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REGULAR RESEARCH ARTICLE

Balanced Activation of Striatal Output Pathways by Faster Off-Rate PDE10A Inhibitors Elicits Not Only Antipsychotic-Like Effects But Also Procognitive Effects in Rodents

Akina Harada, PhD, Nidhi Kaushal, PhD, Kazunori Suzuki, PhD, Atsushi Nakatani, MS, Konstantin Bobkov, PhD, John A. Vekich, PhD, Joseph P. Doyle, PhD, Haruhide Kimura, PhD

Neuroscience Drug Discovery Unit, Research, Takeda Pharmaceutical Company Limited, Fujisawa, Kanagawa, Japan (Dr Harada, Dr Suzuki, Mr Nakatani, Dr Kimura); Neuroscience Drug Discovery Unit (Dr Kaushal), and Early Target Discovery (Drs Bobkov, Vekich, and Doyle), Research, Takeda California Inc., San Diego, CA.

A.H. and N.K contributed equally to this work.
J.P.D. and H.K. contributed equally to this work.

Correspondence: Haruhide Kimura, PhD, 26-1, Muraoka-Higashi 2-chome Fujisawa, Kanagawa 251-8555, Japan (haruhide.kimura@takeda.com).

Abstract

Background: Faster off-rate competitive enzyme inhibitors are generally more sensitive than slower off-rate ones to binding inhibition by enzyme substrates. We previously reported that the cyclic adenosine monophosphate concentration in dopamine D_1 receptor-expressing medium spiny neurons (D_1-MSNs) may be higher than that in D_2-MSNs. Consequently, compared with slower off-rate phosphodiesterase 10A inhibitors, faster off-rate ones comparably activated D_2-MSNs but partially activated D_1-MSNs. We further investigated the pharmacological profiles of phosphodiesterase 10A inhibitors with different off-rates.
Methods: Phosphodiesterase 10A inhibitors with slower (T-609) and faster (T-773) off-rates were used. D_1- and D_2-MSN activation was assessed by substance P and enkephalin mRNA induction, respectively, in rodents. Antipsychotic-like effects were evaluated by MK-801- and methamphetamine-induced hyperactivity and prepulse inhibition in rodents. Cognition was assessed by novel object recognition task and radial arm maze in rats. Prefrontal cortex activation was evaluated by c-Fos immunohistochemistry in rats. Gene translations in D_1- and D_2-MSNs were evaluated by translating ribosome affinity purification and RNA sequencing in mice.
Results: Compared with T-609, T-773 comparably activated D_2-MSNs but partially activated D_1-MSNs. Haloperidol (a D_2 antagonist) and T-773, but not T-609, produced antipsychotic-like effects in all paradigms. T-773, but not T-609 or haloperidol, activated the prefrontal cortex and improved cognition. Overall gene translation patterns in D_2-MSNs by all drugs and those in D_1-MSNs by T-773 and T-609 were qualitatively similar.
Conclusions: Differential pharmacological profiles among those drugs could be attributable to activation balance of D_1- and D_2-MSNs. The "balanced activation" of MSNs by faster off-rate phosphodiesterase 10A inhibitors may be favorable to treat schizophrenia.

Keywords: phosphodiesterase 10A, medium spiny neurons, translating ribosome affinity purification, cognition, prefrontal cortex

Significance Statement

In schizophrenia, striatal dopaminergic dysfunction may cause psychosis and cognitive impairment via dysregulation of the cortico-striato-thalamo-cortical circuit. Two striatal output neurons, dopamine D_1 and D_2 receptor-expressing medium spiny neurons (D_1- and D_2-MSNs), express phosphodiesterase 10A (PDE10A). In this study, we observed that, compared with slower off-rate PDE10A inhibitors, faster off-rate ones comparably activated D_2-MSNs but partially activated D_1-MSNs in rodents. Only faster off-rate PDE10A inhibitors produced antipsychotic-like effects in multiple rodent models. Moreover, faster off-rate inhibitors, but not slower off-rate ones or D_2 antagonists, activated the prefrontal cortex and improved cognition in rodents. Overall gene translation patterns by all these drugs in D_2-MSNs and those by faster and slower off-rate PDE10A inhibitors in D_1-MSNs are qualitatively similar. Thus, activation balance of D_1- and D_2-MSNs is the major difference between these drugs. The "balanced activation" of D_1- and D_2-MSNs by faster off-rate PDE10A inhibitors may be favorable to treat schizophrenia.

Introduction

Striatal dopaminergic dysfunction may lead to psychosis and cognitive impairment via dysregulation of the cortico-striato-thalamo-cortical circuit in patients with schizophrenia (Dandash et al., 2017). The striatal output neurons in this circuit are dopamine D_1 receptor-expressing, substance P (SP)-positive medium spiny neurons (D_1-MSNs) and D_2 receptor-expressing, enkephalin (Enk)-positive D_2-MSNs (Gerfen et al., 1990). Blockade of D_2 receptors and the resulting activation of D_2-MSNs by elevation of cyclic adenosine monophosphate (cAMP) levels are believed to underlie clinical efficacy of current antipsychotics (Boyd and Mailman, 2012). Phosphodiesterase 10A (PDE10A) is an enzyme that hydrolyzes both cAMP and cyclic guanosine monophosphate (cGMP) and is highly expressed in both D_1- and D_2-MSNs (Grauer et al., 2009). Therefore, PDE10A inhibitors can activate both D_1- and D_2-MSNs via upregulation of cAMP and cGMP levels. Because activation of D_1-MSNs counteracts excess activation of D_2-MSNs, which causes extrapyramidal symptoms (Ginovart and Kapur, 2012), PDE10A inhibitors are candidates as novel antipsychotics with reduced risk of extrapyramidal symptoms (Kehler and Nielsen, 2011).

In general, faster off-rate competitive enzyme inhibitors are more sensitive to binding inhibition by enzyme substrates than slower off-rate ones. Indeed, a faster off-rate PDE10A inhibitor TAK-063 was more sensitive than a slower off-rate PDE10A inhibitor MP-10 to binding inhibition by cyclic nucleotides (Suzuki et al., 2016). In the rat striatum, >90% of cAMP-positive cells were SP-positive, suggesting higher intracellular cAMP concentrations in D_1-MSNs than in D_2-MSNs (Suzuki et al., 2016). Therefore, partial PDE10A inhibition by TAK-063 compared with MP-10 was expected especially in D_1-MSNs. In fact, by using SP and Enk mRNA as pathway-specific markers, we found that compared with slower off-rate PDE10A inhibitors MP-10 and compound 1 (hereafter T-609), TAK-063 comparably activated D_2-MSNs and partially activated D_1-MSNs in rats (Suzuki et al., 2016).

Like motor control, we observed that excessive activation of D_1-MSNs by a D_1 agonist SKF82958 canceled antipsychotic-like effects of a D_2 antagonist haloperidol. Thus, activation balance of D_1- and D_2-MSNs is critical for PDE10A inhibitors to produce antipsychotic-like effects. We found that TAK-063, but not MP-10 or T-609, dose-dependently produced antipsychotic-like effects in multiple rodent models (Suzuki et al., 2015, 2016). As slower off-rate PDE10A inhibitors induced more robust activation of D_1-MSNs compared with faster off-rate ones, we hypothesized that excessive activation of D_1-MSNs hampered their antipsychotic-like efficacy. We defined the activation pattern of MSNs by TAK-063 as "balanced activation" (Suzuki et al., 2016).

However, this hypothesis assumed that D_2 antagonism and PDE10A inhibition would induce a similar pattern of activation in D_2-MSNs; note that PDE10A inhibition activates both cAMP- and cGMP-pathways, whereas D_2 antagonism modulates canonical G protein pathways (cAMP-pathways) and noncanonical β-arrestin2 pathways in D_2-MSNs (Masri et al., 2008). It has recently been proposed that modulation of β-arrestin2 pathways, but not cAMP-pathways, is the fundamental mechanism of action of antipsychotics (Urs et al., 2017). Further characterization of the downstream effects of D_2 antagonism and PDE10A inhibition on D_2-MSNs would help clarify this hypothesis.

Interestingly, TAK-063 improved cognition in various preclinical models (Shiraishi et al., 2016), whereas MP-10 did not show procognitive effects in novel object recognition task (NORT) using rats (Grauer et al., 2009). Moreover, pharmacological magnetic resonance imaging and/or c-Fos immunohistochemistry studies showed that TAK-063, but not MP-10, affected neuronal activity in the rat prefrontal cortex (PFC) (Wilson et al., 2015; Tomimatsu et al., 2016; Nakatani et al., 2017). PFC is strongly implicated in cognition (Miller, 2000) and connected with the striatum via cortico-striato-thalamo-cortical circuit (Dandash et al., 2017). The balanced activation of D_1- and D_2-MSNs may also be critical for neuronal activity in PFC and cognitive function.

To our knowledge, TAK-063 is the only faster off-rate PDE10A-selective inhibitor reported. In this study, we revealed that T-773, a faster off-rate PDE10A inhibitor with a chemical structure similar to TAK-063 and T-609, also produced balanced activation of D_1- and D_2-MSNs and antipsychotic-like effects in rodents. Moreover, using T-773 and T-609, we investigated whether off-rates of PDE10A inhibitors show activation of PFC and cognitive improvement in rodents. Additionally, using the bacterial artificial chromosome-based translating ribosome affinity purification (bacTRAP) that permits isolation of polysomal mRNAs under translation in specific cell populations in vivo (Doyle et al., 2008; Heiman et al., 2008), combined with RNA sequencing (RNA-seq), we comprehensively analyzed translational responses to haloperidol, T-773, and T-609 in D_1- and D_2-MSNs to gain insight into the difference in downstream effects among D_2 antagonists and faster and slower off-rate PDE10A inhibitors.

Materials and Methods

Animals

Institute of Cancer Research and C57BL/6J male mice were supplied by CLEA Japan Inc. (Tokyo, Japan). Male Sprague–Dawley and Long Evans rats were supplied by Charles River Laboratories Japan, Inc. (Yokohama, Japan). *Drd1*- and *Drd2*-bacTRAP mice (Heiman et al., 2008) had been extensively backcrossed to C57BL/6J strain and maintained in Takeda Pharmaceutical

Company Limited. After an acclimation period of at least 1 week (group-housing, 12-hour-light/-dark cycle, with lights on at 7:00 AM), 7- to 10-week-old animals were used. Different cohorts of animals were used for each experiment. The care and use of the animals and the experimental protocols were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited.

Chemicals

T-773, T-609, and MP-10 succinate were synthesized by Takeda Pharmaceutical Company Limited (Harada et al., 2015b; Yoshikawa et al., 2015; Suzuki et al., 2016). Haloperidol was purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO). These drugs were suspended in distilled water with 0.5% (w/v) methylcellulose. [^{3}H]T-773 was synthesized by Quotient Bioresearch Ltd. (Cambridgeshire, UK). (+)-MK-801 hydrogen maleate (Sigma-Aldrich) and methamphetamine (METH) hydrochloride (Dainippon Sumitomo Pharma Co. Ltd., Osaka, Japan) were dissolved in saline. All compounds were dosed at 10 or 20 mL/kg body weight in mice and at 2 mL/kg body weight in rats.

Dissociation Study Using Mouse Brain Sections

Off-rates of PDE10A inhibitors were evaluated according to the previous report (Suzuki et al., 2016). See the Supplementary Information for details.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

RT-qPCR was performed as reported previously (Suzuki et al., 2016). See the Supplementary Information for details.

Behavioral Testing

Effects of drugs on METH- and MK-801-induced hyperactivity, deficits in prepulse inhibition (PPI), NORT, and radial arm maze (RAM) in rodents were evaluated as previously described (Suzuki et al., 2015, 2016; Shiraishi et al., 2016). See the Supplementary Information for details.

Microdialysis of Striatal Dopamine in Mice

Striatal dopamine efflux was measured in mice as reported previously (Suzuki et al., 2016). See the Supplementary Information for details.

Striatal cAMP and cGMP Measurements in Mice

Striatal cAMP and cGMP contents were measured in mice as previously described (Suzuki et al., 2015). See the Supplementary Information for details.

Counting of c-Fos-Positive Cells in Rat Brain Slices

Counting of c-Fos-positive cells in rat brain slices was conducted as reported previously (Nakatani et al., 2017). See the Supplementary Information for details.

RNA-seq of Samples from *Drd1a* and *Drd2* bacTRAP Mice

Immunoprecipitation samples were prepared as described previously (Heiman et al., 2008). See the Supplementary Information for details.

Statistical Analysis

Bartlett's test was used for testing the homogeneity of variances (parametric data, $P \geq .05$; nonparametric data, $P < .05$). The statistical significance of differences between 2 groups were assessed by Student *t* test (for parametric data) or Aspin-Welch test (for nonparametric data). For comparing dose-dependent effects of drug treatment, the statistical significance was analyzed by 2-tailed Williams' test (for parametric data) or 2-tailed Shirley-Williams test (for nonparametric data). The multiple comparison between vehicle group and each drug treatment group was conducted using 1-way ANOVA followed by Dunnett's test (for parametric data) or Steel's test (for nonparametric data). The multiple comparison between groups was conducted using 1-way ANOVA followed by Tukey's test. See each figure legend for details.

Results

Off-Rate Characterizes PDE10A Inhibitor in Activation Pattern of MSNs, Antipsychotic-Like Effects, and Striatal Dopamine Release

To further support our hypothesis that the off-rates of PDE10A inhibitors would characterize their pharmacological profiles, we comprehensively compared the profiles of faster and slower off-rate PDE10A inhibitors with a similar chemical structure; structural similarity can minimize noise signals derived from their off-targets. T-773 is a specific PDE10A inhibitor, which has been developed as a positron emission tomography tracer for PDE10A (Harada et al., 2015b; Takano et al., 2016) and is structurally similar to T-609 (Figure 1A). Autoradiography studies using mouse brain slices revealed that binding of both T-773 and T-609 in the striatum was reduced in a time-dependent manner (Figure 1B). After 60-minute incubation, the PDE10A occupancy of T-773 (2.79%) was remarkably lower than that of T-609 (54.3%). Thus, the off-rate of T-773 was much faster than that of T-609.

Orally administered T-773 significantly and dose-dependently increased striatal cAMP and cGMP levels in Institute of Cancer Research mice (supplementary Figure 1A). To compare activation patterns of MSNs, we measured striatal SP and Enk mRNA expression levels in mice after administration of haloperidol, T-773, or T-609. Haloperidol significantly and dose-dependently increased Enk, but not SP, mRNA expression (28% increase at 3 mg/kg) (Figure 1C). T-773 and T-609 significantly and dose-dependently increased Enk mRNA expression to a similar extent (37% increase at 10 mg/kg of T-773 and 42% increase at 10 mg/kg of T-609), whereas T-609 increased SP mRNA expression levels to a greater extent than T-773 (95% increase at 10 mg/kg of T-609 and 44% increase at 10 mg/kg of T-773) (Figure 1C). Similar patterns of SP and Enk mRNA induction by T-773 and T-609 were also seen in rats (supplementary Figure 1B) (Suzuki et al., 2016). The expression ratio of SP mRNA to Enk mRNA was dose-dependently decreased by haloperidol (Figure 1D), reflecting its selective activation of D_2-MSNs. The ratio was dose-dependently increased by T-609, but not by T-773 (Figure 1D), reflecting a T-609-induced greater activation of D_1-MSNs.

In the following experiments, the timing of drug administration was determined based on the pharmacokinetic data of the drugs (supplementary Figure 2; supplementary Tables 1–4) (Suzuki et al., 2018). Hyperactivity in rodents treated with an N-methyl-$_D$-aspartate receptor antagonist MK-801 or the dopamine releaser METH is widely used as an animal model of acute psychosis (Bubeníková-Valesová et al., 2008; Grant et al., 2012).

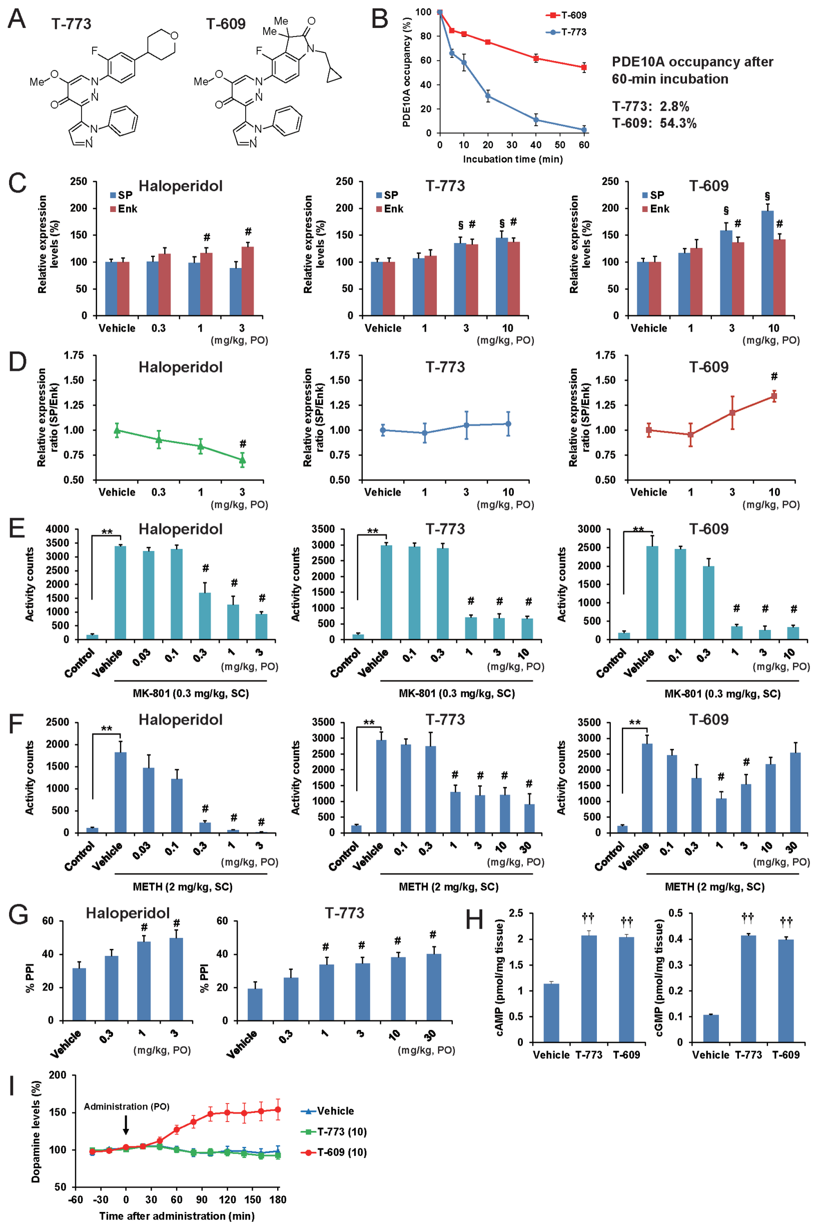


Figure 1. Off-rate characterizes phosphodiesterase 10A (PDE10A) inhibitor in activation pattern of medium spiny neurons (MSNs), antipsychotic-like effects, and striatal dopamine release. (A) Chemical structures of T-773 and T-609. (B) Brain slices from male C57BL/6J mice were treated with T-773 (20 nM) or T-609 (20 nM) to saturate striatal PDE10A and then were incubated with [^{3}H]T-773 (20 nM) to induce time-dependent displacement. Time-occupancy curves of T-773 and T-609 were monitored by binding of [^{3}H]T-773 in the striatum of slices. Data are represented as mean ± SEM (n = 3). (C) Substance P (SP) and enkephalin (Enk) mRNA expression levels in the striatum were evaluated by real-time quantitative polymerase chain reaction 1 hour after oral (PO) administration of haloperidol, T-773, and T-609 in male C57BL/6J mice. Data are represented as mean + SEM (n = 7). §P < .05 (for SP) and #P < .05 (for Enk) vs the vehicle-treated group (2-tailed Shirley-Williams test for haloperidol, 2-tailed Williams' test for T-773 and T-609). (D) Expression ratio of SP mRNA to Enk mRNA was calculated for each drug. Data are represented as mean + SEM (n = 7). #P < .05 vs the vehicle-treated group (2-tailed Williams' test). (E) MK-801 was subcutaneously (SC) administered to male C57BL/6J mice 1 hour after oral treatment with haloperidol, T-773, and T-609, and the locomotor activity of mice was measured during a 2-hour period from MK-801 injection. Data are represented as mean + SEM (n = 4 for control, n = 6 for all other groups). **P < .01 vs the control group (Student t test); #P < .05 vs the vehicle-treated group (2-tailed Shirley-Williams test for haloperidol and T-609, 2-tailed Williams' test for T-773). (F) Methamphetamine (METH) was subcutaneously administered to male C57BL/6J mice 1 hour after oral treatment with haloperidol, T-773, and T-609, and the locomotor activity of mice was measured during a 2-hour period from METH injection. Data are represented as mean + SEM. For haloperidol, n = 5 (0.03 and 3 mg/kg) and n = 6 (other groups). For T-773, n = 4 (control), n = 6 (vehicle), and n = 5 (other groups). For T-609, n = 5 in each group. **P < .01 vs the control group (Aspin-Welch test); #P < .05 vs the vehicle-treated group (2-tailed Shirley-Williams test for haloperidol, 2-tailed Williams' test for T-773, and Dunnett's test for T-609). (G) One hour after oral treatment with haloperidol, T-773, and T-609, male C57BL/6J mice were presented with a 118-db pulse for 40-millisecond with and without 20-millisecond prepulse of 82 db, 100 milliseconds before the 118-db pulse. The reduction of the startle response by presentation of prepulse was calculated as percentages. Data are represented as mean + SEM. For haloperidol, n = 13 (vehicle), n = 14 (0.3 and 3 mg/kg), and n = 15 (1 mg/kg). For T-773, n = 10 (0.3 mg/kg) and n = 9 (other groups). #P < .05 vs the vehicle-treated group (2-tailed Williams' test). (H) Striatal cAMP and cGMP levels were evaluated 1 hour after oral treatment with T-773 and T-609 (10 mg/kg each) in male C57BL/6J mice. Data are represented as mean + SEM (n = 8). The multiple comparison between vehicle group and each drug treatment group was conducted using 1-way ANOVA followed by Dunnett's test (††P < .01). (I) Temporal changes in striatal dopamine efflux were monitored by microdialysis during a 3-hour period from oral administration of T-773 and T-609 in freely moving male C57BL/6J mice. Data are represented as mean ± SEM (n = 5).

Haloperidol, T-773, and T-609 significantly suppressed MK-801-induced hyperactivity in mice and rats (Figure 1E; supplementary Figure 3A) (Suzuki et al., 2015). Haloperidol and T-773 also dose-dependently reduced METH-induced hyperactivity; however, T-609 exhibited U-shaped dose-responses in mice and rats (Figure 1F; supplementary Figure 3B) (Suzuki et al., 2016). C57BL/6J mice exhibit a naturally low PPI (Suzuki et al., 2016) and are used as models for PPI deficits, which have been extensively replicated in schizophrenia (Javitt and Freedman, 2015). Haloperidol and T-773, but not T-609, dose-dependently enhanced PPI in C57BL/6J mice (Figure 1G) (Suzuki et al., 2016). All drugs did not affect startle responses to the pulse (supplementary Figure 4). T-773 and T-609 at 10 mg/kg (PO) comparably increased striatal cAMP ($F_{2,21}$ = 53.1463, P < .0001) and cGMP ($F_{2,21}$ = 468.0818, P < .0001) (Figure 1H); thus, we used 10 mg/kg of T-773 and T-609 in the following studies using mice. We previously reported that MP-10 and T-609, but not TAK-063, markedly enhanced striatal dopamine release attributable to excess activation of D_1-MSNs in rodents (Suzuki et al., 2016). Consistently, T-609, but not T-773, enhanced striatal dopamine release in mice (Figure 1I). These results support our hypothesis that faster off-rate PDE10A inhibitors produce balanced activation of D_1- and D_2-MSNs and potent antipsychotic-like efficacy in multiple paradigms without enhancing striatal dopamine release in rodents.

Faster Off-Rate PDE10A Inhibitors, But Not Slower Off-Rate Ones or a D_2 Antagonist, Improve Cognition and Activate PFC in Rats

TAK-063 improved cognitive function in multiple domains (Shiraishi et al., 2016), including recognition memory and spatial working memory that were evaluated by NORT using naive rats and RAM using rats treated with MK-801, respectively. To investigate whether the relative activation levels of D_1- and D_2-MSNs

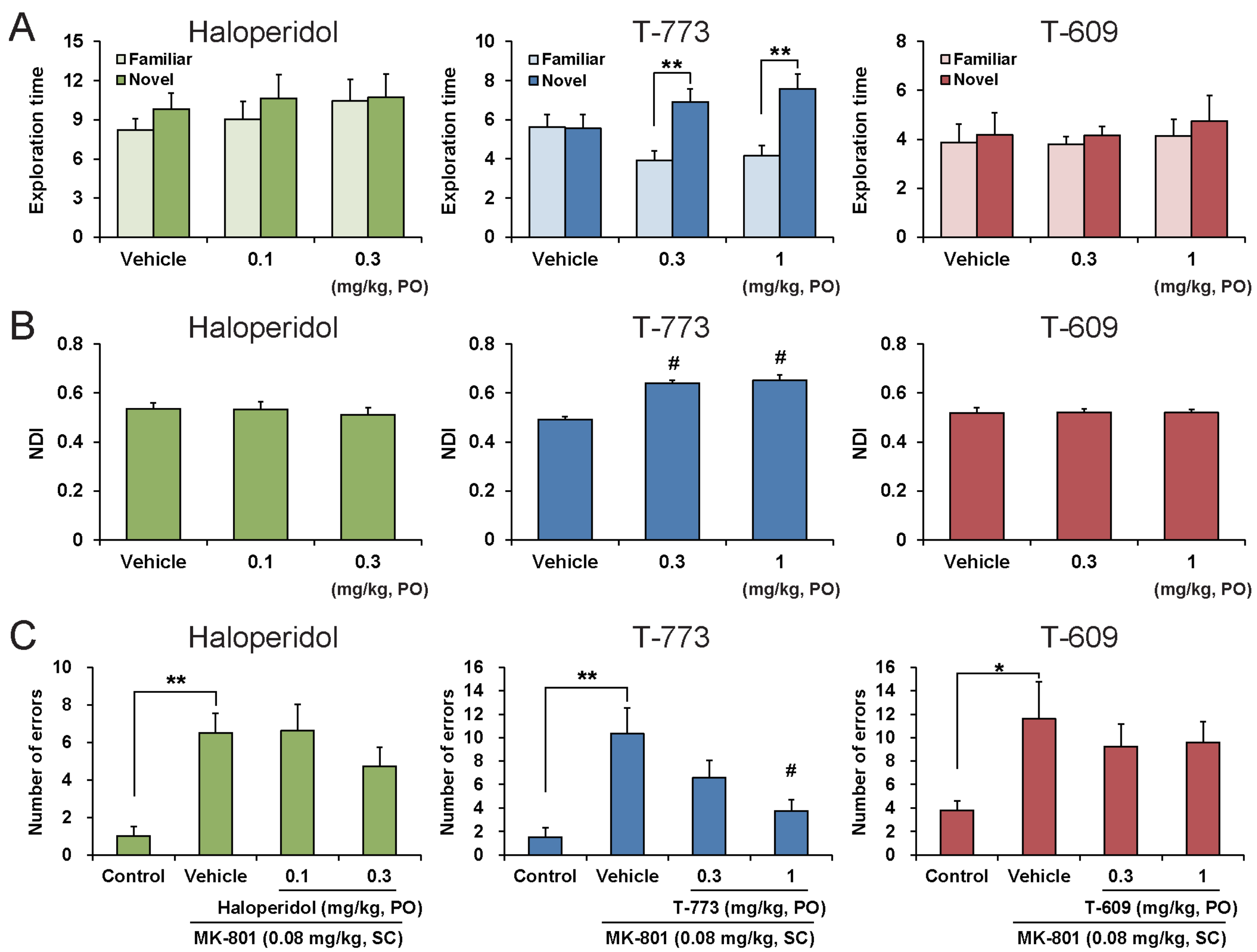
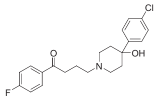
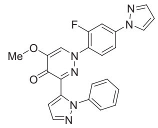
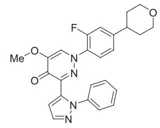
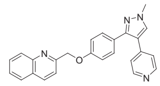
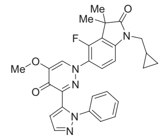


Figure 2. T-773, but not T-609 or haloperidol, improves cognition in rats. (A) In the novel object recognition task (NORT), male Long Evans rats were allowed to explore 2 identical objects for 3 minutes (acquisition trial), and 48 hours later, the rats were allowed to explore 2 objects, the familiar object and a novel object, for 3 minutes (retention trial). Haloperidol, T-773, and T-609 were orally (PO) administered to rats 1, 1, and 2 hours prior to the acquisition trial and exploration times for familiar and novel objects were measured in the retention trial. Data are represented as mean + SEM (n = 10 in each group). **P < .01 (paired t test). (B) In NORT, the novelty discrimination index (NDI) was calculated as the ratio of exploratory time for the familiar object to that for the novel object in the retention trial. Data are represented as mean + SEM (n = 10 in each group). #P < .05 vs the vehicle-treated group (2-tailed Williams' test). (C) In radial arm maze (RAM), male Long Evans rats were trained to find food pellets at the end of all (8) arms during a 5-minute exploration with the number of entry error (an entry into the previously entered arm) ≤2. On the trial day, rats were orally treated with haloperidol, T-773, and T-609 2, 1, and 2 hours prior to the trial and then subcutaneously treated with MK-801 0.5 hour before the trial, and the entry errors for each rat were counted in a 5-minute trial. Data are represented as mean + SEM. For haloperidol, n = 6 (control), n = 18 (vehicle), and n = 17 (0.1 and 0.3 mg/kg). For T-773, n = 6 (control), n = 18 (vehicle), and n = 17 (0.3 and 1 mg/kg). For T-609, n = 5 (control), n = 15 (vehicle), n = 13 (0.3 mg/kg), and n = 12 (1 mg/kg). *P < .05; **P < .01 (Aspin-Welch test); #P < .05 vs the vehicle-treated group (2-tailed Shirley-Williams test).

Table 1. Summary of pharmacological profiles of D_2 antagonist and faster and slower off-rate PDE10A inhibitors.

	D_2 antagonist	PDE10A inhibitor			
	Haloperidol	TAK-063	T-773	MP-10	T-609
Chemical structure					
IC_{50} for recombinant human PDE10A (nM)	No relevance	0.30[a]	0.77[b]	0.10[c]	0.080[d]
Off-rate from PDE10A	No relevance	Fast[e]	Fast	Slow[e]	Slow[f]
Activation of D_1-MSNs (SP mRNA induction)	–	+[e]	+	++[e]	++[f]
Activation of D_2-MSNs (Enk mRNA induction)	+	+[e]	+	+[e]	+[f]
Striatal dopamine release	No data	–[e]	–	+[e]	+[f]
MK-801-induced hyperactivity	+[g]	+[h]	+	+[e]	+
METH-induced hyperactivity	+[f]	+[e]	+	–[e]	–[f]
Low PPI in C57BL/6J mice	+	+[e]	+	–[e]	–[e]
Procognitive effects in rats	–	+[i]	+	–[j]	–
Activation of PFC in rats	–	+[k]	+	–[l]	–

–, no significant effect; +, significant effect; ++, significant effect on SP mRNA induction and significant increase in the expression ratio of SP mRNA to Enk mRNA. D_1-MSNs, dopamine D_1 receptor-expressing medium spiny neurons; D_2-MSNs, dopamine D_2 receptor-expressing medium spiny neurons; Enk, enkephalin; IC_{50}, half-maximal inhibitory concentration; METH, methamphetamine; PDE10A, phosphodiesterase 10A; PFC, prefrontal cortex; PPI, prepulse inhibition; SP, substance P.
a Data from the previous study (Harada et al., 2015a).
b Data from the previous study (Harada et al., 2015b).
c Data were obtained according to the method previously reported (Harada et al., 2015a).
d Data from the previous study (Yoshikawa et al., 2015).
e Data from the previous study (Suzuki et al., 2016).
f Data from both the previous (Suzuki et al., 2016) and present studies.
g Data from both the previous (Suzuki et al., 2015) and present studies.
h Data from the previous study (Suzuki et al., 2015).
i Data from the previous study (Shiraishi et al., 2016).
j Data from the previous (Grauer et al., 2009) and present studies.
k Data from the previous study (Nakatani et al., 2017).
l Data from the previous (Wilson et al., 2015) and present studies.

affect cognition, we evaluated effects of haloperidol, T-773, and T-609 in NORT and RAM in rats.

In the acquisition trial of NORT, the exploratory time was significantly decreased after haloperidol (1 mg/kg), T-773 (3 mg/kg), and T-609 (3 mg/kg) treatment (supplementary Figure 5). Because short exploratory time potentially hampers memory acquisition, we excluded these treatment groups. In the retention trial, exploration time for the novel object was significantly longer than that for the familiar object in T-773, but not T-609 or haloperidol, treatment group (Figure 2A). This resulted in that only T-773 significantly increased the novelty discrimination index (Figure 2B), suggesting enhanced retention of recognition memory. In RAM, T-773, but not T-609 or haloperidol, significantly and dose-dependently reduced the number of MK-801-induced entry errors (Figure 2C), indicating the improvement of MK-801-induced working memory deficits. Moreover, MP-10 did not show procognitive effects in NORT or RAM using rats (supplementary Figure 6A-B). These results suggest that faster off-rate PDE10A inhibitors, but not slower off-rate ones or D_2 antagonists, improve cognition in rodents.

TAK-063, but not MP-10, enhances the neuronal activity in rat PFC (Wilson et al., 2015; Tomimatsu et al., 2016; Nakatani et al., 2017), which is strongly implicated in cognitive function (Miller, 2000) and is connected with striatum via cortico-striato-thalamo-cortical circuit (Dandash et al., 2017). The expression level of c-Fos has been widely used as a marker of neuronal activity (Chung, 2015). To further investigate whether the relative activation levels of D_1- and D_2-MSNs affect neuronal activity in PFC subregions, we compared c-Fos-positive cell numbers in the anterior cingulate cortex (ACC), prelimbic cortex (PrL), and infralimbic cortex (IL) of rats after oral treatment with haloperidol (3 mg/kg), T-773 (10 mg/kg), or T-609 (10 mg/kg) by immunohistochemistry (Figure 3A). Interestingly, T-773, but not T-609 or haloperidol, significantly increased the number of c-Fos-positive cells in the ACC ($F_{3,24}=8.3174$, $P=.0006$) and PrL ($F_{3,24}=5.9459$, $P=.0035$) (Figure 3B-C). Furthermore, MP-10 did not increase the number of c-Fos-positive cells in these regions (supplementary Figure 6C). Thus, faster off-rate PDE10A inhibitors, but not slower off-rate ones or D_2 antagonists, could elicit neuronal activation in rat ACC and PrL.

T-773, T-609, and Haloperidol Induce Qualitatively Similar Gene Translation Patterns in D_2-MSNs

We revealed that faster but not slower off-rate PDE10A inhibitors produce balanced activation of D_1- and D_2-MSNs, neuronal activation of PFC subregions, and antipsychotic-like and procognitive effects in multiple paradigms without inducing striatal dopamine release (Table 1). We hypothesized that the relative activation level of D_1- and D_2-MSNs is key for the pharmacological profiles of PDE10A inhibitors and assumed that D_2 antagonism and PDE10A inhibition would induce a similar activation pattern of D_2-MSNs. To understand overall translational impacts of faster and slower off-rate PDE10A inhibitors and D_2 antagonists

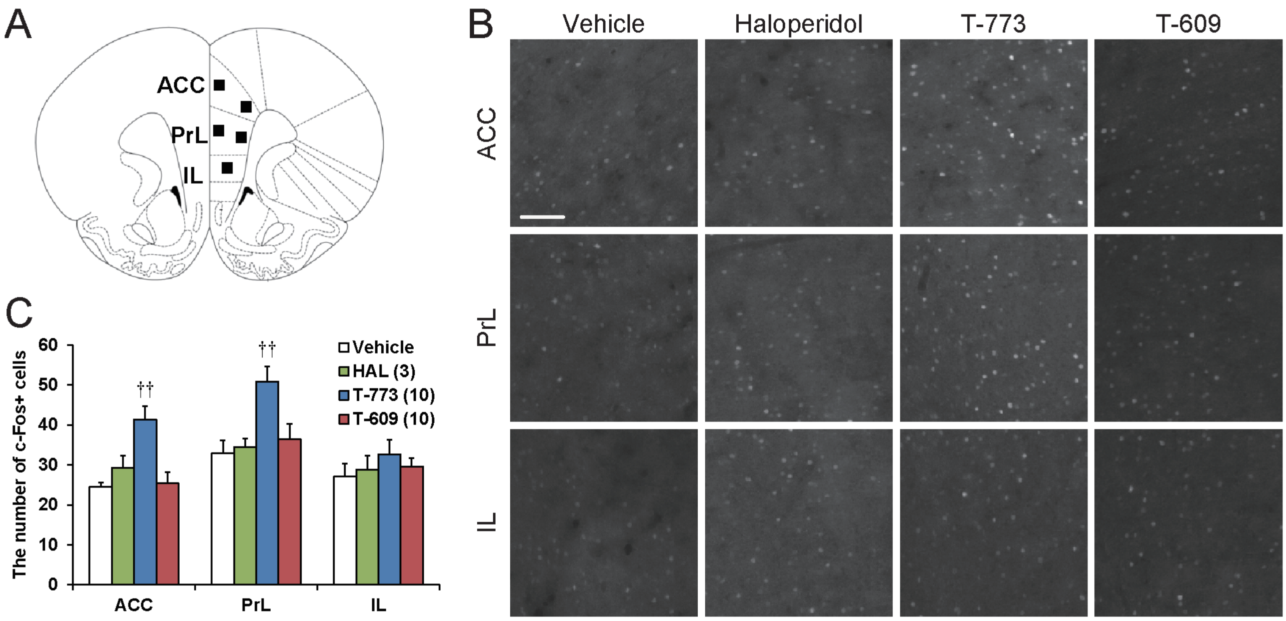


Figure 3. T-773, but not T-609 or haloperidol, activates the prefrontal cortex in rats. (A) The regions of interest (black squares) in the anterior cingulate cortex (ACC), prelimbic cortex (PrL), and infralimbic cortex (IL) in male Long Evans rats were determined with reference to "The Rat Brain in Stereotaxic Coordinates," 4th ed. (Paxinos and Watson, 1998). (B) Representative photographs of immunostaining of c-Fos protein using brain slices from rats transcardially perfused 90 minutes after oral treatment with vehicle, haloperidol (3 mg/kg), T-773 (10 mg/kg), or T-609 (10 mg/kg) for each cortical region. Scale bar = 100 µm. (C) The number of c-Fos-like immunoreactive cells in the rat ACC, PrL, and IL was automatically counted for each treatment group. Data are represented as mean + SEM (n = 7). The multiple comparisons between the vehicle group and each drug treatment group were conducted using 1-way ANOVA followed by Dunnett's test (††$P < .01$).

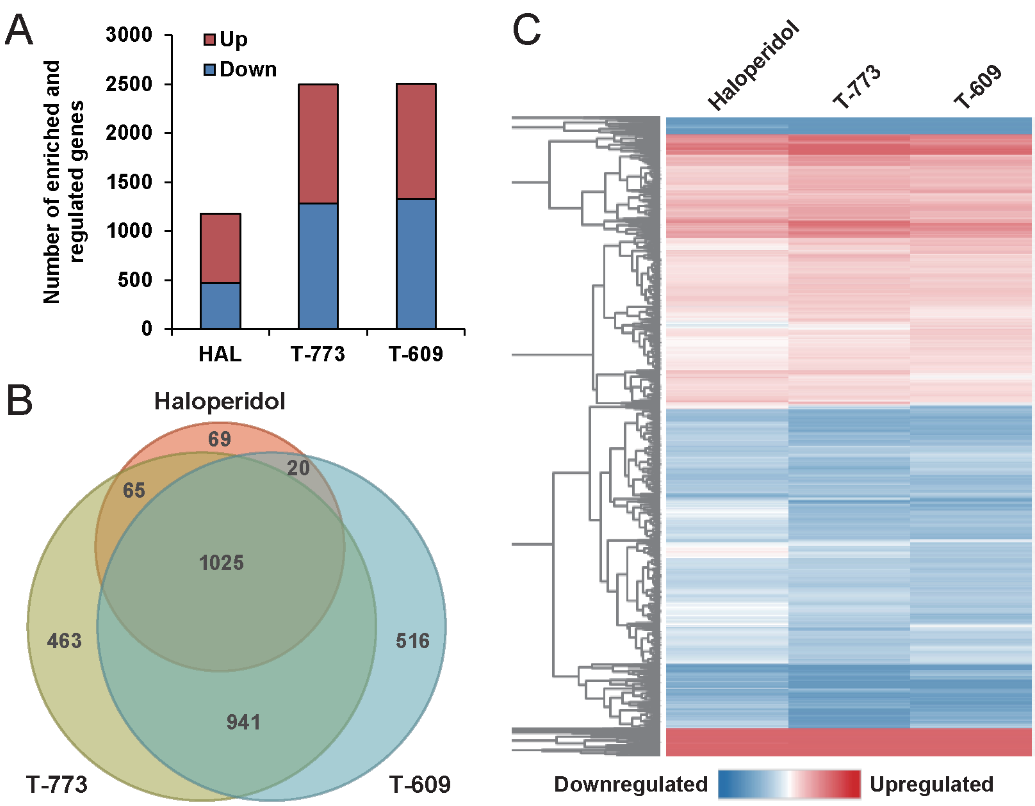


Figure 4. T-773, T-609, and haloperidol induce qualitatively similar patterns of gene translation in dopamine D_2 receptor-expressing medium spiny neurons (D_2-MSNs). (A) RNA sequencing was conducted using striatal tissues dissected from *Drd2*-bacterial artificial chromosome-based translating ribosome affinity purification mice 1 hour after oral treatment with haloperidol (3 mg/kg), T-773 (10 mg/kg), and T-609 (10 mg/kg). The total number of genes enriched and differentially upregulated (red columns) and downregulated (blue columns) by each drug in D_2-MSNs is shown. (B) The overlap of genes enriched and differentially regulated by haloperidol, T-773, and T-609 in D_2-MSNs is represented by Venn diagram. The number of genes is described in each area. (C) Overall gene translation patterns induced by haloperidol, T-773, and T-609 are shown as heatmaps of genes enriched and differentially regulated by these drugs in D_2-MSNs. Red, blue, and white colors represent upregulated, downregulated, and unchanged genes, respectively.

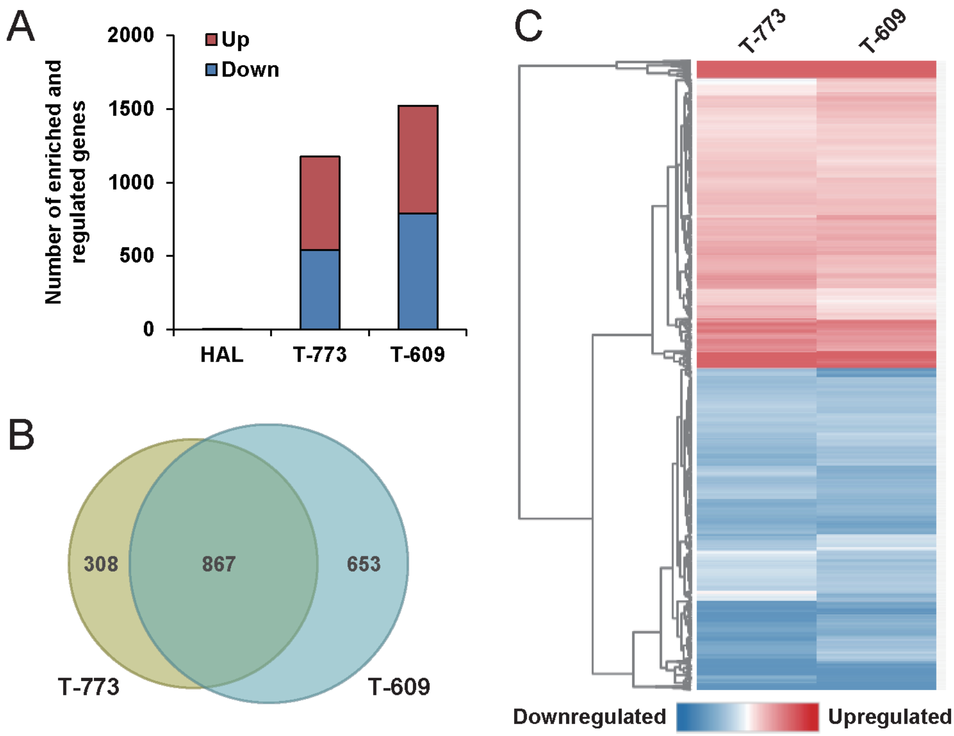


Figure 5. Overall gene translation patterns induced by T-773 and T-609 are qualitatively similar in dopamine D_1 receptor-expressing medium spiny neurons (D_1-MSNs). (A) RNA sequencing was conducted using striatal tissues dissected from *Drd1a*-bacterial artificial chromosome-based translating ribosome affinity purification mice 1 hour after oral treatment with haloperidol (3 mg/kg), T-773 (10 mg/kg), and T-609 (10 mg/kg). The total number of genes enriched and differentially upregulated (red columns) and downregulated (blue columns) by T-773 and T-609 in D_1-MSNs is shown. (B) The overlap of genes enriched and differentially regulated by T-773 and T-609 in D_1-MSNs is represented as Venn diagram. The number of genes is described in each area. (C) Overall gene translation patterns induced by T-773 and T-609 are shown as heatmaps of genes enriched and differentially regulated by these drugs in D_1-MSNs. Red, blue, and white colors represent upregulated, downregulated, and unchanged genes, respectively.

on D_1- and D_2-MSNs, we comprehensively investigated the gene translation patterns in D_1- and D_2-MSNs using *Drd1a* and *Drd2* bacTRAP mice orally treated with haloperidol (3 mg/kg), T-773 (10 mg/kg), or T-609 (10 mg/kg) for RNA-seq studies. T-773, but not T-609, produced dose-dependent effects on METH-induced hyperactivity in *Drd1a* and *Drd2* bacTRAP mice (supplementary Figure 7), suggesting that the distinct response of D_1-MSNs to T-773 and T-609 is preserved in these mice.

In D_2-MSNs, the total number of differentially regulated genes (DRGs) for T-773 (2494 total genes; 1212 upregulated and 1282 downregulated genes) was essentially equivalent to that for T-609 (2502 total genes; 1171 upregulated and 1331 downregulated genes) (Figure 4A). The number of DRGs for haloperidol (1179 total genes; 707 upregulated and 472 downregulated) was less than one-half of those for PDE10A inhibitors. T-773 and T-609 shared a large number of DRGs, and most (87%) genes differentially regulated by haloperidol were also significantly regulated by the PDE10A inhibitors (Figure 4B). The heatmap of all combined DRGs for haloperidol, T-773, and T-609 showed their comprehensive similarity in gene translation patterns in D_2-MSNs (Figure 4C). This suggests that most genes, even those that were not significantly regulated by 1 or more of the drugs, have a similar trend in translational response among all treatments. We did not identify any major class of genes or pathways that was uniquely regulated by any treatment. Notably, β-arrestin2 pathway-related genes, such as *Akt1*, *Akt1s1*, *Gskip*, and *Arrb2* that encode serine/threonine kinase 1, AKT1 substrate 1, GSK3β-interacting protein, and β-arrestin2, respectively (Kovacina et al., 2003; Chou et al., 2006; Beaulieu and Gainetdinov, 2011), were regulated similarly by all treatments (Table 2). These results suggest that D_2 antagonists and faster and slower off-rate PDE10A inhibitors induce a qualitatively similar gene translation pattern in D_2-MSNs.

Gene Translation Patterns Induced by T-773 and T-609 Are Qualitatively Similar in D_1-MSNs

In D_1-MSNs, only 4 DRGs were identified for haloperidol, indicating its selective activation of D_2-MSNs (Figure 5A). The total number of DRGs for T-609 (1520 total genes; 732 upregulated, 788 downregulated) was approximately 1.3-fold larger than that for T-773 (1175 total genes; 633 upregulated; 542 downregulated) (Figure 5A). A large number of DRGs overlapped between T-773 and T-609 in D_1-MSNs (Figure 5B), although the ratio of shared DRGs (intersection of the Venn diagram) to total DRGs (union of the Venn diagram) for D_1-MSNs (47.4%) was approximately 1.4-fold lower than that for D_2-MSNs (64.9%). The heatmap of all combined DRGs for T-773 and T-609 in D_1-MSNs showed a qualitatively similar gene translation pattern between T-773 and T-609 (Figure 5C). Thus, overall translational patterns for T-773 and T-609 in D_1-MSNs are also qualitatively similar.

T-773 More Robustly Enhances Gene Translation Than T-609 in Both D_1- and D_2-MSNs

The gene translation patterns for T-773 and T-609 were qualitatively similar in both D_1- and D_2-MSNs. This could support the

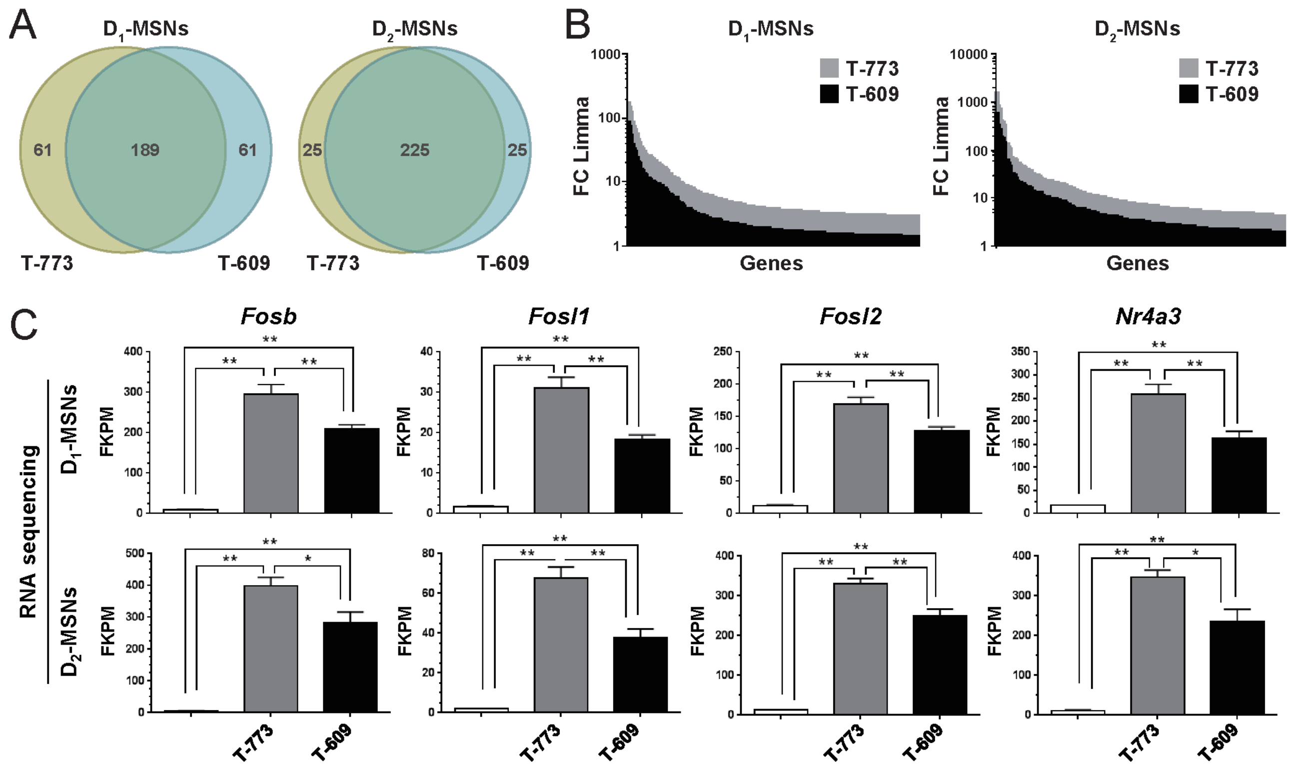


Figure 6. T-773 could more robustly upregulate gene translation in both dopamine D1 receptor- and D2 receptor-expressing medium spiny neurons (D1- and D2-MSNs), compared with T-609. (A) The top 250 upregulated genes by T-773 and T-609 based on fold change linear models for microarray (FC Limma) were extracted from the data of RNA sequencing using *Drd1a*- and *Drd2*-bacTRAP mice and are represented as Venn diagrams for D_1- and D_2-MSNs, respectively. (B) Waterfall graphs were generated from the top 250 upregulated genes by T-773 and T-609 in D_1- and D_2-MSNs, respectively. (C) Multiple neuronal activity-related immediate early genes, which were more robustly upregulated by T-773 than T-609 in both D_1- and D_2-MSNs, were found within the top 20 upregulated genes by T-773 and T-609. The magnitude of translational responses of those genes are represented as fragments per kilobase million (FKPM). Data are represented as mean + SEM (n = 4 in each group). The multiple comparisons between groups were conducted using 1-way ANOVA followed by Tukey's test (*$P < 0.05$; **$P < .01$).

Table 2. Translational responses of β-arrestin2 pathway-related genes to T-773, T-609, and haloperidol in D_2-MSNs.

Gene	Haloperidol	T-773	T-609
Akt1	0.759	0.731	0.627
Akt1s1	0.791	0.747	0.745
Gskip	0.837	0.711	0.692
Arrb2	1.671	1.724	1.671

Data are represented as fold change analyzed by linear models for microarray (FC Limma) compared with vehicle-treated group.

idea that the relative activation level of D_1- and D_2-MSNs, rather than specific downstream signaling in D_1- or D_2-MSNs, is critical for pharmacological differences of PDE10A inhibitors. Therefore, we further analyzed the translational responses to T-773 and T-609 in D_1- and D_2-MSNs, focusing on the magnitude of responses.

We generated lists of the top 250 upregulated genes based on the fold changes in each MSN by each treatment. The 250 genes found for each MSN largely overlapped between T-773 and T-609, and the intersection to union ratio was larger in D_2-MSNs (81.8%) than in D_1-MSNs (60.8%) in accordance with the results for total genes (Figure 6A). The waterfall graphs of the union of the 250 genes for T-773 and T-609 indicated that the magnitude of translational response to T-773 is more robust than that of T-609 in both D_1- and D_2-MSNs (Figure 6B). Immediate early genes are frequently used as markers of neuronal activity and are implicated in neuronal plasticity (Chandra and Lobo, 2017). Within the top 20 upregulated genes, we identified several immediate early genes, such as *Fosb*, *Fosl1*, *Fosl2*, and *Nr4a3*, which were more robustly upregulated by T-773 compared with T-609 in both D_1-MSNs ($F_{2,9}$=90.1, P<.0001; $F_{2,9}$=70.75, P<.0001; $F_{2,9}$=125.1, P<.0001; $F_{2,9}$=67.93, P<.0001, respectively) and D_2-MSNs ($F_{2,9}$=65.72, P<.0001; $F_{2,9}$=61.12, P<.0001; $F_{2,9}$=177.2, P<.0001; $F_{2,9}$=61.86, P<.0001, respectively) (Figure 6C). These genes encode FosB proto-oncogene, FOS like 1, 2, and nuclear receptor subfamily 4 group A member 3, respectively, and are implicated in neuronal plasticity and/or adaptation (Pennypacker et al., 2000; Pönniö and Conneely, 2004; Hebert et al., 2005; Faure et al., 2006; Abe and Takeichi, 2007; Weinberg et al., 2007; Christiansen et al., 2011). RT-qPCR confirmed that T-773 induced more robust upregulation of *Fosb*, *Fosl1*, *Fosl2*, and *Nr4a3* compared with T-609 in both D_1-MSNs ($F_{2,9}$=11.67, P=.0032; $F_{2,9}$=8.344, P=.0089; $F_{2,9}$=19.69, P=.0005; $F_{2,9}$=3.635, P=.0696, respectively) and D_2-MSNs ($F_{2,9}$=22.59, P=.0003; $F_{2,9}$=35.69, P=.0001; $F_{2,9}$=60.47, P<.0001; $F_{2,9}$=21.34, P=.0004, respectively), although not all cases were statistically significant (supplementary Figure 8). These results suggest that T-773 may more robustly upregulate neuronal plasticity and/or adaptation-related genes than T-609 in both D_1- and D_2-MSNs.

Discussion

The present study demonstrates that (1) D_2 antagonism and PDE10A inhibition induce qualitatively similar translational patterns in all classes of genes in D_2-MSNs, including those involved in β-arrestin2 pathways, suggesting that the critical difference between D_2 antagonism and PDE10A inhibition is activation of D_1-MSNs, (2) PDE10A inhibitors with faster and slower off-rates induce qualitatively similar translational patterns in both D_1- and D_2-MSNs. Thus, activation balance of D_1- and D_2-MSNs is the major difference between these drugs; (3) the balanced activation of D_1- and D_2-MSNs by faster off-rate PDE10A inhibitors results in not only potent antipsychotic-like effects but also activation of PFC and procognitive effects in rodents.

D_2 antagonism and the resulting activation of D_2-MSNs are believed to be the fundamental mechanism of action of current antipsychotics such as haloperidol. In fact, haloperidol selectively upregulated Enk mRNA levels (a marker for activation of D_2-MSNs) in mice. PDE10A inhibitors can activate D_2-MSNs via upregulation of cAMP and cGMP levels; therefore, PDE10A inhibitors have been proposed as novel antipsychotics. This hypothesis assumed that D_2 antagonism and PDE10A inhibition would induce a similar activation pattern of D_2-MSNs; note that PDE10A inhibition activates cAMP- and cGMP-pathways, whereas D_2 antagonism modulates cAMP- and β-arrestin2 pathways in D_2-MSNs. In this study, we investigated comprehensive translational responses to haloperidol and faster and slower off-rate PDE10A inhibitors in D_1- and D_2-MSNs using the bacTRAP technique combined with RNA-seq. As expected, haloperidol induced significant translational responses in D_2-MSNs but not in D_1-MSNs, indicating its selective activation of D_2-MSNs. Heatmap analysis demonstrated similar translational patterns between PDE10A inhibitors and haloperidol in D_2-MSNs. Surprisingly, haloperidol, T-773, and T-609 regulated β-arrestin2 pathway-related genes in a similar manner. These results further support that PDE10A inhibition may be a promising approach to treat psychosis in patients with schizophrenia. Although the randomized placebo-controlled study of TAK-063 in patients with an acute exacerbation of schizophrenia did not meet the primary endpoint, the total result was suggestive of its antipsychotic activity: the least-square mean change of the Positive and Negative Syndrome Scale total score from baseline in the TAK-063 group was consistently greater than that in the placebo group at each assessment during the 6-week treatment period (–14.1 for placebo and –19.5 for TAK-063 at week 6; $P=.115$) (Macek et al., 2019). Moreover, nominal significances favoring TAK-063 were observed in the 3 secondary endpoints: Clinical Global Impression (CGI) Scale-Severity of Illness, CGI-Global Improvement, and CGI-Global Improvement responders (Macek et al., 2019). Several factors are likely to have a role in the failure to meet the primary endpoint of the study such as the high placebo response (–8.3 and –14.1 as the least-square mean changes in Positive and Negative Syndrome Scale total scores at week 1 and 6, respectively) with the heterogeneity in placebo response across sites and the imbalance in the proportion of black patients across study arms with the efficacy differences for TAK-063 between black and non-black patients (Macek et al., 2019).

As summarized in Table 1, a D_2 antagonist (haloperidol) and PDE10A inhibitors with faster (TAK-063, T-773) and slower (MP-10, T-609) off-rates showed different pharmacological profiles. The high PDE10A selectivity of those inhibitors (Harada et al., 2015a, 2015b; Suzuki et al., 2016) and the structural similarity between T-773 and T-609 emphasize that the differences are attributable to their distinct off-rate properties rather than their off-target profiles. Compared with slower off-rate PDE10A inhibitors, faster off-rate ones produced the balanced activation of D_1- and D_2-MSNs (comparable activation of D_2-MSNs measured by Enk mRNA induction and partial activation of D_1-MSNs measured by SP mRNA induction). Faster, but not slower, off-rate PDE10A inhibitors showed antipsychotic-like and procognitive effects in multiple paradigms and neuronal activation in PFC without affecting striatal dopamine release in rats and/or mice. The substantia nigra pars compacta is a major source of striatal dopamine and is negatively regulated by inhibitory neurons from the substantia nigra pars reticulata, the projection site of D_1-MSNs (Tepper et al., 1995; Nishi et al., 2011). Excess activation of D_1-MSNs by slower off-rate PDE10A inhibitors may inhibit substantia nigra pars reticulata and subsequently disinhibit substantia nigra pars compacta (supplementary Figure 9). Indeed, striatal injection of a D_1 agonist SKF82958 induced striatal dopamine release in rats (Suzuki et al., 2016). This may explain the enhancement of striatal dopamine release by slower off-rate PDE10A inhibitors. Accumulating evidence suggests that the ACC and PrL have critical roles in cognitive functions in multiple rodent models including NORT and RAM (Seamans et al., 1995; Christoffersen et al., 2008; Weible et al., 2009; Tse et al., 2011; Wartman et al., 2014; Pezze et al., 2015). Therefore, the neuronal activation in the ACC and PrL might contribute to the procognitive effects of faster off-rate PDE10A inhibitors. PDE10A is selectively expressed in striatal MSNs and the expression level in the cortex is 50- to 200-fold lower than that in the rat striatum (Seeger et al., 2003). Indeed, our previous autoradiography studies demonstrated that both $[^3H]$T-773 and $[^3H]$TAK-063 minimally accumulate in rat PFC (Harada et al., 2015a, 2015b). Moreover, TAK-063 increased cAMP levels in the striatum but not in other brain regions, including the frontal cortex in mice (Suzuki et al., 2015). Thus, neuronal activation in PFC and cognitive improvement by faster off-rate PDE10A inhibitors could be derived, not from their direct effects in PFC, but from the balanced activation of D_1- and D_2-MSNs and possibly the subsequent modulation of cortico-striato-thalamo-cortical circuit.

The number of DRGs for T-773 and T-609 were almost equivalent to each other, and the DRGs for the 2 drugs were largely overlapped in D_2-MSNs. Many of the DRGs for T-773 and T-609 were also overlapped in D_1-MSNs, although the overlap was smaller than that in D_2-MSNs: the number of DRGs for T-609 was larger than that for T-773 in D_2-MSNs. This may reflect the excessive activation of D_1-MSNs by T-609 compared with T-773, as indicated by SP mRNA induction. Nevertheless, the heatmap analyses suggest that T-773 and T-609 induce qualitatively similar translational patterns in both D_1- and D_2-MSNs. These results further support our hypothesis that the relative activation level of D_1- and D_2-MSNs based on off-rates of PDE10A inhibitors is critical for their pharmacological differences.

Slower off-rate PDE10A inhibitors induce excessive activation of D_1-MSNs as represented by a robust upregulation of SP mRNA expression, resulting in enhancement of striatal dopamine release. Increased striatal dopamine can deactivate D_2-MSNs but further activate D_1-MSNs, because D_2 agonism reduces intracellular cAMP level, whereas D_1 agonism increases it. Several PDEs such as PDE2A and PDE10A have regulatory GAF-B domains that act as a brake on an excessive increase in cAMP and/or cGMP: binding of these cyclic nucleotides to GAF-B domains allosterically enhances the catalytic activity of those PDEs (Menniti et al., 2006). Recent studies indicated that loss-of-function mutations in GAF-B domains of PDE2A and PDE10A are implicated in childhood-onset chorea, suggesting critical roles of these domains in MSNs in humans (Mencacci et al., 2016; Salpietro et al., 2018). An excessive increase in cAMP and cGMP in D_1-MSNs by slower off-rate PDE10A inhibitors may stimulate such a negative feedback system, resulting in the reduced activity of D_1-MSNs; note that PDE10A-selective inhibitors cannot inhibit other subtypes of activated PDEs. On the other hand, partial activation of D_1-MSNs by faster off-rate PDE10A inhibitors does not induce striatal dopamine release; therefore neither reduction in activity of D_2-MSNs nor deactivation of D_1-MSNs via GAF-B domain stimulation occurs. Although this is merely a speculation and further studies are needed, it may explain the observation that T-773 more robustly upregulated neuronal plasticity and/or adaptation-related genes than T-609 in both D_1- and D_2-MSNs.

In conclusion, the current study demonstrated that PDE10A inhibition and D_2 antagonism induce qualitatively similar downstream effects in D_2-MSNs, and the balanced activation of D_1- and D_2-MSNs by faster off-rate PDE10A inhibitors produce not only antipsychotic-like effects but also procognitive effects probably via PFC activation in rodents. Further studies are warranted to gain a mechanistic insight into PFC activation and cognitive improvement by the balanced activation of D_1- and D_2-MSNs.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Statement of Interest

None.

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