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Adolescent nicotine-induced dendrite remodeling in the nucleus accumbens is rapid, persistent, and D1-dopamine receptor dependent

D. G. Ehlinger · H. C. Bergstrom · J. C. Burke · G. M. Fernandez · C. G. McDonald · R. F. Smith

Abstract Chronic nicotine exposure during adolescence induces dendritic remodeling of medium spiny neurons (MSNs) in the nucleus accumbens (NAcc) shell. While nicotine-induced dendritic remodeling has frequently been described as persistent, the trajectory of dendrite remodeling is unknown. Specifically, no study to date has characterized the structural plasticity of dendrites in the NAcc immediately following chronic nicotine, leaving open the possibility that dendrite remodeling emerges gradually over time. Further, the neuropharmacological mechanisms through which nicotine induces dendrite remodeling are not well understood. To address these questions, rats were co-administered chronic nicotine (0.5 mg/kg) and the D1-dopamine receptor (D1DR) antagonist SCH-23390 (0.05 mg/kg) subcutaneously every other day during adolescence. Brains were then processed for Golgi–Cox staining either 1 day or 21 days following drug exposure and dendrites from MSNs in the NAcc shell digitally reconstructed in 3D. Spine density was also measured at both time points. Our morphometric results show (1) the formation of new dendritic branches and spines 1 day following nicotine exposure, (2) new dendritic branches, but not spine density, remains relatively stable for at least 21 days, (3) the co-administration of SCH-23390 completely blocked nicotine-induced dendritic remodeling of MSNs at both early and late time points, suggesting the formation of new dendritic branches in response to nicotine is D1DR-dependent, and (4) SCH-23390 failed to block nicotine-induced increases in spine density. Overall this study provides new insight into how nicotine influences the normal trajectory of adolescent brain development and demonstrates a persistent form of nicotine-induced neuroplasticity in the NAcc shell that develops rapidly and is D1DR dependent.

Keywords Nicotine · Adolescence · Dendrite morphology · Dopamine · D1 receptor · Dendritic spine

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Introduction

Adolescence is a developmental stage in which the majority of smokers initiate tobacco use (NIDA 2012). Thus, adolescence represents a unique period of vulnerability to the neurobehavioral effects of nicotine (Smith 2003; O'Dell 2009; Baler and Volkow 2011; Gulley and Juraska 2013). Nicotine has consistently been shown to remodel neuron dendrite morphology and change the density of spines (Brown and Kolb 2001; Robinson and Kolb 2004). During adolescence, nicotine-induced dendritic remodeling is considerably more pronounced in the nucleus accumbens (NAcc) shell (McDonald et al. 2005, 2007), a neuronal network hub critical for reward learning (Di Chiara et al. 1999). Dendritic remodeling and changes in spine density have the potential to dramatically alter synaptic connectivity (Chklovskii 2004; Yuste 2011), which may play a role in the emergence and maintenance of nicotine addiction.

While nicotine has consistently been shown to alter dendritic structure, the neuropharmacological mechanisms

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are unknown. Nicotine acts at nicotinic acetylcholine (ACh) receptors located on ventral tegmental area (VTA) dopaminergic neurons (Picciotto et al. 1998) and its influence on the NAcc shell is largely through increased VTA dopaminergic transmission onto the medium spiny neurons (MSNs) (Di Chiara 2000; Zhou et al. 2001; Picciotto 2003). Although dopamine (DA) signaling directly modulates MSN activity during nicotine exposure, and is involved in the neurobehavioral effects of chronic nicotine (Corrigall and Coen 1991; Pierce and Kumaresan 2006; Spina et al. 2006), whether DA signaling is necessary for nicotine-induced dendritic remodeling of MSNs has not been tested. DA signaling at D1-type DA receptors (D1DR) is a likely mechanism for nicotine-induced dendritic remodeling in the NAcc shell due to positive coupling of D1DRs with the cAMP–PKA pathway and their ability to upregulate intracellular signaling pathways in the NAcc that are known to influence dendritic plasticity (Lachowicz and Sibley 1997; Self 2004; Arikkath 2012; Nestler 2013). Importantly, D1DR signaling on D1DR-containing MSNs is thought to play a predominant role in the primary reinforcing effects of drugs of abuse occurring during drug exposure (Lobo and Nestler 2011) and, therefore, has a likely role in the induction of persistent addiction-like behaviors. In contrast, DA signaling at D2-type DA receptors (D2DR) inhibits the cAMP–PKA pathway and these same intracellular processes, and have a predominant role in reinstatement of drug-seeking behavior following a previously established pattern of drug intake (Self 2004; Lobo and Nestler 2011). Therefore, signaling specifically at D1DRs during repeated nicotine exposure represents a likely neuropharmacological mechanism for the induction of nicotine-induced dendrite remodeling of MSNs.

Nicotine-induced dendritic remodeling in the NAcc has frequently been described as persistent (Robinson and Kolb 1999; Nestler 2001) with alterations observed at several relatively remote time-points (21, 30, and 75 days) after dosing (Brown and Kolb 2001; McDonald et al. 2005, 2007). No study to date has characterized dendritic structure in the NAcc *immediately* following nicotine dosing, leaving open the possibility that changes in the structure of dendrites occur gradually over time. A comparison of dendrite remodeling at multiple time-points (early and late) after chronic nicotine dosing is required to determine precisely when plasticity is induced, the trajectory of plasticity and directly address the question of whether nicotine-induced dendrite remodeling is indeed persistent.

Here we sought to characterize the trajectory of MSN dendrite remodeling in the NAcc shell at an early and late time-point following chronic nicotine administration. We also set out to determine whether DA signaling at D1DRs during nicotine exposure is required for dendrite remodeling and changes in spine density. We tested these questions by co-administering adolescent rats with the selective D1DR antagonist R(+)-SCH-23390 hydrochloride (SCH-23390) and nicotine. Brains were then processed for Golgi–Cox staining at either early (1 day) or late (21 days) time-points after dosing and NAcc shell MSN dendrites were fully reconstructed in 3D for morphometric and spine density analysis. We show for the first time that blocking DA signaling at D1DRs during chronic adolescent nicotine exposure prevents dendritic remodeling and that this effect is specific to the dendritic arbor rather than spines. Further, we show increases in dendritic branching and spine density immediately following nicotine exposure and that new dendritic branching, but not spine density, persists into early adulthood.

Materials and methods

Subjects

A total of 64 male Sprague–Dawley rats (Harlan, IN, USA) were used in this study. Rats arrived on postnatal day (P) 21, were randomly assigned to experimental groups and were group-housed 3–4 rats per cage. The animal colony was temperature, humidity, and light controlled (12 h/12 h light/dark cycle, lights on 0700 hours), and rats were given access to food and water ad libitum. All experimental procedures were completed in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (eighth edition; http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf) and the George Mason University Institutional Animal Care and Use Committee.

Drugs

R(+)-SCH-23390 hydrochloride (SCH-23390; Sigma Aldrich, St. Louis, MO, USA) was dissolved in 0.9 % saline and administered subcutaneously (SC) at a dose of 0.05 mg/kg at volume of 1 ml/kg. This dose has previously been shown to disrupt behavioral responses to nicotine while limiting extrapyramidal side-effects (Acquas et al. 1989; Zarrindast et al. 1996). (−)-Nicotine hydrogen tartrate (Nicotine; Sigma Aldrich, St. Louis, MO, USA) was dissolved in 0.9 % saline and pH was adjusted to 7.0. Nicotine was administered SC at a dose of 0.5 mg/kg at volume of 1 ml/kg. This dose administered chronically during adolescence has previously been shown to be rewarding during adolescence (Brielmaier et al. 2007, 2008), alter behavioral responses measured in adulthood (Bracken et al. 2011), and produce dendritic remodeling (Bergstrom et al. 2010).

Fig. 1 Dosing protocol and experimental design. *Black color* represents time period of nicotine exposure, with each *vertical line* representing a single pretreatment/treatment injection. *Blue color* represents length of abstinence for either early (**a**) or late (**b**) time-point groups. *Red color* represents day of sacrifice for tissue staining
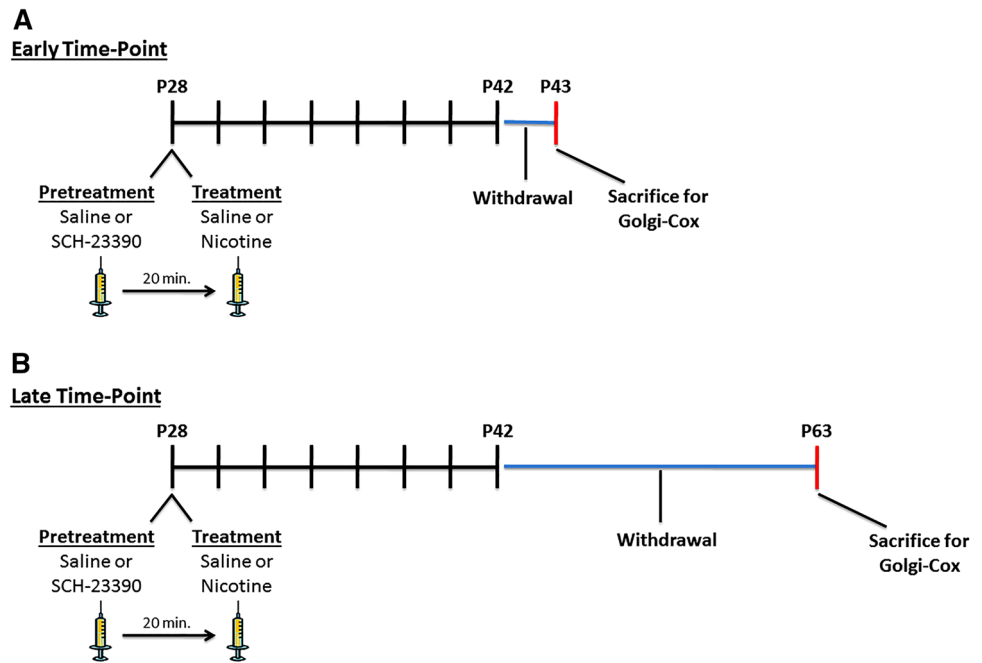
Physiological saline (0.9 % NaCl solution—control) was administered SC at volume of 1 ml/kg.

Experimental protocol

The first goal of this study was to determine the influence of D1DRs on adolescent nicotine-induced dendritic remodeling and spine density in the NAcc shell. We tested this question by administering nicotine or saline (control) during adolescence (P28-42) and co-administering pretreatment injections of either the D1DR antagonist SCH-23390 or saline (control). Animals were then killed for Golgi–Cox staining and quantification of dendrite remodeling and spine density. The age range chosen for drug administration in this study represents a conservative estimate for adolescence (Spear and Blake 1983; Spear 2000), although indices of adolescent brain development in the rat may extend to as late as P60 (Odell 1990; Ojeda and Urbanski 1994). Upon arrival at our facility, animals were randomly assigned to one of four groups (n = 16) that differed on pretreatment drug (saline or SCH-23390) and treatment drug (saline or nicotine) administered. Our four groups consisted of (1) saline (pretreatment)–saline (treatment), (2) SCH-23390–saline, (3) saline–nicotine, and (4) SCH-23390–nicotine. Pretreatment drug was administered (SC) precisely 20 min prior to treatment drug administration (SC). Animals were dosed in their home-cage every other day from P28–P42, for a total of eight injection days within the 14-day period (Fig. 1). This pattern of repeated nicotine exposure has been previously shown to produce dendritic remodeling in the NAcc shell (Brown and Kolb 2001).

A second goal of this study was to determine whether adolescent nicotine-induced dendritic remodeling of NAcc Shell MSNs is present immediately following the cessation of nicotine exposure or not and to assess whether these changes are persistent into adulthood. To assess this, half of these animal subjects were killed for Golgi–Cox tissue staining 1-day following the cessation of drug administration (P43; early time-point; n = 32) (Fig. 1a), while the other half of these animal subjects were killed 3 weeks following the cessation of drug administration (P63; late time-point; n = 32) (Fig. 1b). Therefore, each pretreatment by treatment by time-point group contained eight animal subjects. All animals were used for subsequent neuroanatomical analyses.

Golgi–Cox tissue staining

On either P43 (early time-point) or P63 (late time-point), all animals were deeply anesthetized with a ketamine/xylazine cocktail injected intraperitoneally (IP) and perfused intracardially with 0.9 % NaCl. Brains were immediately extracted and placed into a Golgi–Cox solution, following the recipe of Glaser and Van der Loos (1981), to allow for fixation and impregnation of tissue. After 2 days, brains were placed into fresh Golgi–Cox solution for an additional 12 days. Following Golgi–Cox immersion, brains were stored in a 30 % sucrose solution until vibratome sectioning (200 μm sections). Sections containing the entire rostral–caudal extent of the NAcc shell were placed onto gelatinized slides. Sections were then stained using the protocol of Gibb and Kolb (1998). Briefly, sections

Fig. 2 Location of NAcc shell and MSNs. **a** Brain atlas images representing the rostral to caudal extent of the NAcc shell (*gray highlight*) from which MSNs were sampled (adapted from Paxinos and Watson 2007). Coordinates are relative to bregma. **b** Micrograph of Golgi–Cox stained MSNs image under 20× objective. *Scale bar* 120 µm. **c** Micrograph of a partially reconstructed MSN imaged under 60× objective and *colored* by branch order. *Scale bar* 25 µm
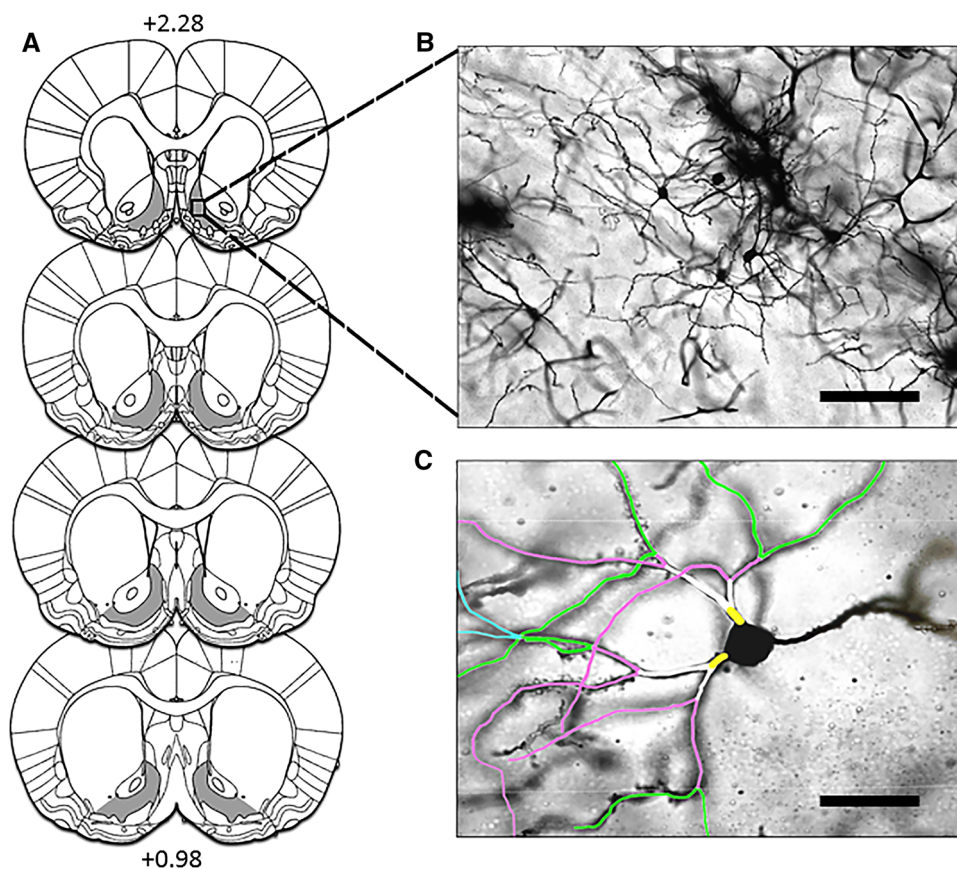
were alkalinized in ammonium hydroxide, developed and fixed using Kodak Rapid Fix, dehydrated through a series of ethanols, and cleared in a solution of 1/3 xylene, 1/3 100 % alcohol, and 1/3 chloroform. Slides were then cover-slipped and stored in the dark for the remainder of the experiment.

Analysis of dendritic structure and spine density

Golgi–Cox impregnated sections containing NAcc shell MSNs were visualized using light-microscopy and MSNs were manually reconstructed in 3 dimensions (3D) using Neurolucida software (Microbrightfield Biosciences, Williston, VT, USA) under 60× objective by experimenters blind to treatment groups. NAcc shell MSNs were selected based on the anatomical boundaries of the NAcc shell as defined in Paxinos and Watson (2007) (Fig. 2a) and identified by soma size (approx. 10–20 µm diameter), multi-polar shape with the presence of three or more primary dendrites attached to the soma, and the presence of dendritic spines on late branch orders (≥3rd order). Only well-stained MSNs that possessed dendrites unobstructed by neighboring cells or blood vessels and that could be followed from soma to terminal tip without interruption were chosen for reconstruction (Fig. 2b, c). 4–6 MSNs

were reconstructed from each animal and were sampled equally between hemispheres within each animal, for a total of 319 reconstructed neurons (early time-point, $n = 168$; late time-point, $n = 151$). Representative MSN reconstructions are presented in Fig. 3. Additionally, spine density was assessed on six neurons per animal, sampled equally between hemispheres, by manual labeling of spines on a reconstructed 40–50 µm segment of a distal branch (≥3rd order branch, at least 60 µm from the soma) ending in a terminal tip (Brown and Kolb 2001). This technique ensures that the diameter of each segment chosen for analysis of spine density is similar and places analysis of spine density at the same location of the dendritic arbor at which nicotine-induced dendritic remodeling occurs on MSNs (McDonald et al. 2005, 2007). Due to limitations of the Golgi–Cox stain, spine type was not assessed in the current study.

Morphometrics on reconstructed MSNs were obtained using Neuroexplorer (Microbrightfield Biosciences, Williston, VT, USA). Morphometric parameters obtained included total dendritic length, total number of bifurcations, and branch-order analysis (centrifugal method; total dendritic length, total number of branches, and average length as a function of branch order). Spine density was assessed as the total number of spines/length of dendritic
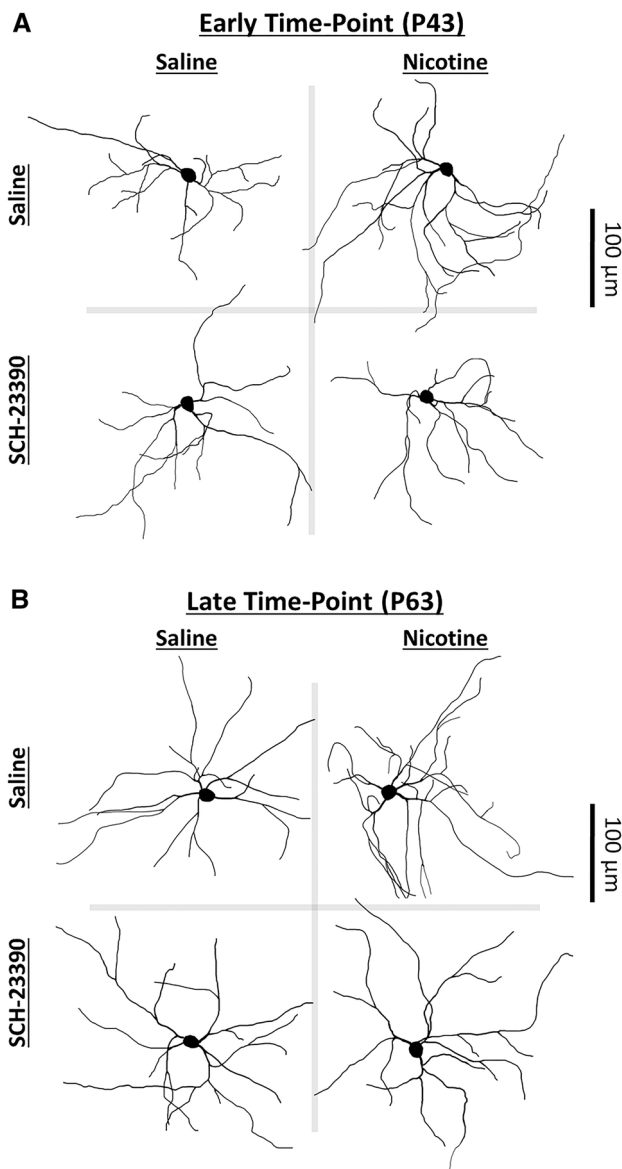
Fig. 3 Representative MSN reconstructions. *y*-axes, pretreatment drug. *x*-axes, treatment drug. All MSNs are within group means (±SEM) for both total length and bifurcations. **a** MSNs from animals killed at 1 day after dosing (early time-point; P43). **b** MSNs from animals killed at 21 days after dosing (late time-point; P63)

segment reconstructed. Finally, the distribution of dendritic material was assessed using 3D Sholl analyses (20 μm increments) with parameters of dendritic length and number of bifurcations.

Data analysis

Prior to statistical analyses, a mean value per animal was obtained for each morphometric parameter based on the 4–6 neurons reconstructed from each subject and for spine density based on the six dendritic segments reconstructed from each subject. For each morphometric parameter, mixed-ANOVA with within-subject factor of distance from the soma (Sholl analyses) or branch-order (branch-order analyses), and between-subject factors of time-point (early vs. late), pretreatment drug (saline vs. SCH-23390), and treatment drug (saline vs. nicotine) were conducted. Following a significant interaction, separate mixed-ANOVAs were conducted within the early and late time-point groups followed by comparisons to test the following hypotheses: (1) nicotine administered alone will induce dendritic-remodeling (saline–saline vs. saline–nicotine), (2) SCH-23390 co-administered with nicotine will prevent nicotine-induced dendritic remodeling (saline–nicotine vs. SCH-23390–nicotine), and (3) SCH-23390 alone will not induce dendritic remodeling (saline–saline vs. SCH-23390–saline). Additionally, we assessed developmental changes in morphometric parameters by comparing early vs. late time-point in each pretreatment-treatment group. Violation of the assumption of sphericity for repeated measures was corrected using the Greenhouse–Geisser correction for degrees of freedom (superscripted letter "a" proceeding an *F* value indicates Greenhouse–Geisser-corrected value for degrees of freedom). To further assess the distances from the soma at which the greatest dendritic remodeling occurs, comparisons between groups were made at specific radii from the soma (*t* test) with significance determined as $p < 0.05$ at consecutive radii (Bergstrom et al. 2010; Ehlinger et al. 2012).

For spine density, three-way ANOVA with between-subjects factors of time-point (early vs. late), pretreatment drug (saline vs. SCH-23390), and treatment drug (saline vs. nicotine) were conducted. Following significant interactions, separate two-way ANOVAs were conducted within the early and extended abstinence groups, followed by comparisons (*t* test, *Bonferroni* correction) to test the previous hypotheses.

Results

Nicotine-induced dendritic remodeling of MSNs

Statistical analyses revealed a significant interaction between radius, pretreatment drug, and treatment drug on both dendritic length ($^{a}F_{(2.9,160.9)} = 8.2$, $p < 0.001$) and bifurcations ($^{a}F_{(4.6,258.4)} = 6.1$, $p < 0.001$). Statistical analyses also revealed an interaction between treatment and time-point on dendritic length ($F_{(1,56)} = 5.5$, $p < 0.05$), and a trend toward an interaction between radius, treatment drug, and time-point on bifurcations ($^{a}F_{(2.9,160.9)} = 2.5$, $p = 0.06$).

For animals co-administered saline (pretreatment) during adolescent nicotine treatment, nicotine significantly induced dendritic remodeling of MSNs, as MSNs from saline–
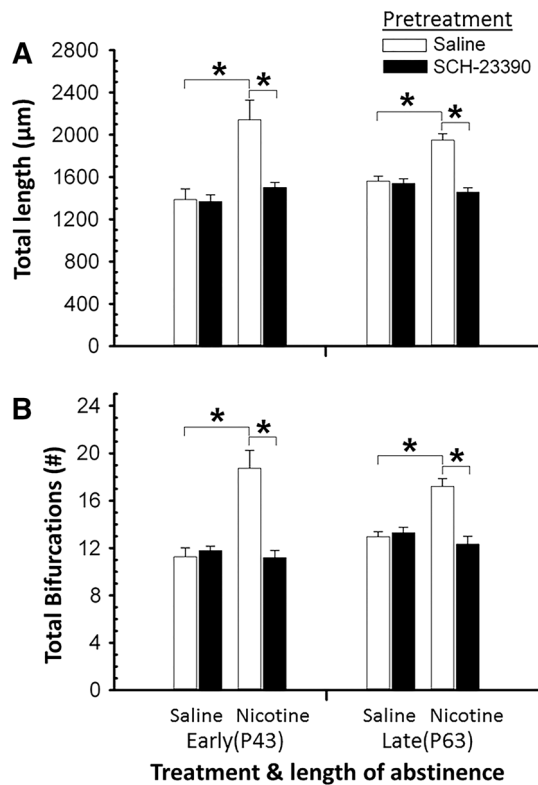
Fig. 4 Influence of adolescent nicotine and co-administered D1DR antagonist on dendritic morphology. **a** Mean (±SEM) total dendritic length of MSNs from early time-point and late time-point animals. **b** Mean (±SEM) total number of bifurcations of MSNs from early time-point and late time-point animals. *$p < 0.01$

nicotine animals display increased total dendritic length when measured at both P43 ($F_{(1,14)} = 12.5$, $p < 0.01$) and P63 ($F_{(1,14)} = 25.4$, $p < 0.001$) compared to saline–saline (control) animals (Fig. 4a). These nicotine-induced increases in total dendritic length were accompanied by a significant nicotine-induced increase in the total number of bifurcations when measured at both P43 ($F_{(1,14)} = 20.0$, $p < 0.01$) and P63 ($F_{(1,14)} = 30.0$, $p < 0.001$) (Fig. 4b), suggesting that the reported increases in total dendritic length are the result of new branch formation and that this dendritic remodeling is persistent for at least 21 days following nicotine exposure. Specifically, Sholl analyses indicated that nicotine-induced dendritic remodeling occurred at radii distal to the soma. At P43, significantly increased dendritic length of MSNs from saline–nicotine animals is present at radii between 40 and 240 μm from the soma ([a]$F_{(1.8,25.3)} = 8.1$, $p < 0.01$) (Fig. 5a) and bifurcations at radii between 40 and 160 μm from the soma ([a]$F_{(3.5,49.4)} = 5.2$, $p < 0.01$) (Fig. 5b). At P63, significantly increased dendritic length is still present at radii between 40 and 140 μm from the soma ([a]$F_{(3.7,51.8)} = 6.9$, $p < 0.001$) (Fig. 5c) and bifurcations at radii between 80 and 100 μm from the soma ([a]$F_{(3.3,45.4)} = 2.8$, $p < 0.05$) (Fig. 5d).

Influence of D1DRs on nicotine-induced dendritic remodeling of MSNs

For animals co-administered with nicotine and SCH-23390 during adolescence, nicotine failed to induce the previously observed dendritic remodeling of MSNs when compared to animals pretreated with saline during nicotine treatment, as MSNs from SCH-23390–nicotine animals display significantly reduced total dendritic length measured at both P43 ($F_{(1,14)} = 11.0$, $p < 0.01$) and P63 ($F_{(1,14)} = 43.9$, $p < 0.001$) (Fig. 4a), as well as significantly reduced total number of bifurcations measured at both P43 ($F_{(1,14)} = 21.8$, $p < 0.001$) and P63 ($F_{(1,14)} = 27.3$, $p < 0.001$) (Fig. 4b), compared to those from saline–nicotine animals. Furthermore, MSNs from SCH-23390–nicotine animals display no difference to those from saline–saline (control) animals. Sholl analyses suggest that these reductions in nicotine-induced dendritic remodeling of MSNs occur at radii distal to the soma. At P43, reduced dendritic length in MSNs of SCH-23390–nicotine animals compared to saline–nicotine animals is exhibited at radii between 40 and 180 μm from the soma ([a]$F_{(2.0,28.3)} = 6.0$, $p < 0.01$) (Fig. 5a), and reduced number of bifurcations at radii between 40 and 100 μm from the soma ([a]$F_{(3.8,53.1)} = 6.9$, $p < 0.001$) (Fig. 5b). At P63, reduced dendritic length is exhibited at radii between 40 and 160 μm from the soma ([a]$F_{(2.6,36.3)} = 9.5$, $p < 0.001$) (Fig. 5c), and reduced number of bifurcations at radii between 80 and 100 μm from the soma ([a]$F_{(3.8,52.6)} = 3.1$, $p < 0.05$) (Fig. 5d). Finally, no difference in total dendritic length or total number of bifurcations was observed between MSNs from SCH-23390–saline and saline–saline (control) animals, nor were there any differences in MSN morphology at any radial distance from the soma between these two groups, indicating that D1DR antagonism prevents nicotine-induced formation of new dendritic branches rather than producing a general reduction in dendritic length or bifurcations.

Between P43 and P63, significantly increased dendritic length was observed at radii distal to the soma in saline–saline and SCH-23390–saline animals, between 100 and 120 μm from the soma in saline–saline animals ([a]$F_{(2.9,41.0)} = 3.6$, $p < 0.05$) and 80 and 100 μm from the soma in SCH-23390–saline animals ([a]$F_{(3.2,45.0)} = 2.7$, $p = 0.05$). These increases in dendritic length are accompanied by a trend toward increased total bifurcations in saline–saline animals ($F_{(1,14)} = 4.0$, $p = 0.064$), and a significant increase in total bifurcations in SCH-23390 animals ($F_{(1,14)} = 6.9$, $p < 0.05$), although not dependent on radial distance from the soma (Figs. 4, 5). Collectively, this suggests an increase in the formation of new dendritic branches in saline treatment (control) animals between P43 and P63. No differences in dendritic length or bifurcations

Fig. 5 Influence of chronic nicotine and co-administered D1DR antagonist on dendritic morphology as a function of distance from the soma. Total dendritic length (mean ± SEM) (**a**, **c**) and number of bifurcations (mean ± SEM) (**b**, **d**) within each 20 μm spherical radius from the soma, in early time-point (**a**, **b**) and late time-point (**c**, **d**) animals. *Significant difference between saline–nicotine group and all other pretreatment-treatment groups ($p < 0.05$ at consecutive radii)
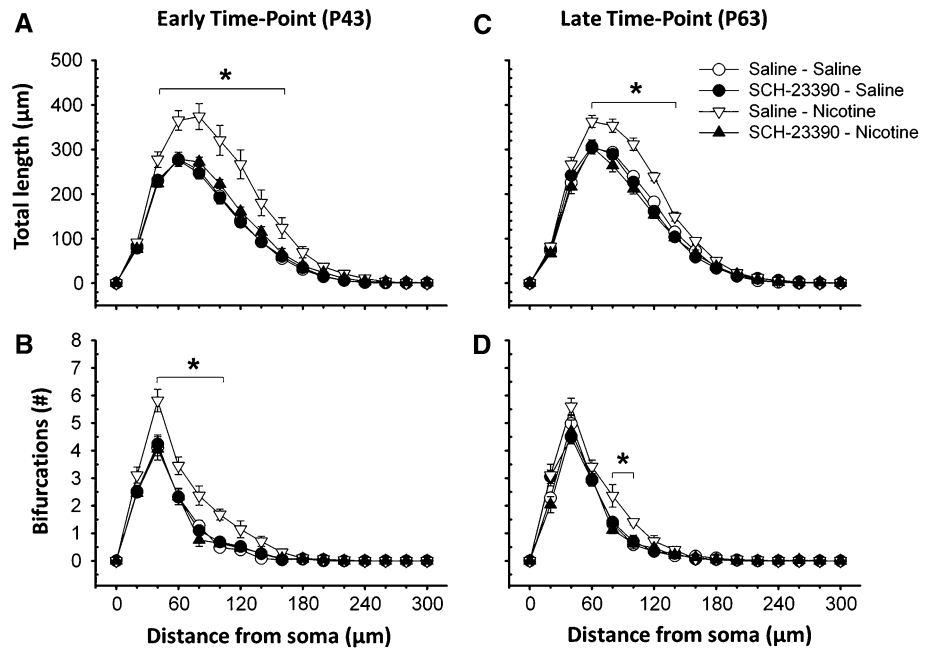

Table 1 Changes in dendritic morphology and spine density through withdrawal (P63–P43)

Data based on branch-order and spine density analyses. All values reflect mean differences between P43 and P63 ± SED.
^ $p < 0.07$, * $p < 0.05$,
** $p < 0.01$

| Parameter	Pretreatment–treatment			
	Saline–saline	SCH23390–saline	Saline–nicotine	SCH-23390–nicotine
Branch order analyses				
Total length (μm)	174.4 ± 111.9	169.7 ± 76.1^	−191.7 ± 231.6	−45.9 ± 62.2
First order	−39.2 ± 27.4	−45.9 ± 19.4*	−43.0 ± 23.4	−53.8 ± 22.2*
Second order	27.3 ± 49.2	67.5 ± 41.1	−4.4 ± 46.6	−67.7 ± 52.2
≥Third order	344.0 ± 137.2*	170.8 ± 70.1*	−144.4 ± 196.7	75.6 ± 85.6
Branches (#)	2.8 ± 1.7	2.9 ± 1.3*	−3.6 ± 3.3	1.8 ± 1.8
First order	−0.58 ± .19**	−0.49 ± 0.31	−0.59 ± 0.26*	−0.51 ± 0.17**
Second order	0.25 ± 0.43	0.40 ± 0.5	0.17 ± 0.47	−0.11 ± 0.41
≥Third order	3.2 ± 1.6^	3.0 ± 1.2*	2.9 ± 3.0	2.4 ± 1.7
Average length (μm)				
First order	−6.4 ± 4.8	−8.0 ± 3.3*	−6.3 ± 4.1	−8.7 ± 5.0
Second order	−1.5 ± 2.6	4.3 ± 3.8	−1.1 ± 4.4	−7.5 ± 5.4
≥Third order	18.8 ± 12.9	13.3 ± 13.5	17.8 ± 19.1	−9.4 ± 10.0
Spine density (#/10 μm)	0.09 ± 0.3	0.42 ± 0.29	−0.72 ± 0.42	−1.3 ± 0.35**

between P43 and P63 were observed in nicotine treatment groups. Additional differences in dendritic morphology and spine density within pretreatment-treatment groups between P43 and P63 are reported in Table 1.

Nicotine-induced alterations in spine density and influence of D1DRs

Representative micrographs of MSN dendritic segments containing dendritic spines are presented for early (P43) (Fig. 6a, left) and late (P63) (Fig. 6b, left) time-point groups. Statistical analyses revealed a significant interaction between treatment drug and time-point on spine density ($F_{(1,56)} = 13.1$, $p < 0.01$), with no main effects nor interactions related to pretreatment drug. When measured at P43, nicotine treatment induced a significant increase in spine density on MSNs ($F_{(1,28)} = 45.2$, $p < 0.001$). However, in contrast to findings on dendritic remodeling, spine density was increased on MSNs from both saline–nicotine ($F_{(1,14)} = 25.4$, $p < 0.001$) and SCH-23390–nicotine ($F_{(1,14)} = 15.0$, $p < 0.01$) animals compared to saline–saline (control) animals (Fig. 6a, right). Further, there was

Fig. 6 Influence of chronic nicotine and co-administered D1DR antagonist on spine density. *Left* representative micrographs of distal dendritic segments of MSNs from early time point (**a**) and late time-point (**b**) animals. Dendritic segments are within group means ± SEM for spine density. *Right* mean (±SEM) spine density of dendritic segments from early time-point (**a**) and late time-point (**b**) animals. *Significant difference between both nicotine (treatment) groups and control ($p < 0.01$)
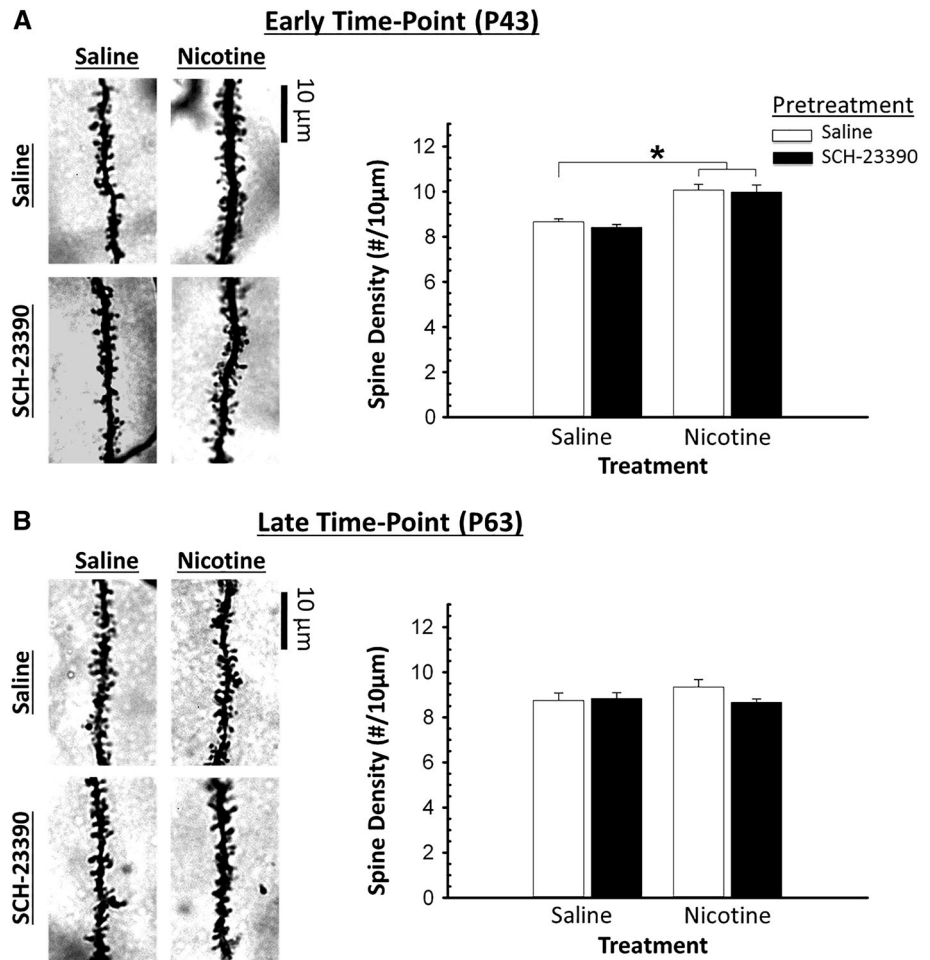
no observable difference in spine density between SCH-23390–saline and saline–saline (control) animals (Fig. 6a, b). Collectively, these results suggest that SCH-23390 does not influence spine density in nicotine or saline treated animals. Furthermore, nicotine's influence on spine density does not persist through 21 days post-nicotine exposure, as no observable differences in spine density are revealed at P63 between any of the pretreatment and treatment groups (Fig. 6b, right).

Discussion

This study aimed to answer two major questions regarding adolescent-nicotine induced dendritic remodeling in the NAcc shell. First, we sought to determine whether nicotine-induced dendrite plasticity is dependent on DA signaling at D1-type DA receptors and second, we sought to characterize the developmental trajectory of adolescent nicotine-induced dendritic remodeling at an early and late-time point following chronic nicotine.

Adolescent nicotine rapidly induces dendritic remodeling and increases spine density of NAcc shell MSNs

While the finding that chronic nicotine exposure results in the formation of new dendritic branches and increased spine density on MSNs in the NAcc shell is consistent with previous literature (Brown and Kolb 2001; McDonald et al. 2005, 2007), here we provide the first evidence of increased branch formation at an early time-point after dosing and increased spine density following adolescent exposure. Immediately following 2 weeks of chronic adolescent nicotine, a substantial (54 %) increase in total dendritic material was observed over a large portion of the distal dendritic arbor and was accompanied by an increase in spine density on distal dendritic branches. Therefore, our results suggest that this form of structural plasticity in NAcc shell MSNs is likely a direct result of nicotine exposure rather than an effect that emerges following cessation of exposure. These alterations to dendritic structure and spine density can be expected to substantially

increase the amount and/or change the pattern of synaptic contact onto these neurons (Stepanyants et al. 2002; Chklovskii 2004).

Importantly, the formation of new dendritic branches is persistent, as increased dendritic branching and total dendritic material are maintained for at least 21 days following cessation of nicotine exposure, supporting the hypothesis that nicotine-induced dendritic remodeling is a long-lasting form of neuroplasticity. Although the magnitude of increased total dendritic length (25 %) observed at this late time-point appears lower than that observed at the early time-point (54 %), it should be noted that this largely reflects continued growth of MSN dendritic length in control animals ($\sim$13 %) rather than a reduction in dendritic length of MSNs from nicotine-treated animals, consistent with the presence of continued neural development during adolescence (Ojeda and Urbanski 1994; Bergstrom et al. 2010; Koss et al. 2014). Previous research has demonstrated that adult nicotine exposure also induces dendrite remodeling in the nucleus accumbens shell when measured at remote time-points (Brown and Kolb 2001; McDonald et al. 2007), and although these alterations are of lower overall magnitude we would expect the overall trajectory of dendrite remodeling to follow a similar pattern as in the present study. However, the continued developmental pattern in NAcc MSNs might be an important factor mediating the enhanced vulnerability of the adolescent brain to nicotine-induced dendritic remodeling and other neurobehavioral effects of nicotine exposure (O'Dell 2009).

Our findings that the nicotine-induced formation of new dendritic branches is present at both early and late time-points suggest that this plasticity occurs during nicotine exposure itself and is persistent over an extended withdrawal period. Therefore, neurochemical alterations taking place during drug exposure are a likely cause for this long-lasting dendritic remodeling. Our data show that DA signaling at D1DRs is one specific neurochemical alteration occurring during nicotine exposure that is necessary for the persistent effect of nicotine exposure on dendrite morphology. It is unknown whether additional neurochemical mechanisms taking place during extended withdrawal are responsible for the maintenance of increased branching. However, our results clearly show that D1DR signaling occurring during nicotine exposure is necessary for the induction of persistent dendrite remodeling. Further research is needed to dissect the precise time-course of adolescent nicotine-induced dendritic remodeling and alteration in spine density, particularly the analysis of dendritic remodeling following different lengths of drug exposure and the neurochemical mechanisms responsible for the maintenance of dendrite remodeling over extended withdrawal.

While the nicotine-induced formation of new dendritic branches is persistent, the nicotine-induced increase in spine density returned to control levels by 21 days post-nicotine exposure. Previous research examining nicotine-induced alterations in spine density following extended withdrawal periods has yielded mixed results (Brown and Kolb 2001; Hamilton and Kolb 2005; Gipson et al. 2013) and could reflect differences related to the nicotine administration protocol, the age of the animal at the initiation of nicotine exposure, or the length of withdrawal or abstinence following nicotine dosing. However, despite a return of spine density to control levels in the present study we would still expect a persistent elevation in the total number of spines per MSN even at the late time-point, as total number of spines per neuron is a function of both spine density and the total amount of dendritic material (Feldman and Peters 1979). Furthermore, it is possible that differences in spine density observed at the 1- and 21-day time-points reflect an altered ratio of spine morphological subtypes. For example, it is possible that a greater quantity of thin/motile spines might be present at earlier time points following nicotine exposure, possibly reflecting immediate changes in circuit connectivity, while fewer but more stable mushroom or stubby spines might be present at later time points, likely reflecting increased stability in the circuit with the passage of time (Kasai et al. 2003). Study of spine morphology at multiple time-points following nicotine exposure represents an important area of future study. Collectively, the persistence of adolescent nicotine-induced formation of new dendritic branches reported in this study is consistent with an altered pattern of synaptic connectivity onto NAcc shell MSNs maintained into adulthood.

Adolescent-nicotine induced dendritic remodeling is D1DR-dependent

When the D1DR antagonist SCH-23390 was co-administered with nicotine, the length and branching of dendritic arbors of NAcc shell MSNs was equivalent to those from controls. There was also no change in MSNs from animals receiving SCH-23390 alone, suggesting that D1DR antagonism prevents nicotine-induced dendrite remodeling rather than producing a general reduction in dendritic branching. Finally, inhibition of nicotine-induced dendritic remodeling was present at both early and late time-points, suggesting that blocking D1DRs during chronic adolescent nicotine exposure produces a persistent blockade of nicotine-induced dendritic remodeling. Collectively, these results suggest that nicotine-induced DA signaling at D1DRs during the time of drug exposure is necessary for long-lasting dendritic remodeling in the NAcc shell. As drug-induced dendritic remodeling is believed to be an important correlate of long-term behavioral alterations

following exposure to drugs of abuse (Robinson and Kolb 2004), we suggest that nicotine-induced dendritic remodeling might best correlate with neurobehavioral indices of nicotine-exposure that are both long-lasting and specifically D1DR dependent.

Potential mechanisms of D1DR-dependent dendrite remodeling

The specific mechanism downstream of D1DR activation that induces dendritic remodeling is unknown. Consistent with the present results, Ren et al. (2010) have shown that cocaine-induced dendritic remodeling in the NAcc is dependent on D1DR and ERK activation, suggesting that intracellular mechanisms similar to those in response to cocaine exposure may underlie the effects of nicotine despite the significantly different pharmacological action of these two substances. Given that the dendritic cytoskeleton is quite stable during the adolescent time-period (Koleske 2013) intracellular signaling or transcription pathways downstream of the D1DR that can reorganize dendritic structure may be particularly important. Through positive coupling of the D1DR with the cAMP–PKA pathway, nicotine up-regulates numerous intracellular signaling and transcription pathways in the NAcc, including CREB (McCarthy et al. 2012), ERK (Valjent et al. 2005), ARC (Schiltz et al. 2005), deltaFosB (Marttila et al. 2006; Soderstrom et al. 2007), and BDNF expression (Perna and Brown 2013). Importantly, each of these intracellular mechanisms has the potential to regulate dendritic plasticity (Redmond et al. 2002; Wayman et al. 2006; Schubert and Dotti 2007; Urbanska et al. 2008; Pitchers et al. 2013) and are also up-regulated following exposure to other drugs of abuse that induce dendritic remodeling (Nestler 2001, 2004; Robinson and Kolb 2004).

Several of the intracellular pathways downstream of D1DR signaling are up-regulated to a greater extent following adolescent nicotine-exposure compared to adult exposure (Schochet et al. 2005; Shram et al. 2007). Further, adolescence represents an important time period in the development of the dopaminergic system in the rat, characterized by increasing D1DR expression and altered D1DR function when compared to adulthood (Huppé-Gourgues and O'donnell 2012; Garske et al. 2013). Although the observed increase in dendritic branching at a late time-point in the present study is similar to findings in adult exposed animals, whether D1DR-signaling uniquely influences magnitude, maintenance or trajectory of adolescent versus adult dendrite remodeling is unknown and represents a valuable avenue for future study. Collectively, our results highlight the need to examine pathways downstream of drug-induced D1DR activation that specifically induce dendritic remodeling and whether the effects of nicotine-induced neuroplasticity via DA signaling at D1DRs are dependent on age of exposure.

It is important to note that in the NAcc, MSNs are largely segregated into primarily D1DR containing and primarily D2DR containing neurons (Lu et al. 1998; Aubert et al. 2000), and it is possible that the pattern or presence of nicotine-induced dendritic remodeling is different for these two cell populations. In the present study, we were unable to separate these two cell types using the Golgi–Cox stain, and our results likely reflect a random sampling from both cell types. While nicotine-induced dendritic remodeling has yet to be compared between D1DR versus D2DR MSNs, cocaine-induced dendritic remodeling appears in both subtypes (Li et al. 2012). However, Lobo et al. (2013) recently showed that increased deltaFosB following chronic cocaine is present in only D1DR MSNs, and Lee et al. (2006) report that cocaine-induced increases in dendritic spine density is maintained through an extended abstinent period only in D1DR MSNs. These findings suggest that for some neuroadaptations psychostimulant drug exposure may preferentially affect one MSN subtype over the other. Therefore, the specific pattern of drug-induced structural plasticity in the NAcc across MSN subtypes and the potential influence of D2DR signaling on drug-induced dendrite remodeling are intriguing areas for future study.

Adolescent nicotine-induced increases in spine density are not D1DR-dependent

In the present study, while co-administration of the D1DR antagonist SCH-23390 prevented nicotine-induced increases in dendritic branching in the NAcc shell, the antagonist failed to block nicotine-induced increases in spine density that were observed at the early time-point. Therefore, while increased DA signaling at D1DRs during chronic adolescent nicotine exposure is necessary for the formation of new dendritic branches, non-D1DR mediated mechanisms may underlie the nicotine-induced increase in spine density in the NAcc shell. Although this has yet to be examined in relation to nicotine, our results are in contrast to a number of recent studies linking intracellular pathways downstream of D1DR activation with cocaine-induced increases in spine density (Lee et al. 2006; Maze and Russo 2010; Ren et al. 2010; Grueter et al. 2013). This suggests that despite general similarities between nicotine and cocaine in their ability to alter dopaminergic function in the NAcc (Pierce and Kumaresan 2006), differences between these two drugs in their specific mechanism of dopamine release, D1DR activation, or drug-induced activity at non-D1DR synapses may contribute to how each drug specifically alters dendritic spine density in the NAcc shell.

Dendritic branch and spine cytoskeletal structure

The observed differences in the influence of D1DRs on nicotine-induced dendritic remodeling versus alteration in spine density likely reflect differences between two uniquely structured neuronal components that differ substantially in both cytoskeletal and synaptic make-up. It is likely that both intracellular and extracellular signals mediating the formation of new dendritic spines differ from those that mediate formation of new dendritic branches (Harris and Kater 1994; Harris 1999). In particular, dendritic spines are unique functional compartments and the primary sites of excitatory synaptic contact, devoid of dendritic microtubules, and enriched in *F*-actin, which provides for both stability and plasticity of spine shape (Smith et al. 1998; Hering and Sheng 2003; Tada and Sheng 2006; Urbanska et al. 2012). In contrast, dendrites contain points of contact onto non-excitatory neurotransmitter receptors, including D1DRs (Hersch et al. 1995), and their shape or plasticity is likely maintained by networks of microtubules and alterations to microtubule-associated proteins (MAPs) (Sánchez et al. 2000; Georges et al. 2008; Urbanska et al. 2012). As the formation and remodeling of new dendritic spines and branches largely depend on the restructuring of these different cytoskeletal elements (Dillon and Goda 2005; Newey et al. 2005; Szebenyi et al. 2005; Penzes et al. 2009), it is possible that in the present study antagonism of the D1DR preferentially affected only those processes related to dendritic remodeling while leaving those responsible for spine formation unopposed. In support of this, previous studies have shown that alteration in spine density and *F*-actin stability can be regulated through activity at AMPA and NMDA-type glutamate receptors (Halpain et al. 1998; Hering and Sheng 2003; Passafaro et al. 2003; Ultanir et al. 2007), which were not blocked during nicotine exposure in the present study, and that D1DR activation can alter MAP expression and dendritic outgrowth (Reinoso et al. 1996; Song et al. 2002). While more research is needed to better identify the specific mechanisms regulating plasticity of dendritic spines versus the dendritic arbor, our results certainly highlight the importance of analyzing both spine density and dendritic remodeling at multiple time-points in future studies of drug-induced dendritic plasticity.

Conclusions

We provide the first evidence that adolescent nicotine-induced dendritic remodeling in the NAcc shell is dependent on nicotine-induced DA signaling at D1DRs. Through increased D1DR activation during nicotine exposure, there is a substantial increase in the formation of new, distally located dendritic branches and increased spine density on MSNs in the NAcc shell. The impact of nicotine on dendritic structure, but not spine density, is persistent as increased dendritic branching is still present following an extended (21-day) withdrawal period. Finally, the influence of nicotine-induced DA signaling at D1DRs on dendritic structure is specific to the formation of new dendritic branches, as nicotine-induced D1DR activation does not influence nicotine-induced increases in spine density. These results provide important insight into the specific neuropharmacological mechanisms that are responsible for drug-induced dendritic remodeling and support evidence that nicotine-induced dendrite remodeling is a long-lasting form of drug-induced neuroplasticity. Given that adolescence is a critical period of brain development, particularly of the dopaminergic system (Tarazi and Baldessarini 2000; Wahlstrom et al. 2010) and a time-period of enhanced vulnerability to the neurobehavioral effects of drug exposure (Baler and Volkow 2011; O'Dell 2009; Smith 2003), we suggest that a D1DR-dependent and long-lasting form of nicotine-induced neuroplasticity is an important neurobehavioral correlate of chronic adolescent nicotine exposure.

Conflict of interest The authors declare no conflict of interest.

References

Acquas E, Carboni E, Leone P, Di Chiara G (1989) SCH 23390 blocks drug-conditioned place-preference and place-aversion: anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? Psychopharmacology 99(2):151–155

Arikkath J (2012) Molecular mechanisms of dendrite morphogenesis. Front Cell Neurosci 6:61

Aubert I, Ghorayeb I, Normand E, Bloch B (2000) Phenotypical characterization of the neurons expressing the D1 and D2 dopamine receptors in the monkey striatum. J Comp Neurol 418(1):22–32

Baler RD, Volkow ND (2011) Addiction as a systems failure: focus on adolescence and smoking. J Am Acad Child Adolesc Psychiatry 50(4):329–339

Bergstrom HC, Smith RF, Mollinedo NS, McDonald CG (2010) Chronic nicotine exposure produces lateralized, age-dependent dendritic remodeling in the rodent basolateral amygdala. Synapse 64(10):754–764

Bracken AL, Chambers RA, Berg SA, Rodd ZA, McBride WJ (2011) Nicotine exposure during adolescence enhances behavioral sensitivity to nicotine during adulthood in Wistar rats. Pharmacol Biochem Behav 99(1):87–93

Brielmaier JM, McDonald CG, Smith RF (2007) Immediate and long-term behavioral effects of a single nicotine injection in adolescent and adult rats. Neurotoxicol Teratol 29(1):74–80

Brielmaier JM, McDonald CG, Smith RF (2008) Nicotine place preference in a biased conditioned place preference design. Pharmacol Biochem Behav 89(1):94–100

Brown RW, Kolb B (2001) Nicotine sensitization increases dendritic length and spine density in the nucleus accumbens and cingulate cortex. Brain Res 899(1–2):94–100

Chklovskii DB (2004) Synaptic connectivity and neuronal morphology: two sides of the same coin. Neuron 43(5):609–617

Corrigall WA, Coen KM (1991) Selective dopamine antagonists reduce nicotine self-administration. Psychopharmacology 104(2):171–176

Di Chiara G (2000) Role of dopamine in the behavioural actions of nicotine related to addiction. Eur J Pharmacol 393(1–3):295–314

Di Chiara G, Tanda G, Bassareo V, Pontieri F, Acquas E, Fenu S, Cadoni C, Carboni E (1999) Drug addiction as a disorder of associative learning. Role of nucleus accumbens shell/extended amygdala dopamine. Ann N Y Acad Sci 877:461–485

Dillon C, Goda Y (2005) The actin cytoskeleton: integrating form and function at the synapse. Annu Rev Neurosci 28:25–55

Ehlinger DG, Bergstrom HC, McDonald CG, Smith RF (2012) Nicotine-induced dendritic remodeling in the insular cortex. Neurosci Lett 516(1):89–93

Feldman ML, Peters A (1979) A technique for estimating total spine numbers on Golgi-impregnated dendrites. J Comp Neurol 188(4):527–542

Garske AK, Lawyer CR, Peterson BM, Illig KR (2013) Adolescent changes in dopamine D1 receptor expression in orbitofrontal cortex and piriform cortex accompany associative learning deficit. PLoS ONE 8(2):e56191

Georges PC, Hadzimichalis NM, Sweet ES, Firestein BL (2008) The yin-yang of dendrite morphology: unity of actin and microtubules. Mol Neurobiol 38(3):270–284

Gibb R, Kolb B (1998) A method for vibratome sectioning of Golgi–Cox stained whole rat brain. J Neurosci Methods 79(1):1–4

Gipson CD, Reissner KJ, Kupchik YM, Smith AC, Stankeviciute N, Hensley-Simon ME, Kalivas PW (2013) Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. Proc Natl Acad Sci USA 110(22):9124–9129

Glaser EM, Van der Loos H (1981) Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi–Nissl stain. J Neurosci Methods 4(2):117–125

Grueter BA, Robison AJ, Neve RL, Nestler EJ, Malenka RC (2013) ΔFosB differentially modulates nucleus accumbens direct and indirect pathway function. Proc Natl Acad Sci USA 110(5):1923–1928

Gulley JM, Juraska JM (2013) The effects of abused drugs on adolescent development of corticolimbic circuitry and behavior. Neuroscience 249:3–20

Halpain S, Hipolito A, Saffer L (1998) Regulation of F-actin stability in dendritic spines by glutamate receptors and calcineurin. J Neurosci 18(23):9835–9844

Hamilton DA, Kolb B (2005) Differential effects of nicotine and complex housing on subsequent experience-dependent structural plasticity in the nucleus accumbens. Behav Neurosci 119(2):355–365

Harris KM (1999) Structure, development, and plasticity of dendritic spines. Curr Opin Neurobiol 9(3):343–348

Harris KM, Kater SB (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. Annu Rev Neurosci 17:341–371

Hering H, Sheng M (2003) Activity-dependent redistribution and essential role of cortactin in dendritic spine morphogenesis. J Neurosci 23(37):11759–11769

Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK, Bolam JP, Ince E, Yi H, Levey AI (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15(7 Pt 2):5222–5237

Huppé-Gourgues F, O'donnell P (2012) D1-NMDA receptor interactions in the rat nucleus accumbens change during adolescence. Synapse 66(7):584–591

Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure–stability–function relationships of dendritic spines. Trends Neurosci 26(7):360–368

Koleske AJ (2013) Molecular mechanisms of dendrite stability. Nat Rev Neurosci 14(8):536–550

Koss WA, Belden CE, Hristov AD, Juraska JM (2014) Dendritic remodeling in the adolescent medial prefrontal cortex and basolateral amygdala of male and female rats. Synapse 68(2):61–72

Lachowicz JE, Sibley DR (1997) Molecular characteristics of mammalian dopamine receptors. Pharmacol Toxicol 81(3):105–113

Lee KW, Kim Y, Kim AM, Helmin K, Nairn AC, Greengard P (2006) Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. Proc Natl Acad Sci USA 103(9):3399–3404

Li J, Liu N, Lu K, Zhang L, Gu J, Guo F, An S, Zhang L, Zhang L (2012) Cocaine-induced dendritic remodeling occurs in both D1 and D2 dopamine receptor-expressing neurons in the nucleus accumbens. Neurosci Lett 517(2):118–122

Lobo MK, Nestler EJ (2011) The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. Front Neuroanat 5:41

Lobo MK, Zaman S, Damez-Werno DM, Koo JW, Bagot RC, DiNieri JA, Nugent A, Finkel E, Chaudhury D, Chandra R, Riberio E, Rabkin J, Mouzon E, Cachope R, Cheer JF, Han MH, Dietz DM, Self DW, Hurd YL, Vialou V, Nestler EJ (2013) ΔFosB induction in striatal medium spiny neuron subtypes in response to chronic pharmacological, emotional, and optogenetic stimuli. J Neurosci 33(47):18381–18395

Lu XY, Ghasemzadeh MB, Kalivas PW (1998) Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. Neuroscience 82(3):767–780

Marttila K, Raattamaa H, Ahtee L (2006) Effects of chronic nicotine administration and its withdrawal on striatal FosB/DeltaFosB and c-Fos expression in rats and mice. Neuropharmacology 51(1):44–51

Maze I, Russo SJ (2010) Transcriptional mechanisms: underlying addiction-related structural plasticity. Mol Interv 10(4):219–230

McCarthy MJ, Duchemin AM, Neff NH, Hadjiconstantinou M (2012) CREB involvement in the regulation of striatal prodynorphin by nicotine. Psychopharmacology 221(1):143–153

McDonald CG, Dailey VK, Bergstrom HC, Wheeler TL, Eppolito AK, Smith LN, Smith RF (2005) Periadolescent nicotine administration produces enduring changes in dendritic morphology of medium spiny neurons from nucleus accumbens. Neurosci Lett 385(2):163–167

McDonald CG, Eppolito AK, Brielmaier JM, Smith LN, Bergstrom HC, Lawhead MR, Smith RF (2007) Evidence for elevated nicotine-induced structural plasticity in nucleus accumbens of adolescent rats. Brain Res 1151:211–218

National Institute on Drug Abuse (2012) Research report series: tobacco addiction. Department of Health and Human Services (US), Washington (NIH publication no. 12-4342)

Nestler EJ (2001) Molecular neurobiology of addiction. Am J Addict 10(3):201–217

Nestler EJ (2004) Molecular mechanisms of drug addiction. Neuropharmacology 47(Suppl 1):24–32

Nestler EJ (2013) Cellular basis of memory for addiction. Dialogues Clin Neurosci 15(4):431–443

Newey SE, Velamoor V, Govek EE, Van Aelst L (2005) Rho GTPases, dendritic structure, and mental retardation. J Neurobiol 64(1):58–74

O'Dell LE (2009) A psychobiological framework of the substrates that mediate nicotine use during adolescence. Neuropharmacology 56(Suppl 1):263–278

Odell WD (1990) Sexual maturation in the rat. In: Grumbach MM, Sizonenko PC, Aubert ML (eds) Control of the onset of puberty. Williams and Wilkins, Baltimore, pp 183–210

Ojeda SR, Urbanski HF (1994) Puberty in the rat. In: Knobil E, Neill JD (eds) The physiology of reproduction, 2nd edn. Raven Press, New York, pp 363–409

Passafaro M, Nakagawa T, Sala C, Sheng M (2003) Induction of dendritic spines by an extracellular domain of AMPA receptor subunit GluR2. Nature 424(6949):677–681

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates, 6th edn. Academic Press/Elsevier, Amsterdam/Boston

Penzes P, Srivastava DP, Woolfrey KM (2009) Not just actin? A role for dynamic microtubules in dendritic spines. Neuron 61(1):3–5

Perna MK, Brown RW (2013) Adolescent nicotine sensitization and effects of nicotine on accumbal dopamine release in a rodent model of increased dopamine D2 receptor sensitivity. Behav Brain Res 242:102–109

Picciotto MR (2003) Nicotine as a modulator of behavior: beyond the inverted U. Trends Pharmacol Sci 24(9):493–499

Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux JM (1998) Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 391(6663):173–177

Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev 30(2):215–238

Pitchers KK, Vialou V, Nestler EJ, Laviolette SR, Lehman MN, Coolen LM (2013) Natural and drug rewards act on common neural plasticity mechanisms with ΔFosB as a key mediator. J Neurosci 33(8):3434–3442

Redmond L, Kashani AH, Ghosh A (2002) Calcium regulation of dendritic growth via CaM kinase IV and CREB-mediated transcription. Neuron 34(6):999–1010

Reinoso BS, Undie AS, Levitt P (1996) Dopamine receptors mediate differential morphological effects on cerebral cortical neurons in vitro. J Neurosci Res 43(4):439–453

Ren Z, Sun WL, Jiao H, Zhang D, Kong H, Wang X, Xu M (2010) Dopamine D1 and N-methyl-D-aspartate receptors and extracellular signal-regulated kinase mediate neuronal morphological changes induced by repeated cocaine administration. Neuroscience 168(1):48–60

Robinson TE, Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11(5):1598–1604

Robinson TE, Kolb B (2004) Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology 47 Suppl 1:33–46

Sánchez C, Díaz-Nido J, Avila J (2000) Phosphorylation of microtubule-associated protein 2 (MAP2) and its relevance for the regulation of the neuronal cytoskeleton function. Prog Neurobiol 61(2):133–168

Schiltz CA, Kelley AE, Landry CF (2005) Contextual cues associated with nicotine administration increase arc mRNA expression in corticolimbic areas of the rat brain. Eur J Neurosci 21(6):1703–1711

Schochet TL, Kelley AE, Landry CF (2005) Differential expression of arc mRNA and other plasticity-related genes induced by nicotine in adolescent rat forebrain. Neuroscience 135(1):285–297

Schubert V, Dotti CG (2007) Transmitting on actin: synaptic control of dendritic architecture. J Cell Sci 120(Pt 2):205–212

Self DW (2004) Regulation of drug-taking and -seeking behaviors by neuroadaptations in the mesolimbic dopamine system. Neuropharmacology 47(Suppl 1):242–255

Shram MJ, Funk D, Li Z, Lê AD (2007) Acute nicotine enhances c-fos mRNA expression differentially in reward-related substrates of adolescent and adult rat brain. Neurosci Lett 418(3):286–291

Smith RF (2003) Animal models of periadolescent substance abuse. Neurotoxicol Teratol 25(3):291–301

Smith Y, Bevan MD, Shink E, Bolam JP (1998) Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 86(2):353–387

Soderstrom K, Qin W, Williams H, Taylor DA, McMillen BA (2007) Nicotine increases FosB expression within a subset of reward- and memory-related brain regions during both peri- and post-adolescence. Psychopharmacology 191(4):891–897

Song ZM, Undie AS, Koh PO, Fang YY, Zhang L, Dracheva S, Sealfon SC, Lidow MS (2002) D1 dopamine receptor regulation of microtubule-associated protein-2 phosphorylation in developing cerebral cortical neurons. J Neurosci 22(14):6092–6105

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24(4):417–463

Spear LP, Brake SC (1983) Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 16(2):83–109

Spina L, Fenu S, Longoni R, Rivas E, Di Chiara G (2006) Nicotine-conditioned single-trial place preference: selective role of nucleus accumbens shell dopamine D1 receptors in acquisition. Psychopharmacology 184(3–4):447–455

Stepanyants A, Hof PR, Chklovskii DB (2002) Geometry and structural plasticity of synaptic connectivity. Neuron 34(2):275–288

Szebenyi G, Bollati F, Bisbal M, Sheridan S, Faas L, Wray R, Haferkamp S, Nguyen S, Caceres A, Brady ST (2005) Activity-driven dendritic remodeling requires microtubule-associated protein 1A. Curr Biol 15(20):1820–1826

Tada T, Sheng M (2006) Molecular mechanisms of dendritic spine morphogenesis. Curr Opin Neurobiol 16(1):95–101

Tarazi FI, Baldessarini RJ (2000) Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. Int J Dev Neurosci 18(1):29–37

Ultanir SK, Kim JE, Hall BJ, Deerinck T, Ellisman M, Ghosh A (2007) Regulation of spine morphology and spine density by NMDA receptor signaling in vivo. Proc Natl Acad Sci USA 104(49):19553–19558

Urbanska M, Blazejczyk M, Jaworski J (2008) Molecular basis of dendritic arborization. Acta Neurobiol Exp (Wars) 68(2):264–288

Urbanska M, Swiech L, Jaworski J (2012) Developmental plasticity of the dendritic compartment: focus on the cytoskeleton. Adv Exp Med Biol 970:265–284

Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, Greengard P, Hervé D, Girault JA (2005) Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc Natl Acad Sci USA 102(2):491–496

Wahlstrom D, White T, Luciana M (2010) Neurobehavioral evidence for changes in dopamine system activity during adolescence. Neurosci Biobehav Rev 34(5):631–648

Wayman GA, Impey S, Marks D, Saneyoshi T, Grant WF, Derkach V, Soderling TR (2006) Activity-dependent dendritic arborization mediated by CaM-kinase I activation and enhanced CREB-dependent transcription of Wnt-2. Neuron 50(6):897–909

Yuste R (2011) Dendritic spines and distributed circuits. Neuron 71(5):772–781

Zarrindast MR, Sadegh M, Shafaghi B (1996) Effects of nicotine on memory retrieval in mice. Eur J Pharmacol 295(1):1–6

Zhou FM, Liang Y, Dani JA (2001) Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 4(12):1224–1229