A One-Dimensional Current Source-Density Analysis is Applicable to the Mouse Accessory Olfactory Bulb

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ABSTRACT. Field potentials evoked in the accessory olfactory bulb (AOB) of the mouse by a vomeronasal organ (VNO) volley were analyzed with current source-density (CSD) techniques. The potentials were recorded with an electrode oriented in the direction perpendicular to the laminae of the centre of the AOB in five tracks of equal spatial lattice for identical coordinates. There were little or no differences in the amplitude or time course of the potentials at equal depth among the five tracks. The CSD profile from a one-dimensional CSD analysis corresponded closely with that of a three-dimensional CSD analysis of a central track in the AOB. Furthermore, VNO-evoked field potentials were recorded in the AOB external plexiform layer while an electrode was being moved in the direction parallel to this layer. The potentials recorded at adjacent points were nearly identical and hence the related one-dimensional CSD calculations revealed no net currents along this layer. These results indicate that a one-dimensional CSD analysis is applicable to the mouse AOB. — **KEY WORDS:** accessory olfactory bulb, current source density, field potential.

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Female mice form an olfactory memory to the pheromones of the mating male, during a critical period after mating. This olfactory memory is of critical biological importance, because it prevents any subsequent exposure to this male's pheromones from initiating neuroendocrine mechanisms that would terminate pregnancy [8]. Pheromones from strange males, for which no memory has been formed, induce pregnancy block [4] by activating the accessory olfactory system by way of the vomeronasal receptors [1, 9]. Previous studies have shown that this memory is located in the accessory olfactory bulb (AOB), which is located at the caudal-dorsal end of the main olfactory bulbs, and most likely involves changes at the mitral-granule reciprocal dendrodendritic synapse [2, 3, 6, 7]. In order to explore the electrophysiological correlates of this olfactory memory, it is necessary to extract synaptic events in the AOB. An attractive method of localizing neuronal activities and determining their sequence is current source-density (CSD) analysis of field potentials [10]. It has been recognized that CSD analysis reveals sites of excitatory synaptic interactions rather than locations of cell somata and provides better temporal and spatial resolution of neuronal events than conventional field-potential analysis [10]. In this study we therefore applied CSD analysis of field potentials to the mouse AOB, which were evoked by ipsilateral vomeronasal organ (VNO). Theoretically, CSD analysis has to be made three-dimensionally, but under certain conditions which are discussed, a one-dimensional analysis can be applied. The purpose of this study was to test whether a one-dimensional CSD analysis is applicable to the AOB, which has a curved shape.

Adult Balb/c female mice were purchased from JAPAN SLC (Hamamatsu) and maintained in light-(lights on 18:00 to 06:00) and temperature- (23 to 25°C) controlled rooms with food (CRF-1, Charles River Japan, Atsugi) and water available *ad libitum*. A total of six animals were used for the experiments. Each animal was anaesthetized with chloral hydrate (400 mg/kg, intraperitoneally). A light,

constant level of anaesthesia was maintained throughout the recording session by means of a continuous intraperitoneal infusion of chloral hydrate solution (1 mg/hr) delivered with a peristaltic pump. After cannulation of the trachea, the animals were placed in a stereotaxic instrument in the supine position. The mucosa of the palate ventral to the vomeronasal capsules was incised at the midline and retracted. Two holes were drilled in the left vomeronasal capsule, in the anterior and posterior sections. A bipolar electrode made of two stainless wires (diameter 0.16 mm), insulated with Epico 1500 except at the tip, was inserted approximately 1 mm into each hole in the capsule and fixed in place with cyanoacrylate glue and dental cement. The animals were then placed in the prone position, with the incisor bar adjusted 5 mm below the centre of ear bars and two holes were drilled in the skull overlying the dorsal surface of the olfactory bulb and the frontal cortex ipsilateral to the VNO implant. Throughout the experiments rectal temperature was maintained at $37 \pm 0.1^\circ\text{C}$ with a feedback-controlled heating blanket and tungsten lamp (ATB-1100, Nihon Koden, Tokyo).

The AOB was orthodromically activated by electrical stimulation of the VNO. Constant current stimuli, consisting of 0.2 ms square pulses with intensity adjusted in the range 0.3–0.6 mA to elicit a near-maximal response, were delivered from an isolated stimulator. Evoked potentials were recorded with glass micropipettes filled with 2% Pontamine Sky Blue dye in 0.5 M sodium acetate. The pipette (resistance: 1–5 M Ω) was advanced at an angle of 50° subtended to the coronal plane passing vertically through the ear bars so as to be oriented in the direction perpendicular to the laminae of the centre of the AOB. Before data collection, the micropipette was advanced several times through the AOB in order to reduce tissue distortion and minimize noise associated with unitary responses. Potentials were recorded during electrode withdrawal to prevent surface dimpling. Evoked potentials were recorded at 100- μm steps. Five consecutive responses

were averaged at each depth (interstimulus interval 5 s). Each series of recording required 15 min to complete. A band pass filter from 0.08 Hz to 3 kHz was used. The potentials were digitized at 2 kHz and analyzed with a personal computer.

Following data collection, iontophoretic ejections of the Pontamine Sky Blue dye (10 μ A negative for 5 min) were made at three different depths to precisely determine the correspondence between CSD profiles and the AOB lamination. The animals were then given an overdose of sodium pentobarbitone (Nembutal) and perfused through the heart with 10% formalin in saline. The brains were removed, fixed, frozen, sectioned (50 μ m) in the sagittal plane, and stained with Neutral Red. The spots were then located microscopically.

The fundamental theoretical principles of CSD analysis have been described in detail elsewhere [10]. Briefly, the extracellular field potential ϕ is related to the active and passive transmembrane currents of cell assemblies of activated neurons by the Poisson's equation:

$$\nabla \cdot \sigma \cdot \nabla \phi = -I_m \quad (1)$$

where σ is the conductivity tensor and I_m is current source density. Equation (1) is based on the assumptions that the extracellular space has the properties of an Ohmic conductor and that the electrical fields are quasi-static. The validity of those assumptions has been verified in brain tissues [5, 12, 13].

Since no striking geometric subdivisions can be detected within the AOB, the conductivity tensor may be reduced to its three principal components. Conductivity gradients over cortical depth proved negligible in the cerebellum [12], which has a clearly distinct layering. Furthermore, it has shown that omission of the conductivity term results in only minor changes in CSD profiles in spite of variations in conductivity along an axis perpendicular to the laminar planes [5, 11, 14, 15]. We therefore assumed that conductivity in the extracellular space is uniform within the AOB. In optimally oriented rectangular Cartesian coordinates, I_m is then given by the second spatial derivative:

$$\sigma_x \frac{\partial^2 \phi}{\partial x^2} + \sigma_y \frac{\partial^2 \phi}{\partial y^2} + \sigma_z \frac{\partial^2 \phi}{\partial z^2} = -I_m(x, y, z) \quad (2)$$

where x , y and z are the coordinates. If translational symmetry in two dimensions (x - y plane) can be assumed in the AOB, field potential changes can be observed only in the z -direction perpendicular to the laminae. Under these conditions, a one-dimensional CSD analysis can be applied [12]. Arguments justifying this assumption are given below. Equation (2) is then reduced to:

$$\sigma_z \frac{\partial^2 \phi}{\partial z^2} = -I_m(z) \quad (3)$$

The second spatial derivatives were actually calculated with a finite difference formula.

$$\frac{\partial^2 \phi}{\partial z^2} \approx \frac{\phi(z+h) - 2\phi(z) + \phi(z-h)}{h^2} \quad (4)$$

where h is the sampling interval (100 μ m in this study).

In three animals, we recorded VNO-evoked field potentials, with a single electrode, in five tracks of equal spatial lattice (spacing = 100 μ m) for identical coordinates within the AOB. The central track was oriented through the centre of the AOB. The remaining four tracks were respectively oriented rostral, caudal, medial and lateral to the central track (see Fig. 1B). The temporal succession of the tracks was chosen randomly. An example is illustrated

Fig. 1. VNO-evoked field potential profiles in five tracks of equal spatial lattice for identical coordinates within the AOB. A: Sagittal section of the mouse brain with electrode track. B: Evoked potential profiles. Frontal, lateral and depth spacings between adjacent recordings are 100 μ m. Average 5 $\times$, bin width 500 μ s, stimulus at 20 ms marked by arrow heads. Note the similarity of the potentials at equal depth. Abbreviations: EPL, external plexiform layer; GL, glomerular layer; GRL, granule cell layer; IPL, internal plexiform layer; VNL, vomeronasal nerve layer.

Fig. 2. Comparison of a one-dimensional CSD calculation (A) of a central track within the AOB with a three-dimensional CSD calculation (B) from five tracks of equal spatial lattice for identical coordinates within the AOB. Differential grid is $100\ \mu\text{m}$. Vertical crosshatching denotes sinks (net inward currents). Data are from the experiment illustrated in Fig. 1. Abbreviations as in Fig. 1.

in Fig. 1. The VNO-evoked potential recorded at the surface of the centre of the AOB showed a negative deflection. The potential increased in amplitude with depth of penetration and reached its maximum in the external plexiform layer after which it decreased in size. As the electrode was advanced further, this potential reversed its polarity at the superficial part of the granule cell layer, achieved its maximal positive amplitude, and then gradually diminished. In comparison of the VNO-evoked potentials at equal depth among the five tracks, there were little or no differences in the amplitude or time course of the potentials (Fig. 1). We compared the CSD profile from a one-dimensional CSD analysis with that of a three-dimensional CSD analysis of a central track in the AOB (Fig. 2). The characteristic feature in both CSD depth profiles is the occurrence of a sink(s) (net inward membrane current) from the glomerular to external or internal plexiform layers, coupled with sources in the vomeronasal nerve and deeper layers. A comparison of the CSD profiles, revealed either by a one- or three-dimensional CSD calculation, demonstrates that both profiles have highly corresponding sink-source distributions (Fig. 2). If this is the case, there should be no substantial net current in any plane parallel to the laminae of the AOB. To confirm this assumption, in three animals, we recorded VNO-evoked field potentials at $100\text{-}\mu\text{m}$ steps while moving the electrode over the external plexiform layer along the longitudinal axis of the AOB, the direction which was orthogonal to the electrode track shown in Fig. 1. An example is illustrated in Fig. 3. The potentials recorded at adjacent points were nearly identical within the AOB (Fig. 3B, left) and hence the related one-dimensional CSD calculations revealed no net currents anteroposteriorly in the external plexiform layer of the AOB (Fig. 3B, right). The results illustrated in Figs. 1–3 indicate that a one-dimensional CSD analysis can be applied to the mouse AOB. The results further indicate that the CSD analysis applied to the central part of the mouse AOB provides better temporal and spatial resolution of neuronal events than conventional field-potential analysis. Whereas VNO-evoked negative potentials extend from the dorsal surface of the

Fig. 3. VNO-evoked potentials and related current source densities measured over the external plexiform layer along the laminae of the AOB. A: Sagittal section of the mouse brain with electrode track. B: Evoked potential profile (left) and CSD profile (right). Evoked potential: Average $5\times$, bin width $500\ \mu\text{s}$, stimulus at arrow head. CSD: Differentiation grid $100\ \mu\text{m}$. Note the negligible sink-source distributions in the CSD profile.

AOB to the internal plexiform layer, the current sinks are primarily restricted to the glomerular and external plexiform layers.

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