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Research Report

# Recovery of network-driven glutamatergic activity in rat hippocampal neurons during chronic glutamate receptor blockade

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ABSTRACT

Previous studies indicated that a long-term decrease in the activity of ionotropic glutamate receptors induces cholinergic activity in rat and mouse hypothalamic neuronal cultures. Here we studied whether a prolonged inactivation of ionotropic glutamate receptors also induces cholinergic activity in hippocampal neurons. Receptor activity was chronically suppressed in rat hippocampal primary neuronal cultures with two proportionally increasing sets of concentrations of NMDA plus non-NMDA receptor antagonists: 100 μM/10 μM AP5/CNQX (*1X cultures*) and 200 μM/20 μM AP5/CNQX (*2X cultures*). Using calcium imaging we demonstrate that cholinergic activity does not develop in these cultures. Instead, network-driven glutamate-dependent activity, that normally is detected in hyper-excitable conditions, reappears in each culture group in the presence of these antagonists and can be reversibly suppressed by higher concentrations of AP5/CNQX. This activity is mediated by non-NMDA receptors and is modulated by NMDA receptors. Further, non-NMDA receptors, the general level of glutamate receptor activity and CaMK-dependent signaling are critical for development of this network-driven glutamatergic activity in the presence of receptor antagonists. Using electrophysiology, western blotting and calcium imaging we show that some neuronal parameters are either reduced or not affected by chronic glutamate receptor blockade. However, other parameters (including neuronal excitability, mEPSC frequency, and expression of GluR1, NR1 and βCaMKII) become up-regulated and, in some cases, proportionally between the non-treated, 1X and 2X cultures. Our data suggest recovery of

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*Abbreviations:* 1X, 100 μM/10 μM AP5/CNQX concentrations or chronically treated culture condition; 2X, 200 μM/20 μM AP5/CNQX concentrations or chronically treated culture condition; 5X, 500 μM/50 μM AP5/CNQX concentrations or chronically treated culture condition; ACh, acetylcholine; AP5, D,L-2-amino-5-phosphonovalerate; CaMK, $Ca^{2+}$/calmodulin-dependent protein kinases; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DIV, day *in vitro*; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; $GABA_AR$, γ-aminobutyric acid A receptor; mEPSC, miniature excitatory postsynaptic current; NMDA, *N*-methyl-D-aspartate; PKA, cAMP-dependent protein kinase; TTX, tetrodotoxin


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the network-driven glutamatergic activity after chronic glutamate receptor blockade. This recovery may represent a form of neuronal plasticity that compensates for the prolonged suppression of the activity of glutamate receptors.



## 1. Introduction

Specification of neurotransmitter phenotype is critical for neural circuit development and is influenced by intrinsic and extrinsic factors, including calcium signaling, cascades of transcription factors and target-derived neurotrophic factors (Goridis and Rohrer, 2002; Landis, 1996; Spitzer et al., 2004). Our previous findings in the rat and mouse hypothalamus *in vitro* and *in vivo* suggested the role of neurotransmitter glutamate in the regulation of cholinergic phenotype in neurons (Belousov et al., 2001; Belousov et al., 2002; Liu et al., 2008). The studies demonstrated that a prolonged (2–3 week) inactivation of ionotropic glutamate receptors increases the number of cholinergic neurons, the expression of acetylcholine (ACh) receptors, and induces ACh-dependent excitatory synaptic activity (Belousov et al., 2001; Belousov et al., 2002). The data suggested the importance of *N*-methyl-D-aspartate (NMDA) receptors and $Ca^{2+}$-dependent signaling in the cholinergic up-regulation and indicated that cholinergic phenotypic properties are induced in glutamate-secreting neurons (Belousov et al., 2002; Liu et al., 2008). In addition, the data implied that the mechanisms for cholinergic regulation have region-specific character. For example, the blockade of ionotropic glutamate receptors induces ACh-dependent excitatory activity in neuronal cultures prepared from the hypothalamus and cerebellum, but not from the neocortex (Belousov et al., 2001; Belousov et al., 2002). We speculated that glutamate-dependent regulation of cholinergic properties contributes to the neurotransmitter phenotype specification in developing neurons (Liu et al., 2008) and also represents a form of compensatory regulation that is expressed in both developing and mature neurons during the prolonged decrease in glutamate synaptic transmission (Belousov et al., 2001).

Here we extended our studies to determine whether or not a prolonged inactivation of ionotropic glutamate receptors also induces ACh-dependent excitatory activity in hippocampal neuronal cultures and, if not, whether other forms of compensatory regulation become expressed. We found that cholinergic activity does not develop in these cultures. Instead, network-driven glutamate-dependent activity, that normally is detected in hyper-excitable conditions, reappears in neurons in the presence of glutamate receptor antagonists. Given that inactivation of glutamate receptors induces homeostatic changes in glutamate-dependent activity in neuronal networks (Thiagarajan et al., 2002; Thiagarajan et al., 2005; Turrigiano, 1998; Turrigiano et al., 2007) and glutamate receptor antagonists are considered for the use in clinical conditions, we studied the dynamics of reappearance of glutamatergic activity during prolonged inactivation of glutamate receptors and the mechanisms responsible for this reappearance.

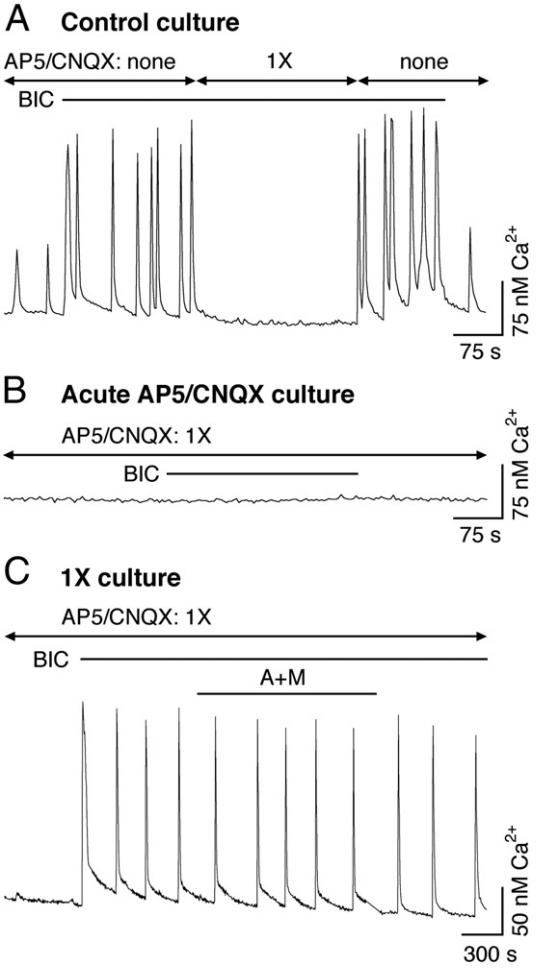


**Fig. 1 – ACh-dependent activity does not develop in hippocampal neuronal cultures after chronic glutamate receptor blockade. Representative $Ca^{2+}$ imaging recordings are shown. (A) Application of bicuculline (BIC) to this non-treated neuron induces large amplitude intracellular $Ca^{2+}$ oscillations that are glutamatergic in nature and are suppressed by AP5/CNQX (also note the presence of low-frequency spontaneous $Ca^{2+}$ oscillations in the background recording that are AP5/CNQX-sensitive too). (B) No bicuculline-mediated $Ca^{2+}$ oscillations are detected in another non-treated neuron shortly after AP5/CNQX administration. (C) The bicuculline-mediated oscillations are expressed in 1X cultures in the continued presence of 100/10 μM AP5/CNQX. These oscillations are not suppressed by ACh receptor antagonists, atropine plus mecamylamine (A+M; 100 μM each) and, therefore, they are not cholinergic in origin. Here and in the following figures, applications of drugs are indicated by bars or arrowed bars. Concentrations: none AP5/CNQX, 0/0 μM; 1X AP5/CNQX, 100/10 μM; BIC, 50 μM.**