Neuropsychopharmacology (2015) 40, 2526–2535

www.neuropsychopharmacology.org

Failure to Recognize Novelty after Extended Methamphetamine Self-Administration Results from Loss of Long-Term Depression in the Perirhinal Cortex

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Exposure to methamphetamine (meth) can produce lasting memory impairments in humans and rodents. We recently demonstrated that extended access meth self-administration results in novel object recognition (NOR) memory deficits in rats. Recognition of novelty depends upon intact perirhinal (pRh) cortex function, which is compromised by meth-induced downregulation of GluN2B-containing N-methyl-D-aspartate (NMDA) receptors. NMDA receptors containing this subunit have a critical role in pRh long-term depression (LTD), one of the primary physiological processes thought to underlie object recognition memory. We hypothesized that meth-induced downregulation of GluN2B receptors would compromise pRh LTD, leading to loss of NOR memory. We found that meth self-administration resulted in an inability to induce pRh LTD following 1 Hz stimulation, an effect that was reversed with bath application of the NMDA receptor partial agonist D-cycloserine (DCS). In addition, pRh microinfusion of DCS restored meth-induced memory deficits. Furthermore, blockade of GluN2B-containing NMDA receptors with Ro 25-6981 prevented DCS restoration of pRh LTD in meth subjects. Thus, targeting pRh LTD may be a promising strategy to treat meth-induced cognitive impairment.
Neuropsychopharmacology (2015) **40,** 2526–2535; doi:10.1038/npp.2015.99; published online 20 May 2015

INTRODUCTION

Despite profound negative consequences, methamphetamine (meth) abuse remains highly prevalent. In humans, chronic meth addiction often includes symptoms of impaired cognitive function in areas of episodic, verbal, and working memory (Scott *et al*, 2007; McCann *et al*, 2008; Salo *et al*, 2009). Several reviews have summarized the effects of repeated meth abuse on cognitive dysfunction and the clinical relevance of memory deficits (Scott *et al*, 2007; Hart *et al*, 2012; Dean *et al*, 2013). In general, meth causes cognitive decline in humans, particularly during early-to-middle adulthood (Dean *et al*, 2013). Importantly, the high incidence of cognitive dysfunction that occurs in chronic pathological meth addiction is distinct from acute or low-dose effects of meth (Wood *et al*, 2014). Also, memory impairment has been positively correlated with relapse probability, suggesting that abstinent meth addicts may reinitiate drug use as a means of alleviating cognitive impairment (Simon *et al*, 2004). Consequently, the mechanisms underlying meth-induced memory dysfunction are an important consideration for developing therapies for meth addiction.

In rodents, both non-contingent and operant meth self-administration (SA) paradigms impair recognition memory (Marshall and O'Dell, 2012). Here we use an extended access meth SA paradigm starting with short access (1 h) sessions and transition into chronic exposure with long access (6 h) sessions. Following meth SA, subjects then go through 7 days of forced abstinence. This paradigm reliably produces significant deficits in novel object and object-in-place recognition tasks not seen with short access (Rogers *et al*, 2008; Reichel and See, 2010; Reichel *et al*, 2011; Reichel *et al*, 2012) that have been postulated to represent key elements of episodic memory (Ennaceur, 2010).

Lesion studies in rodents have demonstrated that the perirhinal (pRh) cortex has an important role in temporal order and spatial memory processes, as do the hippocampus and medial prefrontal cortex (Barker *et al*, 2007; Barker and Warburton, 2011). However, pRh lesions disrupt the neural systems that regulate the discrimination of novel and familiar objects (Kealy and Commins, 2011). Moreover, studies have shown that the extent of pRh damage in lesion studies is positively correlated with the degree of object recognition impairment (Albasser *et al*, 2009). An emerging theory suggests that pRh neuron activity increases during novelty recognition and decreases as familiarity develops,

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Received 9 November 2014; revised 27 March 2015; accepted 6 April 2015; accepted article preview online 13 April 2015

eventually leading to the induction of pRh long-term depression (LTD; Warburton *et al*, 2013)

Behavioral pharmacology studies highlight the importance of pRh glutamatergic transmission in recognition memory as direct intracranial microinfusion of the N-methyl-D-aspartate (NMDA) receptor antagonist, (2R)-amino-5-phosphonovaleric acid, into the pRh just prior to task acquisition inhibits object recognition memory in rodents (Barker and Warburton, 2008; Warburton *et al*, 2013). Neurophysiological recording of the pRh cortex indicates that blockade of ionotropic glutamate receptors may inhibit object recognition memory by preventing the induction of NMDA receptor-dependent LTD (Cho *et al*, 2000; Griffiths *et al*, 2008). Further, neuroimaging of the pRh cortex in Macaques shows that blockade of glutamatergic transmission impairs recognition memory (Malkova *et al*, 2015).

We have previously shown that extended access meth SA followed by 7 days of withdrawal selectively downregulates the expression of the NMDA receptor subunit GluN2B, yet has no effect on expression of the GluN2A subunit, using a whole-cell lysate preparation of the pRh (Reichel *et al*, 2014). GluN2B-containing NMDA receptors mediate the induction of pRh LTD (Massey *et al*, 2004), one of the primary physiological processes believed to underlie recognition memory (Griffiths *et al*, 2008; Massey *et al*, 2008). Here we show that meth SA impaired recognition memory, blocked the induction of pRh LTD, and decreased GluN2B surface expression. Further, pharmacological activation of NMDA receptors with the partial agonist D-cycloserine (DCS) reversed meth-induced memory deficits in object recognition tests and restored the induction of pRh LTD.

MATERIALS AND METHODS

Surgery and SA

Surgical procedures and meth SA were performed as previously described (Reichel *et al*, 2014). Briefly, male Sprague Dawley rats were implanted with intracranial cannulae and/or jugular catheters in a single surgery. Following recovery, subjects self-administered meth hydrochloride (Sigma, St Louis, MO; dissolved in sterile 0.9% saline) in 1-h/day sessions for 7 days and then switched to 6-h/day access for 14 days. A response on the active lever resulted in a meth infusion (20 μg/50 μl bolus) and the 5 s presentation of a stimulus complex (light and tone). Yoked controls received saline whenever matched subjects received meth. All procedures were conducted in accordance with the 'Guide for the Care and Use of Laboratory Rats' (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 2011) and approved by the IACUC of the Medical University of South Carolina.

Novel Object Recognition

Novel object recognition (NOR) was performed as previously described (Reichel *et al*, 2011). In brief, rats were habituated to the test apparatus twice for 5 min without objects. On abstinence day 7, during familiarization, rats explored two identical objects for 3 min. A short-term memory test was conducted 90 min later by allowing rats to explore an object from the familiarization phase and a novel object for 3 min. A long-term test was conducted 24 h after sampling, in which rats were given the familiar object and a different novel object. Object exploration during both familiarization and NOR testing was defined as time spent sniffing or touching the object with the nose but not sitting, leaning, or standing on the object and was scored and reported as time spent with object (seconds; novel or familiar). Using these values, the recognition index was calculated by dividing the time spent with the novel object by the time spent with both objects. Approaches to the object were also quantified in order to calculate an approach score (approaches to novel object over total approaches). Familiar and novel objects, as well as the placement of the objects were counterbalanced. Objects consisted of combinations of a PVC pipe ($6.4 \times 3.8\ cm^2$), a light bulb (8.9 cm), and a plastic bottle (Reichel *et al*, 2014). Total distance traveled in cm (ambulation) was also recorded and reported as a control measure.

Biotinylation and GluN2B/GluN1 Quantification

The surface biotinylation assay was performed as previously described (Knackstedt *et al*, 2014; Schwendt *et al*, 2012). In the aforementioned and present study, pRh tissue punches include a small amount of material from the neighboring entorhinal and ectorhinal cortices (Supplementary Figure S2). Briefly, 300 μm slices were made from 2 mm tissue punches of the pRh area with a tissue chopper and incubated with 1 mg/ml Sulfo-NHS-Biotin (Pierce) for 30 min at 4 °C with gentle shaking. The reaction was quenched by 100 mM glycine in PBS (pH 8.0). Tissue was washed in PBS and then sonicated in RIPA buffer. After incubating on ice, insoluble fragments were removed by centrifugation at 10 000×*g* for 10 min at 4 °C. 500 μg of sample was combined with NeutrAvidin agarose resin and incubated for 2 h at 4 °C with rotation. A capillary-based size sorting system, 'WES' by ProteinSimple (Bio-Techne) was used to quantify protein levels as previously described (O'Neill *et al*, 2006). Briefly, prior to loading the WES plate, lysates (1 μg/μl stocks) were diluted to 0.2 μg/μl by combining 2.5 μl lysate, 2.5 μl master mix and 7.5 μl 0.1 × sample buffer for a total volume 12.5 μl. The reaction mix was then heated at 70 °C for 10 min. 5 μl of the reaction mix was then pipetted into the WES plate resulting in a final concentration of 1 μg in the capillary. Lysate mixture, blocking reagent, antibodies, chemiluminescent substrate, and wash buffer were then dispensed into designated wells on the WES microplate. Stacking matrix load time was set at 20 s and sample load time was set at 10 s. Data were analyzed using Compass software (ProteinSimple) using the 'Gaussian fit' function for quantitative analysis of GluN1, GluN2B, and Calnexin (see Figure 2c). The primary antibodies used were GluN2B (mouse, BD Biosciences, 610416, 1:500), GluN1 (mouse, BD Biosciences, 556308, 1:1000), and Calnexin (rabbit, Enzo Life Sciences, ADI-SPA-860-F, 1:2000). Surface (biotinylated) and intracellular (non-biotinylated) GluN2B signals were normalized to that of calnexin and expressed as a percent averaged saline values.

Microinjections and Histology

Injection cannulae (26 gauge, Plastics One) were positioned 1.5 mm above the pRh region via stereotaxic surgery at a 12° angle. pRh coordinates were − 4.8 A/P, +5 M/L, − 7.5 D/V.

Although this cannulation targets the pRh, it is likely that drug solutions delivered may have been able to diffuse into the nearby ectorhinal and entorhinal cortices. DCS (Sigma) was dissolved in Milli-Q ultra-pure water at 20 mg/ml and rats were given either 0.5 μl/side of vehicle (Milli-Q water) or DCS (10 μg/side). Infusions were bilaterally administered through 33 gauge injectors (projected 1.5 mm past the guide cannula tip) at a flow rate of 0.2 μl/min. Cannula placement was determined following an overdose of Equithesin and perfusion with 4% formaldehyde and coronal slices (100 μm) were made for cresyl violet staining.

Field Recordings

Acute slices containing pRh, entorhinal, and temporal cortex were obtained from meth or yoked-saline rats as previously described (Cho *et al*, 2000). Briefly, brain tissue was rapidly removed, and midsaggital slices were cut at 400 μm thickness in ice-cold dissection artificial cerebrospinal fluid (aCSF) solution, using a previously described aCSF composition (Trantham-Davidson *et al*, 2014). Slices were incubated at 34 °C for at least 1 h before recordings in incubation aCSF. Slices were continuously aerated with 5% carbon dioxide/95% oxygen. After incubation, slices were transferred to a submerged recording chamber, held at 34 °C, and bathed with oxygenated recording aCSF. The pH of all solutions listed above was adjusted to 7.3 using NaOH and osmolarity was measured to be ~300 mOsm.

Extracellular field recordings were made with a Multiclamp 700B amplifier (Axon Instruments, Union City, CA), connected to a computer running Axograph X software and later analyzed offline. The recording electrode was filled with recording aCSF, had a resistance of <1 MΩ and was placed in the superficial layer of pRh, medial to the rhinal fissure. Two tungsten bipolar stimulating electrodes were placed in the superficial layers of the entorhinal and temporal cortices. Stimulation pulses were delivered every 30 s at 0.033 Hz and alternated between the regions using a Grass S88 stimulator and stimulus isolation unit. First, an input–output relationship was obtained by varying the intensity of stimulation from an amount that produced no field excitatory postsynaptic potential (fEPSP) up to an amount that produced a response of maximal amplitude, regardless of further increases in stimulus intensity. The intensity was then dialed down to generate a response that was ~75% of the maximal amplitude. Next, fEPSPs were recorded for 15 min to obtain a baseline amplitude measurement followed by application of low-frequency stimulation (LFS; 900 pulses at 1 Hz) to the entorhinal input previously shown to produce LTD in the pRh cortex (Cho *et al*, 2000; Ziakopoulos *et al*, 1999).

Statistics

Significant differences in time spent with objects during NOR testing, approach, and total ambulation were assessed using two-tailed *t*-tests. Significant differences in the WES protein quantification study were performed with one-tailed *t*-tests. Recognition indexes and field recordings were compared with theoretical means using one-sample *t*-tests. Meth infusions during extended access and GluN2B surface expression assays were analyzed using one-way ANOVA with Bonferroni *post hoc* analyses. Results were considered statistically significant at $p<0.05$. Sample sizes were chosen based on previous results from recognition memory tasks, protein quantification, and field recording studies. Sample exclusion criteria were determined prior to experimentation for technical failures, including failure of jugular catheters during meth SA. During field recordings, recognition memory testing, and WES protein quantification, the investigator was blind to group allocation during experimentation.

RESULTS

Meth SA

Meth subjects readily pressed the active lever for drug and increased responding during 6-h sessions. Yoked-saline controls indiscriminately sampled both levers (Figure 1a). Meth rats displayed escalating drug intake with significant increases in infusions across 6-h sessions (session effect ($F_{(18,234)}=15.38$, $p<0.0001$) with higher intake on days 8–14 of 6-h access compared with the first day (Bonferroni's multiple comparison, $p<0.05$; Figure 1b).

NOR

Following 1 week of abstinence, rats underwent NOR testing. During familiarization, yoked-saline and meth rats displayed similar time spent with either object (light bulb or plastic bottle), demonstrating that both groups of subjects equally explored both objects (Table 1A). Moreover, both groups showed no significant differences in the approach index (novel object/both objects) or total ambulation (cm) during familiarization (Table 1A).

During NOR testing, yoked-saline rats spent significantly longer time with the novel object ($t_{(30)}=5.373$, $p<0.0001$; Figure 1c). Moreover, yoked-saline subjects displayed robust NOR memory (recognition index) compared with chance levels of exploration (a hypothetical mean of 0.5; $t_{(16)}=6.66$, $p<0.0001$; Figure 1d). Direct comparison revealed that meth recognition scores were significantly lower than yoked-saline controls ($t_{(29)}=6.00$, $p<0.0001$; Figure 1d). During NOR testing neither the approach scores (yoked-saline 0.54 ± 0.03 and meth 0.46 ± 0.02) nor ambulation values (distance traveled in cm; yoked-saline 2165 ± 118; meth 2351 ± 142) significantly differed between yoked-saline and meth groups (Table 1A).

GluN2B and GluN1 Expression

In order to test the hypothesis that chronic meth SA decreases surface expression of the NMDA receptor subunits GluN2B and GluN1, we selectively labeled surface receptors in pRh slices with biotin as previously described (Schwendt *et al*, 2012; Knackstedt *et al*, 2014). It is important to note that the pRh punches made for this study contain a small amount of material from the surrounding ectorhinal and entorhinal cortices (see Supplementary Figure S2). We observed a significant decrease in surface expression of GluN2B in rats with a history of extended access to meth ($t_{(19)}=1.891$, $p=0.037$; Figure 2a). However, intracellular (non-biotinylated) GluN2B levels did not significantly differ. Although we observed no significant decrease in surface or intracellular expression of GluN1 in rats with a history of

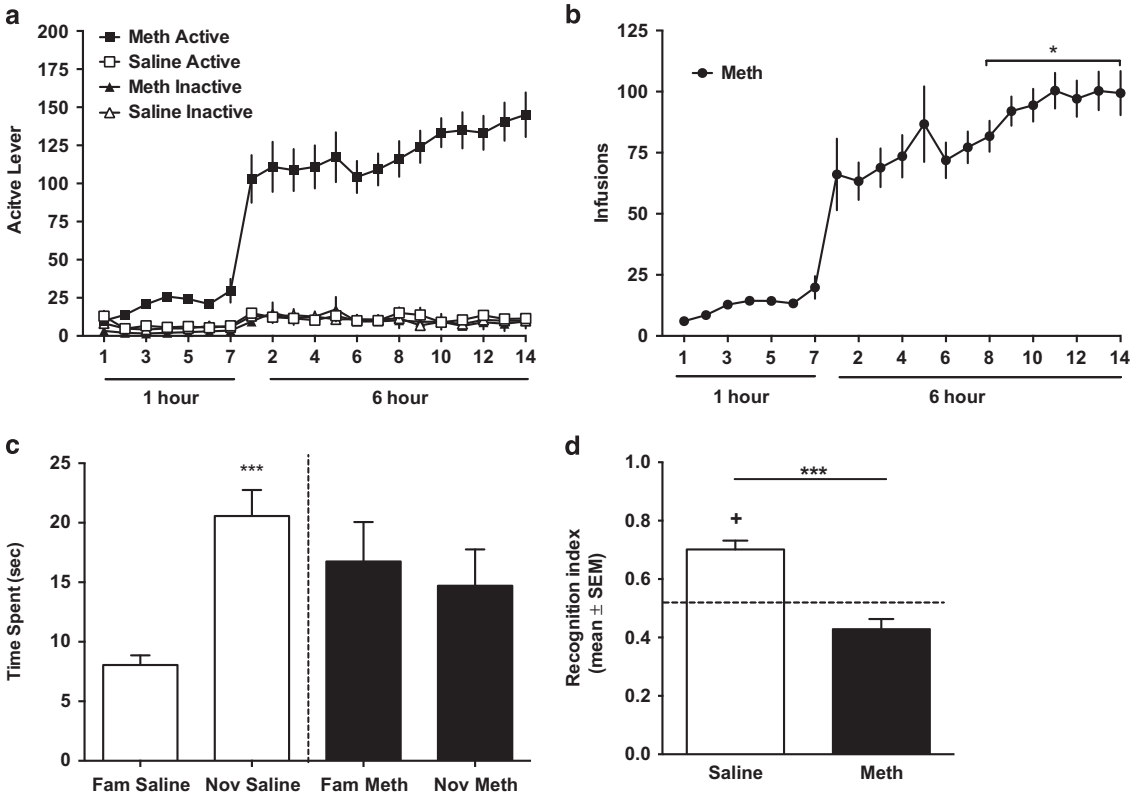

Figure 1 Extended access meth SA produced deficits in NOR. (a) Meth rats readily pressed the active lever for drug and increased responding during 6-h sessions. Yoked-saline controls indiscriminately sampled both levers. (b) Meth rats escalated drug intake, with significant increases in the number of infusions on days 8–14 of 6-h access compared with the first day (*$p<0.05$). (c) Time spent with each object during NOR is shown here with saline rats spending significantly more time with the novel object when compared with the familiar object (***$p<0.001$). (d) Recognition index for meth and yoked-saline controls. Yoked-saline controls displayed robust object recognition memory (+$p<0.001$). In contrast, recognition scores in meth rats did not significantly differ from chance exploration. Direct comparison revealed that meth recognition scores were significantly lower than yoked-saline controls (***$p<0.001$). Meth, methamphetamine; NOR, novel object recognition; SA, self-administration.

extended access to meth (Figure 2b), representative electropherograms used in the quantification of GluN2B by the WES system are shown in Figure 2c.

pRh LTD Following Extended Access to Meth

We hypothesized that a dysfunction of GluN2B-containing NMDA receptors in the pRh cortex leads to a loss of pRh LTD, which may account for the meth-induced loss of recognition memory in rats. To test this, we performed field recordings in the superficial layer of pRh cortex in acute slices obtained from yoked-saline and chronic meth rats. Recordings from chronic meth rats revealed a complete lack of LTD in the pRh cortex using the 1 Hz LFS protocol (the final 20 min of the recording were analyzed *vs* hypothetical mean of 100%; $t_{(19)}=0.77$, $p=0.45$), whereas LTD was robustly elicited in saline controls ($t_{(19)}=31.95$, $p<0.0001$; Figure 3a). The percent change in amplitude (normalized to baseline) was also significantly different when comparing the final 20 min of the meth and yoked-saline control recordings ($t_{(38)}=17.26$, $p<0.0001$). There were no significant differences in fEPSP amplitude between the saline and meth groups during baseline (saline baseline 577.72 ± 189.96 pA; saline post LTD 397.34 ± 111.34 pA; meth baseline 459.78 ± 94.92 pA; meth post LTD 480.59 ± 134.28 pA. In addition, the temporal input pathway served as control stimulation with no changes in fEPSP amplitude observed in either treatment group over the length of the recording, suggesting that the fEPSP was stable over long recording times (data not shown).

pRh LTD and GluN2B Activation

In slices from meth rats, we bath applied the NMDA partial agonist DCS (100 μM), and found that LTD could be restored through DCS facilitation of GluN2A/2B-continaing NMDA receptors ($t_{(19)}=16.75$, $p<0.0001$; Figure 3b). Co-application of DCS with Ro 25-6981 (3 μM), a potent and selective antagonist at GluN2B-containing NMDA receptors (Fischer *et al*, 1997), entirely prevented DCS-mediated restoration of LTD in chronic meth rats (DCS values were significantly greater than baseline; $t_{(19)}=8.72$, $p<0.0001$). Further, percent change in amplitude was also significantly different when comparing the final 20 min of the DCS and DCS+Ro 25-6981 recordings ($t_{(38)}=17.93$, $p<0.0001$; Figure 3b). These data confirm the importance of GluN2B-containing NMDA receptors in the DCS-restoration of LTD in chronic meth rats.

Table 1 NOR Familiarization and Test Data

Time spent	Novel		Familiar		Difference?	Approach score		Difference?	Ambulation (cm)		Difference?
	Mean	SEM	Mean	SEM	Nov *vs* Fam	Mean	SEM	Meth *vs* Saline	Mean	SEM	Meth *vs* Saline
(A) Extended meth SA impairs NOR											
Fam											
Sal, $n=16$	12.52	1.688	11.52	1.633	No: $p>0.05$	0.572	0.02939	No: $p>0.05$	2266	179.7	No: $p>0.05$
Meth, $n=13$	12.69	1.489	9.924	1.552	No: $p>0.05$	0.577	0.04967		2510	160.5	
Test											
Sal, $n=16$	20.57	2.182	8.05	0.8139	**Yes: $p<0.001$**	0.545	0.03879	No: $p>0.05$	2165	118.2	No: $p>0.05$
Meth, $n=13$	14.7	3.056	16.75	3.311	No: $p>0.05$	0.462	0.02065		2351	142.9	
(B) DCS restoration of meth induced NOR deficits											
Fam											
Veh, $n=7$	9.838	0.7988	13.27	1.684	No: $p>0.05$	0.438	0.04133	No: $p>0.05$	3239	215.4	No: $p>0.05$
DCS, $n=7$	9.837	1.78	9.793	3.13	No: $p>0.05$	0.484	0.08194		2687	407.7	
Test											
Veh, $n=7$	7.841	1.123	10.28	1.152	No: $p>0.05$	0.416	0.05442	No: $p>0.05$	3077	278.4	No: $p>0.05$
DCS, $n=7$	15.83	3.327	6.683	1.529	**Yes: $p<0.05$**	0.49	0.07633		3053	538.2	
(C) DCS controls in yoked-saline animals											
Fam											
Veh, $n=8$	16.08	1.64	12.3	2.117	No: $p>0.05$	0.5073	0.05287	No: $p>0.05$	2907	174.8	No: $p>0.05$
DCS, $n=7$	18.9	2.439	15.57	2.974	No: $p>0.05$	0.4232	0.01813		2832	126.1	
Test											
Veh, $n=8$	17.22	3.437	5.735	0.8555	**Yes: $p<0.01$**	0.5521	0.02981	No: $p>0.05$	2712	202.8	No: $p>0.05$
DCS, $n=7$	20.46	2.458	11.7	1.741	**Yes: $p<0.05$**	0.5102	0.04165		2727	203.3	

Abbreviaitons: DCS, D-cycloserine; Fam; familiar; meth, methamphetamine; NOR, novel object recognition; SA, self-administration; sal, saline; veh, vehicle.
Time spent with objects (seconds), approach score (approaches to novel object/total approaches) and total ambulation (cm) for (A) the extended meth SA experiments, (B) DCS restoration of meth induced NOR deficits and (C) DCS/vehicle yoked-saline control experiments. All statistical comparisons were performed using two-tailed *t*-tests. Significant differences are highlighted in blue. Bold values indicate significant differences.

DCS Rescues Meth-Induced Deficits in NOR

We infused either vehicle or DCS (10 μg/side) bilaterally into the pRh in subjects that had undergone extended meth access followed by 1 week of abstinence, immediately following familiarization. During familiarization, vehicle and DCS rats spent similar amounts of time with each object, indicating equal exploration of the test apparatus (Table 1B). Moreover, both groups showed no significant differences in approach or ambulation during familiarization (Table 1B).

As described above, meth rats showed NOR memory deficits 90 min after familiarization, indicated by equal exploration of novel and familiar objects. However, rats that received bilateral pRh DCS infusions spent significantly more time with the novel object ($t_{(12)}=2.50$, $p=0.028$). As expected, meth rats that received DCS infusions had recognition scores that were significantly higher than chance performance ($t_{(6)}=2.81$, $p=0.031$), indicating restoration of NOR memory (Figure 4a). Direct comparison of vehicle and DCS groups revealed a significant increase in recognition scores for DCS-treated animals relative to controls ($t_{(12)}=3.73$, $p=0.003$; Figure 4a). Importantly, during testing, neither approach nor ambulation differed between the vehicle and DCS groups (Table 1B).

A long-term memory test for NOR was conducted at 24 h after the initial infusions of vehicle or DCS. Rats that had previously received DCS showed a nearly significant increase in time spent with the novel object ($t_{(8)}=2.22$, $p=0.0568$), and showed recognition indexes that were significantly higher than a hypothetical mean of 0.5 ($t_{(4)}=2.88$, $p<0.05$). These data indicate some recognition of the novel object even 24 h later. Despite the enduring enhancement of recognition memory in the DCS group, the recognition index did not significantly differ from that of the vehicle group ($t_{(10)}=1.31$, $p=0.22$; Figure 4b). During this later test, neither the approach scores (vehicle 0.54 ± 0.03; DCS 0.51 ± 0.06), nor the ambulation values (vehicle 2804 ± 258; DCS 2718 ± 571) differed between the vehicle and DCS groups. Placements of cannulae used for infusion of vehicle or DCS into the pRh were verified *ex vivo* (Figure 4c).

In a series of control experiments, yoked-saline subjects were given bilateral DCS or vehicle microinfusions prior to NOR testing as described above. During familiarization, vehicle and DCS-treated yoked saline rats displayed no significant differences in time spent with objects, approach index or total ambulation (Table 1C). During NOR testing, both vehicle ($t_{(14)}=3.24$, $p=0.006$) and DCS ($t_{(12)}=2.91$, $p=0.013$) rats spent significantly more time with the novel object (Supplementary Figure S1A) and had recognition indexes that were significantly higher than chance exploration (vehicle: ($t_{(7)}=11.08$, $p<0.0001$), and DCS: ($t_{(6)}=3.06$, $p=0.022$); Supplementary Figure S1B).

DISCUSSION

Previous studies have shown that the pRh cortex has an important role in novelty detection, and indeed exposure to

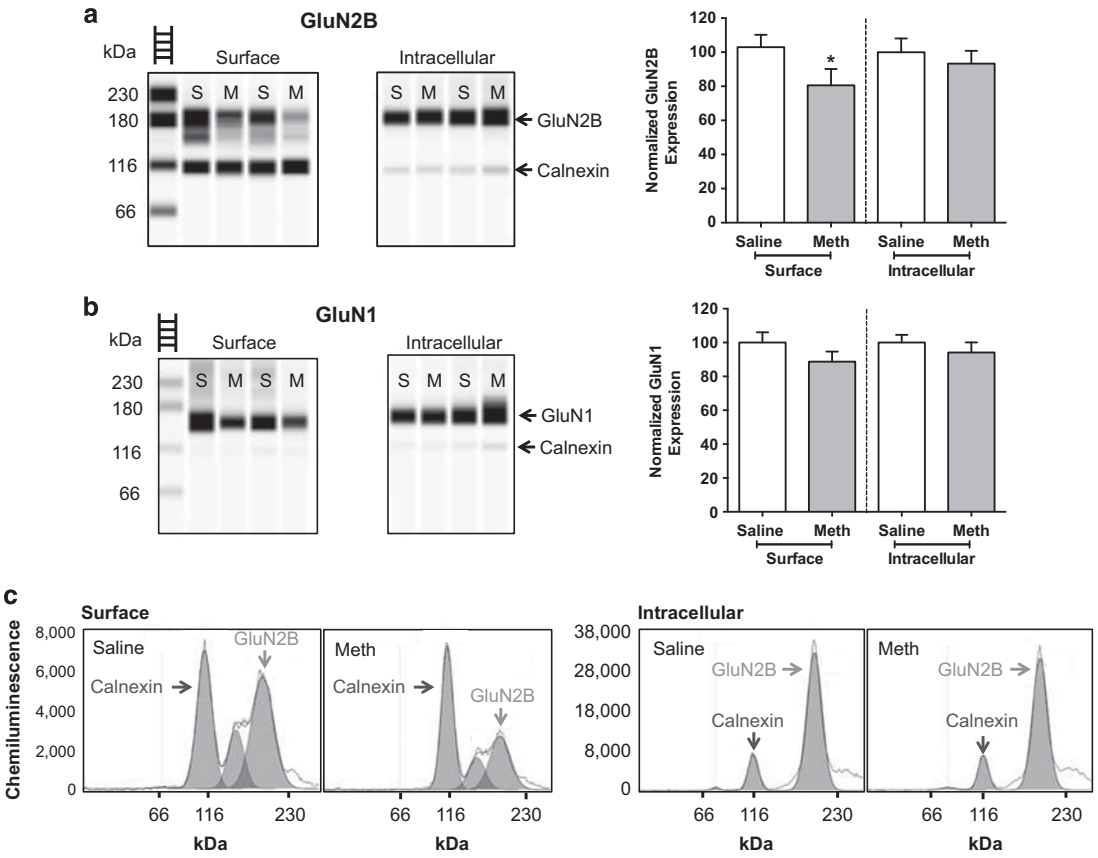

Figure 2 WES quantification of GluN2B and GluN1 surface expression. (a) Representative images of GluN2B immunoreactivity for both the surface (left) and intracellular fractions (right) in saline (S) and meth (M) subjects. We observed a significant decrease in surface expression of GluN2B in subjects with a history of extended access to meth (*$p < 0.05$). Values obtained for intracellular levels GluN2B expression did not significantly differ between the meth and the yoked-saline groups. (b) Representative images of GluN1 immunoreactivity for both the surface (left) and intracellular fractions (right) in saline (S) and meth (M) subjects. Values obtained for surface or intracellular levels GluN1 expression did not significantly differ between the meth and the yoked-saline groups. (c) Representative electropherograms for GluN2B in the surface and intracellular fractions. Signals were quantified using the Gaussian quantification method. The shaded grey regions depict the area under the curves used for quantification of the calnexin, GluN2B and GluN1 signals. Meth, methamphetamine.

novel visual stimuli activates this region in humans (Pihlajamaki *et al*, 2003; Staresina *et al*, 2012). In support of human neuroimaging data, rodent studies show greater activation of pRh neurons following the perception of novelty (Brown and Aggleton, 2001). The current model of novelty encoding involves elevated pRh activity during the perception of novel objects, which gives way to LTD as familiarity develops (Montaldi *et al*, 2006; Barth and Wheeler, 2008). Here we report that extended access meth SA followed by 7 days of withdrawal impaired the ability to induce LTD in the pRh. These data support the hypothesis that meth rats have impaired NOR memory due to an inability to attribute familiarity to a particular object as a result of the loss of synaptic plasticity in the pRh. Although this has not been measured in human meth addicts using neuroimaging approaches, the data presented here suggest that the pRh may be a target area for future exploration in meth addicts.

Consistent with others (Cho *et al*, 2000), we show that NMDA receptors are required for the induction of pRh LTD (Supplementary Figure S3). Further, prior studies showed that blockade of GluN2B-containing NMDA receptors with Ro 25-6981 inhibits the induction of pRh LTD (Massey *et al*, 2004) and that pRh LTD is required for NOR (Griffiths *et al*, 2008). As such, we hypothesize that downregulation of pRh GluN2B-containing NMDA receptors following meth SA and withdrawal is responsible for the lack of pRh LTD and the subsequent inability to recognize novelty during NOR testing. It is also possible that extended access meth SA alters basal glutamate transmission in the pRh, which negatively impacts the ability to engage synaptic plasticity and induce LTD. A similar loss of LTD induction has been observed in the nucleus accumbens following cocaine SA (Martin *et al*, 2006; Moussawi *et al*, 2009; Huang *et al*, 2011). Loss of accumbal LTD occurs due to decreased expression of proteins that mediate homeostatic levels of extracellular glutamate, which acts to regulate synaptic plasticity. The consequent loss of glutamate tone ultimately leads to an inability to induce LTD (Moussawi *et al*, 2009), which can be mitigated by restoring tone on presynaptic metabotropic glutamate receptors (Moussawi *et al*, 2009; Kupchik *et al*, 2012). One alternative interpretation of the results presented here is that DCS treatment alters basal glutamate transmission to allow for the induction of pRh LTD.

We found that extended meth SA blocked both short-term and LTD and we were able to restore LTD with DCS but not

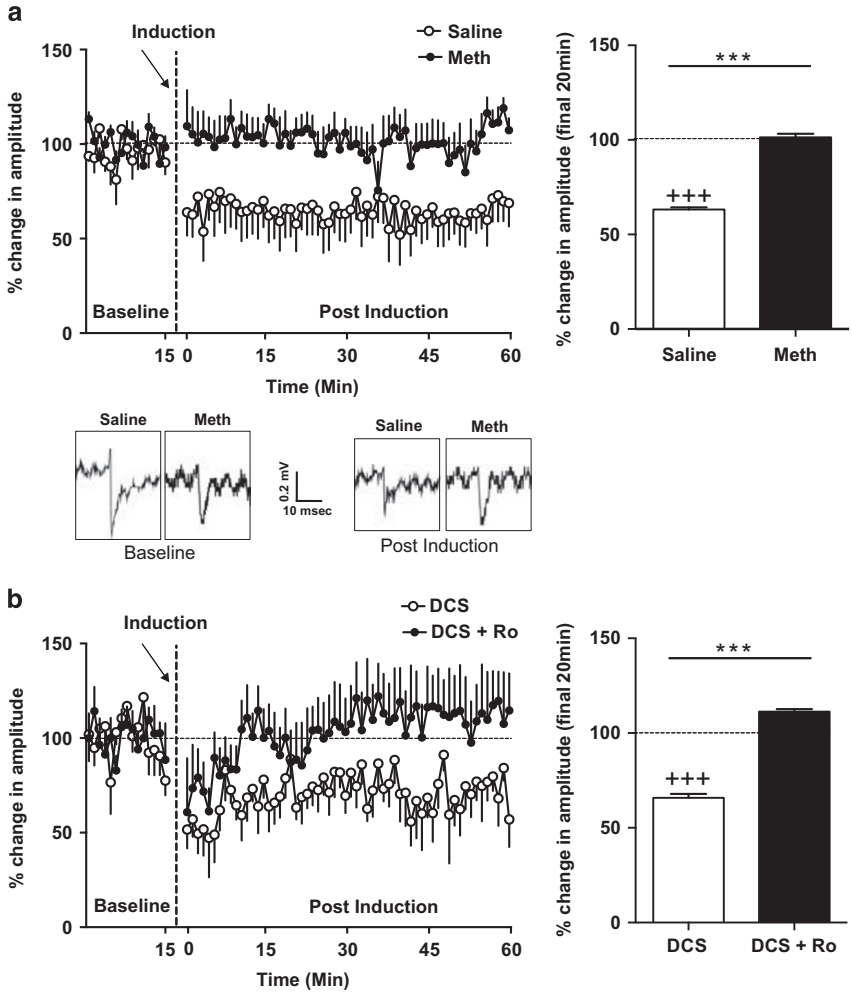

Figure 3 DCS reversed meth-induced inhibition of pRh LTD. Field recordings are shown on the left with averaged activity over the final 20 min of each recording shown in bar graphs on the right (a, b). (a) 1 Hz LFS induced LTD in yoked-saline controls ($+++p<0.001$), whereas chronic meth SA rats show almost no change from baseline ($***p<0.001$ saline *vs* meth). Representative recordings before and after stimulation for both groups are shown below the graph. (b) In slices from meth rats, bath application of the NMDA receptor agonist DCS restored LTD following LFS ($+++p<0.001$). This effect was blocked by the specific GluN2B-containing NMDA receptor antagonist Ro 25-6981 ($***p<0.001$, DCS *vs* DCS+Ro 25-6981), suggesting that LFS-induced pRh LTD is dependent upon the activation of GluN2B-containing NMDA receptors. DCS, D-cycloserine; LFS, low-frequency stimulation; LTD, long-term depression; meth, methamphetamine; NMDA, N-methyl-D-aspartate; pRh, perirhinal.

short-term LTD. This suggests that meth exposure may have additional effects on calcium channels that are not sufficient to prevent LTD but could have other important consequences that will be examined in future studies.

Surface biotinylation experiments revealed that meth-induced disruption of LTD coincides with a diminished presence of surface GluN2B-containing NMDA receptors in the pRh cortex. In the current study, we did not investigate the mechanism of GluN2B-containing NMDA receptor internalization or the fate of internalized receptors. However, recent evidence suggests that psychostimulant-induced internalization of GluN2B-containing NMDA receptors can be caused by proteasomal degradation of GluN2B-anchoring proteins (Mao *et al*, 2009). In the present study, the decrease in GluN2B-containing NMDA receptors at the surface was not accompanied by an increase in the intracellular pool. It is possible that receptor degradation may explain this discrepancy. As the blockade of GluN2B degradation improves synaptic plasticity and memory (Hawasli *et al*, 2007; Lee and Silva, 2009), future studies will investigate whether the persistent character of pRh LTD and memory deficits following meth exposure is related to internalization and degradation of local GluN2B-containing NMDA receptors.

As a non-selective partial agonist of NMDA receptors, DCS does not distinguish between GluN2A- or GluN2B-containing NMDA receptors. We chose DCS to verify our findings from physiological analyses of pRh LTD in the NOR behavioral model due to the fact that no selective agonists of GluN2B-containing NMDA receptors are suitable for microinjection in the brain. Despite the fact that the DCS reversal of meth-induced pRh LTD impairment was prevented by administration of a specific GluN2B antagonist, Ro 25-6981 (Figure 3b), it is important to note that we cannot conclude from the DCS microinfusion experiments that restoration of NOR occurred through a GluN2B-dependent mechanism, only that NOR memory was restored through the non-selective activation of pRh NMDA receptors.

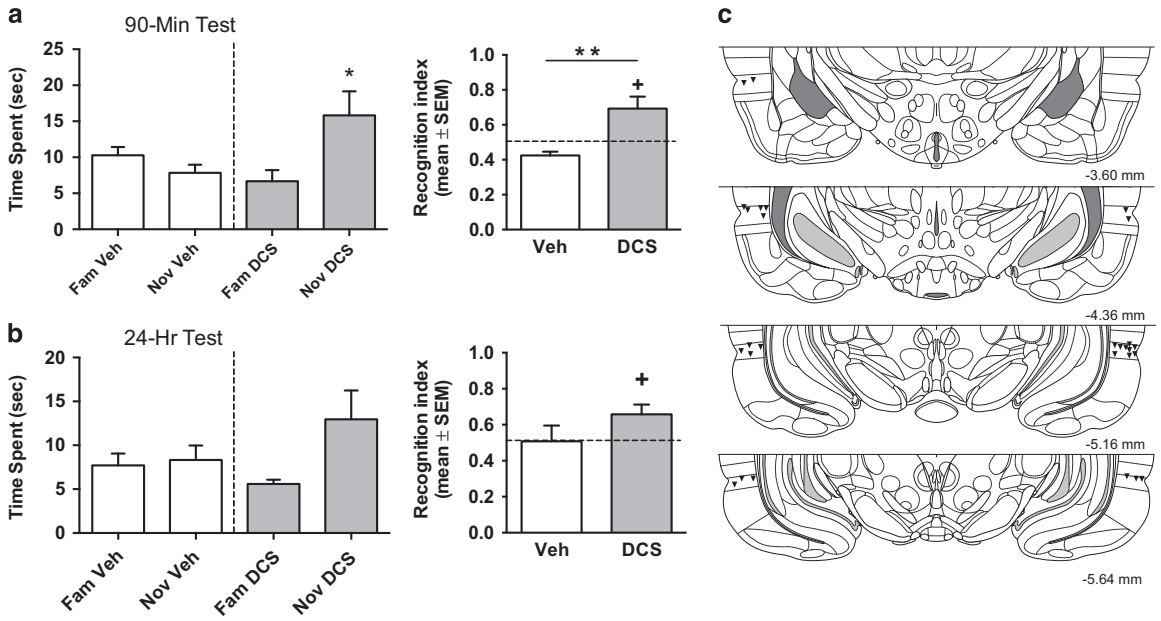

Figure 4 DCS microinfusion into the pRh reversed meth-induced NOR deficits. (a) Meth rats, infused with vehicle, spent similar amounts of time with the familiar and novel object. However, infusion with DCS in the pRh produced a significant increase in time spent with the novel object (*$p<0.05$). Furthermore, meth rats showed significantly lower recognition scores when compared with chance levels of exploration, indicating object recognition memory deficits (+$p<0.05$). In contrast, meth rats that received bilateral perirhinal DCS infusions had recognition scores significantly higher than chance exploration (+$p<0.05$), indicating intact recognition memory. Direct comparison of meth/vehicle and meth/DCS groups also revealed a significant increase in recognition scores for DCS treated subjects relative to controls (**$p<0.01$). (b) 24 h later, meth rats that received vehicle still show equal amounts of time spent with each object, those that received DCS showing a trend toward increased time spent with the novel object. Meth rats that received vehicle also show recognition scores equivalent to chance levels, indicating object recognition memory deficits. In contrast, rats that previously received bilateral perirhinal DCS infusions had recognition scores significantly higher than chance exploration (+$p<0.05$). (c) DCS infusion sites are depicted in serial coronal planes (3.6–5.6 mm posterior to bregma). DCS, D-cycloserine; meth, methamphetamine; NOR, novel object recognition; pRh, perirhinal; SA, self-administration.

The induction of NMDA-dependent LTD requires receptor activation, calcium influx, activation of calcineurin and the internalization of AMPA receptors (Beattie *et al*, 2000). Accordingly, direct inhibition of AP2-mediated AMPA receptor internalization disrupts both pRh LTD and NOR memory (Griffiths *et al*, 2008). As such, down regulation of GluN2B-containing NMDA receptors in the pRh following chronic meth SA may also impact AMPA receptor internalization through this mechanism. Further investigation is required to determine whether the downregulation of GluN2B-containing NMDA receptors and dysregulation of AMPA receptor internalization is indeed the primary mechanism for both the loss of pRh LTD and NOR memory impairment following chronic meth SA.

Here, we demonstrate that extended access meth SA followed by withdrawal resulted in NOR memory deficits and impaired the ability to induce pRh LTD. These deficits are likely the consequence of the downregulation of GluN2B-containing NMDA receptors in the pRh. Further, DCS restored pRh LTD in meth subjects in a GluN2B-dependent manner. Finally, DCS microinfusion into the pRh reversed deficits in NOR memory, consistent with its ability to restore pRh LTD. In light of these data, we conclude that the meth-induced deficit in NOR memory is likely due to the loss of pRh LTD. Our data indicate that the pRh may be a key brain region disrupted by chronic meth exposure. Given that relapse probability is related to episodic memory impairments in meth addicts (Simon *et al*, 2004), these data suggest that pRh LTD can be targeted to restore cognition in meth addicts.

FUNDING AND DISCLOSURE

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Shannon Ghee, Nastassia Bryant, and Sarah Barry for technical assistance. This research was supported by National Institute on Drug Abuse (NIDA) grants DA033049, DA007288, and NIH grant C06 RR015455.

AUTHOR CONTRIBUTIONS

All authors discussed the experimental design, results and implications, and commented on the manuscript. MDS, JP, and CMR contributed to and conducted the behavioral components. MDS, MS, K-CL, and CMR contributed to and conducted the neurochemistry component. HT-D conducted, analyzed, and wrote the electrophysiology part of this work. MDS, HT-D, and CMR wrote the manuscript. JP, RES, and MS gave technical support and conceptual advice, as well as reviewed and edited the manuscript.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)