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Comparison of the effects of bupropion and nicotine on locomotor activation and dopamine release *in vivo*

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ABSTRACT

Bupropion is an atypical anti-depressant that is approved for smoking cessation. In addition to inhibiting dopamine reuptake, bupropion has been reported to block nicotinic acetylcholine receptors *in vitro*, and this action might contribute to its efficacy for smoking cessation. In this study we investigated if nicotinic receptor-mediated responses *in vivo* are decreased in the presence of a behaviorally effective dose of bupropion. In separate experiments we measured locomotor activation and dopamine overflow in the nucleus accumbens core, using *in vivo* microdialysis in freely moving rats. Bupropion (30 mg/kg i.p.) increased locomotor activity, which remained elevated for up to 2 h. Nicotine (0.4 mg/kg s.c.) also increased locomotor activity but for a shorter duration. When given 20 min after bupropion, hyperlocomotion was significantly enhanced, compared to the response to either nicotine or bupropion alone, consistent with the effects of the two drugs being additive. Systemic administration of bupropion (30 mg/kg i.p.) also elicited a significant increase in dopamine overflow (113 ± 16% above basal levels). Nicotine (3 mM; delivered into the nucleus accumbens core via the microdialysis probe) increased dopamine overflow by 126 ± 35%. Nicotine delivered during the response to bupropion resulted in enhanced dopamine overflow of 294 ± 50%, also consistent with the actions of the two drugs being additive. This study suggests that behaviorally effective concentrations of bupropion in the rat do not diminish the effects of nicotine by blocking nicotinic receptors.

1. Introduction

Nicotine is the principal psychoactive component in tobacco smoke, whose reinforcing properties underpin addiction to cigarette smoking [1]. By activating nicotinic acetylcholine receptors (nAChRs), nicotine stimulates dopamine overflow from mesolimbic neurones that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and the prefrontal cortex [2,3]. Nicotine-evoked dopamine release in the NAc plays a central role in the rewarding and locomotor stimulating properties of nicotine that lead to dependence [4–9].

Cigarette smoking remains a major cause of death worldwide, despite increased social awareness and the availability of cessation aids. Nicotine replacement therapy has been the first line drug treatment for smoking cessation. The atypical anti-depressant bupropion (Zyban™) was the first non-nicotinic pharmacotherapy to be approved for smoking

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Abbreviations: DAT, dopamine transporter; NAc, nucleus accumbens; NAc_{core}, nucleus accumbens core; nAChR, nicotinic acetylcholine receptor; VTA, ventral tegmental area

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cessation and it has proved moderately efficacious as an anti-smoking agent [10–12]. The mechanism of action of bupropion that results in increased abstinence rates in smokers remains unclear [13,14]. Bupropion is a weak but relatively selective inhibitor of the dopamine transporter (DAT). Its potency for blocking the noradrenaline transporter is 2–4 times lower than that for DAT, and it exhibits little affinity for the serotonin transport system [15]. Hence its modest inhibition of dopamine and, to a lesser extent, noradrenaline reuptake is considered to be of major importance in combating nicotine addiction, by attenuating the craving for nicotine through its ability to maintain raised extracellular dopamine levels in the brain [16].

Recent studies have shown that bupropion also interacts directly with nAChRs. Bupropion inhibited nAChRs expressed in human neuroblastoma cell lines [17] and *Xenopus* oocytes [18]: the insurmountable blockade, despite increasing agonist concentrations, suggests a non-competitive interaction [17,18]. Systemically administered bupropion has also been reported to block nicotine-induced responses in mice, including anti-nociception, hypolocomotion, hypothermia and convulsions [18].

Of more relevance to the rewarding effects of nicotine, bupropion dose-dependently inhibited nicotine-evoked [^{3}H]dopamine overflow from rat striatal and hippocampal slices *in vitro*, under conditions in which any interaction with DAT or the noradrenaline transporter was avoided by the presence of nomifensine or desipramine, respectively [19]. We recently examined the effects of bupropion on nicotine-evoked [^{3}H]dopamine release from rat striatal synaptosomes and slices in the absence of any other transporter inhibitor [20] and confirmed that in the presence of 10 μM bupropion nicotine-evoked responses were significantly inhibited, consistent with previous reports. However, nicotine-evoked [^{3}H]dopamine release was also affected by sub-micromolar concentrations of bupropion in a manner consistent with inhibition of DAT, indicating a separation between the effective concentrations of bupropion that act on these two targets.

In the present study we aimed to explore the relative contributions of DAT inhibition and nAChR blockade by bupropion at functionally effective concentrations of the drug *in vivo*. Locomotor activation was recorded as an indirect index of dopaminergic activity and extracellular levels of dopamine in the NAc core (NAc_{core}) were measured using microdialysis in freely moving rats. The results indicate that the effects of an effective concentration of bupropion, sufficient to elicit increases in locomotor activation or extracellular dopamine, are additive with nicotine-evoked responses, consistent with a lack of interaction with nAChRs under these experimental conditions.

2. Materials and methods

2.1. Animals and environment

Male Sprague Dawley rats (250–300 g; University of Bath Animal House breeding colony) were used. Prior to the start of an experiment, rats were housed in groups of four per cage in a temperature- and humidity-controlled environment with free access to food and water. Rats were kept on a 12 h light:dark cycle with lights on at 07.00 h. All experiments were undertaken between 9.00 h and 14.00 h. All experiments were carried out within the guidelines of the United Kingdom Animals (Scientific Procedures) Act of 1986.

2.2. Locomotor activity experiments

2.2.1. Experimental design

Rats were transferred to the test room and placed randomly into individual clear-sided, perspex activity test cages (*H.* 185 mm × *W.* 265 mm × *L.* 425 mm) in which they were allowed to acclimatize to the conditions for 1 h.

2.2.1.1. *Experiment 1.* Following acclimatization, rats were given an intraperitoneal (i.p.) injection of 10, 30 or 60 mg/kg bupropion or saline. Locomotor activity was scored automatically for a period of 200 min after injection, by infrared detection beams.

2.2.1.2. *Experiment 2.* Following acclimatization, rats were given an i.p. injection of 30 mg/kg bupropion or saline. After 20 min, rats were challenged with a subcutaneous (s.c.) injection of 0.4 mg/kg nicotine or saline. Locomotor activity was monitored for a further 180 min after the second injection, as described above.

2.2.2. Statistical analysis

Mean activity counts for each treatment group were determined at 20 min intervals. All values are mean ± S.E.M. for $n = 8$ rats per treatment group. Statistical comparisons between drug treated and control groups were carried out by two-way ANOVA for repeated measurements (time × treatment), using StatView (v 5.0.1) for Windows. Where statistical significance was observed, one-way ANOVA with post hoc Dunnett's test for multiple comparisons was used. A p-value of <0.05 was considered statistically significant.

2.3. Microdialysis in freely moving animals

2.3.1. Surgery and microdialysis

Rats were anaesthetized with a mixture of medetomidine 1 mg/kg and ketamine 100 mg/kg, i.p. and transferred to a stereotaxic frame (David Kopf, Topanga, USA). Body temperature was maintained at 37 °C throughout using a homeothermic blanket set. A concentric microdialysis probe (O.D. 0.3 mm) with 2 mm of exposed Hospal membrane tip (manufactured in house) was implanted into the right NAc_{core} (coordinates: anterior: +1.2 mm; lateral: +2.0 mm; ventral: −7.8 mm from Bregma) [21]. The probe and a tether screw (Instech Soloman, UK; placed posterior to the probe) were secured with dental cement, and the wound sealed. Anaesthesia was reversed using atipamezole (1 mg/kg, i.p.). Following surgery, rats were individually housed in circular chambers (I.D. 395 mm × *H.* 360 mm) with the microdialysis probe connected to a liquid swivel (Instech Soloman, UK) and a counter-balanced arm to allow unrestricted movement. Rats were allowed a recovery period of at least 16 h with food and water available *ad libitum*. Probes were continuously perfused

with artificial cerebrospinal fluid (aCSF; 125 mM NaCl, 2.5 mM KCl, 1.18 mM $MgCl_2$, 1.26 mM $CaCl_2$, and 2.0 mM Na_2HPO_4, adjusted to pH 7.4 with 100 mM H_3PO_4) at a flow rate of 1.2 μl/min. After this period, samples were collected at 20 min intervals into 0.3 ml polypropylene sample vials (HPLC Technology, UK) containing 5 μl of perchloric acid (0.1 M).

2.3.2. *Dopamine analysis*

Dopamine in the dialysis samples was quantified by reverse-phase, ion-pair high pressure liquid chromatography coupled with electrochemical detection (HPLC–ECD). Briefly, compounds were separated on a C_{18} reverse-phase column (100 mm × 2.1 mm; Spherisorb ODS; Higgins Analytical). A Bischoff solvent delivery pump with a pulse dampener (PD-120625, Presearch Ltd., Herts., UK) was used to circulate the mobile phase (100 mM NaH_2PO_4, 1 mM EDTA, 1 mM octane sulphonic acid, 12% methanol, pH 4.0) at a flow rate of 0.2 ml/min. The mobile phase was filtered through a 0.22 μm filter (Millipore, Bedford, USA) and degassed under vacuum. Dopamine standards (20 μl) were injected onto the column via a refrigerated (4 °C) Triathlon autosampler. A stock solution of dopamine (1 mM) was prepared by dissolving it in a mixture of equal quantities of deionised water and 0.1 M perchloric acid and stored at 4 °C. A working solution was prepared daily. An Antec-Intro (Leyden, Netherlands) electrochemical detector was used in conjunction with an Antec ''wall-jet'' design cell (VT-03). The cell employs a high density, glassy carbon working electrode (+0.60 V) combined with an Ag/AgCl reference electrode. The electrode signal was integrated using a PowerChrom data acquisition system (AD Instruments, Oxfordshire, UK). The detection limit for dopamine was 1 fmol on column.

2.3.3. *Experimental design*

For each experiment, rats were randomly assigned to one of four treatment groups that received bupropion and nicotine, bupropion alone, nicotine alone, or vehicle only. Four basal samples were collected and then rats were given either bupropion (30 mg/kg, i.p.) or saline, followed by a local infusion of either 3 mM nicotine in aCSF or aCSF alone for 20 min. There were no artefacts associated with the procedure of changing from a syringe containing aCSF to a syringe containing aCSF with or without nicotine. Dialysates were sampled for a further 280 min.

2.3.4. *Histology*

At the end of each experiment, rats were killed with an overdose of sodium pentobarbital and their brains rapidly removed and stored in 4% paraformaldehyde in phosphate buffer for at least 5 days. Serial coronal sections (100 μm) were made using a vibratome and histological verification of probe placement was confirmed with reference to a stereotaxic atlas [21]. Data are reported only from animals where probe membranes were correctly positioned in the NAc_{core}.

2.3.5. *Statistical analysis*

All data shown are mean ± S.E.M. values for at least $n = 6$ rats per treatment group. Basal release measured in four consecutive samples before drug application was averaged and defined as 100%. Results for subsequent samples were calculated as percentages of this average basal release. Statistical comparisons between drug treated and control groups were carried out by two-way ANOVA for repeated measurements (time × treatment), using StatView (v 5.0.1) for Windows. Where statistical significance was observed, one-way ANOVA with post hoc Dunnett's test for multiple comparisons was used. A p-value of <0.05 was considered statistically significant.

2.4. *Drugs and reagents*

(−)-Nicotine hydrogen tartrate, bupropion hydrochloride, dopamine and paraformaldehyde were purchased from Sigma–Aldrich Co. Ltd. (Gillingham, Dorset, UK). For systemic injection, nicotine and bupropion were dissolved in 0.9% saline and administered in a volume of 1 ml/kg. The pH of nicotine and bupropion solutions was adjusted to pH 7.4 before administration. Drug doses are expressed as the free base. All reagents used in HPLC analysis were of HPLC grade. EDTA, methanol, KCl, $MgCl_2$, Na_2HPO_4 and H_3PO_4 were purchased from Fisher Scientific Ltd. (Loughborough, Leics., UK). NaCl, $CaCl_2$, NaH_2PO_4, octane sulphonic acid and perchloric acid were obtained from VWR International Ltd. (Poole, Dorset, UK).

3. Results

3.1. *Locomotor experiment 1: effects of increasing bupropion concentration*

The effect of systemic bupropion (10, 30, 60 mg/kg, i.p.) on locomotor activity, a dopamine-mediated behavior, was examined. Bupropion (10–60 mg/kg) increased locomotor activity, with 30 and 60 mg/kg bupropion producing a significant increase in locomotion ([$F(36, 336) = 8.476$, $p < 0.0001$]), compared to saline-treated controls, over a 200 min post-treatment period (Fig. 1a). The maximal increase in locomotor activity was observed at 20 min following administration of either 30 mg/kg bupropion or 60 mg/kg bupropion, compared to saline-treated controls ($p < 0.001$, $n = 8$); both concentrations increased locomotor activity to a similar extent. Locomotor activity was sustained for longer after the higher dose: it remained significantly elevated for 80 and 200 min following 30 and 60 mg/kg bupropion, respectively. From these data, a dose of 30 mg/kg bupropion was selected for studying the interaction with nicotine-evoked responses.

3.2. *Locomotor experiment 2: comparison of bupropion and nicotine*

To compare the effects of systemic bupropion and nicotine on locomotor activity, rats were pretreated with bupropion (30 mg/kg, i.p.) or vehicle, and challenged with an acute injection of nicotine (0.4 mg/kg, s.c.) or vehicle after 20 min (Fig. 1b). In the absence of nicotine, bupropion (30 mg/kg, i.p.) significantly increased locomotion ([$F(1, 28) = 64.714$, $p < 0.0001$]), compared to saline-treated controls over a 200 min post-bupropion period. The time course and magnitude of the response was comparable to that recorded in the previous experiment (Fig. 1a). Compared to saline-treated controls, systemic nicotine

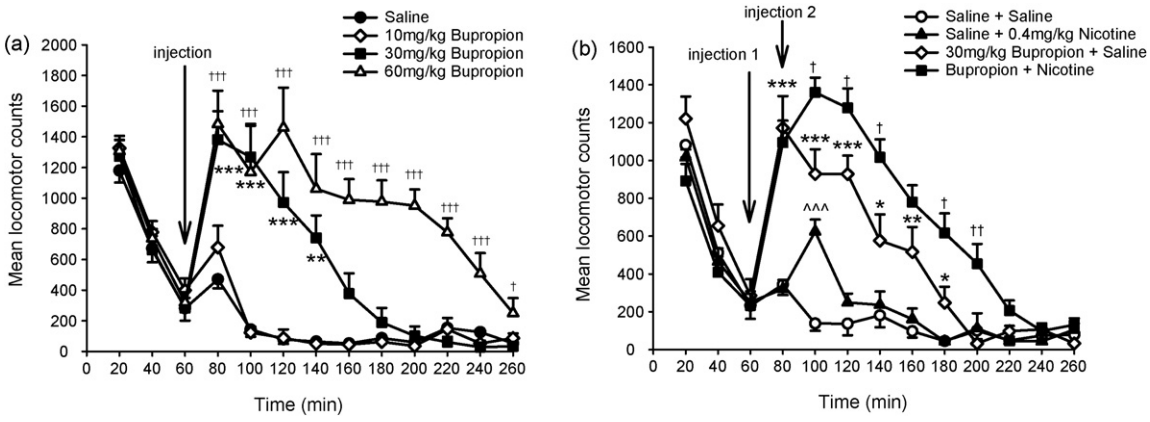

Fig. 1 – Effect of (a) bupropion and (b) bupropion plus nicotine on rat locomotor activity. (a) Rats were placed individually in activity test cages for a 60 min acclimatization period, after which they received bupropion (10, 30, 60 mg/kg i.p.) or saline. Locomotor activity was recorded automatically by infrared detection beams and pooled at 20 min intervals. Data points represent the mean ± S.E.M. locomotor counts, calculated from eight rats per treatment group. To test for a significant difference from saline-treated (control) rats, data were analyzed using a one-way ANOVA with repeated measures and Dunnett's post hoc test ($p < 0.01$, ***$p < 0.001$ for 30 mg/kg bupropion; $^{\dagger}p < 0.05$, $^{\dagger\dagger\dagger}p < 0.001$ for 60 mg/kg bupropion). There was no significant difference in the activity of the different groups of rats during the acclimatization period or following 10 mg/kg bupropion. (b) Rats were placed individually in activity test cages for a 60 min acclimatization period, after which they received bupropion (30 mg/kg i.p.) or saline, followed by nicotine (0.4 mg/kg s.c.) or saline 20 min later. Locomotor activity was recorded automatically by infrared detection beams and pooled at 20 min intervals. Data represent the mean ± S.E.M. locomotor counts, calculated from eight rats per treatment group. To test for a significant difference from control rats, data were analyzed using a two-way ANOVA with repeated measures and Dunnett's post-hoc test ($^{\wedge\wedge\wedge}p < 0.001$ for saline + 0.4 mg/kg nicotine vs. saline + saline; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for 30 mg/kg bupropion + saline vs. saline + saline; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ for 30 mg/kg bupropion + 0.4 mg/kg nicotine vs. 30 mg/kg bupropion + saline). There was no significant difference in the activity of the different groups of rats during the acclimatization period.**

(0.4 mg/kg, s.c.) significantly increased locomotor activity ([$F(1, 28) = 4.923$, $p < 0.05$]), with peak locomotion observed at 20 min following the nicotine challenge. Nicotine-induced hyperlocomotion returned to saline-treated control values over the next 20 min.

In rats pretreated with bupropion, nicotine significantly enhanced bupropion-induced hyperlocomotion compared to bupropion alone (Fig. 1b; [$F(12, 336) = 2.785$, $p < 0.001$]). Maximal locomotor activity was observed at 20 min following the nicotine challenge ([$F(12, 336) = 27.065$, $p < 0.0001$) and this was significantly greater than the locomotor activation in response to bupropion alone ($p < 0.05$, $n = 8$). Locomotor activity remained significantly elevated for 120 min after the nicotine injection.

3.3. *Effects of systemic bupropion and locally applied nicotine on dopamine release*

To further explore the interaction between the effects of bupropion and nicotine, we examined dopamine release from the nucleus accumbens core (NAc_{core}) in freely moving rats. Bupropion (30 mg/kg, i.p.) was administered systemically followed, 20 min later, by nicotine. Because acute systemic administration of nicotine does not elicit robust dopamine overflow from NAc_{core} [7], nicotine (3 mM) was applied locally via the microdialysis probe [22].

Basal levels of dopamine were 25.8 ± 2.2 fmol/20 μl ($n = 37$). These data have not been corrected for recoveries across the dialysis membrane. Local infusion of 3 mM nicotine (t = 20–40 min) in the NAc_{core} significantly increased extracellular dopamine levels, compared to basal values ([$F(6, 17) = 8.238$, $p < 0.0001$]) and compared to non-drug controls ([$F(1, 24) = 5.506$, $p < 0.05$]) (Fig. 2a). Peak dopamine levels (t = 40 min) were 126 ± 35% ($p < 0.01$, $n = 8$) above basal values. Dopamine levels returned to basal values over the next 20–40 min. We have previously confirmed that the increased dopamine overflow in the NAc in response to this concentration of locally applied nicotine (3 mM) is abolished in the presence of mecamylamine [22], consistent with the specific interaction of nicotine with nAChRs.

In the absence of nicotine, bupropion (30 mg/kg) significantly increased extracellular dopamine levels compared to basal values ([$F(5, 17) = 6.598$, $p < 0.0001$]) (Fig. 2b). Peak dopamine levels (t = 40 min) were 113 ± 16% ($p < 0.01$, $n = 8$) above basal values, comparable to the response to 3 mM nicotine. Dopamine levels returned to basal values over the next 20–40 min. Administration of nicotine after bupropion produced a significantly greater increase in extracellular dopamine levels above basal values [$F(7, 17) = 17.569$, $p < 0.0001$]), with peak dopamine levels (t = 40 min) of 294 ± 50% ($p < 0.05$, $n = 9$) above basal values. Compared to rats that received only bupropion, increases in extracellular dopamine were significantly potentiated by nicotine ([$F(17, 408] = 8.213$, $p < 0.0001$]). Elevated dopamine levels were maintained between t = 40–60 min, with an average increase in dopamine of 138 ± 23% above the levels recorded in

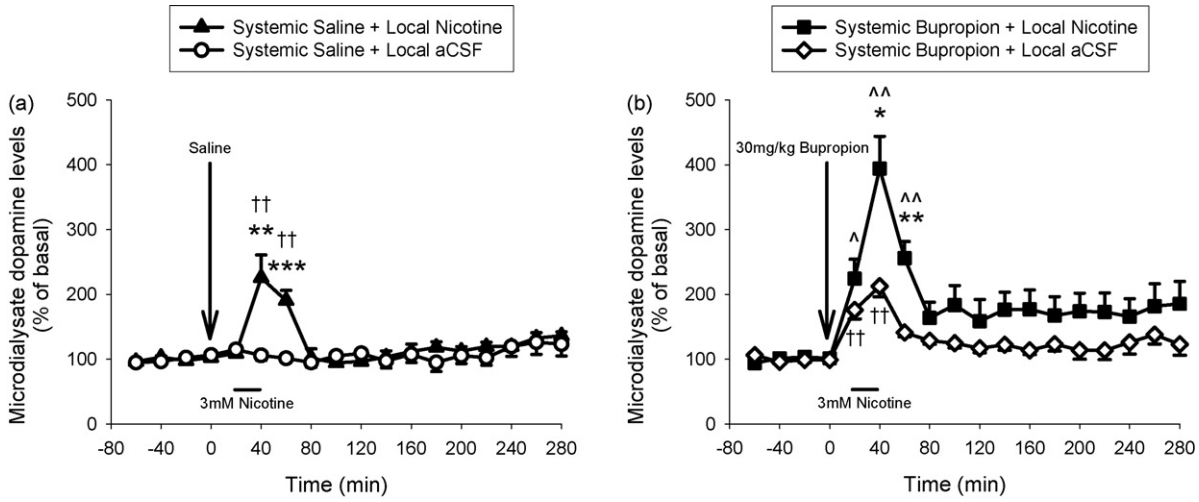

Fig. 2 – Effect of systemic bupropion and locally applied nicotine on dopamine overflow in the NAc_{core}. Rats were prepared for microdialysis as described in Section 2; 20 min (24 μl) serial fractions were collected and dopamine was quantified using HPLC with electrochemical detection. After collection of four stable baseline samples, two groups of rats received a systemic injection of saline, followed by a local infusion of aCSF in the absence or presence of 3 mM nicotine, delivered via the microdialysis probe (a). Another two groups of rats received bupropion (30 mg/kg i.p.) followed by a local infusion of aCSF in the absence or presence of 3 mM nicotine, delivered via the microdialysis probe (b). Dopamine overflow is shown as a percentage of mean ± S.E.M. basal release from at least eight rats per treatment group. To test for a significant difference from basal values, data were analyzed using repeated measures ANOVA and Dunnett's post hoc test [(a) $^{\dagger\dagger}p < 0.01$ for systemic saline + local nicotine, (b) $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$ for systemic bupropion + local nicotine, $^{\dagger\dagger}p < 0.01$ for systemic bupropion + local aCSF;]. To test for a significant difference from control response, data were analyzed using a two-way ANOVA with repeated measures and Dunnett's post hoc test [(a) $^{**}p < 0.01$, $^{***}p < 0.001$ for systemic saline + local nicotine vs. systemic saline + local aCSF, (b) $^{*}p < 0.05$, $^{**}p < 0.01$ for systemic bupropion + local nicotine vs. systemic bupropion + local aCSF;]. Basal levels of dopamine were 25.8 ± 2.2 fmol/20 μl.

animals that received bupropion alone ($p < 0.05$, $n = 8–9$) (Fig. 2).

4. Discussion

The ability of low micromolar concentrations of bupropion to inhibit nAChRs *in vitro* [17,18,20] raises the question of whether this mechanism might contribute to the efficacy of bupropion as a smoking cessation agent. To investigate the relationship between bupropion and nAChRs *in vivo*, we examined the magnitude of responses to nicotine in the presence and absence of bupropion, to ascertain if functional nAChR-mediated responses are compromised by effective concentrations of systemically administered bupropion. In this study we have shown that bupropion increases locomotor activity and also increases extrasynaptic dopamine in the NAc_{core}, as expected. The effects of systemic administration of nicotine on locomotor activity and of locally applied nicotine on dopamine levels were also evident in the presence of bupropion. This suggests that at the concentration studied (a concentration that elicits responses *in vivo*), bupropion's major target is DAT with no significant blockade of nAChRs under the conditions examined.

It has been argued [16] that bupropion can help smokers quit the habit by elevating extracellular dopamine, thus alleviating withdrawal symptoms that arise from nicotine abstinence and attenuating craving by mimicking, to some extent, the neurochemical consequences of nicotine's actions in the reward areas of the brain. Interest in the possibility that concurrent blockade of nAChRs would enhance the efficacy of bupropion by preventing nicotine from exerting its reinforcing effects is encouraged by the success of varenicline. This novel partial agonist at $\alpha4\beta2^*$ nAChR has been advocated for smoking cessation because it combines the ability to promote dopamine release (by partial activation of nAChRs) with the ability to prevent any additional reinforcing effects of nicotine by partially blocking the major subtype of nAChR associated with dopaminergic neurones [23,24]. Such a dual action is suggested to have distinct advantages for smoking cessation.

Locomotor activation provides an indirect index of increased dopamine release in the dorsal and ventral striatum and was employed in the present study to assess bupropion's central actions. Bupropion at 30 mg/kg and 60 mg/kg (but not 10 mg/kg) significantly enhanced locomotor activity, in agreement with previous reports that similar doses of bupropion elicit stereotyped behaviour, including locomotor activation [25]. The intermediate dose of bupropion (30 mg/kg) was selected for further investigation as this provoked a significant and robust response. When administered in conjunction with a systemic injection of nicotine (0.4 mg/kg, optimum for eliciting locomotor responses, [26]), the enhanced locomotor activation is consistent with the effects of the two drugs being additive. Thus at a dose of 30 mg/kg, bupropion does not block the nAChRs through which nicotine exerts its locomotor stimulant properties.

Bupropion (30 mg/kg) also evoked an increase in extracellular dopamine in the NAc, as previously reported [25]. Nicotine, delivered via the microdialysis probe, significantly enhanced dopamine overflow above that seen in response to bupropion alone (Fig. 2). Indeed the responses to nicotine and bupropion appear to be additive, consistent with activation of nAChRs by nicotine and inhibition of DAT by bupropion as the independent mechanisms underlying the measured increase in dopamine. Although it is established that bupropion can inhibit neuronal nAChRs [17,18,19], the effective concentrations required to block those nAChRs responsible for [^{3}H]dopamine release *in vitro* (IC_{50}~1–10 μM; [19,20]) are higher than the concentrations that block DAT (IC_{50} 0.5 μM; [27]). The differential sensitivities of DAT and nAChRs to inhibition by bupropion can explain the present results, which show that DAT blockade can be achieved *in vivo* in the absence of any significant diminution of nAChR responses. The ability of bupropion to antagonise various other nicotine-induced responses in mice *in vivo* [18] could reflect the involvement of more sensitive nAChR subtypes (α3β2/β4*) or the use of higher concentrations of bupropion.

It is generally agreed that somatodendritic nAChRs on mesolimbic neurones make a major contribution to the rewarding properties of nicotine [28]. The present findings that bupropion does not diminish nicotine-evoked dopamine release, based on responses of nAChRs in the terminal field, can be extrapolated to the nicotinic modulation of dopamine release in general as the subunit composition of heteromeric nAChRs of the nigrostriatal and mesolimbic systems is relatively well conserved between soma and terminals. α4β2* nAChR predominate at both sites, although subtle changes in the disposition of α3, α5, α6 and β3 subunits may occur [9,29,30].

As a smoking cessation agent, bupropion is taken chronically for a period of several weeks. In rats, locomotor stimulant effects and the increase in extracellular dopamine in the NAc in response to an acute application of bupropion were enhanced following chronic administration of the drug [31]. The accumulation of drug over time will achieve higher concentrations and these might be sufficient to influence nAChR activity. Moreover, in humans the effects of bupropion may be confounded by its metabolism to yield bioactive products that can also interact with nAChRs [27,32]. Thus more work will be required to tease out the mechanisms responsible for bupropion's efficacy as a smoking cessation drug.

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