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Optogenetics and thermogenetics: technologies for controlling the activity of targeted cells within intact neural circuits

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In recent years, interest has grown in the ability to manipulate, in a temporally precise fashion, the electrical activity of specific neurons embedded within densely wired brain circuits, in order to reveal how specific neurons subserve behaviors and neural computations, and to open up new horizons on the clinical treatment of brain disorders. Technologies that enable temporally precise control of electrical activity of specific neurons, and not these neurons' neighbors — whose cell bodies or processes might be just tens to hundreds of nanometers away — must involve two components. First, they require as a trigger a transient pulse of energy that supports the temporal precision of the control. Second, they require a molecular sensitizer that can be expressed in specific neurons and which renders those neurons specifically responsive to the triggering energy delivered. Optogenetic tools, such as microbial opsins, can be used to activate or silence neural activity with brief pulses of light. Thermogenetic tools, such as thermosensitive TRP channels, can be used to drive neural activity downstream of increases or decreases in temperature. We here discuss the principles underlying the operation of these two recently developed, but widely used, toolboxes, as well as the directions being taken in the use and improvement of these toolboxes.

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Introduction

Throughout the history of neuroscience, technologies for driving or quieting the electrical activity of neurons within a region of the brain have proven important for revealing the causal role that neurons located within, or sending projections through, a brain region play in perception, cognition, and behavior. Such strategies have also revealed many novel targets for the treatment of neural disorders, and even supported new therapeutic modalities for direct medical use. Methodologies capable of such modulation of regional neural activity include electrical neural stimulation [1–4], magnetic stimulation [5], pharmacological modulation [6], infrared light stimulation [7], and ultrasound stimulation [8,9]. In recent years, interest has grown in the ability to manipulate, in a temporally precise fashion, the electrical activity of specific neurons embedded within a densely wired brain region. Even a small volume of neural tissue might contain hundreds of different kinds of neuron (and many kinds of non-neuronal cell), which possess different molecular compositions, morphologies, and connectivities. The ability to control the electrical activity of a specific subset of the neurons embedded in a network would enable the causal assessment of the role which that subset plays in the operation of the network. In addition, different neurological and psychiatric disorders are associated with changes in different cell types, suggesting that the search for new neural targets for treating disorders, and the development of new therapeutic modalities, could benefit from insights on how to alter the electrical activity of specific cells embedded within a dense neural network.

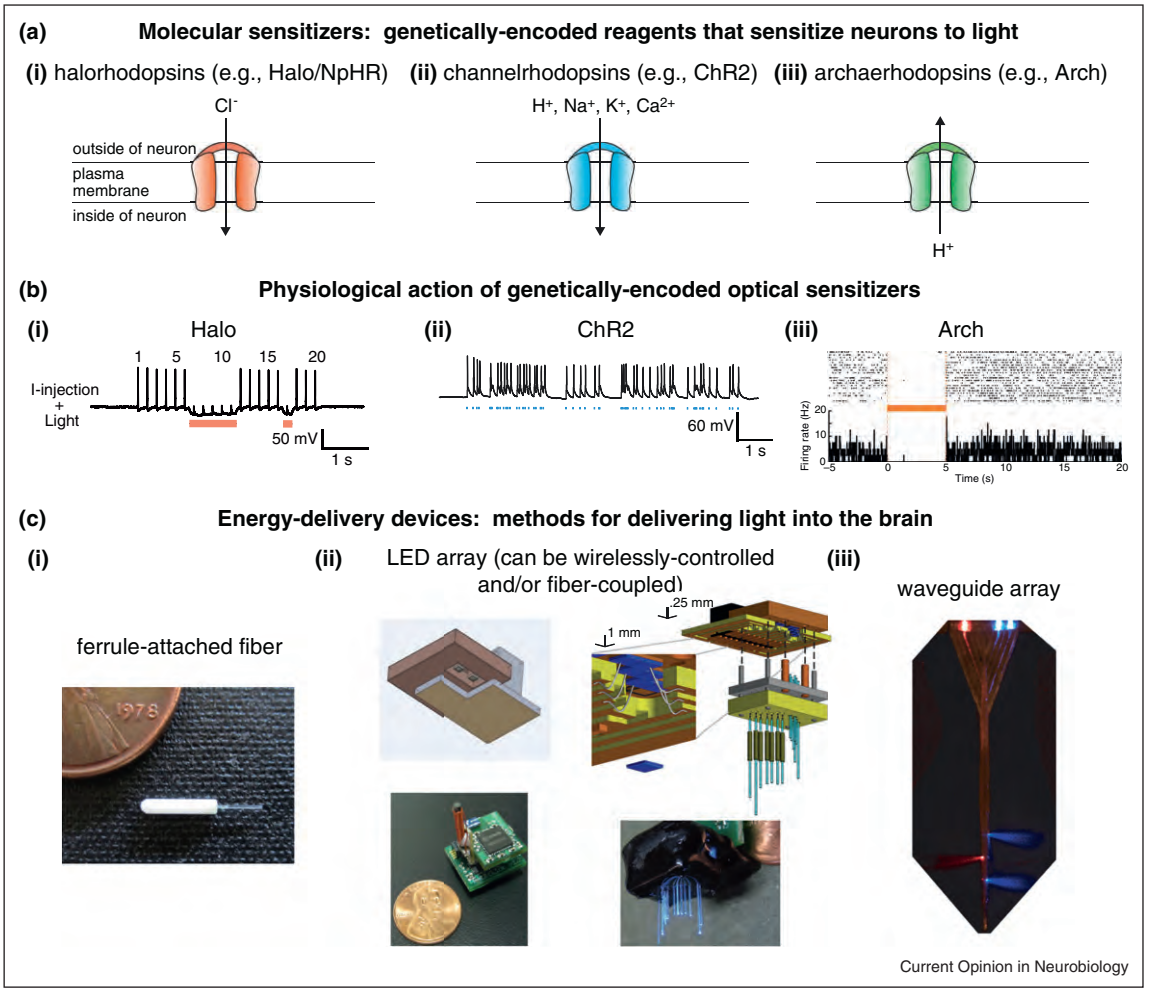
Technologies that enable the temporally precise control of the electrical activity of specific neurons, and not these neurons' neighbors (whose cell bodies or processes might be just tens to hundreds of nanometers away), must possess certain properties. Firstly, they must involve a triggering signal — a form of energy, delivered to the brain, perhaps in a focused fashion — that supports the temporal precision of the control. Secondly, it is important to deliver a molecular sensitizer, capable of responding to the triggering energy and resulting in a precise change in neural voltage, to the specific kind of neuron under investigation. The triggering energy, in such a case, should not significantly interact with the neighboring neurons — an important criterion that, as we shall see below, may require extensive validation to prove. Finally, the molecular sensitizer is most easily targeted to, and enduringly expressed in, a given neuron type if it is genetically encoded, so that the targeting and expression can take advantage of the diversity of viral, transgenic animal, and other gene delivery technologies that are in common use for delivering genes to specific cell types. Here, we describe how these principles have given rise to two toolsets in widespread use, that enable the targeted

control of neurons embedded within intact neural networks — optical neural control tools, and thermal neural control tools. We also discuss the directions being taken by these fields, going into the future.

Optogenetic tools

One set of fully genetically encoded molecular sensitizers, which make the targeted neurons sensitive to being activated or silenced by light, is the set of microbial opsins (Figure 1a), known as the 'optogenetic' toolbox. These molecules are seven-transmembrane proteins found in organisms ranging from archaea to plants, which respond to light by translocating specific ions from one side of the membrane to the other, thus resulting in light sensation or energy production; for this reason, and for their fascinating biophysical properties, they have been studied for over 40 years [10]. Three major classes of these molecules are in use. Light-driven inward chloride pumps, known as halorhodopsins (Figure 1ai), when expressed in neurons, support light-driven neural hyperpolarization. The first of these to be used in neurons, the *N. pharaonis* halorhodopsin [11–15], can support the

Figure 1



Molecular sensitizers and energy-delivery devices, for optical control of neurons. **(a)** Diagrams depicting the responses to light of (i) halorhodopsins (light-driven inward chloride pumps, which hyperpolarize neurons in which they are expressed when illuminated), (ii) channelrhodopsins (light-gated inward nonspecific cation channels, which depolarize neurons in which they are expressed when illuminated), and (iii) archaerhodopsins (light-driven outward proton pumps, which hyperpolarize neurons in which they are expressed when illuminated). **(b)** Electrophysiological data demonstrating the use of (i) the *N. pharaonis* halorhodopsin (adapted from [17]), (ii) the *C. reinhardtii* channelrhodopsin ChR2 (adapted from [21]), and (iii) the *H. sodomense* archaerhodopsin Arch (adapted from [24••]), to mediate the control of neural voltage in response to light. **(c)** Methods for the delivery of light into the brain. (i) Chronically implanted optical fiber, to be inserted into the brain, with ferrule connector that emerges from the brain for easy connection to a corresponding ferrule on an optical fiber coupled to a laser. (ii) Arrays of small raw-die LEDs (*top left*, showing two LEDs from Cree), which can be wirelessly powered and controlled via a small (~1–2 g) radio-powered receiver (*bottom left*). Fibers can also be coupled directly to the LEDs for deep light delivery; fiber tip irradiances can easily exceed 200 mW/mm^2 (*top right, bottom right*, showing a fourteen-LED array designed for targeting bilateral hippocampus). Adapted from [64]. (iii) Microfabricated waveguide arrays for delivery of light to multiple points along the axis of a single, miniature inserted probe (adapted from [65]).

quieting of neural activity in response to yellow light (Figure 1bi) [16,17], although the currents are low in part due to poor protein expression and trafficking, which can be improved through the appending of trafficking and export sequences from potassium channels [18,19••]. Light-driven inward nonspecific cation channels, known as channelrhodopsins (Figure 1aii) [20], when expressed in neurons, support light-driven neural depolarization; the first of these to be used in neurons, the *C. reinhardtii* opsin channelrhodopsin-2, can support the driving of action potentials using brief, millisecond-timescale pulses of blue light (Figure 1bii) [21]. Finally, light-driven outward proton pumps [22,23], such as the *H. sodomense* opsin archaerhodopsin-3 (Figure 1aiii), when expressed in neurons, support powerful light-driven neural hyperpolarization, sufficient to achieve complete shutdown of neurons in the awake behaving mouse, in response to yellow or green light (Figure 1biii) [24••].

The light fluxes, or irradiances, required to activate the majority of these molecules when they are expressed at typical levels, typically fall in the range 0.1–10 mW/mm^2, a range that is safe to use in scientific experiments, but high enough that background light does not typically affect the molecules. Many molecular engineering efforts, involving genome mining and mutagenesis, are underway, resulting in opsins that are more light sensitive (e.g. the archaerhodopsin ArchT [25], the channelrhodopsin CatCh [26•]), higher-amplitude or slower to run down (e.g. several channelrhodopsin mutants and chimeras including H134R, T159C, ChRGR, and ChIEF [27,28,29•,30,31]), faster or slower to turn off after illumination (e.g. several channelrhodopsin mutants including ChETA, SFO, and the D156A mutant [32–34]), color shifted (e.g. Mac, VChR1, and MChR1 [24••,35,36]), or calcium permeability-enhanced (CatCh again [26•]), with new variants arising at a rapid pace. These molecular sensitizers are in widespread use in organisms ranging from *C. elegans* to non-human primate [27,37•,38]. Although many groups use them to perturb neurons, they can be used in glial cells [39,40], as well as in other tissues such as heart [41,42]. Although these molecules require the chromophore all-*trans*-retinal for their operation, this molecule appears to naturally exist at high enough levels in mammals to not require supplementation; for *C. elegans*, Drosophila, and other organisms, dietary supplementation with all-*trans*-retinal is easily achieved.

Other opsin-related light-activated protein cascades, such as the Drosophila phototransduction cascade (the first fully genetically encoded toolset demonstrated to be capable of optical neural control) [43], the rat opsin RO4 [44], and the light-gated mammalian opsin melanopsin, have also been applied to optical manipulation of neural activity [45], although these tools have slower kinetics than the microbial opsins described in the previous paragraph. Still other approaches utilize non-opsin proteins, which require chemical co-factors to be administered; this complicates usage, but can enable biologically defined molecular events to be driven by light. For example, optical chemical uncaging of ligands that bind to defined receptors, which in turn are expressed in defined neuron types, can enable the receptor-expressing neurons to be activated when pulses of UV light uncage the ligands [46,47]. Artificial chromophores that change configuration when exposed to light can also be used; for example, tethering a ligand to a receptor or channel via a light-isomerizable azobenzene linker that is covalently attached to the receptor or channel, can enable that receptor or channel to be activated or blocked when the azobenzene is illuminated [47–50]. Because such methodologies can allow practically arbitrary receptors or channels to be recruited or blockaded, they can result in large and/or well-characterized photomodulatory influences to be driven, but the need for chemicals mandates a method for delivery of the chemicals to the neural structures of interest.

The genes are small enough to fit in most commonly used viral vectors, such as lentiviral vectors, AAV vectors, HSV vectors, and others used in neuroscience [21,51]; in addition, it is possible to make transgenic mice expressing these molecules under a specific promoter [52]; as a result, most of the commonly used methods for gene delivery have been applied to the delivery of these opsins to target specific cell types. One popular method, which takes advantage of the burgeoning number of mice expressing the Cre recombinase in specific cell types within the nervous system, is to use a virus that encodes for an opsin sequence flanked by Cre-dependent recombination sites (lox sites), such that only the specific cells bearing the Cre recombinase will enable the expression of the opsin [53•]. Recently, transgenic mice that bear opsins preceded by transcriptional stop sequences flanked by lox sites have begun to appear [54], so that breeding such a mouse with a mouse that expresses Cre in a desired cell type, will result in offspring that express the opsin in just the desired cell type. Methods for labeling developing neural circuits through electroporation [55], and retrograde labeling of synaptic inputs to a region, have also been demonstrated [19••,56].

The method of energy delivery, for optogenetics, is to deliver light — a strategy that rides the technology development trends in optics that have been driven by fields ranging from telecommunications to displays to medical imaging. *In vitro*, that is, in neural cultures and brain slices, and in transparent organisms such as *C. elegans* and zebrafish, optical stimulation and silencing are easily achieved with conventional microscope optics (e.g. fluorescence lamps, LEDs, and lasers) [21]; micromirror devices, confocal microscopes, and two-photon microscopes have also been used to achieve patterned light

stimulation [55,57]. The development of scanning-based and scan-free methods of delivering light to the periphery of cells, to concentrate light power on the cell membrane where these opsins are located, is enabling effective two-photon cellular photostimulation as well [58,59,60•]. In addition, the ability to target opsins to specific subcellular compartments will enhance the focality of stimulation, both for studying how parts of neurons contribute to overall cellular function, and for enhancing the ability to drive circuit elements in biophysically specified ways [61,62•].

For mammalian *in vivo* use, a variety of methods have been developed to achieve light delivery. Optical fibers can be inserted into the brain chronically, with a connector on the end sticking out of the brain (Figure 1ci), or can be inserted at experiment time down a previously implanted guide cannula [63]. Arrays of inexpensive, small, raw-die LEDs can be made using circuit board fabrication technology, and used to drive sets of surface brain structures, even via compact, low-weight, wireless power and control devices, important for long-term or complex experiments (Figure 1cii, left) [64]. Arrays of optical fibers, directly coupled to arrays of LEDs via optics glue, can enable tiling of deep structures such as the hippocampus with light (Figure 1cii, right). LED brightness is rapidly improving, driven by lighting and other industries; fibers coupled to raw-die LEDs can already achieve irradiances of 200 mW/mm^2 at the fiber tip, for blue light. Microfabricated optical fiber-shaped probes, consisting of arrays of parallel waveguides, enable light delivery to many points along the axis of the probe, enabling more versatile 3D control of brain circuits while minimizing damage [65] (Figure 1ciii).

Although irradiances of 100–200 mW/mm^2 are in common use for 100–200 μm fibers, enabling targeting of ~1–3 mm^3 of tissue depending on the opsin type (careful calibration of light sources is important), it is always critical to do control experiments to insure that heating or other effects of the illumination (e.g. behavioral alerting via the retina) are not affecting the experiment. For multi-light-source devices, characterization of the light spread of the set of sources is important. Another important control experiment is to make sure that endogenous light sensitivity of neurons is not affecting experiments; recently, several sets of neurons were discovered in *C. elegans* and Drosophila to possess intrinsic light sensitivity [66–68], highlighting the importance of control experiments to make sure that the illumination is not directly playing a role in altering neurons of relevance to the experimental scenario.

In the first few years after the first paper on using microbial opsins in neurons, these tools were applied to the activation of defined neurons within neural circuits to see how they influence or mediate wakening [69], learning [70], vision [71,72], somatosensation [73–75], movement [63,76,77], and breathing [78]. Usage of these tools has increased over the years. In the last year or so, optogenetic tools have been used to investigate the role of many different kinds of neuron in physiology and behavior — for example, demonstrating that specific pathways connecting two brain regions, the basolateral nucleus of the amygdala and the nucleus accumbens, when photostimulated, can serve as a reinforcing or rewarding stimulus [79], or that the activation of specific neurons in the piriform cortex, which diffusely receives information from the olfactory bulb, can be transformed into behaviorally meaningful events by associative learning [80]. Although almost all of these papers are focusing on the activation or silencing of populations of hundreds to thousands of neurons, the higher resolution photostimulation methods and protein-localization methods described above will certainly augment the kinds of neural coding questions that can be answered using optogenetic tools in the years to come.

Thermogenetics

An alternative class of genetically encoded molecular sensitizers permits the modulation of neuronal activity in response to temperature changes [81,82••,83••], providing a 'thermogenetic' toolbox. As temperature affects all physiological processes, its use as a stimulus to activate neurons presents special challenges. First, the temperature changes must be small to avoid general effects on neuronal firing and behavior. Thus thermogenetic tools must be exquisitely temperature-responsive. Second, the temperatures must be compatible with the physiology of the organism being manipulated. Since organisms like flies and mice operate at different temperatures, they require different thermogenetic tools. So far, the challenges inherent in thermogenetics have been successfully addressed in *Drosophila*, but still remain for other organisms.

Current thermogenetic approaches exploit two types of molecular tools, one for inhibition and another for activation. The first widely used thermogenetic tool was an inhibitor of neuronal activity based on a temperature-sensitive version of the *Drosophila* Shibire protein, a Dynamin GTPase involved in endocytosis [81]. A single amino acid substitution in Shibire (G273D) creates a dominantly acting protein, $Shibire^{ts1}$, that inhibits endocytosis at temperatures above ~29°C [81,84]. At the synapse, this affects synaptic vesicle recycling and rapidly inhibits chemical transmission [84,85]. The inhibitory effects of $Shibire^{ts1}$ are observed in a wide range of cells and persist throughout the period of temperature elevation. In addition, the onset of inhibition and recovery from inhibition are relatively rapid, within a few minutes of the temperature shift. These favorable properties have led to the widespread and highly successful application of cell-type-specific expression of $Shibire^{ts1}$ as

a method for dissecting neural circuits in *Drosophila* [86]. However, Dynamin affects many vital processes beyond neurotransmitter release including nutrient uptake and receptor-mediated endocytosis, a critical element in Notch, Wnt, Epidermal Growth Factor, and other intercellular signaling pathways [87]. In addition, gap junction-mediated communication should remain operable during Shibire^{ts1}-mediated perturbations. Thus, while Shibire^{ts1} provides a powerful tool for perturbing cellular function, thermogenetic tools that are more specifically targeted to neuronal activity and that modulate electrical as well as chemical communication are desirable.

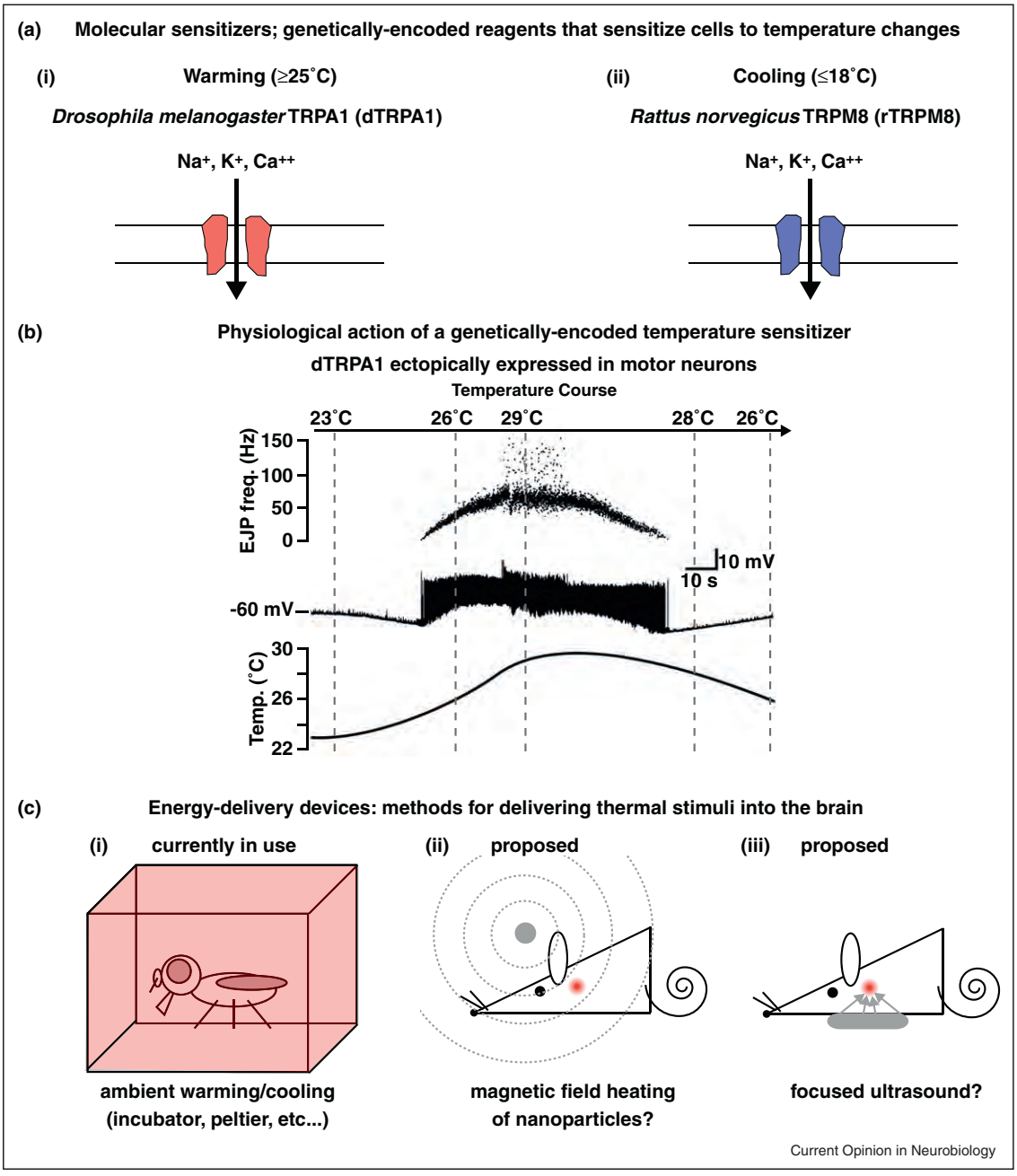
Recently, such a set of thermogenetic tools for modulating neuronal excitability has been developed by exploiting the exceptional thermal sensitivity of thermoTRPs (Figure 2). ThermoTRPs are cation channels of the Transient Receptor Potential (TRP) family whose conductances change dramatically with temperature [88,89]. Their thermal sensitivity is such that a neuron expressing a thermoTRP can switch from silent to robustly active in response to temperature shifts as small as 1–2°C [82••,90]. This permits the specific activation of selected neurons while minimizing other potential thermal effects on circuit properties. Two additional features make thermoTRPs particularly useful as tools for neuronal activation. First, the single channel conductance of a thermoTRP is about three orders of magnitude greater than that of a channelrhodopsin (50–100 pS for TRPs versus 40–60 fS for ChR2 [91,92]). This ~1000-fold greater activity means thermoTRPs drive robust depolarization at lower expression levels. The ability of thermoTRPs to drive robust activation at modest expression levels means even relatively weak promoters can be used in thermogenetics. In addition, low-level expression minimizes potential toxicity associated with expression of exogenous proteins. Second, while getting light into the brain of a small non-transparent animal like a fruit fly is challenging, thermal stimuli can be delivered by ambient warming, which is simple to deliver and non-invasive. Unfortunately, thermoTRP ion selectivity has restricted their role to neuronal activation. The development of a similar set of thermogenetic silencing agents is needed.

To date, two thermoTRP-based tools have been developed for use in *Drosophila*: rat TRPM8 (rTRPM8) [83••] and *Drosophila melanogaster* TRPA1 (dTRPA1) [82••] (Figure 2a). rTRPM8 is a cold-responsive channel activated below ~25°C in heterologous cells [93,94], while dTRPA1 is a heat-responsive channel active above ~25°C [95]. In practice, robust activation of fly neurons using rTRPM8 requires cooling the animal to ≤18°C [83••], a temperature compatible with many behaviors, but which flies normally find aversive [96]. dTRPA1 can activate fly neurons at more moderate temperatures [82••], facilitating its application in behavioral studies (Figure 2b). In fly motor neurons, for example, dTRPA1 drives activation starting at ~25°C [82••,90] (Figure 2b), within the fly's preferred temperature range of ~24–27°C [82••,96]. In practice, the temperatures used to elicit behaviors with dTRPA1 can vary from as low as 25–31°C or higher [97,98,99•,100••,101••]. This variation could reflect differences in dTRPA1 levels resulting from the use of different promoters to drive expression or variations in the intensity with which a given neuron must be stimulated to yield an observable behavior. In addition, these differences could also reflect context-dependent variations in dTRPA1's threshold, as a thermoTRP's threshold can be modulated by voltage, by G-protein and phospholipid signaling and by post-translational modification [89]. Importantly, dTRPA1-mediated activation is both robust and sustained. Physiological measurements detect little desensitization of dTRPA1-mediated motor neuron activation even after 20 min at 27°C [90], and behavioral evidence suggests dTRPA1 can activate neurons in the fly brain for at least three days [97]. Taken together, dTRPA1's ability to activate diverse neurons under the control of a wide variety of promoters, its resistance to inactivation, its responsiveness to moderate temperatures, and the ease of delivering thermal stimuli have combined to make dTRPA1 the most widely used tool for stimulating neuronal activation in *Drosophila*.

While a powerful activation strategy, a limitation of thermogenetics is that it lacks the millisecond temporal resolution of optogenetic tools [102]. In applications to date, thermoTRPs activate neurons over time scales of seconds [82••,90] (Figure 2b), likely reflecting the kinetics of tissue heating and cooling. However, thermoTRPs are capable of much more rapid responses; for example, the mammalian thermoTRP TRPV1 responds with a time constant of ~5 ms to an infrared-laser triggered temperature jump [103]. This raises the interesting possibility of using thermogenetics to achieve the kind of paired stimulation first demonstrated by Claridge-Chang *et al.* [104]. Those investigators used the $P2X_2$ system, in which a channel is activated by laser-stimulated uncaging of caged ATP injected into the fly, to pair activation of a specific set of neurons with exposure to a second stimulus, in their case an odor [104]. One can envision achieving similar effects with thermogenetics by using lasers or other rapid heating approaches to achieve thermoTRP activation.

While thermogenetics, at least as applied to date, has not driven activity patterns with fine temporal structure, high-resolution control of spiking pattern is unnecessary for many applications, including examining the effects of activity over development [99•] or linking activation of specific neurons to behavioral outputs [97,98,100••,101••]. The latter is currently the major application of thermogenetics in *Drosophila*. Its robustness and simplicity has enhanced not only the functional analysis of previously

Figure 2



Molecular sensitizers and energy-delivery devices, for thermal control of neurons. **(a)** Diagrams depicting the responses to changes in temperature of (i) the warmth-activated dTRPA1 channel and (ii) the cold-activated rTRPM8 channel. Both dTRPA1 and rTRPM8 are non-selective cation channels of high conductance that depolarize cells upon appropriate thermal stimulation. **(b)** Electrophysiological data demonstrating the use of dTRPA1 to control a fly motor neuron (adapted from [83••]). EJP: excitatory junction potential of post-synaptic target muscle. **(c)** Methods for delivering thermal stimulation into the brain. (i) Changes in ambient temperature are commonly used to control thermogenetic tools in *Drosophila*. (ii) Magnetic field-mediated heating of $MnFe_2O_4$ nanoparticles has been proposed as a possible strategy for delivering local thermal stimulation within the mammalian brain [106]. (iii) Focused ultrasound is a powerful strategy for localized tissue warming deep in the mammalian brain, and it could potentially be adapted to control thermogenetic tools.

identified neurons, but enabled the development of new approaches for circuit mapping [100••,101••]. These approaches exploit the powerful Gal4/UAS system for driving cell-type-specific expression to express dTRPA1 in various subsets of cells, rendering them temperature-activated. The investigator then tests the function of these cells by warming the flies and examining their behavior. Two recent studies have used this strategy to

dissect the circuitry controlling courtship [100••,101••]. These studies initially confirmed prior work in which remote-control activation of fruitless-expressing neurons using the $P2X_2$ system elicited courtship song [105]. These studies then used TRPA1-based thermogenetic stimulation to sort through ~800 different Gal4 strains and ~475 different genetically mosaic individuals to identify specific subsets of neurons driving specific aspects of courtship behavior [100••,101••]. Not only does the robustness and technical simplicity of TRPA1-mediated neuronal activation greatly facilitate such large-scale thermogenetic circuit mapping, the relatively limited investment in equipment, training and materials required to perform such manipulations makes it readily accessible to a wide range of students and researchers. One anticipates this thermogenetic mapping technique will be extended to study many aspects of fly behavior, leading to significant advances in our understanding of how circuits control behavior.

The extension of thermogenetics to warm-blooded animals like mammals faces two main challenges. First, while heating a fly's brain is easy, heating the brain of an intact mammal requires more sophisticated approaches. One possible solution for local, transient heating of the mammalian brain has recently been described involving radio frequency magnetic-field induced heating of streptavidin-conjugated $MnFe_2O_4$ nanoparticles [106]. A second potential alternative is focused ultrasound. While commonly used to ablate tumors by heating small regions inside the human brain (3–5 mm in diameter), sonication can also be used to achieve moderate warming [107]. While still in their early days, both approaches could potentially allow for highly targeted heating of specific regions of the mammalian nervous system in a minimally invasive fashion (Figure 2c).

A second challenge for mammalian thermogenetics is the lack of appropriate thermogenetic tools. The criterion for mammalian thermogenetic tools is particularly stringent: not only do mammalian nervous systems normally operate over narrower temperature ranges than flies, the gap between normal body temperatures (~37–38°C) and noxious, tissue-damaging temperatures (~43°C) is also narrower. Neither rTRPM8 nor dTRPA1 are compatible with use in mammals as their activation thresholds are well below normal mammalian core body temperatures. To date, the heat-activated rat TRPV1 (rTRPV1) channel has been used for thermogenetic activation of cultured mammalian cells [106]. However, rTRPV1 is normally activated at noxiously high temperatures, >42°C [108]; such temperatures create a suboptimal situation for studying circuit function and behavior. Mammalian TRPV1s also have many endogenous chemical agonists including anandimide, protons and lipoxygenase products [89], further complicating their use as thermogenetic tools. To permit wide application of thermogenetics in mammals, thermoTRPs with more suitable properties are required. One anticipates that both genome mining for new thermoTRPs and mutagenesis of existing thermoTRPs will be helpful in this regard.

Given the advanced state of mammalian optogenetics, is mammalian thermogenetics worth developing? At a practical level, the high conductance of thermoTRPs compared to channelrhodopsins should allow thermoTRPs to activate a wide range of cells at modest expression levels. In addition, the potential of thermogenetic tools to be activated without a need for light source implantation makes them potentially well suited for applications requiring deep brain stimulation. Finally, the potential for truly non-invasive activation also raises the possibility that thermogenetic stimulation could be used in many different areas of a single animal's brain simultaneously or sequentially. Together these approaches could speed and potentially transform the characterization and manipulation of mammalian circuit properties and function, providing a useful complement to optogenetic approaches.

Conclusion

Optogenetic and thermogenetic tools both moved rapidly from introduction to widespread application in basic research, reflecting the tremendous demand for methods of controlling the electrical activity of specific cells embedded within dense neural tissue. Both fields benefited from the discovery of natural molecules that serve as genetically encoded tools that sensitize targeted neurons to specific kinds of energy, and both fields also use forms of energy that are easily deliverable and controllable in laboratory settings. In the near term, many improvements to existing optogenetic and thermogenetic tools would be highly useful. For example, increases in optogenetic tool conductance would further simplify their application, permitting their use with a wider range of promoters and with lower intensity illumination. The development of novel optogenetic tools might also facilitate the creation of transgenic mice strains stably expressing these proteins; to date, the number of transgenic mice expressing these opsins has remained limited. For thermogenetics, the development of delivery methods and molecules compatible with mammalian application are needed. In addition, while thermoTRPs are useful for activation, the development of a complementary set of temperature-gated inhibitors would be extremely useful.

Over the longer term, one can also imagine potentially harnessing other sensory stimuli beyond light and thermal energy, such as mechanical force, magnetic fields, and beyond, to control neural activity, as appropriate molecular sensitizers become available. As such classes of genetic activators and inhibitors become available, they may be useful not only within their individual modalities, but also in combination, enabling investigators to take advantage of the strengths of each toolset and approach. Much like

the developments that followed the introduction of Green Fluorescent Protein (GFP), continual improvements to the molecular toolbox of genetically encoded neuronal activators and inhibitors and ongoing extensions of their applications lie ahead, buoyed not only by ever-expanding genomic discovery efforts but also high-throughput and mutagenic screening approaches. In the future, it is possible that such technologies may even directly support new therapeutic modalities for humans, enabling the control of specific cells embedded within dense neural tissue, for more efficacious, side-effect free, neuromodulation therapies.

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- • of special interest
- •• of outstanding interest

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