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Magnetogenetics: remote non-invasive magnetic activation of neuronal activity with a magnetoreceptor

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Abstract Current neuromodulation techniques such as optogenetics and deep-brain stimulation are transforming basic and translational neuroscience. These two neuromodulation approaches are, however, invasive since surgical implantation of an optical fiber or wire electrode is required. Here, we have invented a non-invasive magnetogenetics that combines the genetic targeting of a magnetoreceptor with remote magnetic stimulation. The non-invasive activation of neurons was achieved by neuronal expression of an exogenous magnetoreceptor, an iron-sulfur cluster assembly protein 1 (Isca1). In HEK-293 cells and cultured hippocampal neurons expressing this magnetoreceptor, application of an external magnetic field resulted in membrane depolarization and calcium influx in a reproducible and reversible manner, as indicated by the ultrasensitive fluorescent calcium indicator GCaMP6s. Moreover, the magnetogenetic control of neuronal activity might be dependent on the direction of the magnetic field and exhibits on-response and off-response patterns for the external magnetic field applied. The activation of this magnetoreceptor can depolarize neurons and elicit trains of action potentials, which can be triggered repetitively with a remote magnetic field in whole-cell patch-clamp recording. In transgenic *Caenorhabditis elegans* expressing this magnetoreceptor in *myo-3*-specific muscle cells or *mec-4*-specific neurons, application of the external magnetic field triggered muscle contraction and withdrawal behavior of the worms, indicative of magnet-dependent activation of muscle cells and touch receptor neurons, respectively. The advantages of magnetogenetics over optogenetics are its exclusive non-invasive, deep penetration, long-term continuous dosing, unlimited accessibility, spatial uniformity and relative safety. Like optogenetics that has gone through decade-long improvements, magnetogenetics, with continuous modification and maturation, will reshape the current landscape of neuromodulation toolboxes and will have a broad range of applications to basic and translational neuroscience as well as other biological sciences. We envision a new age of magnetogenetics is coming.

Keywords Magnetogenetics · Optogenetics · Iron-sulfur cluster assembly protein · Magnetoreceptor · Non-invasive and remote activation · Neuronal activity and circuit

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1 Introduction

The complex neural microcircuits are the essential building blocks of how the brain works, but they are entangled with interdependent different cell types, interconnected wiring

diagrams and internetworked complicated connectome in vivo [1, 2]. Understanding how neural circuits respond to external stimuli, generate electric firing patterns, process information, compute coding, and orchestrate behavior has, therefore, remained a great challenge for neuroscientists [3]. With continuous development and maturation, many neurotechnological toolboxes [4] including optogenetics [5], chemogenetics [6, 7], deep-brain stimulation [8], and functional magnetic resonance imaging (fMRI) [9] have been proven to play an important role in dissecting, perturbing, and modulating interconnected neural microcircuits in the healthy and diseased brain. Among those well-developed neurotechnological toolboxes, both classical deep-brain stimulation and modern optogenetics make it possible to map, monitor, and manipulate physiological and dysfunctional neural microcircuit activity [9, 10]. However, they all have their own limitations or drawbacks. The classical deep-brain stimulation has been successfully used to treat Parkinson's disease and other neurological disorders, but its limitations are the necessity of surgical implant of an electrical wire, the lack of spatial selectivity or specificity, as well as its contradictory effect of low-frequency and high-frequency stimulation on neuronal excitation or inhibition, respectively [11]. Even though the most popular optogenetics could spatiotemporally activate or deactivate neural activity with a millisecond precision [12–14] and has rapidly transformed neuroscience, the side effects from opsin expression patterns, laser-induced heating, abnormal ions distribution caused by overexpressed pumps or channels, and/or undesired network homeostasis can make experimental interpretation very difficult [15]. Both optogenetics and deep-brain stimulation have been used to invasively manipulate the neuronal activity of a specific subregion in the intact mammalian brain through a permanently implanted electric wire or optical fiber during the chronic surgery [9, 16, 17]. As a result, there has been a high demand on a new generation of exclusively noninvasive neuroperturbation and neuromodulation toolboxes for the whole brain at both microcircuit and macrocircuit levels.

In this study, we invented a noninvasive technique named as magnetogenetics thereafter, which combines the genetic targeting of a magnetoreceptor with remote magnetic stimulation. The noninvasive activation of neuronal activity was executed through an iron-sulfur assembly protein, iron-sulfur cluster assembly protein 1 (Isca1) [18–20]. We speculate that this iron-containing magnetoreceptor might form as an iron-sulfur cluster that could bind to cellular plasma membrane through either cytoskeletons or filaments [18, 21, 22]. We found that this magnetoreceptor could evoke membrane depolarization and action potentials, generate calcium influx, and trigger neuronal activity in both HEK-293 and cultured primary hippocampal neurons when activated by a remote magnetic field. We then renamed this revolutionarily highly conserved magnetoreceptor as MAR. The successful combination of remote magnetic stimulation and genetic targeting will, therefore, reshape the landscape of currently available neuroperturbation and neuromodulation toolboxes including optogenetics and deep-brain stimulation. This novel technology makes the exclusively noninvasive dissection of complex brain circuitry as well as the modulation of deep-brain regions possible, opening a new door to noninvasive, remote, and magnetic control of neuronal activities in the intact mammalian brains and biological processes in other organisms.

2 Methods and materials

2.1 DNA constructs

All plasmids were constructed by standard molecular biology procedures and subsequently verified by double-strand DNA sequencing. GCaMP6s and ASAP1 were from Addgene. The AAV-CAG-MAR-P2A-GCaMP6s and Lenti-CAG-MAR-P2A-GCaMP6s were connected via a 2A peptide (P2A) under the chimeric promoter CAG (a combination of the cytomegalovirus early enhancer element and chicken beta-actin promoter). ASAP1 expression plasmid (pcDNA3.1/Puro-CAG-ASAP1) was from Addgene 52519. The AAV-CAG-MAR-P2A-ASAP1 and Lenti-CAG-MAR-P2A-ASAP1 were created with multiple PCR cloning.

2.2 HEK-293 and transfection

HEK-293 cells were maintained and continuously passaged with high-glucose Dulbecco's modified Eagle Medium (DMEM, Gibco/BRL) containing fetal bovine serum (FBS, Life Tech). Transfection was performed using either Lipofectamine-2000 (Life Tech) or classical calcium phosphate transfection.

2.3 Primary neuronal culture and transfection

Rat hippocampus were dissected from embryonic day 18 rats, and primary cultured hippocampal neurons were cultured has been described [23, 24]. Transfection was performed using either Lipofectamine-2000 (Life Tech) or classical calcium phosphate transfection at different days of in vitro culture.

2.4 rAAV production

The rAAV vector was pseudotyped with AAV1 capsid [25]. The chimeric rAAV2/1 was prepared by co-