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INTRACELLULAR *IN VIVO* RECORDING OF INFERIOR COLLICULUS AUDITORY NEURONS FROM AWAKE GUINEA-PIGS

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INTRODUCTION

The state of arousal is an obvious important condition for sensory signal processing. Thus, e. g., receptive fields can vary their size in relation to the arousal level and perhaps to sleep phases. Moreover, cortical receptive fields have been shown to be reduced with the increasing depth of anesthesia (1). Behaviorally related shifts in the unitary activity of auditory subcortical nuclei have been reported in guinea-pigs, without any drug. Dramatic changes in firing rate and pattern of discharge were observed with extracellular recordings, associated with waking and sleep phases as reported in the cochlear nucleus by Peña *et al.* (8) and in the lateral superior olive by Pedemonte *et al.* (7).

The inferior colliculus central nucleus (ICc) is a major auditory integrative center where ascending as well as descending information converges to be processed. The dorsal IC and external nuclei are the first synaptic stations directly reached by corticofugal fibers, thus indirectly connecting to the ICc, although direct terminals in the central nucleus in the monkey have also been reported (2).

An ICc extracellular study, during the sleep-waking cycle, has been reported (4) showing striking neuronal firing shifts and changes in the pattern of discharge, parallel to the behavioral shifts. A more difficult task, the intracellular recording of ICc cells in *in vivo* awake restrained guinea-pigs was our target to settle the physiological cellular basis for the auditory sensory processes.

MATERIAL AND METHODS

Guinea-pigs were chronically implanted (n=10) and prepared for waking state monitoring with in-dwelling nichrome electrodes to record the parietal cortex electrocorticogram, under pentobarbital anesthesia (35 mg/kg, i.p.).

The head was stereotaxically supported and a hole on the skull over the inferior colliculus (IC) region was opened for micropipettes penetration according to Rapisarda and Bachelli (10) atlas (A 0.5, L 1, H 3-6). Two light metal bars to hold the animal and reproduce the stereotaxic position were cemented to the animal's skull in order to facilitate painless recording during natural behavior after a week of recovery. The animal's head was thus firmly held, with the body supported by a canvas sheet leaving the legs free.

The glass micropipettes, with impedances between 50-80 MΩ, were filled with 3MΩ potas-

sium acetate and, in some recordings, mixtured with Sky-blue. Intracellular recordings were amplified through an active bridge circuit, monitored on an oscilloscope (Tektronix 2221) and recorded on a FM tape recorder (Akai-VR10 Instrutech Co) for subsequent off-line processing. Recordings which did not show stable membrane potentials (Vm) of at least -40 mV were not considered.

To avoid any pre-receptorial action on the responses, the middle ear ossicles were removed, leaving the malleus handle attached to the intact eardrum and the stapedial foot-plate connected to the oval window. A polyethylene tube (4 mm dia.) was cemented to the superior recessus; the sound source was then connected to the other end of the short tube forming a "closed" acoustic delivery system (13).

The sound stimuli consisted of square pulses (0.1 ms) generating clicks used to evoke unitary responses. The signals were amplified using an audioamplifier (Scott 2065) and delivered to the middle ear superior recessus by means of ear-phones (Beyer DT48) attached to the cemented polyethylene tube.

Sound stimulus intensity, as measured with a level meter (General Radio 1555-A) was 60 dB SPL at the ear-phone output. A sound-attenuating chamber surrounded the whole apparatus. The micropipette position could be controlled remotely from outside using a hydraulic micromanipulator (Narishige MO 8).

The micropipette position in the ICc was determined through histological studies after electrophoretic Sky-blue staining at each recording site and by use of the stereotaxic coordinates.

Camera lucida drawings of the recording sites were performed.

Data processing. - Post-stimulus time histograms (PSTHs) were generated to characterize the auditory neurons. The student *t*-test was used for statistical validation of the observed changes. All data are presented as means ± S.E. (standard error).

RESULTS

1. *Spontaneous activity and response to current pulses.* - Neuronal intracellular recordings (n=10) in adult guinea-pigs were performed during wakefulness. The averaged resting potential recorded was -52.1 mV ± 4.1, S.E. and the input resistance averages corresponded to 28.1 MΩ ± 1.4, S.E. The action potentials averaged amplitude was 40 mV ± 1.6, S.E. and their duration 0.4 ms ± 0.07, S.E.

The spontaneous subthreshold synaptic potential rate, averaged for two units during 3 min of continuous recording, demonstrated a greater rate of EPSPs than IPSPs (50/min and 26/min, respectively).

The averaged spontaneous firing rate during at least 60 s recording of each unit was 48.3 spikes/s ± 5.2, S.E. (n=10). In Fig. 1 A and B, the spontaneous firing frequency in the upper left raw recording was 31.5 spikes/s; on the right, the frequency histogram showed an oscillating pattern over time (20 s). The firing rate was measured for a fixed period (1 min) including silent as well as spiking moments.

When depolarizing current pulses were injected to a spontaneously discharging neuron (Fig. 1, B), an enhanced and sustained firing was observed. With 100 ms current pulses, very little adaptation, if any, was recorded in awake experiments (Fig. 1, B*).

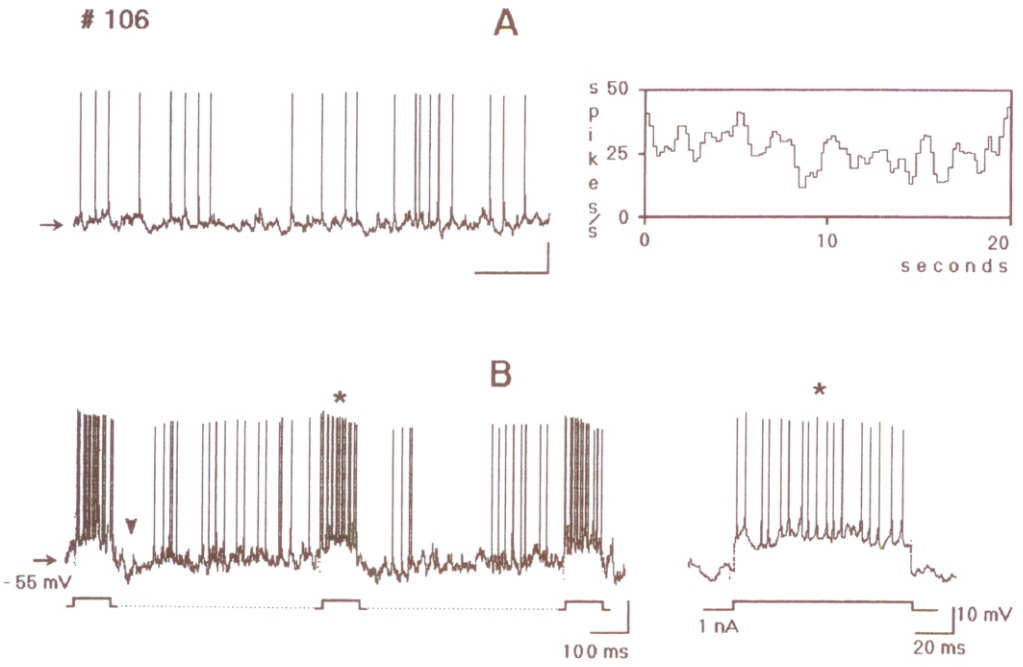

Fig. 1. - *Spontaneous activity during wakefulness (# 106).*

A: left, a raw recording with spikes (31.5 spikes/s) and vigorous synaptic activity. On the right a frequency histogram, 20 s duration.

B: left, during continuous spontaneous firing, three depolarizing current pulses were applied adding increased and sustained descharges.

The arrowhead indicates a subthreshold sequence of synaptic potentials, EPSP-IPSP-EPSP. On the right, a section of the left record (asterisk) with an expanded time scale is shown. The horizontal arrow shows the resting potential, -55 mV.

Injections of intracellular depolarizing current pulses in an otherwise spontaneously firing neuron (Fig. 2), provoked repetitive discharges and, at the cessation of the pulse, a long hyperpolarization of around 100 ms (a, arrowhead).

During the membrane hyperpolarization (injected current), subthreshold EPSPs showed enhanced amplitudes relative to the spontaneous ones (Fig. 2, arrowheads b and d) while other EPSPs reached the threshold, evoking spikes. After the cessation of the hyperpolarizing current pulses (-0.5 nA, -1 nA) and after a nearly total spike inhibition, rebounds exhibited increasing amplitude with increasing current pulse intensity (arrowheads c and e). The rebounds could have a duration of about 100 ms.

2. *Response to sound stimulation.* - The click evoked synaptic potentials duration (average, 55.3 ms ± 1.2, S.E) far outlasted the stimulus duration. The binaural simultaneous and contralateral stimulation evoked an EPSP-IPSP-EPSP sequence or an isolated EPSP; the ipsilateral stimulation may give rise to an initial IPSP or,

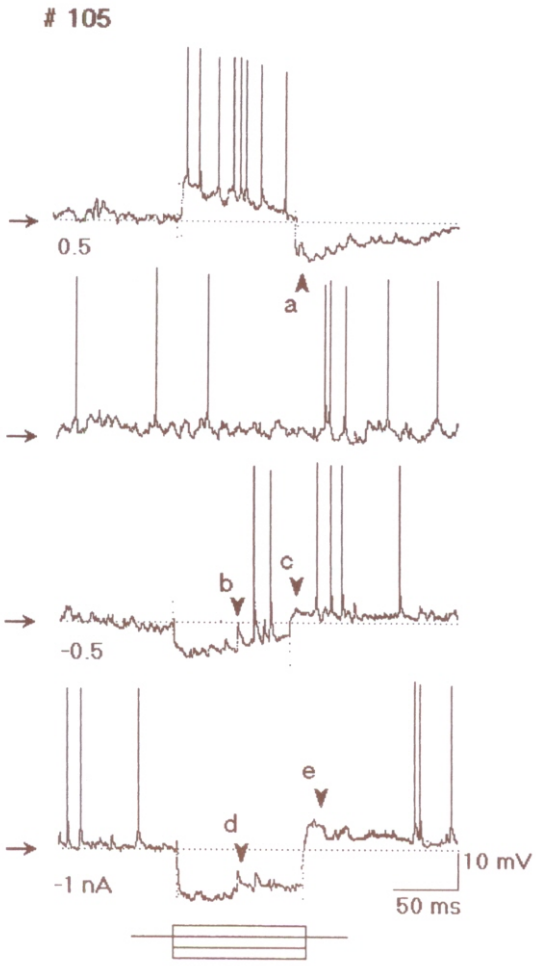

Fig 2. - *Intracellular current injections (# 105).*

The depolarizing current pulse (0.5 nA) evoked repetitive spikes, followed by a long-lasting hyperpolarization (*a*, arrowhead).

The hyperpolarizing current pulses (-0.5 nA, -1 nA) resulted in rebounds increasing in amplitude and duration with higher current intensity (*c*, *e*, arrowheads). When the membrane was hyperpolarized enhanced amplitude EPSPs appeared (*b*, *d*, arrowheads).

The horizontal arrows shows the resting potential, -50 mV.

with higher probability, the sequence previously shown. The latency to the first synaptic potential varied from 4.2 to 7.5 ms, average 6.3 ms ± 0.6, S.E.

In Fig. 3 the responses to click (0.1 ms) stimulation are shown. On the one hand, the binaural sound stimulation evoked a subthreshold EPSP followed by an IPSP and a suprathreshold EPSP producing a spike. The contralateral one evoked a suprathreshold EPSP with an action potential, followed by an IPSP.

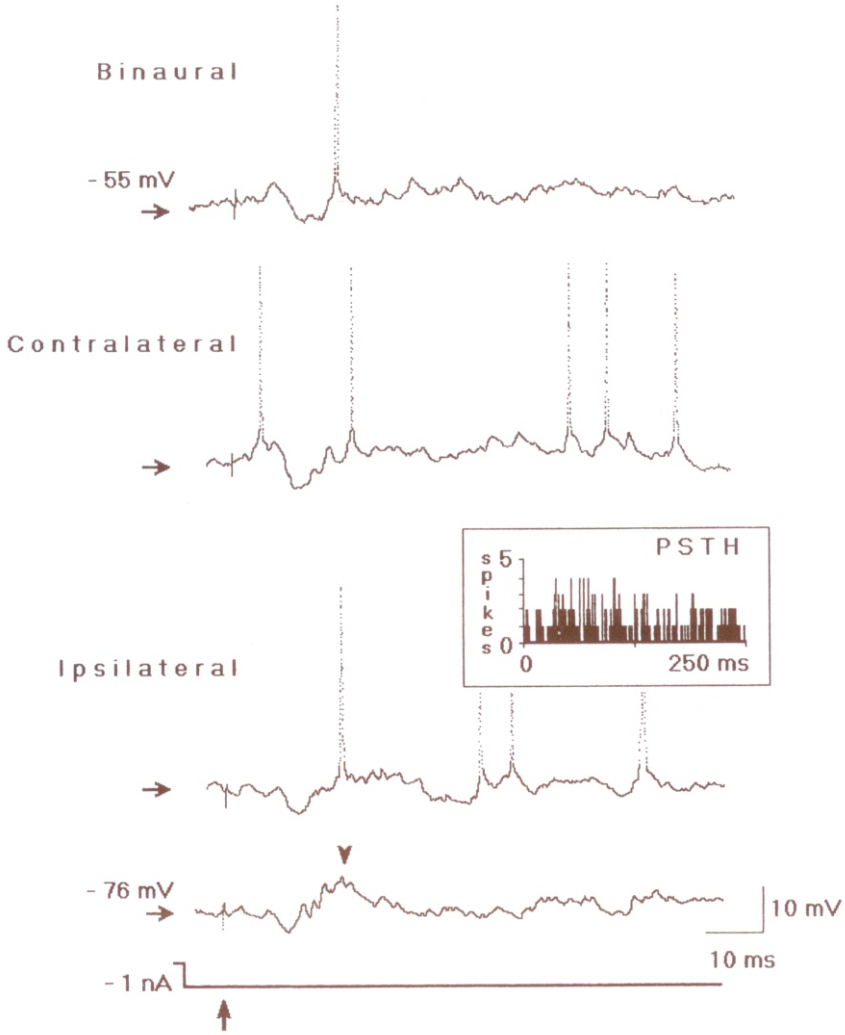

Fig. 3. - *Intracellular responses to click stimulation.*

The binaural stimulation first evoked a subthreshold EPSP followed by an IPSP and a spike discharge. Contralateral stimulation evoked a suprathreshold EPSP and the corresponding spike followed by an IPSP.

Ipsilateral stimulation evoked an IPSP followed by an EPSP and a spike. To demonstrate the existence of an EPSP, hyperpolarizing current was injected (-1 nA). The arrowhead shows a hidden EPSP. The post-stimulus time histogram (PSTH, inset) shows a low probability of discharge at the stimulus onset followed by a higher one, both in close temporal correlation to the synaptic potentials.

The horizontal arrows show the resting potential, -55 mV. Clicks, vertical arrows, 0.1 ms duration, 60 dB SPL.

On the other hand, an IPSP was the first synaptic potential evoked by the ipsilateral click stimulation. It was followed by an EPSP and a spike. The existence of this last EPSP was demonstrated with a -1 nA current injection (lower record-

ing); at a membrane voltage of -76 mV, the isolated EPSP was apparent (arrowhead). Fig. 3 inset shows the low probability of discharge at the stimulus onset (ipsilateral ear stimulation) while an increased firing probability was observed in temporal relation to the EPSP.

DISCUSSION

This report present original data of *in vivo* intracellular recordings of ICc auditory neurons during the waking state of guinea-pigs.

Auditory neurons (n=10) were successfully studied during physiological waking, showing for the first time single cell sensory processing in the ICc without interfering drugs. The technical approach used permitted glass micropipette placement and then the study of the physiological changes in auditory midbrain units during restrained waking behavior.

The membrane characteristics were similar to those recorded under barbiturate anesthesia (5, 12), although during waking the number of spontaneous synaptic potentials (EPSPs and IPSPs) was enhanced as well as the units' firing rate, showing little, if any, adaptation.

That the depolarization following hyperpolarization was an actual EPSP was demonstrated in some neurons by injecting hyperpolarizing current (Fig. 3); however, in its generation a role may be played by the intrinsic characteristics of the neurons' membrane, as observed in Fig. 2 and reported in anesthetized *in vivo* recordings (12).

Spontaneous spike activity was recorded in all the neurons studied. During wakefulness, the action potentials were of the short duration type while in anesthetized preparations a longer action potential duration group was also described (12). The reasons for this discrepancy may be multiple, e.g., the small number of recorded units in the waking state, the higher prabability of recording the largest cells and the acute anesthetized preparation *vs.* the awake, drug-free animal.

After current injection pulses, long duration membrane voltage shifts may occur (Fig. 2), demonstrating intrinsic neuron properties that may partially support the long response phenomena observed with short, 0.1 ms, sound stimulus.

The binaural as well as the contralateral ear stimulation, in all the recorded units, began with an EPSP in agreement with the ICc extracellular activity reported, in which the majority of the neurons responded in the same manner, increasing firing at the stimulus onset. That the ipsilateral sound stimulation first elicited an inhibitory synaptic potential, followed or not by excitatory activity, is also in agreement with extracellular recordings (11).

The waking post-synaptic potentials' duration, in all click stimulated cells studied, consistently outlasted the stimulus (0.1 ms) reaching up an average of 55.3 ms. This intracellular result is in close agreement with extracellular recordings in the ICc, in non-anesthetized guinea-pig neurons, in which the responses of many cells were shown to outlast the tonal stimuli during sleep-waking behavior (4).

As an important midbrain center for auditory processing the ICc showed long sequences of synaptic potentials. The arrival of information from bianural and monaural inputs from lower nuclei, commissural as well as descending paths from the medial geniculate and auditory cortex, must be implicated in the generation of the synaptic potentials, without disregarding the possible action of other neighbour loci as the central gray and the pontine and mesencephalic reticular formation (3, 6, 9). This complex processing may be the first step toward CNS sound in space integration, through a precise sound source location. Such an ICc integrative function of all possible inputs must occur before an output to upper levels is conveyed.

The findings in anesthetized preparations, although of enormous importance, must be confirmed, if possible, with behaving animals.

SUMMARY

Intracellular recordings of identified inferior colliculus (ICc) auditory neurons, were analyzed in *in vivo* awake, chronically implanted guinea-pigs. The passive membrane characteristics as well as the spontaneous and click evoked synaptic potentials and spike activity, were studied.

The injection of current pulses revealed little, if any, adaptation and membrane voltage shifts that outlasted the electrical stimuli.

The spontaneous action potentials, observed in all the units studied, were of the short-duration type. During wakefulness, spontaneous synaptic potentials of higher amplitude were observed in comparison to the anesthetized preparation as well as an enhanced firing rate.

The click evoked synaptic potentials far outlasted the sound (click, 0.1 ms) duration. The binaural, contralateral and ipsilateral sound stimulation evoked different sequences of synaptic potentials and firing. This was mostly in agreement with studies of extracellular recordings from the ICc, in anesthetized and behaving animals.

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