SYNAPSE 61:637–645 (2007)

The Effect of Nicotine on Striatal Dopamine Release in Man: A [^{11}C]raclopride PET Study

ANDREW J. MONTGOMERY,[1]* ANNE R. LINGFORD-HUGHES,[2] ALICE EGERTON,[1] DAVID J. NUTT,[2] AND PAUL M. GRASBY[1]
[1]*MRC-Clinical Sciences Centre, Imperial College, Hammersmith Hospital, London W12 0NN, United Kingdom*
[2]*Psychopharmacology Unit, Dorothy Hodgkin Building, University of Bristol BS1 3NY, United Kingdom*

KEY WORDS nicotine; PET; [^{11}C] raclopride; man

ABSTRACT In common with many addictive substances and behaviors nicotine activates the mesolimbic dopaminergic system. Brain microdialysis studies in rodents have consistently shown increases in extrasynaptic DA levels in the striatum after administration of nicotine but PET experiments in primates have given contradicting results. A recent PET study assessing the effect of smoking in humans showed no change in [^{11}C]raclopride binding in the brain, but did find that "hedonia" correlated with a reduction in [^{11}C]raclopride binding suggesting that DA may mediate the positive reinforcing effects of nicotine. In this experiment we measured the effect of nicotine, administered via a nasal spray, on DA release using [^{11}C]raclopride PET, in 10 regular smokers. There was no overall change in [^{11}C]raclopride binding after nicotine administration in any of the striatal regions examined. However, the individual change in [^{11}C]raclopride binding correlated with change in subjective measures of "amused" and "happiness" in the associative striatum (AST) and sensorimotor striatum (SMST). Nicotine concentration correlated negatively with change in BP in the limbic striatum. Nicotine had significant effects on cardiovascular measures including pulse rate, systolic blood pressure (BPr), and diastolic BPr. Baseline [^{11}C]raclopride binding potential (BP) in the AST correlated negatively with the Fagerström score, an index of nicotine dependence. These results support a role for the DA system in nicotine addiction, but reveal a more complex relationship than suggested by studies in animals. **Synapse 61:637–645, 2007.**

INTRODUCTION

Tobacco addiction is a significant public health problem causing the premature death of an estimated 5 million people world-wide per annum (Doll et al., 2004; Ezzati and Lopez, 2003). Nicotine, the major active component of tobacco products (Stolerman and Jarvis, 1995), has addictive properties, demonstrated by its effects in a variety of experimental paradigms. For example, rodents will repeatedly self-administer nicotine (for example, Corrigall and Coen, 1989), which acts as a positive reinforcer in conditioned place preference paradigms (for example, Fudala et al., 1985).

In common with many addictive substances, the addictive properties of nicotine may be associated with activation of the dopaminergic system (Balfour et al., 2000; Pontieri et al., 1996). Nicotinic receptors are found on mesolimbic and nigrostriatal dopaminergic neurons (Clarke and Pert, 1985) and nicotine given intraperitoneally increases interstitial DA concentrations for example, in the nucleus accumbens (Benwell and Balfour, 1997; Imperato et al., 1986) and caudate (Benwell and Balfour, 1997; Di Chiara and Imperato, 1988) and increases the firing rate of dopaminergic neurones (Grenhoff et al., 1986; Zhang and Sulzer, 2004). Consistent with these observations, infusion of the nicotinic antagonist mecamylamine attenuates the DA increases induced by nicotine (Nisell et al., 1994) that perhaps explains mecamylamine's mechanism of action in treating nicotine addiction (George and O'Malley, 2004). Recent evidence from fast-scan cyclic voltammetry in rat brain

*Correspondence to: Andrew J. Montgomery, MRC-Clinical Sciences Centre Building, Imperial College, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK. E-mail: andrew.montgomery@ic.ac.uk

Received 20 July 2006; Accepted 27 February 2007

DOI 10.1002/syn.20419

Published online in Wiley InterScience (www.interscience.wiley.com).

slices has suggested that the activity state of the dopaminergic neurones determines their response to nicotine (Rice and Cragg, 2004; Zhang and Sulzer, 2004). When in the tonic firing state, nicotine reduced DA release, whereas in the phasic state, the release of DA is enhanced by nicotine, suggesting that nicotine has a role in amplifying signal differences between tonic and phasic states.

Radioligands for DA D_2 receptors, the binding of which are sensitive to the concentration of endogenous neurotransmitter, allow measurement of changes in neurotransmitter concentration in man following pharmacological challenges (Dewey et al., 1993; Laruelle, 2000). PET studies of nicotine administration in primates, using the radioligand [^{11}C]raclopride to index changes in striatal DA, have given contradictory results. Initially nicotine administration was shown to reduce [^{11}C]raclopride binding, indicating increased DA concentration (Dewey et al., 1999) in a single monkey. Subsequently, however, it was suggested that this reduction in [^{11}C]raclopride binding was due to the isoflurane anesthesia rather than a specific nicotine effect, although nicotine did increase DA synthesis (Tsukada et al., 2005). However, a recent study in ketamine anesthetized rhesus monkeys reported 5% reductions in [^{11}C]raclopride binding after nicotine (Marenco et al., 2004). In pigs both high (500 μg/kg) and low (50 μg/kg), dose nicotine exposure was associated with reductions of [^{11}C]raclopride binding (Cumming et al., 2003), although the changes at the low dose did not survive correction for multiple comparisons.

In man, smoking was not shown to decrease [^{11}C] raclopride binding, but there was a significant correlation between reduction in [^{11}C]raclopride binding and increase in self-reported "euphoria" (Barrett et al., 2004). However, the subjective response to smoking is not exclusively nicotine dependent for example, smoking denicotinised cigarettes reduces craving (Brauer et al., 2001). It remains possible therefore the changes in [^{11}C]raclopride binding observed by Barrett et al. (2004) relate to the reward associated with smoking, reward itself having been associated with reductions in [^{11}C]raclopride binding (Small et al., 2003; Zald et al., 2004), rather than nicotine itself. Large reductions in [^{11}C]raclopride binding after cigarette smoking (Brody et al., 2004) were initially reported by a group which more recently found an 8.4% reduction in [^{11}C]raclopride binding, which was significantly related to genotype (dopamine transporter (DAT), catechol-*o*-methyl transferase, D_4 receptor) (Brody et al., 2006).

In this study we measured the response of the DA system to nicotine in a group of smokers using bolus-infusion [^{11}C]raclopride PET. Nicotine was administered directly by nasal spray, instead of via cigarette smoking, to distinguish its effects from smoking reward related changes in the DA system. In view of previous work in man we hypothesized that DA release would correlate with positive subjective mood increases.

METHODS

Volunteers

Volunteers were recruited via advertisement. Inclusion criteria were that volunteers should have been regular smokers for more than 6 months and were currently smoking three or more cigarettes daily. Exclusion criteria were: presence of current or previous psychiatric or neurological disorder; any previous exposure to stimulant drugs other than nicotine. The study was approved by the Hammersmith, Queen Charlotte's and Chelsea and Acton Hospitals Research Ethics Committee. All volunteers provided written informed consent prior to inclusion in the study.

PET scanning

Volunteers were scanned on one occasion with [^{11}C]raclopride administered as a bolus followed by constant infusion with a $k_{bol} = 105$ min (Watabe et al., 2000). Using this k_{bol} 57% of the total administered radioactivity is given as the bolus, with 43% during the infusion. This method of administration avoids the potentially artifactual effects of blood flow change during the scan by establishing a state of equilibrium (Carson et al., 1997). Data was acquired using a Siemans ECAT 966 PET camera (full width half maximum resolution 5.1 mm) (Spinks et al., 2000) over 100 min. Volunteers were asked to abstain from smoking for at least 12 h before scanning. Compliance with this request was monitored by verbal enquiry and a carbon monoxide breath test (excluded if carbon monoxide > 5 ppm). Subjects lay at rest in the darkened, quiet scanning room for the duration of the scan. Nicotine was administered by the scan operator at a dose of 2 mg intranasally from a proprietary dispenser (Nicorette©) 50 min after starting the infusion of [^{11}C]raclopride. This dose of intranasal nicotine produces comparable plasma levels to smoking a cigarette (Sutherland et al., 1992).

Two sampling periods were measured: 38–50 min corresponding to the rest period of the scan and 58–100 min corresponding to the post nicotine period. Head movement was corrected using a frame-by-frame (FBF) realignment procedure based on that described (Montgomery et al., 2006). To reduce the influence of redistribution of radiotracer producing erroneous realignments (Dagher et al., 1998) nonattenuation corrected (non-AC) images were used for realignment. These images have a significant scalp signal compared with attenuation corrected images which provides more information for the realignment algorithm. The non-AC dynamic image was denoised

using a level 2, order 64 Battle Lemarie wavelet (Battle, 1987; Turkheimer et al., 1999). Frames were realigned to a single 5 min frame acquired 25 min postinjection which had a high signal to noise ratio, using a mutual information algorithm (Studholme et al., 1997) and the transformation parameters were then applied to the corresponding attenuation corrected dynamic images. This procedure was applied to all 28 frames to generate a FBF corrected dynamic image. BP was calculated as the ratio (striatal counts/cerebellar counts)-1 in each sampling period. Using this methodology we have recently reported test-retest reliability data for [^{11}C]raclopride scans (Montgomery et al., 2006). Power calculation suggests that with 10 volunteers and a test-retest variability in the limbic striatum (LS) of 9.1%, it is possible to detect a change in BP of 9% with a power of 0.8 and $\alpha = 0.05$.

Region of interest analysis

Striatal and cerebellar regions of interest were defined on a magnetic resonance (MR) scan positioned in standard Montreal neurological institute (MNI) space. The distinction between striatal subregions was based on previously described criteria (Martinez et al., 2003). Subregions examined were LS, associative striatum (AST), and sensorimotor striatum (SMST) with right and left ROI combined. An [^{11}C]raclopride template was constructed in MNI space (Meyer et al., 1999). The [^{11}C]raclopride template was then spatially transformed to individual PET space within SPM 99 (Available at: www.fil.ion.ucl.ac.uk/spm) and the resulting transformation parameters were used to transform the striatal regions of interest into individual space. Time-activity curves for individual regions were generated using image analysis software (Analyze AVW 3.0 Biomedical Imaging Resource, Mayo Foundation).

Other parameters recorded

Volunteers completed the Fagerström nicotine addiction inventory prior to PET scanning (Heatherton et al., 1991). Subjective ratings of feelings ("amused," "happy," "sad," "pain," "tense," "alert," "buzz," "stress," "anxious," "frustrated," "interested," "want a cigarette," "nausea," "fast heart-beat," "dizzy,") were recorded once during rest, and 20 min after nicotine administration. Blood samples for the estimation of plasma nicotine concentration were taken 5 min before scanning commenced and 10 min after the administration of nasal nicotine. Plasma nicotine was estimated using a gas chromatographic method (Feyerabend and Russell, 1990). Blood pressure (BPr) and pulse rate were recorded every 10 min throughout the scan.

TABLE I. Demographic and radiopharmaceutical details of volunteers

Age	30.4 ± 4.7
Gender	7m, 3f
Fagerström score	3.7 ± 2.8
Current cigarettes/day	10.9 ± 6.6
Radioactivity injected (MBq)	309 ± 20
Cold raclopride injected (μg)	6.3 ± 4.7

TABLE II. Visual analogue scores (absolute change) and ROI BP(percentage change)

	Control	Nicotine	Change
Amused	5.0 ± 2.4	4.6 ± 1.8	−0.4 ± 2.5
Happy	6.9 ± 1.5	6 ± 1.9	−0.9 ± 1.5
Sad	7.9 ± 1.7	7.6 ± 2.6	−0.3 ± 2.0
Painful	7.7 ± 2.5	7.7 ± 1.9	0 ± 2.6
Tense	3.6 ± 2.5	3.5 ± 2.2	−0.1 ± 3.5
Alert	6.2 ± 1.8	4.6 ± 2.2	−1.6 ± 3.0
Stresses	3.2 ± 2.1	3.3 ± 2.2	0.1 ± 2.8
Anxious	2.8 ± 1.8	2.7 ± 1.9	−0.1 ± 2.3
Frustrated	8.6 ± 1.5	6.9 ± 2.8	−1.7 ± 2.5
Interested	5.7 ± 2.2	6.3 ± 2.6	0.6 ± 3.2
Want a cig	6.5 ± 2.8	6.3 ± 3.3	−0.2 ± 0.9
Nauseous	8.9 ± 1.6	8.2 ± 2.4	−0.7 ± 2.9
Buzz	8.3 ± 2.7	7.9 ± 2.2	−0.4 ± 2.8
Dizzy	2.5 ± 2.4	3.7 ± 2.6	1.2 ± 3.6
Fast heart	7.6 ± 3.1	6.4 ± 2.7	−1.2 ± 4.2
LS BP	2.1 ± 0.2	2.1 ± 0.3	−0.6% ± 7.2
AST BP	2.4 ± 0.2	2.4 ± 0.2	−1.0% ± 5.0
SMST BP	2.7 ± 0.2	2.7 ± 0.2	1.1% ± 4.5

LS, limbic striatum; AST, associative striatum; SMST, sensorimotor striatum.

Statistical analyses

All values are expressed as mean ± standard deviation. Statistical analyses were performed using the Statistical Package for Social Sciences 12.0 (SPSS Inc., Chicago, IL). Differences across conditions were compared using repeated measures analysis of variance (ANOVA). Post hoc *t*-tests were two-tailed. Correlations between continuous data were assessed using Pearson correlation coefficient, discontinuous data with Spearman rho. Significance was set at the $P < 0.05$ level.

RESULTS

Ten volunteers were recruited for the study. Demographic and radio-pharmaceutical details are shown in Table I. The volunteers' Fagerström score ranged from 0 to 9 with the mean of 3.7. Only one volunteer scored in the 'high dependence' range with the majority (six) scoring in 'low or very low dependence' range. This overall low dependence score is reflected in the relatively low number of cigarettes smoked in a day. [^{11}C]raclopride binding potentials (BP) and VAS scores are in Table II.

Time-activity curves for the four ROIs are shown in Figure 1. There was no significant effect of nicotine on [^{11}C]raclopride BP [$F = 0.00_{1,9}$, $P = 0.99$], with no interaction between region and condition [$F = 0.80_{2,18}$, $P = 0.46$]. There was no main effect of condi-

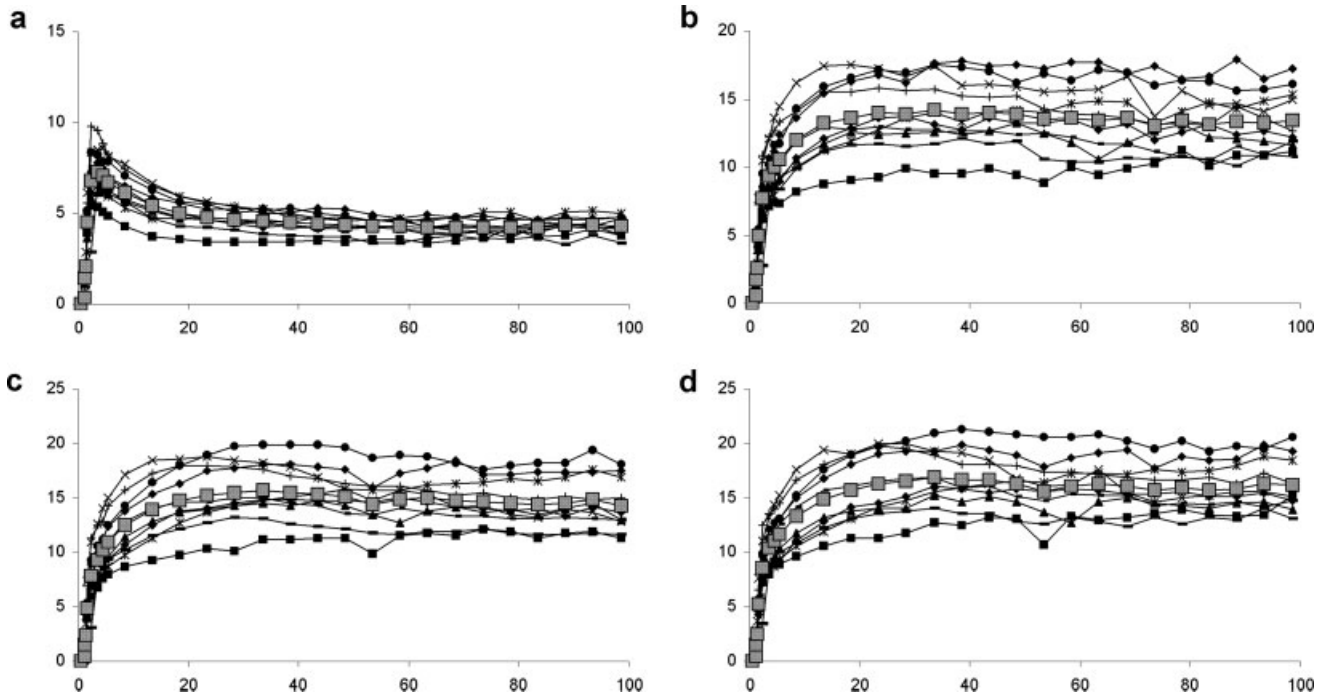

Fig. 1. Time-activity curves for the ROIs. (**a**) cerebellum, (**b**) limbic striatum, (**c**) associative striatum, (**d**) sensory-motor striatum. Filled squares represent mean values, *x*-axis minutes, *y*-axis kBq/ml.

tion (control or nicotine) on VAS scores [$F = 1.44_{1,9}$, $P = 0.26$], with no interaction between score and condition [$F = 0.80_{14,126}$ $P = 0.67$]. Change in AST and SMST [^{11}C]raclopride BP correlated negatively with absolute change in "amused" [$r = -0.84$, $P < 0.01$, and $r = -0.78$, $P < 0.01$] respectively (Fig. 2a) and with absolute change in "happy" [$r = -0.71$, $P = 0.02$, and $r = -0.74$, $P = 0.02$] (Fig. 2b). Individuals who showed reduced [^{11}C]raclopride binding after nicotine tended to show a positive mood response, with the opposite the case with those whose [^{11}C]raclopride binding increased. AST [^{11}C]raclopride BP change also correlated negatively with "alert" [$r = -0.69$, $P = 0.03$] with a trend in the SMST [$r = -0.56$, $P = 0.09$]. Change in LS [^{11}C]raclopride BP did not correlate with any VAS.

Plasma nicotine concentration was significantly increased after nasal administration from 0.8 ± 0.7 ng/ml to 5.8 ± 2.3 ng/ml [$t = -7.18$, $P < 0.01$]. Nicotine concentration 10 min after administration correlated negatively with change of BP in LS [$r = -0.66$, $P = 0.04$] (Fig. 3) but not in other regions. Change in nicotine concentration did not correlate with change in VAS scores.

The Fagerström score inversely correlated with baseline BP in the AST [$r = -0.66$, $P = 0.04$] (Fig. 4) with a trend to a correlation between baseline AST and number of cigarettes smoked per day [$r = -0.58$, $P = 0.08$]. The Fagerström score correlated negatively with change in "want a cigarette" VAS [$r = -0.68$, $P = 0.03$] but did not correlate with change in BP in any region. The results of the correlational analysis between the baseline [^{11}C]raclopride BP and Fagerström score were confirmed at a voxel level using statistical parametric mapping version 2 (SPM2; Available at: www.fil.ion.ucl.ac.uk/spm) (Friston et al., 1995) implemented in Matlab6.5. As this analysis was confirmatory and hypothesis-led, the search area was restricted to voxels within the striatum by application of an explicit spatial mask. Parametric (BP) images were first spatially normalized into MNI space using a [^{11}C]raclopride template (Meyer et al., 1999) and the normalized BP images were then smoothed with a Gaussian filter to 8 mm full-width half maximum. Significance was defined as $P < 0.01$ uncorrected. The inverse correlation between the Fagerström score and the baseline BP was confirmed using SPM; increases in BP were associated with decreases in the Fagerström score bilaterally in the striatum (Fig. 5). Correlations between the number of cigarettes smoked per day and baseline BP did not reach significance at the $P < 0.01$ level using SPM.

There were significant effects of time on pulse rate [$F = 30.37_{10,90}$, $P < 0.01$], diastolic BPr [$F = 21.13_{10,90}$, $P < 0.01$] and systolic BPr [$18.30_{10,90}$, $P < 0.01$] (Figs. 6a and 6b). Increase in nicotine concentration correlated with difference in systolic BPr between rest and nicotine conditions [$r = 0.76$, $P = 0.01$]. Change in diastolic or pulse rate did not correlate with change in nicotine concentration.

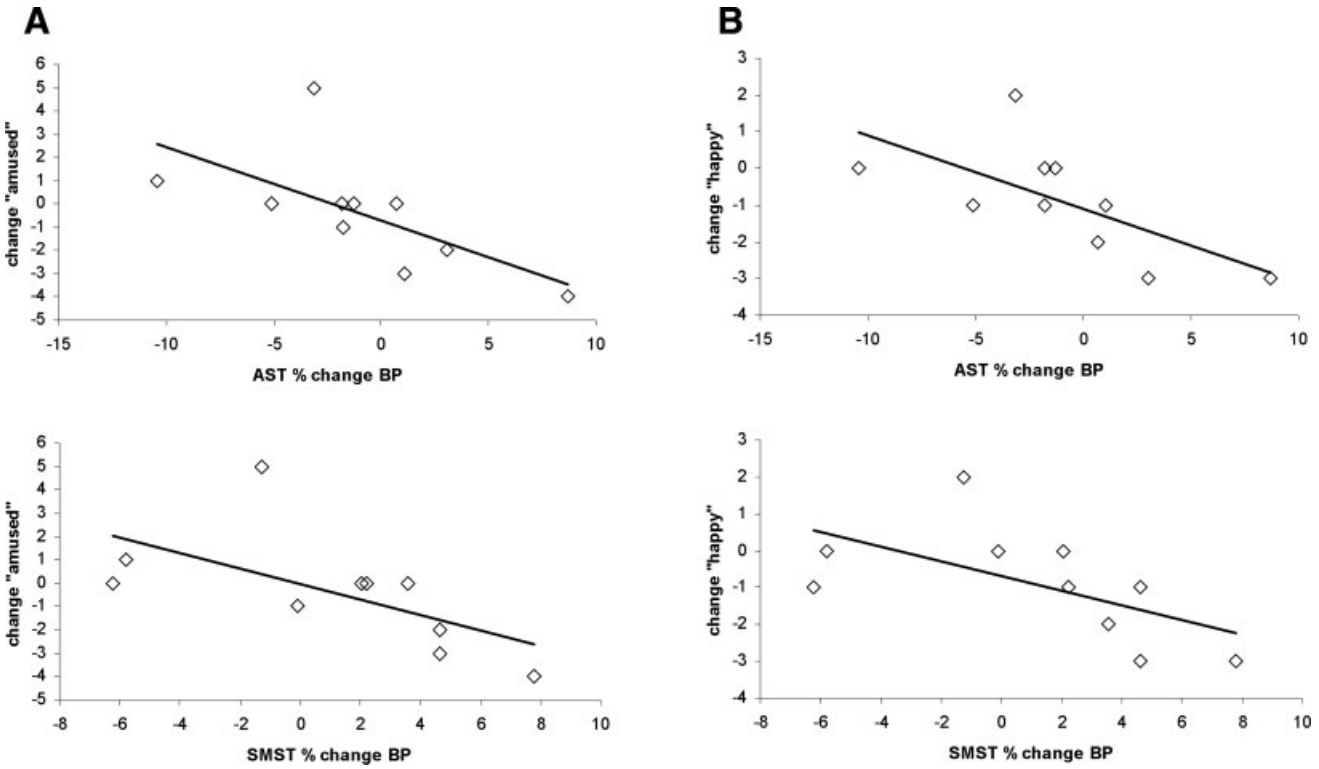

Fig. 2. (**a**) Correlation between change in associative striatum (AST) and sensorimotor striatum (SMST) BP (negative values indicate increased DA release) and change in "amused" VAS (negative values indicate less "amused" after nicotine administration). (**b**) Correlation between change in AST and SMST BP and change in "happy" VAS.

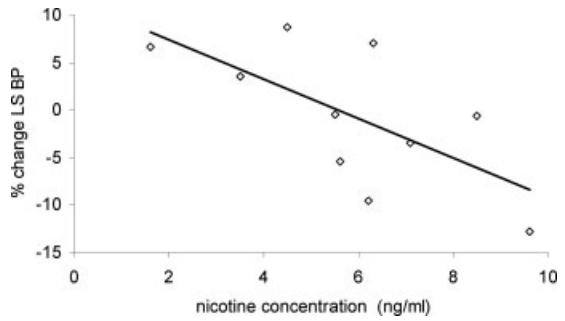

Fig. 3. Correlation between nicotine concentration (ng/ml) and change in LS BP.

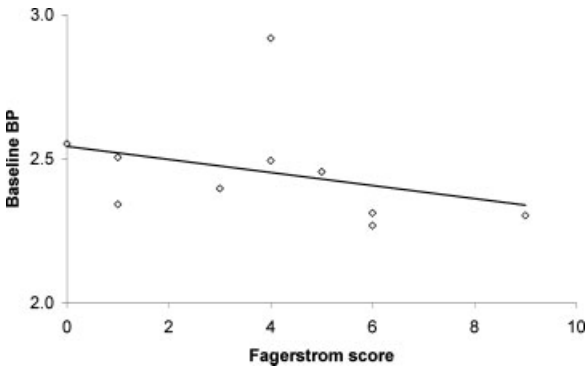

Fig. 4. Baseline BP in the associative striatum (AST) and Fagerström score.

DISCUSSION

In this study we have replicated a previous finding of a correlation between change in mood and change in dorsal striatal [^{11}C]raclopride binding (Barrett et al., 2004) after nicotine exposure. The direction of the [^{11}C]raclopride change was differentiated by the positive or negative subjective response to nicotine, with reductions of [^{11}C]raclopride binding (associated with increased DA release) associated with increased positive mood, and vice versa. We also found that baseline DA D_2 receptor binding correlated negatively with the Fagerström score such that higher or greater dependence was associated with lower DA D_2 receptor levels. As in Barrett et al. there was no overall change in BP across the whole group despite clear individual responses to nicotine. Together, these two studies employing different scanning methodologies and route of administration provide strong evidence that the 'pleasure' from smoking a cigarette is related to DA release in the brain.

The lack of overall change in [^{11}C]raclopride binding, indicative of no group change in striatal DA concentration, is in contrast to the large number of

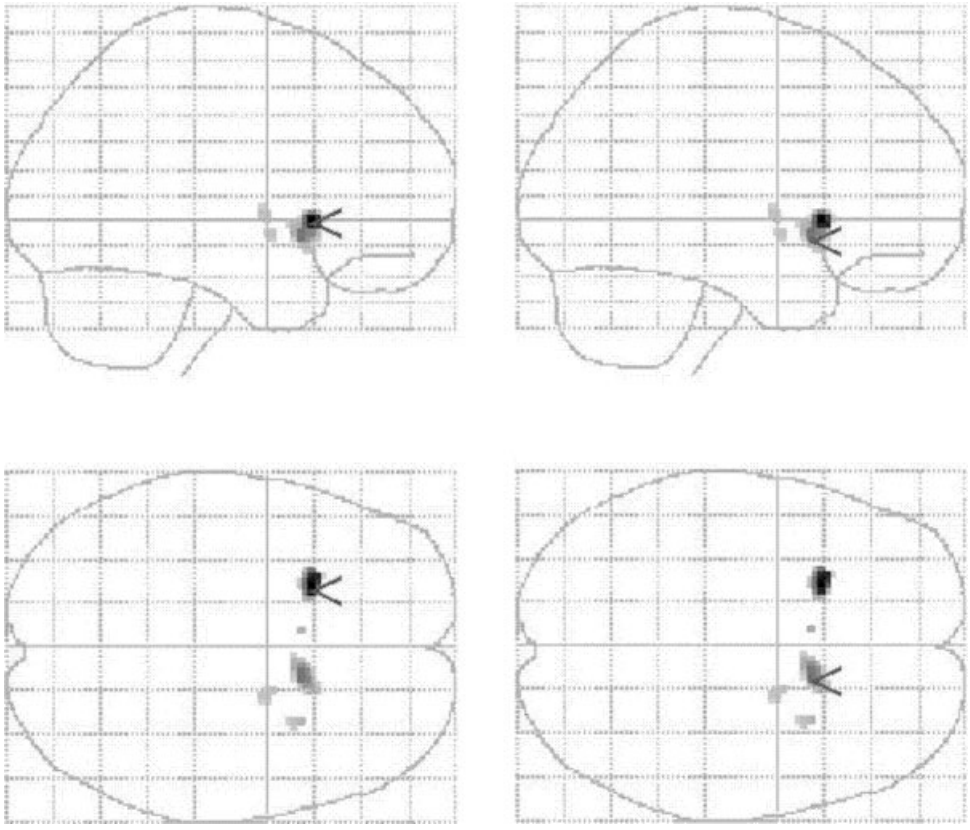

Fig. 5. Glass brain views of SPM analysis of correlation between Fagerström score and BP (upper row sagittal view, lower row axial view). Higher baseline BP was associated with lower Fagerström score bilaterally in the striatum. Specifically, this achieved a significance level of $P = 0.007$ ($z = 2.45$) on the left side (Talairach peak coordinates: $x = -23$; $y = 16$; $z = -1$, left column), and approached significance on the right side ($P = 0.017$; $z = 2.12$) (Talairach peak coordinates: $x = 13$; $y = 12$; $z = -7$, right column).

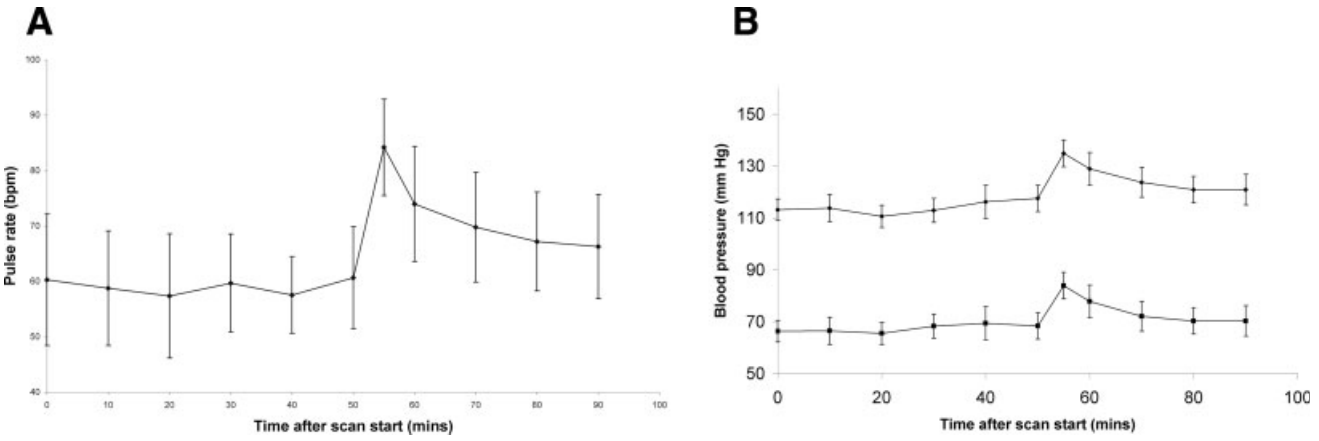

Fig. 6. (**a**) pulse rate (**b**) diastolic and systolic blood pressure. Nicotine was administered at $t = 50$ min. Bars indicate standard deviation.

studies in rodents where increases in DA release, measured with microdialysis, occur after nicotine administration (e.g., (Marshall et al., 1997; Nisell et al., 1994). It is, however, more consistent with PET studies in primates which have shown mixed results. The single study in man to show large reductions in [^{11}C]raclopride binding after smoking (Brody et al., 2004) did not make corrections for head motion during scanning, raising the possibility that the findings were artifactual (Dagher et al., 1998; Montgomery et al., 2006). This inconsistency between rodent and primate studies may partly arise from cross species dosing differences, but may also reflect the fact that rodent studies have typically used much higher doses [e.g., 0.4 mg/kg (Cadoni and Di Chiara, 2000)], while PET studies in primates used smaller doses e.g., 20 μg/kg (Dewey et al., 1999), 32–100 μg/kg (Tsukada et al., 2002, 2005), 10–60 μg/kg (Marenco et al., 2004). Supportive of this suggestion is the result of a PET study in pigs which showed [^{11}C]raclopride dis-

placement after a large dose (500 μg/kg) of nicotine but not after a small one (50 μg/kg) using a parametric map analysis when correction for multiple comparisons was made (Cumming et al., 2003). The dose in our study was in the order of 30 μg/kg. A further complication is the finding that anesthesia potentiates the reduction of [^{11}C]raclopride binding after nicotine. Thus no change was found in [^{11}C]raclopride binding in awake rhesus monkeys after 32–100 μg/kg nicotine i.v., but after isofluorane anesthesia reductions were between 5.7 and 15.9% (Tsukada et al., 2002) and after ketamine changes were between +7.0 and −12.5% (Marenco et al., 2004). In contrast studies in rodents have typically not used anesthesia during the microdialysis (e.g., (Benwell and Balfour, 1997; Cadoni and Di Chiara, 2000). Our data is thus consistent with studies in unanaesthetized primates and pigs using relatively small doses of nicotine.

Further insights into the complexity of the relationship between nicotine and DA release have come from studies using fast-scan cyclic voltammetry to measure synaptic DA concentration. This method, which allows very fine temporal resolution compared to microdialysis, showed reductions of (73 ± 4.8)% in evoked DA release after the application of 50 nM nicotine to striatal brain slices (Zhou et al., 2001). The authors suggest that microdialysis gives a flawed picture of the dynamics of DA release because it measures relatively large volumes of extracellular fluid which relies on diffusion of DA from the synapse. In contrast, fast-scan cyclic voltammetry measures DA released by action potentials. This finding may go some way to explain the difference between studies using microdialysis in rodents and PET studies. The change in PET [^{11}C]raclopride signal does not always correlate well with microdialysis data (Tsukada et al., 1999), and may be better accounted for by intrasynaptic changes. More recently two fast-scan voltammetry studies in rodents have demonstrated that nicotine's effect on striatal DA release is dependent upon the state of the dopaminergic system; specifically whether it is in a phasic or tonic state of firing (Rice and Cragg, 2004; Zhang and Sulzer, 2004). When firing at a tonic rate, nicotine reduced DA release. In contrast, when the dopaminergic neurons were stimulated in a phasic fashion nicotine increased DA release. Thus nicotine served to amplify the difference in DA release between tonic and phasic states of firing. These data suggest that, in the absence of knowledge about the state of firing of dopaminergic neurones, it may not be possible to make predictions about the response of the DA system to the administration of nicotine, and provide a potential explanation for the variability observed in our sample, with individuals displaying very different dopaminergic responses to the same nicotine stimulus.

In our study nicotine was administered by nasal spray which gives a rapid increase in arterial blood nicotine concentrations (Sutherland et al., 1992). The arterial concentrations achieved by this method are comparable with those after smoking a cigarette (Sutherland et al., 1992). The dose of nicotine was necessarily small because of the high incidence of side-effects associated with higher doses, and to emulate the pharmacodynamics of nicotine delivered by cigarette smoking. However, the venous plasma concentrations achieved (5.8 ± 2.3 ng/ml) are close to the arterial plasma concentrations in PET primate studies (e.g., range 8.9–48 ng/ml (Tsukada et al., 2002) and arterial concentrations are higher than venous for 30 min after intranasal spray nicotine (Sutherland et al., 1992). Although the mean blood increase in nicotine concentration was less than the first cigarette of the day, it is similar to the average boost per cigarette during normal daily smoking and is therefore representative of normal smoking practice (Russell et al., 1983). However, the rate of nicotine concentration increase may have been somewhat slower than with smoking.

Despite the pharmacodynamic similarities of our experimental design to normal smoking, it is important to note the significant differences from normal smoking practice. In particular the drug was administered by third party rather than self-administered, a factor previously shown to be important in the release of DA in the nucleus accumbens in rats after cocaine (Hemby et al., 1997). In Barrett et al. nicotine was administered via cigarette smoking which took place throughout the activation scan. Moreover, cigarette smoking is more likely to have activated reward circuitry than intranasal nicotine, and reward associated with nonpharmacological stimuli (Small et al., 2003; Zald et al., 2004), and the anticipation of reward (de la Fuente-Fernandez et al., 2001) is associated with reduced [^{11}C]raclopride binding in man. Indeed, nicotine withdrawal symptoms may be partially alleviated by smoking cigarettes which have had all nicotine removed suggesting that non-nicotine related factors are important in the subjective responses to cigarettes (Brauer et al., 2001). These differences may account for the lack of reduction in "wanting a cigarette" after nicotine in our study. This is in contrast to Barrett et al. who found that craving was reduced by cigarette smoking in the PET scanner (Barrett et al., 2004).

Baseline AST DA D_2 receptor binding was inversely correlated with an index of dependence, the Fagerström score (Fagerström and Schneider, 1989; Heatherton et al., 1991). The volunteers in this study had a relatively wide range of dependence to nicotine (measured by the Fagerström score) compared with other studies in man. This factor may have enhanced our ability to detect the relationship between dependence and baseline D_2 expression. It is not possible to say if D_2 binding is a consequence or a predictor of nicotine dependence, although one SPET study has

not shown any difference between striatal [^{123}I]IBZM binding in smoker and nonsmokers (Yang et al., 2005) suggesting reduced binding is not a sequelae of smoking. However, a PET study in drug-naïve volunteers has shown that low baseline D_2 binding predicts a pleasant response to the DA reuptake inhibitor methylphenidate (Volkow et al., 1999) suggesting some individuals may be at higher risk of subsequent development of drug misuse because of their baseline D_2 receptor number. We are not aware of such work with nicotine, indeed it would be difficult to perform because of the almost universal unpleasant effects of nicotine in naïve individuals. However, use of nicotine may represent a compensatory behavior in people with low baseline DA D_2 receptor binding to augment DA function.

Evidence to support a direct action of nicotine on DA release was obtained in the form of the increase in venous nicotine concentration significantly correlating with change in [^{11}C]raclopride BP in the LS. Nicotine is thought to modulate DA release via actions in the midbrain nuclei as well as within the striatum (Marshall et al., 1997; Nisell et al., 1994; Sziraki et al., 2002). DA release in the striatum is under the control of a number of feedback loops to the midbrain with different subregions having different degrees of modulation (Haber et al., 2000). The LS is under less negative feedback control that the AST and SMST, a factor which has previously been suggested to account for the greater DA release in the LS than other subregions after an amphetamine challenge (Martinez et al., 2003). It is possible that a similar mechanism underlies the absence of a clear relationship between change in [^{11}C]raclopride binding and change in nicotine concentration in the AST and SMST in our study. However, no correlation between self-reported change in mood after nicotine and change in LS BP was found. This result is consistent with Barrett et al. who found the correlation was restricted to the dorsal regions of the striatum (Barrett et al., 2004), and other studies from the same group showing DA release after food and monetary reward (Small et al., 2003; Zald et al., 2004) However, other pharmacological challenges that activate the dopaminergic system have been shown to produce hedonic effects which are associated with changes in the ventral regions of the striatum (Drevets et al., 1999; Martinez et al., 2003). It is thus not possible at this time to define a specific subregion of the striatum which is exclusively associated with the emotional response to rewarding stimuli.

A limitation of the PET study design used was that the order of tasks was fixed because of the long duration of changes in BP found after pharmacological (Carson et al., 2000; Houston et al., 2004) and behavioral (Koepp et al., 1998) manipulations of the DA system. It was therefore necessary to fix the order of tasks with the nicotine condition second. However, an order effect opposing the nicotine effect seems unlikely given the excellent within-scan test-retest reliability achieved with [^{11}C]raclopride studies with the design used here [VS intraclass correlation coefficient (ICC) 0.68, DS ICC 0.79, (Montgomery et al., 2006)].

In summary we have not found a significant reduction of [^{11}C]raclopride binding after exposure to nicotine in a group of regular smokers, the majority of whom were nondependent, in doses which have previously been found to reduce [^{11}C]raclopride binding in anesthetized nonhuman primates. However, we did find a correlation between nicotine levels and reduced [^{11}C]raclopride binding in the LS. We have also, replicated a previous correlation (Barrett et al., 2004) between increase in positive mood state and reduction in [^{11}C]raclopride binding, supporting studies in animals which suggest that DA release has a role in this experience and might be a factor in the addictive properties of nicotine.

REFERENCES

Balfour DJ, Wright AE, Benwell ME, Birrell CE. 2000. The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. Behav Brain Res 113:73–83.

Barrett SP, Boileau I, Okker J, Pihl RO, Dagher A. 2004. The hedonic response to cigarette smoking is proportional to dopamine release in the human striatum as measured by positron emission tomography and [11C]raclopride. Synapse 54:65–71.

Battle G. 1987. A block spin construction of ondelettes. 1. Lemarie functions. Commun Math Phys 7:601–615.

Benwell ME, Balfour DJ. 1997. Regional variation in the effects of nicotine on catecholamine overflow in rat brain. Eur J Pharmacol 325:13–20.

Brauer LH, Behm FM, Lane JD, Westman EC, Perkins C, Rose JE. 2001. Individual differences in smoking reward from de-nicotinized cigarettes. Nicotine Tob Res 3:101–109.

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

Brody AL, Mandelkern MA, Olmstead RE, Scheibal D, Hahn E, Shiraga S, Zamora-Paja E, Farahi J, Saxena S, London ED, McCracken JT. 2006. Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. Arch Gen Psychiatry 63:808–816.

Cadoni C, Di Chiara G. 2000. Differential changes in accumbens shell and core dopamine in behavioural sensitization to nicotine. Eur J Pharmacol 387:R23–R25.

Carson RE, Breier A, de Bartolomeis A, Saunders RC, Su TP, Schmall B, Der MG, Pickar D, Eckelman WC. 1997. Quantification of amphetamine-induced changes in [11C]raclopride binding with continuous infusion. J Cereb Blood Flow Metab 17:437–447.

Carson RE, Channing MA, Vuong B, Watabe H, Herscovitch P, Eckelman WC. 2000. Amphetamine-induced dopamine release: Duration of action assessed with [11C]raclopride in anaesthetised monkeys. In: Gjedde A, editor. Molecular and pharmacologic brain imaging with positron emission tomography. San Diego, CA: Academic Press. p 205–209.

Clarke PB, Pert A. 1985. Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res 348:355–358.

Corrigall WA, Coen KM. 1989. Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology (Berl) 99:473–478.

Cumming P, Rosa-Neto P, Watanabe H, Smith D, Bender D, Clark P, Gjedde A. 2003. Effects of acute nicotine on haemodynamics and binding of [11C]raclopride to dopamine D2/3 receptors in pig brain. Neuroimage 19:1127–1136.

Dagher A, Gunn R, Lockwood G, Cunningham V, Grasby P, Brooks D. 1998. Measuring neurotransmitter release with PET: Methodo-

logical issues. In: Carson R, Daube-Witherspoon M, Herscovitch P, editors. Quantitative functional brain imaging with positron emission tomography. New York: Academic Press. p 449–454.
de la Fuente-Fernandez R, Ruth TJ, Sossi V, Schulzer M, Calne DB, Stoessl AJ. 2001. Expectation and dopamine release: Mechanism of the placebo effect in Parkinson's disease. Science 293:1164–1166.
Dewey SL, Smith GS, Logan J, Brodie JD, Fowler JS, Wolf AP. 1993. Striatal binding of the PET ligand 11C-raclopride is altered by drugs that modify synaptic dopamine levels. Synapse 13:350–356.
Dewey SL, Brodie JD, Gerasimov M, Horan B, Gardner EL, Ashby CR, Jr. 1999. A pharmacologic strategy for the treatment of nicotine addiction. Synapse 31:76–86.
Di Chiara G, Imperato A. 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving animals. Proc Natl Acad Sci USA 85:5274–5278.
Doll R, Peto R, Boreham J, Sutherland I. 2004. Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ. 328:1519.
Drevets W, Price J, Kupfer D, Kinahan P, Lopresti B, Holt D, Mathis C. 1999. PET measurement of amphetamine induced dopamine release in ventral and dorsal striatum. Neuropsychopharmacology 21:694–709.
Ezzati M, Lopez AD. 2003. Estimates of global mortality attributable to smoking in 2000. Lancet 362:847–852.
Fagerström KO, Schneider NG. 1989. Measuring nicotine dependence: A review of the Fagerström Tolerance Questionnaire. J Behav Med 12:159–182.
Feyerabend C, Russell MAH. 1990. A rapid gas-liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. J Pharm Pharmacol 42:450–452.
Friston K, Holmes A, Worsley K. 1995. Statistical parametric maps in functional imaging: A general linear approach. Hum Brain Mapp 2:189–210.
Fudala PJ, Teoh KW, Iwamoto ET. 1985. Pharmacologic characterization of nicotine-induced conditioned place preference. Pharmacol Biochem Behav 22:237–241.
George TP, O'Malley SS. 2004. Current pharmacological treatments for nicotine dependence. Trends Pharmacol Sci 25:42–48.
Grenhoff J, Aston-Jones G, Svensson TH. 1986. Nicotinic effects on the firing pattern of midbrain dopamine neurons. Acta Physiol Scand 128:351–358.
Haber SN, Fudge JL, McFarland NR. 2000. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci 20:2369–2382.
Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. 1991. The Fagerström Test for nicotine dependence: A revision of the Fagerström Tolerance Questionnaire. Br J Addict 86:1119–1127.
Hemby SE, Co C, Koves TR, Smith JE, Dworkin SI. 1997. Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. Psychopharmacology (Berl) 133:7–16.
Houston GC, Hume SP, Hirani SP, Goggi JL, Grasby PM. 2004. Temporal characterisation of amphetamine-induced dopamine release assessed with [11C]raclopride in anaesthetised rodents. Synapse 51:206–212.
Imperato A, Mulas A, Di Chiara G. 1986. Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. Eur J Pharmacol 132:337–338.
Koepp MJ, Gunn RN, Lawrence AD, Cunningham VJ, Dagher A, Jones T, Brooks DJ, Bench CJ, Grasby PM. 1998. Evidence for striatal dopamine release during a video game. Nature 393:266–268.
Laruelle M. 2000. Imaging synaptic neurotransmission with in vivo binding competition techniques: A critical review. J Cereb Blood Flow Metab 20:423–451.
Marenco S, Carson RE, Berman KF, Herscovitch P, Weinberger DR. 2004. Nicotine-induced dopamine release in primates measured with [11C]raclopride PET. Neuropsychopharmacology 29:259–268.
Marshall DL, Redfern PH, Wonnacott S. 1997. Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by in vivo microdialysis: Comparison of naive and chronic nicotine-treated rats. J Neurochem 68:1511–1519.
Martinez D, Slifstein M, Broft A, Mawlawi O, Hwang DR, Huang Y, Cooper T, Kegeles L, Zarahn E, Abi-Dargham A, Haber SN, Laruelle M. 2003. Imaging human mesolimbic dopamine transmission with positron emission tomography. Part 2: Amphetamine-induced dopamine release in the functional subdivisions of the striatum. J Cereb Blood Flow Metab 23:285–300.
Meyer JH, Gunn RN, Myers R, Grasby PM. 1999. Assessment of spatial normalization of PET ligand images using ligand- specific templates. NeuroImage 9:545–553.
Montgomery AJ, Mehta MA, Turkheimer FE, Thielelmans K, Mustafovic S, Grasby PM. 2006. Correction of head movement on PET studies: Comparison of methods. J Nucl Med 47:1936–1944.
Nisell M, Nomikos GG, Svensson TH. 1994. Systemic nicotine-induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 16:36–44.
Pontieri FE, Tanda G, Orzi F, Di Chiara G. 1996. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382:255–257.
Rice ME, Cragg SJ. 2004. Nicotine amplifies reward-related dopamine signals in striatum. Nat Neurosci 7:583–584.
Russell MAH, Jarvis MJ, Feyerabend C, Ferno O. 1983. Nasal nicotine solution: A potential aid to giving up smoking? Br Med J 286: 683–684.
Small DM, Jones-Gotman M, Dagher A. 2003. Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. Neuroimage 19:1709–1715.
Spinks TJ, Jones T, Bloomfield PM, Bailey DL, Miller M, Hogg D, Jones WF, Vaigneur K, Reed J, Young J, Newport D, Moyers C, Casey ME, Nutt R. 2000. Physical characteristics of the ECAT EXACT3D positron tomograph. Phys Med Biol 45:2601–2618.
Stolerman IP, Jarvis MJ. 1995. The scientific case that nicotine is addictive. Psychopharmacology (Berl) 117:2–10. discussion 14-20.
Studholme C, Hill DL, Hawkes DJ. 1997. Automated three-dimensional registration of magnetic resonance and positron emission tomography brain images by multiresolution optimization of voxel similarity measures. Med Phys 24:25–35.
Sutherland G, Russell MAH, Stapleton J, Feyerabend C, Ferno O. 1992. Nasal nicotine spray: A rapid nicotine delivery system. Psychopharmacology 108:512–518.
Sziraki I, Sershen H, Hashim A, Lajitha A. 2002. Receptors in the ventral tegmental area mediating nicotine-induced dopamine release in the nucleus accumbens. Neurochem Res 27:253–261.
Tsukada H, Nishiyama S, Kakiuchi T, Ohba H, Sato K, Harada N. 1999. Is synaptic dopamine concentration the exclusive factor which alters the in vivo binding of [11C]raclopride?: PET studies combined with microdialysis in conscious monkeys. Brain Res 841:160–169.
Tsukada H, Miyasato K, Kakiuchi T, Nishiyama S, Harada N, Domino EF. 2002. Comparative effects of methamphetamine and nicotine on the striatal [(11)C]raclopride binding in unanesthetized monkeys. Synapse 45:207–212.
Tsukada H, Miyasato K, Harada N, Nishiyama S, Fukumoto D, Kakiuchi T. 2005. Nicotine modulates dopamine synthesis rate as determined by L-[β-11C]DOPA: PET studies compared with [11C]raclopride binding in the conscious monkey brain. Synapse 57:120–122.
Turkheimer FE, Brett M, Visvikis D, Cunningham VJ. 1999. Multiresolution analysis of emission tomography images in the wavelet domain. J Cereb Blood Flow Metab 19:1189–1208.
Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Gifford A, Hitzemann R, Ding YS, Pappas N. 1999. Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. Am J Psychiatry 156:1440–1443.
Watabe H, Endres CJ, Breier A, Schmall B, Eckelman WC, Carson RE. 2000. Measurement of dopamine release with continuous infusion of [11C]raclopride: Optimization and signal-to-noise considerations. J Nucl Med 41:522–530.
Yang YK, Yao WJ, McEvoy JP, Chu CL, Lee IH, Chen PS, Yeh TL, Chui NY. 2005. Striatal dopamine D2/D3 receptor availability in male smokers. Psychiatry Res 146:87–90.
Zald DH, Boileau I, El-Dearedy W, Gunn R, McGlone F, Dichter GS, Dagher A. 2004. Dopamine transmission in the human striatum during monetary reward tasks. J Neurosci 24:4105–4112.
Zhang H, Sulzer D. 2004. Frequency-dependent modulation of dopamine release by nicotine. Nat Neurosci 7:581–582.
Zhou F-M, Liang Y, Dani JA. 2001. Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. Nat Neurosci 4:1224–1229.