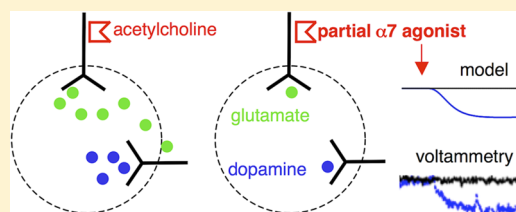


Understanding the Role $\alpha 7$ Nicotinic Receptors Play in Dopamine Efflux in Nucleus AccumbensReinoud Maex,^{*,†} Vladimir P. Grinevich,^{‡,§} Valentina Grinevich,[§] Evgeny Budygin,^{§,||} Merouane Bencherif,[‡] and Boris Gutkin^{†,⊥}[†]Department of Cognitive Sciences, École Normale Supérieure, Paris 75005, France[‡]Targacept Inc., Winston-Salem, North Carolina 27101, United States[§]Wake Forest School of Medicine, Winston-Salem, North Carolina 27157, United States^{||}St. Petersburg State University, St. Petersburg 199034, Russia[⊥]Center for Cognition and Decision Making, National Research University Higher School of Economics, Moscow 101000, Russia

S Supporting Information

ABSTRACT: Neuronal nicotinic acetylcholine receptors (NNRs) of the $\alpha 7$ subtype have been shown to contribute to the release of dopamine in the nucleus accumbens. The site of action and the underlying mechanism, however, are unclear. Here we applied a circuit modeling approach, supported by electrochemical in vivo recordings, to clarify this issue. Modeling revealed two potential mechanisms for the drop in accumbal dopamine efflux evoked by the selective $\alpha 7$ partial agonist TC-7020. TC-7020 could desensitize $\alpha 7$ NNRs located predominantly on dopamine neurons or glutamatergic afferents to them or, alternatively, activate $\alpha 7$ NNRs located on the glutamatergic afferents to GABAergic interneurons in the ventral tegmental area. Only the model based on desensitization, however, was able to explain the neutralizing effect of coapplied PNU-120596, a positive allosteric modulator. According to our results, the most likely sites of action are the preterminal $\alpha 7$ NNRs controlling glutamate release from cortical afferents to the nucleus accumbens. These findings offer a rationale for the further investigation of $\alpha 7$ NNR agonists as therapy for diseases associated with enhanced mesolimbic dopaminergic tone, such as schizophrenia and addiction.

KEYWORDS: $\alpha 7$ -Nicotinic receptor agonist, acetylcholine, desensitization, ventral tegmental area, mesolimbic pathway, modeling



Ligands of neuronal nicotinic receptors (NNRs) are seen as promising therapeutics for a range of CNS disorders, including Alzheimer's disease, Parkinson's disease, schizophrenia, and addiction.^{1–4} NNRs are members of the class of Cys-loop cationic ion channels, yet predicting the systemic effects of their ligands has proven difficult for several reasons: NNRs of varying subunit composition are expressed on different neuron types, locate extrasynaptically, and quickly desensitize to varying degrees in the continued presence of the agonist.

NNRs are abundant in the basal ganglia,⁵ and acute systemic nicotine administration stimulates the efflux of dopamine in rat nucleus accumbens in vivo.^{6,7} Most studies on the cholinergic control of accumbal dopamine release have focused on the effect of activation of different NNR subtypes on the spiking activity of either dopaminergic^{8–10} or GABAergic neurons^{11,12} in the ventral tegmental area (VTA)¹¹ or have measured changes in accumbal dopamine levels after electrical stimulation of midbrain nuclei.¹³ For short, $\alpha 7$ NNRs are thought to exert presynaptic control over inputs to VTA neurons,^{10,14} and consistent with this modulatory role, dopamine neurons of $\alpha 7$ –/– mice showed less prominent changes in their spontaneous or evoked spike patterns than those of $\beta 2$ –/– mice.⁸

More recently, optogenetic stimulation of the accumbal cholinergic interneurons was found to evoke dopamine efflux in vitro without a concomitant change of the spike rate or pattern of the dopamine neurons.^{15,16} Although this accumbal control of dopamine efflux is mediated by preterminal $\beta 2^*$ NNRs on the axons of dopamine neurons,¹⁷ cholinergic stimulation may also activate nearby $\alpha 7$ NNRs located on corticofugal axons and, through the intermediary of ionotropic receptors on dopamine axons, indirectly stimulate dopamine release.^{18,19}

TC-7020 is a selective partial agonist of the homomeric $\alpha 7$ -type NNR²⁰ with an efficacy of 30% and an EC_{50} of 30 nM in rats. Its selectivity for the $\alpha 7$ NNR is evidenced by an IC_{50} of 2 nM, compared with 4200 nM for $\alpha 4\beta 2^*$ NNRs,²¹ and an $IC_{50} > 10 \mu M$ for non-nicotinic receptors.²⁰ In a microdialysis study of a mouse model of schizophrenia, systemic administration of TC-7020 (0.1–1.0 mg/kg ip) normalized the increased striatal extracellular dopamine level.²² The mechanism of action underlying this suppression of dopamine concentration, however, remains elusive. Since microdialysis measures dopamine fluctuations only on a time scale of minutes, these

Received: June 6, 2014

Accepted: August 22, 2014

Published: August 22, 2014



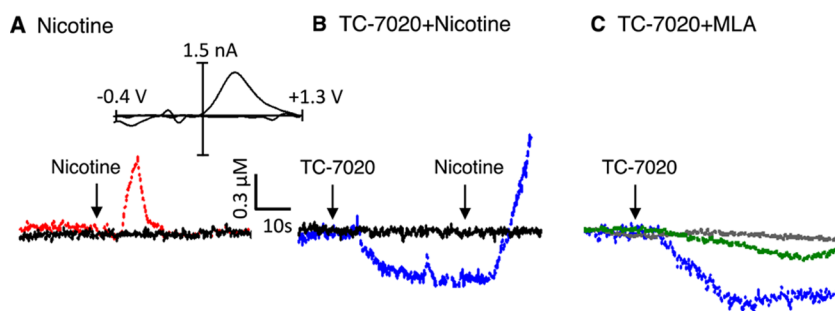


Figure 1. Representative voltammetric recordings of real-time dopamine signaling in nucleus accumbens of anaesthetized rats before and after administration (arrows) of (A) nicotine (0.3 mg/kg iv), (B) TC-7020 (1 mg/kg iv) followed by nicotine, and (C) TC-7020 alone (blue trace) or TC-7020 after pretreatment with MLA (10 mg/kg ip) (green trace) or MLA alone (black trace). Black traces in A and B are controls after saline injection. Inset to panel A is representative background-subtracted voltammogram obtained at peak of response, showing characteristic oxidation and reduction peak potentials (approximately +0.6 V and approximately −0.2 V, respectively) that identify dopamine.

data cannot be effectively used for the computational modeling of the targeted circuitry. Importantly, $\alpha 7$ NNRs rapidly desensitize during agonist exposure, and preterminal receptors interact with glutamatergic transmission in VTA,^{11,23,24} ventral and dorsal striatum,¹⁹ and prefrontal cortex.^{25,26}

In contrast to microdialysis measurements, real-time voltammetry is particularly useful for evaluating the fast dopamine dynamics, due its subsecond-scale temporal resolution. Here, we used a computational modeling approach to understand the observation that TC-7020 acutely reduced dopamine efflux measured by fast-scan cyclic voltammetry (FSCV). In particular, we examine how real-time dopamine signaling depends on the activation and desensitization properties of the $\alpha 7$ NNRs and on their differential distribution on GABA and dopamine neurons or their glutamatergic afferents. The present analysis models only acute effects, up to 1 min after agonist injection, since the circuit may change its dynamics owing to synaptic plasticity²⁷ or adaptation of the expression of other NNR subtypes.²⁸

RESULTS AND DISCUSSION

We first present the *in vivo* voltammetric recording of dopamine efflux in rat nucleus accumbens in response to the iv injection of nicotine and the partial $\alpha 7$ agonist TC-7020. Next we explain how two alternative models, based on desensitization versus activation of the $\alpha 7$ receptor, generated a drop in dopamine release similar to that after TC-7020 injection. Finally, we argue that only one particular implementation of the desensitization model explains the observed response to the combined injection of the positive allosteric modulator PNU-120596.

In Vivo Voltammetry of Dopamine Efflux in Rat Nucleus Accumbens. Intravenous administration of nicotine (0.3 mg/kg) induced a fast and potent increase in accumbal dopamine measured by real-time FSCV in anesthetized rats (Figure 1A). The averaged dopamine concentration was $0.82 \pm 0.08 \mu\text{M}$ ($n = 4$). The effect appeared approximately 7 s after drug administration. Importantly, an identical nicotine-induced dopamine concentration increase had been observed in freely moving rats using the same technique (FSCV).⁷ No changes were observed after saline injection (Figure 1A,B). Using the same experimental design, we explored the effects of the $\alpha 7$ -selective partial agonist TC-7020 (1 mg/kg iv) on real-time dopamine dynamics (Figure 1B,C). In contrast to nicotine, this compound induced a drop in baseline dopamine recordings. This inhibitory effect could be reversed by nicotine (Figure

1B). The TC-7020-induced drop was nearly abolished by pretreatment with MLA (10 mg/kg ip), an $\alpha 7$ nicotinic receptor antagonist (Figure 1C), ruling out the possibility that the observed dopamine changes were nonspecific or non-receptor mediated. No changes were observed when MLA was administered alone (Figure 1C). A previous microdialysis study also had provided clear evidence that systemic (ip) administration of TC-7020 could suppress extracellular dopamine levels on a prolonged time scale.²² Interestingly, in the present experiments, pretreatment with the $\alpha 7$ type-2 positive allosteric modulator PNU-120596 (5 mg/kg, sc),²⁹ rather than enhancing dopamine release, also blocked the effects of TC-7020 on dopamine transmission, through an expected reduction or elimination of desensitization (see Figure 4A). Qualitatively similar observations were made in the dorsal striatum.

Analysis of the Drop in Dopamine Release Produced in Models Based on the Desensitization versus Activation of $\alpha 7$ NNRs. In order to explain these observations, we compared two classes of models based either on the activation or on the desensitization of $\alpha 7$ NNRs. The models differed by the relative distribution of $\alpha 7$ NNRs on dopamine versus GABA-neurons (or their glutamatergic afferents), and as will be shown below, they functioned optimally at different TC-7020 concentrations and different cholinergic tones.

In the first class of models, NNRs were primarily expressed on dopamine neurons and glutamatergic fibers afferent to them (Figure 2A). The channels were located, first, within the VTA on the glutamatergic afferents to dopamine neurons, where they potentiate glutamate release;^{10,24,30} second, on the dendrites and somata of (a subpopulation of) dopamine neurons;^{23,31–33} and, third, within the nucleus accumbens, on the glutamatergic afferents to the medium-sized spiny neurons, where they potentiate glutamate release and subsequent dopamine release through a process involving ionotropic glutamate receptors presumably located on the dopamine axons.^{18,19} The $\alpha 7$ NNRs are notably absent, however, from the dopamine axonal terminals themselves.^{34,35} Since desensitization of receptors at these locations would indeed reduce dopamine release, we further call this class of models the “desensitization model”.

In contrast, when the $\alpha 7$ NNRs were located primarily at the positions highlighted in Figure 3A, they would evoke a drop in dopamine efflux through receptor activation, and this class of models is further referred to as the “activation model”. Its

The Combined Voltammetry-Modeling Results Point to a Desensitizing Action of TC-7020 on $\alpha 7$ NNRs Located on Glutamatergic Afferents in Nucleus Accumbens.

In summary, the present model considered five different sites for the action of TC-7020 on the mesoaccumbal pathway, and hence for the contribution of $\alpha 7$ NNRs to dopamine release. Although models based on NNR desensitization (sites 1–3 in Figure 2A) and NNR activation (sites 4 and 5 in Figure 3A) could both generate a drop in dopamine release such as that observed experimentally, the neutralizing effect of coadministered PNU-120596 was only explained by the desensitization model (receptor resensitization by the allosteric modulator, sites 1–3). Sites 4 and 5 of the activation model further lack experimental support. For site 5, although stimulation of presynaptic metabotropic glutamate receptors (mGluR1) has been shown to reduce dopamine release in mouse striatum *in vitro*,³⁶ mGluR1 agonists were ineffective in dialysis experiments.³⁹ In addition, although local application of the partial $\alpha 7$ agonist JN403 at site 4 has been observed to enhance the spike rate of GABAergic neurons via action on $\alpha 7$ NNRs at glutamatergic terminals¹¹ and optogenetic stimulation of GABA neurons reduced dopamine efflux in nucleus accumbens,⁴⁰ systemic administration of $\alpha 7$ agonists did not affect the rate or spike pattern of dopamine neurons (Figure 7 of ref 41).

This failure of systemically applied $\alpha 7$ agonists to acutely alter the spiking behavior of dopamine neurons also disfavors sites 1 and 2 of the desensitization model (Figure 2A), leaving site 3 as the most likely mesoaccumbal target for TC-7020. Such a contribution of accumbal $\alpha 7$ NNRs to dopamine release was first proposed by Kaiser and Wonnacott.¹⁹ Note that TC-7020 itself does not evoke dopamine release from synaptosomes (V. P. Grinevich and M. Bencherif, unpublished data); hence the mechanism involves crosstalk among cholinergic, glutamatergic, and dopaminergic neurons. That cortico-accumbal glutamatergic neurons could facilitate dopamine release through a presynaptic action on the dopamine terminals, independently of the rate of firing of meso-accumbal dopamine neurons, had been suggested before,⁴² and subsequent studies confirmed the presence of ionotropic glutamate receptors on dopaminergic synaptosomes^{18,43} and of $\alpha 7$ NNRs on glutamatergic terminals.^{44,45} Electrochemical recordings in nucleus accumbens and VTA of freely moving rats showed that low doses of nicotine (30 $\mu\text{g}/\text{kg}$ *iv*) acutely stimulated glutamate efflux.⁴⁶ In the VTA, at least, nicotine-evoked glutamate efflux has been suggested to be mediated through $\alpha 7$ receptors.¹⁴

As shown by the model, the effect of $\alpha 7$ NNR desensitization on dopamine release can only be apparent in the presence of a cholinergic tone. In nucleus accumbens, acetylcholine is released by giant interneurons that fire spontaneously at a rate of 3–10 Hz *in vivo*.⁴⁷ The presence of a cholinergic tone activating preterminal $\alpha 7$ NNRs *in vitro* was demonstrated by the drop of excitatory post-synaptic current (EPSC) frequency in medium-sized spiny neurons after bath application of MLA.⁴⁸ Taken together, these data indicate that $\alpha 7$ NNRs contribute to the cholinergic control of dopamine release in nucleus accumbens, even at baseline receptor recruitment levels, and hence that their acute desensitization indeed will reduce dopamine efflux.

Note that this mechanism has been suggested before, for instance, to underlie the drop in dopamine after intrastriatal infusion of kynurenic acid, which can act as an $\alpha 7$

inhibitor,^{49–51} and for the regularization of enhanced dopamine release in a mouse model of schizophrenia.²² The loss of regulatory action of $\alpha 7$ NNRs has likewise been suggested to underlie to enhanced nicotine-evoked dopamine release in the accumbens of $\alpha 7$ –/– mutant mice.⁵²

Last but not least we have to discard the possibility that TC-7020 did not act by reducing dopamine efflux but by stimulating its reuptake. In the model, a 15% increase of transport velocity would have sufficed to generate a drop in dopamine concentration of the same magnitude as that generated by receptor desensitization. However, in a previous study,²⁰ 10 μM TC-7020 did not show any affinity for the dopamine transporter in a radioligand assay. Neither glutamate⁵³ nor nicotine⁵⁴ interacts with the dopamine uptake transporter. Moreover, such an interaction of TC-7020 with the dopamine transporter would not have explained the effects evoked by MLA (Figure 1C) and PNU-120596 (Figure 4A).

Robustness of the Model. Predicting the systemic effects of a partial agonist requires knowledge of the balance between receptor activation and desensitization and further of the distribution of the receptors at different locations within the circuit.

For the receptor, steady-state can be assumed, since $\alpha 7$ NNRs desensitize on a subsecond time scale. In that case the fraction of conducting receptors is described by the product of the concentration–response curves for activation and desensitization (Figure 5A). The resultant window current has a bell-

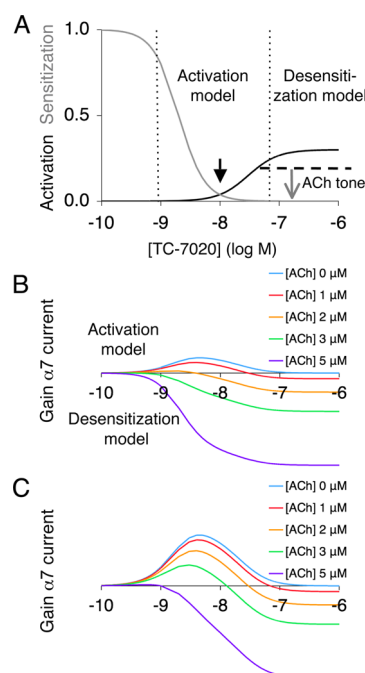


Figure 5. Effect of TC-7020 on acetylcholine-evoked dopamine release summarized. (A) Steady-state activation and desensitization curves for TC-7020 at $\alpha 7$ NNRs. The black arrow indicates the concentration at which TC-7020 is maximally able to activate NNRs without completely desensitizing them. Beyond this concentration, the desensitizing effect on endogenous release (“ACh tone”) predominates (gray arrow). (B) Each trace plots the difference between the NNR current that is generated by the combined presence of TC-7020 and acetylcholine and the NNR current generated by acetylcholine alone. TC-7020 concentration is plotted on the horizontal axis; ACh tone is color-coded. (C) Same data as in panel B, but for a full agonist.

shaped concentration profile (Figure 5B, curve $[ACh] = 0$), and its amplitude increases with the efficacy of the compound (Figure 5C). From the current gained by binding of the exogenous agonist, however, the loss of endogenous current that is due to desensitization must be subtracted. This loss increases with cholinergic tone. The net current diminishes and its peak shifts toward lower agonist concentrations, until at high tone the agonist produces at all concentrations a negative effect (Figures 5B,C).

As stated above, the desensitization and activation mechanisms operate in different concentration regimes, the latter being able to generate a current only at lower ligand concentrations at which a considerable fraction of NNRs did not desensitize yet (black arrow in Figure 5A). Nevertheless in both regimes most of the receptors will desensitize, except when the concentration–response curves for desensitization and activation considerably overlap.⁵⁵ A third mechanism, receptor potentiation, can be considered as a special case of the activation mechanism, operating at agonist concentrations too low to desensitize the receptor but sufficient to open the channel through coagonism with acetylcholine. Although we cannot exclude that this mechanism may be viable within a limited range of concentrations of agonist and acetylcholine (see Supplementary Modeling Results in Supporting Information), it would be incompatible with the effect of coadministration PNU-120596.

The same principles hold for the nicotine response, which was evoked in the model by the activation, and subsequent desensitization, of $\alpha 4\beta 2$ NNRs. Following Figure 5C, such a positive response is easier to obtain when the activation and desensitization curves substantially overlap (see Figure S1C, Supporting Information) and when the efficacy is high and cholinergic tone low.

The second determinant of the response, the relative expression of functional $\alpha 7$ receptors by different neuron types, is difficult to quantify. Although transcription can be measured,^{11,32} most receptors are located intracellularly, and electron microscopy is needed to confirm their subcellular position at the plasma membrane.²³ In addition, electrophysiological responses after local versus systemic application may differ.¹¹ Given that insufficient information is available about the actual distribution of $\alpha 7$ NNRs, we simulated two rather extreme cases with 80% of $\alpha 7$ NNRs located on dopamine neurons or their afferents (the desensitization model, $s = 0.8$) and all of them on the afferents to GABA neurons (the activation model, $s = 0$). Apart from these two cases, the responses from intermediate distributions can be derived as follows. Given that the strength of inhibition from GABA neurons to dopamine neurons in the VTA had a relative weight of 1.5, all $\alpha 7$ responses would virtually disappear in dopamine neurons at a distribution factor s of 0.6 (40% of $\alpha 7$ NNRs on afferents to GABA neurons) since currents of similar amplitude but opposite sign would cancel each other. At still lower values of s , the responses of the dopamine neuron would change sign, and be mirror symmetric about the baseline with respect to those shown before for the desensitization model. (The same reasoning can be applied to the responses of the activation model, with a reduction of response amplitude as s starts rising from 0 and a sign reversal at $s = 0.6$.)

Similar mechanisms of dopamine modulation can take place in other brain regions, for example, in the dorsal striatum. However, since the innervations of the striatum and nucleus accumbens are divergent, some distinction can be expected. In

the Supporting Information, we discuss how the same principles may apply to predict $\alpha 7$ -evoked dopamine release in prefrontal cortex.

Limitations of the Present Study. A final note is needed on the calibration of the two model variables representing the cholinergic and dopaminergic tones, respectively. The cholinergic tone was modeled by a parameter giving the equivalent concentration needed for endogenous acetylcholine to generate a certain steady-state level of receptor activation. Even though many NNRs are located extrasynaptically, the equivalent concentration should not be interpreted as the concentration of acetylcholine in extracellular space, which is in the low nanomolar range.⁵⁶ At best it could represent the acetylcholine concentration close to the synaptic release site, given that the fast $\alpha 7$ NNR responses are presumably generated, at least in neocortex, by classical synaptic transmission.⁵⁷

As for the extracellular dopamine concentration, baseline estimates from voltammetry vary from 20–30 nM⁵⁸ to 73 nM⁵⁹ to 95–220 nM.⁶⁰ Extracellular dopamine concentration may also be spatially heterogeneous, with some patches having concentrations in the low micromolar range.^{51,61} In the present FSCV measurements (Figure 1), no information about quantitative basal dopamine levels was obtained because all data were background subtracted. Drops below baseline have been recorded before,^{51,59} but their magnitude is difficult to quantify, because no dopamine cyclic voltammogram (inset to Figure 1A) can be obtained when the baseline goes down, and small artifacts can be involved in the drop (such as pH changes). Neither can the present model decide on the amplitude of baseline or evoked dopamine responses. The model assumed a baseline dopamine concentration of 50 nM, but some other parameters (including the brain profile of TC-7020 concentration and nicotine concentration after fast iv injection) are poorly constrained. Figure 6 shows how similar realistic dopamine responses can be obtained with a slower clearance of TC-7020 and nicotine and with a baseline dopamine concentration of 337 nM.

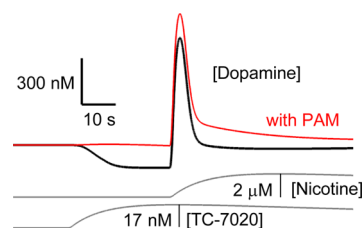


Figure 6. Simulated accumbal dopamine response to TC-7020 and nicotine starting from a baseline $[dopamine]$ of 337 nM. The compounds were administered with a τ_{in} of 10 s and τ_{out} of 200 s. Simulations of the desensitization model with its standard parameters, except for a weaker connection weight from the GABAergic to dopaminergic neurons (1 vs 1.5) and a different cholinergic tone at $\alpha 7$ versus $\alpha 4\beta 2$ NNRs (33 vs 3 μM). The red trace plots the response after pretreatment with PNU-120596.

CONCLUSION

The present study is compatible with a mechanism of glutamatergic control of dopamine efflux in nucleus accumbens, in which neither glutamate nor dopamine requires spikes to be released.⁴² Acetylcholine binding to $\alpha 7$ receptors on glutamatergic terminals promotes the spillover of glutamate to dopaminergic terminals, where binding to ionotropic receptors

leads in turn to dopamine release.¹⁹ Our combined experimental and modeling results indicate that partial agonists such as TC-7020 may suppress dopamine release by desensitizing the $\alpha 7$ receptors.

METHODS

Experimental Procedure. Male Sprague–Dawley rats (Charles Rivers Laboratories, Raleigh, NC) weighing 300–400 g, which were housed two animals per cage with ad libitum food and water in a 12/12 h light/dark cycle, were used in our in vivo voltammetric studies. All procedures were approved by the Wake Forest University and University of North Carolina Animal Care and Use Committees. Experiments were performed on anesthetized animals.

At the day of experiment, naive rats were surgically implanted with indwelling iv catheters under urethane (1.5 g/kg ip) anesthesia using aseptic procedures immediately before voltammetric assessments. A tapered polyurethane catheter was implanted into the right external jugular vein with the catheter exiting the skin behind the ear. A muscle tie served as a tether, preventing the catheter from being dislodged during subsequent voltammetric assessments. Following surgery, the implanted catheter was flushed with 0.2–0.3 mL of sterile 0.9% saline, and the catheter was clamped until later use during the voltammetric experiment. Then rats were head-restrained in a stereotaxic frame, and a carbon fiber electrode (50–100 μm exposed tip length, 7 μm diameter; Goodfellow, Oakdale, PA, USA) was positioned in the nucleus accumbens core (AP + 1.3, L + 1.3, V – 6.7–7.0 mm from bregma) with a Ag/AgCl reference electrode implanted in the contralateral hemisphere. The reference and carbon fiber electrodes were connected to a head-mounted voltammetric amplifier (UNC Electronics Design Facility, Chapel Hill, NC) and voltammetric recordings were made at the carbon fiber electrode every 100 ms by applying a triangular waveform (–0.4 to +1.3 V, 300 V/s). Data were digitized (National Instruments, Austin, TX) and stored on a computer. To confirm the placement of electrodes, a 200 μA current was passed through a stainless steel electrode for 10 s. Brain was removed and postfixed in 10% formalin for at least 3 days. After freezing, 50 μm coronal brain sections were taken and mounted throughout the rostral-caudal extent of the nucleus accumbens. The position of electrodes was assessed by visual examination of coronal sections for electrolytic lesions.

Nicotine (0.3 mg/kg), TC-7020 phosphate (1.0 mg/kg), and saline were administered intravenously as an experimenter-delivered bolus over 4–6 s in a volume of 0.3–0.4 mL. Methyllycaconitine citrate (MLA, 10 mg/kg) and the $\alpha 7$ -selective type-2 positive allosteric modulator PNU-120596 (5.0 mg/kg) were injected intraperitoneally (ip) and subcutaneously (sc), respectively, 30 min before TC-7020 administration.^{62,63} The effect of every compound on dopamine dynamics was replicated on four animals. These results were used for the modeling procedure. TC-7020 was provided by Targacept, Inc., PNU-120596 was purchased from Sigma-Aldrich (St. Louis, MO), and MLA was purchased from Tocris Bioscience (Bristol, U.K.).

Modeling Methods. The mathematical model represented the mesoaccumbens pathway and incorporated both receptor kinetics and network dynamics. Program code was written in XPP.

Receptor Model. Each NNR subtype had an activation and a desensitization gate, resulting in four possible receptor states, with the fraction of conducting receptors being the product of those being active, a , and sensitive, s .⁶⁴ The steady-state concentration–response curves, a_∞ and $(1 - s_\infty)$ for each gate were described as Hill functions (Figure S1, Supporting Information). More particularly, steady-state activation, a_∞ , was calculated as

$$a_\infty = \sum_i w_i \frac{\left(\frac{x_i}{K_i}\right)^{n_i}}{1 + \sum_i \left(\frac{x_i}{K_i}\right)^{n_i}}$$

where x_i is the concentration of the i th agonist, K_i is the agonist's EC_{50} value (half-maximally effective concentration), and n_i is its Hill-exponent (n_{Ha} in Table 1). In this sum, the fraction represents the

Table 1. Parameters of the Hill Functions Used to Model the Receptors' Activation and Desensitization Gates

$\alpha 7$	TC-7020	nicotine	acetylcholine
EC_{50}^a (μM)	0.03	13 ^d	68 ^d
n_{Ha}^b	1.73	1.73	1.73
DC_{50}^a (μM)	0.002	1.3	
n_{Hs}^b	2	2	
E_{max}^c (%)	30	80	100
$\alpha 4\beta 2$	TC-7020	nicotine	acetylcholine
EC_{50}^a (μM)		0.23 ^e	30
n_{Ha}^b		1.05	1.05
DC_{50}^a (μM)		0.061	
n_{Hs}^b		0.5	
E_{max}^c (%)		80 ^f	100

^a EC_{50} (DC_{50}), concentration of half-maximal activation (desensitization). ^b n_{Ha} (n_{Hs}), Hill exponent of activation (desensitization). ^c E_{max} efficacy. ^dSee ref 72. ^eSee ref 68. ^fSee ref 73.

fractional occupancy of the receptor by agonist i , which is multiplied by the agonist's efficacy w_i . For w_p , the E_{max} values from Table 1 were used.

The degree of desensitization ($1 - s_\infty$) was calculated using the same formula, substituting the values of DC_{50} and n_{Hs} for K_i and n_i . Because nicotine and TC-7020 desensitized the receptor completely at high concentrations (see Figure S1A,B, Supporting Information), their weight factor w_i was unity. The w_i of endogenous acetylcholine at the desensitization gate, on the other hand, was set to zero (no desensitization at all) to reflect in vivo the physiological conditions where the transmitter is rapidly hydrolyzed by acetylcholine esterase before desensitization can start.

The acetylcholine parameter in the present model determined the degree of endogenous cholinergic activity, or cholinergic tone, which was assumed to be constant during the course of a recording. The release of acetylcholine not being modeled explicitly, the average tone that it generated was represented by an equivalent concentration constant, $[\text{ACh}]$, which was set so as to generate an average level of receptor activation, relative to which the applied exogenous compounds exerted their action either through further activation, deactivation (competition), or desensitization.

Finally, activation was always fast (time constant 5 ms), whereas the time constant of desensitization varied between minimum and maximum values in a concentration-dependent manner according to the same Hill function as its steady-state⁶⁵ (from 120 s to 50 ms for $\alpha 7$ NNRs; from 600 s to 500 ms for $\alpha 4\beta 2$ NNRs).

Figure S1, Supporting Information, shows the steady-state activation and desensitization curves used in the model for TC-7020 (panel A) and nicotine (panel B) at the $\alpha 7$ NNRs and for nicotine at the $\alpha 4\beta 2^*$ NNRs (panel C). The time traces above each pair of concentration–response curves plot the mean channel current at varying agonist concentrations, illustrating the concentration dependency of the speed of desensitization and the faster desensitization of $\alpha 7$ compared with $\alpha 4\beta 2^*$ NNRs. Table 1 lists the parameters of the Hill functions, which took the same values as in Graupner et al.⁶⁴ except where a new reference is given. Most of these parameters had been taken from Fenster et al.⁶⁶ and Buisson and Bertrand.⁶⁷

Model parameters for the $\alpha 4\beta 2^*$ NNR were identical to those used by Graupner et al.,⁶⁴ except for the lower efficacy (0.8) and a higher affinity (EC_{50} 230 nM) of nicotine, such as is typical of $\alpha 6$ -containing $\alpha 4\beta 2$ NNRs.⁶⁸

The last column of Table 1 also implies that the model's endogenous transmitter, acetylcholine (ACh), activated the receptors without desensitizing them. As stated above, this feature reflects the fundamental difference in kinetics between physiologically released acetylcholine, which is rapidly broken down by ACh-esterase, and exogenous compounds such as nicotine and TC-7020.

Circuit Model. The circuit represented a population of dopaminergic neurons that received inhibition from a local population of

GABAergic interneurons.⁶⁴ The output variables of the model were the mean-field average population activities and the resulting extracellular dopamine concentration. Each population (or its glutamatergic afferents) expressed NNRs of the $\alpha 7$ and $\alpha 4\beta 2^*$ subtypes.

The relative expression of each subtype across the dopamine and GABA neurons was determined by two parameters, r (for $\alpha 4\beta 2$) and s (for $\alpha 7$). A parameter value of 1 indicated that the corresponding NNR subtype was expressed exclusively by the dopamine neuron population (or its glutamatergic afferents); a value of 0.5 meant a balanced distribution across the two neuron populations.

The value of s was the major parameter distinguishing the so-called desensitization and activation models. It determined the relative expression of $\alpha 7$ NNRs on the two neuron populations or their afferents: 80% of $\alpha 7$ NNRs were located on the dopamine neuron or its afferents in the desensitization model ($s = 0.8$) versus all $\alpha 7$ NNRs on the GABA neuron's afferents in the activation model ($s = 0$). The principal locations of $\alpha 7$ NNRs compatible with these two models are as depicted in the respective circuit diagrams of Figures 2A and 3A. Most $\alpha 4\beta 2^*$ NNRs were located on the dopamine neuron in both versions of the model: $r = 0.8$ (0.7) for desensitization (activation) model.

Evidently, the NNRs at the locations depicted in Figures 2A and 3A act all in concert, but for the sake of clarity, we accentuated their relative contributions in the model. A final difference with the circuit model of Graupner et al.⁶⁴ concerned the connection strength from GABAergic onto DAergic neurons, which was enhanced by 50% to favor the activation model.

As described in Graupner et al.,⁶⁴ the action of preterminal $\alpha 7$ NNRs was to enhance glutamate release. Because $\alpha 7$ NNRs may evoke glutamate release in an impulse-independent manner⁶⁹ and because we are faced in this anesthetized in vivo condition with asynchronous inputs from probably tens of afferents, the steady-state terms for spike-evoked and $\alpha 7$ -evoked glutamate release were simply added. Hence, the glutamatergic receptor currents were assumed to be proportional to the amount of glutamate released, with a negative sign for the mGluRs.⁷⁰ Because glutamatergic and dopaminergic terminals often make apposed synapses onto the same medium spiny neuron in nucleus accumbens, diffusion at locations 3 (Figure 2A) and 5 (Figure 3A) of the circuit was assumed to be much faster than the measured responses. Our mean-field approach further implicitly assumed that at the concentrations of spilled-over glutamate, NMDA receptors do not substantially desensitize.

Extracellular Dopamine Concentration. The final outcome of the model was obtained by mapping the mean spike rate of the dopamine neurons onto the variable representing the extracellular dopamine concentration. Although dopamine is particularly released during phasic firing,⁷¹ recent cyclic voltammetry in anesthetized mice showed a fairly linear increase with stimulation frequency.¹⁷ We further assumed a steady-state dopamine concentration of about 50 nM, except in Figure 6 where baseline dopamine concentration was 337 nM. These assumptions allowed us to calculate the dopamine concentration, C , using the following equation:

$$\frac{dC}{dt} = \frac{C_b}{\tau} \left(1 + \frac{r - r_{ss}}{r_{ss}} \right) - \frac{C}{\tau} - V_m \left(\frac{C}{K_m + C} \right)$$

The first term on the right-hand side adds dopamine in proportion to the enhancement in spike rate, r , relative to the steady-state rate, r_{ss} (measured before any drug administration). Dopamine leaks away (second term) and is resorbed by an uptake process with Michaelis–Menten kinetics of maximum rate, V_m , and affinity, K_m (last term). We set V_m to $1.3 \mu\text{M s}^{-1}$, K_m to $0.2 \mu\text{M}$, and τ to 200 ms.⁶⁰ By setting C_b (the basal dopamine concentration in the absence of input or uptake) to 100 nM (500 nM for Figure 6), we obtained a steady-state concentration of about 50 nM.

■ ASSOCIATED CONTENT

§ Supporting Information

Detailed comparative responses of the two models, a consideration of a third mechanism (coagonism), and a discussion of the generalization of our findings to prefrontal cortex. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

V.P.G., E.B., M.B., and B.G. designed and supervised the study. R.M. performed the simulations. V.P.G., V.G., and E.B. performed the experiments. R.M., V.P.G., E.B., M.B., and B.G. wrote the manuscript.

Funding

This work was part of the Collaborative research on NNR/DA integrative pharmacology (PCR-INP10/01), awarded to M.B. and B.G. and was partly supported by IdEx ANR-11-0001-02 PSL* and LabEx ANR-10-LABX-0087 (France). Experimental work was supported by grants from the National Institutes of Health (AA020564) and the Tab Williams Family Endowment Fund to E.B. B.G. acknowledges support from the Basic Research Program of the National Research University Higher School of Economics (Russia).

Notes

The authors declare the following competing financial interest(s): M.B. is an employee and stockholder of Targacept Inc., a biopharmaceutical company involved in research and development of drugs targeting the neuronal nicotinic receptors.

■ ABBREVIATIONS

ACh, acetylcholine; DA, dopamine; FSCV, fast-scan cyclic voltammetry; GABA, γ -amino butyric acid; MLA, methyllycconitine citrate; NNR, neuronal nicotinic receptor; PAM, positive allosteric modulator; PNU-120596, 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea; TC-7020, (2S,3R)-5-methyl-N-[2-(pyridin-3-ylmethyl)-1-azabicyclo-[2.2.2]oct-3-yl]thiophene-2-carboxamide; VTA, ventral tegmental area

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Understanding the role $\alpha 7$ nicotinic receptors play in dopamine efflux in nucleus accumbens

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Supplementary modeling results

Analysis of the drop in dopamine release, as produced in models based on the desensitization versus activation of $\alpha 7$ NNRs

We simulated the responses of two versions of the model to the same stimulation pattern: the partial $\alpha 7$ agonist TC-7020 and nicotine were systemically applied with a 30-s interval (upward arrows in Fig. S2 E). Note that the dopamine response of the model depended on two balances: that between receptor activation and desensitization, and that between excitation and inhibition of the dopamine neuron. As explained in the main text, the desensitization model represented the case where the $\alpha 7$ agonist desensitized NNRs located on the dopamine neuron itself or excitatory terminals afferent to it, with little or no influence from GABA neurons (see Fig. 2A). In the activation model $\alpha 7$ agonists reduced dopamine release by activating NNRs located on glutamatergic afferents to GABAergic interneurons in the VTA (Fig. 3A).

Figure S2 shows the time-course of the key model variables. Most importantly, both the desensitization model (left) and the activation model (right) generated the observed drop in dopamine release after TC-7020 administration, followed by a brisk rise after nicotine injection (Fig. S2 A). A distinctive feature, however, was that the activity of the GABA neurons slightly increased after TC-7020 injection in the activation model (red curve in right panel of Fig. S2 B), whereas it fell concomitantly with the drop in activity of the dopamine neurons in the desensitization model (panel B left). Indeed, in the desensitization model, with 80% of both $\alpha 7$ s and $\alpha 4\beta 2$ s located on the dopamine neuron (or its afferents), the response of the GABA neuron was a scaled-down version of the dopamine neuron's response, and the GABA neuron decreased its spike rate after TC-7020 injection because of desensitization of the residual 20% of $\alpha 7$ s that were located on its glutamatergic afferents. (Note that the baseline levels of activity of the dopamine and GABA neurons in Fig. S2 B are irrelevant for the present study. They can easily be reversed by introducing an additional free parameter, which we avoided for the sake of clarity.)

It is further clear from the fractions of open channels in panels Fig. S2 C that, in both models, the TC-7020 response was exclusively mediated by the $\alpha 7$ NNRs

(desensitization vs activation), whereas the nicotine response was dominated by activation (and subsequent desensitization) of the $\alpha 4\beta 2^*$ channels.

For desensitization of the $\alpha 7$ NNRs to cause a noticeable drop in dopamine release, however, a background receptor activation by acetylcholine was needed (Fig. S2 C, left). In contrast, no cholinergic tone was needed to disclose the dopamine drop in the activation model (Fig. S2 C, right). Finally, the two models used different peak concentrations and time-courses for the applied TC-7020. The reason for using different peak concentrations is that these two models operate in different domains of the dose-response curve (see Fig. 5). In the activation model, the sustained presence of TC-7020 at a low concentration was needed to keep the NNRs activated, without desensitizing them all, during the whole interval of reduced dopamine release. In the desensitization model, in contrast, most of the NNRs completely desensitized and the drop in dopamine release would persist even after complete wash-out of the compound, owing to the slowness of resensitization as compared to activation. We used a faster pharmaco-kinetics for TC-7020 in the desensitization model to illustrate this latter phenomenon (compare Fig. S2 E, left vs right). The responses to slower (faster) kinetics are shown as gray traces. Note also that the response latencies (6-7 s in the experiments) were not modeled explicitly, but assumed to be generated by the systemic blood circulation, and by buffering in the pulmonary capillary bed (1).

Comparative model analysis

The effect of cholinergic tone

The critical effect of the level of the cholinergic tone is illustrated in greater detail in Figure S3. Clearly, a cholinergic tone was essential to the desensitization model (Fig. S3 A), but unnecessary, or even detrimental, in the activation model (Fig. S3 B).

In the desensitization model (Fig. S3 A), a weak or absent tone would reverse the observed effect of TC-7020 into an enhanced dopamine efflux (blue curve around 40 s), because of the default activation of $\alpha 7$ NNRs by partial agonists. In the presence of a higher cholinergic tone, however, TC-7020 did not only contribute less to receptor activation (because of saturation, and competition with acetylcholine), but it caused an even greater loss of acetylcholine-evoked current by desensitizing the

receptor. Stated otherwise, when TC-7020 desensitizes half of the $\alpha 7$ NNRs, it not only reduces its own effect by 50%, but also makes 50% of the receptors unavailable for the background physiological action of acetylcholine, and the latter effect is greater in absolute value the higher the tone. Much of this desensitization effect will go unnoticed in the absence of a cholinergic tone, whence our need of a higher tone in the desensitization model.

The activation model (Fig. S3 B), in contrast, showed an opposite dependency on cholinergic tone: a high tone would unmask desensitization of the $\alpha 7$ NNRs on the GABA neurons' afferents (red trace in inset to Fig S3 B). The resulting drop in activity of the GABA neurons disinhibited the dopamine neurons, so that, after a transient drop, dopamine efflux rose above baseline.

In the following analysis, we focus on the TC-7020 response and disregard the nicotine response, which was generated by identical mechanisms (activation of $\alpha 4\beta 2$ NNRs) in both models alike.

The effect of agonist concentration

Because the drop in dopamine release was generated in the activation model by a window NNR current (in the GABA neuron's afferents), lower TC-7020 doses were needed (peak concentrations from 0.8 to 15 nM; Fig. S4 A, right) than in the desensitization model (from 4 to 37 nM in the left panel of Fig. S4 A). In both models, dopamine efflux dropped faster at higher doses of TC-7020, a consequence of accelerated desensitization and activation, reflecting the nonlinearity of reaction kinetics (see also the current traces in Fig. S1). The depth of the drop was also dose-dependent, but was bounded in the desensitization model, where a substantial tonic $\alpha 4\beta 2^*$ component contributed to baseline dopamine release. The activation model, in contrast, was not able to maintain the drop in dopamine release at high doses of TC-7020 (Fig. S4 A, right, black curve) because the $\alpha 7$ NNRs on the afferents to the GABA neurons started to desensitize after a few seconds, disinhibiting the dopamine neurons.

The effect of agonist efficacy

A distinguishing feature between the models is that in the desensitization model a full $\alpha 7$ agonist evoked a transient peak before the dopamine efflux dropped (Fig. S4 B,

left panel). In contrast, a full agonist caused dopamine concentration only to drop faster and deeper in the activation model without evoking an initial peak (Fig. S4 B, right). In constructing these curves, we assumed that all agonists desensitized the $\alpha 7$ NNR to the same degree, irrespective of their efficacy (2-4). This assumption can be made, since $\alpha 7$ ligands show a poor correlation between activation and residual inhibition or desensitization (M. Bencherif, unpublished data). We also simulated efficacies higher than those reported for currents recorded in vitro ($> 100\%$), because the presence of a receptor reserve may enhance the physiological efficacy in vivo (5). NNRs of the $\alpha 7$ type in particular have been suggested to prolong responses by buffering spilled-over acetylcholine (6, 7).

The effect of co-administration of PNU-120596

PNU-120596 is a type-2 positive allosteric modulator of $\alpha 7$ NNRs, releasing the receptors from desensitization (8). We modelled this effect by shifting the Hill-curve of the desensitization gate towards higher agonist concentrations, leaving the activation gate unchanged. (Positive allosteric modulators of type 2 also shift the activation gate, but this effect is compensated by a concomitantly enhanced endogenous activation by acetylcholine and choline, which enhances the cholinergic tone and partly reduces the net effect of the agonist, see Fig. 5.) In that case, the desensitization model robustly reproduced the experimental cancellation of the TC-7020-induced drop in dopamine release (Fig. S4 C, left panel). The combined administration of ortho- and allosteric compound could even slightly enhance dopamine release, an effect that was readily suppressed by enhancing the cholinergic tone (compare blue and black curves in Fig. S4 C, left).

In the activation model, in contrast, rightward shifting the desensitization curves always amplified the TC-7020 response, instead of neutralizing it (Fig. S4 C, right panel). For the TC-7020 response to be cancelled in the activation model, PNU-120596 should be assumed to interfere with receptor activation, or to block the channel.

The effect of allosteric agonists

Some recently studied allosteric modulators such as 4BP-TSQ, or its (+) enantiomer GAT107, have intrinsic (allosteric) agonist activity (9, 10). Simulating these compounds made the strongest model predictions, as they amplified the drop in

dopamine release in the activation model (Fig. S4 D, red curve right panel), but reversed the drop into a genuine dopamine efflux in the desensitization model (Fig. S4 D, red curve left, see legend for details).

Can $\alpha 7$ ligands potentiate the receptor response through co-agonism with acetylcholine?

As an alternative to receptor desensitization and activation, some partial agonists such as EVP-6124 have been suggested to act through co-agonism with endogenous acetylcholine (11). EVP-6124 would enhance the effect of endogenous acetylcholine at brain concentrations lower than those needed for desensitization or activation. The mechanism involves channel opening after paired binding of one acetylcholine molecule and one molecule of partial agonist at the two available binding sites. In the formulas for receptor occupancy these cross-terms between different ligands are commonly neglected, either because such configurations are very transient or because they do not open the channel. They may, however, explain some of the potentiation effects observed at low concentrations of agonists or antagonists (12, 13).

In the simulations presented below, channel current was calculated using the ad hoc formula

$$\frac{w_x x^{n_x} + 2w_{xy} xy + w_y y^{n_y}}{1 + x^{n_x} + 2xy + y^{n_y} + 2x + 2y}$$

where x and y , representing ACh and TC-7020, are the normalized concentrations (concentration divided by the respective EC_{50} , or DC_{50}), the n 's Hill coefficients and the w 's weight factors. Note that this formula is identical to the formula derived by Cachelin and Rust (12) for the potentiating effect of an antagonist ($w_y=0$), provided the receptor has two non-cooperating binding sites ($n_x=n_y=2$). As before, parameters were set to the values given in Table 1, except for the new free parameter w_{xy} , which expressed the efficacy of channel opening (or desensitization) by co-agonist receptor binding.

We calculated the $\alpha 7$ current to trains of 5-s puffs of acetylcholine, in the presence of varying concentrations of an $\alpha 7$ compound (Figure S5). We first examined this co-agonist mechanism for a competitive antagonist (Fig. S5 A), using as ligand TC-7020 but with zero efficacy and without allowing either acetylcholine or TC-7020 to desensitize the receptor. As in Smulders et al. (14), continuous application of a low dose of antagonist potentiated the responses to puffs of 1 μM acetylcholine. With $w_{xy} = 0.3$ at the activation gate, the response increased by 37% (upper panel in Fig. S5 A). As in Smulders et al., however, potentiation failed when higher doses of acetylcholine were used (lower panel).

We next tried to replicate, using TC-7020 at its 30% efficacy, the observations which Prickaerts et al. (11) made with the partial agonist EVP-6124 (a sequence of potentiation of the puff response, suppression by desensitization, and opening of the channel by the agonist itself). EVP-6124, however, has a very high $\text{EC}_{50}/\text{DC}_{50}$ ratio, and in order to obtain desensitization in the absence of overt activation, the EC_{50} for TC-7020 had to be increased from 30 to 300 nM. Even so, a cross-term weight of $w_{xy} = 20$ at the activation gate gave less than 9% potentiation. If, in contrast, puffed acetylcholine was allowed to desensitize the receptor ($\text{DC}_{50} = 1.3 \mu\text{M}$) in this in vitro condition (absence of cholinesterase), potentiation was readily obtained (Fig. S5 B). It sufficed to set the cross-term weight w_{xy} to zero at the desensitization gate (and at the activation gate as well) to obtain a potentiation of 55% (upper panel in Fig. S5 B). Note that this zero cross-term implied that the co-agonist pair did not desensitize the receptor, whereas each ligand in itself did. Hence, the underlying mechanism is competitive inhibition of the co-agonist pair with pairs of identical ligands, protecting the receptor from desensitization. Note also that the further increase in amplitude after a 60-s interval at 480 s is due to a partial recovery from desensitization. When the partial agonist was applied at higher concentrations (lower panel in Fig. S5 B), desensitization overruled co-agonist potentiation and the response dropped during agonist infusion. At still higher concentrations, the partial agonist caused channel opening on its own and the baseline between puffs rose (not shown).

Although a full study of this mechanism is beyond the scope of the present manuscript, the present results show that there may be a narrow range of concentrations of both acetylcholine and partial agonist at which these ligands would cooperate, as in Fig. S5 A. As for the in vitro experiment, our results suggest that the

potentiation effect is a consequence of reduced desensitization rather than enhanced activation.

Supplementary discussion

Mesocortical versus mesolimbic dopamine release

Can the present desensitization model for the accumbal action of $\alpha 7$ partial agonists be extrapolated to other systems such as prefrontal cortex, where NNRs are also abundantly expressed (15)? This is an important question because $\alpha 7$ ligands such as AZD0328 (16-18), EVP-6124 (11), RG3487 (19) and JN403 (20) have been reported to generate positive cognitive effects at low nanomolar or subnanomolar plasma concentrations (although for JN403 the brain concentration was reported to be higher than its EC_{50} value). $\alpha 7$ NNR knock-out mice, in turn, show deficits in attention and memory (21). The pro-cognitive action of $\alpha 7$ ligands has been ascribed to receptor activation (16, 19, 20, 22), receptor desensitization (2, 23), receptor antagonism (24) or to potentiation of the receptor's response to endogenous acetylcholine (10, 11). Moreover, $\alpha 7$ NNRs have been suggested to be able to exert an effect via intracellular signalling mechanisms, even when they are in a nonconducting (desensitized but strongly agonist-binding) state (25, 26), and in the long run, compensatory mechanisms may come into play, such as up- or downregulation of receptors subtypes (27), and changes in circuit connectivity as a consequence of synaptic plasticity.

According to the model, the acute effects will be determined by ligand-specific and circuit-specific characteristics. Ligand-specific characteristics include, apart from differences in the efficacies to activate and desensitize the receptor and in the amplitude of the window current, also cross-affinities for other receptors. The partial agonist EVP-6124, for instance, is also a functional antagonist of 5-HT₃ receptors (11), through which it may enhance the release of acetylcholine and, secondarily, dopamine (28).

The most important circuit characteristics are the distribution of $\alpha 7$ NNRs over principal neurons versus interneurons and the level of the cholinergic tone. There were two mechanisms through which a partial agonist could *enhance* dopamine efflux in the model. The first one is the desensitization of $\alpha 7$ NNRs positioned primarily on GABAergic neurons or afferents to them. Indeed, a distribution value s less than 0.6 ($> 40\%$ of receptors on GABA-neurons or their afferents) would reverse the drop in [dopamine] in Figs. 2 and S2 (left column) into a [dopamine] rise, at least in the presence of a cholinergic tone. The second mechanism is activation of $\alpha 7$ NNRs

positioned primarily on dopamine neurons or afferents to them, in the absence of cholinergic tone (curve labeled 'ACh 0' in Fig. 2B).

Both these circuit characteristics (tone and receptor distribution) may differ between nucleus accumbens and prefrontal cortex. Whereas $\alpha 7$ NNRs are sparse or absent on accumbal interneurons (29, 30), they are richly expressed on most inhibitory neurons of mouse prefrontal cortex (15) and hippocampus. In addition, whereas the cholinergic tone in nucleus accumbens is determined by intrinsic interneurons (31), prefrontal cortex receives projections from telencephalic basal nuclei, and its cholinergic tone may fluctuate significantly, from very low during slow-wave sleep to high during attention and REM sleep. As noted above and shown in Fig. 5, a low tone will accentuate $\alpha 7$ activation, whereas the window for activation will be narrower and shift to lower agonist concentrations when the tone increases.

No cyclic voltammetry data are available for prefrontal cortex because dopamine signals may be contaminated by noradrenaline, as prefrontal cortex, unlike the basal ganglia, receives a rich innervation from locus coeruleus (32). $\alpha 7$ agonists applied to prefrontal cortex through reverse dialysis, however, were found to evoke dopamine release in anaesthetized rats (33, 34), and this release was enhanced by systemical or local application of PNU-120596 (33). From the failure of local PNU-120596 to raise dopamine on its own in anaesthetized animals, Livingstone et al. (33, 35) concluded the endogenous cholinergic tone to be lower in prefrontal cortex than in the basal ganglia. Note that our desensitization model, which generated a drop in dopamine release in the presence of a high cholinergic tone, also generated dopamine efflux when the tone was low or absent (blue curve in Fig. 2B). In accordance with this, only very small doses of an $\alpha 7$ partial agonist, systemically applied in awake cats, were able to enhance dopamine release in the prefrontal cortex (17); and in behaving monkeys, only low doses of iontophoretically applied agonist enhanced persistent firing in pyramidal cells (18).

To further speculate on this, we suggest that $\alpha 7$ agonists act by activating NNRs when the cholinergic tone is low. For instance, memory consolidation in hippocampus is known to require a low cholinergic tone (36) and under such conditions preterminal $\alpha 7$ NNRs may promote synaptic plasticity (37-40). At higher cholinergic tone, such as that observed during attention, $\alpha 7$ agonists may act by desensitizing NNRs, and hence for instance sharpening the cholinergic transients thought to improve attentional performance (6, 41).

Supplementary figure legends

Figure S1. Concentration-response curves for activation and desensitization of model NNRs.

Fraction of NNRs that are at steady-state in the sensitive (gray curves) and active state (black) for the following compound-receptor interactions: the action of TC-7020 on $\alpha 7$ NNRs (A), nicotine on $\alpha 7$ NNRs (B) and nicotine on $\alpha 4\beta 2$ NNRs (C). Note that the activation curves are plotted full-scale; the actual efficacies relative to acetylcholine are 0.3, 0.8 and 0.8 respectively (Table 1). Above the dose-response curves, time traces of receptor current are drawn for varying concentrations; the relative amplitudes of the currents are indicated by the heights of the corresponding color bars on the dose-response graphs.

Figure S2. Deconstruction of the responses of the desensitization (left) and activation models (right) to TC-7020 and nicotine, administered at 30 s and 60 s respectively (bottom arrows and vertical guide lines). A. Dopamine concentration (nM) in nucleus accumbens (black). The gray traces were calculated with a slower (left) or faster (right) brain-availability of TC-7020 than used in the standard simulations of each model, and correspond to the gray concentration profiles in E. B. Population activities of dopamine (DA) (black) and GABA neurons (red) in the VTA (using arbitrary units). C. Fraction of conducting channels for NNRs of the $\alpha 7$ (blue) and $\alpha 4\beta 2^*$ subtypes (green) (traces identical for DA and GABA neurons). Note that in the right panel the left vertical axis plots $\alpha 7$ opening, the right axis $\alpha 4\beta 2^*$ opening. D, E. Time-courses of the concentrations of nicotine (D) and TC-7020 (E).

Figure S3. The effect of varying cholinergic tone on dopamine release in the desensitization (A) and activation model (B). TC-7020 and nicotine were applied as described in Fig. S2. The insets show the responses of the dopamine neuron in the desensitization model (A) and the GABA neuron in the activation model (B). Tones are expressed as equivalent constant background concentrations (μM) of acetylcholine (ACh). Note that the cholinergic tone determines the baseline levels of activity in the insets, but that this effect has been normalized in the dopamine plots as described in Methods.

Figure S4. Comparative analysis of dopamine release in the desensitization (left column) and activation model (right). As before, TC-7020 and nicotine were applied at 30 s and 60 s, respectively. Since the nicotine response was largely invariant, only the TC-7020 response is shown. A. Effect of varying TC-7020 concentration (peak values given in legend). B. Effect of varying the efficacy of the $\alpha 7$ agonist, for peak agonist concentrations of 11 nM in the desensitization model and 3.9 nM in the activation model. C and D. Effects of applying positive allosteric modulators (PAM) in the model. C. The dose at which the $\alpha 7$ receptor is half-maximally desensitized (DC_{50}) was varied, using the same agonist concentrations as in B. The values in the legend give the factor by which the steady-state desensitization curve was shifted to the right. The black trace on the left used a cholinergic tone of 30 instead of 20 μ M. D. The blue traces are the controls using TC-7020. The green traces had the sensitization gate clamped at unity (modeling complete resensitization by a type-2 PAM). Red traces had in addition the efficacy raised to 100%, modelling the putative effect of a PAM with intrinsic agonist activity (“ago-PAM”).

Figure S5. Co-agonist potentiation at the model $\alpha 7$ NNR. Pulses of acetylcholine of 5 s duration were applied at regular intervals (arrows), before, during and after the application of a competitive agonist (A) or a partial agonist (B) (horizontal bars). A. The antagonist potentiates the response to pulses of 10 μ M acetylcholine (upper trace), but not to 60 μ M pulses (lower trace). Parameters as for TC-7020 but with zero efficacy, and a co-agonist cross-term weight $w_{xy} = 0.3$ at the activation gate. Neither acetylcholine nor the antagonist were allowed to desensitize the receptor. B. A low dose of partial agonist (upper trace) potentiates the response to pulses of 60 μ M acetylcholine, whereas a higher dose of partial agonist (lower trace) suppresses the responses through desensitization. Parameters as for TC-7020 (30 % efficacy), but the EC_{50} had been increased by a factor of 10 (from 30 to 300 nM), as described in the text. Both the partial agonist and acetylcholine were allowed to desensitize the receptor, and the cross-term weight w_{xy} was set to zero at both the activation and desensitization gate.

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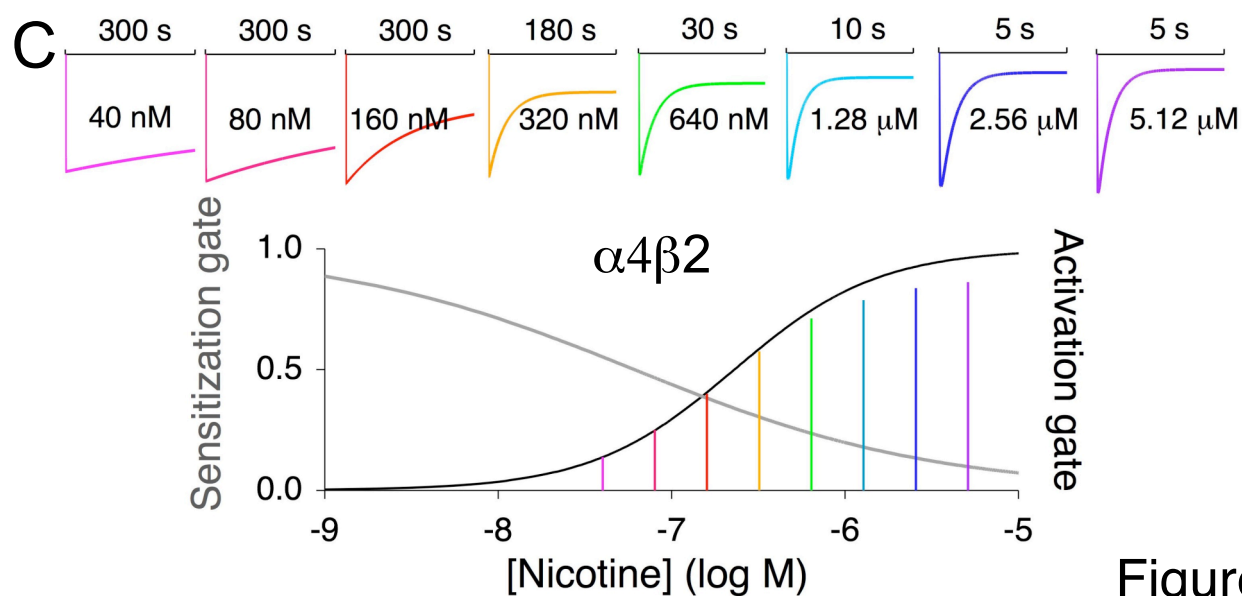
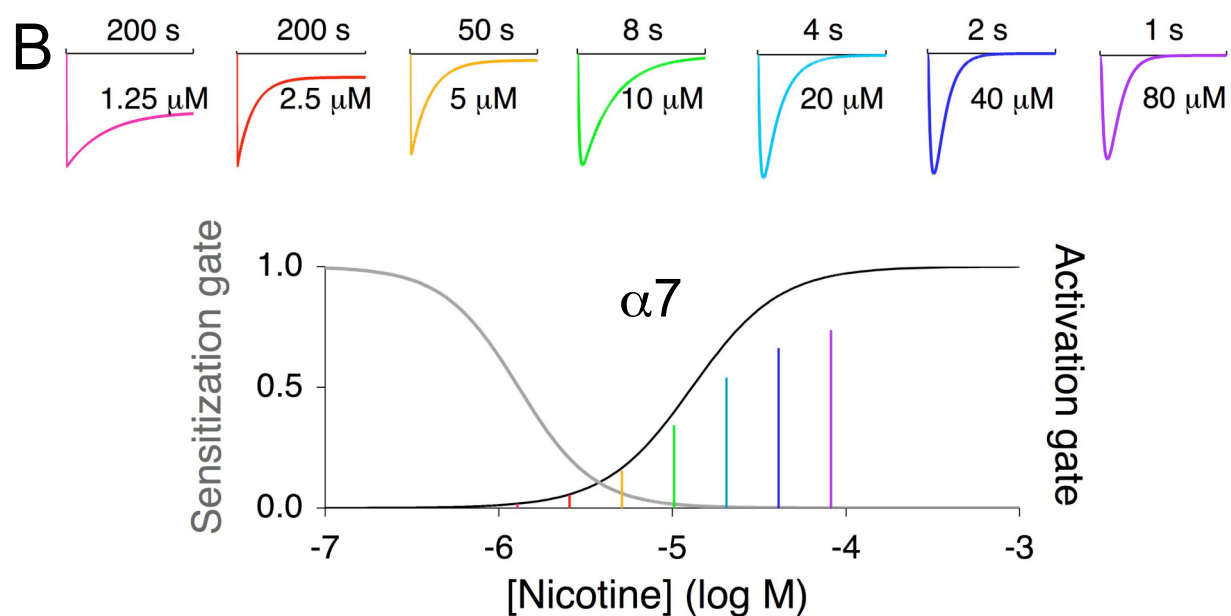
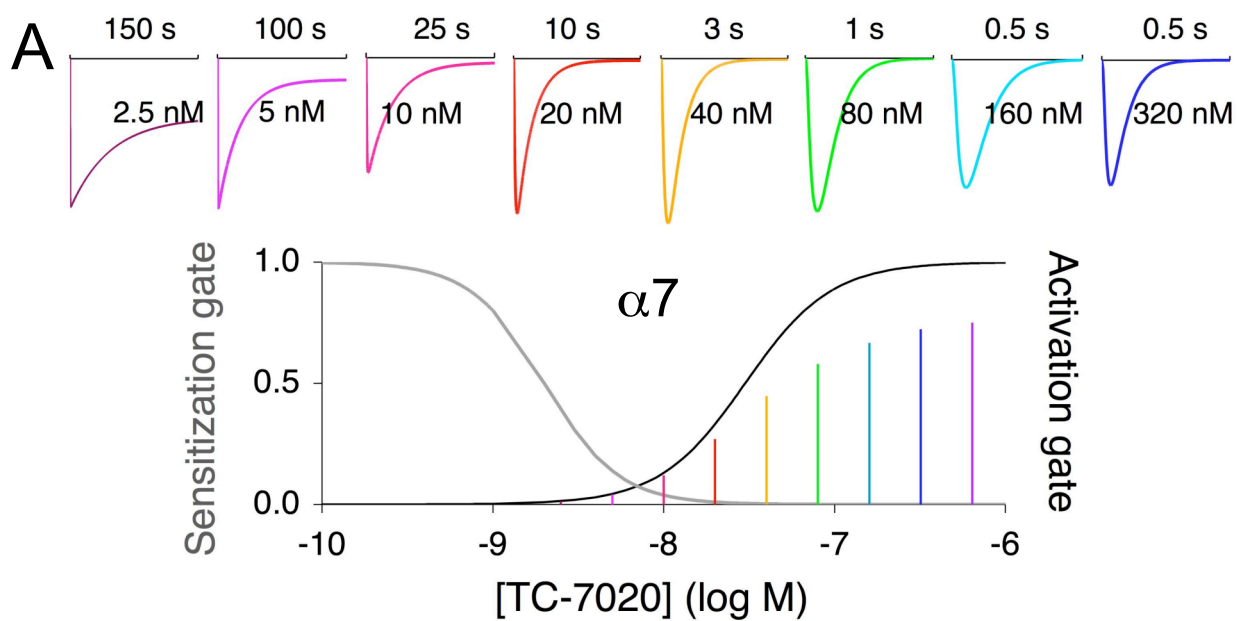


Figure S1

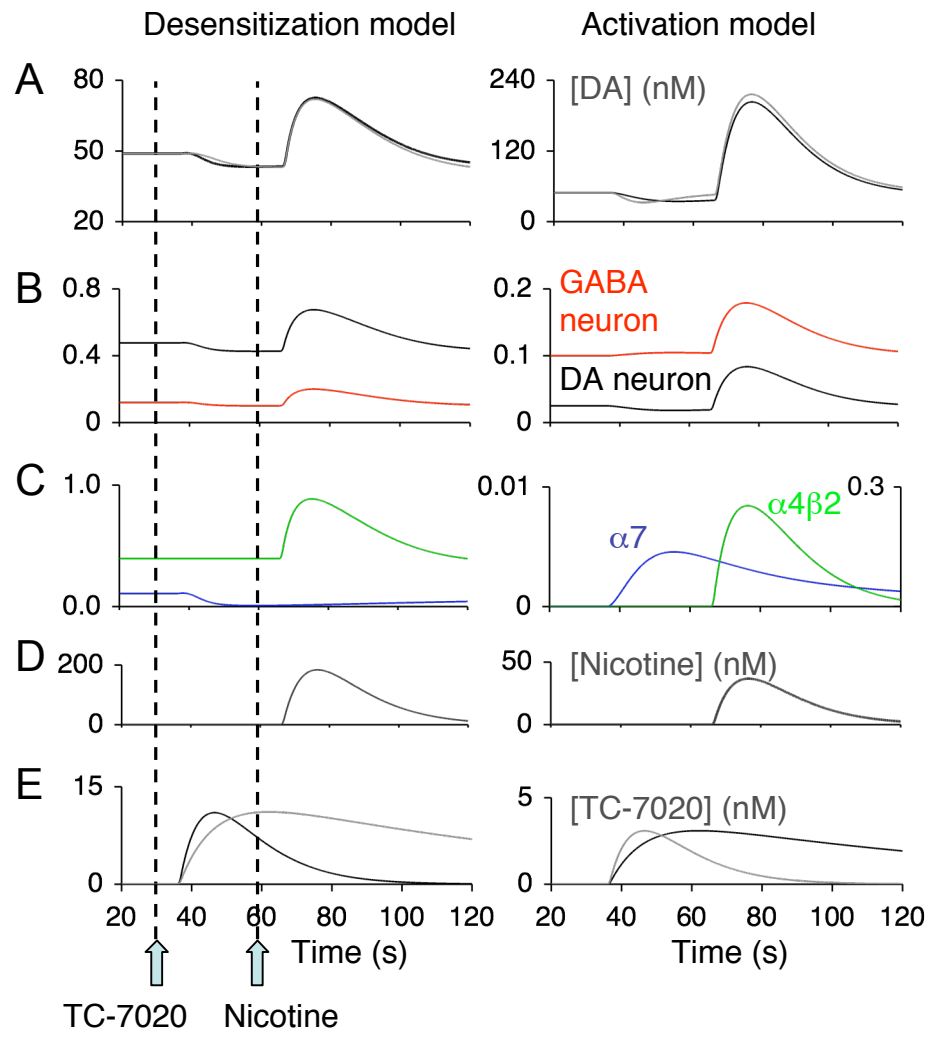


Figure S2

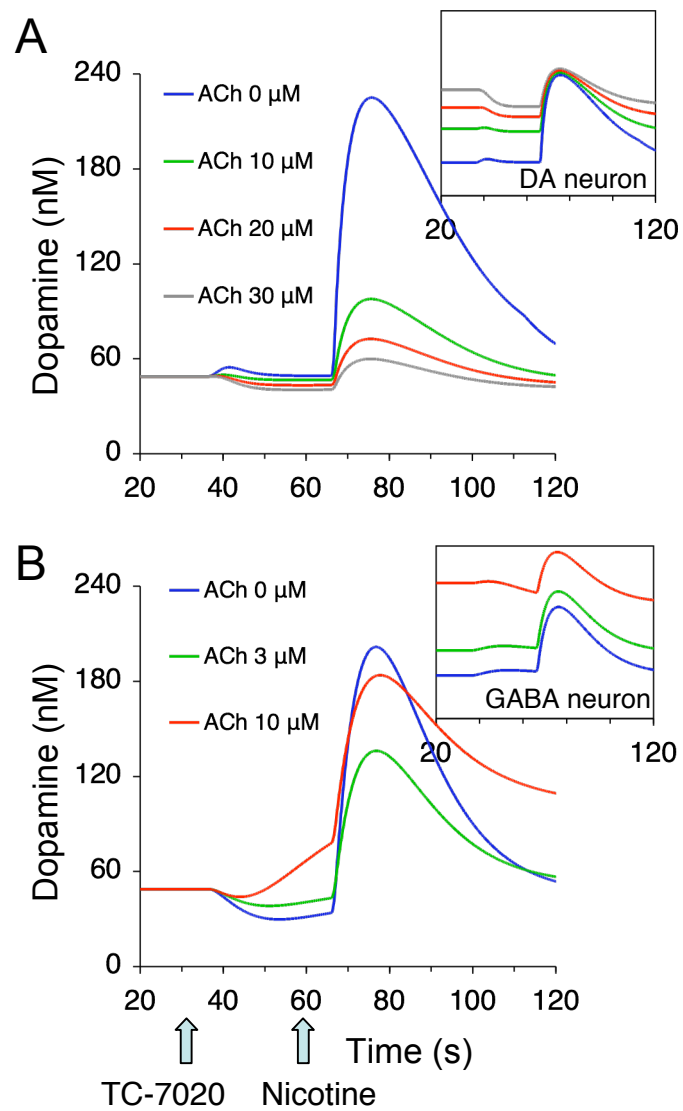


Figure S3

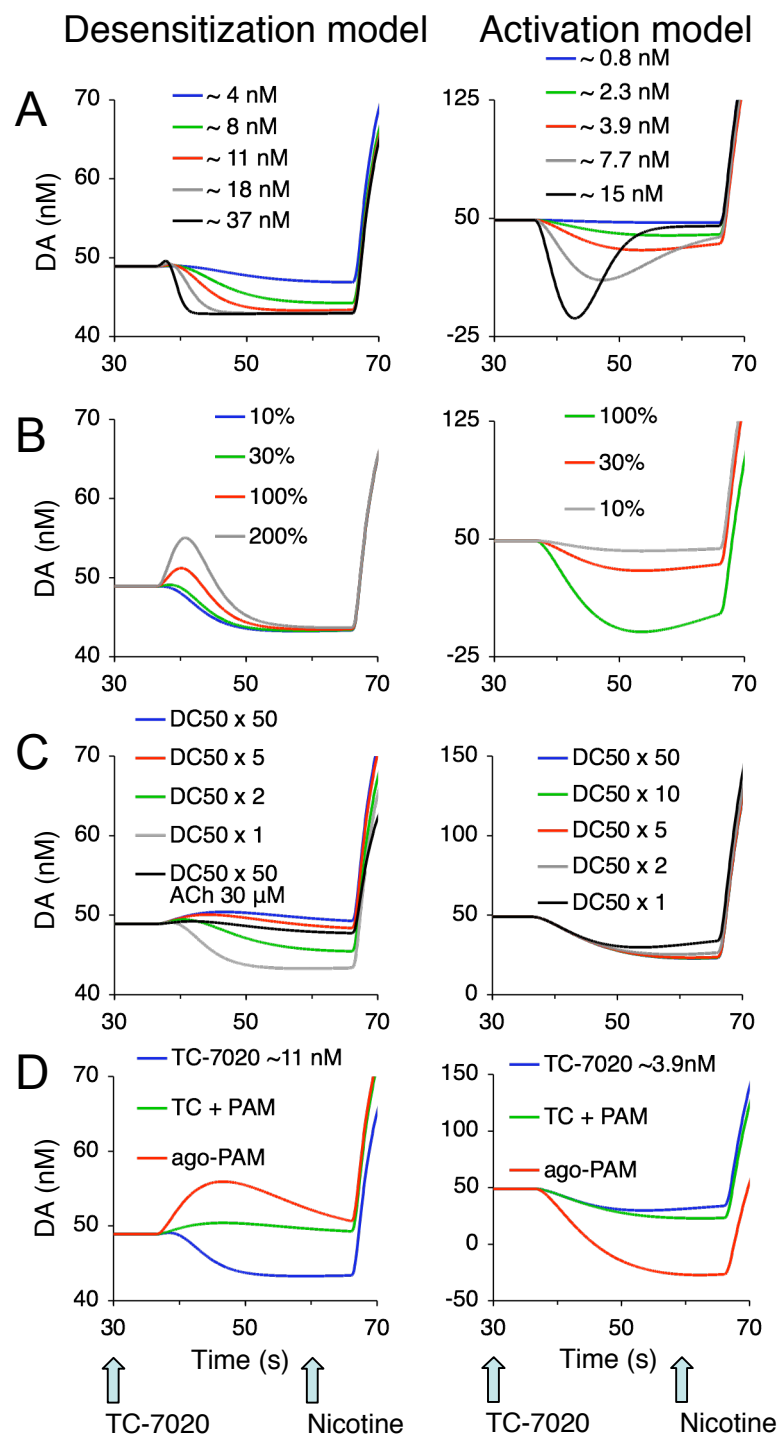


Figure S4

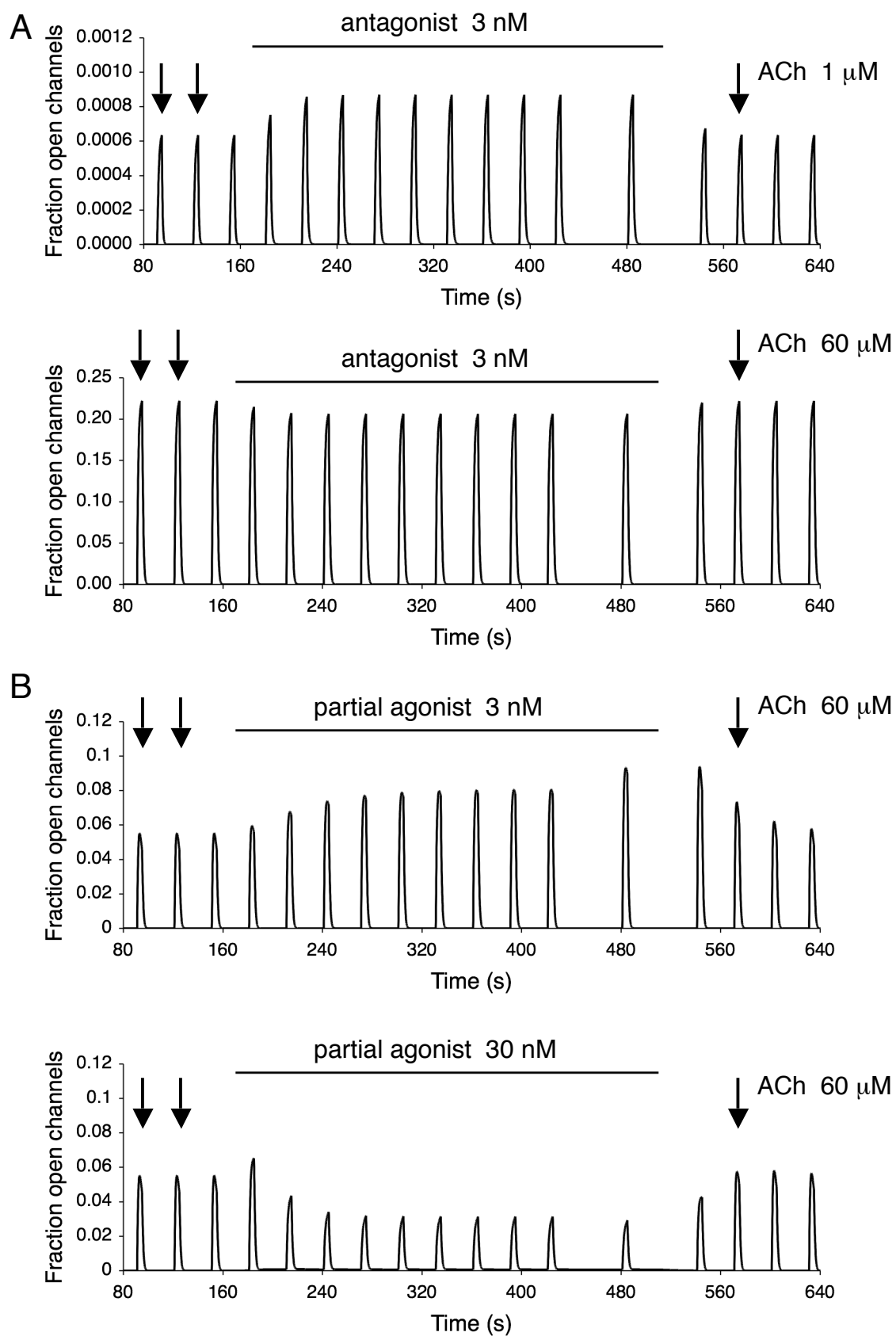


Figure S5