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Chronic exposure to nicotine is associated with reduced reward-related activity in the striatum but not the midbrain

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Abstract

Background—The reinforcing effects of nicotine are mediated by brain regions that also support temporal difference error (TDE) processing, yet the impact of nicotine on TDE is undetermined.

Methods—Dependent smokers (N=21) and matched controls (N=21) were trained to associate a juice reward with a visual cue in a classical conditioning paradigm. Subjects subsequently underwent fMRI sessions in which they were exposed to trials where they either received juice as temporally predicted or where the juice was withheld (negative TDE) and later received unexpectedly (positive TDE). Subjects were scanned in two sessions that were identical except that smokers had a transdermal nicotine (21mg) or placebo patch placed before scanning. Analysis focused on regions along the trajectory of mesocorticolimbic (MCL) and nigrostriatal (NS) dopaminergic pathways.

Results—There was a reduction in TDE-related function in smokers in the striatum, which did not differ as a function of patch manipulation, but was predicted by the duration (years) of smoking. Activation in midbrain regions was not impacted by group or drug condition.

Conclusions—These data suggest a differential effect of smoking status on the neural substrates of reward in distinct dopaminergic pathway regions, which may be partially attributable to chronic nicotine exposure. The failure of transdermal nicotine to alter reward-related functional processes either within smokers or between smokers and controls implies that acute nicotine patch administration is insufficient to modify reward processing, which has been linked to abstinence-induced anhedonia in smokers and may play a critical role in smoking relapse.

Keywords

nicotine; reward; temporal difference error; smoking; fMRI; striatum

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Introduction

Human neuroimaging studies of reward processing suggest network computation mediated through mesocorticolimbic (MCL) dopamine (DA) pathways i.e. those cells of the ventral tegmental area (VTA) that project to the nucleus accumbens (NAcc) and are involved in reinforcement (1–6). Orbitofrontal cortex (OFC) *and* medial prefrontal cortex (mPFC) mediate positive hedonic feelings associated with reward receipt; OFC activation also codes for reward value and is important in learning from unexpected outcomes (7–10). The ventral striatum (VS) is activated at earlier stages of reward processing, concomitant with the attribution of incentive salience to reward-predictive stimuli (11–12), when learning the relationship between an unconditioned stimulus and an impending reward, and when temporal errors occur in the predicted receipt of a reward (13–17). Contingency-dependent changes in striatal activity are mediated by the activation of midbrain dopaminergic (DArgic) neurons in the VTA and substantia nigra (SN) pars compacta and their projections primarily to the ventral and dorsal striatum (18–19), suggesting that signals of this type arise from activity in both MCL *and* nigrostriatal (NS) DA pathways (i.e. SN cell bodies with terminals predominantly in the dorsal striatum), a system involved in habit learning and postulated to play a role in reward and addiction (see 20 for review).

Human, non-human primate, and rodent studies demonstrate phasic increases in DA signaling in the basal ganglia following unexpected natural rewards, a temporal shift in DA response from reward receipt to stimuli predicting reward (21–23) and transient decreases in signaling following the omission of anticipated rewards (13,16–17,24). This suggests a neural basis for temporal difference (TD) learning and error prediction in the structures comprising the basal ganglia. Addictive drugs also produce transient increases in DA signaling (25–26) that become conditioned in humans (27) and may lead to positive TD error (TDE) signals that increase drug value and reinforce drug-seeking behavior (28).

A range of nicotine-related situational states (e.g. withdrawal, expectation, cue-induced reactivity, craving suppression) have been mapped onto regions supporting learning *and* reward (e.g. 29,30–35). Moreover, nicotine exerts its pharmacological effects via high affinity nicotinic acetylcholine receptors (nAChRs), which are widely distributed throughout the brain (36–37), including the cell bodies and axon terminals of MCL and NS DA neurons, suggesting a mechanism for nicotine's reinforcing properties (38–39). Given the role of these pathways in a range of reward processes, nicotine's influence on reward processing may extend beyond motivational aspects of smoking behavior and impact upon TD processing that extends to and modifies other (non-drug) rewards.

Using a simple classical conditioning paradigm (13) we considered: (1) whether being a dependent smoker alters the neurobiology of TDE processing and (2) the impact of acute nicotine administration upon the functional profile associated with TDE. Since withdrawal may have state-dependent effects on reward processing that do not simply reflect the impact of chronic nicotine, these issues were considered in dependent smokers in the absence of a frank withdrawal state. We hypothesized that acute administration of nicotine and chronic exposure to nicotine in dependent smokers would modify TDE processing of a natural reward.

Methods

Participants

Sixty-four right-handed (40) individuals were recruited from the general population. Thirteen subjects were excluded due to data quality issues (primarily head motion) and one subject failed to complete scanning. For the purposes of matching between groups a further

5 participants were excluded prior to analysis. The analysis cohort included adult (>18 years) smokers (N=21) and nonsmoking controls (N=21), matched for age, gender, self-reported race, IQ (41) and years of education (Table 1). Smokers smoked at least 15 cigarettes/day for a minimum of 1 year prior to participation. Controls had no history of smoking.

Exclusion criteria included significant neurological or medical history, any psychiatric history, claustrophobia, pregnancy, and any other contraindication for MRI. With the exception of nicotine dependence in smokers, substance abuse or dependence was exclusionary.

Procedure

This study was approved by the NIDA-IRP IRB and written informed consent was obtained from all subjects. Participants made three study visits: task and procedural training in a mock scanner and two MRI sessions. The sessions were identical for all participants, except that smokers had a 21-mg nicotine or placebo patch (Nicoderm®, GlaxoSmithKline Inc., USA) applied 2h prior to MRI. Session order for smokers was single-blind, randomly determined and counter-balanced between subjects (N=10 nicotine first). Controls were scanned twice without a patch manipulation. Participants were not permitted to consume alcohol or over-the-counter medications for 24h prior to each session and were limited to ½ cup of caffeinated beverage before each scan. Prior to MRI sessions, participants were tested for recent drug use (TRIAGE® urine drug test), alcohol use (Alco-Sensor IV, Intoximeters Inc., USA), pregnancy, and expired carbon monoxide (Vitalograph Breath CO monitor, USA). For the purposes of characterization, participants completed measures of attention, switching (Trails A and B; 42), memory (43), and the Cloninger Temperament and Character Inventory (TCI; 44). Smokers completed a detailed smoking history, including the Fagerstrom Test for Nicotine Dependence (FTND; 45).

Patch administration—Patches were affixed onto the upper back 30mins after the participant's last cigarette and 2h prior to scanning. The time of last cigarette was confirmed by research staff. A 2h delay was chosen to allow for maximal nicotine plasma levels within the nicotine condition and to minimize withdrawal in the placebo condition (42–43). Both patch types were manufactured by the same pharmaceutical company and were identical in appearance and non-nicotine content. Participants were not debriefed regarding session order until they had completed the study. Withdrawal intensity, mood, and nicotine craving were queried before and after scanning using the Parrott mood questionnaire (44) and a 12-item short form of the Tobacco Craving Questionnaire (TCQ; 45,46).

Temporal difference error (TDE)/juice paradigm

Previous applications of this paradigm demonstrate temporal prediction signals in response to predictable gustatory stimuli, i.e. juice (13–14). Prior to training, participants chose a juice flavor (apple, grape, or fruit punch) that was subsequently used for all sessions. To minimize the impact of recent liquid consumption on the palatability of the juice reward, participants refrained from drinking fluids for 2h pre-session. In the mock scanner participants were exposed to training trials (Figure 1A), in which a previously unconditioned yellow light cue (duration=1000ms) was paired with the delayed administration of 0.6ml of juice (delay=6sec; rate=1ml/s). Juice was delivered using a computer-controlled syringe pump (Harvard Apparatus, USA) connected to a mouthpiece via small-bore IV tubing. Participants completed three, 26-trial blocks of learning/training events. During experimental runs participants were exposed to training/'normal' trials (N=58) interspersed with randomly selected 'catch' trials (N=20; Figure 1B), wherein the juice reward was unpredictably and pseudorandomly delayed by 4–7sec. It was anticipated that failing to

deliver the juice reward as predicted would generate a negative TDE (NTDE) signal, whereas the temporally unanticipated receipt of juice would engender a positive TDE (PTDE) signal (Figure 1C).

To determine juice palatability, at the end of each task block participants were asked to rate how much they liked the juice on a visual analogue scale (range +/-400).

Timing paradigm—Since brain regions that might be compromised in smokers overlap with those supporting second range timing function (47), the ability to accurately predict the time between the CS and juice reward was of concern. Therefore, subjects also completed a test of timing function post-MRI. Participants heard four 0.5Hz tones per trial (N=15). The first two tones were separated by a 6sec interval (i.e. equal to the light cue/juice ISI), as were the second and third. However, the interval between the tones three and four varied randomly between 4.5–7.5sec. Participants indicated whether the interval between the last two tones was shorter than, longer than, or equal to the first two intervals.

Functional imaging—Whole-brain echo planar images were acquired on a 3T Siemens Allegra scanner (Erlangen, Germany). Thirty-nine 4-mm slices were acquired in an oblique axial plane (30° to AC-PC) with the following imaging parameters: TR=2000ms, TE=27ms, FOV=220×220mm at 64×64, and flip angle=78°. Total functional scanning time was approximately 27mins. T1-weighted MPAGE structural imaging series with a voxel size of 1mm³ were also acquired.

Blood draw and analysis—Venous blood samples (5ml) were collected from smokers immediately following completion of each MRI session, centrifuged within 2h, and plasma stored at -20°C until analysis. A comprehensively validated liquid chromatography tandem mass spectrometry analysis was employed for simultaneous quantification of nicotine, cotinine, trans-3'-hydroxycotinine, and norcotinine in plasma (48).

Data analysis

Functional imaging data were analyzed using AFNI (49). To correct for head motion, 3D EPI data for each subject were registered to a base volume. The data were inspected for motion using the censor.py application (<http://brainimaging.waisman.wisc.edu/~perlman/code/censor.py>). Strict censoring criteria (i.e. translation>0.3mm or rotation>3° between consecutive TRs) were used to remove unwanted TRs prior to deconvolution. Individuals with a censor rate >25% were excluded. Data time series were analyzed using voxel-wise, multiple regression in which regressors were expressed as a delta function time-locked to event onset and convolved with a hemodynamic response function and its temporal derivative. There were 4 regressors of interest: light cue (*CS*), normal events (*UCS*; juice expected/juice delivered), *NTDE* (juice expected/juice not delivered) and *PTDE* (juice not expected/juice delivered). In addition, six motion parameters were included as regressors of no interest. For each participant and session, a voxel-wise average amplitude change (β) equal to the percentage signal change from baseline was calculated for each event type. The resultant activation maps were registered to a higher resolution (1μl) standard space (50) and spatially blurred using a 4.2mm FWHM Gaussian isotropic kernel.

A priori region of interest (ROI) analyses (Table S5 in the Supplement) focused on the impact of participant group and drug condition on regressor-related activity in regions along the trajectory of MCL and NS pathways. Bilateral ROIs in the SN, striatum (NAcc, caudate and putamen), and mPFC (BA10 & BA32; Figure S1 in the Supplement) were defined using a Talairach template in AFNI. The VTA ROI was defined as a 5mm sphere at its anatomical

locus (Talairach co-ordinates: 0 –16 –7). The mean value across voxels within each ROI was calculated for each participant/regressor and subsequently used as the dependent variable in statistical analyses. To address variability in nicotine metabolism, comparisons within smokers included nicotine plasma concentration as a covariate.

Behavioral data were analyzed in SPSS (SPSS Inc., USA). Analysis of Parrott scores considered the effects of group, drug condition, session, and time (pre- vs. post-scanning; i.e. immediately preceding the first scan vs. directly following completion of the final scan, within session). TCQ analysis examined four craving indices – emotionality (smoking in anticipation of relief from withdrawal/negative mood); expectancy (anticipation of positive outcomes from smoking); compulsivity (an inability to control tobacco use); and purposefulness (intention/planning to smoke).

Results

Parrott and TCQ

Controls vs. Smokers—Participants were more relaxed in session 2, vs. 1 ($F_{(1,39)}=6.48$, $p=0.01$) and were more distracted ($F_{(1,39)}=16.61$, $p<0.001$) and hungrier ($F_{(1,39)}=22.25$, $p<0.001$) by the end of each session. While smokers were more dissatisfied by the end of the scanning session (session 1: $t_{(20)}=-2.88$, $p=0.005$; session 2: $t_{(20)}=-1.83$, $p=0.04$), this difference was absent in controls ($F_{(1,39)}=4.39$, $p<0.05$). Smokers were also more tired at the start of the second session compared to controls ($t_{(40)}=-2.08$, $p=0.02$) but not by the end of the session (Table S1 in the Supplement).

Smokers: Nicotine vs. Placebo—Smokers were more relaxed ($F_{(1,20)}=8.45$, $p=0.009$), content ($F_{(1,20)}=5.81$, $p=0.03$), focused ($F_{(1,20)}=4.11$, $p=0.05$), satisfied ($F_{(1,20)}=4.59$, $p=0.04$) and less hungry ($F_{(1,20)}=5.54$, $p=0.03$) in the nicotine condition. Conversely, following placebo smokers experienced higher smoking expectancy ($F_{(1,20)}=4.76$, $p=0.04$) and purposefulness ($F_{(1,20)}=15.19$, $p=0.001$) scores on the TCQ. These data suggest that the nicotine patch prevented a mild withdrawal state seen in the placebo condition.

There was no interaction between time (pre vs. post-scanning) and drug condition on any measure of mood or craving in smokers (Table S2 in the Supplement).

Nicotine and its metabolites

Blood plasma concentrations of nicotine, cotinine, and norcotinine were higher at the end of the nicotine session, vs. placebo ($t_{\text{NICOTINE}(28)}=11.09$, $p<0.001$; $t_{\text{COTININE}(28)}=4.29$, $p<0.001$; and $t_{\text{NORCOTININE}(28)}=3.49$, $p=0.002$; Table S3 in the Supplement). Hydroxycotinine concentrations did not differ between conditions.

TCI

The TCI provides indices of novelty seeking, harm avoidance, reward dependence, persistence, self-directedness, cooperativeness, and self-transcendence, which purportedly reflect underlying neurobiology (e.g. dopaminergic, serotonergic or noradrenergic activity) (51). Controls and smokers were matched on all these aspects of temperament and character ($p>0.05$).

Cognitive measures (Table S4 in the Supplement)

Performance on all pre-scanning cognitive measures was equivalent between-groups ($p>0.05$). Controls and smokers also performed equally well on the timing task, and accuracy was consistent across conditions for smokers ($p>0.05$). Thus, irrespective of

GROUP or *DRUG CONDITION*, participants were equally able to accurately determine the duration of timing intervals approximating the light/juice ISI.

Juice palatability

Juice palatability did not vary as a function of *GROUP* or *DRUG CONDITION* ($p > 0.05$; mean(s.d.): nicotine=165.90(177.51); placebo=187.24(156.82); controls=238.79(106.65)) and there was no *SESSION*, *GROUP* or *DRUG* effect on the juice rating between the start and end of sessions ($p > 0.05$).

Functional imaging

Effect of acute nicotine—Acute nicotine administration in smokers did *not* alter activity in any *a priori* ROI, compared to the placebo. There was also no *SESSION* effect in control participants. Therefore, our results focus on the average activity across sessions in controls compared with that of smokers averaged across patch conditions. To enhance readability, the results of these between-groups analyses are presented in Table 2.

Effect of Event Type—A main effect of *EVENT TYPE* was noted in all MCL and NS subregions (Figure 2). In the BA10 division of mPFC this was driven primarily by a relative increase for CS vs. NTDE events, while activity in BA32 was equivalent for CS, UCS, and PTDE events but was comparatively reduced following NTDE events.

Striatal activity was also dependent upon *EVENT TYPE*. In the NAcc, activation associated with CS and UCS events exceeded that for NTDE in both cohorts. In putamen activation associated with NTDE events was smaller compared to all other event types, UCS- and PTDE-related activations were equivalent and both exceeded CS-dependent activity. Relatively lower activity corresponding to NTDE vs. all other event types was also seen in the caudate, where there was similarly greater activity for CS vs. UCS events. In accordance with models of TD-learning, this latter effect suggests ‘shift’ in phasic responding from the reward to the predictive stimulus. However, due to experimental design limitations we cannot confirm this. CS-related activity in the caudate was also greater than that in the putamen ($t_{(41)}=3.19$, $p=0.003$) and NAcc ($t_{(41)}=3.95$, $p<0.001$). These data indicate a partial dissociation in reward processing in striatal subregions, with *reward receipt* being processed predominantly in putamen and *TD-learning* effects primarily in the caudate.

The main effect of *EVENT TYPE* was identical in midbrain SN and VTA, where it was manifest as greater activity for CS, UCS and PTDE vs. NTDE events and greater activity for UCS and PTDE vs. CS events.

Effect of group—There was a main effect of *GROUP* in all striatal subregions and BA32, resulting from comparatively reduced activity in smokers (Figure 2).

Group x Event Type Interaction—Only the NAcc demonstrated a *GROUP* x *EVENT TYPE* interaction, which was manifest as less activity associated with PTDE events in smokers, and a relative reduction in activity for PTDE vs. NTDE events in controls only.

Smoking history—To delineate the influence of smoking characteristics, we considered the impact of (1) *DURATION* of smoking (years), (2) age at *FIRST* cigarette, (3) number of cigarettes *PER DAY*, and (4) *FTND* on activity associated with each event type in all ROIs. There was a main effect of *DURATION* in the NAcc ($F_{(1,19)}=5.28$, $p<0.05$) and caudate ($F_{(1,19)}=4.09$, $p=0.05$) and *EVENT TYPE* x *DURATION* interaction effects in BA32 ($F_{(1,19)}=11.02$, $p<0.01$) and putamen ($F_{(1,19)}=3.94$, $p<0.01$). Linear contrasts and *post hoc* bivariate correlations ($p<0.05$; one-tailed) confirmed that TDE-related activity in the caudate

(Figure 3A) and NAcc (Figure 3B) was negatively correlated with *DURATION* and that interaction effects in BA32 (Figure 3C) and putamen (Figure 3D) were due to a negative correlation between *DURATION* and PTDE events. *PER DAY* was negatively correlated with event-related activity in NAcc ($F_{(1,19)}=4.54$, $p<0.05$; Figure 3E), whereas *FIRST* was positively associated with NAcc activity ($F_{(1,19)}=5.17$, $p<0.05$; Figure 3F). *FTND* did not mediate event-related activity in any ROI and none of these factors influenced activity in BA10 or midbrain. These data suggest that in regions where activity was relatively reduced in smokers, functional processing was most consistently influenced by smoking chronicity.

Discussion

Using a classical conditioning paradigm, we observed outcomes indicative of “trait” effects of being a dependent smoker, but not state effects of acute nicotine administration, in TDE/ reward-related activity in regions along the trajectory of MCL and NS DA pathways. This effect was manifest as lower activity in smokers (vs. controls) in striatal and mPFC/BA32 subregions, but *not* the midbrain. Moreover, smoking-related reductions in activity were correlated with the duration of smoking (years) but not the severity of dependence (FTND).

Nicotine and TDE-related activity

Preclinical observations indicate that nicotine acts as both a primary reinforcer *and* enhances the incentive motivational and reinforcing effects of accompanying stimuli (52). However, nicotine’s influence on reward processing in humans may rely less on direct rewarding effects than its ability to modulate the rewarding properties of other stimuli, which may be integral to nicotine addiction and underlie behavioral phenomena such as the increased propensity to smoke while drinking (53). Yet, a direct pharmacological effect of acute nicotine when using a natural (juice) reward was not observed. Rather, differences in TDE-related activity were only seen as a function of group, i.e. smokers vs. non-smokers. This suggests that this stimulus-independent property of nicotine’s influence on reward/ reinforcement might be more related to chronic drug exposure induced neuroplasticity rather than acute nicotine, *per se*.

The lack of an effect of acute nicotine administration implies a trait-like influence of chronic nicotine exposure/smoking status on reward processing. That group differences in reward-related activity were associated with smoking longevity raises the issue of how functional changes in DArgic pathways, especially striatal pathways, may be mediated by repeated nicotine exposure. Human and animal models indicate upregulation of nAChRs following chronic nicotine, an effect perhaps related to desensitization of these receptors (see 54,55 for review). Chronic nicotine enhances $\alpha 4^*$ receptors in striatal pathways (56–57), and selective upregulation of these subunits on GABAergic cells *plus* chronic stimulation by nicotine engenders decreased DArgic function (58). Moreover, $\alpha 4\beta 2^*$ receptors that mediate cholinergic modulation of DA release underlying nicotine reinforcement (59) show decreased availability following both nicotine and smoking (see 54 for review). Thus, lower reward-related activity in smokers may result from selective upregulation of $\alpha 4\beta 2^*$ nAChRs in striatal pathways, coupled with high receptor occupancy levels in non-withdrawn/sated smokers, impacting upon cholinergic- and/or GABAergic-mediated DArgic activity. Moreover, a reduction in the effective availability of $\alpha 4\beta 2^*$ nAChRs in DArgic pathways in smokers may contribute to a baseline shift in neuronal activity, which may have reduced to the potential for event-related increases seen in controls.

The absence of an acute drug effect could simply reflect the nicotine dose used. However, behavioral differences in mood ratings between sessions indicate that nicotine bioavailability post-patch was sufficient to minimize a mild withdrawal. Furthermore, investigations of the time to peak dose for transdermal nicotine suggest that within the time frame of the

experiment (4 hours), a dose would reach maximal dose effects and approach steady-state nicotine levels, even in previously withdrawn individuals (42).

It is intriguing that reward-related activity in these otherwise dependent smokers was not associated with dependence severity (FTND). FTND and other indices of dependence are associated with underlying genetic variability (60–61) that might predict smoking duration, such that individuals with a high genetic load for dependence may be less likely to quit and more likely to smoke for longer compared to those with lower load. If so, correlations between brain activity and smoking chronicity may be mediated by an association of duration and dependence. However, despite behavioral evidence supporting the predictive value of FTND scores for cessation (62), a recent investigation found that polymorphisms predicting dependence were *not* predictive of successfully quitting (63). Moreover, here FTND scores were not correlated with DURATION or FIRST, suggesting that lower reward-related activity in smokers was perhaps the consequence of, not an antecedent to, smoking status.

Smoking, Negative Affect and Reward

Our results extend observations countering the commonly held notion that nicotine provides a means of coping with negative affect. Rather, recent studies suggest that acute nicotine *does not* ameliorate negative affect or reduce subjective reports of stress and anxiety in abstinent *or* non-abstinent conditions and may actually increase the risk of depression (64–66). Group differences reported here may underlie these observations. Indeed, the negative correlation between reward-related activity and smoking duration and the failure of nicotine administration to “normalize” activity in DA pathways support the contention that chronic smoking changes reward processing in a way that is refractory to acute nicotine. This may be a critical factor in the failure of nicotine replacement therapies (NRT) in smoking cessation. If so, NRT alternatives that modulate DArgic activity with a distinct mechanism, such as varenicline and bupropion (67–68), may prove more efficacious.

TDE-related activity

TDE-related signals are thought to originate in the midbrain (69), however, our data did not support this, and instead suggest a pattern of midbrain activity more specific to reward *receipt* than *prediction* or TDE. In contrast, functional changes in the caudate concomitant with reward prediction signals were detected (i.e. CS > UCS), and are supported by recent preclinical evidence (70). Since midbrain TDE signaling to both primary and secondary rewards has been previously reported (71), variability in imaging parameters and effect size could account for this disparity. Alternatively, since VTA and SN provide afferent fibers into the caudate (20), caudate TD signals may be the upstream consequence of learning calculations performed in the midbrain (72). Contradictory to previous observations (13), reward-related activity in the putamen did not code for TD-learning. In light of the considerable overlap in connectivity from midbrain regions to the caudate *and* putamen (20), relative differences in CS/UCS processing between the caudate and putamen may simply reflect a detection limitation due to the experimental paradigm.

Experimental limitations

Since training trials were not imaged, we cannot definitively attribute the CS/UCS response differential in the caudate to a learning-dependent shift in DArgic activity. Furthermore, there was no clear effect of expectedness on striatal activity. Although consistent with an earlier study by our group (73), it contradicts the original investigation using this paradigm (13). Experimental variability, such as differences in the delay between CS and PTDE and in the number/ratio of catch events, may account for this difference. Unique trial types

involving the omission of juice reward (NTDE-only trials) *or* temporally uncoupling juice from the CS (PTDE-only trials) may better delineate expectedness.

Pharmacological manipulation studies using BOLD require additional consideration with respect to non-specific signal transduction interactions. However, since smoking was associated with regionally-specific/task-dependent effects in the absence of global perturbations, between-group differences are unlikely to reflect disease-related vascular alterations (74) but rather functional consequences of alterations in receptor and/or neurotransmitter function at specific locations, especially the striatum. Nonetheless, regionally nonspecific effects cannot be completely precluded and while smoking-related differences are attributed to chronic nicotine intake, cigarettes contain thousands of compounds (75), any number of which could contribute to the observed differences. Yet, 'contaminants' (e.g. CO, tars) might also be expected to have a more global action, arguing for particular nAChR-induced neuroplasticity rather than global neurotoxicity.

Importantly, our experimental paradigm does not allow for the disambiguation of the effects of acute nicotine and withdrawal alleviation. Considering reward function at different time points since last cigarette, using more substantially withdrawn participants, or the administration of acute nicotine to nicotine-naïve individuals would help clarify this issue.

Finally, despite the comparatively robust ANCOVA results, *post hoc* correlations used to confirm the directionality of the relationship between smoking characteristics and activity were potentially underpowered due to the relatively small number of subjects and should be cautiously interpreted.

In sum, reduced TDE-/reward-related processing was seen in striatum and mPFC, but not in the midbrain, of smokers. These effects were related to chronic nicotine exposure and were not amenable to acute, transdermal nicotine delivery. The impact of chronic nicotine exposure on TD-related activity may have implications for the hedonic consequences of smoking cessation and may be highly relevant for the efficacy of treatment strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Temporal difference error (TDE)/juice paradigm. Shown are graphical representations of: **A.** Training events; and **B.** ‘Catch’ trials, which include both negative and positive TDE events. ‘Normal’ trials were a replication of the training trials. **C.** Representation of the predicted change in DArgic function in regions supporting TD learning and TDE before (left) and after (right) training events.

Figure 2.

The impact of event type (CS/UCS/NTDE/PTDE) and group (controls vs. smokers) on the mean signal change in *a priori* anatomical regions of interest along the trajectory of the MCL and NS DA pathways (i.e. mPFC (BA10 & BA32), striatum (i.e. NAcc, caudate and putamen), and midbrain (SN and VTA)). Note: error bars show ± 1 standard error; †=significant main effect of group ($p < 0.05$); *=significant interaction ($p < 0.05$)

Figure 3.

Smoking chronicity and reward-related activity. The duration of smoking (years) had a main effect on reward-related function in **A.** the caudate and **B.** NAcc; both of which were driven by a negative correlation between duration and activity across event types in both regions. There was also an interaction between duration and activity, resulting from a negative correlation involving duration and PTDE-related signal change in **C.** BA32 and **D.** the putamen. Similarly, activity in the NAcc. was positively associated with age at which participant smoked their first cigarette (**E.**) and negatively associated with the number of cigarettes smoked per day (**F.**). All correlations shown here are significant at $p < 0.05$ (one-tailed).

Table 1

Participant demographic information. Note: there was no statistically significant difference between groups in any relevant measure.

	Smokers (N =21)	Controls (N=21)
Age (years: mean (s.d.))	32.90 (9.77)	30.29 (8.74)
Gender (male: female)	8: 13	12: 9
IQ (WASI: mean (s.d.))	106.95 (11.06)	109.16 (13.93)
Education (years: mean (s.d.))	12.90 (2.71)	13.95 (2.54)
Ethnicity (AA: C: As)	7: 14: 0	6: 14: 1
Cigarettes/day (mean (s.d.))	22.55 (5.60)	n/a
Age at first use (years: min-max (mean))	9 – 31 (15.62)	n/a
Years of use preceding study (min-max (mean))	2.5 – 38 (16.36)	n/a
FTND (min-max (mean))	3 – 9 (5.76)	n/a

Note: AA=African American; C=Caucasian; As=Asian/Asian American

Summary statistics from the ROI analysis of reward-related activity in MCL and NS brain regions – Smokers vs. Controls.

<i>Striatum</i>	Caudate			
			CS vs. NTDE: $t_{(41)}=7.19^{***}$	
			UCS vs. NTDE: $t_{(41)}=3.30^{**}$	
		$F_{(1,120)}=4.69^*$ (smokers < controls)	PTDE vs. NTDE: $t_{(41)}=2.63^*$	nil
			CS vs. UCS: $t_{(41)}=2.85^*$	
			PTDE vs. CS: $t_{(41)}=2.40^*$	
			CS vs. NTDE: $t_{(41)}=7.81^{***}$	
			UCS vs. NTDE: $t_{(41)}=8.86^{***}$	
		$F_{(1,120)}=6.94^*$ (smokers < controls)	PTDE vs. NTDE: $t_{(41)}=6.47^{***}$	nil
			UCS vs. CS: $t_{(41)}=4.89^{***}$	
			PTDE vs. CS: $t_{(41)}=2.40^*$	
			CS vs. NTDE: $t_{(41)}=3.36^{**}$	
NAcc		$F_{(1,120)}=5.76^*$ (smokers < controls)	UCS vs. NTDE: $t_{(41)}=2.99^{**}$	$F_{(3,20)}=4.15^*$ (PTDE – smokers < controls; $t_{(40)}=-2.36^*$)
			PTDE vs. NTDE: $t_{(41)}=2.38^*$	
Midbrain	VTA		CS vs. NTDE: $t_{(41)}=3.66^{**}$	
			UCS vs. NTDE: $t_{(41)}=4.30^{**}$	
		nil	PTDE vs. NTDE: $t_{(41)}=3.66^{**}$	nil
			UCS vs. CS: $t_{(41)}=2.64^*$	
			PTDE vs. CS: $t_{(41)}=2.21^*$	
			CS vs. NTDE: $t_{(41)}=5.78^{***}$	
			UCS vs. NTDE: $t_{(41)}=7.43^{***}$	
SN		nil	PTDE vs. NTDE: $t_{(41)}=6.22^{***}$	nil
			UCS vs. CS: $t_{(41)}=3.66^{**}$	
		$F_{(3,120)}=28.78^{***}$		

	GROUP	EVENT TYPE		Interaction
		a priori	post hoc	
mPFC	BA10		PTDE vs. CS: $t_{(41)}=3.48^{**}$	
			CS vs. NTDE: $t_{(41)}=3.46^{**}$	nil
		$F_{(3,120)}=2.87^*$	CS vs. NTDE: $t_{(41)}=5.87^{***}$	
	BA32	$F_{(1,120)}=5.38^*$ (smokers < controls)	UCS vs. NTDE: $t_{(41)}=3.89^{**}$	nil
			PTDE vs. NTDE: $t_{(41)}=3.27^{**}$	

Note:

p < 0.001;
**
p < 0.005;
*
p < 0.05