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PDE10A inhibitors stimulate or suppress motor behavior dependent on the relative activation state of the direct and indirect striatal output pathways

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Abstract

The enzyme phosphodiesterase 10A (PDE10A) regulates the activity of striatal, medium spiny neurons (MSNs), which are divided into a behaviorally stimulating, Gs-coupled D_1 receptor-expressing "direct" pathway and a behaviorally suppressant, Gi-coupled D_2 receptor-expressing "indirect" pathway. Activating both pathways, PDE10A inhibitors (PDE10AIs) combine functional characteristics of D_2 antagonists and D_1 agonists. While the effects of PDE10AIs on spontaneous and stimulated behavior have been extensively reported, the present study investigates their effects on suppressed behavior under various conditions of reduced dopaminergic neurotransmission: blockade of D_1 receptors with SCH-23390, blockade of D_2 receptors with haloperidol, or depletion of dopamine with RO-4-1284 or reserpine. In rats, PDE10AIs displayed relatively low cataleptic activity per se. After blocking D_1 receptors, however, they induced pronounced catalepsy at low doses close to those required for inhibition of apomorphine-induced behavior; slightly higher doses resulted in behavioral stimulant effects, counteracting the catalepsy. PDE10AIs also counteracted catalepsy and related behaviors induced by D_2 receptor blockade or dopamine depletion; catalepsy was replaced by behavioral stimulant effects under the latter but not the former condition. Similar interactions were observed at the level of locomotion in mice. At doses close to those inhibiting D-amphetamine-induced hyperlocomotion, PDE10AIs reversed hypolocomotion induced by D_1 receptor blockade or dopamine depletion but not hypolocomotion induced by D_2 receptor blockade. It is concluded that PDE10AIs stimulate or inhibit motor behavior dependent on the relative activation state of the direct and indirect striatal output pathways.

Abbreviations

AE, adverse event; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CL, confidence limits; EPS, extrapyramidal symptoms; i.v., intravenous; JNJ-42314415, 3-[6-(2-methoxyethyl)pyridin-3-yl]-2-methyl-8-morpholin-4-ylimidazo[1,2-a]pyrazine; MP-10, 2-{[4-(1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)phenoxy]methyl}quinoline; MSNs, medium spiny neurons; PDE10AI, phosphodiesterase 10A inhibitor; PQ-10, 6,7-dimethoxy-4-[3-(quinoxalin-2-yloxy)pyrrolidin-1-yl]quinazoline; RO-4-1284, 2-ethyl-9,10-dimethoxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinolin-2-ol; PDE, phosphodiesterase; PKA, protein kinase A; SCH-23390, 8-chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol; SKF-82958, 6-chloro-1-phenyl-3-prop-2-en-1-yl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol; TP-10, 2-({4-[4-pyridin-4-yl-1-(2,2,2-trifluoroethyl)-1H-pyrazol-3-yl]phenoxy}methyl)quinoline; s.c., subcutaneous.

Introduction

The enzyme phosphodiesterase 10A (PDE10A) modulates signal transduction by catabolizing the intracellular second messengers cAMP and cGMP. PDE10A has higher affinity for cAMP and is more efficient with cAMP as substrate (Fujishige et al. 1999; Soderling et al. 1999). PDE10A is expressed primarily in the brain, particularly in the striatum, nucleus accumbens, and olfactory tubercle, regulating the activity of dopamine-sensitive striatal medium spiny neurons (MSNs) (Loughney et al. 1999; Seeger et al. 2003; Coskran et al. 2006; Sano et al. 2008).

Striatal MSNs are organized into a dopamine D_1 receptor-expressing "direct" (striatonigral) output pathway and a dopamine D_2 receptor-expressing "indirect" (striatopallidal) output pathway (Graybiel 1990, 2000). Action selection and execution takes place via the direct pathway, whereas action suppression is mediated via the indirect pathway (Delong and Wichmann 2007; Wilson 2014). Both pathways integrate behaviorally relevant information received via cortical and thalamic glutamatergic projections with input regarding the salience of this information from mid-brain dopaminergic neurons (Surmeier et al. 2007). Processed information is ultimately fed back to the frontal cortex via both pathways (Delong and Wichmann 2007; Wilson 2014), which act like the brake and accelerator in a car (Graybiel 2000).

All currently available antipsychotics block D_2 receptors and, by reducing dopamine-evoked cAMP decrease via Gi-coupled D_2 receptors in the indirect pathway, they activate only the indirect pathway (Kapur and Mamo 2003; Agid et al. 2008). By increasing cAMP, PDE10A inhibitors (PDEA10Is) activate both the Gi-coupled D_2 receptor-expressing indirect pathway and the Gs-coupled D_1 receptor-expressing direct pathway (Nishi et al. 2008; Strick et al. 2010), thereby combining functional characteristics of D_2 antagonists and D_1 agonists. Accordingly, PDE10AIs have been proposed as a novel type of antipsychotics with a broader therapeutic profile and reduced side effects (Siuciak et al. 2006; Kehler et al. 2007; Menniti et al. 2007; Siuciak 2008). However, PDE10AIs may have effects beyond D_1- and D_2-mediated neurotransmission, also affecting striatal glutamate–NO–guanylyl cyclase signaling (Loughney et al. 1999; Seeger et al. 2003; Coskran et al. 2006; Sano et al. 2008; Grauer et al. 2009).

In order to explore their antipsychotic potential and side-effect liability, PDE10AIs have been studied predominantly for effects on stimulated or spontaneous behaviors. In line with results suggesting that PDE10AIs activate indirect pathway neurons to a greater extent than direct pathway neurons (Threlfell et al. 2009; Nishi et al. 2011), PDE10AIs produce similar effects as D_2 receptor blockers, such as inhibition of spontaneous or stimulant-induced behavior, inhibition of conditioned avoidance behavior and reversal of stimulant-induced sensory gating deficits (Menniti et al. 2007; Schmidt et al. 2008; Grauer et al. 2009; Kehler and Nielsen 2011; Gresack et al. 2013; Megens et al. 2014). Concomitant direct pathway activation explains the relatively low cataleptogenic activity (Schmidt et al. 2008; Grauer et al. 2009; Megens et al. 2014), reduced efficiency against behavioral stimulants in case of concomitant D_1 receptor stimulation (Menniti et al. 2007; Sotty et al. 2009; Gresack et al. 2013; Megens et al. 2014), preferential activity against apomorphine-induced climbing versus apomorphine-induced stereotypy (Grauer et al. 2009), cognition-enhancing effects (Rodefer et al. 2005; Grauer et al. 2009), and socializing effects (Grauer et al. 2009).

The present study investigates effects of PDE10AIs on suppressed behaviors (catalepsy in rats; hypolocomotion in mice) under various conditions of decreased dopaminergic neurotransmission, viz. blockade of D_1 receptors with SCH-23390 (Bourne 2001), blockade of D_2 receptors with haloperidol, or dopamine depletion with RO-4-1284 (Colzi et al. 1993; Filinger 1994) or reserpine. Inhibition of apomorphine-induced behavior in rats and antagonism of D-amphetamine-induced hyperlocomotion in mice are included as measures of reduced D_2 receptor signaling. The following PDE10AIs are tested: JNJ-42314415 (Megens et al. 2014), PQ-10 (Kehler and Kilburn 2009), TP-10 (Schmidt et al. 2008; Kehler and Kilburn 2009), and MP-10 (Grauer et al. 2009; Kehler and Kilburn 2009). TP-10 is not included in all assays because of limited availability. In some assays, the D_1 agonist SKF-82958 (Wang and McGinty 1997) and the D_2 antagonist haloperidol are also included for comparison. Part of the data have been presented elsewhere (Vanhoof et al. 2012; Megens et al. 2014). The present results indicate that PDE10AIs stimulate or suppress motor behavior dependent on the relative activation state of the direct and indirect striatal output pathways.

Materials and Methods

Materials and sources

Company codes refer to the following chemical structures: JNJ-42314415: 3-[6-(2-methoxyethyl)pyridin-3-yl]-2-methyl-8-morpholin-4-ylimidazo[1,2-a]pyrazine; MP-10: 2-{[4-(1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)phenoxy]methyl}quinoline; PQ-10: 6,7-dimethoxy-4-[3-(quinoxalin-2-yloxy)pyrrolidin-1-yl]quinazoline); RO-4-1284: 2-ethyl-9,10-dimethoxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinolin-2-ol; SCH-23390: 8-chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol; SKF-82958: 6-chloro-1-phenyl-3-prop-2-en-1-yl-2,3,4,5-tetrahydro-

1H-3-benzepine-7,8-diol; TP-10: 2-({4-[4-pyridin-4-yl-1-(2,2,2-trifluoroethyl)-1H-pyrazol-3-yl]phenoxy}methyl)quinoline. All compounds were synthesized in our own laboratories, except apomorphine, D-amphetamine (Certa SA, Eigenbrakel, Belgium), reserpine, and SKF-82958 (Sigma-Aldrich-Fluka, Diegem, Belgium).

Compound administration

Reserpine was dissolved in 10% hydroxypropyl-β-cyclodextrin containing ascorbic acid (4:1 ratio relative to reserpine; w:w). The other compounds were dissolved in water (apomorphine, D-amphetamine), acidified with tartaric acid if required (haloperidol, JNJ-42314415, SCH-23390), or in 10% or 20% hydroxypropyl-β-cyclodextrin acidified with tartaric acid (MP-10, PQ-10, SKF-82958, TP-10). The test formulations were stored at room temperature in closed containers protected from light and subcutaneously (s.c.) administered (10 mL/kg).

Animals (species, weight, and sex)

Male Wistar rats (Crl:WI; 220 ± 50 g) and male NMRI mice (Crl:NMRI(Han); 22 ± 5 g) were obtained from Charles River Breeding Laboratories (Sulzfeld, Germany for rats; L'Arbresle, France for mice) and housed under standard laboratory conditions (21 ± 2°C; 45–65% relative humidity; light–dark cycle set at 12 h). According to standard procedures, all animals were fasted overnight (tap water remaining available ad libitum), allowing oral dosing on an empty stomach (not applicable to the present study). The Institutional Ethics Committee on Animal Experimentation approved the experimental protocols, in compliance with Belgian law (Royal Decree on the protection of laboratory animals 6 April 2010).

Test descriptions

Apomorphine-induced agitation in rats

Apomorphine (1.0 mg/kg, i.v., intravenous)-induced agitation was scored (0, 1, 2, or 3) every 5 min over the first hour after injection of apomorphine. Criteria for drug-induced inhibition of agitation were: less than six times score 3 (1.5% false positives), less than six times score ≥2 (0.0% false positives), or less than seven times score ≥1 (0.0% false positives).

Observation test in rats

Catalepsy was scored (0, 1, 2 or 3) by two independent observers at 1, 2, 3, and 4 h after administration of test compound or solvent. The catalepsy scores from the two observers were summed for further evaluation. Criteria

for drug-induced catalepsy: score >2 for slight catalepsy (occurrence in 1800 control observations: 0.0%; 450 rats and four observations per rat) and score 6 for pronounced catalepsy (not observed in controls). Other abnormalities were considered drug-induced, if occurring in a dose-dependent way. The interactions with SCH-23390 (0.63 mg/kg, s.c.), haloperidol (0.63 mg/kg, s.c.), and RO-4-1284 (10 mg/kg, s.c.) were evaluated by coadministering these agents simultaneously with the test formulations.

Reserpine-induced behavior in rats

Reserpine (10 mg/kg, s.c.) induces miosis, palpebral ptosis, and sedation and blocks the tail-pinch response (the biting and grasping response when an artery clamp is put on the rat's tail, 1 cm from the distal end). The pupil diameter (of the right eye; using a microscopic micrometer; expressed in 1/24 mm units) and the latency of the tail-pinch response (s; cutoff time: 120 sec) were measured and palpebral opening (score 0, 1, 2, 3, 4, or 5) and sedation (hypomotility; score 0, 1, 2, 3) were scored 1 h after the reserpine challenge. To assess intrinsic effects of the test compounds, the pupil diameter and the tail-pinch response were also measured just before the reserpine injection. Test compound or solvent was administered 1 h before the reserpine challenge. Based on frequency distributions obtained in a historical control population of solvent-pretreated rats ($n = 400$), the following all-or-none criteria were selected to assess drug-induced effects: (1) *before reserpine injection* – induction of miosis: pupil diameter <11 units (4.3% false positive controls; $n > 400$); induction of mydriasis: pupil diameter >25 units (5.2% false positives); inhibition and blockade of tail-pinch response: latency >45 sec and >120 sec, respectively (5.2% and 0.2% false positives, respectively); (2) *after reserpine injection* – reversal of the tail-pinch response blockade: latency <120 sec (4.1% false positives); reversal of miosis: pupil diameter >8 units (1.1% false positives); reversal of palpebral ptosis: score for palpebral opening >1 (3.2% false positives); and reversal of sedation: score <3 (1.1% false positives).

Locomotor activity in mice

The procedure room was only sparsely lit (3–30 lx) to provide better contrast for the video tracking. Each locomotor activity arena (gray PVC cylinder; height: 40 cm; diameter: 22.5 cm) was placed on an infrared LED (8 × 8 LEDs) lit box (white PVC squared box; 40 × 40 cm^2; height 12.5 cm). An infrared-sensitive tube camera and a white light source (in arena: 4–7 lx) were mounted to the ceiling above the arena to track the animal. Distance travelled was

recorded and analyzed using the Noldus Ethovision XT Video Tracking System (Version 3.1; Noldus, Wageningen, the Netherlands). Locomotion was measured over a 30-min period by introducing the mouse into the motor activity arena 30 min after pretreatment with test compound or solvent. The arena was cleaned after each trial. In order to stimulate or suppress locomotion, following treatments were used: D-amphetamine (5.0 mg/kg, s.c.; −0.5 h), SCH-23390 (0.08 mg/kg, i.v.; 0 h), RO-4-1284 (0.63 mg/kg, s.c.; −0.5 h), or haloperidol (0.31 mg/kg, i.v.; 0 h).

General procedure and statistics

General procedure

All experiments were performed by unbiased trained technicians using coded solutions. Doses were selected from the geometrical series 0.01–0.02–0.04...40.0–80.0–160 mg/kg. Each dose group consisted of at least five animals which were tested in separate daily experimental sessions in order to account for day-to-day variability and to minimize systematic errors. Control injections of solvent were included in each experimental session. In Figures 4–6, dose 0 mg/kg refers to results obtained in solvent-treated control animals that were tested concurrently with the test compounds.

ED$_{50}$ determination

All-or-none criteria for drug-induced effects were defined by analyzing a frequency distribution of a series of historical control data, aiming for less than 5% responders in the control population. The fraction of animals responding to these criteria in animals pretreated with test compound was determined per dose level ($n \geq 5$ in the relevant dose range; at least three doses). ED$_{50}$s (the dose inducing the defined effect in 50% of the tested animals) and corresponding 95% confidence limits were determined according to the modified Spearman-Karber estimate, using theoretical probabilities instead of empirical ones (Tsutakawa 1982). This modification allows estimation of the ED$_{50}$ and its confidence interval as a function of the slope of the log dose–response curve (Lewi et al. 1977).

Time–activity relationship

ED$_{50}$s were plotted versus time after dosing using Graph-Pad Prism® (software version 5.00 for Windows, Graph-Pad Software, San Diego, CA; www.graphpad.com). The ED$_{50}$ at time of peak effect and the onset and duration of action (at four times the peak-effect dose) were estimated from the curve of best fit through these data points as generated by nonlinear regression of the polynomial second order function $y = a + bx + cx^2$. Goodness of fit was determined by calculating R^2 and $S_{y.x}$.

Results

Apomorphine-induced agitation in rats

Inhibition of apomorphine-induced behavior relates to the D$_2$ receptor blocking activity of antipsychotics (Leysen and Niemegeers 1985; Leysen 2000). Apomorphine antagonism was used in the present study as sensitive index for the functional D$_2$ antagonism of PDE10AIs in rats, being measured at doses close to the ED$_{50}$ for striatal PDE10A occupancy (Megens et al. 2014).

The ED$_{50}$s of JNJ-42314415, PQ-10, TP-10, and MP-10 obtained for inhibition of apomorphine-induced behavior at various time intervals after s.c. injection are listed in Table 1 and graphically presented in Figure 1 (solid circles; the other data are discussed below). The PDE10AIs showed a fast onset of action (<0.5 h) and reached their peak effect within 0.5–1 h (nonlinear regression estimates in Table 2). JNJ-42314415, MP-10, and TP-10 were about equipotent and about three to four times more potent than PQ-10 in terms of peak-effect dose. MP-10 showed a somewhat shorter duration (2.8 h) than the other compounds (4.3–4.8 h).

Table 1. ED$_{50}$s (and 95% confidence limits; mg/kg) of the test compounds for inhibition of apomorphine-induced agitation in rats as a function of time after s.c. injection.

| Time (h) | ED$_{50}$s (95% confidence limits; mg/kg, s.c.) | | | |
	JNJ-42314415	PQ-10	TP-10	MP-10
0.5	1.02 (0.75–1.38)	3.6 (2.88–4.4)	1.17 (0.86–1.58)	1.17 (0.86–1.58)
1	1.02 (0.75–1.38)	4.1 (3.0–5.5)	1.02 (0.75–1.38)	0.67 (0.45–1.01)
2	1.77 (1.44–2.18)	6.2 (4.1–9.2)	1.77 (1.18–2.65)	2.69 (1.99–3.6)
4	4.1 (3.0–5.5)	7.1 (4.7–10.6)	4.1 (3.0–5.5)	8.1 (6.0–11.0)
8	8.2 (5.5–12.2)	43 (28.7–64)	12.3 (9.1–16.7)	

Observation tests in rats

Interaction with the D_1 antagonist SCH-23390

In order to study involvement of dopamine D_1 receptors, catalepsy was studied after JNJ-42314415, PQ-10, and MP-10 in rats cotreated or not cotreated with the D_1 antagonist SCH-23390 (0.63 mg/kg, s.c., 0 h). In rats not cotreated with SCH-23390 (Fig. 2: upper panel for each compound; open circles), the PDE10AIs failed to induce catalepsy in a dose-dependent and consistent manner. MP-10 tended to induce slight catalepsy (score >2) around 1.25–2.5 mg/kg (maximum 60–80% responders) but only incidentally induced pronounced catalepsy (score
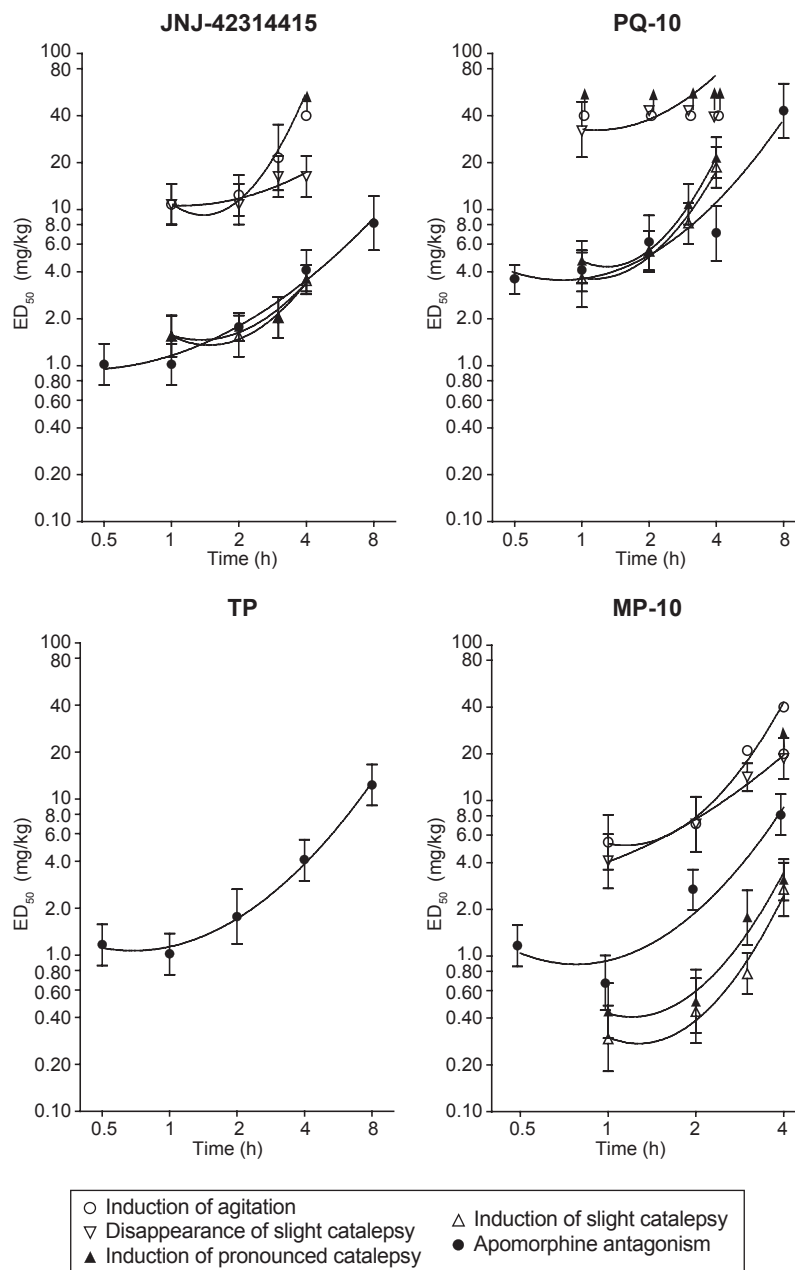

Figure 1. $ED_{50}s$ (95% confidence limits; mg/kg) of PDE10AIs for various effects in rats cotreated with the D_1 antagonist SCH-23390 (0.63 mg/kg, s.c., 0 h) in comparison with the ED_{50} for antagonism of apomorphine-induced behavior in rats not cotreated with SCH-23390. The $ED_{50}s$ have been plotted as a function of time after s.c. injection of the compounds. In the rats cotreated with the D_1 antagonist, PDE10AIs induced (slight and pronounced) catalepsy at doses similar to those required for apomorphine antagonism. At 10-fold higher doses, catalepsy disappeared, being replaced by agitation. The various effects followed a parallel time course. For TP-10 only $ED_{50}s$ for apomorphine antagonism are shown. PDE10AI, phosphodiesterase 10A inhibitor.

6). In rats cotreated with SCH-23390 (Fig. 2: bottom panel for each compound; closed circles), however, the PDE10AIs consistently induced pronounced catalepsy (score 6). The dose–response curves were bell-shaped: catalepsy decreased again at higher dose levels, being replaced by stereotyped sniffing and rearing. The ED_{50}s for the various effects in rats cotreated with SCH-23390 (Table 3) have been plotted as a function of time in Figure 1 in comparison with the ED_{50}s for apomorphine antagonism in rats not cotreated with SCH-23390. The time course for the effects in the rats cotreated with SCH-23390 was found to proceed more or less in parallel with the apomorphine antagonism in the absence of SCH-23390. In the rats cotreated with SCH-23390, the PDE10AIs already induced catalepsy at low doses comparable to those for apomorphine antagonism (Table 4), reflecting a very small safety margin under these conditions. SCH-23390 (0.63 mg/kg, s.c.) alone did not induce catalepsy (score >2 was observed only once in 35 rats tested at four time intervals).

As the functional D_1 agonism of PDE10AIs apparently counteracts the catalepsy that may be expected from their functional D_2 antagonism, it is studied below whether this activity component may also counteract catalepsy and related behaviors induced by other mechanisms, viz. D_2 receptor blockade with haloperidol or dopamine depletion using Ro-4-1284 or reserpine.

Interaction with the D_2 antagonist haloperidol

Figure 3 illustrates the dose-dependent reversal of haloperidol (0.63 mg/kg, s.c.)-induced catalepsy after cotreatment with JNJ-42314415, TP-10, MP-10, and the D_1 agonist SKF-82958. Shown are individual data and median scores per dose group obtained 2, 3, and 4 h after dosing. In solvent-cotreated rats ($n = 321$), haloperidol consistently induced catalepsy, which became more pronounced at later time intervals. Catalepsy scores <2 at 2 h and <5 at 3 h and 4 h occurred in only 6.2%, 4.7%, and

3.1% of these control rats, respectively, and were adopted as critical levels for reversal of haloperidol-induced catalepsy (dotted horizontal lines in Fig. 3). The three PDE10AIs dose dependently reversed the haloperidol-induced catalepsy (Fig. 3, Table 5). The lowest ED_{50} obtained over the three time intervals was 4.7 mg/kg for JNJ-42314415, 2.69 mg/kg for TP-10, and 6.2 mg/kg for MP-10, corresponding to 4.9, 2.5, and 7.0 times, respectively, the ED_{50} for apomorphine antagonism at time of peak effect (Table 2). SKF-82958 reversed haloperidol-induced catalepsy at 1 h with a bell-shaped dose–response curve (estimated ED_{50}: 1.02 mg/kg) but was hardly effective at the later time intervals.

Interaction with the monoamines-depleting agent RO-4-1284

RO-4-1284 (10 mg/kg, s.c.) consistently induced catalepsy and sedation in solvent-cotreated rats ($n = 50$). JNJ-42314415, PQ-10, and MP-10 dose dependently reversed the RO-4-1284-induced sedation and catalepsy, which were replaced by behavioral excitation (Table 6–8; Fig. 4). The effects occurred as doses close to the ED_{50} for apomorphine antagonism (Table 9). The D_1 agonist SKF-82958 tended to reverse the RO-4-1284-induced catalepsy at 2.5 mg/kg without affecting sedation. At this dose, however, the combination with RO-4-1284 resulted in convulsions, spasms and body twitches in two of the five rats tested (one of them died and was not included in Table 10).

Interaction with the monoamines-depleting agent reserpine

Reserpine (10 mg/kg, s.c.) induced palpebral ptosis, sedation, and blocked the tail-pinch response (the grasping and biting response when an artery clamp is put on the distal end of the rat's tail; latency >120 sec). Before the reserpine challenge, the PDE10AIs inhibited the tail-pinch

Table 2. Inhibition of apomorphine-induced agitation in rats: nonlinear regression estimates for the time and dose of peak effect, and for the onset and duration of action after s.c. dosing.

| Compound | Peak effect | | Onset[1] (h) | Duration[1] (h) | R^2 | $S_{y.x}$ |
	Time (h)	ED_{50} (mg/kg)				
JNJ-42314415	0.5	0.95	<0.5	4.3	0.985	0.068
PQ-10	0.83	3.5	<0.5	4.8	0.930	0.163
TP-10	0.68	1.07	<0.5	4.3	0.995	0.044
MP-10	0.77	0.88	<0.5	2.8	0.929	0.217

The values were interpolated from the nonlinear regression lines in Figure 1. Values for R^2 and $S_{y.x}$ show the goodness of fit of the nonlinear regression lines.
[1]Onset and duration of action were assessed at four times the peak-effect dose.

response per se (latency >45 sec) at doses only 1.2–3.2 times the ED_{50} for apomorphine antagonism (Fig. 5, Table 11). The dose–response relations for the inhibition of the tail-pinch response tended to be bell-shaped, except for PQ-10 (tested in a small dose range only). While inhibiting the tail-pinch response before reserpine, the PDE10AIs in contrast dose dependently reversed the reserpine-induced blockade of the tail-pinch response 1 h after reserpine. This effect occurred at 3.3–8.5 times the ED_{50} for apomorphine antagonism. The PDE10AIs even restored normal response latencies (<45 sec) at slightly higher doses (5.0–11 times the ED_{50} for apomorphine antagonism). Reversal of the reserpine-induced tail-pinch response blockade was thus observed at similar doses as those inhibiting the tail-pinch response before reserpine (Table 11). The PDE10AIs also reversed the reserpine-induced sedation (at 0.54–3.2 times the ED_{50} for apomorphine antagonism; Fig. 6), which was replaced by behavioral stimulation (sniffing and rearing) at 1.9–4.9 times the ED_{50} for apomorphine antagonism. The PDE10AIs also tended to reverse the reserpine-induced palpebral ptosis (at 0.96–3.2 times the ED_{50} for apomorphine antagonism) but did not affect pupil diameter up to the highest doses tested (data not shown).

Before the reserpine challenge, the D_1 agonist SKF-82958 inhibited the tail-pinch response in some rats (from 0.16 mg/kg onward; maximum 40% responders), presumably related to behavioral stimulant effects (sniffing, rearing; ED_{50}: 0.44 [0.36–0.55] mg/kg). After the reserpine challenge, SKF-82958 reversed the reserpine-induced sedation (ED_{50}: 0.097 mg/kg; Fig. 6) and the blockade of the tail-pinch response (ED_{50}: 0.112 mg/kg; Fig. 5, Table 11), and tended to normalize the tail-pinch response (estimated ED_{50}: 0.51 mg/kg;) and to reverse the ptosis (from 0.31 mg/kg onward; data not shown) without affecting miosis (ED_{50}: >10 mg/kg; data not shown). The overt behavioral stimulation seen with SKF-82958 before the reserpine challenge was not seen after the reserpine challenge (Table 11). In contrast, the PDE10AIs showed behavioral stimulant effects after but not preceding the reserpine challenge.

Locomotor activity assays in mice

In addition to the above studies at the level of catalepsy in rats, similar studies were performed at the level of locomotion in mice. First, inhibition of D-amphetamine-induced hyperlocomotion was tested as index of functional D_2 antagonism. Subsequently, the ability of PDE10AIs to reverse the hypolocomotion induced by the D_1 antagonist SCH-23390, the monoamines-depleting agent, Ro-4-1284, or the D_2 antagonist haloperidol was evaluated. Total distance travelled in solvent-pretreated

control mice receiving no challenge was 4167 ± 1135 cm (mean $\pm$ SD; $n = 1444$).

Inhibition of hyperlocomotion induced by the dopamine-releasing agent D-amphetamine

In solvent-pretreated control mice, D-amphetamine (5.0 mg/kg, s.c.;−0.5 h) increased distance travelled to $12,764 \pm 4278$ cm (mean $\pm$ SD; $n = 462$). Only 3.0% of these control mice travelled <5500 cm. The PDE10AIs and the D_2 receptor blocker haloperidol reduced the D-amphetamine-induced hyperlocomotion in a dose-dependent manner to normal values <5500 cm (Fig. 7, first column; ED_{50}s in Table 12, first column).

Reversal of hypolocomotion induced by the D_1 antagonist SCH-23390

In solvent-pretreated control mice, SCH-23390 (0.08 mg/kg, i.v., 0 h) decreased distance travelled to 1408 ± 513 cm (mean $\pm$ SD; $n = 279$). Only 1.4% of these SCH-23390-challenged control mice travelled a distance >2500 cm. The PDE10AIs and the D_1 agonist SKF-82958 reversed the SCH-23390-induced hypolocomotion to normal values (>2500 cm; Fig. 7; second column). The ED_{50}s of the PDE10AIs for this effect were similar to those required for inhibition of D-amphetamine-induced hyperlocomotion (Table 12).

Reversal of hypolocomotion induced by the monoamines-depleting agent RO-4-1284

In solvent-pretreated control mice, dopamine depletion with RO-4-1284 (0.63 mg/kg, s.c., −0.5 h) induced pronounced hypolocomotion (total distance [mean $\pm$ SD]: 772 ± 411 cm; $n = 218$). Values >2500 cm were rarely measured (in only 0.4% of the control mice). The tested PDE10AIs and the D_1 agonist SKF-82958 reversed the RO-4-1284-induced hypolocomotion to normal values (>2500 cm; Fig. 7; third column). The dose–response curves of JNJ-42311445 and PQ-10 tended to be bell-shaped and effect size tended to be higher for MP-10 than for JNJ-42311445 and PQ-10. The PDE10AIs were somewhat (two to three times) more potent against Ro-4-1284 than against D-amphetamine or SCH-23390 while SKF-82958 was equipotent in both assays (Table 12).
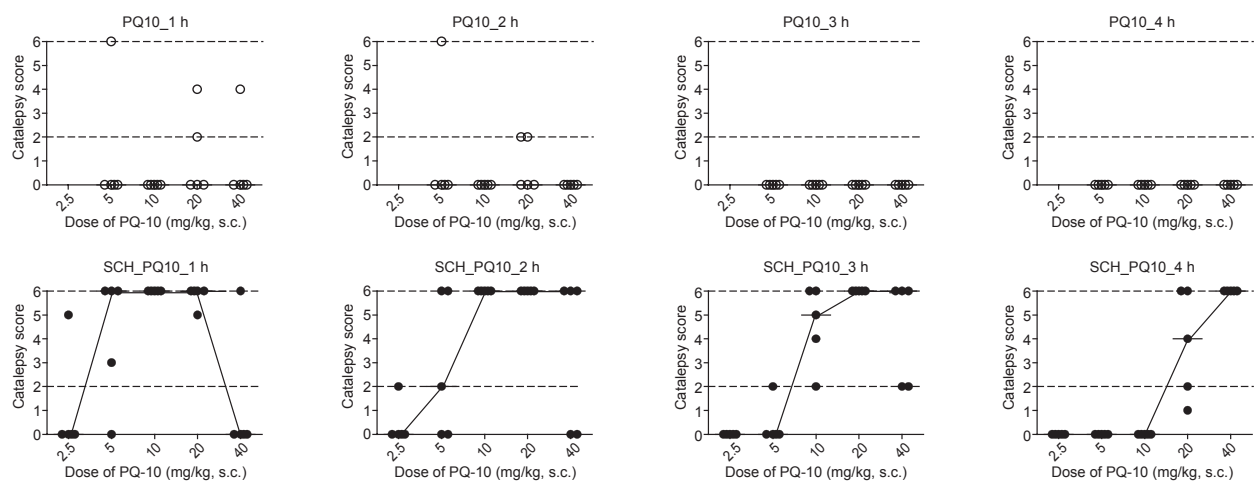
Reversal of hypolocomotion induced by the D_2 antagonist haloperidol

In solvent-pretreated mice, the D_2 antagonist haloperidol (0.31 mg/kg, i.v., 0 h) induced hypolocomotion (total distance [mean $\pm$ SD]: 1257 ± 1370 cm; $n = 1833$).

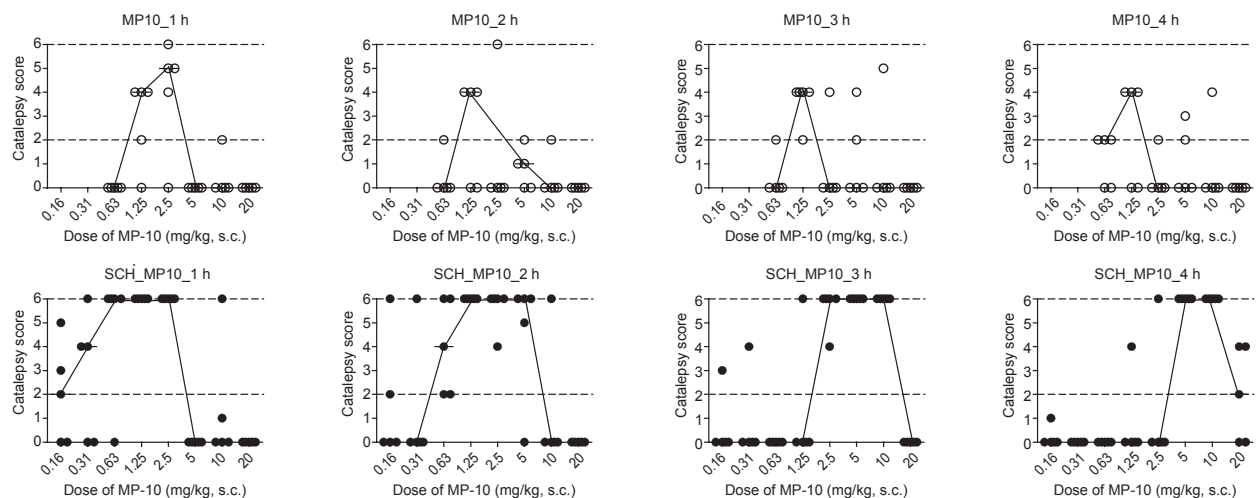**JNJ-42314415**
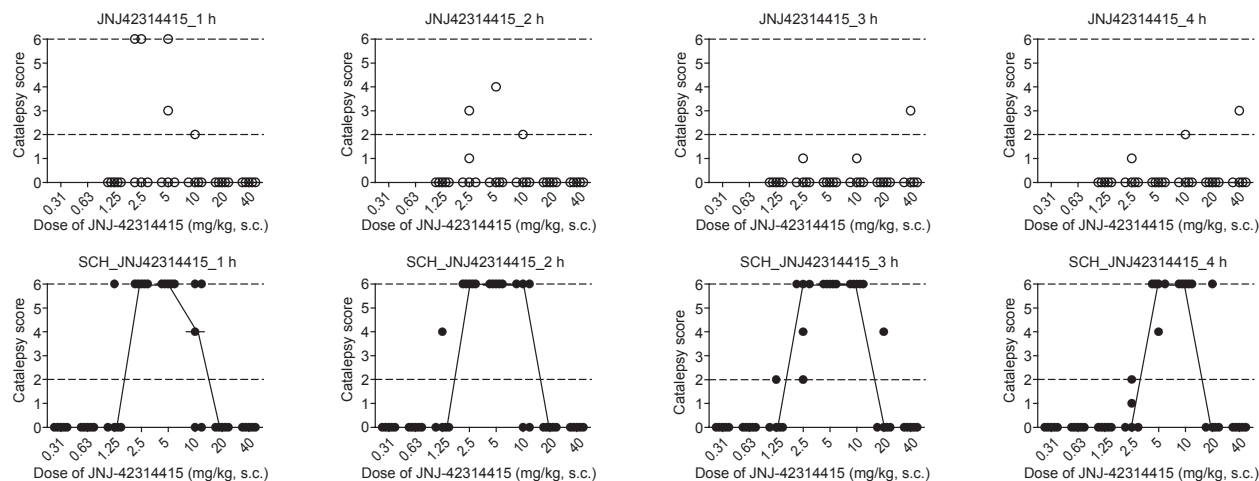
PQ-10

MP-10

Figure 2. Potentiation of PDE10AI-induced catalepsy by cotreatment with the D_1 antagonist SCH-23390. Individual (circles) and median scores (stripes) for catalepsy obtained per dose level 1, 2, 3, and 4 h after s.c. injection of JNJ-42314415, PQ-10, and MP-10 in rats cotreated with SCH-23390 (0.63 mg/kg, s.c., 0 h; bottom panel for each compound; closed circles) or not cotreated (upper panel for each compound; open circles). Median catalepsy scores of successive dose groups have been interconnected. The horizontal dotted lines represent the critical levels adopted for slight catalepsy (score >2) and pronounced catalepsy (maximum score 6). The PDE10AIs consistently induced pronounced catalepsy with bell-shaped dose–response curves in the rats cotreated with the D_1 antagonist, whereas they induced almost no or only slight catalepsy in the rats not cotreated with the D_1 antagonist. PDE10AI, phosphodiesterase 10A inhibitor.

Table 3. $ED_{50}s$ (and 95% confidence limits) for induction of slight and pronounced catalepsy, the subsequent disappearance of catalepsy, and the induction of agitation in rats cotreated with SCH-23390 (0.63 mg/kg, s.c.).

Time (h)	$ED_{50}s$ (95% confidence limits; mg/kg, s.c.)		Disappearance of slight catalepsy (score ≤2)	Induction of agitation
	Induction of catalepsy			
	Slight (score >2)	Pronounced (score = 6)		
JNJ-42314415				
1	1.54 (1.14–2.09)	1.54 (1.14–2.09)	10.8 (8.0–14.6)	10.8 (8.0–14.6)
2	1.54 (1.14–2.09)	1.77 (1.44–2.18)	10.8 (8.0–14.6)	12.4 (9.1–16.7)
3	2.03 (1.50–2.75)	2.04 (1.51–2.76)	16.3 (12.0–22.1)	21.5 (13.3–35)
4	3.5 (2.87–4.4)	3.6 (2.88–4.4)	16.3 (12.0–22.1)	>40
PQ-10				
1	3.6 (2.37–5.3)	4.7 (3.4–6.3)	32 (21.7–49)	>40
2	5.4 (4.0–7.3)	5.4 (4.0–7.3)	43 (–)	>40
3	8.2 (6.0–11)	10.8 (8.0–14.6)	43 (–)	>40
4	18.7 (13.8–25.3)	21.5 (15.9–29.1)	>40	>40
MP-10				
1	0.294 (0.182–0.48)	0.44 (0.297–0.67)	4.1 (2.73–6.1)	5.4 (3.6–8.1)
2	0.44 (0.276–0.72)	0.51 (0.32–0.82)	7.1 (4.7–10.6)	7.1 (4.7–10.6)
3	0.77 (0.57–1.05)	1.78 (1.18–2.65)	14.2 (11.5–17.4)	21 (–)
4	2.69 (1.80–4.0)	3.1 (2.28–4.2)	18.7 (13.8–25.3)	>20

Table 4. $ED_{50}s$ of the PDE10AIs at time of peak-effect for induction of slight and pronounced catalepsy, the subsequent disappearance of catalepsy, and the induction of agitation in rats cotreated with SCH-23390 (0.63 mg/kg, s.c.) in relation to the ED_{50} for inhibition of apomorphine-induced agitation.

Effects	ED_{50} (mg/kg, s.c.) at time of peak effect[1]		
	JNJ-42314415	PQ-10	MP-10
Apomorphine antagonism	0.95 [1.0]	3.5 [1.0]	0.88 [1.0]
Induction of slight catalepsy[2] (score >2)	1.35 [1.4]	3.6 [1.0]	0.30 [0.34]
Induction of pronounced catalepsy[2] (score 6)	1.46 [1.5]	4.3 [1.2]	0.43 [0.49]
Disappearance of slight catalepsy[2] (score ≤2)	10.6 [11]	32 [9.1]	4.0 [4.5]
Induction of agitation[2]	9.2 [9.6]	>40 [>11]	5.2 [5.9]

PDE10AI, phosphodiesterase 10A inhibitor.
[1]$ED_{50}s$ at time of peak effect were interpolated from the nonlinear regression lines plotted in Figure 1.
[2]In animals cotreated with SCH-23390 (0.63 mg/kg, s.c.). Figures between square brackets represent the ratio of the ED_{50} over the ED_{50} for apomorphine antagonism.

Values >2500 cm occurred in only 4.0% of these control mice. The PDE10AIs and the D_1 agonist SKF-82958 were not able to reverse the haloperidol-induced hypolocomotion to values >2500 cm, except for a tendency in the lower dose range of the PDE10AIs (maximum percentage responders: 60% at 10 mg/kg for JNJ-42314415; 40% at 10 mg/kg for PQ-10, 40% at 0.63 mg/kg for MP-10, and 0% for TP-10).

Discussion

PDE10AIs resemble D_2 receptor blockers by, for example, inhibiting spontaneous and stimulant-induced behavior, inhibiting conditioned behavior, and reversing stimulant-induced sensory gating deficits (see Introduction). These effects are mediated by indirect pathway activation, partially counteracted by direct pathway activation when D_1 receptors are concomitantly stimulated (Menniti et al. 2007; Gresack et al. 2013; Megens et al. 2014). By stimulating dopamine D_2 receptors, nonselective dopamine
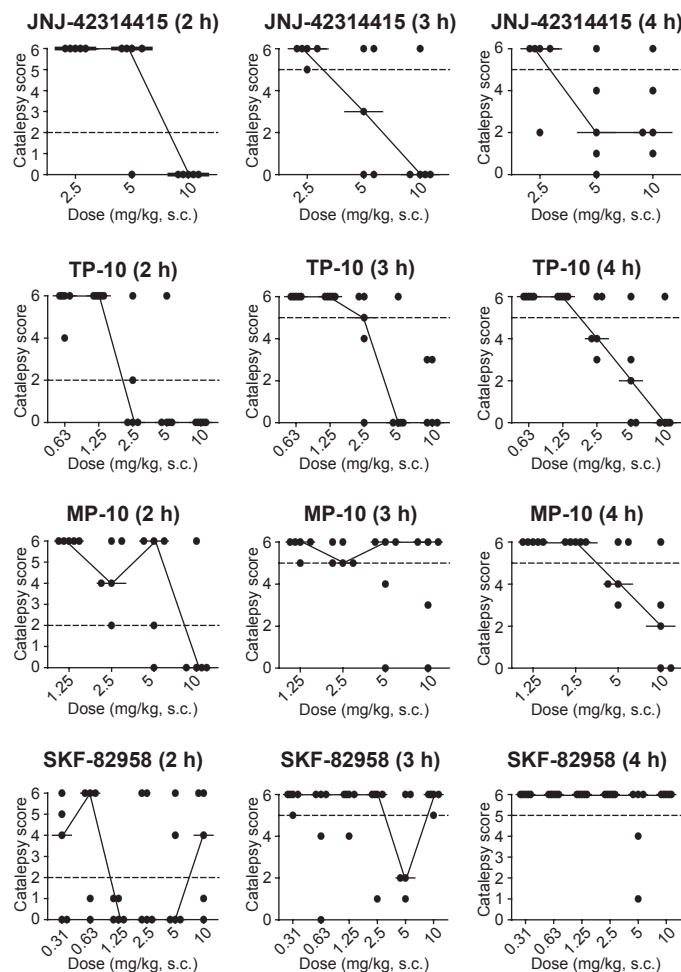
Figure 3. Reversal of haloperidol (0.63 mg/kg, s.c.)-induced catalepsy in rats that were cotreated (at the same time) with the PDE10AIs JNJ-42314415, TP-10, or MP-10 or the dopamine D$_1$ agonist SKF-82958. Shown are individual and median catalepsy scores (symbols and stripes, respectively) per dose group measured 2, 3, and 4 h after dosing. The dotted horizontal lines represent the critical levels adopted for drug-induced reversal of catalepsy (score <2 for 2 h; score <5 for 3 and 4 h) that were used for the determination of the ED$_{50}$ values listed in Table 5. The PDE10AIs dose dependently reduced catalepsy scores to below the critical level. The D$_1$ agonist showed less consistent reversal of catalepsy. PDE10AI, phosphodiesterase 10A inhibitor.

agonists such as apomorphine or endogenous dopamine (e.g., after release by D-amphetamine) deactivate the indirect pathway, thereby reducing behavioral inhibition. By concomitantly stimulating D$_1$ receptors, such compounds also activate the direct pathway, favoring behavioral stimulation. PDE10AIs inhibit the effect of dopamine agonists on the indirect pathway but facilitate their effect on the direct pathway. Acting together, both counteracting effects provide an explanation for the relatively reduced efficacy of PDE10AIs against nonselective dopamine agonists such as apomorphine and dopamine-releasing agents such as amphetamine as compared with their pronounced effects against nondopaminergic stimulants such as NMDA antagonists and antimuscarinics or

D$_2$-selective dopamine agonists such as quinpirole (Menniti et al. 2007; Sotty et al. 2009; Gresack et al. 2013; Megens et al. 2014). Both counteracting activity components may also explain the failure of MP-10 against acute exacerbations of schizophrenia (DeMartinis et al. 2012). The present study investigated the effects of PDE10AIs on suppressed behavior induced by reducing dopaminergic neurotransmission rather than on stimulated or spontaneous behavior. The results show that PDE10AIs can reverse suppressed behavior and even stimulate behavior depending on the relative activation state of the direct and indirect pathways.

Under basal conditions, PDE10AIs have relatively low cataleptogenic activity, due to concomitant direct pathway

Table 5. Dose–response relations and corresponding ED_{50}s for reversal of haloperidol (0.63 mg/kg, s.c.)-induced catalepsy in rats that received test compound simultaneously with haloperidol.

Effect Time (h)	Criterion	Dose (mg/kg, s.c.)						ED_{50} (95% CL; mg/kg)
		0.31	0.63	1.25	2.5	5.0	10	
JNJ-42314415								
2	Score <2				0	1	**5**	6.2 (4.6–8.3)
3	Score <5				0	**3**	**4**	5.4 (3.6–8.0)
4	Score <5				1	**4**	**4**	4.7 (3.1–7.0)
TP-10								
2	Score <2		0	0	**3**	**4**	**5**	2.69 (1.80–4.0)
3	Score <5		0	0	2	**4**	**5**	4.1 (3.0–5.5)
4	Score <5		0	0	**3**	**4**	**4**	3.1 (1.92–5.0)
MP-10								
2	Score <2				0	0	1	**4**	7.1 (4.7–10.6)
3	Score <5				0	0	2	2	≥10
4	Score <5				0	0	2	**4**	6.2 (4.1–9.2)
SKF-82958								
2	Score <2	2	2	**4**	**3**	**3**	2	1.02 (–)
3	Score <5	0	2	1	1	**3**	0	~5.0
4	Score <5	0	0	0	0	2	0	>10

The number of rats responding to the listed criterion is listed per dose level (n = 5 per dose). Values representing more than 50% responders are formatted bold.

Table 6. Dose–response relations and corresponding ED_{50}s for various effects observed over a 4-h period after challenge with RO-4-1284 (10 mg/kg, s.c.) in rats that received test compound simultaneously with RO-4-1284.

Effect Time (h)	Criterion	Dose of JNJ-42314415 (mg/kg, s.c.)							ED_{50} (95% CL; mg/kg)
		0.63	1.25	2.5	5.0	10	20	40	
Reversal of sedation/passivity									
1	Absence (6.0%)[1]	**3**	**5**	**5**	**5**	**5**	**5**	**5**	0.59 (0.44–0.80)[2]
2	Absence (4.0%)[1]	2	2	**5**	**5**	**5**	**5**	**5**	1.03 (0.69–1.54)
3	Absence (2.0%)[1]	2	2	**4**	**4**	**5**	**5**	**5**	1.36 (0.79–2.33)
4	Absence (16%)[1]	1	**3**	2	**4**	**5**	**5**	**5**	1.79 (1.04–3.1)
Reversal of catalepsy									
2	Score <2 (0.0%)[1]	1	0	**4**	**5**	**5**	**5**	**5**	2.05 (1.52–2.78)
3	Score <5 (2.0%)[1]	1	**3**	**5**	**5**	**5**	**5**	**5**	1.03 (0.69–1.54)
4	Score <5 (8.0%)[1]	0	2	**4**	**5**	**5**	**5**	**5**	1.56 (1.04–2.33)
Induction of excitation									
1	Presence (0.0%)[1]	0	0	2	**4**	**4**	**4**	1	4.1 (2.38–7.0)
2	Presence (0.0%)[1]	0	0	**4**	2	**4**	**5**	**3**	3.6 (2.20–5.7)
3	Presence (0.0%)[1]	0	0	2	2	**4**	**5**	2	4.1 (2.53–6.6)
4	Presence (0.0%)[1]	0	0	1	2	**4**	**4**	**5**	6.2 (3.6–10.6)

The number of rats responding to the listed criterion is listed per dose level (n = 5 per dose group). Values representing more than 50% responders are formatted bold.
[1]Percentage of false positives (solvent-pretreated rats responding to the criterion [n = 50]).
[2]ED_{50} estimated assuming no responders at the nontested dose of 0.31 mg/kg.

activation (Kehler et al. 2007; Schmidt et al. 2008; Megens et al. 2014). After D_1 receptor blockade with SCH-23390, however, PDE10AIs induced pronounced catalepsy at low doses comparable to those required for inhibition of apomorphine-induced behavior. Similar levels of catalepsy occur with D_2 receptor blockers only at >10-fold higher dose increments (Langlois et al. 2012). The potentiation of PDE10AI-induced catalepsy by a D_1 antagonist confirms that PDE10AIs suppress central D_2 receptor-mediated neurotransmission very efficiently

Table 7. Dose–response relations and corresponding ED$_{50}$s for various effects observed over a 4-h period after challenge with RO-4-1284 (10 mg/kg, s.c.) in rats that received test compound simultaneously with RO-4-1284.

Effect Time (h)	Criterion	Dose of PQ-10 (mg/kg, s.c.) 0.63	1.25	2.5	5.0	10	20	40	ED$_{50}$ (95% CL; mg/kg)
Reversal of sedation/passivity									
1	Absence (6.0%)[1]		0	0	**5**	**4**	**5**		3.6 (2.89–4.3)
2	Absence (4.0%)[1]		0	2	**3**	**4**	**4**		4.7 (2.38–7.0)
3	Absence (2.0%)[1]		1	2	**3**	2	2		7.1 (–)
4	Absence (16%)[1]		0	2	0	1	**3**		16.2 (–)
Reversal of catalepsy									
2	Score <2 (0.0%)[1]		0	0	1	**3**	**5**		8.2 (5.4–12.2)
3	Score <5 (2.0%)[1]		0	**3**	2	**3**	**5**		4.7 (2.91–7.6)
4	Score <5 (8.0%)[1]		0	1	0	**3**	**4**		10.7 (7.2–16.1)
Induction of excitation									
1	Presence (0.0%)[1]		0	0	0	0	**3**		19 (–)
2	Presence (0.0%)[1]		0	0	0	0	**3**		19 (–)
3	Presence (0.0%)[1]		0	0	1	0	**3**		19 (–)
4	Presence (0.0%)[1]		0	0	0	0	0		>20 (–)

The number of rats responding to the listed criterion is listed per dose level (n = 5 per dose group). Values representing more than 50% responders are formatted bold.
[1]Percentage of false positives (solvent-pretreated rats responding to the criterion [n = 50]).

Table 8. Dose–response relations and corresponding ED$_{50}$s for various effects observed over a 4-h period after challenge with RO-4-1284 (10 mg/kg, s.c.) in rats that received test compound simultaneously with RO-4-1284.

Effect Time (h)	Criterion	Dose of MP-10 (mg/kg, s.c.) 0.63	1.25	2.5	5.0	10	20	40	ED$_{50}$ (95% CL; mg/kg)
Reversal of sedation/passivity									
1	Absence (6.0%)[1]	2	**5**	**5**	**5**				0.68 (0.50–0.91)
2	Absence (4.0%)[1]	2	**5**	**5**	**5**				0.68 (0.50–0.91)
3	Absence (2.0%)[1]	2	**5**	**5**	**5**				0.68 (0.50–0.91)
4	Absence (16%)[1]	1	2	**5**	**5**				1.17 (0.78–1.76)
Reversal of catalepsy									
2	Score <2 (0.0%)[1]	1	**5**	**5**	**5**				0.78 (0.57–1.05)
3	Score <5 (2.0%)[1]	1	**4**	**5**	**5**				0.89 (0.59–1.33)
4	Score <5 (8.0%)[1]	0	2	**5**	**5**				1.35 (1.00–1.82)
Induction of excitation									
1	Presence (0.0%)[1]	0	1	0	**3**				4.7 (–)
2	Presence (0.0%)[1]	0	0	1	**4**				3.5 (2.36–5.3)
3	Presence (0.0%)[1]	0	0	1	**4**				3.5 (2.36–5.3)
4	Presence (0.0%)[1]	0	0	0	2				≥5.0

The number of rats responding to the listed criterion is listed per dose level (n = 5 per dose group). Values representing more than 50% responders are formatted bold.
[1]Percentage of false positives (solvent-pretreated rats responding to the criterion [n = 50]).

when concomitant direct pathway activation is prevented by D$_1$ receptor blockade and implies a risk of increased EPS when combining a PDE10AI with a neuroleptic with associated D$_1$ antagonism (e.g., clozapine or olanzapine; Schotte and Leysen 1998).

The time course for catalepsy in animals cotreated with SCH-23390 was found to proceed more or less in parallel with inhibition of apomorphine-induced behavior in the absence of SCH-23390. This confirms previously reported data on the time course of (mild) catalepsy after
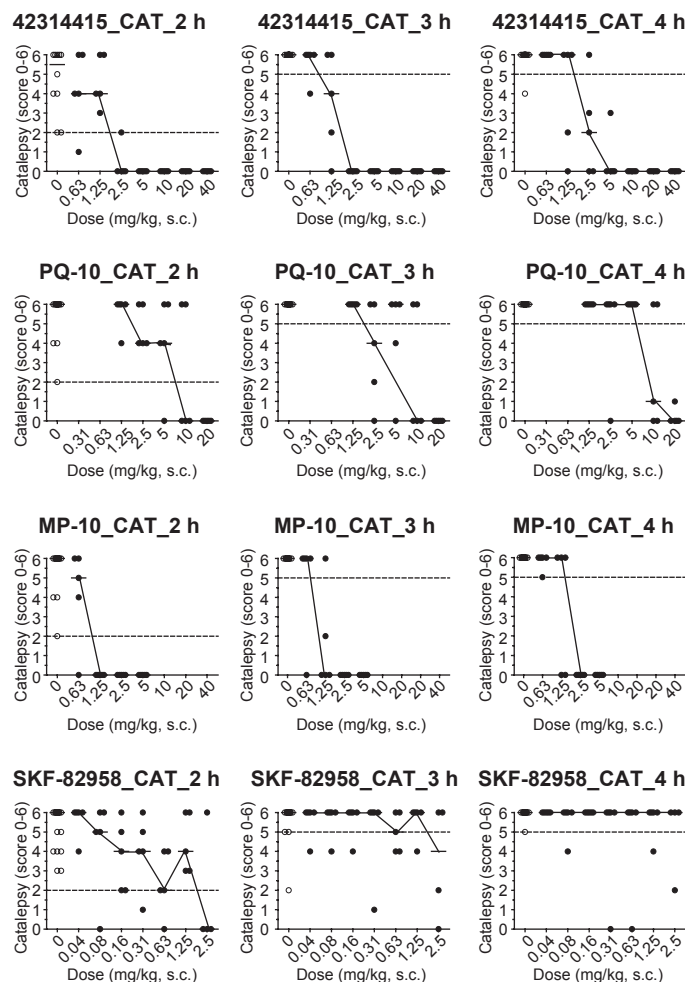
Figure 4. Reversal of Ro-4-1284 (10 mg/kg, s.c.)-induced catalepsy in rats that were cotreated (at the same time) with the PDE10AIs JNJ-42314415, PQ-10, or MP-10 or the dopamine D_1 agonist SKF-82958. Shown are individual and median catalepsy scores (symbols and stripes, respectively) per dose group measured 2, 3, and 4 h after dosing. The dotted horizontal lines represent the critical levels adopted for drug-induced reversal of catalepsy (score <2 at 2 h; score <5 at 3 and 4 h) that were used for the determination of the ED_{50} values listed in Tables 6–8, 10. The PDE10AIs dose dependently reduced catalepsy scores to below the critical level. The D_1 agonist was effective at the 2-h interval and, to a lesser extent, at the 3-h time interval. PDE10AI, phosphodiesterase 10A inhibitor.

PDE10AIs alone (Kehler et al. 2007; Schmidt et al. 2008). However, catalepsy after D_2 receptor blockers intensifies over time with retesting of the animals (Stanley and Glick 1976; Hillegaart et al. 1987), this is apparently not the case after PDE10AIs. The reversal of haloperidol-induced catalepsy by PDE10AIs in the present study apparently conflicts with the reported potentiation of haloperidol-induced catalepsy after the PDE10AI papaverine (Siuciak et al. 2006). When a lower dose of haloperidol was used, however, we also showed PDE10AI-induced potentiation of catalepsy but only at low doses with a bell-shaped dose–response relation (Vanhoof et al. 2012).

The present data show that PDE10AI-induced direct pathway activation also counteracts catalepsy and related behaviors induced by reducing D_2 receptor-mediated neurotransmission via other mechanisms such as blockade of D_2 receptors with haloperidol or dopamine depletion with RO-4-1284 or reserpine. The PDE10AIs were somewhat less potent against haloperidol than against RO-4-1284 or reserpine, presumably because they had to overcome the D_2 receptor blockade by haloperidol, whereas D_2 receptors are functioning normal after dopamine depletion. Moreover, a switch in receptor-mediated signal transduction mechanism to a supersensitive form of D_1-mediated neuronal plasticity has been demonstrated in the dopamine-depleted striatum (Gerfen 2000).

The opposing effects of PDE10AIs in the reserpine test are striking. PDE10AIs inhibit the tail-pinch response

Table 9. $ED_{50}s$ (95% confidence limits; mg/kg) of the PDE10AIs for interactions with RO-4-1284-induced behavior related to their ED_{50} for inhibition of apomorphine-induced behavior.

Compound	JNJ-42314415		PQ-10		MP-10	
	ED_{50} (95% CL)	Ratio[1]	ED_{50} (95% CL)	Ratio[1]	ED_{50} (95% CL)	Ratio[1]
Apomorphine test						
Inhibition of agitation	0.95	[1.0]	3.5	[1.0]	0.88	[1.0]
RO-4-1284 interaction						
Reversal of sedation	0.59 (0.44–0.80)	[0.62]	3.6 (2.89–4.3)	[1.0]	0.68 (0.50–0.91)	[0.77]
Reversal of catalepsy	1.03 (0.69–1.54)	[1.1]	4.7 (2.91–7.6)	[1.3]	0.78 (0.57–1.05)	[0.89]
Induction of excitation	2.05 (1.37–3.1)	[2.2]	14.2 (8.8–22.8)	[4.0]	3.1 (1.91–5.0)	[3.5]

Listed are $ED_{50}s$ obtained at time of peak effect together with the ratio over the ED_{50} for apomorphine antagonism. PDE10AI, phosphodiesterase 10A inhibitor
[1]The ratio over the corresponding ED_{50} for apomorphine antagonism is listed between square brackets.

Table 10. Dose–response relations and corresponding $ED_{50}s$ for various effects observed over a 4-h period after challenge with RO-4-1284 (10 mg/kg, s.c.) in rats that received test compound simultaneously with RO-4-1284.

Effect Time (h)	Criterion	Dose of SKF-82958 (mg/kg, s.c.)							ED_{50} (95% CL; mg/kg)
		0.04	0.08	0.16	0.31	0.63	1.25	2.5[1]	
Reversal of sedation/passivity									
1	Absence (6.0%)[2]	2	1	0	2	**3**	0	0/4	>2.5
2	Absence (4.0%)[2]	1	0	0	2	**3**	0	0/4	>2.5
3	Absence (2.0%)[2]	1	0	0	1	2	0	0/4	>2.5
4	Absence (16%)[2]	0	0	0	0	1	0	1/4	>2.5
Reversal of catalepsy									
2	Score <2 (0.0%)[2]	0	1	0	1	1	0	**3/4**	2.1 (–)
3	Score <5 (2.0%)[2]	1	1	1	1	2	1	2/4	2.5 (–)
4	Score <5 (8.0%)[2]	0	1	0	1	1	1	1/4	
Induction of excitation									
1	Presence (0.0%)[2]	0	0	0	0	0	0	0/4	>2.5
2	Presence (0.0%)[2]	0	0	0	0	0	0	0/4	>2.5
3	Presence (0.0%)[2]	0	0	0	0	0	0	0/4	>2.5
4	Presence (0.0%)[2]	0	0	0	0	0	0	0/4	>2.5

The number of rats responding to the listed criterion is listed per dose level ($n = 5$ per dose group). Values representing more than 50% responders are formatted bold.
[1]One of the five rats tested at 2.5 mg/kg died and was not included.
[2]Percentage of false positives (solvent-pretreated rats responding to the criterion [$n = 50$]).

before reserpine at doses close to the ED_{50} for apomorphine antagonism, presumably via indirect pathway activation. After reserpine, however, only slightly higher doses induce the opposite effect, viz. reversal of the reserpine-induced tail-pinch response blockade, presumably via direct pathway activation. In other words, indirect or direct pathway activation predominates under condition of normal and low dopaminergic neurotransmission, respectively.

The anticataleptic properties of PDE10AIs at conditions of low dopaminergic transmission may suggest therapeutic potential in Parkinson's disease. The anticataleptic effects are mediated via direct pathway activation, an

effect that is shared with D_1 agonists. D_1 agonists have therapeutic potential in Parkinson's disease but suffer from side effects (Mailman et al. 2001; Hurley and Jenner 2006). In view of minimal expression of PDE10A in peripheral tissue (Seeger et al. 2003; Coskran et al. 2006), PDE10AIs are likely devoid of the disturbing peripheral cardiovascular side effects of direct D_1 receptor agonists (Westfall and Westfall 2005).

The results obtained in mice further substantiate the results obtained in rats. Like haloperidol, the PDE10AIs inhibited D-amphetamine-induced hyperlocomotion, attesting to their functional D_2 antagonism. At similar doses, the PDE10AIs also counteracted the hypolocomotion
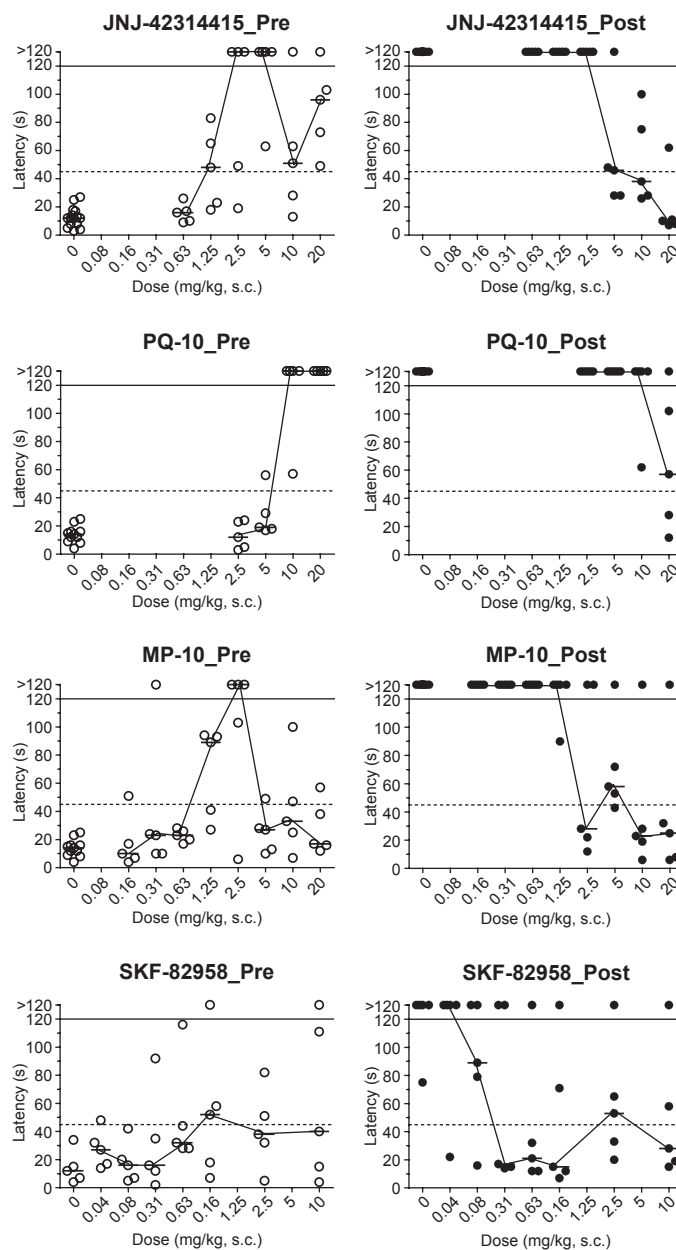
Figure 5. Dose-dependent inhibition of the tail-pinch response measured immediately before reserpine challenge in rats pretreated s.c. with the PDE10AIs JNJ-42314415, PQ-10, or MP-10 or the dopamine D$_1$ agonist SKF-82958 1 h before the reserpine challenge (left panel) and dose-dependent reversal of the blockade of the tail-pinch response measured in the same rats 1 h after reserpine (right panel). Shown are individual and median response latencies (symbols and stripes, respectively) per dose group measured immediately before and 1 h after the reserpine challenge. The dotted and solid horizontal lines represent the critical latencies adopted for inhibition and blockade of the tail-pinch response (>45 sec and >120 sec, respectively) that were used for the determination of ED$_{50}$ values listed in Table 11. The PDE10AIs inhibited the tail-pinch response before the reserpine challenge with an apparently bell-shaped dose–response relation, whereas they reversed the reserpine-induced blockade of the tail-pinch response 1 h after the reserpine challenge. The D$_1$ agonist only tended to inhibit the tail-pinch response before reserpine but efficiently reversed the reserpine-induced blockade of the tail-pinch response 1 h after the reserpine challenge. PDE10AI, phosphodiesterase 10A inhibitor.

induced by the D$_1$ antagonist SCH-23390 or the mono-amines-depleting agent RO-4-1284. Despite their activity against haloperidol-induced catalepsy in rats, PDE10AIs hardly affected haloperidol-induced hypolocomotion in mice. As a similar profile was obtained with the D$_1$ agonist SKF-82958, the effects of PDE10AIs against SCH-23390

Table 11. ED_{50}s (95% CL; mg/kg, s.c.) for inhibition and blockade of the tail-pinch response before reserpine challenge and for reversal of reserpine-induced phenomena (sedation, palpebral ptosis, blockade of the tail-pinch response) 1 h after reserpine challenge in rats pretreated s.c. with the test compounds 1 h before reserpine challenge.

	ED_{50}s (95% confidence limits; mg/kg, s.c.)							
	Before reserpine			After reserpine				
	Tail-pinch response				Tail-pinch response			
Compound	Apomorphine antagonism	Inhibition (>45 sec)	Blockade (>120 sec)	Reversal sedation	Reversal ptosis	Reversal blockade (<120 sec)	Normalization (<45 sec)	Induction excitation
JNJ-42314415	0.95	1.35 (0.90–2.02)	2.70[1] (–)	1.17 (0.78–1.75)	3.1 (1.62–5.9)	4.1 (3.0–5.5)	8.2 (5.0–13.2)	2.35 (1.37–4.0)
PQ-10	3.5	6.2 (4.6–8.4)	8.2 (6.0–11.0)	7.1 (5.8–8.7)	8.2 (5.4–12.2)	14.2 (9.4–21.2)	≥20	10.7 (7.2–16.1)
MP-10	0.88	1.34[1] (–)	60% at 2.5 mg/kg	0.51 (0.32–0.82)	1.78 (0.89–3.6)	3.1 (1.71–5.6)	4.7 (2.73–8.1)	1.78 (0.89–3.6)
SKF-82958	–	0.44[3] (40–60%)	>10	0.097 (0.060–0.157)	40% at 0.31–10 mg/kg	0.112 (0.062–0.203)	0.51 (40–80%)	>2.5[2]

For comparison, ED_{50}s for inhibition of apomorphine-induced behavior (at time of peak effect) have been listed in the first column.

[1]Estimated ED_{50} (bell-shaped dose–response curve).
[2]SKF-82958 induced excitation before reserpine (ED_{50}: 0.44 [0.36–0.55] mg/kg) but not after reserpine.
[3]Estimated ED50 (maximum 40–60% responders).

and RO-4-1284 are most likely related to direct pathway activation.

The minor effects obtained with the D_1 agonist SKF-82958 in rats do not fully support the hypothesis that common direct pathway activation mediates the effects of PDE10AI against the sedation and catalepsy induced by haloperidol, RO-4-1284, and reserpine. SKF-82958 antagonized haloperidol-induced catalepsy only at the 2-h interval but this may be related to short duration of action (Desai et al. 2005). However, the D_1 agonist also reversed the reserpine-induced effects only partially and tended to reverse RO-4-1284-induced catalepsy only at very high doses. The effect on D_1 signaling under these conditions is apparently more efficient with a PDE10AI than that with an orthosteric D_1 agonist. This may be the case because, under dopamine depletion, D_1 receptors become supersensitive resulting from a signaling switch from induction of immediate early genes (such as c-fos) toward ERK/MAP signaling (Gerfen et al. 2002), whereas PDE10AIs induce both immediate early genes and ERK/MAP signaling (Strick et al. 2010).

Behavioral stimulant effects of PDE10AIs were observed in combination with high doses of the D_1 antagonist SCH-23390 or the dopamine-depleting agents RO-4-1284 or reserpine but not in combination with the D_2 receptor blocker haloperidol. Like D_2 receptor blockers, PDE10AIs increase striatal dopamine turnover (Schmidt et al. 2008). In contrast to D_2 receptor blockers, however, PDE10AIs do not directly prevent D_2 receptor stimulation, thereby leaving dopaminergic neurotransmission largely intact.

Thus, the above behavioral stimulant effects of PDE10AIs may arise from progressively increasing activation of the direct pathway overcoming the effect of D_1 receptor blockade and resulting in behavioral stimulation as long as the indirect pathway is not disinhibited by D_2 receptor blockade. The behavioral stimulant effects are observed at doses close to those required for reversal of the cataleptic state, reflecting very efficient direct pathway activation and limited safety margin for anti-Parkinson effects. Concomitant direct pathway activation may also explain the bell-shaped dose–response for inhibition of the tail-pinch response before reserpine. Similar bell-shaped effects on glucose metabolism in the lateral habenula of mice were also attributed to increased direct pathway activation at high PDE10AI doses (Dedeurwaerdere et al. 2011).

Balance between direct and indirect pathway activation is necessary for numerous behaviors including reward processing (Lobo et al. 2010; Beutler et al. 2011). Dopamine D_1 receptors in the direct striatal pathway facilitate several dopamine-dependent functions including appetitive behaviors. Global loss of dopamine D_1 receptors demonstrates its importance for feeding and reward acquisition to the general ability to thrive (Drago et al. 1994; Xu et al. 1994; Wall et al. 2011). In this regard, direct striatal pathway activation by PDE10AIs might improve negative and depressive symptomatology in schizophrenic patients. In view of the monoamine hypothesis of depression (Hirschfeld 2000), the antidepressant potential of PDE10AIs may be supported by the reversal of the effects of monoamines depletion observed in the present study.
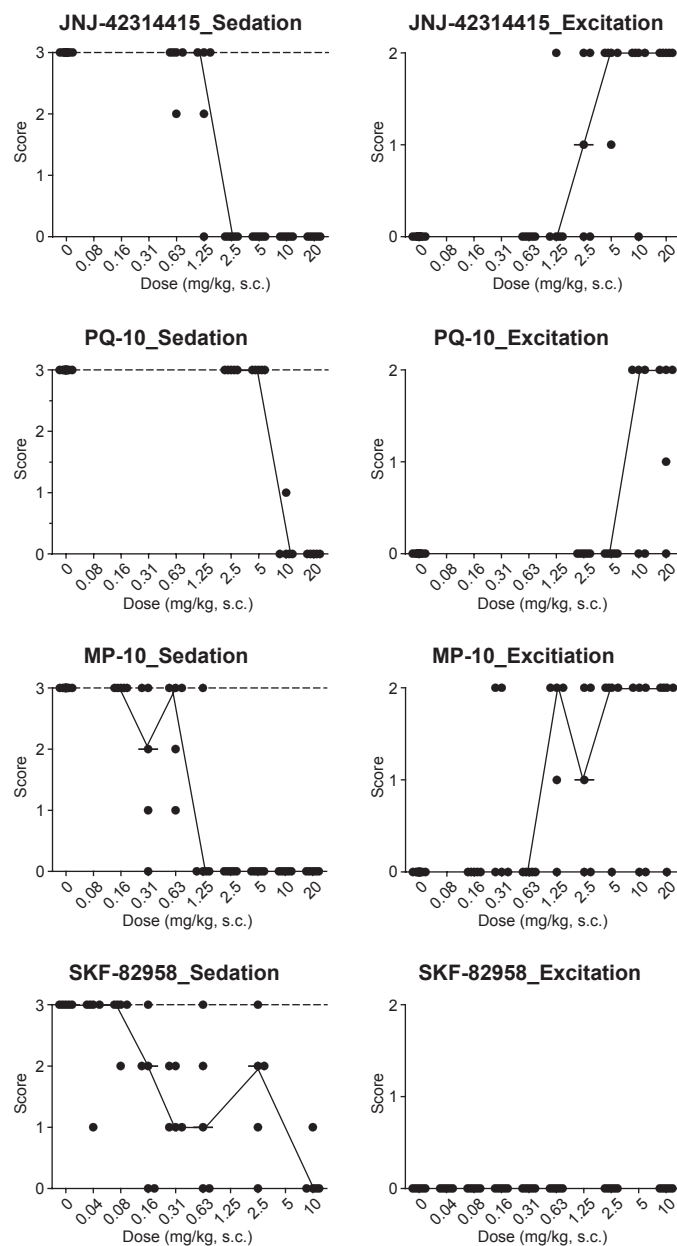
Figure 6. Dose-dependent reversal of reserpine-induced sedation and replacement by excitation. Shown are individual and median scores (symbols and stripes, respectively) for sedation and excitation (measured 1 h post reserpine) in rats pretreated with the PDE10AIs JNJ-42314415, PQ-10, MP-10, and the D$_1$ agonist SKF-82958 1 h before the reserpine challenge. The dotted horizontal lines represent the critical score adopted for reversal of sedation (score <3). In case of excitation, score >0 was used for the determination of ED$_{50}$ values. The PDE10AIs dose dependently reversed the reserpine-induced sedation, which was replaced by excitation. The D$_1$ agonist also reversed the reserpine-induced sedation but without resulting in excitation. PDE10A, phosphodiesterase 10A inhibitor.

Together with previously reported data, the present results indicate that PDE10AIs suppress stimulant behavior via indirect pathway activation, but the effect is counteracted by D$_1$ receptor stimulation and corresponding activation of the direct pathway. In the absence of counteracting direct pathway activation (D$_1$ receptor stimulation), however, this mechanism is very efficient, resulting

in a limited safety margin toward cataleptic side effects. Conversely, PDE10AIs can reverse behavioral suppression (catalepsy, hypolocomotion) via direct pathway activation, but the effect is counteracted by D$_2$ receptor blockade and corresponding disinhibition of the indirect pathway. In the absence of concomitant, counteracting indirect pathway activation (D$_2$ receptor blockade), however, this
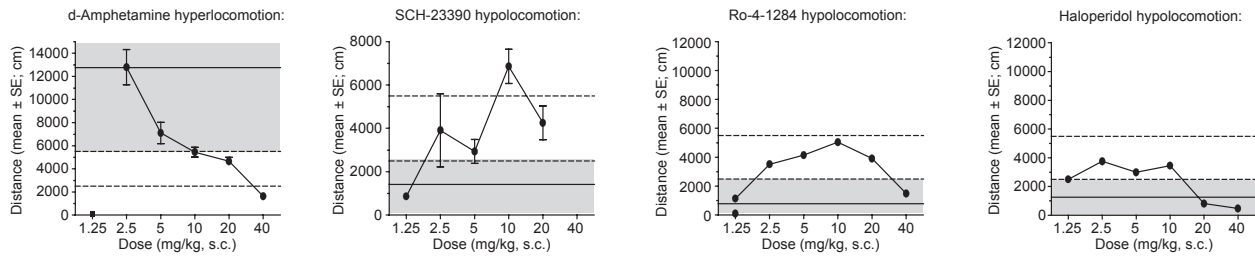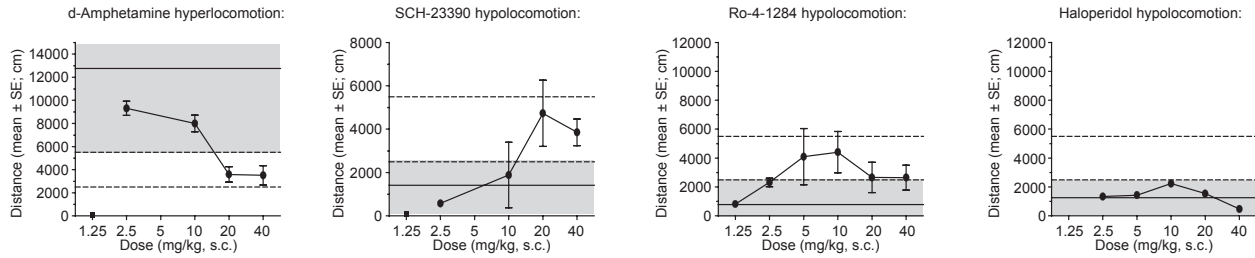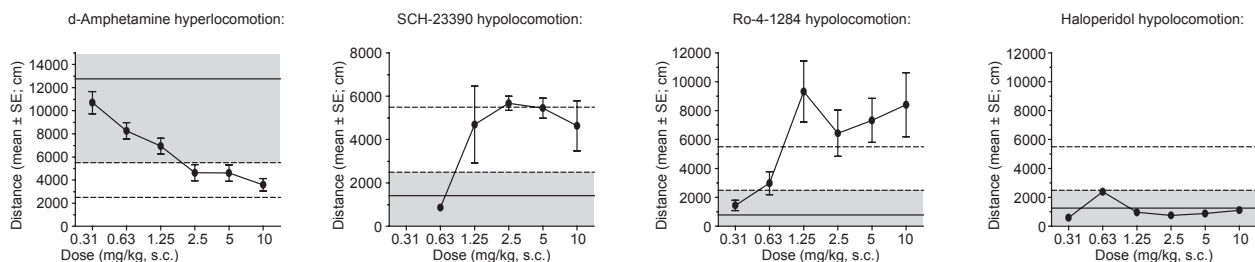
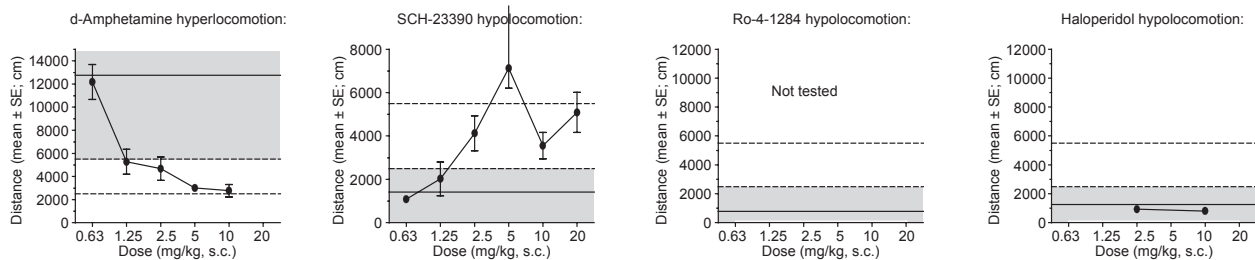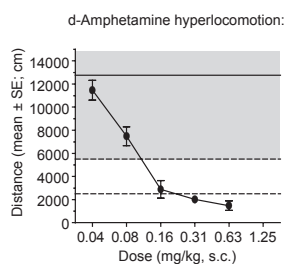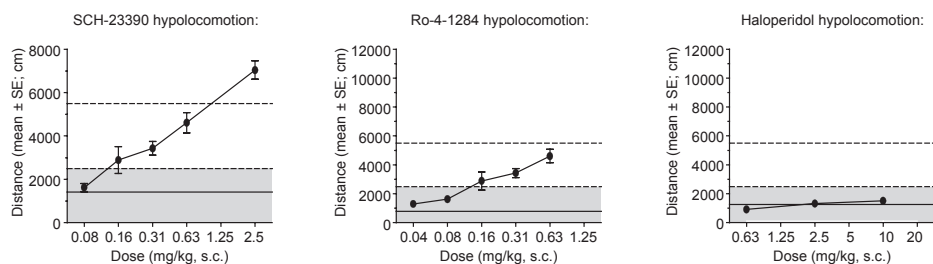**Figure 7.** Activity profile in locomotor activity tests in mice. Dose–response relations for inhibition of D-amphetamine-induced hyperlocomotion and reversal of hypolocomotion induced by the D$_1$ antagonist SCH-23390, the monoamines-depleting agent RO-4-1284 or the D$_2$ antagonist haloperidol 0.5 h after s.c. injection. The solid horizontal line represents the averaged distance obtained in solvent-pretreated control mice, the gray area represents the activity range obtained in challenged solvent-treated control mice, and the area between the two dotted horizontal lines represents the locomotor activity level obtained in nonchallenged control mice. The PDE10AIs dose dependently inhibited D-amphetamine-induced hyperlocomotion and reversed the hypolocomotion induced by the D$_1$ antagonist SCH-23390 or the monoamines-depleting agent RO-4-1284 but hardly affected the hypolocomotion induced by the D$_2$ antagonist haloperidol. PDE10A, phosphodiesterase 10A inhibitor.

Table 12. ED$_{50}$s (95% confidence limits) for inhibition of D-amphetamine-induced hyperlocomotion and for reversal of hypolocomotion induced by the D$_1$ antagonist SCH-23390, the monoamines-depleting agent RO-4-1284, or the D$_2$ antagonist haloperidol measured in mice 0.5 h after s.c. injection.

Compound	ED$_{50}$s (95% confidence limits; mg/kg, s.c., −0.5 h)			
	D-Amphetamine (<5500 cm)	SCH-23390 (>2500 cm)	RO-4-1284 (>2500 cm)	Haloperidol (>2500 cm)
JNJ-42314415	8.1 (5.4–12.2)	7.1 (4.8–10.7)	2.69 (1.99–3.6)	>40[1]
PQ-10	12.4 (9.1–16.7)	16.3 (10.1–26.3)	5.4 (–)	>40
MP-10	1.78 (1.10–2.87)	1.17 (0.86–1.58)	0.58 (0.34–1.01)	>40
TP-10	1.78 (1.19–2.66)	1.78 (1.18–2.65)	No stock	>10
Haloperidol	0.112 (0.075–0.168)	–	–	–
SKF-82958	–	0.148 (0.109–0.200)	0.294 (0.218–0.40)	>10

[1]60% responders at 10 mg/kg.

mechanism is very efficient with a limited safety margin toward behavioral stimulant side effects. In analogy, the brake and accelerator of a car also function more efficiently when used separately rather than simultaneously, yet both are indispensable to adjust speed when out of balance. Further clinical studies have to show which patients may benefit from the dual activity profile of PDE10AIs.

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Disclosure

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References

Agid O, Kapur S, Remington G (2008). Emerging drugs for schizophrenia. Expert Opin Emerg Drugs 13: 479–495.

Beutler LR, Wanat MJ, Quintana A, Sanz E, Bamford NS, Zweifel LS, et al. (2011). Balanced NMDA receptor activity in dopamine D1 receptor (D1R)- and D2R-expressing medium spiny neurons is required for amphetamine sensitization. Proc. Natl. Acad. Sci. USA 108: 4206–4211.

Bourne JA (2001). SCH 23390: the first selective dopamine D1-like receptor antagonist. CNS Drug Rev 7: 399–414.

Colzi A, D'Agostini F, Cesura AM, Borroni E, Da Prada M (1993). Monoamine oxidase-A inhibitors and dopamine metabolism in rat caudatus: evidence that an increased cytosolic level of dopamine displaces reversible monoamine oxidase-A inhibitors in vivo. J Pharmacol Exp Ther 265: 103–111.

Coskran TM, Morton D, Menniti FS, Adamowicz WO, Kleiman RJ, Ryan AM, et al. (2006). Immunohistochemical localization of phosphodiesterase 10A in multiple mammalian species. J Histochem Cytochem 54: 1205–1213.

Dedeurwaerdere S, Wintmolders C, Vanhoof G, Langlois X (2011). Patterns of brain glucose metabolism induced by phosphodiesterase 10A inhibitors in the mouse: a potential translational biomarker. J Pharmacol Exp Ther 339: 210–217.

Delong MR, Wichmann T (2007). Circuits and circuit disorders of the basal ganglia. Arch Neurol 64: 20–24.

DeMartinis N, Banerjee A, Kumar V, Boyer S, Schmidt C, Arroyo S (2012). Results of a phase 2a proof-of-concept trial with a PDE10A inhibitor in the treatment of acute exacerbation of schizophrenia. Schizophr Res 136(Suppl. 1): S262.

Desai RI, Terry P, Katz JL (2005). A comparison of the locomotor stimulant effects of D1-like receptor agonists in mice. Pharmacol Biochem Behav 81: 843–848.

Drago J, Gerfen CR, Lachowicz JE, Steiner H, Hollon TR, Love PE, et al. (1994). Altered striatal function in a mutant mouse lacking D1A dopamine receptors. Proc. Natl. Acad. Sci. USA 91: 12564–12568.

Filinger EJ (1994). Effect of a reserpine-like agent on the release and metabolism of [3H]NA in cell bodies and terminals. Gen Pharmacol 25: 1039–1043.

Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, et al. (1999). Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). J Biol Chem 274: 18438–18445.

Gerfen CR (2000). Molecular effects of dopamine on striatal-projection pathways. Trends Neurosci 23: S64–S70.

Gerfen CR, Miyachi S, Paletzki R, Brown P (2002). D1 dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. J Neurosci 22: 5042–5054.

Grauer SM, Pulito VL, Navarra RL, Kelly MP, Kelley C, Graf R, et al. (2009). Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. J Pharmacol Exp Ther 331: 574–590.

Graybiel AM (1990). Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13: 244–254.

Graybiel AM (2000). The basal ganglia. Curr Biol 10: R509–R511.

Gresack JE, Seymour PA, Schmidt CJ, Risbrough VB (2013). Inhibition of phosphodiesterase 10A has differential effects on dopamine D1 and D2 receptor modulation of sensorimotor gating. Psychopharmacology 231: 2189–2197.

Hillegaart V, Ahlenius S, Magnusson O, Fowler CJ (1987). Repeated testing of rats markedly enhances the duration of effects induced by haloperidol on treadmill locomotion, catalepsy, and a conditioned avoidance response. Pharmacol Biochem Behav 27: 159–164.

Hirschfeld RM (2000). History and evolution of the monoamine hypothesis of depression. J Clin Psychiatry 61 (Suppl 6): 4–6.

Hurley MJ, Jenner P (2006). What has been learnt from study of dopamine receptors in Parkinson's disease? Pharmacol Ther 111: 715–728.

Kapur S, Mamo D (2003). Half a century of antipsychotics and still a central role for dopamine D2 receptors. Prog Neuropsychopharmacol Biol Psychiatry 27: 1081–1090.

Kehler J, Kilburn JP (2009). Patented PDE10A inhibitors: novel compounds since 2007. Expert Opin Ther Pat 19: 1715–1725.

Kehler J, Nielsen J (2011). PDE10A inhibitors: novel therapeutic drugs for schizophrenia. Curr Pharm Des 17: 137–150.

Kehler J, Ritzen A, Greve DR (2007). The potential therapeutic use of phosphodiesterase 10 inhibitors. Expert Opin Ther Pat 17: 147–158.

Langlois X, Megens A, Lavreysen H, Atack J, Cik M, te Riele P, et al. (2012). Pharmacology of JNJ-37822681, a specific and fast-dissociating D2 antagonist for the treatment of schizophrenia. J Pharmacol Exp Ther 342: 91–105.

Lewi PJ, Niemegeers CJE, Gypen LMJ (1977). First hand estimation of the median effective dose (ED50) and its confidence interval, assuming a linear log dose-response function. Department of Global Medical and Scientific Information, Johnson & Johnson Pharmaceutical Research and Development, Janssen Pharmaceutica, Beerse, Belgium. Internal Report N123705/1.

Leysen JE (2000). Receptor profile of neuroleptics. Pp. 57–81. in BA Ellenbroek and AR Cools, eds. Atypical antipychotics. Birkhauser Verlag, Basel, Switzerland.

Leysen JE, Niemegeers CJE (1985). Neuroleptics. Pp. 331–361 in A Lajtha, ed. Handbook of neurochemistry. Vol. 9. Plenum Publishing Corporation, New York, NY.

Lobo MK, Covington HE, III, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, et al. (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. Science 330: 385–390.

Loughney K, Snyder PB, Uher L, Rosman GJ, Ferguson K, Florio VA (1999). Isolation and characterization of PDE10A, a novel human 3′, 5′-cyclic nucleotide phosphodiesterase. Gene 234: 109–117.

Mailman R, Huang X, Nichols DE (2001). Parkinson's disease and D1 dopamine receptors. Curr Opin Investig Drugs 2: 1582–1591.

Megens AAHP, Hendrickx HMR, Hens KA, Fonteyn I, Langlois X, Lenaerts I, et al. (2014). Pharmacology of JNJ42314415, a centrally active PDE10A inhibitor. A comparison of PDE10A inhibitors with D2 receptor blockers as potential antipsychotics. J Pharmacol Exp Ther 349: 1–14.

Menniti FS, Chappie TA, Humphrey JM, Schmidt CJ (2007). Phosphodiesterase 10A inhibitors: a novel approach to the treatment of the symptoms of schizophrenia. Curr Opin Investig Drugs 8: 54–59.

Nishi A, Kuroiwa M, Miller DB, O'Callaghan JP, Bateup HS, Shuto T, et al. (2008). Distinct roles of PDE4 and PDE10A in the regulation of cAMP/PKA signaling in the striatum. J Neurosci 28: 10460–10471.

Nishi A, Kuroiwa M, Shuto T (2011). Mechanisms for the modulation of dopamine d(1) receptor signaling in striatal neurons. Front Neuroanat 5: 43.

Rodefer JS, Murphy ER, Baxter MG (2005). PDE10A inhibition reverses subchronic PCP-induced deficits in attentional set-shifting in rats. Eur J Neurosci 21: 1070–1076.

Sano H, Nagai Y, Miyakawa T, Shigemoto R, Yokoi M (2008). Increased social interaction in mice deficient of the striatal medium spiny neuron-specific phosphodiesterase 10A2. J Neurochem 105: 546–556.

Schmidt CJ, Chapin DS, Cianfrogna J, Corman ML, Hajos M, Harms JF, et al. (2008). Preclinical characterization of selective phosphodiesterase 10A inhibitors: a new therapeutic approach to the treatment of schizophrenia. J Pharmacol Exp Ther 325: 681–690.

Schotte A, Leysen JE (1998). Receptor binding profile of risperidone. Acta Psychiatr Belg 98: 64–75.

Seeger TF, Bartlett B, Coskran TM, Culp JS, James LC, Krull DL, et al. (2003). Immunohistochemical localization of PDE10A in the rat brain. Brain Res 985: 113–126.

Siuciak JA (2008). The role of phosphodiesterases in schizophrenia: therapeutic implications. CNS Drugs 22: 983–993.

Siuciak JA, Chapin DS, Harms JF, Lebel LA, McCarthy SA, Chambers L, et al. (2006). Inhibition of the striatum-enriched phosphodiesterase PDE10A: a novel approach to the treatment of psychosis. Neuropharmacology 51: 386–396.

Soderling SH, Bayuga SJ, Beavo JA (1999). Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. Proc. Natl. Acad. Sci. USA 96: 7071–7076.

Sotty F, Montezinho LP, Steiniger-Brach B, Nielsen J (2009). Phosphodiesterase 10A inhibition modulates the sensitivity of the mesolimbic dopaminergic system to D-amphetamine: involvement of the D1-regulated feedback control of midbrain dopamine neurons. J Neurochem 109: 766–775.

Stanley ME, Glick SD (1976). Interaction of drug effects with testing procedures in the measurement of catalepsy. Neuropharmacology 15: 393–394.

Strick CA, James LC, Fox CB, Seeger TF, Menniti FS, Schmidt CJ (2010). Alterations in gene regulation following inhibition of the striatum-enriched phosphodiesterase, PDE10A. Neuropharmacology 58: 444–451.

Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neurosci 30: 228–235.

Threlfell S, Sammut S, Menniti FS, Schmidt CJ, West AR (2009). Inhibition of Phosphodiesterase 10A Increases the Responsiveness of Striatal Projection Neurons to Cortical Stimulation. J Pharmacol Exp Ther 328: 785–795.

Tsutakawa RK (1982). Statistical methods in bioassay. Pp. 236–243 in S Kotz and NL Johnson, eds. Estimation of relative potency from quantal responses. Encyclopedia of statistical science, Vol. 1. J. Wiley, New York, NY.

Vanhoof G, Megens A, Ver Donck L, Langlois X, Mahieu M, Daems H, et al. (2012). PDE10 inhibitors as neuromodulators. Abstract 42nd Annual Meeting of the Society of Neuroscience, October 13–17, New Orleans, LA.

Wall VZ, Parker JG, Fadok JP, Darvas M, Zweifel L, Palmiter RD (2011). A behavioral genetics approach to understanding D1 receptor involvement in phasic dopamine signaling. Mol Cell Neurosci 46: 21–31.

Wang JQ, McGinty JF (1997). The full D1 dopamine receptor agonist SKF-82958 induces neuropeptide mRNA in the normosensitive striatum of rats: regulation of D1/D2 interactions by muscarinic receptors. J Pharmacol Exp Ther 281: 972–982.

Westfall TC, Westfall DP (2005). Adrenergic agonists and antagonists. Pp. 237–295 in LL Brunton, JS Lazo and KL Parker, eds. Goodman & Gilman's the pharmacological basis of therapeutics. McGraw-Hill Medical Publishing Division, New York, NY.

Wilson CJ (2014). Basal Ganglia. Oxford Univ. Press, Oxford, U.K.

Xu M, Hu XT, Cooper DC, Moratalla R, Graybiel AM, White FJ, et al. (1994). Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D1 receptor mutant mice. Cell 79: 945–955.