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In vitro and *in vivo* measures of evoked excitatory and inhibitory conductance dynamics in sensory cortices

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Abstract

In order to better understand the synaptic nature of the integration process operated by cortical neurons during sensory processing, it is necessary to devise quantitative methods which allow one to infer the level of conductance change evoked by the sensory stimulation and, consequently, the dynamics of the balance between excitation and inhibition. Such detailed measurements are required to characterize the static versus dynamic nature of the non-linear interactions triggered at the single cell level by sensory stimulus. This paper primarily reviews experimental data from our laboratory based on direct conductance measurements during whole-cell patch clamp recordings in two experimental preparations: (1) *in vitro*, during electrical stimulation in the visual cortex of the rat and (2) *in vivo*, during visual stimulation, in the primary visual cortex of the anaesthetized cat. Both studies demonstrate that shunting inhibition is expressed as well *in vivo* as *in vitro*. Our *in vivo* data reveals that a high level of diversity is observed in the degree of interaction (from linear to non-linear) and in the temporal interplay (from push–pull to synchronous) between stimulus-driven excitation (*E*) and inhibition (*I*). A detailed analysis of the *E*/*I* balance during evoked spike activity further shows that the firing strength results from a simultaneous decrease of evoked inhibition and increase of excitation. Secondary, the paper overviews the various computational methods used in the literature to assess conductance dynamics, measured in current clamp as well as in voltage clamp in different neocortical areas and species, and discuss the consistency of their estimations.

Keywords: Excitation; Inhibition; Conductance measurement; Cat visual cortex; Voltage-clamp; *In vivo* patch clamp

1. Introduction

A basic feature in the connectivity of neocortical networks is the profusion of synaptic contacts, established both locally within a given cortical area and across distinct cortical areas (White, 1989). Each pyramidal neuron (the major type of excitatory cell in cortex) receives approximately 10^4 synaptic inputs, of which about 75% are excitatory and 25% inhibitory. Recurrent connectivity between pyramidal cells is expressed, within a given cortical lamina as well as across laminae, as a dense plexus of local horizontal and vertical interconnections. GABAergic inhibitory interneurons, although far less numerous, but having multiple subtypes, seem to control the dynamics of this unstable recurrent excitatory assembly at various target locations (review in Markram et al., 2004; Monyer and Markram, 2004; Silberberg and Markram, 2007). In addition to this dominant interlaced pattern of excitation and inhibition originating from the cortex itself, subsets of both types of cells are directly innervated by excitatory thalamic relay neurons, which are the main source of extrinsic input to the neocortex (Binzegger et al., 2004). Axons from the thalamus make stronger and more frequent excitatory connections onto inhibitory interneurons than onto excitatory cells, and their activation produces robust disynaptic feedforward inhibition of cells that receive concomitant direct thalamocortical excitation (Agmon and Connors, 1992; Cruikshank et al., 2007; Gil and Amitai, 1996). One might therefore expect that the selective firing of any single neuron is the concerted result at any point in time of the dynamic balance between a large numbers of co-active synaptic afferents, mostly intrinsic to cortex.

Indeed, intracellular recordings *in vivo* have revealed consistently that cortical neurons are subjected to an intense ongoing synaptic bombardment (Azouz and Gray, 1999; Bringuier et al., 1997; Paré et al., 1998). Although differences were observed

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depending on the type of anaesthetic used, the resting conductance is generally higher in the intact brain than in partially deafferented networks *in vitro* (review in Destexhe et al., 2003). Thus, neocortical networks most likely operate in a 'high-conductance' state, i.e. with a leak conductance three to five times larger than the resting synaptic conductance (Paré et al., 1998 but see Waters and Helmchen, 2006). This, in turn, is expected to change the integrative properties of the neurons (Bernander et al., 1991; Destexhe and Paré, 1999; Rudolph and Destexhe, 2003), by reducing the apparent membrane time constant and allowing faster transients in membrane potential dynamics.

An important issue in the mammalian sensory neocortex is to determine the functional impact of this high conductance resting state on the processing of sensory information itself. Since the first intracellular recordings in visual cortex (Creutzfeldt and Ito, 1968; Innocenti and Fiore, 1974), many electrophysiological studies have shown that the membrane potential strongly fluctuates in response to visual stimuli. However, no definite canonical generative mechanism has been yet identified since these fluctuations could potentially result from the interplay of a diversity of conductances. For instance, the push–pull arrangement hypothesized in Simple receptive fields supposes that an increase in excitation will correspond to an in-phase decrease in inhibition, and vice versa (Ferster, 1988; Heggelund, 1986). In contrast, the dominance of recurrent circuit architecture predicts that most of the time excitation and inhibition should occur conjointly (Ben-Yishai et al., 1995; Douglas et al., 1995; Somers et al., 1995; Suarez et al., 1995). In addition, these models of visual cortex suggest that response selectivity can arise from recurrent networks operating at high gain. However, such networks operate close to instability and respond slowly to rapidly changing stimuli. Theoretical studies show that divisive inhibition, acting through interneurons that are themselves divisively inhibited, can stabilize network activity for any arbitrarily large excitatory coupling (Chance and Abbott, 2000).

From a theoretical computational perspective, two alternative regimes may be envisioned: (1) the total input conductance of the cell does not change significantly during sensory stimulation. In this case, the ratio between the evoked synaptic conductance and the resting conductance is low or negligible, the excitatory and inhibitory currents add algebraically and the input integration process may be considered as linear; (2) the evoked synaptic conductance increase is in the same range or larger than the resting conductance, leading to a regime where excitatory and inhibitory synaptic inputs interact non-linearly. In other words, if the evoked synaptic input fluctuations are small when compared to the resting conductance, the inputs can be modeled as currents; in the opposite case, the conductance increases must be taken into account.

The amplitude of depolarization and/or hyperpolarization in the evoked voltage response, when recorded in current clamp mode, results from the combined integration of both excitatory and inhibitory inputs and depends on multiple parameters: the voltage at rest, the time constant of the membrane, the leak conductance, the amplitude of excitatory and inhibitory conductances, their kinetics and the degree of temporal overlap between their respective recruitment, as well as their reversal potentials. In order to understand the nature of the full integration process, it is thus necessary to devise methods that allow one to infer, from the current or voltage recordings: (1) the dynamics of the balance between excitation and inhibition (*E*/*I*), (2) the level of conductance increase evoked by the sensory stimulation, and (3) if possible, to characterize the static versus dynamic nature of the non-linear interaction process.

Consensus on these points has been hindered up to now by the fact that different methods have been used *in vitro* and *in vivo* to estimate the *E*/*I* balance, and seldom compared together. A classical method, mostly applicable *in vitro*, consists of dissecting out pharmacologically the excitation from the inhibition and thereafter comparing the relative amplitudes of the remaining components (for example Varela et al., 1999). The disadvantage of this method is that the diffuse blockade of a class of receptors by antagonist bath application disrupts the integrity of the network under study and ignores the impact of all types (pre–pre, pre–post) of interactions between excitation and inhibition. *In vivo* studies rarely rely on iontophoretic approaches (but see Nelson et al., 1994; Sillito, 1975) but usually infer the dominant presence of inhibition and excitation from the peak amplitudes of evoked hyperpolarization and/or depolarisation, respectively (Berman et al., 1991; Ferster, 1986; Pei et al., 1994; Volgushev et al., 1993). Such approaches have been unable to detect the presence of inhibition when concurrent with excitation (see, for a systematic comparative survey, Monier et al., 2003). Thus, although the membrane potential change may reflect in a qualitative way the ratio between excitation and inhibition, it remains impossible from knowledge solely of the mean membrane potential dynamics to deduce the amplitude of the global input conductance change, this measure being crucial to understand the dynamic regime under which the neuronal network operates.

A quantification step, reflecting more directly the functional impact of synaptic input on the spike trigger mechanism, is to measure conductance changes seen at the soma. Detailed simulations have shown that the increase in conductance due to the activity of inhibitory basket cells should be visible from the cell body of pyramidal cells (Koch et al., 1990). These authors estimated that the shunting inhibitory effect would significantly reduce the amplitude of the excitatory postsynaptic potential for somatic input conductance increases larger than 30%. Experimentally, evidence for or against shunting inhibition is still a matter of debate. As early as 40 years ago, large increases in input conductance (up to 300%) were demonstrated in cortical neurons (Dreifuss et al., 1969), both after electrical stimulation of the cortical surface and during exogenous iontophoretic application of GABA. Nevertheless, the first measurements of input conductance performed *in vivo*, using current pulse injection or electrical stimulation of thalamic afferents, revealed only limited relative changes in input conductance (5–20%) during visual stimulation (Berman et al., 1991; Carandini and Ferster, 1997; Douglas et al., 1988; Ferster and Jagadeesh, 1992; Pei et al., 1991). These negative reports were not in agreement with findings *in vitro* where Berman et al. (1989, 1991), using the