Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Pharmacological and Toxicological Methods 51 (2005) 147–151

Journal of Pharmacological and Toxicological Methods

www.elsevier.com/locate/jpharmtox

Original article

Microiontophoresis electrode location by neurohistological marking: Comparison of four native dyes applied from current balancing electrode channels

Péter Kovács, Viktória Dénes, Lóránd Kellényi, István Hernádi*

Department of General Zoology and Neurobiology, Faculty of Natural Sciences, University of Pécs, 6 Ifjúság str., H-7624 Pécs, Hungary

Received 11 February 2004; accepted 22 August 2004

Abstract

Introduction: The re-establishment of electrode position after microiontophoresis with multi-barrelled electrodes has always been a complicated and mostly inaccurate procedure. The present report describes a new method for 'on line' neurohistological marking of each recording position during experimental sessions in which in vivo microiontophoresis was combined with extracellular single unit recording. **Methods:** We tested the effectiveness of four native dyes: pontamine sky blue (PSB), methylene blue (MB), cresyl violet (CV) and toluidine blue (TB). They were ejected from the continuous balancing channels of multi-barrelled microelectrodes during iontophoresis in place of the usual sodium chloride solution. **Results:** The present results are based on observations obtained from 66 neuronal recordings and 42 labelled sites in the rat neocortex. Results indicate that none of the tested native dyes altered the spontaneous firing rate of the neurons, nor did they have any influence on the applied bioactive compounds. Recording positions were detected as small, labelled spots in brain slices (2–12 μm in diameter) at the end of the recording tracks. Labelling was successful with MB and PSB, but not with CV or TB. In addition, MB provided good labelling with a low net ejection current. **Discussion:** The present results suggest that using native dyes, especially MB, instead of sodium chloride in current balancing channels during in vivo microiontophoresis provides fast and accurate confirmation of electrode placement. This method saves the experimenter the time consuming further application of secondary labelling procedures after such experiments. Moreover, the MB-labelling we describe here is focal, enabling a more accurate verification of the site of recording and/or application of various neuroactive compounds. In addition, MB labelled accurately and reliably and with very low ejection currents, presumably due to its smaller molecular weight and higher relative mobility.

Keywords: Methods; Methylene-Blue; Microiontophoresis; Neocortex; Pontamine sky blue; Rat; Single unit recording

1. Introduction

Recently, there has been a new incursion of microiontophoresis in neurophysiological research, presumably because it is still one of the most practical methods for in vivo neuropharmacological investigations (Kirby, Rice, & Valentino, 2000; Ralevic, Thomas, Brunstock, & Spyer, 1999; Sawaguchi, 2001; Yang, Bertram & Coote, 2001). In these studies, identification of the position of the microelectrode tip in the brain is usually a problematic secondary procedure at the end of the experimental session. Formerly, the position of the electrode tip was marked by using Pontamine Sky Blue (PSB) in either the current-balancing channel (Boakes, Bramwell, Briggs, Candy, & Tempesta, 1974) or in a separate channel of the multi-barrelled microelectrode (Lodge, Caddy, Headley, & Biscoe, 1974). Although this method has been approved, it has a few deficiencies: PSB only labels if it is ejected with a rather high anodal current usually in the microA range (430 nA–1200 μA). Therefore, it was usually necessary to terminate

* Corresponding author. Department of Anatomy, University of Cambridge, Downing Street, Cambridge, CB2 3DY, UK. Tel.: +44 1223 339544; fax: +44 1223 333786.

E-mail address: ih226@cam.ac.uk (I. Hernádi).

1056-8719/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.vascn.2004.08.002

the recording session, and start a 3- to 10-min labelling procedure with a high capacity current pump before further advancing the electrode in the track (Boakes et al., 1974; Hernádi et al., 2000; Kirby et al., 2000; Lodge et al., 1974; Ralevic et al., 1999; Yang et al., 2001). In most cases, if several recording sessions were performed in one single electrode track, the total labelling time could last up to 30–50 min. Moreover, this usual method resulted in a relatively large (>100 μm) marked area targeting the placement of the electrode tip with a relatively large uncertainty (Boakes et al., 1974).

In the present report, we aimed to provide a new solution to this issue through performing systematic tests on the marking efficiency of several native dyes.

2. Methods

2.1. Animals and surgical treatment

All experiments were approved by the Animal Care Committee at our Institution (University of Pécs, Hungary) and by international standards (NIH-Guidelines). Twenty-one male CFY rats (Charles River Laboratories, Gödöllő, Hungary) were examined during experiments. Anestesia was induced with a single injection of ketamine (Calypsol, RG, Hungary) 50 mg/ml, 2 ml/kg b.w. Stereotaxic coordinates for the medial wall of the prefrontal neocortex (mPFC) according to Paxinos and Watson (1997) were: AP (from bregma): 3.2–3.5; L:±0.8–1.2, V 1.8–3.8 (from dura).

2.2. Microiontophoresis, single neuron recording and administration of chemical compounds

Seven-barrelled micropipettes were used with tips of 10–15 μm in total diameter (Carbostar-7, Kation Scientific, MN, USA). The impedance of the central, recording channel was 1–3 MΩ (at 50 Hz), whereas the impedance for surrounding "drug-channels" was 30–200 MΩ each. The central barrel contained a 7-μm carbon fibre tip, which was further attached to a silver fibre, served as the recording channel for extracellular firing rate of neurons during the presence of iontophoretically applied bioactive compounds. During microiontophoresis, one of the capillaries was used as a continuous current-balancing channel, and was filled with 2% aqueous solution (dissolved in 0.5 M Na-acetate) of one of the following native dyes: Pontamine Sky Blue (PSB), Methylene-Blue (MB), Cresyl-Violet (CV), Toluidine-Blue (TB) or 0.15 M NaCl solution in control situations. Two channels were filled with GABA (Sigma, 500 mM) and kainic acid (KA, Sigma 50 mM). These compounds were applied for inhibitory and excitatory control of the neural activity, respectively. The remaining three electrode channels were filled with other various bioactive compounds not described in this paper.

We continuously recorded both spontaneous and modulated firing activity of isolated neurons during experimental sessions. Signals were first amplified and filtered, then digitised for analysis and storage purposes via an AD/DA interface unit (Power 1401 with Spike2 software; CED, Cambridge, UK). Labelling took place "automatically" every time the current balancing channel became active. The effects of bioactive compounds were studied elsewhere in previous reports from our laboratory (Kovács & Hernádi, 2002, 2003). All bioactive compounds, except KA, were ejected as cations.

2.3. Labelling procedure

The ejection of all bioactive chemical compounds and the balancing currents was controlled by a high capacity constant current supply (Neurophore BH-2, Medical Systems, USA). A balancing current was always applied automatically when a substance was ejected, and it had the same current intensity with opposite polarity as the ejecting current for bioactive compounds, which was passed through the active electrodes. Labelling (balancing) currents were mostly anodal (from −5 to −100 nA), and

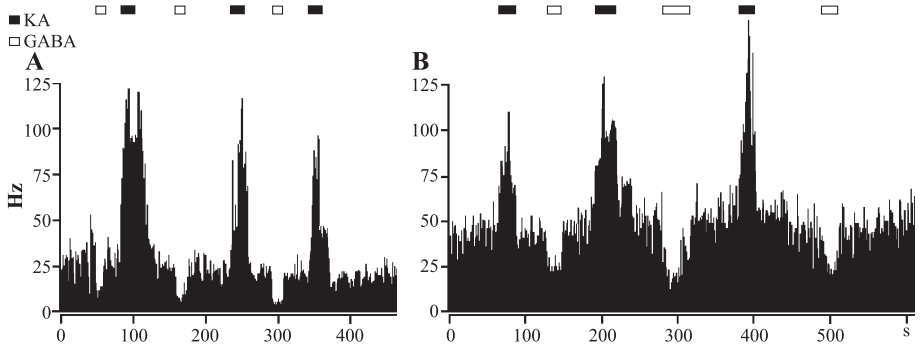

Fig. 1. MB does not alter maintained firing properties of recorded neurons and their responses to bioactive compounds. Frequency histograms from two different single unit recordings using GABA as an inhibitory or kainic acid (KA) as anexcitatory receptor agonist. (A) The current balancing channel contains NaCl solution. (B) The current balancing channel contains MB.