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Loose-patch–juxtacellular recording in vivo—A method for functional characterization and labeling of neurons in macaque V1[☆,☆☆]

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Abstract

We describe a method that uses a modified version of juxtacellular labeling [Pinault D. A novel single-cell staining procedure performed in vivo under electrophysiological control: morpho-functional features of juxtacellularly labeled thalamic cells and other central neurons with biocytin or neurobiotin. J Neurosci Meth 1996;65:113–36], which allows us to functionally characterize and subsequently label single neurons in vivo in macaque V1. The method is generally applicable in acute in vivo preparations. Extracellular recording is made with a patch electrode when the electrode is attached to the cell membrane. Initially a 'blind' search method is used as a guide to obtaining a cell attached configuration that we refer to as a loose-patch (LP). The neuron's receptive field properties are functionally characterized, the neuron is labeled and then characterization is confirmed, all in the LP configuration. There are a number of advantages of the method that we describe over other methods. First, we have found that we can obtain stable extracellular recordings for periods of hours that enable us to make a relatively comprehensive visual functional characterization of a neuron's receptive field properties. Second, because the electrode is closely apposed to the cell we obtain excellent isolation of the extracellular spike. Third, the method provides labeling that gives complete dendritic and axonal filling that survives over a number of days, which is an important feature in acute primate experiments. Fourth, the in vivo method of labeling and reconstructing neurons gives complete three-dimensional structure of the neuron including its intra-cortical axonal arbor. These features overcome known limits of the established methods of studying neuronal morphology including the Golgi stain (limited when adult tissue is used) and in vitro whole cell methods (incomplete axonal filling due to limited slice thickness). They also overcome the known limits of the established method of combined function-morphology studies i.e. intracellular recording in vivo. The modified juxtacellular method provides a reliable alternative to the difficult method of characterization by extracellular recording and subsequent intracellular labeling [Anderson JC, Martin KAC, Whitteridge D. Form, function and intracortical projections of neurons in the striate cortex of the monkey *Macacus nemestrinus*. Cerebral Cortex 1993;3:412–20]. We show the method can be used to record at a range of depths through V1 cortex allowing for sampling of neurons in the different layers and functional subpopulations. Links can then be made with existing knowledge about the anatomical organization of V1, the various morphological classes of neurons found therein, their functional connectivity and visual response properties.

Keywords: Macaque; Striate cortex; Morphology; Functional characterization; Loose-patch; Juxtacellular

1. Introduction

Four main methods have been described to record from neurons to obtain a functional characterization followed by labeling and subsequent morphological identification of the neuronal type and its dendritic and axonal arbors. In the case of neurons in the visual pathway, functional characterization implies a receptive field characterization. Two of these methods use intracellular recording for the whole duration (of recording and labeling), either with sharp electrodes (Azouz et al., 1997; Cardin et al., 2005; Gilbert and Wiesel, 1979; Nowak et al., 2003; Van Essen and Kelly, 1973) or with whole-cell patch electrodes (Hirsch et al., 1995, 1998, 2002, 2003). In the third method, using sharp electrodes, the initial recording is made extracellularly while the neuron's receptive field is functionally characterized. The electrode is then advanced to obtain an intracellular configuration and labeling is performed (Anderson et al., 1993; Martin and

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Whitteridge, 1984a, 1984b). In this method, the receptive field preferences are verified in the intracellular stage. The fourth method is termed juxtacellular recording (Pinault, 1996). In this method a sharp electrode is used to obtain extracellular recording and then moved to be in close apposition to the cell membrane where labeling is obtained by applying a train of current pulses (Pinault, 1996). None of these methods have been widely used in studies of the macaque visual pathway perhaps because the soma sizes are relatively small compared with other species, thus making long stable recordings difficult.

Following the pioneering work of Hubel and Wiesel (1962, 1968), a wide range of studies using extracellular recording in primate V1 have identified numerous properties of receptive fields that can be used to separate functional classes of cells. These properties include color (Dow and Gouras, 1973; Livingstone and Hubel, 1984), spatial frequency (Schiller et al., 1976) and direction selectivity (Orban et al., 1986; Poggio et al., 1985). Quantitative assessment of neuronal firing has refined and extended the range of properties that can be used to define receptive fields. Such properties include spatial frequency (De Valois et al., 1982; Xing et al., 2004), direction selectivity (Hawken et al., 1988), color (Johnson et al., 2001; Lennie et al., 1990), dynamics of orientation tuning (Ringach et al., 1997), stimulus size (Cavanaugh et al., 2002; Levitt and Lund, 1997; Sceniak et al., 1999) and binocular disparity (Cumming and Parker, 1997). Furthermore, recent developments in modeling of the properties of visual neurons make specific predictions about the tuning, dynamics and firing patterns of different neuronal types (McLaughlin et al., 2000; Miller, 2003; Shelley et al., 2002; Troyer et al., 1998).

The morphology of V1 neurons has been studied extensively using a number of methods. Such studies provide reconstructions of neurons and their laminar locations. The labeled neurons are obtained from Golgi stained sections of monkey visual cortex (Lund, 1973, 1987; Lund et al., 1975, 1988; Lund and Wu, 1997; Somogyi et al., 1981) as well as from intracellular fills of neurons both in vivo in macaque striate cortex (Anderson et al., 1993) and cat striate cortex (Gilbert and Wiesel, 1979; Hirsch et al., 1995, 1998, 2002, 2003; Martin et al., 1983; Martin and Whitteridge, 1984a, 1984b; Van Essen and Kelly, 1973) and in vitro in slices of monkey V1 (Katz et al., 1989; Wiser and Callaway, 1996) as well as in sections of fixed tissue (Elston et al., 1999).

Thus, while there is substantial data on the functional properties of macaque V1 neurons on the one hand and on their morphology on the other, there is a relative lack of data that directly relates the two. Although the four methods discussed above can potentially bridge this divide there are limitations on the amount of functional characterization that can be obtained during the period of stable recording, and such experiments have almost exclusively been done successfully in cat striate cortex. In order to relate the properties of identified neurons either to classes of neurons from quantitative extracellular studies or to model neurons we need to obtain measurements of the quantitative properties of their receptive fields along a number of stimulus dimensions as well as the morphological properties. To achieve this goal in the primate V1 we have developed a method that incorporates a number of features from previous methods with the cell-attached recording technique. We call this hybrid method loose-patch–juxtacellular (LP–JC) recording. A major advantage of an in vivo method of characterizing and labeling single neurons is that the complete three-dimensional structure of the neuron and, potentially, its intra-cortical axonal projection, is recovered. Reconstruction of neurons labeled in vitro is limited by the fact that the slice preparations used are typically about 300 μm thick and fibers extending beyond this distance out of the thickness of the sections are lost. With an in vivo method, it may be possible to recover the projections of labeled neurons to their cortical or subcortical targets.

Pinault (1996) described a method of extracellular recording with glass electrodes that could be used to also label a recorded cell. Here, we describe a method that uses a modified version of juxtacellular labeling (Pinault, 1996), which allows us to functionally characterize and subsequently label single neurons in vivo in macaque V1. There are a number of advantages of the method that we describe over other in vivo methods such as whole-cell and sharp electrode intracellular recording. First, using electrodes with tip diameters of 1–2 μm and impedances between 5 and 15 MΩ (1 kHz; patch electrodes), we have found that we can obtain stable extracellular recordings over periods of hours that enable us to make a relatively comprehensive visual functional characterization of a neuron's receptive field properties. This is an advantage over intracellular methods where retaining a stable recording in cortex for hours in vivo is unusual. Second, because the electrode is closely apposed to the cell we obtain excellent isolation of the extracellular spike. Third, the method provides labeling that gives complete dendritic and axonal filling that survives over a number of days, which is an important feature in acute primate experiments.

2. Materials and methods

Initially, we performed a series of preliminary experiments in slices of cortex in vitro. There were three main aims of the in vitro experiments: first, to select the range of electrode tip sizes that did not penetrate the neuron in loose-patch configuration yet showed satisfactory recording of the extracellular action potentials; second, to establish the characteristic changes in electrode impedance when making a 'blind loose-patch' in vivo and to establish initial electrode pressure parameters to obtain loose-patch recordings that do not penetrate the cell membrane while keeping the tip clear; third, to determine the appropriate range of current pulse amplitude and labeling duration to obtain labeling with biocytin (Sigma–Aldrich) and BDA (biotinylated dextran amine; Molecular Probes) and to compare this labeling with that obtained in whole-cell mode. We then used these parameters as a guide for in vivo experiments in the anesthetized paralyzed macaque. The in vitro experiments are described first.

2.1. In vitro experiments

The first and second aims were to choose electrodes and develop a protocol to achieve the loose-patch (cell-attached) configuration. With traditional metal extracellular electrodes (typically in our laboratory—glass-coated tungsten (Merrill and