C96

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

K.M. Walker, A.J. King, B. Ahmed and J.W. Schnupp

Physiology, Anatomy & Genetics, University of Oxford, Oxford, UK

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.

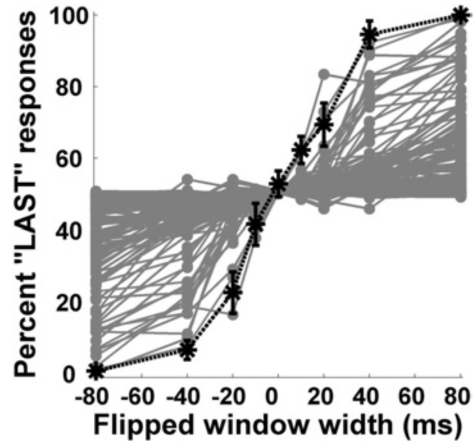

Figure 1. Neurometric functions for all 142 units in our sample are plotted superimposed (grey circles), along with the average psychometric performance (mean ± SEM) of the human listeners on the 2-alternative forced choice (2AFC) task (black asterisks). Negative 'Flipped window widths' indicate that 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.

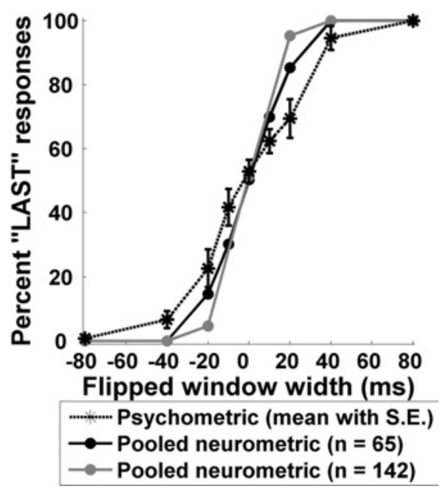

Figure 2. Population neurometrics obtained from the pooled responses of 65 (black circles) and 142 (grey circles) units are shown, along with the human psychometric (mean ± SEM) curve (black asterisks).

Garcia-Lazaro JA, Ahmed B & Schnupp JW (2006). Curr Biol 16, 264-271.

Green DM & Swets JA (1974). Signal detection theory and psychophysics. Kreiger, New York.

Schnupp JWH, Hall TM, Kokelaar RF & Ahmed B (2006). J Neurosci (in press).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C97

Physiological and anatomical evidence for multisensory interactions in auditory cortex

J.K. Bizley[1], F.R. Nodal[1], V.M. Bajo[1], I. Nelken[2] and A.J. King[1]

[1]Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK and [2]Department of Neurobiology, Hebrew University, Jerusalem, Israel

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 (meditomidine 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 ectosyslvian gyruis; 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 ectosyslvian 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.

This work was supported by the Wellcome Trust.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA49

Functional development of sensory hair cells in the mammalian inner ear

G.S. Géléoc and J.R. Holt

Neuroscience, University of Virginia, Charlottesville, VA, USA

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 receptor1 (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.

Géléoc GSG & Holt JR (2003). Nature Neuroscience 6, 1019-1020.

Géléoc GSG, Risner JR & Holt JR (2004). J Neurosci 24, 11148-11159.

McGee JA, Goodyear RJ, McMillan DR, Stauffer EA, Holt JR, Locke KG, Birch DG, Legan PK, White PC, Walsh EJ & Richardson GP (2006). J Neurosci (in press).

Stauffer EA, Scarborough J, Hirono M, Miller ED, Shah K, Mercer JA, Holt JR & Gillespie PG (2005). Neuron 47, 541-553.

Senften M, Schwander M, Kazmierczak P, Lillo C, Shin JB, Hasson T, Gillespie PG, Williams D, Géléoc GSG, Holt JR & Müller U (2006). J Neurosci 26, 2060-2071.

Supported by NIH/NIDCD grants DC006183 (G.S.G.G.), DC05439 and DC03279 (J.R.H.).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA50

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

A. Brandt[1], D. Khimich[1], R. Nouvian[1], E. Gundelfinger[3] and T. Moser[1]

[1]Center for Molecular Physiology of the Brain, University of Goettingen, Goettingen, Germany, [2]Department of Otolaryngology, University of Goettingen, Goettingen, Germany and [3]Dept. of Neurochemistry and Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg, Germany

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. Different from conventional synapses that are driven by action potentials and hence build on strong stimulus-secretion coupling, 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 synaptic vesicles,
-high rates of exocytosis at saturating [Ca^{2+}]
-a 'Ca^{2+} nanodomain' control of release, requiring a close positioning of Ca^{2+} channels and vesicle release sites, which ensure release at high [Ca^{2+}],
-rapidly-gating glutamate receptors depolarizing small postsynaptic elements to threshold.
I will present new findings on hair cell stimulus-secretion coupling, vesicle pool dynamics and their molecular/structural determinants. Combining patch-clamp membrane capacitance measurements, electron microscopy and immunohistochemistry to investigate inner hair cells we obtained estimates for the maximal size of the readily releasable vesicle pool (RRP) and for the number of Ca^{2+} channels at the average ribbon synapse. Operating 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 intensity-defined RRP size. Utilizing mouse genetics we demonstrated that the RRP is strongly diminished in the absence of the synaptic ribbon, which also impairs the synchronous activation of the postsynaptic spiral ganglion neurons. We argue that parallel but statistically independent fusion of several vesicles occurs at the hair cell synapse, reducing the jitter of postsynaptic spike timing.

Supported by German Research Foundation (Center for Molecular Physiology of the Brain) Eurohear.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA51

Regulation of potassium channel phosphorylation by auditory stimuli

L.K. Kaczmarek

Yale University, New Haven, CT, USA

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 different potassium channel subunits in these cells permits accurate phase-locking of their action potentials to different stimulus 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 frequencies can be attributed to the presence of high levels of the Kv3.1b potassium channel, which allows neurons to follow synaptic stimuli at high frequencies. In rats or mice, inhibition of Kv3.1 channels or knockout of the Kv3.1 gene prevents MNTB neurons from following high frequency stimulation (>200 Hz). Nevertheless, high levels of Kv3.1b current degrade the accuracy of action potential timing at lower frequencies of firing. The amplitude 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 phospho-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 frequencies. In vivo acoustic stimulation of animals, or high frequency 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 neurons 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 neurons 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 intracellular sodium are present at the somata of MNTB neurons, and that their biophysical and pharmacological properties match those of Slick and or Slack/Slick channels. Manipulations of the level of KNa current in MNTB neurons, either by increasing levels of internal sodium or by exposure to a pharmacological activator of Slack channels, increases the accuracy of timing of action potentials at high frequencies of stimulation. These findings suggest that KNa channels, like Kv3.1b, influence the fidelity of information transfer through the MNTB and that modulation of these potassium channels constitutes a mechanism that allows neurons to adjust to different frequencies of stimulation.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA52

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

M.S. Malmierca

Cell Biology and Pathology, University of Salamanca, Salamanca, Spain and Auditory Neurophysiology Unit, Institute for Neuroscience, Salamanca, Spain

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 specieal-ized and non-specielized 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 eceives 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.

Casseday JH, Fremouw T and Covey E (2002). The inferior colliculus: Hub of the auditory system. In Integrative Functions in the Mammalian Auditory Pathway, edited by D. Oertel, A.N. Popper & R.R. Fay, pp. 238-318. Springer-Verlag, New York.

Malmierca MS (2003). Int Rev Neurobiol 56, 147-211.

Malmierca MS, Hernández O & Rees A (2005). Eur J Neurosci 21, 2701-2710.

Nelken I (2004). Curr Opin Neurobiol 14, 474-480.

Winer JA & Schreiner CE (2005) The inferior colliculus. Springer, New York.

This study was supported by grants from the Spanish DGES (BFI-2003-09147-02-01) and JCYL-UE (SA040/04).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA53

The dynamics of the construction of auditory perceptual representations - MEG brain imaging in humans

M. Chait

Neuroscience and Cognitive Science Program, University of Maryland, College Park, MD, USA and Equipe Audition/DEC, ENS, Paris, France

The many sound-generating sources within the environment produce an aggregate wave-form that enters the ear. In order to operate in, and make sense of the world, a listener has to be able to separate this input into source-related components, localize them, recognize them, and react appropriately. In this talk, I will discuss auditory perception and specifically what can be learned about perception by investigating how brain responses to sounds unfold over time. During the course of auditory processing, sensory information undergoes a transformation from a representation in purely physical terms to an abstract, behaviorally relevant form - two identical signals may be apprehended completely differently depending on the state of the perceiver. Since perception is inherently intimate and automatic, it is difficult to tap into its constructing stages. In my work, I employ simultaneous psychophysical and brain-imaging tools (Magnetoencephalography; MEG) to study the processes that underlie listener's construction of a representation of the acoustic environment: the mechanisms by which auditory objects are detected and separated from background and the role of top-down processing such as attention. Specifically, I will talk about the temporal dynamics of the cortical systems that subserve the detection of auditory temporal edges. One particular outcome of this research is the discovery of a fundamental asymmetry in how transitions between order and disorder, or between signals with differing statistics, are processed in cortex. I will discuss what this asymmetry implies about processing and how it is related to listener's ability to make sense of an ever changing, complex acoustic world.

This work was supported by R01 DC05660 to David Poeppel.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.