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SPATIO-TEMPORAL CHOLINERGIC MODULATION IN CULTURED NETWORKS OF RAT CORTICAL NEURONS: SPONTANEOUS ACTIVITY

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Abstract—Activation of the cholinergic innervation of the cortex has been implicated in sensory processing, learning, and memory. At the cellular level, acetylcholine both increases excitability and depresses synaptic transmission, and its effects on network firing are hard to predict. We studied the effects of carbachol, a cholinergic agonist, on network firing in cultures of rat cortical neurons, using electrode arrays to monitor the activity of large numbers of neurons simultaneously. These cultures show stable spontaneous synchronized burst firing which propagates through dense synaptic connections. Carbachol (10–50 μM), acting through muscarinic receptors, was found to induce a switch to asynchronous single-spike firing and to result in a loss of regularity and fragmentation of the burst structure. To obtain a quantitative measure of cholinergic actions on cortical networks, we applied a cluster Poisson-process model to sets of paralleled spike-trains in the presence and absence of carbachol. This revealed that the time series can be well-characterized by such a simple model, consistent with the observed $1/f^b$-like spectra ($0.04<b<2.08$). After applying higher concentrations of carbachol the property of the spectra shifted toward a Poisson-process (white) spectrum. These results indicate that cholinergic neurotransmitters have a strong and reliable desynchronizing action on cortical neural activity.

Key words: carbachol, cluster Poisson-process, multi electrode array, 1/*f* fluctuation.

Cortical neurons *in vivo* are continuously bombarded with synaptic inputs, which result from spontaneous activity in the cortical network (Destexhe et al., 2003 and references therein). This background activity *in vivo* causes large conductance changes in the membrane and large-amplitude membrane potential fluctuations (Matsumura et al., 1988; Steriade et al., 2001), so that it modulates not only response variability of spike trains (Shadlen and Newsome, 1998), but also the input/output gain functions (Chance et al., 2002; Mitchell and Silver, 2003) and signal detection sensitivities of neural responses (Hô and Destexhe, 2000). It is well known that the membrane excitability and firing properties of cortical neurons can be significantly modulated by neuromodulators such as acetylcholine (McCormick, 1993) and dopamine (Eytan et al., 2004), which are absent from most *in vitro* preparations (Hasselmo, 1995). Although the exact effects of these neuromodulators are poorly understood, it is likely that, in addition to direct effects on neural responses (Barkai and Hasselmo, 1994), they exert a strong effect on individual neurons through modulation of the spontaneous network activity.

Cholinergic neurons in the basal forebrain provide a diffuse innervation to all cortical regions, and their activation modulates neuronal activity in the cortex (Richardson, 1991). The operation of this cortical cholinergic system has been implicated in a number of important physiological functions, including arousal (Steriade, 2003), sensory processing (Tang et al., 1997; Julianno, 1998), learning (Gorman and Charles, 1991) and memory (Hasselmo et al., 1992; Patil et al., 1998; Kirkwood et al., 1999). The dementia associated with Alzheimer's disease might be related to loss of the cortical cholinergic innervation (Coyle et al., 1983; Sarter et al., 1997). The cortical cholinergic system also plays a critical role in the development of the cortex, since its disruption severely alters the normal development of the cortex (Hohmann and Berger-Sweeney, 1998). In order to understand the mechanisms of these phenomena, it will be necessary to understand how cholinergic agonists modulate concerted firing patterns in cortical circuits.

The actions of cholinergic agonists on individual cortical neurons are now understood in some detail. Activation of muscarinic receptors in cortical neurons, for example, results in the following effects: (i) blockade of calcium-activated potassium currents, of slow voltage-activated potassium ("M") currents, of transient, inactivating potassium ("A") currents, and of non-voltage-dependent "leak" currents (Brown and Adams, 1980; McCormick and Prince, 1986; reviewed in McCormick, 1993), (ii) reduction in voltage-dependent sodium current (Klink and Alonso, 1997), (iii) reduction of several types of calcium current (Allen and Brown, 1993; Howe and Surmeier, 1995; Stewart et al., 1999), (iv) suppression of quantal GABA release, (v) presynaptic inhibition of excitatory transmission (Williams and Constanti, 1988; Hasselmo and Cekic, 1996; Hasselmo and Fehlau, 2001), (vi) postsynaptic suppression of excitatory and inhibitory synapses (Kimura and Baughman, 1997), (vii) prolonged depolarization of some classes of interneurons (Benardo and Prince, 1982; Benardo, 1993),

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Abbreviations: AFR, average firing rate; BW, watching birds; CCh, carbachol; DIV, days *in vitro*; ISI, interspike-interval; LTD, long term depression; LTP, long-term potentiation; MCh, acetyl-beta-methylcholine; MRF, mesencephalic reticular formation; NMDA, *N*-methyl-D-aspartate; PCP, Poisson cluster-process; PS, paradoxical sleep; S.D., standard deviation; S.E., standard error.

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(viii) reduction of Ca^{2+}-dependent slow afterdepolarizing potentials (Klink and Alonso, 1997). Together, these effects produce depolarization, enhanced excitability and reduction in adaptation of spiking of cortical pyramidal cells during long-lasting stimuli. Nicotonic receptors mediate an excitatory synaptic conductance in some cholinergic target cells, including cortical interneurons which secrete vasoactive intestinal peptide, which are believed to control cerebral blood flow (Porter et al., 1999).

However, the synaptic and network effects of cholinergic activation are much less clear. In olfactory cortex, cholinergic activation appears to facilitate LTP (Patil et al., 1998), but to depress synaptic transmission (Hasselmo et al., 1992) and to facilitate LTD in superficial layers (Kirkwood et al., 1999). Kimura and Baughman (1997) have shown that inhibitory transmission and excitatory transmission are both depressed by acetylcholine, but through different subtypes of muscarinic receptor. Cholinergic inputs have several specific actions during early development, including fast, nicotinic excitatory synaptic transmission (Roerig et al., 1997), and a selective enhancement of NMDA-receptor mediated synaptic transmission (Aramakis and Metherate, 1998). A differential action of acetylcholine on two different classes of inhibitory neuron, exciting vertically projecting low-threshold spike cells nicotinically, and inhibiting laterally-projecting fast-spiking cells muscarinically, has led to the suggestion that cholinergic activation of the cortex reduces intralaminar inhibition and promotes intracolumnar inhibition (Xiang et al., 1998). The excitation associated with blocking M-current is often preceded or mitigated by an inhibition, which probably results from excitation of neighboring GABAergic interneurons in the network (McCormick and Prince, 1986). In cortical slices disinhibited by blocking $GABA_A$ receptors with bicuculline, cholinergic agonists promote a theta frequency oscillation (Lukatch and MacIver, 1997), as in rat hippocampus *in vivo* (Cobb et al., 1999). Thus, cholinergic activation, which appears to involve a diffuse release of acetylcholine over wide areas of cortex, has both excitatory actions on cells and broadly inhibitory actions on synapses, and its effects on network activity are hard to predict.

To gain insight into chemical and electrical actions on cortical network activity, a useful model system has been network firing in cultures of rat cortical neurons, using electrode arrays to monitor the activity of large numbers of neurons simultaneously. Such cultures show both evoked and stable spontaneous synchronized burst firing (Robinson et al., 1993; Kamioka et al., 1996) whose propagation is mediated through glutamatergic synaptic projections and modulated by GABAergic projections, and which initiates at multiple sites in the network (Maeda et al., 1995). The strength of this correlated firing is controlled by LTP and LTD in the network (Jimbo et al., 1999); for review, see Marom and Shahaf (2002). It is also known that at early stages of development in cortical cultures, time series of spontaneous burst firing are well-described by simple point processes (Tateno et al., 2002). Here, we study the effects of cholinergic activation on the properties of spontaneously synchronized population bursting, applying the nonhydrolyzable cholinergic agonist, carbachol (CCh), to cortical cultures. To characterize concerted firing properties in the presence and absence of CCh, we examined the temporal and spectral structure of spike trains, using the Poisson cluster-process (PCP) model. This quantitative characterization should help to elucidate the complex actions of cholinergic modulation in the intact cortical networks.

EXPERIMENTAL PROCEDURES

Whole-cell patch recording

Whole-cell current-clamp and voltage-clamp recordings were carried out by a standard method (Sakmann and Stuart, 1995) using a patch-clamp amplifier (Axopatch 1-D; Axon Instruments USA). Whole-cell recording pipettes (Clark GC150T-10 Clark Electromedical Instruments, UK) were filled with the standard intracellular solution: 125-mM potassium methylsulfonate, 5-mM, 10-mM HEPES, 10-mM glucose, 0.2-mM EGTA, 4.5 mM Na-ATP and 0.5 mM Na-GTP (pH 7.2). The pipette electrode resistance was between 4 and 5 MΩ. Data were acquired using pipettes whose series resistance was less than 10 MΩ and series resistance compensation was used.

Electrode arrays and recording setup

The spatial characteristics of network activity were resolved by using electrode-array substrates, which provide high time resolution, are noninvasive, and can be used for long-term recording (Gross, 1979; Pine, 1980; Jimbo et al., 1993). The recording setup was the same as that described by Jimbo et al. (1999, 2003). Since the main frequency components of extracellular spike signals lie between 100 Hz and 6 kHz (Najafi, 1994), sampling at 25 kHz with 16-bit resolution was used for each channel. The arrays had 64 recording terminals within a 1.6×1.3-mm area, and consisted of two rectangular blocks of terminals separated by 500 μm (Jimbo et al., 1999; Tateno and Jimbo, 1999). Each recording terminal in the array covered an area of 30×30 μm square, and the distance between the centers of adjacent sites was 180 μm.

Cell culture

We used a slight modification of the method of Muramoto et al. (1988). Briefly, cortical tissue of E17-18 Wistar rat embryos was finely chopped, digested with 0.02% papain (Boehringer), and mechanically dissociated using trituration. Cells were plated on substrates coated with laminin and poly-D-lysine (Sigma) and cultured in Dulbecco's modified Eagle's medium (Gibco) containing 5% FBS (Hy-clone), 5% heat-inactivated horse serum (Gibco), 2.5 mg/ml insulin (Sigma), and penicillin-streptomycin (5–40 U/ml, Sigma). The medium was conditioned overnight in glia cell cultures prior to use (Baughman et al., 1991), and was changed twice a week. In this paper, all the activity in the cultured networks was recorded from the 40th to the 57th day *in vitro* (DIV). During development the electrophysiological properties of cortical neurons change according to the expression of receptors and voltage-dependent ion channels (Luhmann and Prince, 1991; Burgard and Hablitz, 1993), and rat neocortical neurons show stable patterns of continuous synchronized firing during the period of culture used in the present study (Kamioka et al., 1996; Watanabe et al., 1996). The day before each recording session, the culture medium was exchanged for one without insulin and penicillin–streptomycin.

Estimation of connectivity

Connectivity in the intact cortex is high, and it is estimated that each neuron makes several thousands of connections to other