The dopamine reuptake inhibitor MRZ-9547 increases progressive ratio responding in rats

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Abstract

Drugs that are able to shift effort-related decision making in intact rats towards high-effort response options are largely unknown. Here, we examined the effects of two candidate drugs, MRZ-9547 and its l- enantiomer MRZ-9546 on progressive ratio (PR) responding using two different tasks, a standard PR task that involves increasing ratio requirements and a PR/chow feeding choice task in which animals can lever press for preferred food pellets under a PR schedule or approach freely available less preferred lab chow. Furthermore, we assessed the mechanisms of action of both drugs using in vitro-assay methods and in vivo-microdialysis. Results reveal that MRZ-9547 is a selective dopamine transporter (DAT) inhibitor that moderately stimulated striatal dopamine release. MRZ-9546 was a much less potent DAT inhibitor. Furthermore, MRZ-9547 dose dependently increased the tendency to work for food reinforcement both in the standard PR task and the PR/chow feeding choice task, MRZ-9546 was considerably less active. Relative to MRZ-9547, other DAT-interfering drugs had only moderate (methylphenidate) or marginal (modafinil, d-amphetamine) stimulant effects on PR responding in either task. Collectively, our data demonstrate that the DAT inhibitor MRZ-9547 can markedly stimulate PR responding and shift effort-related decision making in intact rats towards high-effort response options. An analysis of effort-related decision making in rodents could provide an animal model for motivational dysfunctions related to effort expenditure such as fatigue, e.g., in Parkinson’s disease or major depression. Our findings suggest that DAT inhibitors such as MRZ-9547 could be potentially useful for treating energy-related symptoms in neurological or neuropsychiatric disorders.

Introduction

Motivational dysfunctions related to effort expenditure such as fatigue, apathy, anergia and psychomotor slowing are common symptoms in neuropsychiatric and neurological disorders, e.g. major depression, schizophrenia, multiple sclerosis or inflammatory disease (Friedman et al., 2011; Salamone and Correa, 2012). For instance, in a test of effort-related choice, patients with major depressive disorders were less willing to expend effort for rewards than controls (Treadway et al., 2012). Fatigue and anergia are also present in Parkinson’s disease and may be even more deleterious to quality of life than motor dysfunction (Friedman et al., 2011). Yet, the available treatment options rarely cause significant improvements in fatigue in neuropsychiatric and neurological disorders (Harrington, 2012; Kluger et al., 2013). There is evidence that tests of effort-related decision making in rodents could be useful for modeling some motivational dysfunctions in humans and identifying potential drugs for treating effort-related symptoms (Salamone and Correa, 2012). For instance, in a progressive ratio (PR)/chow feeding concurrent choice task in which animals can lever press for preferred food pellets under a PR schedule or approach and consume freely available but less preferred lab chow, a selective adenosine A2A receptor antagonist inclined intact rats to select the increasingly more effortful type of food-seeking behavior (Randall et al., 2012). Moreover, blockade of dopamine (DA) transmission shifted effort-based choice behavior towards the low-cost response option, an effect that can be reversed by co-administration of a selective adenosine A2A receptor antagonist (Randall et al., 2012). In view of these findings, adenosine A2A receptor antagonists have been suggested to be potentially useful for treating energy-related symptoms such as fatigue (Farrar et al., 2007).

Here, we tested the effects of MRZ-9547 (d-(2-(2-Oxo-4-(R)-phenylpyrrolidin-1-yl)-acetamide), and its l-enantiomer MRZ-9546 on effort-related decision making in rats. The racemic form of these compounds referred to as phenotropil has been shown to stimulate motor...
activity in rats (Zvejnieks et al., 2011) and enhance physical capacity and cognition in humans (Malykh and Sadaie, 2010). We examined the effects of MRZ-9547 and MRZ-9546 on PR responding using two different tasks, a standard PR task that involves increasing ratio requirements per reinforcer until an animal ceases lever pressing and a PR/chow feeding choice task in which animals can lever press for preferred food pellets under a PR schedule or approach and consume freely available but less preferred lab chow (Schweimer and Hauber, 2005). Furthermore, we assessed mechanisms of action of MRZ-9547 and MRZ-9546 using in vitro-assay methods and in vivo-microdialysis. For comparison, we included methylphenidate, modafinil and d-amphetamine which, in standard PR tasks, enhanced breakpoints (Poncelet et al., 1983; Mayorga et al., 2000; Young and Geyer, 2010; Mayorga et al., 2000; Young and Geyer, 2010), a putative measure of how much effort an animal is willing to invest to gain access to a reinforcer (Stewart, 1975). However, it is yet unknown in which ways these drugs influence effort-related choice behavior. We hypothesize that MRZ-9547 and its l-enantiomer as well as methylphenidate, modafinil and d-amphetamine are not only able to invigorate PR responding in the standard PR task but also to increase selection of the high-effort over the low-effort response option in the PR/chow feeding choice task.

Methods

All animal experiments were conducted according to the German law of animal protection and approved by the proper authorities.

**MRZ-9546 and MRZ-9547: binding experiments**

For binding experiments, human recombinant dopamine transporter (DAT) was expressed in CHO-K1 cells. Cell membranes were incubated with [125I]RTI-55 (0.15 nM) for 3 h at 4 °C in 50 mM Tris-HCl, 100 mM NaCl, 1 mM leupeptin, 10 μM PMSF pH 7.4. Maximum binding was 0.047 pmol/mg protein. Non-specific binding was determined with 10 μM nomifensine. For functional assays, human recombinant DAT was expressed in CHO-K1 cells. Cells were incubated with [3H]-DA for 10 min at 25 °C in 5 mM Tris-HCl, 7.5 mM HEPES, 120 mM NaCl, 5.4 mM KCl, 1.2 mM CaCl2, 1.2 mM MgSO4, 5 mM D-glucose, 1 mM ascorbic acid, pH 7.1. DA taken up was determined by scintillation counting. MRZ-9546 and MRZ-9547 were dissolved in 0.4% DMSO.

**MRZ-9547 and MRZ-9546: brain extracellular concentrations**

**Animals**

Male Sprague–Dawley rats (Elevage-Janvier, France) weighing 250–300 g were kept up to four per cage (55 × 39 × 27 cm, Tecniplast, Italy) in a room with controlled temperature (21 ± 1 °C), humidity (60 ± 3%) under a 12:12 h light–dark cycle (lights on at 06:00 hours). Standard laboratory food (Altromin, Germany) and water were available ad libitum. At least 6 d after arrival they were subjected to the stereotaxic surgery. After surgery, animals were housed individually in cages (Ehret, Germany, 26 × 18 × 42 cm) with the grid lid elevated by a 5 cm frame.

**Surgery**

Using standard stereotaxic procedures, siliconized guide cannula (MAB 6/14) (MAB, Sweden) were implanted unilaterally in anesthetized animals aiming the caudate-putamen (CPu)(AP: +0.1 mm, L: ±2.6 or ±2.6 mm, DV: ±3.2 mm relative to bregma) with the incisor bar set 3.3 mm under the interaural line according to the atlas of (Paxinos and Watson, 1986). For induction of anesthesia 50 mg/kg pentobarbital i.p. was used followed by 1 ml/kg Metacam™ 10% 1.0 ml. Each rat was given at least 2 d to recover from surgery before starting microdialysis experiments using standard microdialysis techniques and analytical procedures (see Supplementary Methods and Materials).

**Drugs**

MRZ-9547 (Merz Pharmaceuticals, Germany) was dissolved fresh in distilled water for each test day and administered at a volume up to 4 ml/kg i.p. (10, 50, 100 mg/kg, n = 4–5 per dose).

**Data analysis**

Pharmacokinetic variables (e.g. area under curve) given were calculated using Excel add on ‘PK-Solver’ (Zhang et al., 2010).

**MRZ-9547: striatal DA and DOPAC release**

**Animals**

Male Sprague–Dawley rats weighing about 300 g (Harlan, The Netherlands) were housed in plastic cages (60×40×20 cm; max. 5 rats per cage) for 7 d until surgery. Thereafter, animals were housed individually in cages (30 × 30 × 40 cm), food and water were available ad libitum in a room with controlled temperature (20–24 °C), humidity (45–70%) under a 12:12 h light–dark cycle (lights on at 07:00 hours). Post-surgery recovery was at least 48 h.

**Surgery**

Rats were anesthetized using isoflurane (2%, 800 ml/min O2). Bupivacain/epinephrine were used for local anesthesia. Flunixin (1 ml/kg, s.c.) was used to induce pre-/peri-operative analgesia. The animals were placed in a stereotaxic frame (Kopf instruments, USA), and I-shaped probes (Hospal AN 69 membrane, 3 mm exposed surface; Brainlink, The Netherlands) were inserted
into the CPu. Coordinates for the tips of the probes were: posterior (AP)=+0.9 mm to bregma, lateral (L)=+3.0 mm to midline and ventral (V)=−6.5 mm to dura (Paxinos and Watson, 1986). Each rat was given at least 2 d to recover from surgery before starting microdialysis experiments using standard microdialysis techniques and analytical procedures (see Supplementary Materials and Methods). MRZ-9547 was given i.p. at 100 mg/kg.

**Statistical analysis**

Statistical analyses were performed on relative data (per cent change from the baseline) using a two-way analysis of variance (ANOVA) for repeated measurements with treatment and time as main factors followed by a Student–Newman–Keuls post-hoc test (SigmaPlot for Windows v. 12, SPSS Corporation, 2012).

**Behavioral experiments**

**Experiment 1: effects of MRZ-9547 on PR responding**

**Animals**

Male Sprague–Dawley rats (Janvier, France) weighing between 210 and 225 g at the beginning of the experiments were used. They were housed in groups of four animals in transparent plastic cages (55×39×27 cm, Ferplast, Germany) and kept under a 12:12 h light–dark cycle (lights on at 07:00 hours). Throughout the experiments water was available ad libitum and standard laboratory maintenance chow (Altromin, Germany) was restricted to 15 g per animal and day to maintain them at ∼85% of their free-feeding weight. Temperature (22±2 °C) and humidity (50±10%) were kept constant in the animal house.

**Drugs**

MRZ-9547 (Merz Pharmaceuticals, Germany) was dissolved in distilled water fresh for each test day and administered 30 min before behavioral test onset at a volume up to 5 ml/kg i.p. Four treatment groups were included that received either vehicle (n=10) or MRZ-9547 (25 mg/kg, n=10; 50 mg/kg, n=10; 100 mg/kg, n=10).

**Behavioral procedures and apparatus**

Three tests were performed in a consecutive manner on subsequent days, a ‘standard’ PR task, a PR/chow feeding choice task followed by a consumption test. The standard PR task involved increasing ratio requirements per reinforcer until an animal ceased lever pressing (Schweimer and Hauber, 2005), in the PR/chow feeding choice task animals could lever press for preferred food pellets under a PR schedule or approach and consume freely available but less preferred lab chow (Schweimer and Hauber, 2005). In the consumption test the amount of pellets (used in the PR tasks as reward) freely available in a dish ingested under drug/vehicle was measured (for details of behavioral testing see Supplementary Materials and Methods).

**Experiment 2: effects of MRZ-9546 on PR responding**

Unless otherwise noted, the same procedures as in experiment 1 were used. Two treatment groups were included that received either vehicle (n=10) or MRZ-9546 (100 mg/kg, n=10). The day after completion of behavioral experiments, animals assigned to the MRZ-9546 treatment group during behavioral testing received a final drug injection and, 120 min later, a terminal heart function under isoflurane anesthesia. Collected blood samples were analyzed for MRZ-9546 plasma concentrations (see Supplementary Materials and Methods).

**Experiment 3: effects of MRZ-9547 and modafinil on PR responding**

Unless otherwise noted, the same procedures as in experiment 1 were used. MRZ-9547 was dissolved in distilled water. Modafinil ((2-(diphenylmethyl)sulfonyl)acetamide) purchased from Sequoia (UK) was dissolved in 1% w/v methylcellulose (Sigma, Germany) in 0.9% NaCl water fresh for each test day. Drugs administered 30 min before behavioral test onset at a volume of 2 ml/kg i.p. IP injections of 1% w/v methylcellulose (Sigma, Germany) in 0.9% NaCl water (2 ml/kg) served as controls. Five treatment groups were included that received either vehicle (n=11), MRZ-9547 (50 mg/kg, n=11; 100 mg/kg, n=10) or modafinil (32 mg/kg, n=10; 64 mg/kg, n=10). The day after completion of behavioral experiments, animals assigned to treatment groups during behavioral testing received a final injection of MRZ-9547 or modafinil and, 120 min later, a terminal heart function under isoflurane anesthesia. Collected blood samples were analyzed for MRZ-9547 and modafinil plasma concentrations (see Supplementary Materials and Methods).
Experiment 4: effects of MRZ-9547 and methylphenidate on PR responding

Unless otherwise noted, the same procedures as in experiment 1 were used. MRZ-9547 and methylphenidate (Sigma, Germany) was dissolved in distilled water fresh for each test day and administered 30 min before behavioral test onset at a volume of 2 ml/kg. Injections of distilled water served as controls. Four treatment groups were included that received either vehicle (n=11), MRZ-9547 (50 mg/kg, n=10; 100 mg/kg, n=10) or methylphenidate (5 mg/kg, n=10; 10 mg/kg, n=10). The day after completion of behavioral experiments, animals assigned to treatment groups during behavioral testing received a final injection of MRZ-9547 or methylphenidate and, 120 min later, a terminal heart function under isoflurane anesthesia. Collected blood samples were analyzed for MRZ-9547 and methylphenidate plasma concentrations (see Supplementary Materials and Methods).

Experiment 5: effects of MRZ-9547 and d-amphetamine on PR responding

Unless otherwise noted, the same procedures as in experiment 1 were used. MRZ-9547 and d-amphetamine (Sigma, Germany) was dissolved in distilled water fresh for each test day and administered 30 min before behavioral test onset at a volume of 2 ml/kg. Injections of distilled water served as controls. Four treatment groups were included that received either vehicle (n=10), MRZ-9547 (50 mg/kg, n=10; 100 mg/kg, n=10) or d-amphetamine (1 mg/kg, n=10).

Results

MRZ-9547 and MRZ-9546: mechanisms of action

MRZ-9547 turned out to be a DAT inhibitor as shown by displacement of binding of [125I] RTI-55 (IC50=4.82±0.05 μM, n=3) to human recombinant DAT expressed in CHO-K1 cells and inhibition of DA uptake (IC50=14.5±1.6 μM, n=2) in functional assays in the same cells. It inhibited norepinephrine transporter (NET) with an IC50 of 182 μM (one experiment in duplicate). The potencies for the l-enantiomer MRZ-9546 were as follows: DAT binding (Ki=34.8±14.8 μM, n=3), DAT function (IC50=65.5±8.3 μM, n=2) and NET function (IC50=667 μM, one experiment performed in duplicate). (Binding to other targets, see Supplementary Results).

MRZ-9547 and MRZ-9546: brain extracellular concentrations

After i.p. administration, MRZ-9547 (10, 50, 100 mg/kg, n=4–5 per dose) displayed a gradual, delayed and dose-dependent increase in brain concentration (Fig. 1a). The dose of 50 mg/kg resulted in brain concentrations of 22 μM, i.e. slightly above affinity for DAT (see above), whereas at 10 mg/kg concentrations were below this threshold.

MRZ-9547: striatal DA/DOPAC concentrations

MRZ-9547 (100 mg/kg i.p., n=6) moderately, but significantly increased striatal DA concentration (Fig. 1b). When DA reached peak levels, there was a parallel, strong decrease in DOPAC levels which persisted even after DA levels returned to baseline (Supplementary Fig. S1).

Experiment 1: MRZ-9547 effects on PR responding

PR task

MRZ-9547 produced a dose-dependent increase of breaking points (Fig. 2a). In line with this description, an ANOVA revealed a significant effect of treatment (F(3, 36)=19.57, p<0.001). Post-hoc comparisons of drug vs. vehicle treatment groups indicated a significant increase of breaking points in animals treated with MRZ-9547 across all doses relative to vehicle controls (p<0.01, respectively). (For results for lever presses per minute, perseverative lever presses and response latencies see Supplementary Results.)

PR choice task

The breaking points for pressing the lever for preferred pellets differed between treatment groups (F(3, 36)=29.43, p<0.001) (Fig. 2b). Post-hoc comparisons revealed a significant increase of breaking points in animals treated with MRZ-9547 across all doses relative to vehicle controls (p<0.01, respectively). Furthermore, the intake of freely available, less preferred lab chow differed significantly between treatment groups (F(3, 36)=5.0, p<0.01) (Fig. 2c). Post-hoc comparisons showed a significant decrease of lab chow intake in animals treated with MRZ-9547 50 mg/kg (p<0.05) or 100 mg/kg (p<0.01) relative to vehicle controls.

Consumption test

As shown in Fig. 2d, the amount of pellets ingested differed between treatment groups (F(3, 36)=7.40, p<0.001) and post-hoc comparisons demonstrated a significant decrease of pellet intake in animals treated with MRZ-9547 50 and 100 mg/kg relative to vehicle controls (p<0.01, respectively).

Experiment 2: MRZ-9546 effects on PR responding

PR task

MRZ-9546 (100 mg/kg) moderately enhanced breaking points (Supplementary Fig. S2A). Accordingly, an ANOVA revealed a significant effect of treatment (F(1, 18)=5.22, p<0.05). Similarly, MRZ-9546 elicited an increase of lever presses per minutes (F(1, 18)=4.83, p<0.05) but did not alter the number of perseverative

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lever presses \( (F(1, 18)=0.10, \text{n.s.}) \) or response latencies \( (F(1, 18)=0.09, \text{n.s.}) \) (data not shown).

**PR choice task**

The breaking points for pressing the lever for preferred pellets were higher in animals that received MRZ-9546 \( (F(1, 18)=6.81, p<0.05) \) (Supplementary Fig. S2B), whereas their intake of lab chow was moderately lower, an effect that missed significance \( (F(1, 18)=1.92, \text{n.s.}) \) (Supplementary Fig. S2C).

**Consumption test**

As shown in Supplementary Fig. S2D, the amount of pellets ingested was lower in animals that received MRZ-9546 \( (F(1, 18)=4.56, p<0.05) \).

**Plasma concentrations**

The plasma concentrations of MRZ-9546 120 min after administration are: 83.9 ± 3.1; 172.0 ± 4.6; 343.3 ± 11.6 and 673.6 ± 44.9 μM for 25; 50; 100 and 200 mg/kg respectively.

**Experiment 3: MRZ-9547 and modafinil effects on PR responding**

**PR task**

MRZ-9547 but not modafinil increased breaking points (Fig. 3a). In line with this description, an ANOVA showed a significant effect of treatment \( (F(4, 47)=19.27, p<0.001) \). Post-hoc comparisons revealed a significant increase of breaking points in animals treated with MRZ-9547 50 or 100 mg/kg relative to vehicle controls \( (p<0.01, \text{n.s.}) \).
respectively) but not in those treated with modafinil 32 or 64 mg/kg ($p>0.05$, respectively). (For additional results see Supplementary Results.)

**PR choice task**

The breaking points for pressing the lever for preferred pellets clearly differed between treatment groups $F(4, 47)=34.02$, $p<0.0001$ (Fig. 3b). Post-hoc comparisons revealed a significant increase of breaking points in animals treated with MRZ-9547 across both doses relative to vehicle controls ($p<0.001$, respectively) but not in animals treated with modafinil (32, 64 mg/kg; $p>0.05$, respectively). Furthermore, the intake of lab chow differed significantly between treatment groups $F(4, 47)=8.42$, $p<0.001$ (Fig. 3c). Accordingly, post-hoc comparisons
showed a significant decrease of lab chow intake in animals treated with MRZ-9547 50 mg/kg ($p<0.05$) or 100 mg/kg ($p<0.01$), whereas no significant differences were detected in animals treated with modafinil (32, 64 mg/kg; $p>0.05$, respectively).

**Consumption test**

As shown in Fig. 3d, the amount of pellets ingested differed between treatment groups ($F(4, 47)=4.49$, $p<0.01$) and post-hoc comparisons demonstrated a significant or near significant decrease of pellet intake in animals treated with MRZ-9547 100 mg/kg ($p<0.001$) and modafinil 64 mg/kg ($p=0.054$).

**Plasma levels**

MRZ-9547 given at 50 mg/kg was detected in plasma at the concentrations of 245.7±21.2 and 194.6±10.5 μM 30 and 120 min after treatment. For 100 mg/kg corresponding values were 329.6±54.1 and 353.8±28.9 μM. Modafinil at the dose of 32 mg/kg was present in the plasma at the concentrations of 50.6±9.4 and 6.85±1.45 μM for 30 and 120 min, respectively. For 64 mg/kg the respective values were 90.06±24.45 and 14.23±1.14 μM.

**Experiment 4: MRZ-9547 and methylphenidate effects on PR responding**

**PR task**

MRZ-9547 and, to a lesser extent, methylphenidate increased breaking points (Fig. 4a). Accordingly, an ANOVA revealed a significant effect of treatment ($F(4, 46)=49.40$, $p<0.0001$) and post-hoc comparisons revealed a significant increase of breaking points in animals treated with MRZ-9547 50 or 100 mg/kg as well as methylphenidate 10 mg/kg relative to vehicle controls ($p<0.01$, respectively). (For results for lever presses per minute, perseverative lever presses and response latencies see Supplementary Results.)

**PR choice task**

The breaking points for pressing the lever for pellets differed between treatment groups ($F(4, 46)=36.05$, $p<0.0001$) (Fig. 4b). Post-hoc comparisons revealed a significant increase of breaking points in animals treated with MRZ-9547 (50, 100 mg/kg) and methylphenidate (10 mg/kg) ($p<0.001$, respectively). Furthermore, the intake of lab chow differed between treatment groups. However, compared to other experiments, the lab chow intake in vehicle controls was, for unknown reasons, low and the there was only a trend for a significant treatment effect ($F(4, 46)=2.27$, $p=0.07$) (Fig. 4c).

**Consumption test**

As shown in Fig. 4d, the amount of pellets ingested differed between treatment groups ($F(4, 46)=4.63$, $p<0.01$) and post-hoc comparisons demonstrated a significant decrease of pellet intake in animals treated with MRZ-9547 50 mg/kg ($p<0.05$) and 100 mg/kg ($p<0.05$) as well as methylphenidate 5 and 10 mg/kg ($p<0.05$, respectively). Plasma concentrations are given in Supplementary Results.
Experiment 5: effects of MRZ-9547 and d-amphetamine on PR responding

PR task

MRZ-9547 but not d-amphetamine increased breaking points (Fig. 5a). Accordingly, an ANOVA revealed a significant effect of treatment ($F(3, 36)=15.22, p<0.0001$) and post-hoc comparisons revealed a significant increase of breaking points in animals treated with MRZ-9547 50 or 100 mg/kg relative to vehicle controls ($p<0.01$, respectively). (For additional results see Supplementary Results.)

PR choice task

The breaking points for pressing the lever for pellets differed between treatment groups ($F(3, 36)=35.98, p<0.0001$) (Fig. 5b). Post-hoc comparisons revealed a significant increase of breaking points in animals treated with MRZ-9547 (50, 100 mg/kg) and a trend for a significant effect in animals treated with d-amphetamine ($p=0.066$). Furthermore, the intake of lab chow differed between treatment groups ($F(3, 36)=13.86, p<0.001$) (Fig. 5c). Accordingly, post-hoc comparisons showed a significant decrease of lab chow intake in animals treated with either dose of MRZ-9547 ($p<0.01$, respectively) relative to vehicle controls, whereas there was only a trend for a significant effect in animals treated with d-amphetamine ($p=0.08$).

Consumption test

As shown in Fig. 5d, the amount of pellets ingested differed between treatment groups ($F(3, 36)=8.67, p<0.001$) and post-hoc comparisons demonstrated a significant decrease of pellet intake in animals treated across drug/doses relative to vehicle control ($p<0.01$, respectively).

Discussion

The main finding in the present study was that MRZ-9547 turned out to be a selective DAT inhibitor that, relative to other DAT interfering drugs including MRZ-9546, markedly stimulated PR responding and shifted effort-related decision making in intact rats towards high effort response options.

MRZ-9547 and MRZ-9546: mechanisms of action

MRZ-9547 was found to be an effective DAT inhibitor, whereas activity of MRZ-9546 was about 4-fold weaker. Moreover, MRZ-9547 seems to be relatively selective as other targets tested were affected only at 30-fold higher concentrations except NET (over 10-fold difference). After i.p. administration, plasma and brain extracellular fluid (ECF) concentrations of MRZ-9547 displayed a dose-dependent and sustained increase up to 120 min. Notably, plasma levels of MRZ-9546 remained elevated for 120 min after i.p. injection. Furthermore, MRZ-9547 at 100 mg/kg only moderately enhanced striatal DA levels relative to baseline (130%), an increase that is comparable to some other DAT inhibitors such GBR12921 (Hurd and Ungerstedt, 1989) or mazindol (Nakachi et al., 1995) but markedly lower than the increase (400%) produced by the DAT inhibitor methylphenidate (10 mg/kg) (Hurd and Ungerstedt, 1989).

![Figure 5](https://example.com/fig5.png)

**Fig. 5.** Effects of MRZ-9547 (50, 100 mg/kg, i.p.) and d-amphetamine (1 mg/kg, i.p.): (a) on lever pressing in a progressive ratio (PR) task, (b) on lever pressing, (c) chow consumption (c) in a PR chow feeding choice task and (d) on pellet intake in a consumption test. (Means±S.E.M, *p<0.05,* **p<0.01,** different from vehicle controls; in Fig. 1b: d-amphetamine vs. vehicle control, $p=0.06$; in Fig. 1c: d-amphetamine vs. vehicle control, $p=0.08$, Dunnett test).
Drug effects in the PR task

Across experiments, MRZ-9547 consistently and dose-dependently increased the last ratio achieved. Relative to MRZ-9547, the ability of MRZ-9546 to increase the maximal ratio was considerably less pronounced. Consistent with these findings, MRZ-9547 (5–50 mg/kg, i.p.) enhanced locomotor activity to a markedly higher degree than MRZ-9546 (5–50 mg/kg, i.p.) (Zvejniece et al., 2011). Of note, perseverative lever presses and response latencies were not altered by MRZ-9547 and MRZ-9546 suggesting that animals were sensitive to the ratio requirements of the operant schedule and that drugs did not induce motor effects interfering with reward approach. By contrast, modafinil did not alter the last ratio achieved and other performance measures. Modafinil at doses used here produced a large variety of neurochemical and behavioral effects (Eagle et al., 2007), e.g. a reduced food intake (Nicolaidis and De Saint Hilaire, 1993). Consistent with latter observation, in the consumption test (Experiment 4) modafinil (64 mg/kg) reduced food intake suggesting that the dose was behaviorally active. In other words, inappropriate dosing might not account for the lack of effect of modafinil on the last ratio achieved. Moreover, methylphenidate at 10 mg/kg enhanced the last ratio achieved confirming earlier evidence that methylphenidate at a similar dose enhanced breakpoints (Mayorga et al., 2000) By contrast, d-amphetamine (1 mg/kg) had only very subtle effects on the last ratio achieved. This finding is difficult to compare with the literature as earlier studies reported mixed effects in PR tasks, i.e. increased (Poncelet et al., 1983) and decreased (Mobini et al., 2000) breakpoints. Overall, our data are largely consistent with earlier reports (Poncelet et al., 1983) and demonstrate that DAT-interacting drugs can increase in PR tasks the tendency to work for reinforcement to variable degrees. Importantly, breakpoints in PR tasks are not simply a measure of ‘reward’, but predominantly a measure of how much effort an animal will invest to gain access to a reinforcer (Stewart, 1975). DA-related mechanisms, in particular in the nucleus accumbens, are crucial to mediate the tendency to work for reinforcement in PR tasks (Zhang et al., 2003). For instance, DA-related signal transduction activity in the nucleus accumbens, i.e. pDARPP-32(Thr34) expression, was greater in rats with high vs. low lever pressing in a PR task suggesting that accumbens signal transduction activity is related to individual differences in work output (Randall et al., 2012). These findings indicate that a stimulation of mesoaccumbens DA systems may be one likely neurochemical substrate of MRZ-9547 to amplify PR responding.

Drug effects in the PR/chow feeding choice task

Across experiments, MRZ-9547 dose-dependently and consistently inclined rats to select the increasingly effortful type of food-seeking behavior in presence of a low effort response option. In vehicle controls across all experiments, the maximum ratio achieved in the PR/chow feeding choice task was considerably lower than the one achieved in the PR task, probably because less preferred lab chow is freely available. In other words, choosing how much effort to exert under a PR schedule is influenced by the presence or absence of a low effort response option. This effect was also observed in animals treated with 25 and 50 mg/kg MRZ-9547, but not in animals treated with 100 mg/kg MRZ-9547. In other words, cost/benefit-related decision making in animals subjected to low and intermediate doses of MRZ-9547 was sensitive to the presence of a low-cost response option. As pellet intake under MRZ-9547 at 50 and 100 mg/kg was reduced in the consumption test, the enhanced preference for the effortful response option under a PR schedule may not be related to a drug-induced increase of the palatability or incentive value of the pellets. Reduced pellet intake in the consumption test suggests that MRZ-9547 may have appetite suppressant effects as observed with other DAT-interfering drugs, e.g. amphetamine (Holtzman and Jewett, 1971), methylphenidate (Bello and Hajnal, 2006) or modafinil (Nicolaidis and De Saint Hilaire, 1993). In a related PR/chow feeding choice task, a putative appetite suppressant drug, i.e. the cannabinoid CB1 antagonist/inverse agonist AM251, as well as pre-feeding decreased both pellet reinforced lever pressing and lab chow consumption (see Randall et al., 2012 for a detailed discussion). Thus, the effects of appetite suppressant manipulations and DAT interfering drugs with appetite suppressant activity differ markedly in this task suggesting that other factors than appetitive suppression have a major influence on choice behavior. Of note, in the choice test, the total gram amount of pellets and lab chow ingested by rats treated with the highest doses of MRZ-9547, modafinil, methylphenidate or amphetamine did not differ significantly from respective vehicle controls, but in MRZ-9547- and d-amphetamine-treated rats the relative amount of pellets ingested was significantly higher (data not shown). Furthermore, it should be pointed out that, in vehicle controls of Experiment 5, food intake in the choice and consumption test, for unknown reasons, was only moderate. However, as in our other experiments, the last ratio achieved in the choice test was markedly lower than in the PR test, thus reduced chow intake in vehicle controls of Experiment 5 might not have altered their choice behavior.

MRZ-9546 was markedly less active than MRZ-9547 as measured by the maximal ratio achieved in the choice test, whereas modafinil was not effective. By contrast, methylphenidate increased this measure, but seemingly did not reduce the amount of lab chow ingested, an artifact that mainly reflects reduced lab chow ingestion in respective vehicle controls (see also above). Consistent with earlier findings in an effort-related choice task (Cousins et al., 1994), d-amphetamine at the dose tested only moderately increased the last ratio achieved.
Collectively, these data show that among the DAT-interfering drugs tested, MRZ-9547 most potently increased the tendency to work for reinforcement, whereas MRZ-9546 and methylphenidate were markedly less effective. Given that accumbens DA transmission exerts a powerful influence over effort-related choice behavior (Salamone et al., 2012), a stimulation of mesoaccumbens DA systems may be one likely neurochemical substrate of MRZ-9547 to modulate cost/benefit related decision making. Considerable evidence suggests that adenosine A2A receptor antagonists were able to reverse a shift from lever pressing to chow intake induced by concomitant systemic blockade of DA receptors or DA depletion (Salamone and Correa, 2012). Furthermore, the antidepressant bupropion, a drug that can inhibit catecholamine uptake, reversed a shift from lever pressing to chow intake after a tetrabenazine-induced DA depletion (Nunes et al., 2013). Furthermore, in a similar PR/chow choice task as used here, systemic administration of the adenosine A2A receptor antagonist MSX-3 inclined intact rats to select the increasingly more effortful type of food-seeking behavior and decreased chow consumption, an effect that might rely in particular on functional interactions of DA and adenosine systems in the nucleus accumbens (Randall et al., 2012). Relative to MRZ-9547, MSX-3 appears to be less potent to increase the last ratio achieved. However, respective comparisons are limited, e.g., because PR schedules in respective PR/ free feeding choice tasks differ. Thus, a comparative evaluation of drug effects in the same task is required.

**DAT ligands and PR responding**

Overall, these experiments demonstrate corresponding effect sizes of DAT-interfering drugs on the tendency to work for food reinforcement in two related tasks; however, the influence of a given drug on task performance was strikingly different. Because of their effects on extracellular DA levels, one might assume that DAT-interacting drugs would elicit comparable behavioral effects. Yet considerable evidence suggests that this notion is not correct for a number of reasons. First, DAT ligands with distinct chemical structures induce specific conformational changes in the transporter protein that can be differentially transduced by cells, ultimately inducing a unique profile of behavioral effects (Schmitt et al., 2013). Furthermore, depending on the dose, the pattern and magnitude of DA release induced by a given DAT ligand in distinct DA terminal regions can differ considerably (Koda et al., 2010) highlighting the complex behavioral pharmacology of DAT ligands. Although it is conceivable that MRZ-9547, and to a much lesser extent MRZ-9546 and methylphenidate, might invigorate PR responding by interfering with mesoaccumbens DA systems, the specific neurochemical and behavioral mechanisms underlying the particularly prominent effects of MRZ-9547 are yet unclear. Relative to methylphenidate (Koda et al., 2010), the impact of MRZ-9547 on striatal extracellular DA concentrations demonstrated by microdialysis was only moderate implying that there is no simple relationship between DAT ligand evoked DA release and PR responding.

**Clinical significance**

An analysis of effort-related decision making in rodents may not only be important for an understanding of the neural and neurochemical basis of motivational processes but could also provide an animal model for motivational dysfunctions related to effort expenditure (Salamone and Correa, 2012) such as fatigue, apathy, anergia and psychomotor slowing in neuropsychiatric and neurological disorders, e.g., major depression, schizophrenia, multiple sclerosis or inflammatory disease (Friedman et al., 2011; Salamone and Correa, 2012). For instance, in a test of effort-related choice in humans, patients with major depressive disorders were less willing to expend effort for rewards than controls (Treadway et al., 2012). Fatigue and anergia are also common in Parkinson’s disease and may be even more deleterious to quality of life than motor dysfunction (Friedman et al., 2011). Current treatment options rarely cause significant improvements in fatigue (Kluger et al., 2013) and the few available studies on the pharmacotherapy of fatigue in Parkinson’s disease indicate that only methylphenidate had some beneficial effects, whereas L-DOPA had only minimal, modafinil and amphetamine no beneficial effects (Friedman et al., 2011). Our findings indicate that, relative to methylphenidate, MRZ-9547 was able to markedly invigorate progressive ratio responding in rodents and, therefore, could be potentially useful for treating energy-related symptoms such as fatigue or anergia in Parkinson’s disease and possibly other neurological or neuropsychiatric disorders discussed above.

It is well known that DAT ligands such as cocaine are reinforcing (Ritz et al., 1987), hence, a possible concern is their addictive liability. For instance, human imaging studies revealed a relationship between reinforcing effects and degree of DAT blockade by cocaine and methylphenidate after intravenous injection (Volkow et al., 1997). However, there are also DAT inhibitors that show little or no reinforcing properties (Desai et al., 2005; Volkow et al., 2005; Li et al., 2011). Although MRZ-9547 in the racemic form, known as phenotropil, has been examined in human studies (Malykh and Sadaie, 2010), reports on its addictive liability are, to the best of our knowledge, not available. To be reinforcing in humans, DAT inhibitors have to block >50% of DAT in less than 15 min and clear out of the brain rapidly to enable fast repeated administration (Volkow et al., 2005). Our pharmacokinetic data in rats suggest that, relative to cocaine extracellular brain levels (Bradberry et al., 1993; Pettit and Pettit, 1994), MRZ-9547 extracellular brain levels after i.p. administration increased slower and remained elevated...
longer pointing to lower reinforcing effects. However, detailed studies addressing this issue are warranted.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145714000996

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Statement of Interest

WD, RH and BV are employees of Merz Pharmaceuticals, GF is an employee of Brains On-Line. B.V., WH received compensation as a consultant for Merz Pharmaceuticals, SS declares no potential conflict of interest.

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