**A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter**

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**Abstract**

Antipsychotic drugs are effective interventions in schizophrenia. However, the efficacy of these agents often decreases over time, which leads to treatment failure and symptom recurrence. We report that antipsychotic efficacy in rat models declines in concert with extracellular striatal dopamine levels rather than insufficient dopamine D2 receptor occupancy. Antipsychotic efficacy was associated with a suppression of dopamine transporter activity, which was reversed during failure. Antipsychotic failure coincided with reduced dopamine neuron firing, which was not observed during antipsychotic efficacy. Synaptic field responses in dopamine target areas declined during antipsychotic efficacy and showed potentiation during failure. Antipsychotics blocked synaptic vesicle release during efficacy but enhanced this release during failure. We found that the pharmacological inhibition of the dopamine transporter rescued antipsychotic drug treatment outcomes, supporting the hypothesis that the dopamine transporter is a main target of antipsychotic drugs and predicting that dopamine transporter blockers may be an adjunct treatment to reverse antipsychotic treatment failure.

**Introduction**

Antipsychotic drugs (APDs) are a mainstay in the treatment of schizophrenia. The available APDs exhibit a large spectrum of mechanisms and act on receptors of diverse biogenic monoamine neurotransmitters [1]. However, D\(_2\) receptor blockade is a common property of effective APD medications [2]. It is often suggested that the maximal clinical response is achieved when APDs block ~65–75% of D\(_2\) receptors and is negligible when D\(_2\) receptor occupancy is below this therapeutic window [3, 4]. However, a growing body of evidence appears to cast doubt on the causal effect of this mechanism [5]. Although antipsychotic therapeutic efficacy may appear positively associated with receptor blockade at the beginning of the treatment, APDs exhibit declining therapeutic efficacy with long-term treatment even though their receptor occupancy remains fairly stable. Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) studies define the incremental lack of antipsychotic efficacy as one of the main factors of drug discontinuation, which was observed within 18 months after treatment initiation in 75% of patients [6–8]. Related to this acquired pharmacological resistance, ~20% of all schizophrenia patients will never respond to APDs, even if a consistent D\(_2\) receptor occupancy is maintained at the start.
of the treatment within the therapeutic window [9–11]. Additionally, clinical evidence shows that there is indeed a relationship between early response/no-response and long-term antipsychotic outcomes [12]. While the heterogeneous neurobiology underlying schizophrenia symptoms could lead to multiple antipsychotic responses, the pharmacological mechanisms beyond D2 receptors regulating APD treatment efficacy and failure still await clarification. In this study we bridge this gap by modelling APD treatment efficacy and failure in psychopathologically naïve animals. Specifically, we aimed to recapitulate APD-driven neuroadaptations that can contribute to core clinical issues including long-term lack of efficacy, relapse and APD treatment-resistance. To understand the neurobiology of APDs efficacy and failure, we probed the underlying neurochemical and neurophysiological mechanisms and developed a rational pharmacological intervention to ameliorate the loss of APD effects.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Germany) weighing 250–300 g were used for most of the studies. Male C57Bl/6 6-week old mice (Charles River Laboratories, Germany) were used for the electrophysiology studies only. All experiments were performed in accordance with the Animal Protection Law of the Federal Republic of Germany and the European Communities Council Directive of 24 November 1986 (86/609/EEC), and local authorities approved all study protocols.

Drugs and treatment methods

We used clinical equivalent doses of haloperidol (HAL) and olanzapine (OLA), as described in the supplementary material based on previous work [13, 14]. d-amphetamine (AMPH) and the dopamine transporter (DAT) blocker GBR 12909 were administered intraperitoneally (i.p.). GBR 12909 was also administered locally into the CPu (left and right sides) at 20 µg in 1 µl volume per side, according to previously described procedures [15, 16]. A challenge of 100 mM potassium chloride (K+) was delivered for 80 min to the medial prefrontal cortex (mPFC), caudate-putamen (CPu), and nucleus accumbens (NAcc) via reverse dialysis, as previously described [17]. Full methodological procedures are described in the supplementary material.

Behavior

Locomotion, acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) were recorded prior to and after either vehicle (veh) or AMPH (3 mg/kg) injections or in response to a tail-pinch (TP). We monitored the ability of HAL (2 and 14 days) and OLA (2, 6, 14, and 21 days) to inhibit the AMPH-induced behavioral disruptions. Additional methodological details are described in the supplementary material.

Microdialysis and behavior

Microdialysis studies were conducted with behavioral analyses according to previous protocols [13, 14]. Particularly, we investigated the efficacy of HAL (6 and 14 day) and OLA (2, 6, and 21 days) to inhibit dopamine release and locomotion induced by a TP. We also measured the effect of systemic and intrastratal (according to previous procedures [15, 16]) GBR12909 injections in the reversal of HAL treatment failure. Further details are described in the supplementary material.

Western blot analysis and PCR studies

DAT, serotonin (SERT), and noradrenaline transporters (NET) and tyrosine hydroxylase (TH) protein expression levels were measured using standard Western blot analysis in brain samples from rats treated with chronic veh or HAL (see Methods section in supplementary material and Figure S4). We also measured DAT expression levels after OLA treatment (supplementary Figure S5). Brain tissue was acquired at 2, 6, and 14 days of treatment, and the regions of interest (CPu, NAcc, and PFC) were rapidly dissected on ice, according to previous methods [18]. We also determined the nigrostriatal and mesolimbic DAT mRNA expression after veh or HAL treatment. The DAT mRNA expression levels were quantified in freshly dissected portions of striatum using a standard reverse transcription-coupled quantitative real-time PCR (qRT-PCR) procedure and the primer pairs DAT-for-AGCTACCATGCCCTATTGTGG and DAT-rev-ATCAGCACTCCAAACCCAAC (see supplementary material for details).

MicroPET brain study

We determined striatal dopamine D2/3 receptor and DAT availability according to previously described methods using [18F]fallypride [19] and [18F]FP-CMT [20], respectively (see details in the supplementary material). To be conform with the literature we will use “D2,” instead of “D2/3” receptors. We measured striatal dopamine D2 receptor availability after 14 days of treatment with veh or HAL. Image reconstruction was performed as described previously [20, 21], and parametric maps of the binding potential (BPND) were calculated using the simplified reference tissue method (SRTM) [22]. We also measured
the DAT density availability at baseline and on day 14 of HAL treatment (follow-up). Pairs of BFND maps were calculated relative to the cerebellum TAC, as documented in our previous characterization of this DAT ligand [20, 23].

Brain slice electrophysiology study

Veh- or HAL-treated C57BL6 mice were anaesthetized with sevoflurane, and brain slices (250–300 μm) containing the midbrain or dorsal striatum were prepared. Whole-cell recordings of visualized neurons in the substantia nigra pars compacta (SNc) were performed as previously described [17] (see details in the supplementary material).

Neurophotonic study

Primary hippocampal neurons were cultured and transfected with synapto-pHluorin (spH). Synaptic vesicle exocytosis in HAL and veh treatment groups was recorded using a fluorescence microscope as previously described [17]. Further details are described in the supplementary material.

Statistical analyses

Data analyses and a summary of the results are reported in the supplementary material.

Results

Behavioral expression of antipsychotic treatment efficacy and failure

We compared the efficacy of short- (2–6 days) and long-term (6–21 days) treatment with HAL (0.5 mg/kg/d) or OLA (10 mg/kg/d) in the inhibition of AMPH-induced hyperlocomotion and reversal of sensorimotor gating deficits in the PPI of the startle reflex test in rats. To prevent potential experimental biases related to the use of AMPH, we also measured the time-course of APD efficacy in a non-pharmacological model based on the hyperlocomotion induced by a TP. TP stimulates locomotor activity and promotes striatal dopamine release [24], which mimics the effects of AMPH. We found that short-term, but not chronic, HAL and OLA treatments at clinically relevant doses reversed the PPI deficit and blocked AMPH- and TP-induced hyperlocomotion, thus showing the efficacy of APD after short-term treatment and its declining efficacy with long-term treatment in all tests in rats (Fig. 1a–h). These results suggest that the loss of efficacy is a robust consequence of long-term APD treatment in animal models. No clear signs of abnormal motor behavior were observed during daily inspection throughout treatment periods.

D2 receptor binding and extracellular dopamine levels during antipsychotic treatment failure

The occurrence of relapse during APD treatment of schizophrenia is attributed to an excessive potentiation of dopaminergic neurotransmission (dopamine supersensitivity) [25–28]. We hypothesized that concerted changes in receptor density [25, 29–31] and/or sensitivity [27, 32] may reduce APD occupancy at D2 receptors to below the therapeutic threshold. We measured the in vivo availability of striatal D2 receptors using positron emission tomography (PET) with [18F]fallypride in rats undergoing 14 days HAL treatment, i.e., during the HAL loss of efficacy, to investigate this hypothesis. Animals were not challenged with AMPH or TP and the decreased efficacy of HAL in this group of animals was based on the results of the behavioral testing of independent subjects described above. We found that ~69% of striatal D2 receptors were occupied by HAL (Fig. 2a), which indicates that the loss of HAL efficacy observed in the behavioral tests might occur despite constant and significant D2 receptor occupancy by HAL.

We also found that extracellular dopamine levels in rat CPU decreased with prolonged HAL and OLA treatments (Fig. 2b–g) and that individual dopamine levels correlated with the dopamine response to a TP stimulus (Fig. 2h, i). These results demonstrate that APD efficacy in the rat declines in concert with extracellular dopamine levels but not insufficient D2 receptor occupancy by APDs.

Dopamine synthesis, release and clearance capacity during antipsychotic treatment efficacy and failure

Extracellular dopamine concentrations are controlled via physiological mechanisms that establish a balance between release and re-uptake [33]. We investigated adaptations in the mechanisms of dopamine synthesis, release and clearance in relation to APD treatment efficacy and failure. We measured TH and DAT expression using Western blot analysis during HAL and OLA treatment. During treatment failure, we observed increased TH expression in the CPU (Supplementary Figure 1a, c, e, f) but not in the NAcc or PFC compared to vehicle (Supplementary Figure 1b, c, e, f). Also, APD did not alter the group mean of DAT expression in the CPU, NAcc, or PFC (Supplementary Figure 2–3a, c, e). This result may erroneously suggest an absence of effect at this target. However, we detected strong inter-individual APD treatment-induced changes in DAT expression. Therefore, we described this variability by applying a standard procedure to calculate the mean absolute deviation (MAD) [34] using the following formula: individual data value – group mean value [x – (Σx/n)]. The value of each
individual DAT expression at a specific treatment point (i.e., 2, 6, 14, or 21 days) was subtracted from the group mean at the same treatment point.

We found inter-individual DAT changes in the CPu after 14 days of treatment with either APD (Supplementary Figure 2b and Fig. 3b) and in the NAcc with HAL treatment.
HAL (0.5 mg/kg/d) treatment for 6 days reduced AMPH-induced (2 mg/kg) hyperlocomotion ($P < 0.001$, main effect), but no longer after 14 days treatment. N = 7/group. c, d OLA (10 mg/kg/d) treatment for 2 days reduced AMPH-induced (2 mg/kg) hyperlocomotion ($P < 0.001$, main effect), but no longer after 6 days treatments. N = 5/group. e, f HAL (0.5 mg/kg/d) or OLA (10 mg/kg/d) prevented the PPI reduction induced by AMPH (3 mg/kg, vehicle (veh) vs. control (ctrl.), $P < 0.0001$).

Continuous treatment. AMPH-induced (2 mg/kg) hyperlocomotion ($P < 0.001$), but no longer after 6 days treatments. N = 5/7/group. c, d, e HAL (0.5 mg/kg/d) or OLA (10 mg/kg/d) prevented the PPI reduction induced by AMPH (3 mg/kg, vehicle (veh) vs. control (ctl.), $P < 0.0001$), g HAL (0.5 mg/kg/d) treatment for 6 days reduced TP (20 min)-induced hyperlocomotion compared with veh ($P < 0.0001$), but no longer after 14 days treatment. N = 17–18/group. h OLA (10 mg/kg/d) treatment for 2 days reduced TP (20 min)-induced hyperlocomotion compared with veh ($P < 0.05$), but no longer after the continuous treatment. N = 5–20/group. All data are means ± S.E.M *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Statistical significance represents post hoc comparison when not specified (Supplementary Figure 3d). HAL treatment also decreased NET expression in the NAcc (Supplementary Figure 4c).

No other effects of HAL treatment on NET or SERT expression were observed in the CPu, NAcc, or PFC compared with veh (Supplementary Figure 4, 5a–f). HAL and its metabolites directly interact with the DAT and other monoamine transporters [35]. Therefore, we further investigated the effect of APDs on DAT regulation. We estimated the putative coupling of striatal TH and DAT expression, which are coregulated [36]. We found no linear relationship between the TH and DAT expression levels in the control animals (Fig. 3 and Supplementary Figure 6a) or short-term HAL (Supplementary Figure 6b) or OLA treatments (Supplementary Figure 6c). However, long-term treatment with HAL (6–14 days) or OLA (21 days) progressively linearized the TH-DAT relationship, and higher TH levels were associated with increased DAT levels (Fig. 3a–c). This result suggests that APD treatment failure occurs in conjunction with the simultaneous increase in capacity for dopamine synthesis and uptake.

Next, we assessed whether changes in DAT levels occurred due to direct effects of APDs on mRNA expression. We measured DAT mRNA expression using polymerase chain reaction in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) at different HAL treatment times. We found that HAL suppressed DAT mRNA expression in the SNc and VTA after 2 days (Fig. 3e, f) compared with the control treatment. However, the inhibitory effect of HAL in the SNc gradually subsided during ongoing treatment (Fig. 3e). The suppression of DAT expression by HAL persisted until day 6 in the VTA (Fig. 3f). By day 14, DAT mRNA expression showed a modest trend toward regaining normal levels (Fig. 3f). These data suggest that the efficacy of HAL during short-term treatment is associated with a robust suppression of DAT mRNA expression in the SNc and VTA. In contrast, during APD failure, DAT mRNA expression was restored in the nigrostriatal system but not in the mesolimbic dopamine system. This effect suggests a dissociated mechanism in the brain, with dopaminergic projection systems serving to promote APD treatment failure, and the nigrostriatal system exhibiting the most striking changes.

To further investigate the role of the DAT in APD treatment failure, we measured the DAT density in vivo by microPET imaging using [18F]FP-CMT in rats at baseline and after 14 d of HAL treatment. To verify treatment failure at that time, we also measured the TP-induced locomotor activation. We detected increased DAT availability (binding potential; BPND) in striatum at 14 days of HAL treatment (Fig. 3g, h) when treatment failure became evident as a disinhibition of TP-induced locomotion (Fig. 3i) and supplementary Figure 7). DAT BPND correlated with TP-induced locomotion (Fig. 3i), which demonstrates an association of APD efficacy and failure at the behavioral level with a dysregulation of dopamine clearance.

Electrophysiological activity of dopaminergic neurons during antipsychotic treatment efficacy and failure

Extracellular dopamine levels in nigrostriatal and mesolimbic areas also depend on tonic and phasic dopamine neuron activity [37, 38]. Tonic activity is characterized by a spontaneous slow and irregular firing of dopamine neurons, and phasic activity is mediated by burst firing [39, 40]. We measured the spontaneous firing, resistance, and capacitance of dopaminergic neurons from the SNc in brain slice preparations of mice after 6 or 14 days of HAL treatment (i.e., efficacy and failure conditions). Membrane input resistance and membrane capacitance of SNc dopamine neurons were unchanged after either HAL treatment duration (data not shown). Notably, treatment failure after 14 days of HAL treatment coincided with a significant reduction in the firing rate of spontaneously active neurons compared with the vehicle-treated group, but 6 days of HAL treatment did not alter the firing frequency (Fig. 4c). The slowed tonic firing after 14 days of HAL treatment may at least partially account for the reduced extracellular dopamine levels observed in the microdialysis experiments (Fig. 2e). Both drug regimens also affected silent dopamine neurons and shifted their resting membrane potentials to significantly more negative values (Fig. 4d). The HAL-associated hyperpolarization would impede the transition to tonic firing and impair the phasic mode of firing because only spontaneously active dopamine neurons can burst fire [39]. To examine the impact of the HAL-dampened firing frequency of SNc dopamine neurons on the postsynaptic...
side, we monitored the neuronal population responses in CPu, the main projection target. Local electrical stimulation in the CPu evoked responses that consisted of a biphasic field potential, with an early axonal component (fiber volley) and a late synaptic component. The latter component predominantly arose from excitatory

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Fig. 3 Dopamine synthesis, release and clearance capacity during antipsychotic treatment failure. a, b The expression of dopamine transporter (DAT) and tyrosine hydroxylase (TH) is coregulated after 6 days (Pearson r = 0.7438, P < 0.05) and 14 days (Pearson r = 0.9356 P < 0.001) HAL treatment. N = 8/group. c The expression of DAT and TH is coregulated by 21 days olanzapine (OLA) treatment (Pearson r = 0.8107, P < 0.01), but not by the treatment with vehicle (veh). (d) N = 8/group. e Changes in DAT mRNA expression in the substantia nigra (SNc) and in the ventral tegmental area (VTA) after continuous treatment with haloperidol (HAL). HAL treatment for 2 days decreased DAT mRNA expression in the SNc compared with veh (P < 0.05, main effect) and to 6 (P < 0.05) and 14 (P < 0.01) days HAL. N = 7–8/group. Data are means ± S.E.M (*P < 0.05 – vs. veh. **P < 0.01 – vs. 2d HAL treatment). f HAL treatment for 2 and 6 days decreased DAT mRNA expression in the VTA compared to veh (P < 0.05). This inhibitory effect vanished after 14 days. N = 7–8/group. All data are means ± S.E.M (*P < 0.05, **P < 0.01, ***P < 0.001). g DAT density (expressed as mean parametric maps of binding potential (BPND)) in animals scanned first at baseline and after 14 days HAL treatment, as determined by PET imaging with [18F]FP-CMT. h HAL treatment for 14 days increased the mean group of DAT uptake in the caudate-putamen (CPu) compared with baseline (P < 0.001). N = 16/group. Data are means ± S.D. i Relationship between DAT uptake changes (calculated as delta between follow-up and baseline) in the CPu and hyperlocomotion response to tail pinch (TP) (calculated as delta between stimulation and baseline) (pearson r = 0.7167, P < 0.05). N = 9/group. Data are means ± S.D. j Animals with a DATBP < 0.8 were experiencing treatment efficacy compared with the subgroup with a DATBP > 0.8 who were instead prone to antipsychotic treatment failure (P < 0.01). N = 9/group. Data are means ± S.E.M. (*P < 0.05, **P < 0.01, ***P < 0.001). Statistical significance represents post hoc comparison when not specified consistent with the effects of APDs on vesicle pool parameters because HAL treatment reduced stimulated dopamine release in the brain during the treatment efficacy phase but not during treatment failure.

Reversal of antipsychotic treatment failure

APD treatment failure and the generally variable responses to APD treatment are major problems in schizophrenia pharmacotherapy. Having identified the mechanisms that may mediate unfavourable outcomes, we next derived a potential intervention to reverse APD treatment failure and reinstate APD efficacy in behavioral and neurophysiological terms. The present data suggest that if reduced tonic dopamine transmission in the striatum is relevant to the loss of APD efficacy, then a moderate stimulation of tonic dopamine release may restore APD efficacy. We tested this hypothesis in microdialysis studies in freely moving rats. One study measured the extracellular dopamine levels in 14 days HAL-treated and control animals and their locomotor response to TP. We also performed an identical study with an additional treatment of both animal groups with the DAT blocker GBR12909 (10 mg/kg, i.p.) after measuring dopamine at baseline and before recording the TP-induced hyperlocomotion. As observed previously, HAL reduced the extracellular dopamine levels after 14 days of treatment compared with the control treatment (Fig. 6a) in association with the failure of HAL to inhibit the TP-induced hyperlocomotion (Fig. 6b). We replicated the finding of reduced extracellular dopamine during prolonged HAL treatment (Fig. 6c). The co-administration of the DAT blocker moderately increased extracellular dopamine levels in 14 days HAL-treated animals but not in the control group, which reduced the group differences in dopamine levels (Fig. 6d). The combination of the acute DAT blocker and 14 days HAL treatments resulted in a net inhibition of TP-induced hyperlocomotion (Fig. 6e). Therefore, GBR12909 rescued ~40% of the inhibitory effects of HAL (Fig. 6f). This behavioral effect was not confounded by stereotypedies, which may occur when GBR12909 or other psychostimulants are administered after chronic APD regimens [49]. The animals in our conditions exhibited a “fluid” execution of locomotor activity that was not compromised by stereotypes, which would otherwise rigidly dominate the overall behavioral performance. The dose of GBR12909 only modestly increased extracellular dopamine levels (Fig. 6d). These results clearly suggest that a slight potentiation of tonic dopamine levels rescued APD treatment outcomes, which supports the hypothesis that DAT blockers may be used clinically as an adjunct treatment to reverse APD treatment failure.

We also investigated whether the mechanism of this protective effect was mediated in the CPu. We administered GBR12909 (G, 20 μg/μl/side) or vehicle directly into the CPu of rats with 14 days of HAL treatment. Intracerebral vehicle infusion did not alter the usual loss of locomotor inhibition in HAL-treated rats (Fig. 6g), which is indicative of treatment failure. However, the infusion of GBR12909 to the CPu (intra-cuadate, i.c.) inhibited the TP-induced locomotion in 14 days HAL-treated animals (Fig. 6g), which mimicked the behavioral response that is normally observed during HAL treatment efficacy. These data strongly suggest that the dopaminergic innervation of the CPu is a critical site for a pharmacological reversal of APD treatment failure.

Discussion

The present findings elucidate crucial mechanisms of APD therapeutic action and failure and provide a new strategy for failure reversal. First, our model reproduced APD action and failure using clinically relevant APD dosing and treatment regimens. Second, the mechanism underlying APD efficacy was primarily characterized by preserved or increased extracellular dopamine levels in the CPu in association with reduced DAT mRNA expression and relatively preserved expression of TH and of membrane DAT protein. Third, the physiological pattern of APD
efficacy was characterized by preserved tonic firing of SNC dopamine neurons, decreased post-synaptic responses (field potential) and suppression of exocytosis (recycling pool size). The physiological changes underlying APDs efficacy were substantially reversed during APD failure. Specifically, we found that (1) baseline extracellular dopamine
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density changes driven by the APD treatment may be detected, our data strongly suggest that the early inhibition followed by a normalization or, in some cases, an upregulation of DAT expression in striatal tissue constitutes a fundamental component of HAL efficacy and failure, respectively. Accordingly, a previous rat microPET study...
using \(^{123}\text{I}\)-FP-CIT has shown reduced DAT binding only minutes after acute HAL injection [65]. Based on our data, the HAL-driven reduction in DAT binding may occur either directly through reduction of DAT density in the plasma membrane or via suppressed DAT mRNA expression at very early treatment stages. Alternatively, availability changes may reflect radioligand displacement by increased dopamine levels due to the inhibition of DAT expression. It is also possible that sustained brain concentrations of HAL resulting from the treatment with osmotic pumps may block the DAT transporter directly, thus causing an increase in dopamine levels. Studies using neuronal cell culture have shown the likelihood of this possibility [35]. Additionally, the increased dopaminergic tone, driven by the initial HAL treatment, can downregulate DAT membrane expression via the stimulation of the D2 autoreceptors [66].

The present data support pharmacogenomics studies reporting associations between DAT gene variations and clozapine efficacy in treatment resistant patients and in cognitive dysfunctions [67, 68]. Thus, targeting DAT may be a paramount goal of APDs to overcome treatment-resistance in schizophrenia. A second observation supported by our data relates to our recent suggestions about the actual mechanisms of APD action [5]. We argue that the rise and fall of antipsychotic efficacy is driven by dynamic interactions of the endogenous dopamine and presynaptic D2 receptors. Specifically, increased tonic synaptic levels of dopamine in the striatum following the initial treatment with APDs would determine the stimulation of a dopamine D2 receptor reserve, which is defined as the difference between the total number of available D2 receptors (100%) and the proportion of those D2 receptors bound by an APD at a dose within the therapeutic window (60–80% blockade of central D2 receptors). The receptor reserve primarily includes pre-synaptic dopamine receptors, which tonically inhibit dopamine synthesis and release, and may mediate antipsychotic effects [5]. Present results showing an early suppression of mRNA DAT expression (Fig. 3e, f) along with an inhibition of vesicular release during antipsychotic efficacy (Fig. 5f, g) substantiate these interpretations.

By contrast, decreased tonic synaptic availability of dopamine, occurring during chronic APD treatment, is associated with a significant loss of APD efficacy. These effects may reflect a reduced stimulus of the D2 receptor reserve triggered by the initial treatment [5]. Notably, dopamine release in the striatum is closely related to the firing of dopaminergic neurons in the midbrain, which was decreased in this study (Fig. 4c). Furthermore, DAT was upregulated during APD failure (Fig. 3e, h), thus, possibly increasing clearance of extracellular dopamine and contributing to a reduction of synaptic dopamine levels.

Reduced stimulation of D2 autoreceptors would predict that presynaptic neurons will be more sensitive to phasic dopamine release in response to stimulation. Consistent with this proposal we found increased TH expression (Supplementary Figure 1a, d), a potentiation of vesicular release during antipsychotic efficacy and failure outlines quite a different perspective compared to traditional interpretations of antipsychotic mechanisms. The common view is that an “optimal” level of post-synaptic dopamine D2 receptor blockade with antipsychotics attenuates dopaminergic transmission at post-synaptic neurons [4, 71], which ameliorates the positive symptoms of schizophrenia. However, studies show that chronic APD treatment triggers...
adaptations of post-synaptic dopamine D2 receptors, which undergo upregulation [28, 29, 31] and increased sensitivity for local dopaminergic signalling. This circumstance, known as dopamine supersensitivity [25, 72], is the hypothesized mechanism of antipsychotic treatment failure [25, 27, 72].
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Fig. 6 Reversal of antipsychotic treatment failure. a Haloperidol (HAL) treatment for 14d decreased the extracellular dopamine levels in the caudate-putamen (CPu) (P<0.05, main effect) and b is unable to block tail pinch (TP) induced hyperlocomotion (P<0.0001, main effect) compared to vehicle (veh) treated rats. N=7/group. Data are means ± S.E.M. Similar to the previous experiment, HAL decreased the extracellular dopamine levels in the CPu compared with veh (P<0.05, main effect). d The systemic injection of GBR12909 (10 mg/kg, i.p.) replenished the extracellular dopamine to control group levels, and e reversed HAL failure, which was now sufficient to block TP induced hyperlocomotion compared to veh (P<0.0001, main effect). N=5/group. Data are means ± S.E.M. f The co-administration of GBR12909 (10 mg/kg, i.p.) reversed the extracellular dopamine to control group levels, and e reversed HAL failure, which was now sufficient to block TP induced hyperlocomotion compared to veh (P<0.0001, main effect). N=5/group. Data are means ± S.E.M. *P<0.05, **P<0.01, ***P<0.001. Statistical significance represents post hoc comparison when not specified.

Yet, changes in D2 receptor density are not consistently observed in patients previously exposed to antipsychotics [73]. APD treatment failure also occurs independently of any changes in D2 high affinity state receptor density in animal models [27]. In conclusion, whereas reducing the synaptic levels of dopamine has been the primary target of antipsychotics to reduce psychoses, our data suggest an apparent paradox that reduced dopamine signalling at presynaptic D2 receptors is the actual cause of antipsychotic failure. Therefore, reverting dopamine levels in the dopaminergic synapse should reinstate APD efficacy. Here, we demonstrated this to be the case by blocking the clearance of synaptic dopamine using the DAT blocker GBR12909. This dopaminergic intervention may be tested clinically as an augmentation strategy, alternative to increasing APD dosing or switching drugs, and possible abuse potential derived from this combination of treatments may be managed. Genetic variability in DAT expression as well as the age of patients at the start of treatment will affect APD response even if augmentation with a DAT blocker is used, simply because not all individuals will have a sufficient amount of DAT expression to mediate the necessary DAT blocker efficacy. Thus, an intriguing therapeutic strategy may be proposed based on viral expression of DAT in key brain areas to overcome treatment-resistance and equalize the therapeutic antipsychotic response across patients. Our study delineates mechanisms of APD efficacy and failure. It suggests a new strategy for personalized therapeutic approaches to overcome pathways to relapse, including APD tolerance, dopamine supersensitivity psychosis, and treatment resistance.

Limitations of the study

D2 receptors exhibit high and low affinity states for dopamine agonists (D2⁹⁰, D2⁵⁰) [74], which are not distinguished using current antagonist ligands [75]. Therefore, an increased prevalence of D2⁹⁰ activated with APD treatment may lead to the dopamine supersensitivity underlying the antipsychotic loss of efficacy, despite an unaltered absolute total density of D2 receptors and net receptor occupancy. A previous study found that APD treatment failure was associated with a greater prevalence of D2⁹⁰ receptor, but this effect occurred only with high HAL doses [27]. Therefore, the role of the unbalanced D2 affinity state in mediating APD efficacy and failure in animal models remains to be determined.

We report herein that the D2 receptor occupancy to microPET was in the clinical therapeutic range with HAL treatment, even though the drug was no longer effective in independent behavioral testing. Thus, we did not explicitly replicate HAL treatment failure in these animals. Our aim was to verify the D2 receptor occupancy range during 14 days HAL treatment. The use of behavioral or pharmacological manipulations may have been confounding factors in our study. Such interventions can stimulate striatal dopamine release, thus perturbing D2 receptors availability in the HAL occupancy study after 14 days treatment, as shown in previous human PET studies [76].

Dopamine levels decreased after 6 days of HAL treatment, despite continued drug efficacy at that time point in multiple behavioral tests (Fig. 1). However, a decrease in dopamine levels occurred relative to the control group, but not compared with 14 d of HAL, which further lowered dopamine levels. We attribute this effect to possible different temporal kinetics that underlie changes in neurotransmitter levels and behavior. Therefore, reversal of antipsychotic inhibition in behavioral tests may occur with a different time-course than the reversal of extracellular dopamine. Particularly, the dopamine levels may decrease faster than the indices of behavioral APD efficacy. Furthermore, while the use of psychopathological naïve rodents may appear to limit the translational power of the present study, it should be noted that in absence of a clear understanding of the neurobiology of schizophrenia symptoms, which undermines face validity in experimental modelling, our pharmacological model yields evidence for neuroadaptations induced solely by the administration of APDs. These adaptations are very dynamic and time-locked with the onset and offset of antipsychotic efficacy. Nevertheless, the results of the present work may benefit from replications in psychopathological animal models.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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