A neurometric analysis of spike pattern codes for natural and manipulated vocalization stimuli in primary auditory cortex


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We have demonstrated that the temporal discharge patterns of neurons in ferret primary auditory cortex (A1) transmit significant amounts of information about stimulus identity when tested with natural and time-reversed marmoset 'twitter' vocalisations (Schnupp et al. 2006). We have compared the discrimination performance afforded by these spike pattern codes (a 'neurometric' function) to behavioural performance (a psychometric function). Psychophysical data were collected for a two-alternative forced-choice oddity task in which 6 human observers had to distinguish natural twitter recordings from those with local time reversals of 10, 20, 40 or 80 ms width ('flipped twitters'). Listeners' discrimination performance was near perfect when the reversed time windows were 80 ms wide, but declined dramatically for time windows of 20 ms or less. We also recorded responses of 142 A1 neurons to these stimuli in 3 adult ferrets. Anaesthesia was induced by 2 ml/kg intramuscular injection of alphaxalone/alphadolone acetate, and was maintained with intravenous infusions of medetomidine/ketamine at a typical rate of 0.022 and 5.0 mg/kg/h, respectively (as described in Garcia-Lazaro et al. 2006). We used methods derived from signal detection theory (Green & Swets, 1974) to determine if the temporal discharge patterns of A1 responses could discriminate between the natural and flipped twitters. While no individual unit's neurometric matched the psychometric performance curve perfectly (Fig. 1), the neurometric of enveloped or pooled responses of the population of units in our sample closely resembled the psychometric curve (Fig. 2). Therefore, the statistical properties of temporal discharge patterns of distributed populations of A1 neurons show similar stimulus-related effects to the behavioural discrimination of complex stimuli, suggesting that this neural code may underlie the percept of the stimulus. However, neurometrics based on overall spike counts could not account for behavioural performance. Neurometrics performed best when A1 temporal discharge patterns were analysed at a resolution of less than 10-20 ms.

Figure 1. Neurometric functions for all 142 units in our sample are plotted superimposed (grey circles), along with the average psychometric performance (mean ± SEM) of the human listeners on the 2-alternative forced choice (2AFC) task (black asterisks). Negative 'Flipped window widths' indicate that the oddball stimulus was the first of the 3 stimuli presented in the 2AFC trial, while positive values indicate that the oddball stimulus was last in the sequence.

Figure 2. Population neurometrics obtained from the pooled responses of 65 (black circles) and 142 (grey circles) units are shown, along with the human psychometric (mean ± SEM) curve (black asterisks).


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Physiological and anatomical evidence for multisensory interactions in auditory cortex

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Recent studies, conducted almost exclusively in primates, have shown that several cortical areas usually associated with modality-specific sensory processing are subject to influences from other senses. Here we demonstrate using single-unit recordings that visually responsive units are widespread in the auditory cortex of anesthetized with Dormitor (medetomidine hydrochloride 0.1mg/kg), and anaesthesia maintained with intravenous infusion of a mixture of Domitor (0.022 mg/kg/h) and Ketaset (ketamine hydrochloride 5 mg/kg/h). In many cases, these units were also acoustically responsive and frequently transmitted more information in their spike discharge patterns in response to paired visual-auditory stimulation than when either modality was presented by itself. Visually responsive units were present throughout the depth of the cortex. They were particularly common in non-tonotopic areas on the anterior ectosylvian gyrus, where up to 75% (236/315) of units had their responses modulated by visual stimulation. Audio-visual and unimodal visual units were also found within the tonotopic areas including the primary fields located on the middle ectosylvian gyrus; for example, of all neurons tested in the poster pseudosylvian field and primary auditory cortex, respectively, 18% (15/84) and 9% (10/113) were responsive only to visual stimulation and 19% (16/84) and 11% (12/113) had their responses modulated by both visual and acoustic stimulation. Within each auditory cortical field, the pure tone response properties of neurons sensitive to visual stimuli did not differ in any systematic way from those of visually unresponsive neurons. Neural tracer injections revealed the presence of direct inputs from different areas of visual cortex to both primary and non-primary auditory fields, indicating a potential source of origin of the visual responses in auditory cortex. Visual inputs to the anterior bank of the ectosylvian gyrus originated predominantly from areas thought to be involved in processing object motion, whereas inputs to the posterior and middle ectosylvian gyri arose from areas concerned with visual object identification. Moreover, direct projections exist from primary visual to primary auditory cortex. These data suggest that multisensory convergence and integration are features common to all auditory cortical areas.

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Functional development of sensory hair cells in the mammalian inner ear

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We are currently focused on identifying how and when hair cells acquire the remarkable ability of mechanosensitivity and sensory signaling. In particular, we want to know which molecules are involved in the signal transduction cascade and when they are acquired during normal development. To address these questions we have taken several approaches. We have examined the normal development of hair cells from the wild-type mouse inner ear to define the temporal pattern of functional acquisition of signaling components (Géléoc & Holt, 2003; Géléoc et al. 2004). To identify candidate molecules that contribute to essential hair cell functions we have identified temporal correlations between the physiological expression patterns and the expression pattern of hair cells genes using quantitative RT-PCR. To test the hypotheses generated based on these correlations we examine hair cells of mice that carry naturally occurring mutations, as well as transgenic animals including, targeted gene deletions and target gene replacements with mutant genes. In addition, we have pioneered the use of adenoviral vectors to drive expression of dominant-negative constructs to suppress the function of endogenous hair cell proteins; overexpression of wild-type genes to rescue mutant phenotypes; expression of GFP tagged constructs to facilitate protein localization; and suppression of endogenous gene expression using siRNAs. To assay for changes in function we image FM1-43 uptake, an indicator of functional mechanotransduction, and use the whole-cell, tight-seal recording technique in voltage-clamp mode to record transduction currents or voltage-dependent currents. We use a fast piezoelectric bimorph with a submillisecond rise-time to evoke hair bundle deflections. In current-clamp mode we record membrane potential to examine the functional consequences of altered gene and protein expression.

Using these approaches we have identified the physiological consequences of mutations in two structural proteins that are required for integrity of the sensory hair bundle, protocadherin 15 (PCDH15) and the very large G-protein coupled receptor 1 (VLGR1). In the case of PCDH15 (Senften et al. 2006), we found that a naturally occurring mutation, the av3j allele, causes a loss of mechanosensitivity in vestibular and auditory hair cells of early postnatal mice. Localization of the protein and its binding to myosin 7a suggest it may be a component of the extracellular linkages that help maintain the hair bundle in a rigid, upright configuration. Generation of a mouse that carried a targeted deletion of the 7th transmembrane domain of VLGR1 resulted in a lack of FM1-43 uptake and lack of mechanotransduction currents in auditory but not vestibular hair cells (McGee et al. 2006). Immunolocalization of VLGR1 to the base of auditory hair cells suggests it may be component of the ankle link, a linkage required for the normal development and function of hair cells.

To investigate the role of myosin molecules we have used a chemical-genetic strategy and generated a mouse that expresses a mutation in the ATP binding pocket of myosin 1c. The mutation, known as Y61G sensitizes the motor protein to inhibition by an ADP analog, NMB-ADP. We have found that acute application of NMB-ADP disrupts both fast and slow adaptation in vestibular hair cells of mutant but not wild-type mice (Stauffer et al. 2005). Since fast adaptation has been implicated in auditory amplification, we suggest that myosin 1c may be a component of the elusive cochlear amplifier.

To examine the function of voltage-dependent conductances localized to the basolateral membrane, we have generated modified adenoviral vectors and infected hair cells of organotypic cultures. One class of potassium channel, known as KCNQ4, is highly expressed in both auditory and vestibular hair cells and causes a dominant, progressive hearing loss when mutated. To identify the K+ currents carried by KCNQ4 and the function of those currents we generated a vector that expressed GFP as a marker and the dominant-negative form of KCNQ4. We found that the mutant KCNQ4 suppressed the endogenous K+ currents carried by wild-type KCNQ4 in both auditory and vestibular hair cells. In current-clamp mode we found that the cells that expressed the mutant channels had depolarized resting potentials and had larger receptor potentials relative to wild-type controls. Since the conductance is active at rest, we conclude that KCNQ4 functions to maintain hyperpolarized resting potentials and attenuate the hair cell receptor potential. Our presentation will highlight some of these recent findings and the approaches we have taken to understand the hair cell transduction cascade from its origin in the hair bundle to its transmission at the afferent synapse.


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Mechanisms underlying the temporal precision of sound coding at the hair cell synapse

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The hair cell synapse’s precision to code the temporal fine structure of acoustic stimuli is astonishing. For example, our capability to locate sound in space builds on interaural time differ-
ences of sound insertion of only hundreds of microseconds. Diff-
erent from conventional synapses that are driven by action
potentials and hence build on strong stimulus-secretion cou-
p ling, hair cells code sound of extremely different intensities, but
all with a temporal precision that suffices phase locking of the
auditory nerve fibres spiking with tonal stimuli up to the low
kHz range. In fact, even at sound levels that do not yet elicit a
proper onset response of the nerve fibres, fibres preferentially
discharge at a fixed time of the sine cycle.
Biological mechanisms underlying this high temporal precision
of sound coding include:
- short membrane time constant and rapid repolarization due
to massive potassium conductances,
- rapidly gating L-type Ca\(^{2+}\) channels,
- a large and rapidly replenishing pool of readily releasable synap-
tic vesicles,
- high rates of exocytosis at saturating [Ca\(^{2+}\)]
- a 'Ca\(^{2+}\) nanodomain' control of release, requiring a close posi-
tioning of Ca\(^{2+}\) channels and vesicle release sites, which ensure
release at high [Ca\(^{2+}\)],
- rapidly-gating glutamate receptors depolarizing small postsy-
naptic elements to threshold.

I will present new findings on hair cell stimulus-secretion cou-
p ling, vesicle pool dynamics and their molecular/structural deter-
minants. Combining patch-clamp membrane capacitance meas-
urements, electron microscopy and immunohistochemistry to
investigate inner hair cells we obtained estimates for the maxi-
mal size of the readily releasable vesicle pool (RRP) and for the
number of Ca\(^{2+}\) channels at the average ribbon synapse. Oper-
ating in the Ca\(^{2+}\) nanodomain regime, the hair cell responds to
varying stimulus intensities by recruiting different numbers of
Ca\(^{2+}\) channel-release-site units. This results in a stimulus intens-
ity-defined RRP size. Utilizing mouse genetics we demonstrated
that the RRP is strongly diminished in the absence of the synap-
tic ribbon, which also impairs the synchronous activation of
the postsynaptic spiral ganglion neurons. We argue that paral-
ellel but statistically independent fusion of several vesicles oc-
curs at the hair cell synapse, reducing the jitter of postsynaptic spike
timing.

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Regulation of potassium channel phosphorylation by auditory stimuli

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Many auditory neurons fire action potentials at high rates with
high temporal precision. These include neurons of the medial
nucleus of the trapezoid body (MNTB) and anterior ventral
cochlear nucleus (AVCN), which participate in circuits that detect
the locations of sounds in space. The expression of several dif-
ferent potassium channel subunits in these cells permits accru-
rate phase-locking of their action potentials to different stimu-
lus frequencies. Our laboratory has focused on the role of the
voltage-dependent potassium channel subunit Kv3.1b, and on
Slick and Slack, two potassium channel subunits that are present
at high levels in these neurons and that are activated by increases in intracellular sodium ions.

The ability of MNTB and AVCN neurons to fire at high fre-
quencies can be attributed to the presence of high levels of the
Kv3.1b potassium channel, which allows neurons to follow synap-
tic stimuli at high frequencies. In rats or mice, inhibition of Kv3.1
channels or knockout of the Kv3.1 gene prevents MNTB neu-
rons from following high frequency stimulation (>200 Hz). Nev-
evertheless, high levels of Kv3.1b current degrade the accuracy of
action potential timing at lower frequencies of firing. The ampli-
tude of Kv3.1b currents can be regulated by protein kinase C
(PKC), which suppresses current by direct phosphorylation of
serine 503 at the C-terminus of the protein. Using a phosho-
specific antibody to serine 503 of Kv3.1b we find that, in a quiet
auditory environment, Kv3.1b is basally phosphorylated by this
enzyme, providing maximal timing accuracy at low firing fre-
quencies. In vivo acoustic stimulation of animals, or high fre-
quency stimulation of the afferent input of MNTB neurons in
brainstem slices, results in a rapid and reversible decrease in the
level of phosphorylation. This dephosphorylation permits neu-
rions to fire at higher rates, albeit with lower temporal accuracy.
Phosphorylation of Kv3.1b therefore appears to be a mechanism
that rapidly adjusts the intrinsic electrical properties of neu-
rons to the pattern of incoming auditory stimuli.

MNTB and AVCN neurons also express the Slack and Slick genes,
which encode large conductance sodium-activated potassium
channels (KNa channels). Both whole-cell and single channel
recordings have demonstrated that channels gated by intracel-
lular sodium are present at the somata of MNTB neurons, and
that their biophysical and pharmacological properties match
those of Slick and or Slick/Slack channels. Manipulations of the
level of KNa current in MNTB neurons, either by increasing lev-
els of internal sodium or by exposure to a pharmacological acti-
vator of Slack channels, increases the accuracy of timing of action
potentials at high frequencies of stimulation. These findings sug-
gest that KNa channels, like Kv3.1b, influence the fidelity of infor-
mation transfer through the MNTB and that modulation of these
potassium channels constitutes a mechanism that allows neu-
rions to adjust to different frequencies of stimulation.

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The inferior colliculus: the central hub of the auditory nervous system

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A basic role of the auditory system in all mammals is to identify
sounds and use this information to selectively activate neural
systems that focus attention on the sound, or generate a suitable motor response. In the first relay center, i.e. the cochlear nuclear complex, the signals of the cochlear nerve diverge into a number of parallel ascending tracts that converge on the auditory midbrain, the inferior colliculus. In contrast to the role of the superior colliculus within the visual system, the IC is the principal source of input to the auditory thalamus (Malmierca, 2003). Likewise, there is a minimum of three relays in the auditory system, with several stages of convergence and divergence, and at least seven levels of crossing as opposed to the minimum of two relay stations between the periphery and cerebral cortex in the other sensory systems.

The auditory system is unique among sensory systems because it integrates a highly complex network of pathways in the lower brainstem, with a significant amount of processing accomplished in the IC, just prior to the level of the thalamus. The IC probably represents a major output to premotor pathways that initiate or regulate sound-evoked motor behaviour (Casseday et al. 2002).

Of all the brainstem and midbrain auditory structures, the IC has been studied comprehensively by many investigators possibly because it is more easily accessible and highly differentiated than many other parts of the auditory brainstem in both specialized and non-specialized mammals (for detailed reviews see e.g. Malmierca, 2003; Winer & Schreiner, 2005).

The IC is not only the main site of termination for the ascending fibers of the lateral lemniscus but also receives a heavy innervation from the auditory cortex Furthermore, the IC receives crossed projections from its contralateral counterpart (Malmierca et al. 1995) and possesses a dense network of local connections (Malmierca et al. 1995). Thus, the IC occupies a strategic position in the central auditory system and may be considered as a central hub or an interface between the lower auditory pathway, the auditory cortex and motor systems (Casseday et al. 2002).

In this paper, I shall review recent anatomical and physiological experiments which demonstrate that the inferior colliculus is involved in a great diversity of functional roles in the auditory system, and that most of the interesting auditory features might already be extracted from incoming sounds by this midbrain nucleus. Therefore, the inferior colliculus may even be considered as the auditory analog of the primary visual cortex, so that as suggested by Nelken (2004), the role of the auditory cortex might be to organize these features into auditory objects.


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