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Manipulating the munchies in mice

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Abstract

Crystal structure and drug pharmacophore structure-activity data guided a combined mutagenesis and chemical screen to develop ligand-gated ion channels activated by unique chemical agonists. Through genetic engineering, these channels can be used to manipulate neuronal excitability and dissect the neuronal circuitry responsible for complex behaviors.

Behavior is the result of complex information processing by interacting networks of excitatory and inhibitory nerve cells in the brain. Neuroscientists have sought to elucidate how the activity of groups of neurons integrate to produce behavioral outputs such as hunger, thirst, sleep and mating. A key limitation has been the inability to activate or silence specific nerve cells or populations of nerve cells to isolate their functions in intact behaving animals and in in vitro preparations. In a recent issue of *Science*, Sternson and colleagues¹ described the development of a toolbox of genetically engineered ligand-gated ion channels activated by unique exogenous chemicals that are able to penetrate into the brain in waking, behaving animals. Extending this approach to manipulate the excitability of distinct neuron populations in a highly specific manner will greatly facilitate efforts to elucidate the neural circuitry underlying a variety of behaviors.

As a foundation to develop ligand-gated channels that are activated by drugs but not endogenous neurotransmitters, Sternson and colleagues¹ began with the cysteine-loop (Cys-loop) receptor superfamily of neurotransmitter-gated ion channels (Fig. 1a–d). This superfamily includes both excitatory and inhibitory channels, and much is known about their structure and function^{2,3}. Cys-loop receptors are formed by the quasisymmetrical assembly of five homologous or identical subunits around the central channel axis. The subunits are constructed in a modular fashion by three distinct functional domains: an extracellular ligand-binding domain, a membrane-spanning domain that forms the ion channel and an intracellular domain that controls localization to specific regions in the nerve cell and modulation of channel activity by intracellular second-messenger systems^{4–6}. In vertebrates, acetylcholine, serotonin, GABA and glycine are the endogenous neurotransmitters that activate distinct families of Cys-loop receptors. There are two types of membrane-spanning domains in Cys-loop receptors: one that is anion selective in GABA and glycine receptors, and one that is cation selective in acetylcholine and serotonin receptors. Activation of Cys-

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loop receptors with anion-selective channels inhibits the electrical excitability of the target cell, reducing the likelihood that it will fire an action potential. In contrast, activation of cation-selective family members increases the cell's electrical excitability and the probability that it will fire an action potential leading to the release of neurotransmitter onto its target cells.

Starting with a neuronal acetylcholine-subunit homology model, which was based on the structure of nicotine-bound snail acetylcholine-binding protein⁷, Sternson and colleagues modeled the binding of a quinuclidinyl benzamide, PNU-282987, that is known to activate acetylcholine $\alpha 7$ receptors and permeate through the blood-brain barrier⁸. They identified four residues in close proximity to a portion of PNU-282987 where previous pharmacophore studies showed that bulky benzamide modifications were poorly tolerated. They mutated the four residues to each of the other 19 amino acids and screened the resultant 76 mutants for cell-surface expression and channel activation. Forty-three of the mutant channels were functional. These were screened for activation by 71 enantiomerically pure 3-aminoquinuclidine derivatives containing benzamidine substituents at the C2 and C4 positions. Three mutant receptors were identified that were orthogonally activated by three distinct ligands. Additional mutations were added to the three mutants to reduce their affinity for the endogenous ligand, acetylcholine, yielding three 'pharmacologically selective actuator modules' (PSAMs) (Fig. 1e). The affinity of the three ligands was optimized in a subsequent round of synthetic chemistry to yield the three 'pharmacologically selective effector molecule' (PSEM) agonists. Finally, the authors coupled the N-terminal PSAM extracellular domains to the C-terminal membrane-spanning and intracellular domains of the cation-selective 5-HT3A subunit and the anion-selective glycine receptor subunit, both of which form homopentameric channels.

As a proof-of-principle experiment, they expressed the constructs in mouse brain and demonstrated, using electrophysiological recordings from single neurons, that the genetically engineered channels could be activated with the appropriate PSEMs. Finally, they expressed one of the PSAM-inhibitory anion-selective channel constructs in a region of the mouse brain where stimulation increases feeding. Intraperitoneal injection of the PSEM significantly ($P < 0.001$) suppressed feeding but had no effect in control animals. Thus, they demonstrated the utility and function of their constructs in contexts ranging from single-cell electrophysiological recordings to whole-animal behavioral studies.

Neuroscientists have identified many DNA promoters that are expressed in specific subpopulations of neurons in the brain. Using these cell type-specific promoters, it is now possible to create transgenic mice that express either the excitatory or inhibitory PSAM constructs in specific populations of neurons⁹. This represents a notable advance because it allows investigators to modulate the excitability of specific neuron populations as they seek to elucidate their role in shaping complex behaviors.

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Figure 1.

Cys-loop receptor structure and engineered channel strategy. (a) Illustration of the sequence of modular domains in a Cys-loop receptor subunit primary sequence. ECD, extracellular ligand-binding domain; TM, transmembrane ion channel domain; ICD, intracellular domain. (b) Nicotinic acetylcholine (ACh) receptor subunit structure (PDB code 2BG9)10. Orange, ECD; dark blue, TMD; cyan, ICD. Dashed lines indicate extent of membrane. (c) Pentameric acetylcholine receptor quaternary structure. One subunit is color coded as in b. The adjacent subunit is in red, and the other three are in gray. (d) Top view of nicotinic acetylcholine structure. One subunit is color coded as in b, and the others each have a distinct color. The ion channel runs down the central protein axis. (e) Illustration of the strategy used to make constructs. Homopentameric $\alpha 7$ acetylcholine receptor mutagenesis and chemical screening identified mutants with ECDs that recognized distinct exogenous chemicals creating the PSAMs. Chimeras were then constructed by linking the PSAMs to the C-terminal TMD and ICD of either the cation-selective serotonin receptor subunit 5-HT3 or the anion-selective glycine receptor (GlyR) subunit.