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Functional Magnetic Resonance Imaging (fMRI) of the Auditory Cortex: an Event-Related Study Using Pure Tone Stimulation

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ABSTRACT:

Introduction: The use of Functional Magnetic Resonance Imaging (fMRI) in studying central auditory pathways expanded our knowledge of the neurophysiology of hearing. In various studies, an array of diverse auditory stimuli has been used, including pure tones and words, in a "Block" design. Few studies, thus far, have been performed using an event-related design. None of these has used pure tone stimulation.

Subjects and Methods: In eight out of 13 subjects enrolled and scanned in this project, data have been acquired and processed. Binaural pure tone stimulation at 2kHz and one higher frequency at 6, 8, or 12 kHz, at 90 dB SPL was presented to all subjects, using a stroboscopic design in an attempt to eliminate cortical response to scanning noise. In addition, an unusually long time of repetition (TR) interval of 10 seconds was utilized. The aim in data processing was to generate: a) a Peak Height Map (PHM) with selection of areas of stimulation based on the maximal response; b) a statistical map from the raw data of the images.

Results: No consistent morphology was detected in the BOLD event-related curves, corresponding to the percentage change from baseline over time. Such curves revealed significant oscillation with marked dips below baseline, highly suggestive of an irregular noise pattern. Comparing the curves to each other, no discernible shape or similarities were noted.

Conclusion: Attempting to extract both the temporal and spatial characteristics of the cortical response to pure tone stimuli resulted in a dispersal of data over many parameters, therefore "diluting" it. This, in turn, yielded a negative result where the responses were "drowned" revealing but a noise pattern.

ABSTRACT:

Introduction: L'étude du système auditif par l'IRM fonctionelle (IRMf) a contribué de façon importante à nos connaissances de la neurophysiologie de l'ouïe. Plusieurs études ont utilisé des stimulus différents, tels que les tons purs ou les mots, dans le cadre d'un protocol "en Bloc". Toutefois, peu d'études ont utilisé un protocole "event-related". Aucune d'entre elles n'a utilisé des tons purs.

Sujets et Méthodes: Les données de huit des 12 sujets inscrits et soumis à une scanographie, ont été considérées pour l'étude. Une stimulation auditive bilatérale à 2 kHz et une autre fréquence de 6, 8, ou 12 kHz, a 90 dB SPL fut presentée aux sujets, en utilisant un protocole stroboscopique visant a éliminer l'interaction de la réponse du cortex auditif avec celle du bruit de la scanographie. Un long intervalle de 10 secondes entre acquisitions fut utilisé. L'analyse des données a été effectuée sur deux plans: a) la création d'une carte "Peak Height" sélectionnant les régions où la réponse du cortex est maximale; b) la création d'une carte statistique à partir des données des images acquises.

Resultats: On n'a obtenu aucune morphologie cohérente des courbes décrivant la réponse du cortex auditif (% augmentation du signal au dessus du niveau de base) en fonction du temps suivant le stimulus. Les courbes oscillaient avec des descentes importantes en dessous du niveau de base, ce qui est suggestif d'un environnement magnétique bruité. Aucune forme constante ou similarité entre les courbes n'a été observée.

Conclusion: L'effet d'essayer d'extraire simultanément les caractéristiques temporelles et spatiales de la réponse du cortex auditif à un stimulus de ton pur, fut de disperser les données a travers plusieurs paramètres, donc "diluant" ces données. Le résultat final est donc négatif, la réponse du cortex ayant été "noyée" par le bruit magnétique ambiant.

INTRODUCTION

Hearing is a highly complex, fine-tuned and well-evolved sense with intricate anatomy and physiology. Although one of the oldest known and most researched sensory modalities, much remains to be elucidated regarding the extremely complex function and organization of the auditory system. A brief overview of the anatomy, including the neural pathways, and the physiology of the auditory system follows and forms the basis for the ensuing discussion.

Sound waves

Sound is a change in pressure creating particle displacement within an elastic medium. Sound waves are longitudinal waves created by localized, transient changes in air pressure. These pressure changes are initially generated at the source and travel in a spherical three-dimensional fashion outward, while decreasing in intensity as distance from the source increases. The speed of the traveling pressure wave in air is 310 m/s.

Auditory anatomy and physiology (Figure 1)

Sound waves enter the external auditory canal, where they first encounter the eardrum, or tympanic membrane, which separates the external ear, formed by the auricle and external auditory canal, from the middle ear, a chamber, or cavity, containing a chain of three ossicles, connected to the eardrum, among other structures. The induced vibration of the tympanic membrane is transmitted to the ossicular chain, and the stapes, the last ossicle, is in direct contact with the oval window, which is an opening into the inner ear, or cochlea. In the middle ear, several mechanisms ensure the efficient transmission and amplification of the sound signal as it travels to reach the cochlea. These mechanisms are related to the difference in the surface area between the tympanic

membrane and oval window; the lever action of the ossicular chain; the conical shape of the tympanic membrane; and the phase difference between the oval window, covered by the footplate of the stapes, and the round window covered by a membrane. Despite this very efficient system of pressure-transmission, less than half the pressure energy entering the external auditory canal reaches the oval window [1].

The vibration of the stapes-footplate at the oval window, which forms the last step in the transmission through the middle ear, is transmitted to the perilymph-containing scala tympani of the cochlea. This creates a fluid wave, which moves the basilar membrane and the organ of Corti resting upon it. The tectorial membrane covering the organ of Corti is also mobilized by the fluid wave. The stereocilia of the hair cells, the specialized receptor cells of the auditory organ, are in contact with the tectorial membrane (Figure 2). The movement of the tectorial membrane causes movement of the stereocilia. This opens ion channels at the apex of the hair cell resulting in intracellular potential changes and an intracellular cascade leading to the generation of an action potential in the fibers of the auditory nerve connected to the hair cell.

Central Auditory Pathway

The neural pathway processing auditory signals has been elucidated to a significant degree (Figure 3). A brief overview of the relevant aspects follows [2,3].

From the cochlea, the electrical signal, generated by the hair cells is transmitted as an action potential by the cochlear nerve. The cochlear nerve fibers connected to the hair cells are distributed in a tonotopic fashion.

The first relay station is the cochlear nucleus, which receives nerve fibers exclusively from the ipsilateral cochlea and contains five major cell types. Each cell type

maximally responds differently to various parameters of the auditory signal, such as stimulus onset, stimulus offset, and frequency modulation.

From the cochlear nucleus, the majority of the nerve fibers travel to the contralateral **superior olivary complex** (**SOC**). It is estimated that only 20 to 30% of these fibers reach the ipsilateral SOC. Thus the cochlear nucleus is the last center which mainly receives input from the ipsilateral cochlea and auditory nuclei. Auditory nuclei above the SOC can respond by excitatory or inhibitory fashion to stimuli from either ear. Input from the contralateral ear is thought to be excitatory while input from the ipsilateral ear is inhibitory.

The next major relay station is the **inferior colliculus** (**IC**). Fibers travel from the SOC to the IC through a tract called **lateral lemniscus**. In the IC, there are at least 18 different cell types and a minimum of five distinct areas of specialization. This indicates that the IC is more than simply a relay center and is apparently involved in many aspects of the auditory function. The **medial geniculate body** (**MGB**) of the thalamus receives fibers from the IC and projects fibers to the **auditory cortex** (**AC**) [2,3]. The specific function of the MGB has not been clearly elucidated. However, the fact that the thalamus is a major center of modulation of various behavioral processes points to the possibility that the MGB is a center of modulation and modification of the auditory signal being presented to the auditory cortex.

The auditory cortex is located around the area of the Heschl's Gyrus in the Sylvian fissure of the temporal lobe. It consists of a primary auditory cortex, an area termed Brodmann's areas 41 and 42, and several secondary or association areas (Figure 4). It is important to note that although these anatomical landmarks are often mentioned

in association with the auditory cortex, the precise location of the auditory cortex is variable across individuals. Previous studies, using functional imaging techniques, have confirmed the variation in locations of the functional auditory cortex [4,5].

An important feature of the auditory pathway is its tonotopic organization. From the basilar membrane to the auditory cortex, a certain organizational tonotopy is maintained. Multiple studies have demonstrated this tonotopic organization in both animal models [6-8] and human subjects [9-11]. More specifically in each area of the auditory cortex, cells are tonotopically organized in a columnar fashion. Cells in a given column, for instance, may demonstrate tuning at a particular frequency, whereas others may be associated with intensity encoding, while others still may provide inhibitory responses to stimulation of one ear and excitatory responses to the other ear. However, much remains, to be elucidated and ascertained about the specific functions of cell groups in the primary as well as the association auditory cortices.

Finally, it is worth mentioning the existence of an efferent component to the auditory system, consisting of a number of pathways between the higher nuclei, such as the IC and SOC, and the cochlear hair cells. In addition, fibers have been identified, running between the auditory cortex and the MGB on one hand and lower centers on the other [2,3]. It is postulated that the function of the efferent auditory system is to protect the cochlea from loud sounds and continuously fine-tune and modify the function of the cochlear hair cells. However, this remains to be proved and at present, the functions of the efferent auditory system remain largely unknown.

Audiology

The Decibel (dB) is a scale used in the description of sound. It is a logarithmic nonlinear scale. That is, a change from 2 to 4 dB is not equal to a change from 6 to 8 dB. In addition, 4 dB is not twice the intensity or sound pressure as 2 dB. This scale is, in fact, the logarithm of a ratio of two numbers or sounds: a reference value and the value being described. It is therefore a relative measure. That is, 0 dB does not represent the absence of sound. The parameters of the ratio may be sound pressure, intensity, hearing and sensation levels.

Sound Pressure Level (SPL) is a basic measure for all acoustic measurements. The reference is sound pressure. The formula for determining the number of decibels is as follows:

$$\mathbf{dB\ SPL = 10 \log (P_o^2 / P_r^2)}$$

Where P_o is the pressure of the output (sound), and P_r is the reference pressure, usually 20 μPa. Transformed, the formula becomes:

$$\mathbf{dB\ SPL = 20 \log (P_o / P_r)}$$

Hearing Level (HL) is a different scale, which takes into account the fact that the human ear is not equally sensitive to all frequencies. The reference in this case is a normal ear. Zero dB HL at any frequency is defined as the least intensity needed for a normal ear to perceive a sound 50% of the time. For any given frequency, HL is different from SPL and the difference varies across frequencies. For example, the human ear cannot perceive 0 dB SPL at 250 Hz. Rather, a 250 Hz sound must be raised to 26.5 dB SPL before it is

heard. This level is therefore assigned 0 dB HL [1]. The following table shows the SPL level needed for 0 dB HL at various frequencies.

Number of dB SPL needed to equal 0 dB HL at various frequencies (From reference 1).

Frequency	dB SPL
125	47.5
250	26.5
500	13.5
1000	7.5
1500	7.5
2000	11.0
3000	9.5
4000	10.5
6000	13.5
8000	13.0

Functional Magnetic Resonance Imaging (fMRI)

fMRI is a relatively recent imaging modality. Since its introduction in the early nineties, it has proven to be a powerful tool in elucidating the functional significance of physiologic and pathologic states, in the neurosciences in particular. To date, however, its use remains mostly as a research tool allowing an unprecedented look into neurophysiological processes governing multiple sensory and motor functions. fMRI is highly complex technically and its application and interpretation of acquired data require knowledge of, and expertise in physiology, physics, imaging, cognitive sciences, and clinical medicine. Therefore, its use and application necessitates a multidisciplinary approach. By far, the area where fMRI has been most widely studied is in the functional imaging of the brain and central nervous system. It provides a visual representation of the activation of the brain during motor, sensory, and cognitive activity. Numerous studies have been performed examining the cortical response to visual [12], auditory [13,14], and

somatic sensation[15], as well as motor tasks [16]. Although most studies examine the activation of the cortex, others have involved detecting activation in brainstem centers [17]. These studies have used a variety of methodologies and experimental paradigms.

fMRI is a non-invasive procedure that provides a better resolution than other functional imaging modalities, such as Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT), with the added benefit of having no radioactivity. In addition, its cost is inferior to other modalities and, as a result, it is becoming increasingly popular. The physical principles governing fMRI are complex. A basic understanding of these principles is essential in allowing a better understanding of study paradigms and experimental designs. fMRI does not directly measure neural activity by detecting increases in neural metabolism or rates of neural electrical activity. Rather, it measures neural activity indirectly, by sensing changes in blood flow and oxygenation in the local microvascular bed immediately surrounding the stimulated neurons. As a group of neurons is stimulated, action potentials are generated and their metabolism is increased with a corresponding increase in oxygen demand. This, in turn, necessitates an increase in blood flow to the local capillaries with release of oxygen, converting oxyhemoglobin to deoxyhemoglobin. Deoxyhemoglobin contains a significantly more paramagnetic species of iron, due to its four unpaired electrons. It induces a disturbance in the local magnetic field leading to a large magnetic susceptibility effect (the ability of a substance to alter a magnetic field when placed within it). These changes are then detected as a MRI signal [18].

The stimulus is delivered to the subject under examination during the scanning procedure. Various methods and designs have been devised for stimulus delivery.

Auditory stimuli, however, present a particular challenge. The loud noise produced by the MRI equipment, the result of echo-planar imaging acquisitions, presents a complicating factor. These acquisitions by the MRI, following stimulus delivery, detect the above-mentioned functional changes. This loud acquisition noise, however, interacts with the measure of cortical response to the auditory stimulus. The problem, therefore, presents itself in discerning the cortical response to the auditory stimulus in the presence of this loud background noise. There are ways to circumvent this, as will be shown by our experimental design.

The response, marked by a change of oxyhemoglobin to deoxyhemoglobin, is measured as a change in the magnetic field. This is expressed as a blood oxygen level dependent (BOLD) signal. The BOLD signal, detected by the fMRI, is the parameter that reflects the functional response to the delivered stimulus. It consists of a percentage change above a certain baseline, which is determined by the experimental design. For example, in the case of a study using visual stimuli, the baseline can be a white background and the stimulus an image. The response would then be expressed as a percent change in the BOLD signal when going from the baseline (white background) to the stimulus (image). In the case of auditory stimuli, the baseline may be silence or, if comparing the response to one type of acoustic stimulus to that of another, a certain sound. It is important to note that the changes in levels of deoxyhemoglobin measured by the MRI are, by definition, delayed, as they **follow** the vascular response induced by the neuronal activation. The BOLD signal is in fact a measurement of the local hemodynamic changes in the seconds following delivery of a stimulus. Experimental proof of this is

provided by several studies [19-21] where this response was quantitatively measured in the time following stimulus delivery.

The percent change in the BOLD response is visually displayed as a color-coded map, which is then superimposed on the anatomic MRI image acquired at the beginning of the scanning procedure (Figure 5). The color-coding, however, is not the only indicator of the percentage change in BOLD. The actual number is also provided for each voxel. A voxel is the three-dimensional equivalent of a pixel. It is a finite volume in three-dimensional space. In MRI, a voxel represents a certain volume (e.g. 4mm x 4mm x 5mm) in the scanned volume. For example in Figure 5, each colored square represents a voxel of 4mm x 4mm x 5mm of the scanned volume. This display not only provides a visual representation of activation of various areas of the cortex, but also provides an image, which can then be manipulated and subjected to different types of analysis.

Studies using fMRI on the auditory cortex are numerous and examine the cortical response to a variety of stimuli, such as wide band noise, speech, tone bursts, and clicks. Our group has previously used event-related fMRI to evaluate the auditory cortical response to synthetic sounds acoustically similar to speech diphthongs [19]. The problem of background noise generated by the MRI machine was circumvented in this experimental paradigm, which will be discussed in detail below. The results demonstrated bell-shaped curves corresponding to cortical response in the ten seconds following stimulus delivery. Thus a temporal response evaluating the change of neural activation with time can be achieved.

Although studies using pure tone stimulation have been performed [22,23], none has evaluated the temporal response in an event-related design. In our study, pure tone

stimulation has been used in an experimental paradigm developed and validated by Belin et al. [19], and later used in other studies [24,25]. A detailed description of the protocol will be outlined below (see Methodology). Studies of this kind may allow, in the future, the use of fMRI in the study of multiple clinical entities, such as tinnitus, unilateral or bilateral, ototoxicity, noise-induced hearing loss, sudden sensorineural hearing loss, or in the preoperative and postoperative assessment of patients undergoing cochlear implantation.

In general, little is known about the pathophysiology, the underlying changes to the auditory pathway, and the auditory processing in these disorders. Evidently in most cases with similar disorders, hearing is affected in more than one frequency. However, evaluation of hearing using tonal stimuli is a major part of any hearing assessment in the clinical setting. Therefore, generation of correlates between normal subjects and pathologic conditions is an essential step in evaluating and understanding these clinical entities.

METHODS

1) Selection of subjects

Criteria for selection included age over 18 years, no history of ear disease, normal hearing as evaluated by physical examination, pure tone audiometry, and brainstem auditory evoked response (BAER), and no documented psychiatric or psychological disorders. Additional criteria included the absence of head and neck disease, the exclusion of medication known or suspected of causing reversible or irreversible hearing loss, or neurologic disorders or systemic illnesses impairing hearing, the presence of claustrophobia, or ineligibility to undergo MRI scanning.

Physical examination was carried out in all subjects by the same otolaryngologist, to evaluate possible pathology affecting the external auditory canal, tympanic membrane, or middle ear. Normal hearing, as determined by audiometric evaluation, was accepted as a 20 decibels (dB) threshold or better in frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz, which is the standard used in our Speech and Hearing Division. BAER testing was performed to evaluate the auditory pathway. More specifically, waves I to V were evaluated for latency as well as inter-peak latencies between the various curves.

Informed consent was obtained from all subjects by filling a questionnaire fulfilling the requirements of the ethics committees of the Royal Victoria Hospital and the Montreal Neurological Hospital. In addition, each subject filled out the usual screening questionnaire and consent routinely used prior to MRI scanning, confirming the absence of metallic prostheses or implants, and ensuring that female subjects of childbearing age were not pregnant. Subjects enrolled on a purely voluntary basis without financial or other remuneration for their participation.

2) Experimental Protocol

Several authors have devised various methods for circumventing the problem of acquisition noise mentioned above and its effects on the cortical response [26-28]. An acquisition refers to a functional scan following each stimulus delivery, which detects the cortical response to the stimulus. It consists of acquiring a functional MRI scan of 10 slices of a pre-determined region of interest – in our case, a region of the temporal lobe centered around Heschl's gyrus (Fig 5). In acquiring the 10 slices of functional data, the MRI equipment emits a loud noise. This is termed echo-planar imaging (EPI) acquisition noise, and will, for the purposes of this discussion be referred to as "acquisition noise". The origin and acoustic characteristics of this noise will be detailed below.

The experimental protocol used in this study was designed and used in a previous event-related auditory study by Belin et al. [19] and later validated by other studies [24,25] where its use yielded conclusive, consistent results. This protocol ensures minimal interaction with the echo-planar imaging (EPI) acquisition noise by separating the hemodynamic cortical response to the delivered auditory stimulus from the response to the acquisition noise. There are two main features to this protocol, which achieve this goal.

A) Long TR interval.

First, the time interval between two successive acquisitions - TR interval (Time of Repetition interval) - is unusually long (10 seconds). The acquisition itself lasts one second (100 msec per slice), during which a loud acquisition noise is produced. Therefore the time between the end of one acquisition and the start of another is nine seconds of silence during which the stimulus of interest can be delivered. Studies examining the BOLD response in other cortical regions [29,30] have shown that the hemodynamic change

typically returned to baseline within 8 to 9 seconds. Other studies examining the BOLD response of the auditory cortex to the acquisition noise itself have shown a return to baseline within 9 seconds [19,21]. Therefore, the nine seconds between image acquisitions is probably sufficient to allow the hemodynamic cortical response induced by the acquisition noise to return to baseline, before the next acquisition begins. Figure 6-A displays a schematic representation of this concept. The time between the end of one acquisition and the start of the next is 9 seconds. The acquisition noise emitted by the first acquisition will return to baseline, according to studies mentioned above [19,21], within the 9-second time interval. The auditory stimulus of interest is delivered within this 9-second time interval at time "x" seconds **prior** to the next acquisition (Figure 6-B). Therefore, the second acquisition occurs at "x" seconds after the delivered auditory stimulus, and 9 seconds following the first acquisition. At the time of this second acquisition, the response to acquisition noise (EPI noise emitted by the first acquisition) has returned to baseline. Therefore, this second acquisition detects the hemodynamic cortical response to the delivered stimulus, and is minimally "contaminated" by signal change induced by the acquisition noise. This process takes place at each of the 128 a stimulus is delivered, with the only variables being the post-stimulus time "x" and the tonal stimulus delivered. This "stroboscopic" design will be detailed below.

It is important to distinguish the neural response from the hemodynamic response that follows it. Neural stimulation itself is electrical activity that takes the form of action potentials. Action potentials vary in their duration depending on fiber type and size, myelination, number of synapses, and various other physiologic factors [31]. The duration, however, is in the order of milliseconds. The action potential resulting from stimulation

by acquisition noise ends milliseconds after the acquisition noise is heard. Therefore, at the time of stimulus presentation, several seconds following the acquisition noise, these action potentials have long ended. Since neural stimulation is short-lived, there is no overlap between the neural response to acquisition noise with the response to the delivered stimuli.

Conversely, hemodynamic responses are longer lasting, in the order of seconds. As shown in Fig 6-B, there can be an overlap between the hemodynamic responses induced by the acquisition noise, and that induced by the stimulus. However, experiments using short intervals between stimulus delivery have been carried out [32], and suggest that in the typical conditions of a fMRI experiment, the assumption of linear interaction of different hemodynamic responses yield satisfactory results, and constitutes a valid approximation. Thus, the small overlap of the hemodynamic responses induced by the acquisition noise and by the stimulus has probably little effect on the measured cortical response at the time of the next acquisition when the response to acquisition noise has returned to baseline.

B) Stroboscopic design.

The second feature of the protocol is the use of a "stroboscopic" design. As Figure 6B illustrates, the acquisition following the stimulus will detect and measure the cortical response "x" seconds after stimulus delivery. By varying the post-stimulus time "x", a curve can then be constructed quantifying the cortical response for every post-stimulus time. Post-stimulus time is the time between delivery of an auditory stimulus and the acquisition that follows it.

As shown in Figure 7, stimuli were presented to the subject at different delays "x" before each image acquisition, in a randomized order. The stimuli were presented at 0 s (i.e. no stimulus), 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0 seconds before image acquisition. The stimulus presented at 0 s essentially represents the baseline. The auditory stimuli are presented at these variable delays before each acquisition so that the image obtained from each acquisition corresponds to a given time after stimulus delivery, described above and in Figure 6-B as time "x" seconds. For example, as seen in figure 7, the acquisition of the image effected 2.5 seconds after stimulus presentation represents the value on the curve for post-stimulus time 2.5 seconds. This procedure allows for the determination, for each voxel, of the post-stimulus BOLD response as function of time between 2.5 and 6 seconds. Put differently, it allows for the assessment of the cortical response to an auditory stimulus from 2.5 to 6 seconds after stimulus delivery, and this, for each scanned voxel. This means that the stimulus is delivered between 4 (i.e. 10 s – 6 s) and 7.5 (10 s – 2.5 s) seconds following the preceding acquisition. This "stroboscopic" design combined with the long TR interval of 10 s allows for the determination of the shape of the BOLD signal time course while ensuring minimal interaction with acquisition noise.

3) <u>Stimulus Delivery</u>

Stimulus delivery was achieved through headphones fashioned in our laboratory specifically for the purpose of this study. These consisted of the non-paramagnetic components of the plastic framework of regular large headphones with a rubber seal at the point of contact with the subject. Within this casing, a commonly available pisoelectric transducer was placed in a measured symmetrical location. Surrounding the transducers, sponge material cut in squares was placed along the internal surface of the

plastic. This was for the purpose of attenuating sound reverberation. The measured attenuation was approximately 15 dB. This design was ideal for the purpose of delivering pure tone stimuli at relatively high amplitude in contrast to many commercially available designs. In addition, it is magnetically silent. The acoustic output from each headphone was measured using a tonometer to 90 dB SPL and standardized for all frequencies used, to ensure accurate symmetrical position of the transducers and identical output of amplitude. These were placed on the subject's ears and secured tightly in place. This was connected to a high fidelity amplifier/mixer, which in turn was connected to a computer where a Media Control Function software program (Digivox, Montréal) dictated the pseudo-random sequence of stimulus presentation according to the protocol outlined above. The wiring connecting the headphones to the mixer was necessarily non-coiled, as this would cause an artifact due to the generation of a magnetic field within the coils of the wiring. Stimulus delivery was binaural.

The acoustic environment consisted of two types of sound. First, the stimuli delivered consisted of pure tones lasting 500 ms (including 10 ms rise and decay time of amplitude envelope) presented to the subject through headphones at 90 dB SPL. Two different pure tones were presented for each subject. One constant or reference pure tone at 2 kHz and another higher frequency, which varied among subjects. The higher frequency varied from 6 kHz to 11.5 kHz. The reasons for the variability in frequencies will be discussed below. The pure tones were delivered at various delays, as outlined above, before each cerebral volume acquisition (functional acquisition). The event-related BOLD time course was therefore sampled at 0 s (no stimulation), 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0 seconds before volume acquisition. The BOLD sampling performed at 0 seconds is

essentially an acquisition when no stimulus is presented. Therefore it measures the baseline. For each of the two tones presented, sixteen volume acquisitions were performed for each of these eight post-stimulus times (128 volumes for each of the two stimuli) pseudo-randomly intermixed throughout scanning (figure 7). This totaled 8 post-stimulus times x 16 acquisitions/post-stimulus time = 128 acquisitions per stimulus, or 256 per subject.

Second was the scanning or acquisition noise mentioned previously. It consisted of ten very loud "beeps" corresponding to magnetic gradient switching during acquisition. These "beeps" were 833-Hz fundamental frequency and its harmonics. It is an electronic sequence produced by the MRI equipment, which produces the exact sound at the time of every acquisition (every 10 seconds). It has been the subject of multiple studies [26,33,34], which have characterized and quantitatively measured it. The acoustic characteristics of this acquisition noise are entirely determined by the imaging parameters (voxel size, field of view, and number of slices). As each scan in this experiment was performed using the same imaging parameters, this acquisition noise was constant within and across subjects. The aforementioned studies [26,33,34] have established the constant nature of acquisition noise when imaging parameters are held constant across scans. In our study, the acquisition noise was present with each of the 128 acquisitions performed in a scanning session, lasted 1 s and occurred at 10 s intervals, as detailed in the protocol description above. It was partially attenuated by the headphones placed on the patient.

4) Scanning Procedure

Prior to the start of scanning, the subject was instructed to remain as immobile as possible in order to avoid motion artifacts. In addition, the subject was asked to be attentive and "actively listen" to the stimuli rather than simply allow himself/herself to be passively stimulated. It has been proposed that attention-modulated cortical responses to auditory stimuli are more intense than "passive" responses [35].

The scanner used was a 1.5-T Siemens Vision imager. The subject's head was immobilized with the headphones immobile as well on the scanner bed. A high-resolution T1-weighted 3D volume was first acquired for anatomical localization (matrix size 256 x 256 x 170, voxel size 1 x 1 x 1 mm^3). A functional volume was then defined, consisting of ten contiguous 5-mm-thick oblique axial T2 gradient echo EPI images (matrix size 64 x 64, voxel size 4 x 4 x 5 mm^3). The functional volume was oriented along the Sylvian fissure, and centered bilaterally around Heschl's gyri (figure 8). Each cerebral volume acquisition consisted of the aforementioned ten slices following each stimulus delivery. The acquisition of the ten slices was clustered in 1-s-long periods as this has been shown to minimize interaction with scanning noise [36]. Studies comparing the use of continuous sampling – individual acquisitions delivered at regularly spaced intervals of 2-4 seconds – and clustered acquisitions, as used here, have shown that the interaction between the response to acquisition noise and the response to stimuli was minimized by the use of clustered acquisitions [25]. That is, if acquisitions consisted of scanning one slice every 2-4 seconds (Figure 9), rather than the clustered acquisitions we have used (Figure 6-A and 6-B), there would be constant overlap between acquisition noise response and stimulus response, as acquisition noise would be nearly constantly in the background.

Each scanning session consisted of acquisition of 128 functional volumes, for a scanning time of 21 minutes, 40 seconds. Two sessions were carried out for each subject, yielding 256 functional volumes in 43 minutes 20 seconds. Added to this is the time for acquisition of the high-resolution T1-weighted volume for anatomical localization (figure 8), which was approximately 15 minutes, for a total scanning time of nearly one hour.

5) Data Analysis

The data analysis was carried out in two ways.

a) Analysis of fMRI data using a Peak Height Map

The first of the 128 volumes in each session was discarded because of T1 saturation effects. The images obtained were subjected to processing for smoothing Using a 6-mm Gaussian kernel and correcting for motion using in-house dedicated software [37]. The 6-mm Gaussian kernel is a standard for all fMRI studies. The final resolution obtained on the functional scan images depends on this smoothing, as well as on voxel size in the original images (4 mm x 4mm x 5mm), and can be estimated, at the Montreal Neurological Institute, to 5 mm [37]. Previous studies [4,38] using a detailed probabilistic analysis have determined the size of the region of Heschl's gyrus – the primary auditory cortex – to be approximately 3 cm in length, 2 cm in height, and 1 cm in depth. Considering this size, a resolution of 5 mm is acceptable. It is possible to increase the precision of the localization of the signal by decreasing voxel size. However, this would decrease the signal to noise ratio of the measurement. As a result, the resolution and dimensions mentioned are the generally accepted standards in auditory fMRI studies performed at the MNI. The event-related post-stimulus BOLD time course was

generated for each voxel by calculating an average of the 16 acquisitions for each of the eight post-stimulus times. The BOLD was expressed as a percent change from baseline. The baseline was the mean value of the 16 acquisitions at post-stimulus time 0s (no stimulus). Figure 10 shows an example of the BOLD post-stimulus time courses for a given area of 3 x 3 voxels (21 x 21 x 5 mm^3). For each subject, a "Peak Height Map" (PH map) was generated. This consisted of assigning to each voxel a BOLD percentage change which consisted of the difference between baseline (post-stimulus time 0 s) and the maximum of the signal between times 2.5 s and 6.0 s. The formula is as follows:

Peak Height at each voxel = [maximal BOLD % change between 2.5 s and 6.0 s - [baseline].

Various reports in the literature have shown that, for other cortical regions, the peak of the BOLD curve was located between 3 and 5 s post-stimulus. This was extrapolated and used in a previous fMRI study [19] using auditory stimuli and yielded consistent results. Hence, the generation of a PH map was to allow for localization of those voxels with a maximal response to the administered stimulus. Upon computing the PH map, selection of specific voxels for analysis was then performed. The voxel with the highest peak height value was selected from the region of the primary auditory cortex (A1) on each side. The region of the primary auditory cortex was determined based on anatomical criteria. The medial part of Heschl's gyrus, or transverse temporal gyrus, identified on the subject's high-resolution anatomical MR images obtained, was considered to be the site [5,38]. The voxel with the highest Peak Height on each side for each delivered tonal stimulus for each subject was selected. This gave a total of 1 voxel/side x 2 sides/subject x 2 different tones/subject (2 kHz and a higher frequency) = 4

voxels/ subject. The BOLD change event-related curves (figure 10) were then calculated and visualized for a 3 x 3 voxel matrix around the chosen voxel (in the center).

b) Analysis using a Statistical Map

As in the previous analysis, the first volume was discarded. The images were subjected to processing for smoothing. The raw images obtained were subjected to a statistical analysis and the generation of a statistical map. This consisted of performing, for each voxel in the acquisition images, a statistical test of inference. This is akin to a paired t-test, but considers the Gaussian mathematics involved [39]. The voxels where the maximal change from baseline was statistically significant were displayed, visually and numerically. The resulting statistical map visually resembles the PH map. Selection of voxels with the maximal cortical response was then carried out in the fashion described above. The selected voxels were then subjected to a program, which plotted the event-related curve for each voxel and a 3 x 3 voxel matrix around it.

A point regarding terminology is noteworthy. For the purposes of the ensuing results and discussion, it is worthwhile to differentiate between **acquisition noise**, which has been extensively described thus far, and what we will henceforth refer to as "**data noise**" to describe background noise inherent to many experimental measurements. In our case, "**data noise**" relates to background magnetic noise when measurements of changes in the magnetic gradient are being carried out.

RESULTS:

Of 12 subjects enrolled, the scanning and data acquisition was completed for eight. The following table outlines all 12 subjects and their characteristics.

Subject		Age/sex	Frequencies Delivered (kHz)		Reason for exclusion
1	J.P.	31 M	2	11.5	
2	M.H.	28 M	2	6.2	
3	C.G.	29 F	2	8.3	
4	D.S.	34 M	2	11.5	
5	S.S.	28 M	2	8.7	
6	A.Y.	29 M	2	6.2	
7	C.L.	65 M	2	6.7	
8	R.G.	38 F	2	11.2	
9	I.K.	26 F	-	-	Claustrophobia
10	D.Y.	39 F	2	8.1	Mainframe computer crash. Unretrievable data
11	G.S.	36 M	2	8.2	Technical Obstacles Post-scan
12	S.F.	30 M	2	11.0	Mainframe computer crash. Unretrievable data

Data selection was performed according to the above method. When looking at the peak height map or the statistical map, the points of maximal cortical response were not located in the area of the primary auditory cortex. In addition, these points were not clear maxima demonstrating a significant increase in activation in a specific voxel or area compared to adjacent regions. In sum, this pointed to a noise pattern (i.e. magnetic noise, not to be confused with acoustic noise). There were no consistent maxima in the area of the primary auditory cortex. Event-related BOLD curves were generated for the voxels with the maximal cortical response within the region of the primary auditory cortex, as outlined above. These revealed significant oscillation with marked dips below baseline,

highly suggestive of an irregular data noise pattern. No discernible shape or similarities among curves was noted. An example is shown in Figure 11.

DISCUSSION:

A study using fMRI with tonal auditory stimulation had been contemplated in evaluating patients suffering from unilateral tonal tinnitus. The validity of fMRI in the evaluation of the cortical response to tonal stimuli had not yet been established. The need to demonstrate this validity and to establish norms for these cortical responses in normal individuals sparked our study. The purpose of the study was therefore to serve as a template in the evaluation of patients with tinnitus, or other pathological states affecting the auditory system, the pathophysiology of which remains elusive to the clinician. Examples include ototoxicity, sudden sensorineural hearing loss, noise-induced hearing loss, patients post acoustic neuroma resection, and patients with cochlear implants. The choice of stimuli delivered in the study is a reflection of the origin of the study. A population of patients with tonal tinnitus had been previously identified. The pitch of the tinnitus had been determined. It was elected that these patients would undergo fMRI evaluation using pure tone stimuli of the same frequency as the patients' tonal tinnitus. Therefore, in determining the frequency of pure tone stimuli in our study, we attempted to match those frequencies in the evaluation of normal subjects, in the hope of later using this study group as a control group in a study of tinnitus patients. For this reason, a frequency ranging among subjects between 6200 Hz and 11500 Hz was delivered, in addition to a standard frequency of 2kHz. The difference in the cortical response to these different stimuli is highly unlikely to be significant, as the one octave difference is not enough to alter the response to a considerable degree. Previous studies have reported a change in the location of the maximal cortical response in the primary auditory cortex, depending on the stimulus frequency. Two frequencies one octave apart would be

expected to generate cortical responses whose maxima are 2-3 mm apart [40] as demonstrated by electrophysiologic measurements of evoked responses with a high degree of spatial resolution. fMRI studies, being at their beginning, have yet to achieve this degree of resolution. There are studies on tonotopy currently under way attempting to generate a map defining areas of the auditory cortex responding to specific tonal frequencies.

As was pointed out previously, a given Hearing Level (HL) corresponds to a different Sound Pressure Level (SPL) for each frequency. It could therefore be argued that the pure tone stimuli delivered at 90 dB SPL should have been delivered at 90 dB HL. This would likely have been more accurate in evaluating the cortical response. However, as shown in table 1, the difference between SPL and HL for the higher frequencies, as those used in our study, is in the range of 10 to 15 dB SPL, and this, at a 0 dB HL level. This difference narrows as the amplitude increases, for example to the 90-dB range [1]. In addition, this difference, even at a level of 15 to 20 dB is not sufficient to create a significance inter-subject variation in the cortical response [41]. Furthermore, the selection of subjects was such that normal hearing, as defined clinically and audiologically, encompassed a HL from 0 to 20 dB. Therefore, differences of up to 20 dB HL are deemed insignificant and consist of a variation among individuals, rather than an abnormal level. The fact that frequencies delivered were not adjusted to a Hearing Level scale could, nevertheless, be a source of data noise. However, if one looks at the 2 kHz frequency delivered to all subjects, this source of error is eliminated. Indeed, in the case of the 2 kHz stimulus, the same stimulus, at the same intensity level – 90 dB SPL or 98 dB HL [1]– was presented to all subjects. Nevertheless, the results showing BOLD curves

of cortical response to the 2 kHz frequency presented across subjects are not consistent. Taking into account the above considerations, it is safe to conclude that the lack of a significant trend and consistent results is not attributable to the use of SPL instead of HL in stimulus delivery.

Two different methods of data analysis were used. The initial method projected was similar to methods used previously [19]. This involves the generation of the Peak Height (PH) map. There were limitations to this method. Once the PH map is generated, selection of the specific voxels is needed. The basis of selection is the voxels with the maximal cortical response as manifested by the % increase in BOLD. The problem encountered is that the shapes of the curves obtained for the voxels with maximal stimulation was a "sawtooth" configuration with marked increases and dips (Figure 11). Occasionally, adjacent voxels would demonstrate BOLD event-related curves with a smoother, more consistent shape with one maximum. However, these voxels were frequently outside the area of Heschl's Gyrus. After numerous attempts at finding a consistent pattern, we were unable to follow a procedure for selecting the maxima which was consistent and reproducible, and which demonstrated curves with a shape rather than data noise. We therefore redirected our efforts toward another method of analysis. Our approach was to subject the raw data to the generation of a statistical map, as outlined above. However, a similar pattern of data noise was noted on examination of the event-related curves generated.

The method used, devised by Belin et al.[19], is aimed at measuring the BOLD response over time to a single auditory stimulus, while minimizing the problem of interaction with acquisition noise. The use of a long TR interval (10 s) between volume

acquisitions was combined with a stroboscopic method in an attempt to reconstruct the curve of BOLD event-related response as a function of time. The rational behind this method is that the long time intervals between acquisitions allowed for return to baseline or near-baseline of the cortical response to one acquisition before the next acquisition takes place. The advantages of this manipulation have been outlined previously [25] and the results of Belin et al. [19] and others [42] were conclusive in showing a consistent bell-shaped curve of the event-related response to a single stimulus. Their findings corroborated the previously proposed notion that BOLD signal changes in response to acquisition noise follow the same pattern as responses to auditory stimuli. That is, the BOLD signal change returns to baseline within 8-9 seconds. Using the same method, one would therefore expect a similar result.

There are a few differences in this study. First are the sampling intervals, which, in our study were 0.5 seconds or 1.0 seconds apart (at 0 s (no stimulation), 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 6.0 seconds), compared to one-second intervals used previously. Second is the use of a pure tone stimulus. Third is the use of two different stimuli.

The sampling interval does not present a significant modification except to permit a more detailed description of the BOLD response curve. We were interested in the portion of the hemodynamic response around the peak response, which is known to be around 3-5 seconds [19,24,25]. For this reason, we elected to examine the BOLD response between 0 and 6 seconds rather than between 0 and 9 seconds, as in the study of Belin et al. [19].

The use of pure tone stimulation is an important change, which may be a factor responsible for the results obtained as they compare with previous findings. It has been

shown that pure tones stimulate a smaller region of the auditory cortex as compared to wide band or complex sounds [43]. Indeed, pure tones activate the primary auditory cortex whereas the additional activation of association areas is prompted by more complex, more "natural" sounds. Interestingly, the cortical response to acquisition noise has also been demonstrated to be restricted to the primary auditory cortex [26,44]. While this paradigm has been shown to minimize the interaction of the cortical response to scanning noise and the response to acoustic stimulus in the primary auditory cortex [19,24,25], wide band noises were used in the stimulation, as opposed to a pure tone. It is difficult to predict the outcome of the event-related response to pure tones, as this has never been attempted. fMRI studies using pure tones have been published. However, the methodologies used were block designs [45], or average single trials [46]. Although theoretically the experimental protocol we have used minimizes the interaction with scanning noise, there is certainly a possibility, as previously mentioned, that this interaction is occurring and influencing the measured response to stimulus.

The effect of delivering two different stimuli, which in our case were pure tones at two different frequencies, is dividing the total data acquired during scanning by two. Therefore, for each stimulus, the cortical response is measured twice less frequently than if only one stimulus was delivered. To compensate for this, two scanning sessions were performed to multiply the data acquisitions. However, this is not optimal, as it doubles the scanning time, which in turn causes the subject to have a tendency to increase motion and decrease attention to the auditory stimuli. Furthermore, the data obtained from the two scanning sessions cannot be combined in an additive fashion. Therefore, it is not as

powerful. The result is that the cortical response does not manifest itself as strongly and intensely compared to the surrounding noise (magnetic field artifact).

Finally, perhaps the most important factor leading to the results obtained is the spreading and distribution of data acquisition over too many parameters. We attempted to define the cortical response both spatially, in its location within the cortex, and temporally, by measuring the response at eight different time intervals. Consequently, the data acquired was "spread too thin". For example, if we had attempted to define the spatial component only and measured the same 128 acquisitions, but at only one post-stimulus time interval, we would have been much more likely to obtain a visible and measurable response for this particular delay. Other scanning sessions could be performed on the same subject, each measuring the response at a different post-stimulus time. The attempt at extracting both spatial and temporal information, combined with the use of pure tone stimuli, which, as mentioned above, tend to stimulate more limited areas of the cortex, accounts, in large part, for our obtaining results showing a great degree of data noise artifact.

As will be mentioned below, current studies are under way attempting to delineate a tonotopic map of the auditory cortex (Personal communication P. Belin). A tonotopic map is a map of the auditory cortex indicating the location within the cortex that responds to a specific pure tone. The same methodology as the one used in this study is being applied. Pure tone stimulation is being used, and the investigators are obtaining very clear results of specific areas of cortical activation in response to specific pure tones delivered.

STUDY LIMITATIONS

Although the prospective design of the study and its goal are strengths, several limitations are noteworthy. These pertain mainly to the methodology and its feasibility. The need for a multimillion-dollar magnetic resonance imager has a direct bearing on its availability. The shared use and restricted scheduling were definitely obstacles. Recruiting volunteers to be subjected to an hour-long claustrophobic environment without compensation demands a great degree of good will on the part of the subjects. Equipment such as headphones, amplifier, and computer for sound delivery is shared. This, in addition to limiting availability, frequently engenders technical problems. Problems related to the mainframe computer where raw data from the MRI machine is sent and which is further used for processing and analyzing data, is completely out of the experimenter's control. As any mainframe computer equipment, it is subject to errors and crashes, which have grave consequences such as the irretrievable loss of raw data, as outlined in the results. Finally, the financial costs of this project were considerable.

FUTURE DIRECTIONS

Current work is under way, which takes into consideration some of the drawbacks of this study alluded to above. Various studies have been published examining tonotopy in the auditory cortex using fMRI [47,48]. Currently, a study using pure tone stimulation is being undertaken in this context (Personal communication P. Belin). Various frequencies are being used in order to define a tonotopic map of the auditory cortex. However, the data acquisition is concentrated and clustered in order to maximize the amount of data obtained for a given frequency. Post-stimulus time is constant at 6 seconds, where it is known that the BOLD response will be maximal. The results obtained are quite significant, showing intense areas of cortical activation within the primary auditory cortex compared to the surrounding regions (Figure 12).

The time course of the response is not being looked at. However, if one chooses to examine this parameter, it is relatively easy to achieve. As mentioned above, in the discussion, one would simply repeat a similar scanning procedure on the same subject (for example the scanning procedure mentioned here in the study on tonotopic maps), using the same pure tone stimulus, while varying the post-stimulus scanning time at each scanning session. This would not use the stroboscopic design used in this study, which varies the post-stimulus time for each one second scan acquisition, but rather a different scanning session would be performed each time with one constant post-stimulus scanning time. If one chooses to evaluate five different post-stimulus time intervals (e.g. 3, 4, 5, 6, and 7 seconds) then five different scanning sessions would be required of the same subject, each of them taking 128 acquisitions, or more, at one specific post-stimulus time. For example, the post-stimulus time would be 3 seconds for the first session, then a few

days later a second scan would be performed using a post-stimulus time of 4 seconds, and so on.

SUMMARY

Functional MRI is a useful imaging modality offering an unparalleled view into the neurophysiology of cognitive and sensory processes. The study of the cortical response to pure tone stimulation provides an insight into the auditory system. While the results of this study support few conclusions regarding the event-related activation of the auditory cortex to pure tones, much can be learned. The information acquired will contribute significantly to further refining the methodology and devising a study protocol for the study of pure tone stimulation using fMRI. Furthermore, it provides invaluable information regarding the difference between pure tone stimuli and complex sounds when using an event-related design.

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Figure 1
Vertical frontal section of the ear demonstrating the auricle, external auditory canal, middle ear (tympanic cavity), and inner ear, including the cochlea and vestibular organs. The cochlear duct is a membranous structure (blue) within the cochlea. The arrows indicate the course of the sound waves within the chambers of the inner ear.

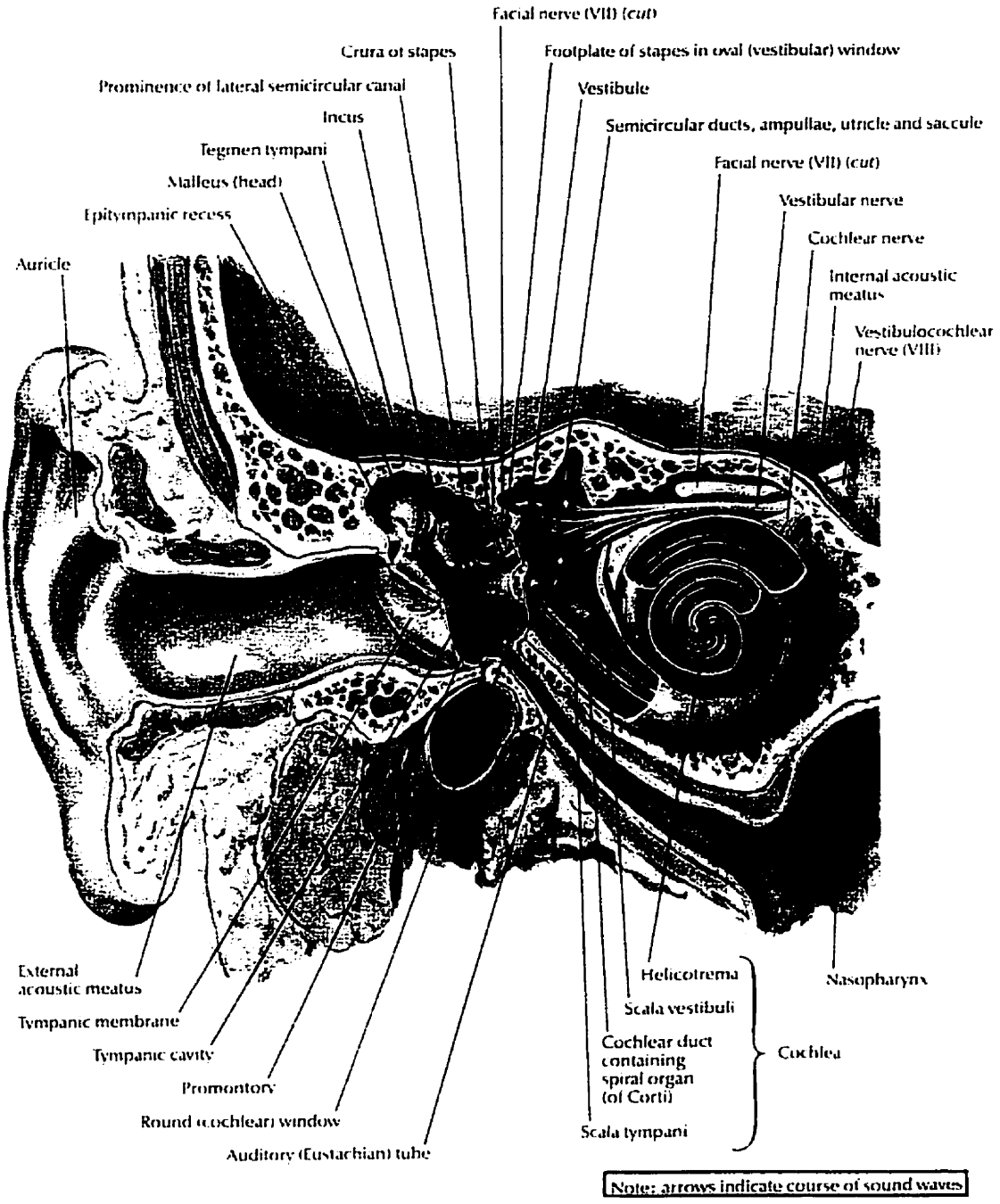


Figure 2
Cross-section of cochlea. Note the organ of Corti within the cochlear duct. As a fluid wave proceeds through the scala vestibuli and scala tympani, the basilar membrane moves with the fluid motion, taking with it the organ of Corti resting upon it. This generates a movement of the stereocilia, which are in contact with the tectorial membrane. This, in turn, opens ion channels, leading to intracellular potential changes which eventually result in an action potential in the nerve fibers supplying the hair cells.

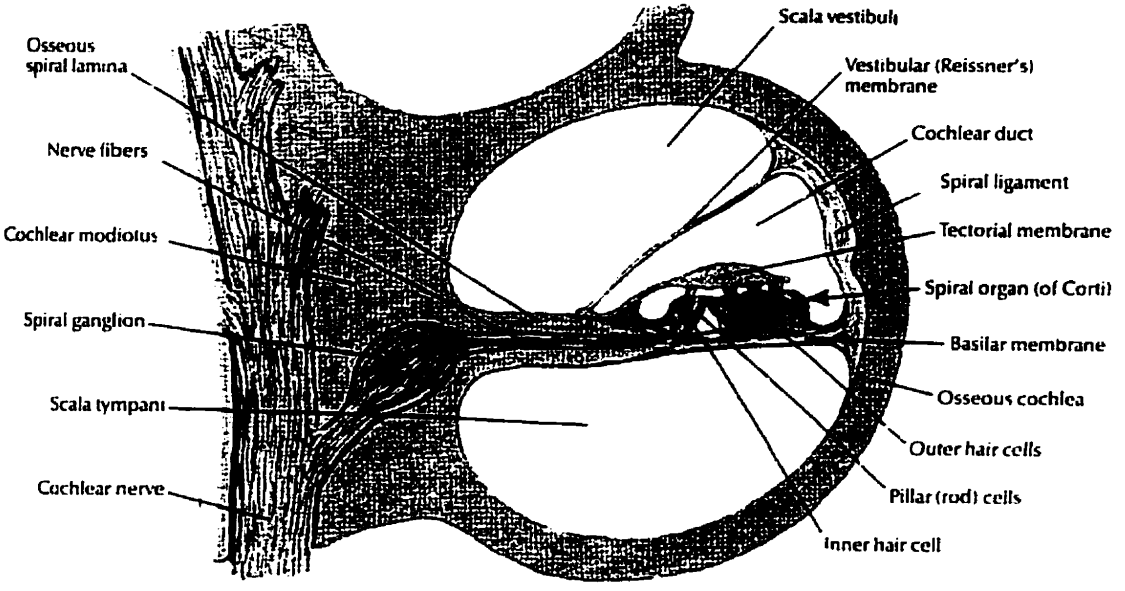


Figure 3
Afferent auditory pathways. This is a highly schematic representation of the ascending auditory pathways from the left cochlea to the auditory cortex. No attempt has been made to accurately represent the disproportionate innervation to the contralateral auditory cortex. **CN**=cochlear nucleus; **SOC**=superior olivary complex; **IC**= inferior colliculus; **MGB**=medial geniculate body; **AC**=auditory cortex.

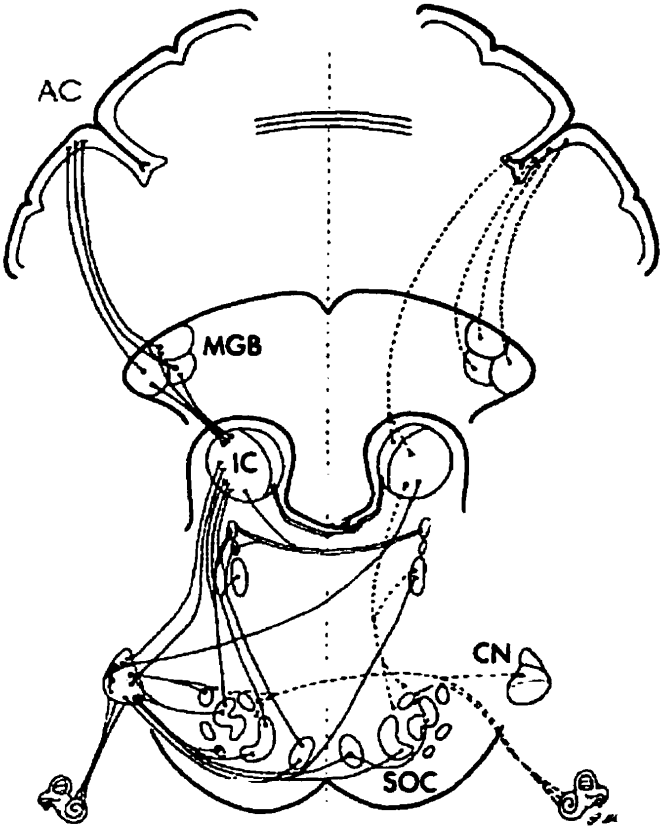


Figure 4
Auditory cortex. Areas 41 and 42 denote the primary auditory cortex while area 22 in this figure denotes the secondary or association auditory cortex.

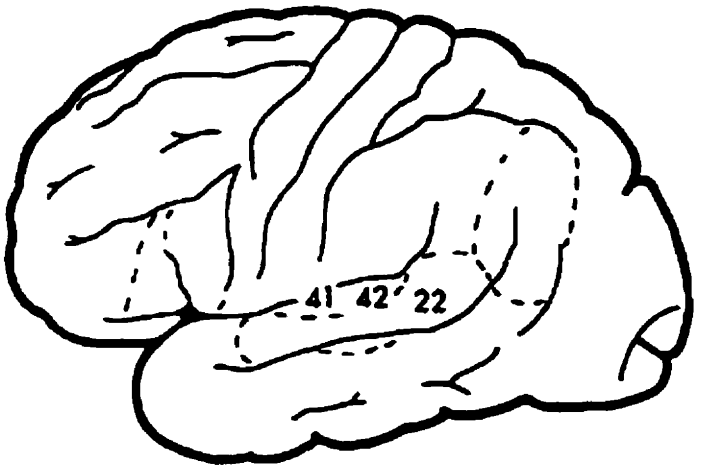


Figure 5
Example of the visual depiction of the functional MRI response. **A**. The color-coded map depicts a color for each 4mm x 4 mm voxel. The color is representative of the percent change in BOLD. **B**. The color-coded map is superimposed on the corresponding anatomical MRI image to delineate the anatomic location of each voxel. The color scale represents values of % change in BOLD signal from 0.12% to 2.0%.

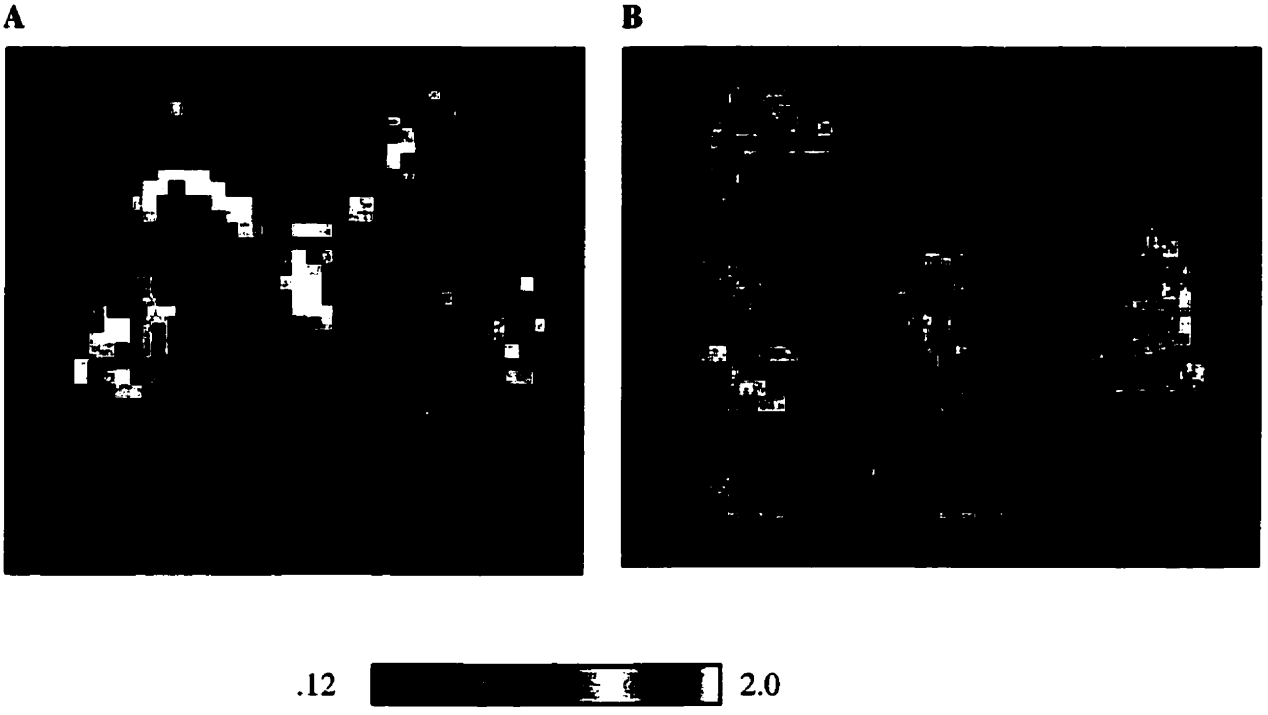
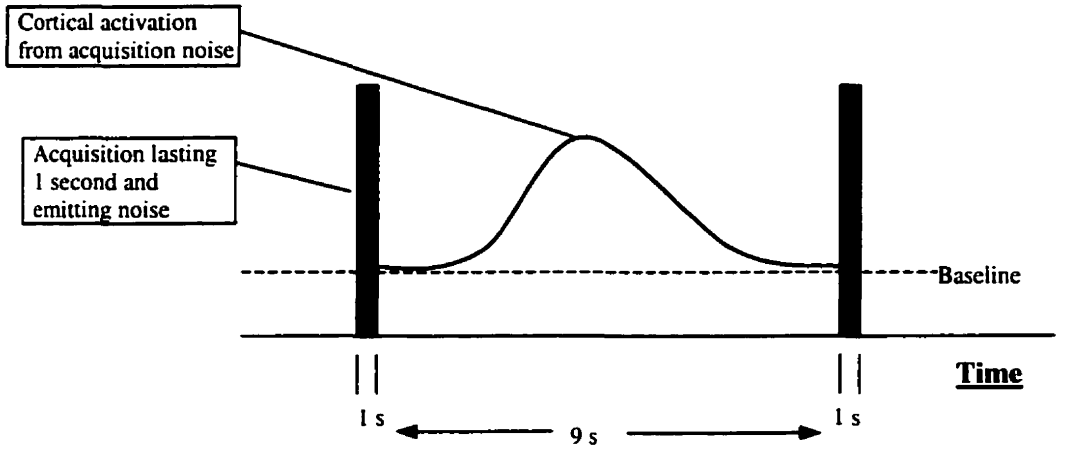


Figure 6

6-A. Schematic depiction of the timing of image acquisition and its attendant cortical activation. The vertical bars represent functional image acquisition. This lasts one second, during which an echo-planar imaging noise (acquisition noise) is emitted by the MRI. This sound engenders a cortical response, depicted by the curve, which returns to baseline within the 9-second time interval prior to the following acquisition.



6-B. As a result of this return to baseline, an auditory stimulus delivered at time "x" seconds, within the time frame between two acquisitions would generate a cortical response free from interference by the response generated by acquisition noise. Please note that this schematic is not to scale and does not attempt to accurately reproduce the shape of the curve of activation nor any values related to cortical activation.

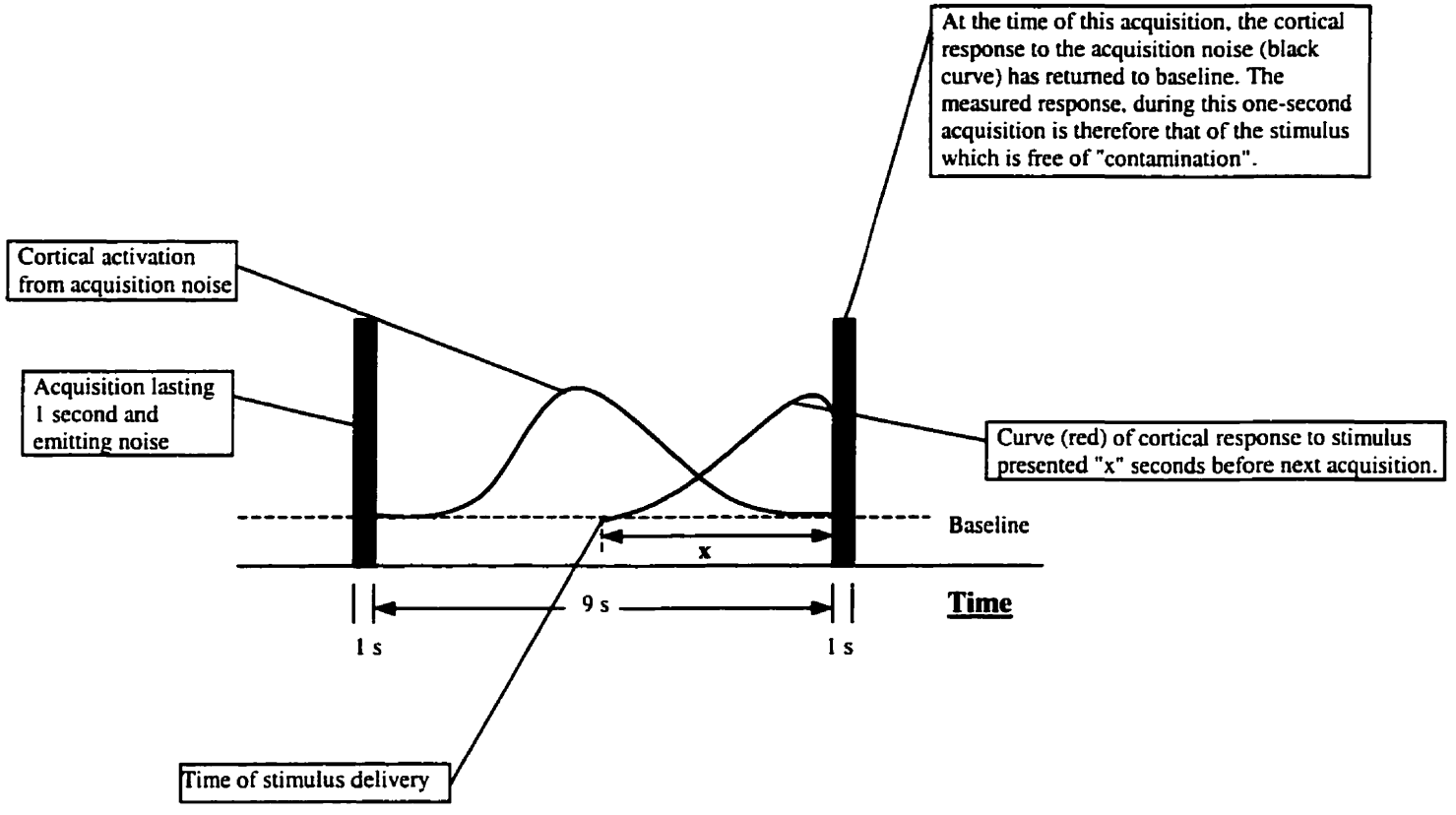


Figure 7
Design of event-related auditory experiment. The top black bars indicate stimulus delivery. The hollow squares at the undersurface of the time line indicate scanning acquisition of the 10 functional slices every 10 seconds.

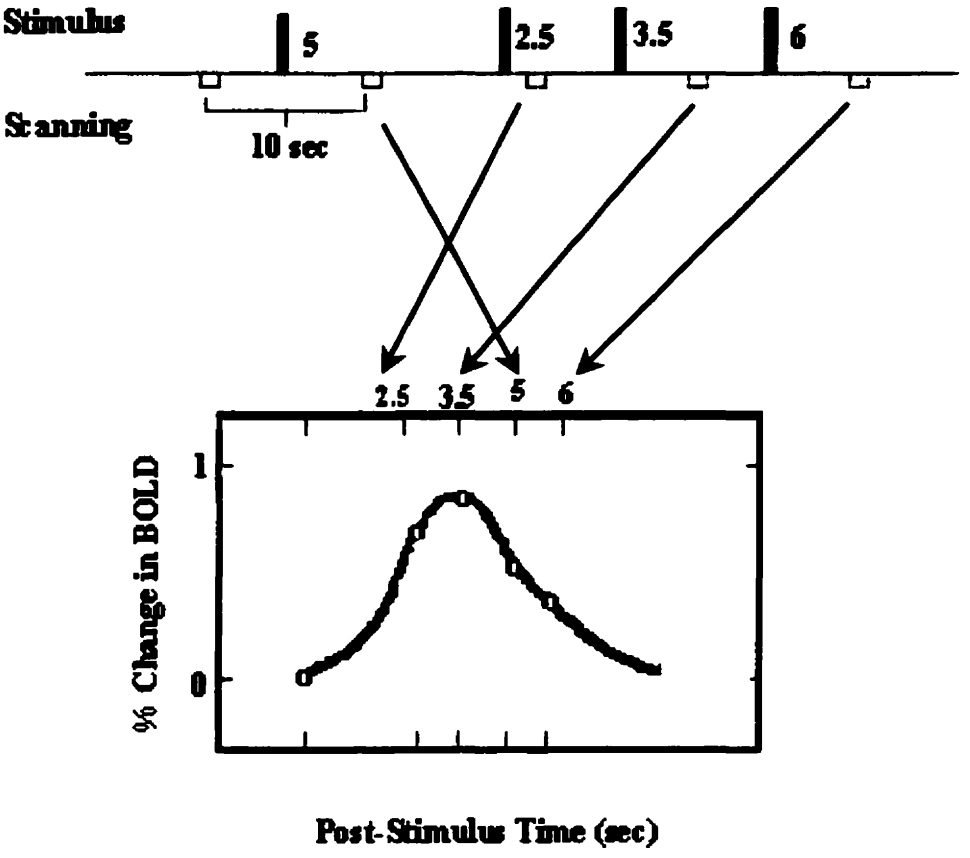


Figure 8
Anatomical localization of functional volume acquired. A T1 sagittal slice of the subject's brain (left) is used to center functional volume (right) around Heschl's gyrus (red cross), in the orientation of the Sylvian fissure).

Figure 9
Schematic depiction of continuous sampling consisting of non-clustered acquisitions. Acquisitions, rather than being clustered and spaced at 10 s intervals as in Figures 6-A and 6-B, are continuously presented at regularly spaced intervals of 2-4 seconds. The cortical response to acquisition noise is represented by the curves following each acquisition.

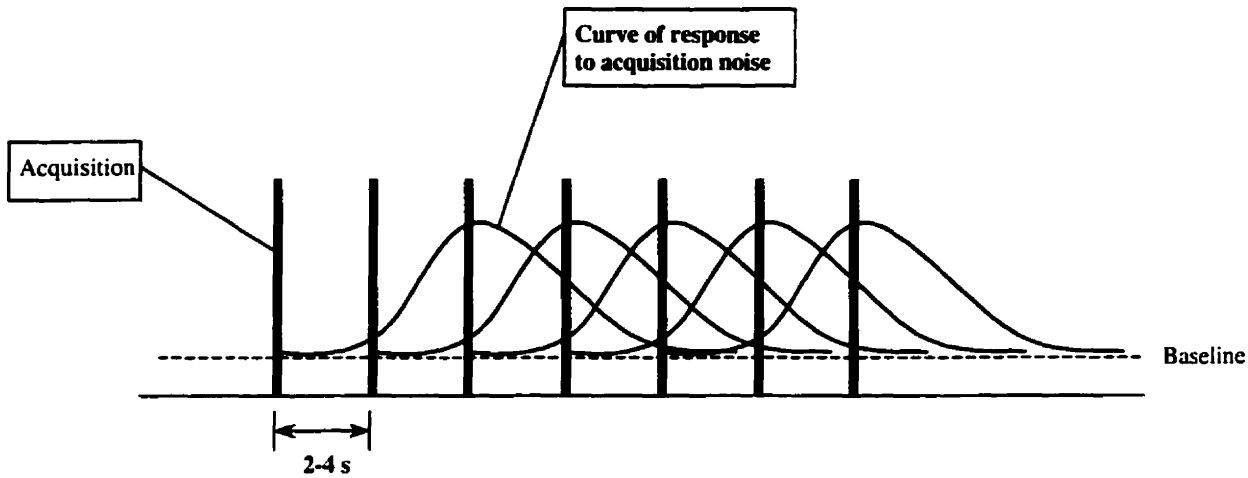


Figure 10

A. Example of a Peak Height Map of one of the 10 slices acquired for a subject post-stimulus. Each voxel is 4 x 4 x 5 mm^3. The colors are a visual representation of the value of the peak height for a given voxel. **B.** The post-stimulus percent BOLD change time course for each voxel in a 3 x 3 voxel array (21 x 21 x 5 mm^3) in an area of the auditory cortex (for a larger magnification of this figure, refer to figure 11). **C.** These "BOLD curves" have on their *x*-axis time 0 s to 10 s, and the y-axis represents the percent change in the BOLD from baseline. The baseline is the blue point marking the 0 s point.

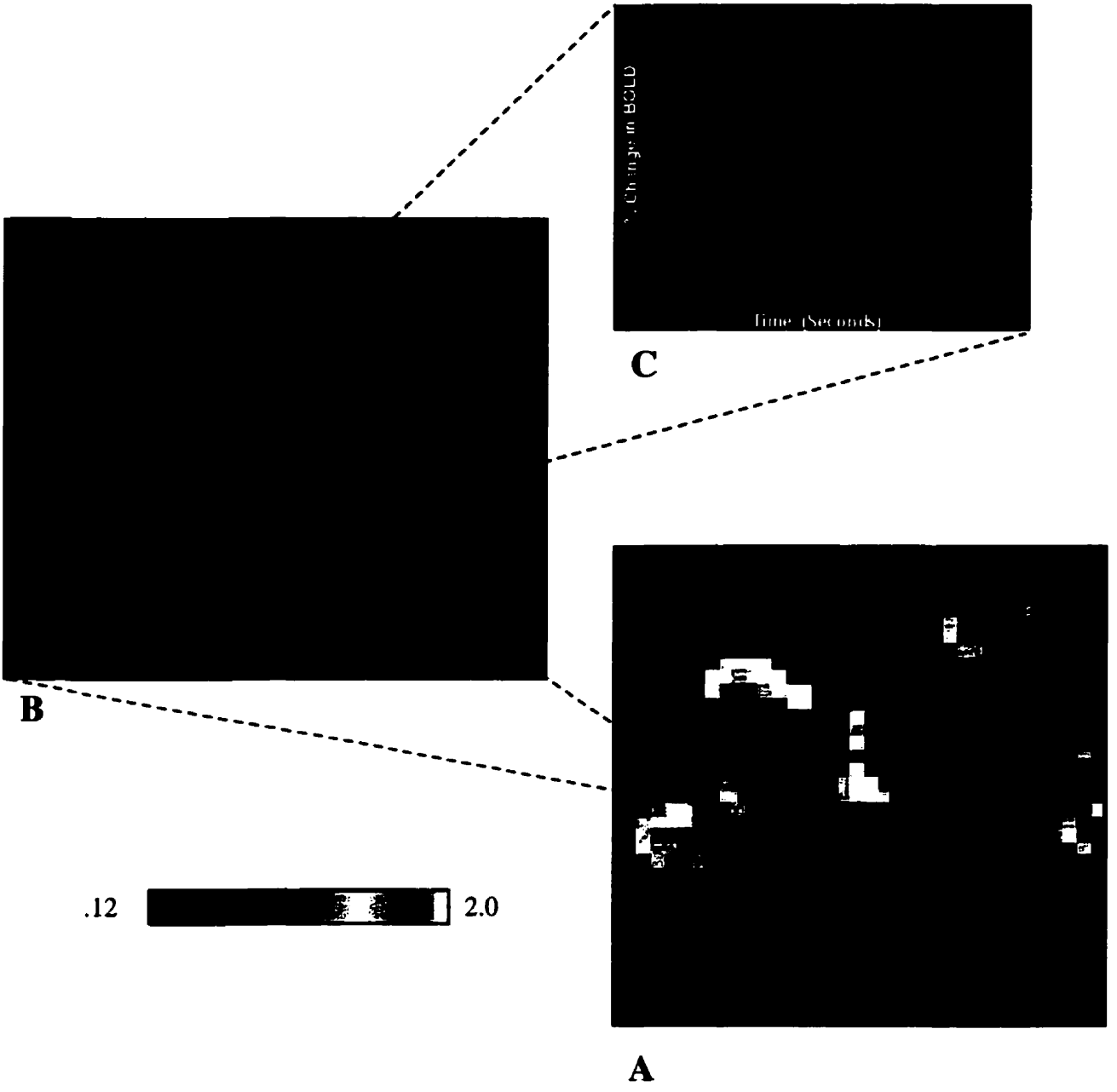


Figure 11
Example of BOLD event-related curves generated from points of maximal stimulation selected from the primary auditory cortex. The red curves represent the response to the 2kHz stimulus, while the yellow represents a higher frequency, 11.5 kHz in this case.

Figure 12
Example of cortical activation seen in response to a pure tone stimulation. Note the area of increased intensity within Heschl's Gyrus, in comparison to the surrounding cortex. A clear intense stimulation is observed. **A**. Functional scan; **B**. Anatomical scan; **C**. Both scans superimposed. Note the position of the purple cross hairs in Heschl's gyrus. Courtesy of P. Belin.

A **B** **C**