Cellular/Molecular

Searching for Presynaptic NMDA Receptors in the Nucleus Accumbens

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The nucleus accumbens shell (NAc) is a key brain region mediating emotional and motivational learning. In rodent models, dynamic alterations have been observed in synaptic NMDA receptors (NMDARs) within the NAc following incentive stimuli, and some of these alterations are critical for acquiring new emotional/motivational states. NMDARs are prominent molecular devices for controlling neural plasticity and memory formation. Although synaptic NMDARs are predominately located postsynaptically, recent evidence suggests that they may also exist at presynaptic terminals and reshape excitatory synaptic transmission by regulating presynaptic glutamate release. However, it remains unknown whether presynaptic NMDARs exist in the NAc and contribute to emotional and motivational learning. In an attempt to identify presynaptically located NMDARs in the NAc, the present study uses slice electrophysiology combined with pharmacological and genetic tools to examine the physiological role of the putative presynaptic NMDARs in rats. Our results show that application of glycine, the glycine-site agonist of NMDARs, potentiated presynaptic release of glutamate at excitatory synapses on NAc neurons, whereas application of 5,7-dichlorokynurenic acid or 7-chlorokynurenic acid, the glycine-site antagonists of NMDARs, produced the opposite effect. However, these seemingly presynaptic NMDAR-mediated effects could not be prevented by application of D-APV, the glutamate-site NMDAR antagonist, and were still present in the mice in which NMDAR NR1 or NR3 subunits were genetically deleted. Thus, rather than suggesting the existence of presynaptic NMDARs, our results support the idea that an unidentified type of glycine-activated substrate may account for the presynaptic effects appearing to be mediated by NMDARs.

Introduction

The forebrain region nucleus accumbens shell (NAc) is an essential brain site for emotion- and motivation-related learning and memory (Cardinal and Everitt, 2004; Kelley, 2004). Serving as plasticity triggers, synaptic NMDA receptors (NMDARs) within the NAc critically contribute to the acquisition of new physiological and pathophysiological emotional and motivational responses (Kelley et al., 1997; Brown et al., 2011). Because of the predominant postsynaptic distribution, most NAc NMDAR-mediated behavioral alterations have been attributed to postsynaptic NMDARs. More recently, evidence from cortical and subcortical regions suggests that NMDARs may also be present at the presynaptic axonal terminals to influence presynaptic release of neurotransmitters (Berretta and Jones, 1996; Sjöström et al., 2003; Bardoni et al., 2004; Yang et al., 2006; Corlew et al., 2007, 2008; Brasier and Feldman, 2008; Larsen et al., 2011). Given the potentially distinct cellular behaviors of presynaptic and postsynaptic NMDARs (Engelman and MacDermott, 2004; Rodríguez-Moreno et al., 2010), it is important to understand whether NMDARs are also expressed presynaptically within the NAc, and if so, what roles they play in NAc-based emotional and motivational learning.

In an attempt to detect presynaptic NMDARs within the NAc, we focused on the NMDAR glycine-binding site, which has been commonly targeted pharmacologically to delineate the behavioral roles of NAc NMDARs (Goldstein et al., 1994; Tricklebank et al., 1994; Wlaź et al., 1994; Kretschmer and Schmidt, 1996; Di Ciano and Everitt, 2001; Huang et al., 2008). NMDARs are thought to exist as tetramers, usually containing obligatory glycine-binding NR1 subunits and glutamate-binding NR2 subunits (Cull-Candy and Leszkiewicz, 2004; Lau and Zukin, 2007). Whereas activation of NR1/NR2 NMDARs requires the binding of both glycine and glutamate, the glycine-site is tonically occupied by endogenous agonists in an unsaturated manner under physiological conditions (Wilcox et al., 1996; Li et al., 2009). In some NMDARs, NR2 subunits are partially or completely substituted by NR3 subunits, which, similar to NR1 subunits, have glycine-, but not glutamate-, binding sites (Chatterton et al., 2002; Low and Wee, 2010). Thus, the glycine site of NMDARs can serve as a regulatory site and a druggable target; NMDAR-mediated primary and secondary responses can be regulated bidirectionally by pharmacological manipulation of NR1- or NR3-located glycine-binding site (Jansen and Dannhardt, 2003; Javitt, 2006; Awobuluyi et al., 2007; Madry et al., 2007).

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Prior results suggest that activation of potential presynaptic NMDARs enhances synaptic transmission by facilitating presynaptic release of neurotransmitters (Berretta and Jones, 1996; Corlew et al., 2007; Brasier and Feldman, 2008; Larsen et al., 2011; but see Bardoni et al., 2004). Our current results show that application of glycine in the presence of glycine receptor antagonist strychnine increased presynaptic glutamate release at excitatory synapses on NAc neurons, whereas application of 5,7-dichlorokynurenic acid (DCKA) or 7-chlorokynurenic acid (7-Cl), the glycine-site antagonists of NMDARs, produced the opposite effect. However, these effects remain intact either in the presence of D-APV, the glutamate-site NMDAR antagonist, or in mice in which NR1 or NR3 subunits were genetically deleted. Thus, the seemingly presynaptic NMDAR-mediated effects of glycine and DCKA were likely mediated by an NMDAR-independent mechanism, possibly through an unidentified type of glycine-activated substrate. These results provide experimental evidence that may help reinterpret some of the prior results related to presynaptic NMDARs.

Materials and Methods

Animals. Male Sprague Dawley rats (Simonsen Labs) were used for most experiments. The NR3A$^{-/-}$ mice and WT controls were generated by the Nakanishi laboratory as previously described (Das et al., 1998). The NR1$^{-/-}$ mice were generated by crossing NR1-floxed mice (generated by the Tonegawa laboratory) (Tsien et al., 1996) with Nex-Cre mice (generated by the Nave laboratory) (Goebbels et al., 2006) as described previously (Ultanir et al., 2007); both types of mice were generously provided by the Ghosh laboratory. The genotypes of all experimental animals were determined by PCR. Mice that were heterozygous for NR1-floxed allele or lack of Cre gene were used as controls and referred to as WT. All protocols were approved by the Washington State University Animal Care and Use Committee.

Preparation of acute brain slices. Four- to 5-week-old rats or 3- to 4-week-old mice were decapitated following isoflurane anesthesia. Coronal slices (300 μm) containing the NAc were prepared on a vibratome (VT1200S; Leica) in 4°C cutting solution containing (in mM): 135 *N*-methyl-D-glucamine, 1 KCl, 1.2 KH_2PO_4, 0.5 $CaCl_2$, 1.5 $MgCl_2$, 20 choline-HCO_3, and 11 glucose, saturated with 95% O_2/5% CO_2, pH adjusted to 7.4 with HCl. Slices were incubated in artificial CSF (aCSF) containing (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 glucose, saturated with 95% O_2/5% CO_2 at 37°C for 30 min and then allowed to recover for at least 30 min at room temperature before experimentation.

Electrophysiological recordings. During recordings, slices were superfused with aCSF that was heated to 31–33°C by passing the solution through a feedback-controlled in-line heater (Warner Instruments) before entering the chamber. Recordings were made under visual guidance (40×, differential interference contrast optics) with electrodes (3–5 MΩ) filled with (in mM): 125 $CsCH_3O_3S$ (or CsCl for recording mIPSCs), 15 CsCl, 5 TEA-Cl, 0.4 EGTA, 20 HEPES, 2.5 Mg-ATP, 0.25 Na-GTP, 1 QX-314, pH 7.3. Picrotoxin (100 μM) (or NBQX, 10–15 μM) was included in the external perfusion aCSF to block $GABA_A$ (or AMPA) receptors. Drugs were either bath-applied (see Figs. 1, 3) or locally superfused onto the slice through a wide-bore pipette (~150 μm) placed near the recording area. In case of local perfusion, a control line containing only bath solution was included.

Excitatory afferents were stimulated by a constant-current isolated stimulator (DS3; Digitimer), using a monopolar electrode (glass pipette filled with aCSF). Stimulus strength was adjusted so that the first EPSC was between 100 and 500 pA. Trains of pulses were generated using Clampex software (Molecular Devices). In some experiments (see Fig. 3), L-glutamate (500 μM dissolved in aCSF) was applied locally through a small-tip pipette (~1 μm diameter) using a Picospritzer (Pressure System IIe, Toohey Co.); a ~5 ms pulse of 20 psi was used to eject the solution. D-APV (50 μM) was included in the puffing pipette solution as well as in the bath to block NMDARs.

For all recordings, series resistance was 8–14 MΩ and was left uncompensated. Series resistance was monitored continuously during all recordings. Cells with a change in series resistance beyond 15% were not accepted for data analysis. Synaptic currents were recorded with a MultiClamp 700A amplifier (Molecular Devices), filtered at 2.6–3 kHz, amplified 5 or 10 times, and then digitized at 20 kHz with a Digidata 1322A analog-to-digital converter (Molecular Devices).

Viral vectors. Recombinant adeno-associated vectors (rAAV) expressing venus-tagged channelrhodopsin-2 (ChR2) H134R (Makinodan et al., 1991) were pseudotyped with AAV1/2 capsid proteins. HEK293T cells were cotransfected with the plasmidspFΔ6 (adenoviral helper plasmid), pRVI (*cap* and *rep* genes for AAV serotype 2), pH21 (*cap* gene for AAV serotype 1 and *rep* gene for serotype 2) and the rAAV plasmid, using linear polyethyleneimine-assisted transfection (Kuroda et al., 2009). The helper plasmids were kindly provided by Dr. M. Schwarz (Max-Planck Institute for Medical Research, Heidelberg, Germany) (Pilpel et al., 2009). Cultures grown in DMEM (Biochrom) with 10% substituted FBS (Biochrom, #S0115) were harvested from 15 by 15 cm dishes after 48 h. rAAV were isolated and purified as described previously (Pilpel et al., 2009). Briefly, HEK293T cells were lysed with sodium deoxycholate and repeated freeze thawing cycles in the presence of Benzonase-Nuclease HC (Novagen). From the supernatant, rAAVs were isolated by iodixanol gradient centrifugation from the 40% and 54% interphase. rAAVs were then desalted by ultrafiltration and filtered through 0.2 μm Millex-GV filter units (Millipore) and stored at 4°C in 500 μl PBS with 10 mM $MgCl_2$ and 25 mM KCl.

Stereotaxic injections. Rats were anesthetized with a mixture of ketamine, xylazine, and acepromazine (0.1 ml/100 mg) and placed in a stereotaxic apparatus (Stoelting). A 26 gauge injection needle was used to bilaterally inject 1 μl (0.2 μl/min) of the AAV-ChR2-YFP solution via a Hamilton syringe into the infralimbic prefrontal cortex [anteroposterior (AP) +3.00, ML ±0.75, DV −4.00], the ventral tegmental area (AP −5.00, ML ±0.90, DV −7.65), or the basolateral amygdala (AP −2.50, ML ±4.80, DV −8.50) using a Thermo Orion M365 pump (Thermo Scientific). Injection needles were left in place for 5 min following injections. Rats were then sutured and allowed for viral expression for ~3 weeks before experimentation.

Drugs. Tetrodotoxin (TTX), DCKA, 7-Cl, D-(−)-2-Amino-5-phosphonopentanoic acid (D-APV), NBQX, bafilomycin A1 (Baf) and cyclothiazide were purchased from Tocris Bioscience. One batch of DCKA and all other chemicals were obtained from Sigma-Aldrich.

Data acquisition and statistics. All data were analyzed off-line. Miniature EPSCs (mEPSCs) were obtained at least 15 min after achieving whole-cell configuration. For analysis, a template was made by averaging ~100 hand-picked miniature events using pClamp9 software (Molecular Devices). Approximately 250–2000 miniature events over a period of 1.5–5 min were analyzed under various conditions. mEPSC decay was fitted by a single exponential to obtain the decay time constant.

For variance-mean (V-M) analysis (see Fig. 4), the variance and mean amplitude were calculated for each of the five EPSCs in the train from ~100 consecutive sweeps once stabilized. The parabola function is $y = A^*X - B^*X^2$, where y is the variance and X is the mean amplitude; A and B can thus be derived through fitting (see Fig. 4, example curves). With mathematical assumptions and simplifications, the average quantal content (Q) = A, $N = 1/B$, and the release probability (Pr) at a particular stimulus in the train: $P_i = X_i^*B/A$. Stimulus artifacts were truncated in evoked synaptic currents for clarity. Mean values are expressed ± SEM. Statistical significance was assessed using one- or two-factor ANOVA with Bonferroni post-tests, or t test as indicated.

Results

In search of presynaptic NMDARs in the NAc, we focused on the NMDAR glycine-binding site, manipulation of which bidirectionally regulates the function of potential NMDARs and produces detectable presynaptically generated alterations during synaptic transmission.

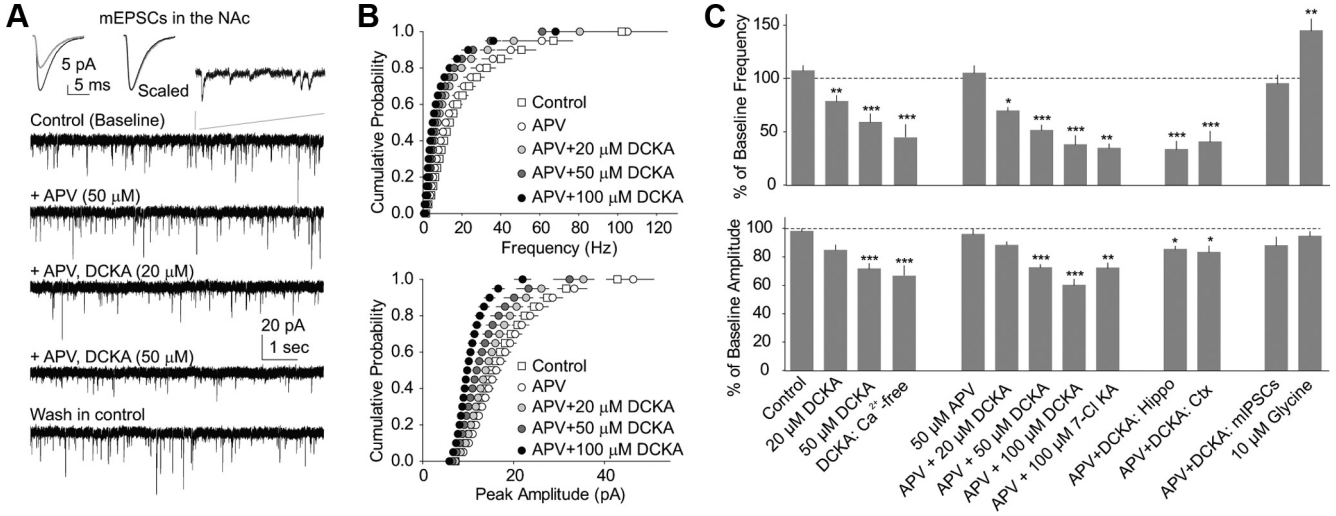

Figure 1. Miniature EPSCs are bidirectionally regulated by glycine-binding site NMDAR agonists and antagonists. ***A***, ***B***, Example traces (***A***) and summarized data in cumulative distribution plots (***B***) show that bath application of D-APV (50 μM) did not affect mEPSCs in NAc MSNs; subsequent application of DCKA in the presence of D-APV reversibly and in a dose-dependent manner decreased the frequency and amplitude of mEPSCs. DCKA was applied at three concentrations (20, 50, and 100 μM) sequentially. Left insets in ***A*** show averaged mEPSCs recorded during control (black) and perfusion of DCKA (gray), and after being scaled to the same peak amplitude. Right inset in ***A*** shows a portion of mEPSCs in an extended time scale. ***C***, Grand summary of the effects of NMDAR glycine-binding site agonists and antagonists on the frequency and amplitude of miniature events. mEPSCs and mIPSCs were recorded at the holding potential −70 mV in aCSF containing 1 μM TTX and 100 μM picrotoxin (for mEPSCs) or 10 μM NBQX (mIPSCs). Controls were obtained from ~5–15 min following stabilization. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Spontaneous glutamatergic synaptic currents are bidirectionally regulated by NMDAR glycine-site agonists and antagonists

In rat NAc medium spiny neurons (MSNs), mEPSCs were recorded under whole-cell voltage-clamp mode at −70 mV in the presence of picrotoxin (PTX, 100 μM) to block $GABA_A$ receptors, TTX (1 μM) to block action potential-dependent release events, and strychnine (1 μM) to block glycine receptors. Application of the NMDAR glycine-site competitive antagonist DCKA reversibly and in a dose-dependent manner reduced the frequency ($F_{(2,19)} = 18.34$, $p < 0.0001$, one-way ANOVA; 20 μM DCKA, 77.4 ± 6.4% of baseline, $p < 0.01$, $n = 5$; 50 μM DCKA, 59.0 ± 7.9% of baseline, $p < 0.001$, $n = 6$, Bonferroni post-test; Fig. 1*C*) and peak amplitude of mEPSCs in NAc MSNs ($F_{(2,19)} = 25.73$, $p < 0.0001$, one-way ANOVA; 20 μM DCKA, 84.5 ± 7.0% of baseline, $p = 0.118$, $n = 5$; 50 μM DCKA, 73.7 ± 3.6% of baseline, $p < 0.001$, $n = 6$, Bonferroni post-test; Fig. 1*C*). Alterations in both the frequency and amplitude of mEPSCs suggest that the effect of DCKA is mediated by both presynaptic and postsynaptic mechanisms (but see discussions below). To affirm the pharmacological effects of DCKA, DCKA from two different vendors (Tocris Bioscience and Sigma) was used and no qualitative difference was observed; nor was there difference observed whether DCKA was dissolved in water or DMSO (in the latter case DMSO was included in equal volume for control conditions). Data were thus combined. Moreover, the transmitter reuptake process or postsynaptic membrane properties do not appear to be involved as the effects of DCKA (50 μM) were not accompanied by detectable changes in either the decay kinetics of mEPSCs (decay τ: control, 5.3 ± 0.5 ms, $n = 6$; DCKA, 5.2 ± 0.5 ms, $n = 6$, $p = 0.300$, paired *t* test; Fig. 1*A* inset), the input resistance (in MΩ: control, 133.7 ± 15.2; DCKA, 132.9 ± 10.8 MΩ, $p = 0.944$, $n = 6$, paired *t* test), or the holding current (in pA: control, −251.2 ± 29.0; DCKA, −253.6 ± 28.3, $p = 0.799$, $n = 6$, paired *t* test).

Surprisingly, application of D-APV (50 μM), the NMDAR glutamate-binding site competitive antagonist, did not change either the frequency ($F_{(4,43)} = 31.65$, one-way ANOVA; $p = 1.000$, Bonferroni post-test) or amplitude ($F_{(4,43)} = 35.15$, one-way ANOVA; $p = 1.000$, Bonferroni post-test; Fig. 1) of mEPSCs. In addition, in the presence of D-APV (50–100 μM), the dose-dependent inhibitory effects of DCKA on the frequency ($p < 0.0001$, Bonferroni post-test; Fig. 1) and peak amplitude ($p < 0.0001$, Bonferroni post-test; Fig. 1) of mEPSCs remained intact. Furthermore, the effects of DCKA were mimicked by another NMDAR glycine-site antagonist 7-Cl (100 μM; frequency: 34.7 ± 4.1% of baseline, $p < 0.01$, $n = 6$; amplitude: 72.1 ± 3.6% of baseline, $p < 0.01$, $n = 6$, paired *t* tests; Fig. 1*C*). These results, although not supporting the hypothesized role of NMDARs, do not exclude this possibility either, because the glycine- and glutamate-binding sites may separately trigger distinct downstream signaling cascades (Nong et al., 2003).

Moreover, when the external Ca^{2+} was removed (bath containing 0 mM Ca^{2+}, 1 mM EGTA, and 1 μM TTX), the effects of DCKA (100 μM) on mEPSCs remained intact ($p < 0.01$, DCKA vs baseline for both frequency and amplitude, $n = 6$; Fig. 1*C*). Thus, the results so far suggest that the DCKA-induced effects in the NAc occur either in the presynaptic release or postsynaptic responsiveness, or both, via a mechanism that can be independent of extracellular Ca^{2+}.

Beyond the NAc, recordings from pyramidal neurons in the hippocampal CA1 region (Fig. 1*C*) and somatosensory cortex layer II/III (Fig. 1*C*) showed a similar decrease in the frequency (hippocampus, $p < 0.001$, $n = 6$; cortex, $p < 0.001$, $n = 4$, paired *t* test) and amplitude of mEPSCs (hippocampus, $p < 0.05$, $n = 6$; cortex, $p < 0.05$, $n = 4$, paired *t* test) in response to the application of DCKA (50 μM). In contrast, miniature IPSCs (mIPSCs) in NAc MSNs were not significantly altered by application of DCKA (100 μM) in either the frequency ($p = 0.103$, $n = 11$, paired *t* test) or peak amplitude ($p = 0.060$, $n = 11$, paired *t* test; Fig. 1*C*). Thus, the effects of DCKA appear to be specific for excitatory synapses and exist across brain regions.

Conversely, application of NMDAR glycine-site agonist glycine (10 μM; in the presence of 1 μM strychnine to block potential glycine receptors) significantly increased the frequency ($p < 0.01$, $n = 11$, paired *t* test), but not the amplitude, of mEPSCs in NAc MSNs ($p = 0.110$, $n = 11$; Fig. 1*C*). Thus far, the results

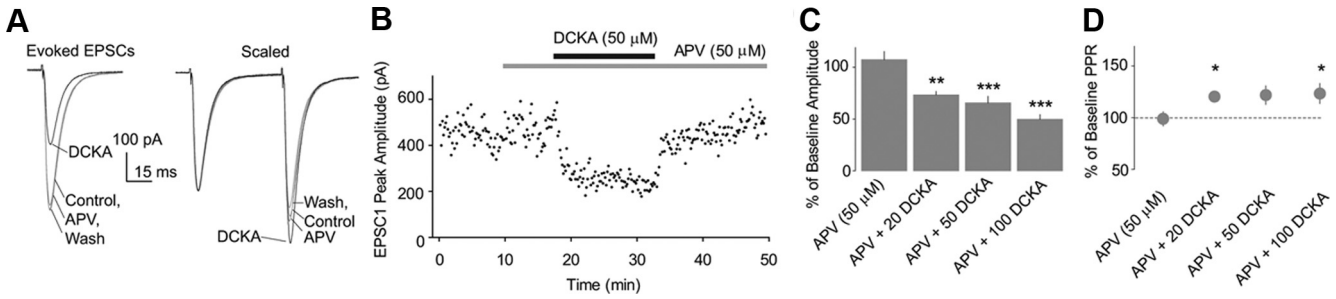

Figure 2. Application of DCKA reduces evoked EPSCs in NAc MSNs. ***A***, Evoked (left) and scaled (right) EPSCs recorded from an example NAc MSN during the periods of control, perfusion of D-APV (50 μM), coperfusion of D-APV and DCKA (50 μM), and wash-out. ***B***, The time course of DCKA-induced alterations in the peak amplitudes of first EPSCs in the example NAc neuron in ***A***. Bars indicate the duration of drug applications. ***C***, Summarized data showing the dose-dependent inhibition of EPSC peak amplitude by DCKA. ***D***, Summarized data showing increases in the PPR concurrently occurred with the DCKA-induced inhibitions of EPSCs in NAc MSNs. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

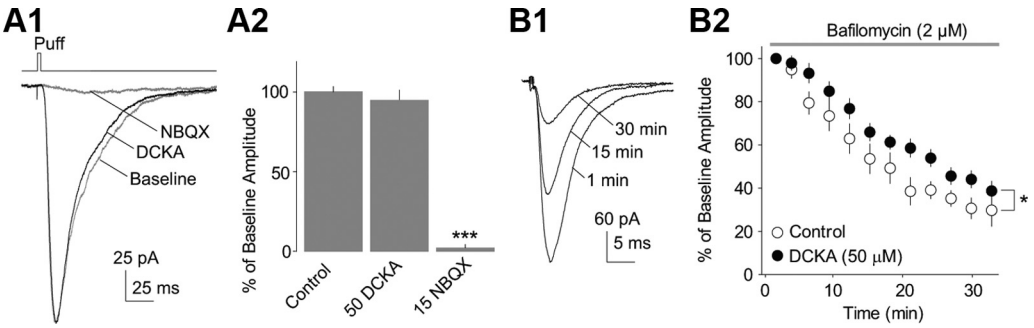

Figure 3. Presynaptic mechanisms dominate in DCKA-induced inhibition of EPSCs. ***A***, Example traces (***A1***) and summarized data (***A2***) showing that glutamate (500 μM) puffing-induced AMPAR-mediated currents were not altered by application of DCKA (50 μM). Recordings were made in the presence of D-APV (50 μM), followed by sequential applications of DCKA and NBQX (15 μM). ***B***, Example traces (from a control cell, ***B1***) and summarized data (***B2***) showing that the peak amplitude of evoked EPSCs gradually decayed in the presence of Baf (2 μM), and the decay rate was significantly slower in the presence of DCKA.

show that pharmacological manipulations that influence the glycine-binding site of NMDARs bidirectionally regulate excitatory synaptic transmission to NAc MSNs via presynaptic and likely also postsynaptic mechanisms. These effects are not dependent on glutamate binding to NMDARs, nor mediated by strychnine-sensitive glycine receptors.

DCKA reduces evoked EPSCs by a presynaptic mechanism

We next examined whether the DCKA-induced effects on spontaneous synaptic activity also occur in action potential-dependent synaptic transmission. Under whole-cell voltage-clamp mode (at −70 mV), AMPAR-mediated EPSCs in NAc MSNs were evoked by local electrical stimulations and were isolated in the presence of PTX (100 μM) and D-APV (50 μM). The evoked EPSCs in NAc MSNs were strongly and reversibly inhibited by DCKA in a dose-dependent manner ($F_{(3,25)} = 19.73$, $p < 0.0001$; Fig. 2*A–C*). Simultaneously, the paired-pulse ratio (PPR) of EPSC peaks (EPSC2/EPSC1) was increased by DCKA (at 20 μM, $p < 0.05$, $n = 5$; at 100 μM, $p < 0.05$, $n = 12$, paired *t* test; Fig. 2*A*, *D*), suggesting that presynaptic alterations contribute to the decrease in EPSCs.

Voltage control error, if prominent, also changes PPR as the peak amplitude changes; it tends to deviate PPR away from 1 as amplitude decreases. Specifically, if PPR is <1 at baseline conditions, a decrease in the peak amplitude would further decrease the PPR if the potential voltage control error is involved. However, this is not the case here because the majority of the recorded neurons that exhibited a PPR <1 showed an increase in PPR by DCKA (8 of 10 cells). Thus, the DCKA-induced increase in PPR likely reflects decreased release probability, which is consistent with its effect on the frequency of mEPSC (Fig. 1).

Presynaptic mechanisms are predominant

Although the above results suggest that both presynaptic and postsynaptic mechanisms are involved in DCKA-mediated synaptic regulation, the results are not unequivocal. For example, a decrease in the frequency of mEPSCs or an increase in the PPR may reflect presynaptic inhibition, but it can also be explained by desensitization of postsynaptic AMPARs (Heine et al., 2008). Meanwhile, a decrease in the amplitude of mEPSCs that is normally interpreted as a decrease in postsynaptic responsiveness can also be explained by a decrease in the quantal content during presynaptic release (Silver, 2003). To better evaluate the presynaptic and postsynaptic contributions to DCKA-mediated effects, we took two additional approaches, which are relatively more specific in detecting presynaptic and postsynaptic alterations, respectively.

To isolate postsynaptic responsiveness, we evoked postsynaptic AMPAR-mediated current by brief local pressure application of glutamate through a puffing pipette directed at the dendritic region of an MSN, thus by-passing presynaptic glutamate release. Puffing glutamate (500 μM) to the dendritic area of NAc MSNs (in the presence of 50 μM D-APV and 100 μM PTX; membrane held at −70 mV) elicited AMPAR-mediated currents, verified as the near-complete inhibition by NBQX (15 μM; 3.0 ± 0.4% of baseline, $p < 0.001$, $n = 7$; Fig. 3*A*), an AMPAR-selective antagonist. Since NBQX was bath-applied, the almost complete inhibition also indicates that bath-applied drugs have sufficient

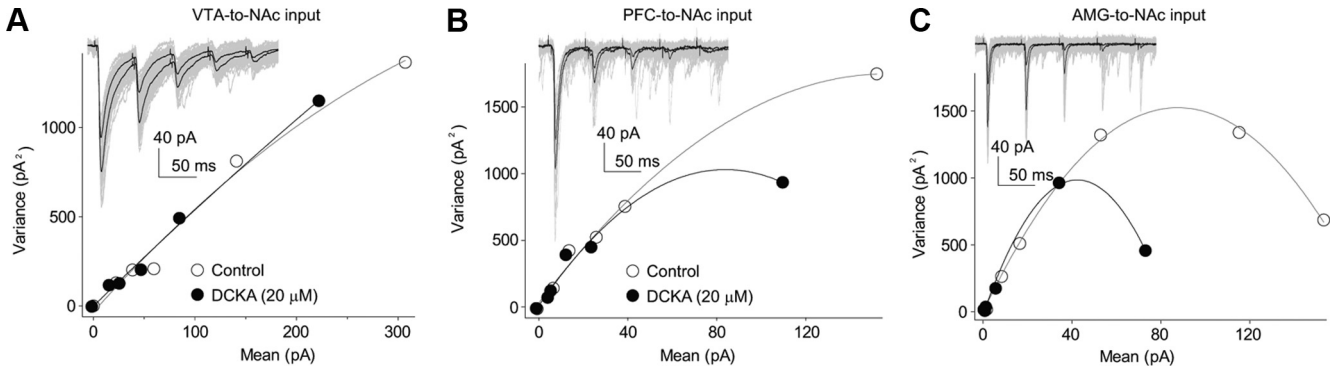

Figure 4. Variance-mean analysis reveals presynaptic effects of DCKA. ***A–C***, Variance-mean plots of example EPSCs recorded from synapses within the VTA-to-NAc (***A***), PFC-to-NAc (***B***), and BLA-to-NAc (***C***) pathways. Insets showing individual (gray) and averaged (black) EPSCs evoked by a repeated train (5 pulses at 20 Hz) generated by channelrhodopsin-based light stimulation.

access to puff-activated AMPARs. Nonetheless, bath application of DCKA (50 μM) did not significantly affect the peak amplitude of puff-evoked currents, albeit a trend of inhibition was observed (90.4 ± 3.7% of baseline peak amplitude, $p = 0.078$, $n = 7$; Fig. 3*A*). This result suggests that the effect of DCKA at the concentrations of 50 μM or lower is not predominantly mediated by direct inhibition of postsynaptic AMPARs. One interpretational caveat is that the nonsynaptically located AMPARs, which can also be activated by puffed glutamate but are insensitive to DCKA, dilute the potential synaptic AMPAR-specific changes. This is not highly likely; given the preferential synaptic distribution of AMPARs (Zamanillo et al., 1999), such a robust reduction (~50%; Fig. 2*A–C*) of synaptic transmission by DCKA, if mediated by postsynaptic AMPARs, should be reflected in the puff-induced current.

We then used presynaptically oriented approaches to examine the effect of DCKA. One potential mechanism is that application of DCKA decreases the Pr of glutamate from presynaptic terminals. In addition to Pr, we also intended to examine the *Q* of presynaptic glutamate vesicles because 7-Cl, an analog of DCKA, has been shown to inhibit presynaptic vesicular glutamate transporters (Bartlett et al., 1998). The first experiment took advantage of the pharmacological properties of Baf, which inhibits vacuolar-type proton pump (V-ATPase) and thus blocks glutamate uptake into synaptic vesicles. In the presence of Baf, neurotransmitter vesicles cannot be refilled once released, and therefore spontaneous or evoked EPSCs would gradually diminish as the transmitter-filled vesicles are consumed during transmission. In the presence of Baf, the time course of decrease in EPSCs depends on the Pr, but not the *Q*. More specifically, synapses with a decreased Pr should exhibit a prolonged diminishing time course, and vice versa. In contrast, synapses with different *Q* but similar Pr should exhibit a similar diminishing time course. As shown in Figure 3*B*, in the presence of Baf (2 μM), the peak amplitude of evoked EPSCs in MSNs gradually diminished over a period of 30 min (aCSF containing 4 mM K^+ was used to accelerate vesicular release). Application of DCKA (50 μM) prolonged the diminishing time course of EPSCs ($F_{(1,78)} = 6.41$, $p < 0.05$, two-way ANOVA; Fig. 3*B*), suggesting a decrease in the Pr by DCKA.

To quantify the DCKA-induced presynaptic alterations, we focused on three key presynaptic quantal parameters, the Pr, *Q*, and number of release sites (*N*), by performing the nonstationary V-M analysis (Clements and Silver, 2000; Silver, 2003; Saviane and Silver, 2007). Briefly, the V-M plot is generated by having the same set of synapses experience different release conditions so as to obtain the mean peak amplitude and the variance of the amplitudes under each condition (Meyer et al., 2001; Scheuss and Neher, 2001; Scheuss et al., 2002). This analysis is feasible only if the different presynaptic release sites behave uniformly as they go through different release conditions. However, in NAc glutamatergic synapses within different afferents exhibit different Prs (Pr = 0.18 ± 0.04, 0.57 ± 0.05, and 0.70 ± 0.03 in VTA, PFC, and amygdala afferents, respectively; see below; Fig. 4), likely resulting in distorted V-M plot. To overcome this, we dissected three glutamatergic afferents, the afferent from the infralimbic PFC, the afferent from the VTA, and the afferent from BLA, by confined expression of channel (ChR2) (Boyden et al., 2005) within each of these three brain regions (see Materials and Methods). The NAc slices were then prepared, with only presynaptic input from the intended brain region expressing ChR2. Thus, a light (at 473 nm) stimulation selectively activated the intended presynaptic input to NAc MSNs.

With this approach, we applied a 5-pulse train (20 Hz) of presynaptic stimuli to induce EPSCs in NAc MSNs (Fig. 4). The train of stimuli presumably activates the recorded synapses repetitively and consistently through different states of release probability, thus, allowing the V-M analysis (Meyer et al., 2001; Scheuss and Neher, 2001; Scheuss et al., 2002). EPSC V-M plot was generated by calculating the mean EPSC amplitude and variance of EPSC amplitudes under each release condition. The V-M plot was then fitted by a parabola function and interpreted assuming binomial statistics of transmitter release (Quastel, 1997; Silver, 2003). To eliminate AMPAR desensitization, which may occur during repeated stimulations and distort the V-M plot (Quastel, 1997; Silver, 2003), cyclothiazide (100 μM) was included in the bath in ~50% of the recordings and results were combined. With this approach, we observed that application of DCKA (20 μM) decreased the Pr and/or *N* of excitatory synapses, but not Q. Specifically, synapses within the VTA projection exhibited a relatively low Pr; application of DCKA decreased the Pr (Pr of EPSC1: control, 0.18 ± 0.04; DCKA, 0.16 ± 0.03; $p < 0.05$, $n = 6$, paired *t* test) and *N* (control, 289 ± 37; DCKA, 205 ± 33; $p < 0.05$, $n = 6$, paired *t* test; Fig. 4***A***). Synapses within the PFC projection exhibited a medium Pr; application of DCKA did not significantly affect the Pr (Pr of EPSC1: control, 0.57 ± 0.05; DCKA, 0.49 ± 0.12; $p = 0.500$, $n = 6$, paired *t* test) but decreased the *N* (control, 15 ± 7; DCKA, 12 ± 7; $p < 0.05$, $n = 5$, paired *t* test; Fig. 4***B***). Synapses within the BLA projection exhibited a relatively high Pr; application of DCKA decreased both the Pr (Pr of EPSC1: control, 0.70 ± 0.03; DCKA, 0.64 ± 0.04; $p < 0.01$, $n = 12$, paired *t* test) and *N* (control, 20 ± 4; DCKA, 15 ± 4; $p < 0.01$,

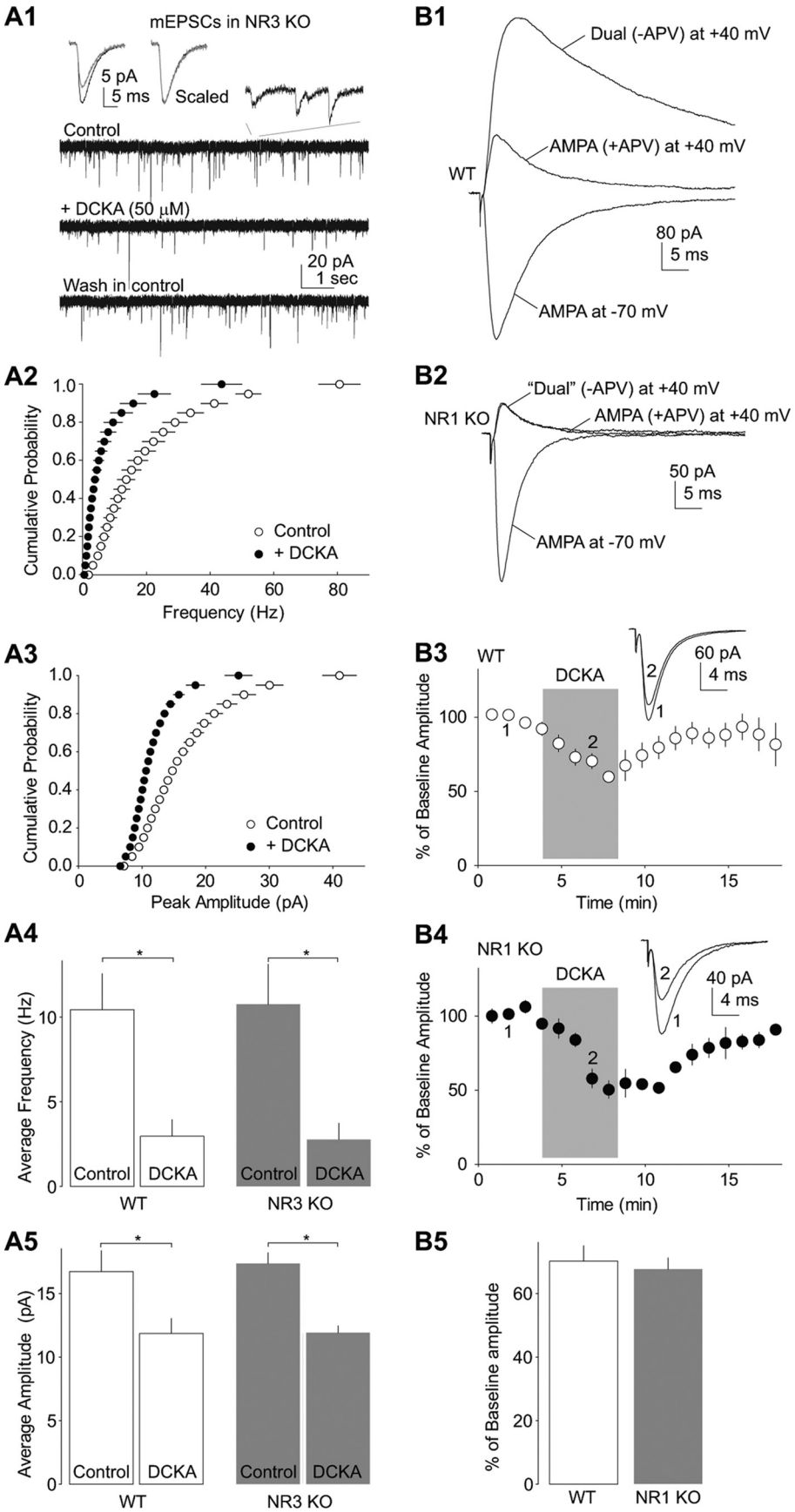

Figure 5. DCKA-induced synaptic inhibition is intact in NR1- or NR3-absent synapses. ***A***, DCKA-induced inhibition of EPSCs in NAc MSNs was intact in NR3 KO mice. Example traces (***A1***) and summarized data in cumulative distribution plots (***A2*** and ***A3***) show that both the frequency and amplitude of mEPSCs in NAc MSNs in NR3 KO mice were decreased by (*Figure legend continues.*)

$n = 12$, paired t test; Fig. 4***C***). A decrease in either Pr or N suggests a presynaptic inhibition. In contrast to the Pr and N, the Q of synapses was not affected by application of DCKA in any of the examined afferent (in pA: VTA: control, 5.1 ± 0.3; DCKA, 5.4 ± 0.4; $p = 0.193$, $n = 6$; PFC: control, 20.7 ± 1.1; DCKA, 20.8 ± 2.8, $p = 0.964$, $n = 5$; BLA: control, 16.6 ± 3.6; DCKA, 18.5 ± 3.6; $p = 0.105$, $n = 12$; paired t test; Fig. 4). In the V-M analysis, the Q is measured as the postsynaptic response (in pA) induced by all glutamate released from a single vesicle (quantum). As such, the lack of change in the Q suggests no change in the vesicular content and postsynaptic AMPARs following application of DCKA. Note that the two effects of DCKA, namely the lack of change in Q and the decrease in the amplitude of mEPSCs, are at odds in terms of presynaptic or postsynaptic interpretation of the DCKA effects (see Discussion). Also note that the relatively small Q in the VTA afferent can be interpreted as smaller vesicular content, weaker postsynaptic AMPARs, or, more likely, relatively distally located synapses.

The effects of DCKA remain intact in NR3A or NR1 KO synapses

The above results suggest that the effects of DCKA and glycine are mediated through the glycine-binding site of NMDARs that are potentially located presynaptically. In an attempt to specify the subtypes of the mediating NMDARs, we first focused on NR3-containing NMDARs. NR3-containing NMDAR is a likely candidate because it has been recently shown to promote neurotransmission by presynaptic mechanisms (Larsen et al., 2011). Furthermore, NR3-containing NMDARs are activated upon agonist binding to their glycine-binding sites without the involvement of glutamate (Chatterton et al., 2002; Low and Wee, 2010). This property is also consistent with our observation that application of the competitive glutamate-binding site antagonists did not prevent the effects of DCKA or 7-Cl (Fig. 1*C*). To test this, we used NR3A KO mice and examined whether the established DCKA-mediated effects on mEPSCs of NAc MSNs were affected. Our results show that the inhibitory effects of DCKA (50 μM) were similar between the KO and WT mice (% of baseline in DCKA: frequency: WT, 27.5 ± 5.2, $n = 5$; KO, 21.4 ± 3.2, $p = 0.221$; $n = 8$; amplitude: WT, 71.3 ± 3.1, $n = 5$; KO, 66.1 ± 4.4, $p = 0.197$, $n = 8$, t test; Fig. 5*A*). These results suggest that NR3A-containing NMDA receptors are not involved in DCKA-induced inhibition of excitatory synaptic transmission to NAc MSNs.

We then attempted to determine whether presynaptic NMDARs are involved in the effects of DCKA in any manner. One way to do this is to deplete all NMDARs and examine whether this manipulation abolishes the effect of DCKA. In addition to bearing glycine-binding sites, NR1 subunits are also obligatory subunits for NMDARs. As such, genetically knocking out NR1 subunits should delete all functional NMDARs at excitatory synapses. Because NR1 KO mice are not viable (Forrest et al., 1994), we used cortical NR1-KO mice instead, in which cortical pyramidal neurons do not express NR1 subunits (Ultanir et al., 2007). In this mouse line, all four transmembrane domains and the C-terminal tail of NR1 subunit are removed; all eight known splice variants of NR1 are expected to be deleted simultaneously (Tsien et al., 1996).

We first confirmed the knock-out efficiency of NR1 in cortical neurons of this mouse line. At postnatal day 21, NMDAR EPSCs were absent in somatosensory cortical layer II/III pyramidal neurons (Fig. 5*B*), suggesting a complete deletion of NR1-containing, and thus likely all types of, NMDARs within cortical pyramidal neurons. Because layer II/III pyramidal neurons within the sensory cortex receive glutamate innervations almost exclusively from within the cortex (Schubert et al., 2007), monosynaptic EPSCs recorded by strictly confining the presynaptic stimulation and postsynaptic recording within layer II/III should be generated from excitatory synapses with no NMDARs on either presynaptic or postsynaptic membranes. Nonetheless, these excitatory synapses with presumably no presynaptic NMDARs were still sensitive to DCKA-induced suppression. In NR1 KO and WT mice, application of DCKA (20 μM) reduced the peak amplitude of evoked AMPAR EPSCs in layer II/III cortical neurons to a similar extent (% of baseline in DCKA: WT, 72.5 ± 5.1, $n = 5$; KO, 67.1 ± 3.2, $n = 10$, $p = 0.208$, t test; Fig. 5*B*). These quantitatively similar effects of DCKA between KO and WT mice suggest that presynaptic and postsynaptic NMDARs are not the mediators of DCKA-induced synaptic inhibition.

Glycine transporters and receptors?

If not NMDARs, DCKA must interact with other molecular substrates to achieve its synaptic effects. Presynaptically located glycine transporters, if inhibited, may decrease excitatory synaptic transmission (Raiteri et al., 2005). However, application of ALX 5407 (2 μM), the selective type I glycine transporter antagonist, did not change the frequency ($F_{(2,18)} = 13.34$; $p = 0.845$, Bonferroni post-test) or amplitude ($F_{(2,18)} = 3.608$; $p = 0.189$, Bonferroni post-test) of mEPSC in NAc MSNs. In the presence of ALX 5407 (2 μM), the inhibitory effects of DCKA (20 μM) on mEPSCs remained (frequency: 73.6 ± 2.4% of baseline, $p < 0.001$, Bonferroni post-test; amplitude: 91.5 ± 2.8% of baseline, $p = 1.000$, Bonferroni post-test; Fig. 6*A*,*E*,*F*). Thus, glycine transporter I is not likely the mediator of the effects of DCKA.

Another potential neuronal substrate for DCKA is the glycine receptor, which also contains the glycine-binding site and exists at presynaptic terminals (Turecek and Trussell, 2001; Kawa, 2003; Lee et al., 2009). Our results show that the effects of DCKA remained intact in the presence of the glycine receptor-selective antagonist strychnine (Fig. 1). Whereas most known glycine receptors are selectively inhibited by strychnine, strychnine-resistant glycine receptors do exist (Han et al., 1997). To explore the potential glycine receptors in DCKA-mediated effects, we took advantage of the pharmacological properties of glycine receptors.

First, D-serine is the endogenous glycine-site agonist of NMDARs. Our prior study shows that bath application of D-serine at 10 μM is sufficient to enhance NMDAR-mediated EPSCs in the slice preparation (Huang et al., 2008). However, D-serine does not interact with all known glycine receptors. Application of D-serine (10 μM) or depletion of D-serine in slices by D-amino acid oxidase (DAAO, 150 μg/ml, bath application for 30 min) did not significantly alter either the frequency ($F_{(3,37)} = 0.2113$, $p = 0.8879$, one-way ANOVA) or amplitude ($F_{(3,37)} = 2.579$, $p = 0.0683$) of mEPSCs in NAc MSNs (Fig. 6*B*,*E*,*F*; D-APV

←

(*Figure legend continued.*) bath-application of 50 μM DCKA to a degree similar to that in WT mice (***A4*** and ***A5***). Left insets in ***A1*** show averaged mEPSCs recorded during control (black) and perfusion of DCKA (gray), and after being scaled to the same peak amplitude. Right inset in ***A1*** shows a portion of mEPSCs in an extended time scale. ***B1***, ***B2***, Example evoked EPSCs from a somatosensory cortical layer II/III pyramidal neuron recorded at −70 and +40 mV from a WT (***B1***) or NR1 KO (***B2***) mouse. Note the absence of APV (50 μM)-sensitive component in the KO mouse. ***B3***, ***B4***, Summarized data showing the normalized peak amplitude of EPSCs was reversibly inhibited by application of DCKA (20 μM) in WT (***B3***) and NR1 KO (***B4***) mice. Shades indicate the duration of drug applications. ***B5***, Summarized data showing that DCKA-induced inhibition of EPSCs was similar between NR1-containing and NR1-absent synapses. *$p < 0.05$.

was not included in the bath). This result suggests that (1) the glycine-binding site compounds that do not affect glycine receptors do not significantly regulate mEPSCs, and (2) activating the glycine-sites of NMDARs by enhancing D-serine tone in the brain slice does not alter (enhance) mEPSCs in NAc MSNs.

Second, glycine is the endogenous glycine-site agonist for both NMDARs and glycine receptors. Application of glycine significantly increased the frequency of mEPSCs in NAc MSNs in the presence of D-APV (50 μM) and strychnine (1 μM) ($p < 0.05$, $n = 6$; no change in amplitude, $p = 0.468$, $n = 6$; t test; Fig. 6*C*,*E*,*F*). Furthermore, although most known glycine receptors do not exhibit an affinity to DCKA (Popik et al., 1995; Han et al., 2004), application of DCKA (50 μM) prevented the enhancing effect of glycine on mEPSCs (frequency, $p = 0.496$, $n = 8$; amplitude, $p = 0.471$, $n = 8$, One-way ANOVA, Fig. 6*D–F*), Thus, if NMDARs, glycine transporters, and strychnine-sensitive glycine receptors are all excluded, this glycine-mediated enhancing effect can be explained to be mediated by an unidentified glycine-binding substrate; it is sensitive to DCKA and glycine, but not D-serine or strychnine.

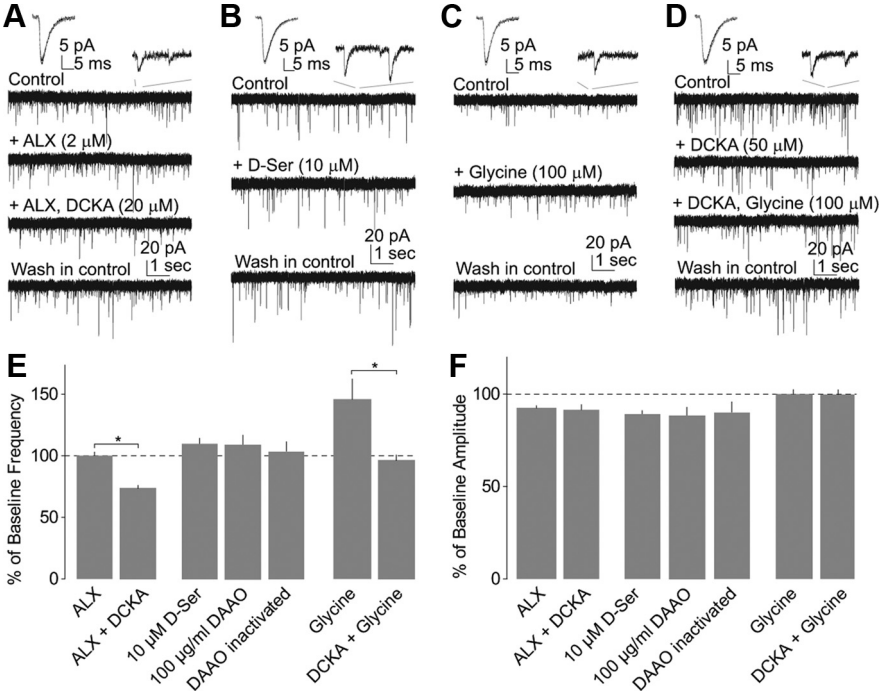

Figure 6. An unidentified glycine-binding substrate regulates excitatory synaptic transmission. ***A***, Example mEPSCs from a NAc MSN in the periods of control, perfusion of glycine transporter 1 antagonist ALX 5407 (ALX, 2 μM), coperfusion of ALX (2 μM) and DCKA (20 μM), and wash-out. ***B***, Example mEPSCs from a NAc MSN in the periods of control, perfusion of D-serine (10 μM), and wash-out. ***C***, Example mEPSCs from a NAc MSN in the periods of control (containing 50 μM D-APV), perfusion of glycine (100 μM), and wash-out. ***D***, Example mEPSCs from a NAc MSN in the periods of control, perfusion of DCKA (50 μM), coperfusion of DCKA and glycine (100 μM), and wash-out. ***E***, Summarized data showing that application of ALX did not prevent DCKA-induced inhibition of the frequency of mEPSCs, application of D-serine did not affect mEPSCs, and application of DCKA prevented the glycine-induced increase in the frequency of mEPSCs in NAc MSNs. ***F***, Summarized data showing that none of the above manipulations significantly altered the amplitude of mEPSCs in NAc MSNs. *$p < 0.05$.

Discussion

Using an approach combining electrophysiological, pharmacological, and genetic tools, the present study was originally set to define potential presynaptic NMDARs in the NAc. However, our results suggest that the seemingly presynaptic NMDAR-mediated effects were indeed mediated by an NMDAR-independent mechanism, possibly through unidentified, strychnine-resistant glycine-activated substrates. These results may help reinterpret some of the previous results related to presynaptic NMDARs.

Presynaptic versus postsynaptic mechanisms

An alteration in presynaptic release of neurotransmitters is often associated with alterations in the frequency of mEPSCs. Application of DCKA exerted a consistent inhibitory effect on the frequency of mEPSCs in neurons from three brain regions (Fig. 1). Furthermore, the presynaptic parameters, Pr or *N*, of excitatory synapses were also decreased by DCKA within the three examined afferents (Fig. 4). These observations suggest the involvement of presynaptic alterations in DCKA-mediated synaptic inhibition. Whereas the Pr and *N* are two relatively independent parameters, they also reciprocally influence each other in the V-M analysis. An extreme example is that when the Pr at some release sites decreases to 0 (thus these sites become inactive), the total *N* will decrease. In this case, if the Pr at active release sites is not altered, the total Pr will remain the same because the V-M analysis only measures active release sites to calculate the Pr. Nonetheless, although synapses within three glutamatergic afferents are inhibited by DCKA presynaptically, the inhibition exhibits distinct presynaptic patterns as the P and *N* were affected differentially (Fig. 4).

In addition to the frequency, application of DCKA also significantly decreased the amplitude of mEPSCs in NAc MSNs (Fig. 1), suggesting the involvement of postsynaptic mechanisms. This interpretation is at odds with the results from the V-M analysis, which show that application of DCKA did not affect the *Q* (Fig. 4). Specifically, a decrease in the amplitude of mESPC suggests reduced vesicular content of glutamate or reduced number/function of postsynaptic AMPARs, either of which should be reflected by a reduction in the *Q*. However, the *Q* was not affected by DCKA within the three examined afferents (Fig. 4). One possibility to reconcile these results is that DCKA-induced reduction in *Q* occurs not in the VTA-, PFC-, and BLA-to-NAc pathways, but in other glutamate afferents. A second possibility is that DCKA does not affect the *Q*, but preferentially decreases the Pr of synapses with high *Q* values. Thus, if the frequency of mEPSCs with large amplitudes is preferentially decreased by DCKA, it would result in a decrease in mean amplitude at the overall level. In the NAc, MSNs receive glutamatergic synaptic inputs from a variety of brain regions, and very often, these synaptic inputs converge at the single MSN level (O'Donnell and Grace, 1995; Finch, 1996; French and Totterdell, 2002, 2003). These glutamatergic inputs are thought to carry different emotional and motivational information and differentially influence the overall functional output of NAc MSNs (Kalivas and McFarland, 2003; Lüscher and Malenka, 2011). The sizes of presynaptic vesicles, and thus likely *Q*, at synapses from different pathways to NAc MSNs are substantially different (French and Totterdell, 2004). Thus, the *Q*, depending on its "size," may influence the regulation susceptibility of different excitatory inputs to the NAc. A

third possibility is that application of DCKA reduces basal multivesicular release. Multivesicular release may preferentially occur at high Pr conditions, and if reduced, the variance of EPSC amplitudes will also be reduced, and predictably with a more robust reduction in EPSCs within larger amplitudes (Fig. 4). If multivesicular release also occurs in mEPSCs (Wall and Usowicz, 1998), suppression of multivesicular release would yield a reduction in the amplitude of mEPSCs. It is not clear, however, how prevalent multivesicular release is in NAc MSNs, although it is the case for dorsal striatal MSNs upon single presynaptic stimulations in young rats (postnatal 15–18 d) (Higley et al., 2009). Nonetheless, these three possibilities suggest that the seemingly postsynaptic effects of DCKA (i.e., changes in the amplitude of mEPSCs) can be explained by presynaptic mechanisms.

It is noteworthy that our results cannot definitively rule out postsynaptic mechanisms. If the function of postsynaptic AMPARs is decreased by DCKA, the amplitude of mEPSCs will be decreased, and because of this decrease, some mEPSCs may become undetectable, resulting in a decrease in the frequency of mEPSCs. Thus, some of the seemingly presynaptic mechanisms can also be explained by postsynaptic mechanisms. However, postsynaptic mechanisms cannot readily explain several apparent presynaptically oriented alterations induced by DCKA, including the change in the Pr, the lack of change in *Q*, the decreased blockade time course of EPSCs by Baf, the change in the PPR of evoked EPSCs, and the lack of change in puff-induced AMPAR-mediated currents. Moreover, in the above presented NAc MSNs (Fig. 1), 3 of 18 neurons exhibited negligible DCKA-induced inhibition in mEPSC amplitude (by 3.7 $\pm$ 0.2%), but these neurons exhibited the same degree of decrease in mEPSC frequency as the population average (by 26.9 $\pm$ 2.9%). Thus, although postsynaptic mechanisms are possibly involved, presynaptic mechanisms likely predominate in DCKA-induced inhibition of excitatory synaptic transmission.

Molecular substrates for DCKA

It has been well documented that both DCKA and 7-Cl are highly selective for the glycine-binding site of NMDARs (Kemp et al., 1988; Baron et al., 1990; Leeson et al., 1991; Mugnaini et al., 1998). Using two transgenic mouse lines (Fig. 5), our experiments largely rule out presynaptic NMDARs in DCKA-induced inhibition of excitatory synaptic transmission. Rather, our results raise the possibility that DCKA and 7-Cl may also have other binding partners in the brain, possibly glycine-binding substrates.

Glycine receptor-mediated regulation of synaptic transmission has been reported at mammalian synapses (Turecek and Trussell, 2001; Kawa, 2003; Lee et al., 2009). For these characterized regulations, the involved glycine receptors are sensitive to strychnine, and activation of these receptors facilitates presynaptic release by inducing depolarization of presynaptic terminals, which, in turn, promotes Ca^{2+} entry through voltage-gated calcium channels (Turecek and Trussell, 2001; Kawa, 2003; Lee et al., 2009). However, these established typical glycine receptors are not likely the potential glycine/DCKA-bound substrates or receptors insinuated in the current study, because the latter ones were DCKA-sensitive but strychnine-resistant, and could function independent of voltage-gated sodium or calcium channels (Fig. 1). Thus, if these glycine/DCKA-bound substrates are glycine receptors, they are atypical receptors with pharmacological properties distinct from those identified previously; they appear tonically and partially active, allowing bidirectional regulations (by DCKA and glycine).

The atypical glycine receptor-oriented scenario is supported by several recent studies. In the salamander retina and HEK cells expressing glycine receptor subunits, application of DCKA inhibits glycine-induced currents in a strychnine-resistant manner (Han et al., 1997, 2004). Thus, the putative glycine receptors that mediate glycine-induced current are atypical as typical glycine receptors are strychnine-sensitive and DCKA-resistant (Han et al., 1997, 2004). Furthermore, in a screening test, the rat cortical tissues are shown to exhibit two clearly dissociable binding sites for either DCKA or 7-Cl; the high affinity site is identified as the glycine-binding site of NMDARs, whereas the low, micromolar-affinity site has not been specified (Mugnaini et al., 1998). This low-affinity site can be atypical glycine receptors suggested by Han et al. (1997, 2004) or other unidentified glycine-binding substrates. In the present study, although DCKA and 7-Cl at 20–100 μM (Kemp et al., 1988; Frankiewicz et al., 2000; Barria and Malinow, 2002; Krasteniakov et al., 2005) would primarily target the high affinity site, partial occupancy of the low affinity site is also possible. Thus, atypical glycine receptors are possible substrates that mediate the observed effect of DCKA. As such, a potential future study would be to identify whether these putative atypical glycine receptors are expressed in the NAc, hippocampus, cortex, and other related brain regions, and if so, whether they are located presynaptically.

Impact of the current findings

Pharmacological activation or inactivation of the glycine site of NMDARs becomes a common means to manipulate NMDAR function in vitro and in vivo. A PubMed search of "glycine site NMDA receptor" reaches >1500 hits (as of 2011), among which ~1/3 involve using DCKA, 7-Cl, glycine, or strychnine in physiological, pharmacological, or behavioral experiments. Our current results indicate that in addition to NMDARs, these glycine-site tools may also interact with unidentified molecular substrates to regulate synaptic transmission in a manner similar to what is mediated by manipulating NMDARs. These findings provide an empirical base to reconsider related prior results with a new, NMDAR-independent interpretation. Furthermore, our results suggest the existence of a putative strychnine-resistant glycine-binding substrate, possibly a novel class of glycine receptors. This putative receptor, if identified, would introduce a new mechanism in regulating excitatory synaptic transmission.

Notes

Supplemental material for this article is available at http://www.vetmed.wsu.edu/research_vcapp/DongLab/publications/supplemental.aspx. Supplemental Materials: Additional data related to Figure 1 in Huang et al., Searching for Presynaptic NMDA Receptor in the Nucleus Accumbens. This material has not been peer reviewed.

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