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CaMKII Activity in the Ventral Tegmental Area Gates Cocaine-Induced Synaptic Plasticity in the Nucleus Accumbens

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Addictive drugs such as cocaine induce synaptic plasticity in discrete regions of the reward circuit. The aim of the present study is to investigate whether cocaine-evoked synaptic plasticity in the ventral tegmental area (VTA) and nucleus accumbens (NAc) is causally linked. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a central regulator of long-term synaptic plasticity, learning, and drug addiction. We examined whether blocking CaMKII activity in the VTA affected cocaine conditioned place preference (CPP) and cocaine-evoked synaptic plasticity in its target brain region, the NAc. TatCN21 is a CaMKII inhibitory peptide that blocks both stimulated and autonomous CaMKII activity with high selectivity. We report that intra-VTA microinjections of tatCN21 before cocaine conditioning blocked the acquisition of cocaine CPP, whereas intra-VTA microinjections of tatCN21 before saline conditioning did not significantly affect cocaine CPP, suggesting that the CaMKII inhibitor blocks cocaine CPP through selective disruption of cocaine-cue-associated learning. Intra-VTA tatCN21 before cocaine conditioning blocked cocaine-evoked depression of excitatory synaptic transmission in the shell of the NAc slices *ex vivo*. In contrast, intra-VTA microinjection of tatCN21 just before the CPP test did not affect the expression of cocaine CPP and cocaine-induced synaptic plasticity in the NAc shell. These results suggest that CaMKII activity in the VTA governs cocaine-evoked synaptic plasticity in the NAc during the time window of cocaine conditioning.
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INTRODUCTION

Learning and drug addiction are associated with similar alterations in synaptic plasticity in the reward circuit (Hyman *et al*, 2006; Kauer, 2004; Nestler, 2002). Single or repeated cocaine intraperitoneal injections induce synaptic potentiation in the ventral tegmental area (VTA) (Borgland *et al*, 2004; Liu *et al*, 2005; Mameli *et al*, 2009; Ungless *et al*, 2001), whereas repeated cocaine intraperitoneal injections or self-administration induced synaptic depression in the nucleus accumbens (NAc) (Kasanetz *et al*, 2010; Kourrich *et al*, 2007; Mameli *et al*, 2009; Ortinski *et al*, 2012; Schramm-Sapyta *et al*, 2006; Thomas *et al*, 2001). An important question in this field is whether drug-induced synaptic plasticity in discrete regions of the reward circuit is causally linked. To establish a causal relationship between cocaine-evoked synaptic plasticity in the VTA and NAc, one can test whether enhancing or blocking synaptic plasticity in the VTA affects synaptic plasticity in the NAc. Previous studies have shown that disruption of metabotropic glutamate receptor (mGluR1) function renders cocaine-evoked synaptic potentiation in the VTA persistent, which in turn triggers lasting synaptic depression in the NAc (Mameli *et al*, 2009). However, it is not known whether blocking synaptic potentiation in the VTA affects cocaine-evoked synaptic depression in the NAc.

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a key regulator of long-term synaptic plasticity, learning and memory (for a review see Coultrap and Bayer, 2012). System analysis of the 'addiction' network suggests that CaMKII is a central node of several signaling pathways implicated in drug-induced synaptic plasticity and addictive behavior (Li *et al*, 2008). Interfering with CaMKII signaling in the NAc or dorsal striatum altered locomotor responses and drug-seeking to cocaine or amphetamine (Anderson *et al*, 2008; Guo *et al*, 2010; Kourrich *et al*, 2012; Liu *et al*, 2009; Loweth *et al*, 2010, 2013; Loweth *et al*; Robison *et al*, 2013; Schierberl *et al*, 2011). Surprisingly, whether CaMKII is required for cocaine conditioned place preference (CPP), which has a strong learning component, remains unexplored. Microinjection of CaMKII inhibitor KN93 into the VTA enhanced the acute locomotor activity to cocaine but attenuated locomotor sensitization to cocaine (Licata *et al*,

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2004). However, KN93 also inhibits L-type Ca^{2+} channels (Gao *et al*, 2006), and blockade of these channels with diltiazem had the same behavioral effect (Licata *et al*, 2004), thus limiting the conclusions about contribution of CaMKII.

Here, we utilized the selective CaMKII inhibitor tatCN21 (Vest *et al*, 2007) to investigate the role of VTA CaMKII in cocaine CPP and cocaine-evoked synaptic plasticity in the NAc. Ca^{2+}/calmodulin-induced autophosphorylation of CaMKII at Thr286 generates a Ca^{2+}-independent, autonomous CaMKII activity (Lou *et al*, 1986; Miller and Kennedy, 1986). In contrast to the traditional CaMKII inhibitors KN93 and KN62 (which only block Ca^{2+}-stimulated activity), tatCN21 blocks Ca^{2+}-stimulated and autonomous CaMKII activity with equal potency (Vest *et al*, 2010) and does not interfere with related kinases such as CaMKIV (Vest *et al*, 2007). We examined whether microinjections of tatCN21 in the VTA affected cocaine CPP and cocaine-evoked synaptic plasticity in the NAc *ex vivo*. The results provide evidence that CaMKII activity in the VTA governs cocaine-evoked synaptic plasticity in the NAc during the acquisition of cocaine CPP.

MATERIALS AND METHODS

Experiment 1: Examining the Effect of VTA CaMKII Blockade on the Acquisition of Cocaine CPP

Sprague–Dawley rats (7–8 weeks old, male, $n = 8$–9 per group) were anesthetized with ketamine (90 mg/kg, intraperitoneally) and xylazine (10 mg/kg, intraperitoneally) and placed in a stereotaxic device (David Kopf Instruments, Tujunga, CA). Guide cannulae (26 G) (Plastics One, Roanoke, VA) were bilaterally implanted 2.8 mm above the VTA at a 10° angle as we have described (Yu *et al*, 2013). Rats were allowed to recover for about 1 week. CPP experiments were performed using three-chamber CPP apparatus (Med Associates, St Albans, VT). The CPP protocol consisted of the following sessions:

Pretest (day 1). Animals were allowed to explore both chambers for 20 min and time spent in each side was recorded. Rats showing unconditioned side preference ($\geqslant$180 s disparity) were excluded ($n = 4$).

Place conditioning (days 2–9). Rats received saline or cocaine conditioning and intra-VTA drug pretreatments. For cocaine conditioning, rats received saline injections (0.9% NaCl, 1 ml/kg, intraperitoneally) on days 2, 4, 6, and 8, and were immediately confined to one chamber for 20 min. On days 3, 5, 7, and 9, rats received cocaine injections (15 mg/kg, intraperitoneally) and were immediately confined to the opposite chamber for 20 min. Saline conditioning was performed as above except that rats received daily saline injections. On days 3, 5, 7, and 9, rats received bilateral intra-VTA infusions of vehicle (0.9% NaCl, 0.5 μl per side), tatCN21 (0.2 μg/μl, 0.5 μl per side) or the same amount of scrambled tatCN21 (tatCtrl) via the preimplanted cannulae 30 min before each place conditioning (Figure 1). In a subset of control experiments, rats received cocaine conditioning as described above, the same amount of tatCN21 or tatCtrl was infused into the VTA bilaterally 30 min before place conditioning on days 2, 4, 6, and 8 (Figure 2). Bath application of tat-CN21 (5–20 μM) for 10–30 min to hippocampal slices was effective in blocking long-term potentiation, whereas intraperitoneal injection of tatCN21 (10 mg/kg) reduced freezing behavior in a fear-learning paradigm in mice (Buard *et al*, 2010; Sanhueza *et al*, 2011). Considering the diffusion limitation in intact tissue of the VTA *in vivo*, we chose a higher dose (0.2 μg/μl, which is approximately 50 μM) and longer pretreatment time (30 min) for intra-VTA infusions of tatCtrl and tatCN21 before place conditioning.

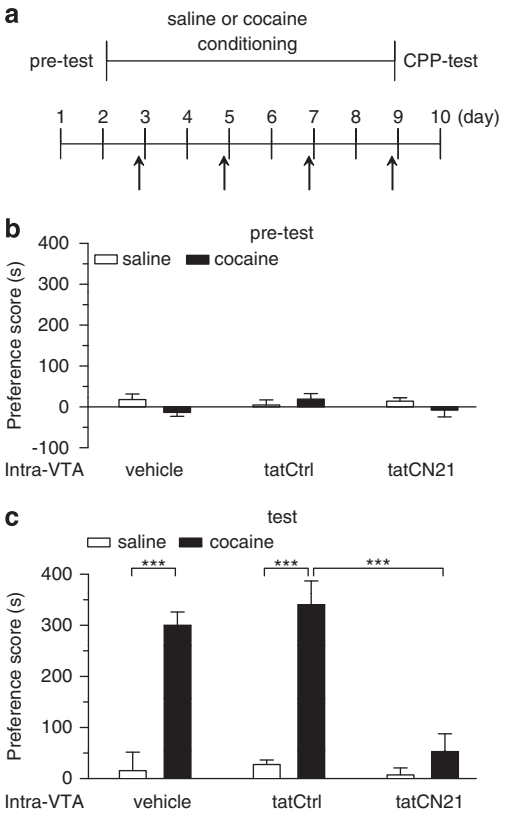

Figure 1 Intra-ventral tegmental area (VTA) microinjections of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) inhibitor tatCN21 in cocaine-paired environment attenuated the acquisition of conditioned place preference (CPP) to cocaine. (a) The timeline of drug treatment and behavioral paradigm. Six groups of rats received saline and cocaine place conditioning once daily for 8 days. Vehicle, tatCtrl or tatCN21 was bilaterally infused into the VTA 30 min before saline- or cocaine-pairing on days 3, 5, 7, and 9. (b) Pretest indicates that rats did not exhibit baseline bias in place preference in all groups ($n = 7$–9; $p > 0.05$). (c) Intra-VTA infusions of tatCN21 blocked CPP to cocaine but did not affect CPP scores in saline-conditioned rats ($n = 7$–9; ***$p < 0.001$, Tukey's *post hoc* test). Error bars indicate SEM.

CPP test (day 10). All of the animals were allowed to explore freely for 20 min between the two sides and time spent on each side is recorded.

The Verification of VTA Cannula Placement

After CPP tests, the VTA cannula placement was verified by Cresyl Violet staining or visual inspection under dissecting microscope as described previously (Yu *et al*, 2013). On the basis of the stereotaxic atlas of Paxinos and Watson

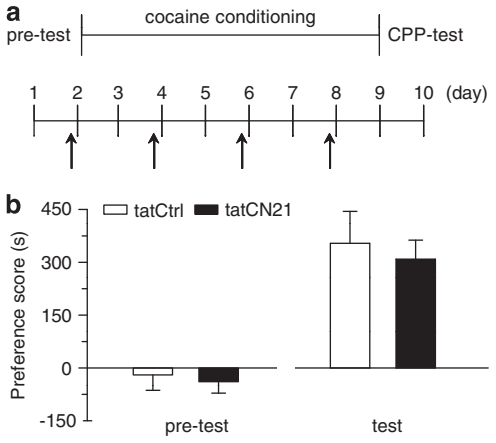

Figure 2 Intra-ventral tegmental area (VTA) microinjections of tatCN21 in saline-paired environment did not significantly affect the acquisition of conditioned place preference (CPP) to cocaine. (a) The timeline of drug treatment and behavioral paradigm. Two groups of rats received cocaine place conditioning once daily for 8 days. TatCtrl or tatCN21 was bilaterally infused into the VTA 30 min before saline- or cocaine-pairing on days 2, 4, 6, and 8. (b) Pretest indicates that rats did not exhibit baseline bias in place preference in all groups ($n = 9$–10; $p > 0.05$). Intra-VTA infusions of tatCN21 did not significantly affect CPP to cocaine compared with intra-VTA infusions of tatCtrl ($n = 9$–10; $p > 0.05$, *t*-test). Error bars indicate SEM.

(Paxinos and Watson, 1997), in total 10 of 136 rats with misplaced cannulae were excluded from this study (from Experiments 1 to 3).

Experiment 2: Examining the Effect of Intra-VTA Infusions of tatCN21 before Cocaine Conditioning on Excitatory Transmission in the NAc Shell *Ex Vivo*

Four groups of rats ($n = 8$–9 per group) received saline and cocaine conditioning and intra-VTA infusions of tatCtrl or tatCN21 as described in Figure 1. One day after the CPP test, the rats were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally) and perfused through the aorta with a cold sucrose-based solution (4–6 °C) containing (in mM): 220 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 7 $MgSO_4$, 26 $NaHCO_3$, and 10 glucose. Coronal midbrain slices (VTA) and striatal slices (NAc) were cut at 250–300 μm thickness in the sucrose-based solution using a vibrating slicer (Leica) and were immediately transferred in oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose. The striatal slice was cut at the midline so that each brain section yielded two symmetric slices. Slices were allowed to recover at room temperature for at least 1 h.

Whole-cell patch-clamp recordings were made from the medium spiny neurons (MSNs) in the shell of the NAc slices, and MSNs were identified visually by their medium-sized, spindle-like cell bodies, large initial axon segment, and electrophysiological characteristics as we have described (Wang *et al*, 2010b). Internal solution consisted of the followings (in mM): 140 Cs-methanesulfonate, 10 HEPES, 1 EGTA, 5 tetraethylammonium chloride (TEA-Cl), 2.5 MgATP, 0.3 Na_2GTP, and 2 $MgCl_2$ at pH 7.2 (with CsOH). Approximate locations for the recordings were: 0.9–2.0 mm anterior to bregma, 6.8–8.2 mm ventral from the dorsal brain surface, and 0.6–1.0 mm lateral to the midline. The coordinates were determined by visual inspection of the slice with a rat brain atlas (Paxinos and Watson, 1997). The neurons were voltage-clamped at the resting membrane potential of MSNs (-80 mV), unless stated otherwise. A bipolar tungsten-stimulating electrode was placed at the prelimbic cortex–NAc border to stimulate preferentially afferent cortical fibers. $GABA_A$ receptor blocker picrotoxin (50–100 μM) was freshly prepared and dissolved into the ACSF through sonication for ~10 min. For the recordings of miniature excitatory postsynaptic currents (mEPSCs), Na^+ channel blocker tetrodotoxin (TTX, 0.5 μM) was added into the ACSF to block action potentials. For measurements of the AMPA receptor/NMDA receptor (AMPAR/NMDAR) ratio, the MSNs were voltage-clamped at $+40$ mV. First, a stable baseline recording of total EPSCs was obtained, NMDAR antagonist (*R*)-CPP (10 μM) was then applied in the bath for 6–10 min to isolate fast AMPAR-mediated EPSCs. Subtraction of AMPAR-EPSCs from the total EPSCs in the same neuron yielded NMDAR-EPSCs. An average of 12–20 EPSCs was collected for the each type of EPSCs.

Experiment 3: Examining the Effects of Intra-VTA Infusion of tatCN21 Just before the CPP Test on the Expression of Cocaine CPP and Excitatory Transmission in the NAc Shell *Ex Vivo*

Rats received cocaine conditioning but no intra-VTA infusion was made during place conditioning ($n = 7$–9 per group). Thirty minutes before the CPP test, vehicle (0.9% NaCl, 0.5 μl per side), tatCtrl (0.2 μg, 0.5 μl per side) or tatCN21 (0.2 μg, 0.5 μl per side) was bilaterally infused into the VTA and their effects on the expression of cocaine CPP was tested. One day after the CPP test, rats were killed and striatal slices were prepared. Whole-cell recordings were made from MSNs in the shell of NAc, and mEPSCs and AMPAR/NMDAR ratios were recorded as described above.

Chemicals

Cocaine hydrochloride, picrotoxin, and all other common chemicals were obtained from Sigma-Aldrich (St Louis, MO). TTX and (*R*)-CPP were obtained from Tocris Bioscience (Ellisville, MO). CaMKII inhibitory peptide tatCN21 (YGRKKRRQRRRKRPPKLGQIGRSKRVVIEDDR) and the scrambled control peptide tatCtrl (YGRKKRRQRRRVKE PRIDGKPVRLRGQKSDRI) were synthesized by CHI Scientific (Maynard, MA).

Statistics

Data are presented as the mean ± SEM. The CPP score was defined as the time spent in the cocaine-paired chamber minus the time spent in the saline-paired chamber. The data of mEPSCs were analyzed using Mini-analysis (Synaptosft, Decatur, GA). The frequency and amplitude of mEPSCs were calculated for at least 5–8 min of recordings. The analysis of mEPSCs was performed with cumulative probability plots (Van der Kloot, 1991). The paired-pulse ratio (PPR) was calculated by dividing the mean amplitude

of the second EPSCs by that of the first EPSCs. Data sets were compared with either Student's *t*-test, one-way, two-way or mixed ANOVA followed by Tukey's *post hoc* analysis. Results were considered to be significant at $p<0.05$.

RESULTS

Intra-VTA tatCN21 Blocked the Acquisition of Cocaine CPP

We examined the effect of intra-VTA infusions of vehicle, tatCN21, and scrambled tatCN21 (tatCtrl) during place conditioning on the acquisition of cocaine CPP in six groups of rats ($n=7$–9 per group). The timeline for baseline preference test (pretest), cocaine or saline conditioning, intra-VTA infusions, and CPP test is described in Figure 1a. During pretest, rats showing unconditioned side preference ($\geqslant$180 s disparity) were excluded. The remaining rats did not exhibit significant difference in the time spent in each chamber ($p>0.05$; Figure 1b). Then, cocaine (15 mg/kg, intraperitoneally) or saline (0.9% NaCl) place conditioning was conducted once daily for 8 days. Vehicle (saline, 0.5 μl per side), tatCN21 (0.2 μg/μl, 0.5 μl per side), or tatCtrl (0.2 μg/μl, 0.5 μl per side) was bilaterally infused into the VTA via preimplanted cannulae 30 min before cocaine- or saline-pairing with a particular chamber on days 3, 5, 7, and 9. Twenty-four hours after the last pairing, CPP was tested without any drug or vehicle administration. Two-way ANOVA revealed that cocaine conditioning ($F_{1,44}=79.57$, $p<0.001$) and intra-VTA drug treatments ($F_{2,44}=16.59$, $p<0.001$) had significant effects on CPP scores and there was a significant interaction between cocaine conditioning and intra-VTA drug treatments ($F_{2,44}=12.45$, $p<0.001$; Figure 1c). Tukey's *post hoc* tests showed that intra-VTA infusions of tatCN21 blocked cocaine CPP ($p<0.001$) but did not significantly affect CPP score in saline-conditioned rats ($p>0.05$), whereas tatCtrl had no significant effect on CPP scores in saline- or cocaine-conditioned rats ($p>0.05$). Thus, intra-VTA tatCN21 blocked CPP to cocaine without inducing CPP or conditioned place aversion in saline-conditioned rats.

During place conditioning, locomotor activities in the conditioning chambers were tracked by infrared photobeam breaks. We compared locomotor activities over the 4 days of cocaine conditioning or correspondent saline conditioning (ie, days 3, 5, 7, and 9). Locomotor activities were analyzed with a mixed ANOVA that included between-subjects factors of place conditioning and intra-VTA infusions and within-subject factor of conditioning days. Cocaine significantly increased locomotor activities over the 4 days of conditioning ($F_{1,168}=46.94$, $p<0.001$), and there was a significant interaction between cocaine and conditioning days ($F_{3,168}=6.84$, $p<0.001$). However, intra-VTA infusions of tatCN21 or tatCtrl before cocaine conditioning did not significantly alter locomotor activities in saline- and cocaine-conditioned rats ($F_{2,168}=0.33$, $p>0.05$) and there was no significant interaction between place conditioning and intra-VTA infusions ($F_{2,168}=1.24$, $p>0.05$) (Supplementary Figure S1).

In the above experiments, tatCN21 or tatCtrl was bilaterally infused into the VTA before cocaine conditioning (ie, days 3, 5, 7, and 9). There is a possibility that intra-VTA tatCN21 disrupts general biological function of VTA neurons, leading to the blockade of cocaine CPP. To exclude this possibility, we examined whether intra-VTA tatCN21 affected cocaine CPP if it was administered before saline conditioning (ie, days 2, 4, 6, and 8). Rats received cocaine conditioning as described above. The same amount of tatCN21 or tatCtrl was bilaterally infused into the VTA 30 min before the rats were placed in the saline-paired chambers (ie, days 2, 4, 6, and 8; Figure 2a). We found that intra-VTA infusions of tatCN21 before saline conditioning did not significantly affect CPP compared with intra-VTA infusions of tatCtrl ($t_{1,18}=0.445$, $p>0.05$, $n=9$–10; Figure 2b). Taken together, these experiments suggest that the CaMKII inhibitor blocks cocaine CPP through selective disruption of cocaine-cue-associated learning.

After the CPP test, the VTA cannula placements in about one-third of rats were verified with Cresyl Violet staining after transcardial perfusion fixation. Figure 3 shows the representative coronal sections containing the VTA and the representative sites of the cannula tips for intra-VTA infusions. Alternatively, fresh midbrain slices were cut in about two-third of rats after transcardial perfusion of sucrose-based solution and VTA cannula placements were verified with visual inspection under dissecting microscope. In total, 10 of 136 rats with misplaced cannulae were excluded from data analyses.

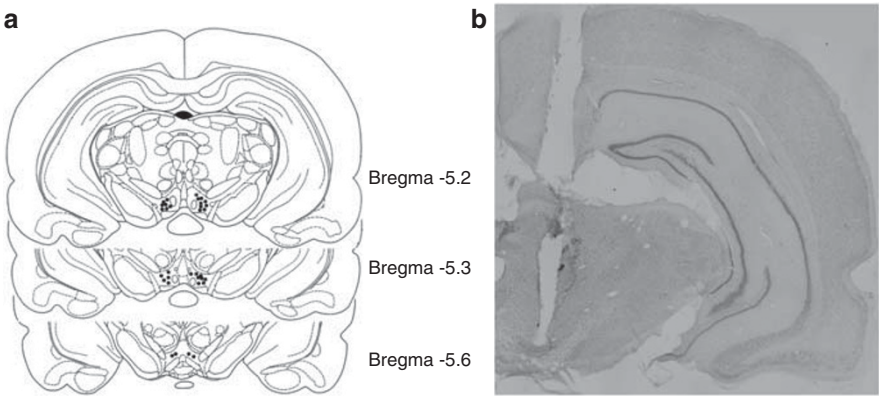

Figure 3 Histological verification of intra-ventral tegmental area (VTA) cannula placements. (a) Representative sites of cannula tips in the VTA. (b) Cresyl Violet-stained coronal section of typical intra-VTA cannula placement.

Intra-VTA tatCN21 Blocked Cocaine CPP-induced Depression of Excitatory Transmission in the NAc shell *Ex Vivo*

Repeated intraperitoneal injections of cocaine and cocaine self-administration decreased AMPAR/NMDAR ratio, an indicator for synaptic strength, in the NAc shell (Kourrich *et al*, 2007; Ortinski *et al*, 2012; Thomas *et al*, 2001). However, it is not known whether cocaine CPP affected excitatory transmission in the NAc, and if so, whether CaMKII blockade in the VTA with tatCN21 affected cocaine CPP-induced synaptic plasticity in the NAc. To address these questions, we examined the effects of cocaine conditioning and intra-VTA infusions of tatCN21 on EPSCs in the NAc shell. Four new groups of rats underwent saline and cocaine conditioning ($n = 8$–9 per group). TatCN21 (0.2 μg, 0.5 μl per side) or tatCtrl (0.2 μg, 0.5 μl per side) was bilaterally infused into the VTA via preimplanted cannulae 30 min before cocaine- or saline-pairing with a particular chamber on days 3, 5, 7, and 9. The experiments were the same as those of Figure 1 except that we omitted the groups that received intra-VTA infusions of vehicle since there was no significant difference between the vehicle and tatCtrl groups. Two-way ANOVA showed that cocaine conditioning and intra-VTA drug treatments had significant effects on CPP scores (cocaine: $F_{1,34} = 27.92$, $p < 0.001$; intra-VTA infusion: $F_{1,34} = 48.95$, $p < 0.001$; cocaine × intra-VTA infusion interaction: $F_{1,34} = 24.86$, $p < 0.001$). Tukey's *post hoc* tests showed that intra-VTA infusions of tatCN21 blocked cocaine CPP (CPP scores for tatCtrl: 320 ± 27 s, $n = 8$; tatCN21: 77 ± 26 s, $n = 9$, $p < 0.001$). One day after the CPP test, the rats were euthanized and living NAc slices were prepared. Whole-cell voltage-clamp recordings were made from MSNs of the NAc shell at a holding potential of −80 mV, near the resting membrane potential of these neurons (Dehorter *et al*, 2009). MSNs are GABAergic neurons that make up the vast majority (90–95%) of cells in the NAc (Meredith, 1999; Zhou *et al*, 2002). We measured the ratio of peak AMPAR- to peak NMDAR-mediated evoked EPSCs from MSNs in the NAc shell in slices prepared from these four groups of mice (Figure 4a and b). The AMPAR/NMDAR ratio has been used as indicator for *in vivo* synaptic potentiation (Thomas *et al*, 2001; Ungless *et al*, 2001). We found that cocaine conditioning ($F_{1,31} = 4.34$, $p < 0.05$) and intra-VTA drug infusions ($F_{1,31} = 8.11$, $p < 0.05$) had significant effects on the AMPAR/NMDAR ratio and there was a significant interaction between cocaine conditioning and intra-VTA drug infusions ($F_{1,31} = 5.42$, $p < 0.05$). Tukey's *post hoc* tests showed that the AMPAR/NMDAR ratio was decreased in cocaine-conditioned rats compared with saline-conditioned rats (both groups received intra-VTA infusions of tatCtrl) ($p < 0.01$), whereas intra-VTA infusions of tatCN21 blocked this decrease in the AMPAR/NMDAR ratio ($p < 0.01$; Figure 4b).

Miniature excitatory postsynaptic currents (mEPSCs) were generated by action potential-independent, quantal glutamate release from presynaptic axonal terminals. mEPSCs provide another good indicator of synaptic strength. mEPSCs were recorded in the MSNs of the NAc shell in these four groups of rats (Figure 5a). Two-way ANOVA revealed that cocaine conditioning and intra-VTA drug infusions had significant effects on the frequency of mEPSCs (cocaine: $F_{1,58} = 7.19$, $p < 0.05$; intra-VTA infusion: $F_{1,58} = 4.7$, $p < 0.05$; cocaine × intra-VTA infusion interaction: $F_{1,58} = 5.69$, $p < 0.05$; Figure 5b) and the amplitude of mEPSCs (cocaine: $F_{1,58} = 4.99$, $p < 0.05$; intra-VTA infusion: $F_{1,58} = 4.01$, $p < 0.05$; cocaine × intra-VTA infusion interaction: $F_{1,58} = 5.93$, $p < 0.05$; Figure 5c). Tukey's *post hoc* tests showed that intra-VTA infusions of tatCN21 blocked cocaine-induced decrease in the frequency ($p < 0.001$) as well as amplitude ($p < 0.01$) of mEPSCs, whereas tatCtrl did not alter mEPSCs of saline- or cocaine-conditioned rats ($p > 0.05$). Cumulative frequency plot analysis showed that the cumulative distribution of interevent intervals was shifted to the right (ie, longer interval and less frequent) in cocaine-conditioned rats, and this shift was blocked by intra-VTA infusions of tatCN21 (Figure 5d and e). The cumulative distribution of mEPSC amplitude was shifted to the left (ie, smaller amplitude) in cocaine-conditioned rats, and this shift was blocked by intra-VTA infusions of tatCN21 as well. Taken together, these results indicate that intra-VTA infusions of tatCN21 blocked cocaine CPP-induced decreases in the frequency and amplitude of mEPSCs in the NAc shell.

A decrease in the amplitude of mEPSCs can be attributed to a decrease in postsynaptic AMPAR number or function, whereas a decrease in the frequency of mEPSCs may be

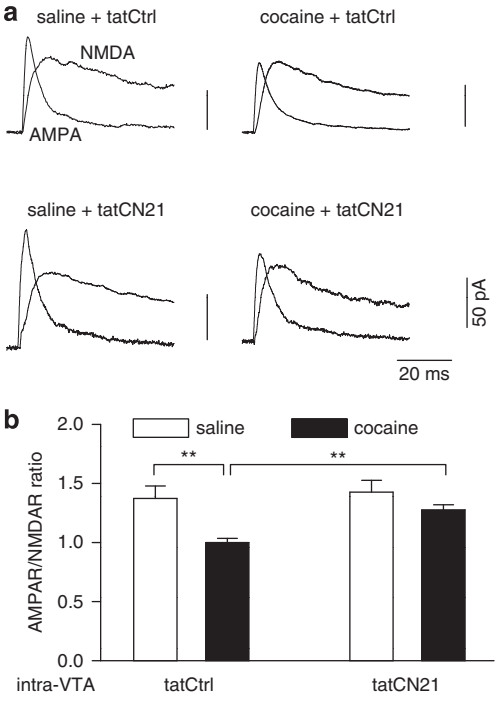

Figure 4 Effects of cocaine conditioning and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) blockade in the ventral tegmental area (VTA) on the AMPAR/NMDAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor/*N*-methyl-D-aspartate receptor) ratio in the nucleus accumbens (NAc) shell *ex vivo*. (a) Representative AMPAR- and NMDAR-mediated excitatory postsynaptic currents (EPSCs) recorded from medium spiny neurons (MSNs) in the NAc shell in slices prepared from saline- and cocaine-conditioned rats that received intra-VTA infusions of tatCtrl and tatCN21. (b) Cocaine conditioning induced the increase of AMPAR/NMDAR ratio ($n = 8$, $^{**}p < 0.01$ *vs* saline conditioning), which was reversed by tatCN21 infusion ($n = 8$, $^{**}p < 0.01$ *vs* tatCtrl). Error bars indicate SEM.

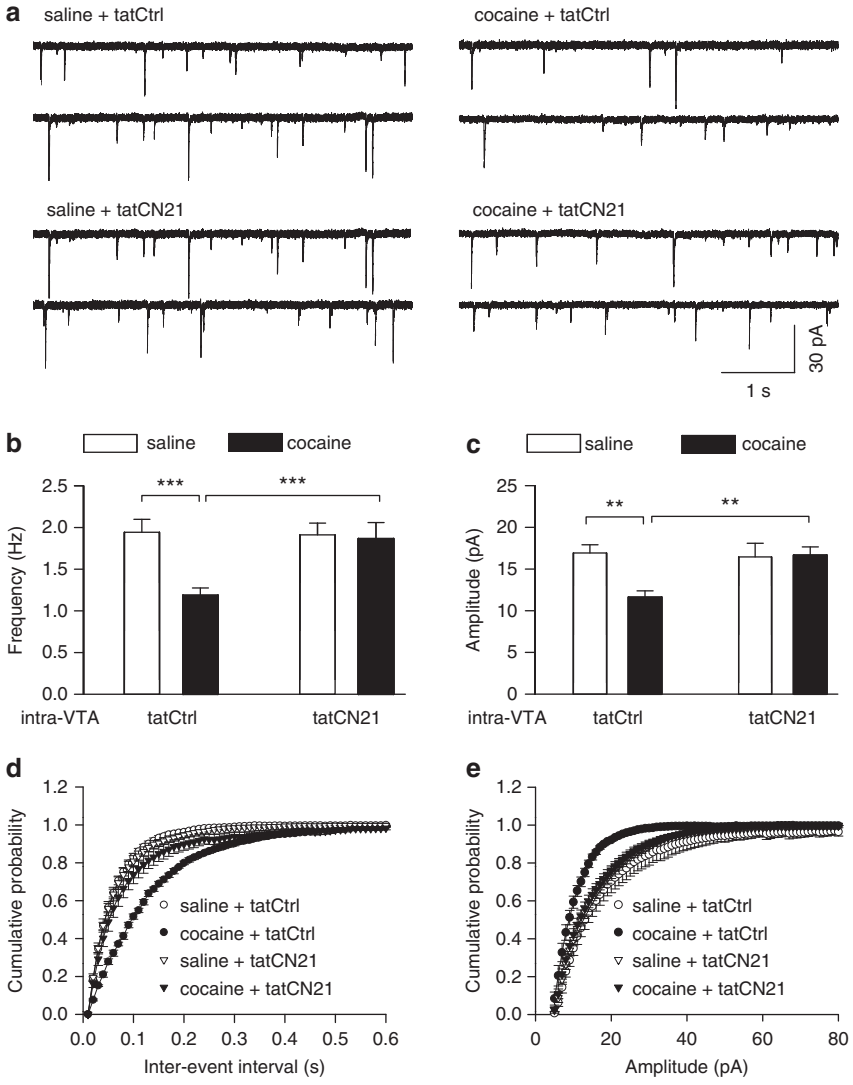

Figure 5 Effects of cocaine conditioning and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) blockade in the ventral tegmental area (VTA) on miniature excitatory postsynaptic currents (mEPSCs) in the nucleus accumbens (NAc) shell *ex vivo*. (a) Representative mEPSCs recorded from medium spiny neurons (MSNs) in the nucleus accumbens (NAc) shell in slices prepared from saline- and cocaine-conditioned rats that received intra-VTA infusions of tatCtrl and tatCN21. (b and c) The averaged frequency (b) and amplitude (c) of mEPSCs in the NAc shell in these four groups of rats. The mean frequency and amplitude of mEPSCs were significantly decreased in cocaine-conditioned rats compared with that in saline-conditioned rats, and this decrease was blocked by intra-VTA tatCN21 (**$p<0.01$, ***$p<0.001$). (d and e) The cumulative probability plots of mEPSC frequency (interevent interval) and amplitude distribution ($n=14$–15).

explained by a decrease in presynaptic glutamate release. However, cocaine conditioning-induced decrease in mEPSC amplitude would, in principle, render smaller mEPSCs below the threshold for detection, which could be misinterpreted as a decrease in the frequency of mEPSCs. To further distinguish between a presynaptic or postsynaptic effect, we recorded PPR of evoked EPSCs at interpulse intervals of 20–400 ms. The change in the PPR suggests a change in presynaptic release probability (Zucker and Regehr, 2002). Two-way ANOVA revealed that cocaine conditioning and intra-VTA drug treatments had significant effects on the PPR at the interpulse intervals of 20 ms (cocaine: $F_{1,32}=4.55$, $p<0.05$; intra-VTA infusion: $F_{1,32}=40.48$, $p<0.001$; cocaine × intra-VTA infusion interaction: $F_{1,32}=52.01$, $p<0.001$; Figure 6a and b) and 50 ms (cocaine: $F_{1,32}=7.86$, $p<0.01$; intra-VTA infusion: $F_{1,32}=18.27$, $p<0.001$; cocaine × intra-VTA infusion interaction: $F_{1,32}=16.52$, $p<0.001$; Figure 6a and b). Tukey's *post hoc* tests showed that intra-VTA infusions of tatCN21 blocked cocaine-induced increases in the PPR at 20 and 50 ms interpulse intervals ($p<0.05$). The PPR at other interpulse intervals (100–400 ms) was not significantly different among these four groups of rats ($p>0.05$). Taken together, the above results suggest that cocaine conditioning decrease the strength of excitatory synaptic transmission via both presynaptic and postsynaptic mechanisms.

Intra-VTA tatCN21 Just before CPP Test did not Affect Cocaine CPP and Cocaine-Evoked Depression of Excitatory Transmission in the NAc shell *Ex Vivo*

We examined whether CaMKII blockade in the VTA just before the CPP test affected the expression of CPP to cocaine in three groups of rats ($n=7$–9 per group). The

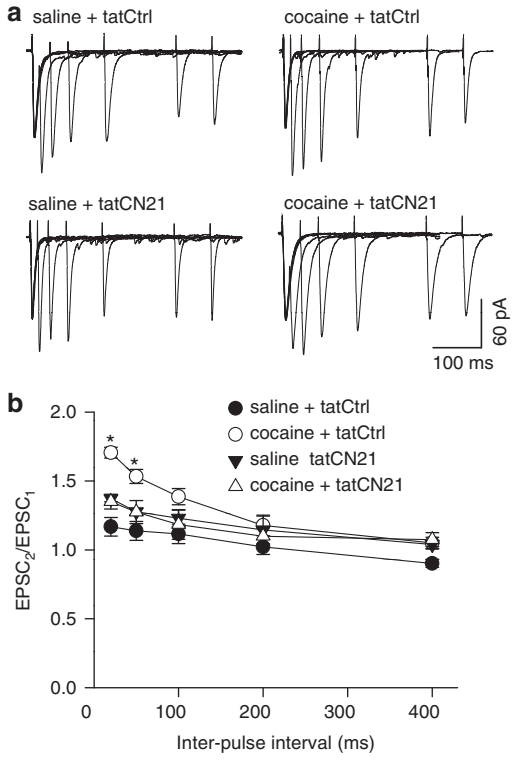

Figure 6 Effects of cocaine conditioning and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) blockade in the ventral tegmental area (VTA) on the paired-pulse ratio ($PPR = EPSC_2/EPSC_1$) of evoked excitatory postsynaptic currents (EPSCs) in the nucleus accumbens (NAc) shell *ex vivo*. (a) Representative traces of paired-pulse EPSCs at 20–400 ms interpulse intervals in all four groups. (b) The PPR at 20 and 50 ms interpulse intervals was significantly increased in cocaine-conditioned rats, and this increase was blocked by intra-VTA infusions of tatCN21 ($n = 8$–9; $^*p < 0.05$).

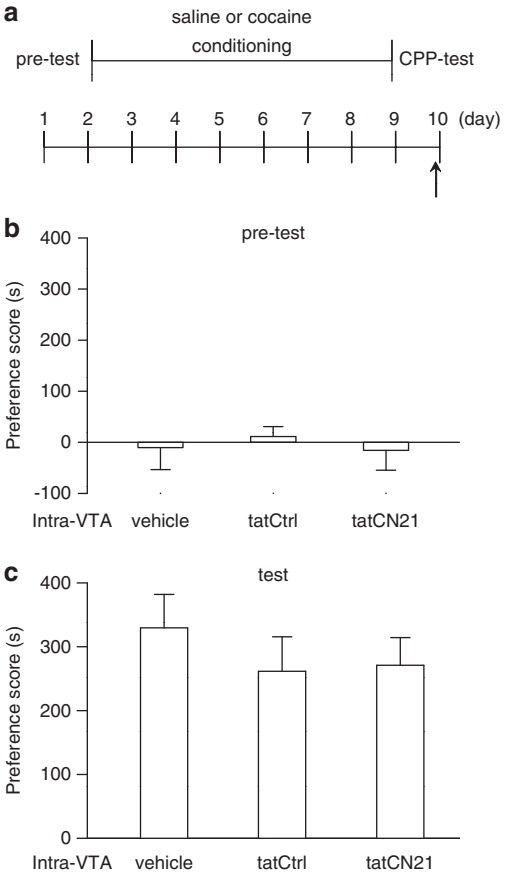

Figure 7 Intra-ventral tegmental area (VTA) microinjections of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) inhibitor tatCN21 just before the conditioned place preference (CPP) test had no significant effect on cocaine CPP. (a) Timeline of drug treatment and behavioral paradigm. Three groups of rats received cocaine conditioning once daily for 8 days. Vehicle, tatCtrl, or tatCN21 was bilaterally infused into the VTA 30 min before CPP test. (b) Pretest indicates that rats did not exhibit baseline bias in place preference ($n = 7$–9; $p > 0.05$). (c) Intra-VTA infusions of tatCN21 or tatCtrl 30 min before CPP test did not affect CPP scores ($n = 7$–9; $p > 0.05$).

timeline for these experiments is shown in Figure 7a. Pretest indicated that rats did not show side preference before drug injections (Figure 7b). Rats received cocaine conditioning but no intra-VTA infusion was made during place conditioning. Thirty minutes before the CPP test, vehicle (0.5 μl per side), tatCN21 (0.2 μg, 0.5 μl per side), or tatCtrl (0.2 μg, 0.5 μl per side) was bilaterally infused into the VTA via preimplanted cannulae. One-way ANOVA indicated that neither tatCN21 nor tatCtrl significantly affected CPP to cocaine compared with vehicle control ($F_{2,\ 23} = 0.030$, $p > 0.05$; Figure 7c). Thus, intra-VTA tatCN21 just before the CPP test did not affect the expression of CPP to cocaine. One day after the CPP test, rats were killed and striatal slices were prepared. We examined whether VTA CaMKII blockade just before the CPP test affected mEPSCs (Figure 8a–c) and the AMPAR/NMDAR ratio (Figure 8d and e) in the MSNs in the NAc shell. The amplitude ($t_{1,28} = 0.323$, $p > 0.05$; Figure 8b) and the frequency ($t_{1,28} = 1.497$, $p > 0.05$; Figure 8c) of mEPSCs and the AMPAR/NMDAR ratio ($t_{1,15} = 0.481$, $p > 0.05$; Figure 8e) were not significant different between tatCN21 and tatCtrl groups. These results indicate that intra-VTA infusions of tatCN21 just before the CPP test have no significant effects on mEPSCs and AMPAR/NMDAR ratio compared with intra-VTA infusions of tatCtrl.

DISCUSSION

The present study showed that inhibition of CaMKII activity in the VTA during the conditioning phase blocked the acquisition of cocaine CPP, as well as cocaine-evoked synaptic depression in the NAc shell. In contrast, inhibition of CaMKII activity in the VTA did not affect established cocaine CPP and cocaine-evoked synaptic plasticity in the NAc shell. Thus, CaMKII activity in the VTA is involved in the acquisition, but not the maintenance, of cocaine-cue memories and neural adaptations in the NAc.

CaMKII Activity in the VTA is Required for the Acquisition of Cocaine CPP

Although the contribution of CaMKII signaling in the NAc to sensitized locomotor responses and drug-seeking behavior to cocaine and amphetamine has been studied extensively (Anderson *et al*, 2008; Guo *et al*, 2010; Kourrich *et al*, 2012; Liu *et al*, 2009; Loweth *et al*, 2010,

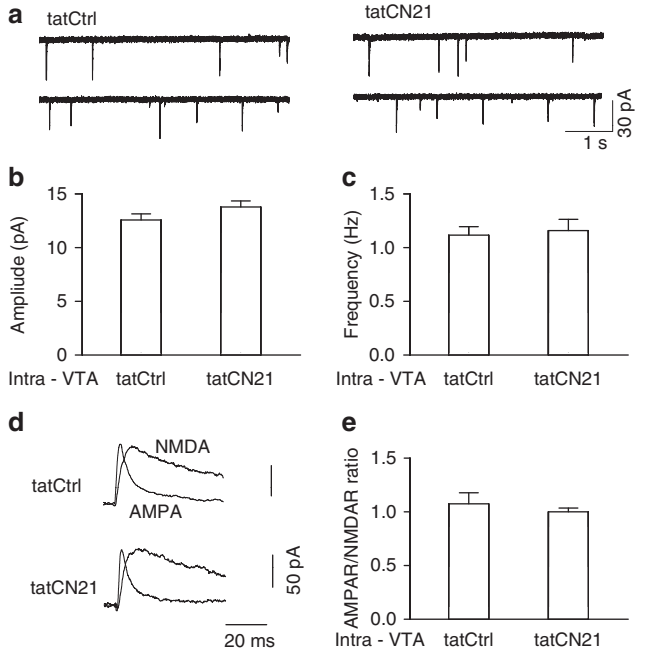

Figure 8 Intra-ventral tegmental area (VTA) microinjections of tatCN21 before the conditioned place preference (CPP) test had no significant effect on miniature excitatory postsynaptic currents (mEPSCs) and AMPAR/NMDAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor/*N*-methyl-D-aspartate receptor) ratio in the nucleus accumbens (NAc) shell *ex vivo*. (a) Representative mEPSCs recorded from medium spiny neurons (MSNs) in the NAc shell in slices prepared cocaine-conditioned rats that received intra-VTA infusions of tatCtrl and tatCN21. (b and c) The averaged frequency (b) and amplitude (c) of mEPSCs in the NAc shell in these two groups of rats. Intra-VTA microinjections of tatCN21 before the CPP test had no significant effect on mEPSCs ($n = 14$–15, $p > 0.05$). (d) Representative AMPAR- and NMDAR-mediated EPSCs in the NAc shell in slices prepared from cocaine-conditioned rats that received intra-VTA infusions of tatCtrl and tatCN21. (e) AMPAR/NMDAR ratio was not significantly different between cocaine-conditioned rats that received intra-VTA infusions of tatCtrl and tatCN21 ($n = 8$, $p > 0.05$).

2013; Robison *et al*, 2013; Schierberl *et al*, 2011; Schramm-Sapyta *et al*, 2006; Wang *et al*, 2010a), relatively little was known about the contribution of CaMKII signaling in the VTA to these behavioral effects (Licata *et al*, 2004). The present study showed that intra-VTA infusions of tatCN21 before cocaine conditioning blocked the acquisition of cocaine CPP (Figure 1), whereas intra-VTA infusions of tatCN21 before saline conditioning did not significantly affect cocaine CPP (Figure 2). In addition, intra-VTA infusions of a scrambled peptide tatCtrl before conditioning in either environment had no significant effect on cocaine CPP. These results provide strong evidence that intra-VTA infusions of tatCN21 block cocaine CPP via selective disruption of the learned association between cocaine and the environment. Given the well-established role of CaMKII in learning and memory (for a review see Coultrap and Bayer, 2012), our results suggest that CaMKII signaling in the VTA has a critical role in the development of cocaine-cue-associated memories.

We found that intra-VTA tatCN21 did not significantly affect cocaine-induced acute increase in locomotor activity, which was measured by photobeam breaks in the conditioning chambers (Supplementary Figure S1). In contrast, previous studies have shown that microinjection of CaMKII inhibitor KN93 into the VTA enhanced the acute locomotor activity to cocaine but attenuated locomotor sensitization in response to repeated cocaine injections. Furthermore, CaMKIIα knockout mice exhibit enhanced locomotor activity to acute cocaine and attenuated locomotor sensitization (Licata *et al*, 2004). Several factors might explain the discrepancy. As mentioned earlier, KN93 also inhibits L-type Ca^{2+} channels (Gao *et al*, 2006), and blockade of these channels with diltiazem had the same behavioral effect (Licata *et al*, 2004). CaMKIIα was lost throughout the brain in CaMKIIα knockout mice, and the behavioral effects cannot be solely attributed to the loss of CaMKII in the VTA. In addition, the conditioning chamber in our study is not optimal for measuring locomotor activity given its small size. During place conditioning, cocaine was given every other day instead of being given daily. The locomotor sensitization induced by cocaine place conditioning is modest under our experimental conditions (see Supplementary Figure S1).

Previous studies have shown that single or repeated intraperitoneal injections of cocaine increases AMPAR/NMDAR ratio in VTA dopamine neurons (Borgland *et al*, 2004; Liu *et al*, 2005; Mameli *et al*, 2009; Ungless *et al*, 2001), suggesting that cocaine induces persistent synaptic potentiation in the VTA. These studies were mostly carried out in midbrain slices prepared from juvenile rats or mice. We did not attempt to make whole cell recordings in the VTA in the present study because of the technical difficulty in making such recordings in midbrain slices prepared from adult rats that received VTA cannulation. Phosphorylation of GluR1 by CaMKII enhances channel conductance of AMPARs and increases synaptic strength (Derkach *et al*, 1999; Kristensen *et al*, 2011). Given that CaMKII has a critical role in long-term synaptic plasticity in many brain regions (for a review see Coultrap and Bayer, 2012), it is highly likely that CaMKII is required for cocaine-induced long-term synaptic plasticity in the VTA. CaMKII-dependent synaptic plasticity in the VTA might contribute to the acquisition of cocaine-cue memories, which provides a putative mechanism for tatCN21-induced blockade of the acquisition of cocaine CPP.

VTA CaMKII Activity Gates Cocaine-Evoked Synaptic Plasticity in the NAc

Repeated intraperitoneal cocaine injections for 5–7 days followed by ~24 h cessation of cocaine injections induced a decrease in AMPAR/NMDAR ratio and occlusion of LTD induction in the NAc shell (Kourrich *et al*, 2007; Mameli *et al*, 2009; Thomas *et al*, 2001) without affecting the frequency and amplitude of mEPSCs (but see Kim *et al*, 2011; Thomas *et al*, 2001), whereas long-term withdrawal from cocaine treatments (10–21 days) causes synaptic potentiation (Kourrich *et al*, 2007) and an increase in the expression of surface AMPARs in the NAc (Boudreau *et al*, 2007; Boudreau and Wolf, 2005). The mechanism underlying the bidirectional changes in synaptic plasticity is not yet clear. Cocaine self-administration (Ortinski *et al*, 2012) and CPP (the present study) are associated with decreases in AMPAR/NMDAR ratio and the frequency and amplitude of mEPSCs and increased the PPR intervals in the NAc shell. Furthermore, we showed that intra-VTA tatCN21 injections blocked cocaine-induced CPP and synaptic depression in

the NAc. Although correlation does not establish causation, we believe that synaptic depression in the NAc is highly relevant to behavioral effects of drugs of abuse. In support of this notion, a previous study has shown that systemic or intra-NAc infusion of GluR2 peptide that blocks AMPAR endocytosis and LTD prevented the expression of amphetamine-induced behavioral sensitization (Brebner *et al*, 2005).

A recent study has shown that disruption of Homer 1b/c–mGluR1 interaction or mGluR1 function in the VTA rendered cocaine-evoked synaptic potentiation in the VTA persistent, which triggered long-term synaptic depression in the NAc shell (Mameli *et al*, 2009), suggesting that cocaine-evoked synaptic plasticity in the VTA and NAc is hierarchically organized. We showed here that intra-VTA infusions of CaMKII inhibitor tatCN21 during the conditioning phase blocked cocaine CPP-induced alterations of AMPAR/NMDAR ratio, mEPSCs and PPR, suggesting that blocking synaptic potentiation in the VTA also blocked cocaine-evoked synaptic depression in the NAc shell. Collectively, these two studies have revealed a causal relationship between cocaine-evoked synaptic potentiation in the VTA and synaptic depression in the NAc.

The mechanisms underlying this long-range modulation of synaptic plasticity remain obscure at present. Given the anatomical segregation of the VTA and NAc, tatCN21 cannot be diffused from the VTA to NAc to block CaMKII in the NAc. Further, AMPAR-mediated synaptic strength in NAc shell is not altered in mice with striatal-specific expression of autonomously active CaMKII (T286D) (Kourrich *et al*, 2012), although overexpression of a CaMKII inhibitory peptide (EAC3I) reduces excitatory transmission in the dorsal striatum (Klug *et al*, 2012). We speculate that modulation of dopamine release may constitute a mechanism by which VTA CaMKII activity governs cocaine-evoked synaptic plasticity in the NAc. This possibility can be tested in future studies by measuring dopamine concentration in the NAc via microdialysis or fast scan cyclic voltammetry. In support of a dopaminergic mechanism, previous studies have shown that cocaine self-administration decreases mEPSCs and AMPAR/NMDAR ratio in the NAc shell, treatment with D_1 receptor agonist SKF38393 normalizes mEPSCs and AMPAR/NMDAR ratio in cocaine-treated rats (Ortinski *et al*, 2012).

The Time Window During which VTA CaMKII Activity Regulates Cocaine-Induced CPP and Synaptic Plasticity in the NAc

We showed that intra-VTA infusions of tatCN21 blocked the acquisition, but not the expression, of cocaine CPP. Because tatCN21 blocks both stimulated and autonomous CaMKII activity with high selectivity (Vest *et al*, 2007), the failure to block the expression of cocaine CPP by tatCN21 cannot be attributed to its inability to block autonomous CaMKII activity. Our results suggest that CaMKII activity in the VTA is required for the learned association between environmental cues and rewarding effects of cocaine but is not necessary for the maintenance of cue-associated memories once such association is established. In contrast, transient inhibition of CaMKII in NAc shell with a dominant-negative CaMKIIα mutant persistently blocked the enhanced locomotor response to and self-administration of amphetamine (Loweth *et al*, 2013). The VTA is considered the gateway for initiation of addictive behavior (Kauer, 2004), and subsequent adaptive changes in target brain regions such as the NAc are likely required for the maintenance of addictive behavior, which might explain why intra-VTA tatCN21 does not affect the expression of CPP, whereas inhibition of CaMKII in the NAc has a lasting effect on addictive behavior of amphetamine.

We found that intra-VTA tatCN21 just before the CPP test did not reverse cocaine conditioning-induced decreases in mEPSCs and AMPAR/NMDAR ratio in NAc shell. We were unable to block cocaine-induced changes in synaptic plasticity in the NAc with a single infusion of tatCN21 just before the CPP test. These results imply that 'addiction' signal is transferred from the VTA to the NAc during the acquisition of cocaine CPP. However, once such transfer has occurred, the VTA is no longer necessary for the maintenance of cocaine-induced synaptic depression in the NAc.

In summary, we have demonstrated a novel role of VTA CaMKII activity in regulating cocaine-induced CPP and synaptic plasticity in the NAc. Our results reveal interesting neuroanatomical associations of cocaine-evoked synaptic plasticity in reward circuit in a behavioral model of drug reward and incentive learning. CaMKII inhibition in the VTA during the conditioning phase blocked cocaine-evoked synaptic depression in the NAc shell, suggesting that cocaine-evoked synaptic plasticity in the VTA and NAc is causally linked. In addition, our data provide evidence for the existence of a time window during which CaMKII in the VTA governs cocaine-evoked synaptic plasticity in the NAc.

FUNDING AND DISCLOSURE

The University of Colorado is currently seeking patent protection for the improved CaMKII inhibitors (PCT/US08/077934 'Compositions and methods for improved CaMKII inhibitors and uses thereof'), even though the authors have no current financial gain from this patent application.

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REFERENCES

Anderson SM, Famous KR, Sadri-Vakili G, Kumaresan V, Schmidt HD, Bass CE *et al* (2008). CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. *Nat Neurosci* **11**: 344–353.

Borgland SL, Malenka RC, Bonci A (2004). Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: electrophysiological and behavioral correlates in individual rats. *J Neurosci* **24**: 7482–7490.

Boudreau AC, Reimers JM, Milovanovic M, Wolf ME (2007). Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. *J Neurosci* **27**: 10621–10635.

Boudreau AC, Wolf ME (2005). Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J Neurosci* **25**: 9144–9151.

Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S *et al* (2005). Nucleus accumbens long-term depression and the expression of behavioral sensitization. *Science* **310**: 1340–1343.

Buard I, Coultrap SJ, Freund RK, Lee YS, Dell'Acqua ML, Silva AJ *et al* (2010). CaMKII 'autonomy' is required for initiating but not for maintaining neuronal long-term information storage. *J Neurosci* **30**: 8214–8220.

Coultrap SJ, Bayer KU (2012). CaMKII regulation in information processing and storage. *Trends Neurosci* **35**: 607–618.

Dehorter N, Guigoni C, Lopez C, Hirsch J, Eusebio A, Ben-Ari Y *et al* (2009). Dopamine-deprived striatal GABAergic interneurons burst and generate repetitive gigantic IPSCs in medium spiny neurons. *J Neurosci* **29**: 7776–7787.

Derkach V, Barria A, Soderling TR (1999). Ca^{2+}/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc Natl Acad Sci USA* **96**: 3269–3274.

Gao L, Blair LA, Marshall J (2006). CaMKII-independent effects of KN93 and its inactive analog KN92: reversible inhibition of L-type calcium channels. *Biochem Biophys Res Commun* **345**: 1606–1610.

Guo ML, Fibuch EE, Liu XY, Choe ES, Buch S, Mao LM *et al* (2010). CaMKIIalpha interacts with M4 muscarinic receptors to control receptor and psychomotor function. *EMBO J* **29**: 2070–2081.

Hyman SE, Malenka RC, Nestler EJ (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci* **29**: 565–598.

Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O *et al* (2010). Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science* **328**: 1709–1712.

Kauer JA (2004). Learning mechanisms in addiction: synaptic plasticity in the ventral tegmental area as a result of exposure to drugs of abuse. *Annu Rev Physiol* **66**: 447–475.

Kim J, Park BH, Lee JH, Park SK, Kim JH (2011). Cell type-specific alterations in the nucleus accumbens by repeated exposures to cocaine. *Biol Psychiatry* **69**: 1026–1034.

Klug JR, Mathur BN, Kash TL, Wang HD, Matthews RT, Robison AJ *et al* (2012). Genetic inhibition of CaMKII in dorsal striatal medium spiny neurons reduces functional excitatory synapses and enhances intrinsic excitability. *PLoS One* **7**: e45323.

Kourrich S, Klug JR, Mayford M, Thomas MJ (2012). AMPAR-independent effect of striatal alphaCaMKII promotes the sensitization of cocaine reward. *J Neurosci* **32**: 6578–6586.

Kourrich S, Rothwell PE, Klug JR, Thomas MJ (2007). Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *J Neurosci* **27**: 7921–7928.

Kristensen AS, Jenkins MA, Banke TG, Schousboe A, Makino Y, Johnson RC *et al* (2011). Mechanism of Ca^{2+}/calmodulin-dependent kinase II regulation of AMPA receptor gating. *Nat Neurosci* **14**: 727–735.

Li CY, Mao X, Wei L (2008). Genes and (common) pathways underlying drug addiction. *PLoS Comput Biol* **4**: e2.

Licata SC, Schmidt HD, Pierce RC (2004). Suppressing calcium/calmodulin-dependent protein kinase II activity in the ventral tegmental area enhances the acute behavioural response to cocaine but attenuates the initiation of cocaine-induced behavioural sensitization in rats. *Eur J Neurosci* **19**: 405–414.

Liu QS, Pu L, Poo MM (2005). Repeated cocaine exposure *in vivo* facilitates LTP induction in midbrain dopamine neurons. *Nature* **437**: 1027–1031.

Liu XY, Mao LM, Zhang GC, Papasian CJ, Fibuch EE, Lan HX *et al* (2009). Activity-dependent modulation of limbic dopamine D3 receptors by CaMKII. *Neuron* **61**: 425–438.

Lou LL, Lloyd SJ, Schulman H (1986). Activation of the multifunctional Ca2+/calmodulin-dependent protein kinase by autophosphorylation: ATP modulates production of an autonomous enzyme. *Proc Natl Acad Sci USA* **83**: 9497–9501.

Loweth JA, Li D, Cortright JJ, Wilke G, Jeyifous O, Neve RL *et al* (2013). Persistent reversal of enhanced amphetamine intake by transient CaMKII inhibition. *J Neurosci* **33**: 1411–1416.

Loweth JA, Singer BF, Baker LK, Wilke G, Inamine H, Bubula N *et al* (2010). Transient overexpression of alpha-Ca^{2+}/calmodulin-dependent protein kinase II in the nucleus accumbens shell enhances behavioral responding to amphetamine. *J Neurosci* **30**: 939–949.

Mameli M, Halbout B, Creton C, Engblom D, Parkitna JR, Spanagel R *et al* (2009). Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc. *Nat Neurosci* **12**: 1036–1041.

Meredith GE (1999). The synaptic framework for chemical signaling in nucleus accumbens. *Ann N Y Acad Sci* **877**: 140–156.

Miller SG, Kennedy MB (1986). Regulation of brain type II Ca^{2+}/calmodulin-dependent protein kinase by autophosphorylation: a Ca^{2+}-triggered molecular switch. *Cell* **44**: 861–870.

Nestler EJ (2002). Common molecular and cellular substrates of addiction and memory. *Neurobiol Learn Mem* **78**: 637–647.

Ortinski PI, Vassoler FM, Carlson GC, Pierce RC (2012). Temporally dependent changes in cocaine-induced synaptic plasticity in the nucleus accumbens shell are reversed by D1-like dopamine receptor stimulation. *Neuropsychopharmacology* **37**: 1671–1682.

Paxinos G, Watson C (1997). The rat brain in stereotaxic coordinates. In *Compact*. 3rd edn. Academic Press: San Diego.

Robison AJ, Vialou V, Mazei-Robison M, Feng J, Kourrich S, Collins M *et al* (2013). Behavioral and structural responses to chronic cocaine require a feedforward loop involving DeltaFosB and calcium/calmodulin-dependent protein kinase II in the nucleus accumbens shell. *J Neurosci* **33**: 4295–4307.

Sanhueza M, Fernandez-Villalobos G, Stein IS, Kasumova G, Zhang P, Bayer KU *et al* (2011). Role of the CaMKII/NMDA receptor complex in the maintenance of synaptic strength. *J Neurosci* **31**: 9170–9178.

Schierberl K, Hao J, Tropea TF, Ra S, Giordano TP, Xu Q *et al* (2011). Cav1.2 L-type Ca(2)(+) channels mediate cocaine-induced GluA1 trafficking in the nucleus accumbens, a long-term adaptation dependent on ventral tegmental area Ca(v)1.3 channels. *J Neurosci* **31**: 13562–13575.

Schramm-Sapyta NL, Olsen CM, Winder DG (2006). Cocaine self-administration reduces excitatory responses in the mouse nucleus accumbens shell. *Neuropsychopharmacology* **31**: 1444–1451.

Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001). Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat Neurosci* **4**: 1217–1223.

Ungless MA, Whistler JL, Malenka RC, Bonci A (2001). Single cocaine exposure *in vivo* induces long-term potentiation in dopamine neurons. *Nature* **411**: 583–587.

Van der Kloot W (1991). The regulation of quantal size. *Prog Neurobiol* **36**: 93–130.

Vest RS, Davies KD, O'Leary H, Port JD, Bayer KU (2007). Dual mechanism of a natural CaMKII inhibitor. *Mol Biol Cell* **18**: 5024–5033.

Vest RS, O'Leary H, Coultrap SJ, Kindy MS, Bayer KU (2010). Effective post-insult neuroprotection by a novel Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) inhibitor. *J Biol Chem* **285**: 20675–20682.

Wang L, Lv Z, Hu Z, Sheng J, Hui B, Sun J *et al* (2010a). Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKIIalpha in the nucleus accumbens is critical for motivation for drug reinforcement. *Neuropsychopharmacology* **35**: 913–928.

Wang W, Sun D, Pan B, Roberts CJ, Sun X, Hillard CJ *et al* (2010b). Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. *Neuropsychopharmacology* **35**: 2249–2261.

Yu F, Zhong P, Liu X, Sun D, Gao HQ, Liu QS (2013). Metabotropic glutamate receptor I (mGluR1) antagonism impairs cocaine-induced conditioned place preference via inhibition of protein synthesis. *Neuropsychopharmacology* **38**: 1308–1321.

Zhou FM, Wilson CJ, Dani JA (2002). Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol* **53**: 590–605.

Zucker RS, Regehr WG (2002). Short-term synaptic plasticity. *Annu Rev Physiol* **64**: 355–405.

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