

NZAP-zeo protein specifically eliminated the cytoplasmic fraction of the viral RNA, with no effect on other cellular mRNAs.

The antiviral construct as originally isolated contained only the 5' one-third of the rZAP coding region, fused to the zeocin resistance gene. To test the function of the full-length protein, the complete ZAP ORF was cloned into an expression plasmid and tagged with a myc epitope at the carboxy terminus, forming pZAP-myc. pZAP-myc was used to transform Rat2 cells, and the effects on Eco-Luc transduction were measured as before. The full-length protein induced a dramatic inhibition of viral vector expression (Fig. 4D). The inhibition was even greater when rZAP and NZAP-zeo were expressed together. These results suggest that the full-length rZAP also acts to negatively regulate viral transcripts.

We tested other constructs to look for forms of ZAP that interfere with the antiviral activity of the wild-type protein. The 5' portion of the gene present in NZAP-zeo was excised from the pBabe-HAZ vector and expressed with a small myc epitope tag substituted for the zeo fusion partner (8). Surprisingly, the NZAP-myc fusion protein reproducibly caused a small increase, rather than a decrease, in the level of luciferase detected after cotransfection with Eco-Luc viral DNA (Fig. 4D). Moreover, this construct almost completely suppressed the inhibition in cells containing NZAP-zeo, restoring normal luciferase expression. Thus, this fragment antagonized the normal rZAP activity. This distinct and opposite behavior of NZAP-myc from NZAP-zeo presumably reflects differences in the size or structure of the fusion partners in these two constructs.

These results show that direct selections for virus-resistant cells (9) can be used to identify new genes with potent antiviral activity. The gene recovered here, rZAP, is sufficient to induce an antiviral state with no apparent effect on cell viability or physiology. ZAP profoundly inhibits the expression of genes carried by retroviral vectors, at the level of the cytoplasmic viral RNA. The presence of four unusual CCCH-type zinc fingers suggests that ZAP interacts directly with the viral RNA. These fingers are found in a small family of RNA binding proteins that includes tristetraprolin (TTP), which negatively regulates the levels of tumor necrosis factor- α (TNF- α) (13), and granulocyte-macrophage colony-stimulating factor mRNAs (14). TTP binds AU-rich sequences in the 3' UTR of the TNF- α mRNA (13, 15) and recruits the exosome to degrade the mRNA (16). rZAP may act in a similar way at sequences found in viral RNAs.

The normal function of rZAP may be to regulate one or more specific cellular mRNAs. However, like PKR, RNase L, and the Mx proteins, rZAP may primarily func-

tion to inhibit viral gene expression and induce an innate immunity to viral infection. The full range of viruses restricted by rZAP is not yet known; however, rZAP potentially blocks replication of Sindbis virus in Rat2 cells (17). Activation of expression of the endogenous gene could help induce immunity and protect individuals from disease caused by viral infections.

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Materials and Methods

Figs. S1 and S2

References

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Spatiotemporal Pattern of Neural Processing in the Human Auditory Cortex

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The principles that the auditory cortex uses to decipher a stream of acoustic information have remained elusive. Neural responses in the animal auditory cortex can be broadly classified into transient and sustained activity. We examined the existence of similar principles in the human brain. Sound-evoked, blood oxygen level-dependent signal response was decomposed temporally into independent transient and sustained constituents, which predominated in different portions—core and belt—of the auditory cortex. Converging with unit recordings, our data suggest that this spatiotemporal pattern in the auditory cortex may represent a fundamental principle of analyzing sound information.

One of the basic operations that the auditory system performs to decode acoustic information is the temporal analysis of sound features and their decomposition into specific neural dis-

charge patterns. The importance of temporal analysis in audition is evident considering that the sensory stream emerges mainly in series over time. Decomposition and integration in the time domain are used for the qualitative and quantitative perceptual analysis of sound information (1, 2), and neural mechanisms specifically dealing with temporal pattern analysis are essential for the processing of complex auditory signals. In animals, neurons of the auditory cortex can be broadly classified into transient and sustained responders. Transient responses typically occur at the onset of a stimulus, whereas sustained responses follow the stimulus (3–9). In the human auditory cortex, the spatiotemporal principles of encoding sound information are not yet fully understood. Magnetoencephalography demonstrated transient and steady-state re-

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sponses (10), and neuroimaging showed a stimulation rate-dependent modulation of the temporal response pattern (11). Here we asked whether transient and sustained neural activity patterns in the human auditory cortex are elicited simultaneously by one and the same stimulus and whether there is a specific pattern of spatial distribution. We combined “magnetization-prepared” functional magnetic resonance imaging (fMRI) and acoustic stimulation by the gradient sounds to study the neural activation after a baseline of silence (9, 12) (fig. S1). Further, we used independent component analysis (ICA) (13) in a hierarchical combination of “spatial” ICA (14) and “temporal” ICA (15) to blindly decompose the evoked blood oxygen level-dependent (BOLD) signal mixtures into constituent spatiotemporal sources (9, 16, 17).

Spatial ICA (14) extracted individually unique maps and associated time courses (Fig. 1) in the supposed primary and secondary auditory cortices (18). The time courses were characterized by an initial peak, then a plateau of persistent and irregularly oscillating signal effects superimposed. This interindividually consistent temporal pattern suggested the presence of at least two concurrent—possibly temporally independent—transient and sustained processes. However, the two presumptive processes concurred in the same spatial components and were mixed with other, less-consistent oscillatory phenomena. Assuming multiple, spatially overlapping, independent signal sources in each imaging voxel, we decomposed by temporal ICA (15) the signals from the spatial ICA regions into maximally temporally independent components (9) and identified a transient and a sustained component (Fig. 2). These concurrent components were blended with the signal mixtures arising from all fields of the auditory cortex. To identify portions with predominantly transient or sustained activity, we mapped the components to the cortical space (9). The distribution of response was characterized by a central stripe-like area (Heschl’s gyri) predominated by sustained responses and a surrounding area (temporal and polar planes, temporal opercula) predominated by transient responses (Fig. 3).

Human neuroimaging studies show transient or sustained temporal patterns of response to different types of acoustic stimuli (11, 19, 20). We demonstrate that the brain can use different temporal codes for one and the same stimulus. On the microscopic level (below the resolution of fMRI), the BOLD signal within an imaging voxel arises from tissue that is presumably composed of single, nearby sources of transient and sustained signals. We cannot determine whether the observed signals were produced by different or the same cell populations, whose response patterns follow a continuum from sharp phasic to robust sustained responses (4). Superposition of varying phasic events, possibly reflecting neural habituation and other adaptation processes, could be related to the

irregular oscillations seen in the sustained phase. This finding is also consistent with the existence of two neuron populations in the monkey auditory cortex, one exhibiting stimulus-synchronized and the other nonsynchronized discharge patterns (8). On the macroscopic level, the predominance of transient and sustained signal sources was reminiscent of the parcellation into core and belt areas of the monkey auditory cortex (21, 22), a distinction that appears to be paralleled in the human brain (23, 24). The specific functional operations associ-

ated with this spatiotemporal response pattern and possible hemispheric differences remain to be elucidated (25). Studies in monkeys and humans showed that the response preference to spectral complexity increases from the core to the belts (2, 23) and suggested that this might be related to “what” and “where” processing streams (26).

Because our imaging parameters range on different temporal and spatial scales than electrophysiological recordings (3–8, 10), they could represent different levels of processing.

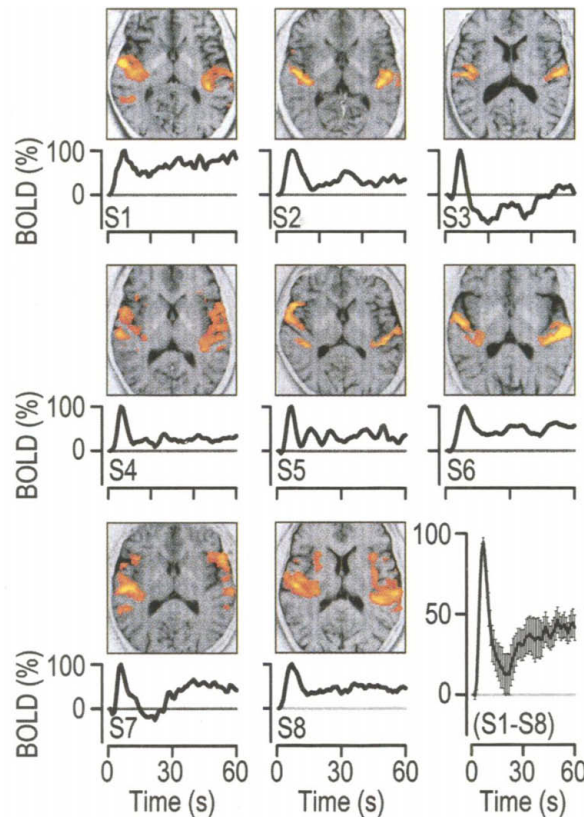


Fig. 1. Spatially independent components and associated time courses of the blood oxygen level dependent (BOLD) signal response to repetitive scanner sounds are projected on individual anatomical slices (radiological convention) positioned through the activation areas of individual subjects (S1 to S8). Spatial ICA blindly decomposed the presumptive primary and secondary auditory cortex. The associated time course was generally characterized by an initial peak at about 5 to 10 s after stimulation onset and evolved into a stationary plateau of activation. The initial transient phenomenon was highly consistent across subjects, whereas the sustained phase was associated with considerable interindividual variation and irregular oscillations. (Bottom right) The mean \pm SE of the individual signals.

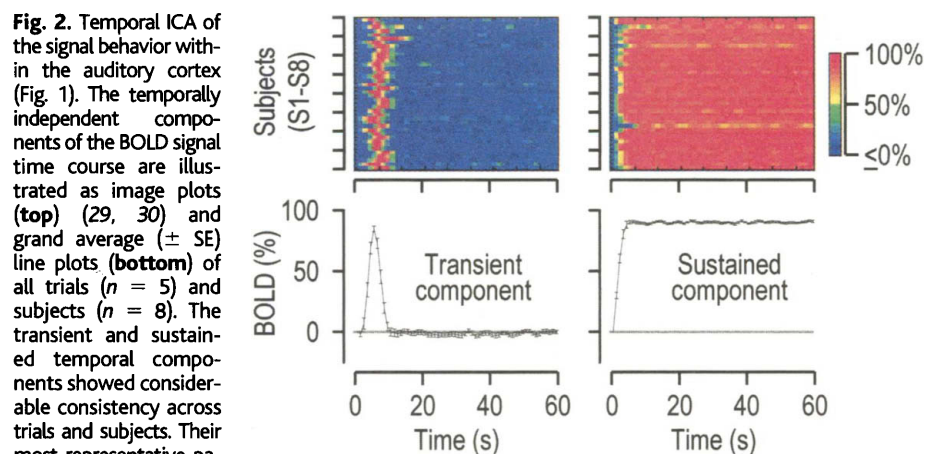
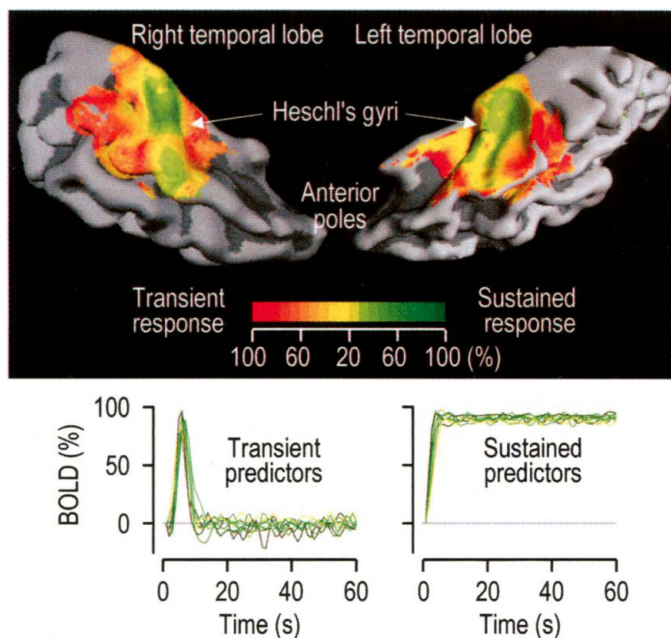


Fig. 2. Temporal ICA of the signal behavior within the auditory cortex (Fig. 1). The temporally independent components of the BOLD signal time course are illustrated as image plots (top) (29, 30) and grand average (\pm SE) line plots (bottom) of all trials ($n = 5$) and subjects ($n = 8$). The transient and sustained temporal components showed considerable consistency across trials and subjects. Their most representative parameters (mean \pm SD) were as follows. Transient: onset time (time to 10% of peak), 2.8 ± 0.19 s; time-to-peak (delay from stimulation start to maximum), 5.1 ± 0.16 s; peak width (time in which signal was $\geq 10\%$ of peak), 4.7 ± 0.22 s. Sustained: onset time, 1.2 ± 0.11 s; rise time (time from 10% of peak to 90% of peak first crossing), $4.9 \pm .89$ s.

Fig. 3. (Top) The relative contribution map of transient and sustained BOLD signal sources across all subjects and trials with the corresponding signals as identified by temporal ICA. **(Bottom)** Signals represent intraindividual averages of the five trials used as predictors within a group multiple regression analysis (31). The functional map is projected on the reconstructed cortical surface of the temporal lobes of a standard brain template. Color coding indicates the relative contribution of the two predictor classes and suggests a spatial continuum between the temporal response patterns. The contribution of the sustained response type becomes less predominant as one moves from the core to the belt areas. There was no notable hemispheric difference in the extension of the predominantly transient and sustained responses.



Our data add to the evidence that the temporal decomposition of neural activity into transient and sustained patterns, or a continuum of them (4), may be a fundamental principle of deciphering auditory information. The mechanisms of upstream propagation of differential neural activity have only been partially unraveled. At the cortical level, the transformation into different temporal response types could be achieved by separate synaptic networks (27). The general rules, however, need to be viewed in light of the auditory network at large. In the thalamo-cortical circuitry, for instance, neural signals undergo radical reconstruction, with some properties preserving fidelity and others being transformed or generated anew in the auditory cortex (28). Temporal signal transformation appears to be a fundamental principle in the auditory system and could be related to different hierarchical levels of sound characterization. This hypothesis becomes particularly perspicuous considering the serial properties of auditory information.

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Materials and Methods

SOM Text

Fig. S1

References

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Representation of the Quantity of Visual Items in the Primate Prefrontal Cortex

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Deriving the quantity of items is an abstract form of categorization. To explore it, monkeys were trained to judge whether successive visual displays contained the same quantity of items. Many neurons in the lateral prefrontal cortex were tuned for quantity irrespective of the exact physical appearance of the displays. Their tuning curves formed overlapping filters, which may explain why behavioral discrimination improves with increasing numerical distance and why discrimination of two quantities with equal numerical distance worsens as their numerical size increases. A mechanism that extracts the quantity of visual field items could contribute to general numerical ability.

The ability to judge the relative quantity of items in the visual field is highly adaptive. Social animals such as primates can make decisions to fight or flee by judging the relative

number of friends versus foes (1–3); in foraging, choosing a larger alternative can contribute to survival (4). These behaviors depend on the capacity to abstract information from sensory inputs and to retain it in memory, neural correlates of which are found in the prefrontal cortex (PFC) (5, 6). To investigate the role of PFC neurons in representing visual quantity, we trained monkeys to judge whether two successive displays contained the same small number of items (Fig. 1A).

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