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Review

Age dependent nicotinic influences over dopamine neuron synaptic plasticity

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ABSTRACT

The dopamine (DA) system of the ventral midbrain plays a critical role as mammals learn adaptive behaviors driven by environmental salience and reward. Addictive drugs, including nicotine, exert powerful influences over the mesolimbic DA system by activating and desensitizing nicotinic acetylcholine receptors (nAChRs) in a subtype-dependent manner. Nicotine induces synaptic plasticity at excitatory synapses onto DA neurons, thereby sending elevated DA signals that participate during the reinforcement of addictive behaviors. While humans and animals of any developmental age are potentially vulnerable to these drug-induced effects, evidence from clinical and epidemiological studies indicates that adolescents have an increased risk of addiction. Although this risk arises from a complex set of variables including societal and psychosocial influences, a contributing factor involves age dependent sensitivity to addictive drugs. One aspect of that sensitivity is drug-induced synaptic plasticity at excitatory synapses onto the dopamine neurons in the ventral midbrain. A single, acute exposure to addictive drugs, including nicotine, produces long-term potentiation (LTP) that can be quantified by measuring the shift in the subtypes of ionotropic glutamate receptors mediating evoked synaptic transmission. This change in glutamatergic transmission is expressed as an increased ratio of AMPA receptors to NMDA receptors at glutamatergic synapses. Age-related differences in the excitability and the nicotine sensitivity within the midbrain dopamine system may contribute to the greater risk of nicotine addiction in adolescent animals and humans.

Contents

1. Introduction

Nicotine is the primary addictive substance in tobacco smoke [1–6], yet many important questions remain regarding its effects on the brain. Nicotine produces a variety of complex effects on several brain regions that are essential for the manifestation of addictive behavior [6–8]. The mesolimbic DA system is a target for addictive drugs that includes the DA neurons of the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc) of

Abbreviations: AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; α-BTX, α-bungarotoxin; ACh, acetylcholine; ADHD, attention-deficit hyperactivity disorder; DHβE, dihydro-β-erythroidine; DA, dopamine; sEPSCs, spontaneous excitatory postsynaptic currents; LTP, long-term potentiation; MLA, methyllycaconitine; NMDA, N-methyl-D-aspartic acid; nAChR, nicotinic acetylcholine receptor; NAc, nucleus accumbens; VTA, ventral tegmental area.

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the ventral striatum [9–12]. A single dose of nicotine induces synaptic plasticity that increases the activity of VTA DA neurons [9,11,13,14]. This plasticity is similar in nature to activity-induced LTP [15,16], and is correlated in animal studies with psychostimulant-induced behavioral sensitization [17] and drug self-administration [18].

Tobacco use, primarily in the form of cigarette smoking, is a behavior that frequently occurs during adolescence. In the United States most smokers begin using before the age of 18 [19–21], and these young people are more likely to make the transition to a daily smoking habit [22–25]. Although several studies using rodent models have examined the effects of addictive drugs during adolescence, relatively little is known about developmental factors affecting the synaptic changes induced by nicotine in the VTA. Here we will describe nicotinic synaptic mechanisms of the mesolimbic DA system, and will describe age dependent differences in the vulnerability to nicotine addiction in animal studies.

2. The role of midbrain dopamine neurons in nicotine addiction

2.1. The mesolimbic dopamine system

The cellular and molecular processes that are targeted by addictive drugs normally reinforce successful behaviors arising from interaction with a changing environment. Our understanding of the nature of DA signaling has evolved from that of a mediator of the experience of reward, to a reinforcer of rewarding behavior [2,26,27]. The concentrations of DA in the nucleus accumbens (NAc) do not scale directly with reward, but rather the DA signal is associated with novelty or gives an indication of deviation of environmental stimuli from the organism's expectations. Thus, among its other roles, DA contributes to the associative learning of survival-related behaviors as an organism develops an internal representation of environmental saliency [27].

As the addiction process progresses, drugs act upon the dopaminergic systems to reinforce behaviors, which involves learning environmental cues leading to or associated with drug-taking [2,3,7,28–36]. Nicotine stimulates midbrain DA neurons by activating nAChRs [10,14,30,37–42], and it stimulates the prolonged release of DA in the NAc [14,33,34,43]. In rodent studies, DA antagonists or critical lesions of the mesolimbic DA system reduce nicotine self-administration [2,29,44–46]. In addition to its effects on midbrain DA neurons, nicotine can also act upon the DA fibers and presynaptic targets to regulate DA signaling in striatal regions [47–49]. Beyond the cellular changes produced by acute drug exposure, chronically administered addictive substances produce additional neural changes. Because those neural changes are not completely characterized, they are called "neuroadaptations", referring to a new neural condition that is reached via homeostatic adjustments to the repeated presence of the drug [6,26,50]. An example is the upregulation of mainly high affinity nAChR subtypes in specific neural locations after chronic nicotine [51].

The behavioral phenomena of drug tolerance and dependence are, in part, explained by the cellular and molecular changes caused by the addictive drug. Neural plasticity of the kind that underlies learning and memory contributes to the long-term motivations and cravings that can persist long after an addict has ceased to self-administer a particular drug. In these cases, re-experiencing environmental cues linked to drug use increase the likelihood of future drug cravings and possibly relapse even after long periods of abstinence [2,6,7,26,28]. Equally important, these cues motivate continued drug use and the maintenance of the addiction. Through their ability to initiate and modulate normal synaptic plasticity mechanisms, addictive drugs induce associated learning and memory that is an inherent and essential component of the overall addiction process.

2.2. Effects of nicotine on midbrain nicotinic receptors

Nicotine activates neurons of the ventral midbrain [7,10,14,37–42,52], and increases DA release to targets including the NAc [14,33,34,43]. The act of smoking cigarettes delivers approximately 50–500 nM nicotine to the brain over the course of seconds to minutes [3,53–55]. Afterwards low concentrations of nicotine persist in the brain for several hours.

Neuronal nAChRs are pentameric ligand-gated ion channels that can be assembled from a combination of subunits: α2–α10 and β2–β4. Thus, a large number of structurally and functionally different nAChR subtypes can result [41,56–60]. The majority of heteromeric neuronal nAChRs are produced by the combination of alpha subunits (α2–α6) and beta subunits (β2–β4). The other major neuronal nAChR subtype contains the α7 subunit (α7* nAChRs), which has rapid activation and desensitization kinetics, and is selectively blocked by α-bungarotoxin (α-BTX) or methyllycaconitine (MLA) [9,61,62]. While relatively high agonist concentrations will cause a rapid desensitization of α7* nAChRs, a low affinity for nicotine means that they are able to maintain a steady-state activation in the presence of low concentrations of nicotine, such as those delivered by cigarette smoke [14,41,63].

In addition to its action at DA neurons in the midbrain, nicotine also produces modulatory affects via nAChRs at axonal, perisynaptic and non-synaptic locations. The DA neurons of the midbrain primarily express nAChRs with relatively slow kinetics and a sensitivity to inhibition by dihydro-β-erythroidine (DHβE) [10,40,41,64]), indicating that the predominant nAChR subtype is composed of β2-containing (β2*) nAChRs. These β2 subunits co-assemble with other nicotinic receptor subunits, specifically α4, α6, and α3 [64–69], a conclusion that is supported by studies using β2-subunit knockout mice in which midbrain DA neurons have significantly diminished nicotinic receptor-mediated currents [40,41]. Although the β2* nAChRs compose the main nicotinic receptor subtype in midbrain DA neurons, the β2-null mice do show a relatively small, MLA-sensitive current with rapid kinetics, indicating a minor amount of α7* nAChR expression [41].

2.3. The influence of nicotine on midbrain synaptic function

Nicotine, at concentrations approximating those experienced by cigarette smokers, modulates excitatory afferent signaling to midbrain DA neurons. The best characterized effect arises from presynaptic nAChRs, as exemplified by nicotine increasing the frequency (but not the amplitude) of spontaneous excitatory postsynaptic currents (sEPSCs) [7,11,14,39,70]. This effect on sEPSC frequency persists during an entire 25 min nicotine delivery period, indicating there is not profound desensitization of the presynaptic nAChRs on the glutamatergic afferents [14]. In addition to enhancing sEPSCs, nicotine increases the amplitude of weakly evoked EPSCs onto DA neurons. The nicotine-induced increase in either spontaneous or weakly evoked excitatory currents persists even after nicotine is removed from the bath solution, suggesting the induction of long-term potentiation [7,11,14,39,70,71]. Similar presynaptic effects mediated by nAChRs have also been demonstrated in other important brain regions, including the hippocampus [7,9,57–59,71–78]. This type of long-lived enhancement of excitatory synaptic transmission onto DA neurons is similar in many respects to the synaptic plasticity that has been linked to learning and memory [79].

Direct nicotine excitation of DA neurons gradually decreases owing to desensitization of the predominant β2* nAChR subtypes. However, concurrent nicotine-mediated modulation of afferent

synaptic activity permits continued excitation, prolonging the microdialysis DA signal measured in the NAc [2,14,29,30,34,43,80–82]. In simplified summary, smoking a cigarette provides an initial dose of nicotine that activates postsynaptic β2* nAChRs on DA neurons and presynaptic α7* nAChRs located on the excitatory glutamatergic terminals that project onto the DA neurons. The β2* nAChRs significantly desensitize after the initial activation, but the α7* nAChRs are not significantly desensitized by the nicotine concentration obtained from a cigarette [7,14,41]. Since α7*-type nAChRs are highly calcium permeable, they often have the combined effect of mediating a direct calcium influx in addition to causing calcium increases indirectly via voltage-gated calcium channels and via release from intracellular Ca^{2+} stores [7,9,11,39,58,70,71,76,77,83,84]. Thus, activation of presynaptic α7* nAChRs increases calcium in glutamatergic presynaptic terminals, facilitating glutamate release and subsequent synaptic excitation of DA neurons despite desensitization of the post-synaptic β2* nAChRs on the DA neurons. After nicotine initially excites VTA DA neurons increasing their action potential firing rate, postsynaptic activity is coupled with a nicotine-induced increase in presynaptic glutamatergic afferent excitation. It is this combination of presynaptic and postsynaptic coincidence that facilitates the formation of LTP [7,11,14,39,70,71].

In contrast to the sustained enhancement of excitatory afferent transmission onto VTA DA neurons, nicotine has a qualitatively different effect on the inhibitory, GABAergic inputs. Nicotinic receptor activation at somatic or preterminal sites, is known to produce local membrane depolarization sufficient to cause or facilitate action potential firing. In this way, bath application of nicotine initially increases action potential firing in GABAergic neurons. However, because of the specific subtypes of nicotinic receptors expressed in these inhibitory neurons (primarily the β2* subtype), sustained exposure to bath-applied nicotine causes nAChR desensitization [7,12,14]. This ultimately reduces local inhibitory tone and increases DA neuron excitability, which increases the likelihood of glutamatergic LTP. The synaptic changes that are brought about by nicotine are very similar to the normal kinds of synaptic plasticity that are thought to contribute to learning and memory. Nicotine influences these normally adaptive pathways, favoring potentiation of synaptic activity related to drug-associated behaviors that are ultimately detrimental to the organism.

3. Nicotine and the adolescent brain

3.1. Vulnerability to nicotine addiction in adolescent humans

More than 60% of young people in the U.S. have tried smoking, and of those, one-half to one-third will make the transition into a daily smoking habit [25]. Commonly those who become regular smokers have their cigarette consumption escalate over a couple of years, but the addiction process proceeds relatively quickly in adolescents [21,85]. About 25% of the adolescents who smoke report symptoms of addiction at the time they establish a monthly smoking habit [85,86]. Nicotine has been reported to have positive effects on mood and behavior, but the initial exposure to cigarette smoke is often perceived as aversive [87–89]. However, when compared to adults, adolescents report more positive effects and fewer aversive effects during their first smoking experience [85].

3.2. Studies in animal models

Accumulating evidence from studies using rodent models continues to show significant age-related differences that may contribute to adolescent vulnerability to nicotine addiction [90]. In studies using young rats, a single dose of nicotine is sufficient to cause meaningful synaptic and cellular changes [11,13,91]. Young rats are also more sensitive than adult rats to nicotine's ability to reinforce intravenous self-administration and place preference conditioning [92]. Nicotine exposure in adolescent rats can also affect how the animal responds to subsequent exposure during adulthood, as this has been shown to increase self-administration later in life [93]. Adolescent rats also show greater levels of immediate early gene induction in the NAc in response to nicotine [94,95].

In making comparisons between rodents and humans, it is important to note that the first 21 postnatal days for the rat or mouse are approximately equivalent to the third trimester of prenatal development in the human [96,97]. During this early postnatal period, the rodent brain is subject to a significant degree of developmentally regulated gene expression, including genes for nAChRs that play key roles in neuronal development [96,98]. The subsequent juvenile and periadolescent periods also display complex changes in nicotinic receptor gene expression in midbrain catecholeminergic neurons. Despite these changes, age-related functional differences between midbrain DA neurons in young and adult mice can be highly specific. As an example of this, a comparison of several basic properties related to neuronal excitability show that the midbrain DA neurons of young and adult C57/BL6 mice are similar under basal conditions (Table 1).

Despite their functional similarities, these same populations of DA neurons show significant differences in excitability when depolarized by direct current injection (Fig. 1). The VTA DA neurons of young mice show a current-frequency relationship that is not statistically different from adult mice (Fig. 1B), but they are capable of sustaining a larger number of action potentials when depolarized (Fig. 1C). This suggests that the same level of excitation may have different effects on DA release in the target regions of these neurons.

Another example of a potentially important difference between young and adult VTA DA neurons is seen in their relative sensitivity to nicotine-induced LTP. Addictive drugs are capable of producing LTP in VTA DA neurons [13,15], and this LTP is correlated with addiction-related behaviors in rodents, including behavioral sensitization [17] and drug self-administration [18]. Acute systemic injections of nicotine produce a shift in the ratio of excitatory glutamate receptor subtypes indicating the induction of LTP [13]. This potentiation is measured experimentally as an increase in the AMPAR/NMDAR current ratio of evoked EPSCs and is consistent with an NMDAR-mediated mechanism [15,99].

While acute nicotine injections will increase the AMPAR/NMDAR ratio in both young and adult C57 mice, a lower dose of nicotine is required in young mice to produce the same magnitude of increase. As shown in Fig. 2, a single injection (1 mg/kg, intraperitoneal) of nicotine given 24 h prior induces an increase in

Table 1
Similar electrophysiological properties of VTA DA neurons in young and adult mice. Values represent mean ± s.e.m. No statistically significant differences were seen between young and adult C57BL/6 mice for each of the properties shown above.

	Firing frequency (Hz)	Resting membrane potential (mV)	Action potential width (ms)	I_h amplitude (pA)	Input resistance (mΩ)
Young (p21–p35) $n = 17$	1.9 ± 0.2	−48.7 ± 0.7	2.2 ± 0.1	−263.6 ± 37.7	245.0 ± 17.9
Adult (p60–p90) $n = 10$	1.7 ± 0.4	−46.1 ± 1.5	2.2 ± 0.1	−302.6 ± 39.0	204.1 ± 27.8

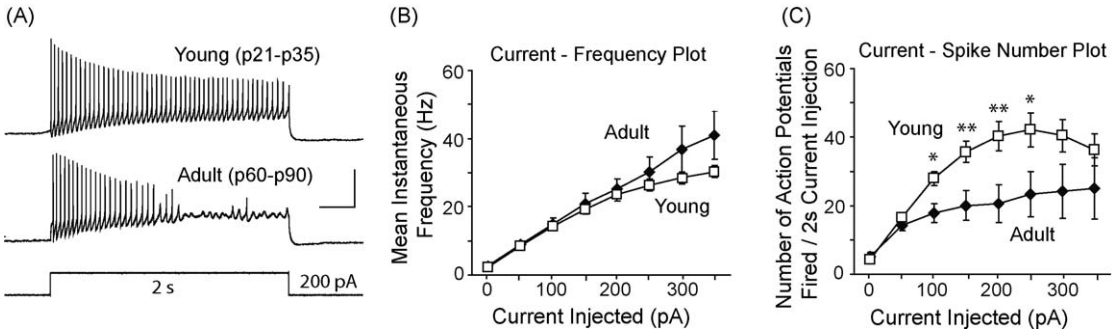

Fig. 1. VTA dopamine neurons from young mice are able to fire more action potentials than adult mice when depolarized as shown. (A) Representative data showing a 200 pA/2 s depolarization in either a young or adult mouse VTA DA neuron. (B) Averaged data shows that both groups fire at similar instantaneous frequencies when initially depolarized. (C) The DA neurons from younger mice are able to fire more action potentials than those of older mice when depolarized by the indicated current injections ($^{*}p < 0.05$, $^{**}p < 0.01$ by repeated measures ANOVA and Tukey's HSD post hoc, $n = 10–17$ per group).

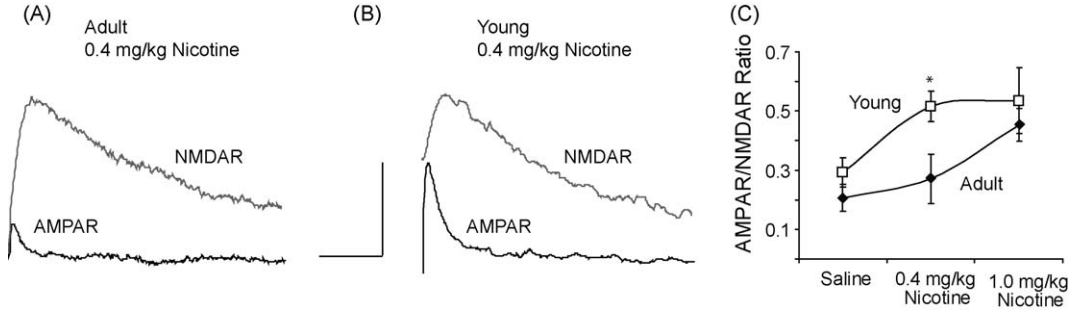

Fig. 2. VTA DA neurons in young mice are more sensitive to nicotine-induced LTP. (A) Representative averaged traces from young (p21–p35) and (B) adult (p60–p90) mice after injection of 0.4 mg/kg nicotine ~24 h prior to recording. The traces are averaged (12–15 sweeps) evoked currents from mouse midbrain DA neurons voltage-clamped at +40 mV in the presence of 100 mM picrotoxin to block $GABA_A$-mediated currents. Black traces indicate the remaining AMPAR component after a 5 min perfusion with 100 μM DL-AP5. Gray traces show a digital subtraction of the AMPAR-mediated currents from the total EPSCs, representing the NMDAR component. The larger AMPA/NMDA ratio is indicative of LTP induction. Scale bars indicate 100 pA/10 ms for the adult and 45 pA/10 ms for the juvenile. (C) Averaged peak amplitude ratios obtained from either young or adult mice 24–48 h after i.p. injection with the indicated dose of nicotine showing a leftward shift in the dose-response relationship for young mice ($^{*}p < 0.05$, young vs. adult at 0.4 mg/kg nicotine by repeated measures ANOVA and Tukey's HSD post hoc, $n = 5–7$ per group).

the AMPAR/NMDAR ratio in both adult and young mice (Fig. 2C). This result suggests LTP induction, and based on control experiments by others [13,15], this expectation is supported for nicotine. In C57 mice, intraperitoneal injection of nicotine at 1 mg/kg corresponds to a peak plasma concentration of approximately 100 ng/ml, with the peak occurring about 5 min post-injection. However, the rapid metabolism of nicotine by mice results in a brief half-life of 6–7 min [100]. The peak nicotine concentration in this case is higher than has been estimated in humans after cigarette smoking (10–50 ng/ml, [101]).

At a lower concentration of nicotine (0.4 mg/kg), a difference is seen between adult and young mice. When we administered nicotine (0.4 mg/kg, i.p.), there was a significantly greater increase in the AMPAR/NMDAR ratio in the young mice: compare Fig. 2A and B with the results summarized in Fig. 2C. This result suggests that the developing brain may be more sensitive to nicotine-induced long-term potentiation, and gives rise to the speculation that this sensitivity may contribute to a greater likelihood of nicotine-induced DA signals that contribute to addiction during adolescence.

With *in vivo* systemic delivery, nicotine will bind to its target receptors in a wide variety of brain regions, including those in the midbrain. While there are multiple nAChR subtypes that are activated and desensitized by nicotine, the particular subtypes that have been shown to be significant for the activation of the VTA dopaminergic system include the $\alpha4\beta2^{*}$ and the $\alpha7^{*}$ subtypes. Studies in mice using radiolabeled nicotine and the α7 selective antagonist α-bungarotoxin have suggested that general patterns of expression resemble the adult brain by 20 days after birth [102]. Also, midbrain mRNA levels for α4β2 and α7 have similarly been reported to be relatively unchanged from adolescence to adulthood in rats [98,103]; but see [104]. However, important brain regions with excitatory projections to the VTA, such as prefrontal cortex, show elevated nAChR expression during adolescence [96], which could contribute to the differences shown in Fig. 2. Furthermore, evidence from rat studies shows increased mRNA levels of the α6 nAChR subunit within the VTA during adolescence [98]. This finding may be particularly relevant in light of the evidence for α6 containing receptors regulating VTA DA neuron firing [105], and nicotine self-administration [106].

Taken together, the literature and current data suggest that adolescent humans have an increased likelihood of attaining smoking behavior and an increased susceptibility of becoming addicted. Animal experiments support the epidemiology studies in humans by showing nicotine to have significant age-related effects on synaptic events and behavioral output. *In vitro* studies also indicate mechanistic differences between young and adult animals in their excitability and in their sensitivity to nicotine-induced synaptic plasticity.

4. Therapeutic implications

The most obvious practical application of the findings described here is to increase efforts to prevent young people from using tobacco. The overall evidence indicates that there is a greater risk of developing a lifelong addiction when nicotine is used during adolescence. Prevention strategies are primarily the domain of policy makers and educators, but there are also intriguing implications for pharmacotherapy. There are some specific examples of drugs that illustrate how developmental factors can

have dramatic effects on the nature of drug therapy. Perhaps the most well known example comes from the clinical use of the stimulant methylphenidate, which reduces the signs and symptoms of attention-deficit hyperactivity disorder (ADHD) in children [107]. Like other stimulant treatments for ADHD, methylphenidate has a paradoxical effect in that it can have calming effects on children, while having the opposite effect on adults.

In the case of developmental factors contributing to nicotine dependence, the first and best approach to the problem is one of prevention, especially in the case of children and adolescents. However, as we gain a better understanding of the specific mechanistic differences between the developing brain and the adult brain, there is the potential for emerging strategies to exploit these differences. As better smoking cessation drugs are developed, this may come in the form of specific drug dosing regimens tailored to adolescents, or even specific compounds with superior efficacy in helping younger smokers to quit. Although some studies have begun to address adolescent issues [108,109], few clinical studies have focused specifically on adolescent responses during smoking cessation therapies. A recent prospective study of young smokers who started between the ages of 12 and 18 years indicated that 1 in 4 reported a desire to quit smoking within a period of a few months, and that the first serious attempt to quit came an average of 2.5 months after the first cigarette [86]. Despite expressing a desire to quit, significant numbers of these smokers had difficulty doing so, underscoring a real need to develop effective approaches to assist this vulnerable population in quitting tobacco use. In light of this, any attempts to use specific drug therapies in adolescent populations will benefit from continued study of the underlying synaptic mechanisms that place the developing brain at greater risk of nicotine addiction.

Conflict of interest

The authors declare that they have no competing financial interests.

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