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In vitro electrophysiology of neurons in subnuclei of rat inferior colliculus

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Abstract

We compared membrane and synaptic properties of neurons in the three major subdivisions of inferior colliculus (IC), central nucleus (ICc, $N = 18$), external cortex (ICx, $N = 38$), and dorsal cortex (ICd, $N = 31$) of slices from rat IC, using intracellular neuronal recording. Three types of responses occurred in each IC subdivision in response to depolarizing currents: on-type ($N = 20$), rapidly-adapting ($N = 11$), and sustained firing ($N = 56$), which was most common. The on-type neurons have lower input resistances and shorter time constants, with wider and lower amplitude action potentials (APs) than sustained neurons. A calcium-mediated 'hump' was often evoked by depolarizing current pulses in ICd neurons (11 of 28), was infrequent in ICx, but was absent in ICc. ICx and ICc neurons often exhibited spontaneous repetitive spike firing, lower repetitive AP firing thresholds, and faster repetitive spike firing than ICd neurons. Calcium-mediated fast after-hyperpolarizations and spike frequency adaptation were regularly seen in IC. Neurons in ICx and ICd, but not ICc, had synaptic responses to stimulation of the collicular commissure (CoIC). In ICx, large epileptiform depolarizing events were often elicited by strong electrical stimulation of CoIC, which was not normally seen in ICd. These results indicate that ICx neurons exhibit a greater degree of synaptic excitability than neurons in ICc or ICd, which may contribute to the proposed role of ICx in pathological IC hyperexcitability.

Key words: Inferior colliculus; Auditory system; Central nucleus; Spike firing; Epilepsy

1. Introduction

The inferior colliculus (IC) is critical for processing auditory information in the mammalian nervous system. Most ascending and descending auditory pathways synapse in the IC. The anatomic subdivisions of the IC in the rat and other mammalian species consist of the central nucleus (ICc), external cortex (ICx), and dorsal cortex (ICd) (Faye-Lund and Osen, 1985; Oliver and Shneiderman, 1991). The ICc is part of the lemniscal (tonotopic) pathway, and is believed to be important in processing and coding auditory information for hearing. The IC cortices surround the ICc and are considered a part of the extralemniscal auditory system. In contrast to ICc, the primary inputs to the IC cortices are not directly from the auditory brainstem nuclei, but from cells in the ICc (ipsilateral and contralateral), IC cortices (contralateral), and auditory cerebral cortical areas (Faye-Lund and Osen, 1985; Faye-Lund, 1986; Games and Winer, 1988; Tokunaga et al., 1984; Smith, 1992; Saldaña and Merchán, 1992). The IC cortices also receive inputs from regions not directly associated with auditory function (Coleman and Clerici, 1987; Oliver and Shneiderman, 1991). The IC cortices are involved in processing auditory information physiologically, for the reflexive orienting responses evoked by acoustic stimulation (Caird, 1991), and pathophysiologically, for genetically-inherited and ethanol withdrawal-induced audiogenic seizures (Ribak et al., 1994; Ribak and Morin, 1995; Chakravarty and Faingold, 1997, 1998). Each IC subnucleus may have a distinctly different function in normal and pathological processing of acoustic information.

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Differences in the electrophysiological properties of neurons in the IC subnuclei may contribute to normal and abnormal auditory processing. Intracellular recording techniques allow for careful observation of neuronal membrane-related phenomena (Li et al., 1993, 1994; Pierson et al., 1989; Smith, 1992; Wagner, 1994, 1996; Yamauchi et al., 1989), but few studies using this technique have compared the specific IC subnuclei. Many important aspects of ICc and IC cortex neuronal physiology are still not clear. In the present study, we characterized IC neurons in the three major subdivisions electrophysiologically and compared some of the basic membrane and synaptic properties of neurons in different IC subregions. We found subregion-specific differences in physiological responsiveness. These differences could be useful in understanding the normal mechanisms of auditory stimulus coding and information processing on a cellular level and may also be valuable for understanding cellular mechanisms underlying pathological dysfunctions involving the IC.

2. Materials and methods

Intracellular recordings were made from IC neurons in brain slices of Sprague-Dawley rats. Adult male rats weighing 250–400 g (11–20 weeks of age) were obtained from Harlan Sprague Dawley, Inc. The methods were similar to those published previously (Li et al., 1994). Briefly, rats were deeply anesthetized with halothane and decapitated. The brain was quickly removed and immersed in cold, oxygenated artificial rat cerebrospinal fluid (ACSF). Transverse cuts were made immediately rostral to the superior colliculus and in the midportion of the cerebellum. A block containing the IC was mounted with cyanoacrylate glue, cerebellum end up, and sliced in 400 μm thick coronal sections using a vibrating tissue slicer. The same type of slice was used for recording from all three subdivisions of IC. The slices used were chosen from an area close to the most caudal portion of the IC commissure. This area includes some commissural fibers, dorsal and external cortices, some lateral lemniscal fibers, and central nucleus (Paxinos and Watson, 1982) (Fig. 1). Slices were kept in an incubation chamber at room temperature for at least 30 min prior to transfer to an interface-type recording chamber at 35°C. The chamber was continually gassed with a warm humidified 95% O_2/5% CO_2 mixture. In the incubation and recording chambers slices were bathed in ACSF with the composition (in mM) NaCl 117.4, KCl 2.0, $MgSO_4$ 1.4, $CaCl_2$ 2.5, KH_2PO_4 1.0, $NaHCO_3$ 26.2, glucose 11.0, and ascorbic acid 1.0 (Rice and Cammack, 1991). Sucrose was substituted for NaCl for the first 30 min of incubation in order to enhance slice viability (Aghajanian and Rasmussen, 1989). The incubation solution was also used for experiments in which the effect of reduced sodium concentration was tested. The ACSF was continually bubbled with 95% O_2/5% CO_2. In the experimental chamber, slices were held at the atmosphere-fluid interface and perfused at a rate of 1.5 ml/min.

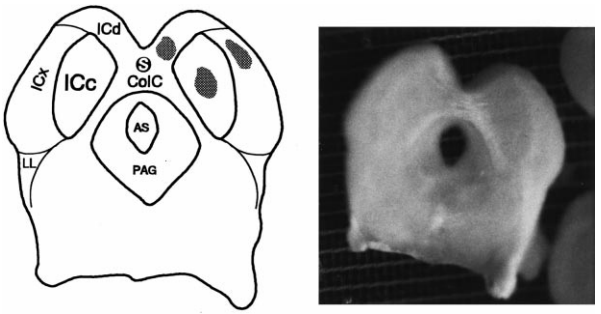

Fig. 1. Inferior colliculus slice. Shown on the right is a photograph of a transverse slice of the IC, and on the left a diagram of this slice. The IC central nucleus (ICc), external cortex (ICx), dorsal cortex (ICd), and commissure (CoIC) are visible in the living slice. Other landmarks include the lateral lemniscus (LL), periaqueductal gray (PAG), and aqueduct of Sylvius (AS). The location of our bipolar stimulating electrode in the CoIC is indicated (circled 's'). The cross-hatched areas in the diagram are the approximate areas from which we recorded.

The locations of IC subdivisions were determined visually according to the atlas of Paxinos and Watson (1982) and descriptions of Faye-Lund and Osen (1985). Neurons with resting membrane potentials more negative than −50 mV, slope input resistance greater than 20 MΩ, and action potentials (APs) peaking at positive membrane potentials were accepted for analysis. A bipolar stimulating electrode (Rhodes Electronics, Woodland Hills, CA) was placed in the fibers of the commissure of IC (CoIC) at the midline. Electrical stimuli were 0.1-ms duration constant-voltage square waves.

Intracellular recording electrodes were pulled from 1.0-mm outer diameter glass pipettes on a Brown/Flaming P87 micropipette puller (Sutter Instrument Co., San Rafael, CA), and filled with 4.0 M potassium acetate. Electrode impedances ranged from 80 to 110 MΩ. Intracellular potentials were recorded with an Axoclamp 2A preamplifier (Axon Instruments, Foster City, CA), low-pass filtered at 3–10 kHz, and digitized for storage on a microcomputer, or displayed on a storage oscilloscope and photographed. Bridge balance was carefully monitored and adjusted when necessary. The intracellular electrodes passed ±1.0 nA of current without significant rectification. Capacity compensation was adjusted to just below the maximum obtainable without oscillation. The pClamp series of programs (Axon Instruments) was used for data acquisition and off-line analysis of digitized data. Photographic data were measured by hand. Data from neurons were grouped and compared using Student's *t*-test, two-factor ANOVA, or χ^2 on incidences. Differences were considered significant if $P < 0.05$. Drugs were applied by bath per-

fusion. The drugs used were bicuculline methiodide, a γ-aminobutyric acid ($GABA_A$) receptor antagonist, *N*-methyl-D-aspartic acid, an NMDA receptor agonist, cobalt chloride, an inorganic voltage-sensitive calcium channel antagonist, and nimodipine, an organic calcium channel antagonist. Drugs were dissolved in ACSF except for nimodipine which was dissolved in ACSF with 0.01% ethanol. Nimodipine was shielded from light during its preparation and use. All chemicals were obtained from Sigma, St. Louis, MO.

The passive electrical properties of IC neurons were studied by injecting a series of hyperpolarizing and depolarizing current pulses (100 ms duration) into neurons at resting membrane potential. Current-voltage (I-V) curves were then generated by plotting the steady-state voltage response of the neuron to injected current. I-V curves were linear from approximately 5–10 mV more positive to 30–40 mV more negative than resting membrane potential in neurons from all three regions, allowing the calculation of neuronal passive membrane resistance from the slope of its I-V curve. The neuronal membrane charging time constant was measured by fitting a single exponential curve to the initial 50 ms of the neuronal response to a small hyperpolarizing current pulse.

Single APs were elicited with brief depolarizing current pulses of 2–5 ms duration. Repetitive firing was studied with 100-ms depolarizing pulses. The firing pattern during long, steady depolarizations was studied by 'manually' injecting depolarizing current using a control on the Axoclamp 2A front panel.

The care and use of animals in this study were approved by the Laboratory Animal Care and Use Committee of Southern Illinois University School of Medicine and the National Institutes of Health.

3. Results

Intracellular recordings were made from 87 neurons in rat transverse IC slices. Thirty-one neurons from the ICd subdivision, 18 neurons from the ICc subdivision and 38 neurons from the ICx subdivision were studied.

3.1. Neuron electrophysiological types

Neurons from each region were classified according to the discharge response patterns seen with large (+1.0 nA, 100 ms duration) depolarizing intracellular current pulses. Three response types were observed: on-type (Fig. 2A, top), rapidly-adapting (Fig. 2A, middle), and sustained (Fig. 2A, bottom). Twenty cells responded with only a single on-type spike (9 of 31 from ICd, 2 of 18 from ICc, 9 of 38 from ICx neurons), even if the current intensities were far above the AP threshold (the maximum current used was +1.5 nA).

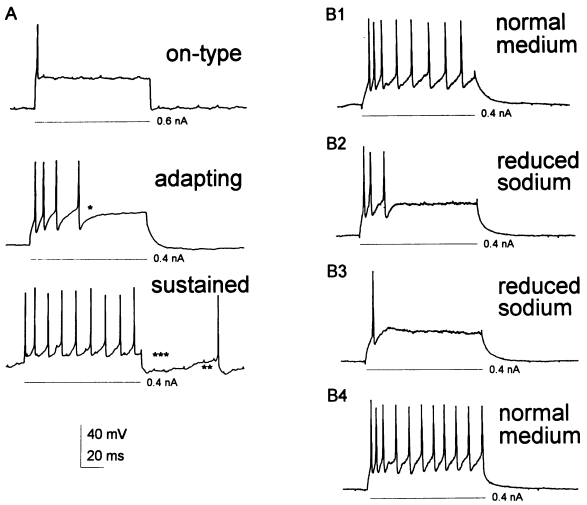

Fig. 2. Cell types of IC based on repetitive firing properties. A: Neurons from the three subdivisions of IC can be classified into three types based on their responses to depolarizing current (100 ms duration current pulses, cell at resting membrane potential). On-type cells (top) lack a fast after-hyperpolarization (AHP). Rapidly adapting cells (middle) have a fast AHP (*) and cease firing during the current pulse. Sustained type cells continue firing throughout the current pulse. Some neurons have spontaneous action potential (AP) firing at resting membrane potential (**). A medium-duration AHP (***) was seen in some neurons, but most neurons lacked it. B: In normal medium this neuron had a sustained firing pattern (B1). Perfusing sodium-reduced solution transformed its firing pattern first into a rapidly adapting pattern (B2, 5 min in reduced sodium) and then into an on-type responding pattern (B3, 8 min in reduced sodium). The sustained firing response reappeared after return to normal ACSF (B4, 15 min after return to normal medium). The duration and intensity of the current stimulus is given below each membrane voltage response. Resting membrane potentials: on-type (−60 mV), adapting (−60 mV), sustained (−70 mV), B (−65 mV).

Eleven neurons had an adapting type of response. With low current intensities only an on-type spike occurred, but if sufficiently depolarized, additional APs occurred, with a gradual increase in the interspike interval and cessation of firing within the first 50 ms (spike frequency adaptation; 3 of 31 from ICd, 2 of 18 from ICc, 6 of 38 from ICx neurons). Adapting neurons were clearly distinguishable from sustained type neurons by their gradual increase in interspike intervals during the pulse, and they fired no APs in the final 50 ms of the pulse, even with the largest current intensities tested. Fifty-six neurons responded with sustained AP firing throughout the 100-ms current pulse (19 of 31 from ICd, 14 of 18 from ICc, 23 of 38 from ICx neurons). One neuron, illustrated in Fig. 3C2, responded with an on/off/on/off pattern. It was classified as a sustained type neuron because firing did not cease in the final 50 ms, and with larger current intensities it fired in a sustained manner without pausing.

Although there was a trend toward less frequent occurrence of on-type responses in ICc, the incidences of these different responses in the subnuclei were not sig-

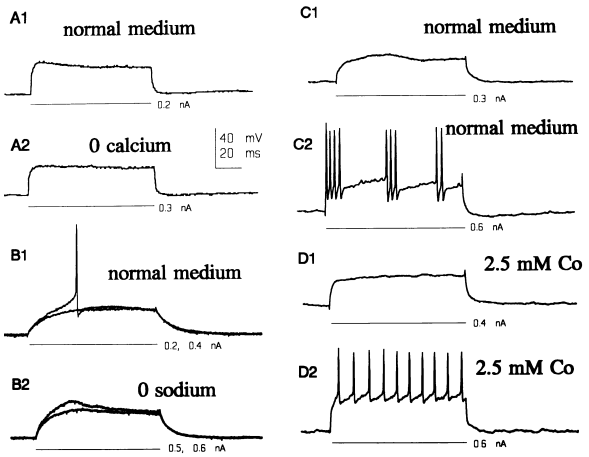

Fig. 3. Calcium dependence of the initial depolarization (hump) during depolarizing pulse injection. A: This neuron showed a hump at the onset of a depolarizing stimulus (A1). The hump was abolished when calcium was omitted from the solution (A2). B: This neuron did not have a hump in normal ACSF (B1), but a hump appeared if sodium APs were blocked by low sodium solution (B2). C–D: In another cell, a hump was recorded with subthreshold stimuli (C1). With larger stimuli AP firing occurred (C2). Although pauses occurred, spike firing did not cease during the final 50 ms of the stimulus, and this cell was classified as a sustained type neuron. The hump was abolished by addition of $CoCl_2$ (D1). The fast AHP and spike frequency adaptation were significantly reduced (D2) by $CoCl_2$, and the cell's response transformed into a typical sustained type of firing pattern. Resting membrane potentials: A1, A2 (−60 mV), B1, B2 (−65 mV), C, D (−78 mV).

nificantly different (χ^2 $P = 0.10$). The on-type and sustained type neuronal responses showed significant differences in several basic membrane properties (Table 1). Except for resting membrane potential, the membrane properties of rapidly adapting type cells were intermediate, and somewhat more similar to those of the sustained firing type. All three cellular types sometimes displayed anode-break spike firing and spontaneous APs at the resting membrane potential.

These different discharge patterns appeared partially to be due to the activation of a sodium conductance. In the two cells tested, substituting a reduced-sodium solution for the normal ACSF turned a sustained-type response into a rapidly adapting or an on-type response (Fig. 2B1–B4). This effect reversed after returning the neurons to normal ACSF.

3.2. Membrane properties

Resting membrane potential, slope resistance, and charging time constant for the three different cell types were measured and compared between the three IC subdivisions. The membrane properties of on-type and rapidly adapting type cells were not statistically different in any of three regions, but there were significant region-specific differences in the membrane properties of the sustained-type neurons. Table 2 shows some differences in the basic membrane properties of sustained response neurons, which were the most numerous cell type in all three subdivisions of IC (64% of all neurons). The average slope input resistance, measured from the I-V curve in the linear segment close to resting membrane potential, was significantly higher in sustained-type neurons from ICx as compared to ICc neurons, but there was no statistical difference when compared to ICd neurons (Table 2). The membrane charging time constants were relatively short (3–7 ms) in sustained-type IC neurons. The mean time constant of ICd sustained-type neurons was longer than that for ICx or ICc neurons.

3.3. Calcium-mediated potentials

With square-wave intracellular current injections subthreshold for AP firing, a slow depolarization or 'hump' was occasionally observed. This hump (Fig. 3A1,C1) was commonly seen in ICd neurons (11 of 28), but was not seen in ICc neurons (0 of 17, $P < 0.05$). Few ICx neurons exhibited this feature (5 of 36). The hump was abolished (2 neurons) in ACSF with no added calcium (Fig. 3A2,A2) and by cobalt ($CoCl_2$, 2 ICd neurons, Fig. 3C1,D1), but not by nimodipine (2 μM, in two ICd neurons, data not shown). Taken together, these data support a major role of a calcium conductance in the generation of this response feature. In three neurons that did not have the hump in normal ACSF, a hump appeared after treatment with

Table 1
Membrane properties of three cell types in inferior colliculus

	On-type			Sustained			Adapting		
	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*
RMP (mV)	−64	11	20	−65	9.6	56	−67	9	11
IR (MΩ)*	44	29	17	60	27	49	50	35	11
TC (ms)*	1.7	1.1	13	5.0	3.0	48	3.4	3.8	8
AP (mV)*	56	15	18	65	16	50	60	9.6	10
AP (ms)*	0.97	0.45	18	0.48	0.20	45	0.88	0.50	10

RMP, resting membrane potential; IR, input resistance; TC, time constant; AP, action potential amplitude in mV or duration in ms. Asterisk (*) indicates significant difference between on-type and sustained-type responses ($P < 0.025$ using *t*-test). *n* indicates the number of neurons in which this measurement was determined.

Table 2
Comparison of membrane properties of neurons in three subdivisions of inferior colliculus

	ICc			ICd			ICx			Statistics			
	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	*P*	cx/cd/dx		
RMP (mV)	−62	8	14	−67	10	19	−64	10	23	ns			
IR (MΩ)	41	2.4	10	56	31	18	68	26	22	+	*	ns	ns
TC (ms)	3.5	2.2	10	7	4.3	16	4.4	2.8	22	+	ns	+	+
AP (mV)	65	11	10	69	16	18	62	15	22	ns			
AP (ms)	0.37	0.2	10	0.42	0.15	14	0.66	0.18	21	+	*	ns	*
Threshold (mV)	10	9	3	31	11	6	21	9	8	+	ns	+	+
'Hump'	0% (0/17)			39% (11/28)			14% (5/36)						
Spontaneous AP	22% (4/18)			0% (0/31)			42% (16/38)						

The first five measures are for sustained-type neurons only, while the last three measures are for all neurons in the subnucleus. Threshold is for action potential firing in mV depolarized from RMP; 'Hump', percentage of neurons having a calcium-mediated hump; Spontaneous AP, percentage of neurons having spontaneous action potential firing at RMP; column *P* indicates overall statistical significance using one-factor ANOVA; S.D., sample standard deviation; *n*, number of neurons tested; ns, not statistically significant. If the ANOVA was significant, *t*-tests compared ICc to ICd (indicated by cd), ICc to ICx (cx), and ICd to ICx (dx). + indicates $P < 0.05$, * indicates $P < 0.01$.

sodium-reduced ACSF (Fig. 3B1,B2).

Adapting type neurons displayed prominent spike frequency adaptation (Fig. 2A, middle). Spike frequency adaptation was reduced by addition of $CoCl_2$ in two adapting type ICd neurons (not shown). In one sustained neuron that displayed a calcium-mediated hump (Fig. 3C1), the firing pattern at low stimulus intensity was changed from an irregular on/off/on/off pattern in normal medium to a more regular firing pattern in $CoCl_2$ (Fig. 3C2,D2).

3.4. Action potentials

Single APs were evoked from IC neurons at their resting membrane potential with depolarizing current pulses (2–5 ms duration). AP height and width at half-height were measured from resting membrane potential to spike peak. AP amplitudes were similar in the three regions. Mean AP width was significantly greater in ICx than in ICc or ICd (Table 2). A fast after-hyperpolarization (AHP) was observed in most neurons of the three IC subdivisions. Only about 10% of neurons lacked a fast AHP, and these were often on-type neurons (7 of 20; Fig. 2A, top). A medium-duration AHP was detected in 20% of IC neurons. The medium-duration AHP usually lasted less than 100 ms (Fig. 2A, bottom). In two neurons tested, the fast AHP was reduced in the presence of cobalt chloride (Fig. 3C2,D2). IC neurons often showed pronounced anode-break excitation. The depolarization following anode break (−1.0 nA currents for 100 ms) lasted from 50 to 100 ms, and sometimes elicited several spikes. Anode-break spike firing occurred significantly more often in ICx neurons (18 of 36) than in the other subnuclei (3 of 29 ICd neurons; 3 of 17 ICc neurons; χ^2 $P < 0.001$). Spontaneous AP firing was seen significantly more often in ICx neurons (16 of 38) than in ICc (4 of 18), and it was never seen in ICd neurons (0 of 31; χ^2 $P < 0.001$, Table 2).

3.5. Repetitive firing in sustained type neurons

The tendency of sustained-type IC neurons to fire repetitive APs with brief stimuli was assessed by injecting 100-ms duration depolarizing current steps into the neuron at resting membrane potential. Sixteen current strengths in 0.1-nA intervals from +0.1 to +1.5 nA were injected, and the number of APs elicited was counted (Fig. 4). Sustained type neurons were more numerous than other types in all three subnuclei, and had somewhat different firing frequency vs. current relationships (f-I curves) in different subnuclei. ICx and ICc neurons had a significantly greater rate of firing for the same strength of current injection compared to ICd ($P < 0.01$, Fig. 4).

3.6. Response to prolonged depolarization

In addition to investigating neuronal responses to

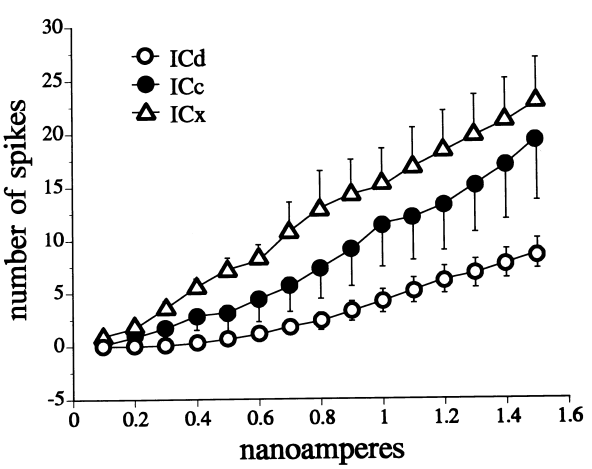

Fig. 4. Mean spike firing frequency vs. current relationship (f-I curves). For any given current intensity, the sustained type cells in ICx and ICc fired more rapidly than ICd neurons ($P < 0.01$, 2-way split-plot ANOVA). Bars indicate standard error of the mean. $N = 14$ ICd, 7 ICc, 18 ICx.

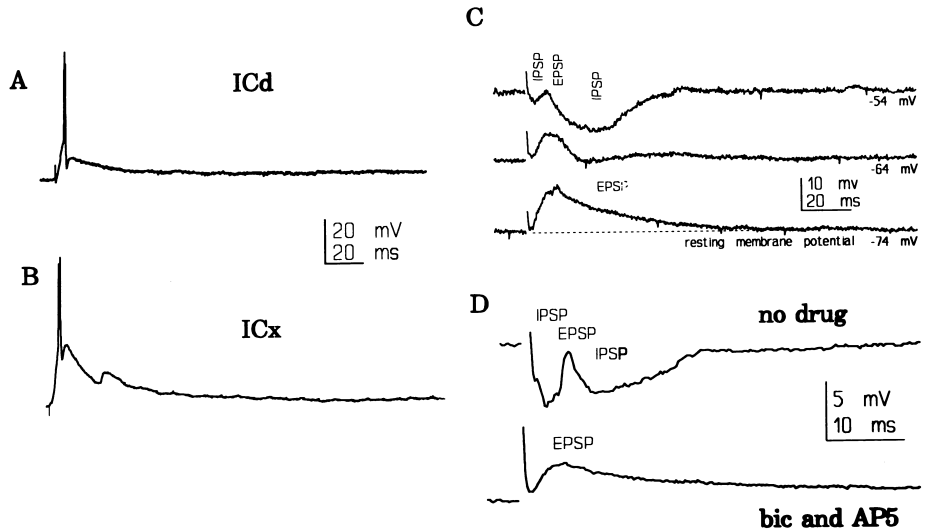

Fig. 5. Consistent differences between ICd, ICc, and ICx responses were seen with stimulation of synaptic pathways. A: In ICd neurons stimulation of the CoIC elicited a single AP followed by a rapid return to resting potential or a prolonged depolarization. B: In ICx, stimulation of CoIC elicited a single AP followed by a prolonged depolarization. In ICc, stimulation of the CoIC did not elicit a response. C: Synaptic response to the same strength of stimulation of the CoIC at the cell's resting potential (−74 mV) and with it held positive to resting potential with steady current injection. At resting potential an IPSP is difficult to detect, but an IPSP/EPSP/IPSP sequence is made clear by depolarization. In general, membrane depolarization allowed demonstration of this sequence of synaptic potentials in every IC subdivision. The dotted line indicates resting membrane potential. D: In another cell, both phases of the IPSP (top trace, in normal ACSF) evoked in an ICd neuron by CoIC stimulation were blocked by bicuculline (bottom trace, in bic 20 μM with 50 μM AP5). Resting membrane potentials: A (−70 mV), B (−60 mV), C (−74 mV), D (−67 mV).

brief stimuli, we determined their response to sustained depolarization. We recorded the threshold for AP firing (Table 2) with steady depolarizing current injection. The cells studied had no spontaneous APs at resting membrane potential. In all subnuclei, if the membrane potential was held slightly negative to the AP threshold, synaptic stimulation usually induced repetitive AP firing. Regular firing of APs was observed when the membrane potential was depolarized to the AP threshold without synaptic stimulation. Upon further depolarization, the frequency of spike firing increased. The threshold for sustained AP firing ranged from 10 to 30 mV depolarized from the resting membrane potential. On average, the threshold for sustained AP firing was significantly closer to the resting membrane potential in ICx and ICc than in ICd ($P < 0.05$).

3.7. Single synaptic responses

We studied the responses of neurons to stimulation of the CoIC. The CoIC connects the two IC hemispheres. The ICx and ICd regions receive inputs from the CoIC, which is formed mainly by fibers crossing the midline from the opposite ICc. Stimulation of the CoIC did not elicit a response in ICc ($N = 0$ of 6 neurons studied). CoIC stimulation did elicit responses in ICx and ICd. When responses were studied at resting membrane potential, most cells had only an excitatory postsynaptic potential (EPSP) at low stimulus intensities, and at higher stimulus intensities fired an AP (Fig. 5A,B). This pattern was seen in 10 of 17 ICd neurons and in 11 of 14 ICx neurons studied. In some of these neurons an inhibitory postsynaptic potential (IPSP) would become apparent at high stimulus intensities, but in most cells the EPSP merely became larger and longer in duration. Prolonged EPSPs were most apparent in ICx neurons (Fig. 5B).

When studied at resting membrane potential, only a minority of neurons in ICd and ICx had a detectable IPSP with CoIC stimulation. These neurons had an IPSP/EPSP/IPSP sequence. However, even neurons that had only an EPSP at resting membrane potential would show the same IPSP/EPSP/IPSP sequence if the cell's membrane was held depolarized by steady current injection (Fig. 5C). This pattern was seen in 8 of the 11 cells tested in this way, with the other 3 of 11 showing only an EPSP even at the most depolarized potentials. Both components of the IPSP were blocked with bicuculline (Fig. 5D, $N = 6$ neurons tested). The complex EPSP and IPSP sequences involved in IC neuronal responses to sound have been examined recently (Smith, 1992; Covey et al., 1996; Pedemonte et al., 1997).

3.8. Epileptiform synaptic responses

In ICd neurons, a single suprathreshold stimulation of CoIC induced only a single AP, even at the maximum stimulus intensity used (15 V). In contrast, ICx neurons often exhibited epileptiform discharges (Fig. 6A,B). If the stimulation strength was sufficient (≤ 1.5

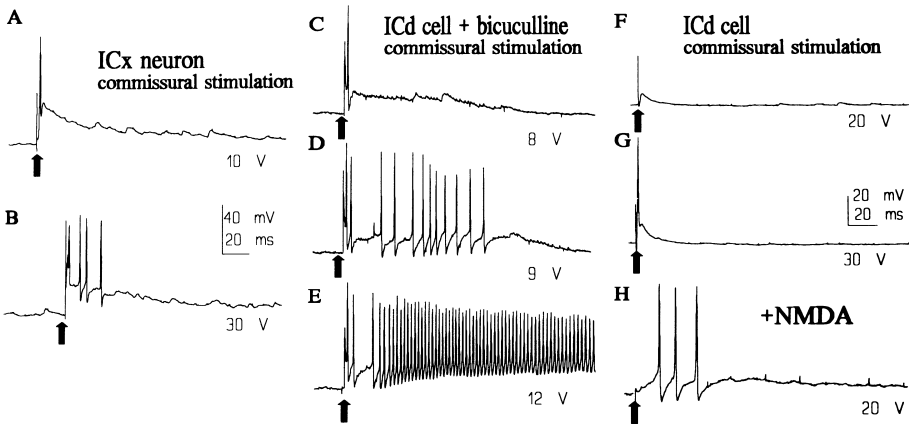

Fig. 6. Epileptiform responses in external cortex neurons. A, B: In ICx neurons synaptic stimulation of the CoIC caused epileptiform burst responses. C–E: Epileptiform burst discharges are demonstrated for an ICd neuron perfused with bicuculline (30 μM). The neuron's response to stimulation of CoIC is shown at three different current intensities. Epileptiform responses were never seen in ICd neurons except after application of bicuculline ($N=6$) or NMDA ($N=4$). F–H: This ICd neuron's response to commissural stimulation was an EPSP (F) or EPSP and AP (G) with larger stimuli. After application of NMDA (H), even small stimuli produced a prolonged depolarization and AP firing. Arrows indicate the time of the synaptic stimulation. The stimulation strength in μA is noted. Resting membrane potentials: A, B (−70 mV); C, D, E (−65 mV); F, G (−65 mV); H (−60 mV).

mA, 0.1 ms was used), CoIC stimulation caused a prolonged membrane depolarization and an epileptiform discharge. This was seen in 43% of ICx neurons (6 of 14 cells studied). The latency to the onset of the depolarization was variable, and often occurred after an initial fast EPSP. The depolarization lasted 100–200 ms and elicited intense AP firing. In other IC neurons lacking this epileptiform activity, similar patterns could be produced, but only after bath perfusion of bicuculline (Fig. 6C–E) or NMDA (Fig. 6F–H) (Li et al., 1994).

ICc neurons did not respond to CoIC stimulation, but synaptic responses could be evoked by lateral lemniscus stimulation in preliminary studies. No ICc neurons exhibited epileptiform responses.

4. Discussion

4.1. Cell types

Neurons in the three IC subnuclei can be classified as on-type, rapidly adapting or sustained-type cells on the basis of their electrical response properties. The three types have different responses to depolarizing current pulses, and on-type neurons have significantly different membrane properties compared to sustained neurons, including membrane input resistance, membrane time constant, and AP width (Table 1). Neurons with an onset response to sound have been found in IC in vivo (Condon et al., 1996; Le Beau et al., 1996; Palombi and Caspary, 1996), and this response pattern has been suggested to be involved in temporal processing of sound, functioning as coincidence detectors and to encode the onset of sounds. Neurons with an on-type response to intracellular current stimulation have been found previously in IC neurons in vitro (Wagner, 1994). In contrast, sustained-type neurons of the IC may integrate features of auditory stimuli without emphasis on temporal acuity. In Wagner's study of mouse ICc neurons, all three electrophysiological types had a multipolar morphology (Wagner, 1994). The mechanisms that underlie the on-type response after injecting depolarizing current are unclear, but may be related to voltage-sensitive sodium channel function, since cells with sustained responses became on-responders in sodium-reduced solution. In on-type neurons, sodium channels may be closed or inactivated rapidly after injecting depolarizing current.

The properties of the ICd neurons (sustained-type) we studied correspond closely to neurons described by Smith (1992) as having a multipolar shape. Our recordings were made from the area which Faye-Lund and Osen (1985) identified as layer three of ICd, and as layer two and three of ICx. They report that ICd and ICx neuronal morphology in the rat is relatively homogeneous, and it is likely that the neurons we studied in IC cortex have a multipolar shape. The rat ICc was described as containing two cell types, disc and multipolar shaped cells, with the latter being larger (Faye-Lund and Osen, 1985). More recently, on the basis of extensive three dimensional reconstructions, Malmierca et al. (1995) have suggested that cell types in ICc are somewhat more homogeneous than the terms 'disc' and 'multipolar' suggest and proposed a new classification of 'flat' and 'less flat' neurons. Taken together, the morphological data suggest that the cellular populations in each subnucleus may be relatively homogeneous. However, the rat neurons impaled in our experiments may be larger on the average than the mouse neurons previously examined (Wagner, 1994), since the ICc neurons

we studied have smaller input resistances but similar time constants. These two differences in intrinsic membrane properties observed in the present study from those described by Wagner may be due to the different animal species employed in the two studies.

4.2. Membrane properties

Several significant differences between the three subdivisions in sustained-type neuronal responses suggest that ICx is the most easily excited area of IC. The differences are of two general kinds: alterations in membrane properties and differences in synaptic transmission. Differences in membrane properties include different membrane input resistances, time constants, repetitive AP firing (f-I curves), spontaneous AP firing, threshold for regular AP firing with steady depolarizing current injection, and frequency of occurrence of calcium-mediated potentials (Table 2). The underlying cause of these differences has not yet been established, but they may indicate differences in neuronal shape, size, or membrane excitability. The higher neuronal input resistance in ICx neurons will increase their tendency to fire APs during depolarizing current injections, by increasing the voltage change produced by a given amount of current. Spontaneous AP firing at resting membrane potential was seen most often in ICx neurons, less often in ICc, and was absent in ICd neurons. The AP firing thresholds in ICx and ICc were significantly closer to the resting membrane potential than in ICd. Since there was no difference in resting membrane potentials, this shift indicates that the spike thresholds of ICc and ICx are lower than ICd. These may result in the different shapes of the f-I curves (Fig. 4) recorded from different regions. Taken together, these results suggest that neurons from ICx have greater membrane excitability than ICc, and ICd is the least excitable.

The movement of calcium is an important factor influencing membrane and intracellular events. We found a calcium-mediated depolarizing potential, or 'hump', in recordings from IC cortex neurons, which was previously observed by Smith (1992). The hump is less frequently seen in ICx neurons and not seen in ICc neurons. Similar calcium-mediated depolarizing 'humps' have been found in other neuronal structures. Disterhoft et al. (1993) reported that nimodipine blockade of the calcium-mediated hump in hippocampal slices enhanced neuronal firing and long-term potentiation. There are at least four kinds of calcium channels in neuronal membranes (Hille, 1992). Although we have not done voltage-clamp recording of calcium currents, the hump in IC neurons may involve N type calcium channels because the depolarization is transient (suggesting rapid inactivation), is not blocked by nimodipine, and has a relatively high threshold (McCormick, 1990) (20–30 mV positive to resting membrane potential). Calcium influx can influence neuronal firing patterns in at least two ways: by causing depolarization through the direct effects of calcium currents, and/or by causing hyperpolarization through activation of calcium-dependent potassium currents (thereby increasing interspike intervals) (Grillner et al., 1991; Manira et al., 1994; McCormick, 1990). In our experiments, abolition of the hump by addition of $CoCl_2$ significantly reduced the fast AHP and spike frequency adaptation. These changes suggest the presence of a calcium-activated potassium conductance in these neurons. The relative lack of an apparent hump in ICx and ICc may relate either to lesser calcium channel activity, or to the fact that fast sodium channel activation occurs before the hump is apparent. Some of our results suggest the latter, since omission of sodium from the bathing medium can unmask a hump in cells in which it was not apparent previously (Fig. 3B). The presence of calcium-activated potassium currents could in part account for the lower firing frequency vs. current (f-I) curves in ICd compared to ICc and ICx, and the greater tendency for the latter structures to rapidly fire APs with steady current depolarization. Since calcium is not the only factor contributing to firing frequency, further experiments would need to be done to resolve this question.

4.3. Synaptic properties and epileptiform activity

Each subdivision in the IC is known to have a specific combination of inputs and intrinsic connections (Faye-Lund and Osen, 1985). Our results are limited to responses arising from stimulation of CoIC, but show that neurons from the three IC subnuclei have distinct synaptic response patterns (Fig. 5). ICx neurons often have an epileptiform response, ICd neurons fire only one AP, and ICc neurons do not respond. These different responses may in part be a consequence of the different input sources to the subdivisions. Commissural inputs to ICc have been described, but in general the ICc receives its major inputs via the lateral lemniscus, including the dorsal nucleus of the lateral lemniscus, which is inhibitory to IC neurons (Li and Kelly, 1992; Faingold et al., 1993a). This pathway uses GABA as its neurotransmitter. In our experiments, ICc neurons did not respond to CoIC stimulation. This could be due to sparse inputs of this pathway, or to damage from the slicing procedure. ICd and ICx both receive input from the ICc (Druga and Syka, 1984; Coleman and Clerici, 1987) ipsilaterally, and contralaterally through the CoIC. Since bicuculline can eliminate both components of the biphasic IPSP seen in IC neurons after CoIC stimulation, the inhibition in this pathway appears to be $GABA_A$ receptor-mediated (Fig. 5D).

In in vitro brain slices from non-epileptic animals, a prominent effect of pharmacological blockade of

$GABA_A$ receptors by bicuculline is spontaneous and evoked epileptiform bursting. This bursting activity is thought to be like in vivo interictal spiking, a hallmark of localization-related (focal) epilepsy (Prince, 1985). We found that epileptiform bursting in normal ICd neurons occurred with synaptic stimulation but only after bath application of bicuculline or NMDA (Li et al., 1994). Similar results were reported previously (Pierson et al., 1989; Smith, 1992). ICc neurons did not exhibit spontaneous or evoked epileptiform bursting. Although ICx neurons did not have spontaneous epileptiform bursting, they often had epileptiform bursts evoked by synaptic stimulation. Unlike ICd, bursting in ICx occurred without application of a convulsant.

The IC is subject to pathological changes that may play an important role in certain dysfunctions such as audiogenic seizures (Chakravarty and Faingold, 1997) and central tinnitus (Szczepaniak and Møller, 1996; Gerken, 1996). The ICc (Faingold et al., 1993b; Ribak and Morin, 1995) and the IC cortices have each been implicated as potential sites for audiogenic seizure initiation (Wada et al., 1970; McCown et al., 1984, 1987; Browning, 1994; Kwon and Pierson, 1997). It has been proposed that audiogenic seizure susceptibility is linked to reduced efficacy of GABAergic neurotransmission and to increased action of glutamate in the IC (Faingold et al., 1986, 1993b; Browning et al., 1989; Faingold and Boersma Anderson, 1991). Epileptiform responses in ICx neurons were observed in the present study, and a previous study observed similar epileptiform responses in ICd neurons of genetically epilepsy-prone rats, which were not seen in normal ICd neurons (Li et al., 1994). Much more prolonged, ictal-like excitatory events occurred in IC neurons when $GABA_A$ inhibition was blocked. The ICx exhibited the highest [^{14}C]-2-deoxyglucose uptake among the three IC subdivisions after seizures induced by electrical stimulation at the ICd-ICx border (McCown et al., 1991). Although we found epileptiform events only in ICx, ICc neurons also seem to have a tendency to repetitive firing (no hump, lower firing threshold, presence of spontaneous AP firing). The resistance of ICd neurons to epileptiform activity may be related to the denser $GABA_A$ receptor distribution that has been found in ICd by receptor binding (Milbrandt et al., 1994). These different features of the IC subnuclei may be especially important for the initiation and propagation of audiogenic seizures. A precise balance in IC neurons between excitation and inhibition is likely to be important in normal auditory function. Imbalance may lead to epileptiform firing patterns. Since GABA-mediated inhibition is less effective in the genetically epilepsy-prone rat IC and other brain sites than in non-epileptic rats (Faingold et al., 1986; Faingold and Boersma Anderson, 1991; Evans et al., 1994; Gould et al., 1995), loss of this inhibitory function may be critical to initiation of audiogenic seizure susceptibility (Faingold and Boersma Anderson, 1991).

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