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Lifetime cigarette smoking is associated with striatal volume measures

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ABSTRACT

Nicotine, the primary addictive component of tobacco, affects the mammalian brain. Smokers' brains have smaller cortical grey matter volumes and/or lower densities compared with non-smokers'. Differences in subcortical structures like the striatum are however, less clear. A high concentration of nicotinic receptors makes the striatum a potential target for nicotine. In addition, striatal nuclei are essential components of the reward/reinforcement pathway involved in addiction. The aim of this study was to explore the relationship between striatal nuclei (caudate, putamen and nucleus accumbens area) volumes and lifetime smoking in a large community-based sample of 'young–old' individuals. Brain volumes were measured using a semi-automated method in 315 participants aged 64–70 years who were selected from a larger randomly sampled cohort and who consented to a magnetic resonance imaging scan. Multiple regression analysis was used to assess the relationship between striatal volumes and cigarette smoking measures while controlling for age, sex, intracranial and total brain volumes and general physical and mental health measures. Greater lifetime use of cigarettes (measured in pack-years) was associated with smaller left nucleus accumbens area volume ($P = 0.018$) and larger left putamen volume ($P = 0.025$). Greater putaminal volume was also associated with a lower age at smoking initiation ($P = 0.004$). In this generally healthy cohort, lifetime use of cigarettes is significantly associated with striatal volume measures. These changes could indicate predisposing factors for nicotine addiction, or an effect of chronic nicotine exposure or a combination of both.

Keywords Brain volume, magnetic resonance imaging, nucleus accumbens, putamen, smoking.

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INTRODUCTION

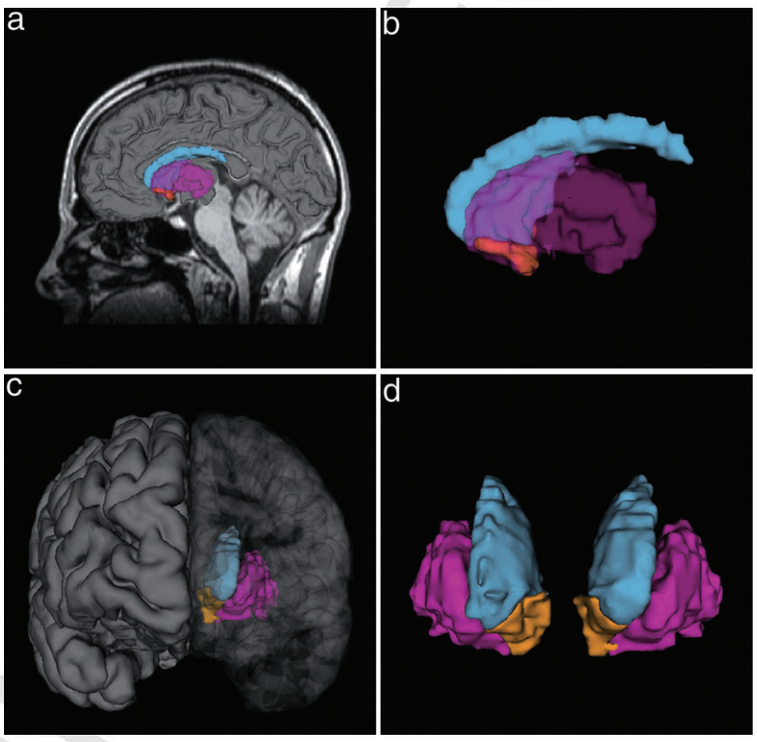
Tobacco use in the form of cigarettes remains a significant source of mortality/morbidity worldwide (Murray 2006). Despite increased awareness of its adverse effects, tobacco use continues largely because of its highly addictive nature. Among the hundreds of compounds present in tobacco products, nicotine, an alkaloid produced by the tobacco plant for its insecticidal effect, is primarily responsible for this addictive property (Benowitz 1988). Nicotine binds with highest affinity to neuronal nicotinic acetylcholine receptors (nAChRs) (Whiting & Lindstrom 1988) and mimics the action of acetylcholine (Gaimarri *et al.* 2007), a neurotransmitter endogenous to the nervous system. In the central nervous system, nAChRs are present both pre- and post-synaptically on different neuronal subtypes and have a neuromodulatory function (Gaimarri *et al.* 2007). This property of neuronal nAChRs allows nicotine to have a secondary effect on virtually all neurotransmitter/neuromodulator systems in the brain (Evans & Drobes 2009). Thus it is not surprising that nicotine's influence encompasses a wide range of cognitive processes including sensory, motor, attention, executive, learning and memory functions (Evans & Drobes 2009). Repeated nicotine exposures complemented by environmental cues produce lasting changes in dopaminergic (DA) signals in the brain reward/reinforcement centres resulting in addiction (Miyata & Yanagita 2001).

Several functional imaging studies have reported nicotine induced changes in human brain activation patterns (Sharma & Brody 2009). However, anatomical and neurochemical changes resulting from nicotine exposure, evident in animals studies (Domino 2008), remain

less explored in humans. In voxel-based morphometric (VBM) studies greater lifetime exposure was correlated with greater brain atrophy in elderly smokers (Longstreth *et al.* 2000) and reduced cortical grey matter volumes/densities in specific cerebral (e.g. frontal and temporal lobes) and cerebellar regions in younger smokers (Brody *et al.* 2004a; Gallinat *et al.* 2006). Group differences in volume/densities in these brain regions were also observed between smokers and non-smokers (Brody *et al.* 2004a; Gallinat *et al.* 2006). Gallinat *et al.* (2006) also reported decreased grey matter volume/density in the thalamus and substantia nigra (SN) in smokers.

Unlike animal studies in which the brain-damaging effect of nicotine exposure is clearly evident, human studies have failed to clarify whether the distinct features of a smokers' brain are predisposing factors for nicotine addiction or the effects of chronic nicotine exposure, or a combination of both (Brody *et al.* 2004a; Domino 2008). Nevertheless, it is interesting that the brain regions reported to have reduced volume/density in smokers also express a rich repertoire of nAChRs. [^{3}H]Nicotine binding studies revealed that in the human brain nAChR density decreases in the following order: thalamus > SN > striatum > cerebral cortex (Court *et al.* 2000). With the exception of the striatum, all these structures were reported to be reduced in grey matter volume/density in smokers (Brody *et al.* 2004a; Gallinat *et al.* 2006). The striatum is a group of subcortical nuclei and can be structurally divided into the caudate (Cau), putamen (Put) and nucleus accumbens (NAc) (or ventral striatum) (Fig. 1). As part of fronto-subcortical neuronal circuits, the striatum plays a critical role in planning, execution, and control of movement, acquisition of motor sequences, learning, reward processing, cognitive functioning and addiction (Raz *et al.* 2003). Previous studies compared only the ventral striatum [the most important region for reward processing (Knutson *et al.* 2000)] and found no significant differences in grey matter volume/density between smokers and non-smokers. Gallinat *et al.* (2006) commented that this might indicate that smoking primarily affects cerebral structures associated with inhibitory control of behaviour (e.g. the prefrontal cortex) than incentive drive (e.g. the ventral striatum). Alternatively, methodological limitation and small sample size could be the underlying reason (Gallinat *et al.* 2006).

Owing to its role in the reward/reinforcement pathway, the striatum is critical for the development of nicotine addiction and with its high nAChR concentration also presents a potential target for nicotine. Furthermore, decrease in striatal nuclei volumes is detrimental for normal brain function. Atrophy of striatal nuclei occurs during normal ageing (Raz *et al.* 2003) and in pathological conditions like Parkinson's disease (PD) (Geng, Li & Zee 2006). In healthy aged individuals decrease in striatal nuclei volumes is associated with severity of gait and balance problems (Rosano *et al.* 2007) while in PD patients, striatal nuclei atrophy is



Colour

Figure 1 Three-dimensional model of the human brain indicating the position of the striatal nuclei. (a) Saggital view of the model superimposed on the magnetic resonance imaging image of head (c) anterior view. Magnified images showing only striatal structures in the saggital (b) and anterior (d) views, respectively. Caudate is indicated in blue, putamen in pink and nucleus accumbens area in yellow

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correlated to the severity of clinical symptoms (Geng *et al.* 2006). In this study we explore the relationship between cigarette use and striatal nuclei volumes in a community-based sample of healthy individuals aged between 64 and 70 years. We are unaware of any previous report that has explored the relationship between these critical subcortical structures and nicotine exposure in a large non-clinical sample in this age group.

METHODS AND MATERIALS

Participants

The study sample was drawn from the PATH Through Life Project designed to investigate the risk and protective factors for normal ageing, dementia and other neuropsychiatric disorder (Jorm *et al.* 2004) in three age groups (20–24, 40–44, 60–64) of randomly selected individuals who were residents of the city of Canberra and the adjacent town of Queanbeyan, Australia, and to be followed-up every 4 years for 20 years. Participants were recruited randomly from the electoral roll, which provides a good representative population sample because enrolment to vote is a legal requirement for all adult Australian citizens. The study was approved by the ethics committees of The Australian National University and The University of New South Wales. All participants gave written informed consent to be included in the PATH project. The present study was focused on the older cohort at the second wave of data collection, which included 2222 individuals aged 64–70 years. During the interview participants provided information about age, sex, years of education, occupation, smoking, substance use, medical, psychiatric, medication history, etc. A randomly selected subsample of 622 participants was offered a magnetic resonance imaging (MRI) scan, which 478 eventually completed. Of those 431 participants took part in the second wave of data collection and after exclusions for a history of stroke ($n = 8$), cognitive impairments ($n = 12$), and poor scan quality or missing data for the variables used in statistical analyses ($n = 96$), 315 participants were available for this investigation. The sample for which MRI data was available (MRI+) did not differ significantly from the remaining cohort (MRI-) in any of the demographic and smoking variables except for years of education ($P = 0.032$) and total years of smoking ($P = 0.014$). The MRI+ sample had greater mean years of education and lower mean total years of smoking.

MRI acquisition

MRI data were acquired on a 1.5 Tesla Gyroscan scanner (ACS-NT, Philips Medical Systems, Best, the Netherlands). T1-weighted 3-D structural MRI images were acquired in coronal plane using Fast Field Echo (FFE) sequence. The scanning parameters were TR = 8.84 ms, TE = 3.55 ms, a flip angle of 8°, matrix size = 256 × 256, slices 160, and the field of view (FOV) 256 × 256 mm. Slices were contiguous with slice thickness of 1.5 mm.

Image analysis

Volumetric segmentation was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). This processing includes 3 motion correction, removal of non-brain tissue using a hybrid watershed/surface deformation procedure (Ségonne *et al.* 2004), automated Talairach transformation, and segmentation of the subcortical structures (including hippocampus, amygdala, Cau, Put, NAc area, ventricles) (Fischl *et al.* 2002, 2004).

Cigarette smoking measures

Participants reported on cigarette use by answering questions such as, 'Do you currently smoke?', 'Have you ever smoked regularly?', 'On an average how many cigarettes you have smoked each day over the time you were smoking?', 'At what age did you start smoking?' and 'At what age did you stop smoking?'. Based on their smoking history participants were grouped as non-smokers (currently not smoking and never smoked regularly) or smokers (including current and ex-smokers). Average number of cigarettes smoked per day (CPD) over the time smoked and age at which smoking started were used to compute total years of smoking and pack-years. Four continuous smoking variables—pack-years, CPD, total years of smoking and age at start of smoking and one categorical variable—smoking status (non-smoker versus smoker) were used in this study. For regression analysis, pack-years, CPD and total years of smoking were entered as continuous variables, with non-smokers having a value of zero.

Brain volume measures

All brain volume measures were entered as continuous variables. Whole-brain measures used were—intracranial volume (ICV), total brain volume (TBV) and total ventricular volume (TVV). Striatal nuclei (Cau, Put NAc) volumes used were uncorrected raw volumes measured separately for left and right hemispheres.

Statistical analysis

All statistical analyses were conducted using SPSS 18 (SPSS Inc., Chicago, IL, USA). Means and standard deviations were computed for all continuous variables of interest. Comparisons between 'non-smoker' and 'smoker' categories were performed using Student's *t*-test

for continuous variables and Pearson's χ^2-square test for categorical variables. Association of each of the continuous cigarette smoking measures (dependent variables) and whole-brain measures or striatal nuclei volumes (independent variables) were assessed by multiple regression while controlling for covariates (age, sex). Cigarette smoking can be affected by a number of factors including education level, physical health and mental health disorders like anxiety and depression (Lawrence, Mitrou & Zubrick 2009). Hence, in addition to age and sex, we included years of education, physical health [RAND-12 physical health scale (Hays, Prince-Embury & Chen 1998)], diabetes and hypertension status, and symptoms of anxiety and depression [Goldberg's scale (Goldberg *et al.* 1988)] as covariates. The inclusion or exclusion of the health variables mentioned above did not alter results of the analyses significantly. Regression models with the health variables included as covariates are reported. As the striatal nuclei volumes used in the analysis were uncorrected raw volumes, we also controlled for ICV and TBV. Analysis of age of smoking initiation was performed for the 'smoker' category only, after controlling for age, sex, ICV and TBV. A high prevalence of comorbid chronic smoking and alcohol dependence has been reported (Meyerhoff *et al.* 2006) and chronic alcohol dependence is associated with smaller brain volumes (Makris *et al.* 2008). We therefore carried out additional regression analyses where we included variables for alcoholic drink consumption. Interaction effects of striatal nuclei volumes × sex and striatal nuclei volumes × alcoholic drink consumption were also tested. In regression analysis the covariates were entered in the model first, followed by the predictors and then the interactions terms. Change in R^2 value between the two models and the P value associated with the R^2 change were noted. Predictors with $P > 0.10$ were progressively removed to generate a reduced model. The threshold of significance was set at $P = 0.05$. As none of the interaction terms contributed significantly models including interaction terms are not reported.

RESULTS

Smoker and non-smoker groups were similar in age, education level, physical health scores, prevalence of hypertension, diabetes and anxiety/depression symptoms (Table 1). There were significant differences in sex ratio between the two groups, with a higher proportion of women being non-smokers ($P = 0.002$). Smokers also consumed significantly ($P < 0.001$) higher amounts of alcoholic drinks per week. For the brain volume measures (Table 2), smokers had significantly higher ICV ($P = 0.029$), TBV ($P = 0.042$) and TVV ($P = 0.005$) while Cau, Put and NAc volumes were similar in both groups. The apparent difference in ICV between smokers and non-smokers disappeared after correcting for sex. However, significant ($P < 0.05$) differences in TBV and TVV remained after correcting for sex and ICV with smokers having smaller TBV and larger TVV compared with non-smokers.

Table 1 Demographic characteristics and cigarette use history of smokers and non-smokers (Mean ± SD for continuous variables and frequency for categorical variables shown).

	Smoker (n = 123)	*Non-smoker (n = 192)*	*d.f.*	t/χ^2	*P*
Age (years)	66.5 ± 1.5	66.7 ± 1.4	313	1.165	0.245
Sex					
Male	77	86	1	9.524	0.002**
Female	46	106			
Education (years)	14.5 ± 2.6	14.2 ± 2.7	313	–0.848	0.397
RAND-12 physical health (Hays *et al.* 1998)	49.49 ± 9.49	49.92 ± 9.33	313	0.415	0.678
Diabetes	12 (9.76%)	18 (9.38%)	1	0.005	0.945
Hypertension	57 (46.43%)	85 (44.27%)	2	0.411	0.814
Goldberg's anxiety score (Goldberg *et al.* 1988)	2.42 ± 2.87	2.26 ± 2.23	313	–0.602	0.548
Goldberg's depression score (Goldberg *et al.* 1988)	1.77 ± 1.87	1.73 ± 1.87	313	–0.176	0.861
Alcoholic drinks/week	10.6 ± 10.4	5.7 ± 7.4	313	–4.924	<0.001**
Pack-years	26.42 ± 25.67				
CPD	19.2 ± 13.4				
Years of smoking	25.7 ± 14.0				
Age at start of smoking	18.7 ± 5.85				

t-tests were performed for continuous variables and χ^2 tests for categorical variables; *Significant at 0.05 level; **Significant at 0.01 level; CPD = Cigarette per day.

Table 2 Brain measures (raw volumes) of smokers and non-smokers (mean ± standard deviation).

	Smoker (n = 123)	*Non-smoker (n = 192)*	*d.f.*	*t*	*P*
ICV (litres)	1.56 ± 0.18	1.52 ± 0.18	313	−2.197	0.029*[a]
TBV (litres)	1.53 ± 0.18	1.49 ± 0.17	313	−2.043	0.042*[b]
TVV (litres)	0.034 ± 0.002	0.028 ± 0.001	313	−2.817	0.005**[b]
N Accumbens volume (ml)					
Left	0.50 ± 0.10	0.51 ± 0.09	313	0.881	0.379
Right	0.54 ± 0.08	0.53 ± 0.08	313	−0.625	0.533
Putamen volume (ml)					
Left	4.89 ± 0.64	4.77 ± 0.60	313	−1.657	0.098
Right	4.89 ± 0.66	4.75 ± 0.58	313	−1.863	0.063
Caudate volume (ml)					
Left	3.36 ± 0.53	3.29 ± 0.48	313	−1.172	0.242
Right	3.70 ± 0.59	3.64 ± 0.53	313	−1.051	0.294

[a]Not significant after corrected for sex; [b]significant after corrected for sex; *Significant at 0.05 level; **Significant at 0.01 level; ICV = intracranial volume; TBV = total brain volume; TVV = total ventricular volume.

Table 3 Multiple regression models for smoking measures with TBV or TVV as predictors.

Cigarette use variables	*TBV*			*TVV*		
	Beta (P)	*R^2 (change)*	*P*	*Beta (P)*	*R^2 (change)*	*P*
Pack-years[a]	**−2.648 (0.000)**	0.125 (0.037[c])	0.000	**0.243 (0.000)**	0.125 (0.037[c])	<0.001
CPD[a]	**−2.312 (0.002)**	0.136 (0.028[c])	0.002	**0.213 (0.002)**	0.136 (0.028[c])	0.002
Years of smoking[a]	**−2.309 (0.003)**	0.064 (0.028[c])	0.002	**0.211 (0.004)**	0.063 (0.027[c])	0.004
Age at start[b]	−0.474 (0.662)	0.050 (0.002)	0.662	0.050 (0.648)	0.050 (0.002)	0.648

[a]Controlled for age, sex, education, physical and mental health measures, intracranial volume; [b]Controlled for age, sex, intracranial volume; [c]0.05 Significant R^2 change from model with only covariates as predictors; TBV = total brain volume; TVV = total ventricular volume.

Means and standard deviations of the cigarette smoking measures—(1) magnitude of lifetime use of cigarettes or pack-years; (2) average number of cigarettes consumed per day (CPD); (3) total years of smoking; (4) age at start of smoking are given in Table 1. We assessed associations between the cigarette use and whole-brain (TBV and TVV) or striatal nuclei volumetric measures by multiple regression while controlling for covariates (see Methods). TBV was negatively and TVV positively associated with pack-years, CPD and total years of smoking (Table 3). No significant associations were observed between age at start of smoking and either of the whole-brain volume measures (Table 3).

Results of regression analyses with smoking measures as dependent variables and Cau, Put, NAc volumes as predictors are presented separately for the left and right hemispheres (Table 4). Significant associations were observed only for left hemispheric volumes, which are described below. After controlling for covariates, NAc and Put but not Cau volumes were significantly associated with lifetime use of cigarettes. After progressively removing variables that did not reach statistical significance, only NAc volume remained as a significant predictor in the reduced model. The association between lifetime use and NAc volume was negative, indicating that heavier cigarette consumption correlated with smaller NAc volume. The pack-year measure of lifetime use includes measures of average daily cigarette use (CPD) and duration (total years of smoking behaviour), both of which independently showed a trend towards a negative association with NAc volume but failed to reach significance (Table 4).

In contrast, the association between lifetime use and Put volume was positive, indicating a correlation of greater use with higher Put volume. CPD and total years of smoking also exhibited positive correlations with Put volume but did not reach statistical significance ($P > 0.1$). In smokers, larger left hemispheric Put volume was significantly associated with a lower age at smoking initiation (Table 4). A similar trend was observed for the right hemispheric Put volume, which did not reach statistical significance ($P = 0.063$).

Although alcoholic drinks used per week was negatively associated ($\beta = -0.019$, $P < 0.001$) with TBV, the associations between striatal nuclei volumes and cigarette smoking variables described above did not change significantly after controlling for alcoholic drink consumption. Smoking status (non-smoker versus smoker)

Table 4 Multiple regression models for smoking measures with striatal nuclei volumes as predictors.

Cigarette use variables	*Model*	*Left*					*Right*				
		Beta (P)					*Beta (P)*				
		NAc	*Put*	*Cau*	R^2 *(change)*	*P*	*NAc*	*Put*	*Cau*	R^2 *(change)*	*P*
Pack-years[a]	Full	**−0.152 (0.021)**	**0.181 (0.025)**	−0.095 (0.210)	0.153 (0.037[d])	0.025	0.027 (0.693)	0.141 (0.105)	−0.120 (0.146)	0.136 (0.011)	0.305
	Reduced	**−0.157 (0.018)**	0.131 (0.063)		0.148 (0.023[d])	0.021					
CPD[a]	Full	−0.118 (0.075)	0.148 (0.068)	−0.095 (0.212)	0.154 (0.018)	0.105	0.000 (0.999)	0.156 (0.070)	−0.121 (0.139)	0.147 (0.011)	0.287
	Reduced[c]	−0.122 (0.064)	0.098 (0.165)		0.150 (0.013)	0.101		0.084 (0.209)		0.141 (0.005)	0.209
Years of smoking[a]	Full	−0.126 (0.069)	0.093 (0.271)	−0.067 (0.405)	0.078 (0.014)	0.221	0.072 (0.314)	0.054 (0.552)	−0.097 (0.258)	0.072 (0.008)	0.503
	Reduced	−0.119 (0.079)			0.074 (0.010)	0.079					
Age at start[b]	Full	−0.117 (0.276)	**−0.400 (0.004)**	0.205 (0.117)	0.126 (0.076[d])	0.023	−0.007 (0.950)	−0.288 (0.063)	0.156 (0.275)	0.083 (0.033)	0.256
	Reduced		**−0.264 (0.015)**		0.098 (0.048[d])	0.015		−0.178 (0.091)		0.073 (0.023)	0.091

[a]Controlled for age, sex, education, physical and mental health measures, intracranial volume and total brain volume; [b]Controlled for age, sex, intracranial volume and total brain volume; [c]After removing left Put from the model, left NAc was no longer significant; [d]0.05 significant R^2 change from model with only covariates as predictors.

was significantly associated with TBV (β = −0.010, $P = 0.017$) and TVV (β = 0.111, $P = 0.018$) after controlling for age, sex, education and health covariates but not with any of the striatal measures.

DISCUSSION

This study detected significant associations between brain volumes and measures of cigarette smoking. We found significant correlations between whole-brain volumes and smoking history. Our results suggest that as lifetime cigarette use increases, TBV decreases with concomitant increase in the volume of brain ventricles. Among the striatal nuclei, significant associations with smoking measures were observed only for left hemisphere with smaller NAc and larger Put volumes associated with greater lifetime cigarette use. NAc volume explains 1.3% of the variance in pack-years in our sample. The relationship between NAc volume and pack-years was not observed in two earlier studies (Brody *et al.* 2004a; Gallinat *et al.* 2006). One possible reason for this is that our study sample size is significantly larger than that of the previous studies. We observed interesting relationships between Put volume and lifetime use of cigarettes and age at start of smoking, which have not been reported in any previous study that we are aware of. Larger left hemispheric Put volume was associated with greater lifetime cigarette use and a lower age of smoking initiation. Age at which smoking is initiated has a large impact on future smoking behaviour. Early initiation was reported to be associated with increased nicotine dependence, greater consumption, longer duration and lower quitting rates (Khuder, Dayal & Mutgi 1999).

In the absence of information on brain volume measures prior to smoking onset, it is not possible to infer the causal relationships between brain volume measures and cigarette use from these associations. However, our result suggesting association between smaller brain volume and greater pack-years is in line with previous reports on increased brain atrophy in smokers (Longstreth *et al.* 2001). In the context of striatal nuclei, a small NAc and a large Put (relative to the whole brain) might indicate vulnerability to nicotine addiction. Conversely, given the evidence supporting the effect of nicotine on the mammalian brain, these features might represent consequences of chronic nicotine exposure. Longitudinal imaging studies tracking smokers during years of active smoking are required to distinguish definitely between these alternatives.

Irrespective of the direction of causality, our finding that NAc volume was negatively associated with lifetime use of cigarettes provides evidence supporting the importance of the NAc in nicotine addiction pathway, as suggested in previous studies (Wise & Bozarth 1987; Koob

1992; Balfour 2004). According to the psychomotor stimulant theory of addiction (Wise & Bozarth 1987), the ability to activate the DA neurons of the ventral tegmental area (VTA) is a characteristic property of all drugs of abuse and is fundamental to their ability to cause dependence. This activation results in a surge of dopamine in the NAc, the primary recipient of the VTA DA terminals (Balfour 2004). Substantial evidence from animal studies demonstrates that nicotine-induced dopamine release in the NAc underlies the reinforcing properties of nicotine addiction (Koob 1992). Positron emission tomography imaging has demonstrated increased dopamine release in human NAc during smoking (Brody *et al.* 2004b). Thus structural differences in this nucleus might represent features of chronic smoking.

The positive correlation between smoking measures and Put volume is in contrast to the trend observed for total brain and other striatal nuclei volumes. Again in the absence of any information on brain volume measures prior to smoking onset, there can be at least two possible explanations for these observations: (1) individuals with larger Put volumes are more likely to become heavy smokers or start smoking at a younger age; (2) greater nicotine exposure owing to greater use or an earlier start protects against age-related putaminal atrophy, so that Put volume declines less in heavy smokers and thus becomes relatively larger compared with non-smokers as they age. While the importance of the Put in development of nicotine addiction is not clear, a neuroprotective role of nicotine is indicated in studies of PD.

A characteristic feature of PD is a selective loss of DA neurons in the SN and loss of dopamine in the striatum resulting in severe motor dysfunction (Shimohama 2009). Putaminal atrophy, evident even prior to any significant loss of SN volume, correlates with severity of motor deficits (Geng *et al.* 2006). Epidemiological studies suggested that cigarette smoking is associated with a decreased risk of developing PD (Quik *et al.* 2009). It is believed that the possible neuroprotective ability of nicotine underlies the negative relation between smoking and PD (Picciotto & Zoli 2008). A neuroprotective effect of nicotine has also been demonstrated on cultured neurons and animal models (Picciotto & Zoli 2008). Both in the rodent and primate Parkinsonian models, nicotine treatment improved function of the lesioned striatum (Quik *et al.* 2009). Moreover, the presence of nicotine prior to but not after damage is neuroprotective (Huang *et al.* 2009). This lends support to the observation that the protective effect of smoking is highest in continuing smokers and progressively decreases in ex-smokers with increasing years after quitting (Ritz *et al.* 2007).

Nicotine has both protective and toxic effects on neurons and different classes of neurons appear to be differentially affected by its damaging and protective properties. The net outcome of nicotine exposure is thus specific to the neuronal subtype. This could be partly mediated by the brain-region specific expression of nAChR subtypes. The neuroprotective effect of nicotine was shown to be mediated primarily by the α-6 containing nAChR subtype (Huang *et al.* 2009), which is expressed in high levels in the SN, VTA and striatum (Gaimarri *et al.* 2007). Hence in the background of global grey matter decrease resulting from nicotine exposure (Longstreth *et al.* 2000, 2001), some brain regions, such as the Put could benefit from the neuroprotective effect of nicotine. It is important to note in this context that in our study Put volumes of smokers only appear relatively larger when brain size is controlled for. This pattern is consistent with protection of the Put from the greater general atrophy that occurs in the brains of heavy smokers.

We observed significant associations only for the left hemisphere. Previous studies have shown that the left hemisphere is more specialized than the right in approach (as distinct from avoidance)-related behaviour, which includes smoking (Demaree *et al.* 2005). Also, smoking-induced dopamine release is significantly higher in the left, but not the right ventral striatum (Brody *et al.* 2004b). Thus, irrespective of whether striatal nuclei volume differences are predisposing factors or outcomes, the left hemisphere is likely to be more important in smoking-related behaviours.

Although we found a significant relationship between brain volumes and lifetime use of nicotine, measured in pack-years and treated as a continuous variable, we did not detect the differences in striatal volumes between smoker and non-smoker categories that have been found in other studies (Brody *et al.* 2004a; Gallinat *et al.* 2006). We believe this is because of a less stringent definition of a 'smoker' used in our study. In earlier studies, only nicotine dependent current smokers were included in the study. As both ex-smokers and current smokers were included in our study, this category had a much broader distribution of nicotine exposures compared with previous studies.

This study has many strengths as well as several limitations. The present investigation was conducted in a cohort based on a larger random sample of the population and it was larger than most studies conducted to date on this topic. Therefore, the present results are more likely to be generalizable. Also, similar findings were found for different measures of smoking behaviour, which suggest that these relationships are reliable and stable. Limitations are that in absence of additional information on age of daily smoking, significant abstinence periods or major changes on cigarette use, the pack-years variable is an imprecise measure of lifetime cigarette use. Smoking was assessed by self-report, which therefore may not be

perfectly accurate. Other factors associated with smoking behaviours such as personality, mood disorders, and physical health may partly underlie the current results. With this in mind we have been particularly careful in controlling for relevant covariates such as physical and mental health status, socio-demographic variables and alcohol consumption. As with alcohol, abuse of other drugs also affects brain structure and is frequently comorbid with cigarette use. Although we collected data on marijuana, ecstasy and amphetamine use, we could not analyse the effect of such comorbid substance abuse in our sample as too few individuals reported use of these drugs. With respect to our study sample, it is possible that the MRI sub-sample might not be as representative of the population as the original random sample from which it was derived. However, as the mean total years of smoking (the only smoking variable significantly different between the two samples) of the MRI sub-sample was lower, this is likely to have decreased and not increased the magnitude of associations we observed. Given the explanatory nature of this study and the controversy regarding correction for multiple comparisons (Rothman 1990) we chose to report the uncorrected *P* values. Hence our analysis needs to be replicated in other samples.

In conclusion, this study provides evidence of associations between striatal nuclei volumes and cigarette smoking. Left hemispheric NAc volume was negatively associated with lifetime use of cigarettes. In contrast, a positive association was observed between left hemispheric Put volume and pack-years and age at start of smoking. Whether these differences in brain structures predispose individuals towards nicotine addiction or are effects of chronic stimulation with nicotine (and/or other chemicals found in tobacco products) remains to be examined.

DISCLOSURE

The authors report no conflicts of interest.

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Authors Contribution

DD, NC, KA and SE developed the concept and design of the study. NC, KA and PS contributed to the acquisition of data. DD and NC analysed the data. DD drafted the manuscript. NC, KA, SE and PS provided critical revision for important intellectual content. All authors critically reviewed content and approved final version for publication.

References

Balfour DJK (2004) The neurobiology of tobacco dependence: a preclinical perspective on the role of the dopamine projections to the nucleus accumbens. Nicotine Tob Res 6:899–912.

Benowitz NL (1988) Drug therapy. Pharmacologic aspects of cigarette smoking and nicotine addition. N Engl J Med 319:1318–1330.

Brody AL, Mandelkern MA, Jarvik ME, Lee GS, Smith EC, Huang JC, Bota RG, Bartzokis G, London ED (2004a) Differences between smokers and nonsmokers in regional gray matter volumes and densities. Biol Psychiatry 55:77–84.

Brody AL, Olmstead RE, London ED, Farahi J, Meyer JH, Grossman P, Lee GS, Huang J, Hahn EL, Mandelkern MA (2004b) Smoking-induced ventral striatum dopamine release. Am J Psychiatry 161:1211–1218.

Court JA, Martin-Ruiz C, Graham A, Perry E (2000) Nicotinic receptors in human brain: topography and pathology. J Chem Neuroanat 20:281–298.

Demaree HA, Everhart DE, Youngstrom EA, Harrison DW (2005) Brain lateralization of emotional processing: historical roots and a future incorporating 'dominance'. Behav Cogn Neurosci Rev 4:3–20.

Domino EF (2008) Tobacco smoking and MRI/MRS brain abnormalities compared to nonsmokers. Prog Neuropsychopharmacol Biol Psychiatry 32:1778–1781.

Evans DE, Drobes DJ (2009) Nicotine self-medication of cognitive-attentional processing. Addict Biol 14:32–42.

Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM (2002) Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355.

Fischl B, Salat DH, van der Kouwe AJW, Makris N, Ségonne F, Quinn BT, Dale AM (2004) Sequence-independent segmentation of magnetic resonance images. Neuroimage 23 (Suppl 1):S69–S84.

Gaimarri A, Moretti M, Riganti L, Zanardi A, Clementi F, Gotti C (2007) Regulation of neuronal nicotinic receptor traffic and expression. Brain Res Rev 55:134–143.

Gallinat J, Meisenzahl E, Jacobsen LK, Kalus P, Bierbrauer J, Kienast T, Witthaus H, Leopold K, Seifert F, Schubert F, Staedtgen M (2006) Smoking and structural brain deficits: a volumetric MR investigation. Eur J Neurosci 24:1744–1750.

Geng D-Y, Li Y-X, Zee C-S (2006) Magnetic resonance imaging-based volumetric analysis of basal ganglia nuclei and substantia nigra in patients with Parkinson's disease. Neurosurgery 58:256–262. discussion 256–262.

Goldberg D, Bridges K, Duncan-Jones P, Grayson D (1988) Detecting anxiety and depression in general medical settings. BMJ 297:897–899.

Hays R, Prince-Embury S, Chen H (1998) RAND-36 Health Status Inventory. San Antonio, TX: The Psychological Corporation.

Huang LZ, Parameswaran N, Bordia T, Michael Mcintosh J, Quik M (2009) Nicotine is neuroprotective when administered before but not after nigrostriatal damage in rats and monkeys. J Neurochem 109:826–837.

Jorm AF, Anstey KJ, Christensen H, Rodgers B (2004) Gender differences in cognitive abilities: the mediating role of health state and health habits. Intelligence 32:7–23.

Khuder SA, Dayal HH, Mutgi AB (1999) Age at smoking onset and its effect on smoking cessation. Addict Behav 24:673–677.

Knutson B, Westdorp A, Kaiser E, Hommer D (2000) FMRI visualization of brain activity during a monetary incentive delay task. Neuroimage 12:20–27.

Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 13:177–184.

Lawrence D, Mitrou F, Zubrick SR (2009) Smoking and mental illness: results from population surveys in Australia and the United States. BMC Public Health 9:285.

Longstreth WT, Arnold AM, Manolio TA, Burke GL, Bryan N, Jungreis CA, O'Leary D, Enright PL, Fried L (2000) Clinical correlates of ventricular and sulcal size on cranial magnetic resonance imaging of 3301 elderly people. The Cardiovascular Health Study. Collaborative Research Group. Neuroepidemiology 19:30–42.

Longstreth WT, Diehr P, Manolio TA, Beauchamp NJ, Jungreis CA, Lefkowitz D (2001) Cluster analysis and patterns of findings on cranial magnetic resonance imaging of the elderly: the Cardiovascular Health Study. Arch Neurol 58:635–640.

Makris N, Oscar-Berman M, Jaffin SK, Hodge SM, Kennedy DN, Caviness VS, Marinkovic K, Breiter HC, Gasic GP, Harris GJ (2008) Decreased volume of the brain reward system in alcoholism. Biol Psychiatry 64:192–202.

Meyerhoff DJ, Tizabi Y, Staley JK, Durazzo TC, Glass JM, Nixon SJ (2006) Smoking comorbidity in alcoholism: neurobiological and neurocognitive consequences. Alcoholism: Clinical and Experimental Research 30:253–264.

Miyata H, Yanagita T (2001) Neurobiological mechanisms of nicotine craving. Alcohol 24:87–93.

Murray S (2006) A smouldering epidemic. Can Med Assoc J 174:309–310.

Picciotto MR, Zoli M (2008) Neuroprotection via nAChRs: the role of nAChRs in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Front Biosci 13:492–504.

Quik M, Huang LZ, Parameswaran N, Bordia T, Campos C, Perez XA (2009) Multiple roles for nicotine in Parkinson's disease. Biochem Pharmacol 78:677–685.

Raz N, Rodrigue KM, Kennedy KM, Head D, Gunning-Dixon F, Acker JD (2003) Differential aging of the human striatum: longitudinal evidence. Am J Neuroradiol 24:1849–1856.

Ritz B, Ascherio A, Checkoway H, Marder KS, Nelson LM, Rocca WA, Ross GW, Strickland D, Van Den Eeden SK, Gorell J (2007) Pooled analysis of tobacco use and risk of Parkinson disease. Arch Neurol 64:990–997.

Rosano C, Aizenstein HJ, Studenski S, Newman AB (2007) A regions-of-interest volumetric analysis of mobility limitations in community-dwelling older adults. J Gerontol A Biol Sci Med Sci 62:1048–1055.

Rothman KJ (1990) No adjustments are needed for multiple comparisons. Epidemiology 1:43–46.

Ségonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B (2004) A hybrid approach to the skull stripping problem in MRI. Neuroimage 22:1060–1075.

Sharma A, Brody AL (2009) In vivo brain imaging of human exposure to nicotine and tobacco. Handb Exp Pharmacol ••:145–171. 4

Shimohama S (2009) Nicotinic receptor-mediated neuroprotection in neurodegenerative disease models. Biol Pharm Bull 32:332–336.

Whiting PJ, Lindstrom JM (1988) Characterization of bovine and human neuronal nicotinic acetylcholine receptors using monoclonal antibodies. J Neurosci 8:3395–3404.

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469–492.

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