Electrical properties of neurons recorded from the rat supraoptic nucleus in vitro

BY W. T. MASON.

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The electrical properties of neurons in the supraoptic nucleus (so.n.) have been studied in the hypothalamic slice preparation by intracellular and extracellular recording techniques, with Lucifer Yellow CH dye injection to mark the recording site as being the so.n. Intracellular recordings from so.n. neurons revealed them to have an average membrane potential of $-67 \pm 0.8$ mV (mean $\pm$ s.e.m.), membrane resistance of $145 \pm 9$ M$\Omega$ with linear current–voltage relations from 40 mV in the hyperpolarizing direction to the level of spike threshold in the depolarizing direction. Average cell time constant was $14 \pm 2.2$ ms. So.n. action potentials ranged in amplitude from 55 to 95 mV, with a mean of $76 \pm 2$ mV, and a spike width of $2.6 \pm 0.5$ ms at 30% of maximal spike height. Both single spikes and trains of spikes were followed by a strong, long-lasting hyperpolarization with a decay fitted by a single exponential having a time constant of $8.6 \pm 1.8$ ms. Action potentials could be blocked by $10^{-6}$ M tetrodotoxin. Spontaneously active so.n. neurons were characterized by synaptic input in the form of excitatory and inhibitory postsynaptic potentials, the latter being apparently blocked when 4 M KCl electrodes were used. Both forms of synaptic activity were blocked by application of divalent cations such as Mg$^{2+}$, Mn$^{2+}$ or Co$^{2+}$. 74% of so.n. neurons fired spontaneously at rates exceeding 0.1 spikes per second, with a mean for all cells of $2.9 \pm 0.2$ s$^{-1}$. Of these cells, 21% fired slowly and continuously at 0.1–1.0 s$^{-1}$, 45% fired continuously at greater than 1 Hz, and the remaining 34% fired phasically in bursts of activity followed by silence or low frequency firing. Spontaneously firing phasic cells showed a mean burst length of $16.7 \pm 4.5$ s and a silent period of $28.2 \pm 4.2$ s. Intracellular recordings revealed the presence of slow variations in membrane potential which modified the neuron's proximity to spike threshold, and controlled phasic firing. Variations in synaptic input were not observed to influence firing in phasic cells.

INTRODUCTION

The in vitro slice preparation originally devised for electrophysiology by Yamamoto & McIlwain (1966) has been successfully used for a range of tissues from the vertebrate central nervous system (for a wide review, see Dingledine et al. 1980). Recently, the technique has also been used for study of the rat hypothalamus (Hatton et al. 1977, 1978a, b; Haller et al. 1978). This preparation is amenable to
both extracellular and intracellular recording and has several advantages over in vivo recording, including the lack of necessity for anaesthetic and the increased stability of an isolated preparation which makes long-term intracellular recording possible.

The magnocellular neurons of the hypothalamus in the supraoptic (so.n.) and paraventricular nuclei (pv.n.) project to the neurohypophysis. Most cell bodies in the so.n. synthesize the neuropeptides vasopressin, oxytocin (Zimmerman 1981; Brownstein et al. 1980) and possibly enkephalin (Sar et al. 1978), which in turn are released from endings in the posterior pituitary into the bloodstream. Action potentials generated in the hypothalamic cell bodies are thought to control the release of these neuropeptides.

Kandel (1964) reported the first intracellular recordings from magnocellular neurons in the anaesthetized goldfish, and subsequently a number of workers have sought to characterize by extracellular recording in vivo (Cross et al. 1975; Hayward 1977; Yagi & Iwasaki 1977) and in vitro (Hatton et al. 1978a; Brimble et al. 1978; Haller et al. 1978) the mechanism by which so.n. neurons control release of vasopressin and oxytocin from the pituitary. However, these data provide little information on the electrical properties of so.n. neurons responsible for neuropeptide release. Indeed, it is a point of controversy whether so.n. neurons are themselves the receptors for humoral factors regulating hormone release, or whether they are merely output neurons that receive regulatory input from other sources. Intracellular recording enables direct investigation of some synaptic interactions between cells, and study of electrical properties of the cell membrane, such as membrane potential, input resistance, spike threshold and underlying mechanisms that control patterning of firing.

The first intracellular recordings from magnocellular neurons in hypothalamic slices were recorded from the so.n. (Hatton et al. 1978b; Mason 1980a, b). They displayed patterns of spontaneous activity similar to those observed in vivo in the anaesthetized animal, and neurons from this preparation responded to small physiological changes in extracellular osmolality. Similar results had previously been obtained by using extracellular recording from cells in the pv.n (Hatton et al. 1978a).

This paper reports the results of electrophysiological studies of the characteristics of so.n. neurons recorded with both intracellular and extracellular techniques. A future paper will report the effects on so.n. neuronal properties of addition of some osmotically active solutes and other substances to the extracellular space of the so.n.

**Methods**

**Slice preparation**

Wistar rats (200–250 g) were stunned and decapitated. The brain was gently lifted away from the cranial cavity to expose the optic nerves. These were cut with iris scissors to avoid pressure on the optic chiasm and disruption of the so.n. The brain was then placed on a Perspex block, and trimmed with a razor blade. The flat surface of the brain was mounted on a brass block, previously coated with cyanoacrylate adhesive (Eastman Kodak) to leave the brain in the desired
orientation, sections usually being cut in either coronal or a sagittal plane. The tissue block was placed into the chamber of a vibratome containing medium at 37 °C (see below) continuously oxygenated with a mixture of 95% O₂ and 5% CO₂. Generally three or four slices of approximately 300 to 400 μm thickness were cut, the total width of cut being approximately 5 mm. In the coronal plane the total travel of the cutting blade was taken from the base of the brain to just beyond the third ventricle, and a cut was made beyond the third ventricle so that the slice was held together at the point of junction. The slice was then transferred into a 5 ml beaker submerged in the vibratome chamber. When all slices had been collected, they were transferred on the end of a fine camel hair brush into the incubation chamber by laying them onto a thin wedge of lens tissue draped across the chamber and supported on a taut piece of nylon mesh. The chamber was similar to many in current use (as Richards & Sercombe 1970), and it was made by the Medical Research Council mechanical workshop at Cambridge. It consisted of an outer chamber, which was heated and gassed and contained medium similar to that with which the slice was perfused. This was found to be necessary as, during the gassing process, gas passed into the internal chamber to aerate the slice was found to carry with it an aerosol of humidifying medium, in this case the isotonic Yamamoto medium.

The perifusing medium entering the chamber was delivered by a Pharmacia LP3 peristaltic pump through the heated outer chamber and into a bubble trap, at a flow rate of 1.0-4.0 ml/min. From here medium passed directly to the underside of the slice, spilled over the inner reservoir of the chamber, and out into an aspirated drain. In the base of the internal chamber into which the slices were mounted were placed three Ag/AgCl electrodes to earth the preparation. To reduce the total dead volume surrounding the slice to about 1.4 ml, a Perspex plug was inserted into the inner chamber, thus reducing turbulence caused by fluid entry. The top of the chamber was partially covered at all times by a large glass cover slip, to maintain maximum humidity.

The entire chamber was mounted on a steel frame to enable positioning of micromanipulators and a Wild stereo microscope. The preparation was illuminated with a Schott dual light fibre optic with focused light. The apparatus was mounted on an Ealing vibration damping table.

Electrophysiological recording

Standard intracellular recording techniques were applied to the slice preparation. Microelectrodes were pulled on a modified Livingstone-type puller (by courtesy of Mr R. Cook, Physiological Laboratory, Cambridge). Microelectrodes were filled with 4 M KCl in early experiments, and later with either 4 M potassium acetate or 4 M potassium acetate containing 100 mM KCl to improve the resistance characteristics. Electrodes were connected to a driven shield preamplifier with a Ag/AgCl pellet, with the initial preamplifier stage mounted close to the preparation. The electrometer used in most experiments was either a WPI M701 or a Dagan 8100 electrometer. The preamplifier section was mounted on a Prior manipulator mounted on a Huxley micromanipulator, for fine positioning and cellular impalement. Signals were amplified x 100, by using high-precision calibrated amplifiers,
and recorded on a Racal Store 4 FMDS tape recorder. Records were transcribed either with a Grass camera directly from the oscilloscope face or onto an SE5000 E.M.I. UV recorder. Analyses of interspike interval histograms were done on a PDP8 computer, with use of a Digital neurophysiological package. Single-shot records were analysed on a Datalab DL501 transient recorder, and played back onto an XY plotter.

Extracellular recordings were made with lower impedance electrodes than those used for intracellular recording and filled with 150 mM NaCl, resulting in tip resistances of 5–50 MΩ, whereas intracellular electrodes usually had resistances of 50–200 MΩ. When intracellular recordings were being made, microelectrodes were lowered onto the slices and small depolarizing currents of 50 ms duration at 10 Hz frequency were passed through the recording electrode. This both enabled the electrode resistance to be balanced with a bridge balance circuit and more importantly the repetitive current stimulation seemed to facilitate impalement and recording stability. Passage of depolarizing current, both extracellularly and intracellularly, also enabled some judgement to be made on the distance of the recording microelectrode from the cell body by the latency of spike response to the depolarizing pulse. A similar judgement could be gained by passage of hyperpolarizing current, which usually resulted in generation of an ‘off’ or ‘anode-break’ response. Accurate estimation of membrane potential during recording from the slice preparation was difficult. Frequently during advance of the electrode through the tissue in increments of approximately 1 μm the potential of the recording electrode drifted and the electrode consequently went slightly out of balance, perhaps owing to blocking of the electrode tip. In most cases the electrode could be cleared and electrical properties regained by shorting the capacity compensation of the electrometer circuit. This led to a high frequency (ca. 100 kHz) oscillation of the recording preamplifier. The same technique also facilitated impalement of single cells, and it was frequently found that a small increase in the apparent electrode resistance, followed by ‘buzzing’ of the microelectrode, would result in cell impalement. Attempts were not made to correct for junction potentials of the recording electrodes, or to evaluate the potential of electrodes. However, large tip potentials usually correlated with poor current-passing properties of the microelectrodes, and such electrodes were always discarded.

Injection of Lucifer Yellow

To evaluate the site and morphology of intracellularly recorded neurons, the highly fluorescent, negatively charged dye Lucifer Yellow CH (Stewart 1978) was used. Of this dye 5 mg were dissolved in 100 μl of 0.1 M LiCl or 0.33 M lithium citrate solution. Following impalement of the cell, the dye was injected by passage of current in a régime of 0.5 s injections of 1 nA hyperpolarizing current at 1 Hz frequency. Current passage over 15–200 s resulted in ejection of adequate amounts of dye to enable observation. Generally, only one cell per son. was injected with dye. Between pulses of dye injection, a small depolarizing (0.5 nA) step current was injected to ensure that the cell was still excitable. After dye ejection, the cell was sometimes tested further, but generally the dye had little effect on membrane
electrical properties. The slice was then immersed in 5% formalin in 0.1 m phosphate buffer, pH 7.2, and photographed in wholemount; this was followed by overnight fixation. After 12–24 h of fixation, the slice was sectioned in 50 μm sections, and cleared with xylene or methyl salicylate. Fluorescence microscopy was done with a Leitz microscope with incident fluorescent excitation and optics suitable for fluorescein detection. Photography was with Kodak Ektachrome film rated at ASA 800.

Solutions

In most experiments a modified Yamamoto’s medium (Yamamoto & Mcllwain 1966) was used which approximated to the normal osmolarity of rat plasma. This medium contained 124 mM NaCl, 5 mM KCl, 1.25 mM KH₂PO₄, 2 mM MgSO₄, 5 mM glucose, 25 mM NaHCO₃ and 2 mM CaCl₂. For experiments where either calcium was omitted or Mg²⁺ or Co²⁺ was added, Ca²⁺-free Yamamoto’s medium was made, and phosphate was also omitted to prevent precipitation of a complex of divalent cation and phosphate. This medium was additionally buffered by the inclusion of 1.25 mM Hepes, and was set to a final pH of 7.4. CoCl₂, MnCl₂ or MgCl₂ was added from stock 1 mM solutions. All media were bubbled with a mixture of 95% oxygen and 5% CO₂ such that final pH was about 7.4. Normal osmolarity of media was measured on a Knauer freezing point depression osmometer, and was in the range 284–292 mosm.

Results

Membrane potential and resistance

Upon impalement of so.n. neurons in isotonic medium (284–292 mosm), a mean resting potential of —67 ± 0.8 mV was found (mean ± s.e. for 82 cells). Measured membrane potentials are shown in figure 1. Cells with a membrane potential less than —50 mV were regarded as either damaged cells or poor impalements, and were excluded from further consideration. Occasionally, cells showing low membrane potential accompanied by small spike amplitude were found to become stable, after 5 or 10 min of recording, the result being a more negative membrane potential. These cells were not tested further until they had reached a steady state.

Membrane input resistance was measured as slope resistance in the hyperpolarizing direction and in the depolarizing direction for subthreshold stimulation. So.n. neurons had a mean slope resistance of 144 ± 9.7 MΩ (mean ± s.e., N = 44). The measured current–voltage curves for these cells were nearly linear for small hyperpolarizing currents (to ca. —40 mV below resting membrane potential) and for depolarizing current to the level of spike threshold. Typical curves for three such cells are shown in figure 2, and a histogram of membrane input resistance is given in figure 3. Current–voltage relations for so.n. neurons indicated non-rectifying membrane properties.

Response to intracellularly injected current

So.n. neurons were excited by passage of depolarizing but not hyperpolarizing current (figure 4), although strong hyperpolarizing currents resulted in an ‘anode
Figure 1. Histogram of intracellularly recorded membrane potential of so.n. neurons in the hypothalamic slice preparation.

Figure 2. Three representative current–voltage relations for neurons in the so.n. slice preparation, showing variability between membrane slope resistance for the three measured neurons. Measurements were made with a single intracellular microelectrode with use of bridge balance to compensate for the electrode resistance.
break' or 'off' spike response (figure 6a). As shown in figure 4, the latency of the spike response varied with the strength of depolarizing current. A notable feature of these cells was their low threshold for spike initiation. Spike threshold correlated with the spontaneous firing rate exhibited by the cell upon impalement. Thus most cells exhibiting spontaneous firing activity greater than 1 spike per second showed a spike threshold within 2–5 mV of the resting potential. Cells exhibiting less than 1 spike per second spontaneous activity, however, lay 10 mV or more from the spike threshold measured for the cell. In the present intracellular recordings, no correlation was found between the membrane potential and input resistance, suggesting that poor seal resistance between membrane and electrode was not a problem. Cell time constants were approximated by a single exponential and were found to be 14 ± 2.2 ms (mean ± s.e., 24 cells).

Passage of long (1 s) depolarizing current pulses was used to examine spike accommodation in s.o.n. neurons. The amplitude of action potentials when the cell was driven by depolarizing current decreased only slightly, i.e. about 15%, for a train of pulses. For strong depolarizing current, cells could be driven to fire at a rate of up to about 50 spikes per second. This upper limit on firing rate appeared to result from the strong after-hyperpolarization that followed action potentials in most of the s.o.n. cells. Firing of neurons could be prevented by step or steady passage of hyperpolarizing current, and firing rate and latency of action potential generation in response to constant current passage was a direct function of current amplitude (figure 5).

**Characteristics of s.o.n. neuron action potentials**

Typical s.o.n. soma spikes recorded intracellularly are shown in figure 6, where they are compared with typical spikes recorded extracellularly. Spike amplitudes...
Figure 4. Example of an intracellularly recorded neuron response to depolarizing and hyperpolarizing constant current injection (50 ms square pulse). A bridge balance has been used to cancel the effective electrode resistance, and the transient arising from the balancing procedure can be seen at the start and end of the square pulse. The magnitude and polarity of the current pulse (I) is given to the right of each trace. Records were captured and stored on a Datalab DL901 transient recorder and transcribed on an XY plotter.
Figure 5. An impaled supraoptic neuron bathed in higher osmolarity medium (318 mosM), showing a high firing rate. The top record shows the neuron in the absence of any current passage. The membrane potential was $-52 \text{ mV}$. In successive records, 1 s steps of hyperpolarizing current were injected to show that, as the neuron moves away from spike threshold, firing slows in response. The amount of current passed ($I$) in each instance is shown on the right.

In good impalements ranged from 55 to 95 mV, with a mean value of $76 \pm 2 \text{ mV}$ (mean ± s.e., $N = 42$). Action potentials were usually associated with a slow depolarization leading to a fast rising phase, with spike width at 30% of maximal spike height being $2.6 \pm 0.5 \text{ ms}$ (mean ± s.e.) for the cells evaluated above. The action potential was followed by a protracted hyperpolarization ($9.3 \pm 1.1 \text{ mV}$).
Figure 6. Intracellular and extracellular recordings from s.o.n. neurons in vitro. (a) This intracellular record shows the effect of current passage on spike generation. The top record shows a 50 ms step of hyperpolarizing current. During the hyperpolarization, the cell does not spike, but cessation of the pulse results in an 'off' spike, or anode-break effect, an effect observed in about 80% of all neurons. In the bottom record, passage of depolarizing current of similar magnitude (0.3 nA) resulted in action potential generation as normal. (b)–(d) Further examples of intracellular records, demonstrating e.p.s.p. summation in different spontaneously active cells, and showing in (d) the time course of the hyperpolarization occurring after spike propagation in s.o.n. neurons, possibly account for the phenomenon of 'recurrent inhibition' noted by the authors. (e) The waveform of an extracellular spike recorded with the high impedance electrodes used in these experiments, also exhibiting a long hyperpolarization following a spike.
Figure 7. (a) Intracellular records from an so.n. neuron in normal medium, showing slow e.p.s.ps summing to lead to spike propagation. This record also demonstrates the slow decay of e.p.s.ps observed in most so.n. neurons. This cell showed a low level of spontaneous activity, being about 12 mV from threshold. (b) Effect of TTX on the same cell as in (a). A 50 ms pulse of depolarizing constant current was passed down the electrode at intervals to test the cell. At time zero, TTX was applied via the perifusate at a final concentration of $10^{-6}$ M. The time, in minutes, after the start of TTX perfusion is given above each record. It can be seen that the toxin slowly blocks spike propagation in the neuron, initially resulting in a broadening of the action potential, and finally leading to complete abolition of the spike after 12–15 min. The bridge balance in these records drifted slightly out of balance over the experimental time course.
(mean ± s.e., N = 42)) which returned to the baseline with an exponential decay time of 8.6 ± 0.8 ms (mean ± s.e. for the above sample).

Action potentials of five so.n. neurons tested were reversibly blocked by the application of $10^{-6}$ M tetrodotoxin (TTX) in the perifusing medium. TTX applied in this fashion reduced spike amplitude and lengthened spike duration (figure 7).

**Postsynaptic potentials**

The spontaneous electrical activity of so.n. neurons was characterized by action potentials preceded by a small depolarizing potential, probably an excitatory postsynaptic potential (e.p.s.p.). These potentials varied widely in amplitude and time course, and appeared to be responsible for spike activation in most cells. As shown in figures 6 and 7, such potentials showed classical summation behaviour which in many cases enabled them to exceed the threshold potential and result in a spike. Many small potentials failed to exceed the measured threshold potential of the cell and these could be analysed for rise and decay time. Characteristics of these potentials were measured in 16 intracellularly recorded neurons and found to have a mean rise time of 1.1 ± 0.2 ms and a decay fitted by a simple exponential of 8.6 ± 1.1 ms. This exponential decay was similar in time course to the exponential decay of the hyperpolarizing phase of the neuron action potential.

In early experiments, microelectrodes were filled with 4 M KCl, and, in 22 intracellular recordings made with these electrodes, neurons with only e.p.s.ps were recorded. Later experiments with use of 4 M potassium acetate electrodes revealed the presence of inhibitory postsynaptic potentials (i.p.s.ps) in so.n. neurons. On some occasions, the two types of potential could be observed to interact, with an i.p.s.p. being superimposed on an e.p.s.p., effectively preventing the e.p.s.p. from eliciting a spike. I.p.s.ps measured in six cells recorded with potassium acetate electrodes showed a time course similar to that of the e.p.s.ps measured in the same cell. Thus leakage of Cl⁻ ions from the KCl microelectrodes into the impaled cells in early experiments probably resulted in a change of reversal potential for the i.p.s.ps, changing them into apparent e.p.s.ps.

In cells showing spontaneous activity, the amplitude of e.p.s.ps could be increased by passage of hyperpolarizing current through the recording electrode in a stepwise fashion. It was not possible to reverse these potentials with depolarizing current as passage of such current led to rapid cell firing.

Perifusion of the slice with solutions containing elevated Mg²⁺ (15 mM) or 2–4 mM Ce²⁺ or Mn²⁺ in Ca²⁺-free solutions abolished both e.p.s.ps and i.p.s.ps in most cells tested. Spontaneous action potentials were also blocked in these cells, but all neurons so tested could still generate action potentials when depolarized with intracellularly injected current, and under these conditions spike amplitudes were not affected. Blockade of postsynaptic potentials by the divalent cations was not absolute. Even after equilibration in such solutions for 1–2 h, a small number of cells could be recorded extracellularly. Although difficult to quantify, some indication of the number of units persisting can be gained from the observation that, in a slice perfused with normal Ca²⁺-containing medium, eight to ten spontaneously firing units were likely to be encountered extracellularly in one electrode pass through a 400 μm section. In preparations blocked with divalent cations.
cation, it was usually necessary to make two to five complete passes to find one unit, but these persisting units were also spontaneously active.

**Quiescent cells in the so.n. slice preparation**

Of all neurons recorded both extracellularly and intracellularly in normal osmolarity Yamamoto's medium, 74% \((N = 124)\) fired spontaneously at rates exceeding 0.1 spikes per second. Of the 26% \((N = 60)\) of neurons recorded extracellularly that did not fire spontaneously, all could be activated by passage of depolarizing current through the recording barrel carrying 150 mM NaCl. Cessation of current passage silenced the cell. Ten out of twelve quiet cells also responded to pressure ejection of 1 mM glutamate from a blunt pipette positioned close to the slice, and ceased to fire as the glutamate diffused away upon termination of the pressure ejection. A number of similar cells (seven of 82) were recorded from intracellularly that, although impaled well and showing membrane potential and resistance within the normal range, did not fire. These neurons had little or no detectable synaptic input in the form e.p.s.ps or i.p.s.ps, and generally lay 15–30 mV away from the threshold potential for spike initiation. All such neurons could, however, be excited to fire normal action potentials upon injection of depolarizing current.

**Spike patterning among spontaneously active neurons**

One-hundred-and-twenty-seven spontaneously firing neurons recorded both extra- and intracellularly from the so.n. have been divided into three arbitrary categories; their overall firing rates are shown in figure 8. The total population of so.n. neurons with activity over 0.1 spikes per second showed a mean firing rate of 2.7 ±0.2 s⁻¹ (mean ± s.e.). The first category consists of slow continually firing neurons with spike frequencies of 0.1–1.0 s⁻¹ and represents 21% of the population. The second category consists of fast continually firing neurons \((> 1 \text{ s}^{-1})\) representing...
Figure 9. For description see opposite.
45% of the population. The remaining category, 34% of the population, is characterized by bursts of activity followed by periods of silence or very low frequency firing. Mean firing rates for these categories of cells are given in table 1, which includes the mean modal interval derived from interspike interval histograms.

**Table 1. Parameters for different categories of spontaneously active supraoptic neuron**

<table>
<thead>
<tr>
<th>category</th>
<th>mean firing rate ± s.e.</th>
<th>mean modal interval</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>continuous firing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>slow (0.1–1.0 s⁻¹)</td>
<td>0.6 ± 0.04</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>fast (1 s⁻¹)</td>
<td>2.5 ± 0.2</td>
<td>53 ± 6</td>
<td>19</td>
</tr>
<tr>
<td>phasic firing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group A</td>
<td>3.1 ± 0.25</td>
<td>42 ± 5</td>
<td>20</td>
</tr>
<tr>
<td>group B</td>
<td>1.5 ± 0.25</td>
<td>162 ± 23</td>
<td>4</td>
</tr>
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Phasic cells could be subdivided into a further category, as characterized by intracellular and extracellular recording. One group comprising 92% of phasic neurons had burst durations with a range of 8–65 s in normal medium. Mean burst lengths ranged from 11 to 80 s (mean of 16.7 ± 4.5 s) and silent periods from 5 to 85 s (mean 28.2 ± 4.2 s). As seen in figure 9, intracellular recordings from these neurons were characterized by phasic oscillations in membrane potential. Superimposed on this were large numbers of e.p.s.p.s which led to spike propagation. In 11 phasic cells specifically examined, the e.p.s.p. frequency did not change during a burst when compared to the frequency in the cell’s silent period, indicating that phasic bursts were regulated by oscillation in membrane potential rather than by a phasic oscillation in synaptic input. The period of phasic firing was terminated by a slow hyperpolarization of the membrane away from spike threshold. The waveform of action potentials and the interspike interval histograms of such phasic cells were similar to those described above for the population of so.n. neurons.

The second type of phasic cell (8%) differed in a number of respects. First, the burst duration and silent duration in these cells were considerably shorter than

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**Figure 9.** This figure demonstrates the two types of phasically firing cells discussed in the text. (a, b) The usual pattern of intracellularly recorded so.n. phasic cell, demonstrating phasic generation of action potentials. Note the slow depolarizations of membrane potential which are superimposed on bursts of firing and which give rise to these bursts, carrying the membrane potential close to threshold. (c) The ‘minority’ phasic cell (8%) recorded intracellularly, which shows lack of slow depolarizations of the membrane potential and shorter bursts. Below each record are given the amplified spike waveform, once again demonstrating that in (a) and (b) the long time course waveform of the magnocellular neuron is observed whereas in (c) the spike is much shorter in duration and does not show the long after-hyperpolarization noted in figure 7. Also shown are the interspike interval histograms for an 8 min record from each cell (middle plot below each figure) and the epoch (activity) histogram for the same record. It can also be seen that (a) and (b) have a much shorter modal interval than the record in (c), emphasizing the very different nature of these neurons.

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in most phasic cells, averaging $2.6 \pm 1.4$ s for the active period and $5.1 \pm 1.8$ s for the silent period (20 burst periods evaluated for each cell). Secondly, three such cells recorded intracellularly appeared devoid of any synaptic input in the form of e.p.s.p.s and were not subject to phasic depolarizations of membrane potential. Finally, the mode of the interspike interval histogram was significantly different ($p < 0.001$), the mean modal interval being 182 ms compared to 52 ms for the larger population of phasic cells.

**Intracellular staining of so.n. neurons**

Thirty-six so.n. neurons were intracellularly recorded from and subsequently injected with Lucifer Yellow. All 36 neurons were magnocellular, with diameters ranging from 12 to 20 µm (figure 10, plate 1). These cells were characterized by a large nucleus, which occupied nearly 80% of the cytoplasm after the fixation procedures used here, a feature borne out by electron micrographs of so.n. neurons. Lucifer-injected so.n. neurons showed two to four processes, which could be followed for 50–300 µm throughout the so.n. and occasionally followed outside the so.n. Continuously and phasically firing cells in this study were indistinguishable morphologically and resembled Golgi-impregnated and horseradish peroxidase-filled cells described in other studies (Armstrong et al. 1980; Dyball & Kemplay 1982). Dendrites were often characterized by thick processes that branched within 20–40 µm of the cell body (figure 10b, c), and these branched elements showed extensive arborization distant from the cell soma. About 15% of all so.n. neurons injected with Lucifer Yellow CH showed dye transfer to one or more additional cell (figure 10a), a phenomenon previously noted in so.n. (Andrew et al. 1981; Mason 1982) and pv.n. (Andrew et al. 1981) and correlated with the presence of gap junctions in the magnocellular nuclei (Andrew et al. 1981; Mason 1982).

**Discussion**

This paper reports a detailed study of magnocellular neurons in the rat so.n., using intracellular recording in the hypothalamic slice preparation to characterize neurons with respect to membrane potential, input resistance, synaptic input, and action potentials. Earlier reports have discussed some intracellular records from cells of so.n. and pv.n. (Dudek et al. 1980; Mason 1980a, b; MacVicar et al. 1982), and extracellular recording techniques have also been applied to the hypothalamic slice preparation (Hatton et al. 1978a; Haller & Wakerley 1980).

Neurons in the so.n. have resting potentials and action potential amplitudes comparable to those found in other mammalian c.n.s. preparations. Membrane slope resistances measured for so.n. neurons, however, are higher than reported for other c.n.s. preparations. Membrane resistances of locus coeruleus neurons (Henderson et al. 1982), hippocampal neurons (Schwartzkroin 1975, 1977; Langmoen & Andersen 1981), olfactory cortex neurons (Scholfield 1978) and neurons of the mesencephalic nucleus of the trigeminal nerve (Henderson et al. 1982) lie in the range of 10–60 MΩ, whereas so.n. neurons have a mean slope resistance of 145 MΩ with a range of 40–350 MΩ. Although the measurements made with all slice preparations from a variety of tissues depended on the use of a bridge balance
Figure 10. Photographs of three so.n. neurons injected intracellularly with Lucifer Yellow CH in lithium citrate (see Methods). (a) A low power micrograph of a typical injection in an so.n. neuron adjacent to the optic chiasm (o.c.). The dotted lines indicate an approximate boundary of the chiasm. The inset shows the injected group of cells, with an arrow marking the neuron actually injected with dye, the other three neurons being stained owing to dye transfer. (b, c) Two separate neurons injected with Lucifer Yellow CH, showing the typical morphology of the magnocellular neurons studied. (c) A dendrite branching some 10–15 μm from the cell body, with a possible axon moving out of the plane of section in the lower right side. All scale bars in this figure represent 15 μm.
with current injection through a single recording electrode, and as such can be prone to some error, such a difference is likely to be significant.

One major difference between the present recordings from so.n. neurons and those described in earlier work is the level of spontaneous activity. The present experiments with use of intracellular recording have revealed firing of so.n. neurons to be regulated by a large amount of synaptic input, as well as dependent on usual properties of excitable cells. This slice preparation of so.n. thus differs from other areas of brain, including hippocampus and locus coeruleus, where low levels of spontaneous activity are usually found in vitro. Spontaneous activity in such slice preparations may vary in the extent of severance of afferent input, and neurons from different c.n.s. areas may also vary with respect to proximity of membrane potential to the threshold for spike initiation. Intracellular recordings of so.n. neurons in coronally or sagittally oriented slices have shown that most neurons have synaptic input in the form of e.p.s.ps and i.p.s.ps and a resting membrane potential within 5-15 mV of spike threshold. In cells with low spontaneous activity, some synaptic input was observed intracellularly, but the difference between membrane and threshold potentials and average e.p.s.p. amplitude determined a low level of neuronal activity. It may therefore prove to be the case that so.n. neurons in the slice receive afferent input either from other intact cells in the slice or from nerve endings that originate elsewhere in the slice but terminate within the so.n.

The hypothalamic slice preparation as described here appears to differ from that used by earlier workers. Reports by Wakerley and colleagues (Brimble et al. 1978; Haller et al. 1978; Haller & Wakerley 1980) suggested that vibratome-cut, coronally oriented sections of hypothalamus containing so.n. had little spontaneous activity, and that cells in this preparation only fired when activated by glutamate iontophoresis. Other workers (Hatton et al. 1977; Hatton et al. 1978a, b; Mason 1980a, b; Armstrong & Sladek 1982) and more recent studies from Wakerley’s group (Noble & Wakerley 1982) have reported that substantial spontaneous activity can be detected in a variety of so.n. and pv.n. preparations. Reasons for this discrepancy could centre on perifusion rate or ionic content of the medium, particularly divalent cations critical for synaptic transmission (Pittman et al. 1981). A further possibility is that Wakerley and co-workers employed distilled water to humidify their slice preparation, whereas the apparatus described here used isotonic medium. Carry-over of water aerosol onto the slice surface could be a major factor in depressing the membrane potential away from the threshold potential, or even osmotically disrupting the neurons themselves.

The patterns of spike activity recorded here from the so.n. neurons in the slice preparation resemble those observed in both so.n. explants (Armstrong & Sladek 1982) and in anaesthetized animals with respect to phasically and continuously firing cells (Dyball & Pountney 1973; Lincoln & Wakerley 1974; Brimble & Dyball 1977; Poulain et al. 1977; Wakerley et al. 1978). The proportion of cells with low spontaneous activity (21%) may reflect some loss of afferent input to the so.n. when the slice is made, but other continuously firing cells with higher firing rates compare well, as a population, with in vivo findings.

For phasic cells the mean burst durations and silent periods noted here are
similar to those reported for other in vitro and in vivo preparations, suggesting that the slice may be a good model for spike-patterning in the s.o.n. By using intracellular recording, it is possible to consider the factors contributing to phasic firing. These are synaptic input in the form of e.p.s.ps, proximity to threshold potential and, most importantly, slow oscillations in membrane potential. The origin of these slow oscillations is uncertain, but they do not appear to be mediated by a chemical synapse as they can persist in low Ca\(^{2+}\) medium in which chemical synaptic transmission has been blocked.

Phasic variations in membrane potential have been well characterized in molluscan neurons (Pinsker 1977; Gainer 1972; Kater & Kaneko 1972; Kononenko 1979), and in vertebrate preparations such as mammalian spinal motoneurons (Krnjević & Lisiewicz 1972) and hippocampal CA3 neurons (Hablitz 1981). In many cases, oscillations in membrane potential appear to be brought about by an activated potassium conductance due to raised intracellular Ca\(^{2+}\), and modulated activity among invertebrate preparations can be mediated by electrical coupling among neuronal populations. Synaptic interactions among s.o.n. neurons do not appear to play a direct role in spike patterning (Leng 1981b), although the presence of gap junctions and transfer of Lucifer Yellow CH suggest the possibility that electrotonic coupling may occur between s.o.n. neurons. Further, Leng & Mason (1982) have reported that vasopressin itself, synthesized in s.o.n. cell bodies, may be a neuromodulator for phasically firing vasopressinergic cells. A final possibility is that oscillations in membrane conductance not examined here may play some role in this patterning.

The phasic patterning of firing observed for s.o.n. neurons in the slice preparation contrasts with the results reported by Gähwiler & Dreifuss (1979) for cultured neonatal s.o.n. These neurons showed bursting activity and silent periods, of the order of a few seconds, whereas workers with whole animals, hypothalamic slices and explants record phasic bursts and silent periods of the order of tens of seconds. There is no good explanation for this difference except that the full electrophysiological properties of s.o.n. neurons may not have developed in neonatal tissue. However, a small proportion (ca. 8%) of s.o.n. phasically firing neurons in the present work did have short burst and silent periods, and were clearly different from most of the phasic cells in that they lacked e.p.s.ps, did not have slow oscillations in membrane potential, and were characterized by a waveform and interspike interval histogram markedly different from most s.o.n. neurons. Indeed, their electrophysiological characteristics strongly resemble those of a population of extracellularly recorded phasically firing neurons in the lateral hypothalamus of anaesthetized rats reported by Leng (1981a), particularly in the shape and modal parameters of the interspike interval histogram. Because the preparation used here does not allow antidromic invasion it is not certain that all neurons recorded project to the neural lobe. Thus, it is tempting to use the criteria of waveform, particularly spike duration, suggested by MacVicar et al. (1982) as a means of identification of magnocellular neurons. On this basis, it may be surmised that the small subpopulation of phasically firing cells described might be considered to be either a parvocellular interneuron or a magnocellular neurosecretory neuron with a non-standard synaptic organization.
It is concluded that the hypothalamic slice preparation provides an interesting model for study of the function of vasopressin and oxytocin neurons in the so.n. This preparation will be used in a later paper to examine the effect of various substances on neuronal activity of so.n. neurons, and specifically to test the hypothesis that the magnocellular neurons of the so.n. region are osmoreceptive, a problem originally addressed by Verney (1947) and Jewell & Verney (1957).

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