

Noise is all around us, whether we pay attention to it or not. We do not often realise that our early ancestors were initially quite unable to hear. Our sense of hearing only arose in an indirect way and its individual components have undergone several changes in function

over the course of more than 400 million years of evolution. Our author BENEDIKT GROTHE and his colleagues at the MAX PLANCK INSTITUTE FOR NEUROBIOLOGY are trying to comprehend the interplay between new developments and changes in function of existing structures by means of a comparative research project.

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As modern town dwellers, we have lost the sense of what a natural summer's night means: noise, or more accurately, a wealth of noisy information which helps secure the survival of many species. In tropical forests, it can sometimes be almost deafening. However, only two groups of animals are responsible for making this noise: insects and vertebrates. Both groups possess acoustic communication systems which have evolved independently of one another. In insects, hearing probably arose in connection with communication within the species – generally with the selection and spatial localisation of a mating partner. Hearing not linked to reproduction tends to be the exception here. For example, some insects can hear bat echo-location calls and attempt to escape from them. Certain parasitic flies find their hosts by recognising and localising the hosts' communication sounds. Whether hearing in vertebrates was developed primarily

to detect prey and avoid predators or as part of a communication system, we do not know – not least because our concept of the evolution of hearing is still changing. Although the object of intense research for well over a century, it has again changed in recent years.

SOUND WAVES MOVE HAIR CELLS

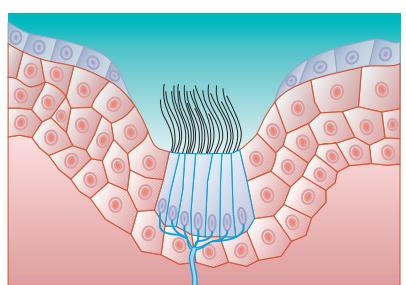
Sound is caused by a vibrating object (transmitter) which sets in motion molecules in the transfer medium, air or water, for example. The sensory cells incorporated into the hearing apparatus must therefore be stimulated by mechanical movement. The mechanoreceptors in-

volved in hearing are hair cells in which the auditory stimulus causes the tiny hairs to deflect in relation to the cell body. This produces electrical potentials in the hair cell which are transmitted to the auditory nerve.

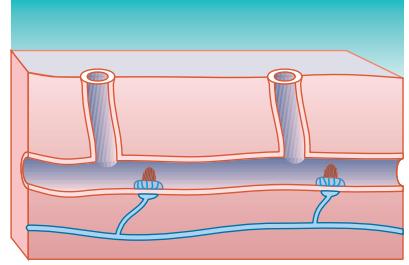
Hair cells are phylogenetically old. Even the first jawless fish had a lateral line organ in which bundles of hair cells could measure local water movements and provide the fish with important information for navigating the water (fig. 1).

Our labyrinthine system also operates according to the same principle: changes in body position accelerate the water column in the semicircular canals. This deflects the tiny hairs and stimulates the hair cells. Both the lateral line organ and the labyrinthine system are old and can be traced back over 440 million years through fossils (fig. 2). So hair cells which, in theory, our ancestors could have used to hear, have been around for a long time – yet our ancestors still could not hear.

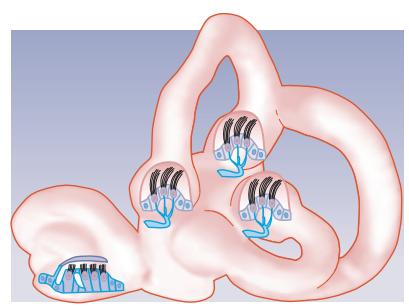
In order to hear, a sound wave must deflect the tiny hairs in relation to the hair cells. The fish and each of



A



B



C

Fig. 1: Hair cells – here on the surface of a zebrafish larva – detect mechanical stimuli triggered by movement or sound and transmit them to the nervous system as a signal.



Photo: T. NICHOLSON, J. BERGER, MPI FOR DEVELOPMENTAL BIOLOGY

Fig. 2: From current detector to sound detector. In the external lateral line organ (A) the hair cells lie exposed on the skin and are deflected, and thus stimulated, by local water currents. In modern-day fish, the bundles of hair cells lie in fluid-filled channels which communicate with the surrounding water via pores (B). The semicircular canals of the labyrinthine system are completely disconnected from the outside world so that only internal fluid movements triggered by the body's movements can cause the hair cells to be stimulated (C). In the auditory organ, sound waves stimulate the hair cells via movements of the organ of Corti (bottom left).

its cells consists to a large degree of water, and the tiny hairs are surrounded by fluid. Underwater sound will make the water, together with the fish, hair cells, and tiny hairs vibrate equally. The fish is virtually transparent for the sound and therefore cannot hear it – at least, not without the aid of a mechanical trick. This consists of introducing into the body a medium in which sound moves at a different speed – like gas, for example. In air, sound waves travel five times slower than in water. Consequently, hearing could only develop in fish with a swim bladder.

Swim bladders are, in all probability, descended from lungs which the early freshwater fish 400 million years ago needed to survive periods of severe lack of oxygen in water by also breathing air. In many cases the lung subsequently became superfluous and developed into the swim bladder which helps the fish to adjust the depth at which it swims in water. This created one of the pre-conditions for hearing. However, the problem of transmitting the vibration of the swim bladder to the inner

ear still remained. And that is cer-

tainly no small matter: when air-borne sound is transmitted from gas to a fluid (like that in the inner ear), more than 98 percent of the energy is reflected at the air-water interface and thus not transmitted further. To overcome this problem, one needs an impedance matching device. This means that the comparatively large, yet weak movement of the air molecules in the swim bladder must be transmitted to a small, yet strong movement of the fluid in the inner ear.

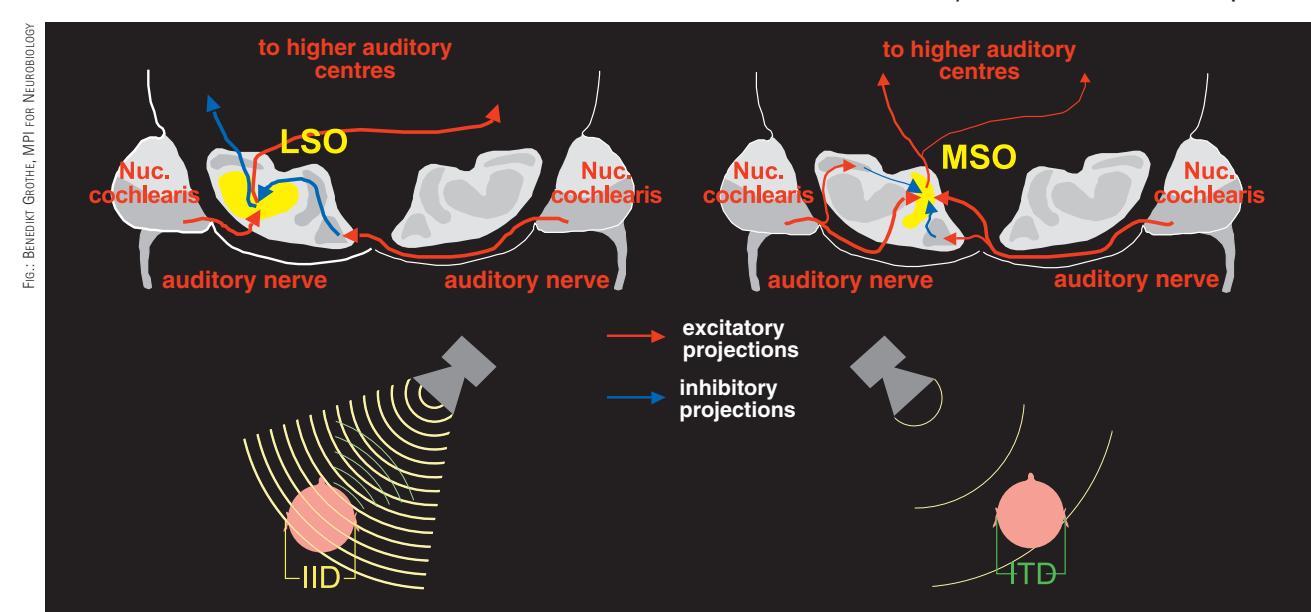
DRUM FOR AN AMPLIFIER

In many fish, this task is performed by the "Weber's ossicles", descendants of ribs of the cervical vertebra. So, fish acquired hearing only by a very indirect evolutionary route. Fish whose ancestors never lived in oxygen-deficient waters – such as the cartilaginous fish (sharks, for instance) – or fish which never left the tropical oxygen-deficient waters and therefore had to rely permanently on lungs as an additional respiratory organ – such as the lung fish – were not able to de-

velop an efficient sense of hearing. Our ancestors who needed lungs to settle the land some 260 million years ago could not hear water – or airborne sound either – and so, consequently, they probably did not make a sound. It was initially quiet on land.

Terrestrial vertebrates certainly do not have the problem of vibrating in phase with airborne sound but they too need effective impedance matching devices. The airborne sound has to be transmitted to the fluid in the inner ear. Yet early vertebrates had no suitable impedance-transforming anatomical structure. Only 120 to 140 million years after vertebrates began populating the land did the eardrum and middle ear develop, allowing for impedance transformation similar to Weber's ossicles in fish. Sound reaching the eardrum could now be transmitted to the oval

Fig. 3 Simplified illustration of the principles of sound localisation. High frequency sounds are reflected by the head creating an interaural intensity difference (IID) (left). These are processes in the lateral superior olive LSO. Low frequency sounds are not reflected. Interaural time differences, ITDs, are the only parameter by which they can be localised. They are encoded in the medial superior olive MSO.



window of the inner ear via the ossicles of the middle ear which act as a lever.

Frogs, reptiles, and birds have one bone in the middle ear which corresponds (is homologous) to our stapes. Mammals, however, have three auditory ossicles: hammer, anvil, and stapes. For over a hundred years, most experts believed that the early amphibians had "invented" a middle ear with one auditory ossicle and that reptiles had inherited this. Mammals, as descendants of reptiles, then incorporated two additional bones into the middle ear. This idea of two additional auditory ossicles being subsequently incorporated without adversely affecting the manner of operation in the process – and thus significantly disadvantaging selection – has always raised fundamental problems. The availability today of a large number of well-preserved fossilised skulls permits an almost perfect reconstruction of the evolution of the inner ear. It suggests

that the middle ears of land vertebrates arose in a number of ways, independently of one another, and the mammalian three-membered middle ear developed at one go and not via a precursor with only one bone in the middle ear. Why amphibians, reptiles (from whom birds inherited the middle ear) and mammals "invented" a middle ear at virtually the same time remains a mystery. A second interesting discovery is that our ancestor's brain did not increase in relative volume, or only marginally, from the early Devonian period 400 million years ago until the point when the middle ear was established. With this came the relative increase in size of the forebrain in mammals and, some time later, also in birds. Whether this link was causal or not must remain open to debate. In any case, whole new dimensions in intra-species communication opened up for both mammals and birds, equipped as they now were with higher neuronal processing capacity and, at the same time, the ability to hear airborne sound. This found its culmination in the development of human speech.

WORLDS OF SOUND WITH HIGHS AND LOWS

In mammals, the sense of hearing which includes not just the ear but a processing apparatus behind it in the form of the ascending auditory pathway, developed under different evolutionary constraints than in other land vertebrates. Since the middle ear of reptiles and birds consists of only one single auditory ossicle, its mechanical options are limited and it cannot transmit high frequencies. Reptiles and most birds cannot detect frequencies above 4 to 6 kHz. Only a few specialists, such as barn owls, have optimised the single-membered middle ear and hear frequencies up to 10 kHz. The more complex mechanism of the mammalian three-membered middle ear

was, from the outset, far better equipped to transmit high frequencies. Reconstructions of the middle ear of early mammals barely the size of a shrew suggest that these animals could hear high frequencies beyond 40 kHz, yet could barely detect low frequencies below 4 kHz. Even today it is still normal for most mammals to hear sounds up to 40 or even 60 kHz but to have poor low frequency hearing.

Our ability to hear low frequencies (to around 30 Hz) probably represents a secondary adaptation, unlike that of frogs, reptiles, and birds which primarily lived in a "low frequency world". Consequently, mammals' hearing can trace a different evolutionary history. This is also reflected in the way in which our hearing solves or, in the course of an individual's development (ontogeny), learns to solve particular tasks. An example of this is the ability to localise low-frequency sounds or, in other words, determine the direction from which the sound is coming.

High frequencies – sound waves with short wavelength – are reflected even by small objects. This creates an "acoustical shadow" behind the object, an area with greatly reduced sound energy. Low frequencies – sound waves with long wavelength – are, on the other hand, only reflected by larger objects. Small objects do not create a sound shadow for them.

As a rule of thumb, the sound is reflected if the wavelength is shorter than the diameter of the object. For humans, this means that frequencies above 1.3 kHz are reflected, producing a "head shadow"; frequencies below 1.3 kHz are not reflected and no "head shadow" is created. This cut-off frequency is proportionately higher in smaller mammals. We use the "head shadow" to ascertain the position of a sound wave in the horizontal plane. If a sound comes from the front, then it reaches both our ears with the same intensity. How-

ever, if it comes from the side, because of the "head shadow" it reaches the ear which is turned away with considerably less intensity than the ear turned towards it. Differences in intensity can sometimes be so large that the acoustic pressure on one ear is over one thousand times higher than on the other. This is known as interaural intensity difference, or IID for short.

RACE TO THE EARDRUM

Since all mammals can hear comparatively high frequencies, they experience this "head shadow" at most frequencies they can hear, and in some cases (like some bats) across their entire frequency range. Accordingly, the ability to use interaural intensity differences to localise a sound source is also found in all mammals. To our knowledge, this ability can be considered as phylogenetically old – probably as old as the ability to hear airborne sound. Consequently, all mammals so far studied have in their auditory path an identical, comparatively straightforward subtracting mechanism, which determines the intensity difference and assigns it to a position in space (fig. 3).

The ability to hear low frequencies is relatively unimportant for the majority of small mammals. They can manage quite well without it. However, when the dominant dinosaurs died out 65 million years ago, a number of ecological niches became free, and increasingly large mammals developed. For them it is beneficial, especially with an increasing radius of action, to be able to hear deeper frequencies as well, since these carry further. We all know the phenomenon at an open air festival of only being able to hear the bass from a distance and not hearing the high frequencies until we get closer. Low frequency hearing allows wide-range acoustic communication, and predators or prey can be detected earlier.

of which our nervous system is normally capable. We can, for instance, only detect gaps in continuous noise if their duration is at least two to three milliseconds (thousandths of a second), hence significantly longer than ITDs.

A neuronal mechanism capable of operating with extreme precision is required in order to detect interaural time differences. This is anchored in the circuitry and cellular properties of the neurons in the so-called medial superior olive (MSO), one of the early stations on the ascending auditory pathway on both sides of our brainstem. These neurons do indeed display the required sensitivity, although the underlying mechanism has not yet been fully explained. The MSO is particularly striking from an anatomical point of view: firstly because of

Fig. 4: Immunohistochemically marked neurons in the medial superior olive (MSO). Double staining for a dendritic marker (MAP2; blue) and glycine receptors (yellow).

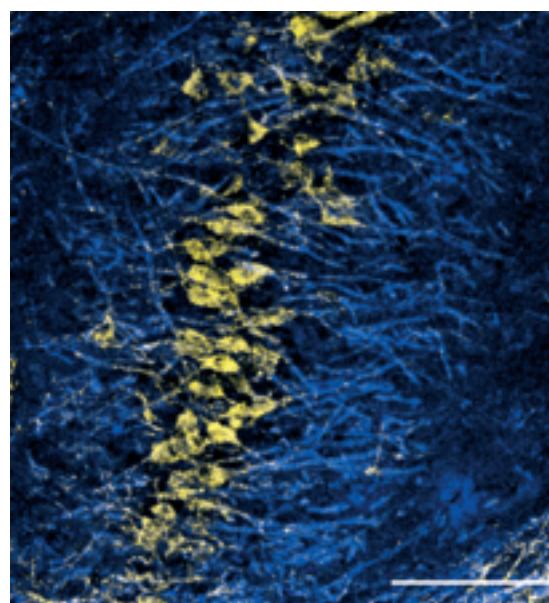


FIG.: BENEDIKT GROTHE, MPI FOR NEUROBIOLOGY

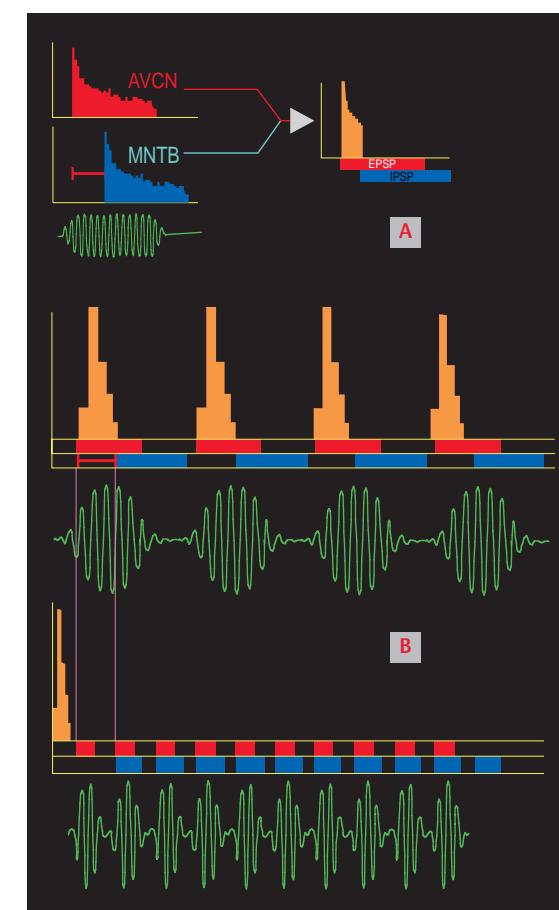


Fig. 5: Interplay between excitation and inhibition in the MSO. A: Both the excitatory (red) and the inhibitory input (blue) are activated by a sound (green). Inhibition is delayed however. This only allows the MSO cell to respond to the onset of the sound stimulus (yellow, the bars represent the accumulated action potential). It is then virtually switched off by the delayed inhibition. B: If a sinusoidal amplitude-modulated sound is presented, the MSO cell responds to each modulation cycle as long as the modulation frequency is low (long period; e.g. 100 Hz modulation frequency). At high modulation frequencies (e.g. 300 Hz), the delayed inhibition overlaps the next excitation. The cell then only responds to the first period.

its bipolar neurons with the dendrites (processes for receiving signals from other neurons) pointing in opposite directions; secondly because of the arrangement of its cell bodies in one single plane (fig. 4). It was initially described only in large mammals capable of hearing low frequencies and reliant on analysing ITDs for sound localisation. At first, using purely anatomical methods, researchers could not find a comparable structure in mammals unable to hear low frequencies like mice or bats.

SEARCH FOR THE RELAY STATION IN THE BRAIN

Only by combining several modern anatomical and physiological methods was it possible to solve the mystery of the apparent absence of an MSO in other mammals: based on the in- and output of neuronal cells, their morphology and cytochemistry, and also their physiological characteristics, it can now be demonstrated that small mammals which only hear

high frequencies – like bats – also have an MSO, regardless whether they use ITDs to localise low frequency sound or not. This obviously raises the question whether the role of the MSO is actually restricted to processing interaural time differences.

The MSO of “ITD users” certainly appears to be modified in two ways: firstly, the cell bodies are only arranged in one plane here (which is why the MSO could not initially be found in other mammals) and secondly, they display a better time resolution.

Using a comparative research approach, we try not only to explain the phylogenetic and ontogenetic processes which played a part in the development of this mechanism, but also to create a basic understanding of the connection between structure and function. This is why in our laboratory, we are examining not only bats that do not use ITDs for sound localisation, but also gerbils which

rely on low frequency hearing and ITD processing.

By using earphones in animal experiments, any interaural time differences between the two ears can be produced, even unnatural ones. With the help of artificially expanded ITDs we were able to show that MSO neurons of bats react to these artificial interaural time differences. However, to affect the response of bat MSO neurons, we had to produce interaural time differences of several hundred microseconds or even several milliseconds, ITDs which naturally never occur in bats (and not even in humans). Because of the bats' small head size, the time difference between the sound reaching the left and right ear can only range between zero (straight ahead) and about 30 microseconds at most (90 degrees to the side in both directions). The time resolution of bat MSO neurons is therefore neither sufficient to encode ITDs relevant to the bat, nor can they do much with the ITDs which we hear. Their neuronal sensitivity to ITDs is obviously an epiphenomenon of their time processing mechanisms which are designed for quite different auditory analyses.

How do MSO neurons operate? We must first begin by saying that there are generally two kinds of input in the nervous system, excitatory and inhibitory inputs. Supraliminal signals which reach a neuron via an excitatory input cause the neuron to produce an electrical signal, the action potential. Signals received via an inhibitory input oppose the excitatory input: they can suppress production of the action potential. An MSO neuron normally receives four inputs, one excitatory and one inhibitory from each ear. Our research has shown that the temporal interplay between the excitatory and inhibitory inputs determines whether and how an MSO neuron responds to an acoustic stimulus.

If we first consider just the input coming from one ear (fig. 5): the excitatory and inhibitory inputs have an almost identical path. They are both active while the sound is maintained. However, the neuronal responses – and this is crucial – occur at different times. There is a 1 to 5 millisecond delay between the excitatory and inhibitory inputs. This causes the MSO cell to be excited briefly at the start of the stimulus (sound on) and to develop an action potential, to then be “switched off” by the delayed inhibition. The delay before inhibition occurs also means that, when the stimulus is switched off (sound off), the cell cannot respond immediately to a second stimulus. Only when inhibition has stopped can the MSO cell be excited again. It follows from this that an MSO cell can only respond to long modulation periods (e.g. low amplitude modulation frequencies). At high frequencies the delayed inhibition overlaps the next excitation. The cell then only responds to the first stimulus period. Neuronal filtering properties like those of the MSO cells play a decisive part in analysing complex signals, such as speech recognition.

So far, we have only considered one excitatory and one inhibitory input coming from one ear. If we add the two inputs coming from the opposite ear, then at first sight it gets really complicated. However, our model calculations and experiments clearly show that, in the MSO, the interaction between all four inputs ultimately produces a basic capability to detect and analyse interaural time differences. The problem, however, that had to be resolved during phylogeny was how to reach the microsecond time resolution necessary to process naturally occurring ITDs.

Our research on MSO cells of mammals which are not “ITD users” shows that inhibitory inputs display a certain inertia. Even stimuli lasting

only several hundred microseconds result in an inhibitory influence amounting to milliseconds. This explains the ITD resolution, for example, of MSO neurons in bats that is of the same magnitude. In contrast, we found a much more precise inhibition with a time course of only a few hundred microseconds in the MSO neurons of gerbils which can hear low frequency sounds and use ITDs to localise them (fig. 6).

Our hypothesis is that it was precisely this improvement in the time course of the inhibitory inputs which was crucial for the evolution of ITD processing. In fact, our experiments indicate not only that the presence of temporally very precise inhibition is necessary to obtain satisfactory ITD resolution, but also that it fine-tunes the ITD sensitivity of MSO cells to the biologically relevant range.

FINE TUNING IN GERBILS

How do the inhibitory MSO inputs of gerbils (or other low frequency hearing mammals including man) differ from those of bats or other mammals which only hear high frequencies? Inhibitory MSO inputs are glycine-based, i.e. inhibition is brought about by the neurotransmitter glycine and accordingly, the membrane of the MSO neurons contains glycine receptors which are anchored there by the molecule gephyrin. The immunohistochemical evidence for these receptors and their anchoring molecules and thus their spatial distribution displays interesting correlations with enhanced ITD resolution (fig. 7): in the gerbil, the glycine receptors are restricted to the cell bodies of the MSO cells. They are only occasionally found on the dendrites.

However, in bats (whose MSO has a time resolution totally inadequate for processing ITDs), opossums or rats, which all cannot hear low frequencies at all or only poorly, these

receptors are found equally on the cell body and along the dendrites of MSO neurons. It is our hypothesis that this extensive distribution results in inhibition being prolonged, whereas restricting the receptors to the cell body shortens it.

Interestingly, we found the distribution of inhibitory, glycinergic inputs in juvenile gerbils before they begin to hear (around 12 days after birth) to be just as diffuse as in adult “non ITD users” such as bats. Only in the days after hearing begins do the inhibitory inputs shift to the dendrites. Interestingly, this does not occur automatically, but is conditioned by experience and can thus be manipulated or prevented. Our interpretation is that the fine-tuning conditioned by experience in the first few days after hearing develops helps to make the MSO neurons into a real ITD detector, and that this development basically reflects the phylogeny of ITD processing in mammals.

BENEDIKT GROTHE

Fig. 6: The responses of an MSO neuron of a bat (top) and a gerbil (bottom) to auditory stimuli presented with different ITDs. Both neurons show changes in discharge rate depending on ITDs. However, the ITDs occurring in the bat (blue area) are too short to produce a change in the cell's response, hence only artificial ITDs affect the cell. In the gerbil MSO, ITD sensitivity falls within the physiologically relevant range of ITDs (blue area).

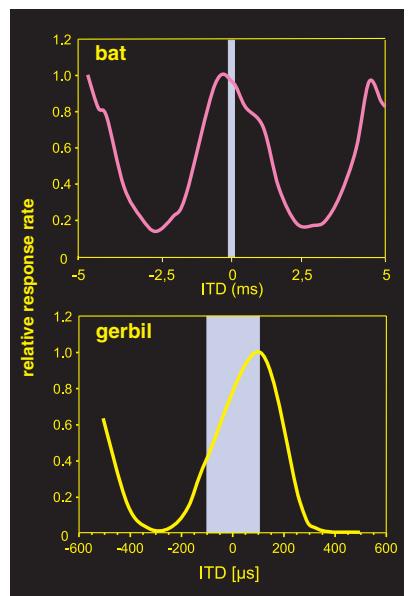


Photo: Okarma

Fig. 7: Distribution of inhibitory and excitatory inputs on the cell bodies and dendrites of MSO neurons in “non ITD users” such as juvenile gerbils or bats (top) compared with “ITD users” such as adult gerbils (bottom).

