

Influence of Rat Central Thalamic Neurons on Foraging Behavior in a Hazardous Environment

Mohammad M. Herzallah,^{1,2} Alon Amir,¹ and Denis Paré¹

¹Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, New Jersey 07102, and ²Palestinian Neuroscience Initiative, Al-Quds University, Jerusalem, Palestine 20002

Foraging entails a complex balance between approach and avoidance alongside sensorimotor and homeostatic processes under the control of multiple cortical and subcortical areas. Recently, it has become clear that several thalamic nuclei located near the midline regulate motivated behaviors. However, one midline thalamic nucleus that projects to key nodes in the foraging network, the central medial thalamic nucleus (CMT), has received little attention so far. Therefore, the present study examined CMT contributions to foraging behavior using inactivation and unit recording techniques in male rats. Inactivation of CMT or the basolateral amygdala (BLA) with muscimol abolished the normally cautious behavior of rats in the foraging task. Moreover, CMT neurons showed large but heterogeneous activity changes during the foraging task, with many neurons decreasing or increasing their discharge rates, with a modest bias for the latter. A generalized linear model revealed that the nature (inhibitory vs excitatory) and relative magnitude of the activity modulations seen in CMT neurons differed markedly from those of principal BLA cells but were very similar to those of fast-spiking BLA interneurons. Together, these findings suggest that CMT is an important regulator of foraging behavior. In the Discussion, we consider how CMT is integrated into the network of structures that regulate foraging.

Key words: amygdala; anxiety; central medial; fear; foraging; thalamus

Significance Statement

Foraging entails a complex balance between approach and avoidance alongside sensorimotor and homeostatic processes under the control of multiple cortical and subcortical areas. Although the central medial thalamic nucleus (CMT) is connected to many nodes in this network, its role in the regulation of foraging behavior has not been investigated so far. Here, we examined CMT contributions to foraging behavior using inactivation and unit recording techniques. We found that CMT inactivation abolishes the normally cautious foraging behavior of rats and that CMT neurons show large but heterogeneous changes in firing rates during the foraging task. Together, these results suggest that CMT is an important regulator of foraging behavior.

Introduction

In the wild, rodents face a perpetual dilemma: to stay in sheltered areas where resources are scarce or to explore open areas where they might find needed resources but also risk being detected by a predator. Biasing this life-and-death calculus are various factors such as current energetic needs, experience with the environment, and the palatability of potential resources. Thus, foraging presents a rich context to study interactions between approach and avoidance as well as sensorimotor and homeostatic processes (Stephens, 2008; Mobbs et al., 2013; Mobbs et al., 2018).

In keeping with the multiple interacting functional systems recruited during foraging, various cortical (Rushworth and Behrens, 2008; Addicott et al., 2017; Barack and Platt, 2017; Kim et al., 2018; Scholl and Klein-Flügge, 2018) and subcortical sites (Choi and Kim, 2010; Canteras et al., 2012; Amir et al., 2015) regulate foraging behavior (Swanson, 1988). Among the latter, rapidly accumulating evidence implicates thalamic nuclei located near the midline [mediodorsal, central lateral, paraventricular (PVT)] in the regulation of motivated behaviors (Bradfield et al., 2013; Do-Monte et al., 2016; Bradfield and Balleine, 2017; Choi and McNally, 2017; Cover and Mathur, 2021; Mair et al., 2021; Petrovich, 2021). By contrast, another midline thalamic nucleus, the central medial thalamic nucleus (CMT) has received little attention so far, although it projects to many regions implicated in the regulation of motivated behaviors like the medial prefrontal cortex, nucleus accumbens, and, especially, the basolateral amygdala (BLA; Vertes et al., 2012, 2015; Amir et al., 2019a).

Indeed, the BLA appears to exert a particularly profound influence on foraging behavior. For instance, in a seminaturalistic

Received Mar. 8, 2022; revised Apr. 25, 2022; accepted May 2, 2022.

Author contributions: M.M.H., A.A., and D.P. designed research; M.M.H. performed research; M.M.H., A.A., and D.P. analyzed data; M.M.H., A.A., and D.P. wrote the paper.

This work was supported by National Institutes of Health Grant R01-MH-107239 to D.P.

The authors declare no competing financial interests.

Correspondence should be sent to Denis Paré at pare@rutgers.edu.

<https://doi.org/10.1523/JNEUROSCI.0461-22.2022>

Copyright © 2022 the authors

foraging task where rats are confronted with a mechanical predator when they venture out of their nest to forage, rats normally show extremely cautious behaviors, including prolonged periods of hesitation at the edge of their nest and rapid escape when charged by the predator (Choi and Kim, 2010; Amir et al., 2015). Following BLA inactivation, rats quickly leave their nest and seem indifferent to the predator. Unit recordings in the same task revealed that most principal BLA neurons show reduced firing rates when rats initiate foraging and become nearly silent close to the predator (Amir et al., 2015, 2019b). Yet, when rats hesitate at the edge of their nest, BLA cells fire at higher rates if rats eventually abort rather than start foraging or in the presence versus absence of the predator (Amir et al., 2015, 2019b). Together, these results paint a complex picture where the amygdala could represent a hub in a larger network that regulates foraging behavior.

The present study aimed to further our understanding of the network that regulates foraging behavior by studying CMT contributions with inactivation and unit recording techniques. Comparing the results of our CMT and prior BLA unit recordings in the same task (Amir et al., 2015) leads us to consider possible network mechanisms for the role of CMT in the regulation of foraging behavior.

Materials and Methods

To test the hypothesis that CMT regulates behavior in the foraging task, we performed two sets of experiments. In the first, we compared the effects of inactivating BLA, CMT, and neighboring thalamic sites on foraging behavior. In the second, we recorded CMT unit activity while rats performed the foraging task. These two experiments are described below. All procedures were approved by the Institutional Animal Care and Use Committee of Rutgers University, in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Subjects

We used Sprague Dawley rats (Charles River; male; initial body weight, 250–275 g) housed individually and with *ad libitum* access to food and water. Rats were kept on a 12 h light/dark cycle (lights off at 7:00 P.M.), and experiments were conducted during the light phase of the cycle. During the period devoted to the foraging task, rats had restricted access to food to ensure proper motivation. During this period, their body weight was maintained at 90% of normal age-matched values. A total of 29 rats was used for the inactivation experiments and 7 were used for the electrophysiological experiments.

Behavioral experiments

The protocol of these experiments adhered closely to that used by Choi and Kim (2010), who first studied the effect of BLA inactivation in the foraging task. Using the same protocol provided us with a point of comparison for the effects of BLA and CMT inactivation.

Surgery

Figure 1A summarizes the timeline of the behavioral experiments. Under aseptic conditions and deep isoflurane anesthesia, rats were placed in a stereotaxic apparatus with nonpuncture ear bars (Kopf). Rats received atropine sulfate (0.05 mg/kg, i.m.) to facilitate breathing, and their scalp was injected with a local anesthetic (bupivacaine, 0.1–0.3 ml of a 0.125% solution, s.c.) in the area to be incised. Ten minutes later, we made a 0.5 cm scalp incision and a craniotomy above the brain region of interest. Under stereotaxic guidance, rats were implanted with guide cannulae [outer diameter (o.d.), 0.48 mm; inner diameter (i.d.), 0.32 mm; World Precision Instruments] aimed at CMT (only one cannula) or BLA (one cannula per hemisphere). The cannulae were attached to the skull with dental cement and anchoring screws. They contained a dummy cannula to prevent blocking. At the conclusion of the surgery, rats were administered an analgesic with a long half-life (ketoprofen,

2 mg/kg, s.c., daily for 3 d). They were allowed a 7 d recovery period during which they were habituated to handling daily. We used the following stereotaxic coordinates (in mm relative to bregma): BLA: anteroposterior (AP), -2.4 ; mediolateral (ML), ± 5.00 ; dorsoventral (DV), -8.00 ; angle from midsagittal line, 0° ; and CMT: AP, -2.28 ; ML, 0.4 ; DV, -7.40 ; lateromedial angle, 20° .

Foraging apparatus

The foraging apparatus (Fig. 1B1) was rectangular in shape and made of black polycarbonate. It had walls 60 cm high and was divided in two compartments, both 60 cm wide. The first compartment was a small, dimly lit nest (length, 30 cm; luminance, 10 lux) with a water bottle. The second was an elongated and brightly lit foraging arena (length, 245 cm; luminance, 200 lux). The two compartments were divided by a sliding door.

Behavioral procedures

Habituation (days 1–2). Rats were first habituated to the nesting area (3.5 h/d for 2 d). During these sessions, the gateway to the nesting area was closed and rats could consume up to 6 g of food.

Foraging without the predator (days 3–9). After habituation to the nest, rats underwent 7 consecutive days of foraging training without the predator. After 60–90 s in the nest (no food), the door was opened and the rat was allowed to explore the foraging arena and search for a food pellet. On the first trial, the food pellet was placed 25 cm from the nest. After each successful trial, the distance was increased in steps of 25 cm up to 75 cm. Upon successful retrieval of the food pellet and reentry into the nest, the door was closed. 1 min after the animal finished consuming the pellet, the gateway was reopened and another trial began. Each day, rats were required to complete at least three successful trials.

Foraging with predator (days 10–11). During the last 2 d of the experiment, on each trial, we placed a robotic predator at the end of the foraging arena opposite to the nest, but facing it. The predator (Mindstorms, LEGO Systems) was 14 cm tall, 17 cm wide, and 34 cm long. Each time rats approached within ~ 25 cm of the food pellet, the predator surged forward (80 cm at 60 cm/s), snapped its jaw repeatedly (approximately nine times), and returned to its original position. On the first trial of each experimental day, a food pellet was placed 75 cm from the nest. If the rat successfully retrieved the pellet, the distance was increased in steps of 25 cm until the rat failed. When the rats failed at 75 cm, the distance was decreased in steps of 25 cm until the rat retrieved the pellet. If the rat failed at 25 cm, we decreased the distance to 12.5 cm. Trials were separated by 1–2 min intervals, during which rats were in the nest with the door closed.

Drug infusions

On days 10–11, we performed infusions (0.1 μ l/hemisphere) of artificial CSF (aCSF; pH ~ 7.4) or fluorescent muscimol (0.9 nmol/hemisphere) in BLA (bilaterally) or CMT (only one infusion). The dummy cannula was removed. Muscimol was dissolved in aCSF and obtained from Sigma-Aldrich. The composition of the aCSF was as follows (in mM): 126 NaCl, 2.5 KCl, 1 MgCl_2 , 26 NaHCO_3 , 1.25 NaH_2PO_4 , 2 CaCl_2 , and 10 glucose, at pH 7.4 and 300 mOsm. The solution was backloaded in infusion cannulae (o.d., 0.3 mm; i.d., 0.16 mm) using polyethylene tubing connected to 10 μ l microsyringes and was infused at a rate of 0.1 μ l/min using a microdialysis pump (model CMA402, Harvard Apparatus). The infusion cannulae remained in place for ~ 60 s after the infusion. For inserting the infusion cannulae into the guide cannulae, rats were habituated to gentle restraint for 10–15 min daily for 3–5 d before the first infusion. At the end of this habituation period, rats could sit quietly on the investigator's lap and groom for 10–12 min with minimal restraint. Vehicle or muscimol infusions were performed on different days, 24 h apart. To minimize the potentially confounding influence of habituation to the predator, the order of the infusions was counterbalanced in each group.

Analysis of behavior

Behavior was recorded by an overhead video camera at a frame rate of 29.97 Hz. A MATLAB script determined the position and velocity of the

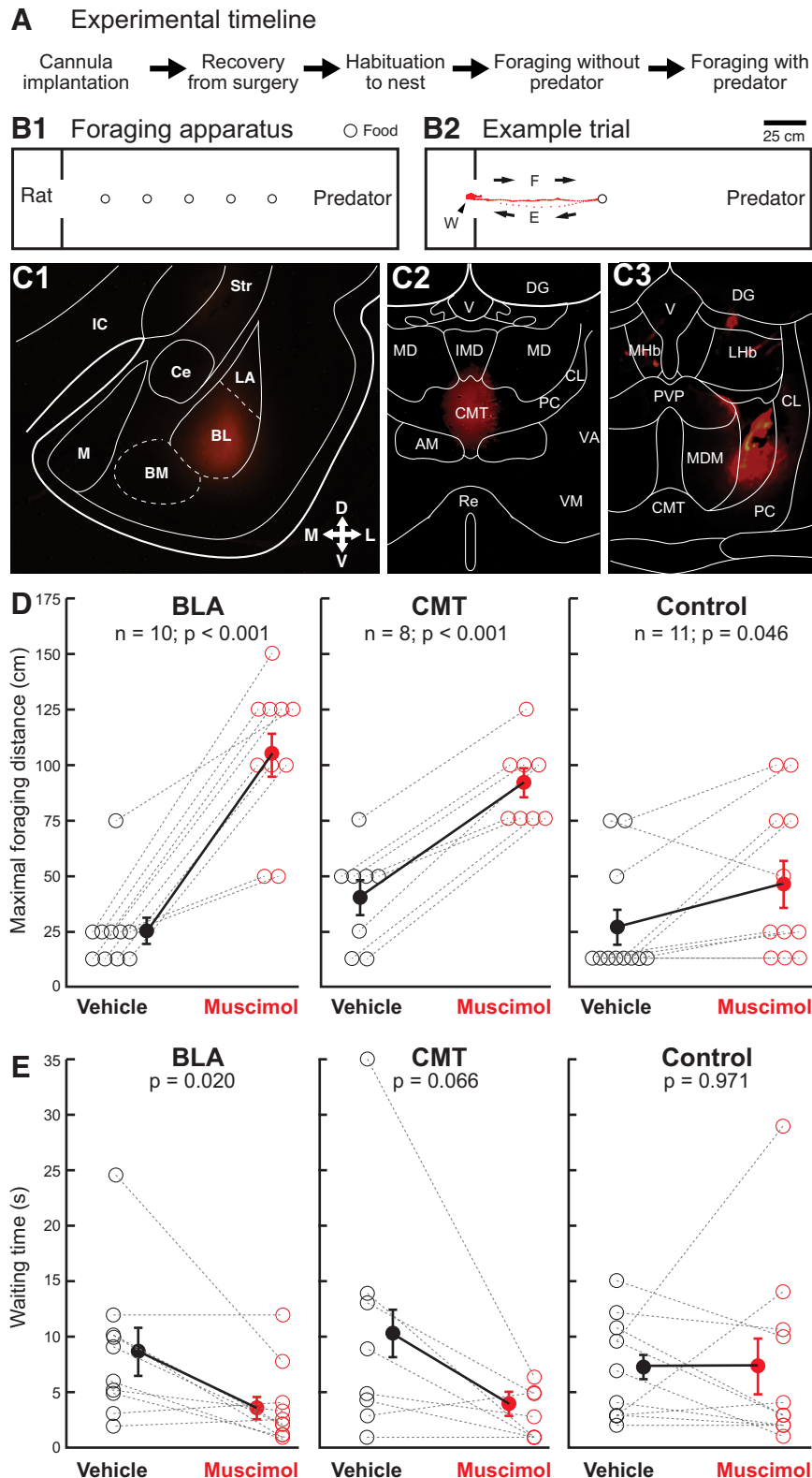


Figure 1. Effect of CMT inactivation on foraging behavior. **A**, Experimental timeline. **B1**, Top view of the behavioral apparatus, which was composed of a small, dimly lit nesting area (left) and a brightly lit and much longer foraging arena (right). The two compartments were separated by a sliding door whose opening marked the start of a trial. On each trial, one food pellet was placed at various distances (circles) from the nest. **B2**, Example trial. Red dots indicate rat position from the waiting phase (W), through the foraging (F) and escape (E) phases. The distance between dots is proportional to the speed of the rat. **C1–C3**, Examples of fluorescent muscimol infusion sites in the BLA (**C1**), CMT (**C2**), and a control site (MD; **C3**). **D, E**, Maximal foraging distance (**D**) or waiting time (**E**) when vehicle (black) or muscimol (red) was infused in the BLA (left), CMT (middle), or control sites (right). Empty symbols, Individual subjects; filled symbols, group averages $\pm$ SEM. Relative to bregma, the

rats by taking advantage of the shifting distribution of light intensity across frames. We analyzed the video files frame-by-frame to identify when the behaviors of interest occurred, including when rats started waiting at the door threshold, and when they started foraging, retrieved a food pellet, escaped, and retreated into the nest (Fig. 1B2). The start of the waiting behavior was defined as when rats extended their snout beyond the door into the foraging arena, and its end as the last frame of stillness before moving entirely out of the nest (including body and tail). The start of foraging behavior was defined as the last frame of waiting stillness before moving entirely out of the nest (including body and tail) and its end as when rats, after approaching the food pellet, started escaping. The start of the escape phase was defined as when rats, after approaching the food pellet, abruptly turned their head (and eventually their entire body) toward the nest and ran back to the nest. This behavior was observed whether the predator was present or not and whether the trial was successful or not. Nest reentry was defined as when rats, after escaping, moved entirely (body and tail) into the nest. Last, on successful trials, we also measured the retrieval interval, defined as the time between the end of the waiting phase and reentry into the nest with the food pellet.

Histology

One day after the completion of the behavioral tests, rats received a bilateral infusion of fluorescent muscimol, as described above. Two hours later, they were overdosed with isoflurane and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Their brains were then stored in 4% paraformaldehyde overnight. A tissue block containing the region of interest was then sectioned in the coronal plane using a vibrating microtome (80 μ m sections). The sections were mounted on gelatin-coated slides, air dried for 2 d in obscurity, coverslipped with DPX Mountant (Sigma-Aldrich), and examined with a fluorescence microscope (model Eclipse E800, Nikon).

Electrophysiological experiments

Surgical procedures

Surgical techniques were identical to those described above with the following exceptions. Under stereotaxic guidance, we aimed silicon probes (NeuroNexus) to CMT. Silicon probes consisted of four shanks (intershank

anteroposterior coordinate in **C1** is -2.4 mm, and in **C2** it is -2.3 mm (Paxinos and Watson, 2007). AM, Anteromedial thalamic nucleus; BM, basomedial nucleus of the amygdala; Ce, central nucleus of the amygdala; CL, centrolateral thalamic nucleus; DG, dentate gyrus; IC, internal capsule; IMD, intermedio-dorsal thalamic nucleus; LHb, lateral habenula; M, medial nucleus of the amygdala; MD, mediodorsal thalamic nucleus; MDM, mediodorsal thalamic nucleus (medial part); MHb, medial habenula; PC, paracentral thalamic nucleus; PVP, paraventricular thalamic nucleus (posterior part); Re, nucleus reuniens; Str, striatum; VA, ventral anterior thalamic nucleus; VM, ventromedial thalamic nucleus.

distance, 200 μm), each with eight recording leads (deinsulated area, 144 μm^2) separated by $\sim 20\ \mu\text{m}$ dorsoventrally. They were attached to Buzsáki-style microdrives (Vandecasteele et al., 2012), such that they could be lowered between recording sessions. Rats were allowed 2–3 weeks to recover from the surgery.

Foraging task

The foraging apparatus was identical to that used in the first experiment, as were the training stages and behavioral scoring procedures. However, after rats were trained on the foraging task in the absence of the predator (days 3–4), on each recording day (days 5–7), we conducted alternating trial blocks with ($n = 10$ –20) or without ($n = 10$ –15) the predator, for a total of 100–120 trials/d.

Histology

At the conclusions of the experiments, while under deep isoflurane anesthesia, electrolytic marking lesions were made on the most dorsal or ventral recording leads, alternating between shanks (10 μA for 16 s), so that lesions marking different shanks could be distinguished. Rats were then perfused-fixed transcardially, and their brains were extracted and sectioned. Sections were then counterstained with a 1% thionine solution. All neurons included in this study were histologically determined to have been recorded in CMT.

Electrophysiological procedures

The data were sampled at 25 kHz and stored on a hard drive. The data were high-pass filtered (cutoff, 300 Hz), followed by a median filter (window, 1.1 ms). To extract action potentials, a threshold was applied to the filtered data. Using principal component analysis on the spike waveforms, the first three components were clustered using KlustaKwik (<http://klustakwik.sourceforge.net/>), and the resulting clusters of spikes were improved using Klusters (Hazan et al., 2006). To separate the clusters, we computed autocorrelograms and cross-correlograms for potential merging or additional splitting. For units to be considered for further analyses, autocorrelograms had to exhibit a refractory period of ≥ 2 ms. Cross-correlograms with a refractory period were merged as this implied that the same neuron was shared between clusters. Cells with unstable action potential waveforms were excluded.

Plotting firing rates as a function of normalized time

Because of trial-to-trial variations in duration, when pooling together data from different trials, firing rates were plotted as a function of normalized time. To do so, a fixed number of time bins was assigned to each task phase (baseline, 20; waiting, 20; foraging, 10; escape, 5), and the firing rates assigned to each bin were determined using linear interpolation. Variations in the duration of each phase determined the range of values that bins could assume. For baseline, bin values ranged between ~ 1 and 2 s. For waiting, bin values ranged between ~ 0.8 and 2.8 s. For foraging, bin values ranged between ~ 0.2 and 1.7 s. For escape, bin values ranged between ~ 0.4 and 2.5 s.

General linear model

We fit the spiking activity of individual units using a regularized regression, group least-absolute shrinkage and selection operator (Lasso) with Poisson distribution (grpreg version 3.2–0 R package; Breheny and Huang, 2015), identical to the generalized linear model (GLM) used by Amir et al. (2019b). The GRPREG R package we used for the GLM implements the k-fold cross-validation algorithm. Data are divided equally into the specified number of folds (seven in our case). One of the data folds is reserved for testing, while the rest of the data folds are used for training. The cross-validation algorithm repeats this process until all data folds have been used for testing.

Spiking was time binned (66.7 ms bins, which corresponds to two video frames). Trials included the following four task phases: door opening when rats were in the nest, waiting at the door, foraging, and escape. Task variables considered in the GLM were door opening, waiting, foraging, escape, food retrieval, predator activation, nest reentry, speed, position, distance from food, trial type (without or with predator), and prior trial outcome (failure or success). These variables were coded differently in the GLM depending on whether they were task phases

(waiting, foraging, escape, nest reentry), trial characteristics (presence or absence of predator; success or failure on prior trial), punctual events (door opening, predator activation, food retrieval), or features that varied continuously during the trials (speed, distance from food, position). Task phases and trial characteristics assumed a value of 1 for the duration of the variable and otherwise 0. Punctual events were marked as 1 when they occurred. Features that varied continuously during the trials assumed a range of values, as determined by the behavior of the rats. Variables were either coded as dummy (waiting, foraging, escape, trial type, prior trial) or convolved with a group of 14 basis functions defined by log time-scaled raised cosine bumps (door opening, food retrieval, predator activation, nest reentry, speed, position, distance from food). The simplified formula that describes the full GLM model is as follows:

$$\text{FR} \sim \text{Waiting} + \text{Foraging} + \text{Escape} + \text{Door Opening}$$

$$+ \text{Food Retrieval} + \text{Predator Activation} + \text{Nest Reentry}$$

$$+ \text{Speed} + \text{Position} + \text{Distance to Food} + \text{Trial Type} + \text{Prior Trial},$$

where FR is firing rate. The full details of the Lasso GLM model development, validation, and assessment of interaction terms are presented in detail in studies by Amir et al. (2019b) and Kyriazi et al. (2018).

Statistical analyses

Data are stated as the average $\pm$ SEM. In the recording experiments, only cells with stable discharge rates and spike waveforms were considered in the analyses. Statistical tests were two tailed. Different procedures were used to assess statistical significance, depending on the data type, as detailed below.

Behavior

In the inactivation experiment, we used a mixed-model ANOVA followed by *post hoc* paired-samples *t* tests to examine the effect of infusion site (BLA, CMT, control) as a between-subject variable, and treatment (vehicle, muscimol) as a within-subject variable. In the unit recording experiments, to compare the incidence of a behavior in two conditions, we computed χ^2 tests for independence with a threshold α value of 0.05. To compare the duration of a behavior in two conditions, we computed a Student's *t* test.

Task-related changes in firing rates

To determine whether individual neurons showed statistically significant task-related variations in firing rates, we computed Kruskal–Wallis one-way ANOVAs and applied a Bonferroni correction of α for the number of neurons considered (0.05/716, or 0.000014). For those cells with a significant ANOVA, we then computed Tukey–Kramer *post hoc* tests with a threshold α of 0.05. To assess the influence of the predator (presence/absence) and prior trial outcome (success/failure) on firing rates, we computed a Friedman test followed by *post hoc* Wilcoxon tests with a Bonferroni-corrected α for the number of comparisons.

GLM normalized peak firing rate modulations

The absolute peak modulation associated with each variable was normalized to baseline firing rates according to this equation: $(\text{Peak} - \text{Baseline})/(\text{Peak} + \text{Baseline})$. We kept the sign of the normalized peak to distinguish cells inhibited or excited by each variable. Normalized peak modulations with values ≤ 0.001 were set to zero because considered noninformative. In the Results section, for each cell sample, we report their average absolute modulation as well as cumulative excitatory and inhibitory modulations (both normalized for baseline firing rates). To compute the average absolute modulation, we summed the absolute value of all the normalized modulations and divided the total by the number of cells included in the sample. To compute the cumulative excitatory and inhibitory modulations, we summed the normalized positive and (separately) negative modulations of all the cells in a given sample. In contrast to the average absolute modulation, the latter index allows a direct comparison of the relative importance of excitatory and inhibitory modulations. We assessed the relationship

between the modulations associated with the variables across cell types by computing a rank-based (Spearman) correlation. To compare the model fit to observed spiking, we used the coefficient of determination (R^2) as follows:

$$R^2 = 1 - \frac{\sum (y_i - f_i)^2}{\sum (y_i - \bar{y})^2},$$

where y_i represents the observed unit spiking and f_i is the model-estimated firing at different time points i , and $\bar{y}$ is the overall average of the observed unit spiking.

Data availability

The full dataset and custom MATLAB code have not been deposited in a public repository because of their size, but are available from the corresponding author on request.

Results

Experiment 1: muscimol infusions in BLA and CMT

Rats were trained to leave a nest-like compartment to retrieve a food pellet in a brightly lit and elongated arena (Fig. 1B1,B2). After training to retrieve food in the absence of the predator, rats were confronted with a mechanical predator, which was located at the other end of the foraging arena, facing rats as they approached the food pellet. Trials started when the gateway to the foraging arena opened. After a variable delay, rats moved to the gateway and paused at the door threshold (waiting phase). Eventually, they left the nest to retrieve a food pellet placed 12.5–150 cm from the nest (foraging phase). Upon retrieving the food pellet (or failing to do so), rats abruptly changed direction and ran to the nest (escape phase). Upon nest reentry (nest phase), the gateway was closed. On predator trials, each time rats came within ~25 cm of the food pellet, the predator surged forward, snapped its jaw repeatedly, and returned to its original position. See <https://www.youtube.com/watch?v=9ASzt21ZdE> for trial examples.

We compared behavior in the foraging task in the presence of the predator 15–30 min after bilaterally infusing the same volume of vehicle (ACSF, 0.1 μ l) or fluorescent muscimol (0.9 nmol) in BLA or CMT. We formed a third (control) group out of CMT rats with incorrect cannula placements, resulting in the following samples: 10 BLA rats, 8 CMT rats, and 11 control rats (Figs. 1C1–C3, examples of fluorescent muscimol infusions, 2, schemes illustrating cannula tip locations in the three groups). Vehicle and muscimol infusions were performed on different days, 24 h apart. To minimize the influence of habituation to the predator, the order of the infusions was counterbalanced in all groups.

Our behavioral protocol adhered closely to that used by Choi and Kim (2010), with one exception: the nest and foraging arena were separated by a sliding door, whose opening signaled the start of a trial. In brief, after habituation to the behavioral apparatus (days 1–2) and training on the foraging task in the absence of the predator (days 3–9), rats received a vehicle or drug infusion 15–30 min before giving them the opportunity to retrieve a food pellet placed in the foraging arena, 75 cm from the nest (days 10–12). When rats advanced within ~25 cm of the food, the predator surged forward. If the rat successfully retrieved the food pellet, the distance was increased in steps of 25 cm until the rat failed. When the rat failed at 75 cm, the distance was decreased in steps of 25 cm until the rat retrieved the pellet. If the rat failed at 25 cm, we decreased the distance to

12.5 cm. Trials were separated by 1–2 min intervals during which rats were in the nest with the door closed.

The dependent variables we monitored were the maximal foraging distance (Fig. 1D) and the amount of time rats hesitated at the door threshold before initiating foraging (hereafter termed “waiting time”; Fig. 1E). For both dependent variables (with Bonferroni-corrected $\alpha = 0.025$), a two-way mixed-model ANOVA with infusion site (BLA, CMT, control) as the between-subject variable and treatment (vehicle, muscimol) as the within-subject variable revealed a significant effect of infusion site on foraging distance ($F_{(1,26)} = 5.258$, $p = 0.012$, $\eta^2 = 0.288$) but not on waiting time ($F_{(1,26)} = 0.116$, $p = 0.891$), a significant effect of treatment (foraging distance: $F_{(1,26)} = 86.911$, $p < 0.001$, $\eta^2 = 0.770$; waiting time: $F_{(1,26)} = 7.948$, $p = 0.009$, $\eta^2 = 0.234$), and a significant interaction between infusion site and treatment on foraging distance ($F_{(2,26)} = 11.887$, $p < 0.001$, $\eta^2 = 0.478$), but not on waiting time ($F_{(1,26)} = 2.381$, $p = 0.112$).

Post hoc paired-samples *t* tests confirmed the observations of Choi and Kim (2010) that muscimol infusions in BLA caused a significant increase ($320 \pm 41\%$) in maximal foraging distance ($t_{(9)} = 7.236$, $p < 0.001$, $d = 2.289$). We also found that BLA inactivation significantly reduced ($-58 \pm 19\%$) waiting times ($t_{(9)} = -2.835$, $p = 0.020$, $d = 0.896$). Of the other groups, only CMT infusions were found to have significant effects: a 123% increase in foraging distance ($t_{(7)} = 8.000$, $p < 0.001$, $d = 2.829$) and a $72 \pm 33\%$ decrease in waiting times ($t_{(7)} = -2.014$, $p = 0.066$, $d = 0.768$).

It seems unlikely that the behavioral impact of muscimol infusions in BLA and CMT was because of nonspecific effects on locomotor behavior. First, the quantity of muscimol we infused is, to our knowledge, the lowest ever used to inactivate an amygdala nucleus in the literature. Second, other than increased risk-taking, no abnormal locomotor behaviors were observed in the muscimol condition. Third, the maximal speed of rats on aCSF versus muscimol trials did not differ in the three groups (BLA: Wilcoxon $T = 9$, $Z = -1.007$, $p = 0.314$; ACSF, 6.3 ± 0.9 cm/s; muscimol, 8.5 ± 1.5 cm/s; CMT: Wilcoxon $T = 8$, $Z = -0.700$, $p = 0.484$; ACSF, 9.6 ± 1.0 cm/s; muscimol, 10.7 ± 1.3 cm/s; control: Wilcoxon $T = 7$, $Z = -0.845$, $p = 0.398$; ACSF, 8.0 ± 1.6 cm/s; muscimol, 7.4 ± 1.6 cm/s).

Experiment 2: unit recordings in CMT during the foraging task

We recorded unit activity in the CMT, while rats ($n = 7$) performed the foraging task. Figure 3A summarizes the experimental timeline. On each recording day, we conducted alternating trial blocks with ($n = 10$ –20) or without ($n = 10$ –15) the predator, for a total of 100–120 trials/d. On each trial, the distance between the nest and food pellet was varied randomly between 12.5 and 150 cm. Thus, on predator trials, the proximity of the food pellet to the predator varied randomly on a trial-by-trial basis. Rats showed signs of increased apprehension on predator trials. Specifically, the proportion of aborted trials, that is trials in which after hesitating at the door threshold, rats retreated into the nest instead of initiating foraging, was ~3.5 times higher in the presence (2.1%) than the absence (0.6%) of the predator (χ^2 test for independence, $\chi^2 = 8.40$, 28 sessions from seven rats, $p = 0.003$). Moreover, when rats did initiate foraging in the presence of the predator, the retrieval interval increased by $265 \pm 34\%$ (paired-samples *t* test; $t_{(27)} = 6.910$, $p < 0.001$, $d = 1.306$) and the proportion of successful trials dropped 42% (χ^2 test for independence; $\chi^2 = 506.74$, 28 sessions from seven rats, $p < 0.001$). Finally, rats spent much longer

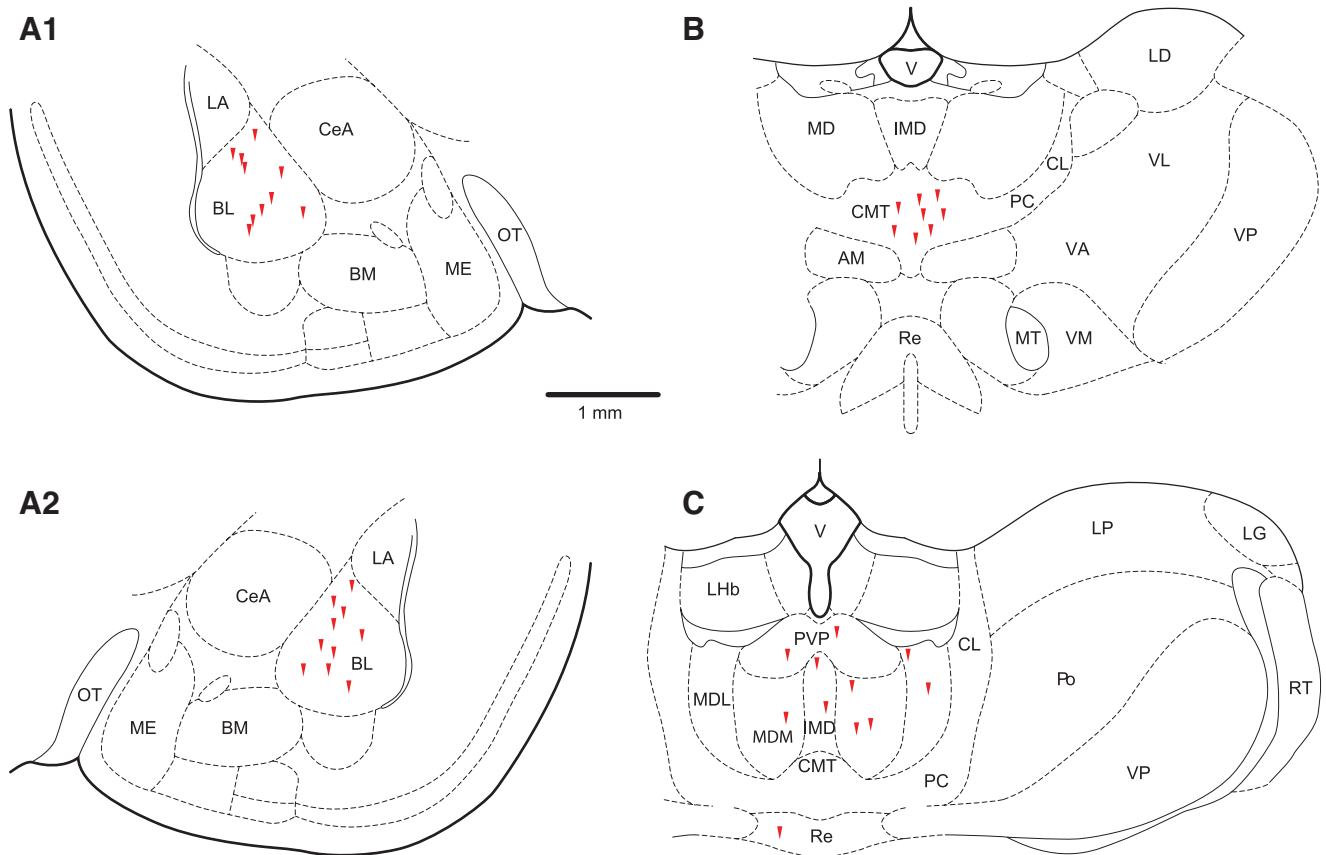


Figure 2. **A1–C**, Location of cannula tips (red arrowheads) in the BLA (**A1**, **A2**), CMT (**B**), and control groups (**C**). Relative to bregma, the anteroposterior coordinate in **A1** and **A2** is -2.4 mm, in **B** is -2.3 mm, and in **C** is -3.6 mm (Paxinos and Watson, 2007).

retrieving the food pellet on predator trials that followed failed trials (61.3 ± 7.7 s) rather than successful trials (24.5 ± 3.1 s; paired-samples *t* test; $t_{(26)} = -6.084$, $p < 0.001$, $d = 1.171$).

Task-related activity of CMT neurons

A total of 716 single units, histologically determined to have been located within CMT (Fig. 3B), were recorded while rats performed the foraging task. We begin by describing the activity of CMT neurons regardless of whether the predator was present or absent. We will return to the impact of the predator in a subsequent section.

Figure 4 shows four representative examples of CMT neurons with obvious task-related activity. For each cell, we provide spike rasters centered on the onset of particular task events and below them, the corresponding average $\pm$ SEM firing rate. As the duration of each behavioral phase varied from trial to trial, we rank ordered the trials based on the timing of events that occurred just before (left column, door opening before waiting) or after (middle column, escape after foraging; right column, nest entry after escape) the behavior of interest. These

A Experimental timeline

Silicone probe implantation → Recovery from surgery → Habituation to nest → Foraging without predator → Foraging with or without predator

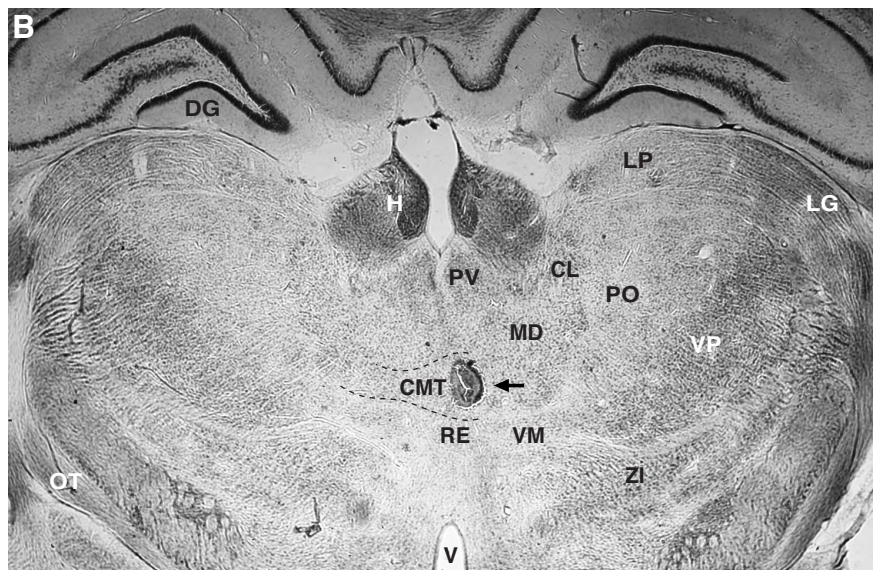


Figure 3. Experimental timeline and histological determination of recording sites. **A**, Main phases of the experiment. **B**, Coronal section counterstained with thionine. Arrow, Electrolytic lesion performed at the conclusion of the experiment to mark the last recording site.

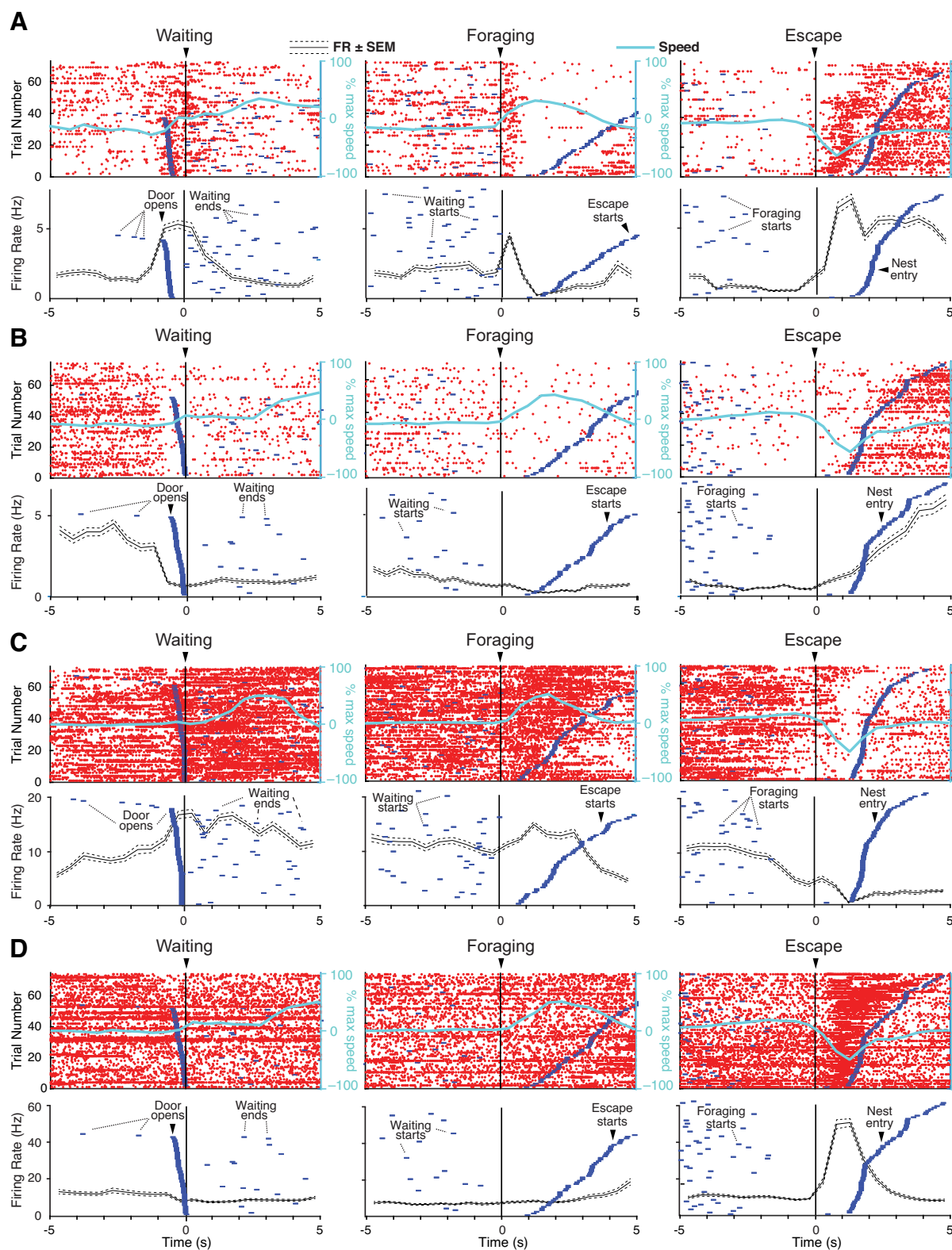


Figure 4. Examples of CMT unit activity during the foraging task. **A–D**, Activity of four different CMT neurons around the onset of waiting (left column), foraging (middle column), and escape (right column). Top row, Rasters where red dots mark spike times and each horizontal line corresponds to a trial. Bottom row, Average $\pm$ SEM firing rates across all trials depicted in the rasters. In the rasters, trials are rank ordered based on the timing of events that occurred just before (left column, door opening before waiting) or after (middle column, escape after foraging; right column, nest entry after escape) the behavior of interest. These events are indicated by blue tick marks. Cyan lines, Rats' average speed (upward and downward deflections indicate movements away from vs toward the nest, respectively).

Table 1. CMT Cells with statistically significant changes in firing rates in the foraging task

	Door opening	Waiting	Foraging	Escape	Predator activation	Nest entry
CMT ($n = 716$)	193 (27.0%)	130 (18.2%)	199 (27.8%)	229 (32.0%)	162 (22.6%)	177 (24.7%)
↑ FR	69 (9.6%)	86 (12.0%)	117 (16.3%)	113 (15.8%)	89 (12.4%)	100 (14.0%)
↓ FR	124 (17.3%)	44 (6.1%)	82 (11.5%)	116 (16.2%)	73 (10.2%)	77 (10.8%)

We computed Kruskal–Wallis one-way ANOVAs on the firing rates of CMT neurons ($n = 716$) across the various task phases. Of the 633 cells with a significant ANOVA, 337 had firing rates that differed significantly between baseline and one or more task phases (Tukey–Kramer *post hoc* test, $p < 0.05$). The table lists the number and proportion of significant cells for each task phase. Because many cells had significant changes in firing rates in more than one task phase, the sum of the cells across the different task phases is >337 .

events are indicated by blue tick marks. Finally, the cyan lines in Figure 4 indicate the average speed of the rats (upward and downward deflections indicate movements away from vs toward the nest, respectively).

As shown in Figure 4, the task-related activity of CMT neurons was extremely variable, with some cells showing transient increases in firing rates in relation to most task events (Fig. 4A), others exhibiting persistent firing rate reductions from the start of the trials until rats returned to the nest (Fig. 4B), and others displaying more differentiated activity profiles (Fig. 4C,D). Although three of the neurons shown in this figure (Fig. 4A,C,D) appear to be strongly modulated by locomotion speed, a closer examination reveals that they have an ambiguous relation to speed. That is, their firing rates changes in relation to some locomotor behaviors, but to not others. For instance, the firing rate of the cell shown in Figure 4A increases transiently at the onset of foraging but then drops markedly for the rest of the foraging period. By contrast, the firing rate of this same cell increased during the locomotion related to escape. As to the neuron shown in Figure 4C, its firing rate also has an ambiguous relation to running speed, but opposite to that of the cell shown in Figure 4A (it increases during foraging and drops during escape). Last, the firing rate of the cell shown in Figure 4D does not increase during foraging but rises very strongly during escape. To address this question further, we analyzed CMT firing rates while rats moved around with no predator or food. Consistent with the above, when rats started moving from complete immobility, no significant change in firing rate was seen at the population level. Similarly, plotting firing rates as a function of running speed in the same conditions revealed no significant correlation (average absolute Spearman $\rho = 0.035$; data not shown).

To determine whether each neuron showed statistically significant task-related variations in activity (excited or inhibited), we computed Kruskal–Wallis one-way ANOVAs on firing rates (Bonferroni-corrected threshold α of 0.05/716, or 0.000014) during the baseline (in the nest with the door closed), waiting, foraging, and escape periods. Overall, 88% of CMT cells (633 of 716) showed significant task-related activity ($p < 0.000014$). Tukey–Kramer *post hoc* tests with a threshold α of 0.05 revealed that 337 of the 633 cells had a significantly different firing rate in at least one of the task phases relative to baseline.

Table 1 shows the distribution of these 337 cells, specifically how many of them increased or decreased their firing rates relative to baseline in the various task phases. A cursory look at this table reveals that CMT cells are heterogeneous: neurons with increased or decreased activity levels relative to baseline are seen across all task phases. Whereas a higher proportion of cells had increased rather than decreased firing rates in the waiting, foraging, and nest reentry phases, the opposite was seen for door opening. For escape, our sample was evenly split between increased and decreased activity levels. Of note, many CMT neurons (229 of 337 or 32% of the total 716 neurons) had

Table 2. Number of cells with firing rates significantly different from baseline in one or more task phases

	Number of task phases				
	1	2	3	4	5
CMT ($n = 716$)	108 (15.1%)	85 (11.9%)	73 (10.2%)	48 (6.7%)	23 (3.2%)
↑ FR	49 (6.8%)	46 (6.4%)	46 (6.4%)	24 (3.4%)	9 (1.3%)
↓ FR	59 (8.2%)	39 (5.4%)	27 (3.8%)	24 (3.4%)	14 (2.0%)

Same statistical procedures as in Table 1 were used.

significantly different firing rates in two or more task phases relative to baseline (Table 2).

A better appreciation of cell-to-cell variations in task-related activity can be gained by inspecting Figure 5A1, which shows the activity of all cells with a significant Kruskal–Wallis ANOVA ($n = 633$), z scored based on variations in firing rates during the baseline period. Cells were rank ordered from least (top, blue) to most active (bottom, yellow) based on their activity during the foraging phase, and the same order was kept in the other phases. Figure 5A2 plots their average ($\pm$ SEM) firing rates. Comparing the color distributions in the different phases (Fig. 5A1) reveals that activity during foraging is a poor predictor of that in other task phases. In fact, rank correlations between z -scored activity in the various phases are consistently low, in the -0.2 to 0.2 range (Fig. 5B1). The lack of consistent association between CMT activity in the different phases is highlighted in Figure 5B2, which plots the ranks of all significant cells in the waiting, foraging, escape, and nest phases, but color coded based on their activity during waiting. Nevertheless, as a group, CMT cells increased their firing rates (z score, 0.5–0.7) in all phases of the foraging task until rats returned to their nest (Fig. 5A2).

In an attempt to identify subsets of CMT neurons with distinct profiles of task-related activity, we used unsupervised clustering (*kmeans* function in MATLAB). Using raw firing rates, the optimal number of identified clusters by gap statistics was always near the maximum number of tested clusters. Separately averaging the activity of cells within each cluster failed to reveal distinct patterns of task-related activity (data not shown). Similarly inconclusive results were obtained when clustering was conducted on normalized firing rates (z scored based on variations during the baseline period).

Influence of the predator and prior trial outcome

As mentioned above, rats showed signs of increased apprehension on trials conducted in the presence of the predator. That is, they waited longer before initiating foraging and foraged more tentatively, especially if they had failed to retrieve the food on the prior trial. To examine the neuronal correlates of these behavioral variations, we compared the z -scored activity of CMT cells on no-predator versus predator trials, rank ordering the cells based on their activity in the foraging phase of no-predator trials, and keeping the same ordering in the other conditions (Fig. 6A). Predator trials were subdivided into two groups, depending on

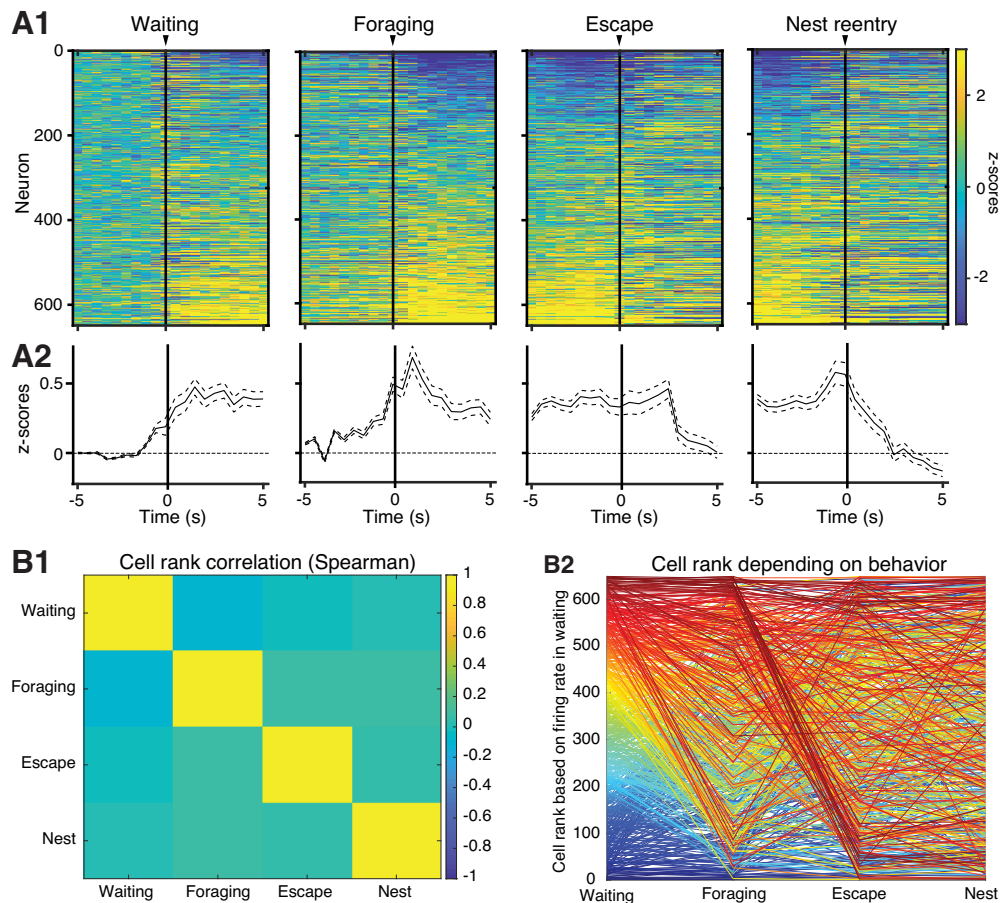


Figure 5. Variations in the activity of CMT neurons during the different phases of the foraging task. **A1**, Activity of all CMT neurons with significant task-related activity ($n = 633$) during the foraging task. Firing rates were z scored based on the activity of the cells during the baseline period (in the nest with door closed). Cells were rank ordered from least (blue) to most active (yellow) based on their activity during the foraging phase and the same order was kept for the other phases. **A2**, Average $\pm$ SEM of z-scored firing rates for all cells in the corresponding panel. **B1**, Matrix of rank correlations of CMT cells ($n = 633$) between the different task phases. **B2**, Relationship between the rank of the same CMT cells in the different task phases. Cells were rank ordered from most (top, red) to least active (bottom, blue) based on their activity in the waiting phase. The color coding of each cell was then kept for the other behavioral phases.

whether rats had failed or succeeded to retrieve the food pellet on the prior trial (failed trials were rare in the absence of the predator). As there were large differences in phase duration across trial types, we plotted the data of Figure 6A as a function of relative time, by distributing a fixed number of samples evenly across each of the phases of interest.

This analysis revealed that not only were the relative activity levels of CMT cells different across different phases on the same trial type (Fig. 5A1), they also differed in the same phase of different trial types (Fig. 6A,B). Moreover, the average z-scored activity of CMT cells was lower on predator than on no-predator trials, particularly if rats had failed the prior predator trial (Fig. 6A,B). A Friedman test confirmed that there was a significant difference in firing rate between different trial types ($\chi^2_r = 34.831$, $df = 2$, $n = 195$, $p < 0.0001$). *Post hoc* pairwise comparisons using Wilcoxon tests (with Bonferroni-corrected $\alpha = 0.017$) revealed significantly higher CMT firing rates in no-predator trials compared with the other two trial types (no-predator prior success vs predator prior success: Wilcoxon $T = 5068$, $Z = 3.772$, $p < 0.001$; no-predator prior success vs predator prior failure: Wilcoxon $T = 5160$, $Z = 4.200$, $p < 0.001$), but no difference between the two subtypes of predator trials. The difference across trial types can be attributed to changes in the kurtosis of the CMT firing rate distributions as opposed to changes in skewness

(Fig. 6C1–C3). Predator presence and failure in the preceding trial increased the peakedness of the firing rate distributions.

In the above analyses, the data of each trial type was z scored to activity during the corresponding baseline period. As a result, differences in task-related activity could be because of changes in baseline firing rates across trial types. To test this possibility, we computed a repeated-measures ANOVA on baseline firing rates. However, we found no effect of trial type on baseline firing rates (Friedman $\chi^2_r = 29.964$, $df = 2$, $n = 649$, $p = 0.878$).

Last, we repeated the above analyses for the current trial type (Fig. 6D) to determine whether trial success or failure was associated with different activity levels earlier in the trial, such as during the waiting phase. The results were very similar to those obtained in the prior trial analyses. As with the prior trial, a Friedman test confirmed that there were significant differences in firing rate between different trial types ($\chi^2_r = 37.05$, $df = 2$, $n = 195$, $p < 0.0001$). *Post hoc* pairwise comparisons using Wilcoxon tests (with Bonferroni-corrected $\alpha = 0.017$) revealed significantly higher CMT firing rates in no-predator trials compared with the other two trial types (no-predator success vs predator success: Wilcoxon $T = 3364$, $Z = -4.158$, $p < 0.001$; no-predator success vs predator failure: Wilcoxon $T = 3499$, $Z = -3.529$, $p < 0.001$). However, the difference between firing rates on successful versus failed predator trials did not reach the Bonferroni-corrected significance threshold

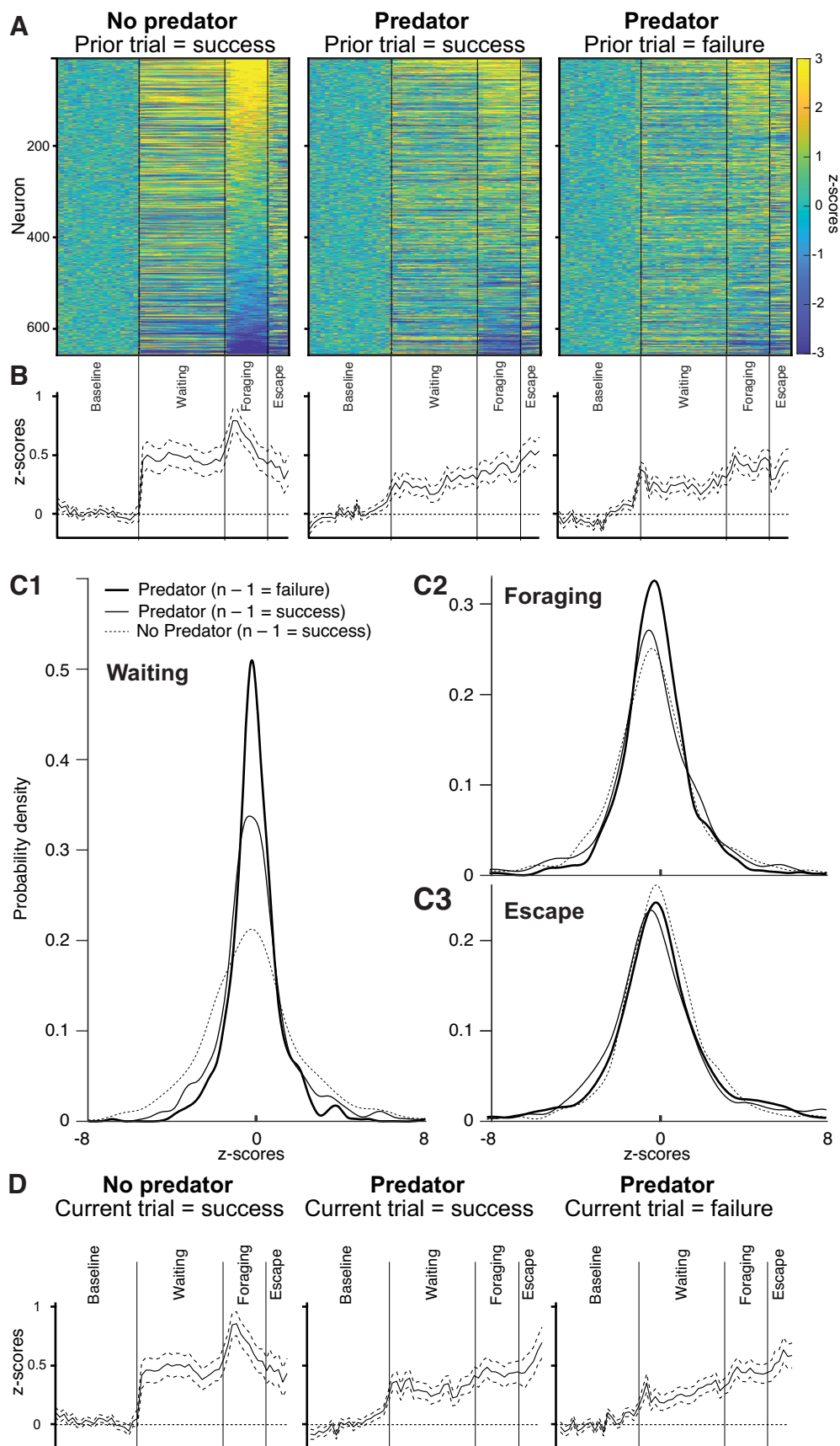


Figure 6. Variations in the activity of CMT neurons as a function of trial type (normalized time). **A**, Activity of all CMT neurons with significant task-related activity during the foraging task ($n = 633$) in no-predator (left) or predator (middle and right panels) trials. The predator trials were further subdivided as a function of prior trial outcome (success, middle; failure, right). Too few failed trials were available for the no-predator condition. In each panel, firing rates were z scored based on the activity of the cells during the baseline period (in the nest with door closed), and the cells were rank ordered based on their activity on no-predator trials during the foraging phase. The same rank order was kept for the other two panels. To allow for comparisons

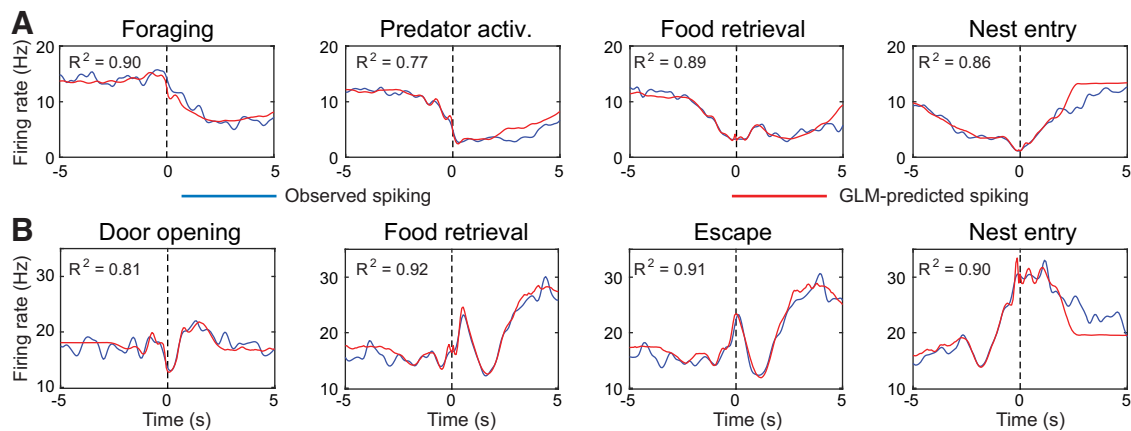


Figure 7. GLM-estimated coding of CMT neurons. **A, B**, Two different cells. Actual (blue lines) and GLM-predicted (red lines) firing rates during all available trials. In both cells, we show actual and predicted firing rates for the four variables with the largest β -coefficients.

($\alpha = 0.017$) for waiting (Wilcoxon $T = 329$, $Z = -2.1775$, $p = 0.029$) and foraging (Wilcoxon $T = 103$, $Z = -0.1134$, $p = 0.910$).

Disentangling the correlates of CMT activity

Overall, the above analyses indicate that CMT neurons exhibit large but variable firing rate fluctuations during the foraging task. However, identifying which factors drive these variations is not trivial. Indeed, as foraging trials unfold, a number of temporally overlapping variables fluctuate in addition to the task phases, such as the speed and position of the rats. To help us quantify the relative influence of these factors, we fit the activity of each CMT unit with a group least absolute shrinkage and selection operator (Lasso) GLM. This type of GLM exploits variations in the timing and duration of relevant variables to determine the encoding preference of neurons. Specifically, we considered the speed, position, distance from the food pellet, influence of task phases (baseline, door opening, waiting at the door, foraging, predator activation, escape, nest entry), trial types (with or without predator), and prior trial outcome (failure, success) of the rats. Critically, this type of GLM promotes the dimensionality reduction of correlated data and permits sparsity in the identification of the factors linked to neuronal activity (Tibshirani, 1996; Yuan and Lin, 2006; Breheny and Huang, 2015).

Figure 7 compares the actual firing rates (blue traces) of two CMT cells to those predicted by the model (red traces) for their task variables with the highest β -values. As in these representative examples, the output of the GLM generally matched observed firing rates (see R^2 values in the top left corner of each graph). Now at a population level, Figure 8A shows frequency distributions of β -values in CMT cells ($n = 716$) for all the variables considered in the GLM (labels at the top left of the histograms), excluding neurons with zero β -values. Excitatory and inhibitory modulations are plotted to the right and left of the origin of the histograms, respectively. Note that we excluded

neurons with any β -values equal to zero for each variable separately. Hence, if for one variable, the β -value of a neuron was zero but for another it was different from zero, we only excluded the β -values of the neuron of the first variable. The β -value of the neuron of the second variable was included.

For comparison, the same data are provided for a sample of principal BLA neurons ($n = 599$) we recorded previously during the foraging task (Amir et al., 2015; Fig. 8B). For both cell types, at the top right of each graph, we provide the number of cells with β -values equal to zero, of cells with modulations different from zero, and the average absolute β -value for the variable under consideration. In both cell types, note variations in the dispersion of β -values and in the asymmetry of the distributions. For instance, the β -values of CMT cells for “door opening” and “nest entry” show much more variability than for “speed” and “distance from food” (Fig. 8A). Yet, CMT distributions (Fig. 8A) were generally narrower than BLA distributions (Fig. 8B), indicating a lower modulation of CMT than BLA neurons by the variables considered. Moreover, CMT distributions are generally more symmetric than BLA distributions, which are typically skewed to the left, betraying a preponderance of negative β -values in BLA neurons.

These contrasting features are also manifest in Figure 9, which plots for CMT cells ($n = 716$; Fig. 9A1,A2), principal BLA neurons ($n = 599$; Fig. 9B1,B2), and fast-spiking BLA cells (Fig. 9C1,C2; $n = 71$; also recorded in the study by Amir et al., 2015), their average absolute modulation (Fig. 9A1,B1,C1) as well as their cumulative excitatory (blue) and inhibitory (red) distributions (Fig. 9A2,B2,C2) for all the variables considered in the GLM. Variables were rank ordered from the highest to lowest absolute modulations, in the three cell types separately. Although the variables with the lowest absolute modulations are nearly identical in the three cell types (trial type, prior trial, position, distance from food, speed), there are major differences between the other variables. First, the magnitude of β -values is two to three times higher in principal BLA neurons than in CMT or fast-spiking BLA cells (Fig. 9, compare B1, and A1, C1, axis range). This difference is likely related to the much higher spontaneous firing rates of CMT and fast-spiking BLA neurons compared with principal basolateral nucleus of the amygdala (BL) cells (in the nest with door closed: CMT, $n = 716$, 6.09 ± 6.30 Hz; fast-spiking BLA cells, $n = 71$, 23.66 ± 13.66 Hz; principal BLA cells, $n = 599$, 0.48 ± 1.23 Hz; Kruskal-Wallis ANOVA, $df = 2$, $\chi^2 = 891.65$, $p < 0.0001$). As a result, task-related changes in firing

despite trial-to-trial variations in the duration of the different behavioral phases, the data are plotted as a function of normalized time. **B**, Average of z-scored firing rates $\pm$ SEM for all cells in the corresponding panel. **C1–C3**, Kernel density estimation of z-scored and time-relative firing rates in the same trial types as shown in **A** (see legend at top of **C1**) and during different task phases (**C1**, waiting; **C2**, foraging; **C3**, escape). **D**, Same analysis as in **B** but for the current trial.

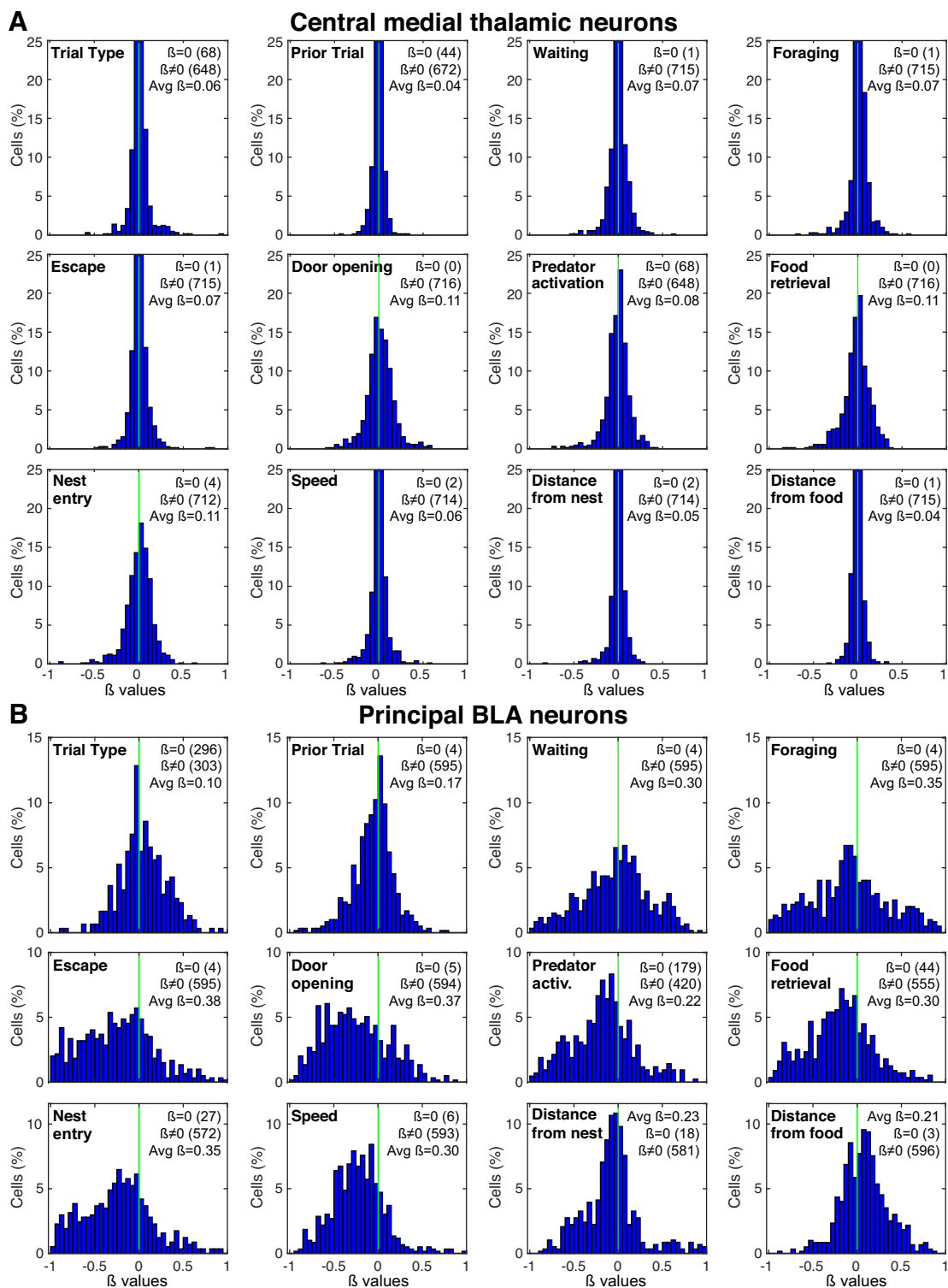


Figure 8. Generalized linear model-estimated coding of 12 variables by CMT and principal BLA neurons. **A**, CMT cells ($n = 716$). **B**, Principal BLA neurons ($n = 599$) recorded in a prior study (Amir et al., 2015). All panels are the frequency distributions of firing modulation (x -axis; β -values) by the variables indicated at the top left of each graph. On the top right of each graph, we list (from top to bottom) the number of cells with β -values equal to zero ($\beta = 0$), the number of cells with β -values different from zero ($\beta \neq 0$), and the average absolute β -values (Avg β).

rates are proportionally much larger in principal BLA cells. Second, whereas inhibitory firing rate modulations are generally higher than excitatory ones in principal BLA cells (Fig. 9B2), CMT, and fast-spiking BLA cells show no such imbalance (Fig. 9A2,C2). Third, while the variables with the highest modulations were similar in CMT and fast-spiking

BLA neurons (door opening, food retrieval, nest entry, predator activation), they differed markedly from those dominating the activity of principal BLA neurons, particularly escape and foraging.

Consistent with the qualitative similarities between the GLM results obtained in CMT and fast-spiking BLA neurons, the

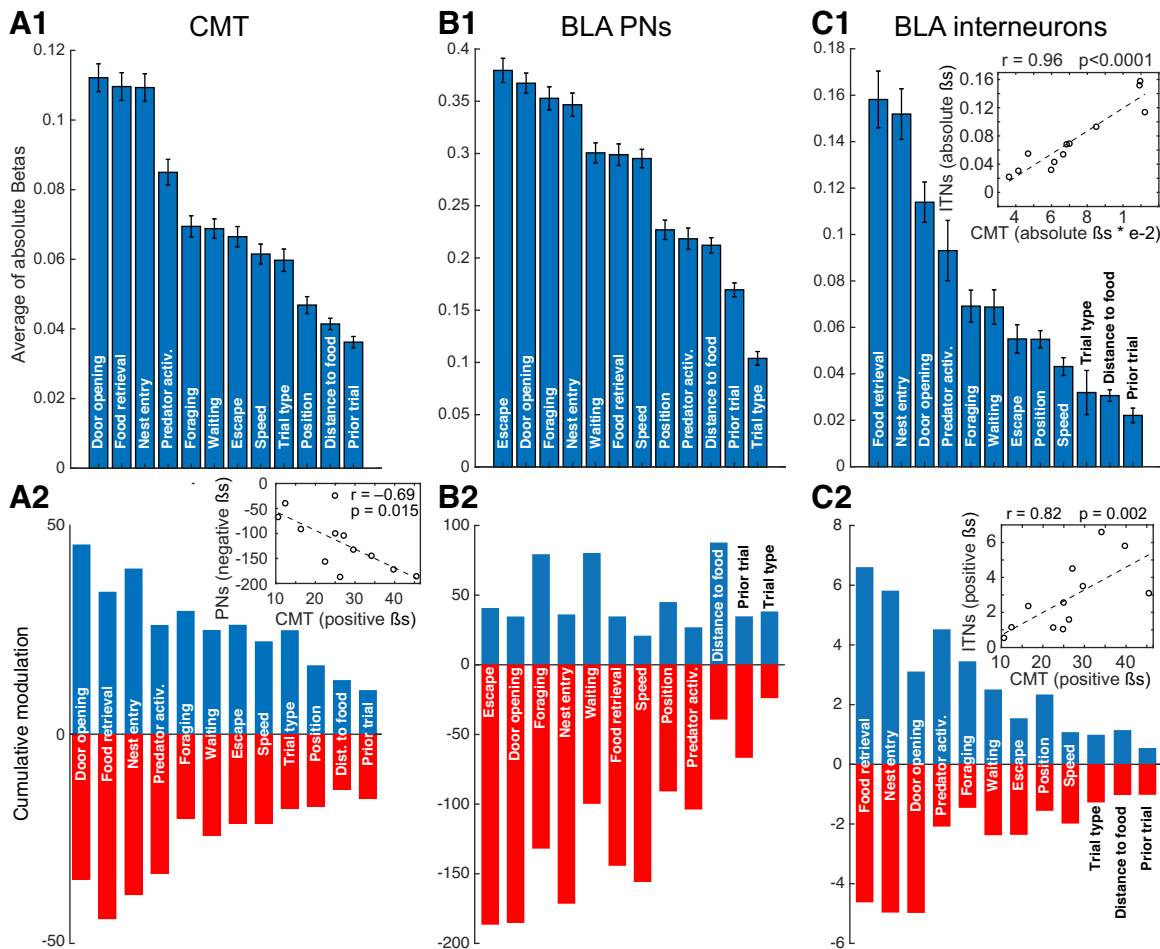


Figure 9. **A1–C2**, Multidimensional coding by CMT and BLA neurons. CMT cells (**A1, A2**), principal BLA neurons (PNs; **B1, B2**) and fast-spiking BLA neurons (ITNs; **C1, C2**) recorded in a prior study (Amir et al., 2015). **A1, B1, C1**, Average of absolute β -values associated with the 12 different variables considered, rank ordered by the magnitude of firing rate modulation. **A2, B2, C2**, Sum of positive (blue) and negative (red) modulations, ordering the variables as in **A1, B1**, and **C1**. Inset in **C1** plots the averages of absolute β -values of BLA ITNs (y-axis) versus those of CMT neurons (x-axis) for the 12 different variables considered. Inset in **A2** plots the sum of the negative modulation of BLA PNs (y-axis) versus the sum of positive modulation of CMT neurons (x-axis) for the same variables. Inset in **C2** plots the sum of positive modulations of BLA ITNs (y-axis) versus that of CMT neurons (x-axis) for the same variables. Spearman's r and associated p values are indicated for each inset.

average absolute β -values associated with the 12 variables were highly correlated in these two cell types (Fig. 9C1, inset; Spearman $r = 0.96$, $p < 0.0001$). And so were the positive β -values associated with these variables (Fig. 9C2, inset; Spearman $r = 0.82$, $p = 0.002$). Opposite to this, a negative correlation was found between the positive β -values of CMT cells and the negative β -values of principal BLA neurons (Fig. 9A2, inset; Spearman $r = -0.69$, $p = 0.015$).

Discussion

In support of the hypothesis that CMT regulates foraging behavior, we observed that inactivation of CMT with muscimol increased foraging distances and decreased waiting times, similar to the effects of BLA inactivation. Moreover, many CMT neurons showed large changes in activity during the foraging task. However, in most task phases, our sample was split between neurons with decreased or increased discharge rates, with a modest bias for the latter. In addition, while GLM analyses revealed that CMT neurons encode many of the same task variables as principal BLA cells, the nature and relative importance of the activity modulations seen in CMT neurons differed markedly from those of principal BLA cells. By contrast, the GLM revealed

striking similarities between the β -values of CMT cells and fast-spiking BLA interneurons. Below, we consider the significance of these findings for the contributions of CMT neurons to foraging behavior. Further, we propose a hypothetical account for the interaction between CMT and BLA neurons during foraging.

Limitations of the foraging task

A limitation of the present study is the use of a pseudopredator, which imperfectly reproduces the features of an actual predator, particularly with respect to its olfactory cues (Martinez et al., 2011; Bindi et al., 2018). Although the pseudopredator does not fully capture the presentation of natural threats, it did alter the behavior of the rats as would be expected from an actual predator. That is, rats showed signs of increased apprehension on predator trials: they waited longer before initiating foraging, failed to retrieve the food on a higher proportion of trials, and foraged more tentatively, especially if they had failed the prior trial. Critically, we could detect changes in the activity of CMT neurons in relation to these behavioral variations. Hence, it appears that the foraging task captures essential features of prey-predator interactions, justifying its use here and the interpretation of the results.

CMT neurons have large but heterogeneous activity correlates in the foraging task

Consistent with relay neurons in other thalamic nuclei (Steriade, 1993), CMT neurons fired tonically (median firing rate, ~5 Hz) during quiet wakefulness. In the foraging task, most CMT neurons (88%) displayed significant changes in firing rates in relation to one or more task events. However, in each task phase, the firing rate of many CMT neurons increased while others showed the opposite. Moreover, activity changes developing during any particular task phase were poor predictors of those occurring in other phases.

At the population level, this heterogeneity resulted in modest task-related activity. Specifically, average CMT firing rates increased ~0.5 *z* scores from baseline to waiting, remained elevated at or slightly above this level (*z* score, 0.7) through foraging and escape, only returning to, and eventually below, baseline levels after reentry into the nest. Moreover, in the presence of the predator, the overall increase in the firing rates of CMT cells was attenuated, especially if the rat failed the preceding trial. As such, the variability of the predator's influence (present vs absent, and success vs failure) did not impact the heterogeneous activity of CMT neurons, only their overall relative firing rates.

CMT neurons encode multiple task features

As trials unfold in the foraging task, multiple overlapping variables potentially contribute to alter neuronal activity. These include factors such as the speed, position in the apparatus, proximity to the food, and predator of the rats, as well as the recent experience of the rats in the task. Disentangling the relative influence of these multiple factors is especially difficult around the time of predator activation, when rats suddenly switch from foraging to escape. Since perievent histograms of firing rates cannot dissociate these overlapping factors, we used a GLM, allowing us to assess the influence of each variable while factoring out that of the others by taking advantage of variations in the timing and duration of relevant variables.

This approach revealed that CMT cells encode many task features (door opening, food retrieval, nest entry, predator activation), mainly corresponding to stimuli in the foraging task. That CMT neurons concomitantly represent multiple types of information is consistent with the functionally diverse inputs they receive. Indeed, CMT is the recipient of afferents from the superior colliculus (Krout et al., 2001), parabrachial nucleus (Krout and Loewy, 2000), and many high-order cortical areas (Vertes et al., 2015), most of which also project to BLA (for review, see McDonald, 1998).

While the activity of CMT and BLA neurons was modulated by a similar array of task features, the relative importance and the nature of these modulations differed. In CMT and fast-spiking BL interneurons, inhibitory and excitatory modulations were balanced, with a small preference for excitation. This contrasted with principal BL neurons where most variables were prevalently associated with inhibitory modulations (Amir et al., 2019b).

Impact of CMT inputs on BLA activity in the foraging task

The role of the thalamus in motivated behavior has received comparatively little attention, possibly because it is thought to support general functions like arousal or the transfer of sensory information (Bradfield et al., 2013; James et al., 2021). However, rapidly accumulating data implicate the

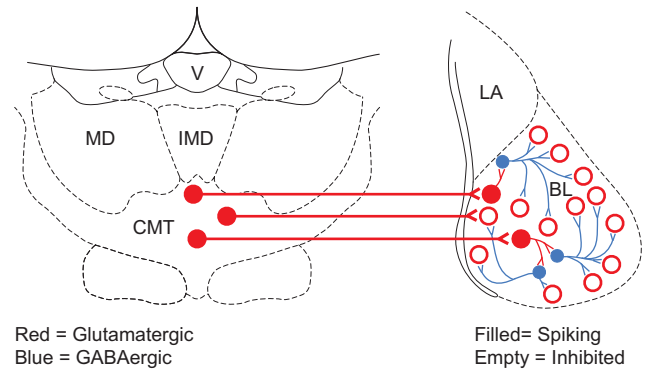


Figure 10. Hypothetical model of the influence of the CMT over the BLA. Red and blue indicate glutamatergic or GABAergic processes, respectively. Filled and empty circles represent spiking and inhibited neurons, respectively. Our results suggest that when CMT neurons fire, they recruit a minority of principal BLA cells. In turn, these neurons recruit GABAergic interneurons through their intrinsic axon collaterals. Ultimately, the recruitment of GABAergic interneurons causes the inhibition of most principal BLA neurons.

mediodorsal (Mair et al., 2021), intralaminar (Bradfield et al., 2013; Bradfield and Balleine, 2017; Cover and Mathur, 2021), and PVT (Do-Monte et al., 2016; Choi and McNally, 2017; Petrovich, 2021) thalamic nuclei in a variety of motivated behaviors. While the contribution of these thalamic nuclei to motivated behaviors are generally thought to depend on their projections to cortex (Saalmann, 2014) or striatum (Bradfield et al., 2013; Smith et al., 2014; Bradfield and Balleine, 2017), PVT projections to the amygdala play an important role, particularly with respect to conditioned fear responses. Indeed, inactivation of PVT neurons attenuate conditioned fear responses (Padilla-Coreano et al., 2012; Penzo et al., 2014, 2015; Do-Monte et al., 2015), an effect mediated by PVT projections to the central lateral nucleus of the amygdala (Penzo et al., 2015). Combined with our results, these findings raise the possibility that multiple thalamic inputs regulate foraging behavior via projections to different amygdala nuclei.

Indeed, CMT sends a very dense glutamatergic projection to BLA (Vertes et al., 2012), which mainly targets principal cells (Amir et al., 2019a; Ahmed et al., 2021). However, relative to other extrinsic afferents to BLA (LeDoux et al., 1991; Brinley-Reed et al., 1995; Smith et al., 2000; Woodson et al., 2000; Unal et al., 2014), CMT inputs form far fewer synapses with inhibitory interneurons (Amir et al., 2019a), a conclusion supported by our recent physiological study *in vitro* (Ahmed et al., 2021). Based on these results, CMT neurons are not expected to recruit much feedforward inhibition in BLA cells. However, when CMT inputs fire principal BLA cells, they strongly recruit feedback interneurons (Ahmed et al., 2021). Together, these findings raise the possibility that during the foraging task, CMT inputs fire a few principal BLA neurons, leading to the recruitment of local feedback interneurons and the consequent inhibition of a high proportion of principal cells (Fig. 10).

Two of our GLM results are consistent with this model. First, despite comparing data from separate cohorts of rats, we found a nearly perfect correlation between the firing modulations of CMT and fast-spiking BLA interneurons (Fig. 9C1,C2, insets). Second, the modulation of CMT and principal BLA neurons was inversely correlated (Fig. 9A2, inset). Also supporting this hypothetical model, fluctuations

in the population activity of CMT neurons during the foraging task were opposite to that of principal BLA neurons. That is, whereas the average firing rate of CMT cells increased during foraging and escape, most principal BLA neurons showed the converse (Amir et al., 2015, 2019b). Mirror image firing rate fluctuations were also observed on nest reentry, when the firing rates of CMT cells decreased, whereas those of principal BLA neurons increased (Amir et al., 2015, 2019b). Finally, whereas CMT neurons showed significantly decreased firing rate elevations on predator trials relative to no-predator trials, principal BLA neurons showed the opposite (Amir et al., 2015).

If the above model is correct and the CMT-driven inhibition of principal BLA cells is a necessary condition for rats to initiate foraging, how come CMT inactivation results in increased risk-taking? There are two nonexclusive possible explanations, highlighting the complexity of the network that regulates foraging behavior. First, in addition to BLA, CMT projects to the dorsal striatum, nucleus accumbens, and multiple cortical areas (anterior cingulate, prelimbic, orbital and insular cortices; Vertes et al., 2012). Thus, it is also possible that CMT inactivation affects foraging behavior indirectly, through a disfacilitation of these other sites. Second, since CMT projects to BLA and most of the cortical targets of CMT also project to BLA (McDonald, 1998), CMT inactivation is expected to cause a major reduction in the excitatory drive to BLA neurons. In turn, this “functional deafferentation” may cause a reduction in the firing rate of BLA neurons, of comparable magnitude to that seen on the initiation of foraging. Overall, our findings lend themselves to several interpretations. Much more work will be needed to untangle the functional interactions among CMT, amygdala, cortex, and striatum during foraging.

References

- Addicott MA, Pearson JM, Sweitzer MM, Barack DL, Platt ML (2017) A primer on foraging and the explore/exploit trade-off for psychiatry research. *Neuropsychopharmacology* 42:1931–1939.
- Ahmed N, Headley DB, Paré D (2021) Optogenetic study of central medial and paraventricular thalamic projections to the basolateral amygdala. *J Neurophysiol* 126:1234–1247.
- Amir A, Lee SC, Headley DB, Herzallah MM, Paré D (2015) Amygdala signaling during foraging in a hazardous environment. *J Neurosci* 35:12994–13005.
- Amir A, Paré JF, Smith Y, Paré D (2019a) Midline thalamic inputs to the amygdala: ultrastructure and synaptic targets. *J Comp Neurol* 527:942–956.
- Amir A, Kyriazi P, Lee SC, Headley DB, Paré D (2019b) Basolateral amygdala neurons are activated during threat expectation. *J Neurophysiol* 121:1761–1777.
- Barack DL, Platt ML (2017) Engaging and exploring: cortical circuits for adaptive foraging decisions. In: *Nebraska symposium on motivation*, pp 163–199. Cham, Switzerland: Springer.
- Bindi RP, Baldo MVC, Canteras NS (2018) Roles of the anterior basolateral amygdalar nucleus during exposure to a live predator and to a predator-associated context. *Behav Brain Res* 342:51–56.
- Bradfield LA, Balleine BW (2017) Thalamic control of dorsomedial striatum regulates internal state to guide goal-directed action selection. *J Neurosci* 37:3721–3733.
- Bradfield LA, Hart G, Balleine BW (2013) The role of the anterior, mediodorsal, and parafascicular thalamus in instrumental conditioning. *Front Syst Neurosci* 7:51.
- Breheny P, Huang J (2015) Group descent algorithms for nonconvex penalized linear and logistic regression models with grouped predictors. *Stat Comput* 25:173–187.
- Brinley-Reed M, Mascagni F, McDonald AJ (1995) Synaptology of prefrontal cortical projections to the basolateral amygdala: an electron microscopic study in the rat. *Neurosci Lett* 202:45–48.
- Canteras NS, Mota-Ortiz SR, Motta SC (2012) What ethologically based models have taught us about the neural systems underlying fear and anxiety. *Braz J Med Biol Res* 45:321–327.
- Choi EA, McNally GP (2017) Paraventricular thalamus balances danger and reward. *J Neurosci* 37:3018–3029.
- Choi JS, Kim JJ (2010) Amygdala regulates risk of predation in rats foraging in a dynamic fear environment. *Proc Natl Acad Sci U S A* 107:21773–21777.
- Cover KK, Mathur BN (2021) Rostral intralaminar thalamus engagement in cognition and behavior. *Front Behav Neurosci* 15:652764.
- Do-Monte FH, Quiñones-Laracuente K, Quirk GJ (2015) A temporal shift in the circuits mediating retrieval of fear memory. *Nature* 519:460–463.
- Do-Monte FH, Quirk GJ, Li B, Penzo MA (2016) Retrieving fear memories, as time goes by. *Mol Psychiatry* 21:1027–1036.
- Hazan L, Zugaro M, Buzsáki G (2006) Klusters, NeuroScope, NDManager: a free software suite for neurophysiological data processing and visualization. *J Neurosci Methods* 155:207–216.
- James MH, McNally GP, Li X (2021) Editorial: role of the thalamus in motivated behavior. *Front Behav Neurosci* 15:720592.
- Kim EJ, Kong MS, Park SG, Mizumori SJY, Cho J, Kim JJ (2018) Dynamic coding of predatory information between the prelimbic cortex and lateral amygdala in foraging rats. *Sci Adv* 4:eaar7328.
- Krout KE, Loewy AD (2000) Parabrachial nucleus projections to midline and intralaminar thalamic nuclei of the rat. *J Comp Neurol* 428:475–494.
- Krout KE, Loewy AD, Westby GW, Redgrave P (2001) Superior colliculus projections to midline and intralaminar thalamic nuclei of the rat. *J Comp Neurol* 431:198–216.
- Kyriazi P, Headley DB, Paré D (2018) Multi-dimensional coding by basolateral amygdala neurons. *Neuron* 99:1315–1328.e5.
- LeDoux JE, Farb CR, Milner TA (1991) Ultrastructure and synaptic associations of auditory thalamo-amygdala projections in the rat. *Exp Brain Res* 85:577–586.
- Mair RG, Francoeur MJ, Gibson BM (2021) Central thalamic-medial prefrontal control of adaptive responding in the rat: many players in the chamber. *Front Behav Neurosci* 15:642204.
- Martinez RC, Carvalho-Netto EF, Ribeiro-Barbosa ER, Baldo MV, Canteras NS (2011) Amygdalar roles during exposure to a live predator and to a predator-associated context. *Neuroscience* 172:314–328.
- McDonald AJ (1998) Cortical pathways to the mammalian amygdala. *Prog Neurobiol* 55:257–332.
- 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. *J Neurosci* 33:9866–9872.
- Mobbs D, Trimmer PC, Blumstein DT, Dayan P (2018) Foraging for foundations in decision neuroscience: insights from ethology. *Nat Rev Neurosci* 19:419–427.
- Padilla-Coreano N, Do-Monte FH, Quirk GJ (2012) A time-dependent role of midline thalamic nuclei in the retrieval of fear memory. *Neuropharmacology* 62:457–463.
- Paxinos G, Watson C (2007) *The rat brain in stereotaxic coordinates*, Ed 6. San Diego: Academic Press.
- Penzo MA, Robert V, Li B (2014) Fear conditioning potentiates synaptic transmission onto long-range projection neurons in the lateral subdivision of central amygdala. *J Neurosci* 34:2432–2437.
- Penzo MA, Robert V, Tucciarone J, De Bundel D, Wang M, Van Aelst L, Darvas M, Parada LF, Palmiter RD, He M, Huang ZJ, Li B (2015) The paraventricular thalamus controls a central amygdala fear circuit. *Nature* 519:455–459.
- Petrovich GD (2021) The function of paraventricular thalamic circuitry in adaptive control of feeding behavior. *Front Behav Neurosci* 15:671096.
- Rushworth MF, Behrens TE (2008) Choice, uncertainty and value in prefrontal and cingulate cortex. *Nat Neurosci* 11:389–397.
- Saalmann YB (2014) Intralaminar and medial thalamic influence on cortical synchrony, information transmission and cognition. *Front Syst Neurosci* 8:83.
- Scholl J, Klein-Flügge M (2018) Understanding psychiatric disorder by capturing ecologically relevant features of learning and decision-making. *Behav Brain Res* 355:56–75.
- Smith Y, Paré JF, Paré D (2000) Differential innervation of parvalbumin-immunoreactive interneurons of the basolateral amygdaloid complex by cortical and intrinsic inputs. *J Comp Neurol* 416:496–508.

- Smith Y, Galvan A, Ellender TJ, Doig N, Villalba RM, Huerta-Ocampo I, Wichmann T, Bolam JP (2014) The thalamostriatal system in normal and diseased states. *Front Syst Neurosci* 8:5.
- Stephens DW (2008) Decision ecology: foraging and the ecology of animal decision making. *Cogn Affect Behav Neurosci* 8:475–484.
- Steriade M (1993) Central core modulation of spontaneous oscillations and sensory transmission in thalamocortical systems. *Curr Opin Neurobiol* 3:619–625.
- Swanson LW (1988) The neural basis of motivated behavior. *Acta Morphol Neerl Scand* 26:165–176.
- Tibshirani R (1996) Regression shrinkage and selection via the lasso. *J R Statist Soc B* 58:267–288.
- Unal G, Paré JF, Smith Y, Paré D (2014) Cortical inputs innervate calbindin-immunoreactive interneurons of the rat basolateral amygdaloid complex. *J Comp Neurol* 522:1915–1928.
- Vandecasteele M, Royer S, Belluscio M, Berényi A, Diba K, Fujisawa S, Grosmark A, Mao D, Mizuseki K, Patel J, Stark E, Sullivan D, Watson B, Buzsáki G (2012) Large-scale recording of neurons by movable silicon probes in behaving rodents. *J Vis Exp* 4:e3568.
- Vertes RP, Hoover WB, Rodriguez JJ (2012) Projections of the central medial nucleus of the thalamus in the rat: node in cortical, striatal and limbic forebrain circuitry. *Neuroscience* 219:120–136.
- Vertes RP, Linley SB, Hoover WB (2015) Limbic circuitry of the midline thalamus. *Neurosci Biobehav Rev* 54:89–107.
- Woodson W, Farb CR, LeDoux JE (2000) Afferents from the auditory thalamus synapse on inhibitory interneurons in the lateral nucleus of the amygdala. *Synapse* 38:124–137.
- Yuan M, Lin Y (2006) Model selection and estimation in regression with grouped variables. *J R Stat Soc B* 68:49–67.