Chronic Nicotine Exposure Produces Lateralized, Age-Dependent Dendritic Remodeling in the Rodent Basolateral Amygdala

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KEY WORDS dendrites; prefrontal cortex; infralimbic; adolescent; cerebral hemispheres; functional laterality; Sprague–Dawley rats; development

ABSTRACT This study investigated the dendritic morphology of neurons located in the right and left basolateral amygdala (BLA) and infralimbic (IL) cortex following chronic nicotine exposure during adolescence or adulthood. Sprague–Dawley rats were administered subcutaneous injections of nicotine (0.5 mg/kg; free base) or saline three times per week for 2 weeks (six total injections). The dose period began on either postnatal day (P) 32 (adolescent) or P61 (adult). Twenty days following the end of dosing, brains were processed for Golgi-Cox staining, and dendrites from principal neurons in the BLA and pyramidal neurons in the IL were digitally reconstructed in three dimensions. Morphometric analysis revealed a contrasting pattern of BLA dendritic morphology between the adolescent and adult pretreatment groups. In the adult control group, basilar dendritic length did not differ with respect to hemisphere. Nicotine induced robust hemispheric asymmetry by increasing dendritic length in the right hemisphere only. In contrast, adolescent nicotine exposure did not produce significant alteration of basilar dendritic morphology. There was, however, an indication that nicotine eliminated a naturally existing hemispheric asymmetry in the younger cohort. At both ages, nicotine produced a reduction in complexity of the apical tree of principal neurons. Chronic nicotine did not affect the dendritic morphology of pyramidal neurons from the IL in either age group, indicating another dimension of anatomical specificity. Collectively, these data implicate the BLA as a target for lasting neuroplasticity associated with chronic nicotine exposure. Further, hemispheric differences in dendritic morphology were uncovered that depended on the age of nicotine exposure, a finding that underscores the importance of considering laterality when investigating neurodevelopmental effects of drug exposure. **Synapse 64:754–764, 2010.**

INTRODUCTION

Exposure to nicotine has the potential to produce neuroadaptations that persist well beyond cessation. Notably, chronic nicotine produces robust remodeling of dendrites from projection neurons located in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAcc) that persists weeks following administration (Bergstrom et al., 2008; Brown and Kolb, 2001; McDonald et al., 2005, 2007). Another brain region where nicotine may be expected to induce significant dendritic remodeling is the basolateral amygdala (BLA).

Nicotine is well-known to alter the activity of BLA neurons. The BLA possesses a high density of nicotine-binding $\alpha 7$ and $\beta 2$ nicotinic acetylcholine recep-

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tors (Hill et al., 1993; Seguela et al., 1993), receives dense cholinergic innervation from the basal forebrain (Woolf, 1991), and is a central target for dopaminergic projections from the ventral tegmental area (Muller et al., 2009). Chronic nicotine alters BLA molecular signaling (Pandey et al., 2001; Tzavara et al., 2002) and gene expression (Konu et al., 2001; Li et al., 2004) and promotes facilitation of BLA synapses (Huang et al., 2008; Jiang and Role, 2008), findings that implicate the BLA as a substrate for neural plasticity associated with nicotine exposure. Despite compelling evidence for nicotine-induced alteration of neural activity in the BLA, it is unknown whether the dendritic morphology of neurons in the BLA is affected by prior chronic nicotine exposure. To address this question, we carried out morphometric analysis of BLA principal neurons following chronic nicotine exposure.

Hemispheric specialization of cognitive and emotional processing is well documented in the rodent brain. In particular, numerous studies have demonstrated hemispheric lateralization in amygdala functioning (Adamec, 1999; Adamec et al., 2005; Andersen and Teicher, 1999; Coleman-Mesches and McGaugh, 1995a,b; Lalumiere and McGaugh, 2005; Sullivan et al., 2009a,b). Dendritic remodeling may also be lateralized. This suggestion seems plausible given the evidence that environmental stress and maternal deprivation can produce unilateral structural alteration in the prefrontal and somatosensory cortices, respectively (Czeh et al., 2008; Perez-Cruz et al., 2007, 2009; Pinkernelle et al., 2009). In light of the considerable evidence for hemispheric specialization of amygdalar function, we evaluated whether nicotine's impact on dendritic morphology was biased with respect to hemisphere.

The BLA possesses reciprocal connectivity with the IL cortex (Hoover and Vertes, 2007), and it has recently been shown that nicotine alters the extinction of conditioned fear (Tian et al., 2008), learning that depends on inhibitory drive from the IL (Milad and Quirk, 2002). We therefore included IL dendrites in our morphological analysis to assess the anatomical specificity of nicotine's impact on dendritic morphology.

As smoking most often begins in adolescence (Nelson et al., 2008) and it is now well understood that adolescent nicotine exposure produces long-term neurobehavioral outcomes that differ from comparable adult exposure (Adriani et al., 2003; Brielmaier et al., 2007; Iniguez et al., 2008; Schochet et al., 2005; Smith, 2003; Smith et al., 2006), both adolescent and adult pretreatment groups were evaluated.

MATERIALS AND METHODS

Animals and drugs

Male Sprague–Dawley rats (n = 60) were ordered from a commercial supplier (Harlan Sprague Dawley, Indianapolis, IN) and arrived at our facility at either postnatal day (P) 21 or P50 to acclimate to our colony room. After arrival, rats were group-housed by age (five per cage) with a 12-h light cycle (lights on at 0700) and ad libitum access to food and water. All dosing took place during the light phase. Experiments were carried out in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the George Mason University Animal Care and Use Committee.

Rats were administered nicotine (0.5 mg/kg; free base) or saline control via subcutaneous (s.c.) injection three times per week for 2 weeks (six total injections) during either adolescence (n = 30; P32–43) or adulthood (n = 30; P60–71). Injections were used in the present study rather than a continuous infusion regimen utilized in previous studies from our lab to equate the mg/kg difference in mass between the developing adolescent and adult age groups. Further, smoking cigarettes during adolescence has been described as intermittent, low-intensity (Karp et al., 2005) with interspersed periods of not smoking (Wellman et al., 2004). Therefore, the intermittent, relatively low dose schedule of nicotine administration chosen for this experiment mimics consumption patterns of adolescent human smokers. It could also be argued that interspersed injections provide periods of withdrawal which may be a key element in the development of nicotine dependence (Le Foll and Goldberg, 2006).

Seventeen days following dosing, all groups were behaviorally tested for fear conditioning and fear extinction on three consecutive days (Bergstrom, 2009). The day following behavioral testing, a subset of rats from the adolescent (n = 14; P63) and adult (n = 14; P92) groups were randomly chosen for sacrifice for Golgi-Cox staining. Therefore, morphological analysis of BLA and IL dendritic morphology was conducted 20 days following cessation from nicotine dosing. An extended nicotine-free period was included in the experimental design to ensure that all rats were of adult age at the time of morphological analysis (>P60). The accepted age range for adolescence in rats conservatively ranges from P28 to P45 (Spear, 2000), and most neurobehavioral systems reach maturation by P60 and are considered adults beyond this age (Herlenius and Lagercrantz, 2004).

Neuroanatomy

Rats were deeply anesthetized with a ketamine/xylazine cocktail and perfused intracardially with 0.9% saline solution. Brains were placed into Golgi-Cox solution for 14 days in the dark at room temperature. The Golgi-Cox solution was prepared according to the recipe of Glaser and Van der Loos (1981). Following Golgi-Cox immersion, brains were stored in a

30% sucrose solution until vibratome sectioning (200 μm). To differentiate the hemispheres, a sagittal cut was made with a razor blade along the prefrontal and parietal cortices of the right hemisphere. Sections were processed using the protocol of Gibb and Kolb (1998) with modifications. Briefly, sections were alkalinized in ammonium hydroxide, developed and fixed using Kodak Rapid Fix, dehydrated through a graded series of ethanols, and cleared in a solution of 1/3 xylene, 1/3 alcohol (100%), and 1/3 chloroform. Golgi-Cox-impregnated neurons were visualized using light microscopy and reconstructed in three dimensions using Neurolucida software (MBF Biosciences, Williston, VT). To eliminate bias, all neurons were traced by an experimenter blind to pretreatment or age group. Neuronal tracing was conducted under a 60× objective. Neurons were selected for tracing only if they were well impregnated and possessed unobstructed dendrites that could be followed without interruption. IL morphological analysis was restricted to pyramidal neurons located between Bregma 3.72 and 2.52 mm (Paxinos and Watson, 2005). IL pyramidal neurons were selected from Golgi-stained tissue based on the location of the IL anatomical boundaries relative to major landmarks (genu of the corpus collosum and lateral ventricle) and cortical distance from the ventral surface of the brain. IL pyramidal neurons were randomly sampled between 250 and 650 μm from the pia surface because the IL cortex is narrow with fewer, clearly distinguishable layers compared to the adjacent prelimbic (PL) cortex (Izquierdo et al., 2006). To rule out artifactual morphological differences resulting from differential sampling across layers, soma-to-pia distances were compared across pretreatment group and age. Cells with a prominent, single apical tree extending from the apex of the soma toward the pia surface of the cortex and two or more basilar dendritic trees extending from the base of the soma and dendritic spines were chosen for reconstruction. BLA morphological analysis was restricted to principal neurons located between Bregma −2.04 mm and −3.36 mm (Paxinos and Watson, 2005). In this experiment, the BLA was defined as the lateral, basal, and accessory basal nuclei (LeDoux, 2000). The contour of the BLA was readily identified by the external capsule which branches into two fiber tracts that make up its borders. Principal neurons in the BLA were differentiated from stellate neurons based on several morphological criteria including spines, an "apical-like" dendritric tree, and biconical dendritic radiation (Sah et al., 2003).

Morphometric analysis

Quantitative morphological measurements were extracted using NeuroExplorer software (MBF Biosciences, Williston, VT). Morphometric parameters included total length and number of bifurcations (nodes) for both apical and basilar trees. The distribution of total dendritic length and bifurcations along the extent of the dendritic tree was determined by implementing a sholl analysis. In the sholl analysis, the total length and number of bifurcations were measured between equidistant concentric spheres radiating from the center of the soma that each measured 20 μm in radial distance. The sholl analysis reflected the complexity of the dendritic tree. Somatic surface area (μm^2) and perimeter measurements were taken at their approximate maximum by tracing at the level in which the soma appears the largest (Duque et al., 2007). Soma-to-terminal length for the apical tree was also determined. In addition, the hemisphere from which each neuron originated was recorded to assess hemispheric differences in morphology. For each animal, two to six neurons were reconstructed. A mean value based on two to six neurons was calculated for each subject. Group values for each parameter (total length and bifurcations) were based on the mean values from each subject. Age (adolescent vs. adult), pretreatment (nicotine vs. control), and hemisphere (right vs. left) were the between-groups factors. To examine the distribution of dendritic material across the extent of the dendrite, an ANOVA with radial distance from the soma (radius) as the within-group factor (repeated measures) was conducted. Violation of the assumption of sphericity was corrected using the highly conservative Greenhouse–Geisser correction for degrees of freedom. Significant interactions led to follow-up comparisons using independent samples *t*-test at distinct radii from the soma. To avoid spurious values due to multiple comparisons or an overly conservative posthoc test, differences were considered to be significant only if the *P*-values for three or more consecutive points were <0.05 (Luck, 2005). Comparisons of mean values were carried out with one-way ANOVA. All data were presented as group means ± SEM. A superscripted letter "a" indicates a Greenhouse–Geisser-corrected value throughout text. Differences were considered significant at $P < 0.05$. All statistical tests were carried out using SPSS software.

RESULTS

Body weight

In the adolescent group, ANOVA (age × pretreatment) with repeated measures (day) revealed a pretreatment × day interaction ($^aF[2.2, 60.7] = 3.3$; $P < 0.05$), with rats in the nicotine group weighing less than the control group over injections days. The difference in the body weight percentage change between the pretreatment groups was similar for the adult (2.5%) or adolescent (1.7%) group, suggesting

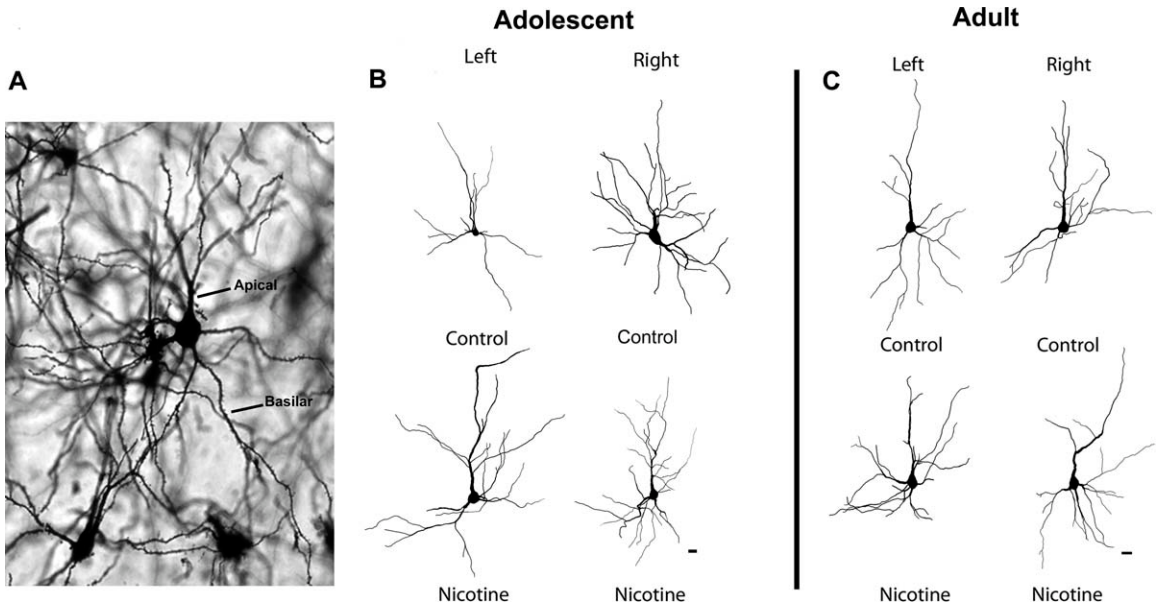

Fig. 1. **A**: Representative photomicrograph of a Golgi-Cox-stained BLA principal neuron with an example of an apical and basilar dendrite. Reconstructions of BLA principal neurons from the adolescent (**B**) and adult (**C**) groups. These nicotine (bottom panels) and control neurons (top panels) from the left (left panels) and right hemisphere (right panels) were selected because they are representative of basilar dendritic lengths near their respective group means. Scale bar represents 20 μm.

that nicotine promoted equivalent body weight loss over injection days for both age groups.

BLA morphometric analysis

For the BLA, two to six neurons were reconstructed from each adolescent (nicotine, $n = 5$; control, $n = 8$) and adult (nicotine, $n = 7$; control $n = 7$) pretreated animal. A total of 54 neurons were reconstructed from the adolescent (nicotine, $n = 26$; control, $n = 28$) and 55 from the adult (nicotine, $n = 27$; control, $n = 28$) group (109 total cells reconstructed; Fig. 1).

BLA basilar dendrites

ANOVA (age × pretreatment × hemisphere) with repeated measures (radius) revealed a significant interaction of age, pretreatment, hemisphere, and radius for total length ($^aF[2.7, 85.8] = 3.9$; $P = 0.014$) and a nearly significant four-way interaction for number of bifurcations ($^aF[3.8, 118.3] = 2.4$; $P = 0.06$).

In the adult group, subsequent ANOVA (pretreatment × hemisphere) with repeated measures (radius) revealed a pretreatment × hemisphere × radius interaction ($^aF[2.9, 43.1] = 3.5$; $P = 0.025$). Follow-up analysis in the control group revealed no difference in the total length of basilar dendrites between hemispheres. Nicotine induced asymmetric remodeling of dendrites between hemispheres for both total length ($^aF[2.5, 184] = 2.5$; $P = 0.003$) and bifurcations ($^aF[3.5, 168] = 4.0$; $P = 0.01$), with the right hemisphere exhibiting greater total length ($F[1, 8] = 21.0$; $P = 0.002$) and bifurcations ($^aF[1, 7.7] = 19.4$; $P = 0.002$) relative to the left hemisphere. Differences were most pronounced at 60–120 μm from the soma for total length (Fig. 2). Together, these data reveal hemispheric symmetry of dendritic morphology from the BLA for adult rats from the control condition and hemispheric asymmetry for rats with prior nicotine exposure.

In the adolescent group, ANOVA (pretreatment × hemisphere) with repeated measures (radius) revealed a significant interaction of hemisphere × radius for total length ($^aF[2.3, 37.5] = 3.1$; $P = 0.049$). Subsequent analysis of pretreatment groups independently showed a trend toward hemispheric asymmetry in the control condition ($P = 0.08$). This asymmetry appeared to be eliminated in the nicotine condition (Fig. 3).

To assess the possibility for differences in dendritic remodeling between age groups that occurred independently of nicotine pretreatment, analysis of controls between age groups was conducted. Results revealed a significant decrease in overall dendritic length ($F[1, 18] = 5.4$; $P = 0.032$) and a trend toward a decrease in bifurcation number ($F[1, 18] = 4.4$; $P = 0.051$) in adults relative to adolescents. Similarly, for the apical tree, there was an overall reduction in total length ($F[1, 18] = 5.2$; $P = 0.035$) in the adult relative to the adolescent group (Fig. 4). There was no interaction of age × hemisphere for both the basilar and apical trees, suggesting that the decrease in dendritic length observed between the adult and adolescent group was similar between the hemispheres.

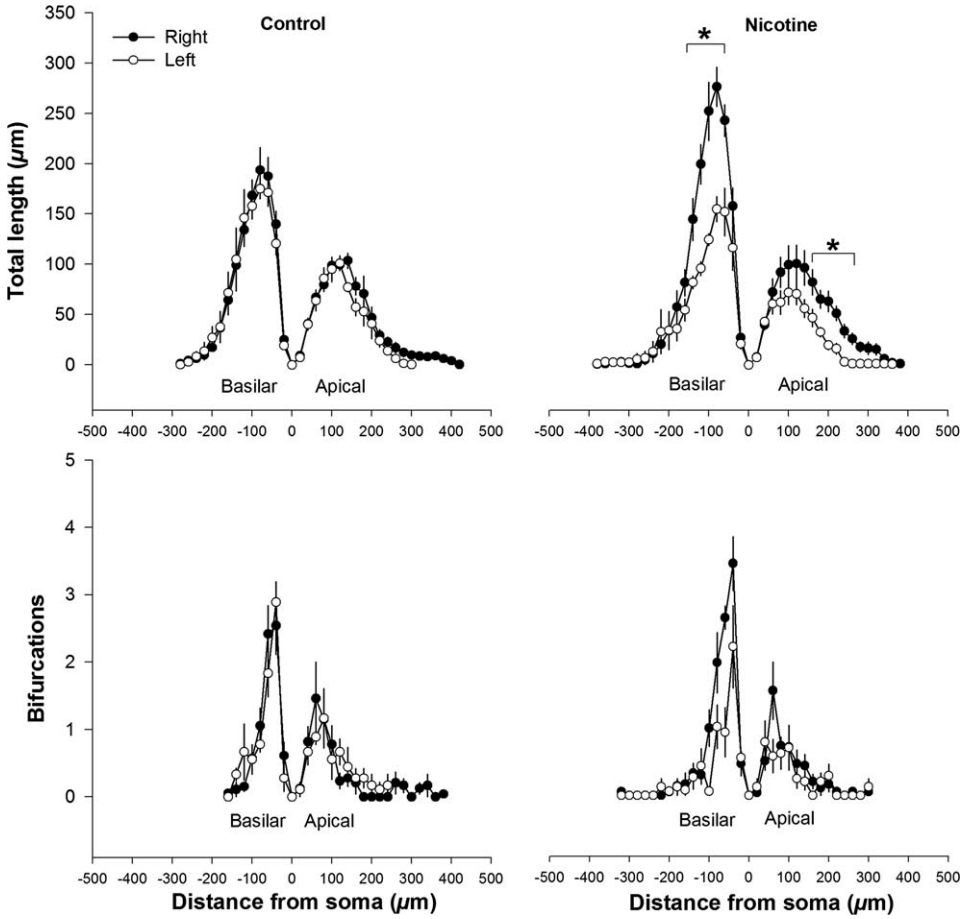

Fig. 2. Nicotine during adulthood induced asymmetry in BLA basilar dendritic morphology by a selective increase in the complexity of dendrites in the right hemisphere ($P < 0.01$). In contrast, there was a reduction in the length of apical dendrites in the left hemisphere relative to right. Hemispheric differences in basilar dendritic length were confined to distances from 60 to 120 μm from the soma. Differences in apical dendritic length emerged at the distalmost distance from the soma (140–240 μm). Basilar and apical dendrites are plotted on the left and right as a function of distance from the soma, respectively. Data points (mean ± SEM) represent the total of all dendritic material at the respective distance from the soma center (bin size was 20 μm) for each hemisphere. *$P < 0.05$ for three or more consecutive points.

Between-hemisphere (interhemispheric) and within-hemisphere (intrahemispheric) main effects of pretreatment can be found in Tables I and II, respectively.

BLA apical dendrites

For the apical tree, ANOVA (age × pretreatment × hemisphere) with repeated measures (radius) for total length indicated a main effect for hemisphere ($F[1, 37] = 5.5$; $P = 0.03$) and a trend toward a pretreatment effect ($F[1, 37] = 4.1$; $P = 0.051$). Analysis of apical bifurcations revealed a main effect of pretreatment only ($F[1, 37] = 6.2$; $P = 0.02$). These results indicated that nicotine decreased the complexity of apical dendrites relative to control and that dendrites in the right hemisphere exhibited greater total length than dendrites in the left hemisphere, regardless of pretreatment or age of dosing (Figs. 3 and 4).

IL morphometric analysis

For the IL, two to four neurons were reconstructed from each adolescent (nicotine, $n = 6$; control, $n = 7$) and adult (nicotine, $n = 6$; control, $n = 5$) pretreated animal. A total of 45 neurons were reconstructed from the adolescent (nicotine, $n = 19$; control, $n = 26$) and 46 from the adult (nicotine, $n = 25$; control, $n = 21$) group (91 total cells reconstructed; Fig. 5).

Pyramidal neurons in the IL cortex were randomly sampled from Layers III–V with a range of 245–650 μm from the pia surface. To address the possibility that group differences in dendritic morphology may have been due to a sampling bias with respect to cortical depth, the distance from the soma to the pia surface was measured for each neuron and compared across groups. Mean distance (mean ± standard error of the mean) to the cortical surface did not vary across pretreatment (nicotine, $M = 448.2 \pm 14.2$; con-

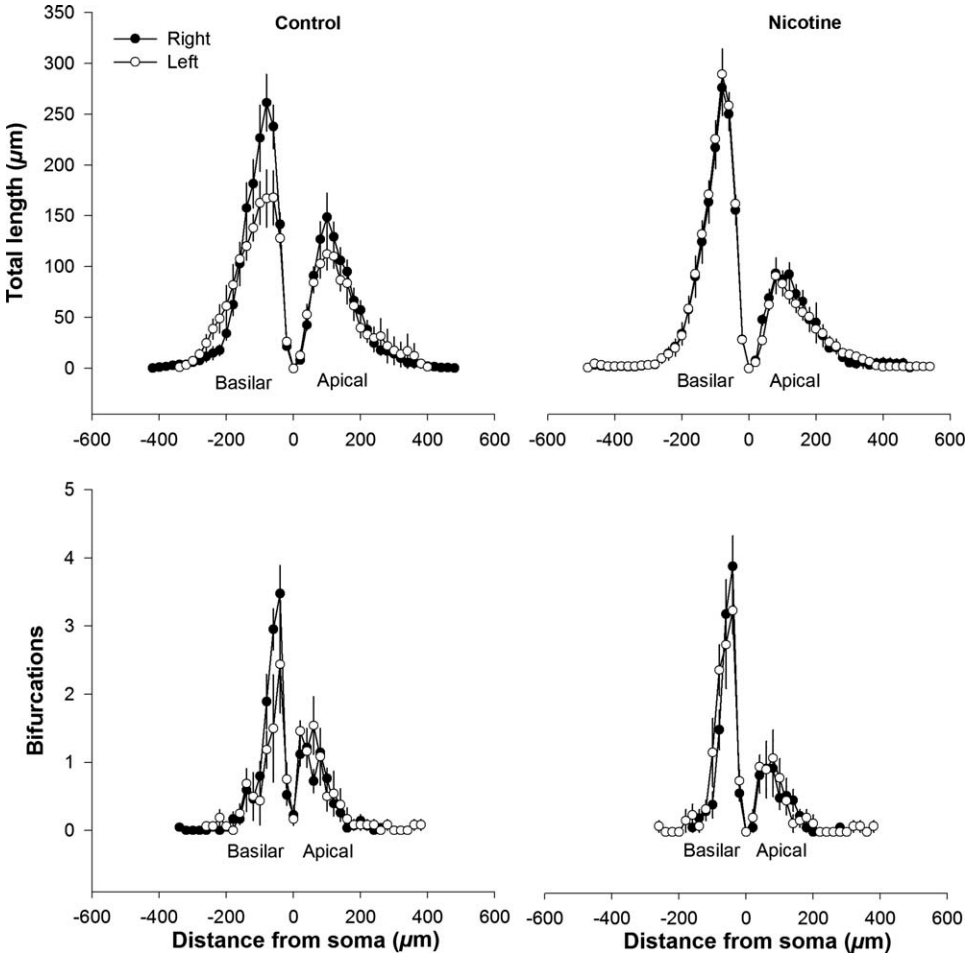

Fig. 3. Nicotine during adolescence appears to eliminate asymmetry in the dendritic morphology of BLA principal neurons, although this result only approached statistical significance. There was a significant decrease in the total length of apical dendrites in the nicotine compared to control group ($P < 0.05$). Basilar and apical dendrites are plotted on the left and right as a function of distance from the soma, respectively. Data points (mean ± SEM) represent the total of all dendritic material at the respective distance from the soma center (bin size was 20 μm) for each hemisphere. *$P < 0.05$ for three or more consecutive points.

trol, $M = 452.4 \pm 12.0$) or hemisphere (right, $M = 468.54 \pm 12.9$; left, $M = 432.8 \pm 12.7$). There was, however, a sampling difference with respect to age ($F[1, 115] = 5.2$; $P < 0.05$), with neurons from the adolescent group having a greater mean cortex-to-pia distance.

IL basilar dendrites

No group differences were detected for IL basilar dendrites.

IL apical dendrites

No group differences were detected for IL apical dendrites. For the nicotine group, although it appears that the apical tree from the adolescent group differed by hemisphere (Fig. 5), there was no significant interaction of hemisphere × distance from the soma. Furthermore, subsequent analysis of individual points along the dendritic tree failed to meet the criteria established above for consecutive significant differences ($P < 0.05$) at three or more points.

Soma size

There were no differences in soma perimeter (71.2 ± 8.8 μm) or area (367.4 ± 85.6 μm^2) across all groups.

DISCUSSION

Chronic nicotine induced a long-lasting alteration of dendritic morphology in the BLA. These are the first data to characterize drug-induced changes in the morphology of dendrites from the BLA. Further, nicotine-induced alteration of dendritic morphology in the BLA was found to be lateralized. To our knowledge, this is the first evidence for drug-induced morphological changes localized to a single hemisphere. The pattern of dendritic remodeling depended on the age of nicotine exposure, a finding supported in other corti-

colimbic brain regions relating to the conditioned reinforcing effects of drugs of abuse (Koob, 2009), including the NAcc (McDonald et al., 2007) and PL cortex (Bergstrom et al., 2008).

The most prominent effect of chronic nicotine exposure on BLA dendritic morphology was in the adult dose group (Fig. 2). The total length and bifurcation number of both basilar and apical dendrites was equivalent between hemispheres in controls. Nicotine induced hemispheric asymmetry by selectively increasing both basilar dendritic length (61.5%) and bifurcation number (79.7%) from principal neurons located in the right BLA. Apical dendritic morphology was also altered between hemispheres with an indication that nicotine reduced the complexity of left hemisphere BLA dendrites relative to the right. These results suggest a bidirectional influence of nicotine on basilar and apical BLA dendritic segments that depended on the hemisphere from which the neuron originated. In contrast to adults, there was an indication that nicotine eliminated hemispheric asymmetry, although this pattern only approached statistical significance (Fig. 3). Like the adult group, nicotine during the adolescent period reduced apical dendritic length. Together, the present results highlight a number of highly specific anatomical alterations in BLA dendritic morphology that include a bidirectional pattern of dendritic remodeling between basilar and apical dendritic segments that depend both on the age of nicotine exposure and on the hemisphere from which the neuron originated. Specificity of nicotine's impact on basilar versus apical segments supports findings for pyramidal neurons in the PL cortex (Bergstrom et al., 2008; Brown and Kolb, 2001). Corresponding changes were not detected in the IL cortex, indicating yet another dimension of anatomical specificity for dendritic remodeling in response to nicotine. Anatomically specific modification of dendritic architecture in response to nicotine is in line with findings in other corticolimbic brain regions. In the NAcc shell, robust modification of dendrites was observed in medium spiny projection neurons, but not in the aspiny cholinergic interneurons (McDonald et al., 2007). In the PL cortex, a pattern of nicotine-induced dendritic remodeling emerged that differed depending on a putative subpopulation of pyramidal neuron (Bergstrom et al.,

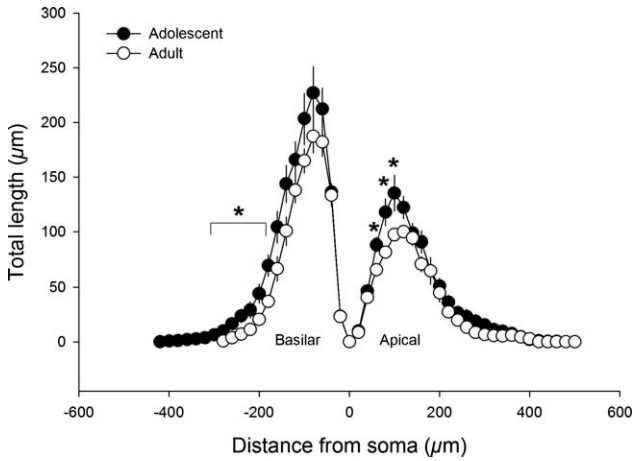

Fig. 4. BLA dendrites were longer in the younger (P63) compared to older (P92) group. Differences were localized to 180–280 μm from the soma for basilar dendrites and 60–100 μm from the soma for apical dendrites. Basilar and apical dendrites are plotted on the left and right as a function of distance from the soma, respectively. Data points (mean ± SEM) represent the total of all dendritic material at the respective distance from the soma center (bin size was 20 μm). *$P < 0.05$ for three or more consecutive points.

TABLE I. Interhemispheric (between) differences in pretreatment for the total length of dendrites (mean ± SEM)

		Control				Nicotine			
		Adolescent		Adult		Adolescent		Adult	
		L	R	L	R	L	R	L	R
BLA	Basilar	1299 ± 64	1500 ± 187	1056 ± 163	1087 ± 92	1537 ± 204	1524 ± 59	942 ± 61	1521 ± 93
	Apical	932 ± 143	1016 ± 86	668 ± 76	823 ± 69	708 ± 105	761 ± 98	498 ± 55	890 ± 109
IL	Basilar	1118 ± 103	1165 ± 87	1224 ± 91	1150 ± 126	1007 ± 132	1232 ± 203	1358 ± 229	1331 ± 98
	Apical	977 ± 119	1236 ± 192	1040 ± 110	872 ± 83	845 ± 88	1097 ± 150	973 ± 80	868 ± 114

Gray cells reflect statistically significant differences between mean values ($P < 0.05$).

TABLE II. Intrahemispheric (within) differences in pretreatment for the total length of dendrites (mean ± SEM)

		Adolescent				Adult			
		Left		Right		Left		Right	
		Nicotine	Control	Nicotine	Control	Nicotine	Control	Nicotine	Control
BLA	Basilar	1537 ± 204	1300 ± 64	1524 ± 59	1501 ± 187	942 ± 61	1056 ± 163	1566 ± 134	1088 ± 92
	Apical	708 ± 105	932 ± 143	761 ± 98	1016 ± 86	498 ± 55	667 ± 76	891 ± 109	823 ± 67
IL	Basilar	1006 ± 132	1117 ± 103	1232 ± 203	1166 ± 87	1358 ± 229	1224 ± 91	1332 ± 98	1150 ± 126
	Apical	845 ± 88	977 ± 119	1097 ± 150	1236 ± 192	973 ± 80	1030 ± 158	868 ± 114	871 ± 83

Gray cells reflect statistically significant differences between mean values ($P < 0.05$).

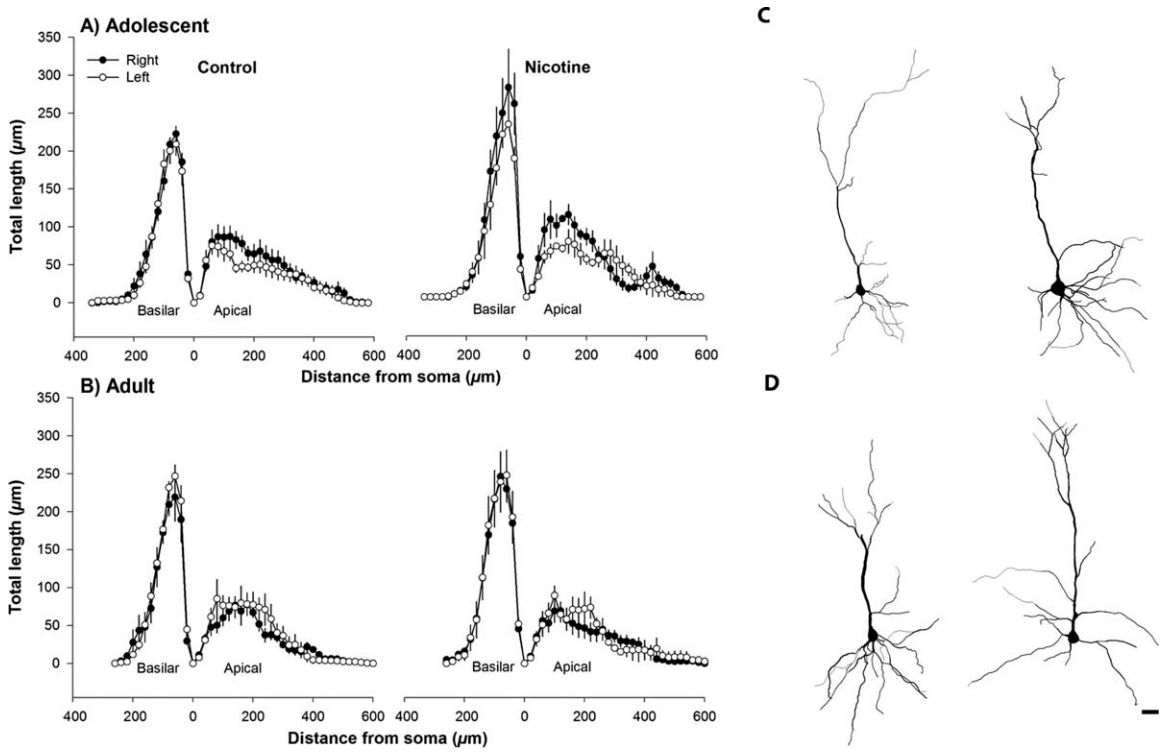

Fig. 5. There was no lasting effect of nicotine in the IL cortex of rats dosed during adolescence (**A**) or adulthood (**B**). Data points (mean ± SEM) represent the total dendritic length at the respective distance from the soma center (bin size was 20 μm). Representative reconstructions of IL pyramidal neurons from the adolescent (**C**) and adult (**D**) dose groups were selected because they are representative of dendritic lengths near their respective group means. Scale bar represents 20 μm.

2008). Further, Brown and Kolb (2001) demonstrated that, while dendrites in the NAcc and PL exhibited robust remodeling in response to nicotine, dendrites in the parietal cortex were shown to be unaltered. Collectively, these data indicate a highly selective form of dendritic remodeling in response to nicotine that is localized to corticolimbic brain regions implicated in nicotine addiction.

Dendrites in the right BLA appeared to be the most sensitive to nicotine-induced structural remodeling (Tables I and II). This result is especially intriguing given numerous findings for a right amygdala bias in emotion-related tasks (Adamec, 1999; Adamec et al., 2005; Andersen and Teicher, 1999; Baker and Kim, 2004; Coleman-Mesches and McGaugh, 1995a,b; Lalumiere and McGaugh, 2005; Sullivan et al., 2009a,b). The functional consequences of lateralized dendritic remodeling are unclear (Czeh et al., 2008).

Age-dependent differences

Lasting age-dependent responsiveness to nicotine is well supported in the preclinical literature and probably relates to the unique neurodevelopmental profile of the adolescent brain. The amygdala in particular undergoes considerable developmental changes through adolescence. Both structural and neurochemical maturation occurs during the adolescence period including dendritic pruning (Zehr et al., 2006), increased acetylcholinesterase activity (Berdel et al., 1996), and continued development of efferent connectivity with the mPFC (Cunningham et al., 2002, 2007). These developmental changes appear to render amygdala circuitry particularly vulnerable to nicotine's pharmacodynamic action, producing long-term outcomes that differ from comparable adult-aged exposure (Smith, 2003). The precise ontogenetic mechanisms that underlie nicotine-induced dendritic remodeling are unknown.

An unexpected finding from the present work was the apparent retraction of BLA dendrites in the older relative to younger group (Fig. 4). Some work suggests that development of the amygdala may extend beyond what is typically defined as adolescence (P28–42) (Spear, 2000). Amygdala volume was found to increase well into young adulthood in mice (Koshibu et al., 2004), a pattern similar to that observed in humans (Giedd et al., 1996). Connectivity with the IL also increases into young adulthood (P65) or later (Cunningham et al., 2002). As whole amygdala volume and connectivity increase, it was recently shown that principal neuron and glia number decrease from

P35 to P90 (Rubinow and Juraska, 2009). Collectively, these data implicate a relatively late wave of neurodevelopmental events in the amygdala that extends well past what is classically considered the adolescent period. Here, it was found that BLA dendritic complexity decreases through young adulthood. In animals not exposed to nicotine, basilar and apical dendritic length was decreased (24.5 and 21.7%, respectively) in the older (P92) compared to younger (P63) group (Fig. 4). No developmental differences were found for dendrites in the IL (Fig. 5), suggesting that continued morphological differences through early adulthood may be brain region specific.

Infralimbic cortex dendritic morphology

Nicotine did not influence dendritic morphology of pyramidal neurons in the IL cortex (Fig. 5), a finding in contrast to observations in the adjacent PL cortex (Bergstrom et al., 2008; Brown and Kolb, 2001). It is important to note that the dosing regimen in the present experiment (intermittent s.c.) differed from that of Bergstrom et al. (2008; continuous infusion) and Brown and Kolb (2001; intermittent interperitoneal). However, subregion-specific nicotine-induced reorganization of mPFC dendritic morphology might be expected since the PL and IL cortices each possess differing cytoarchitecture (Van Eden and Uylings, 1985), connectivity (Gabbott et al., 2005; Hoover and Vertes, 2007), and functionality (Counotte et al., 2008; Marquis et al., 2007; Peters et al., 2009; Van den Oever et al., 2008). Clearly, more work is required to delineate how chronic nicotine may impact the IL cortex. It could be argued that nicotine-induced structural plasticity was confined to a specific IL cortical layer. Future work should address the possibility for layer-specific dendritic alteration in the IL cortex in response to nicotine.

Stress effects

For rats dosed as adults, structural changes were generally characterized as hypertrophic, with increased basilar dendrite complexity in response to nicotine. Numerous experiments have demonstrated dendritic hypertrophy in the BLA following both stress and corticosterone regimens (Johnson et al., 2009; Mitra et al., 2009; Mitra and Sapolsky, 2008; Vyas et al., 2002, 2004, 2006). Based on this work, it could be argued that stress, rather than the pharmacological action of nicotine per se, could have contributed to BLA dendritic hypertrophy. However, it should be noted that in addition to the BLA, stress has reliably been shown to restructure apical dendrites of neurons from the IL cortex (Goldwater et al., 2009; Izquierdo et al., 2006; Perez-Cruz et al., 2007, 2009). In the present work, we found no significant impact of nicotine on IL dendritic morphology, suggesting that putative stress resulting from repeated nicotine injections and withdrawal was not sufficient to induce structural alterations in IL dendritic morphology.

It could also be argued that fear learning or an interaction of nicotine and fear learning prior to morphological analysis of the BLA and IL contributed to the observed pattern of dendritic remodeling. To our knowledge, no work has yet demonstrated changes to dendritic morphology following fear conditioning or extinction, although fear conditioning has been shown to increase spine number in the lateral amygdala (Radley et al., 2006). If an interaction of nicotine and fear conditioning occurred, the effect between groups would most likely be equivalent and not have contributed to the between-pretreatment group differences since all rats underwent fear conditioning and extinction.

Cellular mechanisms mediating dendritic alteration

Altered synaptic activity has the potential to trigger dendritic remodeling (Parrish et al., 2007). Evidence for altered GABAergic (Barazangi and Role, 2001) and glutamatergic (Barazangi and Role, 2001; Girod et al., 2000; Jiang and Role, 2008) synaptic signaling has been indicated in the BLA after nicotine. Although not directly demonstrated in the rodent BLA but a finding common across systems is that increased glutamate release initiates changes in intracellular calcium. Calcium influx through *N*-methyl-D-aspartic acid (NMDA) receptors and postsynaptic voltage-dependent calcium channels has the potential to influence the dendritic cytoskeleton through new gene transcription (Flavell and Greenberg, 2008). The action of neurotrophins is another mechanism for dendritic growth and branching of BLA principal neurons. Although the neurotrophic effects of long-term nicotine are established (Mudo et al., 2007), no studies to date have directly demonstrated increased neurotrophic action in the amygdala following chronic nicotine exposure.

Consequences of basolateral amygdala dendritic reorganization

The BLA is generally thought to be involved in the acquisition and consolidation of emotional memory (McGaugh, 2004). Modifications to BLA dendritic morphology has the potential to significantly impact the physiology, connectivity, and information-processing of BLA neurons (Connors and Regehr, 1996; Hernandez-Echeagaray et al., 2004; Krichmar et al., 2006; Liu and Aghajanian, 2008; Stepanyants and Chklovskii, 2005). Thus, it might be expected that modifications to BLA dendrites would impact behavior that depends on BLA functioning. Indeed, there is

some evidence to suggest that prior nicotine produces lasting alterations of emotional memory that depend on BLA functioning, including auditory fear conditioning (Smith et al., 2006) and extinction (Tian et al., 2008). However, it is important to note that dendritic hypertrophy has not yet been found to be a predictor of classical fear conditioning performance, but rather has been shown to correlate with anxiety-related behaviors (Mitra and Sapolsky, 2008; Vyas et al., 2002, 2006). Another role for the BLA is in the regulation of stress hormone secretion (Herman et al., 1996). Mitra and Sapolsky (2008) theorized that greater elaboration of dendrites in the BLA might be expected to increase BLA excitation to the hypothalamic-pituitary-adrenal (HPA) axis and thus enhance the stress response. Persisting anxiety/stress as a consequence of smoking represents an important contributing factor to nicotine addiction (Picciotto et al., 2002).

Overall, the findings presented here implicate the BLA as a target for persisting neuroadaptative changes associated with chronic nicotine exposure. Nicotine-induced changes of BLA dendrites depended on the hemisphere from which the neuron originated, highlighting the importance of laterality when investigating drug effects.

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REFERENCES

Adamec RE. 1999. Evidence that limbic neural plasticity in the right hemisphere mediates partial kindling induced lasting increases in anxiety-like behavior: Effects of low frequency stimulation (quenching?) on long term potentiation of amygdala efferents and behavior following kindling. Brain Res 839:133–152.

Adamec R, Blundell J, Burton P. 2005. Role of NMDA receptors in the lateralized potentiation of amygdala afferent and efferent neural transmission produced by predator stress. Physiol Behav 86(1/2):75–91.

Adriani W, Spijker S, Deroche-Gamonet V, Laviola G, Le Moal M, Smit AB, Piazza PV. 2003. Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. J Neurosci 23:4712–4716.

Andersen SL, Teicher MH. 1999. Serotonin laterality in amygdala predicts performance in the elevated plus maze in rats. Neuroreport 10:3497–3500.

Baker KB, Kim JJ. 2004. Amygdalar lateralization in fear conditioning: Evidence for greater involvement of the right amygdala. Behav Neurosci 118:15–23.

Barazangi N, Role LW. 2001. Nicotine-induced enhancement of glutamatergic and GABAergic synaptic transmission in the mouse amygdala. J Neurophysiol 86:463–474.

Berdel B, Morys J, Maciejewska B, Narkiewicz O. 1996. Acetylcholinesterase activity as a marker of maturation of the basolateral complex of the amygdaloid body in the rat. Int J Dev Neurosci 14:543–549.

Bergstrom HC. 2009. Lateralized dendritic correlates of enhanced conditioned fear retrieval following cessation from chronic nicotine exposure in adolescent and adult rats (doctoral dissertation). Retrieved from http://digilibgmuedu:8080/xmlui/handle/1920/4511.

Bergstrom HC, McDonald CG, French HT, Smith RF. 2008. Continuous nicotine administration produces selective, age-dependent structural alteration of pyramidal neurons from prelimbic cortex. Synapse 62:31–39.

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 29:74–80.

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.

Coleman-Mesches K, McGaugh JL. 1995a. Differential effects of pretraining inactivation of the right or left amygdala on retention of inhibitory avoidance training. Behav Neurosci 109:642–647.

Coleman-Mesches K, McGaugh JL. 1995b. Differential involvement of the right and left amygdalae in expression of memory for aversively motivated training. Brain Res 670:75–81.

Connors BW, Regehr WG. 1996. Neuronal firing: Does function follow form? Curr Biol 6:1560–1562.

Counotte DS, Spijker S, Van de Burgwal LH, Hogenboom F, Schoffelmeer AN, De Vries TJ, Smit AB, Pattij T. 2008. Long-lasting cognitive deficits resulting from adolescent nicotine exposure in rats. Neuropsychopharmacology.

Cunningham MG, Bhattacharyya S, Benes FM. 2002. Amygdalocortical sprouting continues into early adulthood: Implications for the development of normal and abnormal function during adolescence. J Comp Neurol 453:116–130.

Cunningham MG, Bhattacharyya S, Benes FM. 2007. Increasing interaction of amygdalar afferents with GABAergic interneurons between birth and adulthood. Cereb Cortex 18:1529–1535.

Czeh B, Perez-Cruz C, Fuchs E, Flugge G. 2008. Chronic stress-induced cellular changes in the medial prefrontal cortex and their potential clinical implications: Does hemisphere location matter? Behav Brain Res 190:1–13.

Duque A, Tepper JM, Detari L, Ascoli GA, Zaborszky L. 2007. Morphological characterization of electrophysiologically and immunohistochemically identified basal forebrain cholinergic and neuropeptide Y-containing neurons. Brain Struct Funct 212:55–73.

Flavell SW, Greenberg ME. 2008. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annu Rev Neurosci 31:563–590.

Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ. 2005. Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol 492:145–177.

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

Giedd JN, Vaituzis AC, Hamburger SD, Lange N, Rajapakse JC, Kaysen D, Vauss YC, Rapoport JL. 1996. Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: Ages 4–18 years. J Comp Neurol 366:223–230.

Girod R, Barazangi N, McGehee D, Role LW. 2000. Facilitation of glutamatergic neurotransmission by presynaptic nicotinic acetylcholine receptors. Neuropharmacology 39:2715–2725.

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 4:117–125.

Goldwater DS, Pavlides C, Hunter RG, Bloss EB, Hof PR, McEwen BS, Morrison JH. 2009. Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. Neuroscience 164:798–808.

Herlenius E, Lagercrantz H. 2004. Development of neurotransmitter systems during critical periods. Exp Neurol 190 Suppl 1:S8–21.

Herman JP, Prewitt CM, Cullinan WE. 1996. Neuronal circuit regulation of the hypothalamo-pituitary-adrenocortical stress axis. Crit Rev Neurobiol 10(3/4):371–394.

Hernandez-Echeagaray E, Starling AJ, Cepeda C, Levine MS. 2004. Modulation of AMPA currents by D2 dopamine receptors in striatal medium-sized spiny neurons: Are dendrites necessary? Eur J Neurosci 19:2455–2463.

Hill JA Jr, Zoli M, Bourgeois JP, Changeux JP. 1993. Immunocytochemical localization of a neuronal nicotinic receptor: The beta 2-subunit. J Neurosci 13:1551–1568.

Hoover WB, Vertes RP. 2007. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149–179.

Huang YY, Kandel ER, Levine A. 2008. Chronic nicotine exposure induces a long-lasting and pathway-specific facilitation of LTP in the amygdala. Learn Mem 15:603–610.

Iniguez SD, Warren BL, Parise EM, Alcantara LF, Schuh B, Maffeo ML, Manojlovic Z, Bolanos-Guzman CA. 2008. Nicotine exposure during adolescence induces a depression-like state in adulthood. Neuropsychopharmacology 34:1609–1624.

Izquierdo A, Wellman CL, Holmes A. 2006. Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice. J Neurosci 26:5733–5738.

Jiang L, Role LW. 2008. Facilitation of cortico-amygdala synapses by nicotine: Activity-dependent modulation of glutamatergic transmission. J Neurophysiol 99:1988–1999.

Johnson SA, Wang JF, Sun X, McEwen BS, Chattarji S, Young LT. 2009. Lithium treatment prevents stress-induced dendritic remodeling in the rodent amygdala. Neuroscience 163:34–39.
Karp I, O'Loughlin J, Paradis G, Hanley J, Difranza J. 2005. Smoking trajectories of adolescent novice smokers in a longitudinal study of tobacco use. Ann Epidemiol 15:445–452.
Konu O, Kane JK, Barrett T, Vawter MP, Chang R, Ma JZ, Donovan DM, Sharp B, Becker KG, Li MD. 2001. Region-specific transcriptional response to chronic nicotine in rat brain. Brain Res 909(1/2):194–203.
Koob GF. 2009. Dynamics of neuronal circuits in addiction: Reward, antireward, and emotional memory. Pharmacopsychiatry 42 Suppl 1:S32–41.
Koshibu K, Levitt P, Ahrens ET. 2004. Sex-specific, postpuberty changes in mouse brain structures revealed by three-dimensional magnetic resonance microscopy. Neuroimage 22:1636–1645.
Krichmar JL, Velasquez D, Ascoli GA. 2006. Effects of beta-catenin on dendritic morphology and simulated firing patterns in cultured hippocampal neurons. Biol Bull 211:31–43.
Lalumiere RT, McGaugh JL. 2005. Memory enhancement induced by post-training intrabasolateral amygdala infusions of beta-adrenergic or muscarinic agonists requires activation of dopamine receptors: Involvement of right, but not left, basolateral amygdala. Learn Mem 12:527–532.
LeDoux JE. 2000. Emotion circuits in the brain. Annu Rev Neurosci 23:155–184.
Le Foll B, Goldberg SR. 2006. Nicotine as a typical drug of abuse in experimental animals and humans. Psychopharmacology 184(3/4):367–381.
Li MD, Kane JK, Wang J, Ma JZ. 2004. Time-dependent changes in transcriptional profiles within five rat brain regions in response to nicotine treatment. Brain Res Mol Brain Res 132:168–180.
Liu RJ, Aghajanian GK. 2008. Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: Role of corticosterone-mediated apical dendritic atrophy. Proc Natl Acad Sci USA 105:359–364.
Luck SJ. 2005.Plotting, measurement, and analysis. In: Gazzaniga MS, editor. An introduction to the event-related potential technique. Cambridge, MA: The MIT Press. p 248–249.
Marquis JP, Killcross S, Haddon JE. 2007. Inactivation of the prelimbic, but not infralimbic, prefrontal cortex impairs the contextual control of response conflict in rats. Eur J Neurosci 25:559–566.
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 385:163–167.
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.
McGaugh JL. 2004. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 27:1–28.
Milad MR, Quirk GJ. 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70–74.
Mitra R, Sapolsky RM. 2008. Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. Proc Natl Acad Sci USA 105:5573–5578.
Mitra R, Adamec R, Sapolsky R. 2009. Resilience against predator stress and dendritic morphology of amygdala neurons. Behav Brain Res 205:535–543.
Mudo G, Belluardo N, Fuxe K. 2007. Nicotinic receptor agonists as neuroprotective/neurotrophic drugs. Progress in molecular mechanisms. J Neural Transm 114:135–147.
Muller JF, Mascagni F, McDonald AJ. 2009. Dopaminergic innervation of pyramidal cells in the rat basolateral amygdala. Brain Struct Funct 213:275–288.
Nelson DE, Mowery P, Asman K, Pederson LL, O'Malley PM, Malarcher A, Maibach EW, Pechacek TF. 2008. Long-term trends in adolescent and young adult smoking in the United States: Metapatterns and implications. Am J Public Health 98:905–915.
Pandey SC, Roy A, Xu T, Mittal N. 2001. Effects of protracted nicotine exposure and withdrawal on the expression and phosphorylation of the CREB gene transcription factor in rat brain. J Neurochem 77:943–952.
Parrish JZ, Emoto K, Kim MD, Jan YN. 2007. Mechanisms that regulate establishment, maintenance, and remodeling of dendritic fields. Annu Rev Neurosci 30:399–423.
Paxinos G, Watson C. 2005. The rat brain in stereotaxic coordinates. New York: Academic Press.
Perez-Cruz C, Muller-Keuker JI, Heilbronner U, Fuchs E, Flugge G. 2007. Morphology of pyramidal neurons in the rat prefrontal cortex: Lateralized dendritic remodeling by chronic stress. Neural Plast 2007:46276.
Perez-Cruz C, Simon M, Czeh B, Flugge G, Fuchs E. 2009. Hemispheric differences in basilar dendrites and spines of pyramidal neurons in the rat prelimbic cortex: Activity- and stress-induced changes. Eur J Neurosci 29:738–747.
Peters J, Kalivas PW, Quirk GJ. 2009. Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 16:279–288.
Picciotto MR, Brunzell DH, Caldarone BJ. 2002. Effect of nicotine and nicotinic receptors on anxiety and depression. Neuroreport 13:1097–1106.
Pinkernelle J, Abraham A, Seidel K, Braun K. 2009. Paternal deprivation induces dendritic and synaptic changes and hemispheric asymmetry of pyramidal neurons in the somatosensory cortex. Dev Neurobiol 69:663–673.
Radley JJ, Johnson LR, Janssen WG, Martino J, Lamprecht R, Hof PR, LeDoux JE, Morrison JH. 2006. Associative Pavlovian conditioning leads to an increase in spinophilin-immunoreactive dendritic spines in the lateral amygdala. Eur J Neurosci 24:876–884.
Rubinow MJ, Juraska JM. 2009. Neuron and glia numbers in the basolateral nucleus of the amygdala from preweaning through old age in male and female rats: A stereological study. J Comp Neurol 512:717–725.
Sah P, Faber ES, Lopez De Armentia M, Power J. 2003. The amygdaloid complex: Anatomy and physiology. Physiol Rev 83:803–834.
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 135:285–297.
Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. 1993. Molecular cloning, functional properties, and distribution of rat brain alpha 7: A nicotinic cation channel highly permeable to calcium. J Neurosci 13:596–604.
Smith RF. 2003. Animal models of periadolescent substance abuse. Neurotoxicol Teratol 25:291–301.
Smith LN, McDonald CG, Bergstrom HC, Brielmaier JM, Eppolito AK, Wheeler TL, Falco AM, Smith RF. 2006. Long-term changes in fear conditioning and anxiety-like behavior following nicotine exposure in adult versus adolescent rats. Pharmacol Biochem Behav 85:91–97.
Spear LP. 2000. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417–463.
Stepanyants A, Chklovskii DB. 2005. Neurogeometry and potential synaptic connectivity. Trends Neurosci 28:387–394.
Sullivan RM, Duchesne A, Hussain D, Waldron J, Laplante F. 2009a. Effects of unilateral amygdala dopamine depletion on behaviour in the elevated plus maze: Role of sex, hemisphere and retesting. Behav Brain Res 205:115–122.
Sullivan RM, Dufresne MM, Waldron J. 2009b. Lateralized sex differences in stress-induced dopamine release in the rat. Neuroreport 20:229–232.
Tian S, Gao J, Han L, Fu J, Li C, Li Z. 2008. Prior chronic nicotine impairs cued fear extinction but enhances contextual fear conditioning in rats. Neuroscience 153:935–943.
Tzavara ET, Monory K, Hanoune J, Nomikos GG. 2002. Nicotine withdrawal syndrome: Behavioural distress and selective up-regulation of the cyclic AMP pathway in the amygdala. Eur J Neurosci 16:149–153.
Van den Oever MC, Goriounova NA, Wan Li K, Van der Schors RC, Binnekade R, Schoffelmeer AN, Mansvelder HD, Smit AB, Spijker S, De Vries TJ. 2008. Prefrontal cortex AMPA receptor plasticity is crucial for cue-induced relapse to heroin-seeking. Nat Neurosci 11:1053–1058.
Van Eden CG, Uylings HB. 1985. Cytoarchitectonic development of the prefrontal cortex in the rat. J Comp Neurol 241:253–267.
Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. 2002. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci 22:6810–6818.
Vyas A, Pillai AG, Chattarji S. 2004. Recovery after chronic stress fails to reverse amygdaloid neuronal hypertrophy and enhanced anxiety-like behavior. Neuroscience 128:667–673.
Vyas A, Jadhav S, Chattarji S. 2006. Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. Neuroscience 143:387–393.
Wellman RJ, DiFranza JR, Savageau JA, Dussault GF. 2004. Short term patterns of early smoking acquisition. Tob Control 13:251–257.
Woolf NJ. 1991. Cholinergic systems in mammalian brain and spinal cord. Prog Neurobiol 37:475–524.
Zehr JL, Todd BJ, Schulz KM, McCarthy MM, Sisk CL. 2006. Dendritic pruning of the medial amygdala during pubertal development of the male Syrian hamster. J Neurobiol 66:578–590.