

Acute effect of the anti-addiction drug bupropion on extracellular dopamine concentrations in the human striatum: An [^{11}C]raclopride PET study

Alice Egerton^{a,b,c,*}, John P. Shotbolt^{a,b,d}, Paul R.A. Stokes^{a,b}, Ella Hirani^e, Rabia Ahmad^e, Julia M. Lappin^{a,b,c}, Suzanne J. Reeves^{a,b,c}, Mitul A. Mehta^{a,b,c}, Oliver D. Howes^{a,b,c}, Paul M. Grasby^{a,b}

^a Psychiatric Imaging, Medical Research Council Clinical Sciences Centre, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

^b Division of Neurosciences and Mental Health, Imperial College London, UK

^c Institute of Psychiatry, King's College London, London SE5 8AF, UK

^d GlaxoSmithKline Clinical Imaging Centre, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

^e MDX Research, GE Healthcare, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

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ABSTRACT

Bupropion is an effective medication in treating addiction and is widely used as an aid to smoking cessation. Bupropion inhibits striatal dopamine reuptake via dopamine transporter blockade, but it is unknown whether this leads to increased extracellular dopamine levels at clinical doses in man. The effects of bupropion on extracellular dopamine levels in the striatum were investigated using [^{11}C]raclopride positron emission tomography (PET) imaging in rats administered saline, 11 or 25 mg/kg bupropion i.p. and in healthy human volunteers administered either placebo or 150 mg bupropion (Zyban® Sustained-Release). A cognitive task was used to stimulate dopamine release in the human study. In rats, bupropion significantly decreased [^{11}C]raclopride specific binding in the striatum, consistent with increases in extracellular dopamine concentrations. In man, no significant decreases in striatal [^{11}C]raclopride specific binding were observed. Levels of dopamine transporter occupancy in the rat at 11 and 25 mg/kg bupropion i.p. were higher than predicted to occur in man at the dose used. Thus, these data indicate that, at the low levels of dopamine transporter occupancy achieved in man at clinical doses, bupropion does not increase extracellular dopamine levels. These findings have important implications for understanding the mechanism of action underlying bupropions' therapeutic efficacy and for the development of novel treatments for addiction and depression.

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Introduction

Bupropion is an effective medication in smoking cessation and has a good safety and side effect profile (Aubin, 2002; Hurt et al., 1997; Jorenby et al., 1999). In addition to its original indication for treatment of depressive disorders, bupropion may also be effective in the treatment of methamphetamine addiction and pathological gambling (Dannon et al., 2005; Elkashef et al., 2008). Elucidation of the pharmacological features of bupropion which most contribute to its clinical efficacy may aid development of novel treatments for smoking cessation and other disorders of addiction.

The precise pharmacological mechanisms that underlie the therapeutic effects of bupropion are unclear (Dwoskin et al., 2006; Paterson, 2009; Warner and Shoaib, 2005). Bupropion weakly inhibits monoamine reuptake to presynaptic terminals through dopamine

transporters (DAT), and, to a lesser extent, noradrenaline transporters (Ascher et al., 1995; Damaj et al., 2004; Ferris and Beaman, 1983). Via interaction with vesicular monoamine transporter-2, bupropion increases sequestration of cytoplasmic dopamine to vesicles (Rau et al., 2005). At similar concentrations to those which inhibit monoamine transporter function, bupropion also acts as a noncompetitive inhibitor of nicotinic acetylcholine receptors (Fryer and Lukas, 1999; Miller et al., 2002; Slemmer et al., 2000).

In rats, microdialysis studies show that acute, systemic, bupropion administration reproducibly and dose-dependently increases striatal extracellular dopamine levels (Bredeloux et al., 2007; Brown et al., 1991; Gazzara and Andersen, 1997; Li et al., 2002; Nomikos et al., 1989; Sidhpura et al., 2007). It has been suggested that increases in striatal dopamine concentrations following bupropion administration may help combat the anhedonia associated with withdrawal from nicotine (or other addictive drugs) and anhedonia in depression (Paterson et al., 2007; Paterson, 2009; Warner and Shoaib, 2005; Shiffman et al., 2000). However, what is unclear is whether therapeutic doses of bupropion are sufficient to increase extracellular dopamine levels in the human striatum.

* Corresponding author. Neuroimaging P067, Department of Psychological Medicine and Psychiatry, Institute of Psychiatry, King's College London, SE5 8AF, UK. Fax: +44 207 848 0976.

E-mail address: Alice.Egerton@iop.kcl.ac.uk (A. Egerton).

In man, this question has been addressed indirectly using molecular imaging with dopamine transporter radioligands to estimate the degree of DAT occupancy which occurs following repeated bupropion treatment (Argyelán et al., 2005; Kugaya et al., 2003; Learned-Coughlin et al., 2003; Meyer et al., 2002) or acute administration of the bupropion active metabolite hydroxybupropion (Volkow et al., 2005). Overall, these studies indicate that, in man, only a small proportion – at most, 20–25% – of striatal DAT sites are occupied at clinical doses of bupropion. This observation has led to proposals that DAT inhibition alone does not explain the therapeutic efficacy of bupropion (Meyer et al., 2002; Kugaya et al., 2003; Paterson, 2009; Warner and Shoaib, 2005).

A more direct approach is to investigate the effects of bupropion administration on extracellular dopamine concentrations in the human striatum. Using positron emission tomography (PET) in combination with the D2/3 dopamine receptor radiotracer [^{11}C]raclopride, it is possible to index changes in extracellular dopamine levels in both man and experimental animals, as [^{11}C]raclopride competes with dopamine for D2/3 receptor binding (Laruelle, 2000). As bupropion has negligible affinity at D2/3 dopamine receptors and therefore will not compete with [^{11}C]raclopride directly (Ferris and Beaman, 1983), this noninvasive imaging approach is viable for assessing bupropion-induced changes in extracellular dopamine concentrations in man.

As the relationship between microdialysis and [^{11}C]raclopride PET measures of extracellular DA is complex (Laruelle, 2000), we performed an initial [^{11}C]raclopride PET study in rats to confirm whether bupropion-induced increases in dopamine concentrations are detectable using [^{11}C]raclopride PET. Following positive confirmation, this approach was translated to man in order to determine whether the dose of bupropion used in the UK to aid smoking cessation (150 mg Zyban[®] Sustained-Release) increases extracellular dopamine concentrations in the human striatum.

We investigated the effects of bupropion on striatal dopamine levels while volunteers completed a spatial planning task, previously shown to decrease striatal [^{11}C]raclopride binding potential in healthy volunteers (Lappin et al., 2009), as increases in extracellular dopamine concentrations following dopamine reuptake inhibition are most apparent when dopamine release is stimulated (Volkow et al., 2002). This approach was also selected as stimulation of dopamine release via administration of a behavioral task in combination with dopamine reuptake inhibition by bupropion would additionally provide a relatively safe method of probing striatal dopaminergic function in clinical populations in future studies.

Methods

Initial animal study

Doses of 11 and 25 mg/kg bupropion i.p. were selected for the initial study in rats. Microdialysis studies have previously shown increases in extracellular dopamine levels in the rat within this dose range (Bredeloux et al., 2007; Brown et al., 1991; Li et al., 2002; Nomikos et al., 1989; Sidhpura et al., 2007) and the 11 mg/kg dose is equivalent to the 150 mg human dose as calculated using dose-scaling factors (Mordenti and Chappell, 1989).

All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines. Under isoflurane anesthesia, 14 adult male Sprague–Dawley rats (Harlan Olac, UK) (body weight: mean \pm S.D. = 315 ± 46 g) were administered either vehicle (0.9% saline, $n=5$), 11 mg/kg bupropion (Sigma, UK) ($n=3$) or 25 mg/kg bupropion ($n=6$) i.p. 30 min prior to [^{11}C]raclopride injection. Rats were positioned in a stereotaxic frame and PET data were acquired using a quad-HIDAC (high-density avalanche chamber) small animal tomograph (Oxford Positron Systems). [^{11}C]Raclopride was administered via a previously

catheterized lateral tail vein. The mean \pm SD injectate was 0.311 ± 0.032 mCi (11.5 ± 1.2 MBq) with an associated stable content of 0.68 ± 0.23 nmol/kg. Emission data were acquired in list mode for 60 min.

To reconstruct scan sinograms, list mode emission data were binned into 0.5 mm isotropic voxels using filtered back-projection (Hamming filter, 0.6 cutoff), resulting in a spatial resolution of ~ 0.5 mm full width at half-maximum (FWHM) (Myers and Hume, 2002). Image volumes were then transferred into ANALYZE (www.analyzedirect.com) (Robb and Hanson, 1991). Using a volume of interest (VOI) template (Hume et al., 2001), data were sampled from the dorsal striatum (2×140 voxels) and cerebellum (764 voxels). Data analysis was limited to calculation of the specific binding ratio (SBR: the ratio of specifically bound radiotracer (striatum) to free and nonspecifically bound radiotracer (cerebellum), minus 1) during a single 40-min time frame, beginning 20 min after [^{11}C]raclopride injection, in order to improve count statistics (Hume et al., 2001). Previous studies have shown that [^{11}C]raclopride takes ~ 20 min to reach dynamic equilibrium in isoflurane-anesthetized rats and that the striatum/cerebellum ratio remains unchanged from 20 to 60 min after [^{11}C]raclopride injection (Hume et al., 1996) and ratio data acquired in the 20- to 60-min time frame correlates well with individual binding potential measurements derived from time-activity curves (Houston et al., 2004).

We estimated DAT occupancy under the same experimental conditions as used above: anaesthetized rats were administered vehicle (0.9% saline), 11 mg/kg bupropion or 25 mg/kg bupropion i.p. Thirty minutes later, ~ 10 μCi [^3H]cocaine (Perkin Elmer Life Sciences, UK) was administered i.v., and rats were euthanized 20 min following [^3H]cocaine administration. The striata and cerebellum were dissected out, solubilized (Soluene[®]-350, PerkinElmer, UK), and counted for ^3H using a LKB scintillation counter with automatic quench factor (Beckman, UK). Counts were normalized against standards, and data were calculated as percent injected activity per gram of tissue, normalized for body weight, giving 'uptake units.' The cerebellum, which contains a very low level of dopamine transporters (Panagopoulos et al., 1991), was used to represent free and nonspecifically bound [^3H]cocaine. Data are expressed as the striatal:cerebellar SBR. Percentage occupancy of dopamine transporter sites following bupropion administration was calculated as:

$$\text{Occupancy} = \left(\left[\text{SBR}_{\text{vehicle}} - \text{SBR}_{\text{bupropion}} \right] / \text{SBR}_{\text{vehicle}} \right) \times 100$$

Human study

Participants

Ten healthy participants were recruited by public advertisement (80% male; 90% right handed; average age: 47 ± 6.7 years; age range 37–58 years). Nine of the 10 subjects were nonsmokers; the single participant who smoked consumed ~ 10 cigarettes/day. None of the participants were currently taking any prescribed medication. All participants gave their written, informed consent to be included in the study. Exclusion criteria were pregnancy, any contraindication to PET imaging, current or previous neurological, psychiatric or medical illness including head injury, and alcohol or other recreational drug use or dependency according to DSM-IV criteria. The absence of illicit drugs was confirmed by a urine drugs screen. The study was approved by Hammersmith and Queen Charlotte's and Chelsea Research Ethics Committee, London, UK and the Administration of Radioactive Substances Advisory Committee.

Study design

Each participant underwent three [^{11}C]raclopride PET scans, performed on separate days and administered in a predetermined randomized order. The scan conditions were as follows: (A) Baseline: subjects were administered placebo and the data were acquired at

rest; (B) Placebo_Task: subjects were administered placebo and data were acquired as subjects performed a spatial planning task; (C) Bupropion_Task: subjects were administered bupropion and data were acquired as subjects performed a spatial planning task. Bupropion hydrochloride (150 mg Zyban® Sustained Release Tablets, GlaxoSmithKline) and placebo tablets were administered 2.5 h prior to [^{11}C]raclopride injection, in order that PET data acquisition coincided with peak bupropion plasma levels (Hsyu et al., 1997). All tablets were consumed in the presence of one of the investigators. The participants, but not the study investigators, were blind to the contents of the tablet. Although blood samples were taken mid-way through the scan to assay plasma bupropion levels, these data are not available for technical reasons. The spatial planning task was an adapted one-touch Tower of London task (Owen, 1997) presented on a computer touch-screen during the scan, as previously described (Lappin et al., 2009).

PET image acquisition

Data were acquired on an ECAT HR+ 962 scanner (CTI/Siemens) in three-dimensional mode, with an axial field of view of 15.5 cm. Head movement was monitored and minimized using a light head-strap. A 10-minute transmission scan was performed prior to radiotracer injection to correct for attenuation and scatter. The spatial planning task commenced 5 min before radiotracer injection. [^{11}C]raclopride was administered as a bolus injection followed by constant rate infusion with a K_{bol} of 85 min (Stokes et al., 2009). The total administered activity was 10.72 ± 0.36 mCi (396.8 ± 13.3 MBq) per scan, with an associated stable content of 2.175 ± 1.355 μg .

Image analysis

Head movement was corrected using frame-by-frame (FBF) realignment. Nonattenuation corrected images were used to optimize the FBF realignment process (Dagher et al., 1998). The nonattenuation corrected images were denoised using a level 2, order 64 Battle Lemarie wavelet filter (Turkheimer et al., 1999). A mutual information algorithm (Studholme et al., 1996) was applied for frame realignment to a single frame acquired 40 min post-injection, in which there was a high signal-to-noise ratio. Transformation parameters were applied to the corresponding attenuation-corrected dynamic images to generate a movement-corrected dynamic image.

Striatal and cerebellar regions of interest (ROIs) were defined on an atlas (Hammers et al., 2003) in Montreal Neurologic Institute (MNI) space. Striatal ROIs comprised the sensorimotor, associative and limbic functional subdivisions (Martinez et al., 2003). An [^{11}C]raclopride template (Meyer et al., 1999) was spatially transformed into the individual PET space of each FBF-corrected dynamic image within SPM5 (Wellcome Department of Cognitive Neurology, London; www.fil.ion.ucl.ac.uk/spm), and the resulting transformation parameters were then used to transform the ROI map into individual PET space.

A weighted average add image for the 40–85 min steady-state time period was generated from each FBF-corrected dynamic image using in house software, written in Matlab (version 5; The MathWorks, Inc, Natick, MA). The transformed ROI map was used to sample counts from the steady-state add image using ANALYZE software. Binding potential (BP_{ND}), the ratio at equilibrium of specifically bound radioligand to that of nondisplaceable radioligand in tissue (Innis et al., 2007), was calculated as the ratio of total radioactivity counts in the striatal subdivisions compared to the cerebellum, minus 1, during the 40- to 85-min steady-state sampling period.

Statistical analysis

In the rat study, the effects of 11 and 25 mg/kg bupropion on striatal [^{11}C]raclopride SBR and striatal DAT occupancy were deter-

mined using two-tailed independent sample *t*-tests. For the human study, differences in the amount and specific activity of injected [^{11}C]raclopride across conditions were explored using analysis of variance. [^{11}C]raclopride BP_{ND} values in the associative, sensorimotor and limbic subdivisions were compared across the three scan conditions using repeated measures analysis of variance (ANOVA), with scan condition and side (left or right) as within-subjects factors. Potential effects of scan order on [^{11}C]raclopride BP_{ND} were explored using the same approach. Body surface area (BSA) was calculated for each participant using the formula $\text{BSA} = (W^{0.425} \times H^{0.725}) \times 0.007184$, where *W* is weight in kilograms and *H* is height in centimeters (DuBois and DuBois, 1916). Relationships between BSA and percentage change in [^{11}C]raclopride BP_{ND} in the bupropion_task compared to placebo_task condition were explored using Pearson's correlation coefficient. All statistical analysis was performed in SPSS 16.0 (Chicago, IL), and the threshold for statistical significance was set at an alpha level of 0.05. All data are reported as mean \pm standard deviation.

Results

Initial rat study

Fig. 1 illustrates the images that were obtained in control and bupropion-treated rats using the quad-HIDAC tomograph system. In Fig. 1, the reduction in [^{11}C]raclopride SBR following the higher dose of 25 mg/kg bupropion compared to control values is clearly visible. Individual SBR values obtained in the striatum of control, 11 mg/kg bupropion and 25 mg/kg bupropion-treated animals are presented in Table 1. In the dorsal striatum, pre-treatment with both 11 and 25 mg/kg bupropion significantly reduced [^{11}C]raclopride SBR (11 mg/kg $t_{(6)} = 3.203$; $p = 0.019$; 25 mg/kg bupropion $t_{(6.58)} = 9.157$; $p < 0.001$). These decreases in SBR were to the magnitude of $6 \pm 3\%$ following 11 mg/kg bupropion and $23 \pm 7\%$ following 25 mg/kg bupropion.

Table 1 also presents the [^3H]cocaine dopamine transporter occupancy data that were obtained at doses of 11 and 25 mg/kg bupropion in the rat. 25 mg/kg bupropion produced significant occupancy of the dopamine transporter ($t_4 = 5.984$; $p = 0.004$) and there was a trend for the same effect at the lower dose of 11 mg/kg ($t_4 = 2.678$; $p = 0.055$). These values corresponded to $35 \pm 18\%$ dopamine transporter occupancy with 11 mg/kg bupropion and $60 \pm 11\%$ dopamine transporter occupancy with 25 mg/kg bupropion.

Human study

Spatial planning accuracy offline (mean \pm S.D. = $74.4 \pm 22.7\%$; range = 50–100%) and in the scanner following placebo administration (mean \pm S.D. = $77.3 \pm 19.6\%$; range = 43.8–96.3%) were correlated ($r = 0.745$; $p = 0.013$). Planning accuracy in the scanner following bupropion administration (mean \pm S.D. = $76.3 \pm 15.2\%$; range = 50–91.3%) and placebo administration also correlated ($r = 0.879$; $p = 0.001$). Bupropion did not significantly affect planning accuracy in the scanner ($t_{(9)} = 0.329$; $p = 0.750$). No significant correlations were apparent between planning accuracy and age.

There was no significant difference in either the amount of [^{11}C]raclopride radioactivity injected ($p > 0.36$) or associated stable content ($p > 0.21$) across the three scan conditions. Similarly, scan order did not influence BP_{ND} in any of the striatal subdivisions (sensorimotor: $F_2 = 1.167$; $p = 0.334$; associative: $F_2 = 0.326$; $p = 0.726$; limbic: $F_2 = 0.801$; $p = 0.464$). As there were no significant associations between age and BP_{ND} in the whole striatum or any of the striatal subdivisions, age was not used as a covariate in subsequent analysis. Planning accuracy did not correlate with [^{11}C]raclopride BP_{ND} in any of the striatal subdivisions under either the Placebo_Task or Bupropion_Task condition.

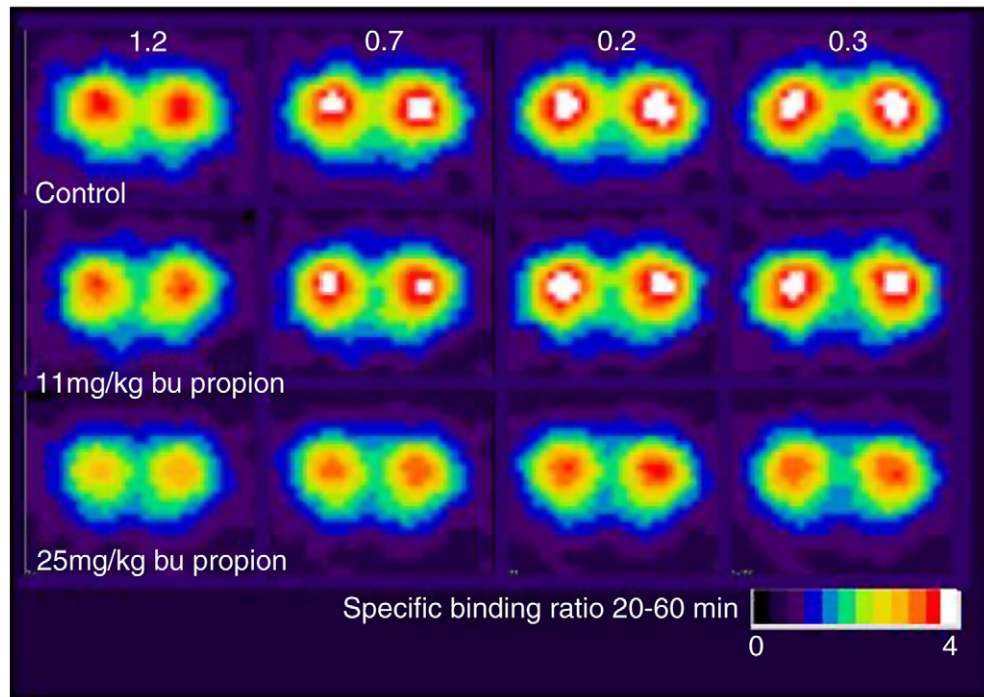


Fig. 1. Mean [^{11}C]raclopride coronal SBR images obtained in rats treated with saline (control), 11 or 25 mg/kg bupropion. The images represent addimages of data collected 20–60 min after [^{11}C]raclopride injection. Distances from bregma (mm) are indicated along the top of the figure.

The BP_{ND} values that were obtained in each of the three scan conditions are presented in Table 2. In the associative striatum, there was a significant overall effect of scan condition ($F_2 = 4.021$; $p = 0.036$) and side ($F_1 = 44.895$; $p < 0.001$) on [^{11}C]raclopride BP_{ND} but no significant condition by side interaction ($F_2 = 1.031$; $p = 0.297$). Post hoc analysis revealed a significant ($4.4 \pm 5\%$) increase in [^{11}C]raclopride BP_{ND} in the associative striatum in the Bupropion_Task compared to Placebo_Task condition ($F_2 = 4.021$; $p = 0.036$), but this did not survive correction for multiple comparisons ($p = 0.081$). Individual BP_{ND} values in the associative striatum in the Placebo_Task and Bupropion_Task conditions are presented in Fig. 2. Change in [^{11}C]raclopride BP_{ND} in the associative striatum in the Bupropion_Task compared to Placebo_Task condition was not significantly correlated with BSA ($r = 0.462$; $p = 0.179$). There was no significant difference in [^{11}C]raclopride BP_{ND} in the associative striatum in the Baseline compared to Placebo_Task conditions ($F_1 = 1.279$; $p = 0.287$).

No significant effects of scan condition on [^{11}C]raclopride BP_{ND} were apparent in the sensorimotor ($F_2 = 2.919$; $p = 0.080$) or limbic ($F_1 = 0.213$; $p = 0.810$) striatal subdivisions. As in the associative striatum, there were significant effects of side (left or right) on BP_{ND} in the sensorimotor ($F_1 = 48.074$; $p < 0.001$) and limbic subdivisions ($F_1 = 15.36$; $p = 0.004$) but no significant condition by side interactions were detected.

Discussion

Using [^{11}C]raclopride PET, we sought to determine whether bupropion administration increases extracellular dopamine levels in the rat and human striatum. In rats, bupropion administration decreased striatal [^{11}C]raclopride specific binding, consistent with increases in extracellular dopamine concentrations resulting from inhibition of dopamine reuptake. However, when this approach was translated to man, bupropion administration did not decrease striatal [^{11}C]raclopride BP_{ND} , indicating that extracellular dopamine levels were not increased to levels detectable using this approach. These results indicate that, in man, bupropion's therapeutic efficacy is unlikely to principally derive from marked increases in striatal dopaminergic transmission.

The decreases in [^{11}C]raclopride SBR which we report in anaesthetized rats are accordant with the increases in extracellular dopamine concentrations that are detected using microdialysis following administration of similar doses of bupropion in awake animals (Brown et al., 1991; Li et al., 2002; Nomikos et al., 1989; Sidhpura et al., 2007). While the relationship between dopamine release and change in [^{11}C]raclopride binding potential varies according to the pharmacological nature of the challenge stimulus (Schiffer et al., 2006; Tsukada et al., 1999), previous studies

Table 1

Striatal [^{11}C]raclopride SBR and [^3H]cocaine SBR in control rats and following administration of 11 or 25 mg/kg bupropion i.p. All data were acquired in anaesthetized animals. [^{11}C]raclopride SBR was determined from summed PET data acquired 20–60 min following [^{11}C]raclopride administration. [^3H]cocaine SBR was determined from *ex vivo* dissection data collected 20 min following [^3H]cocaine administration. Bupropion produced significant ($p < 0.05$) increases in extracellular dopamine concentrations, as indexed by change (Δ) in [^{11}C]raclopride SBR compared to control values, and significant occupancy of dopamine transporter sites as indexed by difference in [^3H]cocaine SBR compared to control values.

[^{11}C]raclopride SBR (mean \pm S.D.)						
Control	11 mg/kg bupropion	Significance	Δ SBR	25 mg/kg bupropion	Significance	Δ SBR
3.25 \pm 0.07	3.05 \pm 0.11	$p = 0.019$	6 \pm 3%	2.52 \pm 0.18	$p < 0.001$	23 \pm 7%
[^3H]cocaine SBR (mean \pm S.D.)						
Control	11 mg/kg bupropion	Significance	Occupancy	25 mg/kg bupropion	Significance	Occupancy
2.00 \pm 0.26	1.30 \pm 0.37	$p = 0.055$	35 \pm 18%	0.81 \pm 0.22	$p = 0.004$	60 \pm 18%

Table 2
 $[^{11}\text{C}]$ raclopride BP_{ND} values (mean \pm S.D.) in the functional subdivisions of the human striatum under each scan condition. AST: associative striatum; SMS: sensorimotor striatum; LS: limbic striatum. Effect of condition denotes overall effect of scan condition (Baseline, Placebo_Task or Bupropion_Task) in repeated measures ANOVA, Effect of side denotes overall effect of side (left or right) in repeated measures ANOVA. * $p < 0.05$.

	Baseline	Placebo_Task	Bupropion_Task	Effect of condition	Effect of side
Left AST	2.16 \pm 0.15	2.15 \pm 0.15	2.24 \pm 0.12	$p = 0.036^*$	$P < 0.001^*$
Right AST	2.32 \pm 0.17	2.26 \pm 0.17	2.36 \pm 0.12		
Left SMS	2.52 \pm 0.14	2.52 \pm 0.18	2.55 \pm 0.18	$p = 0.080$	$P < 0.001^*$
Right SMS	2.78 \pm 0.17	2.73 \pm 0.19	2.88 \pm 0.17		
Left LS	2.28 \pm 0.15	2.29 \pm 0.16	2.32 \pm 0.22	$p = 0.810$	$p = 0.004^*$
Right LS	2.14 \pm 0.16	2.11 \pm 0.12	2.15 \pm 0.10		

combining microdialysis and $[^{11}\text{C}]$ raclopride PET in animals following administration of the dopamine transporter inhibitor methylphenidate, estimate that a 1% change in $[^{11}\text{C}]$ raclopride BP relates to a 17% change in dopamine concentration as measured using microdialysis (Schiffer et al., 2006). Applying this relationship to the current data, the 6% and 22% change in SBR observed at 11 mg/kg and 25 mg/kg bupropion respectively, would translate to a 102% and 374% change in dopamine concentration as measured using microdialysis. These values are in the range of the percentage increases in striatal dopamine concentrations that have been reported using microdialysis in rats after administration of bupropion at similar doses (Brown et al., 1991; Li et al., 2002; Nomikos et al., 1989; Sidhpura et al., 2007) and therefore validate the use of $[^{11}\text{C}]$ raclopride PET imaging to measure changes in striatal dopamine concentrations following bupropion administration.

In the human study, we did not observe any significant decreases in $[^{11}\text{C}]$ raclopride BP_{ND} in the striatum following bupropion administration, despite the presence of a behavioral task applied to stimulate dopamine release and therefore maximize the influence of dopamine reuptake inhibition. We therefore conclude that bupropion administration does not markedly increase striatal extracellular dopamine concentrations. Indeed to the contrary, in the associative striatum we detected a small increase in $[^{11}\text{C}]$ raclopride BP_{ND} , consistent with decreases in extracellular dopamine concentrations, although this did not survive correction for multiple comparisons.

In rats, decreases in $[^{11}\text{C}]$ raclopride BP_{ND} occurred at 11 and 25 mg/kg bupropion i.p. The 11 mg/kg bupropion dose is equivalent to the human dose of 150 mg as simply estimated using dose-scaling factors (Mordenti and Chappell, 1989). However, the extensive metabolism of bupropion to the active metabolites hydroxybupropion and threohydrobupropion in man (Schroeder, 1983), occurs to a far lesser extent in rats (Suckow et al., 1986). We did not compare the plasma concentrations of bupropion and its metabolites that were achieved in the rat and human subjects, although, as brain concentrations of bupropion may be some order of magnitude higher

than those measured in plasma (Schroeder, 1983; Suckow et al., 1986), interpretation would be limited. A better indication of dose equivalence is provided by comparing the degree of striatal DAT occupancy resulting from bupropion administration in rats and man. Here, the lowest (11 mg/kg) dose of bupropion investigated in the rat was estimated to occupy at least 35% of DAT sites; in contrast, previous data show the levels of DAT occupancy achieved in man following chronic bupropion dosing are, at most, ~20–25% (Argyelan et al., 2005; Kugaya et al., 2003; Learned-Coughlin et al., 2003; Meyer et al., 2002). This suggests that higher levels of DAT occupancy were achieved in the rat than the human study, which might explain why significant decreases in $[^{11}\text{C}]$ raclopride BP_{ND} following bupropion were observed in rats, but not in man.

In the animal literature, the central effects of bupropion are often investigated using doses of 10 mg/kg or more. The results of the present study and those previously examining DAT occupancy following bupropion administration in man (Argyelan et al., 2005; Kugaya et al., 2003; Learned-Coughlin et al., 2003; Meyer et al., 2002), suggest that investigation of the effects of bupropion within a lower dose range would be of increased relevance to human, clinical situation.

The human study was powered (0.8) to reliably detect a 5% change in $[^{11}\text{C}]$ raclopride BP_{ND} between scan conditions, based on both previous published data (Mawlawi et al., 2001), and unpublished data acquired in-house on the same scanner. It is unlikely that lack of power precluded observation of decreases in $[^{11}\text{C}]$ raclopride BP_{ND} , as in both the associative and sensorimotor striatal divisions $[^{11}\text{C}]$ raclopride BP_{ND} was actually increased rather than decreased in 8 of 10 volunteers in the Bupropion_Task compared to Placebo_Task condition. Although we scanned volunteers 2.5 h after bupropion administration to coincide with peak bupropion plasma concentrations, bupropion metabolite concentrations peak approximately 6 h following bupropion administration (Hsyu et al., 1997). This raises the possibility that scanning at a later time point, when dopamine transporter occupancy may have been higher, may have revealed differential effects on $[^{11}\text{C}]$ raclopride BP_{ND} . It is also possible that repeated administration of bupropion is required to increase striatal dopamine concentrations in man; this hypothesis could be tested using a similar $[^{11}\text{C}]$ raclopride PET approach to the present study. However, the low levels of dopamine transporter occupancy observed in man following repeated bupropion administration (Argyelan et al., 2005; Kugaya et al., 2003; Learned-Coughlin et al., 2003; Meyer et al., 2002) suggest that this is unlikely.

In contrast to our previous study (Lappin et al., 2009), in this sample we did not detect significant decreases in striatal $[^{11}\text{C}]$ raclopride BP_{ND} during the spatial planning task. Differences between the two studies may explain this. In particular the current but not previous study used placebo tablets, which, as subject expectation may alter dopamine levels, may have masked an effect (Yoder et al., 2008). Furthermore spatial planning accuracy was poorer and more variable in the current sample both offline ($74 \pm 23\%$ versus $90 \pm 10\%$) and within the scanner ($77 \pm 20\%$ versus $90 \pm 4\%$), although the age range of subjects in the two studies was similar (mean 47 ± 7 years, range 37–58 years in the present study, mean 53 ± 9 years, range 39–68

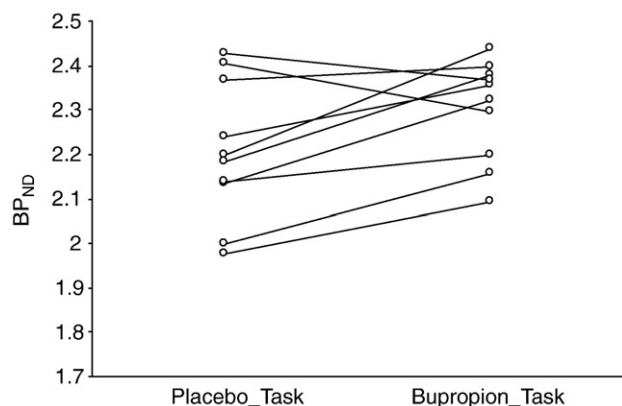


Fig. 2. Individual $[^{11}\text{C}]$ raclopride BP_{ND} in the associative striatum in the Placebo_Task and Bupropion_Task conditions. Bupropion administration significantly increased BP_{ND} ($p = 0.028$).

years in Lappin et al., 2009). This suggests that ability to observe dopamine release during behavioral tasks using [^{11}C]raclopride PET may be particularly sensitive to the precise experimental conditions. We conclude that application of this task, with or without concurrent dopamine reuptake inhibition, does not provide a robust approach to probing striatal dopamine function in man.

In conclusion, as acute administration of bupropion administration did not result in detectable increases in extracellular dopamine concentrations in the human striatum, this study does not support the involvement of striatal dopamine in the clinical efficacy of bupropion.

Disclosure/Conflicts of Interest

This study was performed in collaboration with GlaxoSmithKline UK.

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