

# Activation of NR2A-Containing NMDA Receptors Is Not Obligatory for NMDA Receptor-Dependent Long-Term Potentiation

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Activation of NMDA receptors (NMDARs) within the CNS represents a major signal for persistent alterations in glutamatergic signaling, such as long-term potentiation (LTP) and long-term depression. NMDARs are composed of a combination of NR1 and NR2 subunits, with distinct NR2 subunits imparting distinct characteristics on the receptor. One particular NR2 subunit, NR2A (NR $\epsilon$ 1), has been proposed to play an integral role in LTP induction in the hippocampus and cortex. Here, we report studies investigating the role of NR2A in LTP induction in the dorsolateral bed nucleus of the stria terminalis (dlBNST). The putative NR2A-specific inhibitor NVP-AAM077 (AAM077) has been used previously to demonstrate the dependence of cortical and hippocampal LTP on NMDARs containing NR2A subunits. We report here the same sensitivity of LTP to pretreatment with AAM077 (0.4  $\mu$ M) in the dlBNST. However, inconsistent with the conclusion that LTP in the dlBNST is NR2A dependent, we see intact LTP in the dlBNST of NR2A knock-out mice. Because we also see blockade of this dlBNST LTP in NR2A knock-out mice after pretreatment with AAM077, we conclude that the antagonist is targeting non-NR2A subunit-containing receptors. Using a variety of cultured cell types, we find that AAM077 (0.4  $\mu$ M) can attenuate transmission of NR2B subunit-containing NMDARs when preapplied rather than coapplied with an agonist. Therefore, we conclude that NR2A is not obligatory for the induction of LTP in the dlBNST. Furthermore, our data demonstrate that care must be exercised in the interpretation of data generated with AAM077 when the compound is applied before an agonist.

**Key words:** glutamate; synaptic transmission; synaptic plasticity; NMDA receptor; ion channels; long-term potentiation

## Introduction

We reported previously that NMDA receptor (NMDAR)-dependent long-term potentiation (LTP) can be induced through high-frequency stimulation in the dorsolateral portion of the bed nucleus of the stria terminalis (dlBNST), a region thought to be important in stress/anxiety regulation of reward/addiction-related behaviors (Weitlauf et al., 2004). Recent evidence suggests that NMDAR-dependent LTP is specifically dependent on NMDARs containing the NR2A (NR $\epsilon$ 1) subunit. Although a deficiency in LTP has been reported in the hippocampus of NR2A knock-out mice (Sakimura et al., 1995), the primary evidence for the subunit specificity of LTP has been the sensitivity of hippocampal and cortical LTP to inhibition by an NR2A-

selective antagonist, NVP-AAM077 (AAM077; 0.1–0.5  $\mu$ M) (Liu et al., 2004; Mallon et al., 2004; Massey et al., 2004).

We report here that bath application of AAM077 (0.4  $\mu$ M) also attenuates NMDAR-dependent LTP in the dlBNST. However, inconsistent with the conclusion that this LTP is NR2A subunit dependent, we find that LTP in the dlBNST of NR2A knock-out mice is unchanged compared with wild-type littermates. This finding led us to more closely examine the specificity of AAM077 for NR2A subunit-containing NMDAR-mediated transmission in a variety of cell types. We report here that, much like other NR2-specific inhibitors, AAM077 produces time-dependent antagonism such that specificity for NR2A subunit-containing NMDARs is lost under conditions of prolonged exposure.

## Materials and Methods

**Field potential recordings.** Male C57BL/6J or NR2A knock-out mice (6–8 weeks of age; The Jackson Laboratory, Bar Harbor, ME) were decapitated under isoflurane, and their brains were removed quickly and placed in ice-cold artificial CSF [ACSF; in mM: 124 NaCl, 4.4 KCl, 2.0 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>, 1 NaH<sub>2</sub>PO<sub>4</sub>, 10 glucose, and 26 NaHCO<sub>3</sub>, pH 7.2–7.4, 290–310 mOsm]. Hemisected coronal slices (300  $\mu$ m) were prepared with a Pelco (Redding, CA) vibratome.

Slices containing anterior portions of the dlBNST (bregma, 0.26–0.02

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mm) (Franklin and Paxinos, 1997) were selected using the internal capsule, anterior commissure, and stria terminalis as landmarks. After dissection, slices were transferred to an interface recording chamber in which they were perfused with heated ( $\sim 29^{\circ}\text{C}$ ), oxygenated (95%  $\text{O}_2$ –5%  $\text{CO}_2$ ) ACSF at a rate of  $\sim 2$  ml/min. Slices were allowed to equilibrate in ACSF for at least 1.5 h before experiments began. A bipolar stainless steel stimulating electrode and a borosilicate glass recording electrode filled with ACSF were placed in the dBNST to elicit and record an extracellular field response. Baseline responses to a stimulus (50  $\mu\text{s}$ ) at an intensity that produced  $\sim 40\%$  of the maximum response were recorded for at least 20 min at a rate of 0.05 Hz. AAM077 (Novartis Laboratories, Basel, Switzerland) was bath applied where indicated. To elicit LTP, two trains of 100 Hz, 1 s tetanus were delivered with a 20 s intertrain interval at the same intensity as baseline test pulses.

**Hippocampal neuron and human embryonic kidney cell culture and transfection.** The culture protocol for hippocampal neurons was as described by Bekkers and Stevens (1991) and Chen and Lambert (2000), with minor modifications. A 35 mm culture dish was coated with 0.15% agarose (A-9918; Sigma, St. Louis, MO) and air dried. For microisland culture, a mixture of poly-D-lysine (P-6407; Sigma) at 0.4 mg/ml and collagen (354236; BD Biosciences, Bedford, MA) at 0.8 mg/ml was sprayed on the agarose-coated dish to make microdots. Culture dishes used for dispersed hippocampal primary cultures were not coated with agarose but instead were coated over their entire surface with 0.1 mg/ml poly-D-lysine. Neuronal cultures were prepared using the hippocampus taken from postnatal day 0 Sprague Dawley rats or NR2A knock-out mice. The hippocampus was dissected in minimum essential medium (MEM; 11090-081; Invitrogen, Grand Island, NY), collected, and dissociated with papain (PAP2; Worthington, Lakewood, NJ) at  $\sim 35$  U/ml for 30 min to 1 h at  $37^{\circ}\text{C}$ . Small pieces of the hippocampus were triturated in culture medium (MEM supplemented with 2% B-27; 17504-044; Invitrogen, Grand Island, NY), 0.1% mito serum extender (355006; BD Biosciences), 5% fetal bovine serum (FBS; 16000-044; Invitrogen, Grand Island, NY), 0.6% dextrose (D-9434; Sigma), 1 mM sodium pyruvate (11360-070; Invitrogen, Grand Island, NY), and 0.5 mM glutamine with penicillin–streptomycin (10378-016; Invitrogen, Grand Island, NY) and centrifuged at  $100 \times g$  for 5 min. The pellets were resuspended in the culture medium and plated at a density of  $\sim 50,000$  cells in a microdot 35 mm dish or at 250,000 cells in dispersed cultures. Cultures were maintained for 3–9 d in a  $37^{\circ}\text{C}$  humidified incubator with 5%  $\text{CO}_2$ .

Human embryonic kidney (HEK) 293 cells (CRL-1573; American Type Culture Collection, Manassas, VA) were grown in HEK cell culture medium (MEM Eagle, 30-2003; American Type Culture Collection) supplemented with 10% FBS. Cells were dissociated with trypsin–EDTA (25300-054; Invitrogen, Grand Island, NY) at 0.5 mg/ml and plated in a 35 mm dish at a density of  $4 \times 10^5$  cells/ml. Cells were transfected with NR1–1a plus NRA or NR2B and green fluorescent protein cDNAs at a ratio of 2:3:1 (3  $\mu\text{g}$  of total plasmid per dish) using a calcium phosphate transfection kit (K2780-01; Invitrogen, Carlsbad, CA). After transfection, 100  $\mu\text{M}$  ketamine (K-2735; Sigma) and 200  $\mu\text{M}$  D,L-2-amino-5-phosphonopentanoic acid (APV; A-5282; Sigma) were added to the HEK culture medium, and cells were maintained in a  $37^{\circ}\text{C}$  humidified incubator at 5%  $\text{CO}_2$ . Whole-cell patch-clamp experiments were performed within 48–72 h after transfection.

**Whole-cell patch-clamp recording.** Whole-cell patch-clamp recording was performed at room temperature using a Multiclamp 700A (Molecular Devices, Union City, CA) amplifier. Culture dishes were superfused with external solution (150 mM NaCl, 2.5 mM KCl, 2.5 mM  $\text{CaCl}_2$ , 10  $\mu\text{M}$  glycine, 10 mM HEPES, and 10 mM dextrose, pH adjusted to 7.4 with NaOH and osmolarity adjusted to  $\sim 335$  mmol/kg with sucrose). To record NMDAR-mediated EPSCs, autaptic hippocampal neurons were voltage clamped at a holding potential of  $-70$  mV and depolarized to  $+10$  mV using a 1–2 ms pulse at 0.1 Hz. AMPA receptor (AMPA)-mediated EPSCs were evoked by paired (interval of 50 ms) 1 ms depolarization steps to 10 mV at a rate of 0.1 Hz. Before the evoked pulse, neurons were hyperpolarized to  $-80$  mV for 10 ms to monitor changes in input and series resistances. Patch pipettes had a range of resistance from 1 to 3  $\text{M}\Omega$  when filled with an internal solution containing the following (in mM): 150 K-gluconate, 1.4  $\text{CaCl}_2$ , 2  $\text{MgCl}_2$ , 10 EGTA, 10 HEPES, 3

$\text{Na}_2\text{ATP}$ , and 0.2  $\text{Na}_2\text{GTP}$ , pH adjusted to 7.35 with KOH and osmolarity adjusted to  $\sim 325$  mmol/kg with sucrose. The NMDAR antagonists AAM077 (0.4  $\mu\text{M}$ ) and ifenprodil (3  $\mu\text{M}$ , I-2892; Sigma) were applied to cells using a stepper motor-driven rapid solution exchange apparatus (Warner Fast-Step Perfusion system; Warner Instruments, Hamden, CT). In experiments examining autaptic synaptic currents, antagonists were applied continuously to the neuron beginning at least 15 s before evoking an EPSC, and antagonist application was maintained between EPSCs. NMDAR- or AMPAR-mediated currents were isolated by adding 5  $\mu\text{M}$  2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (0373; Tocris, Ellisville, MO) or 50  $\mu\text{M}$  APV, respectively, to the external solution.

To examine AAM077 and ifenprodil effects on NMDAR expressed in HEK 293 cells, transfected cells were voltage clamped at a holding potential of  $-60$  mV. After establishing the whole-cell mode, cells were lifted and series resistance (up to 10  $\text{M}\Omega$ ) was compensated by 80%. The intracellular recording solution for HEK cell experiments contained (in mM) 140 CsCl, 10 EGTA, 2 ATP, and 10 HEPES. The intracellular solution pH was adjusted to 7.2 with CsOH, and osmolarity was adjusted to 315 mmol/kg with sucrose. Solutions of 100  $\mu\text{M}$  NMDA and/or antagonists (0.4  $\mu\text{M}$  AAM077 or 3  $\mu\text{M}$  ifenprodil) were applied to cells for 5 s using a stepper motor-driven solution exchange apparatus every minute. In the “simultaneous application protocol,” antagonists were not present before the drug application and were only applied during the 5 s agonist applications. In contrast, in the “pretreatment protocol,” antagonists were applied for at least 30 s before combined agonist and antagonist application. This protocol yields antagonist application periods similar to the application protocol used in experiments on autaptic hippocampal neurons. Signals were filtered using a Bessel filter at 2 kHz, and data were acquired at 5 kHz with pClamp 8 software (Molecular Devices).

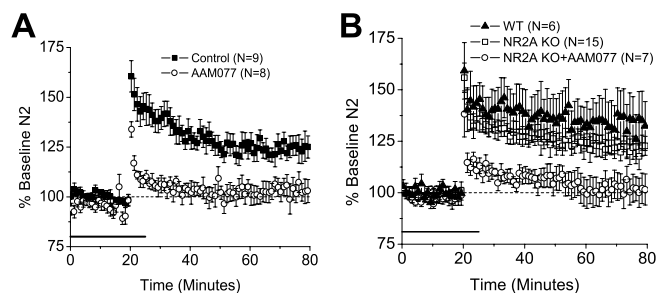
**Data analysis.** For LTP experiments, each point represents the peak response of the N2 averaged from three traces for each minute. This value was plotted as a percentage of the averaged response from a 20 min baseline phase preceding drug application and/or tetanus. The N1 was measured in a similar manner; experiments in which the N1 changed by  $>20\%$  were discarded. To measure for significant effects, responses over a 5 min time span were averaged for each experiment. These were compared with averaged responses from other time points within the experiment or at the same time point across experimental conditions using paired and independent Student's *t* tests, respectively. A minimum criteria of  $p < 0.05$  was used to determine significance.

For experiments on HEK 293 and hippocampal dispersed culture cells, the percentage of control current amplitude during antagonist applications was calculated as the average amplitude for all responses during antagonist application over the mean of the current amplitudes obtained before and after antagonist treatment. To estimate antagonist effects on current just after the onset of agonist application, we made isochronal measurements of current amplitude at the time point when peak current was observed in the absence of antagonist. Steady-state current was measured at a time point just before the end of the 5 s agonist application. In a subset of experiments, full recovery from ifenprodil inhibition was not examined. In these experiments, the percentage of control was defined as current amplitude during ifenprodil application divided by values obtained before antagonist application. Inhibition by ifenprodil was of similar magnitude regardless of the method of measurement. In experiments on autapses, only inhibition of peak current amplitude was measured. This was accomplished by taking the ratio of the average EPSC amplitude in the presence of the antagonist to the averages of EPSCs evoked before and after antagonist application. The AMPAR-mediated paired-pulse ratio (PPR) was determined by calculating the ratio of the amplitude of the second EPSC peak to that of the first EPSC. The results were normalized to the values obtained before and after antagonist application.

## Results

### NMDAR-dependent LTP in the BNST persists in the absence of the NR2A subunit

In slices prepared from C57BL/6J mice, we induced LTP with two 100 Hz, 1 s trains with a 20 s intertrain interval. Potentiation was significant and lasted  $>1$  h (Fig. 1A, filled squares). We showed



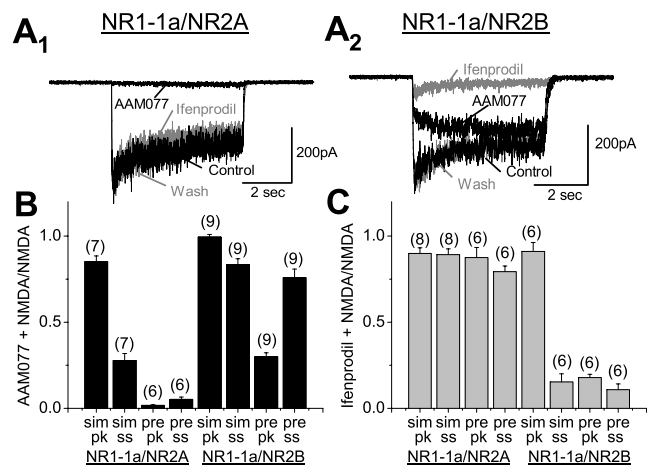
**Figure 1.** NMDAR-dependent LTP in the dIBNST does not require NR2A subunit-containing receptors. **A**, Averaged time course of synaptic field potentials after high-frequency stimulation (21 s trains, 100 Hz) in coronal slices containing the dIBNST from adult C57BL/6J mice. Pretreatment with AAM077 (0.4  $\mu$ M) for 30 min significantly attenuated LTP (open circles) compared with interleaved controls (filled squares) ( $p < 0.05$ ). **B**, LTP in the dIBNST was unaffected in NR2A knock-outs (open squares) compared with wild-type (WT) littermates (filled triangles) ( $p = 0.28$ ). LTP in NR2A knock-outs was blocked by pretreatment with AAM077 (0.4  $\mu$ M; open circles) ( $p < 0.05$ ).

previously using the same tetanus protocol that this LTP is abolished by the NMDAR antagonist DL-AP-5 (Weitlauf et al., 2004). Similar to previous experiments (Liu et al., 2004; Mallon et al., 2004; Massey et al., 2004), bath application of AAM077 (0.4  $\mu$ M) for a period of 30 min attenuated this dIBNST LTP ( $n = 8$ ;  $p < 0.05$  compared with controls) (Fig. 1A, open circles). Based on these data, we predicted that LTP would be disrupted in the dIBNST of NR2A knock-out mice. We found, however, that dIBNST LTP in NR2A knock-out mice was unchanged compared with wild-type littermates ( $n = 15$ ;  $p = 0.28$  compared with wild-type littermates) (Fig. 1B, open squares). Moreover, we found that pretreatment with AAM077 (0.4  $\mu$ M) attenuated dIBNST LTP induction in NR2A knock-out mice in a manner similar to that observed in wild-type mice ( $n = 7$ ;  $p < 0.05$  compared with knock-out controls) (Fig. 1B, open circles).

#### Preapplication of AAM077 produces substantial inhibition of peak currents evoked by activation of NR2B-containing receptors

Previous literature reported a 100-fold higher specificity of AAM077 for NR2A relative to NR2B (Auberson et al., 2002; Liu et al., 2004). These measurements were taken when varying concentrations of the antagonist were coapplied with glutamate to modulate the induction of NMDAR-mediated currents in oocytes expressing human NR1-1a/NR2A or NR1-1a/NR2B cRNA. Based on these data, a concentration of 0.4  $\mu$ M should effectively inhibit NR2A-containing receptors while essentially sparing NR2B-containing receptors. It is important to note, however, that other NR2 subunit-specific antagonists display time-dependent inhibition kinetics. In particular, the NR2B subunit-specific antagonist ifenprodil displays much greater inhibition of NR2B-containing NMDARs when it is applied before agonist application (Legendre and Westbrook, 1991; Tovar and Westbrook, 1999) (Fig. 2C). Because our LTP studies in brain slices require preapplication of the antagonist, we tested the idea that AAM077 may produce time-dependent inhibition of NR2B-containing subunits using HEK cells expressing recombinant receptors containing rat NR1a/NR2A or NR1-1a/NR2B subunits.

Similar to previous studies, we see attenuation of the steady-state (ss) current and, to a lesser degree, the peak (pk) current from NR1-1a/NR2A receptors in response to AAM077 (0.4  $\mu$ M) application (Fig. 2B) (Liu et al., 2004). However, the attenuation of each current was much more complete when the HEK cells

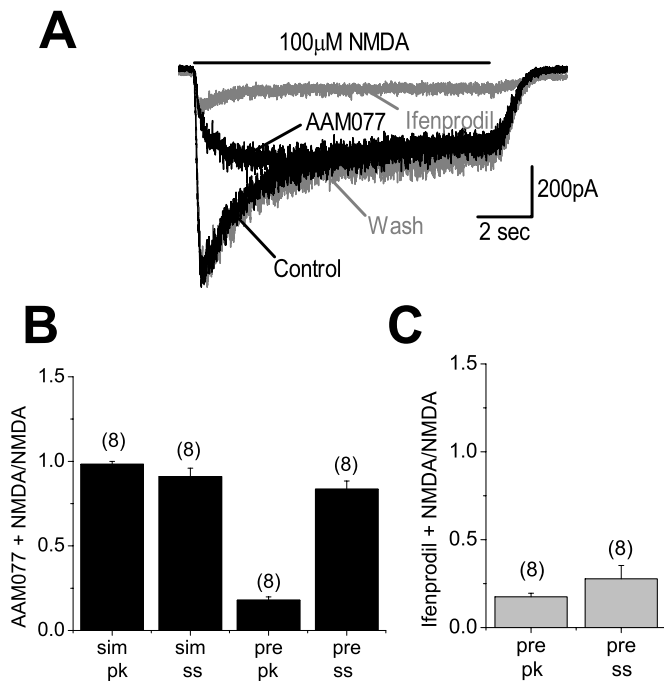


**Figure 2.** Attenuation of NMDA-induced currents by NR2-specific inhibitors in HEK cells expressing recombinant NMDARs. **A**, Representative traces of NMDA-induced currents in HEK cells expressing NR1-1a/NR2A (**A**<sub>1</sub>) and NR1-1a/NR2B (**A**<sub>2</sub>) under control conditions, after preapplication of AAM077 (0.4  $\mu$ M), after preapplication of ifenprodil (3  $\mu$ M), and after wash-out. **B**, Averaged amount of attenuation of NMDA-induced currents by AAM077 (0.4  $\mu$ M) in HEK cells expressing NR1-1a/NR2A (left) and NR1-1a/NR2B (right). **C**, Averaged amount of attenuation of NMDA-induced currents by ifenprodil (3  $\mu$ M) in HEK cells expressing NR1-1a/NR2A (left) and NR1-1a/NR2B (right). Values are expressed as the ratio of the response in the presence of NMDA plus antagonist compared with the response in the presence of NMDA alone. The numbers of averaged cells are indicated in parentheses above the bars. sim, Simultaneous application of antagonist and NMDA; pre, preapplication of antagonist 30 s before NMDA; pk, peak current (measured at the time point at which control currents reach maximum amplitude); ss, steady-state current.

were pretreated with AAM077 30 s before application of NMDA (Fig. 2A<sub>1</sub>,B, pre) compared with those that received simultaneous application of agonist and antagonist (Fig. 2B, sim). Consistent with previous reports, we see only negligible attenuation of NMDA-induced currents in cells expressing NR1-1a/NR2B receptors when AAM077 (0.4  $\mu$ M) is coapplied with the agonist (Fig. 2B, sim). However, we see significant attenuation of the pk current through these receptors under conditions of antagonist pretreatment (Fig. 2A<sub>2</sub>,B, pre). To control for nonspecific drug application effects, we also measured the amount of attenuation seen in the presence of the NR2B subunit-specific antagonist ifenprodil. As predicted, ifenprodil (3  $\mu$ M), whether preapplied or coapplied, had a minimal effect on pk and ss currents in cells expressing NR2A-containing NMDARs (Fig. 2C, left). Significant attenuation of both pk and ss currents was only seen under conditions of preapplication in cells expressing NR2B-containing NMDARs (Fig. 2A<sub>2</sub>,C, right).

#### Preapplication of AAM077 dramatically reduces NMDAR-mediated EPSCs in autaptic synapses from NR2A knock-out mice

To further verify that AAM077 loses its specificity for NR2A-containing NMDARs under conditions of pretreatment, we repeated our experiments in cultured hippocampal neurons under two conditions that effectively removed NR2A subunit-containing NMDARs. First, we used dispersed cultures of rat hippocampal neurons during a time at which NR2B subunit-containing receptors are the predominant receptor subtype (3–4 d *in vitro*). This predominance of NR2B-containing receptors was verified by blocking >75% of the ss and pk currents in these cells with preapplication of ifenprodil (3  $\mu$ M) (Fig. 3A, C). Preapplication of AAM077 (0.4  $\mu$ M) also attenuated the pk current by >75% (Fig. 3A, B). This treatment had little effect on the ss cur-



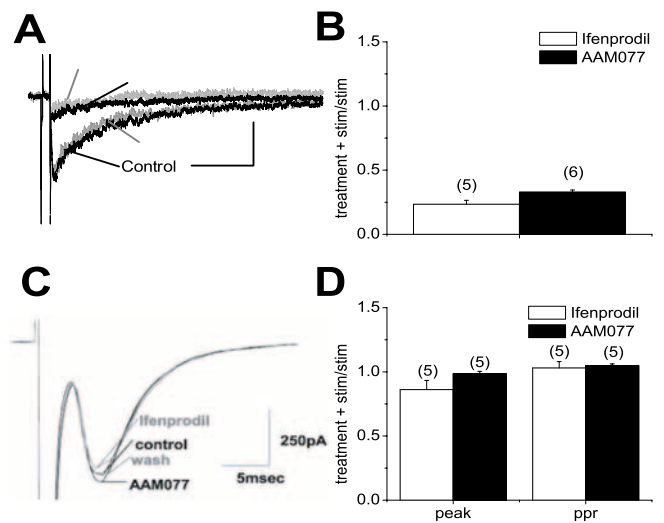
**Figure 3.** Attenuation of NMDA-mediated current by NR2-specific inhibitors in dispersed cultured hippocampal neurons (3–4 d *in vitro*). **A**, Representative traces of NMDA-induced currents under control conditions, after preapplication of AAM077 (0.4  $\mu$ M), after preapplication of ifenprodil (3  $\mu$ M), and after washout. **B**, Averaged amount of attenuation of NMDA-induced currents by AAM077 (0.4  $\mu$ M). **C**, Averaged amount of attenuation of NMDA-induced currents by ifenprodil (3  $\mu$ M). The numbers of averaged neurons are indicated in parentheses above the bars. Data were obtained from three separate culture dishes from two separate litters for each condition. sim, Simultaneous application of antagonist and NMDA; pre, preapplication of antagonist 30 s before NMDA; pk, peak current (measured at the time point at which control currents reach maximum amplitude); ss, steady-state current.

rent (Fig. 3A, B). As before, simultaneous application of AAM077 (0.4  $\mu$ M) with NMDA had little effect on the pk and ss currents (Fig. 3B). Second, we used autaptic neuron cultures produced from the hippocampus of NR2A subunit protein knock-out mice. In these experiments, instead of directly applying agonist over a time frame of several seconds, activation of NMDARs was achieved by depolarization-evoked release of glutamate from presynaptic sites to produce a synaptic NMDAR-dependent EPSC. Like ifenprodil, pretreatment with AAM077 led to an ~70% reduction of these synaptically evoked NMDAR-mediated EPSCs (Fig. 4A, B, left).

To test for nonspecific effects on glutamatergic transmission, we also measured the effect of ifenprodil and AAM077 on AMPAR-mediated synaptic transmission in the autaptic neuron cultures from the hippocampus of NR2A knock-out mice. To isolate AMPAR-mediated currents, we stimulated the neurons in the presence of APV (50  $\mu$ M). Under these conditions, we see no significant effect on these currents when pretreated with either ifenprodil (3  $\mu$ M) or AAM077 (0.4  $\mu$ M) (Fig. 4C, D). Furthermore, we see no change in PPR after pretreatment with either inhibitor, suggesting that neither drug affects the release probability of glutamate (Fig. 4D).

### Discussion

In total, our data support two main conclusions. First, at the antagonist concentrations used here as well as in previous studies (0.1–0.5  $\mu$ M) (Liu et al., 2004; Mallon et al., 2004; Massey et al., 2004), the high degree of specificity of AAM077 for NR2A-



**Figure 4.** Attenuation of NMDAR-mediated, but not AMPAR-mediated, transmission by NR2-specific inhibitors in autaptic cell cultures from the hippocampus of NR2A knock-out mice. **A**, Representative traces of synaptically evoked NMDAR-mediated EPSCs under control conditions in the presence of AAM077 (0.4  $\mu$ M), in the presence of ifenprodil (3  $\mu$ M), and after washout. **B**, Averaged amount of attenuation of synaptically evoked NMDAR-mediated EPSCs by ifenprodil (3  $\mu$ M; gray bar) and AAM077 (0.4  $\mu$ M; black bar). **C**, Representative traces of synaptically evoked AMPA-mediated EPSCs under control conditions, in the presence of AAM077 (0.4  $\mu$ M), in the presence of ifenprodil (3  $\mu$ M), and after washout. **D**, Averaged amount of attenuation of synaptically evoked AMPA-mediated EPSCs (peak and paired-pulse ratios) by AAM077 (0.4  $\mu$ M; closed bars) and ifenprodil (3  $\mu$ M; open bars). The numbers of averaged neurons are indicated in parentheses above the bars. Data were obtained from a single culture for each condition.

NR2B-containing NMDARs only occurs under conditions of co-application of agonist and antagonist, or perhaps preapplication of agonist. When the antagonist is preapplied (as it is in most brain slice experiments such as our LTP studies), selectivity is reduced. This, taken with the observations that AAM077 also has a high affinity for NR2C subunit-containing receptors (Feng et al., 2004; Y. P. Auberson, personal communication) compromises the interpretation of results with this compound. The effect of AAM077 on NMDA-induced currents from NR2B-containing receptors obtained in HEK cells and dispersed hippocampal cultures was primarily on the peak rather than the steady-state portion of the current. This peak current is thought to be in some ways analogous to synaptically induced NMDAR-mediated EPSCs. Consistent with this, we saw marked attenuation of synaptically evoked currents from NR2B-containing receptors obtained in autaptic neurons prepared from NR2A knock-out mice.

Second, our results indicate that NR2A is not obligatory for NMDAR-dependent LTP in all regions of the CNS. We found that LTP in the BNST in slices prepared from adult NR2A knock-out mice was indistinguishable from that observed in wild-type mice. Although it is possible that developmental compensation may occur in the NR2A knock-out mice, NMDAR LTP in area CA1 is disrupted in this line (Sakimura et al., 1995). Furthermore, evidence suggests that the loss of NR2A is not compensated by maintenance of NR2B-containing subunits in the synapse during development (Townsend et al., 2003). Our data are consistent with a number of other studies indicating a lack of general requirement for the NR2A subunit in NMDAR-dependent LTP. For example, although LTP is disrupted in area CA1 in adult mouse slices prepared from NR2A knock-out mice, LTP in these knock-outs is intact in slices prepared from 2- to 3-week-old mice

(Ito et al., 1996). Furthermore, NR2B-specific antagonists reduce LTP induced by pairing protocols in the visual cortex (Yoshimura et al., 2003) (but see Massey et al., 2004). Finally, transgenic overexpression of NR2B resulted in enhancement of LTP in area CA1 (Tang et al., 1999).

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