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Research Paper

# Cholinergic responses of acoustically-characterized cochlear nucleus neurons: An *in vivo* iontophoretic study in Guinea pig

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ABSTRACT

The responses of guinea pig cochlear nucleus neurons to *in vivo* iontophoretic application of various neurotransmitter agonists were recorded with extracellular multi-barrelled electrodes. Where possible, neurons were physiologically identified using strict criteria. Emphasis was placed on the action of cholinergic agonists in relation to the possible action of olivocochlear collateral innervation. Excitatory responses (increase in action potential firing) to glutamate were confirmed in a number of neuronal response types. Application of acetylcholine (ACh) or the broad spectrum cholinergic agonist carbachol produced reliable excitatory responses in about 47% of neurons (n = 29 out of 61 neurons). The remaining neurons were unresponsive to cholinergic agonists and no inhibitory responses were observed. Cholinergic responses were more common in dorsal cochlear nucleus (DCN) (73% of 30 neurons tested) than in ventral cochlear nucleus (VCN) (23% of 31 neurons). Of the total neuron sample in which cholinergic responses were investigated, 41 neurons were able to be categorized according to established acoustic response features. Excitatory responses to cholinergic agonists were seen in "Pauser-buildup" (Pb) and "Transient chopper" (Ct) response types. Primary-like neurons (PL and Pn) as well as "Onset chopper" (Oc) neurons (n = 6) were unresponsive to either ACh or carbachol. Oc neurons also did not show any effect on their acoustic responses. Robust cholinergic responses were also seen in several VCN and DCN neurons that were either unresponsive to sound, or had acoustic response properties that did not fit standard classification. The results suggest a relatively more robust cholinergic innervation of DCN compared to VCN. The excitatory cholinergic responses of some Ct neurons and the lack of effect on Oc neurons are consistent with previous results in mouse brain slice studies, but are in conflict with reports of medial olivocochlear collateral excitatory responses in onset-type neurons *in vivo*. The results also indicate that a number of neurons of unknown identity may also receive cholinergic input.



## 1. Introduction

The mammalian cochlear nuclear complex receives descending cholinergic innervation from several sources. Superior olivary complex inputs comprise the collateral projection of medial olivocochlear (MOC) efferent neurons (Benson and Brown, 1990; Brown and Benson, 1992; Brown et al., 1988, 1991), and a separate direct projection from the ventral nuclei of the trapezoid body (Sherriff and Henderson, 1994). Tegmental areas also are known to supply cholinergic input to both the dorsal (DCN) and ventral cochlear nucleus (VCN) (Mellott et al., 2011). The importance of these descending systems in the modulation of ascending auditory information is still a matter of conjecture and the specific neuronal targets that receive cholinergic input within the diverse populations of cell types in the cochlear nuclear complex, is not firmly established. Anatomical studies show that MOC collaterals make excitatory synaptic contacts with dendrites of multipolar neurons in VCN (Benson and Brown, 1990; Benson et al., 1996). Two major populations of multipolar cells in VCN are the D-stellate, thought to correspond to Onset Chopper (Oc) neurons and the T-stellate, thought to correspond to the transient chopper (Ct) and sustained chopper (Cs) neurons (Oertel and Fujino, 2001; Oertel et al., 1990; Smith and Rhode, 1989; Smith et al., 2005). *In vivo* electrophysiological studies using both intra and extracellular recordings, indicate that onset type and Oc neurons are excited by activation of

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**Abbreviations**

| | |
|---|---|
| ACh | acetylcholine |
| CAP | compound action potential |
| CF | characteristic frequency |
| CN | cochlear nucleus |
| Cs | sustained chopper |
| Ct | transient chopper |
| DCN | dorsal cochlear nucleus |
| MOC | medial olivocochlear |
| Oc | onset chopper |
| Pb | pauser-buildup |
| PL | primary-like |
| Pn | primary-like with notch |
| PSTH | peristimulus time histogram |
| VCN | ventral cochlear nucleus |

olivocochlear collaterals (Mulders et al., 2002, 2003, 2007, 2009).

However, in mouse brain slices, excitatory responses to application of cholinergic agonists have only been reported in T-stellate cells and bushy cells (Fujino and Oertel, 2001; Oertel and Fujino, 2001). The latter authors specifically report a lack of cholinergic responses in D-stellate neurons, which evidence suggests correspond to Oc neurons (Oertel et al., 1990; Smith and Rhode, 1989). The present study attempts to resolve some of these conflicts by combining *in vivo* recording of cholinergic responses of cochlear nucleus (CN) neurons with detailed classification of their acoustic response types.

## 2. Methods

### 2.1. Animals

Eighteen pigmented guinea pigs were used in this study. All anaesthetic and surgical methods conformed to the Code of Practice of the National Health and Medical Research Council of Australia and were approved by the Animal Ethics Committee of the University of Western Australia. All procedures, anaesthetic and surgical methods have been reported in detail previously (Vogler et al., 2011, 2014). Briefly, animals received a subcutaneous injection of 0.1 ml atropine followed by an intraperitoneal injection of pentobarbitone sodium, 30 mg/kg and a 0.15 ml intramuscular injection of Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone). Anaesthesia was maintained with full Hypnorm doses every hour and half doses of Nembutal every 2 h. A tracheostomy was performed and animals were artificially ventilated using a mixture of 95% $O_2$ and 5% $CO_2$ (carbogen). Animals were mounted in hollow ear bars, and acoustic stimuli were delivered to the left ear using a closed calibrated sound system (½" condenser microphone Bruel and Kjaer, type 4134 driven in reverse). Body temperature was maintained between 37.5 and 38 °C by a thermostatically-controlled heating pad. After exposure of the left cochlea a silver wire electrode was placed on the round window and a compound action potential (CAP) audiogram was routinely determined for each animal for the frequency range 4–24 kHz in order to establish normal hearing (Johnstone et al., 1979). Pure tone stimuli and the broad band noise search stimulus were synthesized by a computer equipped with DIGI 96 soundcard connected to an analog/digital interface (ADI-9 DS, RME Intelligent Audio Solution). This interface was also used to collect single neuron spikes for response classification.

### 2.2. Recording and iontophoresis

The left CN was exposed by a posterior craniotomy and aspiration of part of the cerebellum overlying the DCN. A recording and iontophoresis microelectrode assembly (Carbostar-4, Kation Scientific) was introduced into the CN at an angle of approximately 45° from the vertical plane. 4% Agar in 0.9% saline was used to fill the craniotomy and reduce respiratory pulsations of the brainstem. The electrode was advanced using a stepping motor microdrive and broad band noise was used as an acoustic search stimulus. For all neurons the depth from the DCN surface was recorded and the characteristic frequency (CF) was determined. Where single-to-noise ratio and stability of recording permitted, the neuron response type was classified as follows. Spontaneous firing rate was measured using a 10 s sample. Peri-stimulus time histograms (PSTHs) were then acquired using up to 250 repetitions (4/s) of a 50 ms CF tone 20 dB above threshold. For onset chopper neurons (Oc) neurons, a comparison of the response to a CF tone and broad band noise was also made. Response type classification was based on previously published criteria (Winter and Palmer, 1995; Young et al., 1988). Primary-like (PL), transient (Ct) and sustained (Cs) chopper neurons were distinguished from each other using regularity analysis of the discharge during the tone burst as described previously (Winter and Palmer, 1995; Young et al., 1988). Neurons were classified as Oc if they showed no spontaneous firing rate, two clear onset peaks with only weak sustained firing to tones 20–40 dB above CF threshold, and a strong sustained firing in response to noise stimuli that was more robust than the response to CF tones of matched intensity. Onset "L" (OL) neurons were distinguished according to previously described criteria (Winter and Palmer, 1995) by their absence of spontaneous firing, broad tuning, single narrow onset peak and a ratio of peak-to-sustained firing in their PSTH of greater than 10. Fig. 1A–E shows examples of typical PSTHs for 4 of the classical response categories.

A number of neurons could not be classified with certainty because of a less than optimal signal-to-noise ratio, especially at the onset of CF tones, where multiunit activity could contaminate the recording and prevent accurate triggering. These neurons were dubbed "unclassified". In addition to unclassified neurons, there were also neurons that did not fit the standard classification criteria, despite an adequate signal-to-noise ratio in the recording. An example of these "unidentified" neurons is shown in Fig. 1F. This neuron, recorded in DCN, showed a rather sluggish onset response, followed by a marked inhibition below the pre-stimulus spontaneous firing rate during the remainder of the tone burst and no evidence of a buildup in firing above spontaneous firing rate in contrast to typical pauser buildup (Pb) neurons (Fig. 1C). Other neurons in the "unidentified" category, were unresponsive to acoustic stimulation and were only detected by a non-zero spontaneous firing rate.

The central glass insulated carbon fiber of the electrode assembly was used for recording and three separate iontophoresis barrels were used connected to a microiontophoresis pump (Union-200, Kation Scientific). One barrel contained the drug of interest, a second barrel contained 200 mM NaCl for delivering control injections and the third barrel containing 200 mM NaCl was used as the current return path. In a small number of early experiments, glutamate and glycine, known excitatory and inhibitory transmitters in the CN, were used to test the overall feasibility of the iontophoretic methods. In the case of glutamate and glycine, the drug barrel was connected to the negative current output of the current source during injections of varying amplitudes of current. In the case of ACh and carbachol applications, the drug barrel was positive. Injection current amplitudes between 10 and 60 nA were tested, depending on the occurrence of artifacts (see section on