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A novel suction electrode recording technique for monitoring circadian rhythms in single and multiunit discharge from brain slices

Timothy M. Brown, Jon R. Banks, Hugh D. Piggins *

Faculty of Life Sciences, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK

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Abstract

The study of spontaneous neuronal discharge for several days in vitro poses substantial technical difficulties not readily addressed by existing methodologies. Here we describe a novel method for recording bioelectrical signals from brain slices, using media-filled, glass micropipettes stabilized at the recording interface by negative pressure. Such electrodes are durable, economical and easily constructed using standard laboratory equipment. Through these suction electrodes, we monitored ~24 h oscillations in spontaneous single and multiunit discharge from acutely prepared adult mouse suprachiasmatic nuclei (SCN) slices for up to 4 days in vitro, with very high success rates. Neuronal oscillations exhibited all the characteristics previously determined in SCN recordings using other techniques. Importantly, our electrode assembly enables SCN activity in acutely prepared brain slices to be monitored for substantially longer than with other methodologies. In summary, our data suggest that suction electrodes are likely to provide a favorable alternative to existing methods for recording long-term neuronal activity from brain slices.

Keywords: Electrophysiology; Hypothalamus; Circadian; In vitro; Spike sorting; Glutamate; Suprachiasmatic nuclei

1. Introduction

Neurons of the dominant circadian clock located in suprachiasmatic nuclei (SCN) of the hypothalamus regulate near-24 h oscillations of cellular activity in many regions of the mammalian brain (Abraham et al., 2005; Amir et al., 2004; Tousson and Meissl, 2004; Yamazaki et al., 1998; Zhao and Rusak, 2005). The study of such oscillations in vitro poses substantial technical difficulties regarding the stability of the electrophysiological recording interface and long-term maintenance of neuronal viability. In recent years, multi-electrode recording of electrical activity rhythms from cultured SCN neurons (pooled from multiple donor animals) or tissue explants has gained popularity as a method for studying neuronal oscillations for days or even weeks in vitro (Bos and Mirmiran, 1990; Herzog et al., 1997; Welsh et al., 1995). However, due to the lengthy culture procedures and use of neonatal animals in such multi-electrode studies, some authors have questioned their relevance to the physiology of the intact adult SCN (Bouskila and Dudek, 1995; Herzog et al., 2004). Further it is very difficult to relate the results of studies using such techniques to the behavioral phenotype of an individual animal.

Two techniques have been widely employed to record rhythms of spontaneous neural discharge from adult rodent SCN brain slices: (1) the activity of single units within the slice are recorded for short periods of time (5–10 min) using a glass micropipette and a population average built up by constant repositioning of the electrode (Biello et al., 1997; Davies and Mason, 1994; Gillette and Prosser, 1988; Green and Gillette, 1982; Groos and Hendriks, 1982; McArthur et al., 2000; Shibata et al., 1982), or (2) the activity of multiple SCN neurons are recorded simultaneously for long periods of time using a stationary, usually metal, electrode (Albus et al., 2002; Bouskila and Dudek, 1993; Gribkoff et al., 1998; Mrugala et al., 2000).

Each of these techniques has substantial limitations. The 'population sampling method' is labor intensive, requires the experimenter's continual presence, and induces tissue damage through repeated slice-penetrations by the electrode. In contrast, the multiunit recording method requires only minimal maintenance by the experimenter and relies instead on extreme stability of the tissue chamber conditions, as small perturbations can compromise the experiment. For this reason, multiunit recordings longer than 48 h are very difficult to obtain and are very rarely reported (Gribkoff et al., 1998). Furthermore, it is dif-

* Corresponding author. Tel.: +44 161 275 3897; fax: +44 161 275 3938.
E-mail address: hugh.piggins@manchester.ac.uk (H.D. Piggins).

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ficult to obtain consistent long-term recordings in vitro using the standard metal multiunit electrodes (Coogan and Piggins, unpublished observations; Tcheng and Gillette, 1996). An additional drawback of both of the above methodologies is that they do not allow the activities of individual cells to be monitored for long enough to detect or analyze circadian rhythms (Schaap et al., 2003).

Here we report a novel application of glass suction electrode assemblies for producing long-term recordings from adult brain slices. These electrodes can be easily constructed from standard laboratory equipment, are extremely economical, and enable the spontaneous discharge of individual neurons and clusters of cells in acute adult brain slices to be monitored for up to 4 days in vitro.

2. Methods

2.1. Electrode construction

The body of the electrode assembly was constructed from a 1 ml syringe (BD, Oxford, UK), with the plunger removed. The tip of the syringe and ~33 mm from the base were removed using a scalpel blade (Fig. 1A) and a small hole (~1 mm diam.) was made in one side of the syringe using a heated 21 gauge needle. A 200 μl pipette tip, prepared by removing 5 mm from the tip and 27 mm from base, was pushed into the syringe until it was wedged tightly in place, protrouding ~13 mm from the end of the pipette (Fig. 1B).

A 1 m length of twin screened coaxial audio cable (XS94C; Maplin Electronics Ltd., Barnsley, UK) was prepared by removing 6 cm of the outer plastic sheath along with the exposed copper grounding wires. One of the individually screened cables was fed through the hole in the electrode body until it protruded through the tip (Fig. 1C). A 2 cm length of 0.25 mm diameter, non-teflon-coated, silver wire (Clark Electromedical Instruments, Reading, UK) was soldered to the cable running inside the electrode body to form the recording electrode and an 8 cm length of 0.20 mm diam. silver wire soldered to the external cable to form the reference electrode. Before use both electrodes were immersed in 2 M NaCl and current applied, using the silver wire as anode, until an even coating of AgCl was achieved. The cables connecting the recording and indifferent electrodes were attached to the electrode body using a small amount of epoxy resin, sealing the small hole in the syringe (Fig. 1D). The cabling was further secured to the electrode assembly using a small section of rubber tubing (7.9 mm outer diameter, 6.4 mm inner diameter) and a syringe attached to the rear of the electrode using silicone tubing (4.8 mm outer diameter, 1.6 mm inner diameter).

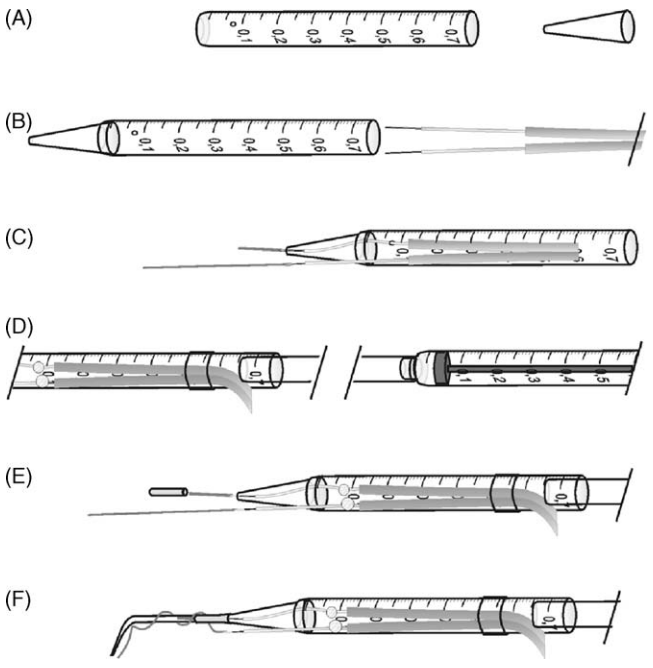

Fig. 1. Steps in the assembly of a suction electrode. See electrode construction in Section 2 for details.

Electrode tips were fabricated from glass micropipettes made from thin-walled glass (PG150T; Harvard Apparatus Ltd., Eddenbridge, UK) using a Flaming/Brown P-97 horizontal puller (Sutter Instrument Co., Novato, USA). Heating and cooling parameters were adjusted to produce pipettes with long shanks (~1.5 cm). The proximal and distal tips of the micropipettes were removed by making a small scratch in the glass with a diamond tipped pen (Fischer Scientific, Loughborough, UK) and gently pushing away from the scratch to achieve a clean break, resulting in a pipette ~3 cm long with a ~100 μm (inner) diameter tip. A spirit burner was used to flame-polish cut-ends of the pipette and bend its shank to an angle of ~45°, by heating the neck and gently pushing the tip until it was at the desired angle. Electrode tips were then attached to the electrode body using a small length (~7 mm) of flexible rubber tubing (1.8 mm outer diameter, 1.5 mm inner diameter) and the indifferent electrode was wrapped loosely around the pipette and angled so the end was positioned close to the glass tip (Fig. 1E and F).

During recording the electrode was filled with artificial cerebrospinal fluid (aCSF: see below) from the tissue chamber and, providing the tip was rinsed thoroughly (1 M HCl followed by distilled water) at the end of each experiment, could be re-used indefinitely.

2.2. Brain slice preparation and maintenance

Adult male mice (C57 BL6/J) were group housed under a 12 h:12 h light dark cycle (LD), at an ambient temperature of 22 ± 1 °C. Food and water were available ad libitum. Zeitgeber time (ZT) 0 was defined as lights on and ZT 12 as lights off. Animals were maintained under these conditions for >2 weeks prior to experimental procedures. All scientific procedures were carried out in accordance with the UK Animal (Scientific Procedures) Act 1986.

Slices were prepared during the early subjective day (ZT 1–2) and maintained using methods previously described (Cutler et al., 2003; McArthur et al., 2000), with minor modifications to promote slice survival. Mice were anaesthetized with halothane (AstraZeneca, Macclesfield, UK) followed by cervical dislocation and decapitation. The brain was then removed and placed in 4 °C aCSF (pH 7.4) of composition (in mM): NaCl 124, KCl 2.2,