


# Medial prefrontal cortical neurotransmitters reactive to relapse-promoting and relapse-suppressing cues in male rats trained to self-administer cocaine or alcohol

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## ABSTRACT

Environmental cues signaling drug availability (S+) vs. omission (S-) each recruit specific prefrontal cortical neurons to promote vs. suppress drug seeking in rats, suggesting similarly cue-specific neurotransmission regulates such behavior. We here determined extracellular neurotransmitter fluctuations in the infralimbic (IL) and prelimbic (PL) cortices of rats reactive to S+ vs. S-. For this, male rats were trained to recognize both S+ and S- within the context of either cocaine or alcohol self-administration and then subjected to S+ vs. S- cue-tests during which animals engaged in active drug seeking vs. suppression of this behavior. In cocaine-trained rats, serotonin, taurine and adenosine in PL were preferentially modulated during the S+ (vs. S-) cue-test, while glutamate in PL was preferentially modulated during the S- (vs. S+) cue-test. In alcohol-trained rats,  $\gamma$ -aminobutyric acid (GABA) in IL was preferentially modulated during the S+ cue-test, while histamine in PL as well as glutamate and dopamine in IL were preferentially modulated during the S- cue-test. In summary, prefrontal neurotransmissions reactive to drug discriminative cues are dependent on cue types (S+ vs. S-), brain regions (IL vs. PL) and drugs used for cue-conditioning (cocaine vs. alcohol), thereby suggesting cocaine- and alcohol-seeking are each regulated by distinct neurochemical processes.

## Introduction

Discriminative environmental stimuli signaling drug availability (S+), along with stress and drugs (priming), trigger drug craving in patients with substance use disorders, and reinstate extinguished drug seeking in rats [1–3]. Conversely, environmental cues signaling drug omission (S-) counter relapse-promotion by drug availability cues, stress and drug priming in rats [4–7]. Thus, rats trained to recognize S+ and S- serve to study brain mechanisms that actively promote and suppress drug seeking.

S+ and S- are each known to activate specific functional units of neurons (neuronal ensembles/engram cells) in IL – the ventral subregion of the medial prefrontal cortex (mPFC) – to promote and suppress

relapse [7–9]. While such cue-specific prefrontal neuroactivity is likely mediated by similarly cue-specific neurotransmission, prefrontal neurotransmitters reactive to S+ and/or S- remain unknown.

Glutamate is the primary source of cortical activation, and infralimbic neurons receive this excitatory neurotransmitter from diverse cortical and subcortical regions [10]. Infralimbic neurons also receive GABA from local inhibitory interneurons, dopamine from the ventral tegmental area (VTA), serotonin from the raphe nucleus, norepinephrine from the locus coeruleus, and acetylcholine from the brainstem. Additional neurotransmitters present in mPFC include, but not limited to, adenosine, aspartate, serine, glycine, histamine and taurine, and S+ and S- could engage any combinations of these neurotransmitters to modulate prefrontal neuroactivity. Since S+ and S- exert their opposing

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actions on relapse similarly across rats trained with cocaine or alcohol [2,3,7,11], cue-specific – but not drug-specific – prefrontal neurotransmissions likely regulate cocaine and alcohol seeking.

We here explored the possibilities above in male rats trained to recognize both S+ and S- within the context of cocaine or alcohol self-administration, and used microdialysis to collect extracellular neurotransmitters from IL and the adjacent PL similarly implicated in drug seeking regulation [12]. Since S+ and S- could engage multiple prefrontal neurotransmitters, we used high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze adenosine, aspartate, dopamine, GABA, glycine, glutamate, histamine, norepinephrine, serine, serotonin and taurine in each dialysate sample [13,14]. However, these 11 neurotransmitters likely represent a fraction of neurochemicals regulating drug seeking. While we used male rats to isolate the effects of S+/S- from the possible effects of estradiol cycle, the interaction between S+/S- and female sex hormones on prefrontal neurotransmission remains to be determined.

## Materials and methods

All behavioral procedures were based on our previous studies [7,13] and conducted at Scripps Research in accordance with the National Institute of Health (USA) Guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee. Neurotransmitter analyses were conducted at Virginia Tech. Data analyses were conducted at Scripps Research, Virginia Tech and Mayo Clinic. Detailed methods are described in the Supplementary Material.

### Subjects

Male Long Evans rats were assigned for cocaine or alcohol discriminative cue-conditioning, as “cocaine-trained” vs. “alcohol-trained” rats. They were further randomly assigned to one of the two experimental groups defined by the brain region (IL or PL) for microdialysis.

### Chemicals

Cocaine was obtained from the Drug Supply Program from the National Institute on Drug Abuse, NIH, USA. Alcohol was purchased from Sigma Aldrich. Chemicals for LC-MS/MS were purchased from Sigma Aldrich or VWR.

### Surgery

Rats received bilateral microdialysis guide cannulae in IL or PL. The coordinates for IL/PL were +3.2/+3.2 mm (anterior),  $\pm 0.5/\pm 0.5$  mm (medial/lateral) and  $-4.6/-2.8$  mm (ventral) to guide cannulae tips from the bregma to accommodate probes with 1.0 mm active membrane. Rats assigned to cocaine discriminative cue-conditioning also received intravenous catheters.

### Behavioral procedures

Rats were trained/tested during the dark phases in operant conditioning chambers based on [2,7,9]. Both cocaine and alcohol discriminative cue-conditioning experiments consisted of three experimental phases:

I. Self-administration training: Each rat was placed in an operant conditioning chamber. Insertion of active and inactive levers began once daily drug self-administration sessions started, during which active lever-presses resulted in cocaine (1.0 mg/kg/infusion, intravenous) or alcohol (20 % in water, 0.2 ml, oral) under FR1. Each drug delivery was paired with light-cue illumination during which active lever-presses were recorded without scheduled consequences.

Each cocaine and alcohol self-administration session lasted 120 min, and 60 min. Rats underwent a minimum of 14 sessions (Fig. 1A and Fig. 1B).

II. Discriminative cue-training: Each rat was trained to recognize both almond and orange scents as S+ and S-. The odor-cue assignment was counterbalanced between subjects. Each rat underwent alternating S+ and S- training sessions to lever press for either cocaine or alcohol (preceded/accompanied by S+) or no cocaine or no alcohol (preceded/accompanied by S-) for 60 min. The S+/S- odor was introduced 30 min prior to insertion of active/inactive levers. Each rat underwent a minimum of 60 training sessions (30 each for S+ or S-; Figs. 1C and D).

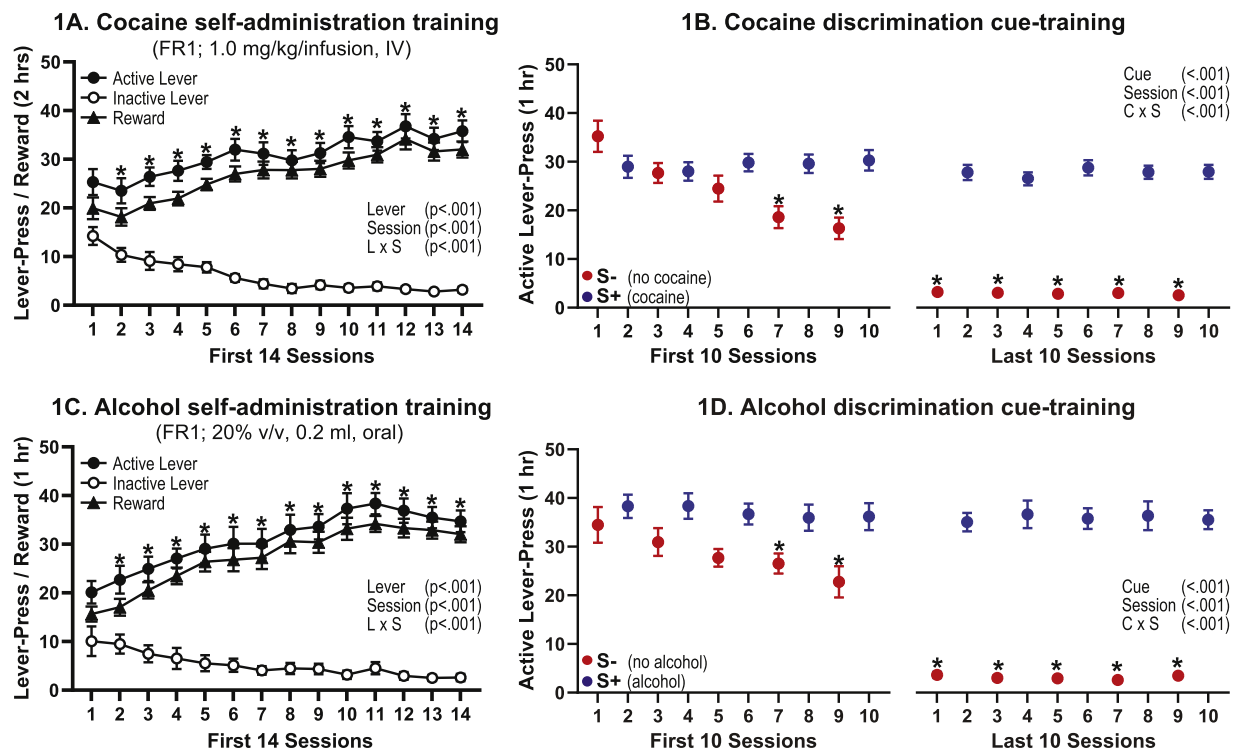
III. Discriminative cue-test: Each rat received microdialysis probes into either IL or PL and placed in an operant chamber overnight for probe equilibration and then underwent two once daily cue-tests (S+ and S- cue-tests). Each cue-test consisted of 4 experimental periods: [1] 60-min ‘Baseline’ period without S+ or S- and active/inactive levers, [2] 30-min ‘cue-exposure (Cue)’ period with S+ or S- but without active/inactive levers, [3] 60-min ‘cue-exposure + lever-pressing (Cue + Lever)’ period with S+ or S- and active/inactive levers, and [4] 30-min ‘re-baseline (Rebase)’ period following removal of the S+ or S- odor and retraction of operant levers. Microdialysis samples were collected every 15 min throughout these 4 periods, lever-presses were recorded during the ‘Cue + Lever’ period. After completing both cue-tests, rats were euthanized, and their brains collected to validate probe placements (Fig. 2A and Fig. 3A).

### Neurochemical analysis

Neurotransmitter qualification/quantification was based on previously developed methods [13,14] using LC-MS/MS. Extracellular neurotransmitter concentrations were expressed as percentages of baseline values: the mean concentration of the 4 samples collected during the 60-min ‘baseline’ period was defined as 100 %.

### Statistics

Data from cocaine- and alcohol-trained rats were separately analyzed using GraphPad Prism and SPSS. Within cocaine- or alcohol-trained rats, data from rats with microdialysis probes in IL and PL were combined for behavioral analyses but were analyzed separately for neurochemical analyses. Total numbers of active vs. inactive lever-presses per session during the first 14 days of self-administration training were analyzed using 2-way repeated measures ANOVA with “Lever” (12 levels) and “Day” (14 levels) as the within-subject variable. Total numbers of active-lever presses during the first and last 10 days of discriminative cue-training, which corresponded to the first and last 5 S+/S- training sessions, were analyzed using 2-way repeated measures ANOVA with “Cue” (2 levels) and “Session” (10 levels) as the within-subject variables. Total numbers of active lever-presses during the discriminative cue-tests (60 min totals) were analyzed using Student’s *t*-test (paired). Total numbers of active lever-presses during the “Cue + Lever” period (60 min) of these cue-tests (15 min totals) were analyzed using 2-way repeated measures ANOVA using Cue (2 levels) and Time (4 levels) as the within-subject variables. Each neurotransmitter was separately analyzed by 2-way repeated measures ANOVA with “Time” (12 levels) and “Cue” (2 levels: S+ and S-) as the within-subject variable. Significant ANOVA results were followed with post-hoc Fisher’s Least Significance Difference (LSD) tests and area under the curve (AUC) analyses. Detailed exclusion criteria for behavioral and neurotransmitter analyses are described in Supplementary Methods. The final numbers of cocaine- and alcohol-trained rats kept for statistical analyses were  $N = 25$ , 17 for the behavioral analyses,  $N = 9$ –13, and 5–9 for the neurotransmitter analyses (see Supplementary Tables 1–2 for details). For all cases, differences were considered significant when  $p < 0.05$  (two-



**Fig. 1.** Cocaine and alcohol self-administration training (A/B) and discrimination cue-training (C/D). Rats were trained to lever-press for cocaine and alcohol before undergoing discrimination cue-training to learn S+ and S-. Statistical results are described in Table 1.  $N = 25/17$  (cocaine/alcohol). \*,  $p < 0.01-0.001$  (vs. inactive lever).

tailed).

## Results

All statistical results are presented in Tables 1–5, Supplementary Tables 3–4 or described below. Significant findings are presented in Figs. 1–3 and described below:

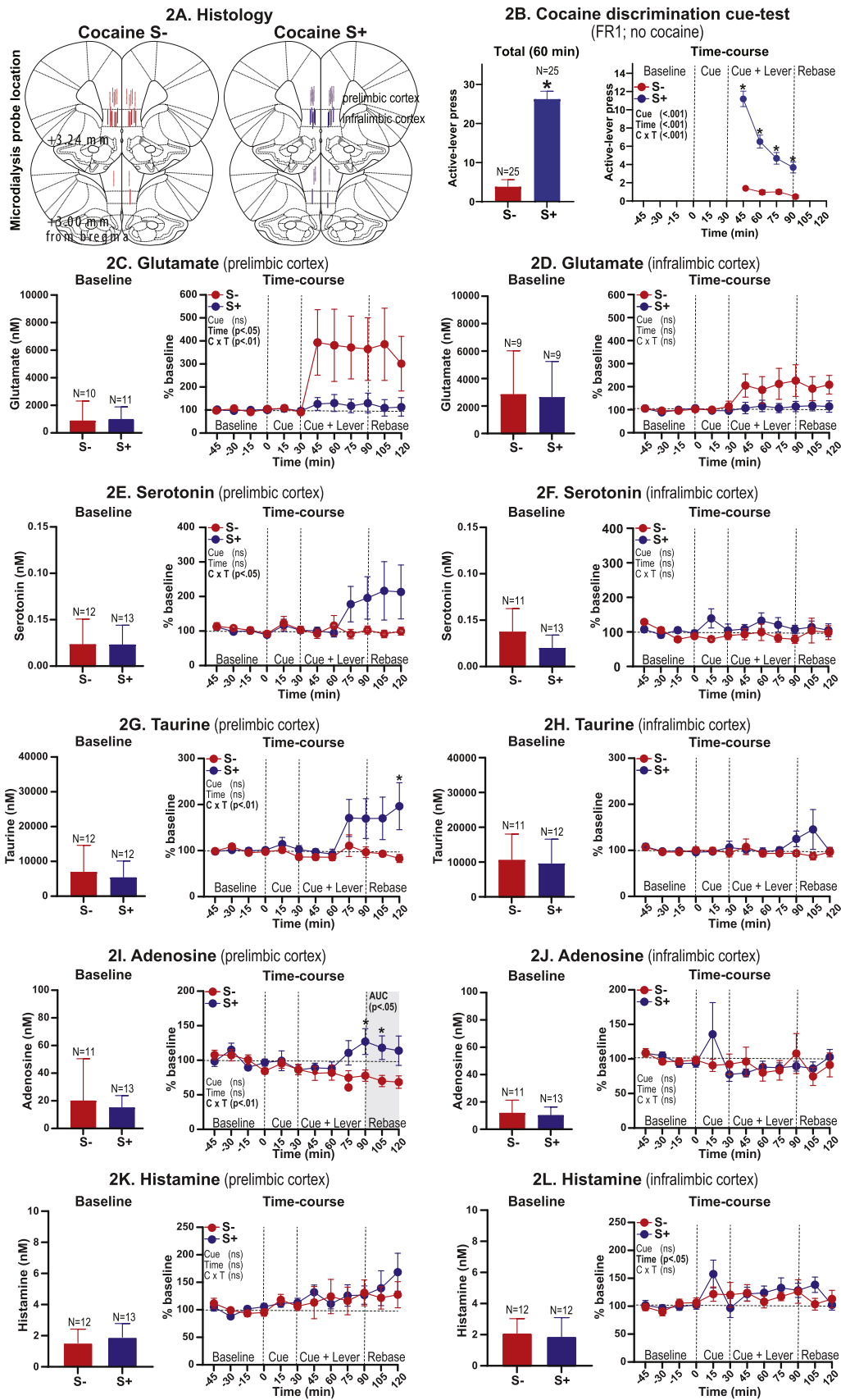
During the self-administration training, rats learned to lever-press for cocaine or alcohol (Fig. 1A and Fig. 1C). Two-way repeated measure ANOVA on active vs. inactive lever-presses by cocaine-trained rats across the first 14 days of training revealed a significant effect of Lever and Lever  $\times$  Day interaction (Table 1). Two-way repeated measure ANOVA on active vs. inactive lever-presses by alcohol-trained rats across the first 14 days of training revealed a significant effect of Lever and Lever  $\times$  Day interaction (Table 1). During the discriminative cue-training, rats learned to dissociate S+ and S- signaling availability and omission of cocaine or alcohol (Fig. 1B and Fig. 1D). Two-way repeated measure ANOVA on lever-presses by cocaine-trained rats across the first and last 10 days of cue-training revealed a significant effect of Cue, Day, and Cue  $\times$  Day interaction (Table 1). Two-way repeated measure ANOVA on lever-presses by alcohol-trained rats across the first and last 10 days of cue-training revealed a significant effect of Cue, Day and Cue  $\times$  Day interaction (Table 1). During the discriminative cue-tests, cocaine-trained ( $t_{(24)}=11.20, p < 0.001$ ) and alcohol-trained rats ( $t_{(16)}=7.67, p < 0.001$ ) lever-pressed significantly more during the S+ vs. S- cue-tests (60 min, total; Fig. 2B and Fig. 3B – left panels) as previously reported [2,3]. Two-way repeated measure ANOVA on lever-presses by cocaine-trained rats during the “Cue + Lever” phase (60 min) revealed a significant effect of Cue, Time and Cue  $\times$  Time interaction (Table 1). Two-way repeated measure ANOVA on lever-presses by alcohol-trained rats during the “Cue + Lever” phase (60 min) revealed a significant effect of Cue, Time and Cue  $\times$  Time interaction (Table 1).

In PL of cocaine-trained rats, ANOVA revealed (Table 2) significant effects of Time ( $p < 0.05$ ) and Cue  $\times$  Time ( $p < 0.01$ ) interaction for

glutamate (Fig. 2C), significant Cue  $\times$  Time interaction ( $p < 0.01-0.05$ ) for serotonin (Fig. 2E), taurine (Fig. 2G) and adenosine (Fig. 2I). In IL of cocaine-trained rats, ANOVA revealed (Table 3) significant effects of Time ( $p < 0.05$ ) for histamine. Thus, in cocaine-trained rats, S+ vs. S- actions on prefrontal glutamate, serotonin, taurine and adenosine represent cue-specific neurotransmissions. In PL of alcohol-trained rats, ANOVA revealed (Table 4) significant effects of Cue ( $p < 0.05$ ) and Cue  $\times$  Time interaction for histamine (Fig. 3I). In IL of alcohol-trained rats (Table 5), ANOVA revealed significant effects of Cue ( $p < 0.05$ ) and almost significant Cue  $\times$  Time interaction ( $p = 0.09$ ) for glutamate, significant Cue  $\times$  Time interaction ( $p < 0.05$ ) for dopamine (Fig. 3F), significant effect of Cue ( $p < 0.05$ ) and Cue  $\times$  Time interaction ( $p < 0.05$ ) for GABA (Fig. 3H), and significant effect of Time ( $p < 0.05$ ) for aspartate (Fig. 3L). Thus, in alcohol-trained rats, S+ vs. S- actions on prefrontal histamine, and infralimbic glutamate, dopamine and GABA represent cue-specific neurotransmissions.

Despite these significant ANOVA results, post-hoc Fisher’s LSD tests comparing baseline (–45–0 min) and subsequent (15–120 min) time-points revealed no significant difference for any neurotransmitters/brain sites/cues in cocaine- or alcohol-trained rats. These negative results are likely due to relatively large individual variabilities, relatively unstable baseline levels, and relatively moderate sample sizes. Nevertheless, Fisher’s LSD tests comparing S+ vs. S- in cocaine-trained rats revealed significant cue-specific modulation of taurine (at 120 min) and adenosine (at 90/105 min) in PL (Fig. 2G and Fig. 2I). Fisher’s LSD tests comparing S+ vs. S- in alcohol-trained rats revealed significant cue-specific modulation of glutamate (at 90 min) and GABA (at 45/60/70/90 min) in IL (Fig. 3D, Fig. 3F and Fig. 3H) and histamine (at 30/75/105/120 min) in PL (Fig. 3I and Fig. 3K). However, despite significant Cue  $\times$  Time interactions revealed by ANOVA ( $p < 0.01-0.05$ ), Fisher’s LSD tests did not reveal any significant differences for prefrontal glutamate and serotonin in cocaine-trained rats (Fig. 2C and Fig. 2E).

AUC analyses to further clarify cue-specific neurotransmitter modulations within the “Cue”, “Cue + Lever” and “Rebase” periods for



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**Fig. 2.** Extracellular neurotransmitter fluctuations in PL vs. IL of cocaine-trained rats during discriminative cue-tests. Microdialysis samples were collected from PL or IL (2A) during the “Baseline”, “Cue (only)”, “Cue+Lever” and “Rebase” periods of the S+ vs. S- cue-tests. Rats were allowed to lever-press during the Cue+Lever period (2B). 12 neurotransmitters were qualified and quantified in each dialysate sample. All statistical results are described in Tables 1–3, and only neurotransmitters with significant effects are depicted here: glutamate (2C/2D), serotonin (2E/2F), taurine (2G/2H), adenosine (2I/2 J) and histamine (2K/2 L). Data are presented as mean  $\pm$  SEM. \*,  $p < 0.05$ – $0.001$  (vs. S+/S-). \*\*AUC, area under curve.

cocaine-trained rats (Supplementary Figure 3) revealed S+ and S- differentially modulated prefrontal adenosine during the “Rebase” period (Fig. 2I). Similarly, AUD analyses for alcohol-trained rats (Supplementary Figure 4) revealed S+ and S- differentially modulated infralimbic glutamate during the “Cue + Lever” period (Fig. 3D), infralimbic dopamine during the “Cue” period (Fig. 3F), infralimbic GABA during the “cue + lever” period (Fig. 3H), prefrontal histamine during the “Cue” and “Rebase” periods (Fig. 3I). However, despite significant “Cue x Time” interactions revealed by ANOVA ( $p < 0.01$ – $0.05$ ), AUD analyses did not reveal any significant cue-specific neurotransmitter modulations for prefrontal glutamate, serotonin and taurine in cocaine-trained rats (Fig. 2C, Fig. 2E and Fig. 2G).

## Discussion

As previously reported [2,3], rats trained to self-administer cocaine or alcohol actively lever-pressed when exposed to S+, but not S-, during the discriminative cue-tests under an extinction (drug free) condition (Fig. 2B and Fig. 3B). Such cue-specific modulation of drug seeking is presumably due to learning processes known as conditioned excitation/inhibition and/or positive/negative occasion setting [15]. Since S+ and S- each recruit specific prefrontal neurons to promote and suppress drug seeking [8,9], similarly cue-specific neurotransmission may underlie such cue-specific behavioral modulation.

### S+ reactive prefrontal neurotransmitters

Of the four cue-specific neurotransmissions in cocaine-trained rats (determined by ANOVA and/or post-hoc LSD/AUC analyses), prefrontal serotonin, taurine and adenosine (Fig. 2E, Fig. 2G and Fig. 2I) were preferentially modulated – likely increased – during the S+ (vs. S-) cue-test. Taurine [16] and adenosine [17] are released from neurons and astrocytes in mPFC. While prefrontal taurinergic and adenosinergic circuitries are not yet characterized, prefrontal serotonin was likely released from serotonergic projection from the raphe nucleus. Regardless of the anatomical source, cue-specific modulation of prefrontal taurine, adenosine, and serotonin during the S+ cue-test occurred later in the “Cue + Lever” period and during the “Rebase” period, and thus would not account for cue-specific prefrontal [18] and behavioral (Fig. 2B) activation by S+ [19]. Therefore, prefrontal neurotransmissions mediating cue-specific neurobehavioral modulations by S+ in cocaine-trained rats remain to be elucidated. Since adenosine [20] and serotonin [21] are both implicated in neuroplasticity whereas taurine is neuroprotective [22], preferential increases in these neurotransmitters during the “Rebase” period may reflect consolidation of S+ ‘cue-extinction’ memory.

In alcohol-trained rats, infralimbic GABA (Fig. 3G) was the only neurotransmitter preferentially modulated – likely decreased – during the S+ (vs. S-) cue-test. Local GABAergic interneurons are the primary source of cortical GABA and since this preferential decrease in GABA occurred during the “Cue + Lever” period, the current finding links inhibition of these inhibitory neurons to behavioral activation by S+ (Fig. 3B). Indeed, the GABA(A) receptor agonist acamprosate, which would compensate decreased infralimbic GABAergic transmission during the S+ cue-test in alcohol-trained (but not cocaine-trained) rats, is an FDA-approved medication for alcohol use disorder [23] with a minimal treatment efficacy against cocaine use disorder [24].

It was surprising that glutamate in PL or IL of cocaine- or alcohol-trained rats were not increased during the S+ cue-test (Figs. 2C,

Fig. 2D and Fig. 3C, Fig. 3D), considering the wealth of literature implicating prefrontal glutamate in cue-provoked drug seeking [19]. Consistent with our results collected 2 days after the last cocaine or alcohol self-administration, a similar lack of cue-evoked prefrontal glutamate was observed 3 days after the last cocaine self-administration despite active cue-provoked cocaine seeking [25]. However, 30 days after the last cocaine self-administration, cue-evoked prefrontal glutamate accompanied enhanced cue-provoked cocaine seeking [25]. Thus, cue-evoked prefrontal glutamate likely underlies cue-provoked drug seeking during protracted – but not early – phase of abstinence. Indeed, pharmacological manipulation of glutamatergic transmission in PL or IL decreased cue-provoked cocaine seeking 30 days after – but not 3 days after – the last cocaine self-administration [26].

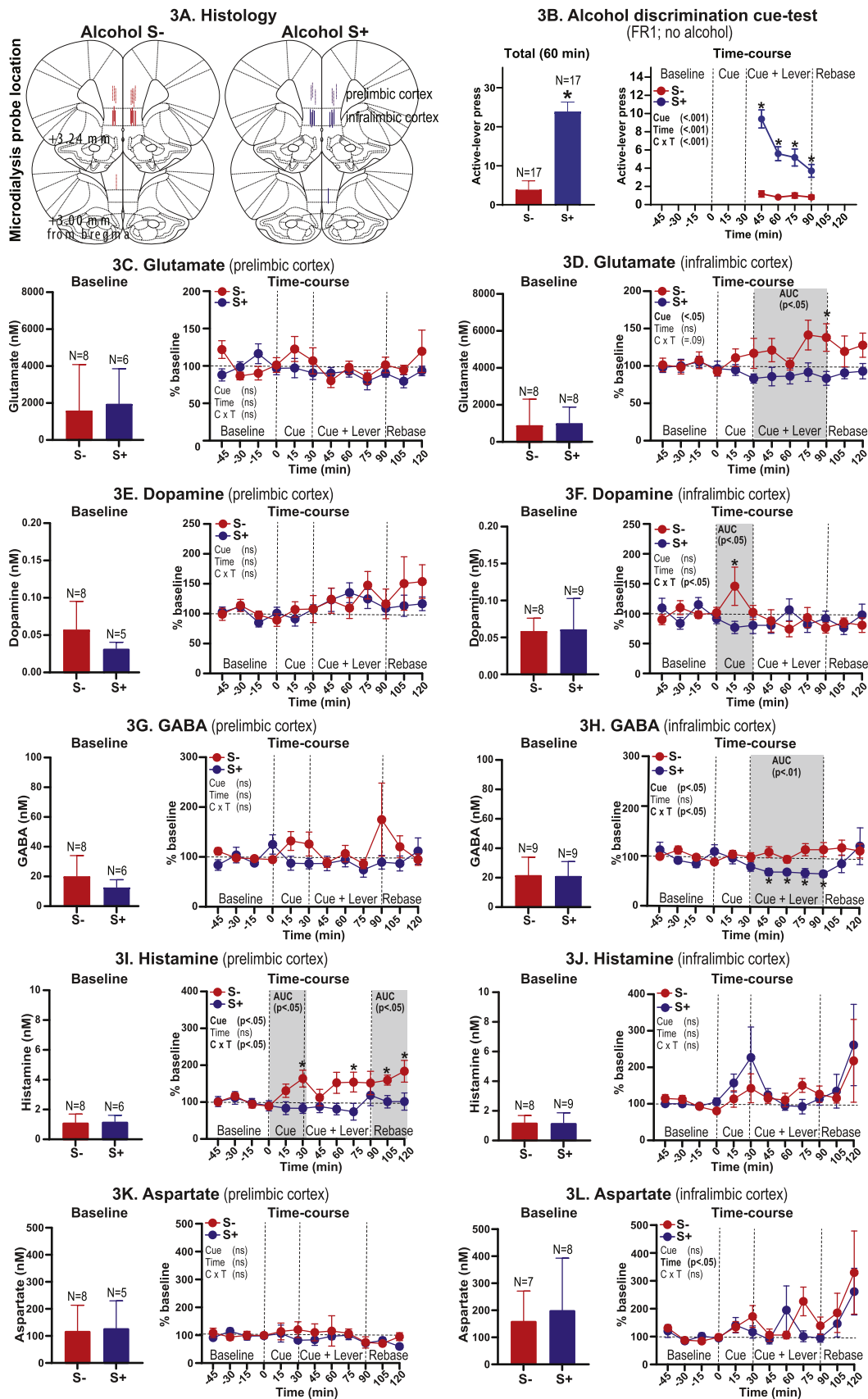
### S- reactive prefrontal neurotransmitters

Of the four cue-specific neurotransmissions in cocaine-trained rats, prefrontal glutamate (Fig. 2C) appears to be the only neurotransmitter in PL or IL preferentially modulated – likely increased – during the S- (vs. S+) cue-test. In alcohol-trained rats, infralimbic (but not prefrontal) glutamate (Fig. 3D) appears to be preferentially modulated – likely increased – during the S- (vs. S+) test. While the reason for these PL vs. IL differences remains to be elucidated, significant modulation of prefrontal glutamate in cocaine- or alcohol-trained rats was observed during the “Cue + Lever” period. Such cue-specific neurotransmission suggests minimal lever pressing during the S- cue-test in cocaine- or alcohol-trained rats (Fig. 2B and Fig. 3B) reflects active neurobehavioral processes, and/or frustration with/aversion to a non-drug context. Nevertheless, glutamate agonists may mimic S- to suppress drug seeking in cocaine- or alcohol-trained rats. Indeed, medications to facilitate glutamatergic neurotransmission (e.g., N-acetylcysteine) exert anti-relapse actions against cocaine and alcohol use [27].

In alcohol-trained rats, infralimbic dopamine and prefrontal histamine (Fig. 3F and Fig. 3I) were also preferentially modulated – both likely increased – during the S- (vs. S+) cue-test. Dopaminergic projection from VTA and histaminergic projection from the tuberomammillary nucleus of hypothalamus are likely the source of infralimbic dopamine and histamine. Significant modulations of infralimbic dopamine were observed during the “Cue” period, hence could reflect S- as conditioned inhibitor/negative occasion setter. Indeed, unlike accumbal dopamine, prefrontal dopaminergic transmission is linked to behavioral inhibition, and reduced prefrontal dopamine is linked to increased alcohol relapse risk [28]. Within mPFC, D1 dopamine receptors are primarily expressed on pyramidal neurons and D2 dopamine receptors on interneurons; dopamine acting on these targets could both increase pyramidal excitability and reduce inhibitory tone, leading to disinhibition. These combined actions may have enabled activation of mostly pyramidal S-reactive “anti-relapse” neurons in IL [7] and suppress alcohol seeking. Significant modulations of prefrontal histamine were observed during the “Cue”, “Cue + Lever” and “Rebase” periods of the S- cue-test and likely related to the inhibitory role of histaminergic transmission on drug motivated behavior [29]. Moreover, H1/H2 histamine receptor-mediated excitatory transmission may account for prefrontal neuroactivity linked to drug-seeking suppression [18].

## Conclusion

Our study demonstrates that prefrontal neurotransmissions reactive to drug discriminative cues are dependent on cue types (S+ vs. S-), brain



**Fig. 3.** Extracellular neurotransmitter fluctuations in PL vs. IL of alcohol-trained rats during discriminative cue-tests. Microdialysis samples were collected and analyzed from PL or IL (3A), and rats were allowed to lever-press (3B), as described for Fig. 2. All statistical results are described in Tables 1, 4, 5, and only neurotransmitters with significant effects are depicted here: glutamate (3C/3D), dopamine (3E/3F), GABA (3G/3H), histamine (3I/3J) and aspartate (3K/3L). Data are presented as mean  $\pm$  SEM. \*,  $p < 0.05$ – $0.001$  (vs. S-/S+).

**Table 1**  
Statistics for behavioral procedures.

Behavioral task	Factors	F-value	P-value
Self-administration training (cocaine-trained rats)	Lever x Day	F (13, 672) = 8.556	<0.001
	Day	F (13, 672) = 0.672	0.792
	Lever	F (1, 672) = 1413	<0.001
Self-administration training (alcohol-trained rats)	Lever x Day	F (13, 448) = 6.590	<0.001
	Day	F (13, 448) = 1.338	0.187
Discriminative cue-training (cocaine-trained rats)	Lever	F (1, 448) = 972	<0.001
	Cue x Time	F (9, 480) = 28.20	<0.001
	Time	F (9480) = 33.00	<0.001
Discriminative cue-training (alcohol-trained rats)	Cue	F (1, 480) = 419.1	<0.001
	Cue x Time	F (9, 320) = 23.70	<0.001
	Time	F (9, 320) = 22.30	<0.001
Discriminative cue-tests (cocaine-trained rats)	Cue	F (1, 320) = 629.8	<0.001
	Cue x Time	F (3, 188) = 15.76	<0.001
	Time	F (3, 188) = 24.41	<0.001
Discriminative cue-tests (cocaine-trained rats)	Cue	F (1, 188) = 218.8	<0.001
	Cue x Time	F (3, 128) = 5.089	<0.001
	Time	F (3, 128) = 6.689	<0.001
Discriminative cue-tests (cocaine-trained rats)	Cue	F (1, 128) = 129.5	<0.001

**Table 2**  
Statistics for neurotransmitters in PL of cocaine-trained rats.

Neuro-transmitter	Factors	F-value	P-value
Adenosine	Cue x Time	F (11, 253) = 2.602	<0.01
	Time	F (3.448, 79.30) = 1.345	0.264
	Cue	F (1, 23) = 4.011	0.057
Aspartate	Cue x Time	F (11, 209) = 0.7136	0.725
	Time	F (3.153, 59.91) = 0.3672	0.787
	Cue	F (1, 19) = 0.1632	0.691
Dopamine	Cue x Time	F (11, 220) = 1.174	0.306
	Time	F (4.765, 95.30) = 1.236	0.299
	Cue	F (1, 20) = 0.1293	0.723
GABA	Cue x Time	F (11, 253) = 1.072	0.384
	Time	F (2.916, 67.07) = 0.5341	0.656
	Cue	F (1, 23) = 1.557	0.225
Glutamate	Cue x Time	F (11, 209) = 2.953	<0.01
	Time	F (1.294, 24.59) = 4.038	<0.05
	Cue	F (1, 19) = 3.241	0.088
Glycine	Cue x Time	F (11, 231) = 0.6660	0.770
	Time	F (3.688, 77.46) = 0.7381	0.559
	Cue	F (1, 21) = 0.2650	0.612
Histamine	Cue x Time	F (11, 253) = 0.4476	0.933
	Time	F (3.193, 73.44) = 1.787	0.154
	Cue	F (1, 23) = 0.2451	0.625
Norepinephrine	Cue x Time	F (11, 231) = 0.7941	0.646
	Time	F (4.634, 97.32) = 0.7908	0.550
	Cue	F (1, 21) = 0.001247	0.972
Serine	Cue x Time	F (11, 231) = 0.4566	0.928
	Time	F (2.847, 59.79) = 0.5437	0.645
	Cue	F (1, 21) = 0.02662	0.872
Taurine	Cue x Time	F (11, 242) = 2.435	<0.01
	Time	F (1.566, 34.46) = 2.457	0.112
	Cue	F (1, 22) = 4.019	0.057
Serotonin	Cue x Time	F (11, 253) = 1.926	<0.05
	Time	F (1.892, 43.51) = 1.426	0.251
	Cue	F (1, 23) = 2.152	0.156

**Table 3**  
Statistics for neurotransmitters in IL of cocaine-trained rats.

Neuro-transmitter	Factors	F-value	P-value
Adenosine	Cue x Time	F (11, 264) = 0.7981	0.642
	Time	F (3.235, 77.64) = 1.111	0.352
	Cue	F (1, 24) = 0.06614	0.799
Aspartate	Cue x Time	F (11, 209) = 0.6429	0.791
	Time	F (3.068, 58.29) = 2.028	0.119
	Cue	F (1, 19) = 0.5194	0.480
Dopamine	Cue x Time	F (11, 231) = 0.7956	0.644
	Time	F (6.546, 137.5) = 0.7625	0.611
	Cue	F (1, 21) = 1.293	0.268
GABA	Cue x Time	F (11, 253) = 0.7809	0.659
	Time	F (3.550, 81.64) = 0.7856	0.524
	Cue	F (1, 23) = 0.7825	0.386
Glutamate	Cue x Time	F (11, 176) = 1.690	0.079
	Time	F (2.166, 34.66) = 2.817	0.070
	Cue	F (1, 16) = 3.166	0.094
Glycine	Cue x Time	F (11, 231) = 0.8428	0.597
	Time	F (2.029, 42.61) = 1.425	0.252
	Cue	F (1, 21) = 0.1022	0.752
Histamine	Cue x Time	F (11, 242) = 1.077	0.380
	Time	F (5.687, 125.1) = 2.547	<0.05
	Cue	F (1, 22) = 0.3578	0.556
Norepinephrine	Cue x Time	F (11, 253) = 0.5394	0.876
	Time	F (3.691, 84.90) = 2.052	0.100
	Cue	F (1, 23) = 1.020	0.323
Serine	Cue x Time	F (11, 242) = 0.6397	0.794
	Time	F (5.847, 128.6) = 0.5723	0.748
	Cue	F (1, 22) = 6.114e-005	0.994
Taurine	Cue x Time	F (11, 242) = 1.297	0.227
	Time	F (2.054, 45.18) = 0.6567	0.527
	Cue	F (1, 22) = 1.335	0.260
Serotonin	Cue x Time	F (11, 242) = 1.082	0.376
	Time	F (5.388, 118.5) = 0.7027	0.633
	Cue	F (1, 22) = 2.234	0.149

**Table 4**  
Statistics for neurotransmitters in PL of alcohol-trained rats.

Neuro-transmitter	Factors	F-value	P-value
Adenosine	Cue x Time	F (11, 121) = 1.073	0.389
	Time	F (1.809, 19.90) = 1.438	0.260
	Cue	F (1, 11) = 4.149	0.067
Aspartate	Cue x Time	F (11, 121) = 0.3282	0.978
	Time	F (3.107, 34.18) = 0.6521	0.592
	Cue	F (1, 11) = 0.4321	0.525
Dopamine	Cue x Time	F (11, 121) = 0.4694	0.919
	Time	F (3.625, 39.87) = 1.319	0.281
	Cue	F (1, 11) = 0.2977	0.596
GABA	Cue x Time	F (11, 121) = 0.9544	0.492
	Time	F (1.616, 17.78) = 0.6679	0.495
	Cue	F (1, 11) = 1.258	0.286
Glutamate	Cue x Time	F (11, 132) = 1.253	0.259
	Time	F (3.648, 43.78) = 1.264	0.299
	Cue	F (1, 12) = 0.8370	0.378
Glycine	Cue x Time	F (11, 121) = 1.117	0.354
	Time	F (2.870, 31.57) = 0.3401	0.788
	Cue	F (1, 11) = 1.576	0.235
Histamine	Cue x Time	F (11, 132) = 1.978	<0.05
	Time	F (4.813, 57.75) = 1.778	0.134
	Cue	F (1, 12) = 5.920	<0.05
Norepinephrine	Cue x Time	F (11, 132) = 0.8696	0.572
	Time	F (2.407, 28.89) = 0.5234	0.631
	Cue	F (1, 12) = 1.178	0.299
Serine	Cue x Time	F (11, 121) = 0.5880	0.836
	Time	F (2.027, 22.29) = 0.7581	0.482
	Cue	F (1, 11) = 0.1331	0.722
Taurine	Cue x Time	F (11, 121) = 1.394	0.184
	Time	F (1.776, 19.53) = 1.030	0.367
	Cue	F (1, 11) = 3.193	0.102
Serotonin	Cue x Time	F (11, 132) = 1.223	0.278
	Time	F (1.252, 15.02) = 1.404	0.264
	Cue	F (1, 12) = 0.1509	0.705

**Table 5**  
Statistics for neurotransmitters in IL of alcohol-trained rats.

Neuro-transmitter	Factors	F-value	P-value
Adenosine	Cue x Time	F (11, 165) = 0.7756	0.664
	Time	F (1.124, 16.86) = 0.959	0.352
	Cue	F (1, 15) = 0.7184	0.410
Aspartate	Cue x Time	F (11, 143) = 0.7023	0.735
	Time	F (2.386, 31.02) = 3.251	<0.05
	Cue	F (1, 13) = 0.6048	0.451
Dopamine	Cue x Time	F (11, 165) = 2.107	<0.05
	Time	F (5.500, 82.49) = 0.9745	0.443
	Cue	F (1, 15) = 0.2997	0.592
GABA	Cue x Time	F (11, 176) = 1.884	<0.05
	Time	F (4.087, 65.39) = 1.311	0.275
	Cue	F (1, 16) = 5.490	<0.05
Glutamate	Cue x Time	F (11, 154) = 1.623	0.097
	Time	F (5.478, 76.70) = 0.7179	0.624
	Cue	F (1, 14) = 6.571	<0.05
Glycine	Cue x Time	F (11, 165) = 0.8206	0.620
	Time	F (2.921, 43.81) = 1.060	0.375
	Cue	F (1, 15) = 0.04305	0.838
Histamine	Cue x Time	F (11, 165) = 0.4156	0.948
	Time	F (2.102, 31.53) = 2.192	0.126
	Cue	F (1, 15) = 0.2008	0.661
Norepinephrine	Cue x Time	F (11, 154) = 0.6506	0.783
	Time	F (3.467, 48.54) = 0.6895	0.583
	Cue	F (1, 14) = 0.003986	0.951
Serine	Cue x Time	F (11, 165) = 0.9278	0.515
	Time	F (1.289, 19.34) = 0.6595	0.464
	Cue	F (1, 15) = 0.5116	0.485
Taurine	Cue x Time	F (11, 176) = 0.2648	0.991
	Time	F (1.900, 30.41) = 1.997	0.155
	Cue	F (1, 16) = 1.046	0.322
Serotonin	Cue x Time	F (11, 165) = 1.812	0.056
	Time	F (1.636, 24.54) = 1.643	0.216
	Cue	F (1, 15) = 0.05992	0.810

regions (IL vs. PL) and drugs used for cue-conditioning (cocaine vs. alcohol), thereby suggesting cocaine and alcohol seeking are each regulated by distinct neurochemical processes. Future studies should elucidate the causal role of these cue-specific prefrontal neurotransmissions in regulating drug seeking. Future studies should also elucidate the prefrontal neurocircuitries responsible for these cue-specific neurotransmissions.

#### CRediT authorship contribution statement

**Hermine Nedelescu:** Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation. **Cristina Miliano:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Grant E. Wagner:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Ayla M. Carroll:** Investigation, Data curation. **Genna L. De Ness:** Investigation. **Tony M. Kerr:** Validation, Supervision, Investigation, Formal analysis, Data curation. **Richard Nana Abankwah Owusu Mensah:** Writing – original draft, Validation, Formal analysis. **Eisuke Koya:** Writing – review & editing. **Ann M. Gregus:** Writing – original draft, Funding acquisition, Formal analysis. **Friedbert Weiss:** Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Matthew W. Buczynski:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nobuyoshi Suto:** Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Nobuyoshi Suto reports financial support was provided by National Institutes of Health. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Supplementary materials

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#### Data availability

Data will be made available on request.

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