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Crucial Role of Dopamine D2 Receptor Signaling in Nicotine-Induced Conditioned Place Preference

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Abstract

Nicotine in tobacco causes psychological dependence through its rewarding effect in the central nervous system (CNS). Although nicotine dependence is explained by dopamine receptor (DR) signaling together with nicotinic acetylcholine receptors (nAChRs), the synaptic molecular mechanism underlying the interaction between dopamine receptor and nAChRs remains unclear. Since reward signaling is mediated by dopamine receptors, we hypothesized that the dopamine D2 receptor (D2R), in part, mediates the synaptic modulation of nicotine-induced conditioned place preference (CPP) in addition to dopamine D1 receptor. To investigate the involvement of D2R, wild-type (WT) and dopamine D2 receptor knockout (D2RKO) mice were assessed using the CPP task after induction of nicotine-induced CPP. As expected, D2RKO mice failed to induce CPP behaviors after repeated nicotine administration (0.5 mg/kg). When kinase signaling was assessed in the *nucleus accumbens* and *hippocampal* CA1 region after repeated nicotine administration, both Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) and extracellular signal-regulated kinase (ERK) were upregulated in WT mice but not in D2RKO mice. Likewise, nicotine-induced CPP was associated with elevation of pro- brain-derived neurotropic factor (BDNF) and BDNF protein levels in WT mice, but not in D2RKO mice. Taken together, in addition to dopamine D1 receptor signaling, dopamine D2 receptor signaling is critical for induction of nicotine-induced CPP in mice.

Keywords Nicotine dependence · Dopamine D2 receptor · Conditioned placed preference · Brain-derived neurotrophic factor

Abbreviations

AcS	Nucleus accumbens shell
ACC	Anterior cingulate cortex
α4β2nAChR	Alpha 4 beta 2 nicotinic acetylcholine receptors
α7nAChRs	Alpha 7 nicotinic acetylcholine receptors
BDNF	Brain-derived neurotropic factor
CaMKII	Calcium/calmodulin-dependent protein kinase II
CHRNA4	Nicotinic acetylcholine receptor (Nachr) A4 subunit
CPP	Conditioned placed preference
CREB	cAMP response element binding
D2RKO	Dopamine D2 receptor knockout
DLS	Dorsolateral striatum
D1R	Dopamine D1 receptors
D2R	Dopamine D2 receptors
ERK	Extracellular signal-regulated kinase
LTP	Long-term potentiation
MSN	Medium spiny neurons
NAc	Nucleus accumbens
NTRK2	Neurotrophic tyrosine kinase receptor 2
pCREB	Phosphorylation cAMP response element binding
PK	Phosphokinase C

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PVDF	Polyvinylidene difluoride
PtC	Parietal association cortex
VTA	Ventral tegmental area
WT	Wild type

Introduction

Nicotine is an active compound and the main addictive material in tobacco products. Nicotine dependence symptoms are characterized by compulsive use, craving, tolerance from continued use, and withdrawal upon cessation [1]. Nicotine interacts with nicotinic acetylcholine receptors (nAChRs), which are pentamers composed of α2, α4 α7, α10, and β2–β4 subunits [2]. The receptors play a critical role in drug addiction through stimulation of synaptic activity in the *hippocampus*, *amygdala*, *ventral tegmental area* (VTA), and *nucleus accumbens* (NAc) [3, 4]. The α7 homo-oligomer and α4β2 hetero-oligomer are the two major subtypes of nAChRs in the mammalian brain [2]. Both of these receptors regulate nicotine dependence, but chronic nicotine exposure selectively upregulates the density of α4β2 to elevate nicotine addiction in rats [5]. Like α4β2 hetero-oligomer, the α7 homo-oligomer is also involved in nicotine dependence, as evidenced by the high expression of alpha 7 nicotinic acetylcholine receptor (α7nAChR) on VTA in a nicotine-dependence rat model [6].

Nicotine also increases the firing rate of midbrain dopamine neurons by stimulation of α4β2nAChRs to promote nicotine dependence via the dopamine receptor [4]. Dopamine receptors are classified into two subfamilies: D1-like (D1 and D5) and D2-like (D2, D3, and D4) [7, 8]. In neuronal cocultures from coronal rat brain slices containing either NAc or VTA, nicotine stimulation increases cAMP response element binding (CREB) gene expression via stimulation of both dopamine D2 receptor (D2R) and dopamine D1 receptor (D1R) [9]. Stimulation of both receptors activates cAMP and Ca^{2+} signaling through $G\alpha_s$/olf in D1R and Gi-stimulated Gβγ function in D2R, respectively [10]. D1R on NAc facilitates drug reward, and exclusive genetic enhancement of the D1R gene in either the NAc core or the NAc shell in mice is sufficient to promote motivation to work to obtain the reward in a progressive ratio task or for motor learning [11]. Additionally, stimulation of D1R in the postnatal NAc promotes psychostimulant-induced behavior sensitization via activation of PKA pathways, which enhance the phosphorylation levels of GluA1 in the mice brain [12, 13]. The pivotal role of D1R in nicotine dependence can be confirmed because administration of the D1R antagonist SCH-23390 in NAc, ACC, and PtA inhibits nicotine-induced behavior, including nicotine dependence in mice [14, 15]. Nicotine stimulates both α4β2nAChRs at dopaminergic terminals and α7nAChRs at glutamatergic terminals. Furthermore, activation of α7nAChRs results in the release of glutamate and activation of α4β2nAChRs and NMDA/AMPA receptors by glutamate synergistically induces robust dopamine release and activates dopamine D1 and D2 receptors [16] to enhance CREB binding to the CRE element (DynCREs) of the PD promote; DynCRE3 is preferentially inhibited by SCH23390 administration [17]. Thus, the pivotal role of D1R has been well documented.

However, the functions of D2R in nicotine dependence remain unclear, although some studies have provided evidence. For example, chronic nicotine administration in a rat elevates the dopamine D2 receptor level [18]. In in vitro studies using GST-fusion proteins containing IL3 (GST-D2R-IL3), D2R was shown to bind to Ca^{2+}/calmodulin-dependent protein kinase alpha (CaMKIIα) via the D2R IL3 domain [19], thereby affecting long-term memory of nicotine dependence because CaMKII is essential for learning formation particularly in CA1 region [20, 21]. D2R shows an inviolable effect in reward processing of drugs and natural stimuli such as food and it mediates approach–avoidance tendencies in smokers [22]. Takeuchi et al. showed that in the NGD2L cell, quinpirole, a D2R agonist, enhances the Ca^{2+} intracellular level and then activates the nuclear isoform of (CaMKII)δ3 to increase exon-4 brain-derived neurotropic factor (BDNF) gene expression through D2LR; in addition, the isoform of (CaMKII)δ3 in rat *subtantia nigra* modulates the exon-2 BDNF gene expression through a CREB phosphorylation pathway [23, 24]. Therefore, elucidation of mechanism of D2R-mediated nicotine dependence is important.

A dose-dependent reduction in serotonin (5-HT) uptake was observed in two of the B cell lines after exposure to BDNF, and BDNF expression in the brain is managed by serotoninergic and dopaminergic neurotransmitters, which are involved in nicotine addictive behavior [25, 26]. With regard to the serotoninergic system, a dose-dependent reduction of 5-HT uptake was observed in B lymphocyte 5-HTTLPR cell culture treated with BDNF [26]. BDNF from dopaminergic neurons is critical for D3R expression in the NAc as a part of substance addiction [25]. Additionally, *hippocampal* BDNF was regulated by various neurotransmitters that are sensitive to nicotine administration, and it can probably serve as a critical molecular target for the behavior-modulating effects of nicotine [27, 28]. In Sprague–Dawley rats, the administration of a serotonin agonist reduced the BDNF mRNA level in the *hippocampus* [27], providing evidence of the interaction between the serotoninergic system and BDNF. Stimulation of the $GABA_A$ receptor activates the voltage gate Ca^{2+} in immature *hippocampus* cell culture to increase the BDNF mRNA level in a GABAergic system [28]. Further studies showed that BDNF in the rat *hippocampus* is upregulated by the activation of muscarinic receptors and correlated with acetylcholine release [29].

In the present study, we confirm that the activation of nAChRs within dopaminergic neurons induces a nicotine reinforcing signaling pathway through D2R in WT mice and provide new evidence for the role of the dopamine D2R signal and BDNF expression in nicotine reinforcement using dopamine D2 receptor knockout (D2RKO) mice. We describe the mechanism underlying D2R-mediated nicotine dependence in mice. These results suggest that D2 receptor is a target for therapeutics of nicotine dependence.

Materials and Methods

Animals

Male C57BL/6JJmsSIC mice as WT mice aged 8 weeks (20–30 g) purchased from SLC (Hamamatsu, Japan) and male D2RKO mice aged 8 weeks (20–30 g) obtained from the Laboratory of Pharmacology, School of Pharmacy, Tohoku University were used in all experiments. The following protocol was used to generate D2RKO mice. A mutation was generated in the DA D_2 receptor gene using homologous recombination in embryonic stem cells and a targeting vector to delete the entire exon 7 and the 5′ half of exon 8, which is the region encoding the majority of the putative third intracellular loop, the last two transmembrane domains, and the carboxy terminus. Blastocyst injection was used to generate chimeric mice of the heterogenous 129/Sv × C57BL/6J background. F_1 heterozygous mice sired by the chimeras were interbred to generate F_2 mice of the genotypes $D_2^{-/-}$. Backcrossing F_2 mice to the C57BL/6J mouse strain for five generations resulted in the incipient congenic N_5 mice of $D_2^{-/-}$ genotypes used here. The genotype of the mice was confirmed using PCR [30]. Mice were housed in a room with a 12/12-h light/dark cycle (lights on at 09:00). Room conditions were temperature controlled at 22.0 ± 2 °C with a relative humidity of 55% ± 5%. Mice had free access to food and water. All experimental animal procedures were approved by the Committee on Animal Experiments at Tohoku University, and studies were conducted in accordance with committee guidelines. Every effort was made to minimize suffering and limit the number of animals used.

Experimental Design

Induction of CPP by Nicotine

Two groups of male mice (C57BL/6JJmsSIC) were treated using saline 0.9% i.p. ($n = 6$) or nicotine 0.5 mg/kg i.p. ($n = 8$). All mice were acclimatized to the conditioned place preference (CPP) box for 5 days, after which, the preconditioning test was performed. Further, treatment was administered to the mice 28 days after the preconditioning. Briefly, mice in the vehicle group received a saline injection (i.p.) and were confined to the designated compartment for up to 30 min per day. Mice in the nicotine group received a nicotine injection (0.5 mg/kg, i.p.) followed by placement in the nicotine-paired compartment for 30 min. The CPP test was performed twice on day 15 and day 29 after the conditioning phase. The place preference score of each mouse was recorded to determine reinforcing effect of nicotine on CPP. Thereafter, the mice were sacrificed on day 29, and the NAc and CA1 regions were dissected out from the mice brains; immunoblotting was performed to examine the D1R ($n = 5$) and D2R ($n = 5$) expression levels.

D1 and D2 Receptor Antagonist Alleviates Nicotine-Induced CPP

WT mice were divided into four groups according to the treatment administered: saline 0.9% i.p. ($n = 6$), nicotine 0.5 mg/kg i.p. ($n = 7$), nicotine 0.5 mg/kg i.p. + SCH23390 0.03 mg/kg s.c ($n = 6$), and nicotine 0.5 mg i.p. + eticlopride-HCl 0.03 mg/kg ($n = 7$). Eticlopride-HCl and SCH23390 were administered subcutaneously 30 min before nicotine injection. The behavior test was performed following the protocol for nicotine induction of CPP in the abovementioned experiment.

Nicotine CPP Test for WT and D2RKO Mice

Mice were divided to four groups: WT vehicle ($n = 9$), WT nicotine ($n = 10$), D2RKO vehicle ($n = 8$), and D2RKO nicotine ($n = 8$). Behavioral assessments were conducted using CPP methods as described above. Mice were sacrificed after the preference score assessment on day 29; the NAc and CA1 regions were dissected out from the mice brains, and immunoblot analysis was performed to examine the level of CaMKII ($n = 6$), extracellular signal-regulated kinase 1/2 (ERK1/2) ($n = 6$), PKCα ($n = 8$), D1R ($n = 5$), D2R ($n = 5$), CREB ($n = 5$), pro-BDNF ($n = 5$), BDNF ($n = 5$), alpha 4 nicotinic acetylcholine receptors (α4nAChRs) ($n = 6$), and α7nAChRs ($n = 6$). Immunostaining of phosphorylated CaMKII (pCaMKII) ($n = 4$) and phosphorylated ERK (pERK) ($n = 4$) was performed to support the immunoblotting results. The experimental protocols are shown in Fig. 1a.

Drug Administration

NaCl (Wako) 0.9% was dissolved in double-distilled water, and nicotine hydrogen tartrate 0.5 mg/kg (Sigma Aldrich), eticlopride-HCl 0.03 mg/kg (Sigma Aldrich), and SCH23390 0.03 mg/kg (Sigma Aldrich) were dissolved in saline solution. Mice were given nicotine or saline intraperitoneally once daily for 28 consecutive days. Eticlopride-HCl and SCH23390 were administered subcutaneously 30 min before nicotine injection.

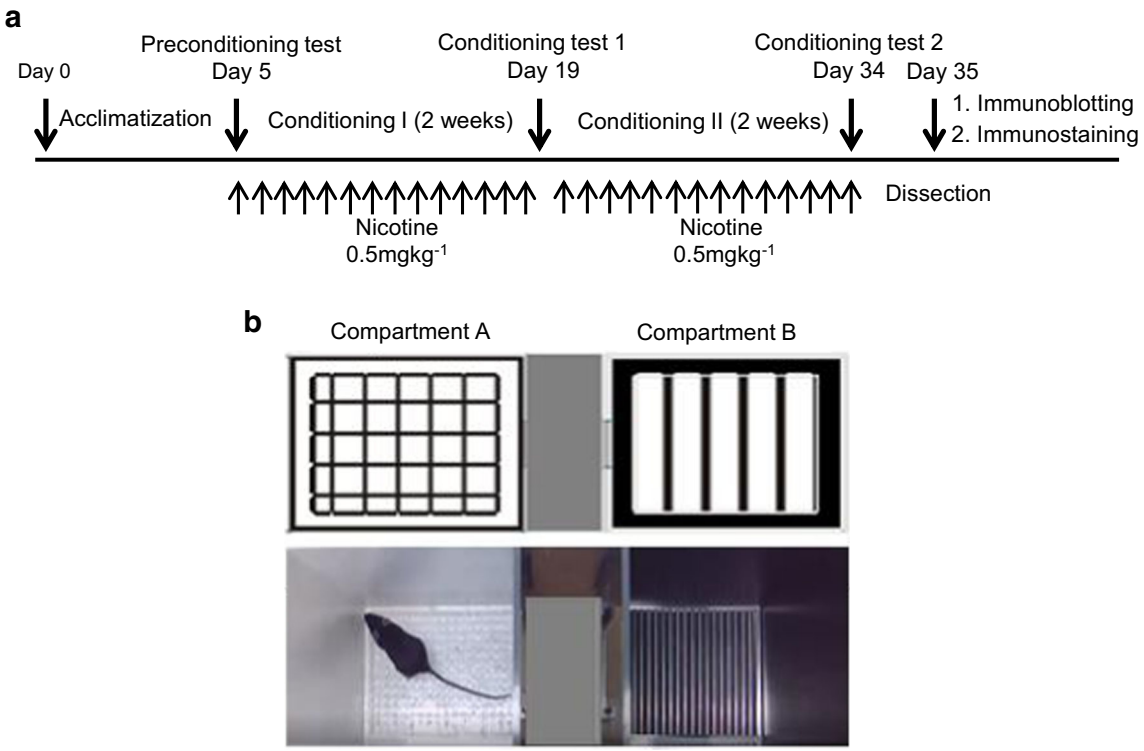


Fig. 1 Experimental design and CPP apparatus. **a** Scheme of the whole experiment. Mice were habituated to the CPP apparatus for 5 days, followed by a preconditioning test to determine the nicotine-paired compartment. The preference test was performed twice after 14- and 28-day conditioning, and the mice were sacrificed after preference score conditioning assessment on day 29. *Nucleus accumbens* and CA1 regions were dissected and assessed using immunoblot analysis. **b** Visualization of the behavior test on the CPP box: The apparatus for the conditioned place preference test consisted of three compartments: The middle (neutral) compartment was gray. Compartment A was white with a quadrangular sieve (mesh) floor, while compartment B was black with stainless steel floors and separated by two guillotine doors

CPP Test

The CPP test was performed using a biased design with two phases: habituation (5 days) and conditioning (28 days).

Acclimatization

Acclimatization was performed for 5 days prior to preconditioning. This process was designed to remove any environmental stress and included the weighing and testing rooms, CPP apparatus, and drug administration.

Preconditioning Test

The preconditioning test was used to determine which compartment should be drug- or nicotine-paired. The compartment with the lower ratio preference was used [31]. One day after habituation, each mouse was placed in the gray compartment for 5 min with the guillotine door closed. Following this, the guillotine door was opened, and the mouse could access all compartments for 15 min. Time spent in each compartment was recorded, and the preference ratio was calculated using the following formula:

$$\text{Preference ratio} = \frac{\text{Sojourn time in related compartment (s)}}{\text{Total time spent in all compartments (s)}} \times 100\%$$

Conditioning

Mice were conditioned for 28 days. Each day, they were injected with nicotine followed by confinement in the designated compartment. The nicotine compartment was paired with the least preferred side of the compartment [31]. Four hours later, the same procedure was repeated with saline administration for all groups. Mice were then confined to the opposite compartment of the apparatus. The next day, the conditioning preference ratio was calculated to assess nicotine dependence. On days 15 and 29 of conditioning, the conditioning preference ratio for each mouse was measured to confirm nicotine dependence using the following formula:

$$\text{Preference ratio} = \frac{\text{Sojourn time in related compartment (s)}}{\text{Total time spent in all compartments (s)}} \times 100\%$$

CPP Apparatus

The apparatus for the CPP test consisted of three compartments measuring 12.7 cm × 46.5 cm × 12.7 cm (width × length × height) in size. The middle compartment was gray, called the neutral compartment. The two conditioning compartments differed in color and floor texture. Compartment A was white with a quadrangular sieve (mesh). The other

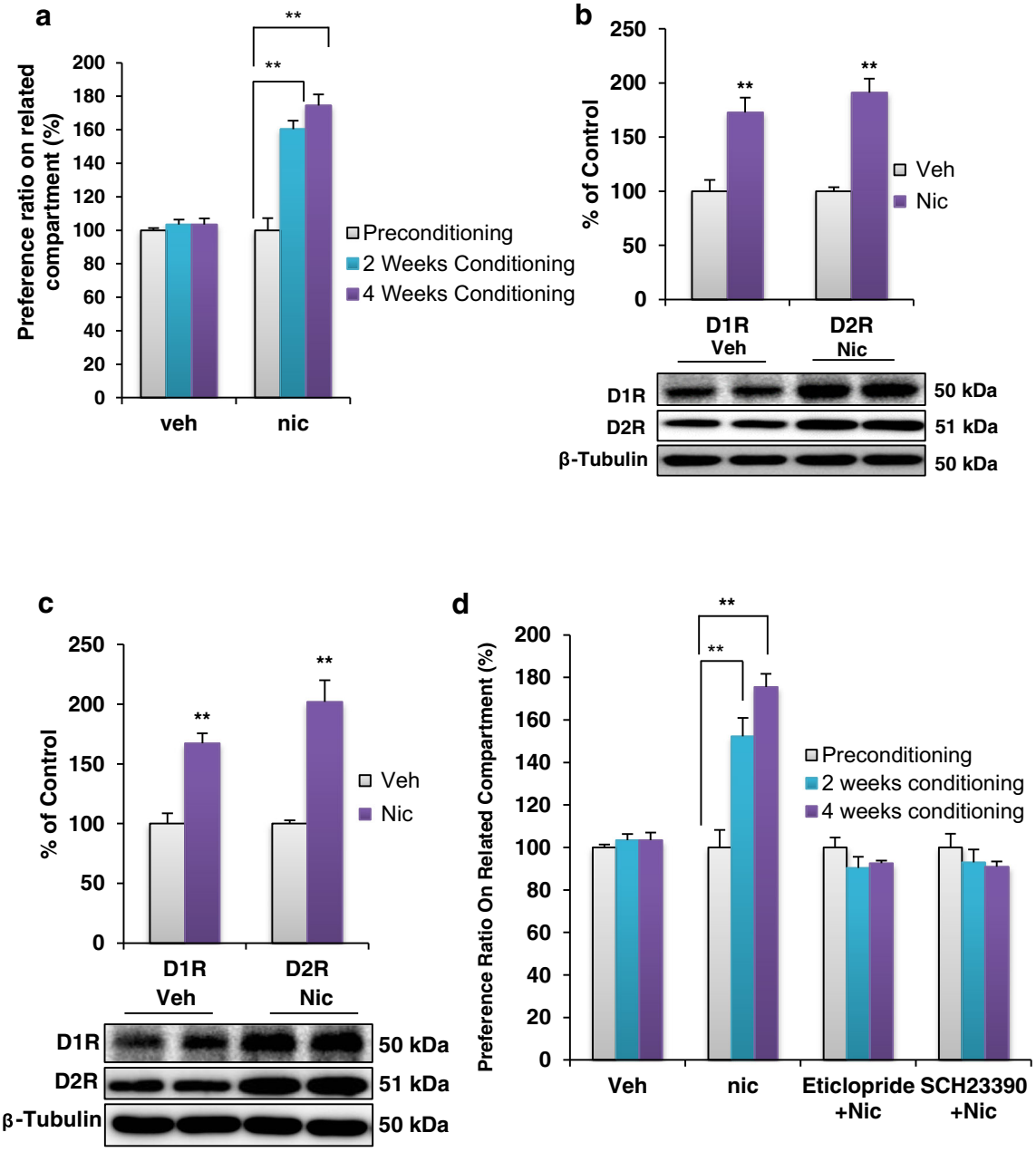


Fig. 2 D1R and D2R are involved in nicotine-induced CPP. **a** Nicotine administration at 0.5 mg/kg generates nicotine CPP, which is characterized by elevation of the preference ratio for 2 weeks in the nicotine injection group ($n = 8$) in comparison with the pretreatment condition. Equal preference score obtained on saline administration for 14 and 28 consecutive days ($n = 6$). Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by multiple comparisons between baseline condition and the results obtained 2 weeks and 4 weeks after nicotine administration post hoc Tukey tests. $^{**}p < 0.01$. **b**, **c** Nicotine addiction was characterized by the strong D1R and D2R expression on the NAc and CA1 regions in the nicotine treatment group ($n = 5$) but not in the vehicle group ($n = 5$). Data are presented as mean ± SEM and were analyzed using Student's t test. $^{**}p < 0.01$ compared to the vehicle group. **d** Inhibition of D1R by SCH23390 ($n = 6$) and D2R with eticlopride ($n = 7$) successfully suppressed the nicotine-induced CPP behavior as a key symptom for curing nicotine-induced behavioral addiction. Data are presented as mean ± SEM and were analyzed using one-way ANOVA followed by multiple comparisons between the values obtained before injection of nicotine and those obtained after nicotine administration for 2 weeks and 4 weeks. Post hoc Tukey test was used as an advance analysis method. $^{**}p < 0.01$

compartment (B) was black with stainless steel floors. Each compartment was separated by two doors (Fig. 1b).

Locomotor Activity Test

Preconditioning and conditioning tests were recorded using Amcap video recording software (Noël Danjou publisher, USA). The video was analyzed by video tracking software using Python version 3.4.6 (Python Software Inc., USA). The data show the total distance traveled in the CPP box for 15 min by the mice from the WT vehicle, WT nicotine, D2RKO vehicle, and D2RKO nicotine groups, each of which consists of six mice.

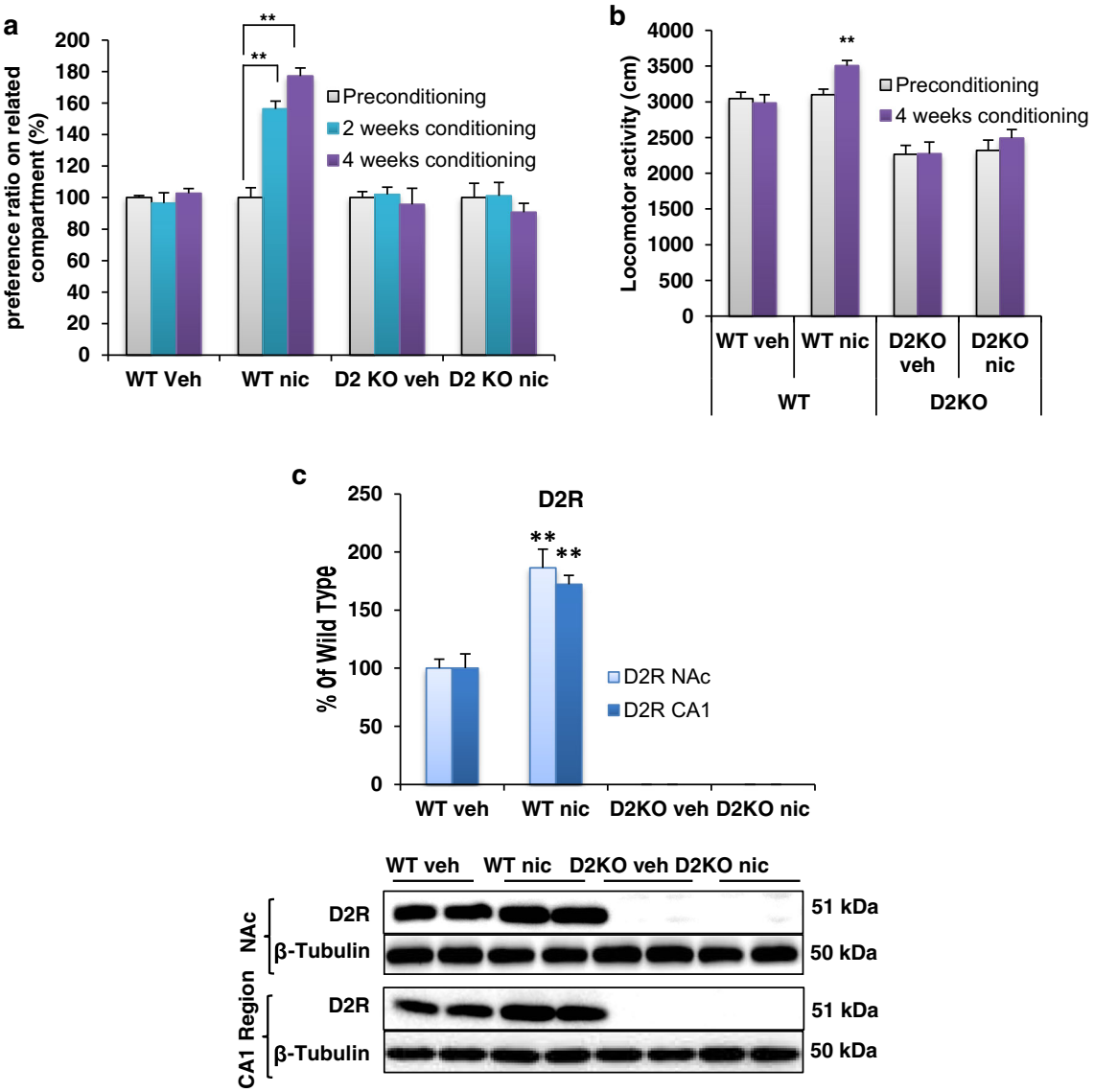


Fig. 3 D2R deficiency abolishes nicotine-induced CPP. **a** Nicotine induces CPP in WT mice but not in D2RKO mice. Nicotine administered intraperitoneally at 0.5 mg/kg for 14 and 28 consecutive days to WT mice ($n = 10$) generates nicotine-induced CPP compared to WT-saline mice ($n = 8$). Nicotine does not affect behavioral activity in D2RKO mice ($n = 8$) compared to the D2RKO saline mice ($n = 8$) as the vehicle group. Data are presented as mean ± SEM values, with $^{**}p < 0.01$ when compared with preconditioning treatment. **b** Nicotine modulates locomotor activity in wild-type mice, whereas D2RKO mice show no deterioration of motor activity with nicotine ($n = 6$ for each group). Data are presented as mean ± SEM, $^{**}p < 0.01$ when compared with prenicotine treatment. **c** Nicotine influenced the manifestation of the dopamine D2 receptor in the *nucleus accumbens* and *hippocampal* CA1 region. Expression of dopamine both in NAc and CA1 is increased by nicotine, and the expression in D2RKO mice without manifestation of the dopamine D2 receptor on the western blot result both in D2RKO-veh and in D2RKO-nic group. Data are presented as mean ± SEM, $n = 5$. $^{**}p < 0.01$ compared with WT-veh. Comparisons between two experimental groups were calculated using the unpaired Student's *t* test

Immunoblot Analysis

Mice were sacrificed after preference score conditioning assessment, and the NAc and CA1 regions were dissected from the brain. The tissues were stored in liquid nitrogen temporarily and then stored at − 80 °C until use. Western blot analysis was performed as described. NAc and CA1 region samples were homogenized in 200 μl of homogenizing buffer containing 50 μm Tris–HCl (pH 7.4), 0.5% Triton-X-100, 4 μm EGTA, 10 μm EDTA, 1 μm Na_3VO_4, 40 μm sodium pyrophosphate, 50 μm NaF, 100 μm calyculin A, 50 μg/ml leupeptin, 25 μg/ml pepstatin-A, 50 μg/ml trypsin inhibitor, and 1- mm dithiothreitol (DTT). Centrifugation for 10 min at 15000 rpm at 4 °C was used to remove insoluble particles. After measuring protein concentration in supernatants using Bradford's solution, samples were boiled in a 100 °C incubator for 3 min in Laemmli buffer [32].

The samples containing equivalent amounts of protein were subjected to SDS–polyacrylamide gel electrophoresis (PAGE) at 500 V and 40 mA. Proteins were transferred to an Immobilon polyvinylidene difluoride (PVDF) membrane (pore = 0.45 μm) (Millipore) for 2 h at 70 V. After blocking with TTBS solution (50 mm Tris–HCl, pH 7.5, 150 mm NaCl, and 0.1% Tween 20) containing 5% of fat-free milk powder for 1 h at room temperature, membranes were incubated overnight at 4 °C with anti-phospho-CaMKII

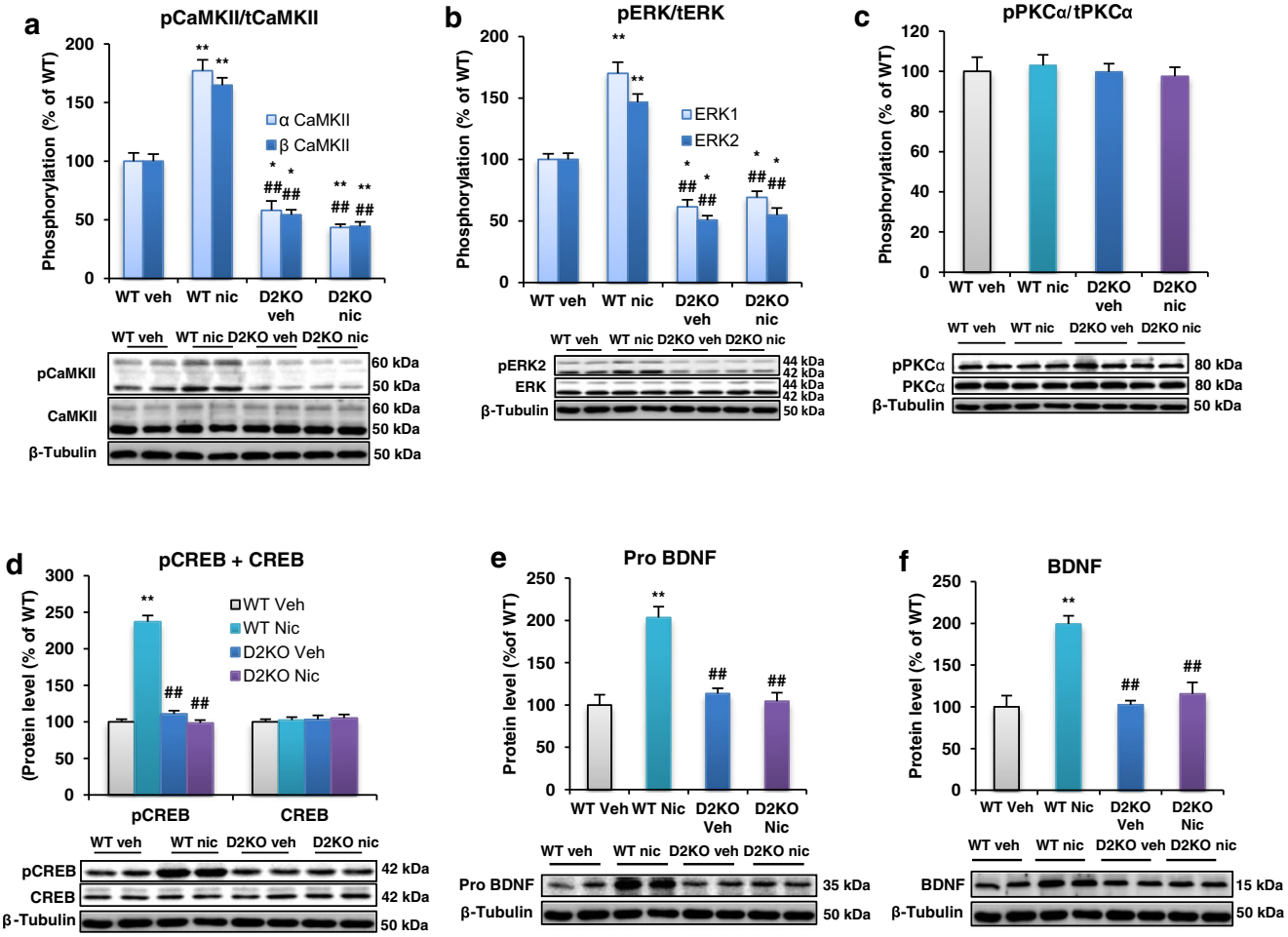


Fig. 4 CaMKII and ERK phosphorylation changed after nicotine-induced CPP in the *nucleus accumbens*. **a** Quantitative analysis of pCaMKII/tCaMKII and expression of pCaMKII and tCaMKII ($n = 6$) in WT and D2RKO mice after 28 days' exposure of nicotine 0.5 mg/kg. **b** Calculation analysis of pERK1/2/tERK1/2 and expression of both pERK1/2 and tERK1/2 in WT and D2RKO mice (each group $n = 6$) after use of nicotine 0.5 mg/kg for 28 days. **c** Quantification of pPKCα/PKCα and manifestation of pPKCα/PKCα in WT and D2RKO mice ($n = 8$). **d** Representative number of pCREB and CREB ($n = 5$), **e** pro-BDNF ($n = 5$), and **f** BDNF ($n = 5$) and their expression in WT-saline, WT-nicotine, D2RKO saline, and D2RKO nicotine groups after nicotine or saline administration for as long 28 days. Data are presented as mean ± SEM values and were analyzed using one-way analysis of variance followed by multiple comparisons between the vehicle (saline) and nicotine groups using post hoc Tukey tests. $^{*}p < 0.05$ compared with WT-veh; $^{**}p < 0.01$ compared with WT-veh; $^{\#}p < 0.05$ compared to WT-nic; $^{\#\#}p < 0.01$ compared to WT-nic

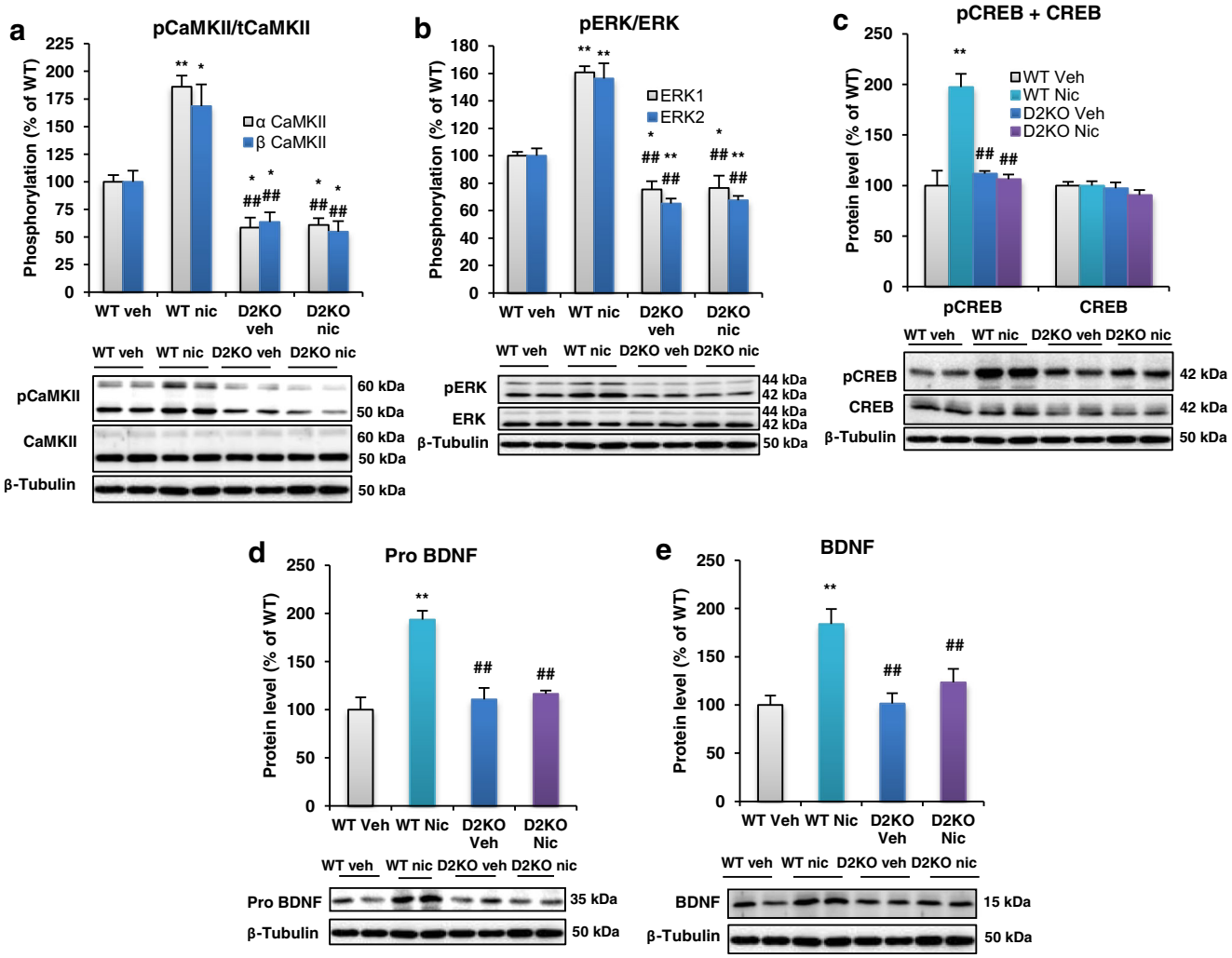
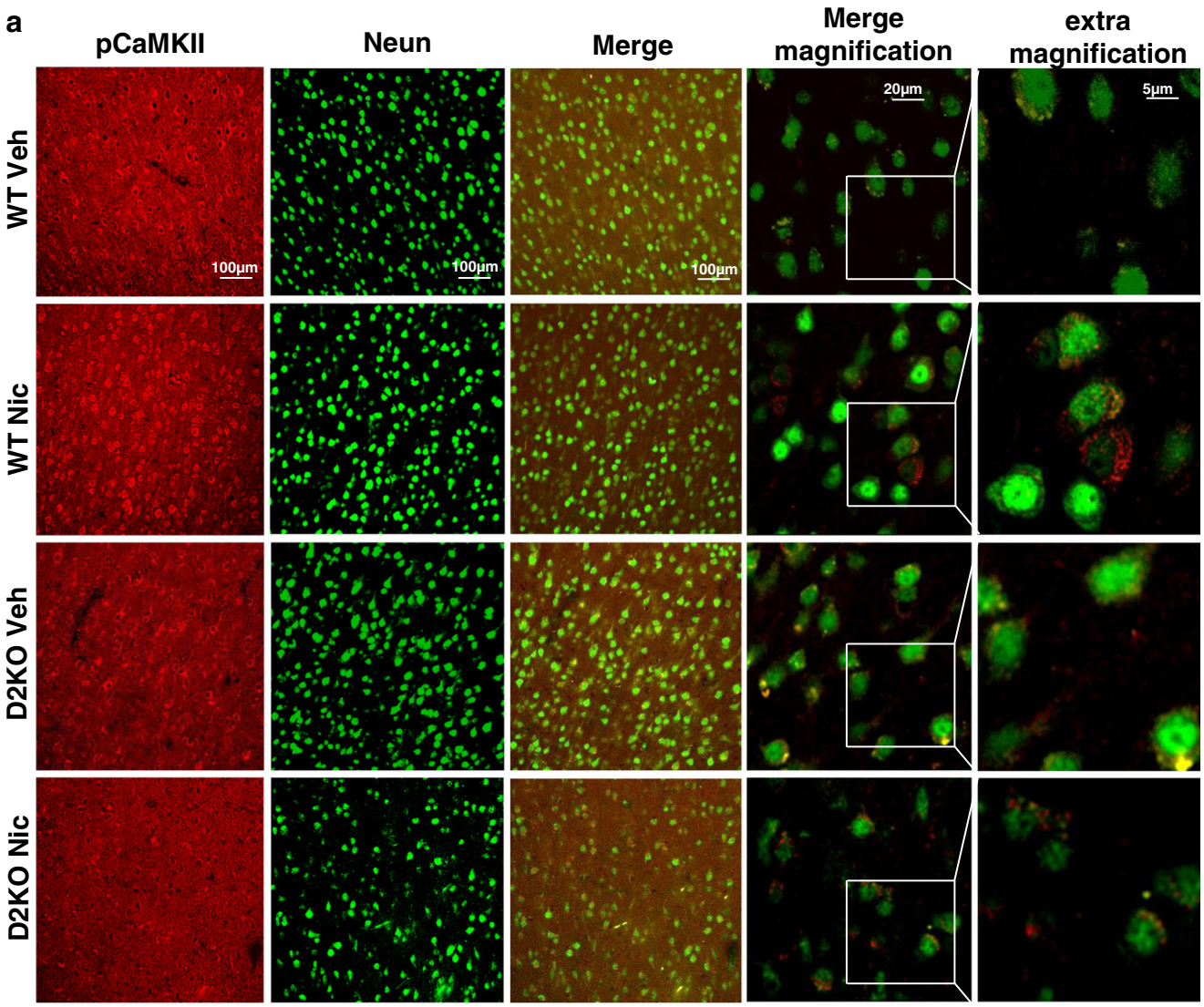


Fig. 5 CaMKII and ERK phosphorylation changed after nicotine-induced CPP in the *hippocampal* CA1 regions. **a** Calculation ratio of pCaMKII and tCaMKII accompanied with the expression bands of pCaMKII and tCaMKII ($n = 6$). **b** Quantification of pERK1/2/tERK1/2 together with the band expression of pERK1/2 and tERK1/2 ($n = 6$). Quantitative analysis of **c** pCREB and CREB, **d** pro-BDNF, and **e** BDNF based on the nature of each protein band after 28 days administration of nicotine 0.5 mg/kg in WT mice ($n = 5$) and D2RKO mice ($n = 5$) compared to WT-saline ($n = 5$) and D2RKO saline ($n = 5$) as the vehicle groups. Data are presented as mean ± SEM, which were assessed using one-way ANOVA followed by multiple comparisons using post hoc Tukey test. $^{*}p < 0.05$ compared with WT-veh; $^{**}p < 0.01$ compared with WT-veh; $^{\#\#}p < 0.01$ compared to WT-nic

(1:5000) or total CaMKII (1:5000), anti-phospho-ERK (1:2000; Cell signaling), anti-total ERK (1:1000; Cell signaling), anti-phospho-PKCα (1:1000; Millipore), anti-total PKCα (1:1000; Millipore), anti-D1R (1:1000; Millipore), anti-D2R (1:1000; Millipore), anti-phospho-CREB (1:1000; Cell signaling), anti-total CREB (1:1000; Cell signaling), anti-pro-BDNF (1:1000; Millipore), anti-BDNF (1:1000; Cell signaling), anti-α4nAChRs (1:1000; Santa Cruz), and anti-α7nAChRs (1:1000; Santa crus). Images of the band were developed using an ECL immunoblotting detection system (Amersham Biosciences, Piscataway, NJ, USA) and visualized on X-ray film (Fuji Film, Tokyo, Japan). Autoradiographic films were scanned for densitometry analysis (Lasergraphics; Irvine, CA, USA) and

Fig. 6 Nicotine administration changes CaMKII autophosphorylation in the *nucleus accumbens*. **a** Twenty-eight days of nicotine administration modifies the number of pCaMKII in the NAc region, as assessed by immunohistochemical methods. **b** Quantification of pCaMKII cells in the NAc tissue from WT and D2RKO mice injected with either saline 0.9% or nicotine (0.5 mg/kg) for 28 consecutive days. Immunostaining revealed an escalation in the pCaMKII number in WT-nicotine treatment ($n = 4$) compared to the WT-saline group ($n = 4$). In contrast, in D2RKO mice, equivalent pCaMKII numbers were obtained both in saline ($n = 4$) and nicotine ($n = 4$) groups. Consistent with the pCaMKII expression on WB, confocal microscopy showed that the number of pCaMKII cells in D2RKO mice (vehicle or nicotine) is lower than that in WT-veh or WT-nic. **c** ImageJ analysis for neun cell as a marker for neurons showed identical numbers of neun cells in all treatment groups. Data are presented as mean ± SEM and were assessed by one-way ANOVA followed by multiple comparisons using the post hoc Tukey test. $^{*}p < 0.05$ compared with WT-veh; $^{**}p < 0.01$ compared with WT-veh; $^{\#\#}p < 0.01$ compared with WT-nic

analyzed quantitatively using Image Gauge version 3.41 (FujiFilm, Tokyo, Japan). Densities of phosphoproteins were normalized to densities of the respective total proteins using conventional antibodies.



Immunohistochemistry

Immunohistochemical analysis of the NAc brain region was conducted by the PFA method [33]. Mice brains were washed with PBS and perfused using PFA 4% in phosphate buffer 0.1 M. Brain slices with a thickness of 50 μm were obtained for the experiment (D.S.K. microslicer, DTK.100, Dosaka Em. Co., Ltd., Japan), cleaned in PBS, treated with 0.1% Triton-X in PBS for 3 × 30 min and 0.3% H_2O_2 in PBS for 10 min, and blocked with 3% bovine serum albumin in PBS for an hour. The brain slices were incubated with the first antibody in blocking buffer solution for 3 × 24 h. Primary antibodies were mouse monoclonal antibody against pCaMKII (1:250, Invitrogen), rabbit monoclonal antibody against pERK (1:400, Cell Signaling), mouse monoclonal antibody against neun (1:500, Millipore), and rabbit monoclonal antibody against neun (1:500). After washing with PBS, sections were incubated with the secondary antibody for a day: Alexa flour 594-labeled anti-mouse (IgG) (1:500, Invitrogen) for pCaMKII, Alexa flour 594-labeled anti-rabbit (IgG) (1:500, Invitrogen) for pERK1/2, Alexa flour 488-labeled anti-mouse for neun (Millipore), and Alexa flour 488-labeled anti-rabbit for neun (Abcam). Brain slices were cleaned with PBS and transferred to a glass objective, after which the sections were mounted with a drop of vectashield (Vector Laboratories Inc.). Immunofluorescence images were captured by a confocal laser-scanning microscope (Nikon Eclipse 80i, Japan), and observations were recorded using Nikon EZ-C1 3.80 viewer software (Nikon Company, Japan). The number of neuron cells was counted using ImageJ software (NIH, MD, USA).

Data Analysis and Statistical Evaluation

Data were presented as mean ± SEM, and calculations were performed using one-way analysis of variance followed by multiple comparisons between the vehicle and experimental groups. Post hoc Tukey multiple comparison tests were performed using Minitab 14 software (Minitab Inc., UK), and comparisons between the two experimental groups were made using the unpaired Student's *t* test. $P < 0.05$ was considered to indicate a significant difference in statistical analyses.

Results

Nicotine Promotes CPP Through D1 and D2 Receptor Activations

Nicotine administration induced preference (Fig. 2a), as shown by the elevation of preference ratio values to 156.4% and 177.2% on days 14 and 28 after continuous nicotine injection, in comparison with the values obtained in the nicotine preadministration condition in normal vehicle-treated mice ((F 51, 8 = 0.00056) $p < 0.01$) [34]. Since D1R and D2R mediate nicotine dependence [16, 35], we analyzed the levels of these receptors. Nicotine-induced CPP was associated with elevation of D1 and D2 receptor levels in both NAc and CA1 regions (Fig. 2b, c). D1 and D2 receptor levels in the NAc and CA1 regions increased to 172.7% and 167.2%, respectively ($p = 0.027$, $p < 0.05$ in NAc and $p = 0.019$, $p < 0.05$ in the CA1 region). Likewise, D2R expression increased to 190.8% and 196.9% in the NAc and CA1 regions, respectively ($p = 0.0042$, $p < 0.01$ in NAc and $p = 0.006$, $p < 0.05$ in the CA1 region vs. the vehicle-treated group).

To confirm the involvement of both D1R and D2R in nicotine-induced CPP, SCH23390, a D1R antagonist, and eticlopride, a D2R antagonist, were administered 30 min prior to nicotine injection. Nicotine-induced CPP was substantially blocked by eticlopride at both 2 and 4 weeks (by 90.3% and 92.5%, respectively, vs. the preconditioning level of 100%) (Fig. 2d) ($F = 1.61$; $p = 0.226$ in 2 weeks; $p = 0.401$ in 4 weeks, $p > 0.05$, not significant vs. the vehicle group). The same trend was shown by subcutaneous administration of the D1 antagonist SCH-23390 (0.03 mg/kg) prior to nicotine injection ($F = 0.8162$; $p = 0.4608$; $p = 0.618$ for 2 weeks; and $p = 0.461$; $p > 0.05$ not significant vs. the vehicle group).

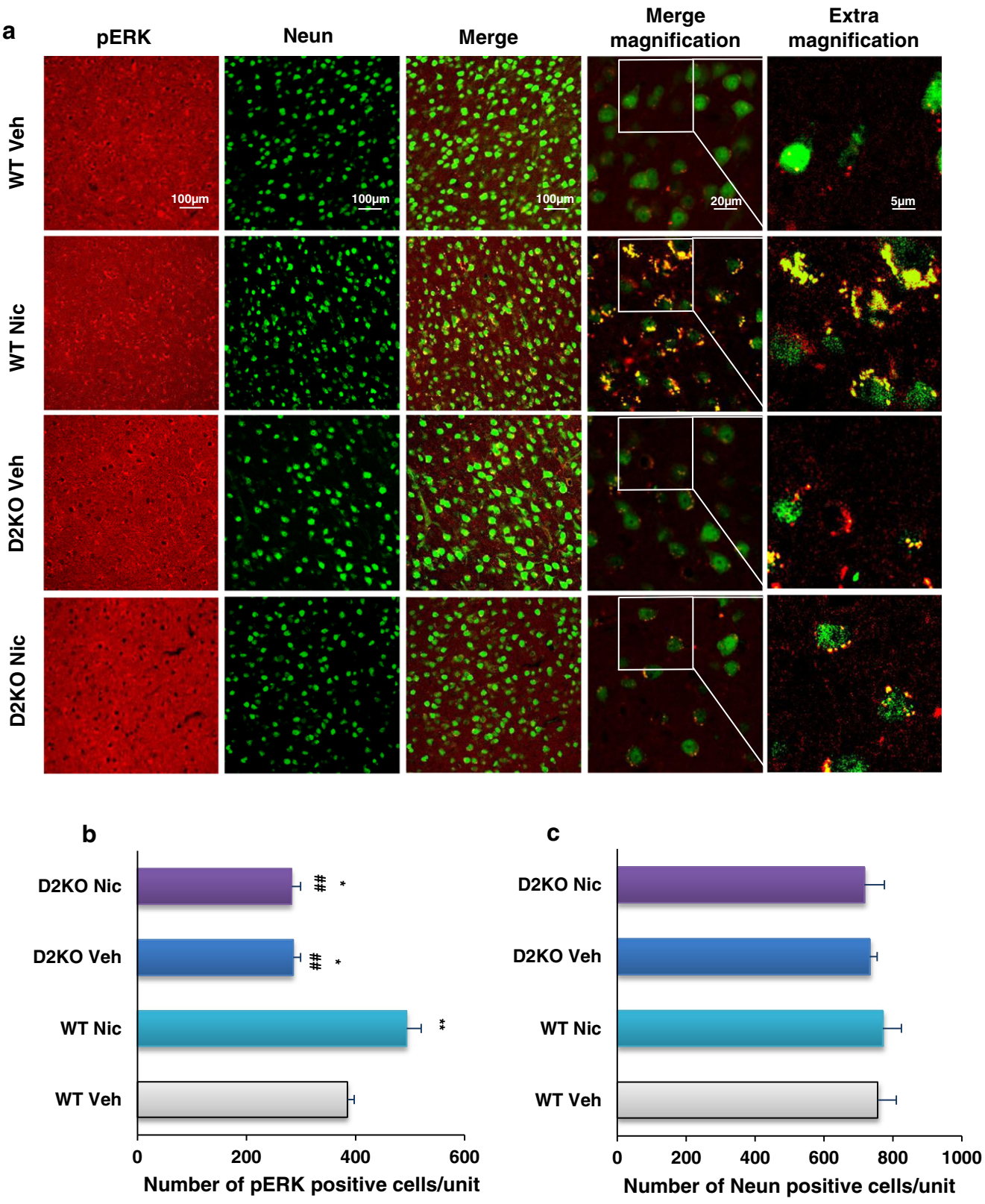
Nicotine-Induced CPP Was Abolished in D2RKO Mice

We next analyzed the pivotal role of D2R in nicotine-induced CPP. Nicotine-induced CPP was completely abolished in D2RKO mice. Repeated administration of nicotine 0.5 mg/kg for 28 days failed to enhance the CPP score in D2RKO mice (90.7%) compared to 100% in WT mice ((F 0.5283 = 0.5972) $p > 0.05$) (Fig. 3a). Consistent with nicotine-induced CPP, nicotine stimulated locomotor activity in WT mice ($p = 0.0037$; $p < 0.01$ vs. vehicle) [34], but no changes were observed in D2RKO mice ($p = 0.383$; $p > 0.05$).

Nicotine administration elevated D2R expression. The D2R protein levels in the NAc were elevated 1.86-fold

Fig. 7 Nicotine administration changes ERK phosphorylation in the *nucleus accumbens*. **a** The diverse appearance of pERK on NAc slices observed using immunostaining analysis. **b** The amount of pERK escalated significantly after nicotine injection compared to saline injection as a vehicle in WT mice ($n = 4$), even though the pERK existence was not modified in D2RKO mice ($n = 4$). D2R knocked out the gene in mice, yielding a reduction in pERK in comparison with WT-veh or WT-nic. **c** Neun cell numbers remained unchanged both in WT and D2RKO mice treated with vehicle and nicotine. Data are presented as mean ± SEM and calculated by one-way ANOVA followed by multiple comparisons using post hoc Tukey test. $^{*}p < 0.05$; compared with WT-veh; $^{\#\#}p < 0.01$ compared to WT-nic

compared to the naïve condition at 28 days after nicotine administration ($p = 0.0045$; $p < 0.01$ vs. WT-vehicle). Likewise, the addiction-induced expression of D2R was elevated in the CA1 region. Elimination of D2 receptor in D2RKO mice revealed no signals of D2R in D2RKO mice in both regions (Fig. 3c).



Protein Kinase Signals Are Elevated After Nicotine-Induced CPP in NAc

Since NAc is a part of the central dogma in dopamine-mediated nicotine dependence [36], we analyzed CaMKII and ERK signaling. We examined the phosphorylation of CaMKII and ERK1/2, CREB, and the levels of these proteins in the NAc (Fig. 4a, b, d, e, f). Nicotine administration for 28 days successfully increased α CaMKII (177.1%), β CaMKII (164.8%), ERK1 (169.9%), ERK2 (146.4%), phosphorylation cAMP response element binding (pCREB) (237.4%), pro-BDNF (203.4%), and BDNF (199.1%) levels in the NAc of wild-type mice compared to WT-vehicle group (100%) ($p < 0.01$). In contrast, D2RKO mice did not show any effect on the protein levels of CaMKII, ERK, PKCα, pCREB, pro-BDNF, and BDNF in the nicotine group treatment compared to the vehicle treatment group. Nicotine did not alter the PKCα signal in either the WT mice or the D2RKO mice group after nicotine exposure for 28 days ($p = 0.899$; $p > 0.05$ WT-veh vs. WT-nic) (Fig. 4c).

Interestingly, phosphorylation of CaMKII and ERK in D2RKO mice in both D2RKO vehicle group (αCaMKII = 58% and βCaMKII = 54.3%; $p < 0.05$ vs. WT-veh; $p < 0.01$ vs. WT-nic) and D2RKO nicotine group (αCaMKII = 43.6% and βCaMKII = 44.4%; $p < 0.01$ vs. WT-nic or WT-veh) significantly decreased in comparison with the values in the WT-vehicle group and WT-nicotine group. Phosphorylation ERK1 = 75.5% and ERK2 = 65.2% D2RKO vehicle and also phosphorylation ERK1 = 76.4% and ERK2 = 67.5% on D2RKO nicotine are significantly diverse with the WT-vehicle $p < 0.05$ vs. D2RKO veh and $p < 0.01$ vs. D2RKO nicotine. Taken together, the findings indicate that signal pathways including CaMKII, ERK, pCREB, pro-BDNF, and BDNF in the NAc in wild-type mice may be critical for nicotine dependence.

Protein Kinase Signals in the *hippocampal* CA1 Are Elevated after Nicotine-Induced CPP

Consistent with the elevation in NAc signal, nicotine elevated the protein kinase activities in the CA1 region in WT mice (Fig. 5). CaMKII phosphorylation, ERK phosphorylation, CREB phosphorylation, and pro-BDNF and BDNF levels were enhanced by nicotine. Phosphorylation of α-CaMKII and β-CaMKII increased by 1.9-fold and 1.7-fold, respectively ($p < 0.01$ vs. WT-vehicle) (Fig. 5a). Likewise, phosphorylation of ERK1 and ERK2 increased by 160.8% and 156.4%, respectively, compared to WT-vehicle treatment ($p < 0.01$; Fig. 5b). The elevation of CREB phosphorylation (197.3%) (Fig. 5c), pro-BDNF level (193.5%) (Fig. 5d), and BDNF level (183.9%) (Fig. 5e) in comparison with the WT-veh group (100%; $p < 0.01$) was also evident in WT mice.

Unlike the WT mice, D2RKO mice showed significant reduction of CaMKII phosphorylation (αCaMKII = 58.4% and βCaMKII = 63.6% in vehicle-treated D2RKO mice compared to αCaMKII = 60.9% and βCaMKII = 54.6% in WT-vehicle) ($p < 0.05$ vs. WT-veh; $p < 0.01$ vs. WT-nic). The ERK phosphorylation in vehicle-treated D2RKO mice was lower than that in WT mice (phospho-ERK1 = 75.5%, phospho-ERK2 = 65.2% of WT mice) ($p < 0.05$ vs. WT-veh; $p < 0.01$ vs. WT-nic). Nicotine treatment in D2RKO mice did not significantly elevate ERK phosphorylation (phospho-ERK1 = 76.5% phospho-ERK2 = 67.5%) ($p < 0.05$ vs. WT-veh; $p < 0.01$ vs. WT-nic). Altogether, aberrant CA1 region cues occurred in a nicotine-dependent acquisition. Likewise, nicotine-induced elevations of pCREB, pro-BDNF, and BDNF levels were totally abolished in D2RKO mice (Fig. 5c, d, e).

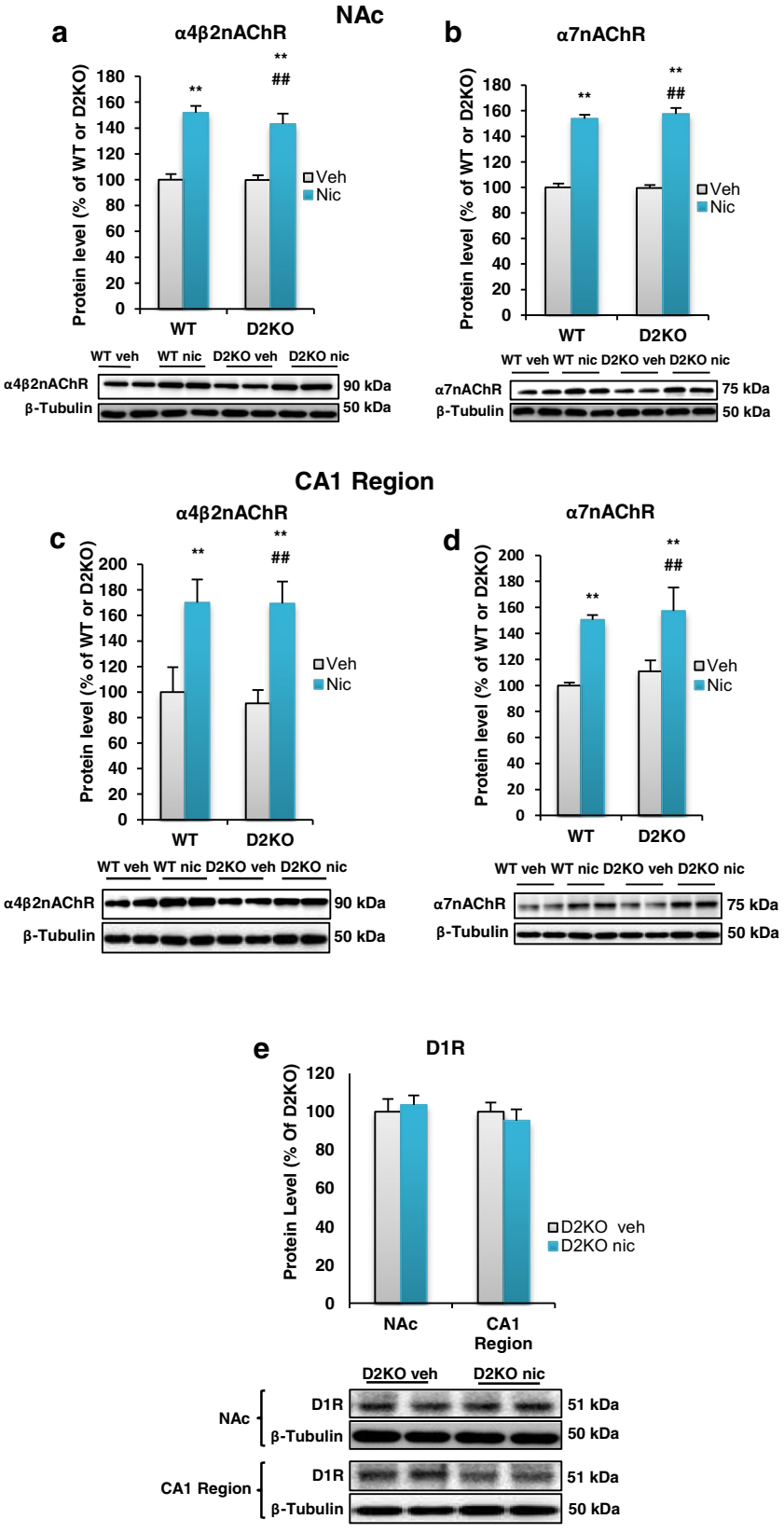
Immunohistochemical Signals of CaMKII and ERK Are Elevated After Nicotine-Induced CPP in NAc

To further confirm the activation pCaMKII and pERK in the NAc, we carried out immunohistochemical analyses. As shown in Fig. 6, nicotine increases the pCaMKII-positive cell number in the NAc (471 cells/1.69 mm^2), which showed an obvious change comparable to the vehicle (340 cells/1.69 mm^2 in WT mice; $p = 0.00373$; $p < 0.01$ WT-veh vs. WT-nic). Nicotine did not alter the total expression of pCaMKII in D2RKO mice ($p = 0.89$; $p > 0.05$) shown on Fig. 6b (D2RKO-veh = 250 cells/1.69 mm^2 and D2RKO-nic = 245 cells/1.69 mm^2).

Likewise, in Fig. 7, we confirmed the pivotal roles of pERK in regulating nicotine addiction. pERK cells were extremely enhanced after administration of nicotine for 28 days (WT-nic = 493 cells/1.69 mm^2; WT-veh = 385 cells/1.69 mm^2) $p = 0.0055$; $p < 0.01$ vs. WT-vehicle (Fig. 7a, b). In contrast, D2RKO mice showed no change phenomena for pERK cell numbers induced by nicotine injection (D2RKO-veh = 286 cells/1.69 mm^2, D2RKO-nic = 282 cells/1.69 mm^2; $p = 0.89$; $p > 0.05$; Fig. 7b D2RKO-veh vs. D2RKO-nic). Fascinatingly, the extra magnification of both pCaMKII and

Fig. 8 Activation of nAChRs generates signal transmission through the D2 receptor, not the D1 receptor, in D2RKO mice. Nicotine exposure for 28 days first stimulates activity of **a** α4β2nAChRs ($n = 6$) and **b** α7nAChRs ($n = 6$) not only in WT mice but also in D2RKO mice, as shown by the expression of this receptor in the *nucleus accumbens*. **c** α4β2nAChRs and **d** α7nAChRs of the CA1 region show an increase of expression due to 4 weeks nicotine administration. Data are presented as mean ± SEM and were assessed by one-way ANOVA followed by multiple comparisons using post hoc Tukey test. *$p < 0.05$ compared with the vehicle-treated group; **$p < 0.01$ compared with the vehicle-treated group; ##$p < 0.01$ compared to the WT-vehicle group. **e** Quantification of dopamine D1 receptor expression in the *nucleus accumbens* and CA1 region in D2RKO mice, with no significant change in D1 receptor expression after D2RKO mice nicotine treatment compared to that after D2RKO vehicle group treatment. Data are presented as mean ± SEM and were assessed by Student's *t* test, $p > 0.05$

pERK molecules and the fluorescence of molecules are tremendous on nicotine treatment against vehicle group on WT mice (Figs. 5a and 6a). Contrary, D2RKO mice block the nicotine-induced phosphorylation of both CaMKII and ERK.



Involvement of nAChR Signal on Activation of Dopamine D2 Receptor Transmission

Activation of muscarinic and nicotinic acetylcholine receptors in the nucleus accumbens core is necessary for the acquisition of drug reinforcement [37]. Further research using fast-scan cyclic voltammetry on *Drosophila melanogaster* larva showed that Ach induces dopamine release catalyzed by nAChR [38]. Therefore, nAChRs are critical for D1 or D2 signaling. We examined the expression of α4β2nAChR and α7nAChR in NAc. Nicotine elevated not only α4β2nAChR but also α7nAChR in either WT mice treated by nicotine or D2RKO mice nicotine group in both NAc and CA1 regions (Fig. 8). In the NAc area, the α4β2nAChR expression levels in the WT-nicotine (151.9%) and D2RKO groups (143.1%) were much higher than those in the vehicle treatment groups for mice of each group ($p < 0.01$ vs. WT-vehicle; Fig. 8a) [39]. Consistent with the α4β2nAChR, the α7nAChR in the NAc region was elevated by nicotine administration in WT mice (153.8%) and D2RKO mice (157.6%) as compared to the vehicle groups (100%) ($p < 0.01$ vs. WT-vehicle) (Fig. 8b) [40]. Additionally, the concentrations of α4β2nAChR and α7nAChR in the CA1 region were also elevated in wild-type mice (170.01% and 150.4%) and D2RKO mice (169.3% and 157.3%) and rapidly changed in comparison with the WT-vehicle (Fig. 8c, d). Evaluation of the effect of nicotine on D1R was performed by measuring the expression levels of D1R in D2RKO mice (Fig. 8e). Nicotine failed to increase the D1R levels in both NAc and CA1 regions in D2RKO mice (103.6% and 95.4% respectively compared to D2RKO vehicle; $p > 0.05$ vs. vehicle treatment group).

Discussion

Nicotine is a neuroactive compound and the addictive agent in tobacco. In the present study, the administration of nicotine 0.5 mg/kg for 14 and 28 days could induce CPP in mice. Consistent with the results of a previous study [41], nicotine administration for 28 days resulted in upregulation of nAChR in mice. Chronic nicotine exposure results in long-term homeostatic regulation of nAChRs in the NAc, wherein an increase in the binding affinity of nicotine plays a key role in the adaptative cellular processes that leads to addiction [42]. The acute administration of nicotine does not significantly affect the nAChRs. In fact, the beneficial cognitive effects of acute nicotine administration have implications for initiation of smoking and maintenance of tobacco dependence [43]. Nicotine enhances dopamine (DA) release in the mouse striatum, where its release is correlated with nicotine dependence [44]. The released dopamine activates D1R and D2R (Fig. 2a, c) by different neurons in the rat striatum [45]. Nicotine stimulation activates D2R in the indirect pathway neurons by activating α4β2nAChRs at dopaminergic terminals [16]. On the other hand, nicotine also stimulates D1R signaling in the direct pathway neurons by activating α4β2nAChR at dopaminergic terminals [45] and α7nAChR at glutamatergic terminals [6]. Selective deletion of α4 subunits from dopaminergic neurons on α4 (α4-DA) null mice abolished nicotine CPP [46], indicating that the effects of α4nAChR-induced changes in GABAergic tone on dopaminergic neurons by desensitization were essential for nicotine reward [47]. Co-localization of α7nAChR and D_2R within some of the same somatodendritic profiles in VTA suggests that there may be convergent signaling between the α7-subunit and D_2R G protein pathways in postsynaptic neurons of the mice brains [48]. Blockade of both D1R and D2R prevents nicotine addiction [49].

Consistent with the results of previous studies, the D1R antagonist SCH23390 and D2R antagonist eticlopride blocked nicotine-induced dependence in the present study. SCH23390 infusion into the *accumbens* shell (AcS), *parietal association cortex*, and *granular insula* in rats reduced nicotine self-administration by 50 to 75% [14, 15]. Nicotine-induced generation of new dendritic branches and spine is completely blocked by SCH23390 in rat MSN [50]. Eticlopride reduces the nicotine effect of enhancing conditioned reinforcement behavior [51] and inhibits the luciferase activation of LacZ/CRE-Luc by nicotine in neurons cultured from VTA and NAc cells of 17-day-old embryonic Sprague–Dawley rats [9]. The projection of dopamine neurons from the VTA to NAc is crucial in the behavioral sensitization and the rewarding/reinforcing effect of nicotine [34]. The D2RKO mice exhibit a slow response for acquisition of the place-learning task in spatial task training, in which reward prediction is associated with the place where the substances were administrated [52]. Locomotor activity was enhanced by nicotine in WT mice but not in D2RKO mice (Fig. 3b). Taken together, the pivotal roles of D1R and D2R have been documented in nicotine-induced CPP and nicotine-enhanced locomotor activity, as reported [53].

CaMKII activation is correlated with LTP formation by nicotine [54]. Parkinson disease with dopamine-deficit conditions causes memory deterioration [55]. Fukunaga reported that D2R stimulation causes elevation of intracellular Ca^{2+} levels, thereby enhancing CaMKII activity in neuronal culture [56]. The increased CaMKII autophosphorylation in immunohistochemical analyses proved the prominent feature of elevated CaMKII activity by nicotine dependence in the present study (Fig. 5). The nicotine dependence was associated with not only increased CaMKII autophosphorylation but also increased ERK phosphorylation in the NAc of wild-type mice (Figs. 5a and 6d). The intracellular Ca^{2+} elevation induces activation of tyrosine kinase, PYK2, and in turn the activation of RAS upstream of ERK1/2 [57]. In the present study, D2R KO mice showed deficits for the nicotine effect on

ERK1/2 phosphorylation. Therefore, D2R is required for transactivating the tyrosine kinase to generate ERK modulation in addition to CaMKII [57, 58]. We further demonstrated that activation of these kinases promotes phosphorylation of CREB in the NAc and CA1 region. Indeed, CREB (Ser 133/143) is phosphorylated by CaMKII [59] and CREB (Ser133) is phosphorylated by ERK1/2 [60]. CREB activation in NAc may be essential for BDNF expression in nicotine-induced CPP as reported [61].

BDNF knockout heterozygous mice showed that BDNF is required for the rewarding effect in drug abuse such as alcohol and cocaine consumption [62, 63]. Nicotine stimulation increases in *hippocampal* BDNF expression contribute to synaptic rearrangement involved in the development and maintenance of nicotine addiction in humans [64]. Notably, nicotine does not alter BDNF expression in D2RKO mice, suggesting that D2R stimulation is essential for BDNF expression through CaMKII and ERK activation [65]. Likewise, α4β2nAChR in coordination with D2R controls BDNF expression after nicotine treatment in mouse NAc [66], while α4nAChR transactivates tyrosine kinase B (TrkB) [67]. BDNF–TrkB signaling mediates the neuronal

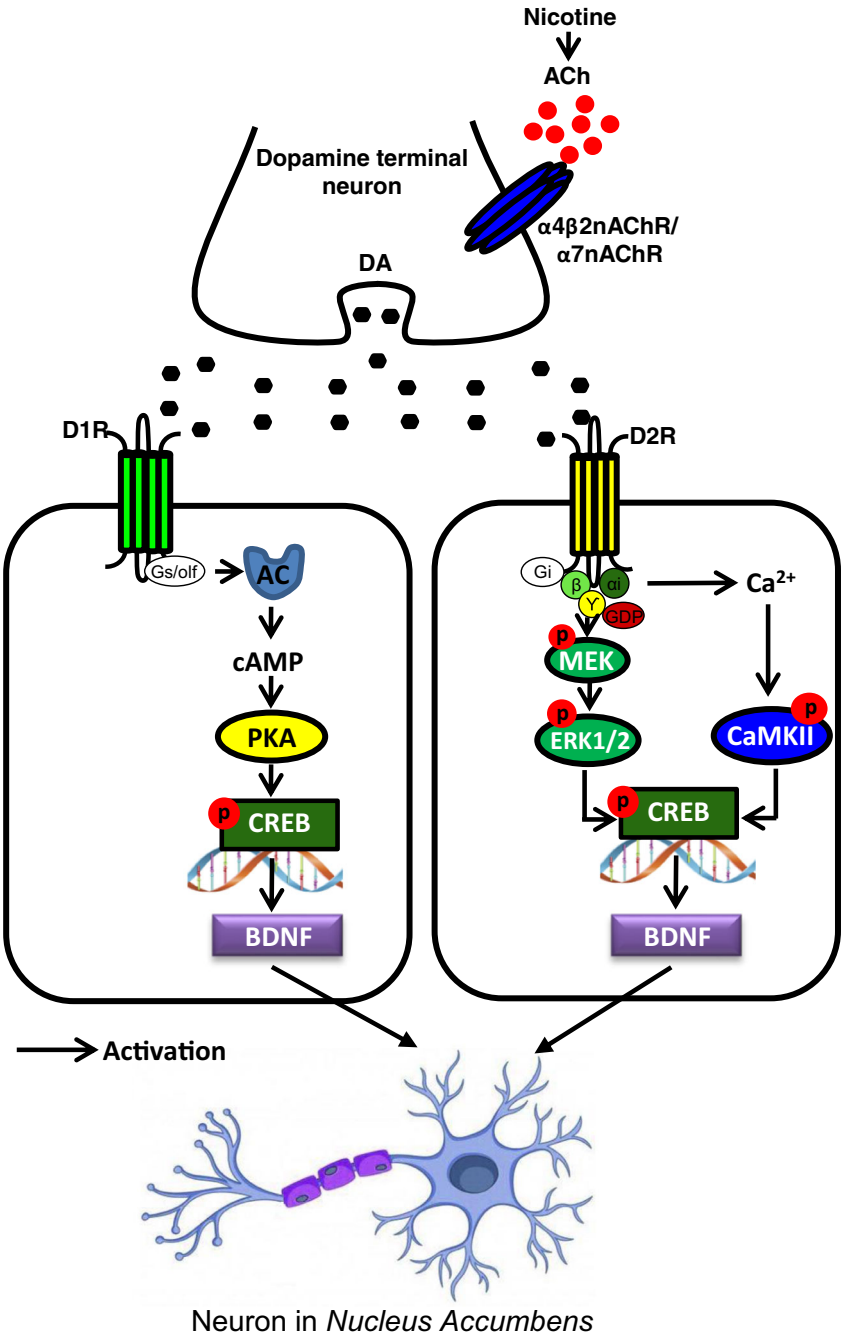


Fig. 9 The mode of D1R and D2R actions on BDNF expression in nicotine dependence. Activation of α4β2nAChRs and α7nAChRs in the dopaminergic terminals enhances DA release. D2R activation stimulates phosphorylation of MEK and the upstream activity of ERK1/2 for influencing the phosphorylation of CREB in core cells. Additionally, D2R activation elevates intracellular calcium to enhanced CaMKII. CaMKII migrates to the nucleus and increases phosphorylation of CREB followed by activation of pro-BDNF. pro-BDNF is a precursor of BDNF and is catalyzed by proteolytic enzyme change to become mature BDNF. D1R stimulation possibly causes synaptic rearrangement through the PKA pathway in the different cell types

structural changes caused by addictive drugs [68], although the main intracellular pathways activated by BDNF–TrkB signaling include MEK–ERK, PI3K–Akt–mTORC1, and PLCγPKC pathways [69].

In other evidence, knock in of the BDNF Val66Met polymorphism gene in mice reduced nicotine-mediated anxiety-like behavior following withdrawal of nicotine and abolished the anxiolytic effect of chronic nicotine consumption [70]. The BDNF Val66Met polymorphism is related to deterioration of *hippocampal* function in humans by fMRI technique [71]. Genotype interaction between BDNF Val66Met polymorphisms, a gene of BDNF and smoking status on serum BDNF, suggests that BDNF Val66Met polymorphism gene did not govern the association between smoking and serum BDNF in humans [72].

In conclusion, nicotine-induced CPP through α4β2nAChR and α7nAChR on dopaminergic neurons [73], wherein activation of both D1R and D2R is crucial for the reinforcing a nicotine effect on nicotine-induced CPP. Activations of CaMKII, ERK1/2, pCREB, pro-BDNF, and BDNF pathways through D2R are vital steps to generate nicotine-induced CPP in addition to D1R (Fig. 9). In conclusion, our results shed new light on D2R in the mechanism of nicotine-induced CPP. These findings provide new perspectives for pharmacological modulation of D2R-induced nicotine addiction.

Conflict of Interest The authors declare that they have no conflict of interest.

Authors Contribution K.F. and G.W. conceived and coordinated the study and wrote the paper. G.W. performed and analyzed the experiment shown in figures. Y.S. provided technical assistance. K.F. and G.W. reviewed the results. All authors approved the final version of the manuscript.

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Compliance with Ethical Standards

All experimental animal procedures were approved by the Committee on Animal Experiments at Tohoku University, and studies were conducted in accordance with committee guidelines.

References

1. Benowitz NL (1999) Nicotine addiction. Prim Care 26:611–631
2. Changeux J-P (2010) Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. Nat Rev Neurosci 11: 389–401. https://doi.org/10.1038/nrn2849
3. Leslie FM, Mojica CY, Reynaga DD (2013) Nicotinic receptors in addiction pathways. Mol Pharmacol 83:753–758. https://doi.org/10.1124/mol.112.083659
4. De Biasi M, Dani JA (2011) Reward, addiction, withdrawal to nicotine. Annu Rev Neurosci 34:105–130. https://doi.org/10.1146/annurev-neuro-061010-113734
5. Nguyen HN, Rasmussen B, Perry DC (2003) Subtype-selective up-regulation by chronic nicotine of high- affinity nicotinic receptors in rat brain demonstrated by receptor autoradiography. J Pharmacol Exp Ther 307:1090–1097. https://doi.org/10.1124/jpet.103.056408.able
6. Nomikos GG, Schilström B, Hildebrand BE, Panagis G, Grenhoff J, Svensson TH (2000) Role of alpha7 nicotinic receptors in nicotine dependence and implications for psychiatric illness. Behav Brain Res 113:97–103. https://doi.org/10.1016/S0166-4328(00)00204-7
7. Missale C, Nash SR, Robinson SW et al (1998) Dopamine receptors: from structure to function. Physiol Rev 78:189–225. https://doi.org/10.1152/physrev.1998.78.1.189
8. Gingrich JA, Caron MG (1993) Recent advances in the molecular biology of dopamine receptors. Annu Rev Neurosci 16:299–321. https://doi.org/10.1146/annurev.ne.16.030193.001503
9. Inoue Y, Yao L, Hopf FW et al (2007) Nicotine and ethanol activate protein kinase A synergistically via G. Pharmacology 322:23–29. https://doi.org/10.1124/jpet.107.120675.jority
10. Baik J-H (2013) Dopamine signaling in reward-related behaviors. Front Neural Circuits 7:1–16. https://doi.org/10.3389/fncir.2013.00152
11. Gore BB, Zweifel LS (2013) Genetic reconstruction of dopamine D1 receptor signaling in the nucleus accumbens facilitates natural and drug reward responses. J Neurosci 33:8640–8649. https://doi.org/10.1523/JNEUROSCI.5532-12.2013
12. Chao SZ, Lu W, Lee HK, Huganir RL, Wolf ME (2002) D1 dopamine receptor stimulation increases GluR1 phosphorylation in postnatal nucleus accumbens cultures. J Neurochem 81:984–992. https://doi.org/10.1046/j.1471-4159.2002.00877.x
13. Mangiavacchi S, Wolf ME (2004) D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. J Neurochem 88:1261–1271. https://doi.org/10.1046/j.1471-4159.2003.02248.x
14. Kutlu MG, Burke D, Slade S, Hall BJ, Rose JE, Levin ED (2013) Role of insular cortex D1 and D2 dopamine receptors in nicotine self-administration in rats. Behav Brain Res 256:273–278. https://doi.org/10.1016/j.bbr.2013.08.005
15. Hall BJ, Slade S, Allenby C, Kutlu MG, Levin ED (2015) Neuroanatomic mapping of dopamine D1 receptor involvement in nicotine self-administration in rats. Neuropharmacology 99:689–695. https://doi.org/10.1016/j.neuropharm.2015.03.005
16. Hamada M, Higashi H, Nairn AC, Greengard P, Nishi A (2004) Differential regulation of dopamine D1 and D2 signaling by nicotine in neostriatal neurons. J Neurochem 90:1094–1103. https://doi.org/10.1111/j.1471-4159.2004.02574.x
17. McCarthy MJ, Duchemin AM, Neff NH, Hadjiconstantinou M (2012) CREB involvement in the regulation of striatal prodynorphin by nicotine. Psychopharmacology 221:143–153. https://doi.org/10.1007/s00213-011-2559-y
18. Novak G, Seeman P, Le FB (2010) Exposure to nicotine produces an increase in dopamine D2 high receptors: a possible mechanism for dopamine hypersensitivity. Int J Neurosci 120:691–697. https://doi.org/10.3109/00207454.2010.513462
19. Zhang SF, Xie CL, Wang Q, Liu ZG (2014) Interactions of CaMKII with dopamine D2 receptors: roles in levodopa-induced dyskinesia in 6-hydroxydopamine lesioned Parkinson's rats. Sci Rep 4:1–6. https://doi.org/10.1038/srep06811
20. Elgersma Y (2004) Mouse genetic approaches to investigating calcium/calmodulin-dependent protein kinase II function in

plasticity and cognition. J Neurosci 24:8410–8415. https://doi.org/10.1523/JNEUROSCI.3622-04.2004
21. Irvine EE, von Hertzen LSJ, Plattner F, Giese KP (2006) αCaMKII autophosphorylation: a fast track to memory. Trends Neurosci 29:459–465. https://doi.org/10.1016/j.tins.2006.06.009
22. Zlomuzica A, Machulska A, Roberts S, von Glischinski M, Rinck M, Lester KJ, Eley TC, Margraf J (2018) The dopamine D2 receptor mediates approach-avoidance tendencies in smokers. Eur Arch Psychiatry Clin Neurosci 268:261–268. https://doi.org/10.1007/s00406-017-0793-y
23. Takeuchi Y, Fukunaga K, Miyamoto E (2002) Activation of nuclear Ca(2+)/calmodulin-dependent protein kinase II and brain-derived neurotrophic factor gene expression by stimulation of dopamine D2 receptor in transfected NG108-15 cells. J Neurochem 82:316–328
24. Kamata A, Takeuchi Y, Fukunaga K (2006) Identification of the isoforms of Ca2+/calmodulin-dependent protein kinase II and expression of brain-derived neurotrophic factor mRNAs in the substantia nigra. J Neurochem 96:195–203. https://doi.org/10.1111/j.1471-4159.2005.03531.x
25. Guillin O, Diaz J, Carroll P, Griffon N, Schwartz JC, Sokoloff P (2001) BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization. Nature 411:86–89. https://doi.org/10.1038/35075076
26. Mössner R, Daniel S, Albert D, Heils A, Okladnova O, Schmitt A, Lesch KP (2000) Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF). Neurochem Int 36:197–202. https://doi.org/10.1016/S0197-0186(99)00122-9
27. Vaidya V, Marek GJ, Aghajanian GK, Duman RS (1997) 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. J Neurosci 17:2785–2795
28. Berninger B, Marty S, Zafra F et al (1995) GABAergic stimulation switches from enhancing to repressing BDNF expression in rat hippocampal neurons during maturation in vitro. Development 121:2327–2335. https://doi.org/10.1242/dev.00351
29. Knipper M, da Penha Berzaghi M, Blöchl A et al (1994) Positive feedback between acetylcholine and the neurotrophins nerve growth factor and brain-derived neurotrophic factor in the rat hippocampus. Eur J Neurosci 6:668–671. https://doi.org/10.1111/j.1460-9568.1994.tb00312.x
30. Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R et al (1997) Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron 19:103–113. https://doi.org/10.1016/S0896-6273(00)80351-7
31. Carboni E, Vacca C (2002) Conditioned place preference. Drugs Abus Neurol Rev Protoc 79:481–498
32. Yabuki Y, Fukunaga K (2013) Oral administration of glutathione improves memory deficits following transient brain ischemia by reducing brain oxidative stress. Neuroscience 250:394–407. https://doi.org/10.1016/j.neuroscience.2013.07.017
33. Yabuki Y, Takahata I, Matsuo K, Owada Y, Fukunaga K (2018) Ramelteon improves post-traumatic stress disorder-like behaviors exhibited by fatty acid-binding protein 3 null mice. Mol Neurobiol 55:3577–3591. https://doi.org/10.1007/s12035-017-0587-2
34. Risinger FO, Oakes RA (1995) Nicotine-induced conditioned place preference and conditioned place aversion in mice. Pharmacol Biochem Behav 51:457–461. https://doi.org/10.1016/0091-3057(95)00007-J
35. Laviolette SR, Lauzon NM, Bishop SF, Sun N, Tan H (2008) Dopamine signaling through D1-like versus D2-like receptors in the nucleus accumbens core versus shell differentially modulates nicotine reward sensitivity. J Neurosci 28:8025–8033. https://doi.org/10.1523/JNEUROSCI.1371-08.2008
36. Pontieri FE, Tanda G, Orzi F, Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382:255–257
37. Crespo JA (2006) Activation of muscarinic and nicotinic acetylcholine receptors in the nucleus accumbens core is necessary for the acquisition of drug reinforcement. J Neurosci 26:6004–6010. https://doi.org/10.1523/JNEUROSCI.4494-05.2006
38. Pyakurel P, Shin M, Venton BJ (2018) Nicotinic acetylcholine receptor (nAChR) mediated dopamine release in larval Drosophila melanogaster. Neurochem Int 114:33–41. https://doi.org/10.1016/j.neuint.2017.12.012
39. Benowitz NL (2010) Nicotine addiction. N Engl J Med 362:2295–2303. https://doi.org/10.1056/NEJMra0809890
40. Brunzell DH, McIntosh JM (2012) Alpha7 nicotinic acetylcholine receptors modulate motivation to self-administer nicotine: implications for smoking and schizophrenia. Neuropsychopharmacology 37:1134–1143. https://doi.org/10.1038/npp.2011.299
41. Govind AP, Vezina P, Green WN (2009) Nicotine-induced upregulation of nicotinic receptors: underlying mechanisms and relevance to nicotine addiction. Biochem Pharmacol 78:756–765. https://doi.org/10.1016/j.bcp.2009.06.011
42. Besson M, Granon S, Mameli-Engvall M, Cloez-Tayarani I, Maubourguet N, Cormier A, Cazala P, David V et al (2007) Long-term effects of chronic nicotine exposure on brain nicotinic receptors. Proc Natl Acad Sci 104:8155–8160. https://doi.org/10.1073/pnas.0702698104
43. Heishman SJ, Kleykamp BA, Singleton EG (2010) Meta-analysis of the acute effects of nicotine and smoking on human performance. Psychopharmacology 210:453–469. https://doi.org/10.1007/s00213-010-1848-1
44. Mclaughlin I, Dani JA, De Biasi M (2015) The neuropharmacology of nicotine dependence. 24:99–123. https://doi.org/10.1007/978-3-319-13482-6
45. Wonnacott S, Kaiser S, Mogg A, Soliakov L, Jones IW (2000) Presynaptic nicotinic receptors modulating dopamine release in the rat striatum. Eur J Pharmacol 393:51–58. https://doi.org/10.1016/S0014-2999(00)00005-4
46. McGranahan TM, Patzlaff NE, Grady SR et al (2011) 4 2 nicotinic acetylcholine receptors on dopaminergic neurons mediate nicotine reward and anxiety relief. J Neurosci 31:10891–10902. https://doi.org/10.1523/JNEUROSCI.0937-11.2011
47. Wooltorton JRA, Pidoplichko VI, Broide RS, Dani JA (2003) Differential desensitization and distribution of nicotinic acetylcholine receptor subtypes in midbrain dopamine areas. J Neurosci 23:3176–3185
48. Garzón M, Duffy AM, Chan J, Lynch MK, Mackie K, Pickel VM (2013) Dopamine D2 and acetylcholine α7 nicotinic receptors have subcellular distributions favoring mediation of convergent signaling in the mouse ventral tegmental area. Neuroscience 252:126–143. https://doi.org/10.1016/j.neuroscience.2013.08.008
49. Dani JA (2003) Roles of dopamine signaling in nicotine addiction. Mol Psychiatry 8:255–256. https://doi.org/10.1038/sj.mp.4001284
50. Ehlinger DG, Burke JC, McDonald CG et al (2017) Nicotine-induced and D1-receptor-dependent dendritic remodeling in a subset of dorsolateral striatum medium spiny neurons. Neuroscience 356:242–254. https://doi.org/10.1016/j.neuroscience.2017.05.036
51. Guy EG, Fletcher PJ (2014) Responding for a conditioned reinforcer, and its enhancement by nicotine, is blocked by dopamine receptor antagonists and a 5-HT2Creceptor agonist but not by a 5-HT2Areceptor antagonist. Pharmacol Biochem Behav 125:40–47. https://doi.org/10.1016/j.pbb.2014.08.006
52. Tran AH, Tamura R, Uwano T, Kobayashi T, Katsuki M, Matsumoto G, Ono T (2003) Dopamine D2 receptor-knockout changed accumbens neural response to prediction of reward associated with place in mice. Int Congr Ser 1250:493–508. https://doi.org/10.1016/S0531-5131(03)00966-X

53. Kelly MA, Rubinstein M, Phillips TJ et al (1998) Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. J Neurosci 18:3470–3479. https://doi.org/10.1523/jneurosci.4936-09.2010
54. Kutlu MG, Gould TJ (2016) Nicotinic modulation of hippocampal cell signaling and associated effects on learning and memory. Physiol Behav 155:162–171. https://doi.org/10.1016/j.physbeh.2015.12.008
55. Picconi B (2004) Abnormal Ca2+-calmodulin-dependent protein kinase II function mediates synaptic and motor deficits in experimental parkinsonism. J Neurosci 24:5283–5291. https://doi.org/10.1523/JNEUROSCI.1224-04.2004
56. Fukunaga K, Shioda N (2012) Novel dopamine D2 receptor signaling through proteins interacting with the third cytoplasmic loop. Mol Neurobiol 45:144–152. https://doi.org/10.1007/s12035-011-8227-8
57. Tahara S, Fukuda K, Kodama H, Kato T, Miyoshi S, Ogawa S (2001) Potassium channel blocker activates extracellular signal-regulated kinases through Pyk2 and epidermal growth factor receptor in rat cardiomyocytes. J Am Coll Cardiol 38:1554–1563
58. Luttrell LM, Daaka Y, Lefkowitz RJ (1999) Regulation of tyrosine kinase cascades by G-protein-coupled receptors. Curr Opin Cell Biol 11:177–183
59. Chiamulera C, Di Chio M, Tedesco V et al (2008) Nicotine-induced phosphorylation of phosphorylated cyclic AMP response element-binding protein (pCREB) in hippocampal neurons is potentiated by agrin. Neurosci Lett 442:234–238. https://doi.org/10.1016/j.neulet.2008.07.025
60. Wu G-Y, Deisseroth K, Tsien RW (2001) Activity-dependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. Proc Natl Acad Sci 98:2808–2813. https://doi.org/10.1073/pnas.051634198
61. Brunzell DH, Mineur YS, Neve RL, Picciotto MR (2009) Nucleus accumbens CREB activity is necessary for nicotine conditioned place preference. Neuropsychopharmacology 34:1993–2001. https://doi.org/10.1038/npp.2009.11
62. Hall FS, Drgonova J, Goeb M, Uhl GR (2003) Reduced behavioral effects of cocaine in heterozygous brain-derived neurotrophic factor (BDNF) knockout mice. Neuropsychopharmacology 28:1485–1490. https://doi.org/10.1038/sj.npp.1300192
63. Hensler JG, Ladenheim EE, Lyons WE (2003) Ethanol consumption and serotonin-1A (5-HT1A) receptor function in heterozygous BDNF (+/−) mice. J Neurochem 85:1139–1147. https://doi.org/10.1046/j.1471-4159.2003.01748.x
64. Kenny PJ, File SE, Rattray M (2000) Acute nicotine decreases, and chronic nicotine increases the expression of brain-derived neurotrophic factor mRNA in rat hippocampus. Brain Res Mol Brain Res 85:234–238
65. Hasbi A, Fan T, Alijaniaram M, Nguyen T, Perreault ML, O'Dowd BF, George SR (2009) Calcium signaling cascade links dopamine D1–D2 receptor heteromer to striatal BDNF production and neuronal growth. Proc Natl Acad Sci 106:21377–21382. https://doi.org/10.1073/pnas.0903676106
66. Peterson DJ, Gill WD, Dose JM, Hoover DB, Pauly JR, Cummins ED, Burgess KC, Brown RW (2017) The effects of nicotine in the neonatal quinpirole rodent model of psychosis: neural plasticity mechanisms and nicotinic receptor changes. Behav Brain Res 325:17–24. https://doi.org/10.1016/j.bbr.2017.02.029
67. Beuten J, Ma JZ, Payne TJ, Dupont RT, Lou XY, Crews KM, Elston RC, Li MD (2007) Association of specific haplotypes of neurotrophic tyrosine kinase receptor 2 gene (NTRK2) with vulnerability to nicotine dependence in African-Americans and European-Americans. Biol Psychiatry 61:48–55. https://doi.org/10.1016/j.biopsych.2006.02.023
68. Ohira K, Hayashi M (2009) A new aspect of the TrkB signaling pathway in neural plasticity. Curr Neuropharmacol 7:276–285. https://doi.org/10.2174/157015909790031210
69. Kumar V (2005) Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. J Neurosci 25:11288–11299. https://doi.org/10.1523/JNEUROSCI.2284-05.2005
70. Lee BG, Anastasia A, Hempstead BL, Lee FS, Blendy JA (2015) Effects of the BDNF Val66Met polymorphism on anxiety-like behavior following nicotine withdrawal in mice. Nicotine Tob Res 17:1428–1435. https://doi.org/10.1093/ntr/ntv047
71. Egan MF, Kojima M, Callicott JH et al (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function and its val/met polymorphism in human memory and hippocampal function and suggest val/met exerts these effects by impacting intracellular. Cell 112:257–269. https://doi.org/10.1016/j.solener.2017.10.050
72. Jamal M, Van der Does W, Elzinga BM et al (2015) Association between smoking, nicotine dependence, and BDNF Val66Met polymorphism with BDNF concentrations in serum. Nicotine Tob Res 17:323–329. https://doi.org/10.1093/ntr/ntu151
73. Faure P, Tolu S, Valverde S, Naudé J (2014) Role of nicotinic acetylcholine receptors in regulating dopamine neuron activity. Neuroscience 282:86–100. https://doi.org/10.1016/j.neuroscience.2014.05.040