NEUROTECHNIQUES

Dark matter

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Researchers selectively inhibit neurons with targeted drug sensitivity.

Despite the directions in *Age of Aquarius*, it is difficult to let the sun shine in mammalian brains. Recently, researchers reported photoactivated ion channels that may help functionally dissect neural circuits. However, most mammalian brains are opaque and would require surgical implantation of light sources. Now Wulff *et al.* report functional dissection of neural circuits in mice with targeted sensitivity to a GABA_A receptor agonist in a recent article in *Nature Neuroscience*.

The zolpidem-sensitive GABA_A receptors localize to cerebellar Purkinje cells in PC-γ2-swap mice. Image reproduced from Nature Neuroscience.

Almost all neurons express GABA_A receptors, which contain two , two and one ² subunit. Zolpidem (marketed as the sleep aid Ambien) is a GABA_A receptor agonist that acts at a binding site formed by and ² subunits. In the ² subunit, phenylalanine 77 (F77) is necessary for zolpidem binding, and GABA_A receptors with isoleucine at position 77 bind GABA but not zolpidem.

The authors generated mice insensitive to zolpidem except in cerebellar Purkinje cells. They generated zolpidem-insensitive ²I277lox mice by substituting a codon encoding isoleucine for the codon encoding phenylalanine in the GABA_A receptor ² subunit gene *Gabrg2* and engineering adjacent loxP recombination sites. They crossed these mice to mice expressing Cre recombinase under the control of the *L7* promoter to generate mice lacking GABA_A receptors in cerebellar Purkinje cells (PC-Δ²). The authors tagged the wild-type zolpidem-sensitive allele with green fluorescent protein (GFP) and targeted it to cerebellar Purkinje cells with the *L7* promoter. They crossed mice expressing this allele with PC-² mice to generate PC-²-swap mice.

PC-²-swap mice showed ²F77 expression and zolpidem sensitivity in cerebellar Purkinje cells. In the molecular layer of the cerebellum, GFP localized to GABA_A receptor ¹ subunit-positive cells that were apposed to glutamic acid decarboxylase-positive (GABAergic) nerve terminals. In cerebellar slices, Purkinje cells showed miniature inhibitory postsynaptic currents (mIPSCs) with similar properties in PC-²-swap, ²I277lox and wild-type mice, suggesting that the I77² subunit does not disrupt normal GABAergic transmission. Zolpidem had no effect on mIPSCs from ²I277lox mice. However, zolpidem increased the amplitude and timecourse of mIPSCs in cerebellar Purkinje cells but not stellate

cells from *PC-2-swap* mice, suggesting that zolpidem sensitivity was restricted to cerebellar Purkinje cells.

Zolpidem quickly modified behavior in *PC-2-swap* mice. Cerebellar Purkinje cells are important in learning to balance on a rotating rod called a rotarod. *PC-2-swap* and *2177lox* mice performed similarly on the rotarod. Within 1-5 minutes of zolpidem treatment, *PC-2-swap* mice showed cerebellar deficits on the rotarod, falling faster than *2177lox* mice.

Therefore, zolpidem can quickly deactivate components of neural circuits expressing engineered GABA_A receptors. Could a similar system activate these same circuit components? The *DMCM* and *CCM* are inverse GABA_A receptor agonists that decrease receptor function and are ineffective in *2177* mice. According to the authors, zolpidem and an inverse agonist could bidirectionally modulate the activity of neurons expressing engineered *2* subunits, helping to identify neuron function in neural circuits.

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1. Wulff, P. *et al.* From synapses to behavior: rapid modulation of defined neuronal types with engineered GABA_A receptors. *Nature Neuroscience* **10**, 923–929 (2007). | [Article](#) |