Effects of Acetylcholine on Spontaneous and Tone Evoked Neuronal Discharges in Auditory Cortex

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Abstract

Anticholinesterases disrupt normal brain function with resultant impairment of psychological and behavioral functions but cholinergic involvement in information processing in cerebral cortex is not understood. This investigation provides an initial characterization of the effects of cholinergic agents on spontaneous and tone evoked discharges of single neurons in the auditory cortex of the unanesthetized cat. Calibrated tone pulses were presented before, during, and after micropressure application of one or more agents. ACh or MCh altered spontaneous or evoked discharges or both in 80% of 140 neurons tested. ACh was more likely than MCh to produce effects, suggesting that ACh may interact with nicotinic as well as muscarinic receptors. Spontaneous discharges were usually depressed by ACh but increased by MCh. Agonists modified evoked discharges in 80-90% of neurons, facilitating responses to tone onset and offset but having different effects on discharges during tone presentation. Agonists often had different effects on spontaneous vs. evoked discharges within the same neuron, and their effects were dose-dependent. Atropine and scopolamine applied alone had effects opposite to MCh and could also block the effects of either ACh or MCh. These results suggest that ACh, acting via muscarinic receptors, has a structured modulation of information processing in auditory cortex.

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Acetylcholine (ACh) is released in the cerebral cortex under physiological conditions but its functional role is unknown. Several findings support the view that ACh, to some extent, modulates information processing within cerebral cortex: (a) cortical release of ACh depends on the state of arousal, a major variable in information processing performance; (b) cholinergic projections are diffuse and widespread, suggestive of a global regulatory function; (c) ACh in the brain promotes long lasting changes in neuronal excitability, a characteristic well-suited for the modulation of information processing; (d) cholinergic agents modify learning and memory in a variety of tasks and species.

The role of ACh may be advantageously studied in sensory neocortex because (a) the general function and structure of sensory cortex is known, (b) foundational data from sensory physiology are at hand and (c) sensory stimuli processed under normal physiological conditions can be presented under controlled conditions. The present experiment was undertaken to provide an initial survey of the effects of cholinergic agents upon spontaneous and evoked activity to controlled pure tone stimulation in the primary auditory cortex of the unanesthetized cat. Because previous reports of physiological plasticity in auditory cortex have noted that learning may differentially affect background vs. evoked discharges, and various components of evoked discharges, special attention was devoted to these parameters of cellular activity.

Data were obtained from healthy adult male cats, prepared for later recording under general anesthesia (sodium pentobarbital, 45 mg/kg, ip). Burr holes were placed in the calvarium overlying primary auditory cortex and a threaded cylinder, for a traumatic attachment to a head holder, was affixed to a pedestal of dental acrylic. Recordings and application of cholinergic agents were via multibarrel micropipettes (5-10 megohms) in the waking animal under muscle blockade, with maintenance of respiration, fluid balance, temperature and with continual monitoring of EEG, expired CO^2 and pupillary diameter and reactivity.

The following agents were applied by micropressure: ACh (2M in distilled water), beta methacholine (MCh, 10-20 mM) atropine (.2-.4 M), and scopolamine (15 mM). The acoustic delivery system was calibrated for each recording session. Tones (0.1-30kHz, 20-80db, 300 ms, 5 ms rise/fall) were delivered to the ear contralateral to the recording site. The responses of single neurons to sequences of 25 tones (isofrequency, isointensity, approx. 1-1.5 min. each sequence) were obtained before, during and after application of drugs. Drugs were always applied 10-15 sec. preceding the beginning of a tonal sequence. Neuronal discharges were processed by a computer which recorded the occurrence of spikes and stimuli. Spontaneous discharges were obtained for periods of 500 ms preceding each acoustic stimulus. Evoked discharges were analyzed independent of spontaneous discharges by subtracting spontaneous activity from discharges elicited by acoustic stimuli during presentation of tone.

The actions of cholinergic agents were tested on 148 neurons, the large majority of which (n=122, 82%) responded to tones. Neurons that were affected by acoustic stimulation responded in a manner characteristic of auditory cortex. In most cases (87%) this consisted of a response to tone onset, either alone ("on only") or followed by discharges during

("through" responses) or after the stimulus ("off" responses) or both. We refer to the latter pattern as "on complex."

Cholinergic agonists altered spontaneous or evoked discharges, or both, in the majority of the 140 neurons tested (n=112, 80%). ACh was more likely to be effective than was MCh (88% vs. 72%). Modification of both spontaneous and evoked discharges usually required several seconds to become evident and the effects often outlasted application of agonists for 0.5-5 minutes. Spontaneous discharge rate was altered in the majority of neurons (n=98, 70%); MCh was more likely to increase spontaneous activity (59%) than was ACh (38%).

Acoustically evoked activity was modified in the vast majority of the 115 neurons tested: ACh, 92%: MCh, 82%. The most prominent effect was modification of the "on" discharges: ACh (65%), MCh (62%).

Many neurons (n=65) had "on complex" responses to tones, the evoked discharges of which were altered by agonists in almost all cases (59/65, 91%). ACh (34/36, 94%) and MCh (25/29, 86%) were equally effective [X^2=.5,p>.4]. Both agonists could change the three components: "on," "through" and "off." However, their effects were not the same.

As pointed out above, ACh and MCh generally facilitated "on" responses; this was the case regardless of whether the "on" response occurred in isolation ("on only") or was followed by other responses ("on complex"). For the latter type, the agonists also facilitated "off" discharges evoked at the end of tone stimulation. "Through" responses were facilitated by MCh but depressed by ACh (X^2=14.07, p<.007). Since spontaneous activity also was generally facilitated by MCh and depressed by ACh, their effects on "through" responses were similar to their effects on spontaneous activity.

In the majority of cases, agonists had <u>different</u> effects on various response components within complex responses (21/39, 54%). This was due to the fact that "through" responses were affected differently than were "on" and "off" responses; these responses to acoustic change generally were affected the same way. Therefore, ACh can have a very high degree of specificity upon complex evoked discharges.

The effects of agonists on spontaneous and evoked activity were often different. A within-cell analysis revealed that this was the case except for spontaneous and "through" responses, which were the same more often than expected by chance (binomial test: ACh, p<.05; MCh, p<.01). The increase or decrease of spontaneous and/or evoked activity in the presence of either cholinergic agonist were dose-dependent.

In summary, the effects of agonists tended to be the same for spontaneous and "through" responses on the one hand, and for "on" and "off" responses on the other hand. This relationship was found both for across-neuron and within-neuron partitioning of the data. MCh facilitates spontaneous and evoked discharges while ACh facilitates "on" and "off" responses and depresses spontaneous and "through" discharges.

The muscarinic antagonist atropine could block the effects of ACh and MCh on both spontaneous and evoked activity, regardless of whether the changes produced by ACh or MCh were increases or decreases in spontaneous or evoked discharges. Blocking by atropine was found both for cases in which atropine itself had no discernable effects and in which its isolated effects were opposite to that of the agonist.

In addition to antagonism, atropine applied in the absence of an agonist could alter spontaneous (57% of 72 neurons) and evoked (71% of 66 neurons) activity. As with the application of agonists, atropine could increase or decrease spontaneous or evoked activity. Scopolamine produced similar effects for the small number of neurons studied (n=9).

Of particular importance, the effects of atropine upon spontaneous and evoked discharges were opposite to the effects of MCh, but did not have this relation to the effects of ACh. Thus, MCh increased spontaneous (59%) and evoked (63%) activity, while atropine depressed spontaneous (80%) and evoked (60%) activity.

These results provide strong evidence that ACh functions in a neuromodulatory manner in auditory cortex. ACh and MCh produce complex, dose-dependent modification of spontaneous and evoked discharges. Atropine and scopolamine are effective blockers of the actions of either ACh or MCh regardless of the nature of their effect, i.e. increase or decrease in discharge rate. The fact that muscarinic antagonists were more effective against MCh than ACh suggests that ACh may engage both nicotinic and muscarinic type receptors in auditory cortex. The ability of MCh to modify neuronal discharge, as well as antagonism of MCh and ACh effects by atropine or scopolamine suggests that cholinergic agonists interact with a muscarinic type receptor in auditory cortex. Furthermore, atropine itself was capable of either increasing or decreasing spontaneous or evoked discharge rate, suggesting a tonic release of ACh, and action at muscarinic receptors, in auditory cortex of the unanesthetized animal.

The effects of cholinergic agonists and antagonists reported here are probably the consequence of applied cholinergic agents in contrast to any direct effect of pressure ejection because: (a) only neurons with stable waveform were included; (b) there was no relationship between agent-produced acceleration or deceleration of discharge rate and pressure; (c) agonists and antagonists could produce opposite effects on the same neuron when ejected by similar pressures;(d) agonist effects were often blocked by antagonists, and the blocking action of antagonists was observed regardless of the direction of agonist-induced change; (e) both effects entailed an increase in total pressure required for drug ejection, however discharges were suppressed in one instance and accelerated in the other; (f) cholinergic agents could have a selective and exclusive influence on either spontaneous or evoked activity recorded from the same neuron.

The complexity associated with cortical processing of acoustic information as well as the complexity of ACh in auditory cortex, is illustrated by the observations that neither ACh nor MCh produce uniform effects on discharge rate, but rather (a) caused either increases or decreases, (b) independent effects on spontaneous and evoked activity and (c) selective effects on particular components of complex evoked discharges. Although the detailed function of ACh in auditory cortex remains to be determined, the fact that cholinergic agonists act in concert on responses to changes in the acoustic environment (i.e., "on" and "off" responses) on the one hand, and affect spontaneous and "through" responses the same way, on the other hand, indicates a structured neuromodulatory role.

Since previous studies indicate that ACh can produce atropine-sensitive increases and decreases in membrane potential of central neurons, the heterogeneity of muscarinic effects in auditory cortex might be related to such differential actions. This heterogeneity of muscarinic response could be the result of a complex distribution of muscarinic receptors on pyramidal and intrinsic neurons of auditory cortex. An additional, but not necessarily exclusive, factor may be the heterogeneity of the muscarinic receptor. Synaptic or agonist-induced depolarizations may be mediated by the M_1 subtype of muscarinic receptor whereas, muscarinic synaptic or agonist-induced hyperpolarization has been suggested to be mediated via M_2-type receptors. Thus, muscarinic action in cortex may not be mediated by a single muscarinic binding site and consequent intracellular mechanism. More detailed pharmacological analyses are an important next step.

The pattern of response to acoustic stimuli, involving periods of brief or sustained increase in discharge rate, as well as periods in which discharge rate is suppressed, likely reflects complex anatomic and physiologic local circuitry at the cortical level. Current evidence suggests that medial geniculate afferents do not release ACh at their terminals in auditory cortex. Thus it is unlikely that ACh functions as a direct mediator of acoustically evoked discharge in auditory cortex. More likely, ACh may function with the local anatomy and physiology to contribute to cortical phenomena such as receptive field specificity, the encoding of stimulus temporal features, and learning-induced physiological plasticity. The finding that cholinergic agonists do not have uniform effects on acoustic responses, but rather could differentially effect the various features of the discharge, indicates that ACh may be involved in subtle modification of cortical sensory processing. The current evidence suggests that ACh may function, either directly or indirectly, to modulate the influence of thalamic afferents on neurons of auditory cortex. At the present time the presynaptic or postsynaptic (or both) site of ACh action can not be determined. The influence of ACh may be via selective action on presynaptic terminals of thalamic, association, or local circuit neurons as well as directly on neocortical pyramidal neurons.