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SUMMATION OF EXCITATORY POSTSYNAPTIC POTENTIALS IN ELECTRICALLY-COUPLED NEURONES

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Abstract—Dendritic electrical coupling increases the number of effective synaptic inputs onto neurones by allowing the direct spread of synaptic potentials from one neurone to another. Here we studied the summation of excitatory postsynaptic potentials (EPSPs) produced locally and arriving from the coupled neurone (transjunctional) in pairs of electrically-coupled Retzius neurones of the leech. We combined paired recordings of EPSPs, the production of artificial excitatory postsynaptic potentials (APSPs) in neurone pairs with different coupling coefficients and simulations of EPSPs produced in the coupled dendrites. Summation of the EPSPs produced in the dendrites was always linear, suggesting that synchronous EPSPs are produced at two or more different pairs of coupled dendrites and not in both sides of any one gap junction. The different spatio-temporal relationships explored between pairs of EPSPs or APSPs produced three main effects. (1) Synchronous pairs of EPSPs or APSPs exhibited an elongation of their decay phase compared to singe EPSPs. (2) Asymmetries in the amplitudes between the pair of EPSPs added a "hump" to the smallest EPSP. (3) Modelling the inputs near the electrical synapse or anticipating the production of the transjunctional APSP increased the amplitude of the compound EPSP. The magnitude of all these changes depended on the coupling coefficient of the neurones. We also show that the hump improves the passive conduction of EPSPs by adding low frequency components. The diverse effects of summation of local and alien EPSPs shown here endow electrically-coupled neurones with a wider repertoire of adjustable integrative possibilities.

Key words: synaptic integration, EPSP, electrical synapse, summation, leech.

Dendritic electrical coupling increases the number of effective synaptic inputs onto neurones of vertebrates and invertebrates by allowing the flow of chemically-induced synaptic potentials from the coupled neurones (Marder and Eisen, 1984; De-Miguel et al., 2001; Zsiros et al., 2007). This transjunctional flow of synaptic potentials contributes to the production of action potentials in the coupled neurone (Garcia-Perez et al., 2004), synchronous firing (Zsiros et al., 2007) and to the regulation of the timing in networks producing rhythmic activity (Marder and Eisen, 1984). In a similar and well-known phenomenon, the flow of photoreceptor-evoked responses between electrically coupled horizontal cells in the retina of vertebrates produces an extension of the visual field (Naka and Rushton, 1967; Kaneko, 1971). For these reasons, it is predictable that the redistribution of dendritic synaptic currents across electrical synapses causes the summation of local and alien currents arriving from the coupled neurone. Although many effects of this type of summation may be predicted on the basis of passive cable theory (Bennett, 1977), a detailed description of transjunctional excitatory postsynaptic potential (EPSP) summation in an experimental preparation displaying the phenomenon remains unavailable. Here we explored summation of pairs of synaptic potentials, each produced in one coupled neurone, by using pairs of Retzius neurones of the leech *Haementeria officinalis*.

The pair of Retzius neurones in each leech ganglion is coupled by non-rectifying electrical synapses (Hagiwara and Morita, 1962; Eckert, 1963) formed between pairs of proximal dendrites (García-Perez et al., 2004). The somatic steady-state coupling coefficient of Retzius neurones varies from one animal to another within a large range, thus suggesting the existence of extensive physiological modulation (De-Miguel et al., 2001). The changes in the coupling coefficient reflect mostly the coupling resistance value, since the space constant of the coupled dendrites is very similar from one neurone pair to another (Garcia-Perez et al., 2004). The coupled dendrites of both neurones are supplied by a common (and yet unidentified) chemical input, which produces single and synchronous EPSPs of varying amplitudes in the absence of external stimulation (Eckert, 1963; De-Miguel et al., 2001). The space constant of the coupled dendrites duplicates their 50-μm length and summation of two or more EPSPs produced in the coupled dendrites of both neurones contributes to produce action potentials at frequencies below 1 s^{-1} (Garcia-Perez et al., 2004). A second source of EPSPs is produced in non-coupled dendrites upon the activation of polysynaptic inputs onto both Retzius neurones from three types of mechanosensory (P, T and N) neurones innervating the skin (Velázquez-Ulloa et al., 2003). Depending on which types of mechanosensory neurones are activated, these pathways produce responses ranging from subthreshold series of EPSPs to bursts of action potentials (Szczupak and Kristan, 1995; Velázquez-Ulloa et al., 2003). A simplified circuit diagram

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Abbreviations: APSPs, artificial excitatory postsynaptic potentials; EPSPs, excitatory postsynaptic potentials.

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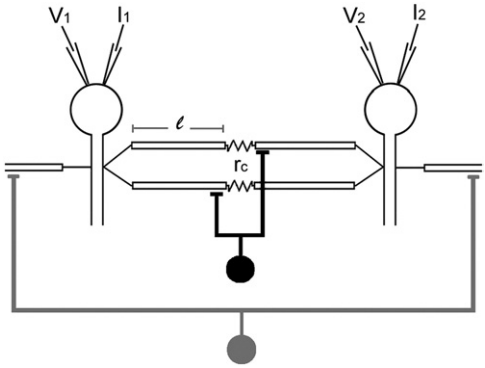

Fig. 1. Simplified diagram of the pair of electrically-coupled Retzius neurones, their excitatory inputs and the experimental array. The somata (large circles) are isopotentially connected to the primary axon, from which coupled and non-coupled dendrites emerge. The electrical synapses are represented by resistors (r_c). The coupled dendrites of both neurones receive chemical excitatory inputs from a common interneurone (black), which releases variable amounts of transmitter, producing single or synchronous EPSPs in both neurones. The electrotonic length of these dendrites is 0.5 and most chemical inputs have been predicted at a distance of 0.85 of the dendritic length (l). Mechanosensory neurones supply common inputs onto the non-coupled dendrites through different sets of interneurones, represented in grey. Independent electrodes used for injecting current (I) into the somata and recording voltage (V) are also represented.

of a pair of coupled Retzius neurones and the synaptic inputs described here is presented in Fig. 1. In addition, Retzius neurones are electrically coupled to other serotonergic neurones in the ganglion (Lent and Frazer, 1977).

Here we studied the summation of pairs of local and alien EPSPs. The effects of the coupling coefficient were analysed from simultaneous recordings of synaptic activity and also by producing pairs of artificial excitatory postsynaptic potentials (APSPs) with different time and amplitude relationships in the soma of pairs of neurones with different coupling coefficients, since in our hands it has not been possible to manipulate pharmacologically the coupling coefficient of these neurones by use of agents that affect electrical synapses in other species (De-Miguel et al., 2001). Our study was complemented by quantitative model simulations of the summation of EPSPs produced by inputs onto the coupled dendrites.

EXPERIMENTAL PROCEDURES

Preparation and dissection

Experiments were carried out in isolated ganglia from adult leeches *Haementeria officinalis* at room temperature (20–25 °C). Animals were collected from the wild and were handled and cared according to the guide of the National Institutes of Health for care and use of laboratory animals, with approval of the local animal care committee. We minimized the number of animals used and their suffering. Individual segmental ganglia (except for ganglia 5 and 6, which are specialised in controlling the reproductive behaviour) were dissected out as described by De-Miguel et al. (2001) and pinned in Sylgard-coated (Dow Corning Corp., Mexico City, Mexico) dishes containing leech Ringer composed of (mmol l^{-1}): NaCl, 115; KCl, 4; $CaCl_2$, 1.8; glucose, 11; Tris maleate 10 all from Sigma (Sigma-Aldrich, Mexico City, Mexico), buffered to pH 7.4. Retzius neurones were identified by their characteristic size and position in the ganglion.

Intracellular recordings

Microelectrodes for intracellular recordings and stimulation were made with borosilicate glass of 1 mm external diameter and 0.75 mm internal diameter pulled in a P97 puller (Sutter Instruments, Novato, CA, USA). The microelectrodes were filled with 3 mol l^{-1} KCl, having resistances of 18–25 MΩ. Independent electrodes were used to inject current and to record voltage. Voltage recordings were made by using intracellular amplifiers (Almost Perfect Electronics, Basel, Switzerland) coupled to an additional custom-designed amplifier with offset control. Recordings were filtered using a custom-designed Bessel filter with a cutoff frequency of 400 Hz, which did not affect the rise time of synaptic potentials (De-Miguel et al., 2001). Data were acquired by an analog-to-digital board Digidata 1200 (Axon Instruments, Union City, CA, USA) at a sampling frequency of 20 kHz using Axoscope software (Axon Instruments) and stored in a PC.

Production of APSPs

The results of the summation of APSPs were obtained from six selected pairs of neurones having steady state coupling-coefficients between 0.18 and 0.62, and membrane time constants of 22–26 ms, estimated from the exponential fit to the decay phase of synchronous APSPs, since these measurements are dominated by the membrane time constant of the soma and are independent of the coupling coefficient of the neurones. The membrane potential of the neurones was held at −60 mV by injection of negative DC current. The steady-state coupling coefficient (V_2/V_1) was measured from the voltage responses of both neurones to 200 ms hyperpolarizing current pulses into either of them. The coupling coefficient of EPSPs or APSPs was calculated from the maximum amplitudes of the transjunctional and the local voltage signals.

Somatic APSPs of 1.5 or 3.0 mV amplitudes were produced by adjusting the amplitude of 0.5 ms duration intracellular current pulses in the interval between 2.8 and 9.30 nA (Fig. 1 of the supplemental material). There were variations in the amount of current injected to produce similar APSP amplitudes between neurone pairs due to the coupling coefficient value, since it contributes to the input impedance of each neurone pair. The APSPs in each neurone pair had rise times of 2.43±0.5 ms (n=12 neurones) and decay times determined by the membrane time constants and by the coupling coefficient of the neurones (Rörig et al., 1996; Edwards et al., 1998). The signal to noise ratio of the voltage recordings was improved by averaging 10 consecutive APSPs. Voltage–current relationships of APSPs were calculated to test for linear membrane responses within a peak voltage interval of 1.0–5.0 mV (see supplemental material). Experiments were also performed in the presence of tetrodotoxin (TTX; 100 nm), which in this leech species blocks sodium currents (Johansen and Kleinhaus,1987) or cobalt (1.8 mM) to block calcium currents (see supplemental material).

Nomenclature

We used a convention to express our data, where V_1 were the voltage recordings from the reference neurone (in which the EPSP was produced), and V_2 were the voltage recordings from the coupled neurone. EPSPs or APSPs arriving from the coupled neurone were called alien or transjunctional. The EPSPs or APSPs were named single when produced in only one neurone although they had spread also to the soma of the coupled neurone; synchronous when produced in both neurones within 3 ms time lags; symmetric when they had the same amplitude; asymmetric when their amplitudes were different or asynchronous when the interval (Δt) between their production in the two neurones was >3 ms.