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Nicotine modulation of adolescent dopamine receptor signaling and hypothalamic peptide response

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Abstract

Adolescence is a sensitive developmental period for limbic and dopamine systems that coincides with the typical age for onset of tobacco use. We have previously shown that a 4-day, low-dose nicotine (0.06 mg/kg) pretreatment enhances locomotor and penile response to the D2-like agonist, quinpirole (0.4 mg/kg), in adolescent but not adult rats. The present study is designed to determine mechanisms underlying this effect. Nicotine enhancement of adolescent quinpirole-induced locomotion was mediated by D2 receptors (D2Rs) since it was blocked by the D2R antagonist, L-741,626, but not by the D3R and D4R antagonists, NGB 2904 and L-745,870. Enhancement of quinpirole-induced erectile response was blocked by both L-741,626 and NGB 2904, indicating involvement of D3Rs. Whereas D2R binding was unaffected by adolescent nicotine pretreatment, effector coupling in the striatum was increased, as determined by GTPγS binding. Nicotine pretreatment enhanced quinpirole-induced c-fos mRNA expression in the hypothalamic paraventricular and supraoptic nuclei in adolescents only. Adolescent nicotine pretreatment enhanced c-fos mRNA expression in corticotropin releasing factor (CRF) cells of the paraventricular nucleus, and enhancement of penile erection was blocked by the CRF-1 receptor antagonist, CP 376,396. These findings suggest that adolescent dopamine and CRF systems are vulnerable to alteration by nicotine. This is the first evidence for a role of CRF in adolescent erectile response.

Keywords

penile erection; locomotion; paraventricular nucleus; tobacco; corticotropin releasing factor; oxytocin

1. Introduction

In adolescence the brain undergoes a massive reorganization that sets the stage for adulthood (Crews et al., 2007). Neurotransmitter systems are substantially remodeled, most notably

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dopamine, which plays a critical role in sensation seeking, reward and decision-making (Steinberg, 2008; Ernst et al., 2009; Wahlstrom et al., 2010). The cellular distributions of dopamine receptors shift markedly during the transition to adulthood, as do their functional roles (Chen et al., 2010; O'Donnell, 2010).

Functional changes in dopamine systems have been proposed as a neural basis for behaviors that facilitate adolescent transition to independence (Steinberg, 2008; Wahlstrom et al., 2010). Risk taking increases in adolescents, as does vulnerability to psychiatric disorders including drug abuse. Eighty percent of smokers try their first cigarette before age 18 (CDC, Youth and Tobacco 2012). Teen smokers are also more likely to use other drugs of abuse (Lai et al., 2000) and to engage in risky behaviors such as unprotected sex (Duncan et al., 1999). These observations suggest that neural processes underlying adolescent appetitive behaviors may be vulnerable to changes induced by nicotine, the main psychoactive component of tobacco.

Rodent models of adolescence, conservatively defined as postnatal day (P) 28 to P42 (Spear, 2000), have shown nicotine to have unique effects during this developmental period. Adolescent rats and mice are more sensitive to the rewarding effects of nicotine and less sensitive to its aversive effects (Vastola et al., 2002, Belluzzi et al., 2004; Breilmaier et al., 2007; Shram et al. 2008). We have also shown that brief, 4-day exposure to a low dose of nicotine, which results in blood levels equivalent to that from smoking 1–2 cigarettes, enhances the initial reinforcing effects of cocaine, methamphetamine, and alcohol in adolescents but not adults (McQuown et al., 2007; Dao et al., 2011). Nicotine pretreatment also produces an adolescent-specific enhancement of both cocaine-induced locomotor sensitization (McQuown et al., 2009) and acute locomotor activity and penile erection induced by the D2-like agonist, quinpirole (Dao et al., 2011). These findings support the concept that nicotine may have unique effects on maturing dopamine systems in adolescent brain.

Quinpirole is a non-selective D2-like agonist with affinities for D2 receptors (D2R), D3R, and D4R (Gehlert et al., 1992; Seeman and Van Tol, 1994). To further understand the unique mechanisms of nicotine's actions on adolescent dopamine systems, we have used a behavioral pharmacology approach to evaluate which D2-like receptor(s) mediate enhancement of quinpirole effects. In order to relate the *in vivo* effects of drug action to activation of underlying neural circuitry, we have also examined the effects of nicotine exposure on quinpirole-induced expression of the immediate early gene, c-fos, in forebrain dopamine terminal regions of the brains of behaviorally tested animals. Our findings provide critical insight into possible mechanisms underlying unique actions of nicotine on adolescent brain.

2. Materials and Methods

2.1 Animals

Male Sprague Dawley rats were obtained from Charles River at P17 and housed with a dam until weaning (P21). Weaned juveniles and adults (P74) were group housed in an AALAC-accredited vivarium on a 12-hour light-dark cycle with food and water available *ad libitum*. No more than one animal per litter per experimental group was used to avoid potential litter effects. For age comparisons, adult and adolescent rats were run in parallel. All procedures were in compliance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. Efforts were made to minimize pain and suffering, and the number of animals used.

2.2 Drugs

(−)-Nicotine di-(+)-tartrate was purchased from Sigma (St. Louis, MO); (−)-quinpirole hydrochloride, L-741,626 (3-[[4-(4-Chlorophenyl)-4-hydroxypiperidin-l-yl]methyl-1 H-indole), NGB 2904 (*N*-[4-[4-(2,3-Dichlorophenyl)-1-pipe razinyl]butyl]-9*H*-fluorene-2-carboxamide), L-745,870 (3-(4-[4-Chlorophenyl]piperazin-1-yl)-methyl-1*H*-pyrrolo[2,3-*b*]pyridine trihydrochloride), and CP 376,395 (*N*-(1-Ethylpropyl)-3,6-dimethyl-2-(2,4,6-trimethylphenoxy)-4-pyridinamine hydrochloride) from Tocris Biosciences; and propofol from Abbot Laboratories (Chicago, IL). (−)-Nicotine di-(+)-tartrate was pH adjusted to 7.2–7.4 and doses were calculated as free base. All other drug doses were calculated as salt. Drugs were filtered through sterile filters (Millipore Millex Sterile Filters, 0.22 pore, 3.3mm diameter) prior to use.

2.3 Nicotine Pretreatment

Animals were implanted with a chronic catheter into the right external jugular vein (Belluzzi et al., 2005), which was flushed daily with sterile heparinized saline. Catheter patency was tested after drug pretreatment by administration of propofol (5 mg/kg, i.v.), with data discarded from any animal that did not display rapid (5–10 sec) anesthesia.

The general experimental design is as described in McQuown et al. (2007) and shown in Figure 1. For dose-response experiments, two intravenous injections of nicotine (2 × 7.5, 15, or 30 μg/kg/0.1 ml), or saline, spaced 1 min apart, were administered daily for 4 consecutive days during early adolescence (P28–31) or adulthood (P86–89). For all other experiments, adolescent or adult rats were pretreated for 4 consecutive days with either nicotine (2 ×30 μg/kg) or saline. Injections were spaced 1 min apart to reduce neurotoxic effects and nicotinic receptor desensitization. This daily nicotine dose yields peak serum levels of approximately 30 ng/ml in both adolescents and adult (Cao et al., 2007), which is well within the range of the average smoker (Benowitz et al. 2009).

2.4 Behavior

Locomotion was measured as described previously (Dao et al., 2011), using four identical open field activity chambers (43.2 × 43.2 × 30.5 cm) connected to a common interface and computer (MED Associates, Inc., St. Albans, VT). Horizontal movement was recorded by 16 photobeams per side evenly spaced along each wall of two adjacent sides. Prior to testing, animals were placed in the locomotor apparatus for a 30 min habituation period, after which they received an intraperitoneal (i.p.) injection of saline or quinpirole (0.4 mg/kg) and monitored for locomotion during the subsequent 30 min. In some experiments rats were also given a single i.p. injection of L-741,626, a D2 receptor antagonist (Bowery et al., 1996; 0, 2, or 5 mg/kg), NGB 2904, a D3 receptor antagonist (Yuan et al., 1998; 0 or 1 mg/kg), L-745,870, a D4 receptor antagonist (Kulagowski et al., 1996; 0 or 1 mg/kg), or CP 376,395 a CRF-1 receptor antagonist (10 mg/kg) 20 min prior to saline or quinpirole injection. Where single doses of antagonist were used, these have been shown to be effective in prior in vivo studies (Giardino et al., 2012; Xi and Gardner, 2007; Yan et al., 2012). Immediately after locomotor testing, animals were scored for erectile response, using a scoring scale (0–3), based on degree of penile erection: 0 = none; 1 = mild; 2 = moderate; 3 = high. In some cases, animals were then decapitated, brains were collected, immediately flash frozen in 2-methylbutane at −20°C and stored at −70°C until use. For some animals, trunk blood was also collected to determine corticosterone levels using a commercial RIA kit (ICN, Costa Mesa, CA, USA).

2.5 Anatomy

Tissue preparation—Tissue sections were cut on a cryostat at −20°C at a thickness of 20μm, and mounted onto glass slides coated with poly-L-lysine. Sections to be processed for c-fos mRNA were fixed with 4% paraformaldehyde for 1 hr at room temperature followed by 3x 5 min washes in 0.1M phosphate-buffered saline (PBS). All slides were then dried with desiccant and stored at −20°C until processed.

[^{35}S]GTPγS autoradiography—Quinerolane-stimulated [^{35}S]GTPγS (Perkin Elmer, Boston, MA) autoradiography was performed as described by Newman-Tancredi et al. (2001). Sections were preincubated in assay buffer (in mm: Tris/HCl, 50; $MgCl_2 \cdot 6H_2O$, 3; EGTA, 0.2; NaCl, 100; pH7.4 at 25°C) for 10 min then incubated for a further 15 min with 2 mM GDP. Agonist-stimulated activity was determined by incubating alternate sections in [^{35}S]GTPγS (0.04 nM) with quinerolane (100 μM) for 2 hr. Non-specific binding was assessed in the presence of quinerolane (100 μM) and raclopride (10 μM) in the assay buffer. After incubation, slides were rinsed twice for 3 min in ice-cold buffer (50 mm Tris/HCl, pH 7.4) followed by one rinse in deionized water. Slides were dried and exposed to Kodak Biomax film together with [^{14}C] standards.

In situ hybridization—[^{35}S]-labeled UTP (Perkin Elmer, Boston, MA) was used to synthesize cRNA riboprobes for c-fos in the sense and antisense orientation from a pGEM-3Z plasmid containing a 680bp fragment of c-fos cDNA between T7 and SP6 promoter sites. Digoxigenin-labelled cRNA probes for corticotropin releasing factor (CRF) and oxytocin (OXY) were synthesized in antisense orientation using digoxigenin-labeled rUTP and appropriate transcription enzymes. cDNAs were kindly provided by Dr. Stanley J. Watson, University of Michigan.

Brains from each experimental group were collected and processed simultaneously for hybridization of c-fos mRNA as described Winzer-Serhan et al. (1999) and Dao et al. (2011). Slides were then dehydrated and apposed to BioMax film with ^{14}C standards. Sections processed for co-localization of c-fos and peptide mRNAs were incubated with digoxigenin-labeled and ^{35}S-labeled c-fos riboprobes as described in Loughlin et al. (2006). After film development, slides were coated with 3% parlodion in isoamylacetate and dipped in liquid NTB2 emulsion.

Anatomical analysis—A computer-based image analysis system, MicroComputer Imaging Device (MCID, Imaging Research, St. Catherine, Ontario, Canada) was used to analyze autoradiographic films. A calibration curve of optical density against radioligand concentration (dpm/mg tissue) was constructed using [^{14}C] brain paste standards (Broide et al., 1996). Specific hybridization was calculated by subtracting values of radioactivity in sections hybridized with sense probe from those hybridized with antisense. Specific binding was calculated by subtracting values of non-specific binding from that of total.

Analysis of double-labeled cells was as described in Loughlin et al. (2006). Coexpression of c-fos with CRF or OXY mRNA was determined by light microscopic analysis using an Olympus BX50 microscope (Scientific Instruments, Temecula, CA) and transillumination system (Micro Video Instruments, Inc., Avon, MA). Double-labeled cells were identified as cells expressing CRF or OXY (cell-sized deposits of visible digoxigenin reaction product) and c-fos mRNA (silver grain counts more than threefold over background) and were expressed as a percentage of the total number of dig-labeled cells.

2.6 Statistics

Locomotor and neurochemical data were analyzed by 1, 2- or 3-way ANOVA, with significant main or interaction effects being further analyzed by one-way ANOVA or *t* tests with Bonferroni-adjusted *post hoc* comparisons. Erectile response data were analyzed by nonparametric analysis of penile erection mean rank scores with Kruskall-Wallis test. Significant effects were further analyzed by Tamhane's T2 post-hoc analysis of mean rank for each treatment group.

3. Results

3.1 Dose-dependent nicotine enhancement of adolescent quinpirole-induced locomotor activity

A dose-response analysis was undertaken to determine the minimum dose of nicotine needed to enhance quinpirole-induced locomotion in adolescent animals. We have confirmed our prior finding (Dao et al., 2011) that nicotine pretreatment produces an age-specific increase in quinpirole-induced horizontal locomotor activity (Figure 2). We now also show that this effect is dose-dependent, with significant interaction effects of time x age x pretreatment dose [$F(15, 440)=2.096$, $p<0.05$] and time x age x quinpirole dose [$F(5, 440)=15.685$, $p<0.0005$]. When split by age, a significant interaction of time x pretreatment dose x quinpirole dose [$F(15, 265)=2.399$, $p<0.05$] was observed in adolescents, but there was no effect of pretreatment in adults. Significant effects of nicotine pretreatment were seen in adolescents at both 30 and 60 μg/kg/day doses, but not 15 μg/kg/day (Figure 2A).

3.2 Roles of D2-like receptors in quinpirole actions

Antagonist studies were undertaken to determine the pharmacology of the dopamine receptors involved in adolescent nicotine enhancement of quinpirole-induced locomotor and penile responses. In initial studies, a D2R antagonist, L-741,626, was used (Figures 3 & 4). For locomotor activity (Figure 3), there was a main effect of quinpirole [$F (1,101) = 31.373$, $p <0.005$], interactions of quinpirole × pretreatment [$F (2,101) = 12.92$, $p<0.005$] and quinpirole × L-741,626 dose [$F (2,101) = 12.92$, $p<0.005$]. L-741,626 dose-dependently reduced quinpirole-induced locomotion in both nicotine pretreatment and control groups (Figure 3), suggesting the involvement of D2Rs in both quinpirole-induced locomotion and its enhancement by nicotine. L-741,626 also significantly inhibited spontaneous locomotor activity at the 5 mg/kg dose, but not at the lower dose.

Penile response in nicotine-pretreated adolescents was also reduced by L-741,626 (Figure 4). Kruskall-Wallis analysis revealed a significant difference in penile erection between treatment groups ($p < 0.001$). Post-hoc analysis showed that nicotine pretreatment enhanced quinpirole-induced penile erection ($p<0.005$) and that L-741,626 dose-dependently reduced this effect. In contrast to locomotor responses, L-741,626 did not block penile response in saline pretreated controls.

Although L-741,626 is considered to be a selective D2R antagonist, it also has some affinity for D3 and D4 receptors (Kulagowski et al., 1996; Collins et al., 2007). Therefore, the D3R and D4R antagonists, NGB 2904 and L-745,870, respectively, were used to further evaluate the pharmacology of the receptors mediating nicotine enhancement of quinpirole responses (Figures 5 & S1). For the D3R antagonist, NGB 2904, there was an overall effect of nicotine pretreatment [$F (1,54) = 14.822$, $p <0.001$], but not of antagonist, on locomotor response (Figure 5A). NGB 2904 did, however, block nicotine enhancement of penile response ($p < 0.05$; Figure 5B). The D4R antagonist L-745,870 did not significantly influence locomotor or penile response in either nicotine pretreated adolescents or controls (Figure S1).

3.3. Nicotine pretreatment enhances the coupling efficiency of D2 receptors

Studies were undertaken to determine whether the observed locomotor effects reflected increased D2R number or enhanced efficacy in nicotine pretreated adolescent rats. There were no significant differences in D2R binding site densities in adolescents pretreated with nicotine compared to saline controls (Table S1). In contrast, D2R signaling, as measured by [^{35}S]GTPγS binding, was significantly enhanced by nicotine pretreatment during adolescence in dorsal striatum [$F(1,14)=4.59$; $p=0.05$] and nucleus accumbens core [$F(5, 70)=3.99$, $p = 0.003$], but not in nucleus accumbens shell (Figure 6).

3.4 Adolescent nicotine pretreatment enhances quinpirole-induced c-fos mRNA expression in hypothalamic nuclei

Brains of behaviorally tested animals were examined for regional changes in mRNA expression of the immediate early gene, c-fos, to establish possible neural mechanisms underlying behavioral response in adolescents and adults. Brain areas in which significant effects were found are illustrated in Figures S2 and S3. Quinpirole-induced elevations in c-fos mRNA levels in the bed nucleus of the stria terminalis, anterioventral thalamus, medial amygdala and dentate gyrus were significantly higher in adolescents than in adults (Figure S2). However, there was no effect of nicotine pretreatment on c-fos mRNA expression in these regions. In the hypothalamic paraventricular nucleus (PVN), supraoptic nucleus (SON), and central nucleus of the amygdala (CeA), there were also significant effects of age [$F(1,50) = 5.229$, $p<0.05$; $F(1, 52) = 14.063$, $p<0.005$; $F(1,53) = 28.039$, $p<0.0005$, respectively] on quinpirole induced c-fos mRNA expression. However, there were also age-specific effects of nicotine pretreatment in these regions, as demonstrated by a significant pretreatment × quinpirole interaction for adolescents in the PVN [$F(1, 34) = 5.567$, $p<0.05$] and SON [$F(1, 36) = 6.589$, $p<0.05$] and an overall significant effect of pretreatment [$F(1,16) = 5.352$, $p<0.05$] and quinpirole [$F(1,16) = 172.254$, $p<0.0005$] for adults in the CeA (Figure 7). Quinpirole induced significant increases in cfos mRNA expression in all three nuclei in both adolescents and adults. However, nicotine pretreatment enhanced quinpirole-induced cfos expression in the PVN and SON only in adolescents, while decreasing cfos expression in adult CeA.

Since dopamine activation of OXY cells in the PVN has been implicated as a mechanism for penile erection (Baskervile et al., 2009, Melis and Argiolas, 2011), we used double-labeling in situ hybridization to determine which cell types in PVN and SON were specifically affected by nicotine pretreatment during adolescence (Figure 8). CRF and OXY mRNA expression within the PVN was concentrated in the medial parvocellular region and lateral magnocellular division; double-labeled cells were mainly localized to the medial parvocellular division. There was an overall effect of quinpirole [$F(1,13)=32.07$, $p<0.0001$] and nicotine pretreatment [$F(1,13)=5.31$, $p=0.038$] on activation of CRF cells in the PVN. Quinpirole significantly increased the number of CRF cells expressing c-fos mRNA, and this effect was further enhanced by nicotine pretreatment (Figure 8A). In contrast, there was a significant effect of quinpirole [$F(1,13)=26.07$, $p<0.0001$], but not nicotine on c-fos mRNA expression in OXY cells of the PVN (Figure 8B).

Cells that expressed OXY were evenly distributed in the SON (Figure 8C), and quinpirole increased the number of c-fos/OXY double-labeled cells in this region [$F(1,13)=32.07$, $p<0.0001$] with a significant nicotine pretreatment x drug interaction [$F(1,13)=10.82$, $p=0.006$]. In nicotine pretreated adolescents, quinpirole significant increased the number of cell expressing of c-fos mRNA when compared to vehicle [$F(1,13)=8.57$, $p=0.012$]. There was also a trend towards significant nicotine enhancement of the number of OXY cells expressing c-fos following quinpirole treatment ($p=0.074$).

3.5 Role of CRF

Further studies were undertaken to examine a possible role of CRF in nicotine enhancement of adolescent quinpirole-induced penile or locomotor response. Although adolescent nicotine pretreatment enhanced quinpirole activation of CRF cells in the PVN, it did not enhance quinpirole activation of the hypothalamic-pituitary-adrenal (HPA) axis as measured by plasma corticosterone levels (Fig S5). However, pharmacological analysis did implicate CRF in nicotine enhancement of adolescent quinpirole-induced responses. Whereas the CRF-1R antagonist, CP 376,395, did not block locomotor response in either saline- or nicotine-pretreated adolescents (Fig. 9A), it abolished nicotine enhancement of quinpirole-induced erectile response ($p=0.005$; Fig 9B).

4. Discussion

Adolescence is marked by a remodeling of dopamine systems that may contribute to heightened appetitive drive (Wahlstrom et al., 2009). Tobacco use during early adolescence has also been associated with increased risk-taking behavior, including drug use and sexual activity, which may result from biological sensitization of neural appetitive systems (Hanna et al., 2001). Nicotine pretreatment enhances locomotion and penile erection induced by quinpirole, a D2-like agonist, in adolescent but not adult rats (Dao et al., 2011). We have used pharmacological and neurochemical approaches to further assess mechanisms underlying nicotine enhancement of quinpirole-induced responses in adolescents. Our findings confirm the involvement of distinct mechanisms underlying nicotine enhancement of locomotor and penile response. Whereas increased locomotor activity reflects enhanced D2R signaling, increased penile response reflects the recruitment of D3R and CRF-1R.

4.1 Dopamine receptor antagonists

Although quinpirole increases locomotion only slightly in adults, its effects are much more profound in adolescents, as has been shown previously (Frantz & Van Hartesveldt, 1999; Dao et al., 2011). However, since this drug has affinity for all D2-like receptors, including D2R, D3R, and D4R (Titus *et al*., 1983; Sokoloff *et al.*, 1992; Gehlert et al. 1992; Seeman and Van Tol, 1994), we used more selective pharmacological agents to assess the mechanisms underlying quinpirole-induced locomotion in adolescents and its enhancement by nicotine. L-741,626, an antagonist with a 10-fold preferential affinity for D2R over D3R (Bowery et al., 1996; Millan et al., 2000), dose-dependently antagonized quinpirole-induced locomotion in nicotine-pretreated animals and in saline-pretreated controls. Neither NGB 2904 nor L-745,870, D3R and D4R antagonists, respectively, reduced quinpirole-induced locomotion in either saline- or nicotine-pretreated adolescents, further confirming the role of D2Rs in adolescent locomotor response.

In contrast, different receptor mechanisms underlie nicotine enhancement of adolescent erectile response. Whereas L-741,626 dose-dependently inhibited nicotine enhancement of quinpirole-induced erection, it did not block baseline response. The D3R antagonist, NGB 2904, also blocked nicotine enhancement of quinpirole-induced penile erection, whereas the D4R antagonist, L-745,870 had no effect. Since the D2 antagonist L-741,626 does have affinity for D3Rs in the dose range used (Collins et al., 2007), these combined findings suggest that nicotine enhancement of quinpirole-induced erectile response is mediated by the D3 receptor type. This finding is consistent with that of other studies in adult rodents (Ferrari and Giuliani, 1995; Collins et al., 2009).

4.2 Dopamine receptor - effector coupling

As with prior studies using a more extensive pretreatment regimen (Collins et al., 2004), we have found that nicotine exposure during adolescence does not affect D2R density.

However, using [^{35}S] GTPγS, we have shown that adolescent nicotine pretreatment significantly enhances D2-like receptor G-protein coupling in adolescent nucleus accumbens core and dorsal striatum, but not in nucleus accumbens shell. Similar to past reports, the signal to noise ratio of this biochemical assay did not allow us to assess receptor coupling in other brain regions (Newman-Tacredi, et al. 2001; Bailey et al., 2008); thus, we cannot determine whether D2-like receptor signaling is affected elsewhere. We also do not know whether this effect is age-specific as we did not test GTPγS function in adults. We have previously shown that acute and subchronic nicotine treatment induces age-specific increases in c-fos mRNA expression in adolescent nucleus accumbens shell (Dao et al., 2011). Furthermore, other recent studies that used more prolonged and higher dose nicotine treatments have shown adolescent nicotine exposure to induce changes in accumbal expression of BDNF and D1R and D3R mRNAs (Perna and Brown, 2013; Wheeler et al., 2013). These findings, combined with prior studies that have shown D2Rs in the nucleus accumbens and cortico-accumbens pathway to change expression and function markedly during the transition from adolescence to adulthood (Teicher et al., 1995; Tarazi et al., 1998; Benoit-Marand et al., 2008; Huppe-Gourgues et al., 2012), suggest changes in striatal and accumbal dopaminergic function as a possible mechanism for adolescent-specific effects of nicotine and quinpirole on locomotion.

4.3 Nicotine pretreatment effects on regional c-fos mRNA expression

Using c-fos mRNA expression as a marker of recent neuronal activity (Flavell and Greenberg, 2008), we have examined regional mechanisms underlying nicotine enhancement of quinpirole actions in adolescents. Although quinpirole significantly increased c-fos mRNA expression in several brain regions of both adult and adolescent rats, this effect was much more robust in adolescent bed nucleus of the stria terminalis, anterioventral thalamus, medial amygdala and dentate gyrus. However, neural activation in these brain regions was not sensitive to nicotine pretreatment. Adolescent nicotine pretreatment only enhanced quinpirole-induced c-fos mRNA expression in the PVN and SON, hypothalamic regions that are known to play a role in circuits that underlie motivated behavior, locomotion and sexual reproduction (Swanson et al., 2000).

Although dopamine activation of OXY cells in the PVN has been shown to be critical for adult penile erection (Baskerville et al., 2009; Melis & Argiolas, 2011), we found that adolescent nicotine pretreatment enhanced quinpirole-induced c-fos expression in PVN CRF cells. Adolescent nicotine pretreatment also increased c-fos mRNA expression in OXY neurons of the SON but not the PVN. Quinpirole has been reported to have a direct depolarizing effect on SON neurons with firing patterns consistent with that of OXY cells in explants from juvenile rats (Yang et al., 1991). Since OXY has been implicated as a facilitator of social behaviors (Gordon et al., 2011), nicotine pretreated adolescents may be expected to exhibit social interactions distinct from that of controls, a possibility that should be examined in future studies.

4.4 CRF-1R mediation of erectile response

D2-like agonists, such as quinpirole, can activate the CRF cells in the parvocellular region of the PVN that regulate the HPA axis stress response (Eaton et al., 1996), resulting in peripheral corticosterone secretion (Borowsky & Kuhn, 1992). Whereas we found that quinpirole did increase plasma corticosterone levels in adolescents, this action was not enhanced by nicotine pretreatment. This may result from a ceiling effect caused by quinpirole challenge, with no further enhancement possible in nicotine-pretreated rats. It is possible, however, that nicotine enhances quinpirole activation of a subset of PVN cells that do not regulate corticosterone release.

CRF cells have been shown to project to areas within the brain, such as the ventral tegmental area and locus coeruleus (Reyes et al., 2005; Wise and Morales 2010), that regulate reward and arousal. We therefore examined the in vivo effects of a CRF-1R antagonist, CP 376,395, to further understand the mechanism behind nicotine enhancement of adolescent of quinpirole-induced locomotor and penile response. Although CRF-1R activation has been shown to be critical for cocaine -induced locomotor activity in adults (Lu et al., 2003), the CRF-1R antagonist did not block quinpirole-induced locomotor response in either saline- or nicotine-pretreated adolescents. However, unexpectedly, CRF-1Rs did mediate adolescent nicotine enhancement of quinpirole-induced penile response. This is the first report of an involvement of CRF-1Rs in penile erection. This finding contrasts with that of earlier studies that have implicated OXY neurons in the PVN as mediators of D2-induced erectile response in adults (Baskervile et al., 2009, Melis and Argiolas, 2011). It is not yet clear whether or not this novel CRF mechanism is unique to the adolescent period before full sexual maturation has occurred.

4.5 Conclusion

This study provides further experimental evidence for the negative impact of brief nicotine exposure on adolescent brain. We have previously shown that nicotine enhancement of quinpirole-induced locomotion, but not penile erection, is mediated by 5-HT1A receptors (Dao et al., 2011). We now show that this reflects an increase in D2R signaling. Further studies will be required to elucidate the exact mechanisms underlying this effect, but the findings add to a growing body of literature showing that D2Rs have distinct roles in adolescent brain. We have also shown that adolescent nicotine pretreatment potentiates quinpirole-induced penile erection via D3R activation and resulting hypothalamic CRF neuronal response. In contrast to a recent study of nicotine-cocaine interactions in adult mice (Levine et al., 2011), the enhancing effects of nicotine on adolescent D2R and D3R signaling are evident after brief pretreatment and do not require the continued presence of the drug. Thus, our findings provide strong support for the concept, derived from epidemiological studies (Duncan et al., 1999, Lai et al. 2000), that tobacco use during early adolescence may enhance appetitive behaviors, including sexual activity and drug use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Tested mechanisms of nicotine enhanced D2R-like function in adolescent rats.
- Enhanced quinpirole-induced locomotion is mediated by D2R.
- Enhanced quinpirole-induced penile response is mediated by D3R.
- Nicotine increased quinpirole activation of CRF and oxytocin cells in hypothalamus.
- CRF-R1 mediates enhanced penile response.

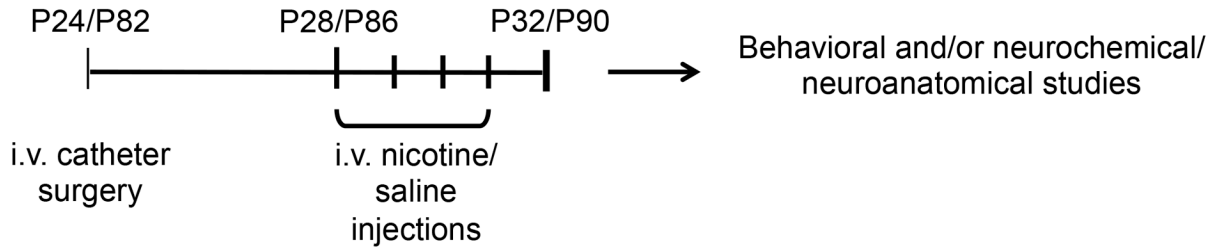

Figure 1.
Experimental timeline

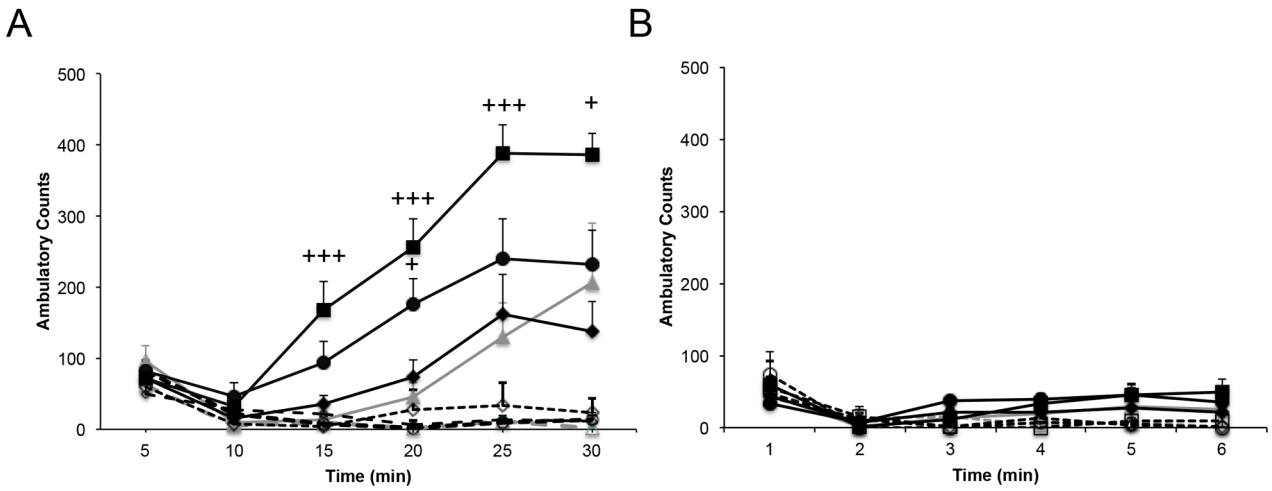

Figure 2. Effect of nicotine pretreatment dose on quinpirole-induced locomotor activity in adolescent and adult rats

Mean ± SEM ambulatory counts of rats pretreated with saline (triangle), or with 15 (diamond), 30 (circle), or 60 (square) μg/kg/day nicotine as A) adolescents (P28-P31) or B) adults (P86-P89) and given either vehicle (dashed line) or quinpirole (solid line) on P32 or P90. Nicotine pretreatment induced a dose-dependent enhancement of quinpirole-induced locomotor activity in adolescent rats. + $p < 0.01$, +++ $p < 0.0005$. n =7–9/group.

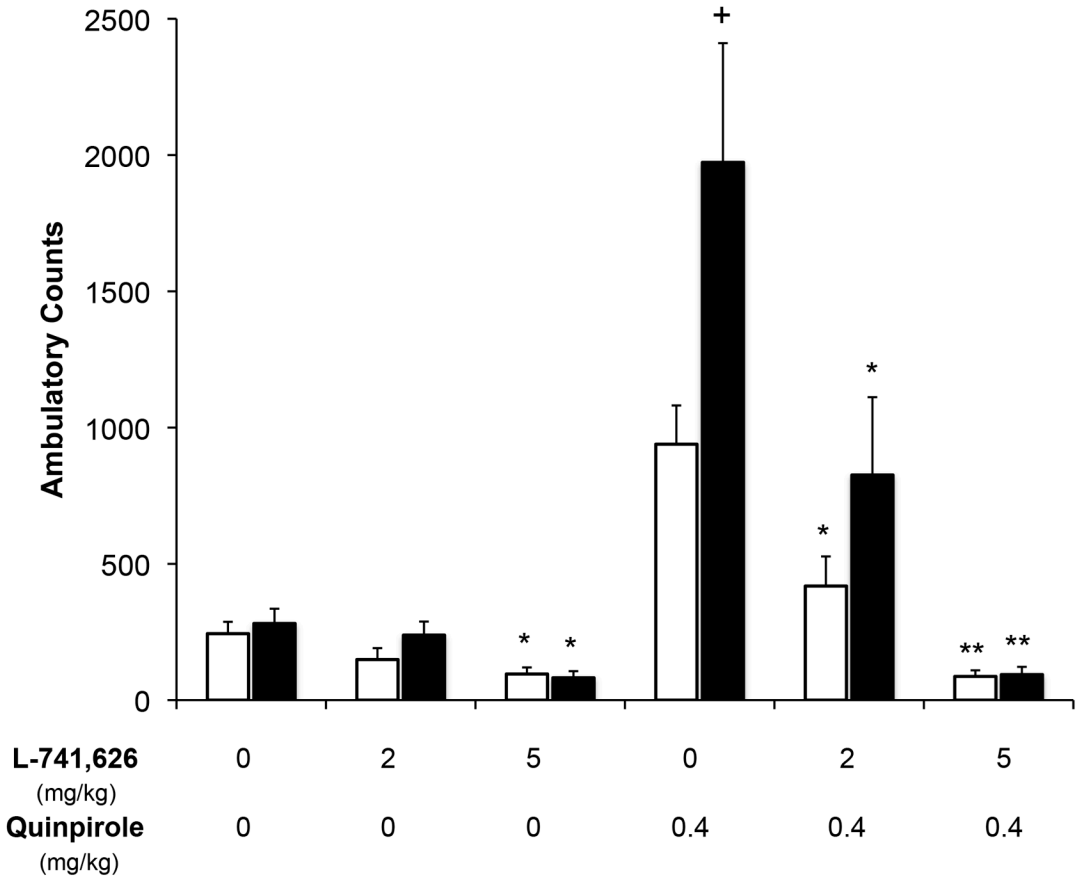

Figure 3. L-741,626 blocks quinpirole-induced locomotion in both saline- and nicotine-pretreated adolescents

The bars represent mean (± SEM) total ambulatory counts for saline- (open bars) and nicotine-pretreated (black bars) rats. The D2R antagonist, L741,626 (0, 2 and 5 mg/kg, i.p.) was given 20 min before an i.p. injection of quinpirole (0.4 mg/kg) or vehicle. + $p < 0.05$ vs. saline pretreated, * $p < 0.05$, ** $p < 0.005$, significant antagonist inhibition. n = 9–12 per group.

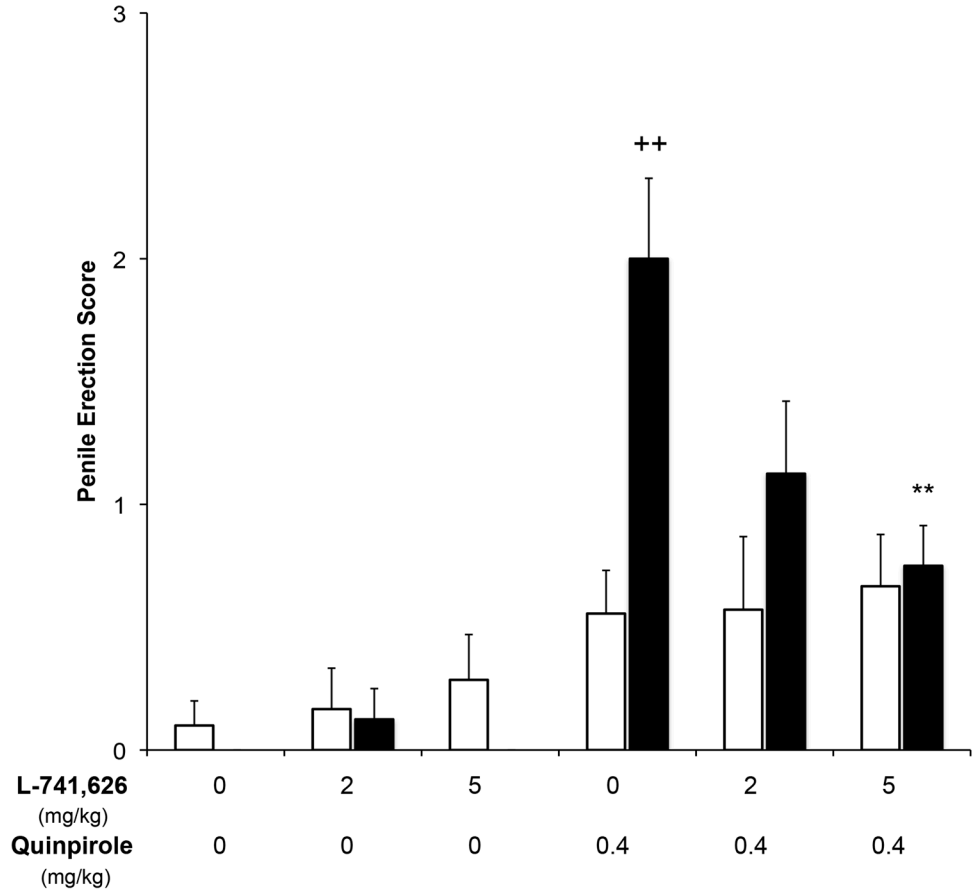

Figure 4. L-741,626 blocks nicotine enhancement of quinpirole-induced penile erection in adolescent rats

The bars represent mean penile erection score (± SEM) for saline- (open bars) and nicotine-pretreated (black bars) rats. The D2R antagonist, L741,626 (0, 2 and 5 mg/kg, i.p.) was given 20 min before an i.p. injection of quinpirole (0.4 mg/kg) or vehicle. ++ $p < 0.005$ vs. saline pretreated, ** $p < 0.005$, significant antagonist inhibition. n = 6–9 per group.

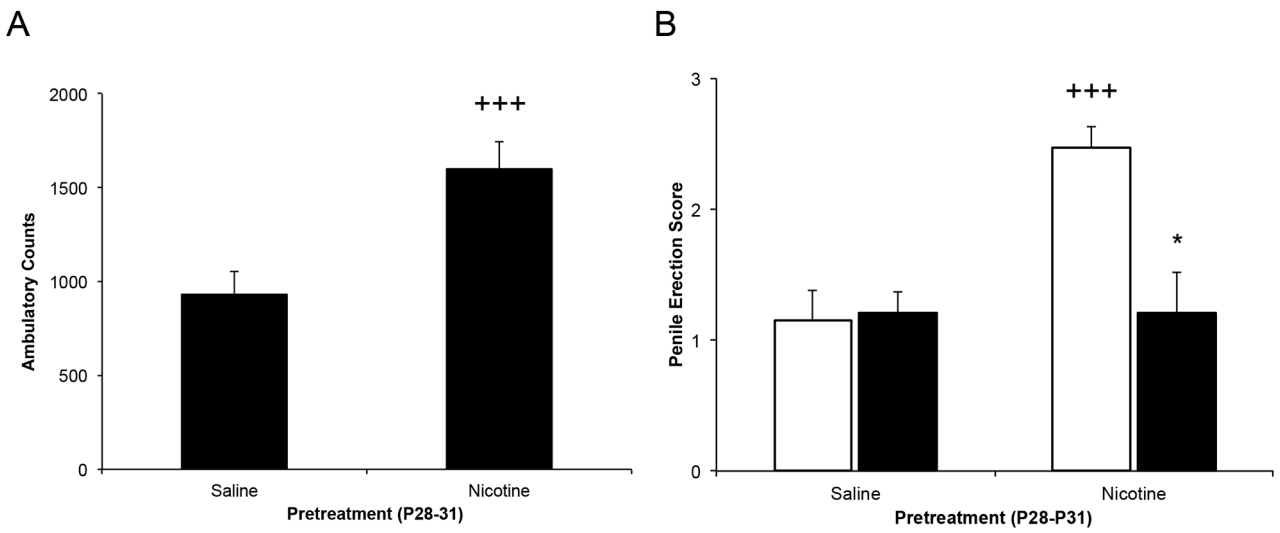

Figure 5. NGB 2904 reduces quinpirole-induced penile response, but not locomotion, in nicotine-pretreated adolescent rats

Data represent the mean (± SEM) of ambulatory counts (A) and penile score(B). Since there was no significant effect of the D3R antagonist, NGB 2904, on locomotor response, data are meaned across antagonist dose. Penile scores are shown separately for rats given vehicle (open bars) or NGB 2904 (black bars). +++ $p=0.001$ vs. Saline; * $p<0.05$ significant antagonist inhibition n=13–15 per group.

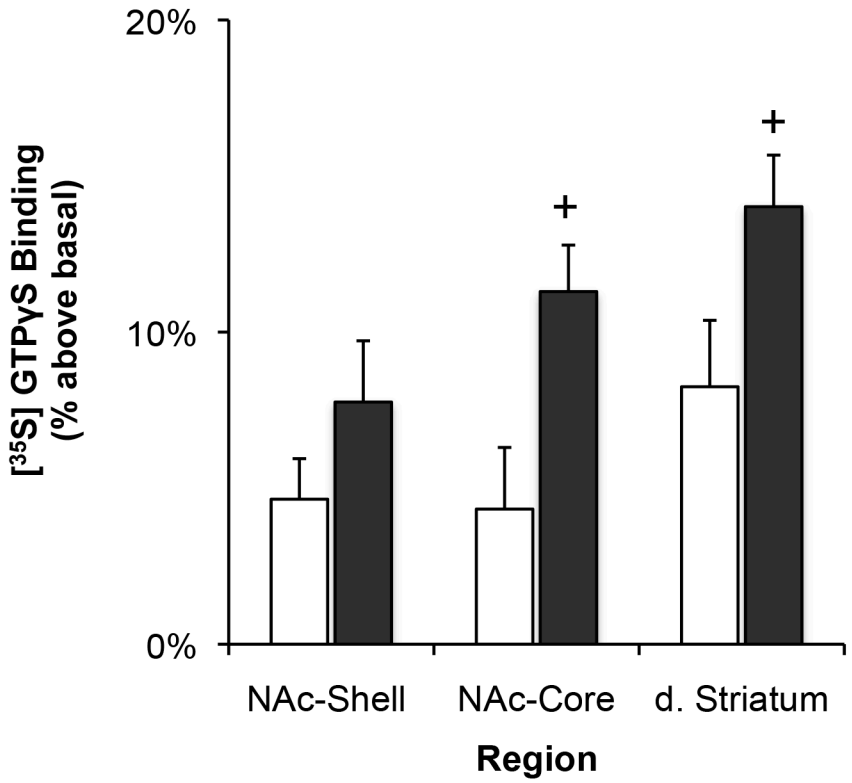

Figure 6. Effect of adolescent nicotine pretreatment on D2R function in adolescent rats

The bars represent mean (± SEM) of specific D2R-mediated [^{35}S] GTPγS binding in striatal regions of saline- (open bars) and nicotine-pretreated (black bars) adolescent rats.

* $p \leq 0.05$ vs. saline pretreated controls. n = 5–6 per group.

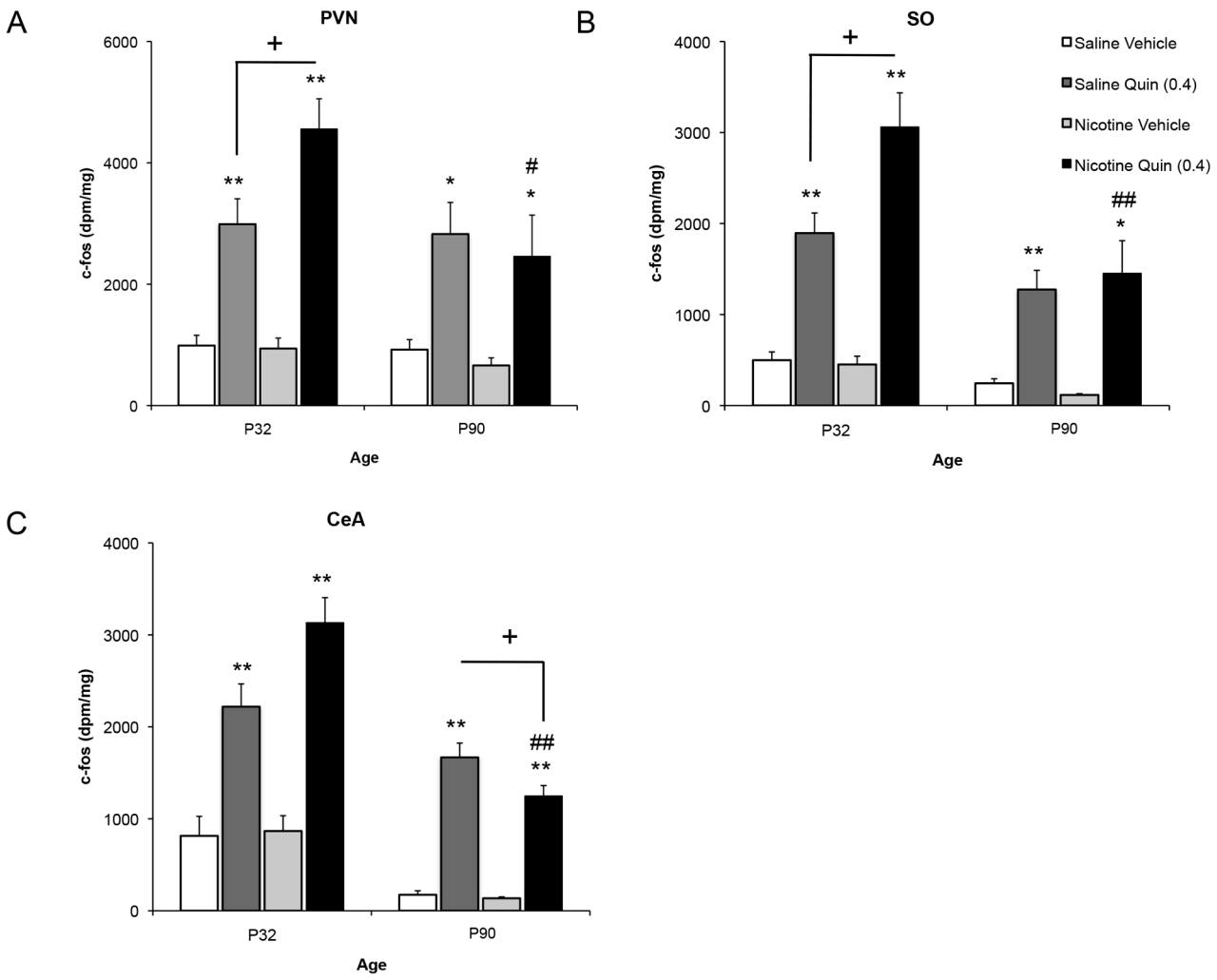

Figure 7. Effect of age and nicotine pretreatment on quinpirole-induced c-fos mRNA expression

Quinpirole increased c-fos expression in the PVN (A), SON (B) and CeA (C) at both ages. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$. Age differences were observed in all nuclei; # $p < 0.001$, ## $p < 0.0001$ vs. adolescents. Adolescents showed pretreatment effects, with nicotine enhancing quinpirole-induced c-fos expression in PVN and SON. Adult showed decreased c-fos mRNA expression in the CeA as a result of nicotine pretreatment. + $p \leq 0.05$. n = 5–10 per group.

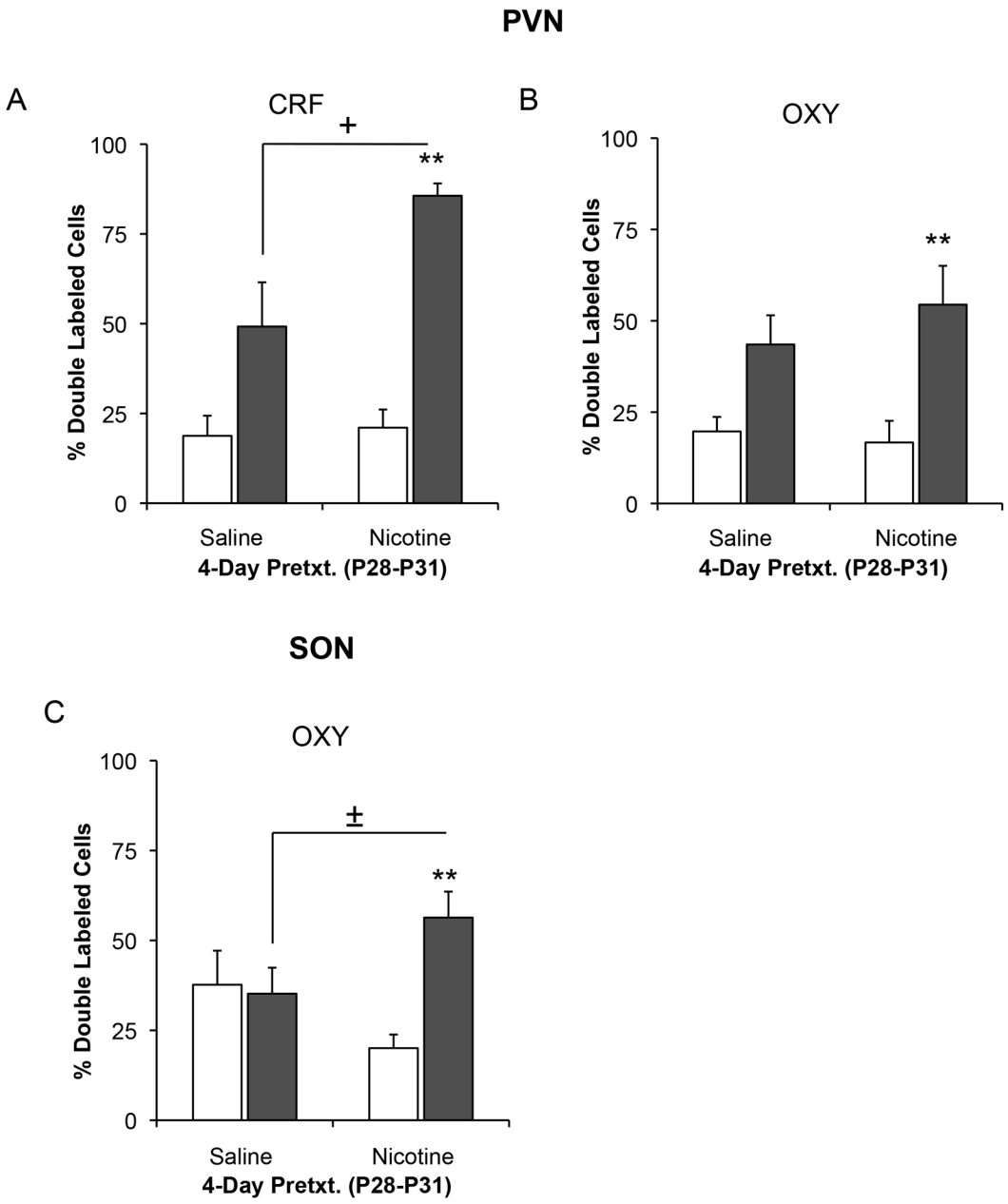

Figure 8. Identification of neurons that express quinpirole-induced c-fos mRNA in the adolescent PVN and SON

Data represent double-labeled cells expressed as a percentage of total CRF (A) and OXY (B) mRNA-containing cells in the PVN, and OXY cells in SON (C). ** $p < 0.001$ vs. vehicle. + $p < 0.05$ vs. saline, ± $p = 0.074$ vs. saline. Data are expressed as mean ± SEM. n = 4–6 per group.

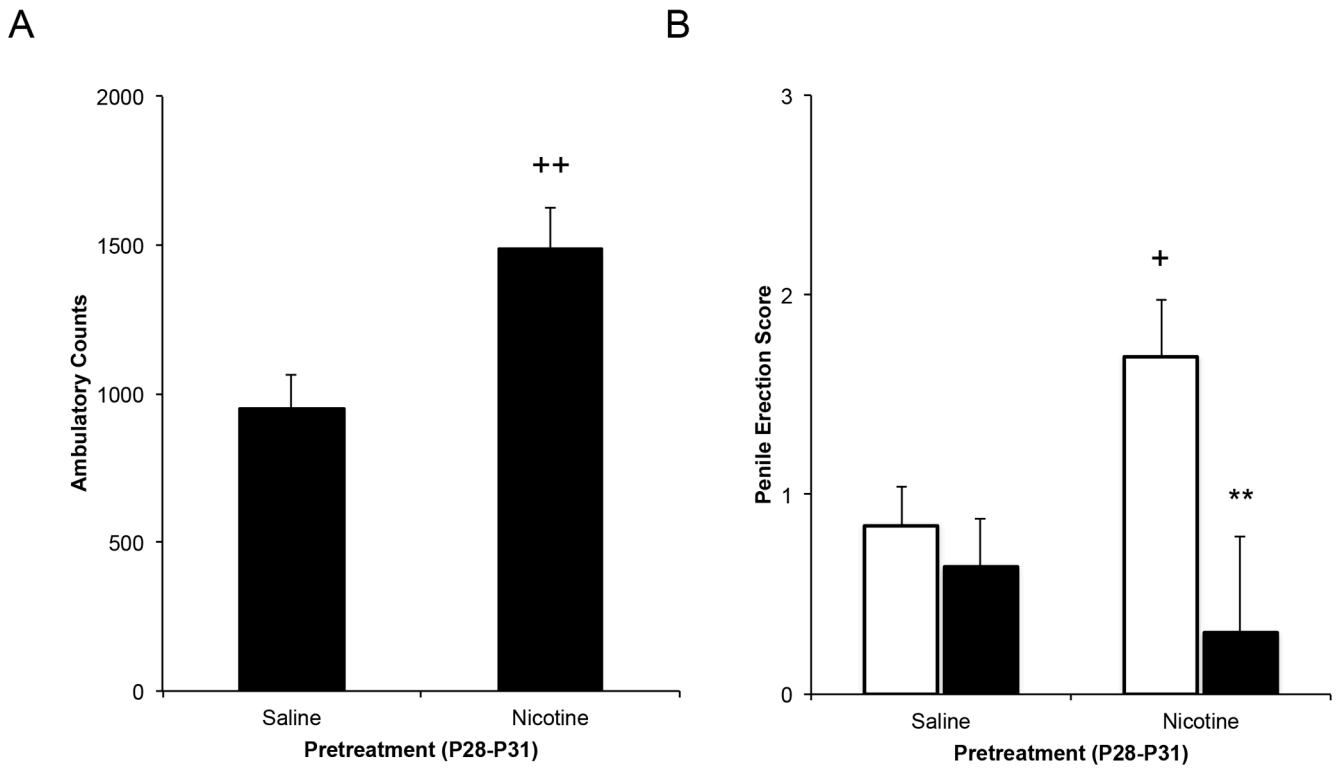

Figure 9. CRF-1R mediation of quinpirole-induced penile response in adolescents

Data represent the mean (± SEM) of ambulatory counts (A) and penile score (B). Since there was no significant effect of the CRF-1R antagonist, CP 376,395, on locomotor response, data are meaned across antagonist dose. Penile scores are from rats given vehicle (open bars) or CP376396 (black bars). + $p<0.05$, ++ $p<0.005$ vs. Saline; **$p=0.005$, significant antagonist inhibition; n=13–15 per group.