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# Nicotine-induced neuroplasticity counteracts the effect of schizophrenia-linked neuregulin 1 signaling on NMDAR function in the rat hippocampus

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ABSTRACT

A high rate of heavy tobacco smoking among people with schizophrenia has been suggested to reflect self-medication and amelioration of cognitive dysfunction, a core feature of schizophrenia. NMDAR hypofunction is hypothesized to be a mechanism of cognitive dysfunction, and excessive schizophrenia-linked neuregulin 1 (NRG1) signaling through its receptor ErbB4 can suppress NMDAR function by preventing Src-mediated enhancement of NMDAR responses. Here we investigated whether chronic nicotine exposure in rats by subcutaneous injection of nicotine (0.5–1 mg/kg, twice daily for 10–15 days) counteracts the suppressive effect of NRG1β on NMDAR-mediated responses recorded from CA1 pyramidal cells in acute hippocampal slices. We found that NRG1β, which prevents the enhancement of NMDAR responses by the Src-family-kinase-activating peptide pYEEI in naive rats, failed to block the effect of pYEEI in nicotine-exposed rats. In naive rats, NRG1β acts only on GluN2B-NMDARs by blocking their Src-mediated upregulation. Chronic nicotine exposure causes enhanced GluN2B-NMDAR responses via Src upregulation and recruits Fyn for the enhancement of GluN2A-NMDAR responses. NRG1β has no effect on both enhanced basal GluN2B-NMDAR responses and Fyn-mediated enhancement of GluN2A-NMDAR responses. Src-mediated enhancement of GluN2B-NMDAR responses and Fyn-mediated enhancement of GluN2A-NMDAR responses initiate long-term potentiation (LTP) of AMPAR synaptic responses in naive and nicotine-exposed CA1 pyramidal cells, respectively. These results suggest that NRG1β suppresses LTP by blocking Src-mediated enhancement of GluN2B-NMDAR responses, but has no effect on LTP in nicotine-exposed rats. These effects of chronic nicotine exposure may counteract the negative effect of increased NRG1-ErbB4 signaling on the cellular mechanisms of learning and memory in individuals with schizophrenia, and therefore may motivate heavy smoking.



## 1. Introduction

Despite the fact that smoking tobacco causes serious health problems, many people continue to smoke, and individuals suffering from schizophrenia or other mental illnesses that can cause cognitive deficits are particularly likely to be heavy smokers (Ziedonis et al., 2008). Understanding the mechanistic basis of this association may provide novel insights into the etiology and treatment of cognitive dysfunction.

Chronic nicotine exposure, which improves hippocampal memory (Levin et al., 1992; Kenney and Gould, 2008), acts via Src tyrosine kinase signaling to enhance the response of *N*-methyl-D-aspartate receptors (NMDARs) in CA1 pyramidal cells (Yamazaki et al., 2006a, b). Interestingly, this effect of nicotine is mimicked by two acetylcholinesterase inhibitors, donepezil and galantamine (Ishibashi et al., 2014), which are currently used for treatment of cognitive deficits associated with Alzheimer's disease (AD). Furthermore, administration of these cholinergic drugs has also

*Abbreviations:* ACSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; AMPAR, α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor; AP5, 2-amino-5-phosphonovaleric acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EPSCs, excitatory postsynaptic currents; EPSPs, excitatory postsynaptic potentials; LTP, long-term potentiation; nAChR, nicotinic acetylcholine receptors; NMDAR, *N*-methyl-D-aspartate receptor; NRG1, neuregulin 1; NRG1β, neuregulin 1β; PBS, phosphate-buffered saline; PSD-95, post-synaptic density protein 95 kD; SFK, Src-family kinase.

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been shown to improve cognition in schizophrenic patients (Levin et al., 2006; Gray and Roth, 2007; Buchanan et al., 2008). Thus, the observed enhancement of NMDAR function might be responsible for the enhanced cognition in AD and schizophrenic patients.

The two major NMDAR subtypes, GluN2A-NMDAR and GluN2B-NMDAR, exist as a macromolecular complex (Husi et al., 2000) and their function can be affected by interactions among many proteins linked to various pathways (Salter and Kalia, 2004). This macromolecular complex appears to be a convergence point for schizophrenia susceptibility genes and pathways that may affect NMDAR function and signaling (Hahn, 2011; Snyder and Gao, 2013). Indeed, increasing evidence indicates that NMDAR hypofunction in the hippocampus-prefrontal cortex pathway is a potential mechanism underlying cognitive dysfunction in schizophrenia patients (Snyder and Gao, 2013). Among schizophrenia susceptibility genes and pathways, excessive neuregulin 1 (NRG1)-ErbB4 signaling has been shown to participate in NMDAR hypofunction (Hahn et al., 2006; Shamir et al., 2012) by preventing Src-mediated enhancement of NMDAR function (Pitcher et al., 2011; Salter and Pitcher, 2012). Thus, Src is a point of convergence for both nicotine-initiated and schizophrenia-linked signaling, and the effect of schizophrenia-linked signaling is the exact opposite of what occurs with chronic nicotine exposure. In this study, we investigated whether nicotine-induced neuroplasticity counteracts the suppressive effects of the schizophrenia-linked signaling on NMDAR function.

## 2. Materials and methods

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with protocols approved by the Institutional Animal Care and Use Committee of the University of California at Irvine and the Animal Research Committees of Yamagata University.

### 2.1. Chronic nicotine treatment

The current study was designed based on our previous results demonstrating that chronic nicotine exposure in Sprague-Dawley rat pups (starting at around postnatal day 21) by subcutaneous injection of nicotine (0.5–1 mg/kg nicotine base, twice daily for 10–15 days) causes the enhancement of NMDAR-mediated responses via Src upregulation in adolescence (Yamazaki et al., 2006a, b; Ishibashi et al., 2014). Therefore, to achieve the same effect of nicotine in adolescence, the same treatment regimen was used in the present study. Smoking several cigarettes delivers an acute dose of 60–300 nM nicotine in the venous blood (Benowitz et al., 1990) and approximately 600 nM nicotine in the arterial blood (Henningfield et al., 1993), which better represents the level of nicotine in the brain. Plasma concentrations of nicotine reach peak values (2.2 μM) within 5–10 min after nicotine (1 mg/kg) administration into the femoral vein of rats, decrease to 0.9 μM 20 min after administration and are maintained at this level for the next 40 min (Sastry et al., 1995). Because the plasma half-life of nicotine in rats is about 45 min and it is about 2 h in humans (Matta et al., 2007), the dose used for subcutaneous injection (intended to be absorbed slowly) in the current study should produce blood levels of nicotine similar to those found in heavy smokers. Nicotine injection induced seizures in most of the pups, which were usually brief, and the effect of nicotine became progressively weaker by continuing daily injection of nicotine. Nicotine-induced enhancement of NMDAR-mediated responses was mimicked by a muscarinic ACh receptor agonist and acetylcholinesterase inhibitors without inducing seizures, and co-administration of the m1 antagonist pirenzepine prevents the effect of nicotine without blocking nicotine-induced seizures (Ishibashi et al., 2014). These observations suggest that the effect of nicotine is mediated by increased release of ACh via the activation of nicotinic acetylcholine receptors (nAChRs) and involves m1 muscarinic receptor activation through ACh, but not nicotine-induced seizures.

### 2.2. Electrophysiological recording

Ninety minutes after the last injection of phosphate-buffered saline (PBS) or nicotine, transverse hippocampal slices were prepared and maintained at 30–32 °C in artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.25 $NaH_2PO_4$, 2 $MgSO_4$, 2.5 $CaCl_2$, 22 $NaHCO_3$, and 10 glucose, and oxygenated with 95% $O_2$ and 5% $CO_2$. Although in the present study we harvested brains approximately 90 min after the last nicotine injection, the enhanced NMDAR-mediated responses were also observed in hippocampal slices from brains harvested 12 h after the last nicotine injection, suggesting that the observed change in NMDAR function is not an acute effect of nicotine. Furthermore, the enhanced NMDAR responses were also observed in slices kept for 6 h in ACSF (Yamazaki et al., 2006b; Ishibashi et al., 2014), suggesting that the effects elicited in vivo are maintained in slices.

We made whole-cell patch-clamp recordings from CA1 pyramidal cells from control (naive or PBS-treated) and nicotine-exposed rats. Pyramidal cells were visualized for whole-cell recording with an infrared differential interference contrast microscope (Axioskop, Zeiss, Germany), using a 40 × water-immersion objective. Patch electrodes were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL, USA) using a micropipette puller (P-97, Sutter Instrument, Novato, CA, USA). The electrodes had a resistance of 5–7 MΩ after being filled with pipette solution containing (in mM) 117 Cs-methanesulfonate, 10 HEPES, 0.5 EGTA, 2.8 NaCl, 5 TEA-Cl, 5 QX-314, 2.5 Mg-ATP, and 0.3 Na-GTP, adjusted to pH 7.3 with CsOH. For current-clamp recordings, pipette solution contained (in mM) 140 K-gluconate, 10 HEPES, 0.5 EGTA, 10 NaCl, 1 $MgCl_2$, 2 Mg-ATP, 0.2 Na-GTP, and 5 QX-314, adjusted to pH 7.3 with KOH. Excitatory synaptic responses were evoked by stimulation of the Schaffer collateral input to CA1 pyramidal cells. NMDAR-mediated excitatory postsynaptic currents (EPSCs) were recorded from CA1 pyramidal cells voltage-clamped at −30 to −40 mV in the presence of the non-NMDAR antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM), and the $GABA_A$ receptor antagonist, bicuculline (10 μM) as described previously (Yamazaki et al., 2006a, b). The peptides [Src ($p60^{c-Src}$, Upstate, Charlottesville, VA, USA), Src-family activating phosphopeptide (pYEEI; Invitrogen-Biosource, Camarillo, CA, USA), Src inhibitor peptide (40–58) (custom synthesis), Fyn (Millipore, Dundee, UK and Signalchem, Richmond, Canada), Yes (Signalchem, Richmond, Canada), and Lyn (Signalchem)] were directly applied into the pyramidal cells by diffusional exchange through the patch pipettes and neuregulin 1β (NRG1β) (Shenandoah Biotechnology, Warwick, PA, USA) was bath-applied. To monitor the change of the synaptic NMDAR-mediated EPSCs, the mean amplitudes recorded 30–35 min after establishment of whole-cell configuration in the absence or presence of peptide were calculated and expressed as a percentage of the mean amplitudes during the first 5–10 min (baseline responses). When current responses did not stabilize within 10 min after achievement of whole-cell configuration, the experiments were stopped and the data were discarded.

AMPAR-mediated excitatory postsynaptic potentials (EPSPs) were recorded from current-clamped pyramidal cells to monitor LTP induction in the presence of 10 μM bicuculline using hippocampal slices with a surgical cut made between the CA1 and CA3 regions as described previously (Yamazaki et al., 2006b), and the intensity of stimulation was set to produce about 30% of maximum