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# Suppression of the dorsomedial striatum impairs reversal learning in response to probabilistic uncertainty and disrupts corticostriatal function in mice

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ABSTRACT

People with schizophrenia often show impairments in cognitive flexibility, which is a key aspect of executive function, and this contributes to poor functional outcomes. Neuroimaging studies in schizophrenia have implicated dysfunction within the dorsomedial striatum (DMS), which is a subcortical brain region integral to reversal learning, i.e., a form of cognitive flexibility involving adapting behaviour in response to changing reward contingencies. Given its integral role in these processes, understanding the biological and cognitive consequences related to DMS dysfunction may inform therapeutic strategies to mitigate cognitive inflexibility. This study employed a serial reversal learning paradigm to examine decision-making under probabilistic uncertainty and investigated the effects of DREADDs-based chemogenetic inhibition of the DMS on performance and the subsequent activity of corticostriatal circuitry in male C57BL/6J mice. Inhibition of DMS activity impaired reversal learning under probabilistic uncertainty, specifically disrupting reward-based decision-making strategies such as by reducing win-stay behaviour. Furthermore, DMS inactivation led to diminished corticostriatal network co-activation during reversal learning, with pronounced reductions in the efficiency of anterior cingulate, orbitofrontal, and insular cortices—cortical regions crucial for flexible learning. These results suggest that inhibiting DMS function disrupts behavioural adaptation as well as corticostriatal co-activation, a mechanism that potentially contributes to the cognitive inflexibility observed in schizophrenia. By elucidating the neural substrates of reversal learning deficits, this study provides insights into the mechanisms by which DMS inhibition affects cognitive flexibility and highlights potential targets for therapeutic intervention in schizophrenia-relevant cognitive impairments.

## 1. Introduction

Compromised cognitive flexibility, the inability to adapt mental processes to generate appropriate behavioural outcomes, is a core transdiagnostic characteristic for a range of neurodevelopmental and psychiatric conditions. For schizophrenia in particular, deficits in cognitive flexibility are well recognized, with mounting evidence indicating a correlation between worse functional outcomes and clinical relevance (Chang et al., 2019; Keefe and Kahn, 2017; Kesby et al., 2021; Starzer et al., 2024). Cognitive impairments can emerge during adolescence before the onset of psychotic symptoms, and cognitive inflexibility may be a potential therapeutic target for schizophrenia (Wykes et al., 2007), but may also be a future biomarker for psychiatric disorders (Chu et al., 2024). A recent systematic review of clinical studies advised that cognitive inflexibility reflects a stable trait rather than a 'state' in chronic schizophrenia, and highlighted lasting frontal cortex dysfunction (Aumer et al., 2024), but the question of how these regional deficits evolve during the progression of the disorder remains unanswered. Furthermore, dorsomedial striatum (DMS) dysfunction has been observed in schizophrenia (McCutcheon et al., 2019), indicating that disruptions in cortico-striatal circuitry may be one of the primary drivers of inflexible behaviour (Conn et al., 2020). Typical assessments in humans use attentional set-shifting and reversal learning paradigms (among others) to determine the capacity for subjects to flexibly alter

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behaviour in uncertain environments, with cross-species translated versions often used in preclinical animal research (Chudasama and Robbins, 2006; Conn et al., 2020; Flintoff et al., 2024; Kehagia et al., 2010; Kesby et al., 2018). The use of chemogenetic techniques in animal models that discretely alter regional activity provide researchers with the unique ability to correlate neural activity with readouts of learning and task performance. While this does not attempt to capture the full presentation of schizophrenia like other developmental and genetic preclinical models (developmental vitamin D deficiency, maternal immune activation, etc.), precisely manipulating DMS function allows for a deeper understanding of the potential circuit-level mechanisms that may contribute to cognitive inflexibility in schizophrenia. Findings from using this approach would also extend to other disorders with known DMS dysfunction such as obsessive-compulsive and eating disorders (Kesby et al., 2018; Kesby and Turner, 2024).

Optimal functioning of the frontal cortex and its downstream striatal targets (i.e., dorsomedial and ventral) are paramount to cognitive flexibility (Izquierdo et al., 2017). While considerable research has focused on the ventral striatum in cognitive flexibility due to its role in processing reinforcer value that drives behavioural output (i.e., stimulus-outcome reward learning), research within the schizophrenia field has shifted focus to the crucial role of the DMS in driving instrumental learning (i.e., the formation of action-outcome associations) and overall goal-directed action (Morris et al., 2015). This is because the DMS is required to integrate limbic information with action selection, and its activity is directly modulated by a range of frontal cortex inputs and the substantia nigra. Therefore, the DMS is perfectly placed as an 'integrative hub' (Haber, 2016), responsible for either driving actions to obtain positive outcomes or inhibiting actions when the outcome is no longer desirable. Since numerous neuroimaging studies in people with schizophrenia point to a DMS dysfunction, the potential that this initiates a progressive and consequential impact on corticostriatal inputs and cognitive flexibility is clear (Kesby et al., 2021). While it is difficult to determine the direct causal relationship between DMS and cognitive flexibility in humans, preclinical animal studies are starting to provide strong evidence to support these theoretical assumptions.

Preclinical animal studies can contribute to a deeper understanding of DMS function in cognitive flexibility by determining the precise roles of neural circuitry and neurotransmitter systems in driving optimal decision-making behaviour (Clarke et al., 2011). For example, inactivation of the DMS in wild-type rats has demonstrated roles in differing types of risk/reward decision-making (Schumacher et al., 2021), with inactivation impacting the flexible adaptations to probabilistic reward contingencies and increasing risky decisions when odds are disadvantageous. More circuit-specific manipulations of prefrontal cortical projecting neurons to the DMS have resulted in impairments in both goal-directed action and reversal learning, through its putative role in controlling behavioural inhibition (Terra et al., 2020). Additionally, manipulations of nigrostriatal circuitry (i.e., substantia-nigra projecting neurons into the DMS) in mice, impair associative learning processes, action selection, and the overall ability to reverse behaviour under probabilistic uncertainty (Conn et al., 2023; Young et al., 2022). Thus, the multifaceted behavioural functions of the DMS are governed by the complex interaction of neurotransmitter systems that promote activity-dependent synaptic plasticity of corticostriatal circuits fundamental to learning.

To identify the potential network-based mechanisms in which cognitive flexibility is impaired in conditions where DMS function is affected, such as in schizophrenia, discrete manipulation of DMS activity in animals during flexible learning is essential. Therefore, in this study we aimed to determine the detrimental effects of acutely suppressing DMS activity in mice during reversal learning under probabilistic uncertainty. Using a constitutively inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) approach, administration of low dose clozapine-N-oxide (2 mg/kg) was used to selectively disrupt the activity of the dorsomedial striatum during performance. This dose was selected for its known capacity to substantially reduce neuronal activity without effecting the behavioural outcomes. For example, using this same paradigm, Young et al. (2022) demonstrated no behavioural impact of CNO in DREADDs negative mice, using both chronic (8 mg/kg daily) and acute (2 mg/kg) administration of CNO. In combination with activity-dependent immunohistochemistry, we were able to determine the impact of DMS suppression on behaviour and broader corticostriatal network activity during performance. As such, suppression of the DMS during reversal learning was associated with a reduction in corticostriatal co-activation that was biased towards specific reductions in corticostriatal inputs essential for adaptation to uncertainty, as seen in human studies of schizophrenia.

## 2. Materials and methods

### 2.1. Subjects and housing

For detailed protocols see the Supplementary Methods. 10-week-old male C57BL/6JArc (N = 36) mice (Animal Resources Centre, WA, Australia) were used. Mice were housed in ventilated cages (Optimice, Animal Care Systems, CO, USA) and maintained at 21 ± 2 °C (~60 % humidity) with a 12-h light/dark cycle (lights on 0700h). Mice were housed in groups of two (with perforated split dividers) per cage. During behavioural testing, mice were food restricted to 90–95 % of their free-feeding body weight, were fed daily at least 1h after testing, and water was provided *ad libitum*. All procedures were performed with approval from the University of Queensland Animal Ethics Committee.

### 2.2. Stereotaxic surgery

Transfection was achieved by bilaterally microinjecting 0.5 μl of a hM4D(Gi) DREADDs virus (pAAV5-hSyn-hM4D(Gi)-mCherry; Addgene, USA) into the striatum. Either the DMS (AP+0.11; ML±1.25; DV−2.90) or the nucleus accumbens (ACB; AP+1.60; ML±1.80; DV−4.37 @ a 10° angle) was targeted. ACB injections led to a limited number of mice with sufficient bilateral expression for inclusion, as such the data from within-subject experiments is not discussed in this manuscript (but can be found in Table S1). As our focus was on the DMS (Fig. 1), ACB targeted mice were also included in the saline controls for the final test day and cFos analysis. Site of viral injections did not impact behaviour for the saline controls (see Table S2). In total, 14 mice were included in the DMS within-subject testing, and 8 saline and 11 DMS compared for cFos activity. Eight mice failed to reach baseline criterion for behavioural testing in sufficient time and were kept in the homecage. Six received saline and two received CNO on the final day of testing to verify inhibition of DREADDs expressing cells (see Fig. S1).

### 2.3. Operant testing

One week after surgery, mice were food restricted and trained/baselined on the probabilistic reversal learning (PRL) protocol as done previously (Young et al., 2022; and see supplementary methods). Mice were tested for 60min at the same time daily during the light cycle (0900–1700h) to align with standard laboratory practices required by the Queensland Brain Institute's animal husbandry. While mice are nocturnal, they are able to adapt to daytime testing, and many validated cognitive paradigms, including the same procedure published by Young et al. (2022) yield reliable results during the light phase (across multiple treatment and control cohorts). Briefly, PRL testing was conducted in eight Plexiglas operant chambers (model ENV-307A, Med Associates Inc.). The chambers featured two nose-poke holes and a reward magazine located on the opposite wall, which could deliver strawberry milk. The target nose-poke was defined as that with the highest reward rate. No punishment followed an unrewarded response, rather punishment/loss reflects a lack of expected reward. After 6 consecutive target nose pokes, the target and non-target reward contingencies were

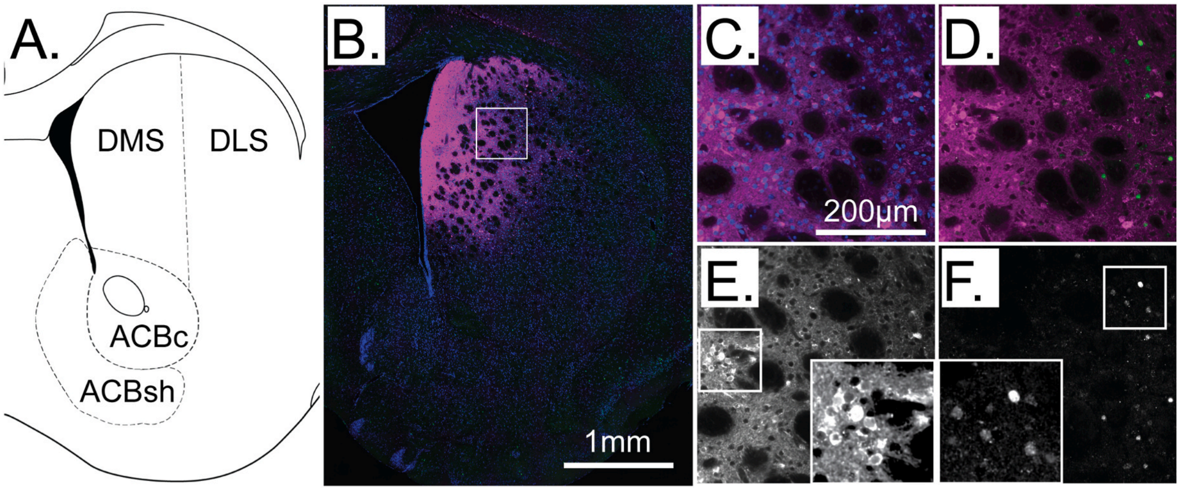


**Fig. 1. DREADDs expression in the dorsomedial striatum (DMS).** Striatal regions were categorized as DMS, dorsolateral striatum (DLS), and nucleus accumbens core (ACBc) and shell (ACBsh) (**A**). Example DREADDs expression (magenta) in the DMS with nuclei (DAPI) in blue and cFos in green (**B**). Zoomed in panels with DREADDs/DAPI (**C**), DREADDs/cFos (**D**), and grayscale images of DREADDs (**E**) and cFos (**F**). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reversed. Behavioral measures included the total number of trials, latency to respond and the number of reversals/100 trials. The proportion of win-stay and lose-shift strategies were also determined, where the subject repeats a choice after a rewarded outcome ("win-stay") and switches to a different choice following an unrewarded outcome ("lose-shift"), respectively, to measure sensitivity to reward-based feedback. Additionally, the number of perseverative errors, i.e., when the animal repeats a previously rewarded action after contingencies have reversed, were also examined (errors in the first 6 trials after a reversal).

### 2.4. Experimental design

For DREADDs activation, mice received injections of 2 mg/kg CNO (clozapine-N-oxide; AK Scientific; USA) diluted in a 0.5 % dimethyl sulfoxide and 0.9 % saline vehicle, administered 40min prior to testing based on established timings of DREADDs activation (Roth, 2016). The study was conducted in two phases (see Table 1 for complete timeline and details). The first was a within-subject Latin-square design whereby mice were tested at each contingency twice (target reward %: nontarget reward % - 80:20, 80:40 and 70:30) and administered either saline or CNO in a counterbalanced order. On the last day of testing, to allow for cFos quantification, mice were separated into two equally performing groups (Table S3) and administered saline or CNO and tested at an 80:20 contingency.

**Table 1**
Experimental timeline and details.

| Manipulation | Days | Cumulative days | Details |
|---|---|---|---|
| Surgery | 1 | 1 | DREADDs surgeries |
| Rest | 7 | 8 | Recovery |
| Training/ baselining | 45 | 53 | Instrumental training.<br>Deterministic reversal learning.<br>Probabilistic reversal learning. |
| Contingencies 80:20/80:40/ 70:30 | 11 | 64 | CNO and Saline counterbalanced.<br>Within-subject.<br>Test days separated by 1 baseline day. |
| Baseline | 2 | 66 | |
| cFos test day | 1 | 67 | CNO or Saline.<br>Perfusion 60 min after testing. |

### 2.5. Tissue collection and immunohistochemistry

On the final day of testing, mice were perfused with 4 % paraformaldehyde (PFA) 60 min after completion of testing to determine cFos expression. 50 μm coronal sections spanning the rostro-caudal extent of the brain were collected using a vibratome (VT1000s, Leica Microsystems, Germany), in a 1:6 series and stored at 4 °C in a 0.1 M sodium phosphate buffer solution until processed for immunofluorescence. Brain sections were stained for cFos, mCherry and DAPI.

### 2.6. Imaging and analysis

Three cortical and four striatal sections were imaged per mouse using an Olympus UPLSAPO 30x/1.05 NA silicone-immersion objective on a spinning disk confocal microscope (SpinSR10; Olympus, Japan) built around an Olympus IX3 body and equipped with an ORCA-Fusion BT sCMOS camera (Hamamatsu Photonics K.K., Japan) and controlled by Olympus cellSens software.

Image preparation was completed using ImageJ (Schneider et al., 2012) and atlas guides fitted to each section using the Bigwarp plugin (Bogovic et al., 2016). Atlas guides were modified from the Allen Scalable Brain Atlas (Bakker et al., 2015). RGB values for each region were converted to unique intensity values to assign all nuclei a regional identifier. Custom values were assigned for the DMS, dorsolateral striatum (DLS) and ACB (core and shell) as these were not defined in the original atlas. Nuclei were segmented with Cellpose (Stringer et al., 2021) and images sets (nuclei objects, cFos channel, mCherry channel and fitted atlas guide) fed into Cellprofiler v4.1 for quantification (Stirling et al., 2021). cFos + cells were determined as those with >1.1-fold mean cFos intensity when compared to the mean intensity of *all cells* per image (Young et al., 2022). All analyses were subsequently conducted in R (version 4.1.2 [Platform: x86_64-w64-mingw32/x64 (64-bit)] on Windows 10 v22H2). Regional correlation maps of corticostriatal areas were constructed and analysed using igraph (Csárdi and Nepusz, 2006) and qgraph (Epskamp et al., 2012) packages. After quantification, the DMS was the primary site of DREADDs expression (Table S4). There was also limited expression in the DLS and motor area due to the needle tract.

### 2.7. Statistical analysis

Analyses were performed using SPSS Statistics (version 26; IBM

Corp.) and R (version 4.1.2). Behavioural and cFos data were analysed using analysis of variance and repeated-measures analysis of variance when within-subject factors were present. Where appropriate Greenhouse-Geisser corrections were applied, and Šídák corrections were used for post-hoc analyses. For cFos network maps, Pearson correlation coefficients were calculated in R and adjusted for multiple comparisons using the Benjamini-Hochberg procedure. Statistical significance was defined as $p < 0.05$ and data are presented as mean ± standard error of the mean (SEM).

## 3. Results

### 3.1. DMS inhibition reduces win-stay but not lose-shift

We have previously shown that amphetamine treatment and viral increases in dopaminergic inputs to the DMS decrease lose-shift use and can impair reversal learning (Young et al., 2022). These outcomes show both contingency- and loss-specific outcomes similar to those with early psychosis (Baker et al., 2023). The present study, focussed on DMS inhibition, further highlights the importance of including multiple contingencies to improve the sensitivity to detect reversal learning outcomes. For example, for reversal performance there was a significant *contingency* × *CNO* interaction (Fig. 2A; $F_{(2,26)} = 4.7, p < 0.05$) with DMS inhibition impairing reversal performance at 80:40 compared with saline ($p < 0.001$). DMS inhibition also reduced win-stay use overall (Fig. 2B) with a significant main effect of *CNO* ($F_{(1, 13)} = 8.6, p < 0.05$). But a significant interaction of *contingency x CNO* ($F_{(2,26)} = 6.5, p < 0.01$) indicated this was driven by significant decreases at 80:40 ($p < 0.001$) and 70:30 ($p < 0.01$) compared with saline. In contrast, lose-shift was not impacted by DMS inhibition (Fig. 2C). The number of perseverative errors were also significantly greater after DMS inhibition compared with saline (Fig. 2D; $F_{(1,22)} = 8.0, p < 0.05$). However, DMS inhibition globally decreased the number of completed trials (Fig. 2E; $F_{(1, 13)} = 8.8, p < 0.05$) and response latency (Fig. 2F; $F_{(1, 13)} = 6.8, p < 0.05$). These outcomes highlight that DMS inhibition slowed motor responses in general but nevertheless, specific contingency-dependent alterations in performance and win-stay use were observed. The 80:40 contingency (with high misleading reward) was particularly sensitive at detecting impairments after DMS inhibition. In contrast to outcomes after stimulation of dopamine release using amphetamine and locally via DREADDs (Young et al., 2022), or in those with early psychosis (Baker et al., 2023), no changes in lose-shift use were observed.

Amphetamine treatment specifically increases DMS neuronal activation, and this was associated with decreases in lose-shift and perseverative errors (Young et al., 2022). Therefore, we were interested in exploring how corticostriatal systems are impacted by DMS inhibition during reversal learning. Using a between-subject design we paired behavioural testing at the 80:20 contingency with cFos quantification and analysis. The behavioural results showed a similar pattern to the within-subject design with DMS inhibition significantly decreasing reversal performance (Fig. 3A; $F_{(1,18)} = 6.3, p < 0.05$), win-stay (Fig. 3B; $F_{(1,18)} = 5.6, p < 0.05$) and increasing perseverative errors (Fig. 3D; $F_{(1,18)} = 5.2, p < 0.05$). Again, no effect of DMS inhibition was observed for lose-shift (Fig. 3C) and there were no significant differences in trials completed (Fig. 3E) or response latency (Fig. 3F).

### 3.2. DMS inhibition reduces corticostriatal network co-activation during reversal learning

Unlike for amphetamine treatment (Young et al., 2022), we observed little change in corticostriatal cFos activity after DMS inhibition (Table S5). The exception was for the nucleus accumbens shell (ACBsh), in which the number of cFos positive cells were increased after DMS inhibition. This isolated outcome may represent a compensatory

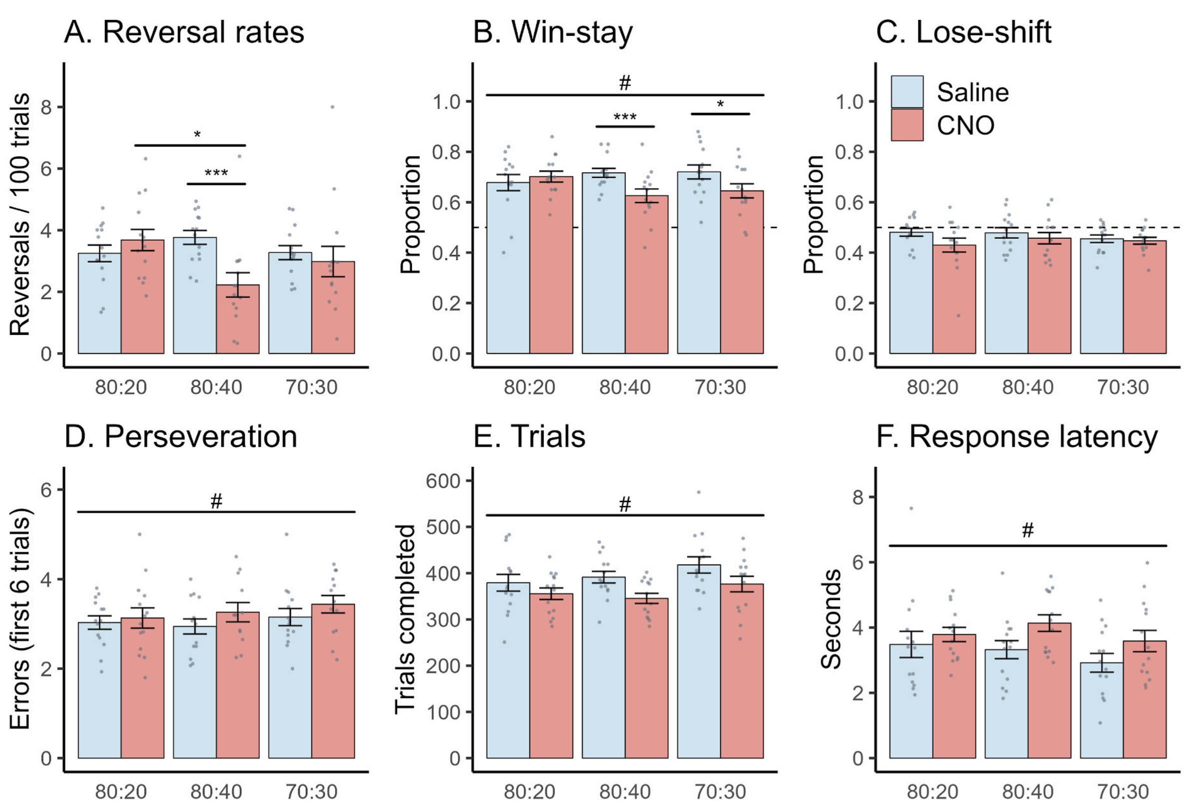


**Fig. 2. Behavioral outcomes after within-subject DMS inhibition at multiple probabilistic contingencies.** DMS inhibition significantly reduced reversal performance at the 80:40 contingency (**A**) and win-stay use at 80:40 and 70:30 (**B**). In contrast, lose-shift use was not impacted by DMS inhibition (**C**). DMS inhibition led to global increases in the number of perseverative errors after a reversal (**D**), decreased the number of trials completed (**E**), and increased response latency (**F**). Data are displayed as the mean ± standard error (N = 14). *$p < 0.05$, ***$p < 0.001$, #main effect of CNO $p < 0.05$.

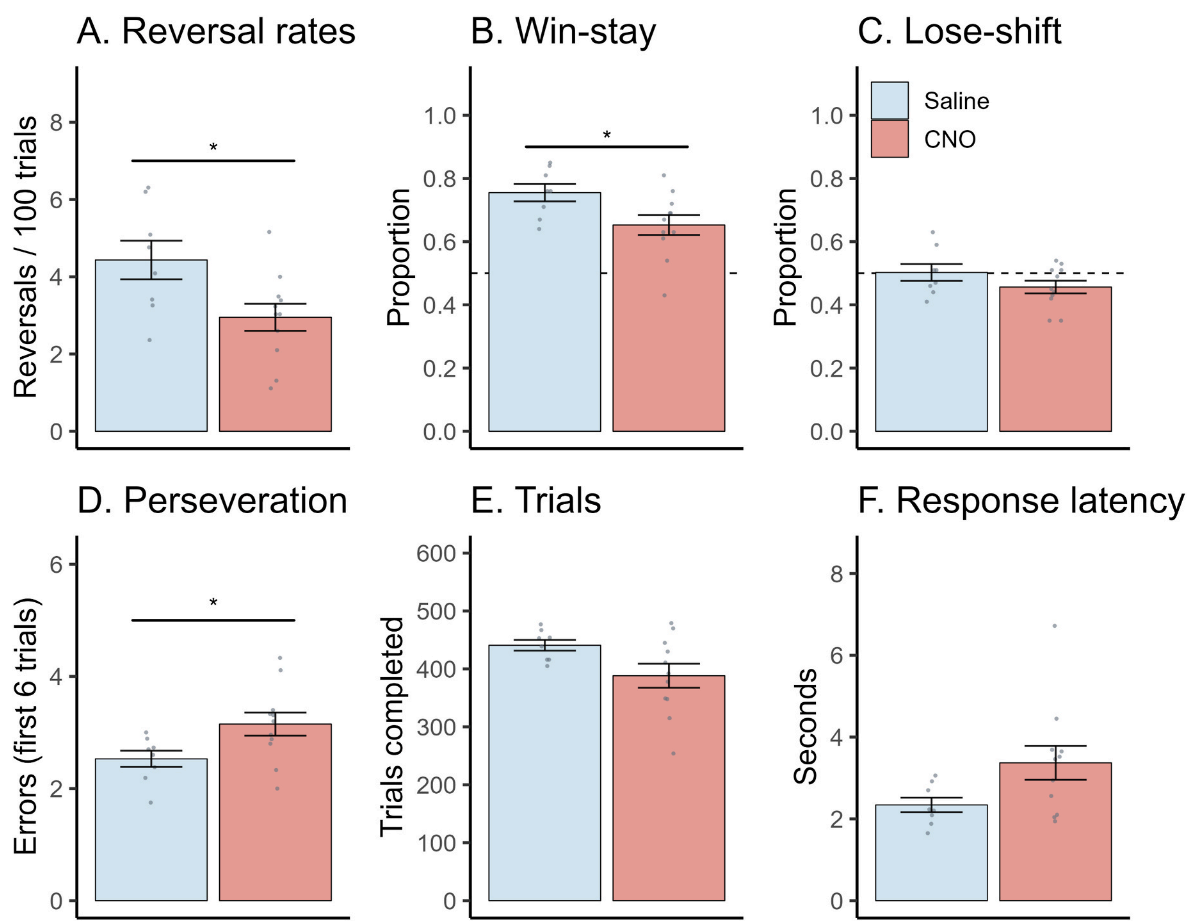


**Fig. 3. Behavioral outcomes at the 80:20 contingency after DMS inhibition on the final cFos test day.** DMS inhibition significantly reduced reversal performance (**A**) and win-stay use (**B**) but not lose-shift use (**C**). DMS inhibition also increased in the number of perseverative errors after a reversal (**D**) but did not significantly alter the number of trials completed (**E**) or response latency (**F**). Data are displayed as the mean ± standard error (N = 8–11). *$p < 0.05$.

response to DMS inhibition. Nevertheless, we were interested in how DMS inhibition impacted overall network efficiency and function during reversal learning. Therefore, we produced correlation network maps of the normalised cFos intensity across corticostriatal regions after saline (Fig. 4A) and DMS inhibition (Fig. 4B). Significant correlations were corrected using the Benjamini-Hochberg adjustment and filtered for Pearson correlation coefficients of at least 0.3 to remove spurious relationships (correlations between all areas can be seen in Table S6/7). Although visible differences in these maps were clear, to obtain objective values we examined the Eigenvector centrality scores to determine the network efficiency, and made informal comparisons between regions. All regions demonstrated a high network efficiency after saline, with qualitatively small decreases observed for the DLS and infralimbic cortex (Fig. 4C). In contrast, although the overall pattern was the same after DMS inhibition, the largest changes in efficiency were observed in four areas: the anterior cingulate cortex (ACC), lateral and ventrolateral orbitofrontal cortex (ORBl and ORBvl, respectively) and anterior insula cortex (Al; Fig. 4D). The ACC's efficiency was approximately half of that after saline, and the latter three regions were no longer associated with the network. This loss of network co-activation and efficiency is further highlighted by DMS inhibition significantly reducing the number of average connections per region (Fig. 4E; $t_{10} = 5.6, p < 0.001$). The dense innervation of the DMS by the ORBl and ACC (Hunnicutt et al., 2016; Young et al., 2022) support the specific effects of DMS inhibition on co-activation in these areas. Moreover, we have previously shown that after amphetamine treatment, cFos activity in DMS, ORBl and win-stay use are all positively associated (Young et al., 2022). Therefore, the likely impact of DMS inhibition is to impair integration of ACC and ORBl (and potentially ORBvl) inputs, which in turn decrease win-stay use. This is further supported by principal components analyses showing positive loadings between the DMS, ACC and ORBl with win-stay after saline treatment, and the opposite relationship after CNO treatment (see Fig. S2). Future studies are required to causally confirm these specific pathways and connectivity patterns using manipulations such as optogenetics.

## 4. Discussion

Suppression of DMS activity during probabilistic reversal learning in mice led to an impairment in reversal performance and revealed alterations in strategies used to flexibly guide decision-making behaviour. Specifically, reductions in reversal rate and the use of win-stay, but not lose-shift, strategies were observed. While these findings were specific to the type of strategy used, overall motoric effects of DMS suppression were also observed with reductions in the number of completed trials and increases in the latency to respond. In terms of the impact of on network co-activation, DMS suppression reduced the number of average connections per region with large changes in the efficiency of four cortical areas: the ACC, ORBl, ORBvl and AI. The ACC's efficiency was approximately halved by suppression, and the latter three regions were no longer associated with the network. As the DMS is densely innervated by the ORBl and ACC, suppression of activity likely impairs the integration of ACC and ORBl corticostriatal inputs, that in turn decreases win-stay behaviour, which compromises optimal reversal performance and cognitive flexibility.

Since the striatum is the primary input structure modulating basal

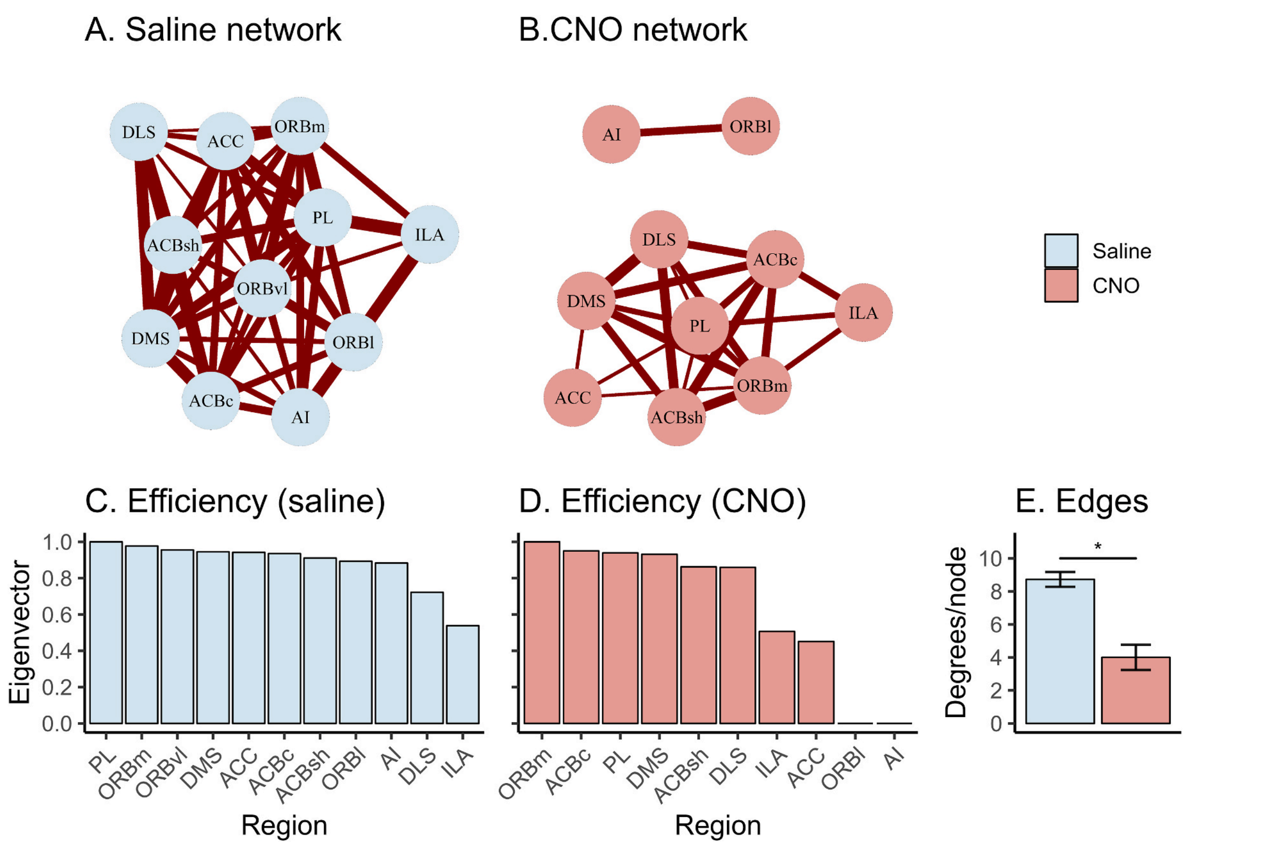


**Fig. 4. Network analyses of cFos activity in corticostriatal regions during reversal learning and DMS inhibition.** The correlation matrices after saline (**A**) or CNO treatment (**B**). Inhibition of the DMS led to a much sparser network of relationships between areas. This was reflected in the eigenvector centrality scores after saline (**C**) and CNO treatment (**D**), with the ACC, ORBl, ORBvl and AI all showing decreased co-activation after DMS inhibition. Moreover, the average edges/ connections (i.e., degrees) per region (i.e., node) was also significantly decreased after DMS inhibition (**E**; mean ± standard error, *$p < 0.001$), indicating that DMS inhibition reduced the correlated activity in corticostriatal networks

ACC, anterior cingulate; ACB, accumbens (shell [sh] and core [c]); AI, anterior insula; DMS, dorsomedial striatum; DLS, dorsolateral striatum; ILA, infralimbic; ORB, orbitofrontal (medial [m], lateral [l] and ventrolateral [vl]); PL, prelimbic.

ganglia function (i.e., motor control, learning, and executive function), inhibition of DMS activity clearly compromises decision-making processes and cognitive flexibility. This is likely due to DMS suppression directly disrupting the capacity for learning-dependent synaptic plasticity of medium spiny neurons that form the direct and indirect output pathways controlling goal-directed action. However, the observed alterations in general motor behaviour may also indicate that task motivation was impacted by DMS inactivation. Reductions in the number of trials completed and increases in response latencies, could signal general motoric impairments commonly observed after DMS impairments (Durieux et al., 2012). Yet despite the changes in responding, the deleterious cognitive effects of DMS inhibition were nuanced, with more uncertain conditions such as the 80:40 probabilistic contingency, more sensitive at detecting reversal learning impairments. Moreover, the use of win-stay strategies were specifically impaired, rather than performance more generally (i.e., lose-shift use). Furthermore, we observed no differences between groups on the initial discrimination (see Table S8/9), indicating these outcomes were specific to reversal learning and flexibility. Together this suggests that the impairments in cognitive flexibility after DMS inhibition were independent of motor and motivational effects.

The ability to learn from previously rewarded actions (i.e., positive reinforcement) was susceptible to DMS inhibition, while learning from negative reinforcement (shifting after a loss) remained intact. This was particularly sensitive to increased uncertainty, suggesting that inhibition of the DMS alters the ability to perform the task accurately as the outcome value of differing actions become more uncertain. This makes heuristic sense with the role of the DMS in evidence accumulation (Yartsev et al., 2018), which becomes increasingly important in more uncertain environments. While reinforcement learning is heavily guided by midbrain dopamine projections to the striatum, higher order cognitive (i.e. executive) function governed by cortical regions are required to monitor changes in reward value and the mapping of effort and reward to related action sequences. Thus, disrupting DMS function can produce behavioural phenotypes that mimic those after disruption of relevant cortical areas (Clarke et al., 2008), indicating that that the integration of these specific inputs has likely been compromised. The co-activation data from this study supports this argument, with an overall reduction in co-activity between corticostriatal circuits. More explicitly, the network activity data revealed that DMS suppression induced a complete functional disconnect with the ORBl (and ORBvl) and AI, and a large decrease in co-activity with the ACC. These outcomes are in line with the dense inputs from these areas to the DMS (Gehrlach et al., 2020; Hunnicutt et al., 2016; Young et al., 2022). Moreover, studies from nonhuman primates and humans have even demonstrated strong overlap of ORB and ACC inputs in the DMS (Haber et al., 2020), highlighting a 'hub' in the DMS for functionally integrating these specific inputs to drive goal-directed action. Therefore, it is not surprising that inhibition of the DMS reduces the correlation of activity between the DMS and these cortical regions.

Alongside the anatomical justification for these cortical inputs, they are particularly relevant to cognitive flexibility and reversal learning. The ORBl has long been implicated in reversal learning (Izquierdo, 2017; Izquierdo et al., 2017), particularly during the early phase after a reversal. For example, ORBl inactivation in rats increases perseverative errors (Hervig et al., 2020), with similar outcomes observed after manipulations of the DMS (Clarke et al., 2008; Young et al., 2022). Recent work has highlighted that inhibition of the ORBl also decreases win-stay and may be particularly important in more uncertain environments (Aguirre et al., 2024; Romero-Sosa et al., 2024), which supports the outcomes from this study. Similarly, the ACC has been associated with managing uncertainty during reversal learning. For example, ACC activity in humans has been associated with unexpected outcomes during probabilistic, but not deterministic, reversal learning (Habiby Alaoui et al., 2021). It has also been included in networks associated with learned uncertainty in adaptive learning more generally (Soltani and Izquierdo, 2019). Importantly, work in monkeys has shown that lesions of the ACC specifically impair the ability to correctly respond to rewards during a reversal (Chudasama et al., 2013), similar to the win-stay deficits observed here. Although a role for the AI in reversal learning has not been thoroughly established, human imaging studies suggest it may work in partnership with the ACC, as global areas involved in managing oversight of uncertain tasks more generally (Yaple and Yu, 2019). These studies, in combination with the data presented here, indicate that DMS suppression is preventing the integration of core corticostriatal inputs, which in turn impairs performance under conditions with probabilistic uncertainty. Taken together, these data provide a potential network-level mechanism for how specific DMS dysfunction leads to alterations in corticostriatal patterns of activity that may drive cognitive inflexibility in schizophrenia.

The finding that DMS inhibition increased activity in the ACBsh is curious given the lack of direct connectivity between the DMS and ACBsh, as well as this being the only region altered in our analyses. This increase in activity may be a compensatory response to impaired corticostriatal function. For example, inhibition of the ACBsh has been shown to reduce win-stay in rats during probabilistic uncertainty (Dalton et al., 2014). However, optogenetic activation of midbrain projections to the ACBsh also impair performance, but only when activated after losses (Zühlsdorff et al., 2024). A potential circuit-based mechanism that could lead to alterations in ACBsh activity is that inhibiting the DMS may affect DLS/motor function since these two subregions have been shown to work in parallel during choice-based learning of action-outcome (Bergstrom et al., 2018). As such, while the ACBsh does not project directly to the dorsal striatum, alterations in activity could modulate its function indirectly through dopaminergic circuits, thalamocortical loops, and behavioural control mechanisms, especially when motivational states or reward contingencies are shifting such as those utilised in this task. The hippocampus also projects to the ACBsh to provide contextual and spatial information. This circuit is important for context-dependent reward learning, helping to guide goal-directed behavior based on environmental cues and spatial memory, essentially shaping motivational responses in a context-sensitive manner. While the hippocampus was not included in the cFos analysis due to primary focus being on the associative regions rather than limbic, a future direction would be to delve further into whether DMS inactivation could also modulate hippocampal activity. Given the many feedback loops in the basal ganglia network, changes in ACBsh activity could be the result of multiple potential mechanisms.

A clear caveat of this study is the use of acute chemogenetic inhibition of the DMS in adulthood. Experimental variables (day, specific mice etc.) interacting with acute administration may have contributed to our observations of win-stay deficits at 80:20 on the final cFos day compared with at 80:40 during the within-subject experiment. However, this may also be due to greater number of DREADDs transfected cells in the mice administered CNO for the cFos experiment (see Table S4). Nevertheless, this highlights that within-subject testing and including a variety of probabilistic environments (i.e., uncertainty) improves the ability to detect nuanced differences in behaviour. This could be especially important when considering human studies of complex neuropsychiatric disorders. Furthermore, acute manipulations do not recapitulate the neurodevelopmental dysfunction observed in schizophrenia (i.e., also observed in prodromal phases). But they provide a useful neurobiological perspective on what may influence changes in cognitive flexibility. Evidence suggests a progressive change in reward processing after first-episode psychosis, mirrored by greater diversity and severity of cortical dysfunction (Kesby et al., 2021). Increases in DMS dopamine function at, and prior to, onset have been consistently demonstrated in schizophrenia (Egerton et al., 2013; Howes et al., 2011; McCutcheon et al., 2018). We have shown that increasing dopamine systems globally and in the DMS of mice impairs reversal learning, specifically lose-shift use (Young et al., 2022). This supports work in those with early psychosis showing similar changes in lose-shift use and changes in punishment sensitivity (Baker et al., 2023; Montagnese et al., 2020). In contrast, studies in those with chronic schizophrenia consistently observe problems with reward learning and win-stay use (Deserno et al., 2020; Katthagen et al., 2020; Reddy et al., 2016; Suetani et al., 2022; Waltz et al., 2013). Taken together, this current study suggests that decreased function of the DMS can lead to alterations in reward learning resulting in impaired decision-making performance, reflecting neurobiological features that have been observed in people with schizophrenia during goal-directed actions (Morris et al., 2015). Therefore, it may be that early increases in DMS dopamine function drive changes in the response to loss (and less obvious impairments in reward-oriented tasks). This progressively compromises corticostriatal networks and reduces DMS activity, leading to more specific impairments in reward processing.

This work delivers a translational framework that combines the discrete manipulation of DMS activity during performance with the consequential impact on the activity of broader corticostriatal networks. The outcomes of this study highlight the role of probabilistic uncertainty as an important feature of reversal learning, supporting recent work showing that increased uncertainty improves detection of behavioural outcomes in preclinical and clinical samples (Aguirre et al., 2024; Baker et al., 2023; Dalton et al., 2014; Habiby Alaoui et al., 2021; Soltani and Izquierdo, 2019; Young et al., 2022). Overall, the role of DMS dysfunction in driving broader corticostriatal network problems has implications not only for schizophrenia but for a range of psychiatric disorders where alterations in corticostriatal circuitry and cognitive flexibility have been observed.

## CRediT authorship contribution statement

**Kyna-Anne Conn:** Writing – original draft, Methodology, Investigation. **Joyosmita Das:** Investigation. **Suzy Alexander:** Software, Methodology, Investigation. **Thomas HJ. Burne:** Writing – review & editing, Supervision, Methodology, Conceptualization. **James P. Kesby:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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## Declaration of competing interest

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2025.110568.

## Data availability

Data will be made available on request.

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