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PRIMARY eye care

Visual Perception, Virtual Avatars and Blood Vessel Leakage

For this issue of Primary Eye Care Quarterly we are highlighting some new findings which add to our understanding of visual perception. This process which starts with photons hitting the retina and ends with 'seeing' is a complex process in which the brain's fundamental task is to reconstruct relevant information about the world from the light that hits the eyes. Because this process is so complex the way that neurons react to images is far from simple and not fully understood. We have also included information about a new discovery regarding angiopoietin-like 4, a signaling protein that is already well known to be a blood vessel growth factor with roles in heart disease, cancer and metabolic diseases, now shown at play in diabetic macular oedema.

What the Brain Likes to 'See'

In a study published this month in the journal *Nature Neuroscience, r*esearchers at Baylor College of Medicine and the University of Tübingen in Germany describe how they have developed a novel computational approach to accelerate finding these optimal sensory stimuli despite the mainly non-linear nature of sensory processing and the high dimensionality of the input to the process. Their work has uncovered some aspects of vision that were not previously expected to occur.

The Nature Neuroscience paper describes how the researchers novel approach was based on 'inception loops', a closed-loop experimental paradigm combining in vivo recordings from thousands of neurons with in silico nonlinear response modeling. The resulting deep-learning-based model accurately predicted thousands of neuronal responses produced by a biological brain to arbitrary visual stimuli.

The newly developed artificial neural networks, functioning like a 'virtual avatar' of a set of biological neurons, enabled the research team to synthesize new images that made particular biological neurons in a mouse population respond very strongly.

"We want to understand how vision works. We approached this study by developing an artificial neural network that predicts the neural activity produced when an animal looks at images. If we can build such an avatar of the visual system, we can perform essentially unlimited experiments on it. Then we can go back and test in real brains with a method we named 'inception loops,'" said senior author Dr. Andreas Tolias, professor and Brown Foundation Endowed Chair of Neuroscience at Baylor.

To make the network learn how neurons respond, the researchers first recorded a large amount of brain activity using a mesoscope, a recently developed large scale functional imaging microscope.

"First, we showed mice about 5,000 natural images and recorded the neural activity from thousands of neurons as they were seeing the images," said first author Dr. Edgar Y. Walker, former graduate student in the Tolias lab and now a postdoctoral scientist at Unviersty of Tübingen and Baylor. "Then, we used these images and the corresponding recordings of brain activity to train a deep artificial neural network to mimic how real neurons responded to visual stimuli."

"To test whether the network had indeed learned to predict neural responses to visual images like a living mouse brain would do, we showed the network images it had not seen during learning and saw that it predicted the biological neuronal responses with high accuracy," said co-first author Dr. Fabian Sinz, adjunct assistant professor of neuroscience at Baylor and group leader at the University of Tübingen.

"Experimenting with these networks revealed some aspects of vision we didn't expect," said Tolias, founder and director of the Center for Neuroscience and Artificial Intelligence at Baylor. "For instance, we found that the optimal stimulus for some neurons in the early stages of processing in the neocortex were checkerboards, or sharp corners as opposed to simple edges which is what we would have expected according to the current dogma in the field."

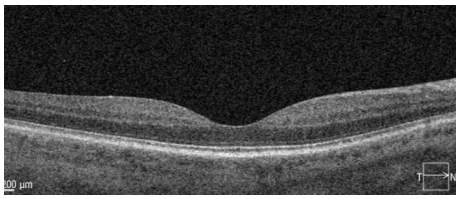
"We think that this framework of fitting highly accurate artificial neural networks, performing computational experiments on them, and verifying the resulting predictions in physiological experiments can be used to investigate how neurons represent information throughout the brain. This will eventually give us a better idea of how the complex neurophysiological processes in the brain allow us to see," Sinz said.

Other contributors to this work include Erick Cobos, Taliah Muhammad, Emmanouil Froudarakis, Paul G. Fahey, Alexander S. Ecker, Jacob Reimer and Xaq Pitkow.

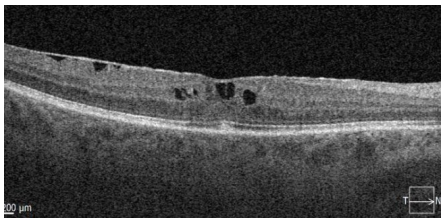
Helper protein worsens diabetic eye disease

In a recent study using mice, lab-grown human retinal cells and patient samples, Johns Hopkins Medicine scientists say they found evidence of a new pathway that may contribute to degeneration of the light sensitive tissue at the back of the eye. They found that angiopoietin-like 4, a signaling

protein that is already well known to be a blood vessel growth factor with roles in heart disease, cancer and metabolic diseases, is at play in diabetic macular oedema.



NORMAL MACULA



MACULA OEDEMA

Current therapies for this disease block the protein VEGF, which contributes to abnormal blood vessel growth. However, because the treatment is not adequate for more than half of patients with diabetic macular oedema, investigators have long suspected that more factors drive vision loss in these patients. In the new study, the Johns Hopkins researchers say they found compelling evidence that angiopoietin-like 4 is at play in macular oedema. The signalling protein is already well known to be a blood vessel growth factor with roles in heart disease, cancer and metabolic diseases, of which diabetes is one.

A report on the findings was published Sept. 23 in The Journal of Clinical Investigation by Akrit Sodhi, M.D., Ph.D., associate professor of ophthalmology at the Johns Hopkins University School of Medicine and the Johns Hopkins Wilmer Eye Institute, who led the team, in collaboration with Silvia Montaner, Ph.D., M.P.H., at the University of Maryland. Dr Sodhi was intrigued by angiopoietin-like 4 after finding, in previous studies, elevated levels of this protein in the eyes of people with a variety of vision-related diseases. In the new study, Sodhi and his team found that angiopoietin-like 4 acts both independent of, and synergistically with, VEGF activity, and they identified a potential way to block it.

The investigators made their discoveries by exposing human blood vessel tissue cells grown in the lab to low levels of VEGF and angiopoietin-like 4. Knowing that low levels of these factors individually did not generally create an effect, the researchers were surprised to find that in combination, low-level VEGF and low-level angiopoeitin-like 4 had a synergistic effect on vascular cell permeability, and doubled the leakage from retinal vessels in mice.

"This told us that you can have sub threshold levels of both molecules, where neither alone is enough to do anything, but together, produce a huge effect," says Sodhi.

The amplifying effect led the researchers to believe that VEGF and angiopoietin-like 4 might share a protein receptor within vascular cells. However, similar experiments revealed that angiopoietin-like 4 also increases blood vessel formation independently of VEGF. "This could explain why some patients continue to experience vision loss despite treatment with current anti-VEGF therapies," says Sodhi.

To test this, the team looked to see whether the angiopoietin-like 4 protein bound to one of VEGF's receptors in lab-grown human vascular cells. They found that angiopoietin-like 4 did not bind to the classic VEGF receptor that is a target of current anti-VEGF medicines, but another less studied one called neuropilin. With the newly identified receptor, the researchers next sought to learn whether a lab-grown version of the receptor could block angiopoietin-like 4 before it was able to interact with blood vessel cells.

To do that, they injected a soluble fragment of the neuropilin receptor into the eyes of mice pharmacologically treated to mimic human diabetes, resulting in a twofold increase in retinal vascular leakage. The treated diabetic mice showed approximately half of the blood vessel leakage as mice who did not receive the treatment, similar to the non-diabetic mice.

To further explore the new receptor-based treatment's potential value for human patients, the researchers grew human blood vessel cells in the lab in fluid samples collected from the eyes of patients with diabetic macular oedema, to replicate the conditions and growth factors found naturally inside of the patients' eyes. One group of such cells was exposed to the soluble receptor neuropilin. The researchers say they observed a marked decrease in the diabetic macular oedema cells treated with the receptor compared to untreated cells.

"This gives us some confidence that this approach will work in human eyes as well," says Sodhi, although he cautions that clinical use of a treatment based on their findings will require many more years of research.

Next, the researchers hope to take a look at the molecular interactions between angiopoietin-like 4 and the neuropilin receptor. Doing so, says Sodhi, will allow them to create a refined match that can bind up as much vision-threatening angiopoietin-like 4 in the eye as possible. **Sodhi also hopes the team's discovery will have value in treating cancer and cardiovascular disease, the courses of which also are influenced by uncontrolled blood vessel growth.**

Other researchers involved in this study include Monika Deshpande, Kathleen Jee and Jordan Vancel of the Johns Hopkins University School of Medicine, and Tao Ma, Deepak Menon, Aumreetam Dinabandhu, Daoyuan Lu and Silvia Montaner of the University of Maryland. The research was supported by the National Eye Institute (5R01EY025705) and Research to Prevent Blindness.

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