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INTRINSIC PROPERTIES OF NEURONES IN THE DORSAL COCHLEAR NUCLEUS OF MICE, *IN VITRO*

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SUMMARY

1. Intracellular recordings were made from the dorsal cochlear nucleus (DCN) in slices of the cochlear nuclear complex. Probably the larger and most frequent cells were impaled.

2. The steady-state current–voltage (*I–V*) properties of all cells impaled were non-linear. The *I–V* curve was steepest in the voltage range depolarized from the resting potential and most shallow when the cell was hyperpolarized from rest by more than about 10 mV. Thus, the inwardly rectifying *I–V* characteristics of cells in the DCN distinguish them from those of ventral cochlear nuclear neurones (Oertel, 1983).

3. When depolarized with current, most cells fired trains of large, all-or-none action potentials. The undershoot after single spikes comprised an initial, fast component followed by a second, slower wave. A few cells (15%) generated bursts of smaller, graded spikes in addition to the large ones.

4. Repetitive firing evoked by depolarizing pulses of current was followed by an after-hyperpolarization whose magnitude depended on the strength and duration of the preceding current pulse.

5. Blocking the large action potentials with tetrodotoxin (TTX) revealed Ca^{2+}-dependent spikes in all cells examined.

6. The steady-state *I–V* relationship became linear in the presence of TTX, suggesting that a persistent Na^+ conductance probably mediates the inward rectification seen above the resting potential.

7. Muscarine at micromolar concentrations excited cells and increased their input resistance.

INTRODUCTION

Temporal and intensive features of acoustic signals are represented by the timing of action potentials in the auditory nerve (Kiang, Watanabe, Thomas & Clark, 1965). Single fibres of the nerve make synapses in both the dorsal and ventral divisions of the cochlear nuclear complex (Lorente de Nó, 1933, 1981; Fekete, Roullier, Liberman & Ryugo, 1984). *In vivo*, many cells in the ventral cochlear nucleus (VCN) respond to sound with firing patterns that reproduce those recorded from primary afferents

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(Godfrey, Kiang & Norris, 1975*b*; Shofner & Young, 1985; Rhode & Smith, 1986*b*). The physiological properties of these cells allow them to follow the timing of their inputs (Oertel, 1983, 1985; Wu & Oertel, 1984). In contrast, the relationship between the temporal structure of the stimulus and the modulation of firing rate recorded in

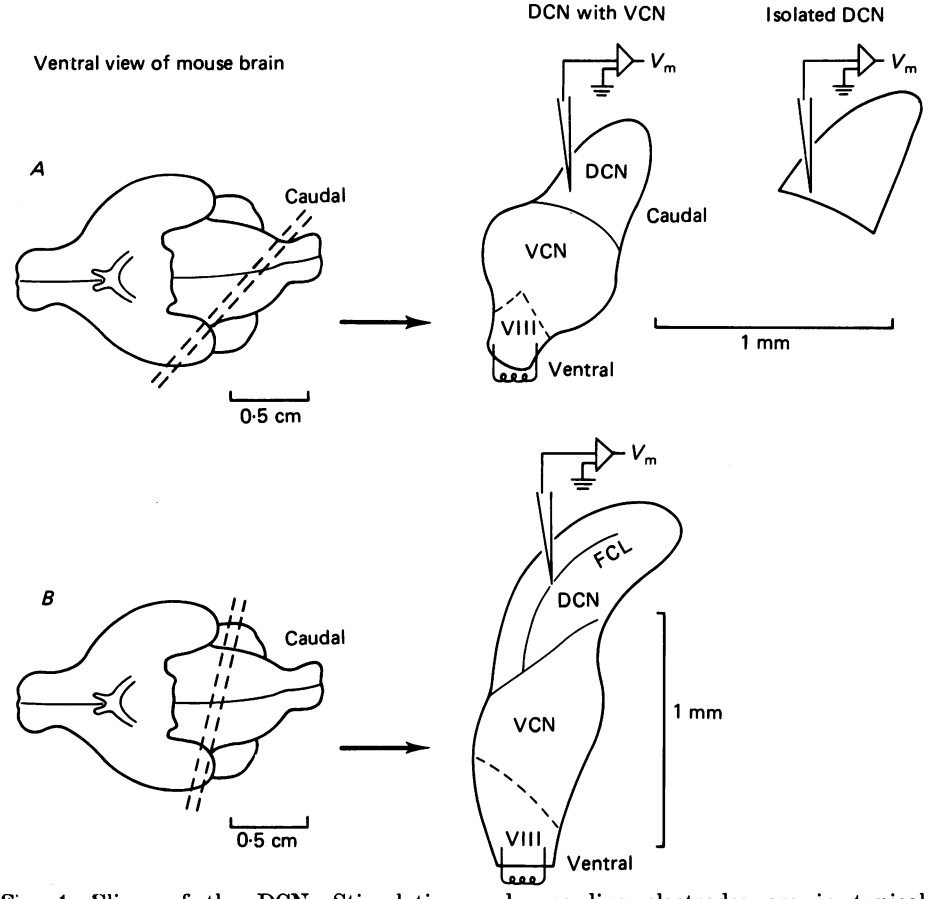

Fig. 1. Slices of the DCN. Stimulating and recording electrodes are in typical configurations. *A*, tangentially cut slices with the pial surface facing upwards. The slice on the left includes the root of the auditory nerve with parts of the VCN and DCN; the one on the right contains only the DCN. *B*, slice cut normal to the laminae of the DCN and parallel to its long axis. The fusiform cell layer is abbreviated to FCL.

the dorsal cochlear nucleus (DCN) differs from that in the nerve (Godfrey *et al.* 1975*b*; Shofner & Young, 1985; Rhode & Smith, 1986*b*). For example, compared to responses in the VCN, firing patterns of cells in the DCN are poorly synchronized with the phase of tonal stimuli, even when the frequency of the tone is low. We have made intracellular recordings from slices of the DCN and found that neurones there express a variety of conductances that can alter the amplitude and duration of responses to synaptic input.

METHODS

Mice between the ages of 17 and 28 days were used for the experiments. An animal was decapitated and its head immersed in warm (30 °C), oxygenated saline. The skull was opened and the brain removed, taking care not to damage the cochlear nuclear complex or to stretch the eighth nerve. A ventral to dorsal cut through the pons was made parallel to the caudolateral surface of the DCN and just anteromedial to the cochlear nuclei. The caudal half of the brain was removed from the bathing medium and its cut surface mounted on a dry Teflon block with cyanoacrylate glue. This assembly was replaced in saline and the tissue sliced with an oscillating microtome (Fredrick Haer). Usually the first cut was made just above the cochlear nuclei, parallel to their surface, and the second 100–400 μm underneath. In some experiments the first cut was made about 50 μm below the pial surface and perpendicular to the laminae of the DCN. Only one slice was cut from each animal. Slices were placed in a glass-bottomed chamber with a volume of about 0·3 ml which allowed saline to flow both above and underneath the tissue (Oertel, 1985). Sometimes slices which contained only the DCN were used. A schematic diagram of the preparations used for this and the companion study (Hirsch & Oertel, 1988) is presented in Fig. 1. The temperature was maintained at 35 °C and the flow rate at about 12 ml/min. The preparation was viewed through a dark-field condenser. Six slices, from six different animals, were cut parallel to the surface of the DCN and fixed, sectioned and stained. They were seen to contain parts of all three layers of the DCN.

Recording and stimulation

Glass micropipettes were filled with 4 M-potassium acetate and had resistances of 100–200 MΩ. After filling they were coated with dichlorodimethyl silane. Intracellular recordings were made using standard techniques. The membrane potential was monitored and intracellular current delivered through a bridge circuit with a Dagan Single Electrode System. Voltage and current traces were displayed on a chart recorder and oscilloscope, from which they were photographed.

Two insulated tungsten electrodes whose tips were about 50 μm in diameter were used to stimulate the nerve as indicated in the text. Stimuli were 100 μs shocks of variable voltage and polarity.

Solutions

The normal saline was composed of (mM): NaCl, 124; KCl, 5; KH_2PO_4, 1·2; $CaCl_2$, 2·4; $MgSO_4$, 1·3; $NaHCO_3$, 26; glucose, 10; saturated with 95% 0_2 and 5% CO_2; it had a pH of 7·4. Calcium-free saline was made by replacing Ca^{2+} with Mg^{2+}. When tetraethylammonium ion (TEA^+) was included it was substituted for Na^+. When Ba^{2+} was used, phosphate was omitted and sulphate was replaced with chloride. All drugs used were obtained from Sigma. All experimental solutions were perfused through the recording chamber.

RESULTS

The results presented here were obtained from fifty-nine acceptable impalements in thirty-two slices. Acceptable cells had input resistances greater than 20 MΩ when measured between rest and 10 mV below, membrane potentials ranging from −55 to −70 mV (mean −61 mV), and action potentials larger than 55 mV. Recordings from such cells were often stable over hours. Poorly impaled cells had one or more of the following features: low input resistances, resting potentials that were depolarized or hyperpolarized, and small action potentials.

Spontaneous firing

At rest, the cell from which the records in Fig. 2*A* were obtained discharged large, fast, action potentials. Hyperpolarizing the cell with direct current silenced its activity and failed to reveal spontaneous excitatory postsynaptic potentials (EPSPs) (Fig. 2*B*). This behaviour was typical, though levels of activity varied from

cell to cell. During individual impalements the rate of firing fluctuated and seemed sensitive to small changes in membrane voltage and input resistance. Often, in addition to large, fast action potentials, damaged neurones spontaneously produced bursts, similar to those evoked by current (Fig. 3), or small (about 5 mV), fast spikes.

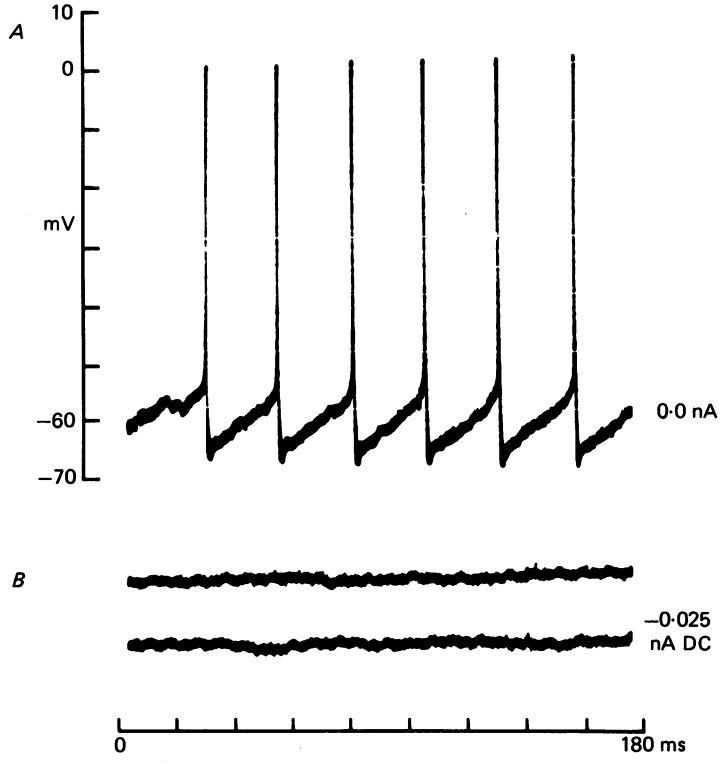

Fig. 2. Spontaneous action potentials. The top trace shows spontaneous firing produced when the cell was at its resting potential. Below are consecutive traces recorded when the cell was silenced by −0·025 nA of direct current.

Current–voltage relationships

Responses to injected current are shown in Fig. 3. Weak pulses of depolarizing current initiated a gradually rising, regenerative depolarization that outlasted the pulse and triggered a spike (Fig. 3*A*, right). Larger injections of depolarizing current evoked trains of large, fast action potentials which overshot 0 mV. The undershoots following each action potential consisted of an initial fast component followed by a slower one. The extent to which the second component overlapped the first varied among cells. Though all cells produced large, all-or-none action potentials, 15% (nine out of fifty-nine) also generated bursts of smaller graded spikes superimposed on a slow depolarization. The presence of such bursts is the only noticeable feature that distinguished the cell in Fig. 3*B* from the one in Fig. 3*A*.

The relationship between the amount of injected current and the change in voltage it produced is shown at the left of Fig. 3*A*. The slope was less steep in the hyperpolarizing than the depolarizing direction and was most shallow when the

intracellular voltage was hyperpolarized by about 10 mV or more from rest (Fig. 3*A*, top left). During injection of hyperpolarizing pulses, the rate at which the intracellular voltage reached the steady-state increased with the magnitude of the pulse. The time constants of the responses to pulses of −0·2 nA and −0·5 nA were measured graphically (Fig. 3*A*, bottom left). The time constant for the small pulse (6·6 ms) was

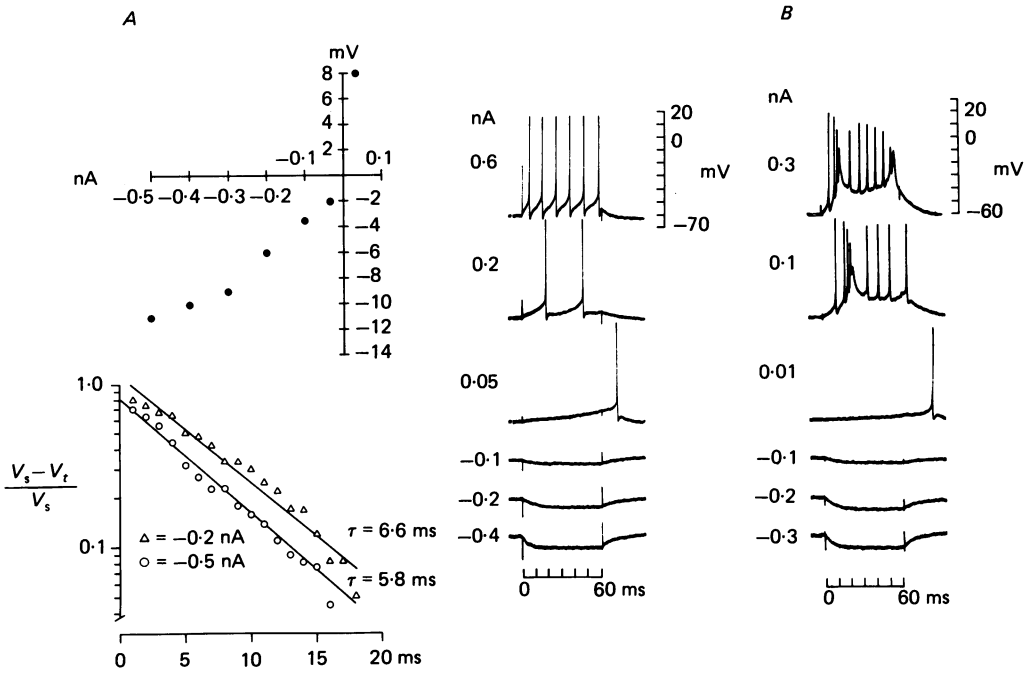

Fig. 3. *A*, *I–V* relationship of a cell which fired regularly. On the right are examples of the traces from which the graphs on the left were made; the amount of current injected is printed at the beginning of each trace. On the upper left is a plot of the voltage change at the end of a pulse as a function of injected current. On the lower left is plot showing that the rate of change of voltage evoked by hyperpolarizing pulses was exponential and increased with current strength. For the graph on the lower left, V_s is the intracellular voltage measured at the end of a pulse and V_t is the voltage when time $= t$ ms; $t = 0$ at the beginning of each pulse. *B*, oscillographs showing the responses of a bursting cell to injection of current pulses.

longer than that for the large pulse (5·8 ms). The change in intracellular voltage was exponential in each case. For a sample of ten randomly chosen cells, the mean time constant (mean ± standard deviation) of the response to an injection of −0·05 to −0·25 nA pulse of current was 8·7 ± 2·1 ms and the mean time constant of the response to −0.3 nA, or more negative, pulse was 5·4 ± 0·9 ms.

The non-linearities in the *I–V* curve indicate the presence of voltage-dependent conductances. We will present evidence that the increase in the slope resistance with depolarization, the inward rectification, reflects the activation of a Na^+ current. The decrease in the slope resistance with hyperpolarizations of more than 10 mV, and the concomitant decrease in the time constant, suggest that voltage-sensitive conductances are turned on or off by hyperpolarization; we have not, however, studied the underlying mechanisms.

After-hyperpolarizations

A hyperpolarization of the membrane followed activity evoked by injection of depolarizing pulses of current. Figure 4*A* shows records from a cell which discharged at a steady rate at rest and more frequently when depolarized with pulses of current.

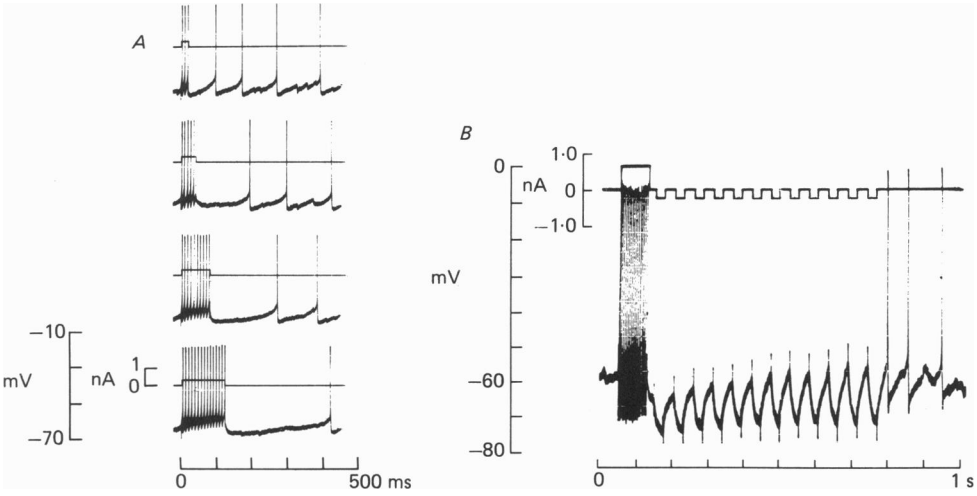

Fig. 4. After-hyperpolarizations. *A*, spontaneous firing was silenced by a hyperpolarization whose size depended on the duration of previous pulses of depolarizing current. Action potentials were attenuated by the amplifier in these traces. *B*, record from another cell shows the response to trains of hyperpolarizing pulses of current delivered after the depolarizing pulse. Input resistance was reduced during the after-hyperpolarization.

A hyperpolarization that silenced spontaneous activity followed each pulse. The extent of this after-hyperpolarization grew with the duration of the preceding depolarization and could last hundreds of milliseconds. In another cell, current pulses of constant amplitude were delivered during the after-hyperpolarization (Fig. 4*B*). The voltage deflections caused by the initial pulses were smaller than those evoked later as the cell repolarized, indicating that an increase in membrane conductance is associated with the after-hyperpolarization.

Ionic conductances

To obtain information about the ionic conductances which mediate the membrane's *I–V* properties, recordings were made in solutions with altered compositions. These experiments provide evidence for the presence of various Na^+, Ca^{2+}, and K^+ conductances.

All cells tested fired both Na^+ and Ca^{2+} action potentials. The traces in Fig. 5 were recorded from a cell which fired only large, fast spikes in normal saline. Tetrodotoxin (TTX) at 1 μM reversibly blocked these, indicating that they are mediated by a Na^+ current. When 15 mM-TEA^+ was added to the bath, we could evoke an action potential which was reversibly removed by eliminating Ca^{2+}. Tetraethylammonium was not required to drive TTX-resistant spikes in cells which fired bursts of graded action potentials.

The results presented in Figs 6 and 7 reveal the presence of a persistent Na^+ current that activates a few millivolts positive to the resting potential. After replacement of Ca^{2+} with Mg^{2+} in the superfusing medium, the cell recorded in Fig. 6*A* lost the ability to generate slow bursts of action potentials (Fig. 6*A b*) and then began to fire spontaneously as the membrane potential gradually decreased. Abruptly, when the membrane potential had become depolarized by about 3 mV, the

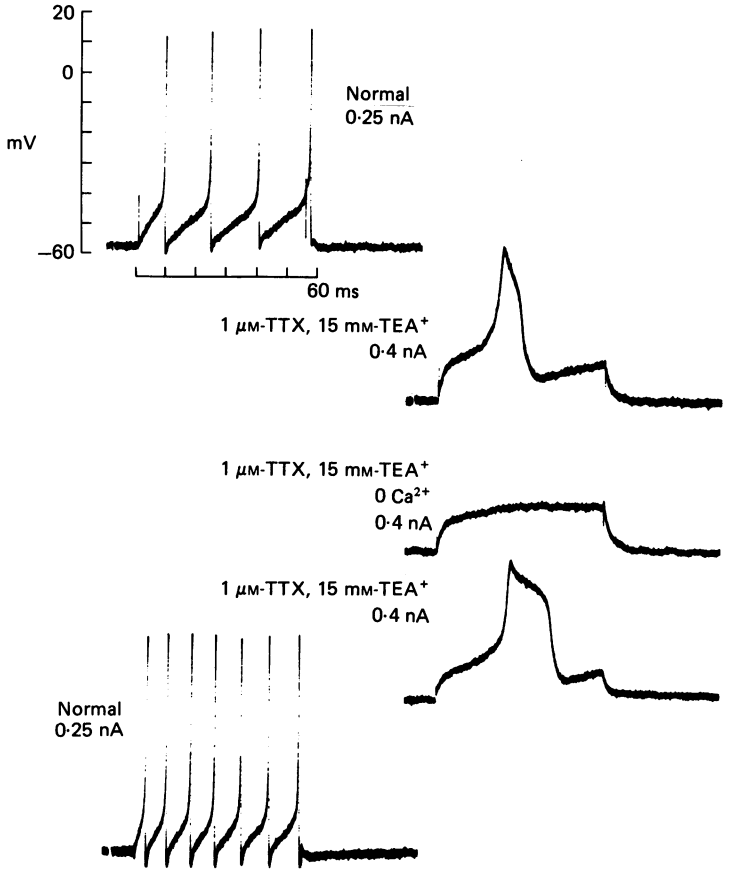

Fig. 5. Cells generate both Na^+ and Ca^{2+} spikes. Traces are shown sequentially with the composition of the superfusate and amount of injected current printed at the beginning of each. When Na^+ action potentials were blocked by TTX, Ca^{2+} spikes could be generated in the presence of TEA^+. All changes were reversible.

membrane potential jumped to a depolarized plateau during which conductance increased greatly (Fig. 6*A c*). After the reintroduction of Ca^{2+} the intracellular voltage stepped back to rest.

To determine whether the events observed in the absence of Ca^{2+} reflected a disruption of the balance between K^+ and Na^+ conductances, we added TEA^+ or 4-aminopyridine (4-AP) to medium containing standard concentrations of divalent cations. Low concentrations (0·5–5·0 mM) of 4-AP are thought to block the A-current selectively (Segal, Rogawski & Barker, 1984); TEA^+ ($\leqslant$ 20 mM) depresses delayed rectification (Segal *et al.* 1984) and a fast Ca^{2+}-dependent K^+ current (Brown &

Griffith, 1983). The results of such tests, obtained from two different cells, are presented in Fig. 6*B*. Addition of either agent evoked a jump to the elevated plateau as did the removal of Ca^{2+}.

Figure 7 compares the changes in membrane potential produced by injected current in the presence or absence of TTX. Application of the toxin removed the

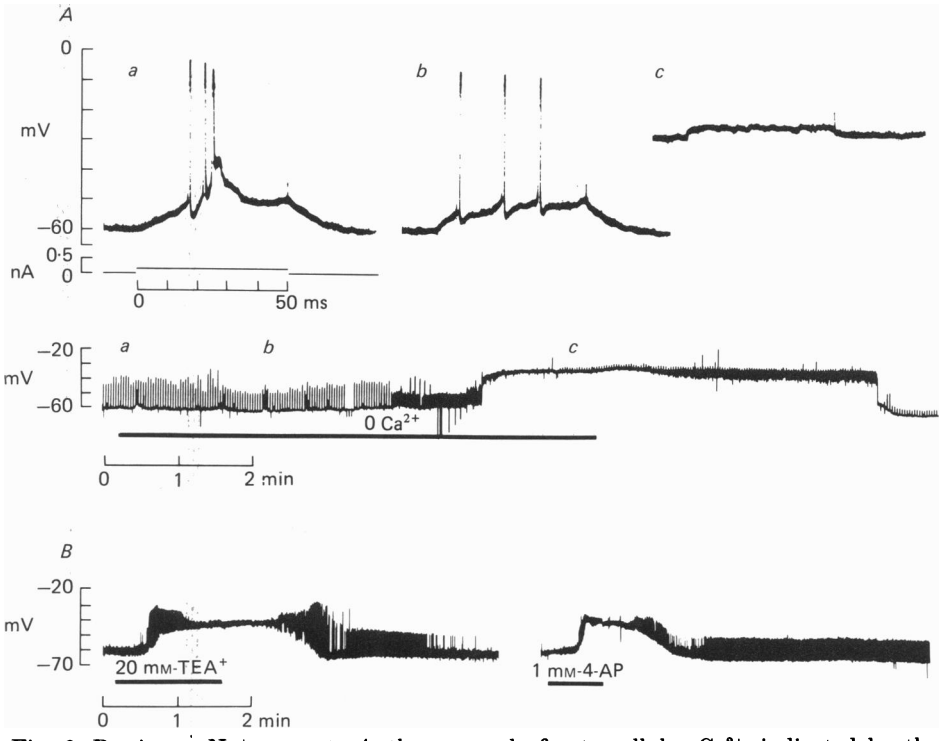

Fig. 6. Persistent Na^+ current. *A*, the removal of extracellular Ca^{2+}, indicated by the heavy bar, raised the membrane potential from the resting level to a depolarized plateau. Current pulses were injected regularly before and during the removal of Ca^{2+}. Lettered points on the bottom record correspond to the traces shown above, on an expanded time scale. Each of the expanded records shows a response to the injection of a 0·3 nA pulse of current. *B*, application of 20 mM-TEA^+ and 1 mM-4-AP also evoked the depolarized plateau; records are from two different cells. Action potentials were attenuated by the chart recorder.

inward rectification, making the *I–V* curve linear within 10 mV of the resting potential. Thus the voltage range in which the slow, regenerative depolarization is activated (see Fig. 3) and the persistent Na^+ current is expressed (Fig. 6*A*) overlaps with the TTX-sensitive leg of the *I–V* curve.

Application of the cholinergic agonist, muscarine, causes a depolarization and decrease in K^+ conductance in various vertebrate neurones (Constanti & Brown, 1981; Halliwell & Adams, 1982). The chart in the top panel of Fig. 8 shows that 0·5 μM-muscarine produced a slight depolarization, during which input resistance increased by 30% and action potentials were generated. Raising the concentration of the drug tenfold caused a larger depolarization and a 60% increase in input

resistance (bottom panel). Inclusion of 100–400 μM-Ba^{2+} to block M-channels (Halliwell & Adams, 1982) mimicked the effects of muscarine (not shown).

The voltage-sensitive properties of the membrane shape synaptic input (Fig. 9). A shock to the nerve root evoked a single EPSP. When the membrane potential was maintained at rest, the EPSP was large and long-lasting (50 ms); however, when

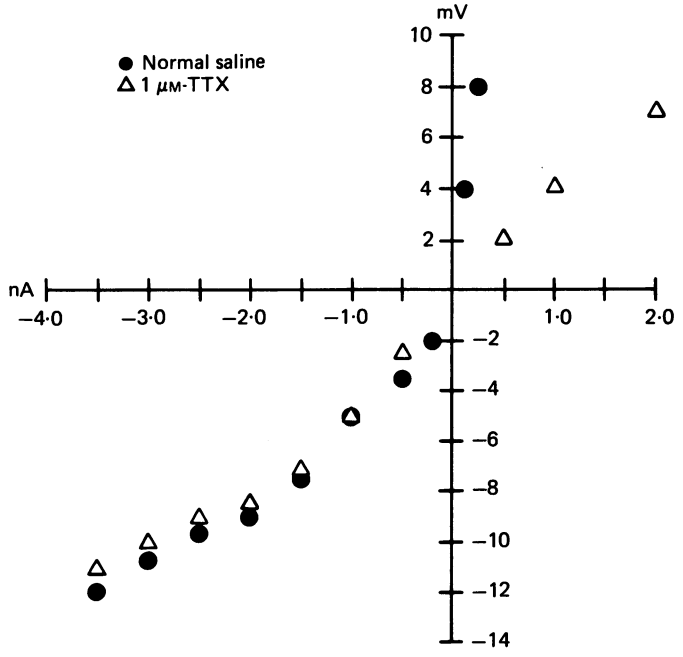

Fig. 7. TTX suppresses inward rectification. Superimposed *I–V* curves obtained from a single cell when the slice was bathed in the standard saline (●) and in medium containing 1 μM-TTX (△).

elicited while the cell was hyperpolarized by 7 mV with a pulse of current it was shorter in height and length (10 ms). That is, the EPSP was prolonged when it traversed the threshold for activation of the regenerative depolarization.

DISCUSSION

Inward currents

Almost all cells in the DCN fire spontaneously. Because suppression of spontaneous activity with hyperpolarizing current reveals no underlying EPSPs it is probably generated by the membrane *per se*, not synaptic input. Studies *in vivo* also indicate that spontaneous firing is produced by mechanisms intrinsic to the DCN. Lesion of the cochlea silences the VCN but not the DCN (Koerber, Pfeiffer, Warr & Kiang, 1966; Evans & Nelson, 1973*b*) and spontaneous activity persists after surgical isolation of the DCN from the rest of the brain stem (Evans & Nelson, 1973*a*).

When depolarized with current, most cells in the DCN fire rhythmic trains of large, fast action potentials; some others generate bursts of graded spikes superimposed on a slow depolarization. Results of experiments with horseradish peroxidase-filled

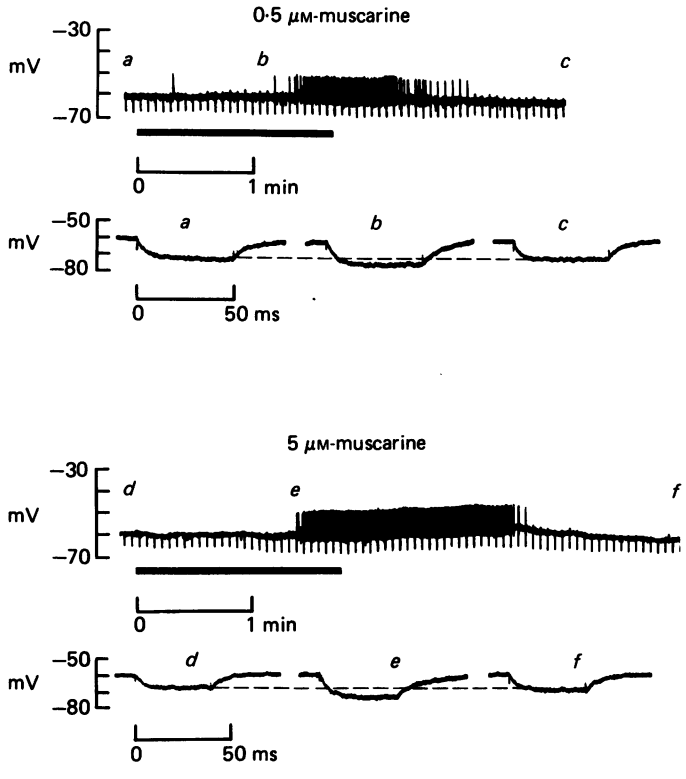

Fig. 8. Muscarine excites cells and decreases the membrane conductance. Application of muscarine for the period indicated by the bars caused firing. Injected pulses of current (−0·5 nA), show that input resistance increased in the presence of muscarine. Action potentials were attenuated by the chart recorder. The small letters above the chart records correspond to the traces shown at a faster sweep speed below.

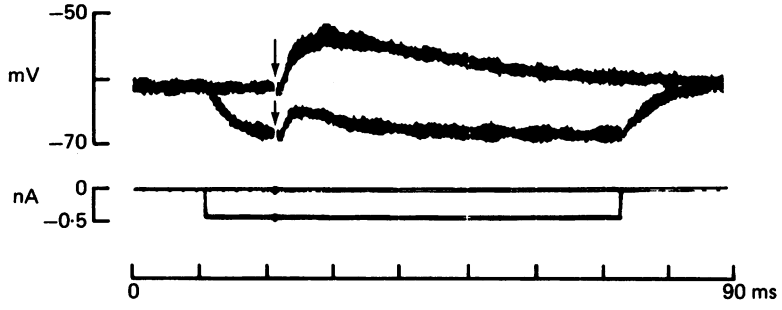

Fig. 9. Hyperpolarization of the membrane with a pulse of current reduced the height and duration of the EPSP. The synaptic response was evoked by shocks to the auditory nerve (arrows); 10^{-6} M-strychnine was included in the bath to block IPSPs. Three traces are superimposed at each condition.

electrodes show that the group of cells which fire only large, fast action potentials includes both projection neurones and local interneurones. As yet, no bursting cells have been injected with dye (S. H. Wu & D. Oertel, in preparation).

Addition of TTX to block the large, fast, Na^+ spikes and of TEA^+ to depress K^+ conductances always reveals Ca^{2+} action potentials. Intradendritic recordings in

hippocampus show that Ca^{2+} spikes are generated in the dendritic arbor where they provide a means to amplify distal excitatory input (Benardo, Masukawa & Prince, 1982). This may be the case in the DCN as well.

In addition to Na^+ and Ca^{2+} action currents, DCN neurones express a persistent Na^+ current that becomes regenerative at membrane potentials positive to rest but below threshold for firing. When TTX is not present in the bath, reduction of the resting K^+ conductance by removing Ca^{2+} or adding TEA or 4-AP causes a jump to a depolarized plateau during which membrane conductance is high. Because the plateau persists in the absence of Ca^{2+}, is mediated by an increase in conductance and is suppressed by TTX, it is almost certainly generated by Na^+. Presumably K^+ currents keep the level of the plateau below the equilibrium potential for Na^+. In the DCN, the persistent Na^+ current is strong; initiating it almost always drives the intracellular voltage across threshold for firing.

A persistent inward current activated near rest could amplify synaptically evoked depolarizations (Fig. 9) as has been shown by others (Stafstrom, Schwindt, Chubb & Crill, 1985). As well as its effect on synaptic integration, the persistent Na^+ current may increase the rate of repetitive firing (Stafstrom *et al.* 1985) and oppose outward currents which cause adaptation.

Outward currents

A long-lasting hyperpolarization mediated by an increase in conductance follows trains of spikes evoked by depolarizing pulses of current. The magnitude of the after-hyperpolarization increases with the duration and amplitude of the preceding depolarization. After-hyperpolarizations provide cells with an endogenous means of modulating their responses to prolonged or intense synaptic depolarization. Long-lasting intervals of suppression often follow the excitation of DCN neurones by sound (Gerstein, Butler & Erulkar, 1968; Starr & Britt, 1970; Evans & Nelson, 1973*a*,*b*; Godfrey, Kiang & Norris, 1975*a*; Young & Brownell, 1976; Rhode, Smith & Oertel, 1983). Starr & Britt (1970) suggested that this could be mediated at the level of the single cell. Our results support this possibility.

Synaptic efficacy and rate of firing may also be modified by reduction of the membrane conductance. Closure of cation-selective channels normally active at rest could be responsible for the excitation and concomitant decrease in conductance we observe in muscarine (Tokimasa, 1985). A cholinergic projection from the superior olivary complex terminates in the cochlear nuclei (Osen & Roth, 1969; Comis & Davies, 1969), and the action of acetylcholine *in vivo* can be excitatory in the DCN (Comis & Whitfield, 1968). In addition, there is biochemical evidence that acetylcholine acts as a transmitter in the DCN (Godfrey & Matschinsky, 1981; Whipple & Drescher, 1984; Frostholm & Rotter, 1986). Muscarine-sensitive channels are also associated with conductance changes mediated by neuroactive peptides (Constanti & Brown, 1981; Adams, Brown & Constanti, 1982); antibodies to many of these peptides bind to cells and processes in the DCN (Ljungdahl, Hokfelt & Nilsson, 1978; Tachibana, Rothman & Guth, 1979; Fallon & Seroogy, 1984).

Comparison of the dorsal and ventral cochlear nuclei

Temporal patterns of primary afferent discharge are important in the processing of acoustic information. In the VCN, neurones respond to changes in the membrane potential with one of two stereotyped profiles (Oertel, 1983, 1985; Wu & Oertel, 1984). Depolarization of one class of cell causes a large increase in conductance, reducing the time constant of the membrane above the resting potential. The EPSPs in these cells repolarize rapidly, lasting only about 2 ms. The I–V properties of the other type are more linear, permitting summation of synaptic potentials in time. In this second group the EPSPs recorded last about 5 ms. Thus certain cells in the VCN are better suited to preserve the timing of their inputs, and others to modify it.

The membrane properties of DCN cells could serve to alter temporal information to a greater degree than either type of neurone in the VCN. Those EPSPs large enough to activate the persistent Na^+ current outlast EPSPs recorded in the ventral nucleus by ten times or more. Prolonged after-hyperpolarizations, which are prominent in the DCN but not VCN, may provide another means of extending the effects of synaptic input, by decreasing excitability. Lastly, the dual undershoots of cells in the DCN could, by increasing the refractory period, limit the frequency at which cells relay their inputs. Action potentials of cells in the VCN are followed by single, fast undershoots. *In vivo*, maximal discharge rates are more rapid, and phase locking more secure in the VCN than in the DCN (Rhode & Smith, 1986*a*,*b*).

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