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Vulnerability to nicotine self-administration in adolescent mice correlates with age-specific expression of α4* nicotinic receptors

Anthony Renda, Nora Penty, Pragya Komal, Raad Nashmi*

Department of Biology, Centre for Biomedical Research, University of Victoria, Canada

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ABSTRACT

The majority of smokers begin during adolescence, a developmental period with a high susceptibility to substance abuse. Adolescents are affected differently by nicotine compared to adults, with adolescents being more vulnerable to nicotine's rewarding properties. It is unknown if the age-dependent molecular composition of a younger brain contributes to a heightened susceptibility to nicotine addiction. Nicotine, the principle pharmacological component of tobacco, binds and activates nicotinic acetylcholine receptors (nAChRs) in the brain. The most prevalent is the widely expressed α4-containing (α4*) subtype which mediates reward and is strongly implicated in nicotine dependence. Exposing different age groups of mice, postnatal day (P) 44–86 days old, to a two bottle-choice oral nicotine self-administration paradigm for five days yielded age-specific consumption levels. Nicotine self-administration was elevated in the P44 group, peaked at P54–60 and was drastically lower in the P66 through P86 groups. We also quantified α4* nAChR expression via spectral confocal imaging of brain slices from α4YFP knock-in mice, in which the α4 nAChR subunit is tagged with a yellow fluorescent protein. Quantitative fluorescence revealed age-specific α4* nAChR expression in dopaminergic and GABAergic neurons of the ventral tegmental area. Receptor expression showed a strong positive correlation with daily nicotine dose, suggesting that α4* nAChR expression levels are age-specific and may contribute to the propensity to self-administer nicotine.

1. Introduction

The age associated with the highest risk of developing an addiction is adolescence (Schramm-Sapyta and Walker, 2009; Stanis and Andersen, 2014; Tyas and Pederson, 1998). Up to 90% of smokers begin before the age of 18 (Chassin et al., 1990). Even more alarming is the inverse correlation between cessation success and age of initiation (Chen and Millar, 1998; Coambs et al., 1992). There are a number of complex factors which correlate with this enhanced risk of nicotine dependence. Genetic factors predict nicotine dependence (Saccone et al., 2009) and sociological factors such as history of abuse (Anda et al., 1999) may affect reinforcement and experience based cortical development, a phenomenon which peaks during adolescence and may influence the propensity towards addiction (Crews et al., 2007). Adolescents respond more powerfully to rewarding stimuli (Schramm-Sapyta and Walker, 2009) including nicotine (Brielmaier et al., 2007; Kota et al., 2007; Vastola et al., 2002), while aversion (Shram et al., 2006) and physical withdrawal (Kota et al., 2007; O'Dell et al., 2006) are reduced in adolescents. These factors may contribute to adolescent susceptibility to nicotine dependence, although the cellular and molecular basis is not clearly understood.

In order to accurately study this critical age, we must treat it as a transitional period rather than a rigid series of steps. We have investigated groups of mice postnatal day (P) 44–86 days old, which corresponds to the periods of periadolescence through adulthood (Adriani et al., 2004; Spear and Brake, 1983). Substantial neurodevelopmental molecular changes occur during this time period and the makeup of the periadolescent brain is substantially different compared to both its younger and older counterparts. Differences in the adolescent molecular profile in brain regions and neurotransmitter systems associated with addiction are purported to have a role in adolescent susceptibility to the effects of abusive substances (Spear, 1987). Notably, dopamine receptor levels are elevated during periadolescence then

* Corresponding author. University of Victoria, Department of Biology, PO Box 3020, Station CSC, Victoria, BC, V8W 3N5, Canada.
E-mail address: raad@uvic.ca (R. Nashmi).

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decline into adulthood, with rat cortical D1 and D2 receptor levels peaking during P40–60 and then showing significant reduction by P80 (Andersen et al., 2000).

Nicotine, the addictive component of tobacco, is an exogenous ligand of nicotinic acetylcholine receptors (nAChRs). Endogenously activated by acetylcholine (ACh), nicotinic receptors are pentameric ligand-gated ion channels. The two most prevalent subtypes in the brain are the widely expressed heteropentameric α4β2 nAChRs (Marks et al., 1992; Whiting et al., 1987) and the homopentameric α7 nAChRs (Clarke et al., 1985; Gotti et al., 2009; Perry et al., 2002). The α4β2 subtype mediates normal cognitive behaviours such as attention, as mice unable to express the β2 nAChR subunit can have their diminished attentional performance rescued by lentiviral re-expression (Guillem et al., 2011). Moreover, tobacco users experience increases in attention (Rusted and Warburton, 1992) as well as learning and memory (Decker et al., 1993; Gould, 2006) – both behaviours linked to the activity of α4β2 nAChRs. These receptors also control endogenous reward and tolerance, as β2 KO mice show suppressed dopaminergic transmission, a known mechanism providing reward (Zhou et al., 2001). Additionally, extremely low doses of nicotine administered to mice with hypersensitive α4-containing (α4*) nAChRs selectively activate α4* receptors and produce behavioural reinforcement and tolerance (Tapper et al., 2004). While self-administration of nicotine is absent in both α4 and β2 KO mice, selective re-expression of the α4 and/or β2 subunit in the ventral tegmental area (VTA) of KO mice re-establishes self-administration (Maskos et al., 2005; Pons et al., 2008). Consequently, α4β2 nAChRs are strongly implicated in the disease of addiction that arises when the brain's reward circuitry is hijacked by abusive substances.

Previous results suggest that the level of α4* nAChR expression in the brain influences the magnitude of nicotine self-administration (Renda and Nashmi, 2014). Notably, nAChRs in the adolescent rat brain peak during adolescence and decline into adulthood (Azam et al., 2007; Counotte et al., 2012; Doura et al., 2008). Here we investigate the age-dependent expression level of α4* nAChRs in the brains of genetically altered knock-in mice containing a yellow fluorescent protein (YFP) tagged to the α4 nAChR subunit (α4YFP) (Nashmi et al., 2003, 2007). Having a fluorescent indicator of a receptor under the control of its native promoter in conjunction with spectral confocal imaging allows us to accurately image and quantify the expression levels of α4YFP nAChR subunits at sub-micron resolution in specific neuronal subtypes in the different brain regions important for reward and cognition (Nashmi et al., 2007; Renda and Nashmi, 2012, 2014).

The α4YFP knock-in mice were also exposed to nicotine via drinking water in a previously established, non-invasive, two bottle-choice self-administration paradigm (Renda and Nashmi, 2014) in order to examine age-dependent nicotine consumption. The choice to administer nicotine is crucial here as it allows us to study the molecular correlates of a dose directly dependent on the age of the animal. Likewise, free-choice also allows the mice to administer nicotine in an episodic manner, as overnight abstinence may contribute to the reinforcing properties of nicotine (Perkins et al., 1994).

Here we examine oral nicotine self-administration in peri-adolescent and adult mice and correlate these consumption patterns to age-dependent α4* nAChR expression levels in the medial perforant pathway of the hippocampus as well as in dopaminergic and GABAergic neurons of the VTA. Our results support the hypothesis that nicotinic receptors exhibit peak expression during adolescence and also that the level of α4* nAChRs influence the propensity to self-administer nicotine. Together, these findings may provide a key contributing mechanism of adolescent vulnerability to nicotine dependence.

2. Methods

2.1. Animal care and breeding

All procedures performed on animals were conducted in compliance with the guidelines for care and use of animals provided by the Canadian Council on Animal Care Use and all protocols were approved by the University of Victoria Animal Care Committee. Homozygous male α4YFP knock-in mice were used in all experiments (Nashmi et al., 2007; Renda and Nashmi, 2012, 2014). In these mice, the M3-M4 loop of the α4 nAChR subunit contains a yellow fluorescent protein (YFP) gene. The strain is back-crossed over 10 generations to C57BL/6J and the α4* receptor (* denotes that the receptor contains other subunits in addition to α4) functions and expresses normally in every respect (Nashmi et al., 2007). Pups were weaned at postnatal day (P) 20 and at P30 were segregated by sex into groups of 2–5 littermates. Mice were housed on a 12 h light/dark cycle at 22 °C and given a standard laboratory diet and water *ad libitum*, except where noted in behavioural experiments.

2.2. Two bottle choice nicotine self-administration

Two bottle choice nicotine self-administration was performed as described in Renda and Nashmi (2014) on individually housed mice who have been acclimated to individual housing for between six and ten days prior to their age-dependent onset of the two bottle choice assay (Fig. 1). Once reaching the appropriate age, mice were presented with two bottles constructed from 50 ml plastic centrifuge tubes fitted with rubber stoppers containing metal spouts. One choice of drinking water contained the vehicle (reverse osmosis water (roH_2O) sweetened with 0.2% saccharine (Sigma-Aldrich, cat# 109185)), while the other contained the vehicle plus 200 μg/ml (−)-nicotine (Sigma, cat# N3876). The metal cage top is divided into three equal sections. During self-administration, the nicotine and vehicle bottles occupy the leftmost two positions, with food occupying the remaining portion. Although it has been shown that C57BL/6J mice do not have an inherent preference for the bottle situated in either the position closest to the wall or closest to the food (Bachmanov and Reed, 2002; Renda and Nashmi, 2014), we nevertheless randomized the position of the bottles so that an equal number of mice had their nicotine-containing bottle in either location. In order to control for false-positive self-administration due to taste testing and provide an environmental cue which has been shown to enhance nicotine self-administration (Caggiula et al., 2002), the spout of each bottle was labeled black or white. The colour of the nicotine-containing bottle was randomized so that an equal number of mice drank from a nicotine-containing bottle with either colour of spout. Bottle position and spout colour were constant for each animal for the entirety of the experiment. During periods of abstinence, the mice were given access to one bottle with an unlabeled spout and containing only unsweetened roH_2O. The bottle is located in the portion of the cage top occupied by food in the self-administration periods; the food then occupies the remaining portion. The mice are given an initial period of 5 days of two bottle choice, followed by either perfusion for quantitative fluorescence imaging (described below) or a 3 day period of abstinence. The initial abstinence is followed by periods of 4 days of self-administration and 3 days abstinence.

Nicotine consumption was recorded every 24 h as a daily dose (mg nicotine/kg mouse) and also as the amount of fluid consumed from the nicotine bottle as a percentage of total daily fluid intake. To minimize the effect of handling-stress on behavior, the mice were weighed on the first day of self-administration and, if intended for imaging, mice were weighed again immediately prior to