Received: 20 February 2023 | Revised: 31 July 2023 | Accepted: 18 August 2023

DOI: 10.1111/ejn.16137

RESEARCH REPORT

EJN European Journal of Neuroscience FENS WILEY

Bed nucleus of the stria terminalis GABA neurons are necessary for changes in foraging behaviour following an innate threat

Annie Ly[1] | Alexandra Barker[1] | Hayden Hotchkiss[1] | Emily D. Prévost[1] | Dillon J. McGovern[1] | Zachary Kilpatrick[2] | David H. Root[1]

[1]Department of Psychology and Neuroscience, University of Colorado Boulder, Boulder, Colorado, USA

[2]Department of Applied Mathematics, University of Colorado Boulder, Boulder, Colorado, USA

Correspondence
David H. Root, Department of Psychology and Neuroscience, University of Colorado Boulder, 2860 Wilderness Place, Boulder, CO 80301, USA.
Email: david.root@colorado.edu

Funding information
Boettcher Foundation, Grant/Award Number: Webb-Waring Biomedical Research Award; Brain and Behavior Research Foundation, Grant/Award Number: 2020 NARSAD Young Investigator Award; CU Boulder, Grant/Award Number: ABNexus; National Institute of Mental Health, Grant/Award Numbers: MH125569, MH132322; National Institute on Drug Abuse, Grant/Award Number: DA047443

Edited by: Mathias Schmidt

Abstract

Foraging is a universal behaviour that has co-evolved with predation pressure. We investigated the role of the bed nucleus of the stria terminalis (BNST) GABA neurons in robotic and live predator threat processing and their consequences in post-threat encounter foraging. Both robotic and live predator interactions increased BNST GABA neuron activity. Mice were trained to procure food in a laboratory-based foraging apparatus in which food pellets were placed at incrementally greater distances from a nest zone. After mice learned to forage, they were exposed to a robotic or live predator threat, while BNST GABA neurons were chemogenetically inhibited. Post-robotic threat encounter, mice spent more time in the nest zone, but other foraging parameters were unchanged compared with pre-encounter behaviour. Inhibition of BNST GABA neurons had no effect on foraging behaviour post-robotic threat encounter. Following live predator exposure, control mice spent significantly more time in the nest zone, increased their latency to successfully forage, and significantly altered their overall foraging performance. Inhibition of BNST GABA neurons during live predator exposure prevented changes in foraging behaviour from developing after a live predator threat. BNST GABA neuron inhibition did not alter foraging behaviour during robotic or live predator threats. We conclude that these results demonstrate that while both robotic and live predator encounters effectively intrude on foraging behaviour, the perceived risk and behavioural consequences of the threat are distinguishable. Additionally, BNST GABA neurons may play a role in the integration of prior innate predator threat experience that results in hypervigilance during post-encounter foraging behaviour.

KEYWORDS
bed nucleus, foraging, GABA, predator, threat

Abbreviations: BNST, bed nucleus of the stria terminalis; GABA, gamma-aminobutyric acid; VGaT, vesicular GABA transporter.

1 | INTRODUCTION

From nematode to mammal and all remaining subjects in kingdom Animalia, foraging for food is a shared behaviour (Iwanir et al., 2016; Mobbs et al., 2013; Silston et al., 2021; Stephens et al., 2007). Foraging for food encompasses a multitude of neural systems: hunger, satiety, sensorimotor integration, attention, navigation, exploration and risk assessment, to name a few (Stephens et al., 2007). While there are many factors involved in foraging behaviour, a hunger drive exists to motivate food consumption that leads to satiation (Hull, 1943). However, foraging is not a behaviour that exists in a vacuum in which animals navigate environments to satiate hunger (Stephens & Krebs, 1986). A limitation for most animals is that they are at risk of predation, and predator threat influences foraging behaviour (Lima & Dill, 1990).

Predation provides a major evolutionary pressure for animals to develop highly conserved defensive behaviours while foraging. One example of a defensive foraging behaviour seen across fish, birds and mammals is the avoidance of open spaces (Lima, 1998b). For example, when a wooden model of a hawk was flown over black-capped chickadees, they preferred to carry their food back to shelter rather than consume it in the open, where there was a perceived predator threat (Lima, 1985). Another instance of a defensive foraging behaviour, particular to nocturnal animals, is the avoidance of bright light, such as natural moonlight and artificial light (Clarke, 1983; Kotler, 1984; Lockard & Owings, 1974; Price et al., 1984; Wolfe & Tan Summerlin, 1989). Avoidance is an especially crucial defensive behaviour during foraging when the threat of predation is uncertain or ambiguous, particularly after a prior predator experience. Therefore, animals must evaluate the relationship between food attainment and predation risk (Fanselow et al., 1988; Lima, 1998a; Lima & Dill, 1990).

The bed nucleus of the stria terminalis (BNST) is an extended amygdala structure that is strongly implicated in the processing of uncertain or ambiguous threats (Bruzsik, Biro, Sarosdi, et al., 2021; Davis et al., 2010; Duvarci et al., 2009; Goode et al., 2019, 2020; Hammack et al., 2015) and feeding (Jais et al., 2020; Kocho-Schellenberg et al., 2014; Roman et al., 2012). We hypothesized that the BNST plays an important role in foraging behaviour post-threat encounter when the threat is absent yet uncertain. The BNST is heterogeneous in neuronal cell types but is a predominantly GABAergic brain region (Bota et al., 2012; Siletti et al., 2022; Welch et al., 2019).

We first observed that BNST GABA neurons increased their neuronal activity in response to interactions with live or robotic predators. To investigate the role of GABA BNST neurons in foraging behaviour after a predator threat, we used a chemogenetic approach to inhibit GABA BNST neurons during predator exposure and assessed behavioural changes in a foraging task following the predator experience. Many laboratory-based foraging paradigms exist for rodents (Blanchard & Blanchard, 1989; Choi & Kim, 2010; Fanselow et al., 1988; Pellman et al., 2017; Stacher Hörndli et al., 2019; St-Cyr et al., 2018; Troxell-Smith et al., 2016). Here, we compared the effects of a semi-natural 'robogator' predator threat (Choi & Kim, 2010) and a live predator threat on post-encounter foraging behaviour, as well as identified the role of GABA BNST neurons.

We found that both robogator and live predator threats disrupted post-encounter foraging behaviour, but the live predator threat was more efficacious. While chemogenetic inhibition of BNST GABA neurons during robogator threat had no effect on post-encounter foraging, inhibition of BNST GABA neurons during live predator threat prevented changes in foraging that were observed post-encounter. BNST GABA neuron inhibition did not affect foraging behaviour during robotic or live predator threats. We interpret these results to show that while both robotic and live predator threats effectively intruded on ongoing foraging behaviour, live predator threats resulted in more pronounced disruptions of post-encounter foraging than robotic threats. Additionally, our results have indicated that BNST GABA neurons may play a role in the integration of prior innate predator threat experience with hypervigilance as a behavioural consequence.

2 | MATERIALS AND METHODS

2.1 | Animals

Vesicular GABA transporter-internal ribosome entry site (VGaT-IRES)::Cre knock-in mice (B6J.129S6(FVB)-*Slc32a1*$^{tm2(cre)Lowl}$/MwarJ; Stock #028862) were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred at the University of Colorado Boulder ($n = 31$, 4–5 months old; 16 females and 15 males). Mice were group-housed by sex (4–5 mice/cage) under a reversed 12 h:12 h light/dark cycle (lights on at 10:00 PM) with access to water ad libitum. Sixteen mice were used for foraging behaviour experiments, eight mice were used for c-Fos histology, and seven mice were used for calcium fibre photometry recordings. For the duration of the foraging experiments, mice were weighed daily and fed to maintain 85% of their body weight. Food-restricted mice were fed after the foraging task. All experiments were performed during the dark phase of the light cycle. The

experiments described were conducted in accordance with the regulations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Colorado Boulder.

2.2 | Surgery

Mice were anaesthetised in a gasket-sealed induction chamber at 3% isoflurane gas. After confirming a surgical plane of anaesthesia, isoflurane was continuously delivered at 1%–2% concentration while the mouse was secured in the stereotactic instrument. AAV8-hSyn-DIO-hM4D(Gi)-mCherry (Addgene, $n = 8$, four males, 4 females) or AAV8-hSyn-DIO-GFP (Addgene, $n = 8$, four males, four females) were injected bilaterally into the BNST (5×10^{12} titre, 350 nL volume per hemisphere; 100 nL/min rate; +.3 mm anteroposterior, ±.6 mm mediolateral, −4.1 mm dorsoventral coordinates from bregma) using an UltraMicroPump, Nanofil syringes, and 35-gauge needles (Micro4; World Precision Instruments, Sarasota, FL). Syringes were left in place for 10 min following injections and slowly withdrawn. For calcium fibre photometry experiments, mice were injected unilaterally into the BNST with AAV1-hSyn-FLEX-GCaMP6m (Addgene, $n = 7$, three males, four females) using the same parameters as previously described. After the syringe was withdrawn, an optic fibre (400-μm cor diameter, .66 NA, Doric Lenses) was implanted in the BNST (+.3 mm anteroposterior, ±.6 mm mediolateral, −3.9 mm dorsoventral coordinates from the bregma) and secured with skull screws and dental cement. Mice were given 3 days of postoperative care and allowed 3–4 weeks of recovery before experimentation.

2.3 | Histology

Mice were anaesthetised with isoflurane and perfused transcardially with .1 M phosphate buffer (PB), followed by 4% (w/v) paraformaldehyde in .1 M PB, pH 7.3. Brains were extracted and cryoprotected in an 18% sucrose solution in .1 M PB at 4°C overnight. Brains were cryosectioned to obtain coronal slices with BNST (30 μM). These coronal brain slices were mounted onto gelatin-coated slides and imaged for green fluorescent protein (GFP) or mCherry fluorescent expression on a Zeiss widefield Axioscope. Off-target or no fluorophore (GFP or mCherry) expression mice were excluded from the analysis.

For c-Fos histology, mice were exposed to five footshocks (.5 mA, 500 ms, 60 s inter-shock interval) and perfused 90 min afterwards. As part of the perfusion protocol, mice were anaesthetised with isoflurane and perfused transcardially with .1 M PB, followed by 4% (w/v) paraformaldehyde in .1 M PB, pH 7.3. Brains were extracted and cryoprotected in an 18% sucrose solution in .1 M PB at 4°C overnight. Brains were cryosectioned to obtain coronal slices with BNST (30 μM). Brain sections containing BNST were incubated with blocking buffer solution (4% bovine serum albumin and .3% Triton X-100 in .1 M PB, pH 7.3) for 60 min, followed by incubation with mouse anti-GFP (1:500, Takara Bio, 632380), rabbit anti-mCherry (1:500, Takara Bio, 632496) and guinea pig anti-c-Fos (1:500, Synaptic Systems 226308) at 4°C overnight. Sections were washed in PB and incubated with donkey anti-mouse Alexa488 (1:200, Jackson ImmunoResearch, 715545150), donkey anti-rabbit Alexa594 (1:200, Jackson ImmunoResearch, 711585152) and donkey anti-guinea pig (1:200, Jackson ImmunoResearch, 706605148) for 120 min. These coronal brain slices were mounted onto gelatin-coated slides, coverslipped with ProLong DAPI diamond mounting medium (Invitrogen, P36971) and imaged for GFP, mCherry and c-Fos fluorescent expression on a Nikon A1R confocal (20X). All GFP-positive, mCherry-positive and c-Fos co-expressing cells within the BNST were counted in Adobe Photoshop between +.38 and −.22 mm from the bregma. Cells were counted by scorers who were blinded to condition, and cells were only counted when they were also DAPI-positive (4',6-diamidino-2-phenylindole).

2.4 | Calcium fibre photometry recordings

GCaMP6m was excited at two wavelengths (465 and 405 nm isosbestic control) with amplitude-modulated signals from two light-emitting diodes reflected off dichroic mirrors and then coupled into an optic fibre (Barker et al., 2017; McGovern et al., 2021, 2022). The GCaMP signal and the isosbestic control signal were returned through the same optic fibre and acquired using a femtowatt photoreceiver (Newport, Irvine, CA), digitized at 1 kHz, and then recorded by a real-time signal processor (Tucker–Davis Technologies).

2.5 | Foraging apparatus

A custom-made apparatus was built with a foraging zone (100 cm length × 25 cm width × 40 cm height) and a nesting zone (15 cm length × 25 cm width × 40 cm height), which were separated by a black Plexiglas gate that could be lifted up and down. The interior of the foraging zone was painted white, while the nesting zone

remained black Plexiglas. LED strip lights around the foraging perimeter maintained brightness at 40 lx, while the interior of the nesting zone was approximately 4 lx. A camera was positioned above the foraging apparatus to generate a video that was used with ANY-maze software (30 Hz, Stoelting Co., Wood Dale, IL) to track the subject's centre point in real time.

2.6 | Robotic and live predator

A four-wheel robot car (Shenzhen Yahboom Technology Co., Ltd.) with a programmable Micro:bit V1.5 board, a mechanical front-facing claw, and an infrared motion sensor module was assembled to mimic a previously described robotic predator design, herein referred to as a robogator (Choi & Kim, 2010; Kim et al., 2018). The dimensions were 24 cm length × 15 cm width × 12.5 cm height. The Micro:bit board was programmed using the Microsoft MakeCode online platform. The robotic predator was programmed to detect a moving object at a distance of less than 10 cm. Once motion was detected, the LED screen of the Micro:bit board flashed three times before the robotic predator surged forward 20 cm, snapped its mechanical claw three times and retreated back to its original location.

A Long-Evans female rat (8 months old) was placed inside a clear plastic cage (28 cm length × 15 cm width × 23 cm height) with a small circular opening on one side, and the filter on the cage top was removed. The rat was able to move freely within the cage to react to the presence of the mouse but was confined to prevent direct interaction.

2.6.1 | Behavioural procedure

Prey–predator interaction

For calcium fibre photometry recordings during prey–predator interactions, an open field box (40 cm length × 40 cm width × 35 cm height, Stoelting Any-Box) was used. Either the robotic or live predator was placed inside a clear plastic cage as previously described, and the cage was placed at the centre of the open field. Each recorded mouse was allowed to freely explore the zone surrounding the predator cage for 10 min. Each mouse was exposed to both the robotic and live predators. The order of exposure was counterbalanced across 2 days, such that half were exposed to the robotic predator and the other half were exposed to the live predator on 1 day and reversed on the next day.

For the analysis of calcium fibre photometry recordings, custom-written MATLAB scripts were used and are available at www.root-lab.org/code. The behavioural timestamps of an interaction were manually extracted. A prey–predator interaction was defined as any time that the mouse's nose was in contact with the predator cage or the mouse reared against the cage. The isosbestic signal (405 nm) and the GCaMP signal (465 nm) were downsampled (10×), and peri-event time histograms were created between −5 s and +10 s surrounding each interaction event. For each trial, the data were detrended by regressing the 405 nm signal on the 465 nm signal. The generated linear model was used to create a predicted 405 nm signal that was subtracted from the 465 nm signal to remove movement, photo-bleaching, and fibre bending artifacts (Barker et al., 2017). Baseline normalized maximum z-scores were taken from −1 to −.01 s relative to the interaction onset. Interaction normalized maximum z-scores were taken from 0 to +2 s relative to the interaction onset.

Foraging baseline

Mice underwent 2 days of habituation to the nesting zone only of the foraging apparatus for 15 min with some home cage nesting material and 20 food pellets (45 mg, grain-based; F0165, Bio-Serv).

After habituation, mice were allowed to explore the foraging zone and consume a food pellet located at discrete, incremental distances. A single foraging session consisted of three trials, defined by the distance of the food pellet in relation to the nesting zone entrance: 30, 50 and 70 cm. Every trial began with the opening of the Plexiglas gate and ended with the mouse back in the nesting zone after consuming the pellet. When a mouse consumed the pellet and returned back to the nesting zone, the Plexiglas gate was lowered, and the mouse was rewarded with an additional food pellet in the nesting zone. The mouse was given 1 min before the next trial began, and the Plexiglas gate was lifted. If a mouse failed to retrieve a food pellet within 5 min in any trial, the session would end with the mouse not receiving another opportunity to forage until the next session. Mice underwent training until there was no session effect on foraging latency across the last four consecutive sessions. Therefore, mice underwent 18 baseline foraging sessions.

Foraging circa- and post-robotic predator exposure

Mice were exposed to the robotic predator in a single session in the last trial, in which the food pellet was located 70 cm away from the nesting zone. Mice were intraperitoneally injected with a behaviourally-subthreshold dose of clozapine (Caymen Chemical, .1 mg/kg) to activate the hM4Di receptor at least 10 min before the foraging session. The robotic predator was positioned behind the 70-cm mark where the food pellet was located. The trial

ended after the mouse consumed the pellet and returned to the nesting zone, or 5 min after the trial began, with no successful consumption of the food pellet. Following 24 h, mice completed two consecutive post-encounter foraging sessions without the presence of the robogator. These post-encounter sessions were identical in structure to baseline foraging. No clozapine was administered in the foraging sessions post-robotic predator exposure.

Return to foraging baseline

After the post-encounter sessions, mice were allowed to explore the foraging zone and consume a food pellet located at discrete, incremental distances in a similar manner to the initial foraging training. This re-training to baseline foraging was conducted over five sessions.

Foraging circa- and post-live predator exposure

After a return to baseline foraging, mice were exposed to a live predator in a single session in the last trial, in which the food pellet was located 70 cm away from the nesting zone. Mice were intraperitoneally injected with clozapine (.1 mg/kg) at least 10 min before the foraging session. The live predator was positioned behind the 70-cm mark, with the small circular opening pointed towards the nesting zone. The trial ended after the mouse consumed the pellet and returned to the nesting zone, or 5 min after the trial began, with no successful consumption of the food pellet.

Foraging post-live predator exposure

Mice completed two consecutive foraging sessions after the live predator encounter. These sessions were identical in structure to baseline foraging. No clozapine was administered in the foraging sessions post-live predator exposure.

Analyses

Statistical analyses were performed in R version 4.0.5. For fibre photometry experiments, baseline and interaction maximum z-scores for robotic threat or live threat were compared with a within-subjects *t*-test. Maximum interaction z-scores for robotic threats and live threats were compared with a within-subjects *t*-test. For foraging behaviour, groups of eight mice were used for behavioural analysis to achieve 80% statistical power with an alpha level of .05. When multiple variables existed for comparison, data were fitted to a linear mixed effects model such that treatment (GFP and hM4D(Gi)), sex (females and males), session (1–18), trial (30, 50, and 70 cm), predator (robogator and live predator) and predator encounter (pre-, circa- and post-encounter) were the fixed effects. Individual mice were treated as a random effect. Linear mixed effects model analyses were conducted using R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017). These models were evaluated with F-tests on Type III sums of squares on the defined fixed effects and their interactions. Post-hoc significance analysis was conducted through comparisons of estimated marginal means using the R package emmeans (Lenth, 2022). Data were fitted to a linear mixed effects model to account for possible unbalanced data and to lower both Type I and Type II errors (Yarkoni, 2022; Yu et al., 2022). For analysis of foraging latency by trial, hazard ratios (HRs) were calculated from a Cox-proportional hazards regression model using the R package survival (Therneau, 2022). For analysis of the locomotor linear path before and after predator threat exposure, the y-coordinate (width of the foraging apparatus) for the centre point of all subjects was averaged across 10-cm-bins of the x-coordinate (length of the foraging apparatus). The averages were used to fit a linear model, in which treatment (GFP and hM4D(Gi)), trial (30, 50 and 70 cm), x-coordinate (1–10 bins) and predator encounter (pre- and post-encounter) were the linear predictors. In order to compare the effect of predator exposure and BNST GABA chemogenetic inactivation on multiple foraging parameters, all foraging parameters (foraging latency, last nest exit, first nest exit, distance travelled, nest time and nest exits) were normalized on a scale of 0–1 within the foraging parameter and trial (30, 50 and 70 cm). We defined overall foraging performance as the polygon area calculated from the shape of all parameters plotted on a polar plot. Normalization $(((x-\min(x))/(\max(x)-\min(x))=z_{(behavioral,\ trial)})$ by foraging parameter in addition to trial was due to statistical analysis revealing significant differences in baseline foraging as a result of trial. The polygon area for each subject was calculated to fit a linear mixed effects model and run post-hoc significance analysis, as previously described. When applicable, outliers were detected by the Grubbs test and removed from analysis. All data are reported as mean ± standard error of the mean (SEM), and all significant comparisons are indicated by an asterisk in the figures.

3 | RESULTS

3.1 | Baseline foraging behaviour in a novel environment

Throughout the foraging sessions, mice were trained to retreat back to the nesting zone after consuming a food pellet at discrete and incrementally greater distances from the nesting zone (Figure 1a). In the first several sessions, mice did not successfully forage equally across the

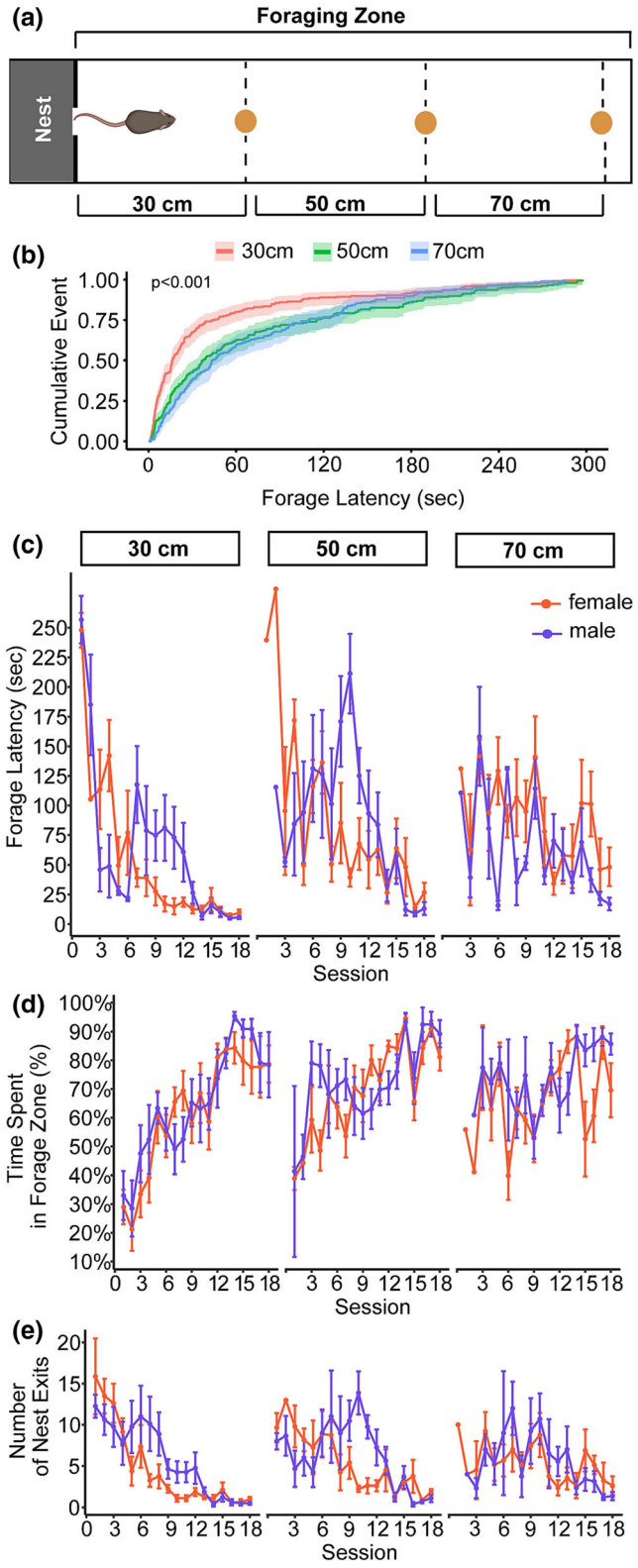

FIGURE 1 Foraging performance of mice in a novel environment. (a) Illustration of the foraging apparatus and placement of the food pellet for each trial in a session. (b) Significant differences in latency to successfully forage by trial, illustrated by Cox-proportional hazards regression model. No significant sex differences in the last four consecutive sessions were observed for (c) foraging latency, (d) time spent in foraging zone and (e) number of nest exits.

distances of the food pellet locations. Compared with the 30 cm trial, mice took significantly longer to successfully forage at the 50 cm (HR = .63; 95% confidence interval [CI] = .53–.77; $p < .001$) and 70 cm (HR = .65; 95% CI = .54–.79; $p < .001$) distances (Figure 1b). For the first 12 consecutive sessions, food consumption latency varied with a session-sex interaction effect ($F(11,326) = 26.9$, $p = .005$). In the last four consecutive sessions, there was neither a significant difference in behaviour due to sex nor a significant interaction with the session or trial (Figure 1c). A main effect of the trial remained on foraging latency, but this effect was likely attributed to the increasing distances the mice must traverse away from the nesting zone to obtain the food pellet (main effect [trial]: $F(2,185) = 16.9$, $p = .0002$). There was a significant difference across the first 12 consecutive foraging sessions for total time spent in the foraging zone (main effect [session]: $F(11,338) = 85.4$, $p < .0001$). This effect is likely due to the mice spending increasingly more time in the foraging zone rather than the nesting zone before procuring and consuming the food pellet. In the last four consecutive sessions, neither a significant sex difference nor a significant interaction with the session or trial was observed with time spent in the foraging zone (Figure 1d). Lastly, there was a significant difference in the number of nest exits across the first 12 consecutive foraging sessions (main effect [session]: $F(11,338) = 72.9$, $p < .0001$). As mice learned to forage over days, the number of nest exits was significantly reduced. In the last four consecutive sessions, there was neither a significant sex difference nor an interaction with the session or trial regarding the number of nest exits (Figure 1e). Thus, by the end of training, male and female mice had learned to traverse the foraging zone to retrieve the food pellets back to the nest.

3.2 | BNST GABA neurons are activated in response to robotic and live predator interaction

We next determined if BNST GABA neurons respond to interactions with an enclosed predator in a novel

environment. To do so, we virally expressed the Cre-dependent calcium sensor GCaMP6m in the BNST of VGaT::Cre female and male mice (Figure 2a). Changes in VGaT neuron GCaMP6m activity were recorded while mice freely explored around a cage that contained either a robotic or live predator (Figure 2b). All mice were exposed to both a robotic and live predator across 2 days, and the order of exposure was counter-balanced. GCaMP6m calcium activity was averaged across interactions for each mouse during robotic predator exposure (Figure 2c) and live predator exposure (Figure 2d). Interactions were determined as the times in which the mouse either had nose-to-cage contact or reared against the predator cage. There was no significant difference in predator interactions between robotic and live predator threat types, $t(6) = .089$, $p > .05$ (Figure 2e). BNST GABA neuron activity was significantly increased from baseline by both robotic and live predator interactions: robotic: $t(6) = -4.468$, $p = .004$; live: $t(6) = -3.882$, $p = .008$ (Figure 2f). There was no difference in GCaMP signalling between robotic and live predator threat interactions ($t(6) = .998$, $p > .05$) or between exposure days ($t(6) = .693$, $p > .05$). These data suggest that a robotic predator and a live predator similarly induce calcium-dependent neural activity of BNST GABA neurons and that there was no effect of first versus second threat exposure (Figure 2g).

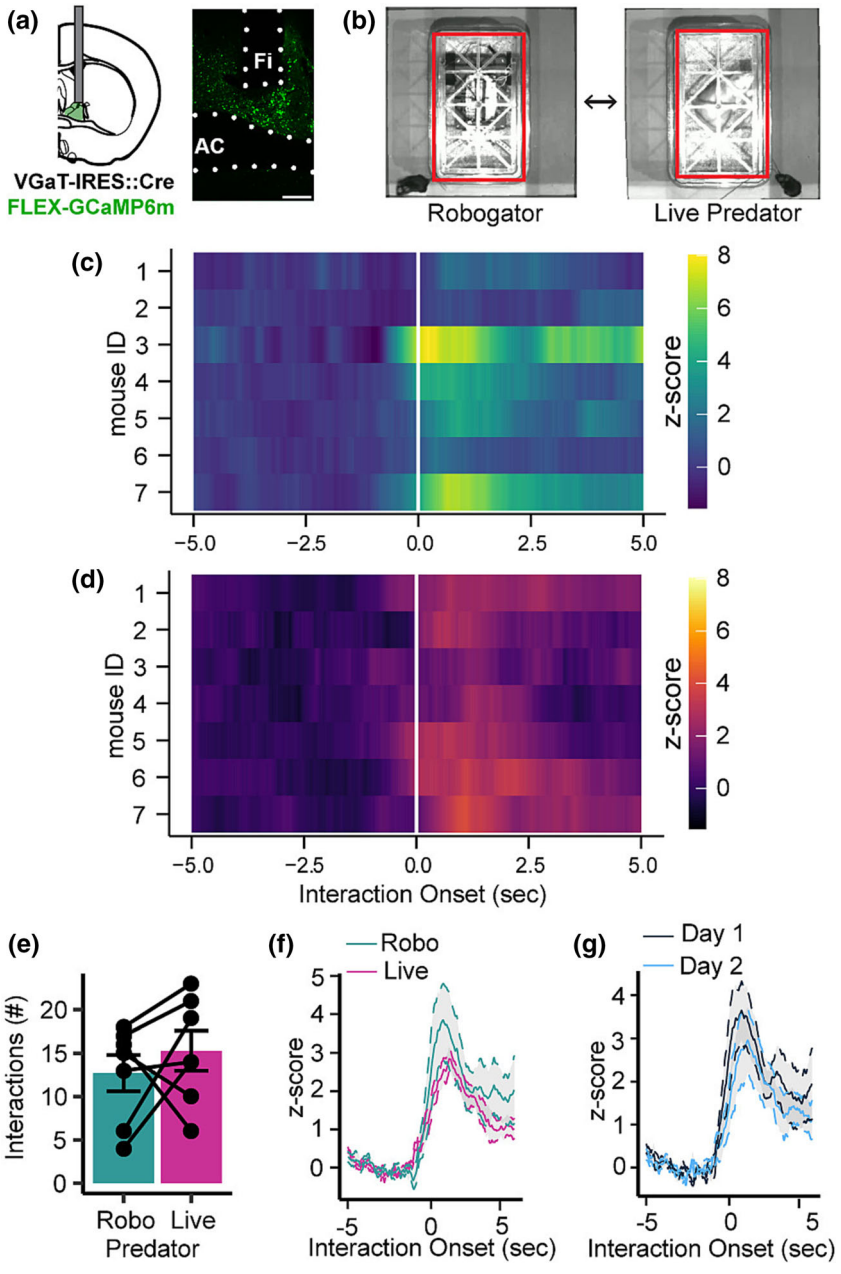

FIGURE 2 Bed nucleus of the stria terminalis (BNST) GABA neural activity in response to interactions with a robotic and live predator. (a) Vesicular GABA transporter-internal ribosome entry site (VGaT-IRES)::Cre mice were injected in BNST with AAV1-hSyn-FLEX-GCaMP6m. Example histology of GCaMP expression. Scale bar = 200 μm. (b) Interaction zones with predator (in red) viewed from above. Heatmap of BNST GABA GCaMP signalling for individual mice in response to interaction with (c) robotic predator and (d) live predator. (e) Averaged total number of prey–predator interactions. Averaged signal trace between (f) predators and (g) days of exposure.

3.3 | Robotic and live predator threats produce similar changes in foraging behaviour, but BNST GABA inhibition during imminent predator threat exposure has no effect on foraging

Prior to testing the role of BNST GABA neurons in the interaction of threat and foraging behaviour, we tested whether hM4D(Gi) was capable of reducing BNST GABA neuronal activity. VGaT-IRES::Cre mice were bilaterally injected in BNST with Cre-dependent adeno-associated viruses encoding the inhibitory designer receptor (hM4D (Gi)) or fluorophore control (GFP) to exclusively transfect BNST GABA neurons (Figure 3a–c). The majority of expression was localized to the anterior subregions of the BNST, although some posterior labelling was also observed. GFP and hM4D(Gi) mice were then intraperitoneally injected with a behaviourally-subthreshold dose of clozapine (.1 mg/kg) (Gomez et al., 2017) to chemogenetically inactivate BNST GABA neurons at least 10 min prior to receiving five footshocks (Figure 3d). Five footshocks were selected as the method for chemogenetic inhibition validation based on prior work showing that aversive stimuli reliably activate BNST neurons (Ventura-Silva et al., 2012; Xu et al., 2012). Mice were euthanized and perfused 90 min later, and virally-labelled BNST neurons were evaluated for c-Fos co-expression. BNST GABA neurons with hM4D(Gi) co-expressed significantly less c-Fos than GFP BNST GABA neurons ($t(6) = -7.45$, $p = .005$) (Figure 3e). Thus, our results indicate that hM4D(Gi) was capable of reducing BNST GABA neuron activity.

After baseline foraging behaviour was achieved, mice were injected with clozapine (.1 mg/kg) to

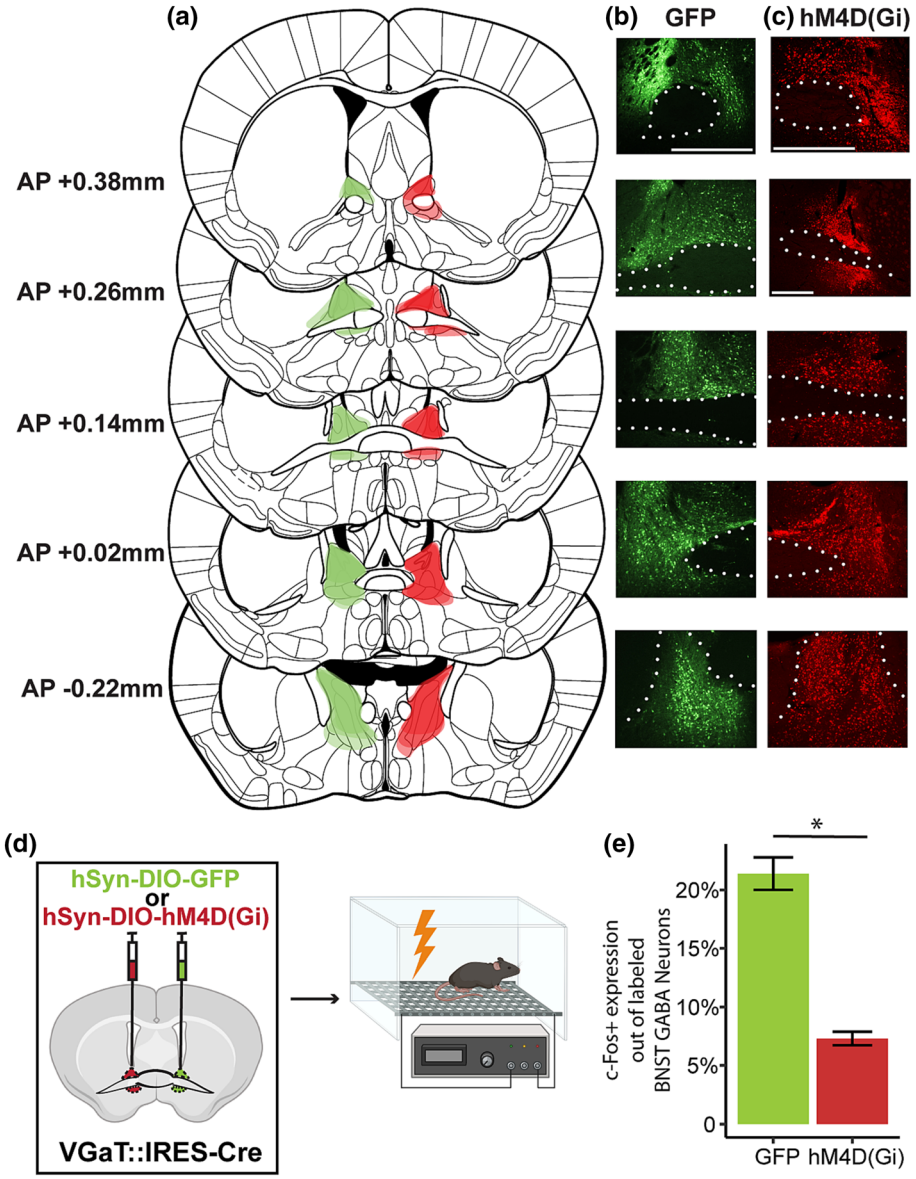

FIGURE 3 Histological verification of chemogenetic expression and targeting. (a) Schematic depicting localization of fluorescence across the bed nucleus of the stria terminalis (BNST). Representative images of each anteroposterior (AP) coordinate for (b) green fluorescent protein (GFP) in green and (c) hM4D(Gi)-mCherry in red. Scale bar = 100 μm. (d) Experimental depiction of chemogenetic verification by five .5 mA footshocks. (e) Significantly fewer hM4D(Gi)-labelled BNST GABA neurons co-expressed with c-Fos than GFP-labelled BNST GABA neurons.

chemogenetically inactivate BNST GABA signalling and challenged to forage under robotic or live predator threat (Figure 4a). Foraging behaviour under a predatory threat changed based on multiple parameters. The latency to successfully forage by consuming the food pellet was also significantly increased under both predator threats (main effect [pre- and circa-encounter]: $F(1,56) = 4.51$, $p = .03$) (Figure 4b). Additionally, mice spent significantly more time in the nest circa-predator exposure (main effect [pre- and circa-encounter]: $F(1,56) = 8.51$, $p = .005$), regardless of whether the predatory threat was live or robotic (Figure 4c,d). Similarly, mice made more exits from the nest during predator threat, but there was no difference between live or robotic threat (main effect [pre- and circa-encounter]: $F(1,56) = 9.36$, $p = .003$) (Figure 4e,f). While robotic and live predator threats similarly changed foraging behaviour, chemogenetic inactivation of BNST GABA signalling failed to change any foraging behaviour during the threat when the predator was present.

3.4 | BNST GABA inhibition does not change foraging behaviour following robotic predator exposure

After predator exposure and chemogenetic inhibition, we examined foraging behaviour 48 h post-encounter in the absence of a threat (Figure 5a). For the robotic predator, across all trials, there was no significant effect of predator encounter or chemogenetic inactivation of BNST GABA neurons on foraging latency (Figure 5b). However, mice spent significantly more time in the nest after the robotic predator, regardless of BNST GABA inactivation (main

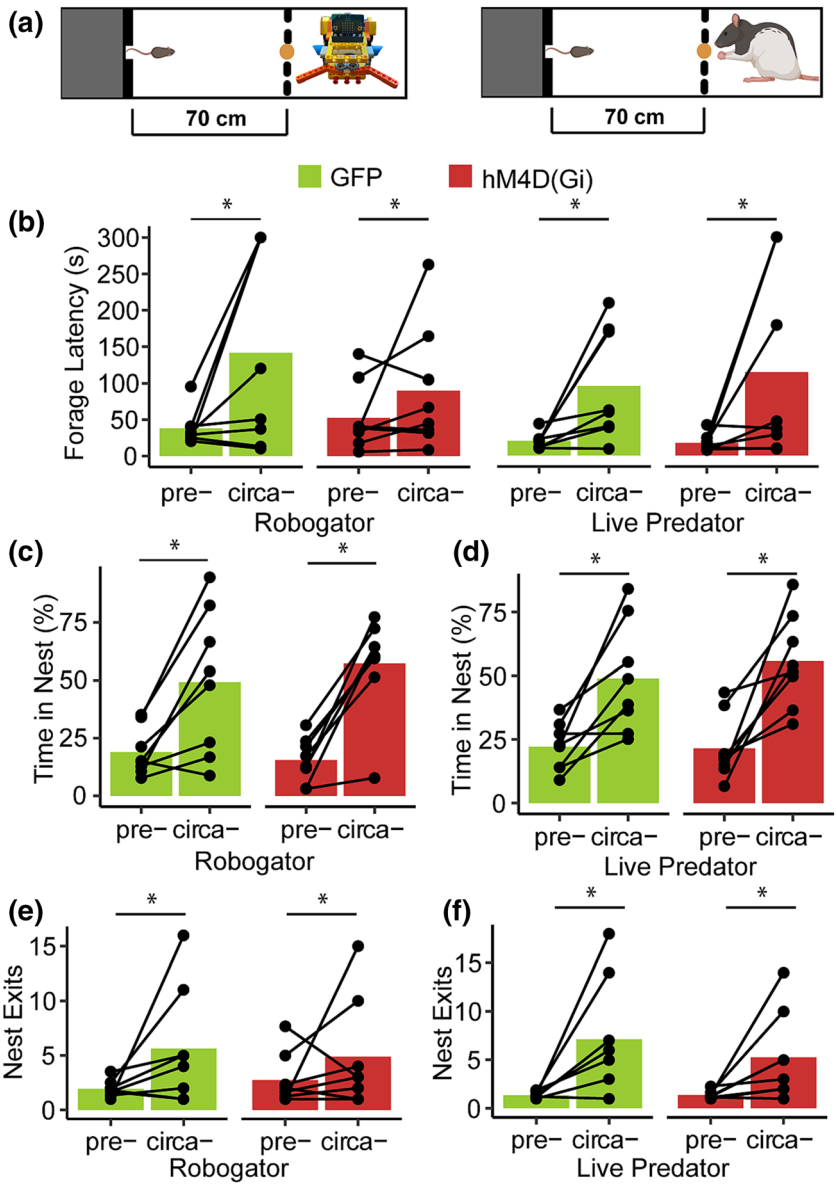

FIGURE 4 Foraging behaviour in the presence of a predator threat with chemogenetic inactivation of BNST GABA signalling. (a) GFP and hM4D(Gi) mice were injected with clozapine and exposed to a predator threat. (b) Foraging latency increased circa- robogator and live predator. Time spent in nest increased circa- (c) robogator and (d) live predator. Nest exits increased circa- (e) robogator and (f) live predator. (g) The first latency to exit the nest increased circa- robogator and live predator, and mice took longer to exit the nest with a live predator present than a robotic predator.

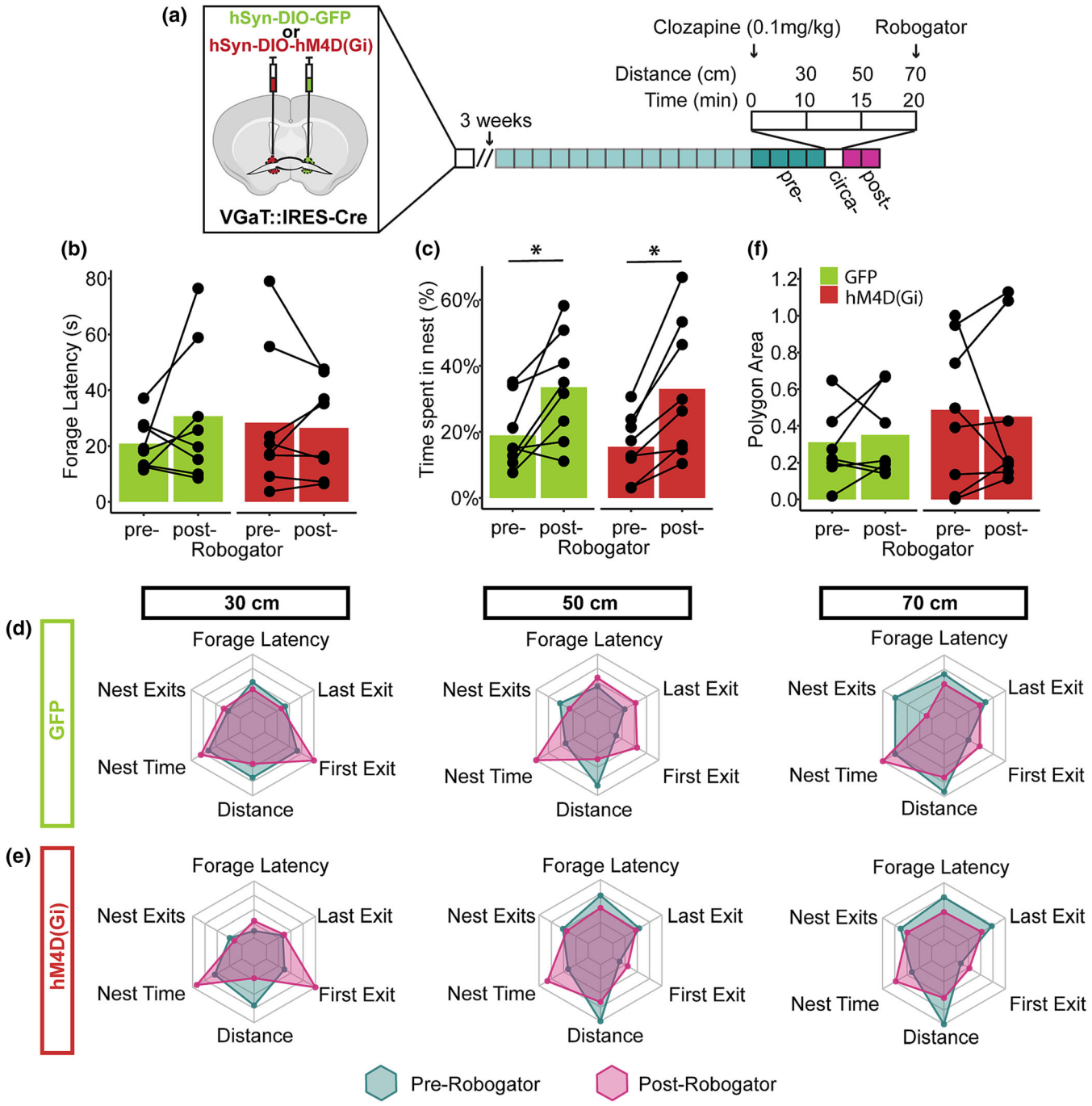

FIGURE 5 Foraging performance post-robogator encounter and chemogenetic inactivation of bed nucleus of the stria terminalis (BNST) GABA signalling. (a) Experimental timeline. (b) No significant differences in foraging latency by treatment or robogator encounter. (c) Mice spend more time in the nest after robogator encounter, regardless of chemogenetic inactivation. Foraging performance by trial before and after robogator exposure for (d) green fluorescent protein (GFP) mice and (e) hM4D(Gi) mice. The maximum axis for each radar plot is equal to the maximum average out of the foraging parameters. (f) No changes in overall foraging performance by treatment or robogator encounter.

effect [encounter]: $F(1,236) = 15.4$, $p < .001$) (Figure 5c). We next examined multiple foraging parameters, such as foraging latency, number of nest exits, time spent in the nest, distance travelled, first nest exit latency and last nest exit latency. Each variable was normalized on a scale of 0–1 by robogator encounter (pre- and post-encounter) and by trial (30, 50 and 70 cm) for GFP and hM4D(Gi) mice (Figure 5d,e). Across all foraging parameters, by calculating the polygon area of each mouse before and after the robotic predator, there was no significant effect of either

the robogator or the chemogenetic inactivation of BNST GABA neurons (Figure 5f). These results indicate that time spent in the nest after an encounter with the robotic predator was the primary behaviour change observed. Additionally, any post-encounter foraging changes were not augmented with BNST GABA inhibition.

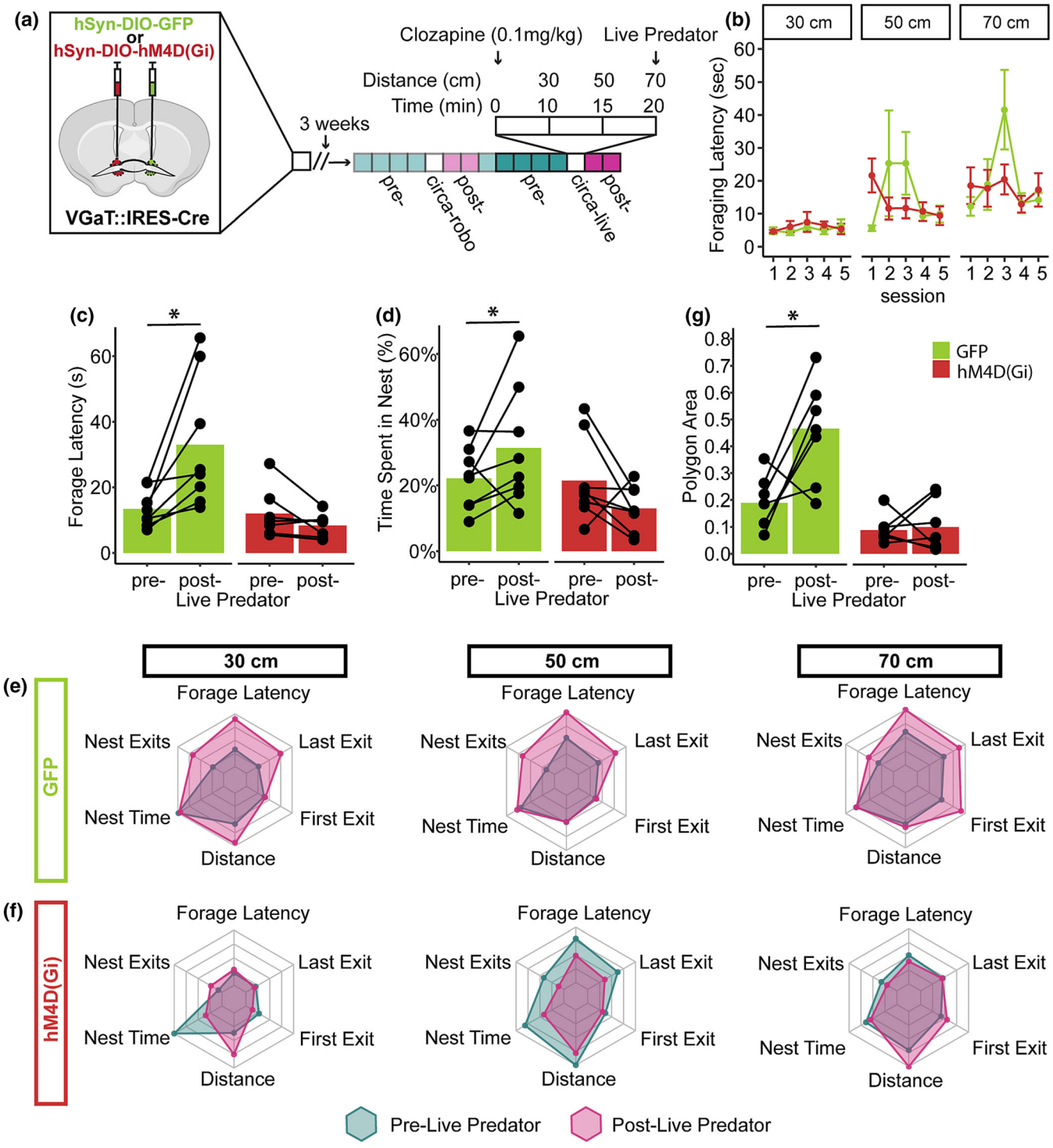

FIGURE 6 Foraging performance post-live predator encounter and chemogenetic inactivation of bed nucleus of the stria terminalis (BNST) GABA signalling. (a) Experimental timeline. (b) No treatment group differences in foraging latency pre-live predator encounter. Significant effect of chemogenetic BNST GABA inactivation on (c) foraging latency and (d) time spent in nest. Foraging performance by trial before and after live predator exposure for (e) green fluorescent protein (GFP) mice and (f) hM4D(Gi) mice. (g) Significant effect of chemogenetic BNST GABA inactivation on overall foraging performance after live predator exposure.

3.5 | BNST GABA inhibition prevents post-live predator changes in foraging

After assessing robogator post-encounter foraging behaviour, mice were re-trained to forage with no group differences in foraging latency before being exposed to a live predator (Figure 6a,b). In contrast to the robogator threat, foraging latency following the live predator threat depended on the interaction between live predator exposure and BNST GABA neuron inactivation (main effect [encounter]: $F(1,334) = 16.9$, $p < .001$; encounter x treatment, $F(1,332) = 5.93$, $p = .01$) (Figure 6c). In the absence of a live predator, but after the experience of encountering one, GFP mice showed significantly increased foraging latency, and this effect was absent in hM4D(Gi) mice (comparisons of estimated marginal means of pre- x GFP versus post- x GFP, $p = .0003$). Time spent in the nest also showed a significant interaction between live predator exposure and BNST GABA neuron inactivation (main effect [encounter]: $F(1,334) = 7.04$, $p = .008$; encounter x treatment, $F(1,332) = 12.9$, $p < .001$) (Figure 6d). GFP mice showed a significant increase in time in the nest post-encounter, and this effect was blocked in hM4D (Gi) mice (comparisons of estimated marginal means of pre- x GFP versus post- x GFP, $p = .04$).

To understand the effect of predator exposure and BNST GABA chemogenetic inactivation on multiple foraging parameters, we normalized selected foraging parameters (foraging latency, last nest exit, first nest exit, distance travelled, nest time and nest exits) on a scale of 0–1 within foraging parameters and trials (30, 50 and 70 cm). We defined overall foraging performance as the polygon area calculated from the shape of all parameters plotted on a polar plot. Across all foraging parameters, we found that live predator exposure had a significant effect on overall foraging performance post-encounter that depended on an interaction between live predator exposure and chemogenetic inactivation of BNST GABA neurons (main effect [encounter]: $F(1,458) = 551.5$, $p < .001$; encounter x treatment, $F(1,456) = 238.5$, $p < .001$) (Figure 6e–g). Post-hoc analysis identified that the foraging performance of GFP mice was significantly different between the pre- and post-live predator encounter, and this effect was not observed in hM4D(Gi) mice (comparisons of estimated marginal means of pre- x GFP versus post- x GFP, $p < .001$). These results suggest that BNST GABA inactivation prevented significant changes that are normally the result of prior live threat experience.

3.6 | Live predator exposure alone induces significant changes in foraging behaviour

The significant changes in foraging behaviour incurred after live predator exposure may have been due to the influence of a prior threat experience with the robotic predator. We hypothesized that live predator exposure without prior robotic predator exposure was sufficient to induce post-encounter changes in foraging behaviour. To test this, mice were trained to forage for several sessions until there was no statistical session difference in foraging latency in the last three sessions within each

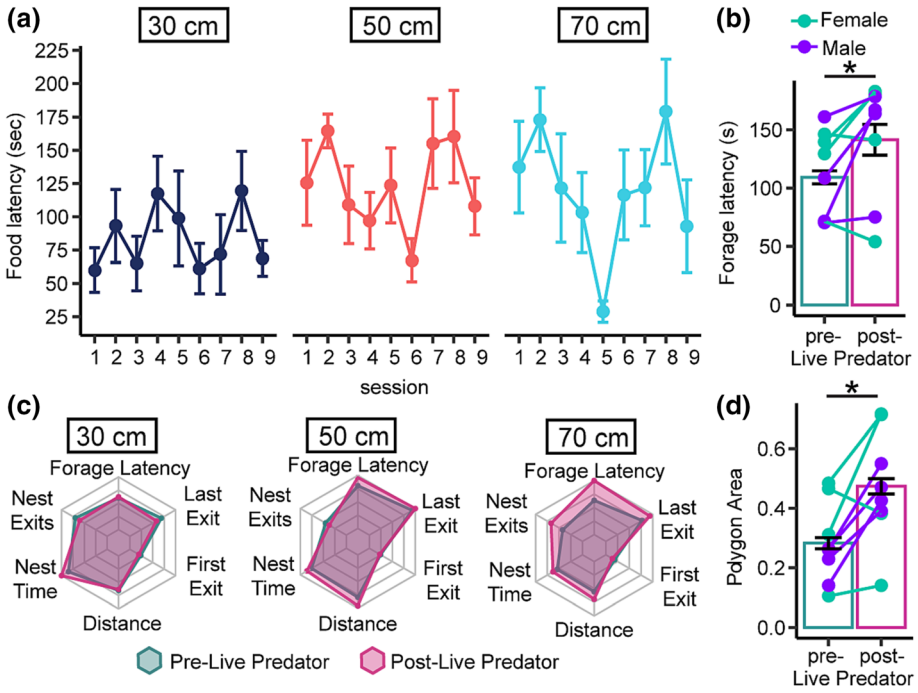

FIGURE 7 Change in foraging behaviour following only live predator experience. (a) Foraging latency across training. (b) Change in foraging latency after live predator exposure. (c) Foraging performance by trial before and after live predator exposure. (d) Significant effect of live predator exposure on overall foraging performance when food is 70 cm away from nest.

trial distance (Figure 7a). Mice were then exposed to the live predator threat when the food was located 70 cm away from the nest. Post-encounter foraging latency was significantly increased after live predator exposure (main effect [encounter]: $F(1,64) = 5.99$, $p = .01$) (Figure 7b). Examining multiple foraging parameters, there were no significant changes in foraging behaviour when the food was located 30 and 50 cm away from the nest (Figure 7c). However, live predator exposure significantly changed foraging performance when the food was located 70 cm away from the nest (main effect [encounter]: $F(1,85) = 153.1$, $p < .001$) (Figure 7d). Together, live predator exposure without prior robotic predator threat experience is sufficient to alter post-encounter foraging behaviour.

3.7 | Locomotor path does not change after predator exposure and BNST GABA inhibition

Confrontation with a predator threat can change the distance travelled and trajectory path of the prey (Choi & Kim, 2010; Kim et al., 2015; Zambetti et al., 2022). We assessed changes in locomotor activity to determine the acute effects of predator exposure and BNST GABA inhibition on foraging. Based on linear modelling of the tracked centre point of the mice, there was no effect of predator exposure on the estimated linear path taken while foraging ($r(118) = 14.3$, $p = .57$). Neither was there a difference between trials ($r(118) = 5.24$, $p = .77$). Overall, there was also no interaction effect of predator exposure and BNST GABA inhibition on locomotor path ($r(118) = -10.6$, $p = .50$) (Figure 8a–d). Similar to robotic predator exposure, there was no effect of live predator exposure ($r(108) = 16.4$, $p = .54$), trial ($r(108) = 8.67$, $p = .66$) or interaction effect of live predator and BNST GABA inhibition ($r(108) = -11.7$, $p = .50$) on locomotion afterwards (Figure 8e–h). In summary, chemogenetic inhibition of BNST GABA neurons did not alter the post-encounter locomotor path for either robotic or live predator experiences.

4 | DISCUSSION

Predation risk not only impacts foraging behaviour in the presence of a predator but also in the immediate

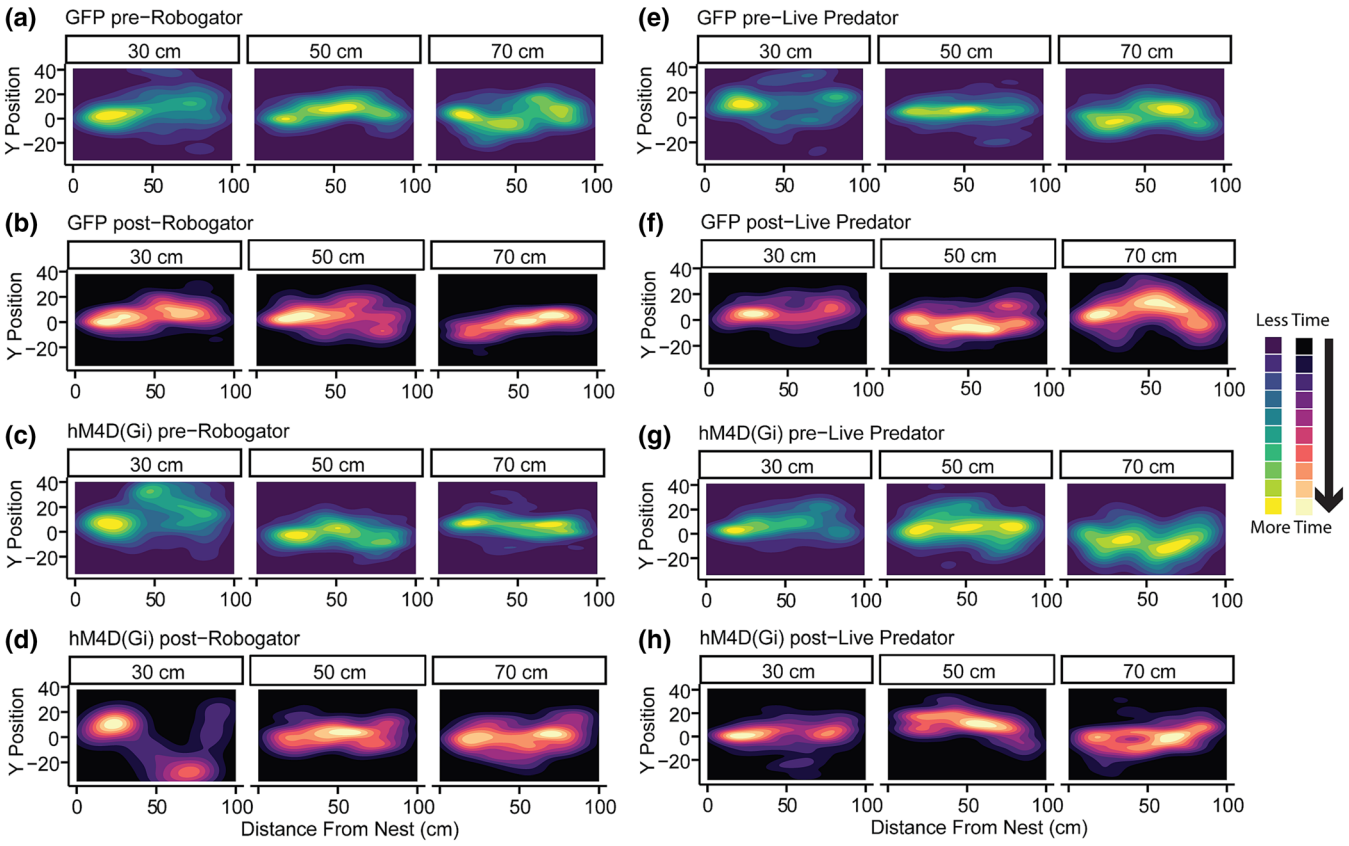

FIGURE 8 Locomotor path patterns before and after predator threat exposure. Averaged linear path taken by green fluorescent protein (GFP) mice (a) before robogator and (b) after robogator. Averaged linear path taken by hM4D(Gi) mice (c) before robogator and (d) after robogator. Averaged linear path taken by GFP mice (e) before live predator and (f) after live predator. Averaged linear path taken by hM4D (Gi) mice (g) before live predator and (h) after live predator.

aftermath of a close encounter. Using a laboratory-based foraging task, we evaluated foraging performance before, during, and after two distinct predator threats, as well as identified the role of BNST GABA neurons in modulating these behaviours. During initial foraging training, mice took significantly longer to procure food at greater distances away from the nest. The increased foraging latency is consistent with an innate avoidance of bright, open spaces in rodents, concomitant with an increasing distance from a dark enclosure (Clarke, 1983; Kotler, 1984; Lima, 1998b; Lockard & Owings, 1974; Price et al., 1984; Wolfe & Tan Summerlin, 1989). Importantly, by the completion of training, mice were able to efficiently navigate and retrieve the food pellet.

Previous research in laboratory rats observed sex differences in foraging strategies involving male rats spending more time in open, novel environments compared with female rats (Jolles et al., 2015; Pellman et al., 2017; Zambetti et al., 2019, 2022). We found no sex differences in mice across foraging success, foraging latency, time spent in the foraging zone and nest exits. While there was a session–sex interaction effect in the first 12 sessions of initial training on foraging latency, there were no interactions or main effects of sex following extensive training.

It is well established in field studies that animals change their foraging behaviour under predation risk (Lima & Dill, 1990; Lima, 1998a, 1998b). Particularly, they will flee when a predator reaches a distance that is perceived as threatening to survival, a concept coined by ethologists as the flight-initiation distance (Fanselow, 2022; Walther, 1969). Fanselow and Lester (1988) articulate the psychological perception of threat in their predator imminence continuum theory. One advantage of robotic predator threat is the ability to programme a reliable predator–prey interaction, thus making the stimulus consistent across subjects (Choi & Kim, 2010). Here, we found that foraging behaviour was significantly disrupted during robotic and live predator threats. Others have revealed similar findings using either a robotic or live predator threat to show interference in successful foraging (Amir et al., 2015; Blanchard & Blanchard, 1989; Kim et al., 2018). Importantly, we found no difference in time spent in the nest, foraging latency, and nest exits between robogator and live predator threats, suggesting that a robotic predator threat is just as effective as a live predator in disrupting ongoing foraging behaviour.

While the BNST has been shown to be involved in unpredictable threat detection (de Araujo Salgado et al., 2023; Hon et al., 2023; Williford et al., 2023), GABAergic neurons, which are the predominant cell type in the BNST (Bota et al., 2012; Siletti et al., 2022; Welch et al., 2019), have yet to be examined during a predator threat. Here, we compared BNST GABA neuronal activity (GCaMP) during predator interactions with a robotic threat and a live threat. BNST GABA GCaMP activity increased during interactions with both the robotic and live predators, and there were no significant differences in signalling between the two predator types. These results suggest that BNST GABA neurons are similarly activated in response to both a robotic and a live predator threat. However, when we chemogenetically inactivated BNST GABA signalling during robotic and live predator threats, we found no effect on foraging behaviour. Thus, BNST GABA neuron signalling does not appear to be involved in foraging behaviour during the circa-strike threat processing of either a robotic or live predator. One reason for the lack of involvement of BNST during a circa-strike threat may be that during a circa-strike, the threat is certain. In contrast, post-encounter foraging in the absence of the threat, where we found a role of BNST GABA neurons following a live predator threat, is a highly uncertain threat context. This result is consistent with prior BNST inactivation experiments that have shown the necessity of the BNST for fear expression in uncertain contexts (Fendt et al., 2003; Goode et al., 2020; Waddell et al., 2006; Walker & Davis, 1997). However, because our viral expression included both dorsal and ventral BNST subregions and that these subregions may support opposing anxiety-related states (Daniel & Rainnie, 2016; Gungor & Pare, 2016), it is possible that the effects observed herein represent a global BNST net effect. Further investigations of BNST subdivisions will be required to determine their roles in foraging performance during the circa-strike and post-encounter stages.

Predator–prey interaction models suggest that even a brief predator exposure after chronic low-risk foraging may result in an overestimated defensive behaviour response, such as hypervigilance (Lima & Bednekoff, 1999). In fact, a brief predator exposure can be followed by a long latency period before animals resume activity levels comparable to before the predator encounter (Sih, 1992). Post-encounter, we found that there was no significant change in foraging latency with the robotic predator threat, but mice spent significantly more time in the nest. Others have reported similar observations after a robotic predator experience; while not exactly measuring time spent in a nest, rats appeared to display far more 'pause-then-retreat' behaviour rather than 'pause-then-approach' after a robotic predator encounter (Walters et al., 2019). Pauses are likely part of a repertoire of defensive behaviours to better assess the uncertain risk of predation (Bednekoff & Lima, 1998; Blanchard & Blanchard, 1989). Spending more time in the nest while foraging latency remained unchanged suggests that the mice were spending more time in a safe

zone to avoid predation risk. However, while the robotic predator threat significantly increased time spent in nests, it did not impact overall foraging performance. It is possible that other measures, such as food intake over time, could be affected, as others have shown (Kim et al., 2014). Chemogenetic inactivation of BNST GABA neurons had no effect on the increased time spent in the nest post-robotic threat encounter.

In contrast to robotic predators, post-live predator encounters resulted in a constellation of changes in foraging behaviour. Following a live predator encounter, control mice showed prolonged foraging latency, increased time spent in the nest, and an overall reduction in foraging performance compared with pre-encounter levels. However, mice with BNST GABA neuron inhibition during live predator exposure showed no change in foraging latency, time spent in the nest, or overall foraging performance post-encounter. Thus, we interpret these results as indicating that inactivation of BNST GABA neurons rescued post-encounter behavioural changes. One limitation to this interpretation is that the live predator threat was a second threat experience that followed the robotic threat experience. In other words, the changes in post-encounter foraging behaviour following a live predator threat may have been influenced by the initial robotic threat experience. However, we found in a separate cohort of mice that post-encounter foraging performance was significantly disrupted by a single live predator experience, indicating prior robotic predator experience is not necessary for post-encounter changes in foraging. Nevertheless, it is still possible that an initial robotic experience had some influence on live threat post-encounter foraging because we observed altered foraging behaviour only at the 70 cm distance following a single live threat experience, while we observed altered foraging at 30, 50 and 70 cm distances with a live threat experience that had previously experienced robotic threat.

When comparing live and robotic predator threats, our results suggest that the robotic threat was effective in disrupting foraging behaviour circa-encounter; however, it was not as salient of a threat experience as a live predator post-encounter. This data indicate that the robotic predator threat may not be sufficient to recruit BNST GABA neurons when the mice were food-seeking under pressure, and thus, BNST GABA neurons were not required for the increased time spent in the nest post-encounter. In contrast, with its greater impact on multiple foraging parameters, a live predator may present an innate threat than a robotic predator, particularly under foraging conditions. Indeed, rat aggression towards mice is observed as predatory behaviour (Horovitz et al., 1966; Ilchibaeva et al., 2017; Tulogdi et al., 2015). In addition, close proximity to a rat elicits a flight response in mice (Deng et al., 2016; Esteban Masferrer et al., 2020; Reis, Lee, et al., 2021; Reis, Liu, et al., 2021; Tobias et al., 2023; Wang, Schuette, La-Vu, et al., 2021; Wang, Schuette, Nagai, et al., 2021). Thus, this innate threat experience required BNST GABA neurons for the consequential behavioural response in post-encounter foraging when the predator was no longer present.

Notably, different subregions of the BNST have opposing effects on anxiety-like behaviour that appear highly dependent on the projection site (Giardino et al., 2018; Jennings et al., 2013; Kim et al., 2013). While anatomically distinct in location, subregions of the BNST largely overlap in their synapses with regions associated with energy homeostasis, such as the lateral hypothalamus and arcuate nucleus (Dong & Swanson, 2003, 2004, 2006a, 2006b). The inactivation of BNST GABA neurons during an innate predator threat while foraging may have disinhibited regions regulating energy homeostasis, such that an animal does not recall the threat post-encounter and proceeds with regular foraging behaviour. This would support research on the role of BNST in contextual fear memory (Goode et al., 2020; Hammack et al., 2015; Luyck et al., 2020; Sullivan et al., 2004; Urien & Bauer, 2022; Williams & Lattal, 2020; Williford et al., 2023). It would also support prior research demonstrating that BNST subdivisions and cell types may have specialized functions in behavioural output and threat processing (Bruzsik, Biro, Zelena, et al., 2021; de Araujo Salgado et al., 2023; Duque-Wilckens et al., 2016; Giardino et al., 2018; Jaramillo et al., 2020; Jennings et al., 2013; Kim et al., 2013; Maita et al., 2023; Mazzone et al., 2018). Further research will be necessary to investigate the participation of BNST in hypothalamic regions in the regulation of defensive responses while foraging.

Additionally, we analysed the locomotor path pre-encounter and post-encounter of robotic and live predators threat to examine the involvement of BNST GABA neurons in locomotor behaviours under threat. We found no effect of predator exposure or influence of BNST GABA neurons on the locomotor path. The lack of BNST GABA neuron influence on locomotor path suggests that BNST GABA signalling plays no role in foraging path after predator threat.

In summary, we found that a live predator threat induced more pronounced changes in post-encounter foraging than a robotic predator threat, and BNST GABA neurons were required in the post-live predator encounter changes in foraging. A parsimonious interpretation of the difference between robotic and live predator experiences in eliciting changes in foraging behaviour may be that not all predator threat experiences are perceived as equal. Consistent with this interpretation, animals respond differently to predator species depending on the

perceived psychological risk (Fanselow & Lester, 1988; Walther, 1969). Given that GABA BNST activation is sufficient to produce anxiety-like behaviour in mice (Jia et al., 2022; Mazzone et al., 2018; Singewald et al., 2003), it is conceivable that BNST GABA neurons are required to induce an anxiety-like phenotype post-live predator threat encounter. It remains to be seen if BNST inactivation during the post-encounter, instead of the circa-strike encounter, would similarly ablate an adaptive defensive response. The observations made here may shed more light on the ethological behaviours of mice that distinguish the multiple components of the predator imminence continuum theory: pre-encounter, circa-strike, and post-encounter, as well as the role of the BNST on discrete characteristics of a predator threat experience.

AUTHOR CONTRIBUTIONS

Annie Ly conceived this project. Annie Ly, Alexandra Barker, Hayden Hotchkiss, Emily D. Prévost and Dillon J. McGovern performed or analysed data. Zachary Kilpatrick and David H. Root contributed critical reagents or analysis. Annie Ly and David H. Root wrote the manuscript with the contributions of all authors.

ACKNOWLEDGMENTS

Some components of figures were created in BioRender.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ejn.16137.

DATA AVAILABILITY STATEMENT

Supporting data are available.

ORCID

David H. Root https://orcid.org/0000-0002-1927-2175

REFERENCES

Amir, A., Lee, S.-C., Headley, D. B., Herzallah, M. M., & Pare, D. (2015). Amygdala signaling during foraging in a hazardous environment. *The Journal of Neuroscience*, *35*, 12994–13005. https://doi.org/10.1523/JNEUROSCI.0407-15.2015

Barker, D. J., Miranda-Barrientos, J., Zhang, S., Root, D. H., Wang, H. L., Liu, B., Calipari, E. S., & Morales, M. (2017). Lateral preoptic control of the lateral habenula through convergent glutamate and GABA transmission. *Cell Reports*, *21*, 1757–1769. https://doi.org/10.1016/j.celrep.2017.10.066

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Bednekoff, P. A., & Lima, S. L. (1998). Randomness, chaos and confusion in the study of antipredator vigilance. *Trends in Ecology & Evolution*, *13*, 284–287. https://doi.org/10.1016/S0169-5347(98)01327-5

Blanchard, R. J., & Blanchard, D. C. (1989). Antipredator defensive behaviors in a visible burrow system. *Journal of Comparative Psychology*, *103*, 70–82. https://doi.org/10.1037/0735-7036.103.1.70

Bota, M., Sporns, O., & Swanson, L. W. (2012). Neuroinformatics analysis of molecular expression patterns and neuron populations in gray matter regions: The rat BST as a rich exemplar. *Brain Research*, *1450*, 174–193. https://doi.org/10.1016/j.brainres.2012.02.034

Bruzsik, B., Biro, L., Sarosdi, K. R., Zelena, D., Sipos, E., Szebik, H., Török, B., Mikics, E., & Toth, M. (2021). Neurochemically distinct populations of the bed nucleus of stria terminalis modulate innate fear response to weak threat evoked by predator odor stimuli. *Neurobiol Stress*, *15*, 100415. https://doi.org/10.1016/j.ynstr.2021.100415

Bruzsik, B., Biro, L., Zelena, D., Sipos, E., Szebik, H., Sarosdi, K. R., Horvath, O., Farkas, I., Csillag, V., Finszter, C. K., Mikics, E., & Toth, M. (2021). Somatostatin neurons of the bed nucleus of stria terminalis enhance associative fear memory consolidation in mice. *The Journal of Neuroscience*, *41*, 1982–1995. https://doi.org/10.1523/JNEUROSCI.1944-20.2020

Choi, J.-S., & Kim, J. J. (2010). Amygdala regulates risk of predation in rats foraging in a dynamic fear environment. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 21773–21777. https://doi.org/10.1073/pnas.1010079108

Clarke, J. A. (1983). Moonlight's influence on predator/prey interactions between short-eared owls (Asio flammeus) and deermice (Peromyscus maniculatus). *Behavioral Ecology and Sociobiology*, *13*, 205–209. https://doi.org/10.1007/BF00299924

Daniel, S. E., & Rainnie, D. G. (2016). Stress modulation of opposing circuits in the bed nucleus of the stria terminalis. *Neuropsychopharmacology*, *41*, 103–125. https://doi.org/10.1038/npp.2015.178

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: Role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology*, *35*, 105–135. https://doi.org/10.1038/npp.2009.109

de Araujo Salgado, I., Li, C., Burnett, C. J., Rodriguez Gonzalez, S., Becker, J. J., Horvath, A., Earnest, T., Kravitz, A. V., & Krashes, M. J. (2023). Toggling between food-seeking and self-preservation behaviors via hypothalamic response networks. *Neuron*. https://doi.org/10.1016/j.neuron.2023.06.006

Deng, H., Xiao, X., & Wang, Z. (2016). Periaqueductal gray neuronal activities underlie different aspects of defensive behaviors. *The Journal of Neuroscience*, *36*, 7580–7588. https://doi.org/10.1523/JNEUROSCI.4425-15.2016

Dong, H.-W., & Swanson, L. W. (2003). Projections from the rhomboid nucleus of the bed nuclei of the stria terminalis: Implications for cerebral hemisphere regulation of ingestive behaviors. *Journal of Comparative Neurology*, *463*, 434–472. https://doi.org/10.1002/cne.10758

Dong, H.-W., & Swanson, L. W. (2004). Organization of axonal projections from the anterolateral area of the bed nuclei of the stria terminalis. *Journal of Comparative Neurology*, *468*, 277–298. https://doi.org/10.1002/cne.10949

Dong, H.-W., & Swanson, L. W. (2006a). Projections from bed nuclei of the stria terminalis, anteromedial area: Cerebral

hemisphere integration of neuroendocrine, autonomic, and behavioral aspects of energy balance. *The Journal of Comparative Neurology*, *494*, 142–178. https://doi.org/10.1002/cne.20788

Dong, H.-W., & Swanson, L. W. (2006b). Projections from bed nuclei of the stria terminalis, dorsomedial nucleus: Implications for cerebral hemisphere integration of neuroendocrine, autonomic, and drinking responses. *Journal of Comparative Neurology*, *494*, 75–107. https://doi.org/10.1002/cne.20790

Duque-Wilckens, N., Steinman, M. Q., Laredo, S. A., Hao, R., Perkeybile, A. M., Bales, K. L., & Trainor, B. C. (2016). Inhibition of vasopressin V1a receptors in the medioventral bed nucleus of the stria terminalis has sex- and context-specific anxiogenic effects. *Neuropharmacology*, *110*, 59–68. https://doi.org/10.1016/j.neuropharm.2016.07.018

Duvarci, S., Bauer, E. P., & Paré, D. (2009). The bed nucleus of the stria terminalis mediates inter-individual variations in anxiety and fear. *The Journal of Neuroscience*, *29*, 10357–10361. https://doi.org/10.1523/JNEUROSCI.2119-09.2009

Esteban Masferrer, M., Silva, B. A., Nomoto, K., Lima, S. Q., & Gross, C. T. (2020). Differential encoding of predator fear in the ventromedial hypothalamus and periaqueductal grey. *The Journal of Neuroscience*, *40*, 9283–9292. https://doi.org/10.1523/JNEUROSCI.0761-18.2020

Fanselow, M. S. (2022). Negative valence systems: Sustained threat and the predatory imminence continuum. *Emerging Topics in Life Sciences*, *6*, 467–477. https://doi.org/10.1042/ETLS20220003

Fanselow, M. S., & Lester, L. S. (1988). *A functional behavioristic approach to aversively motivated behavior: Predatory imminence as a determinant of the topography of defensive behavior evolution and learning* (pp. 185–212). Lawrence Erlbaum Associates, Inc.

Fanselow, M. S., Lester, L. S., & Helmstetter, F. J. (1988). Changes in feeding and foraging patterns as an antipredator defensive strategy: A laboratory simulation using aversive stimulation in a closed economy. *Journal of the Experimental Analysis of Behavior*, *50*, 361–374. https://doi.org/10.1901/jeab.1988.50-361

Fendt, M., Endres, T., & Apfelbach, R. (2003). Temporary inactivation of the bed nucleus of the stria terminalis but not of the amygdala blocks freezing induced by trimethylthiazoline, a component of fox feces. *The Journal of Neuroscience*, *23*, 23–28. https://doi.org/10.1523/JNEUROSCI.23-01-00023.2003

Giardino, W. J., Eban-Rothschild, A., Christoffel, D. J., Li, S.-B., Malenka, R. C., & de Lecea, L. (2018). Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. *Nature Neuroscience*, *21*, 1084–1095. https://doi.org/10.1038/s41593-018-0198-x

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., Ellis, R. J., Richie, C. T., Harvey, B. K., Dannals, R. F., Pomper, M. G., Bonci, A., & Michaelides, M. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science*, *357*, 503–507. https://doi.org/10.1126/science.aan2475

Goode, T. D., Acca, G. M., & Maren, S. (2020). Threat imminence dictates the role of the bed nucleus of the stria terminalis in contextual fear. *Neurobiology of Learning and Memory*, *167*, 107116. https://doi.org/10.1016/j.nlm.2019.107116

Goode, T. D., Ressler, R. L., Acca, G. M., Miles, O. W., & Maren, S. (2019). Bed nucleus of the stria terminalis regulates fear to unpredictable threat signals. *eLife*, *8*, e46525. https://doi.org/10.7554/eLife.46525

Gungor, N. Z., & Pare, D. (2016). Functional heterogeneity in the bed nucleus of the stria terminalis. *The Journal of Neuroscience*, *36*, 8038–8049. https://doi.org/10.1523/JNEUROSCI.0856-16.2016

Hammack, S. E., Todd, T. P., Kocho-Schellenberg, M., & Bouton, M. E. (2015). Role of the bed nucleus of the stria terminalis in the acquisition of contextual fear at long or short context-shock intervals. *Behavioral Neuroscience*, *129*, 673–678. https://doi.org/10.1037/bne0000088

Hon, O. J., Flanigan, M. E., Roland, A. V., Caira, C. M., Sides, T., D'Ambrosio, S., Lee, S., Simpson, Y., Buccini, M., Machinski, S., Yu, W., Boyt, K. M., & Kash, T. L. (2023). Corticotropin releasing factor in the bed nucleus of the stria terminalis modulates the behavioral consequences of unpredictable threat. *bioRxiv*, 2023.2004.2010.535848.

Horovitz, Z. P., Piala, J. J., High, J. P., Burke, J. C., & Leaf, R. C. (1966). Effects of drugs on the mouse-killing (muricide) test and its relationship to amygdaloid function. *International Journal of Neuropharmacology*, *5*, 405–407. https://doi.org/10.1016/0028-3908(66)90005-0

Hull, C. L. (1943). *Principles of behavior: An Introduction to behavior theory*. Appleton-Century-Crofts.

Ilchibaeva, T. V., Tsybko, A. S., Kozhemyakina, R. V., Konoshenko, M. Y., Popova, N. K., & Naumenko, V. S. (2017). The relationship between different types of genetically defined aggressive behavior. *Journal of Ethology*, *35*, 75–81. https://doi.org/10.1007/s10164-016-0493-5

Iwanir, S., Brown, A. S., Nagy, S., Najjar, D., Kazakov, A., Lee, K. S., Zaslaver, A., Levine, E., & Biron, D. (2016). Serotonin promotes exploitation in complex environments by accelerating decision-making. *BMC Biology*, *14*, 9. https://doi.org/10.1186/s12915-016-0232-y

Jais, A., Paeger, L., Sotelo-Hitschfeld, T., Bremser, S., Prinzensteiner, M., Klemm, P., Mykytiuk, V., Widdershooven, P. J. M., Vesting, A. J., Grzelka, K., Minère, M., Cremer, A. L., Xu, J., Korotkova, T., Lowell, B. B., Zeilhofer, H. U., Backes, H., Fenselau, H., Wunderlich, F. T., … Brüning, J. C. (2020). PNOC (ARC) neurons promote hyperphagia and obesity upon high-fat-diet feeding. *Neuron*, *106*, 1009–1025.e1010. https://doi.org/10.1016/j.neuron.2020.03.022

Jaramillo, A. A., Williford, K. M., Marshall, C., Winder, D. G., & Centanni, S. W. (2020). BNST transient activity associates with approach behavior in a stressful environment and is modulated by the parabrachial nucleus. *Neurobiology of Stress*, *13*, 100247. https://doi.org/10.1016/j.ynstr.2020.100247

Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., Kash, T. L., & Stuber, G. D. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature*, *496*, 224–228. https://doi.org/10.1038/nature12041

Jia, X., Chen, S., Li, X., Tao, S., Lai, J., Liu, H., Huang, K., Tian, Y., Wei, P., Yang, F., Lu, Z., Chen, Z., Liu, X.-A., Xu, F., & Wang, L. (2022). Divergent neurocircuitry dissociates two components of the stress response: Glucose mobilization and anxiety-like behavior. *Cell Reports*, *41*, 111586. https://doi.org/10.1016/j.celrep.2022.111586

Jolles, J. W., Boogert, N. J., & van den Bos, R. (2015). Sex differences in risk-taking and associative learning in rats. *Royal Society Open Science*, *2*, 150485. https://doi.org/10.1098/rsos.150485

Kim, E., Kim, E. J., Yeh, R., Shin, M., Bobman, J., Krasne, F. B., & Kim, J. J. (2014). Amygdaloid and non-amygdaloid fear both influence avoidance of risky foraging in hungry rats. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20133357. https://doi.org/10.1098/rspb.2013.3357

Kim, E. J., Kong, M.-S., Park, S. G., Mizumori, S. J. Y., Cho, J., & Kim, J. J. (2018). Dynamic coding of predatory information between the prelimbic cortex and lateral amygdala in foraging rats. *Science Advances*, *4*, eaar7328. https://doi.org/10.1126/sciadv.aar7328

Kim, E. J., Park, M., Kong, M.-S., Park, S. G., Cho, J., & Kim, J. J. (2015). Alterations of hippocampal place cells in foraging rats facing a "predatory" threat. *Current Biology*, *25*, 1362–1367. https://doi.org/10.1016/j.cub.2015.03.048

Kim, S.-Y., Adhikari, A., Lee, S. Y., Marshel, J. H., Kim, C. K., Mallory, C. S., Lo, M., Pak, S., Mattis, J., Lim, B. K., Malenka, R. C., Warden, M. R., Neve, R., Tye, K. M., & Deisseroth, K. (2013). Diverging neural pathways assemble a behavioural state from separable features in anxiety. *Nature*, *496*, 219–223. https://doi.org/10.1038/nature12018

Kocho-Schellenberg, M., Lezak, K. R., Harris, O. M., Roelke, E., Gick, N., Choi, I., Edwards, S., Wasserman, E., Toufexis, D. J., Braas, K. M., May, V., & Hammack, S. E. (2014). PACAP in the BNST produces anorexia and weight loss in male and female rats. *Neuropsychopharmacology*, *39*, 1614–1623. https://doi.org/10.1038/npp.2014.8

Kotler, B. P. (1984). Risk of predation and the structure of desert rodent communities. *Ecology*, *65*, 689–701. https://doi.org/10.2307/1938041

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1–26. https://doi.org/10.18637/jss.v082.i13

Lenth, R. V. (2022). emmeans: Estimated marginal means, aka least-squares means. R package.

Lima, S. L. (1985). Maximizing feeding efficiency and minimizing time exposed to predators: A trade-off in the black-capped chickadee. *Oecologia*, *66*, 60–67. https://doi.org/10.1007/BF00378552

Lima, S. L. (1998a). Nonlethal effects in the ecology of predator-prey interactions: What are the ecological effects of anti-predator decision-making? *Bioscience*, *48*, 25–34. https://doi.org/10.2307/1313225

Lima, S. L. (1998b). Stress and decision making under the risk of predation: Recent developments from behavioral, reproductive, and ecological perspectives. In A. P. Møller, M. Milinski, & P. J. B. Slater (Eds.), *Advances in the study of behavior* (pp. 215–290). Academic Press.

Lima, S. L., & Bednekoff, P. A. (1999). Temporal variation in danger drives antipredator behavior: The predation risk allocation hypothesis. *The American Naturalist*, *153*, 649–659. https://doi.org/10.1086/303202

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology*, *68*, 619–640. https://doi.org/10.1139/z90-092

Lockard, R. B., & Owings, D. H. (1974). Moon-related surface activity of bannertail (Dipodomys spectabilis) and fresno (D. Nitratoides) kangaroo rats. *Animal Behaviour*, *22*, 262–273. https://doi.org/10.1016/S0003-3472(74)80078-3

Luyck, K., Arckens, L., Nuttin, B., & Luyten, L. (2020). It takes two: Bilateral bed nuclei of the stria terminalis mediate the expression of contextual fear, but not of moderate cued fear. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *101*, 109920. https://doi.org/10.1016/j.pnpbp.2020.109920

Maita, I., Bazer, A., Chae, K., Parida, A., Mirza, M., Sucher, J., Phan, M., Liu, T., Hu, P., Soni, R., Roepke, T. A., & Samuels, B. A. (2023). Chemogenetic activation of corticotropin-releasing factor-expressing neurons in the anterior bed nucleus of the stria terminalis reduces effortful motivation behaviors. *Neuropsychopharmacology*. https://doi.org/10.1038/s41386-023-01646-9

Mazzone, C. M., Pati, D., Michaelides, M., DiBerto, J., Fox, J. H., Tipton, G., Anderson, C., Duffy, K., McKlveen, J. M., Hardaway, J. A., Magness, S. T., Falls, W. A., Hammack, S. E., McElligott, Z. A., Hurd, Y. L., & Kash, T. L. (2018). Acute engagement of Gq-mediated signaling in the bed nucleus of the stria terminalis induces anxiety-like behavior. *Molecular Psychiatry*, *23*, 143–153. https://doi.org/10.1038/mp.2016.218

McGovern, D. J., Ly, A., Ecton, K. L., Huynh, D. T., Prévost, E. D., Gonzalez, S. C., McNulty, C. J., Rau, A. R., Hentges, S. T., Daigle, T. L., Tasic, B., Baratta, M. V., & Root, D. H. (2022). Ventral tegmental area glutamate neurons mediate nonassociative consequences of stress. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-022-01858-3

McGovern, D. J., Polter, A. M., & Root, D. H. (2021). Neurochemical signaling of reward and aversion to ventral tegmental area glutamate neurons. *The Journal of Neuroscience*, *41*, 5471–5486. https://doi.org/10.1523/JNEUROSCI.1419-20.2021

Mobbs, D., Hassabis, D., Yu, R., Chu, C., Rushworth, M., Boorman, E., & Dalgleish, T. (2013). Foraging under competition: The neural basis of input-matching in humans. *Journal of Neuroscience*, *33*, 9866–9872. https://doi.org/10.1523/JNEUROSCI.2238-12.2013

Pellman, B. A., Schuessler, B. P., Tellakat, M., & Kim, J. J. (2017). Sexually dimorphic risk mitigation strategies in rats. *Eneuro*, *4*(1), ENEURO.0288-0216.2017. https://doi.org/10.1523/ENEURO.0288-16.2017

Price, M. V., Waser, N. M., & Bass, T. A. (1984). Effects of moonlight on microhabitat use by desert rodents. *Journal of Mammalogy*, *65*, 353–356. https://doi.org/10.2307/1381183

Reis, F. M. C. V., Lee, J. Y., Maesta-Pereira, S., Schuette, P. J., Chakerian, M., Liu, J., La-Vu, M. Q., Tobias, B. C., Ikebara, J. M., Kihara, A. H., Canteras, N. S., Kao, J. C., & Adhikari, A. (2021). Dorsal periaqueductal gray ensembles represent approach and avoidance states. *eLife*, *10*, e64934. https://doi.org/10.7554/eLife.64934

Reis, F. M. C. V., Liu, J., Schuette, P. J., Lee, J. Y., Maesta-Pereira, S., Chakerian, M., Wang, W., Canteras, N. S., Kao, J. C., & Adhikari, A. (2021). Shared dorsal periaqueductal gray activation patterns during exposure to innate and conditioned threats. *The Journal of Neuroscience*, *41*, 5399–5420. https://doi.org/10.1523/JNEUROSCI.2450-20.2021

Roman, C. W., Lezak, K. R., Kocho-Schellenberg, M., Garret, M. A., Braas, K., May, V., & Hammack, S. E. (2012). Excitotoxic lesions of the bed nucleus of the stria terminalis (BNST) attenuate the effects of repeated stress on weight gain: Evidence for the recruitment of BNST activity by repeated, but not acute,

stress. *Behavioural Brain Research*, *227*, 300–304. https://doi.org/10.1016/j.bbr.2011.11.010

Sih, A. (1992). Prey uncertainty and the balancing of antipredator and feeding needs. *The American Naturalist*, *139*, 1052–1069. https://doi.org/10.1086/285372

Siletti, K., Hodge, R., Mossi Albiach, A., Hu, L., Lee, K. W., Lönnerberg, P., Bakken, T., Ding, S.-L., Clark, M., Casper, T., Dee, N., Gloe, J., Keene, C. D., Nyhus, J., Tung, H., Yanny, A. M., Arenas, E., Lein, E. S., & Linnarsson, S. (2022). Transcriptomic diversity of cell types across the adult human brain. *bioRxiv*, 2022.2010.2012.511898.

Silston, B., Wise, T., Qi, S., Sui, X., Dayan, P., & Mobbs, D. (2021). Neural encoding of perceived patch value during competitive and hazardous virtual foraging. *Nature Communications*, *12*, 5478. https://doi.org/10.1038/s41467-021-25816-9

Singewald, N., Salchner, P., & Sharp, T. (2003). Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. *Biological Psychiatry*, *53*, 275–283. https://doi.org/10.1016/S0006-3223(02)01574-3

Stacher Hörndli, C. N., Wong, E., Ferris, E., Bennett, K., Steinwand, S., Rhodes, A. N., Fletcher, P. T., & Gregg, C. (2019). Complex economic behavior patterns are constructed from finite, genetically controlled modules of behavior. *Cell Reports*, *28*, 1814–1829.e1816. https://doi.org/10.1016/j.celrep.2019.07.038

St-Cyr, S., Abuaish, S., Spinieli, R. L., & McGowan, P. O. (2018). Maternal predator odor exposure in mice programs adult offspring social behavior and increases stress-induced behaviors in semi-naturalistic and commonly-used laboratory tasks. *Frontiers in Behavioral Neuroscience*, *12*, 136. https://doi.org/10.3389/fnbeh.2018.00136

Stephens, D. W., Brown, J. S., & Ydenberg, R. C. (2007). *Foraging: Behavior and ecology*. University of Chicago Press. https://doi.org/10.7208/chicago/9780226772653.001.0001

Stephens, D. W., & Krebs, J. R. (1986). *Foraging theory*. Princeton University Press.

Sullivan, G. M., Apergis, J., Bush, D. E., Johnson, L. R., Hou, M., & Ledoux, J. E. (2004). Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific cue-conditioned fear stimulus. *Neuroscience*, *128*, 7–14. https://doi.org/10.1016/j.neuroscience.2004.06.015

Therneau, T. M. (2022). *A package for survival analysis in R*. R Package.

Tobias, B. C., Schuette, P. J., Maesta-Pereira, S., Torossian, A., Wang, W., Sethi, E., & Adhikari, A. (2023). Characterization of ventromedial hypothalamus activity during exposure to innate and conditioned threats. *European Journal of Neuroscience*, *57*, 1053–1067. https://doi.org/10.1111/ejn.15937

Troxell-Smith, S. M., Tutka, M. J., Albergo, J. M., Balu, D., Brown, J. S., & Leonard, J. P. (2016). Foraging decisions in wild versus domestic Mus musculus: What does life in the lab select for? *Behavioural Processes*, *122*, 43–50. https://doi.org/10.1016/j.beproc.2015.10.020

Tulogdi, A., Biro, L., Barsvari, B., Stankovic, M., Haller, J., & Toth, M. (2015). Neural mechanisms of predatory aggression in rats-implications for abnormal intraspecific aggression. *Behavioural Brain Research*, *283*, 108–115. https://doi.org/10.1016/j.bbr.2015.01.030

Urien, L., & Bauer, E. P. (2022). Sex differences in BNST and amygdala activation by contextual, cued, and unpredictable threats. *Eneuro*, *9*(1), ENEURO.0233-0221.2021. https://doi.org/10.1523/ENEURO.0233-21.2021

Ventura-Silva, A. P., Pêgo, J. M., Sousa, J. C., Marques, A. R., Rodrigues, A. J., Marques, F., Cerqueira, J. J., Almeida, O. F., & Sousa, N. (2012). Stress shifts the response of the bed nucleus of the stria terminalis to an anxiogenic mode. *The European Journal of Neuroscience*, *36*, 3396–3406. https://doi.org/10.1111/j.1460-9568.2012.08262.x

Waddell, J., Morris, R. W., & Bouton, M. E. (2006). Effects of bed nucleus of the stria terminalis lesions on conditioned anxiety: Aversive conditioning with long-duration conditional stimuli and reinstatement of extinguished fear. *Behavioral Neuroscience*, *120*, 324–336. https://doi.org/10.1037/0735-7044.120.2.324

Walker, D. L., & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *The Journal of Neuroscience*, *17*, 9375–9383. https://doi.org/10.1523/JNEUROSCI.17-23-09375.1997

Walters, C. J., Jubran, J., Sheehan, A., Erickson, M. T., & Redish, A. D. (2019). Avoid-approach conflict behaviors differentially affected by anxiolytics: Implications for a computational model of risky decision-making. *Psychopharmacology*, *236*, 2513–2525. https://doi.org/10.1007/s00213-019-05197-0

Walther, F. R. (1969). Flight behaviour and avoidance of predators in Thomson's gazelle (Gazella Thomsoni Guenther 1884). *Behaviour*, *34*, 184–220. https://doi.org/10.1163/156853969X00053

Wang, W., Schuette, P. J., La-Vu, M. Q., Torossian, A., Tobias, B. C., Ceko, M., Kragel, P. A., Reis, F. M. C. V., Ji, S., Sehgal, M., Maesta-Pereira, S., Chakerian, M., Silva, A. J., Canteras, N. S., Wager, T., Kao, J. C., & Adhikari, A. (2021). Dorsal premammillary projection to periaqueductal gray controls escape vigor from innate and conditioned threats. *eLife*, *10*, e69178. https://doi.org/10.7554/eLife.69178

Wang, W., Schuette, P. J., Nagai, J., Tobias, B. C., Cuccovia, V., Reis, F. M., Ji, S., de Lima, M. A. X., La-Vu, M. Q., Maesta-Pereira, S., Chakerian, M., Leonard, S. J., Lin, L., Severino, A. L., Cahill, C. M., Canteras, N. S., Khakh, B. S., Kao, J. C., & Adhikari, A. (2021). Coordination of escape and spatial navigation circuits orchestrates versatile flight from threats. *Neuron*, *109*, 1848–1860.e1848. https://doi.org/10.1016/j.neuron.2021.03.033

Welch, J. D., Kozareva, V., Ferreira, A., Vanderburg, C., Martin, C., & Macosko, E. Z. (2019). Single-cell multi-omic integration compares and contrasts features of brain cell identity. *Cell*, *177*, 1873–1887.e1817. https://doi.org/10.1016/j.cell.2019.05.006

Williams, A. R., & Lattal, K. M. (2020). Involvement of the bed nucleus of the stria terminalis in initial conditioning and rapid reconditioning following extinction of contextual fear. *Behavioral Neuroscience*, *134*, 177–186. https://doi.org/10.1037/bne0000358

Williford, K. M., Taylor, A., Melchior, J. R., Yoon, H. J., Sale, E., Negasi, M. D., Adank, D. N., Brown, J. A., Bedenbaugh, M. N., Luchsinger, J. R., Centanni, S. W., Patel, S., Calipari, E. S.,

Simerly, R. B., & Winder, D. G. (2023). BNST PKCδ neurons are activated by specific aversive conditions to promote anxiety-like behavior. *Neuropsychopharmacology*, *48*, 1031–1041. https://doi.org/10.1038/s41386-023-01569-5

Wolfe, J. L., & Tan Summerlin, C. (1989). The influence of lunar light on nocturnal activity of the old-field mouse. *Animal Behaviour*, *37*, 410–414. https://doi.org/10.1016/0003-3472(89)90088-2

Xu, H. Y., Liu, Y. J., Xu, M. Y., Zhang, Y. H., Zhang, J. X., & Wu, Y. J. (2012). Inactivation of the bed nucleus of the stria terminalis suppresses the innate fear responses of rats induced by the odor of cat urine. *Neuroscience*, *221*, 21–27. https://doi.org/10.1016/j.neuroscience.2012.06.056

Yarkoni, T. (2022). The generalizability crisis. *Behavioral and Brain Sciences*, *45*, e1. https://doi.org/10.1017/S0140525X20001685

Yu, Z., Guindani, M., Grieco, S. F., Chen, L., Holmes, T. C., & Xu, X. (2022). Beyond t test and ANOVA: Applications of mixed-effects models for more rigorous statistical analysis in neuroscience research. *Neuron*, *110*, 21–35. https://doi.org/10.1016/j.neuron.2021.10.030

Zambetti, P. R., Schuessler, B. P., & Kim, J. J. (2019). Sex differences in foraging rats to naturalistic aerial predator stimuli. *iScience*, *16*, 442–452. https://doi.org/10.1016/j.isci.2019.06.011

Zambetti, P. R., Schuessler, B. P., Lecamp, B. E., Shin, A., Kim, E. J., & Kim, J. J. (2022). Ecological analysis of Pavlovian fear conditioning in rats. *Communications Biology*, *5*, 830. https://doi.org/10.1038/s42003-022-03802-1

How to cite this article: Ly, A., Barker, A., Hotchkiss, H., Prévost, E. D., McGovern, D. J., Kilpatrick, Z., & Root, D. H. (2023). Bed nucleus of the stria terminalis GABA neurons are necessary for changes in foraging behaviour following an innate threat. *European Journal of Neuroscience*, *58*(7), 3630–3649. https://doi.org/10.1111/ejn.16137