Amygdala Inputs to the Ventral Hippocampus Bidirectionally Modulate Social Behavior

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Behavioral/Cognitive

Amygdala Inputs to the Ventral Hippocampus Bidirectionally Modulate Social Behavior

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Impairments in social interaction represent a core symptom of a number of psychiatric disease states, including autism, schizophrenia, depression, and anxiety. Although the amygdala has long been linked to social interaction, little is known about the functional role of connections between the amygdala and downstream regions in noncompetitive social behavior. In the present study, we used optogenetic and pharmacological tools in mice to study the role of projections from the basolateral complex of the amygdala (BLA) to the ventral hippocampus (vHPC) in two social interaction tests: the resident-juvenile-intruder home-cage test and the three chamber sociability test. BLA pyramidal neurons were transduced using adeno-associated viral vectors (AAV) carrying either channelrhodopsin-2 (ChR2) or halorhodopsin (NpHR), under the control of the CaMKIIα promoter to allow for optical excitation or inhibition of amygdala axon terminals. Optical fibers were chronically implanted to selectively manipulate BLA terminals in the vHPC. NpHR-mediated inhibition of BLA-vHPC projections significantly increased social interaction in the resident-juvenile intruder home-cage test as shown by increased intruder exploration. In contrast, ChR2-mediated activation of BLA-vHPC projections significantly reduced social behaviors as shown in the resident-juvenile intruder procedure as seen by decreased time exploring the intruder and in the three chamber sociability test by decreased time spent in the social zone. These results indicate that BLA inputs to the vHPC are capable of modulating social behaviors in a bidirectional manner.

Key words: amygdala; ChR2; hippocampus; NpHR; optogenetics; social

Introduction

Major psychiatric disorders, such as depression, autism, schizophrenia, and social anxiety disorder, share impaired social interaction as a distinctive feature (American Psychiatric Association, 2013). However, little is known about the neural circuitry regulating adult social interaction. Previous studies using pharmacological interventions or lesions have suggested an involvement of the amygdala in social behaviors and social aggression in both nonhuman primates (Kling, 1974; Kling and Steklis, 1976; Machado et al., 2008) and rodents (Bunnell et al., 1970; Jonason and Enloe, 1971; Sanders and Shekhar, 1995a, b; Gonzalez et al., 1996). In vivo electrophysiology recordings revealed changes in neuronal activity in the basolateral amygdala (BLA) during social interaction behaviors, such as increased firing in the BLA related to the augmentation of general social behavior (Katayama et al., 2009). However, the way the amygdala interacts with other downstream regions in a social context is poorly understood.

One downstream region of the amygdala that has been implicated in social interaction in rodents is the ventral hippocampus (vHPC), which shares reciprocal connections with the BLA (O’Donnell and Grace, 1995; Pikkarainen et al., 1999). Lesion studies provided the first evidence of an involvement of the vHPC in social interaction (Cadogan et al., 1994; Deacon et al., 2002; McHugh et al., 2004) and defensive behaviors (Pentkowski et al., 2006). Furthermore, studies using selective lesion methods have suggested that the vHPC, but not the dorsal hippocampus, is required for social interaction during a resident—intruder test (McHugh et al., 2004). Despite these previous findings, it remains unclear whether the BLA interacts with the vHPC to modify social behavior.

Our recent work demonstrated that the activation of BLA projections to the central nucleus of the amygdala (Tye et al., 2011) or the vHPC (Felix-Ortiz et al., 2013) mediates opposing effects on anxiety-related behaviors. However, the role of specific BLA projections in social behavior has not previously been explored. To selectively control projections from the BLA to specific downstream targets, we took advantage of the cellular specificity and temporal precision of optogenetics. This allowed neural activation or inhibition on a timescale relevant to social approach in two well-validated behavioral procedures (resident—intruder and the three chamber sociability test) (File and Pope,
Inhibition of BLA terminals projecting to the vHPC with NpHR increases social interaction in a resident–juvenile intruder procedure. BLA glutamatergic neurons were transduced with either NpHR-eYFP (n=7) or eYFP alone as a control (n=8). Yellow light was delivered (3 min, constant) via bilateral optical fibers implanted in the vHPC, after ~7–8 weeks of viral incubation. A, Left, Coronal brain schematic indicating the site of viral injection into the BLA. Right, Coronal schematic indicating optic fiber location into the vHPC. Top, Experimental timeline. B, Left, Schematic indicating the home-cage resident–juvenile (3–4 weeks) intruder behavioral procedure. Right, Schematic indicating the 3 min epochs with 24 h, counterbalanced between the social tasks. Different intruders were used for each epoch. C, NpHR mice spent significantly more time performing social interaction than eYFP mice during the yellow light illumination epoch. *p = 0.046. D, No significant effect of light stimulation or group was detected on freezing. E, NpHR mice spent less time (seconds) exploring their home cage during light stimulation compared with eYFP control mice. * p = 0.035. F, No significant effect of light stimulation or group was detected on freezing behavior in the presence of a juvenile intruder. G, Percentage of total time (3 min), showing social interaction, self-grooming, cage exploration, and freezing. Data are mean values. Error bars indicate SEM.

Figure 1.
testing arena to allow animals to interact and move freely while receiving laser stimulation. The patch cord was connected to a 594 nm laser or a 473 nm laser (OEM Laser Systems) with an FC/PC adapter. All laser output was manipulated with a Master-8 pulse stimulator (A.M.P.I.). For mice in Figure 1 (bilateral optogenetic inhibition of BLA-vHPC projection using NpHR3.0), bilateral inhibition through two implanted chronic optic fibers connected to the optic patch cords (Doric) received 10 mW (~35.35 mW/mm²) of constant yellow light generated by a 594 nm DPSS Laser (OEM Laser Systems). For mice in Figures 2, 3, and 4, unilateral activation using ChR2(H134R) was achieved by delivering 10 mW (~35.35 mW/mm²) of blue light in a high-frequency train (20 Hz, 5 ms pulses) generated by a 473 nm DPSS laser (OEM Laser Systems).

**Behavioral assays**
All tests were performed during the dark phase, and animals were allowed to acclimatize to the behavioral testing room for at least 1 h before the beginning of testing.

**Social interaction assay**
Male juvenile mice were used instead of adults to exclude any effect of mutual aggression. Social interaction in the home cage was examined as described previously (Winslow, 2003). A single mouse was allowed to explore freely for 1 min (habitation) in its home cage. A novel juvenile (3–4 weeks old) male C57BL/6 mouse was introduced to the cage and allowed to explore freely for 3 min (test session). All behaviors were video recorded and analyzed by an experimenter blind to the testing condition using ODLog software (Macropod software). The overall score of social interaction included behaviors, such as body sniffing, anogenital sniffing, direct contact (pushing the snout or head underneath the juvenile’s body and crawling over or under the juvenile’s body), and close following (<1 cm). Nonsocial behaviors were also represented in an overall exploration score, which included walking, rearing, freezing, and self-grooming. Each mouse underwent two social interaction tests separated by 1 h, with one intruder paired with optical stimulation and one with no stimulation. Groups were counterbalanced for order of light stimulation.

**Three chamber sociability test**

**Apparatus.** A different cohort of animals was subjected to the three chamber sociability test (Fig. 4). The three compartment testing apparatus consisted of a Plexiglas rectangular box (57.15 cm × 22.5 cm × 30.5 cm), without a top. The center compartment was smaller (8 cm × 22.5 cm) than the two end compartments, which were of equal size (24.5 cm × 22.5 cm). Inverted custom made wire cups (diameter 8 cm) were placed in each side of the end compartments during testing sessions (discussed below) and housed the juvenile stimulus mouse. The apparatus and wire cups were thoroughly cleaned with 70% ethanol between sessions and after each test mouse.

**Sociability procedure.** In the first session, a test mouse was placed in the middle compartment and allowed to habituate to the apparatus for 10 min, and the first 5 min was used for analysis. In the second 5 min session, a stimulus juvenile (3–4 weeks) mouse was placed in an inverted wire cup in the side designated as the social compartment, and an empty inverted wire cup was placed in the side designated as the nonsocial compartment. The test mouse was placed in the middle chamber for 1–2 min while the experimenter placed the juvenile mouse inside the inverted cup. The test mouse was then left to explore both chambers for 5 min. The side designated for the location of the enclosed stimulus mouse was randomly assigned in a counterbalanced fashion throughout the 2 d experiment. Behavioral tests were recorded by a video camera, and the EthoVision XT video tracking system (Noldus) was used to track mouse location, velocity, and movement of head, body, and tail. All measurements were made relative to the mouse body. The amount of time test mice spent in each compartment, the amount of time they explored (sniffing) within a 2 cm vicinity of the inverted cups, and their transitions between compartments were measured. Each mouse underwent the above sociability task twice, separated by 24 h, with one session paired with optical stimulation.
and one with no stimulation. Groups were counterbalanced for order of light stimulation as well as side assigned as the social zone.

**Pharmacology**
A 26 gauge stainless steel internal cannula (PlasticsOne) projecting 0.5 mm beyond the tip of the guide cannula was connected to a syringe pump (Harvard Apparatus). The AMPA receptor antagonist and the NMDA receptor antagonist were infused into the vHPC in a volume of 0.5 μl at a rate of 0.1 μl per minute. The internal cannula was withdrawn 5 min after the end of infusion, and animals underwent social testing 30 min after drug infusion. Testing took place over 4 d, and each day an individual mouse received only one drug, counterbalanced for treatment and stimulation (laser ON or OFF) order. Glutamate receptor antagonist consisted of a mixture of 22 μl of NBQX and 38 μl of AP5 (Tocris Bioscience), dissolved in 0.9% saline, and prepared freshly on each day of the experiment.

**Immunohistochemistry**
To use c-fos expression as a readout for neural activity, mice expressing ChR2(H134R)-eYFP or eYFP were stimulated in vivo inside their home cage for 3 min (with the same stimulation protocol used for behavioral testing) 90 min before death. All mice were anesthetized with sodium pentobarbital and transcardially perfused with ice-cold 4% PFA in 1× PBS, pH 7.3. The brain was extracted and postfixed in 4% PFA overnight, followed by transfer to 30% sucrose in 1× PBS. Brains were sectioned into 40-μm-thick coronal sections using a sliding microtome (HM430; Thermo Fisher Scientific) and stored in PBS at 4°C before being processed using immunohistochemistry. Sections were blocked in Triton X-100 0.3%/PBS and 3% normal donkey serum for 1 h at room temperature, followed by incubation with primary antibody (rabbit anti-c-fos 1:500; Calbiochem) for 17–20 h at 4°C. Sections were then washed 4 times with PBS followed by mounting on microscope slides with PVD-DABCO (Sigma). The number of c-fos-positive cells were counted by experimenters blind to the experimental conditions. Mice showing eYFP somata expression in the cortex were excluded from analysis.

**Confocal microscopy**
Confocal fluorescence images were acquired using an Olympus FV1000 confocal laser scanning microscope using a 10×/0.40 NA or a 40×/1.30 NA oil-immersion objective. Serial z-stack images covering a depth of 10 μm through multiple sections were acquired using the image analysis software (Fluoview, Olympus). The number of c-fos-positive cells were counted by experimenters blind to the experimental conditions. Mice showing eYFP somata expression in the cortex were excluded from analysis.

**Statistics**
A two-way ANOVA was used to examine group differences followed by Bonferroni post hoc tests. We performed a Bonferroni correction for multiple comparisons by multiplying the p value for each individual test by the number of comparisons made. For all results, significance threshold was placed at p = 0.05 and p < 0.01. All data are shown as ± SEM.

**Results**
**Optogenetic inhibition of BLA inputs to the vHPC increases social behaviors**
Using AAV5 under the control of the CaMKIIα promoter, we transduced BLA pyramidal neurons with an enhanced version of halorhodpsin (eNpHR3.0) (Gradinaru et al., 2010). In experimental groups, BLA projection neurons were transduced with NpHR fused to an eYFP (AAV5-CaMKIIα-NpHR-eYFP), whereas control animals received the same viral vector carrying the fluorophore alone (AAV5-CaMKIIα-eYFP). To inhibit NpHR-expressing BLA axon terminals in the vHPC, we bilaterally implanted optical fibers above the vHPC to allow for the delivery of 594 nm light to the pyramidal layer of the vHPC (Fig. 1A). The mice were then tested on a well-validated social interaction test, the resident–intruder procedure (File and Pope, 1974; Winslow, 2003; Silverman et al., 2010; Himmler et al., 2013). Each mouse was tested on two separate days, with different juvenile intruders (3–4 weeks old), to allow for within-subject and within-session comparisons as well as group comparisons. Each mouse had 1 min of habituation in their home cage followed by a 3 min epoch of either light-off (OFF) or
light-on (ON) illumination using constant illumination with 594 nm light. During the testing epochs, the residents were presented with a juvenile intruder. Groups were counterbalanced for order of light stimulation (Fig. 1B).

Mice in the NpHR group showed significantly greater total investigation of the juvenile intruder, reflecting an increase in social behaviors, relative to eYFP mice during the ON epoch (Fig. 1C; two-way ANOVA demonstrating group × light epoch interaction, $F_{(1,13)} = 12.89, p = 0.0033$; Bonferroni post hoc analysis, corrected for multiple comparisons, $p = 0.046$). No significant differences were found in stereotypical behaviors, such as self-grooming (Fig. 1D; two-way ANOVA did not detect a group × light epoch interaction, $F_{(1,13)} = 0.02, p = 0.8993$). Mice in the NpHR group also displayed a decrease in time spent performing home-cage exploration during the ON epoch (Fig. 1E; two-way ANOVA demonstrating group × light epoch interaction, $F_{(1,13)} = 7.12, p = 0.0193$; Bonferroni post hoc analysis, corrected for multiple comparisons, $p = 0.035$), perhaps explained by the increased attention to the juvenile intruder. No significant differences were found in freezing (immobilization) behavior (Fig. 1F; two-way ANOVA did not detect a group × light epoch interaction, $F_{(1,13)} = 0.52, p = 0.4839$). Each epoch session lasted 3 min. Figure 1G shows the overall time mice spent engaging in different behaviors for experimental and control groups. NpHR mice spent significantly more time exploring the intruder than eYFP controls. These data demonstrate that silencing BLA inputs to the vHPC increased the time spent on social interaction.
Optogenetic activation of BLA inputs to the vHPC decreases social behaviors

We transduced BLA pyramidal neurons with a channelrhodopsin-2 (ChR2)-eYFP fusion protein in experimental animals and eYFP alone in control animals along with a unilaterally implanted optical fiber over the vHPC on the ipsilateral hemisphere (Fig. 3A) before testing in the resident–intruder procedure (Fig. 3B). We used the same procedure as in the previous experiments, but with the illumination epoch consisting of a continuous train at 20 Hz of 473 nm (blue) light pulses, with 5 ms pulse duration. ChR2 mice showed reduced investigation of the juvenile intruder, reflecting a reduction in social interaction (Fig. 3C; two-way ANOVA demonstrating group × light epoch interaction, $F_{(1,14)} = 9.04, p = 0.0094$; Bonferroni post hoc analysis, corrected for multiple comparisons $p = 0.034$). ChR2 mice also showed a significant increase in self-grooming compared with eYFP controls (Fig. 3D; two-way ANOVA demonstrating group × light epoch interaction, $F_{(1,14)} = 14.90, p = 0.0017$; Bonferroni post hoc analysis, corrected for multiple comparisons, $p = 0.0008$). No differences were found in home-cage exploration (Fig. 3E; two-way ANOVA did not detect a group × light epoch interaction, $F_{(1,14)} = 0.47, p = 0.8497$) and freezing behavior (Fig. 3F; two-way ANOVA did not detect a group × light epoch interaction, $F_{(1,14)} = 0.29, p = 0.5975$). Figure 4G shows the overall time ChR2 and eYFP groups spent engaging in different behaviors during the test. As shown above, ChR2 mice spent less time exploring the intruder compared with eYFP controls.

To evaluate other aspects of social behavior, we also tested a different group of mice on the three chamber sociability test (Fig. 4). To allow for within-subject comparisons in addition to between-group comparisons, we tested each mouse on two separate days on the three chamber sociability test with different juvenile intruders (3–4 weeks) each day (Fig. 4A). Each test had 5 min of habituation in the testing arena. Representative ChR2 mouse tracks are shown in Figure 3B. No differences were found in the time spent in the right zone versus the left zone of the testing chamber (Fig. 4C; two-way ANOVA did not detect a group × light epoch interaction, $F_{(1,14)} = 1.69, p = 0.2146$; and Fig. 4D; two-way ANOVA did not detect a group × light epoch interaction, $F_{(1,14)} = 0.09, p = 0.7646$). No differences were found in distance traveled (Fig. 4E; two-way ANOVA did not detect group × light epoch interaction, $F_{(1,14)} = 0.93, p = 0.3503$). The habituation period was followed by one 5 min epoch of either a baseline epoch without illumination (OFF) or illumination using continuous train at 20 Hz of 473 nm (blue) light pulses, with 5 ms pulse duration (ON). During the testing epochs, the test mouse was presented with a juvenile intruder inside an inverted cup for which a representative animal track from the ChR2 group is shown (Fig. 4F). Groups were counterbalanced for order of light stimulation. Mice in the ChR2 group displayed a decrease in time spent in the social zone upon illumination (ON epoch) relative to eYFP controls (Fig. 4G; two-way ANOVA demonstrating group × light epoch interaction, $F_{(1,14)} = 9.04, p = 0.0094$; Bonferroni post hoc analysis, corrected for multiple comparisons, $p = 0.033$). ChR2 mice also showed a lower social/nonsocial ratio during the ON epoch, which indicates that during light stimulation ChR2 mice spent more time investigating the nonsocial zone (Fig. 4H; two-way ANOVA demonstrating group × light epoch interaction, $F_{(1,14)} = 8.02, p = 0.0133$; Bonferroni post hoc analysis, corrected for multiple comparisons, $p = 0.023$). Furthermore, we simultaneously analyzed locomotor activity across epochs and did not detect any changes during the ON epoch relative to the OFF epoch as measured by distance traveled (Fig. 4I; two-way ANOVA did not detect a group × light epoch interaction, $F_{(1,14)} = 0.03, p = 0.8728$).

**Figure 5.** Activation of BLA axon terminals in the vHPC using ChR2 increases c-fos expression in the pyramidal layer of vHPC. Blue represents DAPI; green, eYFP; red, c-fos. **A.** Confocal image of the BLA of representative ChR2 animal. **B.** Confocal images of the BLA from two representative mice. Representative ChR2:BLA-vHPC animal (left) and representative eYFP:BLA-vHPC mice (right). **C.** Percentage of DAPI-positive (+) cells expressing eYFP or c-fos in the BLA ($n = 8$ ChR2 mice and $n = 8$ eYFP mice). No differences between groups were found in c-fos+ or eYFP+ cells in the BLA. **D.** Confocal image of the vHPC from representative ChR2 mice. **E.** vHPC confocal images of two representative mice. Representative ChR2:BLA-vHPC animal (left) and representative eYFP:BLA-vHPC mice (right). **F.** Percentage of DAPI-positive (+) cells expressing eYFP or c-fos in the vHPC ($n = 8$ ChR2 mice and $n = 8$ eYFP mice). Compared with eYFP:BLA-vHPC controls, light stimulation of BLA terminals in the vHPC increased the percentage of c-fos+ cells in the vHPC of ChR2:BLA-vHPC group. Data are mean ± SEM. **p = 0.0028.**
Optogenetic stimulation of BLA axon terminals in the vHPC increases c-fos expression in the vHPC, but not in BLA somata

To test for the effects of blue light illumination on neuronal activation, we used the immediate early gene c-fos as a readout for neural activity. A confocal image of the BLA from a representative ChR2 mouse is shown in Figure 5A, demonstrating basal c-fos expression (red) within the BLA. Confocal images of the pyramidal layer in vHPC CA1 from representative ChR2 and eYFP mice are shown in Figure 5B. We did not observe a change in BLA somata c-fos expression induced by illumination of BLA terminals in the vHPC relative to eYFP control mice (Fig. 5C; unpaired Student’s t test, df = 14, t = 0.4515, p = 0.6307). Although light stimulation does not alter basal activity on the BLA, we also show that c-fos expression was increased in the pyramidal layer of the vHPC CA1 extending to ~1.5 mm below the fiber tip (Fig. 5D, E). Quantification of c-fos demonstrates a significant increase of cell activity on the vHPC during light stimulation (Fig. 5F; unpaired Student’s t test, df = 17, t = 3.166, p = 0.0028). Confocal images were also taken from each animal to confirm the viral injection site (Fig. 6A) and optic fiber position (Fig. 6B).

BLA excitatory projections to the vHPC are sufficient to mediate changes in social behaviors

Although our data suggest that excitation of glutamatergic inputs from the BLA to the vHPC can reduce social behaviors, the illumination of Chr2-expressing terminals in the vHPC could induce depolarization of axons of passage and/or back-propagating action potentials to BLA somata (Petreanu et al., 2007). To control for this possibility, we combined in vivo pharmacological manipulations with our in vivo optogenetic manipulations during the resident–intruder procedure (Fig. 7). Once again, we expressed ChR2 in BLA neurons but unilaterally implanted a guide cannula rather than an optic fiber to deliver either saline or glutamate receptor antagonists to the vHPC ~30 min before testing and laser stimulation on the resident–intruder procedure (Fig. 7A). To allow for a within-subject comparison, we tested each animal four times on different days, with unilateral administration of either saline or a combination of the AMPA receptor antagonist NBQX (22 mM) and NMDA receptor antagonist AP5 (38 mM), counterbalanced for order. This enabled comparison of saline trials to glutamate receptor antagonist mixture (GluRX) trials (Fig. 7B). Different intruders were used for each day of testing.

In saline trials, mice replicated the light-induced reduction of social interaction on the resident–intruder procedure; but after treatment with glutamate receptor antagonists, the light-induced changes in social interaction were attenuated (Fig. 7C; two-way ANOVA demonstrating group × light epoch interaction, F(1,14) = 10.37, p = 0.0062; Bonferroni post hoc analysis, corrected for multiple comparisons, p = 0.048). Furthermore, the light-induced increase in grooming evident in the saline group was also attenuated by the GluRX treatment (Fig. 7D; two-way ANOVA demonstrating group × light epoch interaction, F(1,14) = 9.62, p = 0.0078; Bonferroni post hoc analysis, corrected for multiple comparisons, p = 0.0356). No differences were found between groups in locomotor activity shown by distance traveled (Fig. 7E; two-way ANOVA did not detect a group × light epoch interaction, F(1,14) = 0.56, p = 0.4674) or freezing behavior (Fig. 7F; two-way ANOVA did not detect a group × light epoch interaction, F(1,14) = 1.97, p = 0.1827). Figure 7G shows the overall time ChR2:GluRX and ChR2:saline groups spent engaging in different behaviors during the test. As shown above, the light-induced changes were replicated in the ChR2:saline group and attenuated with the presence of the GluRX. After behavioral testing, animals were processed to allow for histological verification of injection site (Fig. 8A) and cannula placement (Fig. 8B).

Discussion

Our results demonstrate a critical role for BLA projections to the vHPC in bidirectionally modulating social interaction. Additionally, activation of this pathway increases self-grooming while decreasing social interaction. These findings provide broad implications for the involvement of this pathway in behaviors relevant to autism spectrum disorders, obsessive–compulsive disorder, and social anxiety, and represent a potential new target for therapeutic development.

Strong evidence suggests a functional role for the amygdala in social processing or social cognition in humans (Killgore and Yurgelun-Todd, 2005; Schultz, 2005; Bickart et al., 2011), as well as in social behavior in animals (Ferguson et al., 2001; Amaral, 2002). However, support for a role of the vHPC has been less
Figure 7. Activation of BLA inputs to the vHPC is sufficient to mediate changes in social interaction without affecting the ability to explore the environment. A, Glutamate receptor antagonists (GluRX:AP5, purple) or saline (black) were unilaterally infused into the vHPC using the same guide cannula subsequently used for light delivery via an acutely inserted optical fiber. Top, Experimental timeline. Middle, Sagittal brain schematic indicating viral injections into the BLA. Right, Sagittal schematic of removable optical fiber used for light delivery 30 min after GluRX delivery. B, Top, Testing took place over 4 consecutive days. Schematic of 3 min epochs for treatment and stimulation (ON or OFF epoch) order. Novel juvenile intruders were used for each session. C, GluRX attenuated the light stimulation effect. GluRX:ChR2 mice (n=8) spent significantly more time (seconds) performing social interaction than Saline:ChR2 mice (n=8). *p = 0.048. D, GluRX:ChR2 mice also spent significantly less time performing self-grooming compared with Saline:ChR2 mice. *p = 0.036. E, No significant effect of light stimulation or group was detected for freezing during the blue light illumination epoch. F, Significant difference in social interaction was detected between treatment and stimulation order. GluRX:ChR2 mice spent significantly more time (seconds) performing social interaction than Saline:ChR2 mice. *p = 0.003. G, Percentage of total time (3 min), showing social interaction, self-grooming, cage exploration, and freezing. Data are mean ± SEM.

Figure 8. Histologically verified placements of viral injections and optical fiber tips in ChR2 animals used for pharmacology experiments. A, Coronal sections of the BLA with coordinates reflecting anteroposterior distance from bregma. Center of viral injections in the BLA for all mice expressing ChR2 (n = 8; purple circles). B, Coronal sections of the vHPC with coordinates from bregma. Location of cannulae above the pyramidal layer of vHPC for ChR2 animals (purple crosses). It has been reported that increases in 5HT and cAMP are observed in the vHPC after social interaction (Cadogan et al., 1994). However, their study used a novel arena, which may have been anxiogenic, which is in contrast to the home-cage social interaction test but similar to the three chamber sociability test. Although these changes appear distinct from our observation of increased c-fos expression, our findings show that the same photostimulation that reduced social interaction showed increased vHPC activity and agree with this report in terms of linking social interaction to changing neural dynamics within the vHPC. In another study, oxytocin was recently shown to increase the signal-to-noise ratio of CA1 pyramidal neurons in mice in an ex vivo preparation (Owen et al., 2013), providing one possible mechanism underlying the ability of oxytocin to reduce social deficits associated with autism spectrum disorders (Guastella et al., 2013).
al., 2010) and to ameliorate the symptoms of obsessive–compulsive disorder (Ansseau et al., 1987) in humans.

Despite these findings, there has been contention about whether the vHPC has a critical role in social interaction. Specifically, although ibotenic acid lesions in neonatal rats produced dramatic changes in social interaction when the rats were tested in adulthood in the absence of changes in general anxiety-related behaviors, no such changes in social interaction were observed when the lesions were performed in adult rats (Sams-Dodd et al., 1997; Becker et al., 1999). This result suggests that the vHPC does not play an active role in social interaction in adulthood. However, these lesions were nonspecific and may have targeted multiple circuits in the vHPC with opposing functions in social interaction, thereby producing a zero sum effect on behavior. Furthermore, in these studies, the lesions were performed 2 weeks before testing, and compensatory mechanisms in the adult brain may have contributed to the lack of change in social interaction observed. In contrast to this nonspecific ablation of the vHPC region, in this study we transiently modulated the activity across a specific subpopulation of synapses in the vHPC without causing any permanent damage. Whereas in vivo electrophysiological studies have been performed in the BLA (Wang et al., 2011) and vHPC during anxiety (Adhikari et al., 2010), and recordings have also been performed in the BLA during social behaviors (Katayama et al., 2009), our findings provide the first evidence that BLA inputs to the vHPC have a causal relationship with social behavior. Furthermore, the neural encoding dynamics of vHPC-projecting BLA neurons have yet to be revealed during social interaction.

In our previous work, we identified two projections originating from the BLA that showed opposing effects on anxiety-related behavior (Tye et al., 2011; Felix-Ortiz et al., 2013). Here, we extend these findings by showing that BLA projections to the vHPC not only mediate anxiety-related behaviors but also mediate social behaviors. We speculate that the dual function of this pathway may contribute to the high rate of comorbidity of autism and anxiety disorders (Kim et al., 2000; Amaral and Corbett, 2008), as individuals with both autism and anxiety-related symptoms could have a perturbation in this pathway.

References

Felix-Ortiz and Tye • Projection-Specific Manipulation of Social Behaviors