Two-photon microscopy reveals new insights into the auditory cortex

Scientists at the Hebrew University have succeeded in using an advanced method of functional imaging, called two-photon calcium imaging, to monitor the activity of dozens of neurons in the auditory cortex of living mice simultaneously. The auditory cortex is a major player in the processing of sounds by the brain. The results of the study, recently published in the journal *Nature Neuroscience*, are surprising. The primary auditory cortex, A1, may be more heterogeneous than previously thought, with neighboring neurons responding very differently to the same sounds. This may help the auditory cortex in processing complex sounds, such as tone combinations, an issue that is still poorly understood.

Gideon Rothschild designed this eye-catching cover for an issue of *Nature Neuroscience* to illustrate the results of his study, in collaboration with Adi Mizrahi and Israel Nelken. Every candy represents a neuron in the auditory cortex, and candies of different color respond to different tones. The broad tonal organization of the primary auditory cortex is represented by the gradual change in colors over the entire picture, but within small areas, neighboring neurons may be listening to different tones, as shown by the different colored candies within small patches of this picture.

Two-photon calcium imaging has not been used before to understand the activity of neurons in the auditory cortex. Unlike other methods, such as electrophysiology, imaging allows monitoring of the activity of dozens of precisely localized neurons simultaneously, allowing the scientists to build an exact image of the responses of groups of neurons to the same stimulus.

Sensory brain areas process information in large networks of neurons. In general, within these networks the spatial location of each neuron is important, as it defines the kind of processing the neuron is involved in - which types of sensory input it processes, and at what level of complexity. This coordinated neuronal activity has important implications for information processing and eventually for behavior. Thus, measuring the activity of populations of neurons whose precise locations are known is fundamental to understanding how neuronal populations code sensory information. The Hebrew university scientists discovered that the local organization of the auditory cortex is more sparse and heterogeneous than previously thought. Local groups of neurons were not organized to respond to one best frequency. Neighboring neurons also differed in the way they responded to varying levels of loudness of the tones. Less than half of the monitored neurons responded selectively to a narrow range of frequencies. The rest of the responding neurons responded to many different combinations of frequency and sound level. These neurons might be involved in something other than simple pure-tone coding, such as the processing of complex sound features.
This image, created using two-photon calcium imaging, shows a group of neurons in the auditory cortex, at a depth of 381 micrometers below the pia, the innermost layer of the membrane around the brain. In this image, neurons are colored green, while the orange and yellow cells are glia support cells. The brighter the color, the more calcium is present in the cell, and the more active it is. The blue size bar at the bottom represents an actual size of 10 micrometers. One micrometer is a millionth of a meter.

The full research article can be found [here](https://elsc.huji.ac.il/content/two-photon-microscopy-reveals-new-insights-auditory-cortex).

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