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Research report

Role of the D3 dopamine receptor in nicotine sensitization

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HIGHLIGHTS

- Changes in DRD3-related mRNAs imply greater availability of this receptor early in NIC exposure.
- NIC-induced changes in DRD3 and locomotor sensitization are temporally dissociated.
- DRD3s are required for the induction of locomotor sensitization in adolescent rats.

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ABSTRACT

Adolescent cigarette use is associated with reduced quitting success and continued smoking in adulthood. Interestingly, polymorphisms of the dopamine D3 receptor (DRD3) gene have been associated with smoking behavior, and the receptor is expressed in an age- and brain region-dependent manner that suggests relevance to addiction. Here, we investigate the possible role of dopamine-related receptors, including DRD3 and an intriguing splice variant known as D3nf, in nicotine-induced sensitization. In adolescent and adult male rats, we examined (1) alterations occurring in dopamine receptor-related mRNAs (DRD1, DRD2, DRD3 and D3nf) at two time points during a sensitizing regimen of nicotine and (2) whether DRD3 antagonism either during the initial treatment (induction) or at a later challenge exposure (expression) is able to block nicotine sensitization. Nicotine-induced changes were seen for DRD3 and D3nf mRNAs in the nucleus accumbens shell early in repeated exposure in both age groups. DRD3 antagonism only blocked the induction of sensitization in adolescents and did not block the expression of sensitization in either age group. Adolescents and adults showed opposite DRD1 mRNA responses to nicotine treatment, while no age- and nicotine-related changes in DRD2 mRNA were observed. These data reveal important age-dependent regulation of DRD1- and DRD3-related mRNAs during the course of nicotine exposure. Furthermore, they highlight a requirement for DRD3 signaling in the development of adolescent nicotine sensitization, suggesting it may represent an appropriate target in the prevention of nicotine dependence initiated at this age.

1. Introduction

Nicotine remains one of the most heavily used pharmacological substances in the United States, with over 25% of people over the age of 12 reporting use of a tobacco product in the previous month during 2013 [1]. Furthermore, 88% of adult daily cigarette smokers began smoking in adolescence [2], and age of smoking initiation is positively correlated with likelihood of smoking cessation [3,4], suggesting that cigarette use at an early age may be especially risky for long-term addiction liability. The persistence of addiction is notable, as even after lengthy drug abstinence, there is a very low rate of long-term therapeutic success.

Polymorphisms in the dopamine D3 receptor (DRD3) gene have been linked to smoking behavior in humans [5]. Unlike the other dopamine receptors, which are relatively ubiquitous in brain [6], DRD3 expression is highly restricted and includes a region known for its prominent role in drug action, the nucleus accumbens (NAc) [7–9]. The ontogenetic profile of DRD3s also differs considerably from dopamine receptor D1 (DRD1) and D2 (DRD2), which show substantial expression during early development in the rat [10,11] and reach adult levels prior to adolescence [11]. In contrast, DRD3 binding is barely detectable in the NAc prior to adolescence [12,13] and during adolescence remains below adult levels [12]. The unique profile of this receptor is intriguing, given that adolescence is a period of increased susceptibility to the effects of drugs of abuse (for review, see [14]).

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Here we consider the role of DRD3 and one of its splice variants, D3nf, in nicotine locomotor sensitization, a phenomenon of increasing locomotor activation that occurs in rodents repeatedly exposed to certain drugs of abuse, particularly psychostimulants. Since sensitized behaviors can be elicited by a single exposure long after drug cessation in both humans [15] and rats [16], the phenomenon is considered an important component of addiction and a model of the longevity of human addiction in the rat. The DRD3 splice variant, D3nf, is of interest because it is able to dimerize with the full-length DRD3, prevent dopamine binding and cause receptor internalization [17,18]. Notably, DRD3 and D3nf have been implicated in psychostimulant sensitization previously; however, their putative roles remain unclear [19,20].

Our goal in this study is two-fold: (1) to determine the effect of repeated (sensitizing) nicotine dosing regimens on DRD1, DRD2, DRD3 and D3nf splice variant mRNA expression in adolescent and adult rats and (2) to determine whether pharmacological antagonism of DRD3 during the induction or expression of nicotine locomotor sensitization prevents sensitization and/or alters the expression of these mRNAs in either age group. We hypothesized that we would see a downregulation of DRD3 mRNA (and/or an upregulation of D3nf mRNA) over the course of nicotine treatment, consistent with a previous theory that proposes DRD3-specific tolerance may contribute to sensitization [20]. We also hypothesized that blocking dopamine access to DRD3s (and thereby possibly preventing DRD3-specific tolerance) during the induction, but not during the expression, of nicotine sensitization would inhibit this drug-induced behavior.

2. Materials and methods

2.1. Animals and drugs

Male Long–Evans rats (Harlan Laboratories, Indianapolis, IN) were shipped to our facility just after weaning (approximately postnatal day, or P, 23) or as adults (~P66). Rats were group-housed (4/cage) by age in transparent, Plexiglas cages lined with TEK-Fresh bedding (Harlan Laboratories, Indianapolis, IN) under a 12-h light cycle (lights on at 07:00) with ad libitum access to standard rat chow and water. Rats were randomly assigned to treatment groups for each study within their age group. Drugs were administered subcutaneously at the following doses/concentrations: nicotine ("NIC"; 0.5 mg/kg, free base), saline ("SAL"; 0.9%), GR 103691 ("ANT"; 2.0 mg/kg; Tocris Bioscience, Minneapolis, MN) and vehicle ("VEH") at a volume of 1 mL/kg. The selective DRD3 antagonist GR 103691 was dissolved in a minimal amount of pure acetic acid, and then NaOH in distilled water was slowly added while stirring to reach the desired volume and pH approaching 5.0. The VEH control consisted of comparable quantities of acetic acid and NaOH in distilled water.

Dosing began at either P35 or P80. The adolescent exposure age was selected based on preliminary work in our laboratory showing strong sensitization when pre-exposure occurred at P35–37 compared to an older (P49–51) age, and which was not accompanied by low baseline locomotor values seen in slightly younger (P28–30) animals (unpublished observations). In order to avoid the added effects of contextual conditioning, all dosing took place in the home cage environment, except for the last day of dosing, when drugs were administered in the open-field apparatus room. All procedures were approved by the Institutional Animal Care and Use Committee at George Mason University and followed NIH laboratory animal guidelines.

2.1.1. Experiment 1: length of exposure study

Rats received one injection of NIC ($n = 40$) or SAL ($n = 40$) daily for either two ($n = 40$) or seven ($n = 40$) consecutive days. On the following day (Day 3 or 8), all rats received an NIC injection and were placed in the open-field apparatus.

2.1.2. Experiment 2: D3 receptor antagonist study

For induction of sensitization, rats received one injection of NIC ($n = 80$) or SAL ($n = 80$) daily for six consecutive days (Days 1–6). After 3 weeks of withdrawal, all rats received an NIC "challenge" injection, and expression of sensitization was measured in the open field (Day 27). For half of the animals, either GR 103691 or VEH was administered 30 min prior to each of the six daily injections (induction). The other half of the animals received either GR 103691 or VEH 30 min prior to the NIC injection given before open field testing (expression). Behavioral timelines are shown in Fig. 1.

2.2. Locomotor testing

Behavioral testing took place during the light phase. Locomotor activity (distance traveled, centimeters) was assessed for 30 min in an open field (white Plexiglas, $42 \times 42 \times 30\,cm^3$) on the last day of dosing or on the challenge date, as described. Video was recorded from overhead and distance traveled was measured automatically in 5-min bins (Videotrack system, Viewpoint Life Sciences, Inc., Montreal, Quebec). On each of three consecutive days prior to testing, rats received a SAL injection and were placed in the chamber for 30 min to habituate them to the environment. Where applicable, habituation trials were given several hours apart from normal daily dosing.

2.3. In situ *hybridization*

Animals were sacrificed by decapitation on the day following behavioral testing. Brains were quickly removed and immediately fresh frozen on powdered dry ice and stored at −80 °C prior to cryosectioning (16 μm sections) onto gelatin-subbed slides, which

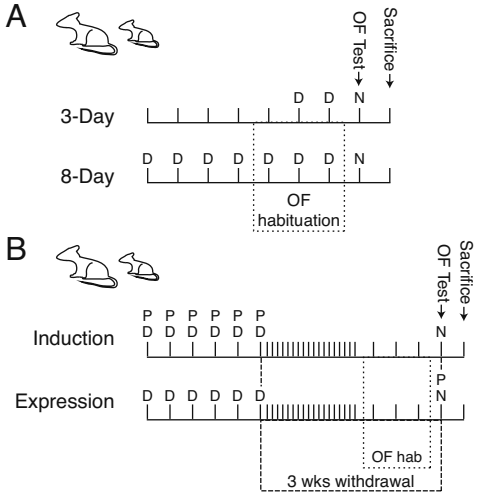

Fig. 1. Timelines for dosing and behavioral testing. (A) Experiment 1. Adolescent and adult rats received 1x/daily s.c. doses ("D") of nicotine or saline for either 2 or 7 days in the home cage environment, and on the next day were treated with nicotine (0.5 mg/kg, free base) before testing in an open field. (B) Experiment 2. Adolescent and adult rats received 1x/daily s.c. doses ("D") of nicotine or saline for 6 days. Three weeks later, all rats were challenged with nicotine (0.5 mg/kg, free base) before testing in an open field ("OF Test"). Half of the animals received either vehicle or GR 103691 pretreatment ("P") 30 min prior to dosing each day during initial exposure (induction), and the other half received pretreatment at the challenge time point (expression). In both experiments, rats were sacrificed the day following the open field test.