Controlling the Elements: An Optogenetic Approach to Understanding the Neural Circuits of Fear

Supplemental Information

Description of All Amygdala Related Optogenetic Studies

Tang W, Ehrlich I, Wolff SB, Michalski AM, Wolfl S, Hasan MT, *et al.* (2009): Faithful expression of multiple proteins via 2A-peptide self-processing: a versatile and reliable method for manipulating brain circuits. *J Neurosci* 29:8621-8629.

-Demonstrated functional ChR2 and Halorhodopsin expression in lateral amygdala neurons from a construct which used 2A peptide bridges to express both of these proteins off of the same promoter.

Johansen JP, Hamanaka H, Monfils MH, Behnia R, Deisseroth K, Blair HT, LeDoux JE (2010): Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. *Proc Natl Acad Sci U S A* 107:12692-12697.

-Expressed ChR2 in pyramidal cells in the lateral amygdala and demonstrated in-vivo control of neural activity using light in amygdala neurons. In behavioral experiments, the authors were able to produce behavioral fear conditioning by pairing an auditory stimulus with optogenetic activation of lateral amygdala (LA) pyramidal neurons in place of an actual shock unconditioned stimulus.

Ciocchi S, Herry C, Grenier F, Wolff SB, Letzkus JJ, Vlachos I, *et al.* (2011): Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature* 468:277-282.

-Elucidated the functional subcircuitry of central nucleus of the amygdala (CE) during fear conditioning. This study showed that fear conditioning induces differential changes in auditory conditioned stimulus (CS) processing in CE neurons and electrophysiologically defined two subclasses of neurons in the lateral division of the CE (CEl). In addition, this work showed that neural activity in the medial division of the CE (CEm) is necessary for expression of previously learned fear responses and activation of CEm neurons is sufficient to produce freezing behavior. Furthermore, this work demonstrated that CEl neural activity is necessary for the acquisition of fear conditioning.

Haubensak W, Kunwar PS, Cai H, Ciocchi S, Wall NR, Ponnusamy R, *et al.* (2010): Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature* 468:270-276.

-Determined the local anatomical connectivity and functional contribution of a molecularly defined subset of CEl neurons. This work showed that protein kinase Cδ (PKCδ) expressing neurons corresponded to a an electrophysiologically identified subclass of CEl neurons and that

PKCδ+ cells inhibited fear output neurons in the CEm. Finally, this study demonstrated that inhibiting neural activity in PKCδ+ cells enhanced learned fear responses.

Tye KM, Prakash R, Kim SY, Fenno LE, Grosenick L, Zarabi H, *et al.* (2011): Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature* 471:358-362.

-Showed that optogenetic excitation of B neuron projection terminals to CEl reduced anxiety-related behaviors and that optogenetic inhibition of these terminals was anxiogenic. Importantly, no effect on anxiety behaviors resulted from manipulations of the cell bodies of these projections in the B. This work was the first to demonstrate an effect of optogenetic manipulation of a specific subset of synaptic inputs to a brain region, and not the cell bodies from which these inputs originated, on behavior.

Morozov A, Sukato D, Ito W (2011): Selective suppression of plasticity in amygdala inputs from temporal association cortex by the external capsule. *J Neurosci* 31:339-345.

-Used optogenetics to stimulate and induce long-term potentiation (LTP) at either anterior cingulate cortex (ACC) or temporal association cortex (TeA) inputs to the LA and found that TeA-LA, but not ACC-LA, LTP was under the control of feedforward inhibitory networks. This work demonstrated pathway specific recruitment of inhibitory networks during the induction of synaptic plasticity in the LA.

Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, *et al.* (2011): Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature* 475(7356):377-80.

-Showed that optogenetic stimulation of B inputs to the nucleus accumbens (NAcc) reinforced lever pressing behavior and that inhibition of B-NAcc inputs reduced cued reward seeking behavior. This effect did not occur when cortical inputs were stimulated demonstrating pathway specific modulation of reward seeking behavior.

Considerations and Caveats

While optogenetics has the potential to revolutionize the study of the nervous system, there are some things to consider when using this technique. For example, lack of tissue specificity and low expression levels can be issues when using minimal tissue specific promoters in combination with viral transduction (1-3). To address these potential problems, it is essential to test any new promoter/virus combination in vivo to determine both its tissue specificity and its infection efficacy. This problem can at least be partially avoided by the use of transgenic animals, which can give high opsin expression in defined neuronal populations. However, such an approach lacks the brain area specificity offered by virus-based methods. Anatomical selectivity

and high expression levels can be obtained, in principle, when using conditional viral vectors in Cre mouse lines. It should be considered, however, that neuronal subpopulations targeted in this manner are often still heterogeneous. In the long run, intersectional strategies, based on more than one molecular marker, may help to refine the targeting of opsins to specific cell types.

Light delivery to large brain structures can also present a problem for the use of optogenetics, especially when using fiber optic approaches to target deep brain structures. While ongoing work is attempting to address this problem (4,5), it is important to keep this in mind when designing optogenetic studies.

In case bidirectional control of neuronal activity is required in the same neurons, it is necessary to use viruses for co-expression of different opsins (see (6)). However, each construct has to be validated for appropriate co-expression. This is especially true for internal ribosome entry site (IRES)-based co-expression constructs, while 2A-based solutions may be more reliable. Furthermore, if the opsins are expressed in different neuronal populations, they have to be carefully chosen to ensure minimal overlap of their activation spectra.

Although the functionality of the optogenetic approach has been shown several times, even in vivo, it should always be tested whether the targeted cells indeed respond to the light stimulus. Electrophysiological recordings in vivo are the most direct way to test this. Furthermore, it is ideal not only to manipulate neuronal activity and to analyze the behavioral effects, but to monitor the light-induced physiological effects in both the transfected and the non-transfected cells. This facilitates much stronger conclusions about the function of defined circuit elements in behavior.

When using optogenetics to identify extracellularly recorded neurons in vivo, it has to be verified that the observed light responses are caused by direct stimulation of the recorded cell rather than indirect network effects. Especially, ChR2-mediated excitation of an entire cell population can cause indirect activation of non-expressing cells which may look similar to direct light-induced activation. A first criterion to address this issue is the latency of the light-response. However, this may also be misleading, since response latency can depend on levels of ChR2 expression and strength and stability of illumination. Therefore, additional criteria (e.g., spike waveform, cross-correlations, spontaneous firing, etc.) can be used to complement the optogenetic identification. Alternatively, one should consider using inhibitory opsins for cell identification because indirect effects are less likely to occur in this case.

Supplemental References

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