

Supplemental Material

Research Articles | Behavioral/Cognitive

Stage-dependent effects of “too little and too much” medial prefrontal activity on reversal learning in rats: functional inhibition impairs early, whereas neural disinhibition impairs late reversals

<https://doi.org/10.1523/JNEUROSCI.1930-25.2026>

Received: 14 October 2025

Revised: 30 July 2026

Accepted: 7 August 2026

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Supplemental Results

Comparison of baseline mPFC LFP and multi-unit parameters with those recorded in our previous study (Pezze et al., 2014)

To assess reproducibility of mPFC LFP and multi-unit measures across our studies, we compared baseline values (mean $\pm$ SEM) of these measures recorded from isofluorane-anaesthetised Vgat-Cre rats in the present study to those that we recorded previously in isofluorane-anaesthetised Lister hooded rats (Pezze et al., 2014). In the previous study, we had baseline values from three different prospective mPFC microinfusion groups, and we averaged these baseline values across these three groups for the purpose of the present comparison. Within-burst firing rates, number of bursts within a 5-min block, mean burst duration, and inter-burst interval were comparable in the present study (181.57 $\pm$ 26.23 spikes/s, 134.3 $\pm$ 5.86, 223.0 $\pm$ 47.0 ms, and 5.37 $\pm$ 1.46 s respectively) and our previous study (187.15 $\pm$ 24.35 spikes/s, 132.2 $\pm$ 35.76, 307.5 $\pm$ 53.0 ms, and 6.625 $\pm$ 0.44 s respectively). In contrast, baseline overall firing rate and AUC of PSD in the present study were lower compared to our previous work (11.54 $\pm$ 2.21 vs. 20.68 $\pm$ 5.65 spikes/s and 0.007 $\pm$ 0.0007 μ V² vs. 0.015 $\pm$ 0.004 μ V², respectively). Overall, these comparisons indicate good agreement of mPFC recordings across our two studies. As to the numerical differences noted for some measures, it is unclear whether these reflect strain differences or methodological differences (including an indwelling infusion cannula in the previous study and different microwire arrays).

mPFC infusion

Reversal stage	Saline (0.9%)			Picrotoxin (300ng)			Muscimol (62.5ng)		
	Omissions	Lat _{cor} (s)	Lat _{inc} (s)	Omissions	Lat _{cor} (s)	Lat _{inc} (s)	Omissions	Lat _{cor} (s)	Lat _{inc} (s)
	<i>n</i> =15	<i>n</i> =15	<i>n</i> =15	<i>n</i> =14	<i>n</i> =12	<i>n</i> =12	<i>n</i> =15	<i>n</i> =15	<i>n</i> =15
SD	9.00±5.66	0.99±0.07	1.31±0.09	108.92±17.91	2.64±0.18	2.94±0.19	2.40±0.84	1.17±0.09	1.32±0.10
R1	(0.33±0.16)	(1.08±0.14)	(1.15±0.23)	(14.5±1.41)	1.98±0.25	2.75±0.21	(0.53±0.19)	(1.18±0.12)	(1.75±0.23)
	1.13±0.36	0.94±0.10	1.10±0.11	65.14±13.15			3.20±0.86	1.15±0.09	1.24±0.12
R2	(0.07±0.07)	(0.99±0.15)	(0.96±0.09)	(17.7±1.22)	2.24±0.27	1.92±0.12	(0.13±0.09)	(1.06±0.17)	(1.08±0.10)
	0.53±0.17	1.07±0.11	1.06±0.11	58.36±7.65			2.80±0.67	1.20±0.11	1.26±0.08
R3	(0.27±0.21)	(0.94±0.10)	(1.03±0.19)	15.6±1.94)	1.65±0.19	1.81±0.23	(0.33±0.27)	(1.20±0.16)	(1.53±0.32)
	1.07±0.55	0.96±0.10	0.99±0.10	49.50±11.24			1.87±0.92	1.20±0.13	1.57±0.22

Supplemental Table S1 Legend

Table S1. Reversal experiment 1: *omissions and response latencies following mPFC saline, picrotoxin and muscimol*. Values (mean $\pm$ SEM) are shown for the different task stages (spatial discrimination, SD; reversals 1 to 3, R1 to 3). Values during the 20 reminder trials at the beginning of each reversal stage are shown in brackets. Response latencies are indicated separately for correct responses (Lat_{Cor}) and incorrect responses (Lat_{Inc}). Sample sizes contributing to the mean values are indicated at the top of each column. Note, only one rat in the picrotoxin group completed at least one correct and one incorrect response and, therefore, this group was excluded from the analysis of reminder-trial response latencies and no reminder trial response latencies are shown for this group. Moreover, two rats in the picrotoxin group did not make at least one incorrect response across all task stages and had to be excluded completely from the latency analysis, resulting in a reduced sample size of n=12 (instead of 14) for the latency means.

mPFC infusion

Infusion series	Saline (0.9%)			Picrotoxin (300ng)			Muscimol (62.5ng)		
	Omissions	Lat _{Cor} (s)	Lat _{Inc} (s)	Omissions	Lat _{Cor} (s)	Lat _{Inc} (s)	Omissions	Lat _{Cor} (s)	Lat _{Inc} (s)
	<i>n</i> =13	(<i>n</i> =12)	(<i>n</i> =12)	<i>n</i> =13	(<i>n</i> =12)	(<i>n</i> =12)	<i>n</i> =13	(<i>n</i> =12)	(<i>n</i> =12)
		<i>n</i> =13	<i>n</i> =13		<i>n</i> =13	<i>n</i> =13		<i>n</i> =13	<i>n</i> =13
Series 1	(0.15±0.10)	(0.66±0.08)	(0.87±0.17)	(13.62±6.77)	(2.65±0.27)	(2.42±0.15)	(0.23±0.17)	(0.67±0.10)	(0.76±0.14)
	0.46±0.18	0.59±0.05	0.59±0.05	34.77±8.29	2.37±0.35	2.99±0.39	1.00±0.36	0.66±0.10	0.86±0.14
Series 2	(0.31±0.21)	(0.65±0.09)	(0.70±0.19)	(2.77±0.79)	(2.77±0.36)	(2.39±0.44)	(0.15±0.10)	(0.59±0.08)	(1.10±0.27)
	0.38±0.21	0.50±0.04	0.66±0.10	23.54±7.64	1.44±0.18	1.95±0.21	0.31±0.13	0.69±0.06	0.63±0.08

Supplemental Table S2 Legend

Table S2. Reversal experiment 2: *omissions and response latencies following mPFC, saline, picrotoxin and muscimol*. Values (mean $\pm$ SEM) are shown for the two-infusion series (series 1, reversals 5 to 7, R5 to 7; series 2, reversals 8-10, R8 to 10). Values during the 20 reminder trials at the beginning of each reversal stage are shown in brackets. Response latencies are indicated separately for correct responses (Lat_{Cor}) and incorrect responses (Lat_{Inc}). Sample sizes contributing to the mean values are indicated at the top of each column. Note: one rat did not make at least one incorrect response during reminder trials in all three drug conditions in both series and, therefore, was excluded from the analysis of reminder trial response latencies; the resulting sample size for Lat_{Inc} during reminder trials is indicated in brackets.

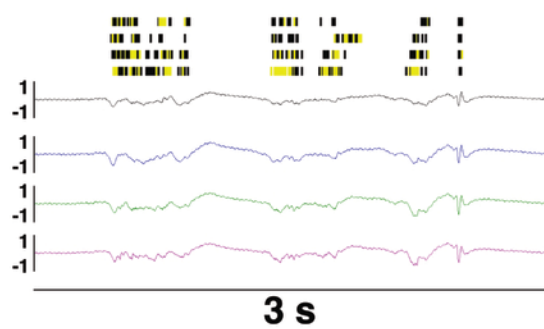
1
2

Systemic injection					
Vehicle (saline, 0.9%)			CNO-2HCl (4.5mg/kg)		
Omissions	Lat _{Cor} (s)	Lat _{Inc} (s)	Omissions	Lat _{Cor} (s)	Lat _{Inc} (s)
<i>n</i> =12	<i>n</i> =12	<i>n</i> =12	<i>n</i> =12	<i>n</i> =12	<i>n</i> =12
(0±0)	(0.70±0.13)	(0.53±0.07)	(1.58±1.08)	(0.90±0.24)	(0.86±0.20)
0.16±0.11	0.63±0.10	0.70±0.11	4.83±3.85	1.00±0.18	1.22±0.26

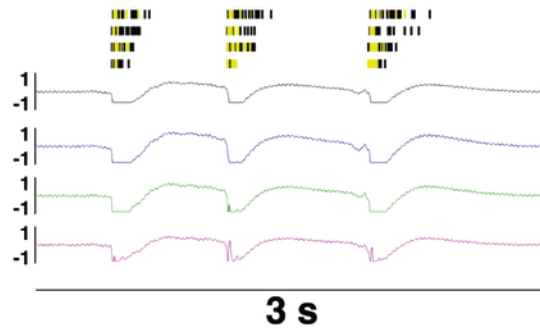
Supplemental Table S3 Legend

Table S3. Reversal experiment 3: *omissions and response latencies following chemogenetic mPFC disinhibition by 4.5 mg/kg CNO-2HCl or saline injection*. Values are shown as mean $\pm$ SEM. Values during the 20 reminder trials at the beginning of each reversal stage are shown in brackets. Response latencies are indicated separately for correct responses (Lat_{Cor}) and incorrect responses (Lat_{Inc}). Sample sizes contributing to the mean values are indicated at the top of each column.

15-min post-saline

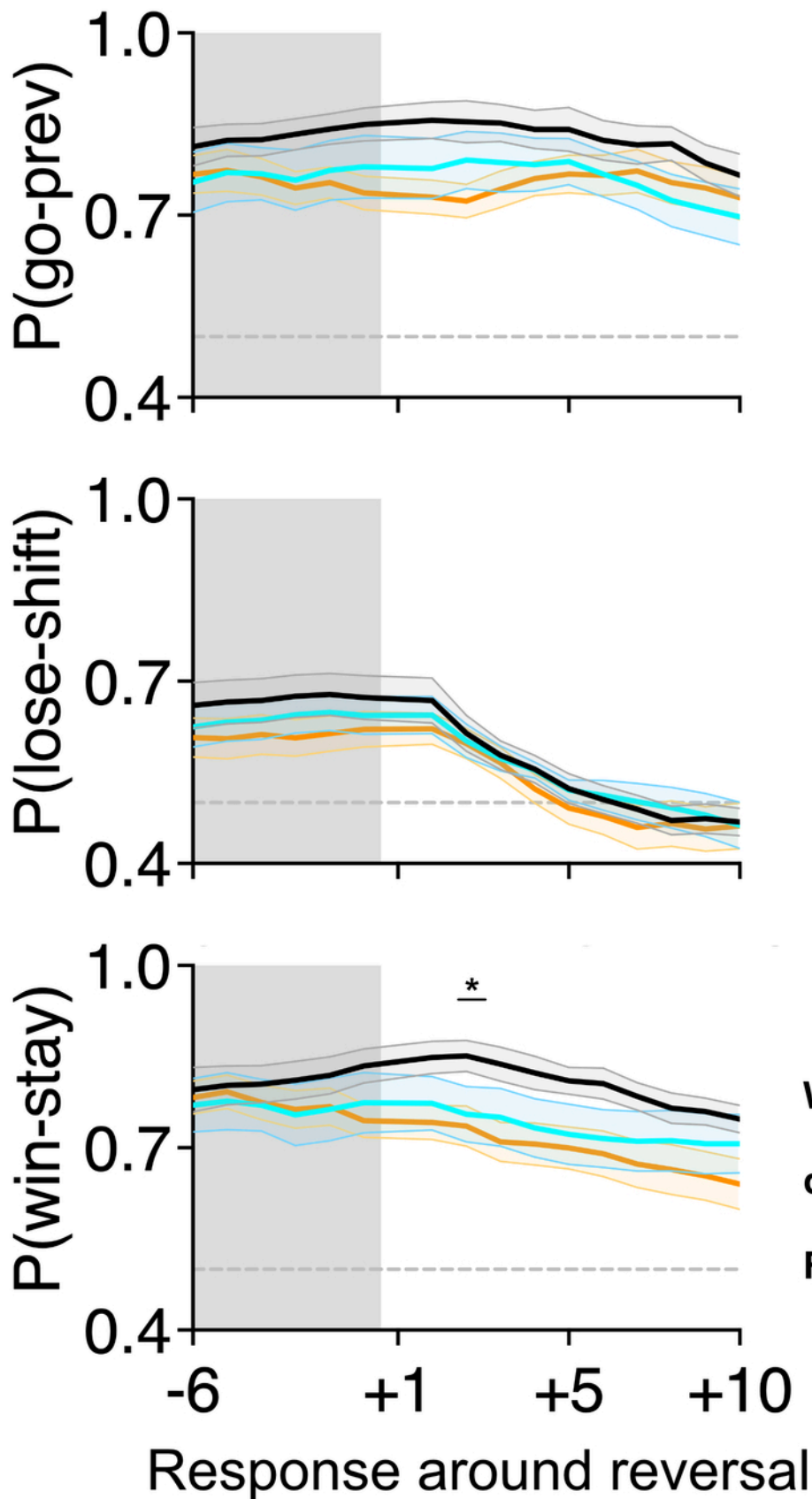


15-min post-CNO2HCl



Supplemental Figure S1 Legend

Figure S1. *Chemogenetic disinhibition by 6.0 mg/kg CNO2HC enhanced LFP spike-wave discharges and intensified burst firing within the mPFC.* Raster plot (top, black vertical lines; yellow shaded areas indicate automatically classified 'bursts') and LFP traces (bottom, colored lines) for one electrode array (electrodes 1-4) during a 3 second period 15-min post-saline (left) or 6.0 mg/kg CNO-2HCl (right). Following mPFC CNO-2HCl, there was a notable increase in potent LFP spike-wave discharges (negative deflection followed by a positive wave), alongside intensification of multiunit burst firing. These changes in mPFC neural activity were similar to those caused mPFC disinhibition by picrotoxin microinfusion in our previous study, including spike-wave LFP discharges and enhanced LFP power and enhanced neural burst firing; Pezze et al., 2014). Note, negative LFP spikes following CNO-2HCl were clipped, because they exceeded our LFP amplitude recording range.



Saline

1.5 mg/kg CNO-2HCl

3.0 mg/kg CNO-2HCl

Supplemental Figure S2 Legend

Figure S2. *Response strategies around reversal following chemogenetic mPFC disinhibition by 1.5 (light blue) and 3.0 mg/kg (gold) CNO-2HCl, compared to saline (black) injection. (Go-previous [top], lose-shift [middle], and win-stay [bottom]). Coloured lines and shaded regions indicate mean $\pm$ SEM. Grey-shaded region indicates reminder trials. Asterisks above datapoints indicate responses where groups significantly differed ($p < 0.05$) following observation of a drug $\times$ response interaction.*