Expression of brain-derived neurotrophic factor in basolateral amygdala inputs to lateral septum is necessary for mice to identify socially novel individuals

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ABSTRACT

BACKGROUND: The lateral septum (LS) is activated by social novelty, and perturbing LS activity impairs social recognition. However, the neural circuits and cell signaling pathways that converge on the LS to mediate social behaviors aren't well understood. Multiple lines of evidence suggest that social behavior is influenced by brain-derived neurotrophic factor (BDNF) signaling through its receptor TrkB. However, whether BDNF-TrkB signaling mediates social behavior by affecting function in LS circuits is not known.

METHODS: We used single-molecule fluorescent *in-situ* hybridization to quantify expression of *Ntrk2* (gene encoding TrkB) in LS. Viral transgenesis was used to induce cre-recombinase mediated TrkB knockdown in LS as well as for ablation and BDNF-depletion in neurons projecting from the basolateral amygdala (BLA) to the LS. We evaluated social recognition behavior using the three-chamber social interaction test, and assessed social novelty-induced c-Fos expression using fluorescence immunohistochemistry.

RESULTS: The majority of GABAergic neurons in LS express TrkB. TrkB knockdown in LS abolishes social novelty recognition, and decreases LS activation in response to social novelty. Ablating BLA-LS projection neurons abolishes social novelty recognition behavior, an effect that is phenocopied by selectively depleting BDNF in this circuit.

CONCLUSIONS: Social novelty recognition in the mouse requires both BDNF expression in BLA-LS projections neurons and intact TrkB signaling in the LS. These data support the hypothesis that BLA-LS projection neurons are a critical source of BDNF for activating LS TrkB signaling to control social novelty recognition.

Keywords: lateral septum, basolateral amygdala, BDNF, social novelty recognition, TrkB

INTRODUCTION

Social deficits are prevalent in many psychiatric and neurodevelopmental disorders, including autism (1, 2) and schizophrenia (3). While behavioral manifestations of altered social behavior in these disorders are highly heterogeneous, deficits in attention to socially salient stimuli, and deficits in social recognition are well documented (3–5). Mice are a highly social species that display robust responses to novel conspecifics, such as attention to, and investigation of, novel individuals (6, 7). The three-chamber social approach task, which can be used to measure the amount of time a mouse spends in close proximity with another mouse, was developed and validated to assess sociability, social recognition, and social novelty preference (8). Although these behaviors can't fully encapsulate the complexities of human social behaviors, these rodent models can be used to study neurocircuitry that is relevant for social behavior impairments observed in human disorders.

The lateral septum (LS) is a predominantly GABAergic region in the basal forebrain that extends across a significant portion of the rostral-caudal axis and is subdivided into a dorsal, intermediate and ventral subregion (9). The LS is a potent modulator of social behaviors in both humans (10–13) and rodents (9, 14). Investigation of the LS in rodents demonstrated its involvement in social saliency (15, 16), social recognition (17–19), and social aggression (20, 21). The LS is also innervated by many regions involved in regulating various social behaviors, including the basolateral amygdala (BLA) (22), ventral hippocampus CA1 area (vCA1) (23), ventral tegmental area (9, 24), as well as the infralimbic and prelimbic cortices (9, 25, 26). However, the inputs to the LS that mediate social recognition are not well understood, and the cell-signaling mechanisms in the LS that transmit this social information remain unclear.

Multiple lines of evidence suggest that the activity-dependent neurotrophin brain-derived neurotrophic factor (BDNF) is required for a variety of social behaviors (27–30). Signaling through its cognate receptor tropomyosin kinase B (TrkB), BDNF regulates dendritic morphology, synapse formation and synaptic plasticity (31–33). Importantly, BDNF plays a critical role in supporting the function of GABAergic neurons (34–38), which rarely synthesize BDNF, but express TrkB (39, 40). GABAergic neurons rely on BDNF released from other cell types to activate TrkB receptor signaling, which is critical for their maturation and physiological function (37, 41–43). In line with its GABAergic composition, the LS is largely devoid of *Bdnf* transcript expression, but many neurons innervating the LS densely express BDNF (44, 45). Previous research has shown that catecholaminergic projections that express BDNF and innervate the LS influence the morphology and gene expression of a subpopulation of calbindin-

expressing neurons in the LS (46). However, whether BDNF-TrkB signaling in the LS is important for the social behaviors mediated by the LS has not been investigated.

We hypothesized that 1) TrkB signaling in LS GABAergic neurons is important for regulating rodent social behavior, and that 2) neuronal projections from BDNF-rich limbic regions serve as a source of BDNF to activate TrkB signaling in LS to control this behavior. Key candidate regions included the BLA and vCA1 because both regions highly express BDNF (44), send projections to the LS (20, 22, 26), and have established roles in regulating social novelty (9, 16, 47) and recognition (23, 48, 49). Indeed, local knockdown of TrkB in the LS demonstrates a role for LS TrkB expression in social novelty and recognition behavior in mice. Moreover, we identify BLA-LS projections as critical for social novelty and recognition behavior in mice, and show that BDNF expression within these projection neurons is required for this behavior. In summary, the data demonstrate a vital role for LS TrkB signaling in social novelty recognition, and suggest that the BLA is a critical source of BDNF for activating LS TrkB receptors to control this behavior.

METHODS AND MATERIALS

Animals

Wild-type mice (C57BL/6J; stock # 000664) and mice carrying a *loxP*-flanked *Bdnf* allele (Bdnf^{tm3Jae}; stock # 004339, referenced in text as BDNF$^{fl/fl}$) were purchased from The Jackson Laboratory. Mice carrying a *loxP*-flanked TrkB allele (strain fB/fB, referenced in text as TrkB$^{fl/fl}$ (50, 51)) were used for some experiments. TrkB$^{fl/fl}$ mice were maintained on a C57BL/6J background. Adult male mice were housed in a temperature-controlled environment with a 12:12 light/dark cycle and *ad libitum* access to food and water. All experimental animal procedures were approved by the Sobran Biosciences Institutional Animal Care and Use Committee.

Behavior

The three-chamber social interaction test arena consisted of three adjacent chambers separated by two clear plastic dividers, and connected by two open doorways. Mice were habituated for 3 consecutive days (10 min per day) prior to testing days. Mice were transferred to the testing room 1 h prior to testing. The test consisted of 5 min of habituation and two 10 min sessions (trial 1 and 2) with a 5 min inter-trial-interval. The subject mouse was kept in the center chamber during habituation. In the first 10 min session (trial 1), the subject mouse was allowed to freely investigate the three chambers. A novel mouse (stranger 1) was placed under an

inverted metal cup in one of the side chambers, and another identical inverted empty cup was placed in the other side chamber. After the trial 1 session, the doorways were closed and the subject mouse was kept in the center chamber for 5 min. In the second session (trial 2), another novel object mouse (stranger 2) was put in the empty cup and the subject mouse freely investigated the three chambers. Age-matched adult C57BL/6J male mice were used as object mice in this study, and housed under the same conditions as the subject mice. Each object novel/stranger mouse was used once a day in the three-chamber social interaction test. Time spent sniffing each cup was scored as interaction time manually in a blind manner using a Stopwatch+ program developed by the Center for Behavioral Neuroscience (cbn-atl.org) at Emory University (52, 53).

For full description of methods, see Supplementary information.

RESULTS

Local knockdown of LS TrkB expression

We used RNAscope single-molecule fluorescent *in-situ* hybridization to quantify expression of the gene encoding TrkB (*Ntrk2* probe) in GABAergic neurons (*Gad1* probe) across the rostral-caudal axis of the LS (Fig. 1A,B). Quantification of these data revealed that in wild-type mice, nearly all LS GABAergic neurons (85-90%) expressed *Ntrk2*, a pattern that was consistent across the rostral-caudal axis (Fig. 1B). Next, we designed a genetic manipulation strategy to knockdown expression of TrkB in the LS. Specifically, we injected a cre recombinase-expressing virus (AAV5-EF1a:Cre) into the LS of mice carrying a floxed TrkB allele (TrkB$^{fl/fl}$ mice) to knockdown TrkB selectively in LS (Fig 1C). As controls, we generated mice that retained intact TrkB expression (TrkB$^{fl/fl}$ mice injected into the LS with a non-Cre expressing virus; AAV5-EF1a:EYFP) (Fig. 1C). Four weeks following injection of the viruses, we micro-dissected the LS and frontal cortex from each mouse, and quantified protein expression of both full-length and truncated TrkB in individual mice (Fig. 1D-F). Expression of cre recombinase in TrkB$^{fl/fl}$ mice resulted in ~86% knockdown of full-length TrkB expression selectively in the LS, but not in the frontal cortex (Fig. 1D,E). Based on predicted sequence translation, removal of the floxed portion of the *Ntrk2* gene in TrkB$^{fl/fl}$ mice is not expected to affect expression of the truncated TrkB isoform (51), and hence it was unsurprising that expression of truncated TrkB remained unchanged across experimental groups (Fig. 1D,F).

Knockdown of LS TrkB abolishes social novelty discrimination

We next used this local knockdown strategy to investigate the necessity of TrkB signaling in social novelty and recognition using the three chamber social interaction task (Fig 2A,B). This task uses a three-chambered box to quantify the time a mouse spends examining socially novel individuals. In trial 1, the mouse is exposed to a novel mouse (stranger mouse 1) in one of the outer chambers while the other outer chamber remains empty. In trial 2, stranger mouse 1 remains in the same chamber (now the familiar mouse) while a new mouse (stranger mouse 2) is placed in the other chamber.

Control mice with intact TrkB expression showed expected social novelty behavior, spending more time investigating the novel mouse in trial 1 and trial 2 (Fig. 2C). However, while mice with LS TrkB knockdown showed expected social novelty behavior in trial 1, these mice spent equal time with the familiar and novel mice in trial 2 (Fig. 2D). To evaluate differences in the ability of control and LS TrkB knockdown mice to discriminate between individuals we generated a discrimination index for trial 2, which is a percentage of the time spent with the novel individual minus the time spent with the familiar individual over the total trial time. The decreased discrimination index in LS TrkB knockdown mice suggests that loss of intact TrkB may impact the ability to discriminate between individual mice (Fig. 2E).

To rule out that the impairment in attention to social novelty discrimination was due to olfactory impairment, both controls and mice with LS TrkB knockdown were subjected to a four trial odor discrimination task (Fig. S1A). LS TrkB knockdown did not impair odor discrimination (Fig. S1B). We also assessed the necessity of TrkB signaling in LS for a number of fear- and anxiety-related behaviors that are known to engage LS circuitry. LS TrkB knockdown did not alter behavior in the elevated plus maze (Fig. S1C), nor did it impact the ability of LS TrkB knockdown mice to acquire, retrieve or extinguish fear memories (Fig. S1D). These data support the hypothesis that the deficits observed following knockdown of LS TrkB are specific to social behavior.

LS TrkB knockdown abolishes socially induced c-Fos Expression

To map neural activity patterns in LS cells following exposure to novel social stimuli, we performed fluorescent immunohistochemistry for c-Fos across the rostral-caudal divide of the LS (Fig. 3A) in wild-type mice exposed to a social stimulus (a novel male mouse) compared to a non-social stimulus (a novel object, conical tube)(Fig. 3B). c-Fos quantification was performed using the open-source biological imaging software Fiji (for detailed description of methods, see Supplementary information and Fig S2A). In the middle portion of the LS, significantly more cells expressed c-Fos in response to social stimuli compared to the non-social stimulus (Fig. 3C).

This effect mapped most strongly to the intermediate and ventral subregions of the LS (Fig. 3D). The number of c-Fos expressing cells in all other subregions within the rostral (Fig. S2B) and caudal (Fig. S2C) portions of the LS remained relatively consistent across the social and non-social conditions suggesting that these effects on cell recruitment in the LS are restricted to the middle portion of the LS.

To investigate whether TrkB signaling contributed to LS-responsivity to socially novel stimuli, we generated an additional cohort of LS TrkB knockdown and control mice using the same viral strategy described above (Fig. 3E). Compared to controls, we detected significantly fewer cells expressing c-Fos in response to novel social stimuli in the middle portion of the LS in LS TrkB knockdown mice (Fig. 3F). All subregions (dorsal, intermediate, and ventral) within the middle portion of the LS displayed blunted c-Fos expression in response to a socially novel individual (Fig. 3G). However, LS TrkB knockdown had no effect on c-Fos expression induced in response to socially novel individuals within the rostral (Fig. S3B) and caudal (Fig. S3C) portions of the LS compared to controls.

BLA projections, but not vCA1 projections, to the LS are necessary for social novelty discrimination

While the LS is critical for controlling responses to social novelty (16–19), the brain regions that transmit information to the LS for proper execution of social novelty discrimination have not been identified. We noted the BLA and the vCA1 as candidate regions because both have established roles in regulating social novelty (9, 16, 47) and recognition (23, 48, 49), and send projections to the LS (22, 26). To determine the importance of projections from the BLA or vCA1 to the LS in controlling social novelty behavior, we used a diphtheria toxin A (DtA)-mediated viral circuit elimination strategy. Specifically, we injected two viruses with retrograde tropism (AAVrg-CB7.CI:EGFP and AAVrg-EF1a:mCherry-IRES-Cre) into the LS bilaterally, causing expression of eGFP, mCherry, and Cre in cells projecting to LS (Fig. 4A). In our regions of interest (separate cohorts for BLA and vCA1) we also injected a virus (AAV5-EF1a:mCherry-FLEX-dtA) expressing mCherry and Cre-dependent DtA (Fig. 4A). In this scenario, cell bodies in the BLA or vCA1 that also sent projections to the LS expressed Cre-recombinase, and hence Cre-mediated expression of DtA in the BLA-LS or vCA1-LS neuronal projections. This manipulation caused elimination of the eGFP signal in those ablated projections, but retention of the mCherry signal in non-projecting cells (Fig. 4B). Control mice with an intact BLA-LS circuit showed expected social novelty behavior, spending more time investigating the novel mouse in trial 1 and trial 2 in the three chamber social recognition task (Fig 4C). Mice with ablated BLA-

LS projections displayed expected social novelty behavior in trial 1, but in trial 2 these mice spent equal time with the familiar and novel mice (Fig. 4D). In addition, mice with intact BLA-LS projection neurons discriminated better than mice with ablated BLA-LS projection neurons according to their relative percentage of time spent with the novel versus familiar mouse in trial 2 (Fig. 4E). Both control mice with an intact vCA1-LS circuit (Fig. 4F) and a vCA1-LS ablated circuit (Fig. 4G) showed the expected social novelty behavior in trial 1 and trial 2. Neither group differed in their ability to discriminate between the novel and familiar mice in trial 2 (Fig. 4H). Our ablation studies demonstrate a role in BLA-LS neurons, and not vCA1-LS neurons, in regulating social novelty discrimination.

Expression of BDNF within BLA projections to the LS is necessary for social novelty discrimination

Neurons in the BLA strongly express BDNF (44), and the BLA sends projections to the LS (22). Hence, BLA-LS neurons are a strong candidate source for providing BDNF to LS TrkB-expressing neurons that regulate social novelty. To assess whether BDNF expression is necessary in BLA-LS projections to control social novelty behavior, we used a viral strategy combining the Flp recombinase (FlpO) and cre recombinase systems in mice carrying a floxed *Bdnf* allele (BDNF$^{fl/fl}$). This strategy allowed us to eliminate BDNF selectively from BLA-LS projections (Fig. 5A). We injected a retrograde virus in the LS (AAVrg-EF1a:Flpo) of BDNF$^{fl/fl}$ mice, causing FlpO expression in all neuronal inputs to the LS. We simultaneously injected a virus (AAV8-EF1a:fDIO-mCherry-P2A-Cre) expressing Flp-dependent mCherry and cre-recombinase in the BLA. Control mice were injected with a virus (AAV8-EF1a:fDIO-mCherry) into the BLA that expressed Flp-dependent mCherry. In the experimental group, BLA neurons projecting to the LS express cre-recombinase in a FlpO-dependent manner. Expression of cre leads to excision of the floxed BDNF allele and Cre-dependent expression of mCherry (Fig. 5B). Control mice with intact BDNF expression in the BLA-LS circuit showed expected social novelty behavior, spending more time investigating the novel mouse in trial 1 and trial 2 in the three chamber social recognition task (Fig. 5C). Mice with knockdown of BDNF expression in BLA-LS projection neurons showed expected social novelty behavior in trial 1, but in trial 2 these mice spent equal time with the familiar and novel mice (Fig. 5D). Moreover, mice with decreased BDNF expression in BLA-LS projection neurons display poorer discrimination between individuals compared to animals with intact BDNF expression in BLA-LS projection neurons according to their relative percentage of time spent with the novel versus familiar

mouse in trial 2 (Fig. 5E). In summary, BDNF expression in BLA-LS neurons is critical for social novelty discrimination.

DISCUSSION

TrkB signaling in the LS critically regulates social novelty behavior

Our findings reveal a novel role for TrkB signaling in the LS in controlling social novelty behavior in mice. The LS is involved in controlling social salience (15, 16) and social recognition (17–19), but little is known about the cellular and molecular mechanisms by which social information is relayed to the LS. We found that knocking down LS TrkB abolished social novelty behavior in the three-chamber social interaction task, suggesting that TrkB expression in the LS is necessary for discriminating socially novel conspecifics from those that are socially familiar. Importantly, the effects of TrkB knockdown did not impair olfactory discrimination or fear learning, highlighting the specificity of LS TrkB signaling in controlling social novelty behavior. The LS is spatially complex, containing dorsal, ventral, and intermediate subregions that span its entire rostral-caudal axis. Importantly, these subregions have functional distinctions, for example the dorsal and ventral LS control different aspects of social aggression in both males (23) and females (54). While elevated c-Fos expression in the LS in response to social stimuli is documented (15, 16), how this activity maps to the spatial topography of the LS has not been rigorously explored. Using c-Fos neural activity mapping, we demonstrated that the intermediate and ventral subregions of the middle portion of the LS are the socially responsive loci. Previous results suggested that social novelty-induced activity in the LS was concentrated more rostrally than caudally (15), which is consistent with our results. In addition, we demonstrated that TrkB expression in the LS is necessary for LS responsiveness to social stimuli. Specifically, recruitment of neural activity in the intermediate and ventral subregions of the middle portion of the LS in response to social stimuli is significantly blunted by LS TrkB knockdown. While our data suggests that TrkB expression in the LS gates the region's response to social stimuli, further work with acute manipulations of TrkB receptors *in vivo* could reveal the fast acting effects of BDNF-TrkB signaling within the LS in controlling social behaviors. This is important, since the chronic effects of TrkB knockdown in LS in the model utilized here could impact the physiological function of TrkB-expressing inhibitory neurons, which could impact the strength of inhibitory synapses. This in turn could impact connectivity within the LS, or inputs to the LS, contributing to the impairments in social behaviors that were observed. Hence, to better define the kinetics and mechanisms by which LS TrkB signaling controls social discrimination, a more

transient approach to blocking TrkB activation (e.g. using TrkB antagonists) could be pursued in follow up studies.

BLA projections to the LS control social novelty behavior

Our data reveal a novel role for direct neuronal projections from the BLA to the LS in controlling social novelty discrimination. The BLA is responsive to socially novel conspecifics (16, 47), and controls social discrimination (49), but the brain regions with which the BLA communicates to regulate these behaviors are not well established. We found that ablation of neuronal projections from the BLA to the LS abolishes social novelty behavior, suggesting that this circuit is necessary for discriminating socially novel conspecifics from those that are socially familiar. The BLA is critical for valence processing in a variety of contexts (55, 56), which may extend to processing social stimuli (57). The BLA receives inputs from multiple regions that can transmit social information, including the olfactory systems of the brain (58). Further work identifying the extended circuits that relay social information to the BLA may help clarify the role of the BLA in controlling social novelty and recognition.

Importantly, ablating projections from vCA1 to the LS did not alter social discrimination behavior. This finding is somewhat surprising as vCA1 has been implicated in controlling responses to social novelty (16) and regulating social memories (23, 48), and LS projections from adjacent subregions of the hippocampus, including dorsal CA2 (dCA2), are critical for controlling social aggression (20). While dCA2 projections to vCA1 are critical for social recognition (23), this social information may not be transmitted from ventral hippocampal circuitry to the LS to control social novelty. Further research may determine if other portions of the ventral hippocampus, such as dCA2, regulate social novelty and recognition, in addition to aggression. Interestingly, activation versus inhibition of BLA projections to the ventral hippocampus bidirectionally controls time spent socializing (57), which may point to the BLA as a central transmitter of social information to limbic circuitry.

BLA-derived BDNF signaling in the LS is critical for social novelty behavior

While our data identify BLA-LS projection neurons as critical for regulating social novelty and recognition behavior, the molecular mechanisms by which these neurons influence signaling in the LS to control this behavior is unknown. Our data demonstrate that TrkB expression in the LS is critical for social novelty and recognition behavior (Fig1D-G), but the source of BDNF for activating LS TrkB receptors is unclear. BDNF is not synthesized within the LS (44), but is highly expressed in BLA neurons (44), and given our data showing that BLA-LS

projection neurons are also critical for this behavior, we reasoned that BDNF produced in BLA-LS projection neurons could be a critical source for activating LS TrkB receptors to control this behavior. Indeed, when we knocked down BDNF expression selectively in BLA-LS projection neurons, we found that this manipulation phenocopied the behavioral deficits caused by local TrkB knockdown in the LS.

Bdnf transcription is controlled by nine different unique promoters, each of which drives expression of a short, non-coding exon that is spliced to a single, common coding exon that produces the mature BDNF protein (27, 59, 60). Disruption of BDNF production from specific *Bdnf* promoters impacts a variety of behaviors, including social aggression (27, 60) and maternal social behaviors (27). For example, our group recently demonstrated that BDNF production from promoters I and II controls maternal behaviors by influencing BDNF-dependent cellular functions in the hypothalamus (27). While BDNF-TrkB signaling in the LS is critical for regulating social novelty and recognition, it remains to be understood which promoters drive *Bdnf* expression in the BLA. Future experiments that determine this information may provide further insight into specific cellular and molecular mechanisms, and therefore specific stimuli, that facilitate the ability of BLA circuitry to control these social behaviors.

Similar to the rodent BLA, the non-human primate BLA (61) and the human BLA (62–64) are implicated in controlling a variety of social behaviors. Better understanding the nature of limbic control over social behaviors, such as social saliency and social memory, which are frequently disrupted in neuropsychiatric and neurodevelopmental disorders, is important to understand the etiology of these disorders, and develop targeted treatments for these symptoms. While the cellular and molecular mechanisms that control these behaviors are complex, given the prominent role of BDNF-TrkB signaling in controlling many facets of synaptic function, it is likely that this pathway contributes to regulating a variety of social behaviors. Supporting this notion, rare genetic disorders that cause BDNF haploinsufficiency as well as BDNF gene variants in humans are linked with aggression as well as other aspects of social functioning (65–67). In addition to BDNF, a recent genome-wide association study of anxiety, which included a large population of human subjects with social anxiety, identified a single nucleotide polymorphism in *NTRK2*, the gene encoding the TrkB receptor as significantly contributing to genetic risk (68). Better understanding the cellular and molecular mechanisms by which BDNF-TrkB signaling exerts control over social behaviors may help us further elucidate the circuit and molecular mechanisms that are relevant to the manifestation of both normal and abnormal social behavior.

Ethological Considerations

A limitation of our studies is that they were conducted exclusively in male mice. While male and female mice show many overlaps in social behaviors, there are various documented differences, including how they establish social hierarchies (69, 70), neural coding of social behaviors (71), and certain aspects of social recognition (72). Hence, direct comparisons of males and females in social novelty and recognition paradigms may be difficult to interpret at the behavioral level. Despite these sex differences in social behavior, there are several reasons why it is important for future studies to examine a potential role for BDNF-TrkB signaling in BLA-LS circuitry in the context of sex-specific social behaviors. First, prevalence rates (73), as well as genetic risk for disorders that often feature social deficits are not equal across sex and gender in humans (74–78). Second, BDNF-TrkB signaling and expression is significantly impacted by sex and hormones (79–81).

Conducting these studies, however, will require careful experimental design that takes into consideration the ethological aspects of female social behaviors. Specifically, the presence of a socially novel individual can signal different information to a male versus a female mouse, and can vary by context. For example, female mice establish different social hierarchies depending on the presence of male mice (70). This hierarchy leads to territoriality and aggression in some females toward males and females, while the non-aggressive females typically were breeders (70), which is remarkable given that female aggression is usually only displayed by postpartum dams in defense of their pups (82). The lack of male mice leads to little to no aggression in female mice. Male mice, on the other hand, typically display territoriality and aggression in male-male interactions (70, 83), but seldom display these behaviors in male-female interactions (70). It is also noteworthy that this female hierarchy and aggressive social behavior is found in instances where mice are housed in larger chambers with more individuals than the typical 4-6 mice per cage housing scheme used in most academic vivariums (70).

Despite the difficulties in making direct comparisons between male and female mice in studies of social behaviors, future studies can implement well designed experiments to study sex-specific differences in social novelty and recognition. For example, studying female social novelty in postpartum mice or dominant female mice raised in complex social structures will elucidate whether the circuits that enables normal male responses to social novelty are also necessary for controlling female social novelty. However, even then, this beckons the question about whether these female-male (or female-female) and male-male responses to social novelty are parallel at an ethological level.

Conclusions

Collectively, the presented data demonstrate a critical role for TrkB signaling in LS neurons in controlling social novelty recognition, and suggest that the BLA is a critical source of BDNF in activating these TrkB receptors to control this behavior. The data provide novel information about the broader role of signaling in amygdala-septal circuits, and provide a foundation for future studies to better delineate how molecular and cellular signaling in these circuits impacts social functioning.

FIGURE LEGENDS

Figure 1. Tropomyosin receptor kinase B (TrkB) is highly expressed in the lateral septum (LS) and can be effectively depleted virally. (A) Illustration depicting the anatomical boundaries across the rostral-caudal axis of the LS. (B) Fluorescent *in-situ* hybridization in sections of mouse LS (n=4) demonstrates *Gad1*-positive cells highly express *Ntrk2* throughout the rostral-caudal divisions. (C) Viral strategy using Cre-induced TrkB knockdown in the LS of TrkB$^{fl/fl}$ mice to quantify extent of TrkB knockdown quantification. (D) Western blot images for full-length TrkB (F-TrkB), truncated TrkB (T-TrkB), and α-Tubulin (aTub). (E) Relative expression levels of F-TrkB protein were significantly lower in the LS of the experimental group (AAV-Cre in LS of TrkB$^{fl/fl}$ mice,n=5) compared to the control group (AAV-eGFP in LS of TrkB$^{fl/fl}$ mice,n=6)(t_9 = 5.015, p = 0.0014). (F) Relative expression levels for T-TrkB protein were similar between experimental and control mice.

Figure 2. Tropomyosin receptor kinase B (TrkB) expression in the lateral septum (LS) is required for social novelty recognition behavior in mice. (A) Description of three chamber social interaction experiment. (B) Viral strategy using Cre-induced knockdown of TrkB expression in the LS of TrkB$^{fl/fl}$ mice prior to social novelty recognition. (C) Mice with intact TrkB expression in the LS (n=7) display intact social novelty recognition behavior. In both trial 1 (t_6 = 10.99, p < 0.0001) and trial 2 (t_6 = 5.921, p = 0.001) control mice spend more time investigating the socially novel mouse. (D) TrkB knockdown in LS (n=11) abolishes the social novelty phenotype observed in trial 2 (t_{10} =1.898, p = 0.088), but not trial 1(t_{10} = 5.615, p = 0.0002). (E) Mice with intact TrkB expression in the LS show better social discrimination between the socially novel and familiar mice in Trial 2 when compared to mice with TrkB knockdown in the LS ($U_{11,7}$ = 12, p = 0.0154).

Figure 3. Tropomyosin receptor kinaseB (TrkB) expression in the lateral septum (LS) is required for social novelty induced c-Fos expression. (A) Illustration depicting the anatomical boundaries across the rostral-caudal axis of the LS, denoting the dorsal (d), intermediate (i), and ventral (v) subregions of the LS. (B) Description of social or non-social cues used to induce c-Fos expression. (C) Induction of c-Fos is significantly increased by social cues in the middle portion of the LS (t_6 = 5.543, p = 0.004), (D) an effect seen specifically in the intermediate (t_6 = 3.697, p = 0.030) and ventral subdivisions (t_6 = 3.131, p = 0.040) of the middle LS. (E) Viral strategy using Cre-induced TrkB knockdown in the LS of TrkB$^{fl/fl}$ mice prior to examining social interaction induced c-Fos expression. (F) Knockdown of TrkB in the LS significantly decreases cell recruitment in the middle portion of the LS following social stimuli (t_9 = 3.069, p = 0.0396), (G) an effect seen across the dorsal (t_9 = 3.049, p = 0.029), intermediate (t_9 = 3.254, p = 0.029), and ventral (t_9 = 2.342, p = 0.044) subdivisions of the middle LS.

Figure 4. Ablating inputs to the lateral septum (LS) from basolateral amygdala (BLA), but not ventral CA1 (vCA1), abolishes social novelty recognition behavior in mice. (A) Cartoon of viral strategy used to selectively eliminate inputs to the LS from either the BLA or vCA1 utilizing retrograde labeling and Cre-mediated expression of diphtheria toxin A (DtA) (left panel). Schematics depict ablation in cohort where BLA-LS projections are targeted (middle panel), and ablation in cohort where vCA1-LS projections are targeted. (B) Representative images show that in the cohort where DtA is expressed in BLA (top row), BLA-LS projections are ablated, while vCA1-LS projections are intact, while in the cohort where DtA is expressed in vCA1 (bottom row), vCA1-LS projections are ablated while BLA-LS projections remain intact . (C) Mice with the BLA-LS circuit intact (n=10) display normal social novelty recognition behavior. In both trial 1 (t_9 = 7.808, p < 0.0001) and trial 2 (t_9 = 5.17, p = 0.0006) they spend more time investigating the socially novel mouse. (D) Ablation of the BLA-LS circuit (n=10) abolishes the social novelty phenotype observed in trial 2 (t_9 = 0.6048, p = 0.56), but not trial 1(t_9 = 12.74, p < 0.0001). (E) Mice with an intact BLA-LS circuit show better social discrimination between the socially novel and familiar mice in Trial 2 than mice with an ablated BLA-LS circuit ($U_{10,10}$ = 17, p = 0.0115). (F) Mice with the vCA1-LS circuit intact (n=8) display normal social novelty recognition behavior. In both trial 1 (t_7 = 8.449, p < 0.0001) and trial 2 (t_7 = 10.5, p < 0.0001) they spend more time investigating the socially novel mouse. (G) Ablation of the vCA1-LS circuit (n=8) does not alter social novelty behavior in trial 1 (t_7 = 5.235, p = 0.0012) or trial 2 (t_7 = 2.979, p = 0.0206). (H) Mice with an intact vCA1-LS circuit and mice with an ablated vCA1-LS circuit show no difference in social discrimination ($U_{9,9}$ = 30, p = 0.8527).

Figure 5. Knockdown of brain-derived neurotrophic factor (BDNF) in basolateral amygdala (BLA) inputs to the lateral septum (LS) abolishes social novelty recognition behavior in mice. (A) Schematic of viral strategy used to deplete BDNF expression in BLA-LS projections in BDNF$^{fl/fl}$ mice. (B) Representative images of viral expression of mCherry within the BLA. (C) Mice with intact BDNF expression in BLA-LS projections (n=9) display normal social novelty recognition behavior. In both trial 1 (t_8 = 5.73, p = 0.0004) and trial 2 (t_8 = 6.842, p = 0.0001) they spend more time investigating the socially novel mouse. (D) BDNF depletion in BLA-LS projections (n=9 mice) abolishes the social novelty phenotype observed in trial 2 (t_8 = 1.763, p = 0.116), but not trial 1(t_8 = 5.456, p = 0.0006). (E) Mice with intact BDNF expression in BLA-LS projections display better social discrimination between the socially novel and familiar mice in trial 2 than mice with BDNF knockdown in BLA-LS projections ($U_{9,9}$ = 9, p = 0.004).

ACKNOWLEDGEMENTS

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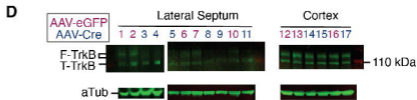54. Oliveira VE de M, Lukas M, Wolf HN, Durante E, Lorenz A, Mayer A-L, *et al.* (2021):
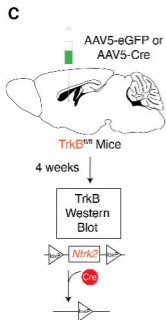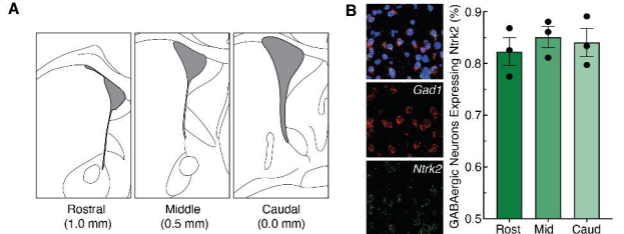
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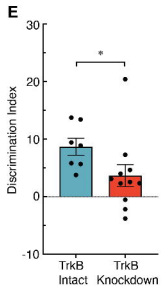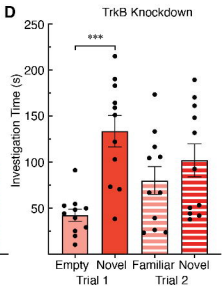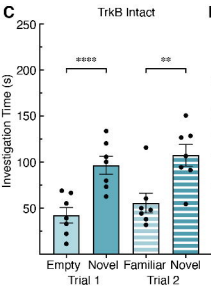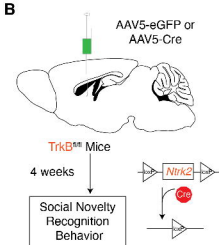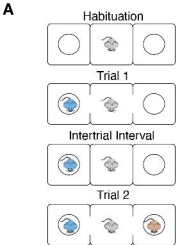
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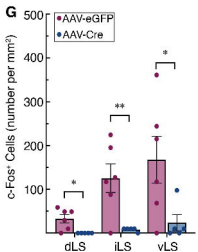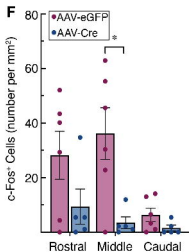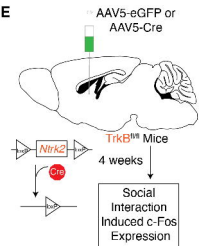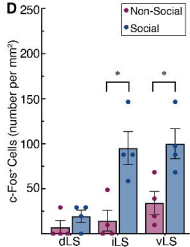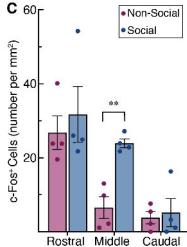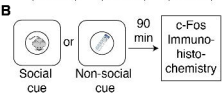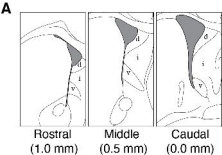
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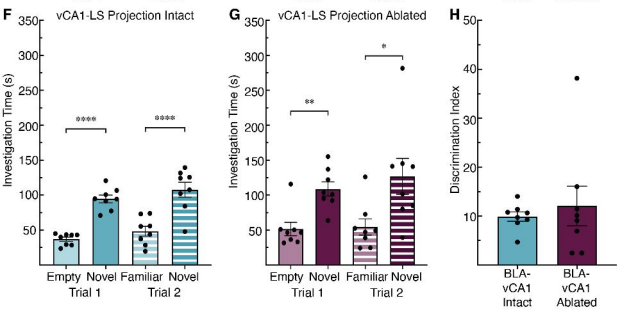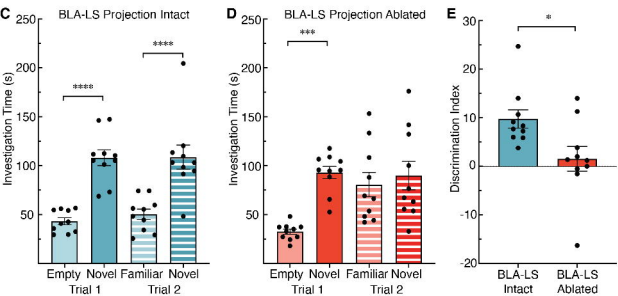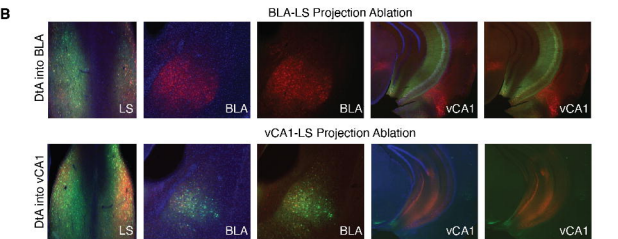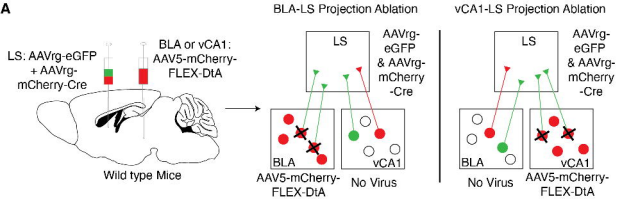84. Maynard KR, Tippani M, Takahashi Y, Phan BN, Hyde TM, Jaffe AE, *et al.* (2020):

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A Rostral (1.0 mm), Middle (0.5 mm), Caudal (0.0 mm)

B
Gad1
Ntrc2

GABAergic Neurons Expressing Ntrk2 (%) — Rost, Mid, Caud

C AAV5-eGFP or AAV5-Cre

TrkB$^{fl/fl}$ Mice

4 weeks

TrkB Western Blot

Ntrc2 / Cre

D AAV-eGFP / AAV-Cre

Lateral Septum — 1 2 3 4 5 6 7 8 9 10 11

Cortex — 12 13 14 15 16 17

F-TrkB
T-TrkB
110 kDa

αTub

E Full-length TrkB

Relative TrkB Levels (%) — AAV-eGFP, AAV-Cre

Lateral Septum, Cortex

$**$

F Truncated TrkB

Relative TrkB Levels (%) — AAV-eGFP, AAV-Cre

Lateral Septum, Cortex

A

Habituation

Trial 1

Intertrial Interval

Trial 2

B

AAV5-eGFP or
AAV5-Cre

TrkB^fl/fl Mice

4 weeks

Social Novelty
Recognition
Behavior

Ntrk2

Cre

C TrkB Intact

Investigation Time (s)

****

**

Empty   Novel   Familiar   Novel
    Trial 1   Trial 2

D TrkB Knockdown

Investigation Time (s)

***

Empty   Novel   Familiar   Novel
    Trial 1   Trial 2

E

Discrimination Index

*

TrkB   TrkB
Intact   Knockdown

A

LS: AAVrg-FlpO

BLA :
AAV8-fDIO-
Cre-mCherry
or AAV8-
fDIO-
mCherry

BDNF^fl/fl Mice

BDNF Intact

LS ——— AAVrg-
FlpO

BLA ——— AAV8-
fDIO-
mCherry

▷—▷ *Bdnf* ◁—◁

BDNF Knockdown

LS ——— AAVrg-
FlpO

BLA ——— AAV8-
fDIO-Cre
mCherry

▷—▷ *Bdnf* ◁—◁
▷—▷ Cre ◁—◁

B

C

BLA-LS Projection
BDNF Intact

Investigation Time (s)

Empty   Novel   Familiar   Novel
  Trial 1   Trial 2

*** (Empty vs Novel, Trial 1)
** (Familiar vs Novel, Trial 2)

D

BLA-LS Projection
BDNF Depleted

Investigation Time (s)

Empty   Novel   Familiar   Novel
  Trial 1   Trial 2

*** (Empty vs Novel, Trial 1)

E

Discrimination Index

**

BLA-LS   BLA-LS
BDNF   BDNF
Intact   Depleted