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BIDIRECTIONAL MODULATION OF ANXIETY-RELATED AND SOCIAL BEHAVIORS BY AMYGDALA PROJECTIONS TO THE MEDIAL PREFRONTAL CORTEX

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Abstract—The basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) modulate anxiety and social behaviors. It remains to be elucidated, however, whether direct projections from the BLA to the mPFC play a functional role in these behaviors. We used optogenetic approaches in behaving mice to either activate or inhibit BLA inputs to the mPFC during behavioral assays that assess anxiety-like behavior and social interaction. Channelrhodopsin-2 (ChR2)-mediated activation of BLA inputs to the mPFC produced anxiogenic effects in the elevated plus maze and open field test, whereas halorhodopsin (NpHR)-mediated inhibition produced anxiolytic effects. Furthermore, activation of the BLA-mPFC pathway reduced social interaction in the resident-intruder test, whereas inhibition facilitated social interaction. These results establish a causal relationship between activity in the BLA-mPFC pathway and the bidirectional modulation of anxiety-related and social behaviors.

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Key words: optogenetics, prelimbic, infralimbic, fear, stress, anxiety disorders.

INTRODUCTION

The basolateral amygdala complex (BLA) is considered to be a crucial neural hub for the modulation of anxiety-related and emotionally-driven behaviors (Davis, 1992; Bremner, 2004; Tye et al., 2011; Dias et al., 2013; Felix-Ortiz et al., 2013; Janak and Tye, 2015; Namburi et al., 2015; Allsop et al., 2014). In humans, the BLA exhibits hyperactivity in most forms of anxiety disorders (Rauch et al., 2003), and in rodents BLA hyperexcitability and hypertrophy is associated with an enduring facilitation of anxiety-like behaviors (Roozendaal et al., 2009; Rosenkranz et al., 2010). Along with a critical role in anxiety, research has established a crucial role for the BLA in the modulation of social behavior (Kling and Steklis, 1976; Katayama et al., 2009; Bickart et al., 2014; Felix-Ortiz and Tye, 2014). Given the common comorbidity between anxiety disorders and social deficits (Stein and Stein, 2008; Kennedy and Adolphs, 2012; American Psychiatric Association, 2013), increasing efforts have been directed to understand BLA mechanisms underlying the regulation of anxiety and social behaviors (Allsop et al., 2014).

Despite substantial research examining the role of the BLA in anxiety-related and social behaviors, there is still much work to do in elucidating how the BLA interacts with downstream structures to modulate these behaviors. Application of optogenetics to manipulate specific projections (Boyden et al., 2005; Deisseroth, 2011; Tye et al., 2011; Tye and Deisseroth, 2012) allows us to map the functional role of discrete neural projections with high cellular and temporal precision. We have already tested the functional role of some BLA targets, such as the central nucleus of the amygdala (CeA) and the ventral hippocampus (vHPC), and found that optogenetically-mediated activation or inhibition of neural transmission from the BLA to either region produces bidirectional changes in anxiety-like behavior (Tye et al., 2011; Felix-Ortiz et al., 2013). In addition, we have observed bidirectional modulation of social behavior by targeting the BLA-vHPC pathway (Felix-Ortiz and Tye, 2014). These findings support the hypothesis that BLA interactions with downstream targets such as the CeA and vHPC are sufficient to alter anxiety, and that distinct projections can contribute opposing forces in guiding anxiety-related behavior.

Recent attention has been given to the medial prefrontal cortex (mPFC), which shares reciprocal projections with the BLA (Pitkänen, 2000; Gabbott et al., 2005; Hoover and Vertes, 2007), and exhibits profound alterations in a wide range of anxiety and social disorders (Milad and Rauch, 2007; Gotts et al., 2012). Electrophysiological recordings have revealed that

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Abbreviations: ACC, anterior cingulate cortex; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; ChR2, channelrhodopsin-2; EPM, elevated plus maze; IL, infralimbic cortex; mPFC, medial prefrontal cortex; NpHR, halorhodopsin; OFC, orbitofrontal cortex; OFT, open field test; PL, prelimbic cortex; vHPC, ventral hippocampus.

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increased excitability in the mPFC correlates with heightened anxiety-related behavior in the open field test and elevated plus maze (Bi et al., 2013), and that some populations of mPFC neurons fire preferentially to the ''anxiogenic'' open arms of the plus maze versus the ''safe'' closed arms, and vice versa (Adhikari et al., 2011). The mPFC, along with the BLA (Likhtik et al., 2014; Likhtik and Paz, 2015), is capable of representing states of high and low anxiety. The mPFC has also been shown to represent social interactions, with some populations of neurons exhibiting increased activity and others showing decreased activity during bouts of social interaction (Jodo et al., 2010). Thus, the mPFC appears to be a key component of the neural circuitry underlying social and anxiety-related behaviors. Although it has been proposed that direct interactions between the BLA and mPFC may be vital for the modulation of anxiety and social behaviors (McClure et al., 2007; Adhikari, 2014), a causal role for BLA projections to the mPFC has yet to be established. Using projection-specific optogenetic approaches in freely-moving mice, we tested how activation or inhibition of BLA projections to the mPFC modulates anxiety-like and social behaviors.

EXPERIMENTAL PROCEDURES

Subjects

All procedures were approved by the Massachusetts Institute of Technology Committee on Animal Care, in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All experiments were conducted on wild-type male C57BL/6 mice aged 6–7 weeks (Jackson Laboratory, Bar Harbor, ME). A total of 43 mice were used in this study. Mice were group-housed in clear Plexiglas homecages with access to food and water *ad libitum*. Mice were maintained on a 12 h reverse light/dark cycle. For social interaction experiments, 3–4 week old juvenile male C57BL/6 mice were used as the social stimuli (intruders).

Surgery

Mice were anesthetized with 1.5–2.0% isoflurane gas/oxygen mixture and mounted on a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA) for viral transduction of the BLA. A midline incision was made down the scalp and craniotomies were made using a dental drill. The stereotaxic coordinates used for BLA transfection were −1.6 mm anterior-posterior (AP), ±3.35 mm medial–lateral (ML), and −4.9 mm dorsal–ventral (DV), relative to bregma. A 10 μl microsyringe with a 33 G needle (Nanofil; WPI, Sarasota, FL, USA) was used to deliver the viral solutions into the BLA at a rate of 0.1 μl/min using a microsyringe pump (UMP3/Micro4; WPI, Sarasota, FL).

For inhibition, bilateral viral transduction of the BLA (0.5 μl per side) with serotype-5 adeno-associated viral vectors (AAV_5) that carried an enhanced third-generation version of the yellow light-sensitive chloride-pump *Natronomonas pharaonis* halorhodopsin (eNpHR3.0), which was fused to the enhanced yellow fluorescent protein (eYFP) and was expressed under the control of the Ca^{2+}/calmodulin-dependent protein kinase II alpha (CaMKIIα) promoter (AAV_5-CaMKIIα-eNpHR3.0-eYFP). For activation, the BLA was transfected unilaterally with similar viruses that coded for the blue light-sensitive cation-pump *Chlamydomonas reinhardtii* channelrhodopsin-2 (ChR2) fused with eYFP (AAV_5-CaMKIIα-ChR2(H134R)-eYFP). Mice in the control groups were transduced with viruses mediating expression of eYFP alone (AAV_5-CaMKIIα-eYFP). All viral aliquots were obtained from the University of North Carolina Vector Core (Chapel Hill, NC, USA). The DNA sequence maps for these viral constructs can be found online at www.optogenetics.org. Following viral infusion, needles were kept at the infusion site for ∼10 min to allow for viral diffusion. They were then slowly withdrawn at an approximate rate of ∼1 mm/min.

Optical fibers were chronically implanted over the mPFC to either inhibit or activate BLA terminals (optical fiber length, 3 mm; 300 μm core; NA = 0.37; Thorlabs, Newton, NJ, USA). Optical fibers were held in stainless steel ferrules (Precision Fiber Products, Milpitas, CA, USA). The stereotaxic coordinates used for unilateral fiber implants were +1.7 mm AP, ±0.3 mm ML, and −1.9 mm DV, relative to bregma. For bilateral implants, fibers were implanted with a 10° angle and the stereotaxic coordinates used were +1.7 mm AP, ±0.9 mm ML, and −2.1 mm DV. Fiber implants were anchored to the skull with a layer of adhesive cement (C&B Metabond; Parkell, Edgewood, NY, USA) and covered with a layer of black dental cement (Ortho-Jet; Lang, Wheeling, IL, USA). The incision was securely closed using sutures. Postoperative recovery was facilitated by maintaining body temperature using a heat lamp and reducing pain with Ketoprofen (5 mg/kg) or Meloxicam analgesic (1.5 mg/kg). ∼6–8 weeks were allowed for viral expression before behavioral testing.

Optical manipulations

Optical fibers were connected to patchcords (Doric; Québec, Canada), which were in turn connected to lasers (OEM Laser Systems; Draper, UT) with FC/PC adapters located over the behavioral testing arenas. Laser output was controlled with a Master-8 pulse stimulator (A.M.P.I.; Jerusalem, Israel). For NpHR experiments, a 100 mW 594 nm DPSS laser was used to deliver 5 mW of constant yellow light. For ChR2 experiments, a 100 mW 473 nm DPSS laser was used to deliver 5 ms pulses of blue light at 5 mW and at a frequency of 20 Hz.

Behavioral assays

All behavioral tests were performed during the active dark phase of the animals. Mice were allowed to acclimate to the testing rooms for at least 1 h prior to experiments.

Elevated plus maze (EPM). The EPM apparatus consisted of two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 30 cm) extending from a central intersection platform (5 × 5 cm). The apparatus