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Review

Optogenetic strategies to investigate neural circuitry engaged by stress

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HIGHLIGHTS

- Review of current techniques for optogenetic neural circuit mapping.
- Strategies for optogenetic manipulation of neural circuit elements *in vivo*.
- Review of optotgenetic techniques used to dissect the BNST.

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ABSTRACT

Optogenetic techniques have given researchers unprecedented access to the function of discrete neural circuit elements and have been instrumental in the identification of novel brain pathways that become dysregulated in neuropsychiatric diseases. For example, stress is integrally linked to the manifestation and pathophysiology of neuropsychiatric illness, including anxiety, addiction and depression. Due to the heterogeneous populations of genetically and neurochemically distinct neurons in areas such as the bed nucleus of the stria terminalis (BNST), as well as their substantial number of projections, our understanding of how neural circuits become disturbed after stress has been limited. Using optogenetic tools, we are now able to selectively isolate distinct neural circuits that contribute to these disorders and perturb these circuits *in vivo*, which in turn may lead to the normalization of maladaptive behavior. This review will focus on current optogenetic strategies to identify, manipulate, and record from discrete neural circuit elements *in vivo* as well as highlight recent optogenetic studies that have been utilized to parcel out BNST function.

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Contents

Abbreviations: AAV, adeno-associated virus; AGRP, agouti-related peptide; ARC, arcuate nucleus of the hypothalamus; Arch, archaerhodopsin; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CaMKIIα, calcium–calmodulin dependent protein kinase IIα; CeA, central nucleus of the amygdala; ChR2, channelrhodopsin-2; CRF, corticotropin releasing factor; DAT, dopamine transporter; GABA, γ-Aminobutyric acid; HSV, herpes simplex virus; LED, light emitting diode; LDT, laterodorsal tegmentum; LH, lateral hypothalamus; LHb, lateral habenula; MeA, medial nucleus of the amygdala; NAc, nucleus accumbens; NpHR, halorhodopsin; NPY, neuropeptide Y; PAG, periaqueductal gray; PBN, parabrachial nucleus; POMC, pro-opiomelanocortin; PRV, pseudorabies virus; PTSD, post traumatic stress disorder; PVH, paraventricular nucleus of the hypothalamus; RG, rabies virus envelop glycoprotein; RMTg, rostromedial tegmental nucleus; SNr, substantia nigra reticulata; TH, tyrosine hydroxylase; TTX, tetrodotoxin; TVA, avian retroviral receptor; Vgat, vesicular GABA transporter; Vglut, vesicular glutamate transporters; W, watts.

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1. Introduction

Stress is defined as the body's response to any demands for change [1]. Although stress can be positive, as it forces an organism to adapt in order to survive, the neurophysiological components of the stress response often times become disturbed in neuropsychiatric illnesses, such as depression, generalized anxiety disorder, post traumatic stress disorder and drug and alcohol addiction [2–5]. Thus, in order to develop novel and effective treatments, a critical understanding of how discrete neural circuit elements are altered through stress-producing stimuli is essential.

The extended amygdala has been implicated in rodent and human studies as a crucial mediator of the behavioral effects of aversive stimuli and both acute and chronic stress [6–10]. The region is comprised of the bed nucleus of the stria terminalis (BNST), the central (CeA) and medial (MeA) nucleus of the amygdala, and the shell of the nucleus accumbens (NAc) [11]. Since the extended amygdala encapsulates many different structures and cell types, which are intermingled within these regions, the neurophysiological properties and behavioral importance of genetically distinct neural circuits mediating stress and anxiety remain elusive. This review will focus on current optogenetic strategies that can be used to examine neural circuit function from synapse to behavior. In addition, this review will also highlight recent optogenetic strategies that have been used to dissect the BNST, a region that has been implicated in the integration and processing of the stress response [10].

2. Optogenetics

Introduction of the light-gated cation channel channelrhdopsin-2 (ChR2) or chloride and proton pumps such as halorhodpson (NpHR) and archaerhodopsin (Arch) into genetically defined neural tissues has revolutionized neuroscience [12–15]. Opsin proteins can be delivered into mammalian brain tissue through viral vectors that contain cell specific promoters, such as calcium–calmodulin dependent protein kinase IIα (CaMKIIα) [16–18]. Additionally, transgenic animals that express cre-recombinase in defined populations of neurons can be injected with viral vectors encoding a cre-inducible opsin protein, to express opsins in only neurons that contain cre-recombinase [19–21]. This approach has been used to target discrete neuronal subtypes in the ventral tegmental area (VTA). For example, infusion of a cre-inducible ChR2 into the VTA of Vgat-cre mice transduced only GABAergic neurons [21]. Thus, promoter driven and cre-dependent viral strategies allow for the precise control of neurochemically discrete cell bodies and terminals in heterogeneous brain tissue. Unlike other neuroscience approaches such as lesions and pharmacology, optogenetics allows for more temporally controlled perturbations of distinct neural circuit pathways in awake and behaving animals. Furthermore, the combination of optogenetic strategies and traditional pharmacological techniques can also increase the precision of neural circuit manipulations [22]. Importantly, optogenetic techniques can be utilized for long term behavioral manipulations, which is critical for identifying novel neural circuits that are involved in chronic stress. For a more thorough review of optogenetic procedures and principles see Refs. [14,22–24]. This review will now focus on several new strategies that utilize optogenetic techniques to assist in the identification and modulation of discrete neural circuits in brain slices and behaving animals.

3. Optogenetic circuit mapping

ChR2-assisted circuit mapping allows for the characterization of functional connectivity between multiple neural substrates in heterogeneous tissue. With ChR2-assisted circuit mapping, axons and terminals from an input brain region can be optically stimulated even when excised from cell bodies, thus allowing for the examination of circuit connectivity in brain slices. In one of the first studies to utilize this procedure, Petreanu et al. [25] transduced pyramidal neurons in layers 2/3 of the somatosensory cortex with ChR2. Presynaptic terminals from this region were then optically stimulated in various cortical output areas to examine their synaptic input onto targeted postsynaptic neurons using slice electrophysiology. In these experiments, the authors found that layers 2/3 pyramidal neurons synapse onto pyramidal neurons in layers 2/3/5/6, a finding that would have been difficult to obtain using traditional electrophysiological techniques. In an elegant series of experiments, Atasoy et al. [19], used ChR2-assisted circuit mapping to functionally dissect subcircuits within the arcuate nucleus of the hypothalamus (ARC) and their projection neurons in order to deconstruct neural circuits that control hunger and feeding. Initially, the authors transduced two populations of neurons: agouti-related peptide (AGRP)- and pro-opiomelanocortin (POMC)-containing cells, within the ARC with a cre-inducible ChR2 to test their functional connectivity. Using optogenetics in conjunction with patch-clamp electrophysiology, the authors demonstrated that AGRP-containing neurons formed functional synapses on POMC-containing neurons within the ARC and that optical stimulation of the ARC^{AGRP}–ARC^{POMC} circuit produced robust inhibitory post-synaptic currents. Furthermore, no synaptic responses were seen in ARC^{AGRP}–ARC^{AGRP}, ARC^{POMC}–ARC^{AGRP}, or ARC^{POMC}–ARC^{POMC} circuits. Additionally, the authors examined the behavioral consequences of stimulating AGRP-containing ARC neurons on two output areas: the paraventricular nucleus of the hypothalamus (PVH) and the parabrachial nucleus (PBN). In these studies, AGRP neurons were transduced with a cre-inducible ChR2 with optical fibers implanted above the PVH and PBN within the same animal. Optical stimulation of the ARC^{AGRP}–PVH pathway produced robust increases in food intake, whereas optogenetic activation of the ARC^{AGRP}–PBN pathway had no effect.

Although the previous studies examined the functional connectivity of neural circuit output regions, optogenetic techniques can also be used to examine synaptic input onto a brain region of interest. For example, viral vectors such as rabies, which allow for the retrograde transport of proteins, can be employed [26]. Watabe-Uchida et al., [27] used an EnvA-pseudotyoed, G-deleted rabies virus to visualize monosynaptic inputs to genetically defined neurons within the VTA in an exquisite series of experiments. In these studies, an AAV coding a cre-inducible avian receptor TVA, required for initial infection, and an AAV encoding rabies virus envelop glycoprotein (RG), necessary for transsynaptic spread, was injected