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Unit activity to click CS changes in dorsal cochlear nucleus after conditioning

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RECORDINGS were made of single unit activity (n = 360 units) from the dorsal cochlear nucleus of cats. Different patterns of activity were elicited by acoustic stimuli before and after Pavlovian conditioning. The peak response to a forward paired click conditioned stimulus (CS) increased whereas that to a backward paired hiss discriminative stimulus (DS) did not. The percentage of units responding to the CS increased from 34% to 46% after conditioning. The findings do not support the widely accepted hypothesis that learning has no effect on transmission through the first brain stem relay of the auditory system and indicate, instead, that the cochlear nucleus can participate in complex adaptive acoustic signal processing.

Key words: Audition; Discriminative conditioning; Learning; Auditory system; Hearing; Cognition

Introduction

Following comprehensive studies in which no changes in background or acoustic (CS) evoked activity of neurons of the cochlear nucleus were found after conditioning in rabbits,[1,2] it was widely accepted that learned adaptations did not occur at this initial level of the brain stem auditory system and that transforms of acoustic signals passing directly through the cochlear nucleus were invariant with respect to learning. This has had far-reaching implications for clinical assessment of hearing disorders, for determining regions of the brain and brain stem involved in processing complex acoustic signals, and for determining loci of the central nervous system essential for learning.[3,4]

Recent findings of short-latency activation of neurons of the rostral thalamus and subcerebellar dentate nucleus by acoustic signals and the sensitivity of that activation to conditioning[5,6] led us to re-examine the possibility that unit activity at the level of the cochlear nucleus might change after conditioning. We measured patterns of activity before and after conditioning an eye blink response to a click stimulus in conscious cats. We focussed on the dorsal portion of the cochlear nucleus because earlier studies[7] suggested that this region might preferentially serve complex acoustic signal processing.

Materials and Methods

Studies were performed in eight adult cats weighing between 2.5 and 3.0 kg. Extracellular and intracellular recordings of unit activity (see reference 8 for criteria) were measured with a Dagan 8100-1, high impedance amplifier and stored on a Vetter FM tape recorder (Model D) at DC to 5000 Hz (< 1% falloff). Electrodes were pulled from 1.5–2.0 mm (o.d.) theta tubing. When filled with 2% biocytin (Sigma) in 2.5 M KCl and connected on both sides with Ag/AgCl wire, the resistances of the electrodes ranged from 40 to 10 MΩ. The animals were surgically prepared under Na pentobarbital anesthesia (35 mg kg^{-1}, intraperitoneally), as described in detail previously,[9,10] to allow later, conscious recording/training sessions using a stereotaxic guide tube[11] and fixation of the head to a stabilizing frame. Penicillin G (150,000 units, i.m.) and benzathine penicillin G (150,000 units, i.m.) were given on the day of surgery, three days later during the recovery period, and at one week intervals thereafter, as needed. During recording/training sessions the bodies of the animals were placed in loose cloth sleeves. The behavior of the animals was continuously observed to evaluate their comfort, and the studies were discontinued if the animals gave any signs of discomfort such as vocalization and hyperactivity. The procedures met APS, USPHS, Society of Neuroscience and University of California guidelines.

Blink conditioning was produced by forward pairing of click as CS with glabella tap and hypothalamic electrical stimulation (interstimulus intervals for tap and onset of four pulse (0.1 ms, 1–5 mA) train of hypothalamic stimulation, 570 and 10 ms respectively; 10 s intertrial interval) followed 4.4 s by hiss as DS. Rationale for stimulation of lateral hypothalamus and parameters of stimulation as well as complete details of locus stimulated and training-testing protocols have been given earlier.[12,13] CS = conditioned stimulus, the click; DS = discriminative stimulus, the hiss. All unit testing was done with CS and DS alone. Training was given after each unit recording to maintain the behavioral state. The conditioned blink response was measured electromyographically from the orbicularis oculi muscles and resembled that found previously.[6]

Physical parameters of the click and hiss have been described earlier[12] from amplified earphone recordings of the stimuli. The clicks were of 70 dB intensity (measured at the ears of the animals with General Radio Company dB meter type 1565-A at standard SPL level of 20 $\mu N\ m^{-2}$), and were generated by a rectangular pulse of 1 ms duration, delivered to a loudspeaker placed 1–2 feet in front of the animals. The hiss was of comparable intensity and longer (100–200 ms duration. During testing, clicks and hisses were presented 4.4 s apart (click preceding hiss) every 10 s. Single unit activity was recorded during presentations of these stimuli. The depth of each unit studied was noted. Spike occurrences were detected with a threshold discriminator (Frederick Haer and Co.[14]). Data were collected at 1 ms intervals and analyzed (PDP 11–44) in histograms of 2 ms bin width for each cell. No differences were found between data obtained from intracellular and extracellular recordings. Thus data from all units were combined when making averages of patterns of activity before and after conditioning.

After making intracellular recordings of spike activity, hyperpolarizing currents (3 nA, 3 min) were passed to introduce biocytin for cell identification. At the conclusion of the studies the animals received lethal doses of Na pentobarbital, and serial sections of perfused brain tissue were examined for electrode tracks and biocytin marked cells.[15]

Results

Peristimulus time histograms of spike occurrences were made with reference to times of click and hiss onset (uncorrected for 1 ms air conduction delay between sound source and ears of animal) for each cell that was studied. Grand averages of the mean post-CS activity were then compiled from all units recorded before (n = 181 units, 8 cats) and after (n = 179 units, 5 cats) conditioning. The results (Fig. 1) showed an increased activity with onset 4–8 ms following delivery of the click-CS after conditioning. The increase was more than 3 standard deviations (s.d.) above the mean of the background activity (measured in the 400 ms preceding click delivery) and 3 s.d. above the peak of the response 4–8 ms after click delivery prior to conditioning (Fig. 1). Increases in activity at this same time period were found in each of the five animals that underwent conditioning. Activity elicited by the forward paired click CS was disproportionately increased after conditioning versus that elicited by the backward paired hiss DS (arrows, Fig. 1). As in previous studies,[6,16] the conditioned blink response was elicited discriminatively by the CS. (The hiss has been shown to be an effective CS for producing blink conditioning when forward paired in other experiments.[13,16]) Smaller increases in late activity evoked 40–160 ms after the click CS were also observed after conditioning (Fig. 1). The results indicate that conditioning affects transmission of acoustic signals used as CSs at the primary level of the brain stem auditory system-i.e., the cochlear nucleus. (These studies did not attempt to investigate the associative sensitivity of the effects of the conditioning procedure, but preliminary results of other investigations [n = 38 units, 3 cats] found no increase in CS-evoked activity in the dorsal cochlear nucleus after backward conditioning with hypothalamic stimulation preceding [by 2.5 s] instead of following the CS. See reference 13 for details of that paradigm.) That conditioning rather than sensitization or other non-specific contextual effects produced the increased activity in the present report is also suggested by the relatively selective increase of response to CS versus DS (Fig. 1).

The percentage of units responding to the CS was also found to be increased after conditioning. Prior to conditioning, 34% of the 181 units tested with click responded with increased discharge. (Responsive units were defined, as in earlier studies,[6] on the basis of increased activity in any 4 ms period during the 160 ms following click presentation ($\geq$ 2 discharges above the peak of twenty 4 ms periods of baseline activity before click delivery).) After Pavlovian conditioning using the same click as a CS, 46% of 179 units tested responded. (Chi sq of difference in numbers of cells = 5.5, $p < 0.03$.)

Marking of cells (Fig. 2) and analysis of electrode tracts indicated that the recordings were obtained from the dorsal cochlear nucleus. Since not every cell was marked, we cannot exclude the possibility that the data included a small number of cells from the ventral cochlear nucleus, but the lower 2 mm of recordings (corresponding to cells recorded primarily from the ventral cochlear nucleus) were not included in the present analyses, and showed different patterns of change after conditioning.

Discussion

The results provide new evidence that changes in spike responses to acoustic signals can occur in neurons of the cochlear nucleus after conditioning. The findings contradict the widely held view that adaptive processing of acoustic signals does not occur at this level of the auditory system. That view was based on studies that did not find changes in cochlear nucleus activity after conditioning in rabbits.[1,2] Also, changes in short latency, acoustically evoked activity were not found after conditioning in units of the inferior colliculus of rabbits[1,2] or rats.[17] Since the inferior colliculus was thought to be an obligatory relay nucleus for all fibers of the primary auditory system ascending from the brain stem, it was concluded that the principal relay nuclei of the auditory system did not undergo the neuronal plasticity that codes learning.[2] Changes in ventral cochlear nucleus discharge found after conditioning in one early study in cats[18] were discounted because of

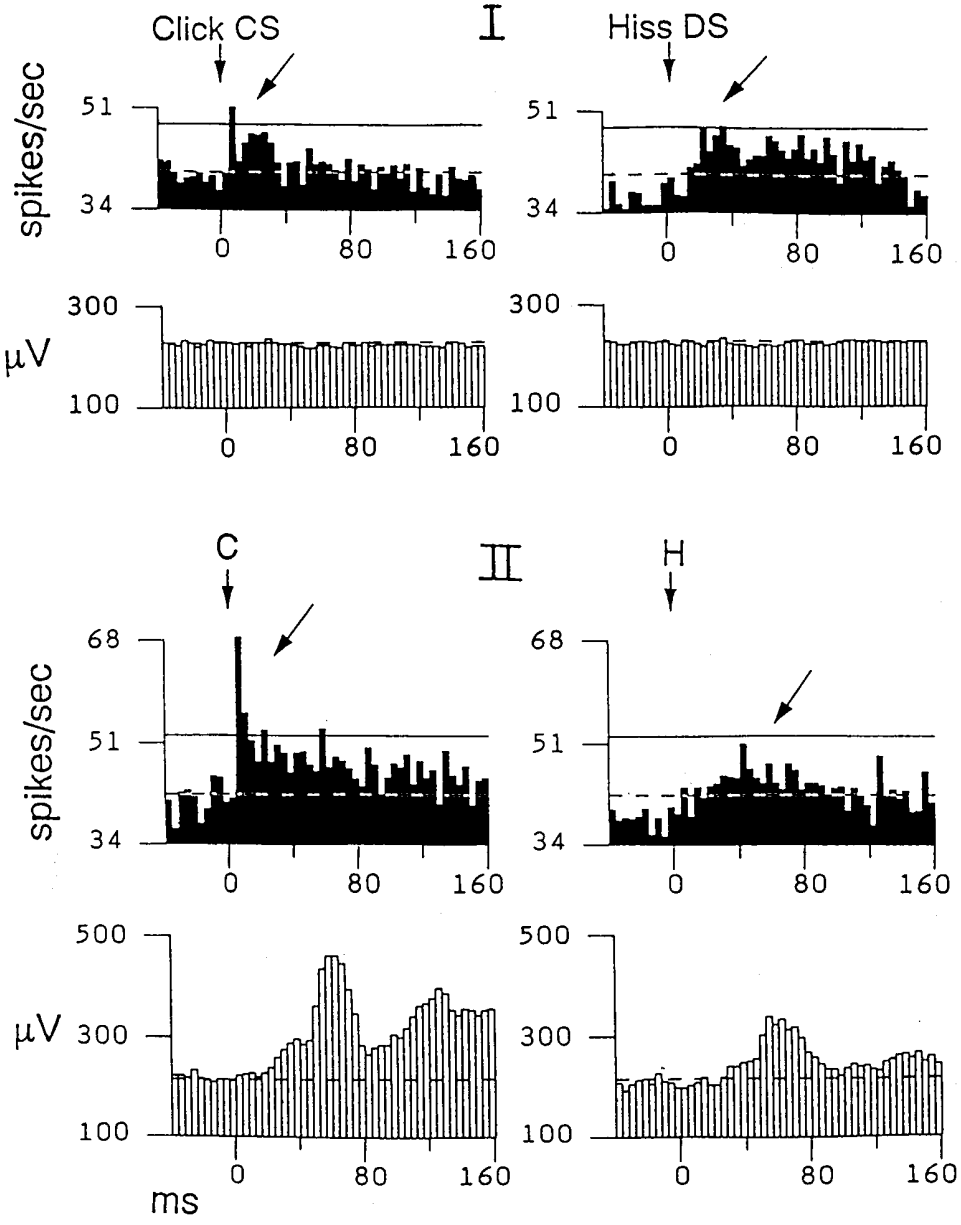

FIG. 1. Histogram averages of spike activity (solid bars) from dorsal cochlear nucleus before (I: average from 181 single unit recordings) and after (II: 179 (different) units) conditioning a blink CR to the click CS. Below (hollow bars) is shown the average of the simultaneously recorded electromyographic activity of left, orbicularis oculis (eyeblink) muscles. The 4–8 ms onset of the unit response to the click CS precedes the onset of muscle activity. The click stimulus was delivered at time 0. Dashed line shows mean baseline level of spike discharge calculated from the 400 ms preceding click delivery. Solid horizontal line is 3 standard deviations of the mean of the baseline spike activity.

uncertainties arising from the multiple unit approach used to measure spike activity. (The studies in rabbits[1,2] also mainly investigated the ventral portions of the cochlear nucleus.)

Modifications of neuronal conductances essential for conditioning have been found in early portions of the sensory pathways of invertebrates.[19] Neuronal adaptations supporting changes in activity at the level of the cochlear nucleus of mammals could influence operations supporting learning at the levels of cerebellum, subcerebellar nuclei, or cerebral cortex.[4,8] Accordingly, some complex forms of hearing disorders such as cortical deafness that have been previously attributed solely to disruption of 'higher' auditory regions could conceivably arise from or be worsened by impairment at the level of the cochlear nucleus. (We specifically have in mind auditory processing disorders analogous to those somatosensory disorders that resemble disruptions found after cortical parietal lobe lesions but occur after dorsal column lesions.[20])

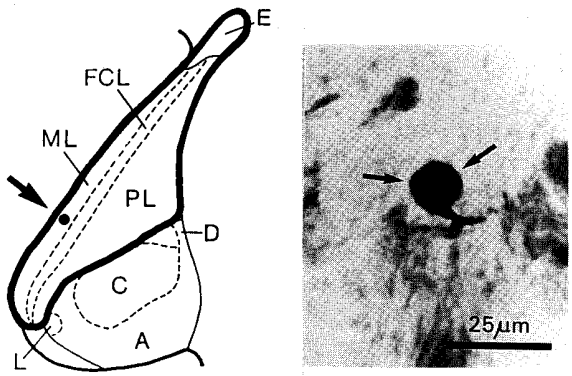

FIG. 2. An intracellularly recorded neuron that was marked with biocytin (right). The location of the neuron in the granular, posteriolateral portion of the molecular layer of the dorsal cochlear nucleus[21] is shown to the left (arrow/dot). Only the single, bifurcating process seen in the photomicrograph was found to emerge from the cell body. Size calibration as shown. (Another, marked, intracellularly recorded neuron was located nearby with morphology resembling that of a fusiform cell.[22]) Abbreviations: medial dorsal granular region of dorsal cochlear nucleus, E; fusiform cell layer, FCL; molecular layer, ML; polymorphic layer, PL; and dorsal (D), central (C), lateral (L) and anterior (A) portions of the ventral cochlear nucleus. Number is posterior (P) stereotaxic coordinate in mm.[11]

Conclusions

We conclude that the activity of neurons at the primary relay of the brain stem auditory pathway, the cochlear nucleus, can be modified by conditioning. Because of this the transforms that process acoustic signals at this level of the nervous system should be thought of as plastic rather than invariant. Adaptations occurring at the cochlear nucleus will transform raw signals before they reach higher processing levels, and may thus transform our perception of the peripheral, physicalistic, auditory universe from a raw, invariant view into an altered and potentially distorted one.

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