**Influence of developmental nicotine exposure on glutamatergic neurotransmission
in rhythmically active hypoglossal motoneurons**

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Abstract

Developmental nicotine exposure (DNE) is associated with increased risk of cardiorespiratory, intellectual, and behavioral abnormalities [in neonates](#), and is a risk factor for [apnea of prematurity, altered arousal responses and](#) Sudden Infant Death Syndrome. Alterations in nicotinic acetylcholine receptor signaling (nAChRs) after DNE lead to changes in excitatory neurotransmission in neural networks that control breathing, including a heightened excitatory response to AMPA microinjection into the hypoglossal motor nucleus. Here, we report on experiments designed to probe possible postsynaptic and presynaptic mechanisms that may underlie this plasticity. Pregnant dams were exposed to nicotine or saline via an osmotic mini-pump implanted on the 5th day of gestation. We used whole-cell patch clamp electrophysiology to record from hypoglossal motoneurons (XIIMNs) in thick medullary slices from neonatal rat pups (N= 26 control and 24 DNE cells). To enable the translation of our findings to breathing-related consequences of DNE, we only studied XIIMNs that were receiving rhythmic excitatory drive from the respiratory central pattern generator. Tetrodotoxin was used to isolate XIIMNs from presynaptic input, and their postsynaptic responses to bath application of L-glutamic acid (glutamate) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) were studied under voltage clamp. DNE had no influence on [total inward current magnitude](#) ~~inward current, peak current amplitude, or the time to reach half-peak amplitude~~ evoked by either glutamate or AMPA. However, [with bath application of AMPA the](#) ~~the~~ amplitude distribution of miniature excitatory postsynaptic currents (mEPSCs) [was shifted to higher values](#) ~~was significantly higher in DNE neurons, while the frequency of mEPSCs was shifted to lower values in DNE neurons under baseline conditions.~~ [DNE had no influence on](#)

mEPSC amplitude or frequency with glutamate application, or under (unstimulated) baseline conditions. Thus, DNE is associated with a small but significant increase in glutamatergic mEPSC amplitude in XIIMNs, consistent with an increase in the strength of quantal synaptic transmission, and a slight decrease in the probability of glutamate release, with both effects mediated by AMPA receptors.

Introduction

According to the Centers for Disease Control (CDC) cigarette smoking is one of the leading causes of preventable disease in the United States, resulting in 1 out of 5 deaths annually (www.cdc.gov/tobacco). Nicotine is the primary neurotoxin in tobacco products (Abreu-Villaca et al., 2003; Eriksson et al., 2001; Ginzel et al., 2007; Pauly and Slotkin, 2008), as it has been strongly associated with altered development of neurons throughout the brain (Eriksson, 1997; Slotkin, 2004), including brainstem neurons involved in the control of breathing (Dwyer et al., 2009; Hafstrom et al., 2005; Muhammad et al., 2012; Pilarski et al., 2011). Despite an overall decline in the use of cigarettes in the U.S. in the last decade, the incidence of nicotine use during gestation remains unaddressed, as nicotine replacement therapy (patch, gum) is commonly prescribed to pregnant women who continue to smoke (Oncken, 2012). Developmental nicotine exposure (DNE) in rodents is a widely accepted model for studying the effects of *in utero* exposure to nicotine (Slotkin, 2004). Chronic nicotine exposure causes widespread desensitization of nicotinic acetylcholine receptors (nAChRs), leading to increased receptor expression, but a reduction in synaptic efficacy (Gentry and Lukas, 2002; Marks et al., 1983; Marks et al., 1985). This paradox results in a “functional loss” of nAChRs and is due to long-term

receptor desensitization. [Consistent with this, our](#) previous work (Pilarski et al., 2012) showed that DNE [caused functional](#) desensitization of nAChRs on hypoglossal motoneurons (XIIMNs). Hypoglossal motoneurons control the activity of the tongue muscles, which play an important role in swallowing and chewing, as well as breathing, especially during sleep (Eisele et al., 2003; Kezirian et al., 2010), and excitatory drive to XIIMNs relies importantly on glutamatergic neurotransmission (Revill et al., 2015; Steenland et al., 2006).

Our laboratory previously reported that DNE decreased excitatory synaptic input in XIIMNs of neonatal rats (Pilarski et al., 2011), and increased the response of XIIMNs to microinjection of glutamate or AMPA into the hypoglossal motor nucleus (Jaiswal et al., 2013). These observations lead to the development of a model that is based on the [idea working hypothesis](#) that the reduced presynaptic excitatory input leads to an increased density, and/or a functional change, in postsynaptic glutamate receptors, as summarized in Fig. 1. Here we focus on two components of this model, namely postsynaptic changes in glutamatergic transmission, and alterations in the quantity and frequency of glutamate release from glutamatergic neurons that synapse on XIIMNs.

We ~~made~~ [used the](#) whole-cell patch-clamp [technique to](#) recordings from rhythmically-active XIIMNs in thick medullary slices. Postsynaptic effects were examined by bath applying glutamate or AMPA in the presence of tetrodotoxin (TTX) and recording the kinetics and magnitude of the evoked inward current. We also examined changes in quantal synaptic transmission by recording the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs), under baseline conditions and also during bath application of glutamate or AMPA. The only significant difference between control and

DNE animals was an increase in the amplitude distribution [and a decrease in the frequency distribution](#) of mEPSCs [during AMPA bath application](#), consistent with an increase in the strength of quantal synaptic transmission [and slight decrease in the probability of glutamate release](#); mechanisms that may explain these observations are addressed in the Discussion.

Materials and Methods

2.1. Animals

We used a total of 50 Sprague-Dawley rats of either sex, aged from postnatal day zero (P0) through postnatal day four (P4). This neonatal period corresponds to a gestational age of 29-34 weeks in humans (Ballanyi et al., 1999). The nicotine-exposed neonates were taken from 19 separate litters; the saline-exposed neonates from 15 different litters; and the unexposed from 3 litters. From each litter, 1-3 neonates were used. All neonates were born via spontaneous vaginal delivery from pregnant adult female rats purchased from Charles River Laboratories (Wilmington, MA). Neonatal rat pups were housed together with their mothers and siblings until they were studied. Dams were housed in the Animal Care Facility at the University of Arizona under a 12: 12 h light/dark cycle (lights on 07:00 h) with water and food available *ad libitum*, in a quiet room at 22°C and 20-30% relative humidity. All protocols were approved by the University of Arizona Institutional Care and Use Committee and conformed to National Institutes of Health guidelines.

2.2. Developmental nicotine exposure

DNE was achieved by subcutaneous implantation of Alzet 1007D mini-osmotic pumps (Alzet Corp., CA, USA) into pregnant dams on gestational day 5 under aseptic conditions, as previously described (Fregosi et al., 2004; Huang et al., 2004; Luo et al., 2007). The pumps allow for fluid infusion into subcutaneous space at a rate of $2.5 \mu\text{L h}^{-1}$ for 28 days. This infusion rate achieves mean nicotine bitartrate delivery of $6 \text{ mg kg}^{-1} \text{ day}^{-1}$ throughout gestation. We previously showed that this regimen produces plasma levels of cotinine, a major metabolite of nicotine with a long half-life (20-24 h), ranging from 60-92 ng/mL (Powell et al., 2015). This is comparable to average cotinine levels (88 ng/mL) found in the umbilical cord blood of newborns whose mothers smoked on average 95 cigarettes/week (Berlin et al., 2010).

2.3. Medullary slice preparation

Pups of either sex were removed from their home cages at random and weighed. Animals were anesthetized by hypothermia until unresponsive to a paw pinch, and decerebrated at the coronal suture. The vertebral column and ribcage were exposed, moved to a dissection dish filled with cold ($4-8^{\circ}\text{C}$) oxygenated (95% O_2 -5% CO_2) artificial cerebrospinal fluid (aCSF), composed of (in mM): 120 NaCl, 26 NaHCO_3 , 30 glucose, 1 MgSO_4 , 3 KCl, 1.25 NaH_2PO_4 , and 1.2 CaCl_2 with pH adjusted to 7.4 and osmolarity adjusted to 300-325 mOsm. The spinal cord and brainstem were glued to an agar block with superglue, rostral surface up, for serial microsectioning in a vibratome (VT1000; Leica). Transverse medullary slices were taken in chilled oxygenated aCSF until the most rostral hypoglossal nerve (XIIIn) rootlets were near the surface of the tissue. A single 700- μm slice was taken to capture the majority of hypoglossal motor nucleus and the preBotzinger complex, which can drive

rhythmic respiration-related bursting in the medullary slice. The slice was then transferred to fresh aCSF that was continuously oxygenated with 95%O₂-5%CO₂ and allowed to equilibrate at room temperature for 1-2 hours before recording. Slices were then transferred to the recording chamber and perfused with oxygenated aCSF. To evoke rhythmic bursting of XIIMNs, the KCl of the perfusate was raised to 9 mM. [We used rhythmic slices because we are interested in motoneurons that play a role in breathing. High potassium is commonly used to evoke rhythmic bursting in brainstem slices, though we realize that some motoneurons that were rhythmic in high potassium may have not been rhythmic when exposed to normal extracellular potassium, though the rhythmic slice does mimic “high respiratory drive”.](#)

2.4. Electrophysiology

XIIMNs were visualized with an Olympus BX-50WI fixed-stage microscope (X40 water-immersion objective, 0.75 N.A.) with infrared and differential contrast optics and a video camera (C25400-07, Hamamatsu). XIIMNs were approached with a glass pipette (4-8 MΩ) pulled from thick-walled borosilicate glass capillary tubes (OD: 1.5 mm, ID: 0.75 mm) and filled with intracellular solution which contained (in mM): 135 K-gluconate, 4 KCl, 10 HEPES, 5 ATP (Mg²⁺ salt), 0.375 GTP, and 12.5 phosphocreatine, with pH adjusted to 7.2 and osmolarity of 275-300 mOsm. The electrophysiology equipment consisted of Digidata 1440 and Multiclamp 700B amplifier (Molecular Devices).

2.5. Drugs

Most drugs were purchased from Sigma (St. Louis, MO, USA). Tetrodotoxin was purchased from R&D (Minneapolis, MN, USA). The drugs were mixed on the day of the experiment in modified aCSF (9 mM KCl) with the following concentrations: tetrodotoxin (TTX, 1 μ M), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, 2.5 μ M), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μ M), and L-glutamic acid monosodium salt hydrate (glutamate, 200 μ M). The drug solutions were oxygenated and were delivered to the recording chamber at a rate of 1.5-2 ml/min, with the temperature controlled at 27°C (TC-324B temperature controller, Warner Instrument Corporation). A summary of drug concentrations and durations of application is provided in Table 1.

2.6. Protocols

A total of 26 control and 24 DNE rhythmically active XIIMNs (1 cell from each animal) were studied under whole cell voltage-clamp ($V_{\text{hold}} = -70$ mV). The slow and fast components of the voltage transient were cancelled. The series resistance was compensated with the bandwidth set at 4 kHz and correction at 60%. A small voltage-step (from -70 to -75 mV) protocol was performed to calculate motoneuron input resistance. This was followed by 1-min recording in current-clamp mode ($I_{\text{hold}} = 0$ pA, pipette offset adjusted) to check the stability of the cell and to measure the resting membrane potential. We then performed a square-pulse current-step protocol to determine the firing threshold. Current steps (800 msec duration) were initiated at 0 pA and ranged from -200 pA to +350 pA in 50pA increments. Then, the cell was switched back to voltage clamp mode ($V_{\text{hold}} = -70$ mV), the rhythmic activity was recorded for 1 min, and TTX was bath applied for 2 min to establish a new, stable baseline. These studies were then followed by bath application of cocktails

containing AMPA + TTX (N=13 control, 11 DNE) or glutamate + TTX (N=13 control, 13 DNE) for 5 min. This was followed by bath application of AMPA + TTX + CNQX, or glutamate + TTX + CNQX for 5 min, followed by a washout with TTX alone (Fig. 2).

Importantly, the addition of CNQX always ~~resulted in~~ abolished the inward current and in cessation of mEPSCs, confirming that ~~they both were mediated by~~ glutamate, largely by AMPA receptors. At the end of each experiment, the small voltage-step protocol (from -70 mV to -75 mV) was repeated to measure the input resistance and confirm that the gigaohm seal was intact.

2.7. Data analysis and Statistics

Analysis of the voltage- and current-clamp data obtained in Clampex (Molecular Devices, Sunnyvale, CA) was performed in Clampfit (Molecular Devices, Sunnyvale, CA) using custom scripts written in Spike2 (CED, Cambridge, UK). The analysis of miniature excitatory postsynaptic currents was done using MiniAnalysis 6.0.7 (Synaptosoft, Decatur, GA). The quantitative data were statistically analyzed using Prism (GraphPad Software, Inc., La Jolla, CA) and SPSS (IBM, Armonk, NY). Unpaired t-tests were used to compare treatment groups for the following variables: input resistance, resting membrane potential, weight, age, holding current, total inward current produced during drug application, inward current produced at 20, 40, 60, and 80% of the total drug application time, peak current amplitude and the time needed to reach 1/2-peak amplitude. Multiple comparisons were corrected with the Bonferroni method. Average mEPSC amplitude and frequency at baseline and during application of AMPA or glutamate were compared between the treatment groups using Two-Way ANOVA. To compare the cumulative

probability distribution of mEPSC amplitude and inter-event interval in control and DNE animals, we used the two-sample Kolmogorov-Smirnov procedure, which tests the hypothesis that two distributions come from different populations. For illustration purposes, data for each neuron were normalized by assigning a value of 1.0 to the largest mEPSC, and the amplitude of each remaining mEPSC was expressed as a fraction of that maximal value. The normalized amplitude of each mEPSC was rank ordered, and the cumulative distribution function was calculated. Data are displayed by plotting the cumulative fraction as a function of normalized amplitude; a cumulative fraction of 0.5 defines the midpoint of the normalized mEPSC amplitude distribution. Data were considered statistically significant if $p \leq 0.05$. Data in all figures and tables are represented as Means $\pm$ SEM, unless stated otherwise.

Results

DNE does not alter AMPA- and glutamate-induced inward currents.

At baseline, the frequency of rhythmic, respiration-related XIIMN bursts did not differ between the groups, although there was a trend for a higher frequency in the DNE group (data not shown, 14.3 ± 2.00 control, 18.0 ± 2.88 DNE, $p = 0.293$). The passive properties of rhythmically active XIIMNs, such as resting membrane potential, input resistance, and baseline holding current also did not differ between the groups (Table 2). The bath application of AMPA or glutamate (concomitantly with TTX in order to assess the direct effect of the agonist on the XIIMN) produced a large and rapid inward current (Fig. 2A-B), with no differences between the treatment groups (Table 2). Similarly, the peak amplitude, as well as time to half-peak amplitude, was the same in controls and DNE preparations

(Table 2), as was the evoked inward current measured at 20, 40, 60, and 80% of the time from the beginning of drug application to the time corresponding to the peak of the inward current (data not shown). Finally, analysis of the slope of the inward current produced in the first 200 seconds following bath application of AMPA or glutamate was calculated for both groups, with no differences noted (data not shown).

DNE alters mEPSC amplitude distribution

The bath application of TTX blocks action potential dependent synaptic inputs onto XIIMNs, but the spontaneous release of neurotransmitter by the presynaptic cells remains intact in the presence of TTX and can be measured as mEPSCs from the postsynaptic cell (Vautrin and Barker, 2003). The cumulative probability distribution for mEPSC amplitude was right-shifted in XIIMNs from DNE animals under these baseline recording conditions ($P < 0.0001$, Fig. 3). In contrast, the frequency of mEPSCs at baseline was not different between the groups (data not shown). Application of either AMPA or glutamate did not result in a significant change of mEPSC frequency or amplitude compared to baseline in either control or DNE groups (data not shown). Thus, DNE increased the amplitude of mEPSCs, but did not affect their frequency or their response to agonists.

Discussion

In the present study we sought to better understand changes in glutamatergic neurotransmission in XIIMNs from neonatal rats that were exposed to nicotine during development. We previously showed that DNE is associated with an exaggerated excitatory response in XIIMNs when AMPA or glutamate was injected into the hypoglossal

motor nucleus (Jaiswal et al., 2013), as well as a reduction in baseline glutamatergic excitatory synaptic input (Pilarski et al., 2011). Thus, DNE may be altering the postsynaptic response of XIIMNs to glutamate, the release of glutamate by presynaptic neurons or both. To test for an effect on the postsynaptic response to glutamate, we applied agonists in the presence of TTX. To examine possible changes in presynaptic parameters, we measured miniature EPSCs. XIIMNs exhibit spontaneous mEPSCs that are mediated by GABA, glycine, or glutamate (Donato and Nistri, 2000). Here we focused specifically on glutamatergic mEPSCs, which we confirmed by abolishing them with CNQX.

Summary of findings. We first tested the hypothesis that DNE caused a change in the postsynaptic sensitivity of XIIMNs to glutamate. Such a change might be caused, for example, by a compensatory increase in the density of postsynaptic receptors, as schematized in Fig. 1. The bath application experiments reject this hypothesis in its most simple form, as the post-synaptic response to glutamate or AMPA in rhythmically active XIIMNs was not altered by DNE. Specifically, we found no influence of DNE on the total amount of inward current produced by bath application of either AMPA or glutamate. Furthermore, although we saw a trend for faster time to half-peak amplitude in cells from the DNE group (Table 2), a finding that could potentially suggest a change in the kinetics of receptors on the postsynaptic cell, this difference was not statistically significant. Secondly, to test whether baseline spontaneous presynaptic release from glutamatergic neurons was altered in DNE pups we measured both the frequency and amplitude of glutamatergic mEPSCs. An effect of DNE on mEPSC frequency would suggest a presynaptic mechanism, whereas an effect on amplitude could reflect either presynaptic or postsynaptic changes. Interestingly, we observed a right-shift in the glutamatergic mEPSC amplitude distribution,

but no effect of DNE on frequency. We consider possible interpretations of these findings below. Finally, we tested the hypothesis that activation of presynaptic glutamate receptors located on the terminals (i.e., glutamate autoreceptors) would change the amplitude or frequency of mEPSCs, and whether or not such an effect would be altered by DNE. Our data provided no support for either hypothesis as no changes in distributions of mEPSC frequency or amplitude were found after the addition of agonists in either control or DNE preparations. These findings indicate that the glutamatergic regulation of presynaptic inputs via glutamate autoreceptors is unlikely.

DNE-mediated increase in mEPSC amplitude: possible mechanisms. As indicated above, under baseline conditions, DNE was associated with a right shift of the normalized mEPSC amplitude distribution (Fig. 3). Although this could be explained by an increase in the number and/or a change in the structure or function of postsynaptic glutamate receptors, our observation that bath application of drugs did not result in a larger or faster inward current in XIIMNs suggests that the shift in the mEPSC amplitude distribution reflects plasticity of the presynaptic release mechanism. Presynaptic release is governed by the neurotransmitter content of presynaptic vesicles (Sulzer and Edwards, 2000), differential vesicle filling and release (Rahamimoff and Fernandez, 1997; Sulzer and Pothos, 2000), and the size of the pore that connects the vesicle and the plasma membrane. Additionally, increased intracellular Ca^{2+} in presynaptic terminals may increase synchronous release of quanta and thus increase mEPSC amplitude (Kirischuk and Grantyn, 2000; Llano et al., 2000), but whether or how DNE alters intracellular calcium kinetics is not clear. Finally, it is plausible that DNE caused a shift towards mEPSCs originating at synaptic sites where quanta are larger. Understanding the molecular

mechanisms behind the DNE-mediated increase in mEPSCs will require thorough investigation, including electron microscopy to confirm structural changes at presynaptic release sites.

We cannot however, confidently exclude the possibility that DNE changed the postsynaptic sensitivity to glutamate to yield larger mEPSCs. Although there were no significant differences between DNE and control preparations in the inward current evoked in XIIMNs by bath-applied agonists, this method of application may obscure changes in postsynaptic sensitivity. Bath application allows compounds to diffuse freely and broadly within the slice affecting multiple neurotransmitter and neuromodulatory systems that may express presynaptic glutamate receptors, which when stimulated could evoke transmitter release (Funk and Greer, 2013). Furthermore, bath application activates all postsynaptic sites simultaneously. We have recently reported that DNE leads to a significant alteration of XIIMN dendritic branching (Powell et al., 2016). Therefore, there may be rearrangement of synaptic locations relative to the cell body, or changes in the subtypes or density of glutamatergic receptors at a subset of postsynaptic sites that lead to increased mEPSC amplitude but not increased overall response of the XIIMNs to bath application of agonists. Our earlier observations using immunocytochemistry revealed an overall reduction in the expression of glutamate receptors in the hypoglossal motor nucleus as a result of DNE (Jaiswal et al., 2013). This may reflect alterations in the branching patterns of XIIMN dendrites that occurs with DNE (Powell et al., 2016). Focal pressure ejection would have confined the drugs to a smaller region, but the inability to precisely replicate drug dose and time of action from cell-to-cell would have created more serious problems in the interpretation of the results.

In conclusion, these experiments contribute to our understanding of how DNE alters the development of excitatory neurotransmission in XIIMNs. Our studies show that previously reported heightened response to microinjection of AMPA or glutamate into the hypoglossal motor nucleus (Jaiswal et al., 2013) may not reflect postsynaptic modification as we hypothesized. Further studies are necessary to elucidate the precise mechanisms underlying this form of nicotine-induced plasticity in the neural networks that control the activity of XIIMNs.

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Table 1. Concentration and duration of drug application used in the experiments outlined in Figure 2. All drugs were dissolved in oxygenated (95% O₂/5% CO₂) aCSF (KCl 9mM), and were bath-applied at 27°C.

Drug(s) Applied	Concentration	Duration (min)
TTX	1 μ M	2 min
AMPA/TTX	2.5 μ M/1 μ M	5 min
CNQX/AMPA/TTX	20 μ M/2.5 μ M/1 μ M	5 min
Glutamate/TTX	200 μ M/1 μ M	5 min
CNQX/Glutamate/TTX	20 μ M/200 μ M/1 μ M	5 min
Wash/TTX	-/ 1 μ M	5 min

Figure Legends

Figure 1. Diagram illustrating possible mechanisms of DNE-induced plasticity of glutamatergic neurotransmission in XIIMNs. (A) The nAChRs on glutamatergic neurons are located on the soma, and also on the presynaptic terminals. When nicotine or ACh binds to nAChRs on the soma of the glutamatergic neuron, sufficient depolarization can evoke action-potential-dependent release of glutamate. When terminal nAChRs are stimulated by nicotine or ACh, vesicular release of glutamate is increased secondary to a rise in intracellular calcium; this reinforces the random, spontaneous vesicular release of glutamate. The XIIMNs express both nAChRs and AMPA receptors. (B) DNE is associated with an increase in nAChRs on both the cell body and terminals, but many of these receptors are in a desensitized state. As explained in the text, the net effect of DNE is a “functional loss” of nAChR-mediated synaptic transmission. This functional loss could lead to both presynaptic (decreased glutamate release) and postsynaptic (upregulation of glutamate receptor expression) plasticity.

Figure 2. (A) Representative voltage-clamp ($V_{\text{hold}} = -70\text{mV}$) recording in a rhythmically active XIIMN demonstrating the timeline of pharmacological treatments. After documenting that the XIIMNs were bursting rhythmically, TTX was applied to block action potential-dependent synaptic transmission, followed by a cocktail of AMPA and TTX, which produced a large inward current. To antagonize the effects of AMPA, a cocktail containing CNQX, AMPA, and TTX was applied, followed by washout in TTX alone. (B) Same as in A, except AMPA was replaced with glutamate in this cell. All drugs were bath-applied. A zoomed-in view of spontaneous respiratory-related bursting is shown in A, while a close up

view of mEPSCs recorded after TTX are shown in B. (C) Zoomed-in view of the mEPSCs recorded after TTX application. For concentrations and durations of drug applications, see Table 1.

Figure 3. Passive properties and quantitative analysis of response to pharmacological activation of postsynaptic ionotropic glutamatergic receptors in XIIMNs. Note that application of AMPA in both groups produced a greater inward current than the application of glutamate. In both experiments addition of CNQX abolished the inward current, suggesting that AMPA-type glutamatergic receptor activation is responsible for the great majority of the inward current that we recorded. All data are represented as the mean $\pm$ SEM. Data were obtained from a total of 11 DNE and 13 control cells in the AMPA experiments, and 13 DNE and 13 control cells in the glutamate experiments. No statistically significant changes between groups were noted.

Figure 4.

Figure 5.

Figure 6. Cumulative fraction of normalized mEPSC amplitudes recorded under baseline conditions (i.e., TTX alone) in 21 control and 19 DNE cells. As indicated in Results, CNQX resulted in the cessation of the mEPSCs confirming that they are glutamatergic. The distribution was shifted to the right in the DNE group, and this difference was statistically significant (*, Kolmogorov-Smirnoff test, $p < 0.0001$). Details on normalization procedure are given in the Methods section.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

AMPA experiments

Glutamate experiments

Figure 6