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Optogenetic strategies to investigate neural circuitry engaged by stress

Dennis R. Sparta[1,2,*], **Joshua H. Jennings**[3,*], **Randall L. Ung**[1], and **Garret D. Stuber**[1,2,3,4,5]

[1]Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

[2]Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

[3]Neurobiology Curriculum, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

[4]Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

[5]Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

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.

Keywords

BNST; optogenetics; stress; circuit mapping

[#]Address correspondence to: Dennis R. Sparta, Ph.D., Department of Psychiatry, UNC Neuroscience Center, University of North Carolina at Chapel Hill, Tel: +1 (919) 843-7140, Fax: +1 (919) 966-1050, dennis_sparta@med.unc.edu.

[*]These authors contributed equally to this work

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[2345]. 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[678910]. 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].

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[12131415]. 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)[161718]. 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[192021]. 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[23142224]. 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.

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 into the VTA or substantia nigra reticulata (SNr) in dopamine transporter (DAT) -cre mice, to transduce only dopaminergic neurons expressing TVA. Approximately 2 weeks after the initial viral cocktail injection, the same mice were injected with the modified rabies virus. Since only neurons that expressed TVA were transduced by the rabies viral construct, inputs to dopamine neurons were visualized and quantified, providing a comprehensive map of midbrain dopaminergic circuitry. Furthermore, rabies viral vectors can also be packaged

with cre and used in combination with optogenetic strategies in order to selectively control presynaptic inputs into a target region. Utilizing these techniques, Chaudhury et al.[28], injected a pseudorabies virus expressing cre (PRV-cre) into the NAc and an AAV coding a cre-inducible ChR2 into the VTA. Consequently, only neurons that expressed cre expressed a functional ChR2. Thus, the authors were able to optically stimulate VTA neurons that project to the NAC to manipulate behavior during a social-interaction task. Opsins can also be tethered to rabies viral vectors for trans-synaptic transduction. Lammel et al.[29] injected a rabies virus coding a cre-inducible ChR2 into the VTA of tyrosine hydroxylase (TH) -cre mice and implanted fibers into two VTA input structures, the laterodorsal tegmentum (LDT) and the lateral habenula (LHb). Due to the retrograde insertion of ChR2, the authors were able to control two distinct inputs onto VTA dopamine neurons. The authors found that optical stimulation of the LDT caused a conditioned place preference, whereas optogenetic activation of the LHb resulted in a conditioned place aversion, consistent with previous data[30]. One caveat in using a rabies viral vector for retrograde transsynaptic labeling, however, is that the virus will cause cell death approximately 2 weeks post transduction[26]. Thus, long-term behavioral manipulations are often not feasible. Herpes simplex viral vectors (HSV), which can also retrogradely transport proteins, such as opsins, may be beneficial for long term *in vivo* applications since cell death from viral infection is significantly lowered when compared to rabies. Additionally, HSV allows for rapid transduction (approx. 24 hr) into cell bodies, whereas AAV vectors take approximately 10–14 days for maximal expression. Figure 1 depicts a schematic summary detailing various strategies discussed for optogenetic circuit mapping.

Optogenetic manipulation of projection neurons *in vivo*

Although a majority of optogenetic experiments have focused on controlling cell bodies to measure a behavioral phenotype[31323334353620], opsins can be trafficked from the soma to axon terminals for pathway specific stimulation or inhibition, as mentioned previously in the experiments conducted by Atasoy et al[19]. In another example, Stamatakis et al.[30] globally transduced lateral habenula (LHb) neurons with ChR2 and implanted a fiber optic above the rostromedial tegmental nucleus (RMTg) in order to stimulate the LHb-RMTg pathway during behavioral tasks. The authors found that optical stimulation of LHb-RMTg neural circuit resulted in active, passive, and conditioned behavioral avoidance, indicating that this pathway is aversive. One drawback for stimulating axon terminals *in vivo* using optogenetic manipulations is that ChR2-activation of axon terminals produces back-propagating action potentials, thereby activating transduced cell bodies, which in turn can activate axons to other brain regions. To minimize the potential contribution of network activity, pharmacological inactivation of ChR2-infected cell bodies, in conjunction with optical stimulation, during behavioral assays can be employed. In a study by Stuber et al.[17], BLA glutamatergic efferents were transduced with ChR2 and a guide cannula was placed above the NAc for optogenetic activation of the BLA-NAc pathway. In these studies, mice reliably nose poked to receive optogenetic stimulation of BLA glutamatergic fibers terminating within the NAc. To further test the specificity of the BLA-NAc pathway in promoting reward seeking, the authors injected lidocaine into the BLA to inactivate ChR2-expressing cell bodies before the start of the nose-poking task. Infusions of lidocaine did not alter the

acquisition or expression of reward-seeking behavior, indicating that the effects observed were not due to the influence of network mediated activity through the BLA. Another limitation of using optogenetic terminal stimulation *in vivo* is that fibers of passage from the transduced brain region may also be activated if they pass through the output target area. Therefore, to demonstrate that the behavioral effects of ChR2 terminal stimulation are specific to the circuit, and not from fibers of passage, traditional pharmacological manipulations can be incorporated with optogenetic strategies. As previously discussed, Atasoy et al.,[19] optically stimulated inhibitory AGRP fibers originating in the ARC within the PVH, which increased food intake. In a separate series of experiments, the authors infused a GABA antagonist into the PVN during ARC^{AGRP}-PVN optical stimulation in a feeding paradigm. This resulted in a significant reduction of food intake, indicating the behavioral necessity of ARC^{AGRP} signaling within the PVN in promoting feeding.

Optogenetic identification of neurons *in vivo*

By combining optogenetics techniques and *in vivo* electrophysiology, real-time identification and recordings of distinct, genetically identified neurons is possible in heterogeneous brain tissue. In a recent study, Cohen et al.[37], recorded the activity of neurochemically distinct neurons in the VTA using optogenetic strategies *in vivo*. Here, the authors utilized the cre-dependent viral strategy in order to selectively target either dopaminergic or GABAergic neurons with ChR2 in DAT-cre and Vgat-cre mice, respectively. By coupling a fiber optic to a multielectrode array, which was implanted in the VTA, the authors revealed the identity of the recorded neurons using ChR2-mediated spiking during a classical conditioning task.

A recent study used a similar optical identification technique in order to identify genetically distinct circuit projection neurons. In these studies, Jennings, Sparta et al.[38] transduced glutamate and GABA neurons with ChR2 in the BNST of Vglut2-cre and Vgat-cre mice, respectively. Additionally, a multielectrode array was implanted in the BNST with an optical fiber above the VTA. By photostimulating terminals in the VTA, antidromic spiking was reliably detected within the BNST based on back propagating action potentials. Latencies in spike detection were then utilized to filter possible transsynaptic spikes from responses due to antidromic activation. Importantly, these experiments were completed in awake and behaving animals, which allowed for the real-time measurement of neuronal activity in optically identified VTA-projecting BNST glutamate and GABA during behavioral assays.

Strategies for optogenetic manipulation *in vivo*

Acutely implanted optical fibers were initially used to optogenetically perturb neural circuits *in vivo*. For this procedure, a fiber optic coupled to a laser was inserted into a guide cannula before any behavioral manipulations. However, these optical fibers often broke inside the cannula and repeated insertion of the optical fiber led to tissue damage. Therefore, the development of implantable optical fibers that can manipulate neural circuit elements with minimal tissue damage or change in light output over time has optimized optogenetic tools with long-term behavioral experimentation. These implanted optical fibers readily interface

with *in vivo* electrophysiological arrays or electrochemical detection electrodes to provide a more thorough probing of neural circuits *in vivo*[39]. Importantly, implantable optical fibers can penetrate into deep brain structures such as the VTA[21]. Light emitting diodes (LEDs) can also be used to deliver light to mammalian tissue. These devices have been used to photostimulate neurons transduced with ChR2 in the barrel cortex during a detection task[40]. Recently, wireless LEDs have been developed, which allow for the transmission of light into the brain without the need to tether an animal to an optical patch cable[41]. These devices weigh between 2–3 g, allow the animal a full range of movement, and can deliver up to 2 W of power. Wentz et al.[41] used wireless LEDs to deliver light *in vivo* to activate ChR2-transuced neurons within the motor cortex during a locomotor assay. One caveat with LEDs is the limited level of depth that light can penetrate into brain tissue. Studies using LEDs for optical stimulation *in vivo* have focused primarily on cortical regions although a recent study has utilized an injectable wireless LED in order to perturb deep brain structures such as the VTA[42]. These microLEDs are approximately 1000 × smaller than traditional LEDs and can provide better spatial resolution. Additionally, these devices can be coupled with electrode arrays that allows for concurrent collection of photostimulation and neuronal spiking from the same brain region. This review will now focus on optogenetic strategies used to dissect the BNST, an integral structure of the stress response.

BNST

The BNST is considered to be a connective locus between amygdaloid stress regions including the BLA and CeA and brain reward centers such as the VTA and NAc[114344454610]. The BNST can be subdivided into many sub-nuclei, including the oval, juxtacapsular, and rhomboid nuclei[47], yet consists primarily of GABAergic neurons[48]. However, neurons expressing vesicular glutamate transporters (Vglut2 and Vglut3) are also evident[464950]. Neuropeptides including corticotropin releasing factor (CRF), neuropeptide Y (NPY), enkephalin, substance P, neurotensin, and dynorphin also comprise many BNST neurons[5152535455565057]. These neuropeptides can be used as selective markers for small subpopulations of cells. However, their role is not well-understood in the regulation of the stress response.

BNST and stress

The BNST has been implicated in integrating and processing stress as well as pathological anxiety states, such as post traumatic stress disorder (PTSD), that result from chronic stress exposure and/or aversive stimuli[58596061626364]. The BNST has been hypothesized to mediate “sustained” fear and/or anxiety after repeated stressors, as well as contextual cues that predict aversive and/or stressful stimuli[65]. Information pertaining to acute or phasic fear responses, on the other hand, is thought to be mediated by other amygdala nuclei, such as the BLA and CeA[6664]. Additionally, the BNST has been linked as a key structure in modulating stress-induced reinstatement of drug seeking[67]. Inactivation of the BNST, using muscimol and baclofen, can block cocaine reinstatement following injections of the pharmacological stressor yohimbine[68]. Interestingly, BNST efferents to brain reward centers such as the VTA can encode stressful stimuli, as VTA-projecting BNST neurons exhibit

increased c-Fos protein levels following cocaine reinstatement and a forced swim stressor[6970].

One intriguing signaling molecule within the BNST that plays a key role in modulating stress is CRF. Stress-producing stimuli can elevate CRF expression in the dorsolateral and ventrolateral regions of the BNST[71727374]. Additionally, overexpression of CRF by a lentivirus in the BNST enhances a conditioned fear response[75]. Consistent with the BNST's role in promoting sustained fear/anxiety, administration of a CRF antagonist into the BNST blocked sustained, but not phasic, fear behaviors[65]. Moreover, intra-BNST injections of CRF antagonists can block both stress-induced reinstatement of cocaine seeking and morphine conditioned place preference[7677]. Interestingly, acute inhibition of the BNST by tetrodotoxin (TTX) injection during stress exposure prevents the expression of stress-induced anxiety[58] and activation of CRF receptors directly in the BNST induces social anxiety[78]. In a recent study, Gafford et al.,[79] knocked out $GABA_A$ α1 subunits in CRF-containing neurons within in the BNST, causing increases in anxiety-like behavior as well as impairments in the extinction of conditioned fear. These behavioral deficits were rescued with an intra-BNST infusion of a CRF antagonist.

Since the BNST comprises many different subnuclei and genetically distinct cell types, parceling out specific neural circuit elements that are altered during or following stress exposure has been extremely challenging. Moreover, little is known about specific BNST projection targets since electrical stimulation of this structure often results in indirect stimulation of other brain regions and fibers of passage. However, by utilizing optogenetic strategies, we now have a contemporary toolkit that will provide a circuit-aided design to determine how genetically defined BNST neural circuits process and respond to stress-producing stimuli.

Optogenetic manipulations of BNST neural circuits

Within the BNST, this type of precise functional circuit mapping described above will be essential for dissecting the plethora of genetically distinct neurons and neural circuit elements that are involved in the stress response, which traditional anatomical tracing and electrophysiology techniques cannot address. Conventional circuit tracing experiments cannot differentiate between distinct cell types in heterogeneous tissue. Additionally, electrophysiology experiments, which utilize electrical stimulation, do not provide precise synaptic connectivity due to stimulation of all cell types as well as fibers of passage. Two recent studies have utilized optogenetic strategies to parcel out distinct BNST circuits involved in reward, stress, and anxiety-related behaviors. Kim et al.[80] globally transduced BNST neurons with NpHR in order to inhibit cell bodies within this region. The authors demonstrated that photoinhibition of the BNST increased open-arm time in an elevated plus maze (EPM), indicative of anxiolysis. Conversely, photostimulation of the BNST with ChR2 resulted in anxiogenesis, as mice spent less time in the open arm of the EPM. In the same study, the authors then examined the role of BLA inputs into the BNST on anxiety-like behaviors. In these experiments, separate groups of mice were injected with a viral vector containing either ChR2 or NpHR into the BLA. Additionally, mice were implanted with optical fibers above the BNST in order to stimulate or inhibit BLA terminals within the

BNST. Surprisingly, the authors found that photostimulation of the BLA-BNST pathway produced anxiolysis, whereas photoinhibition of this pathway resulted in anxiogenesis in both the EPM and respiration rate. In a final series of experiments, the authors examined the behavioral ramifications of stimulating three discrete BNST output regions: LH, VTA, and PBN. Here, the authors reported that photostimulation of BNST efferents in each of the target output regions resulted in a unique behavioral phenotype of the anxiety-like state. Jennings, Sparta et al.[38], demonstrated that parallel yet opposing BNST subcircuits modulate divergent motivational states by utilizing optogenetic techniques. The authors transduced both glutamatergic and GABAergic BNST neurons with ChR2 in Vglut2-cre and Vgat-cre mouse lines, respectively. By antidromically stimulating BNST terminals within the VTA, the authors were able to identify VTA-projecting BNST glutamate and GABAergic neurons during a foot-shock stress paradigm. The authors found that aversive foot shock caused a net enhancement of activity of optically identified VTA-projecting BNST glutamate neurons. In contrast, aversive foot shock resulted in the reduction of activity in optically identified VTA-projecting BNST GABA neurons. In a separate series of experiments, the authors found that photostimulation of BNST-VTA glutamatergic fibers resulted in aversion and anxiety-like behavior, whereas photostimulation of BNST-VTA GABAergic terminals promoted reward-related behaviors and buffered stress induced anxiety. The results from both of these studies indicate that the BNST has a more complex role than what is canonically thought in regard to stress, anxiety, and reward. Figure 2 depicts a schematic of potential BNST output stress neural circuits, which can be manipulated by optogenetic strategies discussed above.

Conclusions

Using optogenetic strategies, we now have the ability to parcel out neural circuits within heterogeneous structures, such as the BNST, in order to examine their contribution to the stress response. With these new techniques, we will come to a greater understanding of BNST neural circuits that are recruited during exposure to stressful stimuli and potentially identify new pharmacological targets for the treatment of neuropsychiatric illness.

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List of acronyms

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

References

1. Selye H. A syndrome produced by diverse nocuous agents. 1936. J Neuropsychiatry Clin Neurosci. 1998; 10:230–231. [PubMed: 9722327]
2. Adamec R. Transmitter systems involved in neural plasticity underlying increased anxiety and defense--implications for understanding anxiety following traumatic stress. Neurosci Biobehav Rev. 1997; 21:755–765. [PubMed: 9415900]
3. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology. 2001; 24:97–129. [PubMed: 11120394]
4. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology. 2008; 33:88–109. [PubMed: 17851537]
5. Sinha R. Chronic stress, drug use, and vulnerability to addiction. Ann N Y Acad Sci. 2008; 1141:105–130. [PubMed: 18991954]
6. Buchel C, Morris J, Dolan RJ, Friston KJ. Brain systems mediating aversive conditioning: an event-related fMRI study. Neuron. 1998; 20:947–957. [PubMed: 9620699]
7. LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. Neuron. 1998; 20:937–945. [PubMed: 9620698]
8. Phelps EA, Delgado MR, Nearing KI, LeDoux JE. Extinction learning in humans: role of the amygdala and vmPFC. Neuron. 2004; 43:897–905. [PubMed: 15363399]
9. Schulz D, Canbeyli RS. Lesion of the bed nucleus of the stria terminalis enhances learned despair. Brain Res Bull. 2000; 52:83–87. [PubMed: 10808077]
10. Walker DL, Davis M. Role of the extended amygdala in short-duration versus sustained fear: a tribute to Dr. Lennart Heimer. Brain Struct Funct. 2008; 213:29–42. [PubMed: 18528706]
11. Alheid GF, et al. The neuronal organization of the supracapsular part of the stria terminalis in the rat: the dorsal component of the extended amygdala. Neuroscience. 1998; 84:967–996. [PubMed: 9578390]
12. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci. 2005; 8:1263–1268. [PubMed: 16116447]
13. Chow BY, et al. High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature. 2010; 463:98–102. [PubMed: 20054397]
14. Yizhar O, Fenno LE, Davidson TJ, Mogri M, Deisseroth K. Optogenetics in neural systems. Neuron. 2011; 71:9–34. [PubMed: 21745635]
15. Zhang F, Wang LP, Boyden ES, Deisseroth K. Channelrhodopsin-2 and optical control of excitable cells. Nat Methods. 2006; 3:785–792. [PubMed: 16990810]
16. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature. 2009; 459:698–702. [PubMed: 19396159]
17. Stuber GD, et al. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature. 2011; 475:377–380. [PubMed: 21716290]
18. Tye KM, et al. Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature. 2011; 471:358–362. [PubMed: 21389985]
19. Atasoy D, Betley JN, Su HH, Sternson SM. Deconstruction of a neural circuit for hunger. Nature. 2012; 488:172–177. [PubMed: 22801496]
20. Witten IB, et al. Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. Neuron. 2011; 72:721–733. [PubMed: 22153370]
21. van Zessen R, Phillips JL, Budygin EA, Stuber GD. Activation of VTA GABA neurons disrupts reward consumption. Neuron. 2012; 73:1184–1194. [PubMed: 22445345]
22. Stuber GD, Mason AO. Integrating optogenetic and pharmacological approaches to study neural circuit function: current applications and future directions. Pharmacological reviews. 2013; 65:156–170. [PubMed: 23319548]
23. Stamatakis, AM. S. G. Optogenetic Strategies to Dissect the Neural Circuits that Underlie Reward and Addiction. Vol. Vol. 2. Cold Spring Harbor Laboratory Press; 2012.

24. Tye KM, Deisseroth K. Optogenetic investigation of neural circuits underlying brain disease in animal models. Nat Rev Neurosci. 2012; 13:251–266. [PubMed: 22430017]
25. Petreanu L, Huber D, Sobczyk A, Svoboda K. Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. Nat Neurosci. 2007; 10:663–668. [PubMed: 17435752]
26. Wickersham IR, et al. Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. Neuron. 2007; 53:639–647. [PubMed: 17329205]
27. Watabe-Uchida M, Zhu L, Ogawa SK, Vamanrao A, Uchida N. Whole-brain mapping of direct inputs to midbrain dopamine neurons. Neuron. 2012; 74:858–873. [PubMed: 22681690]
28. Chaudhury D, et al. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature. 2012
29. Lammel S, et al. Input-specific control of reward and aversion in the ventral tegmental area. Nature. 2012; 491:212–217. [PubMed: 23064228]
30. Stamatakis AM, Stuber GD. Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance. Nat Neurosci. 2012; 15:1105–1107. [PubMed: 22729176]
31. Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Nature. 2007; 450:420–424. [PubMed: 17943086]
32. Adamantidis AR, et al. Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. J Neurosci. 2011; 31:10829–10835. [PubMed: 21795535]
33. Kravitz AV, Tye LD, Kreitzer AC. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci. 2012; 15:816–818. [PubMed: 22544310]
34. Lobo MK, et al. Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. Science. 2010; 330:385–390. [PubMed: 20947769]
35. Tsai HC, et al. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science. 2009; 324:1080–1084. [PubMed: 19389999]
36. Tye KM, et al. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. Nature. 2012
37. Cohen JY, Haesler S, Vong L, Lowell BB, Uchida N. Neuron-type-specific signals for reward and punishment in the ventral tegmental area. Nature. 2012; 482:85–88. [PubMed: 22258508]
38. Jennings JH, et al. Distinct extended amygdala circuits for divergent motivational states. Nature. 2013; 496:224–228. [PubMed: 23515155]
39. Sparta DR, et al. Construction of implantable optical fibers for long-term optogenetic manipulation of neural circuits. Nat Protoc. 2012; 7:12–23. [PubMed: 22157972]
40. Huber D, et al. Sparse optical microstimulation in barrel cortex drives learned behaviour in freely moving mice. Nature. 2008; 451:61–64. [PubMed: 18094685]
41. Wentz CT, et al. A wirelessly powered and controlled device for optical neural control of freely-behaving animals. Journal of neural engineering. 2011; 8:046021. [PubMed: 21701058]
42. Kim TI, et al. Injectable, cellular-scale optoelectronics with applications for wireless optogenetics. Science. 2013; 340:211–216. [PubMed: 23580530]
43. Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. J Comp Neurol. 1993; 338:255–278. [PubMed: 8308171]
44. Georges F, Aston-Jones G. Potent regulation of midbrain dopamine neurons by the bed nucleus of the stria terminalis. J Neurosci. 2001; 21:RC160. [PubMed: 11473131]
45. Georges F, Aston-Jones G. Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. J Neurosci. 2002; 22:5173–5187. [PubMed: 12077212]
46. Jalabert M, Aston-Jones G, Herzog E, Manzoni O, Georges F. Role of the bed nucleus of the stria terminalis in the control of ventral tegmental area dopamine neurons. Prog Neuropsychopharmacol Biol Psychiatry. 2009; 33:1336–1346. [PubMed: 19616054]
47. Ju G, Swanson LW. Studies on the cellular architecture of the bed nuclei of the stria terminalis in the rat: I. Cytoarchitecture. J Comp Neurol. 1989; 280:587–602. [PubMed: 2708568]

48. Cullinan WE, Herman JP, Watson SJ. Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. J Comp Neurol. 1993; 332:1–20. [PubMed: 7685778]

49. Kudo T, Uchigashima M, Miyazaki T, Konno K, Yamasaki M, Yanagawa Y, Minami M, Watanabe M. Three types of neurchemical projections from the bed nucleus of the stria terminalis to the ventral tegmental area in adult mice. journal of neuroscience. 2012; 32:18035–18046. [PubMed: 23238719]

50. Poulin JF, Arbour D, Laforest S, Drolet G. Neuroanatomical characterization of endogenous opioids in the bed nucleus of the stria terminalis. Prog Neuropsychopharmacol Biol Psychiatry. 2009; 33:1356–1365. [PubMed: 19583989]

51. Arluison M, et al. Demonstration of peptidergic afferents to the bed nucleus of the stria terminalis using local injections of colchicine. A combined immunohistochemical and retrograde tracing study. Brain Res Bull. 1994; 34:319–337. [PubMed: 7521777]

52. Gray TS, Magnuson DJ. Neuropeptide neuronal efferents from the bed nucleus of the stria terminalis and central amygdaloid nucleus to the dorsal vagal complex in the rat. J Comp Neurol. 1987; 262:365–374. [PubMed: 3655018]

53. Kash TL, Winder DG. Neuropeptide Y and corticotropin-releasing factor bi-directionally modulate inhibitory synaptic transmission in the bed nucleus of the stria terminalis. Neuropharmacology. 2006; 51:1013–1022. [PubMed: 16904135]

54. Li C, et al. Presynaptic inhibition of gamma-aminobutyric acid release in the bed nucleus of the stria terminalis by kappa opioid receptor signaling. Biol Psychiatry. 2012; 71:725–732. [PubMed: 22225848]

55. Merchenthaler I, Vigh S, Petrusz P, Schally AV. Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain. Am J Anat. 1982; 165:385–396. [PubMed: 6760710]

56. Phelix CF, Paull WK. Demonstration of distinct corticotropin releasing factor--containing neuron populations in the bed nucleus of the stria terminalis. A light and electron microscopic immunocytochemical study in the rat. Histochemistry. 1990; 94:345–364. [PubMed: 2228733]

57. Walter A, Mai JK, Lanta L, Gorcs T. Differential distribution of immunohistochemical markers in the bed nucleus of the stria terminalis in the human brain. J Chem Neuroanat. 1991; 4:281–298. [PubMed: 1718318]

58. Christianson JP, et al. Safety signals mitigate the consequences of uncontrollable stress via a circuit involving the sensory insular cortex and bed nucleus of the stria terminalis. Biol Psychiatry. 2011; 70:458–464. [PubMed: 21684526]

59. Hammack SE, Richey KJ, Watkins LR, Maier SF. Chemical lesion of the bed nucleus of the stria terminalis blocks the behavioral consequences of uncontrollable stress. Behav Neurosci. 2004; 118:443–448. [PubMed: 15113272]

60. Lebow M, et al. Susceptibility to PTSD-like behavior is mediated by corticotropin-releasing factor receptor type 2 levels in the bed nucleus of the stria terminalis. J Neurosci. 2012; 32:6906–6916. [PubMed: 22593059]

61. McElligott ZA, et al. Distinct forms of Gq-receptor-dependent plasticity of excitatory transmission in the BNST are differentially affected by stress. Proc Natl Acad Sci U S A. 2010; 107:2271–2276. [PubMed: 20133871]

62. Koob GF. A role for brain stress systems in addiction. Neuron. 2008; 59:11–34. [PubMed: 18614026]

63. Sullivan GM, et al. Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific cue-conditioned fear stimulus. Neuroscience. 2004; 128:7–14. [PubMed: 15450349]

64. Walker DL, Davis M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. J Neurosci. 1997; 17:9375–9383. [PubMed: 9364083]

65. Davis M, Walker DL, Miles L, Grillon C. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. Neuropsychopharmacology. 2010; 35:105–135. [PubMed: 19693004]

66. Gewirtz JC, McNish KA, Davis M. Lesions of the bed nucleus of the stria terminalis block sensitization of the acoustic startle reflex produced by repeated stress, but not fear-potentiated startle. Prog Neuropsychopharmacol Biol Psychiatry. 1998; 22:625–648. [PubMed: 9682277]

67. Shaham Y, Shalev U, Lu L, De Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl). 2003; 168:3–20. [PubMed: 12402102]

68. Buffalari DM, See RE. Inactivation of the bed nucleus of the stria terminalis in an animal model of relapse: effects on conditioned cue-induced reinstatement and its enhancement by yohimbine. Psychopharmacology (Berl). 2011; 213:19–27. [PubMed: 20827461]

69. Briand LA, Vassoler FM, Pierce RC, Valentino RJ, Blendy JA. Ventral tegmental afferents in stress-induced reinstatement: the role of cAMP response element-binding protein. J Neurosci. 2010; 30:16149–16159. [PubMed: 21123561]

70. Mahler SV, Aston-Jones GS. Fos activation of selective afferents to ventral tegmental area during cue-induced reinstatement of cocaine seeking in rats. J Neurosci. 2012; 32:13309–13326. [PubMed: 22993446]

71. Choi DC, et al. Chronic social stress in the visible burrow system modulates stress-related gene expression in the bed nucleus of the stria terminalis. Physiol Behav. 2006; 89:301–310. [PubMed: 16949112]

72. Funk D, Li Z, Le AD. Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: Relationship to the reinstatement of alcohol seeking. Neuroscience. 2006; 138:235–243. [PubMed: 16359808]

73. Kim SJ, et al. Effects of repeated tianeptine treatment on CRF mRNA expression in non-stressed and chronic mild stress-exposed rats. Neuropharmacology. 2006; 50:824–833. [PubMed: 16504218]

74. Shepard JD, Schulkin J, Myers DA. Chronically elevated corticosterone in the amygdala increases corticotropin releasing factor mRNA in the dorsolateral bed nucleus of stria terminalis following duress. Behav Brain Res. 2006; 174:193–196. [PubMed: 16934343]

75. Sink KS, et al. Effects of continuously enhanced corticotropin releasing factor expression within the bed nucleus of the stria terminalis on conditioned and unconditioned anxiety. Mol Psychiatry. 2012

76. Erb S, Salmaso N, Rodaros D, Stewart J. A role for the CRF-containing pathway from central nucleus of the amygdala to bed nucleus of the stria terminalis in the stress-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl). 2001; 158:360–365. [PubMed: 11797056]

77. Wang J, Fang Q, Liu Z, Lu L. Region-specific effects of brain corticotropin-releasing factor receptor type 1 blockade on footshock-stress- or drug-priming-induced reinstatement of morphine conditioned place preference in rats. Psychopharmacology (Berl). 2006; 185:19–28. [PubMed: 16374599]

78. Lee Y FS, Johnson PL, Shekhar A. Repeated stimulation of CRF receptors in the BNST of rats selectively induces social but not panic-like anxiety. Neuropsychopharmacology. 2008; 33:2586–2594. [PubMed: 18288095]

79. Gafford GM, et al. Cell-type specific deletion of GABA(A)alpha1 in corticotropin-releasing factor-containing neurons enhances anxiety and disrupts fear extinction. Proc Natl Acad Sci U S A. 2012; 109:16330–16335. [PubMed: 22992651]

80. Kim SY, et al. Diverging neural pathways assemble a behavioural state from separable features in anxiety. Nature. 2013; 496:219–223. [PubMed: 23515158]

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.

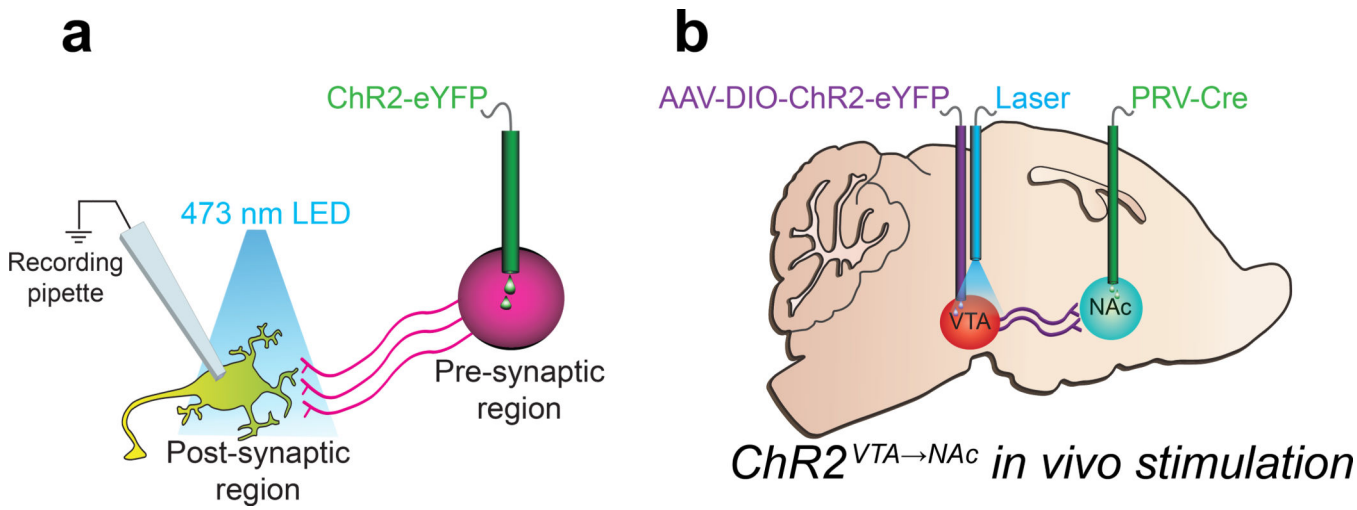

Figure 1. Optogenetic strategies for circuit mapping

a. Schematic detailing how to employ ChR2 assisted circuit mapping in slice electrophysiology. Presynaptic neurons are transduced with ChR2-eYFP. Optical stimulation (473 nm light to optimally activate ChR2) of transduced presynaptic terminals results in the detection of ChR2 mediated currents in the patch clamped postsynaptic neuron. Additionally, the recording pipette can be filled with dye to identify its neurochemical makeup. **b.** Schematic detailing how to employ a rabies viral delivery strategy in order to manipulate VTA neurons terminating in the NAc. Pseudorabies (PRV) that express a functional cre protein is injected into the NAc. PRV-cre is taken up by presynaptic terminals in the NAc. Infusion of a cre-inducible ChR2 into the VTA. Only neurons that express cre will express ChR2. An optical fiber is implanted above the VTA in order to manipulate VTA neurons that project to the NAc.

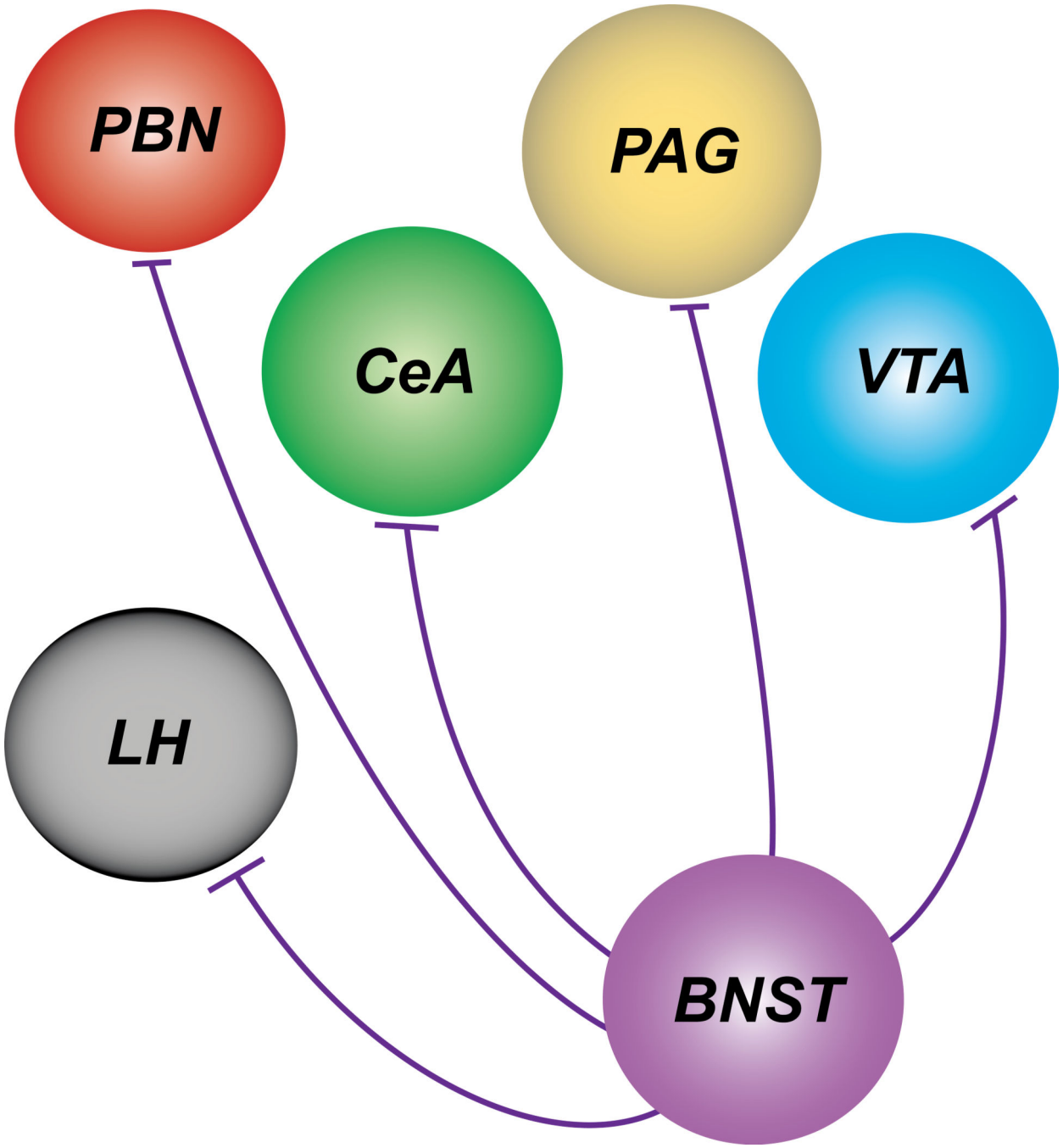

Figure 2. Potential BNST stress neural circuits

The BNST sends projections to the VTA, PAG, CeA, PBN, and LH. These potential stress neural circuits can be analyzed using optogenetic strategies detailed above.