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ORIGINAL ARTICLE

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Sex-dependent differences in the ability of nicotine to modulate discrimination learning and cognitive flexibility in mice

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Abstract

Nicotine, an addictive compound found in tobacco, functions as an agonist of nicotinic acetylcholine receptors (nAChRs) in the brain. Interestingly, nicotine has been reported to act as a cognitive enhancer in both human subjects and experimental animals. However, its effects in animal studies have not always been consistent, and sex differences have been identified in the effects of nicotine on several behaviors. Specifically, the role that sex plays in modulating the effects of nicotine on discrimination learning and cognitive flexibility in rodents is still unclear. Here, we evaluated sex-dependent differences in the effect of daily nicotine intraperitoneal (i.p.) administration at various doses (0.125, 0.25, and 0.5 mg/kg) on visual discrimination (VD) learning and reversal (VDR) learning in mice. In male mice, 0.5 mg/kg nicotine significantly improved performance in the VDR, but not the VD, task, while 0.5 mg/kg nicotine significantly worsened performance in the VD, but not VDR task in female mice. Furthermore, 0.25 mg/kg nicotine significantly worsened performance in the VD and VDR task only in female mice. Next, to investigate the cellular mechanisms that underlie the sex difference in the effects of nicotine on cognition, transcriptomic analyses were performed focusing on the medial prefrontal cortex tissue samples from male and female mice that had received continuous administration of nicotine for 3 or 18 days. As a result of pathway enrichment analysis and protein–protein interaction analysis using gene sets of differentially expressed genes, decreased expression of postsynaptic-related genes in males and increased expression of innate immunity-related genes in females were identified as possible molecular mechanisms related to sex differences in the effects of nicotine on cognition in discrimination learning and cognitive flexibility. Our result suggests that nicotine modulates cognitive function in a sex-dependent manner by alternating the expression of specific gene sets in the medial prefrontal cortex.

Abbreviations: i.p., intraperitoneal injection; nAChRs, nicotinic acetylcholine receptors; PFC, prefrontal cortex; P-PI, protein–protein interaction; RNAseq, RNA sequencing; RRIDs (see scicrunch.org), Research Resource Identifier; TF, transcription factor; VD task, visual discrimination; VDR task, reversal.

Yoshiatsu Aomine and Yuto Shimo contributed equally to this work.

KEYWORDS
cognitive flexibility, protein–protein interaction network, reversal learning, RNA-seq, transcription factor analysis, visual discrimination learning

1 | INTRODUCTION

Nicotine, a highly addictive alkaloid contained in tobacco (Gould, 2023; Jarvik, 1991; Mcgrath-Morrow et al., 2020; Zeidler et al., 2007), has been known to induce various physiological changes via its agonistic effect to nicotinic acetylcholine receptors (nAChRs), heterogeneous cationic channels expressed widely in both neural and non-neuronal tissues such as grail and peripheral cells (Gotti et al., 2006; Hollenhorst & Krasteva-Christ, 2021; Zoli et al., 2018). Exogenous nicotine primarily binds at nAChRs expressed at the presynaptic and preterminal axonal shaft of the neurons in the central nervous system, and this promotes the release of excitatory, inhibitory, and neuromodulatory neurotransmitters.

It has been known that the intake and administration of nicotine have significant impacts on our cognitive and emotional functions (Benowitz, 2008; Kim & Picciotto, 2023). Importantly, previous studies in humans and rodents have demonstrated that nicotine can improve cognitive function. For example, a meta-analysis study focusing on human subjects reported facilitative effects of nicotine treatment or smoking on attention, as well as short-term episodic and working memory (Heishman et al., 2010). Supporting this, several other human studies have also reported enhanced cognitive functions such as sustained attention, computational processing, and memory by nicotine treatment (DeVito et al., 2014; Myers et al., 2008; Warburton et al., 1992). Furthermore, in rodent studies, nicotine improved initial learning in the visual discrimination (VD) task in both rats and three strains of mice (Bovet-Nitti, 1966, 1969), and facilitated the reversal learning in the probabilistic reversal learning tasks in mice (Milienne-Petiot et al., 2018). However, conversely, nicotine has also been reported to impair the reversal learning in VD tasks, suggesting that the effect of nicotine on cognitive function largely depends on the type of cognitive tasks (Cole et al., 2015; Ortega et al., 2013).

It should be noted that sex differences in the effects of nicotine have also been reported in the previous studies. In human studies, compared to males, females typically develop nicotine dependence more easily. Females show a depressive tendency during the dependence more often, and they have a stronger negative affective response during its withdrawal (Hogle & Curtin, 2006; Komiyama et al., 2018; Pogun et al., 2017). In preclinical studies, female rats have exhibited greater nicotine self-administration behavior (Chaudhri et al., 2005) and nicotine-induced locomotor sensitization (Booze et al., 1999; Harrod et al., 2004). Moreover, it has been reported that female rats and mice showed a stronger anxiolytic behavioral response immediately after the acute nicotine administration (Cheeta et al., 2001) and during nicotine withdrawal (Caldarone et al., 2008). Although these previous studies have shown the important sex difference of nicotine's effect on animal behaviors, possible sex differences of the effect on cognitive functions, such as discriminative learning and its reversal, have not been tested.

Among nAChR subunits, the α7 subunit is known to have an essential role for the effect of nicotine to facilitate discrimination learning, and treatment of positive allosteric modulation of the α7 subunit itself is sufficient for enhancing recognition memory and cognitive flexibility at least in male rats and mice (Milienne-Petiot et al., 2018; Nikiforuk et al., 2015). On the other hand, it has also been reported that nicotine impaired cognitive flexibility through the activation of the β2 subunit (Cole et al., 2015). Interestingly, other studies have shown that repeated nicotine injections increased the nAChR binding only in male rats but not in female rats (Koylu et al., 1997; Moen & Lee, 2021). Furthermore, the downstream gene expression of nAChRs is also known to be sex-dependent, especially in the brain regions that have been associated with cognitive control, such as the frontal cortices (Friedman & Robbins, 2022). For example, chronic nicotine administration induced an upregulation of the sphingolipid metabolism-related gene *CERKL* in the frontal lobe of male rats, although this gene was downregulated in the female (Vargas-Medrano et al., 2023). Similarly, gestational nicotine exposure increased and decreased the expression of major myelin genes in the prefrontal cortex (PFC) in male and female mice, respectively (Cao et al., 2013). Although these previous studies have identified sex-dependent changes of the expressions of individual genes by nicotine, it is important to investigate more general changes in nicotine-induced gene expressions and compare between male and female by taking advantage of large-scale and unbiased analysis.

Here, we investigate the effects of subchronic intraperitoneal nicotine administration on visual discrimination learning and cognitive flexibility in male and female mice. VD learning and VD reversal (VDR) learning tasks in the Bussey-Saksida touch screen chamber were chosen as behavioral tests, because of its ability to measure complex cognitive functions with minimal researcher interference and its high translational potential between rodent and human studies (Horner et al., 2013; Macpherson & Hikida, 2018; Nishioka et al., 2023). Furthermore, we also investigate gene expression changes in the PFC induced by short-term or long-term subchronic administration of nicotine in both male and female mice, in order to elucidate the molecular mechanisms that may underlie the ability of nicotine to modulate cognitive performance and its difference between the sexes.

2 | MATERIALS AND METHODS

2.1 | Animals

Male ($n = 40$) and female ($n = 44$) C57BL6/JJcl mice obtained from CLEA Japan Inc (Tokyo, Japan) and aged between 8 and 10 weeks were used for all experiments. No sample size calculations were performed. The sample size was determined based on previous studies (Festing, 2018). Mice were housed on a 12-h light/dark cycle (Light: 0800–2000, Dark: 2000–0800) in a quiet environment with room temperature maintained at 24 ± 2°C and 50 ± 5% humidity. Mice were housed according to sex and in groups of between 2 and 5 mice with ad libitum access to food and water until behavioral experiments. The experiment was conducted without aligning the estrus cycles in the females. All animal experiments complied with institutional guidelines set by Osaka University Institute for Protein Research Animal Research Committee (29-02-1 and R04-01-0).

2.2 | Drugs

The nicotine solution was prepared by dissolving nicotine hydrogen tartrate salt (cat. no. SML1236, Sigma-Aldrich, Missouri, USA) in saline (cat. no. 035081517, Otsuka normal saline, Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan). 0.5 mg/kg solution was prepared by dissolving 0.5 mg of nicotine hydrogen tartrate salt in 10 mL of saline; 0.25 and 0.125 mg/kg solutions were prepared by diluting the 0.5 mg/kg solution two and fourfold with saline. The solutions were i.p. administered to mice at a volume of 0.1 mL per gram of mouse weight. Doses of nicotine were selected based on those previously reported to facilitate VD in mice and rats (Bovet-Nitti, 1966, 1969). Four mice were group-housed in each cage. The dose of nicotine to be administered was determined according to stratified randomization.

2.3 | Touchscreen operant chambers

Pretraining and testing were conducted in touchscreen operant chambers (Model 80 614, Campden Instruments Ltd., Loughborough, UK). A partition plate with two square holes (W:7 cm, H:7.5 cm) in the center separated by a 5 mm space in between was fitted in front of an infrared touch screen to create two distinct panels for touch responses. A reward tray connected to the polyvinyl tube and a peristaltic pump for liquid reward delivery was placed on the opposite side of the chamber. The chamber was always kept dark during experiments, with only three light sources: a touch screen light (panel lights), a light on the top of the reward presentation dish (dish light), and a house light. The touchscreen operant chambers were controlled using ABET II (Lafayette Instrument Co., Indiana, USA) and Whisker (Cambridge University Technical Services Ltd., Cambridge, UK) software. Between each task session, the chamber was cleaned with 70% ethanol.

2.4 | Pretraining

Visual discrimination (VD) Tasks in the Touchscreen Operant System were conducted as previously described with minor modification (Horner et al., 2013; Morita et al., 2016). It consisted of six phases: pretraining (*Initial touch training*, *Must touch training*, *Must initiate training*, *Punish incorrect training*), followed by two test periods (VD task, VD Reversal (VDR) task). Mice were individually housed at least 7 days prior to the start of the experiment (Figure 1a). Food consumption was restricted to maintain mice at 85–90% of their initial free-feeding body weight. Mice were fed every day after the completion of the task session. Food was given immediately after the end of the task, that is, all mice were removed from the chamber and had access to food as soon as they returned to their home cages. Water was always available in the home cage.

In *Initial touch training*, during each trial, a visual stimulus was randomly presented on only one panel (Figure 1b). If the mouse did not touch the panel for 30 s, a "regular" reward (7 μL, 20% condensed milk, Morinaga Milk Industry Co. Ltd., Tokyo, Japan) was delivered. if the mouse touched the panel within 30 s, a "tripled" reward (21 μL) was delivered immediately. Rewards were delivered with panel lights off, reward tray lights on, and with an auditory cue (3 kHz, 1 s). When the mouse nose-poked into the reward tray to collect the reward, the reward tray light was turned off and a 20-s interval (ITI) period was initiated. At the end of the ITI, the next trial was initiated. The stimulus was displayed no more than four times consecutively in the same position (left or right panel). Sessions were terminated after the completion of 30 trials or after 60 min. Mice progressed to *Must touch training* following the completion of 30 trials in 60 min.

In *Must touch training*, rewards (7 μL) were delivered only when the mouse touched the visual stimulus panel. Other conditions were the same as in *Initial touch training*. Mice progressed to *Must initiate training* following the completion of 30 trials in 60 min.

In *Must initiate training*, an additional requirement was added, where the mouse was required to make a nose-poke entry into the reward tray to initiate the trial after the completion of a 20-s ITI. Other conditions were the same as in *Must touch training*. Mice progressed to *Punish incorrect training* following the completion of 30 trials in 60 min.

In the *Punish incorrect training*, when the mice were exposed to the incorrect stimulus, the house light was turned on for 5 s to indicate the wrong response. After the house lights were turned off and 20 s of ITI, a correction trial (image shape and left–right position were the same as in the previous trial) was initiated, and the correction trial was repeated until the mouse touched the correct stimulus. Other conditions were the same as in *Must initiate*

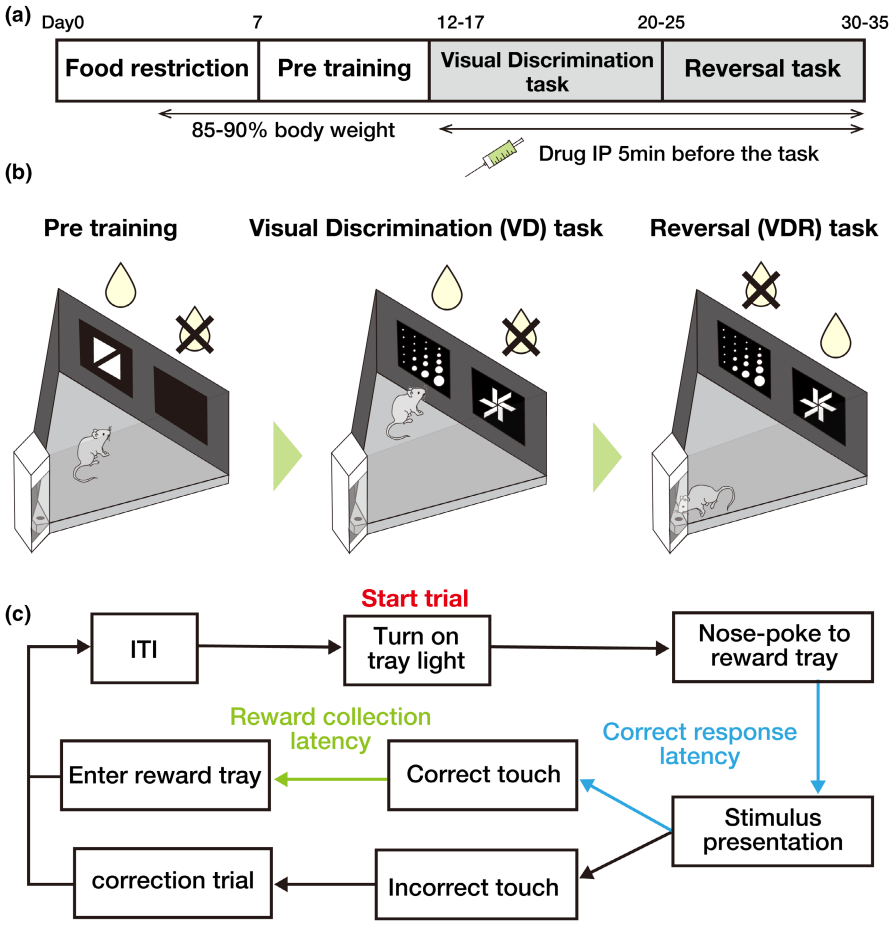

FIGURE 1 Experimental paradigm. (a) In 1 week, mice were confined to a diet that limited their body weight to 85%–90% of their initial weight at day0. IP: Intraperitoneal administration. (b) The schematic diagram of the Bussey-Saksida touchscreen operant chamber and example visual stimuli in the pre-training and each task. (c) Task design for the Visual Discrimination (VD) task and the Reversal (VDR) task. Correct response latency is the time period indicated by blue arrows and reward collection latency is the period indicated by green arrows.

training. Mice progressed to the VD task following the completion of 30 trials in 30 min and achieved at least 23/30 correct responses (76.7%) on two consecutive days (excluding the number of correction trials).

2.5 | Visual discrimination (VD) task

Two different visual stimuli, a dot array pattern and a star pattern, were assigned as either the correct or the incorrect stimulus in a counterbalanced manner among mice and kept consistent throughout VD sessions (Figure 1b). The position (left or right panel) of the correct and incorrect visual stimuli was randomized across trials, with the same stimulus displayed no more than four times consecutively in the same position. Other conditions were the same as in *Punish incorrect training* (Figure 1c). The criterion was defined as the completion of 30 trials in 60 min and the achievement of at least 24/30 correct answers (80.0%) on two consecutive days. In order to compare learning curves between all individuals, all mice performed the task for 8 days before progressing to the Reversal task, regardless of how fast they reached the criterion. Nicotine at different doses (0.125, 0.25, and 0.5 mg/kg), or saline as a control, were administered via intraperitoneal (i.p.) injection to separate experimental groups 5 min prior to each VD session. It should be noted that plasma concentrations of nicotine reach its peak within 5 min after the injection and significantly elevated nicotine level is sustained at least for 1 h. (Jung et al., 1999; Siu & Tyndale, 2007). One female was excluded from the data because it had health problems before the end of the VD task period, and the experiment was terminated.

2.6 | Reversal (VDR) task

In the VDR task, the correct and incorrect stimuli from the previous stage (VD task) were inverted (i.e., if the star pattern stimulus had been correct during the VD task, the dot array pattern stimulus was now correct during the VDR task, and vice versa for the incorrect stimulus; Figure 1b). Other conditions were the same as in the VD task. The criterion was defined as the completion of 30 trials in 60 min and the achievement of at least 24/30 correct answers (80.0%) on two consecutive days. All mice performed this task

for 10 days regardless of how fast they reached the criterion. As with the previous stage, 5 min prior to each VDR session, nicotine at different doses (0.125, 0.25, and 0.5 mg/kg), or saline as a control, were administered via i.p. injection to the same experimental groups as had received them during the VD task. One male was excluded from the data because it had health problems before the end of the VDR task period, and the experiment was terminated.

2.7 | Tissue sampling

Male ($n = 6$) and female ($n = 6$) mice housed under identical conditions to mice used for behavioral experiments were administered daily with a 0.5 mg/kg dose of nicotine or saline as a control. This dose of nicotine was selected because of its largest facilitative or inhibitory effects on the discriminatory behaviors on VD and VDR tasks in male and female, respectively. After 3 and or 18 days, mice were dissected 65 min after nicotine or saline administration, because in the behavioral experiment, we injected nicotine (i.p.) 5 min before the start of VR and VDR training and these training took 60 min on average. After deep anesthesia with isoflurane (3%) and cervical dislocation, brains were removed and immediately cooled and washed in 4°C saline. Brains were sliced into 1-mm thick coronal sections using a brain matrix (cat. no. of BrainSience·idea. CO., Ltd. MBS-S1C, Aster Industries, USA). The medial prefrontal cortex (mPFC; centered on AP = 2.5 mm on anterior side, ML = 0 mm, DV = 2 mm on ventral side, based on bregma (George & Keith, 2019)) was harvested with a 2-mm biopsy punch, immediately placed in 200 μL of RNAlater (Thermo Fisher Scientific, Massachusetts, USA), and stored at 4°C for 24 h, after which time excess RNAlater solution was removed and samples were frozen at −80°C. Three samples were prepared for each condition.

2.8 | Transcriptome analysis (RNAseq)

RNA was extracted from cells using RNeasy plus mini kit (cat. no. 74136, QIAGEN, Venlo, The Netherlands) according to the manufacturer's protocol. Library preparation for RNA sequencing (RNAseq) was performed using a TruSeq stranded mRNA sample prep kit (Illumina, California, USA) according to the manufacturer's instructions. Sequencing was performed on an Illumina NovaSeq 6000 sequencer (RRID:SCR_016387, Illumina) in the 101-base single-end mode. Sequenced reads were mapped to the mouse reference genome sequences (NCBI-RefSeq-GCF_000001635.27_GRCm39) using STAR-2.7.11b. The fragments per kilobase of exon per million mapped fragments (FPKMs) was calculated using RSEM-1.3.3 and TCC-GUI (Su et al., 2019). In TCC-GUI, DEseq2 was used to normalize counts and detect differential expression among genes ($p < 0.05$, $|FC| < 1.1$). Pathway analysis was performed using Enrichr (RRID:SCR_001575; Chen et al., 2013; Kuleshov et al., 2016; Xie et al., 2021). Protein–protein interaction (P-PI) networks and cluster enrichment were analyzed using STRING version 12.0 (RRID:SCR_005223) (Szklarczyk et al., 2023; von Mering et al., 2003, 2005). Transcription Factor (TF) analysis was performed using wPGSA (Kawakami et al., 2016). All results are collected in the supplementary tables (TCC-GUI: Table S2, Enrichr pathway analysis: Table S3, STRING cluster enrichment analysis: Table S4, wPGSA TF analysis: Table S5).

2.9 | Statistical analysis

GraphPad Prism software (RRID:SCR_002798, v.10.2.0, GraphPad Software Inc., California, USA) was used for all statistical analyses. A repeated-measures two-way ANOVA was used for analyzing the percentage of correct responses, the correct response latency, and the reward collection latency. A one-way ANOVA was used to analyze sessions-to-criterion. For all ANOVAs, post-hoc Dunnett's multiple comparisons tests were used for comparisons between nicotine and saline groups. All results of ANOVA are collected in the supplementary table (Table S1). Data were not assessed for normality and no test for outliers was conducted.

3 | RESULTS

3.1 | Nicotine impairs discrimination learning in female, but not male, mice

To examine the effects of nicotine administration on discrimination learning, an important cognitive skill for decision-making, we evaluated the task performance of mice in the VD task. In each session, mice were i.p. administered nicotine or saline 5 min before the start of the task. Male mice administered saline or low (0.125 mg/kg), medium (0.25 mg/kg), or high (0.5 mg/kg) doses of nicotine did not significantly differ in the percentage of correct responses in the VD task (Dose, $F(3, 288) = 0.1402$, $p = 0.9358$; Dose × Days, $F(21, 288) = 0.9014$, $p = 0.5896$; Figure 2a), or in the number of sessions taken to reach the criterion (Dose; $F = 0.5833$, $p = 0.6299$; Figure 2b). Similarly, no significant difference was found between groups in the correct response latency, a measure of locomotion, and in the reward collection latency, a measure of the motivation for the food reward (Figure S1).

In contrast, in female mice, medium and high doses, but not a low dose, of nicotine significantly decreased the percentage of correct responses compared to those administered saline (Dose, $F(3, 312) = 5.992$, $p = 0.0006$; Dose × Days, $F(21, 312) = 0.5884$, $p = 0.9256$; Figure 2c). In addition, high-dose nicotine significantly increased the number of sessions to reach the criterion (Dose, $F = 2.744$, $p = 0.056$; post hoc, saline vs high dose, AdjP = 0.0269; Figure 2d). Similar to male mice, no significant differences were found between groups in the correct response latency and the reward collection latency (Figure S1).

These findings indicate that nicotine impairs VD learning in female mice without affecting locomotion or motivation.

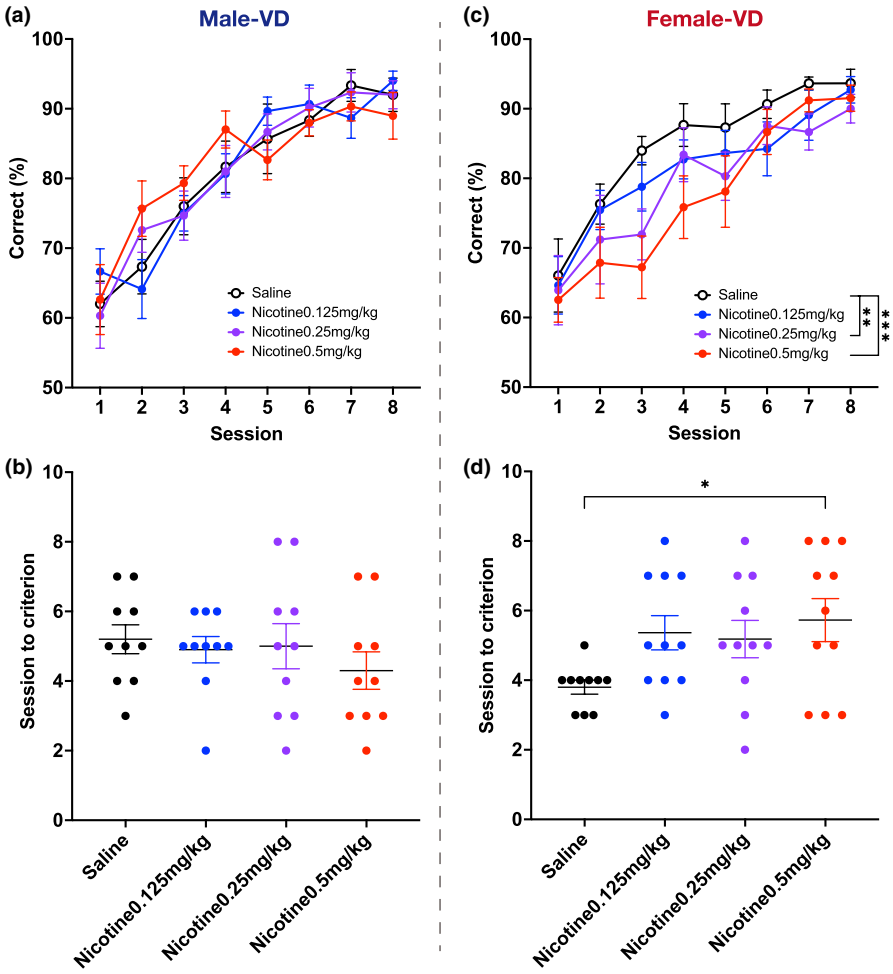

FIGURE 2 Performance on the VD task. Percentage of correct responses in each session and the number of sessions required to reach the criterion in male (a, b) and female (c, d) mice. Data represent the mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ in post-hoc analyses after two-way ANOVA and one-way ANOVA (Male; Saline $n = 10$, Nicotine 0.125 mg/kg $n = 10$, Nicotine 0.25 mg/kg $n = 10$, Nicotine 0.5 mg/kg $n = 10$, Female; Saline $n = 10$, Nicotine 0.125 mg/kg $n = 11$, Nicotine 0.25 mg/kg $n = 11$, Nicotine 0.5 mg/kg $n = 11$).

3.2 | Nicotine increases cognitive flexibility in male mice but decreases it in female mice

Next, to explore the effect of nicotine i.p. administration on cognitive flexibility, and the ability to adaptively shift mental strategies based upon the changing demands of the environment, mice were tested in the VDR task. In this test of reversal learning, the correct and incorrect images from the previous VD task were reversed.

In male mice, high-dose nicotine significantly increased the percentage of correct responses when compared with saline (Dose, $F(3, 230) = 4.579$, $p = 0.0039$; Dose × Days, $F(27, 230) = 0.4542$, $p = 0.9916$; Figure 3a), while low and medium doses of nicotine had no significant effect. Additionally, high-dose nicotine significantly decreased the number of sessions to reach the criterion when compared with saline (Dose, $F = 2.432$, $p = 0.0909$; post hoc, saline vs high dose, AdjP = 0.0378; Figure 3b). No significant differences were found between groups in the correct response latency and the reward collection latency (Figure S1).

In contrast, in female mice, medium-dose nicotine significantly decreased the percentage of correct responses when compared with saline, while low and high doses nicotine dose had no significant effect (Dose, $F(3, 390) = 5.321$, $p = 0.0013$; Dose × Days, $F(27, 390) = 0.5004$, $p = 0.9840$; Figure 3c). No significant effect of nicotine dose was observed on sessions to reach the criterion (Dose; $F = 0.9865$, $p = 0.4091$; Figure 3d). Similarly, no significant differences were found between groups for the correct response latency and the reward collection latency (Figure S2).

Taken together, these results reveal that high-dose nicotine administration increases cognitive flexibility in a VDR task in male mice, while medium-dose nicotine decreases cognitive flexibility in female mice.

3.3 | Genes upregulated after 3 days of nicotine administration

To elucidate cellular alterations resulting from repeated nicotine i.p. administration, as well as their differences in males and females, RNAseq was performed on brain tissue from nicotine-treated mice. Two different time periods were chosen for RNAseq experiments at distinct durations of nicotine treatments. The first time period selected was 3 days of nicotine administration, which served as the short-term treatment condition. This time point was deemed suitable for inducing cognitive impairment in VD performance in female mice. The second time period chosen was days of nicotine administration, which represented the longest duration of nicotine exposure in behavioral experiments. This time period was chosen as the long-term treatment condition. As the high dose (0.5 mg/kg) of nicotine

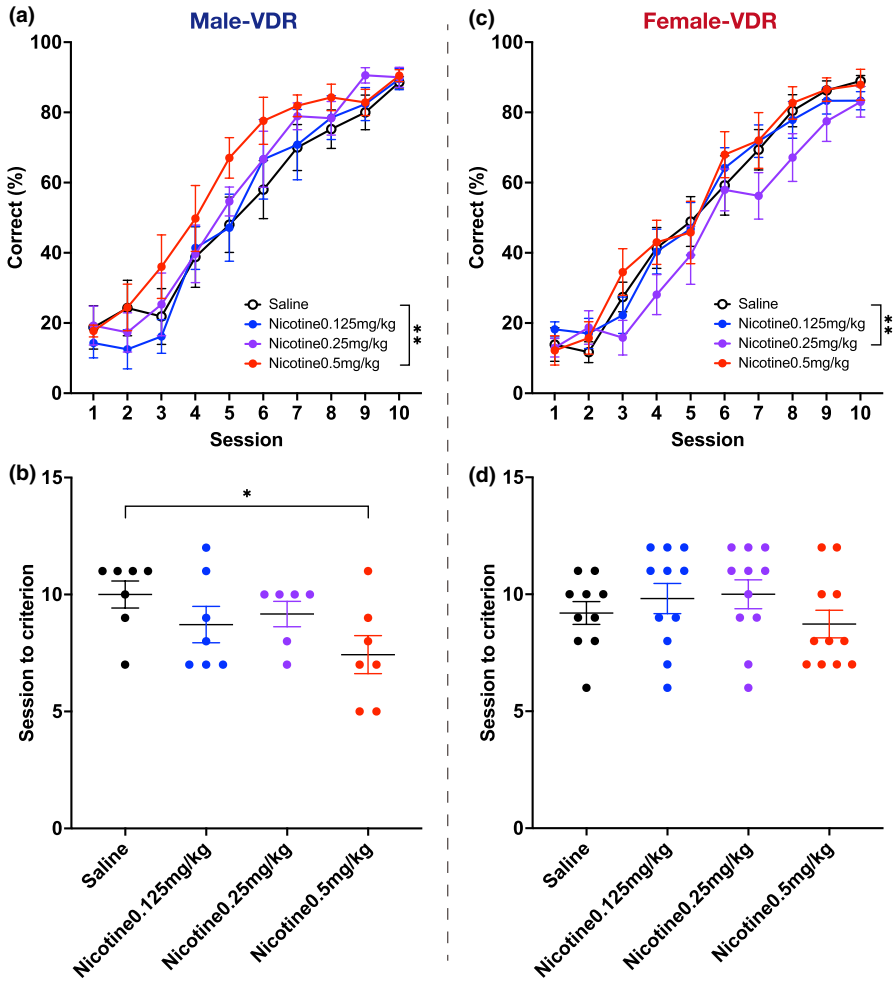

FIGURE 3 Performance on the reversal (VDR) task. Percentage of correct responses in each session and the number of sessions required to reach the criterion in male (a, b) and female (c, d) mice. Data represent the mean ± SEM, $^{**}p < 0.01$ in post-hoc analyses after two-way ANOVA and one-way ANOVA (Male; Saline $n = 10$, Nicotine 0.125 mg/kg $n = 10$, Nicotine 0.25 mg/kg $n = 9$, Nicotine 0.5 mg/kg $n = 10$, Female; Saline $n = 10$, Nicotine 0.125 mg/kg $n = 11$, Nicotine 0.25 mg/kg $n = 11$, Nicotine 0.5 mg/kg $n = 11$).

produced the greatest changes in performance in VD and VDR tasks, this dose was chosen for RNAseq experiments. Finally, the mPFC was chosen as a target for analysis as this brain region has previously been implicated in several cognitive abilities, including discrimination learning and cognitive flexibility (Brigman & Rothblat, 2008; Friedman & Robbins, 2022; Salazar et al., 2004; Wulaer et al., 2020). Thus, differential gene expression in the mPFC was analyzed in four groups, on days 3 and 18 in both males and females. Subsequently, using gene sets with altered expression, P-PI network analysis, pathway analysis, and TF analysis were performed.

P-PI network analysis on the gene set upregulated after 3-day nicotine treatment in male mice generated one large network of six clusters and four small networks. The largest cluster identified by cluster (cluster-by-cluster) enrichment analysis included genes related to "oligodendrocyte differentiation," "axon sheath," and "myelin." The second largest cluster was related to the "PI3K-Akt-mTOR signaling pathway" (Figure 4a, Table S3). No terms were significantly enriched in the pathway analysis when using the two databases (Figure 4b,c). In the female mice, P-PI network analysis generated one large network of three clusters and six smaller networks. In cluster enrichment analysis, "Collagen-containing extracellular matrix" and "PI3K-Akt–mTOR signaling pathway" were enriched in the components of the largest cluster component, "AP-1 transcription factor" and "MAP kinase activation" in the second cluster, and "MHC class II protein complex" in the third cluster (Figure 4d, Table S3). Pathway analysis showed significant enrichment of "Oncostatin M," "Beta-1 integrin cell surface interaction," "BDNF signaling pathway," "Integrins in angiogenesis," and "FRA pathway" in the BioPlanet2019 database, as well as "Integrins in angiogenesis," and "FRA pathway" in Reactome2022 (Figure 4e,f). The female gene set had more significantly enriched terms compared to the male set.

3.4 | Genes upregulated after 18 days of nicotine administration

Using the gene set upregulated after 18-day nicotine treatment in male mice, P-PI network analysis generated two large networks of four clusters and fourteen small networks. Components of the largest clusters were genes related to "AP-1 transcription factor" and "MAP kinase activation" by cluster enrichment analysis (Figure 5a, Table S3). No significant terms were enriched in pathway analysis (Figure 5b,c). In the female mice, the P-PI network analysis generated one large network of twelve clusters and two smaller networks. In cluster enrichment analysis, "Integrin binding," "Growth factor activity," "PI3K-Akt signaling pathway," and

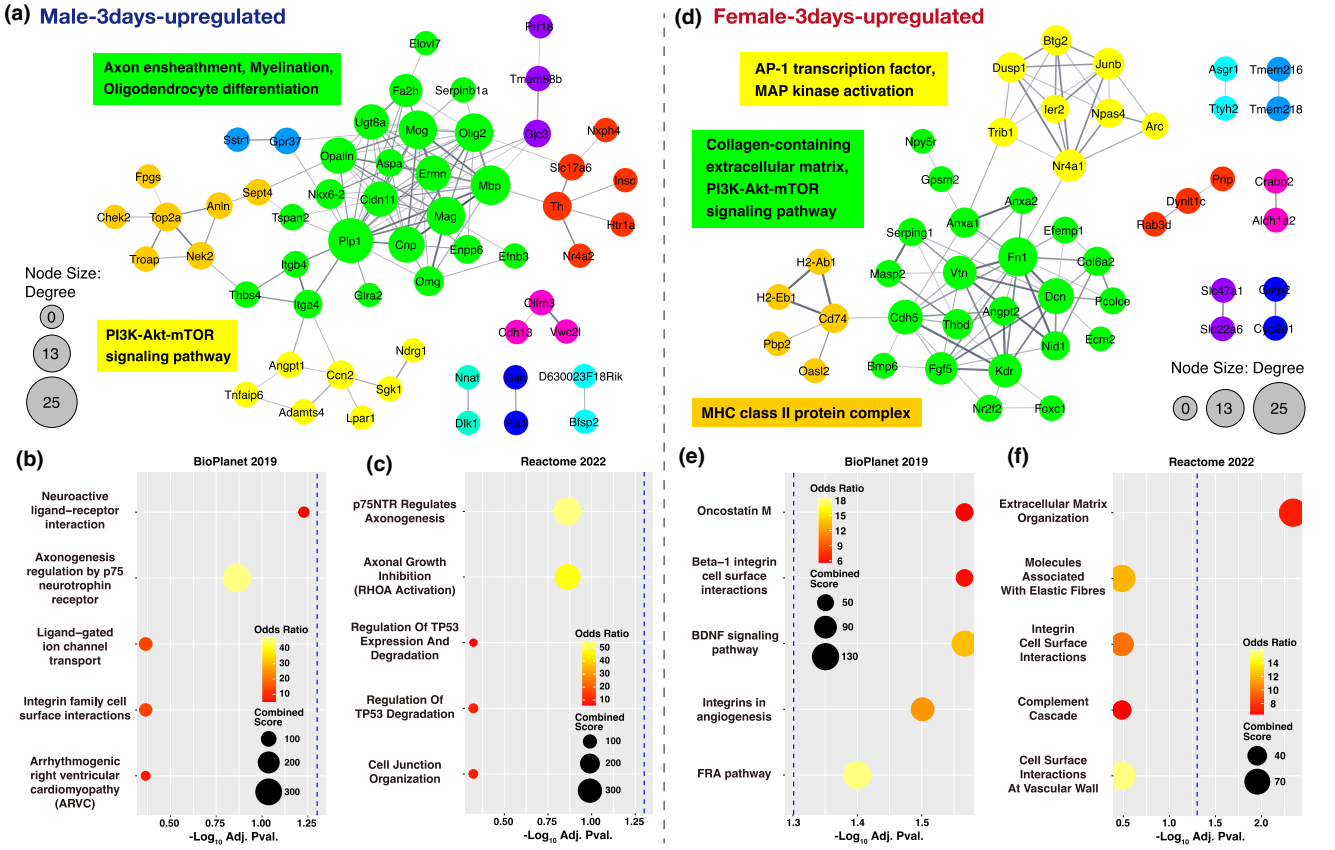

FIGURE 4 Analysis of the gene set upregulated after 3-day nicotine treatment. Results of the P-PI network cluster analysis and pathway analysis in male (a–c) and female (d–f) mice. Cluster and enriched terms for the largest (green), second (yellow), and third (orange) clusters of the P-PI network. The Blue dashed line indicates $p = 0.05$ in the pathway analysis. The results of the cluster enrichment and pathway analyses are summarized in the Tables S3 and S4 (Male; Saline 3 days $n = 3$, Nicotine 0.5 mg/kg 3 days $n = 3$, Female; Saline 3 days $n = 3$, Nicotine 0.5 mg/kg 3 days $n = 3$).

"TGF-beta signaling pathway" were enriched in components of the largest cluster, "Cell Cycle," "Checkpoints," and "Mitotic" in the second cluster, "MHC class II protein complex" in the third cluster, "Innate immune response" in the fourth cluster, and "Myelination" in the fifth cluster (Figure 5d, Table S3). In addition, a small cluster of Fos and Junb, which are AP-1 transcription factors, was found to interact with 6 clusters. Pathway analysis showed significant enrichment of "Interleukin-4 regulation of apoptosis," "BDNF signaling pathway," "Neural crest differentiation," "Transport of glucose and other sugars," "bile salts and organic acids," "metal ions and amine compounds," and "TGF-beta regulation of extracellular matrix" in BioPlanet 2019 (Figure 5e,f). Across all upregulated gene sets, there were more significantly enriched terms in female sets than in the male sets, and an increase of enriched terms as a result of long-term administration was seen only in females.

3.5 | Genes downregulated after 3 days of nicotine administration

Using the gene set downregulated after 3-day nicotine treatment in male mice, P-PI network analysis generated one large network of three clusters and eight small networks. Components of the largest clusters were genes related to "Glutamatergic synapse and Postsynaptic density" and the second cluster included genes related to "Transmembrane receptor protein tyrosine kinase signaling pathway" (Figure 6a, Table S3). "Neural System" was only significantly enriched in Reactome2022 (Figure 6b,c). In the female mice, the P-PI network analysis generated five small networks (Figure 6d, Table S3), and no significant terms were enriched in the pathway analysis (Figure 6e,f). These findings indicate that nicotine has no significant suppressing effect on gene expression in female mice.

3.6 | Genes downregulated after 18 days of nicotine administration

Using the gene set upregulated after 18-day nicotine treatment in male mice, P-PI network analysis generated one large network of nineteen clusters and twelve small networks. In cluster enrichment analysis, "Glutamatergic synapse," "Neurexins and neuroligins," and "Cell junction," "Postsynapse," were enriched in components of the largest cluster, "Transmembrane receptor protein tyrosine kinase signaling pathway" and "Focal adhesion" in the second cluster, and

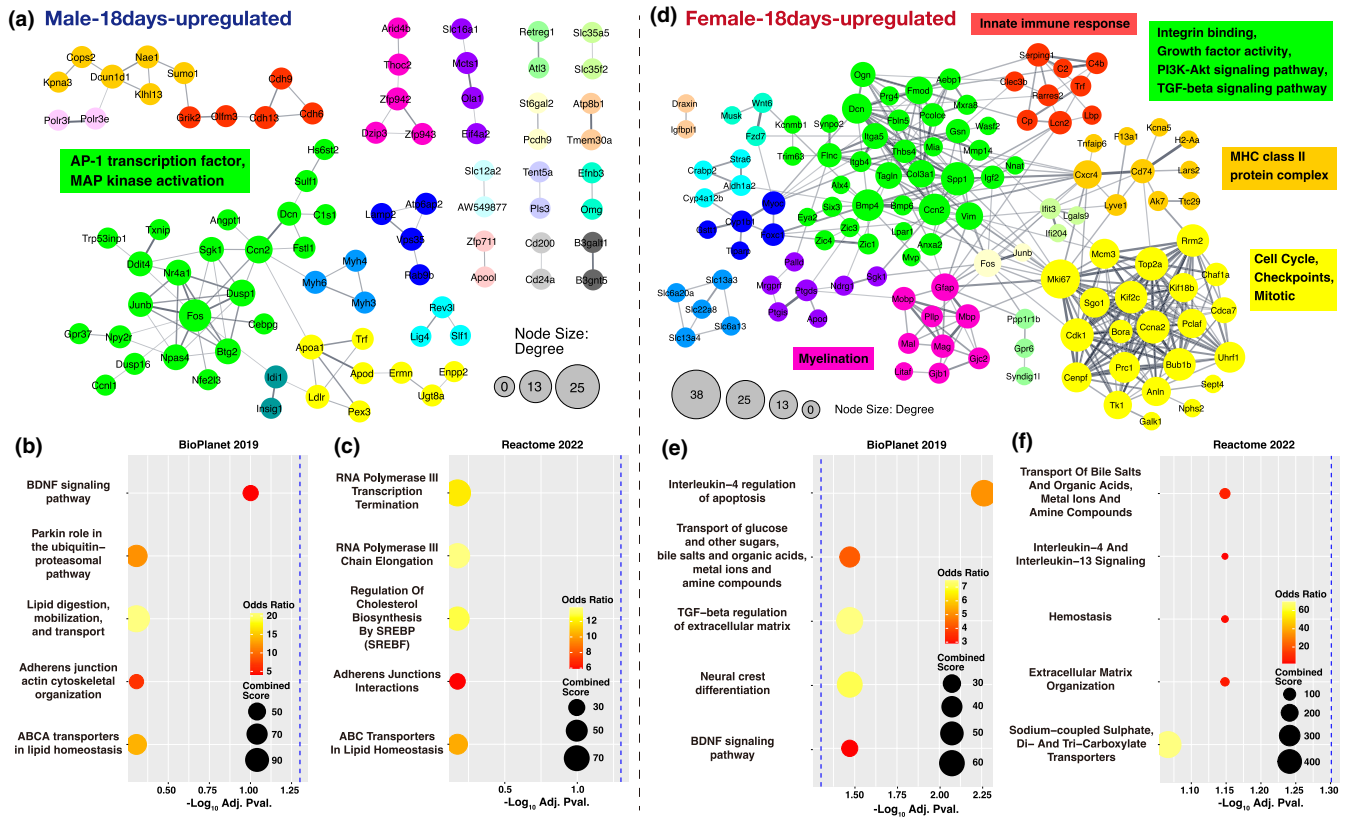

FIGURE 5 Analysis of the gene set upregulated after 18-day nicotine treatment. Results of the P-PI network cluster analysis and pathway analysis in male (a–c) and female (d–f) mice. Cluster and enriched terms for the largest (green), second (yellow), third (orange), fourth (red), and fifth (pink) clusters of the P-PI network. The Blue dashed line indicates $p = 0.05$ in the pathway analysis. The results of the cluster enrichment and pathway analyses are summarized in the Tables S3 and S4 (Male; Saline 18 days $n = 3$, Nicotine 0.5 mg/kg 18 days $n = 3$, Female; Saline 18 days $n = 3$, Nicotine 0.5 mg/kg 18 days $n = 3$).

"Histone deacetylase binding" and "Negative regulation of transcription by RNA polymerase II" in the third cluster (Figure 7a, Table S3). Pathway analysis revealed several pathways that were significantly enriched, including "Neuronal system," "L1CAM interactions," "Interaction between L1-type proteins and ankyrins," "PGC-1a regulation," "Endocytosis," "Neurexins and Neuroligins," "Protein-protein Interactions at Synapses," "Transmission Across Chemical Synapses," and "Activation of NMDA Receptors and Postsynaptic Events" (Figure 7b,c). In the female mice, P-PI network analysis generated only one small network (Figure 7d, Table S3), And no significant terms were enriched in the pathway analysis (Figure 7e,f). Therefore, regardless of the number of doses, nicotine has very little suppressing effect on gene expression in female mice. On the other hand, far more enriched terms were found in the male gene set, and the size of the networks and the number of enriched terms increased as a result of long-term nicotine administration.

4 | DISCUSSION

Here, we investigated the sex-dependent effects of nicotine on discrimination learning and cognitive flexibility in mice for the first time. As a result, we found that nicotine treatment significantly facilitated performance in the VDR task in male mice, while it significantly impaired performance in both VD and VDR task in female mice.

Our finding of the pro-cognitive effect of nicotine in male mice is supported by the previous study, which showed a similar facilitative effect of nicotine on reversal learning in probabilistic tasks in male rats (Milienne-Petiot et al., 2017). However, nicotine's pro-cognitive effects in the male contrast with other previous studies using male mice, in which chronic nicotine administration with osmotic pumps facilitated performance in VD task, but inhibited performance in VDR task (Cole et al., 2015; Ortega et al., 2013). Although the reason for this inconsistency remains unclear, it could be due to differences of the route (i.p. injection vs. oral vs. osmotic pump) and the duration (acute vs. chronic) of nicotine administration. Furthermore, even if the same visual discrimination tasks were used, the difference in the experimental procedures deriving from the specifications of behavioral apparatus might result in the difference in cognitive abilities required for behavioral performance. For example, the effect of nicotine in VD and VDR tasks in previous studies has largely been assessed in the "classic" operant chambers where a single visual cue light located above either correct or incorrect lever guides the choice behaviors (Cole et al., 2015; Milienne-Petiot et al., 2018; Ortega et al., 2013). In this case, animals could earn the reward either by simply approaching or avoiding from single cue (i.e., light

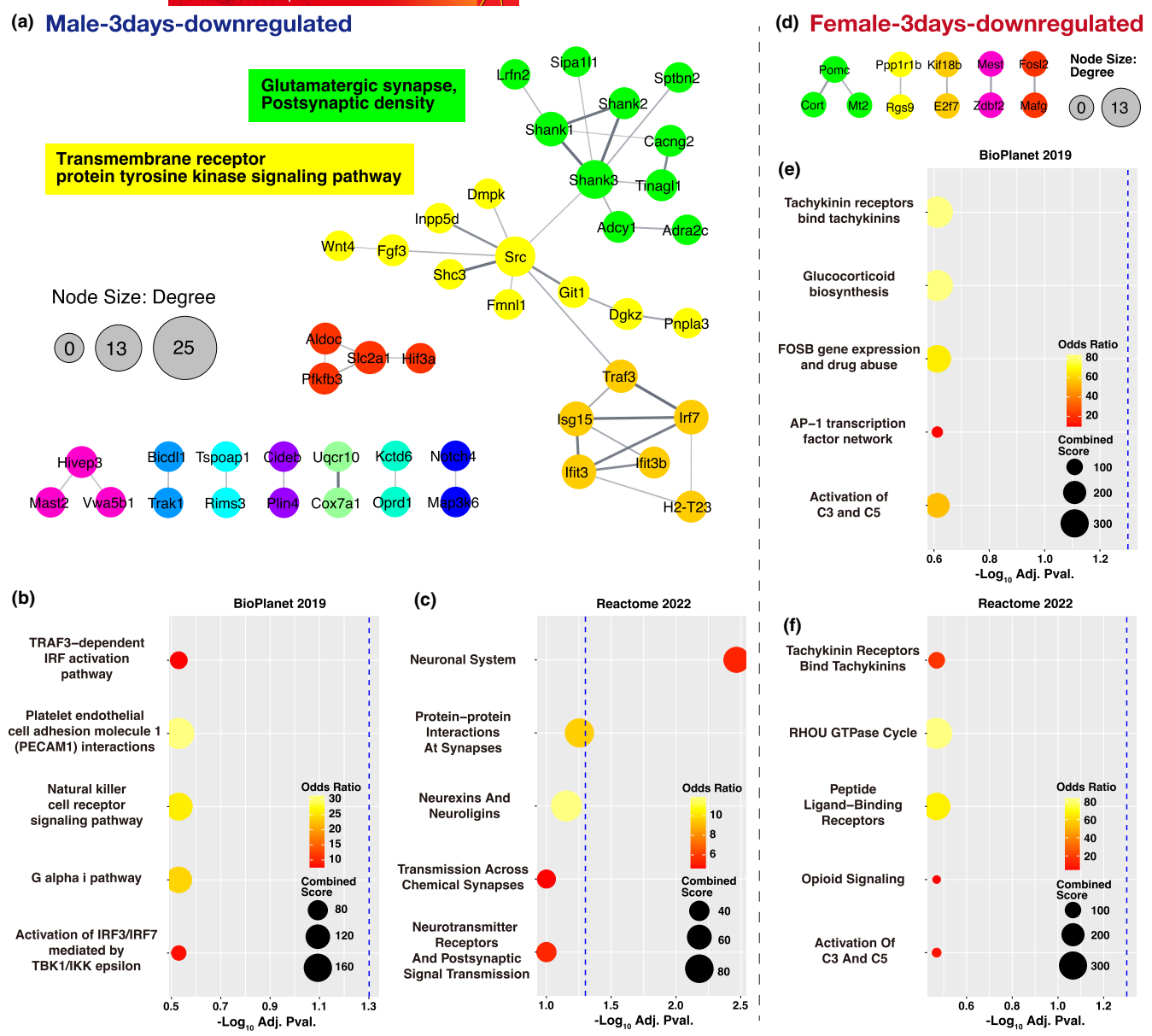

FIGURE 6 Analysis of the gene set downregulated after 3-day nicotine treatment. Results of the P-PI network cluster analysis and pathway analysis in male (a–c) and female (d–f) mice. Clusters and enriched terms for the largest (green) and second (yellow) clusters of the P-PI network. The Blue dashed line indicates $p = 0.05$ in the pathway analysis. The results of the cluster enrichment and pathway analyses are summarized in the Tables S3 and S4 (Male; Saline 3 days $n = 3$, Nicotine 0.5 mg/kg 3 days $n = 3$, Female; Saline 3 days $n = 3$, Nicotine 0.5 mg/kg 3 days $n = 3$).

ON or OFF). However, in our touchscreen chambers, both correct and incorrect visual cues are presented simultaneously. The performance in this task might require greater attentional control to make a correct decision because mice had to discriminate two different visual cues depending on their shape at the same time to guide their choice behavior.

Importantly, in our experiments, nicotine treatment consistently impaired performance in the VD task in female mice, suggesting that the previously reported pro-cognitive effects of nicotine might be limited to male mice, and rather nicotine might have opposite inhibitory effects in females. A previous study using rats reported that the effect of nicotine to increase impulsive choice was much greater in females compared to males (Íbias & Nazarian, 2020), suggesting that nicotine might worsen the discriminative performance in female by increasing short-latency impulsive response in our study. However, this might not be the case, because in our study, there was no significant effect of nicotine on correct response latency in female mice (Figures S1 and S2).

Previous studies have suggested that nicotine affects motivation for food intake. For example, acute and chronic nicotine increased the motivation for sucrose reward in rats (Grimm et al., 2012; Jias & Ellison, 1990; Lacy et al., 2012). On the other hand, other studies have reported that acute self-administration of nicotine or exposure to cigarette smoke decreases the motivation for or intake of sucrose

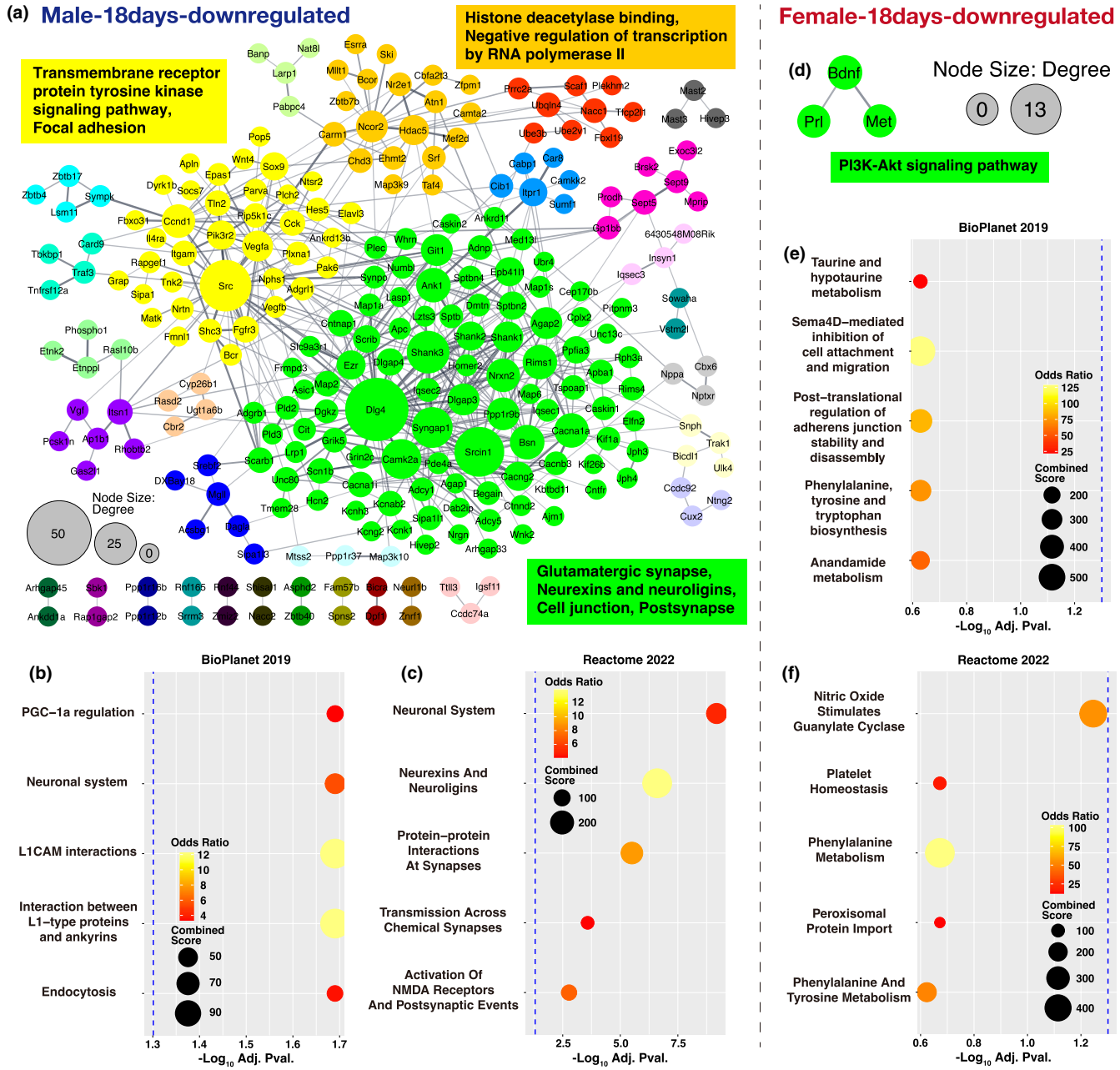

FIGURE 7 Analysis of the gene set downregulated after 18-day nicotine treatment. Results of the P-PI network cluster analysis and pathway analysis in male (a–c) and female (d–f) mice. Clusters and enriched terms for the largest (green), second (yellow), and third (orange) clusters of the P-PI network. The Blue dashed line indicates $p = 0.05$ in the pathway analysis. The results of the cluster enrichment and pathway analyses are summarized in the Tables S3 and S4 (Male; Saline 18 days $n = 3$, Nicotine 0.5 mg/kg 18 days $n = 3$, Female; Saline 18 days $n = 3$, Nicotine 0.5 mg/kg 18 days $n = 3$).

in rats and mice (Bunney et al., 2016; Chen, Vlahos, et al., 2005; Hart et al., 2021). Furthermore, it has also been reported that nicotine pre-exposure did not influence the effects of sucrose to reinforce their seeking behavior in rats (Schwartz et al., 2018). Thus, although the effects seem to be inconsistent, it is possible that nicotine affected discriminative performance by affecting motivation for food in our study. Regarding this point, the analysis of the reward collection latency in our VD and VDR tasks revealed no changes in the motivation for food rewards following nicotine administration in both males and females, suggesting that nicotine-induced changes in performance in VD and VDR tasks in the present study are not attributed to altered motivation (Figures S1 and S2).

To explore the possible alteration in nicotine-induced gene expression underlying the nicotine's sex-dependent effect on cognitive performance, we conducted transcriptomic analysis focusing on the mPFC, a brain region linked with discrimination learning and cognitive flexibility (Brigman & Rothblat, 2008; Friedman & Robbins, 2022; Salazar et al., 2004; Wulaer et al., 2020), in male and female mice treated with nicotine over 3 days or 18 days. Our analyses, specifically the P-PI and pathway analysis, uncovered three

TABLE 1 Three different types of gene sets, whose expressions were distinctively regulated by nicotine administration, and enriched words. The typical genes are hub genes in the P-PI network and cluster.

Gene group	Term	Cluster enrichment analysis	Pathway analysis	Typical genes	Functions
Upregulated in both sex	3 days and 18 days	Regulation of response to external stimulus	PI3K-Akt signaling pathway	Ccn2, DCN	Sex independent effect (early cellular responses)
		AP-1 transcription factor	BDNF signaling pathway	JunB, Fos, Npas4	
Downregulated in male	3 days and 18 days	Modulation of chemical synaptic transmission	Neurexins and neuroligins	Shank1, Shank2, Shank3, Dlg4	Increased performance in the VDR task
		Protein phosphorylation	Transmembrane receptor protein tyrosine kinase signaling pathway	Src, Shc3, Fmnl1, Vegfa, Pik3r2	
Upregulated in female	3 days and 18 days	MHC class II protein complex	Antigen processing and presentation	Cd74, Cxcr4, histocompatibility 2	Impaired performance in the VD and VDR task
	18 days	Nuclear chromosome segregation	Cell cycle Checkpoints, Mitotic	Mki67, Cdk1, Bub1b, Ccna2	
		Innate immune response	Complement and coagulation cascades	C2, C4b, Serping1	
		Myelination	Neural crest differentiation	Gfap, Mbp, Mobp, Mag	

distinct types of gene sets that were regulated by nicotine administration in a sex-specific manner (Table 1). First, there was a gene set whose expressions were upregulated by nicotine across both sexes. Second, there was a gene set whose expressions were downregulated by nicotine specifically in males. Lastly, there was a gene set whose expressions were upregulated by nicotine specifically in females. Our categorization of expressed genes into three groups is also supported by our transcription factor analysis, called wPGSA, which estimates the activities of transcription factors controlling gene expressions (Figure S3). This analysis revealed that more than 30% of the top 30 enriched transcriptional factors in each experimental group were common among all groups. Furthermore, the other 30% of the top 30 enriched transcriptional factors in males and females were specific to each sex.

The first gene set includes the genes upregulated in both sexes by nicotine treatment. Cluster enrichment analysis on the P-PI network showed that nicotine increased the expression of PI3K-Akt signaling pathway-related genes and/or AP-1 transcription factors such as Fos and Junb, regardless of day and sex. Nicotine is known to activate the PI3K-Akt signaling pathway via nAChRs, through which it modulates glucose metabolism, cell cycle progression, and apoptosis (He et al., 2024; West et al., 2003). Nicotine is also known to induce the expression of immediate early genes such as c-fos and junB in various brain regions (Emilio Merlo Nisell et al., 1997; Pich et al., 1997; Schilström et al., 2000). Our results are consistent with these findings. These results indicate that nicotine promotes the PI3K-Akt signaling pathway at downstream of nAChRs, and then activates transcription factors such as Fos and JubB in both males and females. Although activities of these pathways might be involved in some general effect of nicotine, the sex-dependent effect of nicotine on visual discrimination in the current study is not explained.

The second gene set includes the genes downregulated by the nicotine treatments only in males. P-PI network cluster analysis showed that nicotine treatment downregulates specific gene set typified by two large clusters specifically in males; the largest cluster consisted of the genes associated with "glutamatergic synapses" and the second largest cluster consisted of the genes associated with "tyrosine kinase signaling pathway." Pathway analysis also revealed that downregulated genes in males are associated with "neuronal system" supporting the above-mentioned results of P-PI interaction. Importantly, the sizes of these two clusters associated with "glutamatergic synapses" and "tyrosine kinase signaling pathway" were larger in 18-day nicotine treatment group compared with that of the 3-day treatment group suggesting nicotine treatment decreased the expression of genes included in these clusters according to dose or treatment-period dependent manner. The hub genes of the cluster of "glutamatergic synapses" are Shank family, Dlg4 (PSD-95), and Neuroligins. These proteins have been known as the regulators of postsynaptic functions in excitatory synapses, and their dysfunctions have also been known as risk factors for autism (Berkel et al., 2010; Durand et al., 2007; Michael Feyder et al., 2010; Pinto et al., 2010; Sato et al., 2012; Südhof, 2008). PSD-95, Shank, and Neuroligin complex have been known as essential scaffold proteins that are necessary for normal excitatory synaptic function as well as neural plasticity by controlling receptor membrane trafficking and enlargement of postsynaptic structure (Chen, Vinade, et al., 2005; Hayashi et al., 2009; Sala et al., 2001; Südhof, 2008). Mice deficient in Shank, Neuroligin, or PSD-95 complex components have previously been evaluated as mouse models of autism, which showed impaired learning and cognitive flexibility in the selective task, communication, and social behaviors, especially in the context of social interaction (Copping et al., 2017; Feyder et al., 2010; Ponzoni et al., 2019; Qin et al., 2018; Radyushkin et al., 2009; Winkler et al., 2018). Given the roles of these proteins for the consolidation and/or maintenance of acquired memory (Coley & Gao, 2019; Garrido et al., 2022; Xu et al., 2023), it is possible that the nicotine treatment downregulated the expression of the genes

coding these proteins that are important for the maintenance of original associative memory about cue and behavioral response, and then facilitated the acquisition of reversal learning in male mice. Actually, there are previous studies showing that reduction of some components of the PSD-95 protein complex, such a neuroligin-3 or Dlgap2, enhanced cognitive flexibility (Horner et al., 2021). Taken together, our results suggest that the nicotine treatment induces the reduction of scaffold proteins and their associated proteins in the mPFC to facilitate reversal visual discrimination learning.

The third gene set includes the genes upregulated by the nicotine treatments specifically in females. P-PI network cluster analysis showed that nicotine treatment upregulated the large cluster of genes associated with major histocompatibility complex (MHC) class II protein complex in both 3-day and 18-day treatment conditions. The MHC class II protein complex is constitutively expressed in antigen-presenting cells, and intracellular MHC class II molecules bind to antigen and facilitate full activation of adaptive immunity (Liu et al., 2011; Wieczorek et al., 2017). Furthermore, the analysis of the P-PI network in 18-day, but not 3-day, nicotine-treated female mice showed enrichment of "Cell cycle, Checkpoints, Mitotic," "Myelination," and "Innate immune response." In previous studies, nicotine suppressed inflammatory processes in immune cells including microglia by its agonistic effect on the nAChR α7 subunit receptor in male and female mice (De Jonge & Ulloa, 2007; Wang et al., 2003). However, previous studies focusing on female mice showed that nicotine increased the expression of proinflammatory cytokines (Kumar et al., 2024). Importantly, in the present study, the individual analysis of transcriptomics of the five detectable nAChR subunits revealed that α7 subunit expression in female mice was lower than that of males, and the expression in female mice was further reduced after the 18-day nicotine treatment (Figure S4). Taken together, previous studies and our results suggest that nicotine functions as a proinflammatory regulator in female mice that show lower expression level of anti-inflammatory nAChR α7 subunit, and long-term nicotine exposure facilitates inflammation by further reducing nAChR α7 in female mice (Imamura et al., 1994; Neumann et al., 1996). Supporting this, α7 nAChR-deficient mice had higher expression of inflammatory cytokines (Fujii et al., 2007; Zhang et al., 2016). Given that innate immune responses could induce synaptic and circuit dysfunction which increase the vulnerability to cognitive decline and neurodegeneration by aging in humans (Cribbs et al., 2012; Haroon et al., 2017; Turner et al., 2021), it is possible that nicotine inhibits VD performance by activating immune responses in female mice. In addition, our results also showed that long-term nicotine treatment in female mice increased the expression of a common astrocytic marker (Gfap), the factors involved in myelin formation that are mostly expressed in oligodendrocytes (Mbp, Mobp, Mag), and other factors regulating the mitotic cell cycle (Mki67, Cdk1, Bub1b, Ccna2). Interestingly, it has been known that long-term nicotine exposure disrupts cell cycle restriction machinery via the Ras pathway and stimulates G1 cell cycle transition (Chu et al., 2005). Because the neuronal cell cycle is permanently arrested after mitosis (Deneris & Hobert, 2014; Politis et al., 2007), upregulation of cell cycle-related genes by nicotine in female mice might occur in glial cells. These findings suggest that long-term nicotine administration facilitates the actives and cell cycles of glial cells, including microglia, astrocytes, and oligodendrocytes, and impairs the visual discrimination learning in female mice.

In summary, in the present study, we found that subchronic nicotine administration facilitated discrimination learning in male mice, whereas it impaired discrimination and cognitive flexibility in female mice. Furthermore, our transcriptome analysis focusing on the mPFC revealed that nicotine treatment suppressed excitatory postsynaptic-related genes in males but increased the expression of innate immunity-related genes in female mice suggesting these sex-dependent changes in gene expression might underlie the sex-dependent effect of nicotine on the performance of visual discrimination learning. It should be noted that our results do not necessarily guarantee the functional importance of altered gene expression. Therefore, further investigations on focusing intracellular pathways of excitatory postsynaptic mechanism and immunity are necessary to elucidate the actual molecular differences underlying the sex-dependent differences in the effects of nicotine on cognitive functions.

AUTHOR CONTRIBUTIONS

Yoshiatsu Aomine: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; validation; visualization; writing – original draft. **Yuto Shimo:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – review and editing. **Koki Sakurai:** Funding acquisition; methodology; supervision; validation; visualization; writing – original draft. **Mayuka Abe:** Data curation; investigation; resources. **Tom Macpherson:** Funding acquisition; supervision; writing – original draft. **Takaaki Ozawa:** Conceptualization; funding acquisition; project administration; supervision; validation; writing – original draft. **Takatoshi Hikida:** Conceptualization; funding acquisition; project administration; supervision; validation; writing – original draft.

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All experiments were conducted in compliance with the ARRIVE guidelines.

CONFLICT OF INTEREST STATEMENT

All research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Funding agencies had no influence on any part of the experimental design, execution, analysis, and conclusions of this study.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jnc.16227.

DATA AVAILABILITY STATEMENT

All sequencing data and gene count tables are available on DDBJ BioProject (https://www.ddbj.nig.ac.jp/bioproject/index-e.html) accession ID: PRJDB17923 (https://ddbj.nig.ac.jp/search/entry/bioproject/PRJDB17923).

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REFERENCES

Benowitz, N. L. (2008). Clinical pharmacology of nicotine: Implications for understanding, preventing, and treating tobacco addiction. *Clinical Pharmacology and Therapeutics*, *83*(4), 531–541. https://doi.org/10.1038/clpt.2008.3

Berkel, S., Marshall, C. R., Weiss, B., Howe, J., Roeth, R., Moog, U., Endris, V., Roberts, W., Szatmari, P., Pinto, D., Bonin, M., Riess, A., Engels, H., Sprengel, R., Scherer, S. W., & Rappold, G. A. (2010). Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nature Genetics*, *42*(6), 489–491. https://doi.org/10.1038/ng.589

Booze, R. M., Welch, M. A., Wood, M. L., Billings, K. A., Apple, S. R., Mactutus, C. F., Booze, R. M., Welch, M. A., Wood, M. L., Billings, K. A., Apple, S. R., & Behav, C. F. M. (1999). Behavioral sensitization following repeated intravenous nicotine administration: Gender differences and gonadal hormones. *Pharmacology Biochemistry and Behavior*, *64*(4), 827–839.

Bovet-Nitti, F. (1966). Facilitation of simultaneous visual discrimination by nicotine in the rat. *Psychopharmacologia (Berl.)*, *10*, 59–66.

Bovet-Nitti, F. (1969). Facilitation of simultaneous visual discrimination by nicotine in four "inbred" strains of mice. *Psychopharmacologia (Berl.)*, *14*, 193–199.

Brigman, J. L., & Rothblat, L. A. (2008). Stimulus specific deficit on visual reversal learning after lesions of medial prefrontal cortex in the mouse. *Behavioural Brain Research*, *187*(2), 405–410. https://doi.org/10.1016/j.bbr.2007.10.004

Bunney, P. E., Burroughs, D., Hernandez, C., & LeSage, M. G. (2016). The effects of nicotine self-administration and withdrawal on concurrently available chow and sucrose intake in adult male rats. *Physiology and Behavior*, *154*, 49–59. https://doi.org/10.1016/j.physbeh.2015.11.002

Caldarone, B. J., King, S. L., & Picciotto, M. R. (2008). Sex differences in anxiety-like behavior and locomotor activity following chronic nicotine exposure in mice. *Neuroscience Letters*, *439*(2), 187–191. https://doi.org/10.1016/j.neulet.2008.05.023

Cao, J., Wang, J., Dwyer, J. B., Gautier, N. M., Wang, S., Leslie, F. M., & Li, M. D. (2013). Gestational nicotine exposure modifies myelin gene expression in the brains of adolescent rats with sex differences. *Translational Psychiatry*, *3*, e247. https://doi.org/10.1038/tp.2013.21

Chaudhri, N., Caggiula, A. R., Donny, E. C., Booth, S., Gharib, M. A., Craven, L. A., Allen, S. S., Sved, A. F., & Perkins, K. A. (2005). Sex differences in the contribution of nicotine and nonpharmacological stimuli to nicotine self-administration in rats. *Psychopharmacology*, *180*(2), 258–266. https://doi.org/10.1007/s00213-005-2152-3

Cheeta, S., Irvine, E. E., Tucci, S., Sandhu, J., & File, S. E. (2001). In adolescence, female rats are more sensitive to the anxiolytic effect of nicotine than are male rats. *Neuropsychopharmacology*, *25*(4), 601–607. www.acnp.org/citations/Npp

Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., Clark, N. R., & Ma'ayan, A. (2013). Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*, *14*(128), 1–14. https://doi.org/10.1186/1471-2105-14-128

Chen, H., Vlahos, R., Bozinovski, S., Jones, J., Anderson, G. P., & Morris, M. J. (2005). Effect of short-term cigarette smoke exposure on body weight, appetite and brain neuropeptide Y in mice. *Neuropsychopharmacology*, *30*(4), 713–719. https://doi.org/10.1038/sj.npp.1300597

Chen, X., Vinade, L., Leapman, R. D., Petersen, J. D., Nakagawa, T., Phillips, T. M., Sheng, M., & Reese, T. S. (2005). Mass of the postsynaptic density and enumeration of three key molecules. *Proceedings of the National Academy of Sciences*, *102*(32), 11551–11556.

Chu, M., Guo, J., & Chen, C. Y. (2005). Long-term exposure to nicotine, via Ras pathway, induces cyclin D1 to stimulate G1 cell cycle transition. *Journal of Biological Chemistry*, *280*(8), 6369–6379. https://doi.org/10.1074/jbc.M408947200

Cole, R. D., Poole, R. L., Guzman, D. M., Gould, T. J., & Parikh, V. (2015). Contributions of $\beta 2$ subunit-containing nAChRs to chronic nicotine-induced alterations in cognitive flexibility in mice. *Psychopharmacology*, *232*(7), 1207–1217. https://doi.org/10.1007/s00213-014-3754-4

Coley, A. A., & Gao, W. J. (2019). PSD-95 deficiency disrupts PFC-associated function and behavior during neurodevelopment. *Scientific Reports*, *9*(1), 9486. https://doi.org/10.1038/s41598-019-45971-w

Copping, N. A., Berg, E. L., Foley, G. M., Schaffler, M. D., Onaga, B. L., Buscher, N., Silverman, J. L., & Yang, M. (2017). Touchscreen learning deficits and normal social approach behavior in the Shank3B model of Phelan–McDermid syndrome and autism. *Neuroscience*, *345*, 155–165. https://doi.org/10.1016/j.neuroscience.2016.05.016

Cribbs, D. H., Berchtold, N. C., Perreau, V., Coleman, P. D., Rogers, J., Tenner, A. J., & Cotman, C. W. (2012). Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: A microarray study. *Journal of Neuroinflammation*, *9*(179), 1–18. https://doi.org/10.1186/1742-2094-9-179

De Jonge, W. J., & Ulloa, L. (2007). The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. *British Journal of Pharmacology*, *151*(7), 915–929. https://doi.org/10.1038/sj.bjp.0707264

Deneris, E. S., & Hobert, O. (2014). Maintenance of postmitotic neuronal cell identity. *Nature Neuroscience*, *17*(7), 899–907. https://doi.org/10.1038/nn.3731

DeVito, E. E., Herman, A. I., Waters, A. J., Valentine, G. W., & Sofuoglu, M. (2014). Subjective, physiological, and cognitive responses to intravenous nicotine: Effects of sex and menstrual cycle phase. *Neuropsychopharmacology*, *39*(6), 1431–1440. https://doi.org/10.1038/npp.2013.339

Durand, C. M., Betancur, C., Boeckers, T. M., Bockmann, J., Chaste, P., Fauchereau, F., Nygren, G., Rastam, M., Gillberg, I. C., Anckarsäter, H., Sponheim, E., Goubran-Botros, H., Delorme, R., Chabane, N., Mouren-Simeoni, M. C., De Mas, P., Bieth, E., Rogé, B., Héron, D., … Bourgeron, T. (2007). Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nature Genetics*, *39*(1), 25–27. https://doi.org/10.1038/ng1933

Festing, M. F. W. (2018). On determining sample size in experiments involving laboratory animals. *Laboratory Animals*, *52*(4), 341–350. https://doi.org/10.1177/0023677217738268

Feyder, M., Karlsson, R.-M., Mathur, P., Lyman, M., Bock, R., Momenan, R., Munasinghe, J., Scattoni, M. L., Ihne, J., Camp, M., Graybeal, C., Strathdee, D., Begg, A., Alvarez, V. A., Kirsch, P., Rietschel, M., Cichon, S., Walter, H., Meyer-Lindenberg, A., … Holmes, A. (2010). Association of mouse Dlg4 (PSD-95) gene deletion and human DLG4 gene variation with phenotypes relevant to autism Spectrum disorders and Williams' syndrome. *American Journal of Psychiatry*, *12*(167), 1508–1517. https://doi.org/10.1176/appi.ajp.2010.10040484

Friedman, N. P., & Robbins, T. W. (2022). The role of prefrontal cortex in cognitive control and executive function. *Neuropsychopharmacology*, *47*(1), 72–89. https://doi.org/10.1038/s41386-021-01132-0

Fujii, Y. X., Fujigaya, H., Moriwaki, Y., Misawa, H., Kasahara, T., Grando, S. A., & Kawashima, K. (2007). Enhanced serum antigen-specific IgG1 and proinflammatory cytokine production in nicotinic acetylcholine receptor $\alpha 7$ subunit gene knockout mice. *Journal of Neuroimmunology*, *189*(1–2), 69–74. https://doi.org/10.1016/j.jneuroim.2007.07.003

Garrido, D., Beretta, S., Grabrucker, S., Bauer, H. F., Bayer, D., Sala, C., Verpelli, C., Roselli, F., Bockmann, J., Proepper, C., Catanese, A., & Boeckers, T. M. (2022). Shank2/3 double knockout-based screening of cortical subregions links the retrosplenial area to the loss of social memory in autism spectrum disorders. *Molecular Psychiatry*, *27*(12), 4994–5006. https://doi.org/10.1038/s41380-022-01756-8

George, P., & Keith, B. J. F. (2019). *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*. Elsevier.

Gotti, C., Zoli, M., & Clementi, F. (2006). Brain nicotinic acetylcholine receptors: Native subtypes and their relevance. *Trends in Pharmacological Sciences*, *27*(9), 482–491. https://doi.org/10.1016/j.tips.2006.07.004

Gould, T. J. (2023). Epigenetic and long-term effects of nicotine on biology, behavior, and health. In *Pharmacological research* (Vol. *192*, p. 106741). Academic Press. https://doi.org/10.1016/j.phrs.2023.106741

Grimm, J. W., Ratliff, C., North, K., Barnes, J., & Collins, S. (2012). Nicotine increases sucrose self-administration and seeking in rats. *Addiction Biology*, *17*(3), 623–633. https://doi.org/10.1111/j.1369-1600.2012.00436.x

Haroon, E., Miller, A. H., & Sanacora, G. (2017). Inflammation, glutamate, and glia: A trio of trouble in mood disorders. *Neuropsychopharmacology*, *42*(1), 193–215. https://doi.org/10.1038/npp.2016.199

Harrod, S. B., Mactutus, C. F., Bennett, K., Hasselrot, U., Wu, G., Welch, M., & Booze, R. M. (2004). Sex differences and repeated intravenous nicotine: Behavioral sensitization and dopamine receptors. *Pharmacology Biochemistry and Behavior*, *78*(3), 581–592. https://doi.org/10.1016/j.pbb.2004.04.026

Hart, E., Hertia, D., Barrett, S. T., & Charntikov, S. (2021). Varenicline rescues nicotine-induced decrease in motivation for sucrose reinforcement. *Behavioural Brain Research*, *397*, 112887. https://doi.org/10.1016/j.bbr.2020.112887

Hayashi, M. K., Tang, C., Verpelli, C., Narayanan, R., Stearns, M. H., Xu, R. M., Li, H., Sala, C., & Hayashi, Y. (2009). The postsynaptic density proteins Homer and Shank form a polymeric network structure. *Cell*, *137*(1), 159–171. https://doi.org/10.1016/j.cell.2009.01.050

He, Z., Xu, Y., Rao, Z., Zhang, Z., Zhou, J., Zhou, T., & Wang, H. (2024). The role of α7-nAChR-mediated PI3K/AKT pathway in lung cancer induced by nicotine. *Science of the Total environment*, *20*(912), 169604. https://doi.org/10.1016/j.scitotenv.2023.169604

Heishman, S. J., Kleykamp, B. A., & Singleton, E. G. (2010). Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology*, *210*(4), 453–469. https://doi.org/10.1007/s00213-010-1848-1

Hogle, J. M., & Curtin, J. J. (2006). Sex differences in negative affective response during nicotine withdrawal. *Psychophysiology*, *43*(4), 344–356. https://doi.org/10.1111/j.1469-8986.2006.00406.x

Hollenhorst, M. I., & Krasteva-Christ, G. (2021). Nicotinic acetylcholine receptors in the respiratory tract. *Molecules*, *26*(20), 1–17. https://doi.org/10.3390/molecules26206097

Horner, A. E., Heath, C. J., Hvoslef-Eide, M., Kent, B. A., Kim, C. H., Nilsson, S. R. O., Alsiö, J., Oomen, C. A., Holmes, A., Saksida, L. M., & Bussey, T. J. (2013). The touchscreen operant platform for testing learning and memory in rats and mice. *Nature Protocols*, *8*(10), 1961–1984. https://doi.org/10.1038/nprot.2013.122

Horner, A. E., Norris, R. H., McLaren-Jones, R., Alexander, L., Komiyama, N. H., Grant, S. G. N., Nithianantharajah, J., & Kopanitsa, M. V. (2021). Learning and reaction times in mouse touchscreen tests are differentially impacted by mutations in genes encoding postsynaptic interacting proteins SYNGAP1, NLGN3, DLGAP1, DLGAP2 and SHANK2. *Genes, Brain and Behavior*, *20*(1), e12723. https://doi.org/10.1111/gbb.12723

Íbias, J., & Nazarian, A. (2020). Sex differences in nicotine-induced impulsivity and its reversal with bupropion in rats. *Journal of Psychopharmacology*, *34*(12), 1382–1392. https://doi.org/10.1177/0269881120937543

Imamura, K., Suzumura, A., Sawada, M., Mabuchi, C., & Marunouchi, T. (1994). Induction of MHC class II antigen expression on murine microglia by interleukin-3. *Journal of Neuroimmunology*, *55*, 119–125.

Jarvik, M. E. (1991). Beneficial effects of nicotine. *British Journal of Addiction*, *86*(5), 571–575. https://doi.org/10.1111/j.1360-0443.1991.tb01810.x

Jias, L. M., & Ellison, G. (1990). Chronic nicotine induces a specific appetite for sucrose in rats. *Pharmacology, Biochemistry, and Behavior*, *35*(2), 489–491. https://doi.org/10.1016/0091-3057(90)90192-K

Jung, B. H., Chung, B. C., Chung, S.-J., Lee, M.-H., & Shim, C.-K. (1999). Simultaneous GC-MS determination of nicotine and cotinine in plasma for the pharmacokinetic characterization of nicotine in rats. *Journal of Pharmaceutical and Biomedical Analysis*, *20*, 195–202.

Kawakami, E., Nakaoka, S., Ohta, T., & Kitano, H. (2016). Weighted enrichment method for prediction of transcription regulators from transcriptome and global chromatin immunoprecipitation data. *Nucleic Acids Research*, *44*(11), 5010–5021. https://doi.org/10.1093/nar/gkw355

Kim, K., & Picciotto, M. R. (2023). Nicotine addiction: More than just dopamine. *Current Opinion in Neurobiology*, *83*, 102797. https://doi.org/10.1016/j.conb.2023.102797

Komiyama, M., Yamakage, H., Satoh-Asahara, N., Ozaki, Y., Morimoto, T., Shimatsu, A., Takahashi, Y., & Hasegawa, K. (2018). Sex differences

in nicotine dependency and depressive tendency among smokers. *Psychiatry Research*, *267*, 154–159. https://doi.org/10.1016/j.psychres.2018.06.010

Koylu, E., Demirgoren, S., London, E. D., & Pogiin, S. (1997). Sex difference in up-regulation of nicotinic acetylcholine receptors in rat brain. *Life Sciences*, *61*(12), 5–PL190.

Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S. L., Jagodnik, K. M., Lachmann, A., McDermott, M. G., Monteiro, C. D., Gundersen, G. W., & Maayan, A. (2016). Enrichr: A comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research*, *44*(1), W90–W97. https://doi.org/10.1093/nar/gkw377

Kumar, M., Keady, J., Aryal, S. P., Hessing, M., Richards, C. I., & Turner, J. R. (2024). The role of microglia in sex- and region-specific blood-brain barrier integrity during nicotine withdrawal. *Biological Psychiatry Global Open Science*, *4*(1), 182–193. https://doi.org/10.1016/j.bpsgos.2023.08.019

Lacy, R. T., Hord, L. L., Morgan, A. J., & Harrod, S. B. (2012). Intravenous gestational nicotine exposure results in increased motivation for sucrose reward in adult rat offspring. *Drug and Alcohol Dependence*, *124*(3), 299–306. https://doi.org/10.1016/j.drugalcdep.2012.01.025

Liu, X., Zhan, Z., Li, D., Xu, L., Ma, F., Zhang, P., Yao, H., & Cao, X. (2011). Intracellular MHC class II molecules promote TLR-triggered innate immune responses by maintaining activation of the kinase Btk. *Nature Immunology*, *12*(5), 416–424. https://doi.org/10.1038/ni.2015

Macpherson, T., & Hikida, T. (2018). Nucleus accumbens dopamine D1-receptor-expressing neurons control the acquisition of sign-tracking to conditioned cues in mice. *Frontiers in Neuroscience*, *12*(418), 1–6. https://doi.org/10.3389/fnins.2018.00418

Mcgrath-Morrow, S. A., Gorzkowski, J., Groner, J. A., Rule, A. M., Wilson, K., Tanski, S. E., Collaco, J. M., & Klein, J. D. (2020). The effects of nicotine on development. *Pediatrics*, *145*(3), e20191346. https://doi.org/10.1542/peds.2019-1346

Milienne-Petiot, M., Higa, K. K., Grim, A., Deben, D., Groenink, L., Twamley, E. W., Geyer, M. A., & Young, J. W. (2018). Nicotine improves probabilistic reward learning in wildtype but not alpha7 nAChR null mutants, yet alpha7 nAChR agonists do not improve probabilistic learning. *European Neuropsychopharmacology*, *28*(11), 1217–1231. https://doi.org/10.1016/j.euroneuro.2018.08.005

Milienne-Petiot, M., Kesby, J. P., Graves, M., van Enkhuizen, J., Semenova, S., Minassian, A., Markou, A., Geyer, M. A., & Young, J. W. (2017). The effects of reduced dopamine transporter function and chronic lithium on motivation, probabilistic learning, and neurochemistry in mice: Modeling bipolar mania. *Neuropharmacology*, *113*, 260–270. https://doi.org/10.1016/j.neuropharm.2016.07.030

Moen, J. K., & Lee, A. M. (2021). Sex differences in the nicotinic acetylcholine receptor system of rodents: Impacts on nicotine and alcohol reward behaviors. In *Frontiers in Neuroscience*, *15*, 1–14. https://doi.org/10.3389/fnins.2021.745783

Morita, M., Wang, Y., Sasaoka, T., Okada, K., Niwa, M., Sawa, A., & Hikida, T. (2016). Dopamine D2L receptor is required for visual discrimination and reversal learning. *Molecular Neuropsychiatry*, *2*(3), 124–132. https://doi.org/10.1159/000447970

Myers, C. S., Taylor, R. C., Moolchan, E. T., & Heishman, S. J. (2008). Dose-related enhancement of mood and cognition in smokers administered nicotine nasal spray. *Neuropsychopharmacology*, *33*(3), 588–598. https://doi.org/10.1038/sj.npp.1301425

Neumann, H., Boucraut, J., Hahnel, C., Misgeld, T., & Wekerle, H. (1996). Neuronal control of MHC class II inducibility in rat astrocytes and microglia. *European Journal of Neuroscience*, *8*(12), 2582–2590. https://doi.org/10.1111/j.1460-9568.1996.tb01552.x

Nikiforuk, A., Kos, T., Potasiewicz, A., & Popik, P. (2015). Positive allosteric modulation of alpha 7 nicotinic acetylcholine receptors enhances recognition memory and cognitive flexibility in rats. *European Neuropsychopharmacology*, *25*(8), 1300–1313. https://doi.org/10.1016/j.euroneuro.2015.04.018

Nisell, M., Nomikos, G. G., Chergui, K., Grillner, P., & Svensson, T. H. (1997). Chronic nicotine enhances basal and nicotine-induced fos immunoreactivity preferentially in the medial prefrontal cortex of the rat. *Neuropsychopharmacology*, *17*(3), 151–161.

Nishioka, T., Attachaipanich, S., Hamaguchi, K., Lazarus, M., de Kerchove d'Exaerde, A., Macpherson, T., & Hikida, T. (2023). Error-related signaling in nucleus accumbens D2 receptor-expressing neurons guides inhibition-based choice behavior in mice. *Nature Communications*, *14*(1), 2284. https://doi.org/10.1038/s41467-023-38025-3

Ortega, L. A., Tracy, B. A., Gould, T. J., & Parikh, V. (2013). Effects of chronic low- and high-dose nicotine on cognitive flexibility in C57BL/6J mice. *Behavioural Brain Research*, *238*(1), 134–145. https://doi.org/10.1016/j.bbr.2012.10.032

Pich, E. M., Pagliusi, S. R., Tessari, M., Talabot-Ayer, D., van Huijsduijnen, R. H., & Chiamulera, C. (1997). Common neural substrates for the addictive properties of nicotine and cocaine. *Science*, *275*, 83–86. https://doi.org/10.1126/science.275.5296.83

Pinto, D., Pagnamenta, A. T., Klei, L., Anney, R., Merico, D., Regan, R., Conroy, J., Magalhaes, T. R., Correia, C., Abrahams, B. S., Almeida, J., Bacchelli, E., Bader, G. D., Bailey, A. J., Baird, G., Battaglia, A., Berney, T., Bolshakova, N., Bölte, S., … Betancur, C. (2010). Functional impact of global rare copy number variation in autism spectrum disorders. *Nature*, *466*(7304), 368–372. https://doi.org/10.1038/nature09146

Pogun, S., Yararbas, G., Nesil, T., & Kanit, L. (2017). Sex differences in nicotine preference. *Journal of Neuroscience Research*, *95*(1–2), 148–162. https://doi.org/10.1002/jnr.23858

Politis, P. K., Makri, G., Thomaidou, D., Geissen, M., Rohrer, H., & Matsas, R. (2007). BM88/CEND1 coordinates cell cycle exit and differentiation of neuronal precursors. *Proceedings of the National Academy of Sciences*, *115*, 17861–17866.

Ponzoni, L., Sala, C., Verpelli, C., Sala, M., & Braida, D. (2019). Different attentional dysfunctions in eEF2K−/−, IL1RAPL1−/− and SHANK3Δ11−/− mice. *Genes, Brain and Behavior*, *18*(5), e12563. https://doi.org/10.1111/gbb.12563

Qin, L., Ma, K., Wang, Z. J., Hu, Z., Matas, E., Wei, J., & Yan, Z. (2018). Social deficits in Shank3-deficient mouse models of autism are rescued by histone deacetylase (HDAC) inhibition. *Nature Neuroscience*, *21*(4), 564–575. https://doi.org/10.1038/s41593-018-0110-8

Radyushkin, K., Hammerschmidt, K., Boretius, S., Varoqueaux, F., El-Kordi, A., Ronnenberg, A., Winter, D., Frahm, J., Fischer, J., Brose, N., & Ehrenreich, H. (2009). Neuroligin-3-deficient mice: Model of a monogenic heritable form of autism with an olfactory deficit. *Genes, Brain and Behavior*, *8*(4), 416–425. https://doi.org/10.1111/j.1601-183X.2009.00487.x

Sala, C., Pië ch, V., Wilson, N. R., Passafaro, M., Liu, G., & Sheng, M. (2001). Regulation of dendritic spine morphology and synaptic function by shank and Homer. *Neuron*, *31*, 115–130.

Salazar, R. F., White, W., Lacroix, L., Feldon, J., & White, I. M. (2004). NMDA lesions in the medial prefrontal cortex impair the ability to inhibit responses during reversal of a simple spatial discrimination. *Behavioural Brain Research*, *152*(2), 413–424. https://doi.org/10.1016/j.bbr.2003.10.034

Sato, D., Lionel, A. C., Leblond, C. S., Prasad, A., Pinto, D., Walker, S., O'Connor, I., Russell, C., Drmic, I. E., Hamdan, F. F., Michaud, J. L., Endris, V., Roeth, R., Delorme, R., Huguet, G., Leboyer, M., Rastam, M., Gillberg, C., Lathrop, M., … Scherer, S. W. (2012). SHANK1 deletions in males with autism spectrum disorder. *American Journal of Human Genetics*, *90*(5), 879–887. https://doi.org/10.1016/j.ajhg.2012.03.017

Schilström, B., De Villiers, S., Malmerfelt, A., Svensson, T. H., & Nomikos, G. G. (2000). Nicotine-induced fos expression in the nucleus accumbens and the medial prefrontal cortex of the rat: Role of nicotinic and NMDA receptors in the ventral tegmental area. *Synapse*,

36(4), 314–321. https://doi.org/10.1002/(SICI)1098-2396(20000615)36:4<314::AID-SYN8>3.0.CO;2-U

Schwartz, L. P., Kearns, D. N., & Silberberg, A. (2018). The effect of nicotine pre-exposure on demand for cocaine and sucrose in male rats. *Behavioural Pharmacology*, *29*(4), 316–326. https://doi.org/10.1097/FBP.0000000000000357

Siu, E. C. K., & Tyndale, R. F. (2007). Characterization and comparison of nicotine and cotinine metabolism in vitro and in vivo in DBA/2 and C57BL/6 mice. *Molecular Pharmacology*, *71*(3), 826–834. https://doi.org/10.1124/mol.106.032086

Su, W., Sun, J., Shimizu, K., & Kadota, K. (2019). TCC-GUI: A shiny-based application for differential expression analysis of RNA-Seq count data. *BMC Research Notes*, *12*(1), 133. https://doi.org/10.1186/s13104-019-4179-2

Südhof, T. C. (2008). Neuroligins and neurexins link synaptic function to cognitive disease. *Nature*, *455*(7215), 903–911. https://doi.org/10.1038/nature07456

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & Von Mering, C. (2023). The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, *51*(1 D), D638–D646. https://doi.org/10.1093/nar/gkac1000

Turner, J. A., Padgett, C., McDonald, S., Ahuja, K. D. K., Francis, H. M., Lim, C. K., & Honan, C. A. (2021). Innate immunity impacts social-cognitive functioning in people with multiple sclerosis and healthy individuals: Implications for IL-1ra and urinary immune markers. *Brain, Behavior, and Immunity—Health*, *14*, 100254. https://doi.org/10.1016/j.bbih.2021.100254

Vargas-Medrano, J., Carcoba, L. M., Vidal Martinez, G., Mulla, Z. D., Diaz, V., Ruiz-Velasco, A., Alvarez-Primo, F., Colina, G., Iñiguez, S. D., Thompson, P. M., O'Dell, L. E., & Gadad, B. S. (2023). Sex and diet-dependent gene alterations in human and rat brains with a history of nicotine exposure. *Frontiers in Psychiatry*, *14*, 1–14. https://doi.org/10.3389/fpsyt.2023.1104563

von Mering, C., Huynen, M., Jaeggi, D., Schmidt, S., Bork, P., & Snel, B. (2003). STRING: A database of predicted functional associations between proteins. *Nucleic Acids Research*, *31*(1), 258–261. https://doi.org/10.1093/nar/gkg034

von Mering, C., Jensen, L. J., Snel, B., Hooper, S. D., Krupp, M., Foglierini, M., Jouffre, N., Huynen, M. A., & Bork, P. (2005). STRING: Known and predicted protein-protein associations, integrated and transferred across organisms. *Nucleic Acids Research*, *33*, D433–D437. https://doi.org/10.1093/nar/gki005

Wang, H., Yu, M., Ochani, M., Amelia, C. A., Tanovic, M., Susarla, S., Li, J. H., Wang, H., Yang, N., Ulloa, L., Al-Abed, Y., Czura, C. J., & Tracey, K. J. (2003). Nicotinic acetylcholine receptor $\alpha 7$ subunit is an essential regulator of inflammation. *Nature*, *421*(6921), 384–388. https://doi.org/10.1038/nature01339

Warburton, D. M., Rusted, J. M., & Fowler, J. (1992). A comparison of the attentional and consolidation hypotheses for the facilitation of memory by nicotine. *Psychopharmacology*, *108*, 443–447.

West, K. A., Brognard, J., Clark, A. S., Linnoila, I. R., Yang, X., Swain, S. M., Harris, C., Belinsky, S., & Dennis, P. A. (2003). Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. *Journal of Clinical Investigation*, *111*(1), 81–90. https://doi.org/10.1172/JCI200316147

Wieczorek, M., Abualrous, E. T., Sticht, J., Álvaro-Benito, M., Stolzenberg, S., Noé, F., & Freund, C. (2017). Major histocompatibility complex (MHC) class I and MHC class II proteins: Conformational plasticity in antigen presentation. *Frontiers in Immunology*, *8*(292), 1–16. https://doi.org/10.3389/fimmu.2017.00292

Winkler, D., Daher, F., Wüstefeld, L., Hammerschmidt, K., Poggi, G., Seelbach, A., Krueger-Burg, D., Vafadari, B., Ronnenberg, A., Liu, Y., Kaczmarek, L., Schlüter, O. M., Ehrenreich, H., & Dere, E. (2018). Hypersocial behavior and biological redundancy in mice with reduced expression of PSD95 or PSD93. *Behavioural Brain Research*, *352*, 35–45. https://doi.org/10.1016/j.bbr.2017.02.011

Wulaer, B., Hada, K., Sobue, A., Itoh, N., Nabeshima, T., Nagai, T., & Yamada, K. (2020). Overexpression of astroglial major histocompatibility complex class I in the medial prefrontal cortex impairs visual discrimination learning in mice. *Molecular Brain*, *13*(1), 1–12. https://doi.org/10.1186/s13041-020-00710-5

Xie, Z., Bailey, A., Kuleshov, M. V., Clarke, D. J. B., Evangelista, J. E., Jenkins, S. L., Lachmann, A., Wojciechowicz, M. L., Kropiwnicki, E., Jagodnik, K. M., Jeon, M., & Ma'ayan, A. (2021). Gene set knowledge discovery with Enrichr. *Current Protocols*, *1*(3), e90. https://doi.org/10.1002/cpz1.90

Xu, Y., Cui, T. L., Li, J. Y., Chen, B., & Wang, J. H. (2023). Associative memory neurons of encoding multi-modal signals are recruited by neuroligin-3-mediated new synapse formation. *eLife*, *12*, 87969. https://doi.org/10.7554/eLife.87969

Zeidler, R., Albermann, K., & Lang, S. (2007). Nicotine and apoptosis. *Apoptosis*, *12*(11), 1927–1943. https://doi.org/10.1007/s10495-007-0102-8

Zhang, J. C., Yao, W., Ren, Q., Yang, C., Dong, C., Ma, M., Wu, J., & Hashimoto, K. (2016). Depression-like phenotype by deletion of $\alpha 7$ nicotinic acetylcholine receptor: Role of BDNF-TrkB in nucleus accumbens. *Scientific Reports*, *6*, 1–14. https://doi.org/10.1038/srep36705

Zoli, M., Pucci, S., Vilella, A., & Gotti, C. (2018). Neuronal and Extraneuronal nicotinic acetylcholine receptors. *Current Neuropharmacology*, *16*(4), 338–349. https://doi.org/10.2174/1570159x15666170912110450

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