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# The role of 5-$HT_{2A}$ receptor antagonism in amphetamine-induced inhibition of A10 dopamine neurons in vitro

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**Abstract**

The role of the 5-$HT_{2A}$ receptor in modulating amphetamine-induced inhibition of dopamine neuronal firing in A9 and A10 was investigated in rat midbrain slices. The antipsychotic drugs olanzapine and clozapine more potently reversed the amphetamine-induced inhibition in A10 neurons compared to A9 neurons. Risperidone (0.03 and 0.1 μM) reversed amphetamine-induced inhibition of firing activity similarly in A9 and A10. The dopamine D2 receptor antagonist (−)sulpiride (0.05 and 1 μM) reversed the amphetamine (10 μM)-induced inhibition of firing activity in A9 and A10 neurons. The selective 5-$HT_{2A}$ receptor antagonist MDL100907 (0.05 μM), strongly enhanced the reversal of amphetamine-induced inhibition by (−)sulpiride in A10, but its effectiveness was much smaller in A9 dopamine neurons.

We conclude that 5-$HT_{2A}$ receptor antagonism enhanced reversal of amphetamine-induced inhibition by dopamine D2 antagonism in A10, suggesting that dopamine $D_2$ receptor antagonism combined with 5-$HT_{2A}$ receptor antagonism may play a role in antipsychotic drug atypicality.


*Keywords:* Substantia nigra; Ventral tegmental area; Serotonin; Extracellular recording; Antipsychotic drug

## 1. Introduction

Increased activity of the mesoaccumbal dopamine circuitry originating in the ventral tegmental area (A10) has been hypothesized to underlie some of the symptoms of schizophrenia (Arnt and Skarsfeldt, 1998; Pani, 2002). Antipsychotic drugs used to treat schizophrenia also suppress the nigrostriatal system originating in the substantia nigra pars compacta (A9) and have been implicated in extrapyramidal side-effects (Crocker and Hemsley, 2001; Grace et al., 1997; Wong and Van Tol, 2003). The more recently developed atypical antipsychotic drugs such as clozapine and olanzapine have a broader range of receptor affinities and produce less extrapyramidal side-effects compared to the classical antipsychotic drugs (Conley and Kelly, 2002; Grace et al., 1997; Kapur and Remington, 2001; Tarsy et al., 2002). Consequently, much effort has been invested in the development of new antipsychotic drugs that only possess therapeutic efficacy without the unwanted extrapyramidal side-effects. The main approach has been to specifically target the A10 system, avoiding effects on the A9 system.

Amphetamine-induced inhibition of dopamine neurons is used as an in vivo model to determine potential antipsychotic activity (Ellenbroek and Cools, 2000; Stockton and Rasmussen, 1996). Amphetamine, a potent central nervous system stimulant drug with psychotomimetic properties, elevates extracellular dopamine by promoting non-vesicular dopamine release (via the dopamine transporter) and blocking dopamine re-uptake (Byrnes and Wallace, 1997; Jones et al., 1999; Sulzer et al., 1995). The increased extracellular dopamine level inhibits dopamine neuron firing activity via dopamine D2 auto receptors. Reversal of amphetamine-induced inhibition of dopamine neurons is a common property of clinically effective antipsychotic drugs (Bunney et al., 1973; Goldstein et al.,

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1993; Stockton and Rasmussen, 1996; White and Wang, 1983). This property is used as a test to predict therapeutic efficacy (reversal in A10) and extrapyramidal side-effects liability (reversal in A9). In this model classical antipsychotic drugs reverse amphetamine-induced inhibition in both A9 and A10 dopamine neurons, while atypical antipsychotic drugs tend to reverse amphetamine-induced inhibition more potently in A10 than in A9 dopamine neurons. Reversal of amphetamine-induced inhibition by antipsychotic drugs is explained by their dopamine D2 receptor antagonistic properties. However, it is unclear why atypical antipsychotic drugs reverse amphetamine-induced inhibition more potently in A10 dopamine neurons. Atypical antipsychotic drugs also have affinity for the 5-$HT_{2A}$ receptor (Arnt and Skarsfeldt, 1998), therefore it is hypothesized that this receptor is a contributing factor in the observed difference in reversal. This is supported by the observations that 5-$HT_{2A}$ receptor activation can modulate the activity of A10 dopamine neurons (Brodie and Bunney, 1996; Doherty and Pickel, 2000; Olijslagers et al., 2004; Sorensen et al., 1992), while such a role was not observed in A9 dopamine neurons.

In this study we determined if 5-$HT_{2A}$ receptor antagonism could affect reversal of amphetamine-induced inhibition by a dopamine $D_2$ receptor antagonist in vitro in a rat midbrain slice preparation.

## 2. Material and methods

### 2.1. Preparation of brain slices

After decapitation (male Wistar rats; 75–100 g; Harlan, Zeist, The Netherlands) the brain was quickly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl 120, KCl 3.5, $MgSO_4$ 1.2, $NaH_2PO_4$ 1.25, $CaCl_2$ 2.5, D-glucose 10, $NaHCO_3$ 25, ascorbic acid 1 and gassed with a mixture of 95% $O_2$ and 5% $CO_2$. A tissue block was prepared from the brain and coronal slices (350 μm thick) containing both the A9 and the A10 region were cut in ice-cold aCSF with a vibratome (model VT1000S, Leica). Immediately after cutting, the slice was transferred to warm aCSF (35 °C) for 20 min, where after it was stored at room temperature for later use (Werkman et al., 2001). For recording, the slice was transferred to a recording chamber (volume ≈ 1 ml), which was continuously perfused with aCSF (≈2 ml/min) and held at 35 °C. After approximately 30 min of equilibration, extracellular recordings of dopamine neurons in the A9 and the A10 region started.

### 2.2. Extracellular recordings

Extracellular recordings were made with electrodes that were pulled with a micropipette puller (Brown/Flaming P-87; Sutter Instruments, CA, USA) from thin-wall borosilicate glass pipettes (1.5 mm outer diameter, Science Products, Hofheim, Germany) and filled with aCSF. One electrode was placed in A9 and another one in A10 at positions where they each recorded a spontaneously active dopamine neuron. The following criteria had to be fulfilled before a neuron was considered dopaminergic (Olijslagers et al., 2004; Werkman et al., 2001): (i) a regular firing pattern (0.5–8 Hz); (ii) a broad (>2 ms), triphasic action potential; and (iii) quinpirole sensitivity to a concentration below 0.3 μM, resulting in cessation of action potential firing. Neurons in A9 and A10 that fulfill the electrophysiological criteria listed above have previously been identified by immunocytochemistry as dopamine-containing (Grace and Onn, 1989). The extracellular signals were high-pass filtered at 300 Hz and digitized at 4 kHz with an ADC converter under control of a personal computer for off-line analysis.

### 2.3. Drugs

Unless otherwise mentioned drugs were obtained from Solvay Pharmaceuticals (Weesp, The Netherlands). Stock solutions were prepared as follows: 10 mM (−)sulpiride, 10 mM clozapine, 10 mM olanzapine, 10 mM risperidone in 0.01 M HCl. MDL100907 (4-piperidinemethanol) stock (5 mM) was made in dimethylsulfoxide (DMSO). Quinpirole (10 mM, Sigma, St. Lois, MO, USA) and amphetamine (1 mM, Duchefa Farma BV, Haarlem, Netherlands) stock solutions were made in $H_2O$. The solutions were diluted to the final concentrations in aCSF and applied to the slices by superfusion. DMSO concentration never exceeded 0.01%.

### 2.4. Data analysis

An analysis program running on the personal computer detected action potentials by means of template matching. This emphasized the characteristic shape of the extracellular action potentials of the dopamine neurons and allowed quantification of the neuronal activity even if gradual variations in extracellular action potential amplitude occurred. The times of occurrence of action potentials during control periods and during drug applications were determined and used to calculate the firing rate (spikes/s) in bins of 5 s. The mean baseline firing rate of each neuron was determined for at least 2–3 min. Amphetamine-induced reduction of the firing rate in respect to the baseline level was calculated for each neuron. The $IC_{50}$ values were calculated using the fits to the logistic equation of the form:

$$F(C) = \frac{F_0}{1 + (C/\mathrm{IC}_{50})^h}$$

in which $F(C)$ is the firing rate at concentration $C$, $F_0$ the mean baseline firing rate, $IC_{50}$ the concentration that induces

50% reduction in firing rate and *h* is a shape factor analogous to the Hill coefficient, which was set to 1 in all fits.

Values are given as mean±standard error of the mean (S.E.M.) and "n" represents the number of neurons recorded. Statistical comparisons between multiple groups were performed using an analysis of variance (ANOVA) and the post hoc Dunn's Bonferroni test; $P<0.05$ was assumed to indicate a significant difference.

## 3. Results

### *3.1. Electrophysiological properties of A9 and A10 dopamine neurons*

Only neurons that complied with the criteria mentioned in the Methods section, including a complete cessation of the firing on the application of quinpirole (0.3 μM), were used for further experimentation.

The mean baseline firing rate of the dopamine neurons in A9 (mean 2.4 spikes/s, standard deviation 1.0 spikes/s, $n=74$) was higher than that of dopamine neurons in A10 (mean 1.6 spikes/s, standard deviation 1.1 spikes/s, $n=66$, $P<0.001$). This difference confirmed earlier findings (Werkman et al., 2001).

### *3.2. Amphetamine-induced inhibition in A9 and A10 dopamine neurons*

Amphetamine was applied cumulatively in three concentrations (1, 10 and 50 μM; 7 min per concentration, Fig. 1A). The mean firing rate was determined for each concentration and its value normalized to control was plotted as a function of the amphetamine concentration (Fig. 1B). The logistic equation was fitted to the mean data points and the $IC_{50}$ values were calculated. The $IC_{50}$ value obtained for dopamine neurons in A9 ($IC_{50}=8.8\pm3.4$ μM, $n=8$) was similar to the value obtained for neurons in A10 (8.9±1.9 μM, $n=7$, Fig. 1B).

To determine the stability of the amphetamine-induced inhibition a time course experiment was performed. Amphetamine was applied at a single concentration (10 μM) for 30 min and the mean firing activity was determined for 3-min periods. In dopamine neurons from A9 the firing rate was reduced by amphetamine to 22±9% of baseline value ($n=7$, $P<0.001$) while in A10 the reduction amounted to 33±12% of baseline value ($n=9$, $P<0.001$) (Fig. 2), which was not different from A9. The amphetamine-induced inhibition remained stable within 8% throughout the 30 min application period (Fig. 2C,D).

### *3.3. Reversal of amphetamine-induced inhibition by antipsychotic drugs*

In the next series of experiments we investigated whether antipsychotic drugs could interfere with the amphetamine-induced inhibition of firing activity demonstrated in the previous paragraph. The slices were first perfused with aCSF (for 3 min), followed by perfusion with 10 μM amphetamine. During this perfusion with amphetamine, two cumulative concentrations of the antipsychotic drug were applied (4 min per concentration). The first (low) concentration was applied 6 min after the start of amphetamine application, at which time the amphetamine-induced inhibition had reached a stable level.

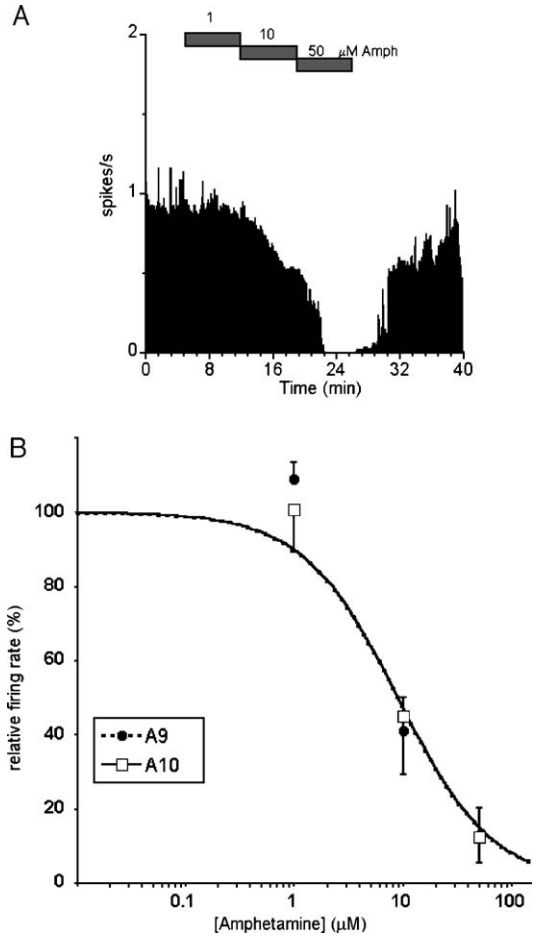



Fig. 1. Amphetamine-induced inhibition of firing activity in A9 and A10 dopamine neurons. (A) Recording of an A9 dopamine neuron. The firing rate was in a concentration-dependent way inhibited during cumulative application of the indicated amphetamine (AMPH) concentrations (7 min per concentration). (B) Normalized firing rate as a function of concentration in A9 and A10 dopamine neurons. Points represent the mean, vertical bars S.E.M. The $IC_{50}$ values were in A9: 8.8±3.4 μM ($n=8$) and in A10: 8.9±1.9 μM ($n=7$).

#### *3.3.1. (−)Sulpiride*

(−)Sulpiride is a selective dopamine D2 receptor antagonist and was used as a representative of the typical antipsychotic drug class. The low concentration (−)sulpiride (0.05 μM) significantly reversed the amphetamine-induced inhibition of A9 dopamine neurons back to 100±10% of baseline firing (compared to amphetamine,

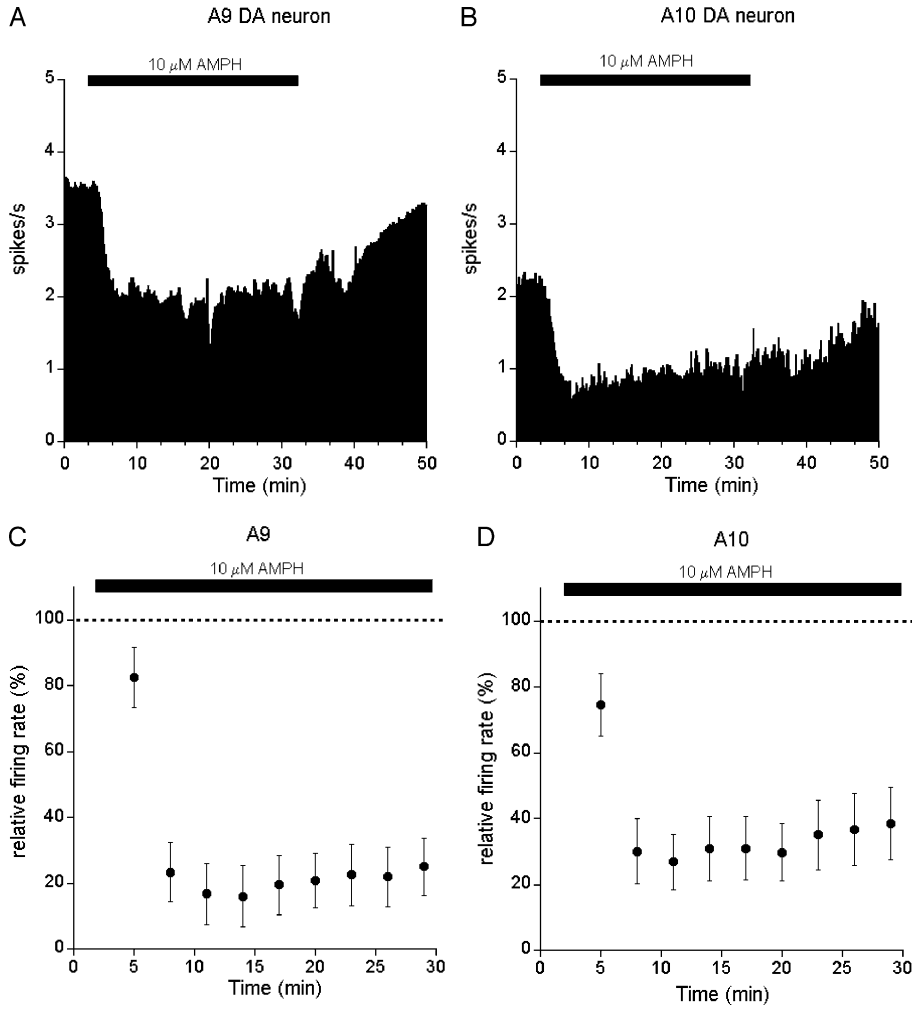



Fig. 2. Time-course of amphetamine-induced inhibition of A9 and A10 dopamine neuron firing rate. Examples of a recorded A9 dopamine neuron (A) and an A10 dopamine neuron (B), during a 30-min perfusion period with 10 μM amphetamine (AMPH). (C) The mean inhibition of A9 dopamine neuron firing activity induced by 30 min of amphetamine application, determined over 3-min intervals ($n=12$). (D) The mean amphetamine-induced inhibition of A10 dopamine neuron firing activity ($n=9$). Bars indicate S.E.M.

$P<0.01$). In dopamine neurons from A10 the amphetamine-induced inhibition was reversed to 93±8% of baseline firing (Fig. 3). The high concentration of 1 μM (−)sulpiride not only reversed the amphetamine-induced inhibition, but it further enhanced firing rate in A9 to 142 ±13% of baseline level ($P<0.01$, compared to amphetamine). In A10 a firing rate of 145±11% could be reached by application of the high concentration of (−)sulpiride (Fig. 3). We could not detect any difference between the effect of (−)sulpiride on dopamine neurons in A9 or those in A10.

### 3.3.2. Interaction of 5-$HT_{2A}$ and dopamine $D_2$ receptor antagonism in reversal of AMPH-induced inhibition

Atypical antipsychotic drugs are not only (weak) dopamine D2 receptor antagonists, but they are also 5-$HT_{2A}$ receptor antagonists. Therefore we tried to determine whether 5-$HT_{2A}$ receptor antagonism could play a role in the difference in reversal in A9 and A10 dopamine neurons induced by atypical antipsychotic drugs that was reported in vivo. The selective 5-$HT_{2A}$ antagonist MDL100907 was applied in the amphetamine-induced inhibition model, either alone or in combination with the selective dopamine D2 antagonist (−)sulpiride.

### 3.3.3. MDL100907

Previously we reported (Olijslagers et al., 2004) that the selective 5-$HT_{2A}$ receptor antagonist MDL100907 does not affect the basal firing rate of dopamine neurons in A9 and A10 (concentrations up to 2 μM). When MDL100907 (0.05 or 1 μM) was applied in the presence of amphetamine, it did not affect the amphetamine-induced inhibition of firing activity (Fig. 4A). In the presence of 0.05 μM MDL100907 and amphetamine, the firing activity was 26±8% of baseline level in A9 ($n=8$) (not different from amphetamine alone: 25±8% of baseline), while in A10 these values were 11±7% and 12±8%, respectively ($n=7$).

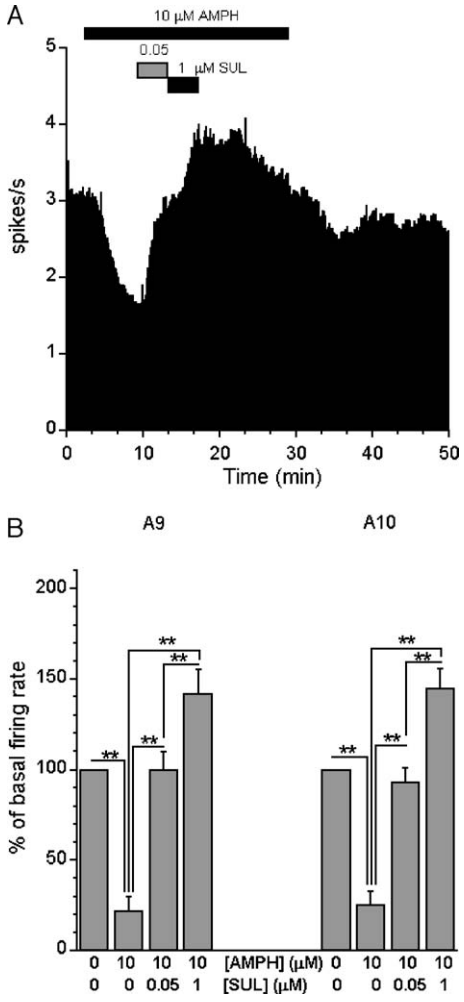



Fig. 3. Reversal of amphetamine-induced inhibition of A9 and A10 dopamine neuron firing activity by the dopamine $D_2$ receptor antagonist (−)sulpiride. (A) Recording of a dopamine neuron in A10. During amphetamine (AMPH) perfusion (10 μM) (−)sulpiride (SUL) was cumulatively applied in two concentrations (0.05 and 1 μM; 4 min per concentration). (B) The low (−)sulpiride concentration already reversed amphetamine-induced inhibition in A9 as well as in A10 (A9, $n=12$; A10, $n=9$). When 1 μM (−)sulpiride was applied, the firing rate exceeded baseline level considerably. Vertical bars indicate S.E.M.; asterisks indicate significance, $^{**}P<0.01$.

### 3.3.4. Combined (−)sulpiride and MDL100907 effects

Next the selective dopamine $D_2$ receptor antagonist (−) sulpiride was used in combination with MDL100907 (0.05 μM) to (partly) mimic the pharmacological profile of atypical antipsychotic drugs. The experimental protocol was as follows: first amphetamine (10 μM) was applied (6 min), followed by amphetamine in the presence of either 0.05 or 1 μM (−)sulpiride for 4 min. Then amphetamine and (−)sulpiride (same concentrations as before) were applied in the presence of 0.05 μM MDL100907 (4 min). In the presence of MDL100907, 0.05 μM (−)sulpiride reversed amphetamine-induced inhibition of A9 dopamine neurons to a level of 98±5% ($n=6$) of baseline firing activity (compared to amphetamine, $P<0.01$), a similar level as the mean reversal by 0.05 μM (−)sulpiride alone (91±6%) (Fig. 4A). In A10 dopamine neurons simultaneous application of 0.05 μM (−)sulpiride and 0.05 μM MDL100907 reversed amphetamine-induced inhibition and further increased firing activity above baseline level to 153±24% ($n=5$). This level was significantly higher compared to the reversal to 96±12% of baseline level observed with 0.05 μM (−)sulpiride alone in A10 dopamine neurons ($P<0.05$) and the reversal by (−)sulpiride and MDL100907 in A9 dopamine neurons ($P<0.05$) (Fig. 4A).

In A9 neurons, 1 μM (−)sulpiride in the presence of 0.05 μM MDL100907 reversed amphetamine-induced inhibition

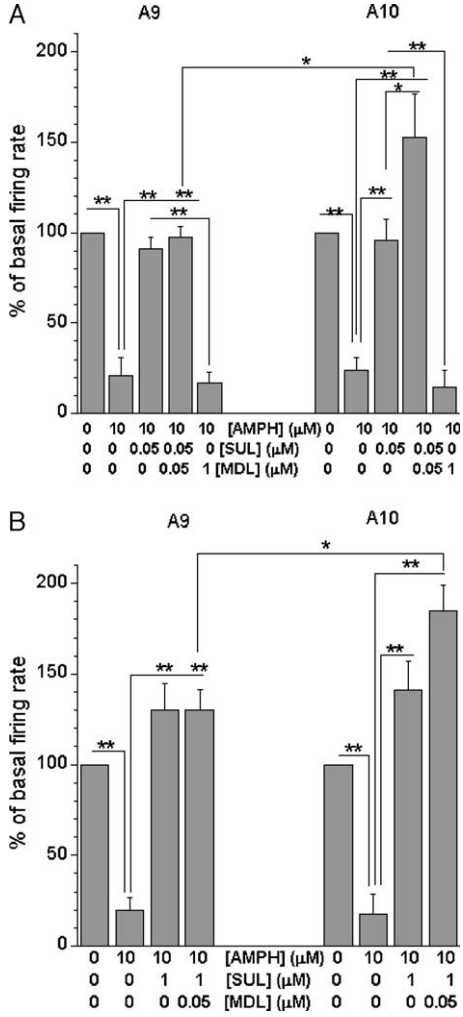
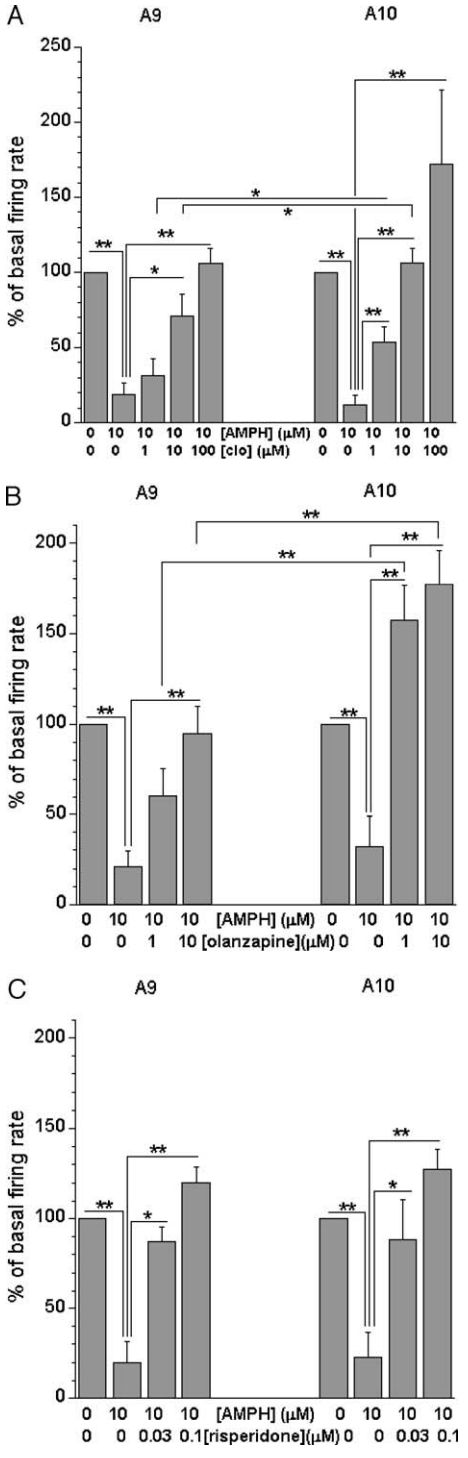



Fig. 4. Reversal of amphetamine-induced inhibition of A9 and A10 dopamine neuron firing activity by (−)sulpiride and the 5-$HT_{2A}$ receptor antagonist MDL100907. During amphetamine (−)sulpiride (SUL) was applied alone, or in the presence of MDL100907 (MDL, 0.05 μM). (A) Reversal of amphetamine (AMPH)-induced inhibition of firing activity by 0.05 μM (−)sulpiride and MDL100907. In the presence of 0.05 μM MDL100907 the reversal induced with (−)sulpiride exceeded baseline level in A10, but not A9 dopamine neurons (A9, $n=6$; A10, $n=5$). A high concentration of MDL100907 (1 μM) alone did not reverse the amphetamine-induced inhibition. (B) The experiment of A now for the (−) sulpiride concentration of 1 μM. Vertical bars indicate S.E.M. (A9, $n=6$; A10, $n=6$). Asterisks indicate significance; $^{*}P<0.05$, $^{**}P<0.01$.

and further increased firing activity above baseline to 130 ±13% (compared to amphetamine, $P<0.01$, $n=6$), a level not different from what was attained by 1 μM (−)sulpiride alone (130±11%) (Fig. 4B). In A10 dopamine neurons, 1 μM (−)sulpiride in combination with 0.05 μM MDL100907 in the presence of amphetamine increased firing activity to 185±14% ($n=6$) of baseline level, which was higher ($P<0.05$) than the reversal by 1 μM (−)sulpiride and MDL100907 in A9 (Fig. 4B), but the difference with the reversal induced by 1 μM (−)sulpiride alone did not reach significance in A10 dopamine neurons.




### 3.3.5. Clozapine

The atypical antipsychotic drug clozapine was applied in concentrations of 1, 10 and 100 μM during perfusion with amphetamine. In A9 dopamine neurons, amphetamine-induced inhibition of firing activity was not reversed by 1 μM clozapine (31±11% of baseline level, $n=10$). In A10 dopamine neurons, 1 μM clozapine did partly reverse amphetamine-induced inhibition (53±10%, $n=9$, compared to amphetamine-induced inhibition, $P<0.01$) (Fig. 5A). 10 μM clozapine reversed amphetamine-induced inhibition in A10 dopamine neurons to baseline firing activity (107±10%, compared to amphetamine, $P<0.01$) (Fig. 5A), while in A9 this concentration of clozapine reversed amphetamine-induced inhibition to only 71±15% (compared to amphetamine, $P<0.01$). The reversal that was induced by clozapine in A10 dopamine neurons was significantly larger than the reversal induced in A9 dopamine neurons for both concentrations used ($P<0.05$). A few cells (A9, $n=3$ and A10, $n=3$) were also tested with 100 μM of clozapine, to see if this concentration could reverse amphetamine-induced inhibition to baseline level in A9 dopamine neurons and/or exceed baseline level. In A9 dopamine neurons, 100 μM clozapine did reverse amphetamine-induced inhibition to baseline level (106±10%, $P<0.05$). In A10 dopamine neurons a trend towards reversal of amphetamine-induced inhibition exceeding baseline was observed (172±50%).

### 3.3.6. Olanzapine

An atypical antipsychotic drug that closely resembles the pharmacological profile of clozapine is olanzapine. In the presence of 1 μM olanzapine, amphetamine-induced inhibition in A10 dopamine neurons was reversed ($P<0.01$) and firing activity further increased to 158 ±19% above baseline ($n=5$), while in A9 no reversal of

Fig. 5. Reversal of amphetamine-induced inhibition of A9 and A10 dopamine neuron firing activity by antipsychotic drugs. (A) The atypical antipsychotic drug clozapine (A9, $n=10$; A10, $n=9$) was applied in concentrations of 1, 10 and 100 μM. In A10 dopamine neurons, the reversal of amphetamine-induced inhibition reached and exceeded baseline level (set at 100%) when 10 and 100 μM ($n=3$) clozapine were used, while in A9 dopamine neurons only 100 μM clozapine reversed inhibition to baseline level ($n=3$). The reversal induced by clozapine was larger in A10 than in A9 for 1 and 1 μM clozapine. (B) Reversal of amphetamine-induced inhibition by 1 and 10 μM olanzapine (A9, $n=9$; A10, $n=4$). In A10 dopamine neurons, the reversal of amphetamine-induced inhibition exceeded baseline level (set at 100%), while in A9 dopamine neurons only 10 μM olanzapine reversed inhibition. The reversal induced by olanzapine was larger in A10 than in A9. (C) Reversal of amphetamine-induced inhibition by 0.03 and 0.1 μM risperidone (A9, $n=5$; A10, $n=5$). Risperidone reversed amphetamine-induced inhibition in A9 and A10 dopamine neurons for both concentrations used. The reversal exceeded the baseline level in A9 and A10 dopamine neurons when 0.1 μM risperidone was used. Vertical bars indicate S.E.M. Asterisks indicate significance; *$P<0.05$, **$P<0.01$.

amphetamine-induced inhibition was observed (Fig. 5B). When 10 μM olanzapine was applied, amphetamine-induced inhibition was reversed to 95±12% of baseline activity in A9 dopamine neurons ($P<0.01$, $n=9$). However, in A10 dopamine neurons this concentration of olanzapine significantly reversed amphetamine-induced inhibition and further increased firing activity to 178±19% of baseline level (compared to amphetamine, $P<0.01$, $n=4$) (Fig. 5B). The firing activity that was induced by olanzapine in A10 dopamine neurons was significantly larger than the firing activity induced in A9 dopamine neurons for both concentrations ($P<0.01$) (Fig. 5B).

*3.3.7. Risperidone*

The atypical antipsychotic drug risperidone was used in concentrations of 0.03 and 0.1 μM. In A9 and A10 dopamine neurons the amphetamine-induced inhibition was reversed in the presence of 0.03 μM risperidone to 87±8% ($n=5$), respectively 88±22% ($n=5$) of baseline level (compared to amphetamine, $P<0.05$) (Fig. 5C). Amphetamine-induced inhibition was reversed to exceed baseline level by 0.1 μM risperidone in A9 and A10 dopamine neurons, to 120±11% and 127±11% of baseline level, respectively (compared to amphetamine, $P<0.01$) (Fig. 5C). The effect of risperidone was not different in A9 and A10 dopamine neurons.

## 4. Discussion

5-$HT_{2A}$ antagonism is hypothesized to be a critical feature of atypical antipsychotic drugs, since most atypical antipsychotic drugs not only share antagonism for dopamine D2 receptors, but also antagonism for 5-$HT_{2A}$ receptors (Meltzer et al., 2003). In addition, we and others have found that 5-$HT_{2A}$ and 5-$HT_{2C}$ receptor activation can modulate dopamine neuron firing rate in A10, but not in A9 (Di Giovanni et al., 2000; Di Matteo et al., 1999; Olijslagers et al., 2004). Also, in vivo amphetamine-induced dopamine release can be enhanced by 5-$HT_{2A}$ receptor activation (Ichikawa and Meltzer, 1995; Kuroki et al., 2003; Sorensen et al., 1992). Therefore, 5-$HT_{2A}$ receptor antagonism might contribute to the difference in reversal of amphetamine-induced inhibition of A9 and A10 dopamine neurons by atypical antipsychotic drugs that has been reported in vivo (Bunney et al., 1973; Goldstein et al., 1993; Stockton and Rasmussen, 1996; White and Wang, 1983). To investigate the role of 5-$HT_{2A}$ antagonism in reversal of amphetamine-induced inhibition, we used the selective dopamine D2 receptor antagonist (−)sulpiride in combination with the selective 5-$HT_{2A}$ receptor antagonist MDL100907.

(−)Sulpiride alone potently reversed amphetamine-induced inhibition in dopamine neurons of both A9 and A10, which is in line with in vivo observations on classical antipsychotic drugs (Goldstein et al., 1993; Meltzer et al., 1989). Increasing the (−)sulpiride concentration to 1 μM further increased firing rate to levels significantly above baseline firing. Increasing the firing activity of A9 and A10 dopamine neurons above baseline is an effect of (−)sulpiride due to blockade of the inhibitory effect of endogenously released dopamine. The (−)sulpiride-induced increase in firing rate has been reported previously (Werkman et al., 2001). However, when 0.05 μM (−)sulpiride was combined with the selective 5-$HT_{2A}$ receptor antagonist MDL100907 (0.05 μM), a marked increase to 153% of baseline level was observed, compared to 0.05 μM (−)sulpiride alone (96% of baseline) in A10 dopamine neurons. Interestingly, this marked increase when MDL100907 was present was not observed in A9 dopamine neurons. In contrast, when a higher concentration (1 μM) of (−)sulpiride was used in combination with MDL100907, no difference could be observed between (−)sulpiride alone or (−)sulpiride together with MDL100907 in either A10 or A9 dopamine neurons. Thus it seems that increasing the degree of dopamine D2 receptor antagonism reduces the effect of 5-$HT_{2A}$ receptor antagonism. When we compare these results to the effects of risperidone, we might be able to explain the observation that this atypical antipsychotic drug reverses amphetamine-induced inhibition similarly in A9 and A10 dopamine neurons. Recalling the in vivo observation that atypical antipsychotic drugs reverse amphetamine-induced inhibition more potently in A10 dopamine neurons, risperidone does not behave accordingly to its atypical profile. Since no in vivo results for amphetamine-induced reversal by risperidone are available, we cannot compare our findings. However, from other in vivo models and the clinic, it seems that risperidone has a more narrow therapeutic window compared to other atypical antipsychotic drugs, possibly due to its potent dopamine $D_2$ receptor antagonism (Arnt and Skarsfeldt, 1998; Tarsy et al., 2002). The high dopamine $D_2$ receptor affinity of risperidone ($K_i=0.4$ nM) is similar to the affinity of (−)sulpiride (0.7 nM), which may be the reason why risperidone- and (−)sulpiride-induced reversal seem alike in our experiments. While risperidone also has a high 5-$HT_{2A}$ receptor affinity ($K_i=0.4$ nM) compared to olanzapine and clozapine ($K_i=1.9$ and 4 nM, respectively) (Arnt and Skarsfeldt, 1998; Markowitz et al., 1999), the more potent dopamine D2 receptor affinity compared to clozapine ($K_i=36$ nM) or olanzapine ($K_i=2$ nM) might impact on the 5-$HT_{2A}$ receptor antagonizing effects. This is in line with our observation that after increasing the concentration of (−)sulpiride in combination with MDL100907 we could no longer detect a difference with (−)sulpiride alone in A10 dopamine neurons.

As for the other two atypical antipsychotic drugs, clozapine and olanzapine, a difference in reversal of amphetamine-induced inhibition between A9 and A10 was observed. Olanzapine induced the most pronounced difference in reversal between A9 and A10 dopamine neurons. Olanzapine (1 and 10 μM) clearly induced a strong reversal of amphetamine-induced inhibition and further increased firing activity above baseline level in A10 dopamine

neurons, while in dopamine neurons from A9 the effect of olanzapine was clearly less. These observations are in agreement with in vivo results (Goldstein et al., 1993; Stockton and Rasmussen, 1996). With clozapine a similar pattern of reversal could be observed. 1 μM clozapine reversed amphetamine-induced inhibition in A10 dopamine neurons, but not in A9 dopamine neurons.

We demonstrated previously that 5-$HT_{2A}$ receptor activation enhances dopamine D2 receptor mediated auto-inhibition in A10, but not A9 dopamine neurons (Olijslagers et al., 2004). We hypothesized that the 5-$HT_{2A}$ antagonistic property of atypical antipsychotic drugs prevents the facilitation of (dopamine D2 receptor-mediated) auto-inhibition in A10. This would enable endogenous 5-HT to facilitate auto-inhibition in A9, thus counteracting the increase in firing activity induced by dopamine D2 receptor blockade, which could be reflected by a lower extrapyramidal side-effects liability. In addition, our present results indicate that 5-$HT_{2A}$ receptor antagonism enhances the effect of dopamine D2 receptor antagonism in A10 dopamine neurons, again eventually resulting in a reduced extrapyramidal side-effects liability.

Our results suggest that combining 5-$HT_{2A}$ receptor antagonism with a dopamine D2 receptor antagonist can enhance reversal of amphetamine-induced inhibition in A10 dopamine neurons more easily than in A9 dopamine neurons. This suggests that the 5-$HT_{2A}$ receptor antagonistic property of atypical antipsychotic drugs plays a role in the more potent reversal of amphetamine-induced inhibition in A10 dopamine neurons.

Besides 5-$HT_{2A}$ receptor antagonism, other mechanisms or neurotransmitter receptors could play a role in determining antipsychotic activity. For instance, the antipsychotic drug amisulpride has no affinities for receptors other than dopamine $D_2$ and $D_3$ receptors, while it behaves as an atypical antipsychotic drug (Leucht, 2004; Scatton et al., 1997). It is also known that amphetamine can release other catecholamines, which could affect firing activity of dopamine neurons.

By using an in vitro approach with good pharmacological accessibility, we were able to determine that 5-$HT_{2A}$ receptor antagonism of atypical antipsychotic drugs plays a role in the more potent reversal of amphetamine-induced inhibition in A10 dopamine neurons, suggesting that antipsychotic activity of antipsychotic drugs can be enhanced by 5-$HT_{2A}$ receptor antagonism.

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