UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

SURF 2015 Research Summary

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Research interest: My current research interest is using various methods in the field of behavioral neuroscience to study the functional role of new neurons in the hippocampus.

Abstract: Given the critical importance of the hippocampus in spatial learning and memory, there is much interest in understanding the functional role of new neurons that are generated in the hippocampus during adulthood. Optogenetics is a novel and powerful method that uses light and light-sensitive proteins to control the activity of neurons. Our lab has created a mouse line whose new hippocampal neurons are inactivated only in response to green light. Temporary, and not permanent, inactivation of these neurons would enable us to more accurately study their functionality in behavior and cognition. In our pilot experiment, we first implanted wireless green uLEDs near the hippocampus of our transgenic mice (N=2). By robustly activating all the neurons in their brain through a seizure, we were able to clearly see a difference in the level of hippocampal activity between the mouse that had its uLED turned on and the mouse that did not. Our results show that we are able to use optogenetic techniques to inactivate new hippocampal neurons in our transgenic mouse line. In the future, we hope to replicate our finding and afterwards, conduct behavioral experiments with them that are aimed at studying the functional role of new hippocampal neurons.

Only in recent scientific history have we revised the idea that the adult brain produces a finite amount of neurons and is unable to generate new neurons after development. The discovery that new neurons are continuously being produced past our developmental period in the adult mammalian brain has created a lot of excitement in the field of neuroscience (Altman & Das, 1965). It is widely accepted that adult neurogenesis happens in two places: olfactory bulb and the dentate gyrus (DG) of the hippocampus (Gage, 2002). Given the critical importance of the hippocampus in spatial learning and memory, there is much interest in understanding the functional role of neurogenesis in the DG of the hippocampus (Squire, 1992; van Praag, 2002).

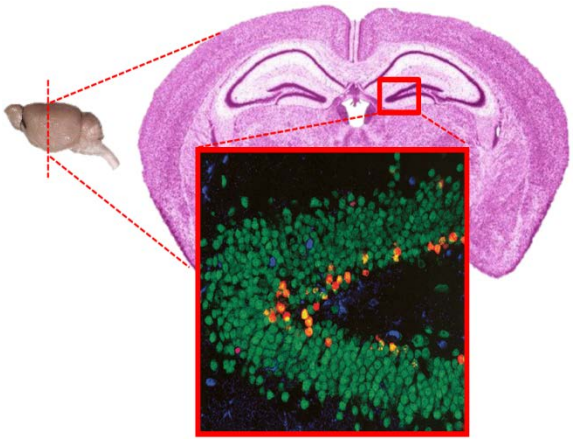
There have been various approaches used to study the functional role of new neurons in behavior and cognition. Most methods, such as x-ray irradiation and toxic drug administration, rely on killing all of the newly generated neurons in rodents and observing any resulting deficits that may occur in their learning and memory (Imayoshi et al., 2011). However, one limitation of these methods is that the brain is very good at adapting to changes (Mustroph et al., 2015). Therefore, it is possible that the results were due to the compensatory mechanism of brain, and not due to the lack of new neurons. In order to study the role of adult hippocampal neurogenesis without ablating new neurons, our lab has incorporated a unique technique called optogenetics, which allows us to temporarily inactivate new neurons and thus giving the brain very little to no time to enact any compensatory mechanisms. Optogenetics is a novel and powerful method that allows us to control neurons through the use of light and light-sensitive proteins. This approach provides the ability to control the activity of neurons similar to how a light switch is used to turn our lamps on and off (Ting and Feng, 2013).

In the past year, we have successfully developed a Cre-Lox transgenic mouse model that is able to express a light-sensitive protein (opsin) specifically in new neurons of the hippocampus

(Sun et al., 2014). This summer, our goal was to surgically implant wireless green uLEDs , developed by the John Rogers group on campus, into our transgenic mice to demonstrate that we can successfully inactivate these new neurons using green light.

In our pilot experiment, we fed our transgenic mice (N = 2) with a special diet containing tamoxifen that enables the expression of the opsins in the new hippocampal neurons. After 4 weeks on the tamoxifen diet, we put them back on a normal diet for a week to allow extra time for the opsins to be expressed within the neurons. Afterwards, we surgically implanted wireless green uLEDs near their hippocampus and gave them two weeks of recovery. On the day of the experiment, one of the mice had its wireless uLED turned on (n=1) while the other did not (n=1) in order to act as a control. Both mice were injected with 15mg/kg of kainic acid, which induces a seizure that robustly activate neurons in the hippocampus (Ben-Ari, 1985). This induced-seizure is a way for us to easily visualize the inactivation of the new neurons as all the other neurons would be in a state of activation. After 30 minutes, we extracted their brains and processed them for analysis.

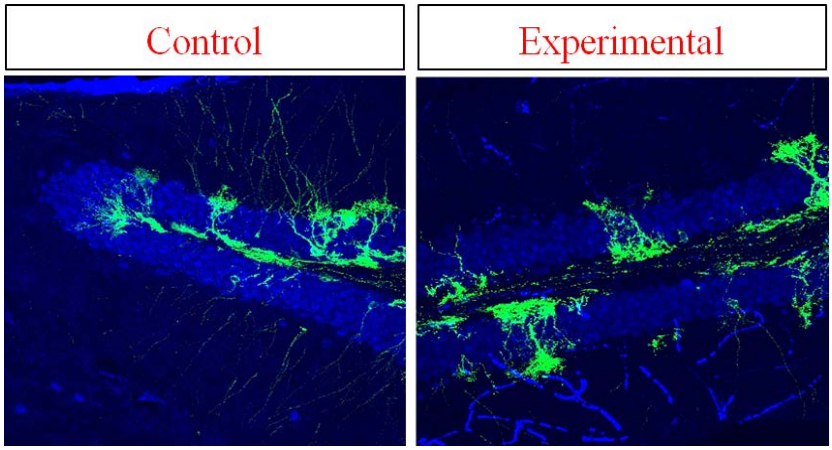
We sectioned the brain tissues coronally (front-to-back) into 40 nm thick slices (Figure 1). We then used various antibodies and chemicals on the brain slices to detect for: 1) opsin-containing neurons and 2) neuronal activity. Staining for the opsin-containing neurons would allow us to confirm that our mouse model only expresses the opsins in new hippocampal neurons, while staining for neuronal activity would indicate whether or not we had successfully caused



(Figure 1) A diagram showing: a mouse brain, a coronal section, and a 40X confocal microscope picture of the dentate gyrus

neuronal inactivation using the uLED. After staining the tissue, we took images and compared the brain slices of the two mice using both confocal and light microscopy.

Figure 2 shows the presence of opsin-expressing neurons in the DG of the hippocampus, where neurogenesis occurs, in both the control (uLED off) and experimental (uLED on) mice. The blue colored cells are neurons, while the green colored cells are neurons that contain the opsins. There was no significant difference in the number of opsin positive neurons between the control and experimental animal.



(Figure 2) A 40X confocal image of the hippocampus (dentate gyrus) of control v.s. experimental mouse. The green colored cells are neurons that contain the light-sensitive proteins (opsins)

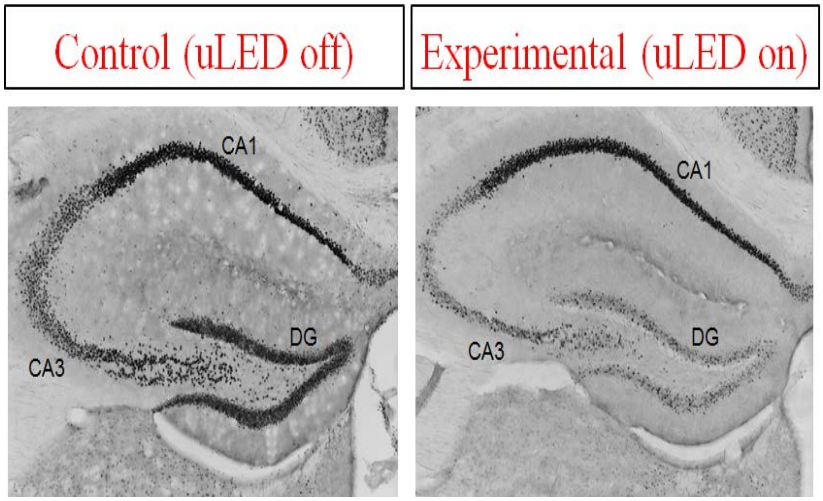


Figure 3) A 10X light microscope image comparing the level of activation of neurons in the hippocampus (dentate gyrus, CA3, and CA1) in the control vs. experimental mouse.

Figure 3 shows the level of neuronal activation in the hippocampus of both control and experimental mice. The control mouse showed robust neuronal activation in the DG, CA3, and CA1 regions of the hippocampus, whereas the experimental mouse, compared to the control mouse, showed much less neuronal activation in the DG and CA3, but not CA1 region of the hippocampus.

Our results show that not only were we able to inactivate the opsin-containing neurons in the DG of the hippocampus, but we also prevented neurons in the downstream CA3 region from being activated by the kainic acid-induced seizure. More importantly, the results from our pilot experiment was able to demonstrate that we can use optogenetics method on our transgenic mice, which will enable us to further study the functional role of new neurons in the hippocampus. This fall, we hope to use a triple immunofluorescence staining technique whereby we can stain for neurons, opsin-expressing neurons, and activated neurons all on the same tissue in order to ensure that none of the opsin-expressing neurons show any sign of neuronal activity in the experimental mouse brain tissues. We are also in the process of replicating our findings using a larger sample size before we can finally use our transgenic line in behavioral experiments aimed at exploring the role that hippocampal neurogenesis plays in learning and memory.

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