Behavioral/Cognitive

Pharmacological Inactivation of the Bed Nucleus of the Stria Terminalis Increases Affiliative Social Behavior in Rhesus Macaques

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The bed nucleus of the stria terminalis (BNST) has been implicated in a variety of social behaviors, including aggression, maternal care, mating behavior, and social interaction. Limited evidence from rodent studies suggests that activation of the BNST results in a decrease in social interaction between unfamiliar animals. The role of the BNST in social interaction in primates remains wholly unexamined. Nonhuman primates provide a valuable model for studying social behavior because of both their rich social repertoire and neural substrates of behavior with high translational relevance to humans. To test the hypothesis that the primate BNST is a critical modulator of social behavior, we performed intracerebral microinfusions of the $GABA_A$ agonist muscimol to transiently inactivate the BNST in male macaque monkeys. We measured changes in social interaction with a familiar same-sex conspecific. Inactivation of the BNST resulted in significant increase in total social contact. This effect was associated with an increase in passive contact and a significant decrease in locomotion. Other nonsocial behaviors (sitting passively alone, self-directed behaviors, and manipulation) were not impacted by BNST inactivation. As part of the "extended amygdala," the BNST is highly interconnected with the basolateral (BLA) and central (CeA) nuclei of the amygdala, both of which also play critical roles in regulating social interaction. The precise pattern of behavioral changes we observed following inactivation of the BNST partially overlaps with our prior reports in the BLA and CeA. Together, these data demonstrate that the BNST is part of a network regulating social behavior in primates.

Key words: **amygdala; BNST; macaque; muscimol; primate**

Significance Statement

The bed nucleus of the stria terminalis (BNST) has a well-established role in anxiety behaviors, but its role in social behavior is poorly understood. No prior studies have evaluated the impact of BNST manipulations on social behavior in primates. We found that transient pharmacological inactivation of the BNST increased social behavior in pairs of macaque monkeys. These data suggest the BNST contributes to the brain networks regulating sociability.

Introduction

The bed nucleus of the stria terminalis (BNST) is a subcortical structure located in the ventral forebrain. It has been described as a component of the "extended amygdala" because of its anatomic and functional similarities to the amygdaloid complex (Alheid and Heimer, 1988; Alheid et al., 1998; Fox et al., 2015). The BNST is densely and reciprocally interconnected with key limbic structures, including the amygdala and the hypothalamus (Silverman et al., 1981; Weller and Smith, 1982; Dong et al., 2001a; Dong and Swanson, 2004; deCampo and Fudge, 2013; Kim et al., 2013; Torrisi et al., 2015; Fudge et al., 2017; Huang et al., 2021) and has been implicated in a range of behavioral functions, including the stress response, extended-duration fear states (i.e., anxiety), and social behavior (Walker et al., 2003; Lebow and Chen, 2016; Lischinsky and Lin, 2020; Flanigan and Kash, 2022).

The BNST, and its contributions to social behavior, has been most extensively studied in rodent models. In both rats and

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mice, aggressive behaviors increase activity within the BNST under various conditions (Haller et al., 2006; Ferris et al., 2008; Lin et al., 2011). Moreover, increasing activity within the BNST using either pharmacological or chemogenetic approaches decreases social interaction (Sajdyk et al., 2008; Emmons et al., 2021). Conversely, pharmacological inhibition of the BNST decreases submissive behavior following social defeat in rats, while chemogenetic inhibition of the BNST increases social novelty preference in mice (Sajdyk et al., 2008; Emmons et al., 2021). No studies to date have examined a role for the primate BNST in social behavior.

Nonhuman primates have a rich social repertoire that includes a variety of complex social behaviors, making them a valuable model system to study the neural substrates of social behavior. Moreover, we have previously found that the effect of focal brain manipulations on behavior diverges, sometimes in surprising ways, between rodents and primates (see Wellman et al., 2016; Aguilar et al., 2018), underscoring the importance of cross-species analyses. Previous work in our laboratory has examined the role of different amygdala subnuclei in social interaction between familiar macaque monkeys; pharmacological disinhibition of the basolateral amygdala (BLA) decreases social behavior, whereas pharmacological inactivation of the BLA or central amygdala (CeA) increases social behavior (Wellman et al., 2016). Given the dense input to the BNST from both the BLA and CeA (Dong et al., 2001a; Fudge et al., 2017; Oler et al., 2017), the robust impact of amygdala manipulations on social behavior in macaques, and the reduced social behavior associated with activation of the BNST in rodents, we hypothesized that inactivation of the BNST would increase affiliative social interaction in macaques.

To test this hypothesis, we performed intracerebral microinfusions of muscimol, a $GABA_A$ agonist, directly into the BNST of rhesus macaques and examined their interaction with a familiar conspecific. We monitored social and nonsocial behavior in dyads following either drug or saline infusion, as we have done in our previously published studies (for details, see Materials and Methods) (Forcelli et al., 2016, 2017; Wellman et al., 2016). We found a selective increase in social contact following muscimol infusion in the BNST. These data provide clear evidence for a role for the BNST in the regulation of social behavior in primates.

Materials and Methods

Subjects. Five adult male rhesus macaque monkeys (*Macaca mulatta*) were used in this study (PI, FR, MA, FI, ME). They were procured from AlphaGenesis and the Manheimer Foundation at the age of 2-3 years, and transferred to Georgetown University, where all experimental procedures were conducted. We used exclusively same-sex dyads to mirror the troupe structure of juvenile male macaques in the wild. Based on their compatibility, they were combined to generate five experimental dyads (Table 1). Three monkeys served as injected subjects (FR, MA, PI). In separate experimental sessions, 2 of them (FR and MA) also served as noninjected partners. The remaining 2 monkeys (FI, ME) served only as noninjected partners.

Monkeys were pair- or group-housed in two to four adjoining cages (size, 61 × 74 × 76 cm each) in a room with a regulated 12 h light/dark cycle. They were maintained on a primate lab diet (Purina Mills, catalog #5049), supplemented with fresh fruit and vegetables. Water was available *ad libitum* in the home cage. Care and housing of the monkeys in the Georgetown University Division of Comparative Medicine met or exceeded the standards as stated in the *Guide for the care and use of laboratory animals* (National Research Council Institute for Laboratory Animal Research, 2011), Institute for Laboratory Animal Research recommendations, and Association for Assessment and Accreditation of Laboratory Animal Care International accreditation standards. The study was conducted under a protocol (#2016-1115) approved by the Institutional Animal Care and Use Committee at Georgetown University.

Table 1. Experimental dyads used for each experiment[a]

Dyad	FR and MA	FR and FI	MA and FR	MA and FI	PI and ME
Symbol	□	◇	⬭	▽	△
No. of muscimol infusions	2	2	2	3	2
No. of control infusions	2	2	2	2	2

[a]The first animal in each dyad denotes the injected animal; the second indicates the noninjected partner. Symbols represent dyads. Numbers indicate the number of drug infusions each injected animal received while being paired with the partner animal, for each condition.

The present experiments began after the animals were extensively socialized and behaviorally trained (including chair training), which continued until the age of 3-4 years. At the time of testing, animals ranged from 4 to 5 years old and weighed between 7 and 12 kg. In addition to the experimental procedures described here, all subjects were assessed for prepulse inhibition (PI, FR, MA, FI, ME) and participated in another study of social behavior (PI, FR, MA, FI, ME). As part of those experiments, all animals received drug infusions in the NAc and the BLA. These data will be published in a separate communication.

Implantation of drug infusion platform and site verification. The monkeys were implanted with stereotaxically positioned chronic infusion platforms, which enabled us to target specific sites within the BNST based on the coordinates assessed by structural MRI scans (Fig. 1). We chose to target the anterior BNST based on previous reports of dense projections from the BLA to this area in rodents (Dong et al., 2001a) as well as evidence for a functional link between this area and anxious temperament in nonhuman primates (Kalin et al., 2005; Fox et al., 2008).

For surgery and postoperative MRI, we followed the procedures as described in detail in our previous studies (Wellman et al., 2005; West et al., 2011; Holmes et al., 2012; DesJardin et al., 2013; Forcelli et al., 2014). Briefly, the infusion platform was implanted under anesthesia and aseptic conditions (Wellman et al., 2005; Forcelli et al., 2016), followed by a postoperative regimen of analgesics and antibiotics determined in consultation with the facility veterinarian. Postoperatively, each monkey received at least one T1-weighted structural MRI scan (0.75 × 0.75 mm in-plane resolution, 1 mm slice thickness) intended to obtain coordinates for infusions in the BNST. Tungsten micro-electrodes (FHC), which were visible on the scan, were used to determine the precise coordinates for drug infusion, as described previously (Holmes et al., 2012).

In Figure 1, the atlas drawing shows the area of intended drug infusion with the yellow area indicating the BNST (Fig. 1*A*). Figure 1*B* shows the placement of individual infusions based on the MRI, and Figure 1*C* shows the placement of a tungsten microelectrode at the dorsal border of the BNST in 2 subjects. Based on electrode placement in the postoperative MRI, we successfully targeted the BNST in all three infused animals.

Intracerebral drug infusions. To transiently inactivate the BNST, the $GABA_A$ receptor agonist muscimol (Sigma-Aldrich) was administered at a dose of 4.5 nmol in a 0.5 µl volume, bilaterally. The concentration was the same as that used in our previous study targeting BLA (9.0 nmol in 1 µl) (Wellman et al., 2005), with a positive effect. However, we reduced the volume and dose as the intended infusion site in the BNST is smaller than that of the BLA.

Drug infusions were conducted as in our previous reports (Wellman et al., 2005; West et al., 2011; Holmes et al., 2012; DesJardin et al., 2013; Dybdal et al., 2013; Forcelli et al., 2014; Malkova et al., 2015) using a removable cannula that was inserted for each infusion in an area determined from the MRI scan through the infusion platform and a grid with holes spaced 2 mm apart in both mediolateral and anteroposterior planes. The entire infusion procedure lasted 10-15 min. At least 48 h elapsed between drug treatments in an individual subject. Control infusions consisted of microinjection of an equivalent (0.5 µl) volume of sterile saline. Following each drug infusion, each dyad was video recorded for subsequent analyses.

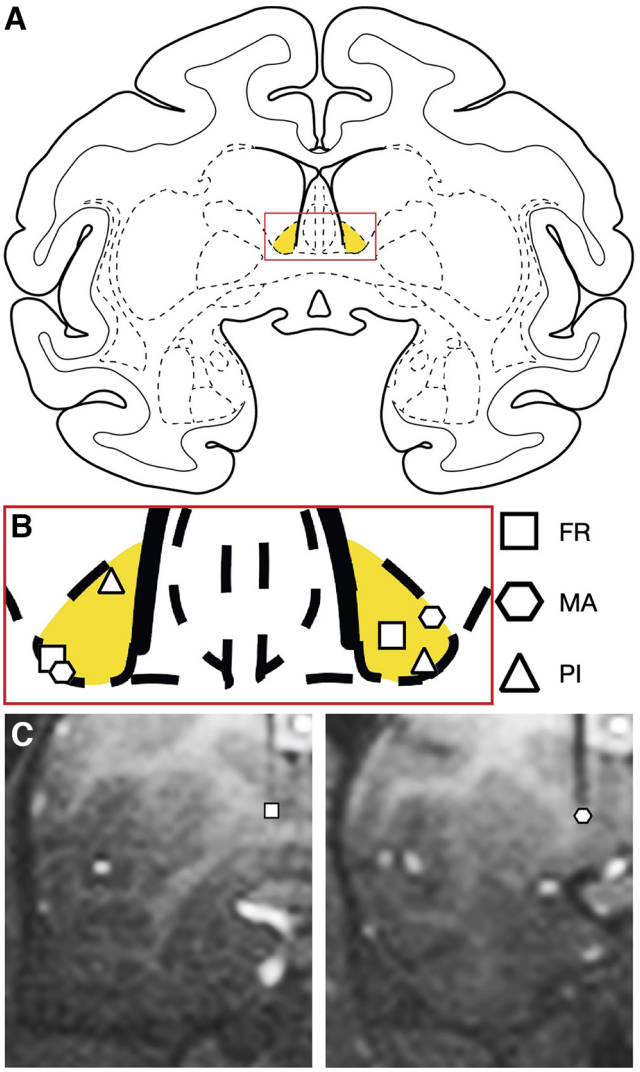

Figure 1. ***A***, A coronal atlas plane showing the area of intended drug infusion. Yellow area represents the anterior BNST. ***B***, Placement of individual infusions in the 3 subjects based on postoperative MRI. ***C***, MRI images from 2 subjects showing the placement of a tungsten microelectrode at the dorsal border of the BNST.

In our prior studies, we reported that injection of 1 µl of the contrast agent gadolinium (5 mM in saline) resulted in an area hypersignal of ~3 mm in diameter (DesJardin et al., 2013; Forcelli et al., 2014; Wicker et al., 2018). This suggests a sphere of drug spread of ~3 mm 1 h after infusion of a 1 µl volume. Moreover, we showed that muscimol effects can be detected within 10 min of drug infusion (Dybdal et al., 2013), and that muscimol infusion into the amygdala produces robust changes in social behavior in the first 15 min of observation. Therefore, we concentrated on behavioral analyses of the first 15 min of the recording. This time period limited the observation to the time before the drug potentially diffused to neighboring areas.

Experimental design. The goal of this experiment was to assess the effects of inactivation of the BNST on social interaction in dyads. As indicated above, we tested five dyads. The animals in each dyad were separated (i.e., the partition between their cage compartments was closed) the evening before the experiment and remained separated until the intended drug (or saline) infusion. Immediately following drug infusion, the infused animal and its uninfused partner were transferred to an observation cage (85 cm wide × 95 cm deep × 101.5 cm tall) in a separate room from their homecage for behavioral observation. Animals were infused with either muscimol or saline (vehicle) in a pseudorandomized order. Each animal received 2 infusions (one subject, MA paired with FI, received three infusions of muscimol) and 2 saline infusions, for each dyad the animals was observed in. The number of infusions per animal is shown in Table 1. All dyads consisted of monkeys that were highly familiar with each other.

Behaviors were analyzed using the annotation platform, BehaviorCloud. A list of behaviors and their definitions are presented in Table 2. These categories were modified from those used previously (Malkova et al., 2010; Wellman et al., 2016; Forcelli et al., 2017). One observer (J.T.J.) analyzed all records, and those analyses were used for statistical processing. An additional observer was trained to achieve a high level of interobserver correlation ($r = 0.9$ or better) and analyzed a subset of videos. Three behavioral classes were used for analyses: general behaviors (nonsocial), social behaviors, and vocalizations. These classes were coded in parallel (e.g., an animal could be in engaging in social contact while also engaging in manipulation). Frequency and duration were recorded for each behavior. Several of the behaviors occurred so infrequently that any effect of drug treatment would be obscured by floor effects. These behaviors included motor stereotypies, approach, mounting, aggression, play, withdrawal, and vocalizations. Only one of the animals (FR) produced any vocalizations and this was restricted to 0-3 affiliative vocalizations per session. Furthermore, while we did not directly quantify anxiety behavior (i.e., scratching, yawning, body shakes, etc.), these behaviors similarly occurred very rarely and thus would be subject to floor effects.

Statistical analysis. For each behavior, the scores were averaged across the repeated sessions, resulting in one data point for muscimol and one for saline for the infused animal in each dyad. Data were analyzed using GraphPad Prism software. Data analyses were performed using a paired t test. p values of <0.05 were considered statistically significant. Behaviors were analyzed independently, as has been reported previously (Bachevalier et al., 2001; Malkova et al., 2010).

Results

Effects of BNST inactivation on social behavior

The effects of muscimol infusion into the BNST on social behavior are shown in Figure 2. Consistent with our hypothesis, inactivation of the BNST significantly increased duration of total social contact (Fig. 2*A*; paired t test, $t = 3.912$, df = 4, $p = 0.02$), an effect observed in all dyads. The estimation plot in Figure 2*A* shows both individual responses and the mean difference in total social contact (149.3 s). No other social behaviors differed significantly between control and treatment conditions. For four of five dyads, there was no solicitation of grooming by the infused animal from the uninfused partner after either saline or muscimol infusion (Fig. 2*B*; paired t test, $t = 1.000$, df = 4, $p = 0.37$). Similarly, the duration of reception of grooming from the uninfused partner remained at or near 0 for three of five dyads in both conditions (Fig. 2*C*; paired t test, $t = 0.246$, df = 4, $p = 0.82$). The duration of grooming of the uninfused partner by the infused animal remained at 0 or very low for four of five dyads (Fig. 2*D*; paired t test, $t = 1.176$, df = 4, $p = 0.30$). Consistent with an overall increase in social contact without a significant increase in grooming behaviors, the duration of passive social contact increased (Fig. 2*E*). This effect approached significance (paired t test, $t = 2.042$, df = 4, $p = 0.11$). The mean difference in passive contact between conditions was 93.46 s. While this did not reach the level of statistical significance, BNST inactivation numerically increased passive social contact in all dyads.

Effects of BNST inactivation on nonsocial behavior

The effects of muscimol infusion into the BNST on nonsocial behavior are shown in Figure 3. Muscimol infusion significantly

Table 2. Operational definitions of general (nonsocial), social behaviors, and vocalizations[a]

Behavior	Description
General (nonsocial)	
Locomotion	Walks, runs, climbs or jumps
Manipulation	Handles, chews, licks, moves, or smells objects or cage parts
Passive while alone	Inactive, stays in one location
Self-directed	Engages in self-directed behaviors, i.e., self-grooms, hugs head, self-grabs and bites, presses face with hands, self-holds, closes fists, self-clutches, sexually self-stimulates, prone, or head on chest
Motor stereotypies	Repeatedly paces, somersaults, circles, swings
Social	
Approach	Initiates social contact; moves body or head toward the conspecific
Mounting	Mounts the conspecific
Aggression	Makes threatening gestures (i.e., mouth threat, head or body lunge, cage shake) toward or hits, grabs, or bites the conspecific
Isolation	Sits alone
Contact	Touches or holds the conspecific
Play	In contact with the conspecific, includes chasing, wrestling, and "rough and tumble" behaviors
Groom conspecific	Subject grooms the conspecific
Solicitation of grooming	Subject presents for grooming
Receives grooming	Subject is groomed by the conspecific
Withdrawal	Moves away from the conspecific when approached
Total social contact	Includes contact, dominance, grooming, and play
Vocalizations	
Affiliative vocalizations	Coo, grunt, girney
Defensive vocalizations	Bark, scream

[a]The behavioral category and description for each of the general (nonsocial), social behaviors, and vocalization behaviors analyzed in the manuscript.

reduced duration of locomotion (paired t test, $t = 5.458$, df = 4, $p = 0.01$; Fig. 3*A*), which was observed in all dyads. No other nonsocial behaviors differed between control and treatment conditions. There was no discernable pattern between control and treatment conditions for passive behavior (Fig. 3*B*, paired t test, $t = 0.049$, df = 4, $p = 0.96$), self-directed behavior (Fig. 3*C*; paired t test, $t = 0.751$, df = 4, $p = 0.49$), or manipulation (Fig. 3*D*; paired t test, $t = 1.684$, df = 4, $p = 0.167$).

Discussion

While the BNST has been of growing interest for its role in social and emotional behaviors, its contribution to social interaction in primates was previously unstudied. Here, we found that bilateral inactivation of the BNST increased social contact between familiar macaque monkeys. Consistent with an increase in social contact, BNST inactivation also decreased locomotion. No other nonsocial behaviors were impacted. The increase in social interaction is consistent with our overarching hypothesis that the BNST, as an output target of the extended amygdala, contributes to the brain networks mediating social behavior.

The BNST is considered part of the extended amygdala based on its physical continuity with the CeA, the similarity of its neurochemical and cytoarchitectonic composition to the amygdala proper, its dense reciprocal connectivity with amygdala subnuclei, and its shared functional characteristics (de Olmos and Heimer, 1999). It is well established that the amygdala is a critical node in the social behavioral network. In rhesus monkeys, disruption in normal amygdala function, either through axon-sparing excitotoxic lesions (Emery et al., 2001; Machado et al., 2008) or transient pharmacological inactivation (Wellman et al., 2016), results in a significant increase in social interaction between familiar monkeys. The BNST receives glutamatergic projections from the amygdala (Dong et al., 2001a; Kim et al., 2013; Fudge et al., 2017; Oler et al., 2017). Therefore, we hypothesized that inactivation of the BNST would increase social contact in a manner similar to that following amygdala lesions or inactivation.

Interestingly, inhibition of the BNST produced a pattern of behavioral changes that differed in part from our prior studies in the BLA and CeA (Wellman et al., 2016). Pharmacological inactivation of the BLA, CeA, or the BNST each produced an increase in total social contact. In the case of the BLA, this was associated with a significant increase in grooming behaviors, including soliciting and receiving grooming. By contrast, inactivation of the CeA increased receiving grooming, but not soliciting grooming. Inactivation of the BNST was without effect on any grooming-related behavior. The increase in total social contact we observed included sitting passively together, grooming, and grooming-related behaviors, but none of these alone reached the level of statistical significance. The impact of BLA, CeA, and BNST inactivation on nonsocial behaviors also differed. While inactivation of the BLA and BNST decreases locomotion, inactivation of the CeA does not. Both the basolateral nuclei and the CeA project to the BNST, the BLA is also a major source of excitatory input to the CeA. Simple monosynaptic connections between these regions cannot explain our findings, as the patterns differed between the structures. This is not surprising, however, given the diverse projection targets of each of these regions. The BLA is robustly interconnected with sensory and prefrontal cortices (McDonald, 1996; McDonald, 1998), while the CeA sends extensive projections to subcortical targets, including the hypothalamus and periaqueductal gray (Price and Amaral, 1981; LeDoux et al., 1988; Rizvi et al., 1991; Petrovich et al., 2001). The BNST projects back to the amygdala, as well as to hypothalamic and brainstem regions (Dong et al., 2001b; Dong and Swanson, 2004, 2006; Oler et al., 2017). Consistent with tracer studies in rodents and primates, functional connectivity studies in humans show high coupling between BNST and amygdala, hippocampus, thalamus, caudate, periaqueductal gray, hypothalamus, and mPFC (Torrisi et al., 2015). Thus, parallel pathways may mediate the different components of the behavior we observed across these studies.

The behavioral effect of BNST inactivation was largely specific to social behavior. There was no difference between conditions for passive behavior while alone, self-directed behavior, or

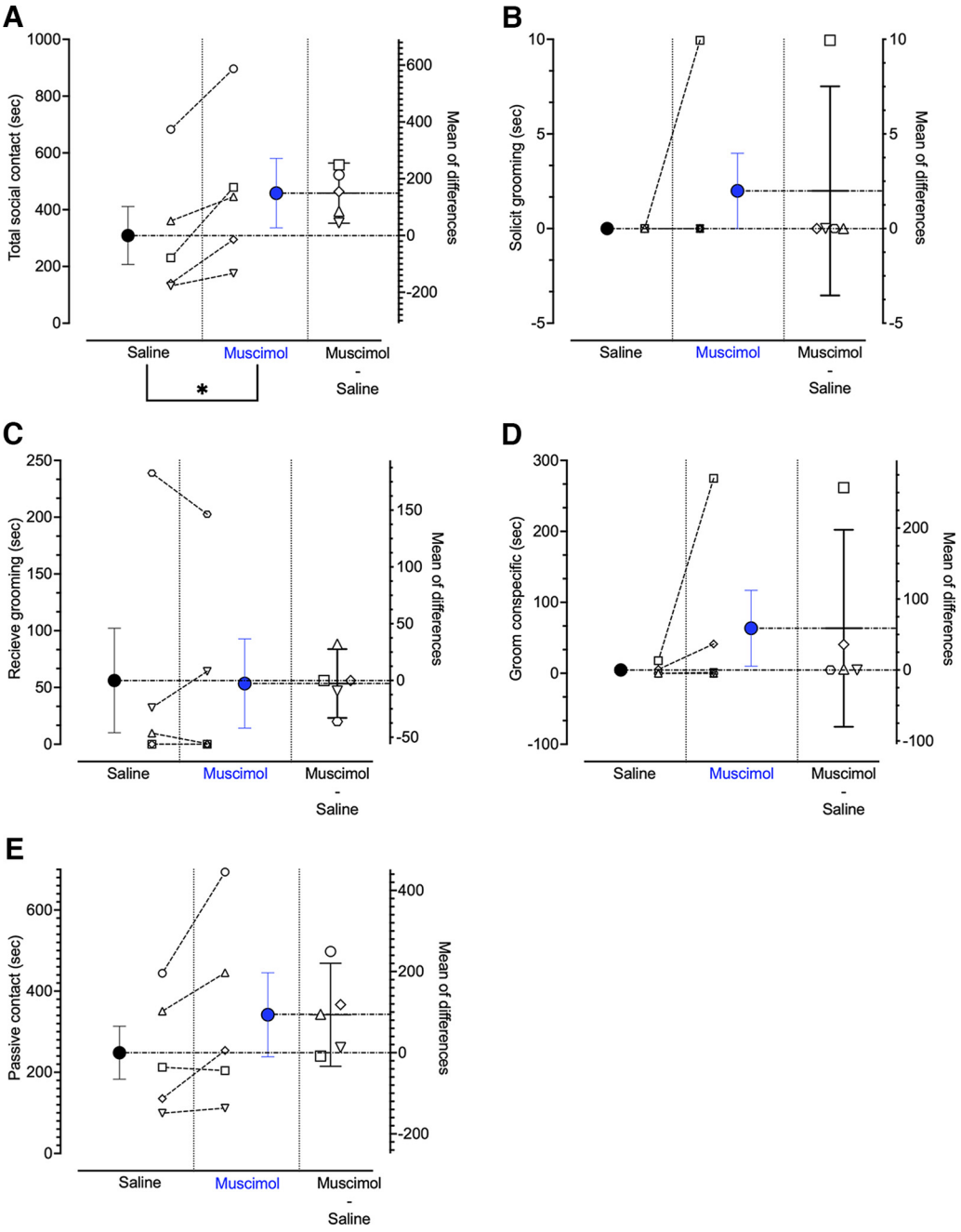

Figure 2. Duration of social behaviors following saline or muscimol infusion into the BNST. Estimation plots for each of the social behavior categories. ***A***, There was a significant increase in duration of social contact following BNST infusion compared with saline. This was associated with a nonsignificant increase in passive contact (***B***). No other behaviors differed significantly between conditions (solicit grooming, ***C***; receive grooming, ***D***; groom conspecific, ***E***). Solid symbols represent the mean of the five dyads ± SEM for each condition. Smaller open symbols represent individual dyads under saline-infused and muscimol-infused conditions. The line connecting these indicates the effect of drug infusions. The plot to the right in each panel represents effect size (mean of differences between conditions with 95% CIs). *$p < 0.05$ (paired t test).

manipulation. While there was a significant decrease in locomotion following BNST inactivation, this effect may be explained by the increase in social contact. In a fixed observation period, if the animals spend more time interacting, it logically follows that they have less opportunity to move about the cage. In support of a lack of direct impact of BNST inactivation on locomotion, previous studies using lesions of the BNST have not reported any motor deficits (Pezük et al., 2006, 2008). Finally, BNST inactivation did not affect the duration of manipulation or self-directed behavior, suggesting that the decrease in locomotion was not caused by motor difficulties.

The BNST consists of at least 18 distinct subnuclei defined by unique cytoarchitecture, chemoarchitecture, gene expression patterns, and connectivity (Bota et al., 2012). Primarily, the BNST is divided into anterior and posterior divisions, both of which are involved in the coordination of social behaviors in rodents (Lebow and Chen, 2016). As discussed previously, we targeted the anterior portion of the BNST based on reports of dense projections from the BLA to this region (Dong et al., 2001a). There are several subregions within the anterior BNST, including the anterolateral, anteromedial, oval, fusiform, juxtacapsular, rhomboid, dorsomedial, ventral nucleus, and magnocellular divisions (Bota et al., 2012). Each subregion has distinct projections within and outside of the BNST (Bota et al., 2012). The differential contributions of each region to social behavior have yet to be adequately described

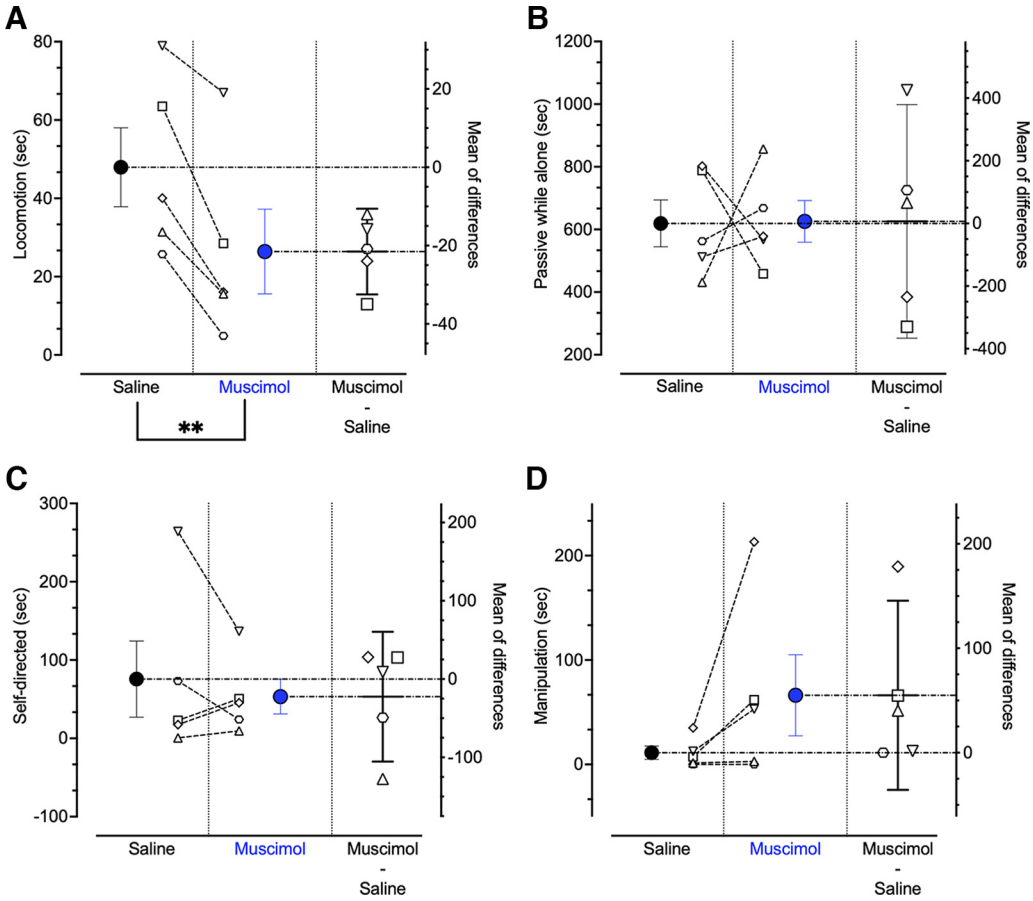

Figure 3. Duration of nonsocial behaviors following saline or muscimol infusion into the BNST. Locomotion (***A***) was significantly decreased following muscimol infusion. There was no difference in duration of passive while alone (***B***), self-directed (***C***), or manipulation (***D***) behaviors between conditions. Solid symbols represent the mean of the five dyads ± SEM for each condition. Smaller open symbols represent individual dyads under saline-infused and muscimol-infused conditions. The line connecting these indicates the effect of drug infusions. The plot to the right in each panel represents effect size (mean of differences between conditions with 95% CIs). $^{**}p < 0.01$ (paired *t* test).

in any species. The resolution of our microinjection approach is such that we were unable to target specific subnuclei within the anterior BNST; this remains an area for future investigation.

While no previous studies in primates have examined a role for the BNST in social behavior, this has been explored in rodent models. Social behaviors, including aggression, maternal care, mating behavior, and social interaction, are modulated by activity within the rodent BNST (Haller et al., 2006; Klampfl et al., 2014; Duque-Wilckens et al., 2016; Bayless et al., 2019; Nordman et al., 2020; Flanigan and Kash, 2022). For example, increasing activity within the BNST decreased social interaction between same-sex partners in mice (Lungwitz et al., 2012). Similarly, in rats, injection of a corticotropin-releasing factor (CRF) agonist into the BNST resulted in a multiweek decrease in social interaction (Lee et al., 2008). Moreover, inhibition of GABA synthesis within the BNST decreased social interaction time in rats (Sajdyk et al., 2008). By contrast, focal inhibition of the BNST produces the opposite response: chemogenetic silencing of the BNST produced an increase in social novelty preference in normal mice, and an increase in social preference in animals with a history of early life stress (Emmons et al., 2021).

In addition to its role in social behavior, the BNST has been implicated in anxiety. A series of studies examining brain metabolism through fluorodeoxyglucose positron emission tomography in rhesus monkeys found increased BNST activity in animals that showed high levels of trait anxiety (Kalin et al., 2005; Fox et al., 2008; Shackman et al., 2017). Moreover, subpopulations of neurons in the CeA that express transcripts associated with high trait anxiety project to the BNST (Kovner et al., 2020). Many human studies have found increased BNST activation in anxiety-provoking contexts (for review, see Avery et al., 2016). For example, BNST activity was found to increase with proximity of a threatening stimulus (tarantula) in healthy subjects (Mobbs et al., 2010). Similarly, BNST activation was found to increase with increasing threat (shock) likelihood in a cohort of healthy individuals, and engagement and tracking of BNST activation were more exaggerated in individuals with higher trait anxiety (Somerville et al., 2010). Of relevance to social vigilance, left BNST activation is enhanced in individuals who display increased empathy for suffering of others (Vekaria et al., 2020). The BNST, along with the CeA, has appreciable populations of CRF expressing neurons (Fudge et al., 2022). It has been suggested that the effects of CRF on anxiety responses (compared with its neuroendocrine function) are likely mediated by the extended amygdala. Indeed, CRFergic neurons project from the CeA to the BNST and likely use GABA as a cotransmitter (Partridge et al., 2016; Fudge et al., 2022). Future studies exploring microinfusion of CRF into the BNST of the primate for impact on social behavior would be of interest.

BNST dysregulation in anxiety-provoking contexts is even more robust in individuals with social anxiety disorder. During a cued anticipation task, unpredictable threat was associated with alterations in BNST connectivity with the amygdala, ventromedial PFC, posterior cingulate cortex, and postcentral gyrus in

individuals with social anxiety disorder (Clauss et al., 2019). Furthermore, individuals with social anxiety disorder displayed increased BNST activation compared with control subjects while anticipating aversive relative to neutral events during a temporally unpredictable threat paradigm (Figel et al., 2019). Although there is a clear role for the BNST (and the amygdala) in anxiety responses, our prior data suggest that effects on anxiety are dissociable from effects on dyadic social interaction. When we treated animals with systemic diazepam, a well-established anxiolytic agent, we found no changes in social interaction (Wellman et al., 2016). This contrasts with the robust effects of GABA-mediated inhibition we report here in the BNST and with our prior studies in the amygdala.

Our data add to the growing literature implicating the BNST as a critical node in the social behavior network. A deeper understanding of the role of the BNST in anxiety and social behavior has implications for the treatment of various human pathologies, social anxiety disorder being of particular note.

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