Nicotine control of dopamine signaling in the Ventral Tegmental Area

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ABSTRACT
Midbrain dopaminergic (DA) neurons signal motivational properties of natural reinforces and addictive drugs. Nicotine, like other drugs of abuse, boosts DA output from the ventral tegmental area (VTA). This increase results from direct stimulation of nicotinic acetylcholine receptors (nAChRs) expressed in the VTA. However, how the DA signal is constructed in the VTA and how nicotine changes this signal remains controversial. In particular, *in vivo* and *in vitro* experimental paradigms reach contradictory conclusions about the key target of nicotine action: direct DA cell stimulation or indirect effects mediated through the GABAergic inter-neurons. We address these issues by building and analysing a computational model of the VTA circuitry and nAChR function, based on known activation and desensitization properties of the nAChR subtypes. We show that the apparent data mismatch between *in vitro* and *in vivo* recordings can be reconciled by differences in the afferent input activity. We find that the GABA cells are principle in causing nicotine dependent DA signals. We pin-point the specific contributions of various nAChRs to the DA signal. Finally, we show that DA neuron response to afferent inputs is altered in the presence of nicotine so as to favor phasic dopaminergic release. These results identify mechanisms by which the VTA mediates the rewarding properties of nicotine that lead to addictive behavior.

KEY WORDS
dopamine, nicotinic acetylcholine receptors, ventral tegmental area, local circuitry, biophysical model

1 Introduction

Addiction to nicotine is a major public health problem. Addiction as a process remains an outstanding problem in molecular, systems, behavioral and cognitive neuroscience. Brain areas involved in motivation, affect, memory and motor function are affected by addictive drugs. Among these the mesocorticolimbic dopamine (DA) system serves a fundamental role in the acquisition of behaviors reinforced by addictive drugs [1, 2]. The relevant DA neurons are found in two nuclei, the substantia nigra pars compacta (part of dorsal action/habit systems) and the ventral tegmental area (VTA) that is part of the meso-limbic tract. The VTA projects ventrally to the Prefrontal cortex, the hippocampus and nucleus accumbens among other structures and signals rewards and motivations [3]. Nicotine preferentially stimulates activity and release from the VTA DA neurons [4] and boots dopamine levels in the nucleus accumbens (NAcc), thus mediating the motivational properties of the drug [5, 6]. Nicotine reinforces self-administration as well as complex addictive behaviors [7]. Genetic knockout studies point to the VTA as key to nicotine addiction [8].

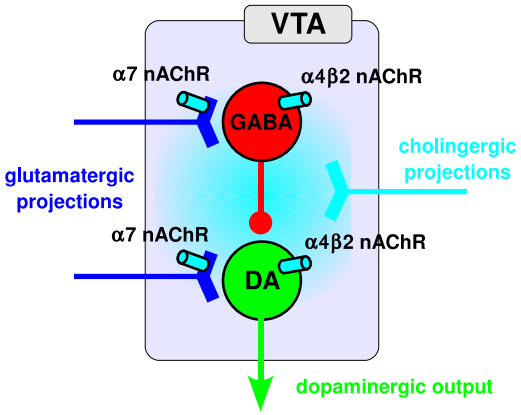

Figure 1. **Scheme of the ventral tegmental area.**

The VTA contains DA and GABAergic cells expressing various nAChR subtypes [9, 10]. The VTA receives major glutamatergic (Glu) inputs from the prefrontal cortex (PFC) that project to the DA [11] and the GABA neurons [12, 13]. Cholinergic inputs from tegmental nuclei [14] to VTA activate nAChRs expressed on GABAergic neurons, dopaminergic neurons and on presynaptic glutamatergic terminals [15, 16, 17]. DA projections from the VTA go to the PFC and to limbic/striatal structures including the NAcc [18, 19]. The GABAergic neurons in the VTA furnish local inhibitory connections [10] and efferents to the brainstem [20] (see Fig. 1). Thus the VTA is an intricate neuronal circuit generating DA signal in response to cortical and ACh inputs.

Under normal conditions, behaviorally relevant stimuli evoke ACh release into the VTA, causing nearly syn-

chronous activation of nAChRs. The rapid delivery and breakdown of ACh precludes significant nAChR desensitization [21]. Nicotine, on the other hand, is not hydrolyzed, *e.g.* in smokers the nicotine concentration is strongly elevated minutes during and after smoking [22]. This activates and desensitizes nAChRs within seconds to minutes [23]. The various subtypes of nAChRs have distinct nicotine affinity/desensitization properties and expression targets: The high affinity $\alpha4\beta2$ subunit containing nAChRs desensitize slowly; low affinity $\alpha7$ nAChRs desensitize rapidly [24, 8]. DA neurons express $\alpha2$ - $\alpha7$ and $\beta2$ - $\beta4$ subunits; GABA neurons express mostly $\alpha4$ and $\beta2$ subunits; $\alpha7$ nAChRs are found on presynaptic terminals of glutamatergic projections to the VTA [15, 16].

Despite data detailing the effects of nicotine at the receptor level, precise mechanisms how the DA response to nicotine is constructed in the VTA remains controversial. *In vitro* recordings from DA neurons conclude that the DA increase is due to disinhibition [15, 25]. Here nicotine transiently boosts GABA transmission to DA cells, followed by $\alpha4\beta2$ nAChR desensitization and therefore disinhibition. The $\alpha4$ nAChR upregulation on VTA GABAergic neurons in response to chronic nicotine further supports the functional importance of this effect [26]. In contrast, *in vivo* studies of wild-type as well as $\alpha7$ and $\beta2$ knockout mice suggest that the key are $\beta2$ nAChRs on DA neurons [27]. These receptors furnish nicotinic excitation to DA cells, increasing the DA activity. This contradiction in the data leaves an important functional lacunae in understanding nicotine action.

In order to untangle the question where is the key site of nicotine action in the VTA, we develop a neuronal network which accounts for the local VTA connectivity as well as the location of the nAChRs. Based on known activation and desensitization properties of nAChR subtypes, we investigate the steady-state activity and the dynamical response of DA neurons to nicotine exposures and changes in afferent input activity. With this approach, we hope to clarify the precise dynamical mechanisms for nicotine action.

2 Mean-field model of the VTA

The activity of the dopaminergic and GABAergic and neuron populations in the ventral tegmental area are characterized by the following differential equations

$$\tau_{\rm D}\dot{\nu}_{\rm D} = -\nu_{\rm D} + \Phi(I_0 + I_{\rm G} + I_{\rm Glu} + I_{\rm ACh}), \quad (1)$$
$$\tau_{\rm G}\dot{\nu}_{\rm G} = -\nu_G + \Phi(I_{\rm Glu} + I_{\rm ACh}). \quad (2)$$

$\nu_{\rm D}$ is the activity of the dopaminergic neuron population and $\nu_{\rm G}$ the activity of the GABAergic neuron population. $I_{\rm Glu}$ and $I_{\rm ACh}$ characterize the excitatory input to the respective neuron population mediated by glutamatergic and cholinergic afferents to the VTA, respectively. $I_{\rm G}$ is the local inhibitory input to DA neurons from GABAergic neurons (see Fig. 1). I_0 is an intrinsic current of DA cells giving rise to the intrinsic spiking of DA cells also present *in vitro* [25]. $\Phi(I)$ is the steady-state current-to-rate transfer function. For simplicity, we assume that $\Phi(I)$ is threshold-linear, *i.e.* $\Phi(I) = I$ if $0 \leq I$ and $\Phi(I) = 0$ otherwise.

The input currents in Eqs. (1) and (2) are chosen such that they vary between 0 and 1. They are given by

$$I_{\rm G} = w_{\rm G}\nu_{\rm G}, \quad (3)$$
$$I_{\rm Glu} = w_{\rm Glu}[\nu_{\rm Glu} + \eta_{\alpha7}]_1, \quad (4)$$
$$I_{\rm ACh} = w_{\rm ACh}\eta_{\alpha4\beta2}, \quad (5)$$

where $w_{\rm x}$ (with x=G, Glu, ACh) refers to the synaptic strength of the respective connection. Cholinergic input activates nicotinic acetylcholine receptors (nAChRs) in the VTA. The activity of $\alpha4\beta2$- or $\alpha7$-subunit containing receptors is given by $\eta_{\rm x}$ (with x=$\alpha7$, $\alpha4\beta2$, see below). Glutamatergic synapses can be activated by upstream activity, $\nu_{\rm Glu}$, or by the $\alpha7$-subunit containing nAChRs located at their presynaptic terminal

$$\nu_{\rm Glu} + \eta_{\alpha7} = \begin{cases} \nu_{\rm Glu} + \eta_{\alpha7} & \text{if } \nu_{\rm Glu} + \eta_{\alpha7} \leq 1, \\ 1 & \text{if } \nu_{\rm Glu} + \eta_{\alpha7} > 1. \end{cases} \quad (6)$$

2.1 nAChR activation and desensitization

The opening and desensitization of nicotinic acetylcholine receptors (nAChRs) is controlled by the endogenous neurotransmitter acetylcholine (ACh) or exogenous ligands such as nicotine. Here, we present a simple description which describes the transitions of the nicotinic acetylcholine receptors between two states of two independent variables: receptor activation and desensitization. This yields four different states: deactivated-sensitive, activated-sensitive, activated-desensitized and deactivated-desensitized. Of those states, three are closed (deactivated-sensitive, active-desensitized and deactivated-desensitized) and only the activated-sensitive is an open state of the receptor in which it mediates an excitatory current. Both, nicotine and acetylcholine evoke the transition from the deactivated-sensitized state to the activated-sensitized state. Since ACh is rapidly removed form the synapse by hydrolization through actylcholinesterase, in our model only nicotine drives desensitization of the receptors. In other words, the transition into the activated-desensitized state is evoked by nicotine only. The receptor recovers slowly from desensitization after removal of nicotine. Deactivation happens rapidly ($\sim$ ms) after removal of Ni/ACh [28].

We model the total activation level of nAChRs by the product of receptor activation and receptor sensitization, *i.e.* $\eta_{\rm x} = a_{\rm x}s_{\rm x}$ with (x= $\alpha4\beta2, \alpha7$). The time course of activation and sensitization variables is given by

$$\frac{dx}{dt} = (x_\infty(Ni, ACh) - x)/\tau_{\rm x}(Ni, ACh), \quad (7)$$

where the $x_\infty(Ni, ACh)$ for activation and sensitization

are given by Hill equations of the form

$$a_\infty(ACh, Ni) = \frac{(ACh + \alpha Ni)^{n_a}}{{EC_{50}}^{n_a} + (ACh + \alpha Ni)^{n_a}}, \quad (8)$$

$$s_\infty(Ni) = \frac{{IC_{50}}^{n_s}}{{IC_{50}}^{n_s} + (Ni)^{n_s}}. \quad (9)$$

EC_{50} and IC_{50} are the half maximum/minimum concentrations of nACh receptor activation and desensitization, respectively. The factor $\alpha > 1$ accounts for the higher potency of nicotine to evoke a response compared to acetylcholine. n_a and n_s are the Hill coefficients of activation and sensitization.

The transition from the closed (deactivated) to the open (activated-sensitized) state is fast compared the other time scales of the system ($\sim$ ms), we therefore simplify the activation time constant, τ_a, to be independent of the acetylcholine/nicotine concentration, *i.e.* $\tau_a(Ni, ACh) = \tau_a = const.$. The nicotine driven desensitization is characterized by a nicotine concentration dependent time constant

$$\tau_s(Ni) = \tau_0 + \tau_{max} \frac{{K_\tau}^{n_\tau}}{{K_\tau}^{n_\tau} + (Ni)^{n_\tau}}. \quad (10)$$

τ_{max} is the recovery time constant from desensitization in the absence of nicotine ($\tau_{max} \gg \tau_0$). τ_0 is minimal time constant at which the receptor can be driven from the sensitive in the desensitized state at high Ni concentrations. K_τ is the Ni concentration at which the desensitization time constant attains half of its minimum. The parameters describing nAChR activation and desensitization are taken from [29, 30, 28, 31, 32]. The model currents evoked by Ni and ACh exposures resemble the ones obtained in experiments.

For simplicity, the above model of nAChR activation and desensitization is not exhaustive of all the details of nAChR function. It is a minimal model describing the key behavior of nAChRs. For example, it is assumed that ACh and Nicotine evoked responses reach the same maximal amplitude and that despite differing potencies Nicotine and ACh dose-response curves can be characterized by the same Hill coefficient. This is approximately true for $\alpha 7$ containing nAChR [33]. ACh evokes however twice the response of Nicotine with $\alpha 4\beta 2$ receptors in a study by [33], but the same response according to [31].

3 Results

We describe the activity of VTA DA and GABAergic neurons by a population activity model. The state variables are the firing rates of the neuronal populations involved. We start with the hypothesis that both, DA and GABAergic neurons, receive excitatory Glu input and express $\alpha 4\beta 2$-containing nAChRs (as shown in Fig. 1). Glu terminals to both cell types express $\alpha 7$ nAChR homomers [17]. Furthermore, GABAergic neurons locally inhibit DA neurons and DA neurons are additionally driven by an intrinsic current leading to regular firing even in the absence of external input [34].

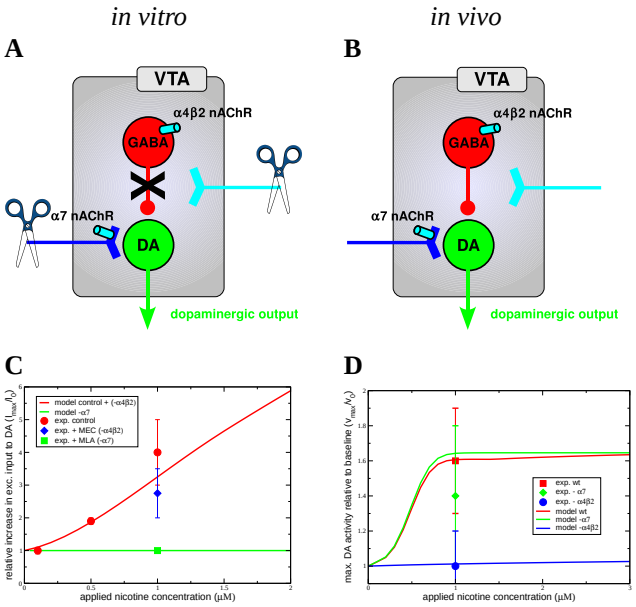

Figure 2. **Relative increase in DA neuron activity in response to nicotine exposure. A** & **B**, Different scenarios experienced during *in vitro* (panel A) and *in vivo* (panel B) experiments: GABAergic transmission is blocked and afferent input strength is reduced in *in vitro* experiments. **C** & **D** Increase of DA activity with respect to nicotine: The maximal increase in DA activity is shown in response to a 1 min exposure to the respective nicotine concentration in the presence of weak (panel C, *i.e. in vitro*) and strong (panel D, *i.e. in vivo*) afferent input strengths.

We use the above described population activity network model to qualitatively account for electrophysiological recordings from DA neurons during nicotine exposures. By doing so, we reduce the model to signaling pathways which are necessary and sufficient to account for the experimental data. We start by considering the *in vitro* results and then move onto the *in vivo* situation.

In vitro, bath application of nicotine causes a robust enhancement of spontaneous excitatory postsynaptic potential (EPSP) frequency onto DA neurons in slices of the VTA [15]. This increase is completely abolished in the presence of methyllycaconitine (MLA), an inhibitor of $\alpha 7$-containing nAChRs, and confirms the presence of $\alpha 7$ containing nAChR presynaptically on glutamatergic terminals onto DA cells. Blocking $\alpha 4\beta 2$ containing nAChRs on the other hand does not significantly change the increase in DA activity [15] (see Fig. 2*A* and *C*). Note that GABAergic transmission is blocked during those experiments. In our model we find that the low affinity of $\alpha 7$-containing receptors for nicotine results in a weak increase of synaptic transmission stemming from the activation of $\alpha 7$-containing nAChR on glutamatergic terminals. It is therefore necessary to choose a low Glu input strength in order to qualitatively reproduce the increase in excitatory input to DA cells shown in Fig. 2*C*. This situation is characteristically for *in*

vitro experiment.

In a further series of experiments the GABAergic neurotransmission in the VTA was left intact in order to study nicotine evoked changes in the frequency of inhibitory postsynaptic potentials (IPSP) [25]. Bath application of nicotine evoked an increase in IPSP frequency recorded in DA cells. The blockade of $\alpha 7$-containing receptors did not show any effect and inhibition of $\beta 2$-containing receptors abolished completely the increase in IPSP frequency. We reproduced the experimental data by assuming that Glu terminals to GABAergic cells express no $\alpha 7$ homomers and $\alpha 4\beta 2$-containing receptors are expressed by GABAergic cells (see Fig. 2). Since the Glu input to GABAergic cells does not appear to make a qualitative difference, we do not account for it in our attempt to distill a minimal model accounting for the experimental data. Again, ACh input is low *in vitro* such that nicotine can further boost the drive to GABAergic cells through activation of nAChRs. This boost in turn leads to an increase in the IPSP frequency. Nicotine also drives the receptors into the desensitized state and the IPSP frequency falls below baseline level after nicotine exposure. This effect is seen in the experiments and is reproduced by the model [25] (results not shown)

We now show that the model accounts for data on nicotine exposures *in vivo*. Importantly, to account for the data we only change the afferent input strength to the VTA, leaving the circuit unchanged (compare Fig. 2*A* and *B*). Here we assume that *in vivo* both the Glu and ACh input levels to the VTA are significantly increased. DA neuron activity rises in response to nicotine in the wild-type animals; this increase is less pronounced in the $\alpha 7$ knockout mice [27]. As described above, the low affinity $\alpha 7$ containing nAChRs show weak activation in response to the applied nicotine doses. On the other hand, the high affinity of the $\alpha 4\beta 2$ containing receptors results in a considerable activation and desensitization at the same nicotine concentration range. Hence nicotine mainly drives the $\alpha 4\beta 2$ receptor into the desensitized state since *in vivo* the strong ACh drive will have already activated the receptor. For this desensitization to result in an increase of DA activity, as observed experimentally, the $\alpha 4\beta 2$ containing receptor has to be mainly expressed on GABAergic neurons, *i.e.* desensitization of $\alpha 4\beta 2$ results in disinhibition of DA neurons. In turn, the increase in DA activity is mostly abolished in the $\beta 2$ knockout animals as we can account for with the model (blue line in Fig. 2*D*). The reduction of DA activity increase in response to nicotine in the $\alpha 7$ knockout animals is not accounted for by the model. We observe a slight increase in the relative peak amplitude instead (see green line in Fig. 2*D*). This increase stems from the reduced baseline activity in the absence of $\alpha 7$ nAChRs. Since the absolute increase in DA activity evoked dominately by $\alpha 4\beta 2$ nAChR activation remains the same, the reduced baseline activity results in a small increase of the relative peak activity of DA neurons.

Finally, we investigate how input via the Glu and/or the ACh projections control the DA activity. In the absence

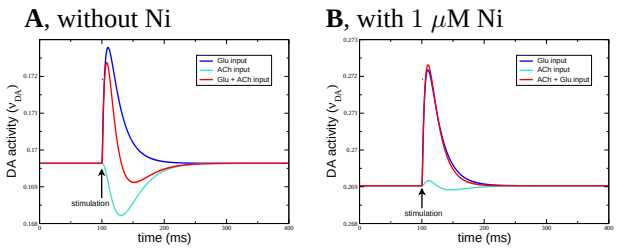

Figure 3. **VTA response to ACh and Glu inputs and its modulation by Ni.** The response in DA neuron activity to 1 ms pluses of Glu (blue lines), ACh (cyan lines) or both (red lines) inputs occurring at $t = 100$ ms. The responses are shown in the absence (panel *A*) and in the presence (panel *B*) of 1 μM nicotine. The pulses are increments of ACh inputs from 5.3 μM to 10 μM and of normalized Glu upstream activity from 0.1 to 1 for 1 ms.

of nicotine, DA neuron activity is augmented in response to transient Glu input and decreased in response to ACh inputs. The DA neuron response to simultaneous input from Glu and ACh pathways is approximately the sum of the individual responses, *i.e.* the activity initially raises above baseline followed by a drop below baseline (see Fig. 1*A*). In the presence of nicotine, the negative drive of ACh input to DA neuron activity is removed while the impact of Glu inputs on DA activity is not affected. For example, while an increase in ACh input alone evokes a decrease of DA activity in the absence of nicotine, it does not change DA activity in the presence of nicotine. On the other hand, an increase in Glu input is translated in an increase of DA activity with or without nicotine (see Fig. 3). Hence nicotine appears to bias the signaling in the VTA toward DA increases.

4 Conclusions

We present a computational model of the VTA circuit that allows to shed light on the mechanisms for nicotine action on dopamine signaling in the VTA. We show that the apparent data mismatch between *in vitro* and *in vivo* recordings can be reconciled by differences in the afferent input strength. We find that the $\alpha 4\beta 2$-containing receptors are principle in causing nicotine dependent DA signals in the nicotine concentration range experienced by smokers. We pin-point that the long lasting increase of DA neuron activity after nicotine exposure is dominately mediated through disinhibition. That means that the local inhibitory drive emerging from GABAergic neurons is reduced due to desensitization of $\alpha 4\beta 2$ receptors dominately expressed on those neurons. We show that the low affinity of $\alpha 7$ containing nAChR exclude those receptors to play an important role in the range of realistic concentrations of nicotine. However, strong cholinergic drive is able to activate those receptors.

Moreover, we predict that changes in DA activity re-

flect the difference in Glu and ACh input changes. Simultaneous inputs from both projections evoke biphasic DA responses. Nicotine changes this computation by affecting primarily ACh inputs, *i.e.* nicotine preferentially removes the negative drive of cholinergic input on dopaminergic signaling. DA responses to coincidental ACh and Glu inputs are therefore dominated by Glu projections and do not reflect the difference between ACh and Glu inputs in the presence of nicotine. These results help to understand how the VTA mediates the rewarding properties of nicotine and therefore may have clinical implications for nicotine replacement therapy.

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