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Visually guided whole cell patch clamp of mouse supraoptic nucleus neurons in cultured and acute conditions

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Stachniak, Teyye J. E., and Charles W. Bourque. Visually guided whole cell patch clamp of mouse supraoptic nucleus neurons in cultured and acute conditions. Am J Physiol Regul Integr Comp Physiol 291: R68–R76, 2006. First published February 9, 2006; doi:10.1152/ajpregu.00830.2005.—Recent advances in neuronal culturing techniques have supplied a new set of tools for studying neural tissue, providing effective means to study molecular aspects of regulatory elements in the supraoptic nucleus of the hypothalamus (SON). To combine molecular biology techniques with electrophysiological recording, we modified an organotypic culture protocol to permit transfection and whole cell patch-clamp recordings from SON cells. Neonatal mouse brain coronal sections containing the SON were dissected out, placed on a filter insert in culture medium, and incubated for at least 4 days to allow attachment to the insert. The SON was identifiable using gross anatomical landmarks, which remained intact throughout the culturing period. Immunohistochemical staining identified both vasopressinergic and oxytocinergic cells present in the cultures, typically appearing in well-defined clusters. Whole cell recordings from these cultures demonstrated that certain properties of the neonatal mouse SON were comparable to adult mouse magnocellular neurons. SON neurons in both neonatal cultures and acute adult slices showed similar sustained outward rectification above −60 mV and action potential broadening during evoked activity. Membrane potential, input resistance, and rapidly inactivating potassium current density (I_{K1}) were reduced in the cultures, whereas whole cell capacitance and spontaneous synaptic excitation were increased, perhaps reflecting developmental changes in cell physiology that warrant further study. The use of the outlined organotypic culturing procedures will allow the study of such electrophysiological properties of mouse SON using whole cell patch-clamp, in addition to various molecular, techniques that require longer incubation times.

vasopressin; oxytocin; magnocellular; neonatal; organotypic

THE HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM regulates the secretion of vasopressin and oxytocin from magnocellular neurosecretory cells (MNC), influencing such diverse functions as fluid balance, reproduction, and behavior (2, 8, 41). Because of the high protein production rates and accessibility of the system, MNCs have been used as a model to study neuropeptide secretion (18). As such, a number of acute preparations have previously been used to study MNC physiology (7, 35, 44). To study molecular and electrophysiological features of MNCs, we need a protracted preparation that permits acute recordings. The principle obstacle to making such recordings is identifying the MNCs in culture to patch the correct cells in live preparations. To this end, we generated two types of mouse organotypic culture, and identified the supraoptic nuclei (SON) in each. We further examined the electrophysiological properties of these SONs and compared them to adult mouse SON.

Visual identification of MNCs is more difficult in neonatal than in adult SON. Magnocellular neurons are readily identifiable in adult SON from acutely dissected tissue. The mature SON is more transparent than the surrounding tissue and is always found slightly lateral and superior to the optic tract. Any large cells (for rat, a cross-sectional area >160 μm²) patched within the nucleus will be MNCs (35). In neonatal rodent tissue, however, the nervous system is smaller and still developing. The optic tract, not yet fully myelinated, is therefore rarely visible as distinct white matter. The difficulty in locating MNCs is further compounded by the fact that after several days in culture, cells may not reside in exactly the same position relative to their initial placement (38).

Previous studies have shown that whole cell (42) or intracellular (43) recordings can be obtained from dispersed hypothalamic cultures, or from cultured punches of tissue derived from fetal rat brain (11, 33). However, because of the dispersed nature of the cell culture, identification of putative MNCs requires the difficult procedure of postfixation immunohistochemistry (e.g., 43). The roller tube organotypic culture method produces SONs of more consistent morphology that are identifiable under visual microscopy (28). However, the necessity of stabilizing such tissue with a plasma clot presumably impedes access and seal formation using patch pipettes. To make patch-clamp recordings from readily identifiable MNCs in culture, it would be desirable to establish a method that would allow visual identification of live MNCs under videomicroscopy, while retaining access by patch pipette.

Recent developments in tissue culture materials have allowed videomicroscopy-guided whole cell patch-clamp recordings to be obtained from neurons in stationary organotypic slice cultures of hippocampus (32). In such cultures, neonatal tissue rests on a translucent membrane that allows light to pass through for live cell imaging; thus it can be visualized with videomicroscopy in the same manner as acute adult slices. In

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this study, we therefore adopted a similar procedure to culture neonatal SON. We show that putative MNCs from our cultures can be visually identified and patched. For comparison purposes, we patched adult mouse MNCs from acute slices, as adult tissue does not survive well in organotypic culture (17). We compare a number of electrophysiological parameters in mouse between neonatal culture and acute adult slice, with the understanding that these parameters presumably change more readily in response to developmental period than to the culturing protocol (16). Consistency between such parameters, for example, the presence of a transiently active potassium current ($I_K$), allows us to draw parallels between recordings made in adult and neonatal rats (37, 46) and our measurements in mice. Conversely, differences observed between adult and neonatal rats may offer opportunities to study developmental changes in physiology. Our results show that organotypic slice cultures offer the opportunity to make visually guided whole cell recordings from mouse MNCs, establishing this model as a useful tool for studying the hypothalamo-neurohypophysial system.

**METHODS**

**Tissue preparation.** All animal protocols were performed in accordance with McGill Animal Care Committee guidelines. To prepare acute hypothalamic slices from adult animals, male mice (6–10 wk old) were anesthetized with halothane and decapitated, and their brains were quickly removed. Coronal sections 350–500 μm thick were cut on a vibratome in mouse artificial cerebrospinal fluid (ACSF; in mM): 130 NaCl, 3 KCl, 1.23 NaH2PO4, 1.48 MgCl2, 2 CaCl2, 26 NaHCO3, and 10 α-glucose, oxygenated with 95% O2-5% CO2 at 0°C, pH 7.3–7.4, 312 mosmol/kg, and placed in a recording chamber for video microscopy and whole cell patch clamp as described below. Mouse ACSF is modified from rat ACSF by increasing the NaCl content by 10 mM to 130 mM, and increasing the osmolality to 312 mosmol/kg, the natural basal serum osmolality observed in this strain of mouse (34, 36).

Neonatal mouse pups (C57BL6, Jackson ImmunoResearch Laboratories, West Grove, PA) 5–8 days old were anesthetized with hypothermia and killed by decapitation. Brains were quickly removed, and tissue containing the SON was dissected out. Our initial survey of neonatal mouse tissue indicated that the SON itself is indistinguishable from the surrounding tissue, and we therefore located the SON using anatomical landmarks. The bifurcation of the anterior and middle cerebral arteries on the ventral surface of the brain was generally used as a reference point. For punch cultures, punches were taken in an area centered ~0.5 mm medial and 0.5 mm caudal to the vascular bifurcation. To obtain tissue punches from this area, an 18-gauge needle with the bevel removed and rounded was filled with culture medium and inserted into the ventral surface of the brain, all the way through. The resulting punch of tissue was transferred to a culture dish, maintaining the dorso-ventral orientation of the tissue relative to an external mark on the dish. For the slice cultures, brains were bisected in the horizontal plane, and then the ventral half was placed on a block of agar ventral side up and sectioned using a scalpel. Coronal sections 350–500 μm thick looked unhealthy presumably because of hypoxia, and sections at 300 μm thinned down to nearly a monolayer with few cells (data not shown). Thus 350 μm sections were used routinely to prepare slice cultures. One or two sections were selected on the basis of identifiable coronal morphology at the ventral surface.

**Culture.** The organotypic culture procedure was similar to that previously described (25). Millicell sterilized culture plate inserts (0.4 μm; model PIMORGS0; Millipore, Billerica, MA) were placed into 35-mm culture dishes on top of 1-ml culture medium [50 ml MEM (GIBCO (Gaithersburg, MD) 42360–032), 25 ml Hank’s balanced salt solution (GIBCO 14175–095), 25 ml horse serum (GIBCO 26050–088), 25 μl penicillin/streptomycin (GIBCO 15140–122), 0.65 g dextrose]. One ml of culture medium was added to the top of the filter to render the membrane translucent, and it was subsequently removed when the tissue was added. The dishes were incubated at 37°C, 5% CO2 for at least 4 days to allow attachment to the filter. The medium was changed every 2 days. Viral transfections with Semliki Forest Virus containing enhanced green fluorescent protein (eGFp, kindly donated by Dr. Keith Murai) were performed by applying 10 μl of concentrated virus (titer = 10$^9$ particles/ml) in PBS on the tissue and incubating for 48 h.

**Immunohistochemistry.** Tissue sections were fixed overnight in PBS containing 4% paraformaldehyde, and then washed in PBS and blocked at room temperature (PBS, 1% normal goat serum and 0.3% Triton X-100, 1 h). Sections were incubated overnight at 4°C with the primary antibodies, PS-38 mouse monoclonal anti-oxytocin (1:100) and VA-4 rabbit polyclonal anti-vasopressin (1:1,000), kindly provided by Dr. Hal Gainer (1, 5). Dishes were then washed in PBS and incubated in labeled goat anti-mouse (rhodamine, 1:200; Chemicon AP124R) and anti-rabbit secondary (fluorescein, 1:200, Chemicon AP132F) for 2 h, washed in PBS, and visualized using confocal microscopy. Alternatively, cultures were treated with PS-38 (oxytocin) and PS-41 (vasopressin, 1:100) mouse monoclonal primaries. These were incubated in biotinylated goat anti-mouse secondary (1:200, Chemicon AP124B) for 2 h, washed in PBS, and placed in ABC reagent (Vector PK-4000) for ½ h, washed, and placed in diaminobenzidine substrate kit (Vector SK-4100) for 3–4 min.

**Electrophysiology.** For electrophysiological recordings, a square piece (~1 × 1 cm) of the millicell filter insert containing the cultured tissue (5 to 9 days in vitro) was cut out using a scalpel, placed in a glass-bottomed recording chamber, and immobilized using a segment (~2 cm) of platinum wire (1 mm OD) placed over the edges of the insert surrounding the tissue. For adult tissue, the slice was immobilized with a C-shaped platinum wire fitted with nylon strings. Both cultured and adult tissues were perfused with ACSF (32°C) delivered at a rate of 2–3 ml/min. Glass capillary patch pipettes (1.2 mm OD, A-M Systems) were pulled on a micropipette puller (P-87; Sutter Instrument) and filled with an internal solution (in mM): 110 K+ gluconate, 1 MgCl2, 1 EGTA, 0.1 CaCl2, 10 KCl, 4 ATP, 1 GTP, with pH adjusted to 7.3 with NaOH, 295 mosmol/kg). Pipette resistance was 3–6 MΩ. Cells were visualized on a black and white monitor using an Olympus BX51WI upright microscope coupled to a Hitachi KP-M1A video camera. Electrodes were visually guided to each cell using a motorized micromanipulator (SD Instruments, Grants Pass, OR) where giga-ohm seals were made to establish whole cell recordings. Under these conditions, pipette series resistance was 10–30 MΩ. Membrane potentials were not corrected for a measured liquid junction error of +2 mV. Whole cell current and voltage was recorded using an Axopatch-1D amplifier (Axon Instruments, Sunnyvale, CA), displayed on an oscilloscope and digitized using pCLAMP 8.0 software. Signals were analyzed offline using Clampfit 8 software (Axon Instruments).

**Electrophysiological analysis.** In silent cells, resting membrane potential was identified as the mean value of membrane voltage recorded when no current was applied. In cells firing spontaneous action potentials, the resting potential was defined as the "baseline potential." During spike trains, baseline potential was measured as the average value observed during flat segments of the membrane potential after recovery of the hyperpolarizing afterpotential and before the gradual increase in voltage that occurred before the next spike. Normalized action potential (spike) broadening was calculated by recording the spike duration at 0 mV of the nth spike in an evoked train divided by the duration of the first spike. Spike threshold was defined as the voltage observed at the end of a 0.1-ms interval, in which the rate of voltage change (dV/dt) exceeded 50 mV/ms before spike discharge. Action potential (AP) and afterhyperpolarization

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(AHP) amplitudes were calculated as the peak positive and negative voltages, respectively, observed during an AP minus the baseline. The electrotonic voltage responses to hyperpolarizing current pulses were averaged and fit with a standard monoexponential function to estimate the membrane time constant (τ). The degree of membrane rectification observed at positive and negative voltages was quantified from steady-state current-voltage (I-V) analysis under voltage clamp in the presence of TTX (0.5 μM). Specifically, negative and positive rectification ratios (RR) were determined by dividing the instantaneous slope conductance observed at −100 mV (negative RR) and −40 mV (positive RR), respectively, by the slope of the I-V relationship in the linear range (i.e., slope between −80 mV and −60 mV). To quantify the I_A, a hyperpolarizing pulse to −120 mV (300 ms) followed by a depolarizing pulse to −30 mV (200 ms) was delivered. Current density was determined by dividing the amplitude of I_A at −30 mV by the whole cell capacitance. To quantify the frequency of miniature excitatory postsynaptic currents (mEPSCs) or miniature inhibitory postsynaptic currents (mIPSCs), the total number of events observed was divided by the total duration of the corresponding recording interval. For measurements of mEPSCs, voltage was held at −60 mV (the reversal potential of mIPSCs under our conditions), whereas for mIPSCs the voltage was held at −40 mV, and outward synaptic currents were counted.

Statistics. Statistical significance was calculated using the Microsoft Excel two-sample t-test assuming equal variances, reporting the two-tailed P value. For comparing action potential parameters within groups across spike number, a one-way repeated-measures ANOVA was carried out on Sigmasat 2.0 (Access Softek), followed by Tukey’s post hoc test. A P value < 0.05 was considered significant. Not significant (NS) indicates a P value > 0.05. All values are reported as means ± SE.

RESULTS

MNCs in punch cultures of mouse hypothalamus. Our first approach consisted of obtaining small tissue punches containing the SON by performing a needle biopsy on anatomically defined positions on the ventral face of the hypothalamus of postnatal mouse brains (Fig. 1A; see METHODS for details). The tissue punches were placed longitudinally on the culture substrate, so that the SON would be located at the most ventral extremity of each explant. Indeed, as illustrated in Fig. 1, B and C, MNCs identified by their immunoreactivity to oxytocin and vasopressin antibodies were routinely observed near the ventral edge of these tissue punches (generally 10 to 100 cells per...
tract and the emergence of the medial aspect of the temporal tation of the tissue created by the lateral extremity of the optic cultured coronal hypothalamic slices. Nonetheless, the inden-

Whole Cell Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Neonatal Culture</th>
<th>n</th>
<th>Adult Slice</th>
<th>n</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>-52±2</td>
<td>17</td>
<td>-43±2</td>
<td>20</td>
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<td>Whole cell capacitance, pF</td>
<td>18.4±1.8</td>
<td>18</td>
<td>10.4±0.5</td>
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<td>Input resistance, MΩ</td>
<td>236±24</td>
<td>16</td>
<td>621±62</td>
<td>24</td>
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<tr>
<td>Specific conductance, pS/pF</td>
<td>284±25</td>
<td>16</td>
<td>213±31</td>
<td>24</td>
<td>NS</td>
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<tr>
<td>Time constant, τ, ms</td>
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<td>11</td>
<td>18.9±2.2</td>
<td>15</td>
<td>NS</td>
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<tr>
<td>Rectification ratio at -100 mV</td>
<td>1.32±0.10</td>
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<td>1.81±0.23</td>
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<td>NS</td>
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<tr>
<td>Rectification ratio at -40 mV</td>
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<td>1.55±0.14</td>
<td>8</td>
<td>NS</td>
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<tr>
<td>I, current density, pA/pF @ -30 mV</td>
<td>9.2±2.0</td>
<td>7</td>
<td>36.2±9.8</td>
<td>7</td>
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<tr>
<td>Action potential parameters</td>
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<td></td>
</tr>
<tr>
<td>Spike threshold, mV</td>
<td>-33.84±1.29</td>
<td>7</td>
<td>-32.36±0.76</td>
<td>14</td>
<td>NS</td>
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<tr>
<td>First spike amplitude, mV</td>
<td>100.8±5.9</td>
<td>7</td>
<td>102.0±2.4</td>
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<td>NS</td>
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<tr>
<td>First spike width, ms</td>
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<td>1.31±0.04</td>
<td>14</td>
<td>&lt;0.0001</td>
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<td>Normalized spike broadening, 10th spike</td>
<td>1.16±0.04</td>
<td>7</td>
<td>1.22±0.04</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Fast afterhyperpolarization peak, mV, relative to baseline</td>
<td>-22.2±3.9</td>
<td>7</td>
<td>-18.8±2.3</td>
<td>14</td>
<td>NS</td>
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<tr>
<td>Synaptic Parameters</td>
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</tr>
<tr>
<td>Miniature EPSP frequency, Hz</td>
<td>5.2±1.4</td>
<td>7</td>
<td>1.2±0.1</td>
<td>8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Miniature IPSP frequency, Hz</td>
<td>1.3±0.5</td>
<td>7</td>
<td>1.0±0.4</td>
<td>8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; NS, not significant.

The emergence of the medial aspect of the indentation in one of the slices or on the SON in adults, was usually observed in 2–3 of the slices. These slices were therefore selected for culturing. After a few days in culture, the third ventricle could still be clearly discerned in these preparations by the active beating of cilia of ependymal cells assembled as a vertical midline structure that extended toward the ventral aspect of the tissue (see sketch in Fig. 1D). When examined by videomicroscopy of live preparations, tight clusters of large neurons were commonly observed on the medial aspect of the indentation in one of the slices or on different sides of two consecutive slices. Indeed, immunohis-
tochemical staining with antibodies directed against vasopres-
in and oxytocin confirmed that such clusters represented MNCs in 92% (11/12) of the sections examined (e.g., Fig. 1F). In general, oxytocin neurons were more numerous. Various types of cells, including MNCs, could readily be transfected using eGFP viral transfer of either punch cultures (e.g., Fig. 1E) or cultured slices. Moreover eGFP-positive neurons could easily be identified by epifluorescence and patch clamped in live preparations (data not shown). We therefore investigated the basic electrophysiological properties of putative MNCs in the SON of neonatal slice cultures and compared them to those of MNCs in slices of adult mouse hypothalamus.

Action potentials in cultured neonatal and adult mouse SON neurons. Whole cell patch clamp recordings were obtained from visually identified SON neurons in cultured neonatal (n = 18 cells) and acutely prepared adult (n = 24 cells) hypothalamic slices. Under whole cell current clamp, both adult and cultured SON neurons were either silent (Vm less than -55 mV), or fired spontaneous action potentials in the 1–10 Hz range. This spontaneous activity likely resulted from a combination of the relatively positive resting membrane potential of the cells (see Table 1) and to the presence of spontaneous excitative synaptic activity (see below). To analyze action potential parameters, spontaneous activity was silenced (if present) by injecting hyperpolarizing current (−5 to −20 pA). After a silent interval of at least 10s, a depolarizing current pulse was then injected to induce repetitive firing at a rate of 10 Hz. The average steady-state firing frequency elicited in SON neurons during these trials was not different in the neonatal (8.2 ± 2.4 Hz) and adult (10.5 ± 2.0 Hz; P > 0.05) preparations. As illustrated in Fig. 2, A–C, action potentials evoked in adult and neonatal SON neurons both displayed a progressive and significant increase in spike duration at the onset of such trains, but the absolute duration of action potentials recorded at all intervals was significantly more prolonged in adult neurons (Fig. 2D). Moreover, the amplitude of the action potential was found to decline significantly during successive spikes elicited in adult but not in neonatal SON neurons (Fig. 2, E and F). In contrast, the amplitude of the postspike AHP was found to decline significantly during successive spikes elicited in neonatal and adult SON neurons (Fig. 2, G and H). All other action potential parameters measured were not significantly different between the cultured and adult cells (Table 1). To further our understanding of these active properties, we went on to examine the underlying whole cell parameters of the SON neurons.

Whole cell parameters in neonatal and cultured mouse SON neurons. As shown in Table 1, neonatal SON neurons displayed a significantly lower resting membrane potential and input resistance than adult SON neurons, whereas input capacitance was significantly higher in neonatal SON neurons compared with adult neurons. We next compared the degree of rectification observed at positive and negative voltages in adult and neonatal SON neurons. For this purpose, cells were voltage-clamped at a holding potential of −70 mV, and steady
state I-V analysis was performed by measuring the steady-state current measured at the end of voltage commands lasting 2 s (Fig. 3A). As shown in Fig. 3B, the mean input conductance of neonatal SON neurons measured at −70 mV was higher than that of adult neurons, consistent with the difference in input resistance reported above. Rectification ratios were then computed as described in the METHODS and compared quantitatively. No significant differences in the amount of rectification were observed between SON neurons recorded in the two preparations, despite the increased input conductance in neonatal cells.

Finally, given its prominent and characteristic expression in rat MNCs (6, 14, 15, 37), we specifically investigated the presence of the transient $K^+$ current $I_A$ in adult and neonatal mouse SON neurons. For this purpose, cells were stepped to −120 mV for a period of 300 ms to remove inactivation, and the voltage was then stepped to −30 mV to elicit $I_A$ (Fig. 4A). While all adult SON neurons displayed $I_A$, only 75% (9/12) of the cells patched in cultured SONs displayed a measurable transient outward current. Furthermore, as shown in Fig. 4B, the peak amplitude of $I_A$ was significantly greater in adult SON neurons than in neonatal SON neurons expressing this current. No significant difference was noted in the time constant of inactivation of $I_A$ in cultured (26.3 ± 4.2 ms) vs. adult (37.9 ± 3.6 ms) cells.

**Miniature synaptic events in neonatal and cultured mouse SON neurons.** To assess possible differences in the density of the synaptic innervation in these two preparations, we measured the average frequency of miniature postsynaptic currents in the presence of TTX (see METHODS for details). As shown in Table 1, neonatal SON neurons displayed a significantly higher frequency of mEPSCs than adult SON neurons. In contrast, no difference was noted in the frequency of mIPSCs.

**DISCUSSION**

Herein, we describe a procedure that produces a consistent, reliable method for mid- to long-term culturing and visually guided recording from mouse SON neurons using the whole cell patch-clamp configuration. Our successful transfection and recording of eGFP-labeled cells illustrates one main advantage of an organotypic culture approach: the ability to maintain access to healthy neuronal cells over a number of days, a time course necessary for genetic manipulation. Our organotypic culture technique enables us to visually identify and patch SON neurons with a high degree of success.

**Peptidergic phenotype of MNCs in organotypic slice culture.** Immunohistochemistry suggests that a larger population of oxytocin-positive than vasopressin-positive neurons exists in cultured SON (Fig. 1F). This is interesting in view of the fact that in the adult mouse SON, it appears that vasopressin neurons dominate the population of MNCs (27). An increased prevalence of oxytocin neurons has been reported previously for rat in both the roller-tube (28) and stationary (25) methods of organotypic culture. The apparent enrichment of oxytocin...
neurons may reflect a slower time course of development of the vasopressin neurons. Indeed, House et al. (25) observed an increase in neonatal mouse of detectable vasopressin but not oxytocin MNCs with increasing days in vitro, possibly due to an increasing cellular expression of vasopressin protein over the course of development (26). Alternatively, the greater number of oxytocin MNCs may represent a decreased survivability of vasopressin neurons in culture. Vasopressin neurons do appear to be more susceptible to axotomy than oxytocin neurons (12), which is certainly a consequence of slice culturing. Decreased survival might also explain why our cultures generated a relatively small number of cells (10–100) per SON.

Identification of MNCs in whole cell recordings. Our observations that cells in the neonatal SON are large cells expressing IA, and clustered in the same area as the immunohistochemically verified MNCs make us confident of their identity as MNCs. However, to definitively categorize the cell type of recorded cells, additional experiments are necessary. The addition of biocytin in the recording pipette has been used successfully to label analyzed cells (3, 23), which can then be costained to confirm their identity as MNCs. In fact, this technique has also been used to distinguish between vasopressin or oxytocin neurons after recordings (3, 23, 28). For the purposes of the present study, we chose not to use such a technique to distinguish between vasopressin and oxytocin neurons, as most of the parameters we examined (including resting membrane potential, input resistance, membrane time constant, action potential amplitude, and width) have been shown to be quantitatively equivalent in rat oxytocin and vasopressin neurons (3). Alternatively, organotypic culture might be combined with transgenic labeling approaches to identify live MNCs during patch recordings. GFP-expressing vasopressin (45, rat) or oxytocin (47, mouse) neurons may make selecting MNCs in culture much easier, provided they can be visualized by epifluorescence.

Electrophysiological properties. The majority of electrophysiological properties analyzed in this study was similar between the cultured neurons and the acute adult neurons, except for a few notable differences, such as input resistance and capacitance. The differences we observed in whole cell capacitance and input resistance between adult and neonatal conditions are qualitatively similar to observations that have been made in acute preparations of adult and neonatal rat SON (9), suggesting that culturing conditions have a marginal impact on these physiological parameters as opposed to age. Indeed, these cells have been reported to undergo an increase in membrane capacitance that peaks around postnatal day 8 (p8) and then subsides, in conjunction with a decrease in membrane resistance that also recovers (9). Because the specific capacitance of biological membranes is fixed at ~1 μF/cm² (21), changes in membrane capacitance indicate a change in total membrane surface area. Therefore, neonatal supraoptic neurons express a significantly greater surface area than their adult counterparts. Because the cell bodies of neonatal and adult MNCs are of equivalent size (unpublished data), the enhanced membrane area of neonatal neurons presumably reflects an increase in the number of dendrites or

Fig. 3. A: representative MNCs from both adult (left) and cultured (right) conditions show a sustained inward rectification below −80 mV and a sustained outward rectification above −60 mV. Voltage-clamp protocols showing 2-s steps from −120 mV to −30 mV from a resting potential of −70 mV are illustrated top. B: The average current-voltage relationships (I-V) from several cells are illustrated, and the rectification can be seen more clearly, where the traces deviate from the linear region (indicated by fit line through −80 to −60 mV). Note that the cultured cells show a higher I-V slope, indicating a higher input conductance.
dendritic arborization. Indeed, Chevaleyre et al. (9) have shown that the higher input capacitance of neonatal SON neurons in acute slices is correlated to an increase in dendritic arborization that occurs transiently around p8. By normalizing the whole cell conductance to the capacitance (Table 1), we find the difference in whole cell conductance is due to a difference in total membrane surface area. Likewise, as there is no significant difference in the mean time constants between the two groups of neurons (Table 1), we conclude there is no difference in the density of leak channels.

Our neonatal mouse neurons show an approximately fivefold increase in EPSC frequency compared with ~1 Hz in adult mouse (Table 1) or adult rat (44). A qualitatively equivalent disparity has been demonstrated in acute sections from adult and p8 rat SON (10). Although this increase appears to be consistent with a more extensive arborization, the lack of change in IPSC frequency suggests a more specific alteration of synaptic density. The organotypic culture system may therefore be useful for studies examining alterations in dendritic arborization, as previously performed in acute neonatal slice (9, 10), and acute adult rat slice (40), as it would allow transfection and visualization of live neurons, while maintaining access for electrophysiological recordings.

$I_A$ and spike broadening. The density of the $I_A$ measured at $-30 \text{ mV}$ is greater in adult cells than in cultured cells (Table 1, Fig. 4). $I_A$ is known to assist in repolarization during the falling phase of an action potential (30, 31), and spike broadening is thought to occur in part due to an inactivation of $I_A$ (24, 37). Neonatal MNC spikes show less absolute broadening in parallel with reduced $I_A$ density but broaden at a similar rate to adult MNCs when normalized to first spike width. One might therefore hypothesize that during postnatal development of the action potential, decreased spike width is compensated for by the reduction in $I_A$ density.

Rectification. Previous studies have shown that the sustained outward rectification (SOR) observed positive to $-60 \text{ mV}$ in adult MNCs plays a role in spike frequency adaptation (4, 19). It has also been suggested that the inward rectification observed below $-80 \text{ mV}$ may be due to the presence of the hyperpolarization activated inward current (13, 20). A number of investigators have reported that the SOR is present to a greater extent in oxytocin than in vasopressin neurons in the rat (22, 39). We observed rectification in the majority of neonatal (57%) and adult (88%) cells, and we separately observed a majority of oxytocin neurons in our cultures. However, we cannot support or refute the SOR hypothesis for the mouse model, as we did not characterize the chemical phenotypes of the neurons from which we recorded. The important questions of whether the differences in electrophysiological properties observed in vasopressin vs. oxytocin cells in adult rats are present in neonatal tissue and whether these differences change over the course of development remain to be studied. Such questions require double labeling of recorded MNCs with markers, such as biocytin.

In conclusion, visually guided whole cell patch-clamp recordings indicate that MNCs in the SONs cultured from neo-

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Fig. 4. A: transiently activated outward currents ($I_A$) at $-30 \text{ mV}$ generated by depolarizing steps from $-120 \text{ mV}$ (protocol inset). The current observed in adult cells was noticeably larger than that found in cultured cells. B: average peak current and average current density at $-30 \text{ mV}$ are reduced in the cultured condition. The time constant $\tau$, as measured by the fit lines shown, did not differ between the two groups. $^*P < 0.05 \text{ vs. adult value.}$
natal mice exhibit a number of properties in common with those in adult mouse SON. Successful recordings and viral transfections of MNCs in culture pave the way for this model system to incorporate genetic, molecular and electrophysiological techniques.

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