

ORIGINAL ARTICLE

Effect of chronic antipsychotic treatment on striatal phosphodiesterase 10A levels: a [¹¹C]MP-10 PET rodent imaging study with *ex vivo* confirmationS Natesan^{1,6}, S Ashworth^{2,6}, J Nielsen^{3,6}, S-P Tang², C Salinas², S Kealey¹, JB Lauridsen³, TB Stensbøl³, RN Gunn^{2,4}, EA Rabiner^{2,5} and S Kapur¹

A number of phosphodiesterase 10A (PDE10) inhibitors are about to undergo clinical evaluation for their efficacy in treating schizophrenia. As phosphodiesterases are in the same signalling pathway as dopamine D₂ receptors, it is possible that prior antipsychotic treatment could influence these enzyme systems in patients. Chronic, in contrast to acute, antipsychotic treatment has been reported to increase brain PDE10A levels in rodents. The aim of this study was to confirm these findings in a manner that can be translated to human imaging studies to understand its consequences. Positron emission tomography (PET) scanning was used to evaluate PDE10A enzyme availability, after chronic haloperidol administration, using a specific PDE10A ligand ([¹¹C]MP-10). The binding of [¹¹C]MP-10 in the striatum and the cerebellum was measured in rodents and a simplified reference tissue model (SRTM) with cerebellum as the reference region was used to determine the binding potential (BP_{ND}). In rats treated chronically with haloperidol (2 mg kg⁻¹ per day), there was no significant difference in PDE10A levels compared with the vehicle-treated group (BP_{ND} ± s.d.: 3.57 ± 0.64 versus 2.86 ± 0.71). Following PET scans, *ex vivo* analysis of striatal brain tissue for PDE10A mRNA (Pde10a) and PDE10A enzyme activity showed no significant difference. Similarly, the PDE10A protein content determined by western blot analysis was similar between the two groups, contrary to an earlier finding. The results of the study indicate that prior exposure to antipsychotic medication in rodents does not alter PDE10A levels.

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INTRODUCTION

Antipsychotic drugs mediate their effects by blocking dopamine D₂ receptors and in doing so they increase intracellular cyclic adenosine monophosphate (cAMP) levels. The duration and amplitude of cyclic nucleotide signalling is regulated by cellular phosphodiesterases (PDEs) that break down cAMP.^{1,2} The brain expresses the most dense and diverse collection of PDEs in the body and the medium spiny neurons of the basal ganglia have the greatest density of PDEs as well as dopamine D₂ receptors.^{3,4} As PDEs are at the heart of the striatal dopamine D₂ signalling pathway, it is possible that chronic D₂ receptor blockade due to antipsychotic medication could alter PDE levels in the brain.

The PDE10A subtype has recently been characterized and has quite a remarkable localization in the brain, being predominantly expressed in the basal ganglia in medium spiny neurons.⁵ Hence, it is a prime therapeutic target for schizophrenia. PDE10A is a dual-substrate PDE hydrolysing both cAMP and cyclic guanosine monophosphate but has higher affinity for cAMP.⁶ A study by Dlaboga *et al*⁷ reported a substantial increase in the level of PDE10A expression in rodent striatum following chronic antipsychotic treatment. In that study, chronic haloperidol and clozapine treatment increased striatal PDE10A protein expression by 118% and 62%, respectively, but acute administration had no effect. There are also several other instances where PDE levels

have been influenced either by the disease process or by concurrent medication.^{8,9} An example in the CNS is the alteration of brain PDE4 iso-enzyme levels due to repeated treatment with antidepressants in the mouse.¹⁰

In preclinical models, inhibition of PDE10A is shown to have potential antipsychotic action.^{11,12} As a number of candidate PDE10A inhibitors are about to undergo clinical trials for the treatment of schizophrenia¹³ and if prior antipsychotic medication were to influence PDE10A levels in the brain, then there is a need to adjust dosing levels to accommodate such a change. Dose calculations are normally derived from preclinical models that have not been exposed to antipsychotic medication and from healthy human volunteers in Phase 1 trials.

Also, the method by which antipsychotics were administered (daily intraperitoneal injections) in the Dlaboga *et al*⁷ study is not entirely consistent with clinical use (where a sustained threshold is maintained). Daily intraperitoneal injections in rodents lead to plasma levels that dip to a negligible level during a 24-h period as most antipsychotics have a half-life of 2–4 h in rodents, whereas the half-life of most antipsychotics in humans is usually 12–24 h.¹⁴ Hence, the present study was designed taking into consideration a delivery method (subcutaneous mini-osmotic pumps) that maintained constant plasma levels to evaluate the effect of chronic antipsychotic treatment on striatal PDE10A levels in rodents.

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Extensive efforts are underway to develop radiotracers to noninvasively image PDEs especially PDE10A using positron emission tomography (PET) and the challenge has been to achieve subtype and splice variant selectivity.^{15,16} In our study, a PDE10A-specific ligand ($[^{11}\text{C}]\text{MJP-10}$)¹⁷ was used to evaluate PDE10A enzyme availability after chronic haloperidol administration, primarily as a translational tool for human imaging. *Ex vivo* tissue analysis for mRNA, protein levels and enzyme activity were also carried out to substantiate the PET findings. mRNA levels of four target genes (*Pde10a*, *Pde1b*, *dopamine D₂ receptor* and *neurotensin*) were chosen as changes in expression of these genes due to chronic antipsychotic treatment have been reported.^{7,18,19} Western blotting was carried out to quantify striatal protein levels. Total and PDE10A-specific cAMP PDE activity in striatum were measured in enzymatic assays to assess potential changes in PDE activity of PDE10A or other PDEs.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Charles River, Kent, UK), weighing 308–456 g, housed under a 12-h light/dark cycle (0700 hours lights on) with food and water available *ad libitum* were allowed to habituate for at least 7 days before performing experimental procedures. The temperature and relative humidity settings at the animal house were 19–21 °C and 40–55%, respectively. At the imaging facility, the temperature and relative humidity settings were 19–23 °C and 45–65%, respectively. The procedures used in the present study were approved by the Animal Ethical Review Committee of Imperial College London, UK and all experimental procedures were carried out in accordance with the Home Office Animals (Scientific Procedures) Act, UK, 1986.

Experimental design

The experiment was carried out in two phases. The first phase ($n = 7$) was a pilot to determine an adequate sample size that would detect a difference of at least 20% of the primary PET parameter BP_{ND} (binding potential) with >80% power ($\alpha = 0.05$, in a two-tail test) in normal rats at baseline conditions. In the second phase, rats were divided into two groups ($n = 6$ per group) and they received either a common vehicle (β -hydroxypropylcyclodextrin, 20% w/v, acidified by ascorbic acid, to pH 6) or 2 mg kg^{-1} per day haloperidol (all chemicals were sourced from Sigma-Aldrich, Gillingham, UK) via subcutaneous osmotic mini-pumps (Alzet[®] Model 2ML4, 28 days; Alzet, Cupertino, CA, USA) for 3 weeks. The dose of haloperidol was at the higher range of clinical dosing based on receptor occupancy studies using our prior results with osmotic mini-pumps.^{14,20} Osmotic mini-pumps were implanted under aseptic conditions on the back flank under isoflurane general anaesthesia (induction 5% isoflurane with oxygen flow rate at 5 l min^{-1} and during anaesthetic maintenance, 1.5–2.5% isoflurane was administered with oxygen at a flow rate of 2–3 l min^{-1} , IsoFlo, Abbott Lab Ltd., Kent, UK). The analgesic carprofen (Rimadyl, Zoetis UK Ltd., London, UK) was administered to each rat at a dose of 5 mg kg^{-1} before the start of surgery. The animals were monitored and body weights recorded at appropriate intervals as part of post-operative care.

PET-computed tomography (CT) scanning was carried out on day 21 following implantation of the osmotic pumps. Vacuous chewing movements (oral dyskinesias) were evaluated by placing each rat on a small elevated box and recording the number of vacuous chewing movements over a 2-min period, before anaesthetizing them for PET-CT scanning.²¹ Under isoflurane anaesthesia, the tail vein and artery of each rat were cannulated for tracer injection and blood sampling, respectively. Blood (up to 0.75 ml) was collected before the start of PET scan. After the completion of PET scan, each animal was killed using a lethal dose of intravenous pentobarbitone (Euthasol Vet, Le Vet B.V., Oudewater, The Netherlands). The striatal brain tissue was immediately dissected and stored at -80°C until analysis could be performed. Plasma haloperidol was measured commercially (St George's, University of London, Tooting, UK) using an analytical technique that used a combination of liquid chromatography and mass spectrometry. Statistical comparisons between the two treatment groups were performed using a two-tailed Welsh *t*-test unless otherwise mentioned.

PET-CT scanning

In both phases of the experiment, dynamic PET scans using the Siemens Inveon DPET/MM PET-CT scanner (Siemens AG, Erlangen, Germany) were acquired under Isoflurane general anaesthesia (1.5–2.5% isoflurane with oxygen at a flow rate of 2–3 l min^{-1}). Each rat was secured on a bed comprising of ear bars, an anaesthetic delivery nose cone and a tooth bar in the scanner. The head was first placed in the field-of-view of the CT scanner and a 20-min CT scan was performed to collect data for attenuation correction and provide structural information. Subsequently, the head was positioned in the field-of-view of the PET scanner. $[^{11}\text{C}]\text{MJP-10}$ was prepared by N-alkylation of the precursor GSK2375586 using cyclotron-produced $[^{11}\text{C}]\text{methyl iodide}$ at IMANOVA as previously described.¹⁷ In the pilot phase a 60-min dynamic PET scan was performed, whereas in the second phase, the majority of the animals underwent a 90-min acquisition following intravenous bolus administration (specific activities 61.64–141.26 GBq μmol^{-1}) of $[^{11}\text{C}]\text{MJP-10}$. The body temperature was maintained throughout the scan using a heating mat and lamp, and the temperature was monitored using a rectal probe. The respiration rate was monitored using a respiration pad.

Conservative striatal and cerebellum regions of interest (ROIs) were drawn in the PET images registered to their corresponding CT images using an approach that balanced partial volume effects and the number of counts within the ROI. The striatum was drawn within +2.28 to -1.20 mm anteroposterior to the bregma and anterior to the interaural coordinates of 11.28–7.80 mm guided by the CT images with reference to the Paxinos Rat Atlas.²² Cerebellum ROIs were drawn within 9.96–13.20 mm posterior to the bregma and 0.96–4.20 mm posterior to the interaural line of the atlas avoiding the vermis. Volumes of ROIs for the striatum and cerebellum were maintained across scans. The ROIs were applied to the dynamic PET data to generate the corresponding time–activity curves (TACs) for the striatum and cerebellum. Tissue radioactivity concentrations were normalized for the radioactive dose injected and for the animal's body mass giving standard uptake values ($(\text{kBq ml}^{-1} \text{ tissue})/(\text{kBq g}^{-1} \text{ body mass})$) with the assumption that 1 cm^3 equals 1 g of tissue. A simplified reference tissue model (SRTM) with the cerebellum as the reference region was applied to the TACs to generate a striatal BP_{ND} value for each scan using the first 60 min of dynamic data for each scan.²³

Quantitative PCR (qPCR)

Total RNA was purified from dissected striata using the NucleoSpin RNA and Protein kit according to the manufacturer's instructions (Macherey-Nagel, Düren, Germany). A measure of 1 μg of each sample based on NanoDrop measurements (NanoDrop products, Wilmington, DE, USA) was used for the first strand cDNA synthesis using random hexamer primers and the TaqMan reverse transcription reagents according to the manufacturer (Life Technologies, Paisley, UK). *Pde10a*, *Pde1b*, *D₂ receptor* and *Neurotensin* (Nts) striatal mRNA were measured.

Quantitative PCR primers were designed by the Primer Blast algorithm (Supplementary Table S1)²⁴ and validated against Mouse Universal Reference Total RNA from Clontech Laboratories (Mountain View, CA, USA). qPCR was performed on a Bio-Rad C1000 Touch thermal cycler with a CFX384 optical reaction module using SsoFastTM EvaGreen Supermix according to the manufacturer's instruction (Bio-Rad, Hercules, CA, USA) starting with 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s and 60 °C for 10 s. Samples were run in technical duplicates and mean quantification cycle (C_q) values were used for further calculations. Eight reference genes (*Alas2*, *Actb*, *B2m*, *Tbp*, *G6pd*, *Ppia*, *Trfc* and *Ubc*) were included and the three most optimal genes (*Ppia*, *Trfc* and *Ubc*) based on the geNorm algorithm (Supplementary Figure S1)²⁵ were used for geometric normalization; however, all eight of the analysed reference genes were fit for normalization as indicated by the relative low variation based on *M* values (Supplementary Figure S1C). Data were normalized to vehicle control and significant differences were based on two-tailed Welsh *t*-tests.

Western blotting

Striatal lysate for western blotting and PDE activity measurement was prepared as follows: dissected striata were homogenized with a Polytron PT 1200E homogenizer (Kinematica AG, Luzern, Switzerland) in 700 μl cold lysis buffer containing 50 mM Tris-HCl (pH 8.0), 1 mM MgCl_2 , 0.5% Triton X-100 and 1% complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA). Samples were left on ice for 15 min, centrifuged for 30 min at 20 000 *g* at 4 °C and the supernatant (striatal lysate) was removed for further testing and frozen in aliquots on dry ice. Protein content of each

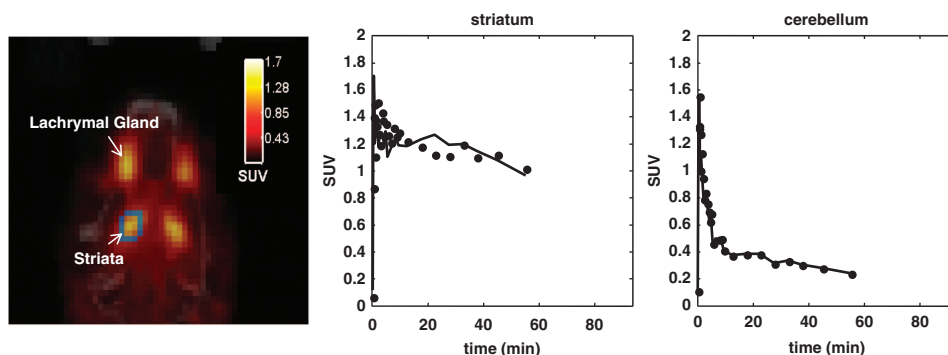


Figure 1. A representative positron emission tomography scan of the head of a rat injected intravenously with [¹¹C]MP-10 and time-activity curves of standard uptake values from its striatum and cerebellum regions.

preparation was measured by Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL, USA); the protein concentration varied from 2.6 to 4.0 μg μl⁻¹.

Total protein (30 μg) of each striatal lysate was mixed with LDS loading buffer (NP0007, Life Technologies) supplemented with 4 M urea and were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 4–12% bis-tris gel (Life Technologies). Rainbow molecular weight markers (Amersham Biosciences, GE Healthcare Europe GmbH, Brøndby, Denmark) were included. Immunoblotting was carried out using standard methods with anti-PDE10A C-17, SC66502 (Santa Cruz Biotechnology, Dallas, TX, USA) diluted 1:1500 and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) AB9484 (Abcam, Cambridge, UK) diluted 1:5000 as primary antibodies. IRDye 800CW donkey anti-goat antibody 926–32214 (LI-COR Biosciences, Lincoln, NE, USA) diluted 1:20 000 and IRDye 680 goat anti-mouse antibody 926–32220 (LI-COR Biosciences) diluted 1:20 000 were used as secondary antibodies. PDE10A signal was quantified in the 800 nm channel and GAPDH in the 700 nm channel on an LI-COR Odyssey Infrared Western Blot Imaging System (LI-COR Biosciences). Differences in intensities between vehicle- and haloperidol-treated rats were evaluated by the two-tailed Welsh *t*-test.

PDE activity measurement

Total and PDE10A PDE activity was assessed on aliquotes of striatal lysate prepared for western blotting. cAMP PDE activity of striatal lysate was measured using a scintillation proximity assay (SPA)-based method as previously described.^{26,27} Lysate (0.05 μl) was used in each 60 μl reaction, which resulted in ≈20% hydrolysis of tritiated cAMP at the end point. Each measurement was performed in quadruplicates and the mean was used for further comparison. Total PDE activity was assessed by subtracting counts per minute for wells with striatal lysate with the background measured as counts per minute in wells without added lysate. PDE10A activity was assessed by subtracting total PDE activity with PDE activity measured in the presence of 250 nM of the selective PDE10A inhibitor MP-10, which has a reported IC₅₀ for PDE10A of 0.18 nM.¹¹ Differences in activity between vehicle- and haloperidol-treated rats were evaluated on the basis of two-tailed Welsh *t*-test.

RESULTS

PET scanning

In the first phase of the experiment, high accumulation of radioactivity was seen in the striatum and lacrimal glands following [¹¹C]MP-10 administration (Figure 1). Striatal TACs for each subject showed an initial high uptake of radioactivity, which was retained over the time-course of the scan (Figure 1). The cerebellum TACs showed a similar initial uptake but more rapid decline in radioactivity concentrations over the same period due to low levels of PDE10A in this region (Figure 1). SRTM with the cerebellum as the reference region was applied to the TACs to generate a striatal BP_{ND} value for each scan. The cerebellum was chosen as the reference region given its very low PDE10A protein expression and supported by negligible levels of displaceable binding of [¹¹C]MP-10 due to cold MP-10 administration in

baboons.^{5,17,28} The average BP_{ND} for seven animals was calculated as 2.8 ± 0.6 (mean ± s.d.). Using these results, the statistical power to measure a 20% change of BP_{ND} using 12 animals (6 in each group, assuming variances in the two groups would be similar) for the next phase was determined to be >80% (two tail Student's *t*-test).

In the second phase, animals tolerated the drug well and there was no significant change in body weight between the two groups. In the drug-treated arm, one animal stopped breathing midway through the scan and could not be revived but with the exception of BP_{ND}, all other parameters were recorded for that animal and included in the data analysis. There was also no statistically significant difference in BP_{ND} between the two groups after 3 weeks of treatment (Table 1). The average injected mass of [¹¹C]MP-10 (mean ± s.d.) was not significantly different between the groups (vehicle 0.30 ± 0.08 versus haloperidol 0.33 ± 0.12 μg kg⁻¹, *P* = 0.71). The average plasma level of haloperidol in the drug-treated group measured after 21 days of treatment on the day of PET scanning was 19.9 ± 5.13 ng ml⁻¹ (mean ± s.d.). Rats in the haloperidol group showed statistically increased vacuous chewing movements compared with the vehicle-treated group (Table 1). The plasma levels of haloperidol and vacuous chewing behaviour indicate that the drug was administered as intended via osmotic mini-pumps.

Pde10a mRNA levels—qPCR

The mRNA levels of Pde10a and other selected transcripts in the striatum were assessed by qPCR (Figure 2). There was no significant change in Pde10a mRNA levels in the striatum after 21 days of chronic haloperidol treatment compared with vehicle (*P* = 0.669). Furthermore, no significant changes were identified in Pde1b mRNA levels (*P* = 0.975) or dopamine D₂ receptor (Drd2) mRNA levels (*P* = 0.728), whereas a significant increase in Nts mRNA levels was observed (*P* < 0.0001).

Table 1. Striatal [¹¹C]MP-10 binding potential, plasma drug level and VCM due to haloperidol treatment

Treatment (n = 6 in each group)	Binding potential (BP _{ND})	Haloperidol plasma levels (ng ml ⁻¹)	VCM
Vehicle	3.57 ± 0.64	b.l.d.	1.33 ± 0.52
Haloperidol	2.86 ± 0.71 ^a	19.9 ± 5.93	8.17 ± 2.93 ^b

Abbreviations: b.l.d., below level of detection (< 1 ng ml⁻¹); VCM, vacuous chewing movement. Values are presented as mean ± s.d. ^aNot statistically significant in comparison to the vehicle group (Welsh *t*-test statistic *P* = 0.12). ^bStatistically significant compared with the vehicle group (Wilcoxon–Mann–Whitney test *U* = 36, *P* < 0.001).

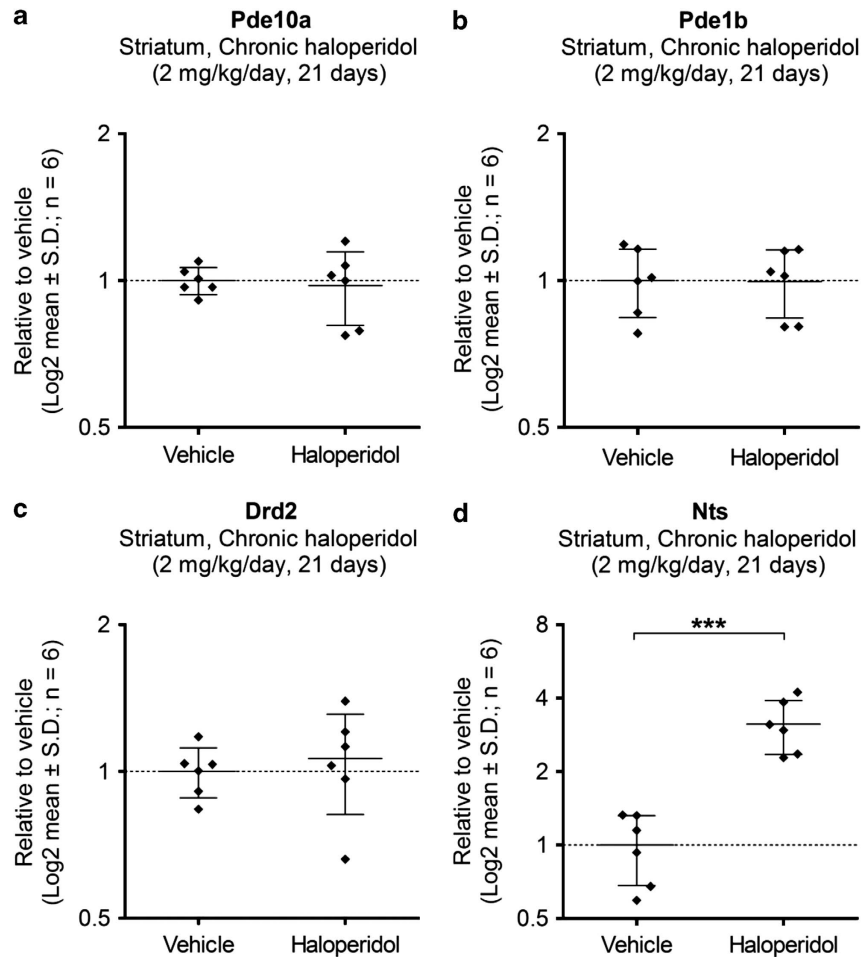


Figure 2. mRNA levels in the striatum after treatment with haloperidol in comparison to vehicle-treated rats: (a) phosphodiesterase 10A (Pde10a); (b) phosphodiesterase 1B (Pde1b); (c) dopamine D₂ receptor (Drd2); and (d) neurotensin (Nts). *** $P=0.0001$ compared with vehicle.

PDE10A protein levels—western blots

Effect of haloperidol treatment on striatal PDE10A protein levels were assessed by western blotting (Figure 3a). PDE10A was measured simultaneously with GAPDH for normalization using the LI-COR Odyssey Western Blot Infrared Imaging system. There was no significant difference in GAPDH-normalized PDE10A expression in vehicle- and haloperidol-treated groups (Figure 3b). Similarly, there was no significant difference in the absolute PDE10A expression between the two groups (data not shown).

Total and PDE10A PDE enzyme activity

The total cAMP PDE activity in striatal lysates was measured as the amount of conversion of [³H]cAMP to [³H]AMP in the presence of a fixed amount of striatal lysate (Figure 4a). It was not significantly different between haloperidol- and vehicle-treated rats ($P=0.35$). PDE10A-specific activity was assessed by inhibiting total cAMP PDE activity in striatal lysates with 250 nM of the selective PDE10A inhibitor MP-10. PDE10A constituted just under half of the total PDE activity in striatal lysate of vehicle-treated animals (Figure 4b). There was no significant difference in striatal PDE10A activity between haloperidol- and vehicle-treated rats ($P=0.86$).

DISCUSSION

The main aim of this study was to verify the earlier observation that antipsychotic treatment induces elevation of striatal PDE10A levels.⁷ Antipsychotic drug action on the dopaminergic system is

of a temporal nature. In the initial phase, blockade of presynaptic dopamine D₂ receptors leads to a surge of dopamine in the synapse and this could lead to activation of the postsynaptic D₁ receptors while postsynaptic dopamine D₂ receptors are blocked.²⁹ Both these actions lead to increase in intracellular cAMP.³⁰ The surge of dopamine due to presynaptic blockade rapidly desensitizes presynaptic dopamine receptors in rodents and this is followed by sustained postsynaptic blockade with synaptic dopamine reaching baseline levels.³¹ Over time, the postsynaptic dopamine D₂ receptors are known to upregulate and become supersensitive owing to chronic blockade both in rodents and in humans.^{31,32} Hence, both intracellular cAMP levels as well as PDE activity could vary depending on the duration of the treatment.

The present experiment was performed in the later phase guided by previous experimentation with chronic antipsychotic dosing in rodents.³¹ After 3 weeks of chronic haloperidol treatment in rodents, the system stabilizes and is ideal for measurements that reflect a chronic phase of antipsychotic treatment.³¹ The PET imaging results showed no evidence of PDE10A enzyme elevation in the whole rodent striatum. The radiotracer ([¹¹C]MP-10) is a selective and potent PDE10A inhibitor and has shown good brain penetration and regional binding in rhesus monkey.^{12,33} The observed kinetics in rats was consistent with those previously observed for MP-10 in primates with rapid washout in cerebellum and prolonged uptake in the striatum. In our data analysis, we quantified [¹¹C]MP-10 binding using a SRTM, a technique that has been previously validated for quantification

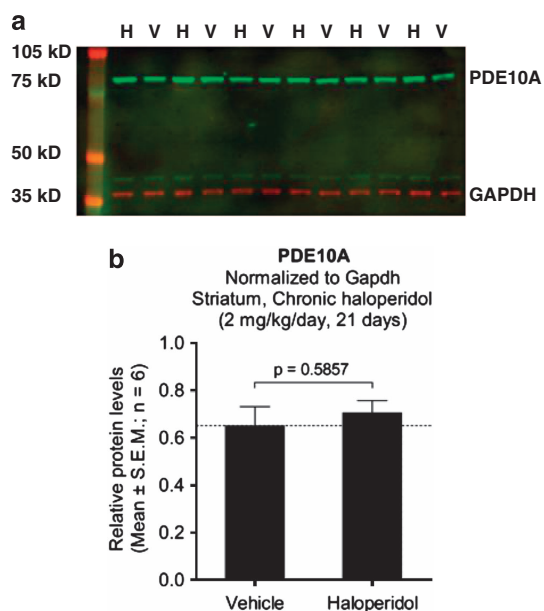


Figure 3. Effect of haloperidol treatment on the striatal PDE10A protein levels assessed by western blotting. **(a)** Two-colour western blot with striatal lysate from the six haloperidol (H)- and six vehicle (V)-treated rats loaded in alternate wells. PDE10A (green) and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; red) were simultaneously measured in the 800- and the 700-nm channels, respectively, using the LI-COR Odyssey Infrared Western Blot Imaging System. **(b)** There was no significant difference in GAPDH-normalized PDE10A expression in the vehicle- and haloperidol-treated groups.

microarray experiment followed by secondary confirmation by *in situ* hybridization of gene expression.¹⁹ A number of previous reports have reported this and even suggest antipsychotic action to be mediated by this neuropeptide system and hence Nts was chosen in this study.^{40–42} The direct target of haloperidol, dopamine D₂ receptor, and Pde10a and Pde1b mRNA as reported by Dlaboga *et al.*⁷ were also tested. Dopamine D₂ receptor mRNA upregulation has previously been reported to be changed by chronic haloperidol treatment, although reports are mixed.^{18,19,31} The lack of dopamine D₂ receptor mRNA may suggest that high and sustained levels of D₂ receptor blockade may not have been achieved but high levels of drug in plasma, increased levels of Nts mRNA and high vacuous chewing movement scores in the haloperidol group are indicators of high and sustained D₂ receptor blockade. We did not find any change in Pde10a, Pde1b or D₂ receptor mRNA levels in the striatum after chronic haloperidol treatment; however, we observed a highly significant increase in the striatal Nts mRNA levels indicating transcriptional activation due to antipsychotic treatment.

In our study, western blot and PET studies did not indicate any change in PDE10A protein content due to drug treatment and similarly Pde10a mRNA levels were unchanged. These results contradict the previous study by Dlaboga *et al.*⁷ and differences in dosing patterns of the antipsychotic drug could be a cause. However, the dosing strategy adopted in this study is closer to clinical reality and hence, our study is likely to be more predictive for patients chronically treated with antipsychotic medication.

PDEs are regulated through a variety of transcriptional and posttranscriptional mechanisms in response to hormonal, environmental or pharmacological stimuli.² The complex PDE machinery in cells and the way it maintains cellular homeostasis is still at a nascent stage of our understanding.⁴³ There exists a delicate

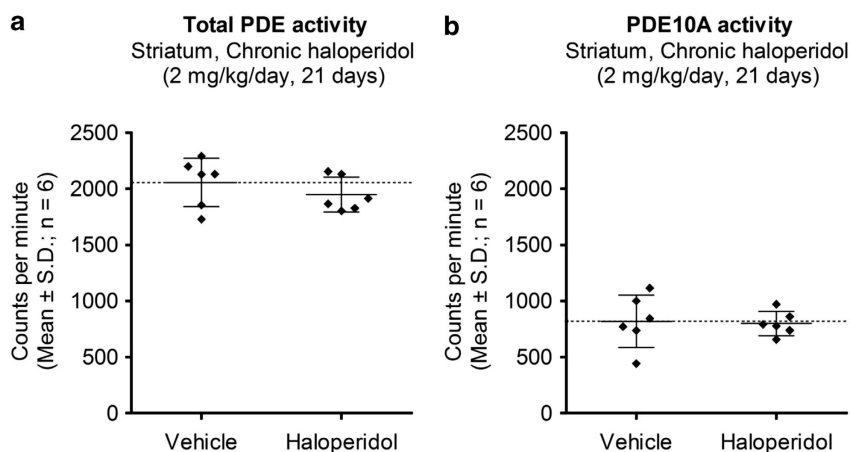


Figure 4. Effect of haloperidol treatment on striatal phosphodiesterase (PDE) enzymatic activity. **(a)** Total PDE activity and **(b)** PDE10A activity indicating no effect of drug treatment.

of this tracer in primates.¹⁷ Good model fits were obtained to the rat striatal data using SRTM. There was no evidence of any alteration in cerebellum levels between groups as assessed by changes in their standard uptake values consistent with the cerebellum as a valid reference region.

Very few transcriptomic studies have used appropriate antipsychotic doses or a chronic dosing strategy that mimics clinical reality and hence, there is very little concurrence in their striatal transcriptomic profiles.^{34–39} Girgenti *et al.*¹⁹ have used haloperidol deconate (a long-acting form of haloperidol) as a depot and this study is the closest to being clinically relevant. They identified Nts as the most robustly upregulated mRNA in rats injected with haloperidol deconate (21 mg kg⁻¹), once weekly, for 4 weeks in a

balance between synthesis and degradation of cyclic nucleotides and hence, a subtle change in the ability of PDEs to break down cyclic nucleotides can have a significant effect in cell signalling.⁴⁴ Their subcellular localization can also have a significant effect in cell signalling.⁴⁵ In the case of PDE10A, it has been shown that the main PDE10A isoform is palmitoylated and that phosphorylation of PDE10A by protein kinase A can interfere with palmitoylation.⁴⁶ Furthermore, PDE10A has a high-affinity cAMP binding site in its N-terminal GAF-domain, but the effect of cAMP binding to this site is unclear.^{26,47} We have observed change neither in the accessibility of MP-10 binding sites *in vivo* nor in the striatal PDE10A enzymatic activity *ex vivo* owing to haloperidol treatment,

but *ex vivo* experimentation may not have captured the intricacies in native tissue.

The other caveats of this study include: (i) both PET as well as *ex vivo* studies were performed using the entire striatum and there could be unrecognized regional variation; (ii) there are reports of two brain penetrating radiometabolites of [¹¹C]MP-10 in rodents, albeit in low quantities, and suboptimal kinetics of the radiotracer in the striatum that could limit the PET interpretation of PDE10A levels and hence, a combination of methods was used in this study;³³ (iii) isoflurane anaesthesia can affect regional cerebral blood flow, metabolic rates and neurotransmission, which could affect tracer kinetics, and this remains a limitation in this study;⁴⁸ (iv) the consequences of drug withdrawal have not been studied; and (v) the study is limited to a single antipsychotic, measured at one time point during the course of treatment.

In any case, the results indicate that striatal PDE10A expression is not altered due to chronic antipsychotic treatment. A proof of concept study by Pfizer in which MP-10 (PF-2545920) was evaluated for its antipsychotic efficacy resulted in negative results.⁴⁹ On the basis of the findings in the present study, it is unlikely that altered PDE10A levels in the patients due to prior exposure to antipsychotics underlie the negative result. However, we also have no knowledge on the baseline PDE10A expression levels in patients diagnosed with schizophrenia. As a next step, we are conducting human PET imaging experiments to answer these questions. The result of this study will help guide clinical trials not only for schizophrenia therapeutics but also for other neuropsychiatric disorders, especially Huntington's disease, where concomitant antipsychotic use is prevalent.⁴⁹

CONFLICT OF INTEREST

S Kapur has received grant support from AstraZeneca and GlaxoSmithKline and has served as consultant and/or speaker for AstraZeneca, Bioline, BMS-Otsuka, Eli Lilly, Janssen (J&J), Lundbeck, NeuroSearch, Pfizer, Roche, Servier and Solvay Wyeth in the past three years. SA, S-PT, CS, RNG and EAR are all employees of Imanova Ltd. RNG has also served as consultant for GlaxoSmithKline, AbbVie, UCB and Roche in the past three years. EAR has also served as consultant for GlaxoSmithKline, Roche, BioTie, Takeda and AbbVie. JN, JBL and TBS are employees of Lundbeck A/S. The remaining authors declare no conflict of interest.

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DISCLAIMER

This article presents independent research and the views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. Some of the data were presented at the XXVth International Symposium on Cerebral Blood Flow, Metabolism and Function and XIth International Conference on Quantification of Brain Function with PET, Shanghai, China, May 20–23, 2013.

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