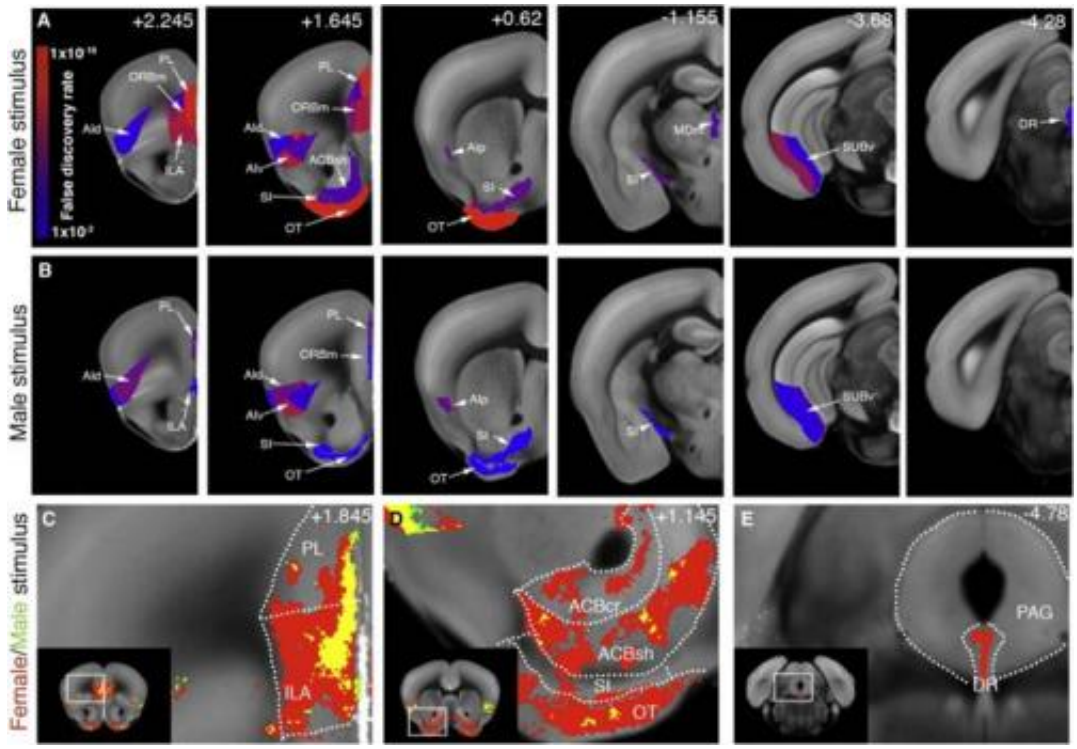
Imaging linking cell activity and behavior shows what it means for mice to have sex in mind

January 5 2015



The top 2 rows (A and B) show differences in cellular activation over time (according to brain areas, which are abbreviated) when male mice interact with females (row A) and other males (row B). Panels C, D, and E show close-ups of particular brain areas involved in motivational behavior (notably, sexual activity) in which male-male contact differed from male-female contact. Credit: Osten Lab, CSHL

Most people have seen fMRI scans of the human brain. These use a technology called functional magnetic resonance imaging to identify portions of the brain that are active while a subject is being scanned. Fuzzy, ill-defined areas that "light up" on the scans indicate where neurons are active, based on magnetic changes in the blood that correlate with activated cells' need for glucose, their energy supply.

Such scans, while roughly indicative of brain activity, have very low resolution. Therefore they are of limited value in parsing how brain cells work individually and in highly complex networks dispersed both locally and brainwide to generate complex thought and behavior.

Today, however, a team of scientists at Cold Spring Harbor Laboratory (CSHL), with collaborators at MIT, Boston University and the Allen Institute of Brain Science, describes the development of a completely automated method to detect the activity of neurons during specific behaviors, at the resolution of individual brain cells throughout the entire mouse brain. The technology can be used to study living, "behaving" animals.

In *Cell Reports*, the team, led by CSHL Associate Professor Pavel Osten, reports a demonstration of the method's effectiveness in determining brain activation patterns when male mice perform two critical tasks: recognizing other individuals and determining the sex of another individual. In concept, this or a similar technology could be useful in figuring out at the cellular level what goes wrong in disorders such as autism and schizophrenia in which social interaction is impaired.

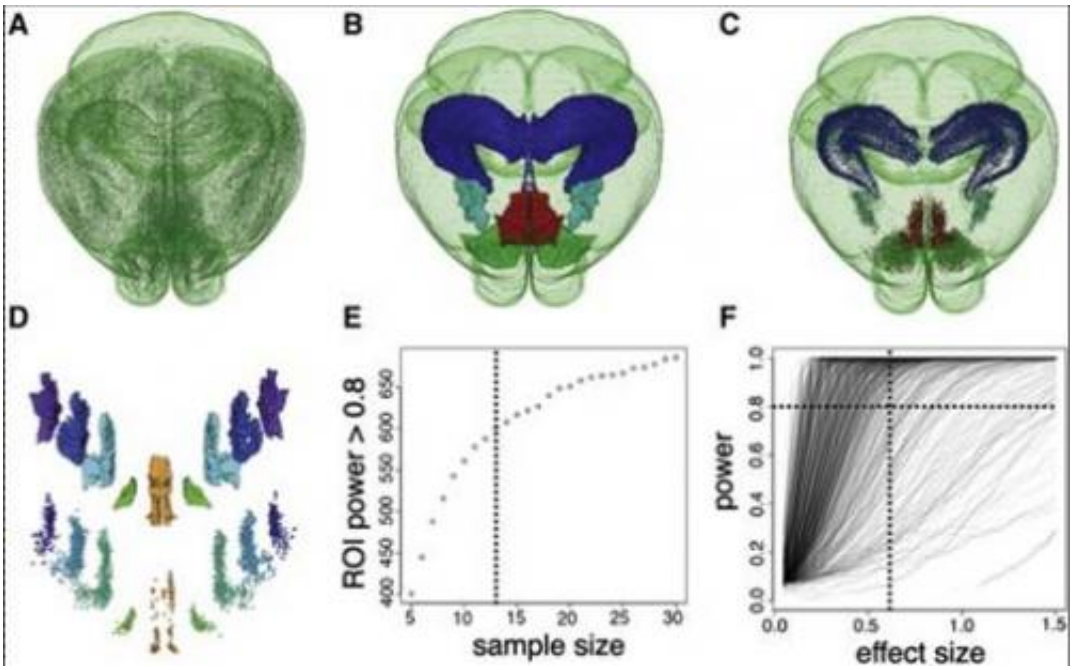
Osten is careful to note that at this point, "our study opens the door to do automated screening of brain activation evoked by specific behaviors in mice." The method uses the presence of a protein called c-fos to indicate where individual neurons are active. The protein is generated by expression of a gene by the same name, which is known to correlate with

neural activity. c-fos protein has been used previously as a marker for neural activity, although in experiments that required considerable labor and were often confined to small portions of the mouse brain.

But mice have approximately 70 million neurons, and knowing what they do in complex brainwide networks is essential if scientists are to obtain a finely detailed understanding of how specific brain areas and the neurons within them function in specific behaviors.

Osten has adapted an automated method he previously developed called serial two-photon (STP) tomography, to scan the full mouse brain in 280 segments. In the current research, a series of behavioral experiments were run featuring a line of mice whose brain cells express a marker called green fluorescent protein (GFP) that glows whenever the c-fos gene is expressed. Cell activity across the brain in these mice was compared in groups of mice exposed to different behavioral stimuli - "social" groups having either male-male or male-female interaction, and various control groups with and without social interaction and/or olfactory stimulation.

The mice in the social group were engaged in tasks that involved sniffing and thereby discriminating among other individuals, whether of the same sex or the opposite sex. In each animal's brain, glowing cells with activated c-fos were detected by computer algorithms and their precise spatial locations mapped to a "reference brain," synchronized with the Allen Mouse Brain Atlas, a public resource published to the web by the Allen Brain Institute. A final portion of the method involves statistical analysis to quantify cellular activation in 3-D space for specific behaviors.



Panels A, B, and C show the results of different stages in data processing, following collection of the whole-brain activation map. Active cells are correlated with brain regions, and ultimately, in panel C it is possible to count the number of individual cells active in each region of interest. The active areas include hippocampus (dark blue; 33,508 active cells) amygdala (light blue; 3035 cells) nucleus accumbens (green; 13,627 cells) and infralimbic cortical area (red; 4,665 cells). Credit: Osten Lab, CSHL

"Very little is known is known about brain areas activated during social recognition and the initial period of sex discrimination, before an animal begins to display the appropriate behavioral response," Osten noted. "When we compared male and female whole-brain activation patterns using our technology and analytical pipeline, we were able to make satisfying side-by-side comparisons."

Male-female as well as male-male interactions entail initial common social behaviors such as anogenital sniffing and something scientists call "close following." Other behaviors are sex-specific including sexual

mounting and fighting.

After performing the battery of 3-D imaging and analysis, the resulting 3-D whole-brain activity maps (and accompanying real-time movies) suggested novel facts. A sex-specific dorsal and ventral activation pattern in two subsections of the olfactory cortex called the piriform and entorhinal cortices was noted. Additionally, specific parts of the brain linked to behavioral motivation were found to be strongly activated during male-female interaction. The study also found social brain regions that were commonly activated by both male and female stimulation, but not by non-social odor stimulation.

Importantly, for the first time, the new imaging method makes it possible to accurately count how many neurons are active in various brain areas during behavior. According to Osten, the average result for all behaviors, brainwide, was that in any activated structure, about 4500-5000 neurons are activated per cubic millimeter of brain space when animals were "behaving" as compared with about 1500 - 2000 fewer activated neurons per structure in control animals that were only being "handled" but were not interacting. This result supports a "sparse activation" theory of brain function which postulates that activation of only a few percent of all the neurons in a given brain area will suffice for complex behavior.

These results being only demonstrations, Osten and colleagues suggest many discoveries lie ahead with further application of the new imaging and processing "pipeline" described in the paper.

More information: "Mapping social behavior-induced brain activation at cellular resolution in the mouse" appears online January 5, 2015 in *Cell Reports*. The authors are: Yongsoo Kim, Kannan Umadevi Venkataraju, Kith Pradhan, Carolin Mende, Julian Taranda, Srinivas C. Turaga, Ignacio Arganda-Carreras, Lydia Ng, Michael J. Hawrylycz,

Kathleen S. Rockland, H. Sebastian Seung and Pavel Osten. The paper can be obtained at: www.cell.com/cell-reports/abstract/S2211-1247%2814%2901043-2