Fabrication and Use of High-Speed, Concentric H\(^+\)- and Ca\(^{2+}\)-Selective Microelectrodes Suitable for In Vitro Extracellular Recording

Nataliya Fedirko, Nataliya Svichar, and Mitchell Chesler

Department of Neurosurgery, and Department of Physiology and Neuroscience, New York University School of Medicine, New York, New York

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Fedirko, Nataliya, Nataliya Svichar, and Mitchell Chesler. Fabrication and use of high-speed, concentric H\(^+\)- and Ca\(^{2+}\)-selective microelectrodes suitable for in vitro extracellular recording. J. Neurophysiol. 96: 919–924, 2006; doi:10.1152/jn.00258.2006. Ion-selective microelectrodes (ISMs) have been used extensively in neurophysiological studies. ISMs selective for H\(^+\) and Ca\(^{2+}\) are notable for their sensitivity and selectivity, but suffer from a slow response time, and susceptibility to noise because of the high electrical resistance of the respective ion exchange cocktails. These drawbacks can be overcome by using a “coaxial” or “concentric” inner micropipette to shunt the bulk of the ion exchanger resistance. This approach was used decades ago to record extracellular [Ca\(^{2+}\)] transients in cat cortex, but has not been subsequently used. Here, we describe a method for the rapid fabrication of concentric pH- and Ca\(^{2+}\)-selective microelectrodes useful for extracellular studies in brain slices or other work in vitro. Construction was simplified compared with previous implementations, by using commercially available, thin-walled borosilicate glass, drawing an outer barrel with a rapid taper (similar to a patch pipette), and by use of a quick and reliable silanization procedure. Using a piezoelectric stepper to effect a rapid solution change, the response time constants of the concentric pH and Ca\(^{2+}\)-electrodes were 14.9 ± 1.3 and 5.3 ± 0.90 ms, respectively. Use of these concentric ISMs is demonstrated in rat hippocampal slices. Activity-dependent, extracellular pH, and [Ca\(^{2+}\)] transients are shown to arise two- to threefold faster, and attain amplitudes two- to fourfold greater, when recorded by concentric versus conventional ISMs. The advantage of concentric ISMs for studies of ion transport and ion diffusion is discussed.

INTRODUCTION

Synchronous neuronal activity in the brain is accompanied by rapid changes in the concentration of extracellular ions. Such ionic shifts have been the focus of many studies that used ion-selective microelectrodes (ISMs). These devices are notable for their small tip size and ability to accurately detect minute ionic changes (Nicholson and Rice 1988). For many ISMs, however, use is limited by a slow speed of response, inherent noise, and propensity to movement artifacts. These drawbacks are especially evident in ISMs that have a very high electrical resistance arising from the incorporation of neutral ion exchangers, such as those selective for H\(^+\) (Ammann et al. 1981) or Ca\(^{2+}\) (Lanter et al. 1982). Their considerable internal resistance, coupled with the capacitance across the glass capillary, results in a long electrical time constant, a corresponding slow response time, and a susceptibility to noise.

Numerous studies have demonstrated that brain extracellular pH (pH\(_e\)) can change rapidly in response to neural activity (reviewed by Chesler 1990, 2003). These experiments primarily relied on pH microelectrodes, based on the protonophore triprolidine, that have response times as long as 1–5 s (Ammann et al. 1981). This speed appears to be far less than optimal because optical studies of pH transients in the interstitial space of hippocampal slices have shown that extracellular alkaline transients can arise within a few tens of milliseconds (Gottfried and Chesler 1996; Tong et al. 2006). These fluorescence-based methods for recording changes in interstitial pH have been cumbersome to implement because extensive averaging was required to overcome noise. Indeed, whereas fluorescent recording methods for intracellular ions are extensively used and have undergone considerable technical evolution, good optical techniques for extracellular ion recording are not generally available. Thus despite their limited speed, conventional ISMs have remained the principal means of studying changes in interstitial pH and shifts in other extracellular ions linked to neural activity (Autere et al. 1999; de Curtis et al. 1998; Tong et al. 2000; Xiong and Stringer 2000).

A method of increasing the speed of ISMs was conceived by Orme, who suggested insertion of a thin, concentric micropipette inside the ion-exchanger column, to within a few micrometers of the tip (Orme 1969). In this conception, the central pipette would serve to reduce the longitudinal resistance of the column and thereby decrease the electrical time constant. Ujec and colleagues first tested this method for the construction of K\(^{+}\)-, Cl\(^-\), and Ca\(^{2+}\)-selective microelectrodes (Ujec et al. 1979, 1981). Later, Pumain et al. (1983) used this concentric modification to speed the response of neutral carrier, Ca\(^{2+}\)-selective microelectrodes and, in a study of cat cortex, discerned rapid, in vivo decreases in extracellular Ca\(^{2+}\) activity ([Ca\(^{2+}\)]\(_e\)) during epileptic discharges.

Apart from the report of Pumain and colleagues (1983) we are not aware of any further use or development of the concentric ISM technique in neurophysiological studies. This is not surprising given the complexity of design. The assemblies described by Ujec et al. (1979, 1981) used a triple-barreled configuration that consisted of a large-diameter ion barrel, a smaller-diameter reference barrel, and a small, independent concentric pipette thread within the ion-selective side. This technique made use of specially fused capillaries of different-diameter glass tubing. In addition, the placement of the assemblies deep within mammalian cortex required a long taper, a factor expected to add to the difficulties of silanization and construction.

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Here we describe an alternative implementation of this concentric ISM technique suitable for use in acute brain slices or other in vitro settings. We show that rapid-responding ISMs for H⁺ and Ca²⁺ can be fabricated using readily available, thin-walled, borosilicate capillary tubing. Construction was greatly simplified by the use of a rapid taper (similar to that of a whole cell patch pipette) and a fast method for silanization of the outer microelectrode. Response times for the pH- and Ca²⁺-selective microelectrodes, measured with a piezoelectric stepper, were shown to be in the range of a few milliseconds. A comparison of conventional versus concentric forms of these ISMs, performed in rat hippocampal slices, demonstrated considerable advantages of the concentric electrodes in recording rapid, stimulus-evoked ion transients.

**METHODS**

**Fabrication and testing of concentric ISMs**

Concentric ion-selective microelectrodes were fabricated from two thin-walled borosilicate glass capillaries of different diameters. The wider capillary had an OD of 2.0 mm and an ID of 1.6 mm (A-M System 6185). This pipette blank was drawn on a Brown–Flaming P-87 puller in a two-step program, to yield a rapid taper, and tip diameter of 2–4 μm. The resulting micropipette was backfilled to the shank with a column of N,N-dimethyltrimethylsilylamine (Fluka 41716). A Teflon suction line was inserted up to the shank of the micropipette, and the tip mounted 2–4 in. from the barrel of a hot air gun as described by Chesler and Kraig (1989). The tip was heated to about 200–300°C for 30–60 s with the suction turned on to prevent reflux of the silane. The micropipette was then refilled with N,N-dimethyltrimethylsilylamine and the procedure repeated. A proton-selective cocktail (Fluka 95291) or a Ca²⁺-selective cocktail (Fluka 21048) was then incorporated into the tip by suction to form a 100- to 200-μm column. It was not necessary to follow the column with a saline solution because electrical contact with the ion exchanger would substantially be made with the inner, concentric pipette. However, we noted that filling most of the remaining volume with a solution of 150 mM NaCl (for pH electrodes) or 100 mM CaCl₂ (for Ca²⁺ electrodes) resulted in faster stabilization and an overall quieter ISM. Because no electrical contact between the ion exchanger and this backfill solution resulted in faster stabilization and an overall quieter ISM. A completed concentric assembly could be fabricated in 10–15 min. The final appearance of a concentric ISM is schematized in Fig. 1A. A photograph of a completed concentric electrode is also shown in the left portion of Fig. 2B.

To test the response time of the concentric ISMs, a piezoelectric stepper was used to shift the placement of two gravity-fed solution streams flowing from broken tip of a double-barreled, theta-glass-micropipette (Fig. 1B). The time to switch concentrations at the tip of the tested electrode was about 1 ms, as recorded by the change in junction potential of a micropipette filled with 150 mM NaCl, alternating between solutions of 150 and 10 mM NaCl (not shown). To test concentric pH microelectrodes, the pair of test solutions passed through the theta-glass barrels consisted of 50 mM phosphate buffers of pH 6.87 or 7.42. For the Ca²⁺-selective ISMs, the corresponding solutions consisted of 150 mM NaCl containing 0.10 or 1.0 mM CaCl₂.

**Fabrication of conventional ISMs**

Conventional pH- and Ca²⁺-selective microelectrodes electrodes were fabricated to enable a comparison with responses of the concentric ISMs in brain slice experiments (see following text). These double-barreled ISMs were made using a modification of the method of Lux (1974), as previously described (Chesler and Chan 1988). In brief, a pair of bound, thin-walled glass capillaries (A-M System 6170, OD 1.5 mm × ID 1.12 mm) was heated, twisted 180°, and then drawn on a vertical pipette puller. The reference barrels were backfilled with 2 M NaCl. The future H⁺-selective barrels were backfilled with 150 mM NaCl buffered to pH 7.42. The future Ca²⁺-selective barrels were filled with 150 mM NaCl plus 150 mM CaCl₂. The tips were broken to a diameter of 4–6 μm, and the ion barrel was rendered hydrophobic by repeated suction and ejection of 4% trimethylchlorosilane (Sigma T-4252) in xylene. A column of proton-selective or Ca²⁺-selective cocktail (Fluka 95291 or 21048, respectively) was then incorporated into the tip by suction to form a 100- to 200-μm column. For pH electrodes, the micropipette was then backfilled with a solution of 3 M KCl containing 50 mM K/Na phosphate buffer at pH 7.42. For Ca²⁺ electrodes, the backfill solution contained 3 M KCl plus 150 mM CaCl₂.

The larger, outer pipette was secured on the stage of an upright microscope and visualized at a power of ×100 or ×400. The smaller-diameter micropipette was attached to an independent micromanipulator and threaded within the larger barrel and through the column of ion exchanger, until its end was 4–6 μm from the tip of the outer micropipette. The inner barrel was then secured around the opening of the outer barrel using dental wax. Electrical contact to the KCl solution of the inner pipette was made with a silver–silver chloride junction. A completed concentric assembly could be fabricated in 10–15 min. The final appearance of a concentric ISM is schematized in Fig. 1A. A photograph of a completed concentric electrode is also shown in the left portion of Fig. 2B.

To test the response time of the concentric ISMs, a piezoelectric stepper was used to shift the placement of two gravity-fed solution streams flowing from broken tip of a double-barreled, theta-glass-micropipette (Fig. 1B). The time to switch concentrations at the tip of the tested electrode was about 1 ms, as recorded by the change in junction potential of a micropipette filled with 150 mM NaCl, alternating between solutions of 150 and 10 mM NaCl (not shown). To test concentric pH microelectrodes, the pair of test solutions passed through the theta-glass barrels consisted of 50 mM phosphate buffers of pH 6.87 or 7.42. For the Ca²⁺-selective ISMs, the corresponding solutions consisted of 150 mM NaCl containing 0.10 or 1.0 mM CaCl₂.

**FIG. 1.** Fabrication and testing of concentric ion-selective microelectrodes (ISMs). A: schematic representation of the tip of a concentric ISM. B: means of testing ISM response time. Rapid movement of the stepper switched the solution flow around the tip of the ISM, which was placed near the visible junction of the solution streams. C: response of a concentric pH microelectrode to a step in pH from 7.42 to 6.87 (50 mM phosphate buffers). D: left: response of a concentric Ca²⁺-selective microelectrode to a step from 0.10 to 1.0 mM CaCl₂. Right: responses of the Ca²⁺-selective microelectrode during repetitive steps between the same 0.10 and 1.0 mM CaCl₂ solutions.
incorporated into the tip by suction. The conventional ISMs were calibrated in phosphate buffers or solutions of CaCl$_2$ (as above) contained in separate beakers. We did not study the speed of conventional ISMs using the piezoelectric stepper because these electrodes were subject to high noise and large movement artifacts on this testing apparatus.

**Brain slice preparation**

Transverse rat hippocampal slices were prepared from anesthetized juvenile (P8–P14) rats of either sex. All procedures were performed with the approval of the New York University School of Medicine Institutional Animal Care and Use Committee. The brain was removed, placed into ice-cold, artificial cerebrospinal fluid (ACSF), and cut into 300-um sagittal slices using a Vibratome. The slices were incubated in ACSF at room temperature for at least 1 h before use. ACSF contained (in mM) 124 NaCl, 3.0 KCl, 3.0 CaCl$_2$, 1.5 MgCl$_2$, 26 NaHCO$_3$, 1.0 NaH$_2$PO$_4$, and 10 D-glucose, pH 7.4 (equilibrated with 95% O$_2$-5% CO$_2$). Hippocampal slices were studied in a submersion-style incubation chamber at 32°C. To better test the response time of the ISMs, we maximized the speed and amplitude of evoked ionic responses by switching to an ACSF solution with zero Mg$^{2+}$ and 100 mM picrotoxin (Gottfried and Chesler 1994). To elicit a rapid shift in pHe or [Ca$^{2+}$]$_e$, the Schaffer collateral fibers were activated with a train of three 400-msec duration, constant-current stimuli at 100 Hz, delivered by a twisted pair of 50-um diameter, Teflon-insulated, platinum-iridium wires.

**Brain slice recordings**

In brain slice experiments, the concentric ISM and a separate reference microelectrode were mounted on a Narishige MD-4 dual micromanipulator. The reference barrel was used to record evoked extracellular field potentials and slow DC potentials that were subtracted from the voltage on the ISM. The reference pipette was pulled from 1-mm glass capillary blanks (A-M System 6010) and was broken to a tip diameter of about 2 um. To enable a more acute angle between the ISM and reference electrode when mounted on the dual micromanipulator, the reference micropipette was heated and bent twice (Fig. 2B) and then backfilled with 2 M NaCl. The reference and concentric microelectrodes were fitted with an Ag–AgCl junction attached to high-impedance head stages. Capacitance neutralization was used to match the electric time constants of the two electrodes, as judged by the rise time of a 1-ms, 1-mV calibration pulse in the common ground circuit. The voltage of the reference barrel was continuously subtracted from the potential on the concentric ion electrode and the output was filtered at 4 kHz. The tip separation of the ISM and reference electrode was adjusted to 5–10 um before the dual assembly was advanced into the slice. ISM recordings were obtained in the CA1 stratum radiatum, at a depth of maximal orthodromic field potential, typically 100–150 um within the tissue. Recordings were obtained from the same region of the slice using conventional, double-barreled ISMs, placed at the same approximate depth as the concentric assembly, using a second, independent micromanipulator (Fig. 2A). Small alterations in depth had little effect on the amplitude and time course of the evoked, extracellular ionic shifts.

**Data analysis**

Statistical data are presented as means ± SE, where n refers to the number of electrodes tested or the number of slices, as indicated. Time constants of the response to a step alteration of pH or Ca$^{2+}$ activity were measured as the time to 63% of the steady-state voltage attained after a rapid solution change from low to high ion activity. Measure-
ments of voltage changes and peak-to-peak noise were made using the cursor function of a Nicolet 3090 digital storage oscilloscope. Comparisons were made with an unpaired, two-tailed Student’s t-test.

RESULTS

Response features of concentric pH microelectrodes

The response time of concentric pH-sensitive microelectrodes was tested by rapidly changing between 50 mM phosphate buffers of pH 7.2 and pH 6.87, using a piezoelectric stepper, as depicted in Fig. 1B. An example of a response to this step change in pH is shown in Fig. 1C. The transition was marked by an initial, negative, downward transient. This transient was noted in most records using the piezoelectric stepper, whether testing pH or Ca$^{2+}$ electrodes, and appeared to be a mechanical artifact arising from the rapid motion of the theta-glass assembly. In the example shown, the mechanical transient was followed by a rapid positive deflection to a new plateau potential corresponding to pH 6.87. In this case, the time constant of the voltage change was 8.6 ms, which was the fastest response noted. For seven concentric pH microelectrodes the mean time constant was 14.9 ± 1.3 ms (range 8.6–19.0 ms).

The slope response of concentric pH microelectrodes was obtained from the difference in steady-state voltages at pH values of 6.87 and 7.42. The mean slope for seven electrodes was 61.8 ± 2.6 mV per unit pH (range 48–67 mV/pH), which was slightly greater than the theoretical Nernst value of 58–59 mV per unit pH. This feature was not related to the rapid solution change because slight super-Nernst slope responses were also noted when the concentric pH electrodes were tested in static solutions contained in beakers. The electrode represented in Fig. 1C happened to have both the highest slope and lowest time constant, although among all seven concentric pH microelectrodes, there was no correlation between the slope and time constant of response. By comparison, the conventional, double-barreled pH microelectrodes used in physiological experiments (below) had a mean slope response of 53.4 ± 1.7 mV per unit pH.

Response features of concentric Ca$^{2+}$-selective microelectrodes

Concentric Ca$^{2+}$-selective microelectrodes were tested by a rapid change of 150 mM NaCl solutions containing 0.10 versus 1 mM CaCl$_2$. An example of a response to this step change in Ca$^{2+}$ concentration is shown in Fig. 1D. On switching from 0.10 to 1.0 mM Ca$^{2+}$, a brief, negative (apparently mechanical) transient was followed by a rapid positive shift in potential to a new plateau. The example shown is from the fastest of these electrodes, which had a response time constant of 3.2 ms. For seven concentric Ca$^{2+}$-selective microelectrodes, the mean response time constant was 5.3 ± 0.90 ms (range 3.2–9.3 ms), which was significantly different from that of the concentric pH microelectrodes ($P < 0.001$). The mean slope response of the same set of electrodes was 28.4 ± 0.40 mV per decade change in Ca$^{2+}$ activity, which was 96% of the theoretical Nernst response. There was no correlation between electrode slope and response time. The consistency of the concentric ISM response during repeated, rapid changes in Ca$^{2+}$ activity is evident in the right panel of Fig. 1D. Conventional, double-barreled Ca$^{2+}$-selective microelectrodes used in physiological experiments (below) had a nearly identical mean slope response of 28 ± 1.1 mV per decade change in Ca$^{2+}$ activity.

 Extracellular recordings in rat hippocampal slices

An advantage of fast responding ISMs should be an ability to better discern the amplitude and time course of rapid, physiological ion responses. To demonstrate and quantify the advantage of the concentric microelectrodes over their conventional counterparts, we conducted paired recordings in the stratum radiatum of rat hippocampal slices, as depicted in Fig. 2A. Transient pH$_e$ or [Ca$^{2+}$]$_e$ responses were evoked by a three-shock, 100-Hz train (duration 20 ms) to the Schaffer collateral fibers.

This orthodromic stimulus train elicited a rapid extracellular alkaline response, as has been described in numerous reports (Carlini and Ransom 1986; Chen and Chesler 1992; Taira et al. 1995; Walz 1989). The alkaline transients recorded by the concentric assembly were far greater in amplitude and much faster than the recordings obtained with the conventional double-barreled pH microelectrodes, as is evident in the pair of records shown in Fig. 2C. The mean amplitude of the alkaline shift was two- to threefold greater and the time to peak, half time to peak, and half time of decay were two- to threefold faster in the concentric microelectrode records ($P < 0.01$ for all parameters, Table 1). In fact, the time course of the pH$_e$ transients recorded with the concentric assembly compared favorably with similar measurements previously obtained us-

### TABLE 1. Characteristics of stimulus-evoked pH$_e$ and [Ca$^{2+}$]$_e$ transients recorded by conventional vs. concentric ISMs

<table>
<thead>
<tr>
<th>H$^+$-Selective Microelectrodes</th>
<th>Amplitude, $\Delta$PH$_e$,</th>
<th>Time to peak, ms</th>
<th>Half-Time to Peak, ms</th>
<th>Half-Time Decay, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>0.016 ± 0.003</td>
<td>1,111 ± 115</td>
<td>421 ± 87</td>
<td>2,428 ± 234</td>
</tr>
<tr>
<td>Concentric</td>
<td>0.04 ± 0.01*</td>
<td>399 ± 36*</td>
<td>186 ± 16*</td>
<td>813 ± 102*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ca$^{2+}$-Selective Microelectrodes</th>
<th>Amplitude, $\Delta$Ca$^{2+}$, mM</th>
<th>Time to peak, ms</th>
<th>Half-Time to Peak, ms</th>
<th>Half-Time Decay, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>0.051 ± 0.013</td>
<td>890 ± 72</td>
<td>316 ± 47</td>
<td>1,974 ± 294</td>
</tr>
<tr>
<td>Concentric</td>
<td>0.18 ± 0.03*</td>
<td>365 ± 23*</td>
<td>197 ± 10*</td>
<td>860 ± 67*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 5$ slices for both H$^+$-selective and Ca$^{2+}$-selective microelectrodes. The amplitude, time to peak, half-time to peak, and half-time of decay were significantly different for the concentric ISMs compared against the respective conventional ISMs (*$P < 0.01$).
ing an extracellular, fluorescent pH probe (Gottfried and Chesler 1996; Tong et al. 2006). In addition to greater speed, a lower inherent noise was evident on the concentric traces, and they were less subject to capacitance-coupled movement artifacts. Respective peak-to-peak noise for these five concentric versus five conventional pH microelectrodes was 0.14 ± 0.02, versus 0.49 ± 0.04 mV.

Similar advantages were apparent in a comparison of \( [\text{Ca}^{2+}]_e \) responses recorded by concentric versus conventional ISMs. This is evident in the example shown in Fig. 2D, where the Schaffer collateral fibers were again stimulated by three shocks delivered at 100 Hz. Compared with the conventional ISMs, the mean amplitude was three- to fourfold greater, and the temporal features more rapid, in the records obtained with the concentric assembly \( (P < 0.01 \) for all parameters, Table 1). The lower noise of the concentric \( \text{Ca}^{2+} \)-selective microelectrode is also evident in the traces of Fig. 2D. Respective peak-to-peak noise for these five concentric versus five conventional \( \text{Ca}^{2+} \) microelectrodes was 0.16 ± 0.03, versus 0.58 ± 0.06 mV.

**DISCUSSION**

Ion-selective microelectrodes that make use of neutral carrier ionophores have been used extensively in electrophysiological experiments (Chesler 1990, 2003; Grafe and Ballanyi 1987; Heinemann and Pumain 1980; Nicholson 1980; Somjen 1980; Sykova and Svoboda 1990). These electrodes are notable for a great selectivity to a particular ion species, but are limited by their high degree of noise and slow response times. These liabilities arise from the high resistivity of the ion-selective cocktails. When incorporated into the narrow tip of a microelectrode, the column of exchanger must be of sufficient length to ensure a good electrical seal against the wall of the micropipette, and therefore typically extends several hundred micrometers. The high resistance of the column, coupled with the capacitance across the glass, results in a long electrical time constant. To overcome this limitation, the use of a concentric shunting pipette was conceived some time ago by Orme (1969). The obvious effect of the central pipette would be a reduction in the longitudinal resistance of the ion-exchange column. Another consequence would be a marked reduction in the distributed capacitance to ground. A decrease in capacitance would result from increased thickness of the dielectric. In the absence of the inner electrode, the dielectric amounts to the thickness of the glass wall of the larger micropipette. With the inner, concentric microelectrode, however, the dielectric constitutes two glass walls and the comparatively large distance between the inner pipette solution and the outer bath. Thus a rationalization of enhanced performance in electrical terms would have to account for the improvements in both the resistance and capacitance of the concentric ISMs. Although these electrical principles are straightforward, the implementation of Orme’s idea has been extremely limited.

In this report, we used the concentric concept of Orme and borrowed aspects of construction from Ujek et al. (1979, 1981), to fabricate concentric ISMs selective for \( \text{H}^+ \) and \( \text{Ca}^{2+} \). The present design benefited from several key modifications. A silanization technique based on that of Chesler and Kraig (1989) was used that proved to be fast and reliable. This method did not require exposure of the glass to silane vapors or to long periods of heating at high temperature, as is sometimes described (Munoz et al. 1983). In principle, the silanization procedure of Lux (1974) used for conventional ISMs could also be used to fabricate the concentric microelectrodes, although we found such ISMs to be relatively unstable.

Another advantage of the present method was that the concentric assemblies could be constructed from commercially available, thin-walled, capillary glass. Final incorporation of the central shunting micropipette was also greatly simplified by drawing the outer electrode with a rapid taper, similar to a patch pipette. This improved the visualization during placement of the inner pipette and lessened the chance of its breakage during insertion.

In the original study of Pumain and colleagues (1983), neutral carrier, concentric \( \text{Ca}^{2+} \)-selective microelectrodes were used to record from cat brain. This required that the entire assembly have a long taper, to enable recordings at cortical depths >1 mm. Use of these electrodes in vivo also required the use of double-barreled capillaries, to provide a reference barrel that could accompany the concentric assembly deep within the brain. The configuration described in this report would clearly be inappropriate for such studies, given the rapid taper of the ISM, and the use of a distinct reference pipette on a dual micromanipulator. The present method, however, is ideally suited for brain slice or single-cell studies, where patch pipettes of similar taper are commonly used.

The response time of the \( \text{Ca}^{2+} \)-selective microelectrodes constructed by Pumain et al. (1983) were neither detailed nor displayed. Their electrodes were said to respond to pressure-ejected solutions in a few milliseconds, suggesting a speed similar to the \( \text{Ca}^{2+} \) electrodes in this report, where a step from 0.1 to 1.0 mM \( \text{Ca}^{2+} \) elicited a nearly Nernst response, with a mean time constant of 5.3 ms. Our \( \text{Ca}^{2+} \)-selective microelectrodes were considerably faster than the \( \text{H}^+ \)-selective counterparts that had a mean time constant of 14.9 ms for a step from pH 7.42 to pH 6.87. The basis for this difference in speed was not explored, but might be expected because of the 40% higher concentration of the charge carrier sodium tetraphenylborate used in the \( \text{Ca}^{2+} \)-selective cocktail.

Some of the concentric pH microelectrodes had a slightly super-Nernst response, leading to a mean slope of 61.8 mV per unit pH. The basis for this small deviation from the Nernst equation is not clear, although this feature would not hinder utility of the electrodes for biological measurements. The original descriptions of tridodecylamine-based \( \text{H}^+ \)-selective cocktail also mentioned a modest, unexplained, super-Nernst behavior of the pH electrodes, but over a more acidic range of pH (Ammann et al. 1981; Schulthess et al. 1981).

The advantage of the concentric ISMs over their conventional counterparts was evident from the comparison of stimulus-evoked transients of \( \text{pH}_e \) and \( [\text{Ca}^{2+}]_e \). These experiments indicated that conventional ISMs considerably underestimate the amplitude and speed of extracellular ionic changes in response to a rapid stimulus. This shortcoming of conventional pH microelectrodes was also evident in comparisons against an extracellular fluorescent pH indicator (Gottfried and Chesler 1996; Tong et al. 2006). These prior fluorescence studies were conducted in hippocampal slice preparations from rats of similar age, using the same ACSF and stimulus parameters as in the present study. It is therefore notable that the amplitude of the evoked alkaline transients estimated with the fluorescent probe was about 0.03–0.04 unit pH, which is comparable to the mean value of 0.04 recorded with
concentric pH microelectrodes in the present study. This suggests that the response speed of the two methods is probably comparable. However, the time course of responses recorded with both techniques could be different because the fluorescence method sampled extracellular pH from a distributed volume of perhaps 10^3 µm³, whereas a concentric pH microelectrode records from a 1,000-fold smaller volume in close proximity of its tip. Thus in studying more local pH changes, concentric pH microelectrodes would have a considerable advantage over the fluorescence technique.

A number of uses for rapid-responding concentric ISMs can be envisaged in addition to the study of activity-dependent ionic shifts in brain slices. Although not useful for recordings deep within tissue, in vivo studies near the surface of the brain would be feasible. In addition, it seems likely that intracellular studies could be performed with the concentric electrodes when studying large cells such as oocytes or snail neurons where a separate impalement with a reference electrode is feasible. Alternate use of concentric ISMs in an extracellular context can be envisaged. In studies of extracellular diffusion in the brain, ISMs for tetramethylammonium (TMA⁺) are typically placed 50–100 µm from a TMA⁺ iontophoresis pipette (Nicholson 1993). The diffusion analysis makes use of conceptualized volume fraction and tortuosity parameters for the intervening interstitial space (Nicholson and Phillips 1981). A faster-responding and quieter ISM may allow for the study of these extracellular diffusion parameters over shorter distances. Faster ISMs might also find use in the study of ionic shifts at the surface of single cells, where inherent noise and the rapid diffusive dissipation of local gradients could render slow-responding ISMs less effective. Used in this manner, concentric H⁺-selective microelectrodes could prove beneficial for the study of bicarbonate or proton transport (Grichtchenko and Boron 2002; Kaila and Voipio 1987; Schwiening et al. 1993).

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