# Peroxisome Proliferator-Activated Receptors-Alpha Modulate Dopamine Cell Activity Through Nicotinic Receptors

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**Background:** Modulation of midbrain dopamine neurons by nicotinic acetylcholine receptors (nAChRs) plays an important role in behavior, cognition, motivation, and reward. Specifically, nAChRs containing β2 subunits (β2-nAChRs) switch dopamine cells from a resting to an excited state. However, how β2-nAChRs can be modulated and thereby how dopamine firing activity is affected remains elusive. Because changes in dopamine cell activity are reflected in the dynamics of microcircuits generating altered responses to stimuli and inputs, factors regulating their state are fundamental. Among these, endogenous ligands to the nuclear receptor-transcription factor peroxisome proliferator-activated receptors type-alpha (PPARα) have been recently found to suppress nicotine-induced responses of dopamine neurons.

**Methods:** We used both in vitro and in vivo electrophysiological techniques together with behavioral analysis to investigate on the effects of modulation of PPARα in Sprague–Dawley rat and C57BLJ/6 mouse dopamine neurons and their interactions with β2-nAChRs. To this aim, we took advantage of a selective reexpression of β2-nAChR exclusively in dopamine cells by stereotaxically injecting a lentiviral vector in the mouse ventral tegmental area.

**Results:** We found that activation of PPARα decreases in vitro both dopamine cell activity and ventral tegmental area net output through negative modulation of β2-nAChRs. Additionally, PPARα activation in vivo reduces both the number of spontaneously active dopamine neurons and nicotine-induced increased locomotion.

**Conclusions:** Our combined findings suggest PPARα ligands as important negative modulators of β2-nAChRs on dopamine neurons. Thus, PPARα ligands might prove beneficial in treating disorders in which dopamine dysfunction plays a prominent role, such as schizophrenia and nicotine addiction.

**Key Words:** Acetylcholine, dopamine neuron, nicotine, patch-clamp, peroxisome proliferator-activated receptor, ventral tegmental area

Dopamine (DA) neurons of the ventral tegmental area (VTA) project to subcortical and cortical structures to form the mesocorticolimbic pathway. The VTA DA neurons detect primary rewards, novel and reward-predicting stimuli (1,2), and respond to these with changes in their firing activity (3), resulting in phasic DA transmission in target regions where the signal is integrated and translated into learned appetitive behaviors (4). VTA DA cell function is shaped in response to both behaviorally relevant events and drugs of abuse, including nicotine. VTA DA cell firing pattern is controlled by extrinsic afferents, among which the excitatory inputs play a major role (5,6). The excitatory projections to the VTA originate from the prefrontal cortex, the bed nucleus of the stria terminalis, and the cholinergic laterodorsal and pedunculopontine tegmental nuclei (7–9). These latter make synaptic contacts with VTA DA cells (10), which express both muscarinic and nicotinic receptors (11,12), the activation of which leads to increases in DA neuron activity (13,14). Importantly, endogenously produced acetylcholine (ACh) controls DA neuron firing pattern and frequency through activation of nicotinic receptors (nAChRs) (15), and an imbalance between DA–ACh function is often associated with diverse brain disorders (7,16–19).

Nicotine-induced increase in DA neuron firing rate can be suppressed by ligands for the peroxisome proliferator-activated receptor-α (PPARα) (20), a family of nuclear receptor transcription factors involved in the modulation of various peripheral physiological responses, such as lipolysis, inflammation, and energy balance (21). PPARα-induced phosphorylation of nAChRs accounts for the suppression of DA neuron responses to nicotine (20). Because the effects of nicotine on DA neurons were enhanced when PPARα were blocked (20), we hypothesized a constitutive interaction between PPARα and tyrosine kinases regulating the function of nAChRs in VTA DA cells. Thus, tonic control of the ratio of phosphorylated/dephosphorylated nAChRs might allow DA neurons to have access to distinct firing patterns, to change their firing frequency, or both.

To explore the possible role of PPARα in the regulation of DA neuron excitability by endogenous acetylcholine, we investigated interactions between nuclear PPARα and surface nAChRs in VTA DA cells both in vitro and in vivo, and the functional consequences of PPARα activation on nicotine-induced increases in spontaneous locomotion, a DA-mediated behavior.

## Methods and Materials

All procedures were performed in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience

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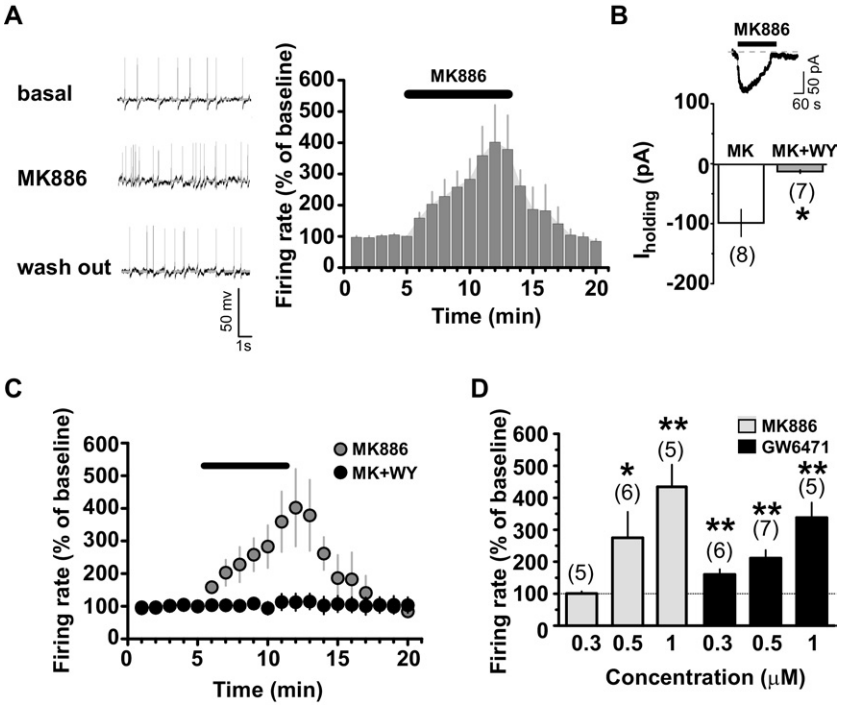


**Figure 1.** Peroxisome proliferator-activated receptors type-alpha (PPARα) blockade activates ventral tegmental area dopamine neurons in vitro. **(A)** MK886 application (.5 μmol/L) increases dopamine neuron spontaneous activity. Current-clamp recording from a dopamine neuron (left panel) and rate histogram depicting MK886 averaged effects (right panel). **(B)** In voltage-clamp mode, MK886 caused an inward current ($V_{hold}$ = −70 mV) blocked by the PPARα agonist WY14643 (.3 μmol/L). **(C)** WY14643 blocked MK886-induced activation of dopamine neurons. **(D)** Summary of dose-related effects of PPARα antagonists on dopamine neuronal frequency. Numbers above bars indicate *n* values. Data are expressed as mean ± SEM. *$p < .05$; **$p < .005$.

and Behavioral Research (National Research Council 2004), EEC Council Directive (219/1990 and 220/1990) and approved by Animalerie Centrale and Médecine du Travail, Institut Pasteur. We made all efforts to minimize pain and suffering and to reduce the number of animals used.

### Electrophysiological Studies In Vitro

Whole cell patch clamp recordings from VTA DA cells were as described previously (22) and Supplement 1. Briefly, male Sprague–Dawley rats (Harlan Nossan, San Pietro al Natisone, Italy) or $\beta2^{-/-}$ and $\beta2^{+/+}$ mice (detailed information follows) were anesthetized with halothane and killed. Recordings were made from horizontal slices superfused with artificial cerebrospinal fluid (ACSF, 37°C) saturated with 95% $O_2$ and 5% $CO_2$ containing (in mmol/L): 126 NaCl, 1.6 KCl, 1.2 $NaH_2PO_4$, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 18 $NaHCO_3$, and 11 glucose. Evoked field potential recordings were as described previously (23) (Supplement 1).

All the drugs were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was <.01%.

### Electrophysiological Study In Vivo

Extracellular single unit recordings from male Sprague–Dawley rat VTA DA neurons were performed as described previously (24) and in Supplement 1. Single unit activity of DA neurons located in VTA was recorded extracellularly with glass micropipettes filled with 2% pontamine sky blue dissolved in .5 mol/L sodium acetate. Single units were isolated and identified according to published criteria (20).

### Mice

A line expressing Cre recombinase under the control of the dopamine transporter promoter, line DAT-Cre (Tronche), was backcrossed for several generations with the B2-nAChR knockout line ACNB2. The resulting mice used in this study were heterozygous for the Cre transgene and homozygous for the B2 knockout, $Cre^{+-}$; $B2^{-/-}$.

### Lentiviral Expression Vector and Stereotaxic Procedure

pTripPDGF lentivector (25) was modified by replacing the eGFP with the β2nAChR sequence. Lentivirus was bilaterally injected (26) at a dose of 230 ng p24 protein/2 μL into the VTA of $\beta2^{-/-}$/DAT-Cre transgenic mice, generated by backcrossing $\beta2^{-/-}$ with DAT-Cre mice (27). Injection of the vector into $\beta2^{-/-}$/DAT-Cre mice leads to recombination and subsequent expression of the β2 subunit exclusively in DA neurons (25).

### Behavioral Study

Mice were pretreated with either WY14643 (40 mg/kg intraperitoneal [IP]) or its vehicle (10 ml/kg IP) 60 min before nicotine administration (.02 mg/kg subcutaneous) and individually tested for motor activity under standardized environmental conditions as previously described (28) (Supplement 1).

### Statistical Analysis

Numerical data are given as mean ± SEM. Data were compared and analyzed by two-way analysis of variance (ANOVA) for repeated measures, or by one-way ANOVA or Student's *t* test, when appropriate.

## Results

### The PPARα Antagonist MK886 Enhances DA Neuron Spontaneous Activity

To characterize the postsynaptic effects on DA neurons, all experiments were performed in the presence of CNQX (10 μmol/L), D-AP5 (100 μmol/L), and picrotoxin (100 μmol/L) to block alpha-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-, *N*-methyl-D-aspartate-, and gamma-aminobutyric $acid_A$-mediated postsynaptic responses. Whole-cell current- and voltage-clamp recordings were performed from medial posterior VTA DA neurons in rat horizontal slices containing the midbrain. The DA cells displayed electrophysiological characteristics that facilitated their identification (29). Figure 1A shows that bath application of the PPARα antagonist MK886 (500 nmol/L, 8 min)