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Brief communication

Tachykinin-1-expressing parasubthalamic nucleus neurons are necessary for odorant-induced appetite suppression

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ABSTRACT

Odorants play a critical role in regulating feeding behavior by signaling potential threats or food sources in the environment. However, the neural mechanisms by which odorants affect feeding are not well understood. Tachykinin-1-expressing neurons in the parasubthalamic nucleus ($PSTN^{Tac1}$ neurons) are critical for reducing food intake in response to internal appetite-suppressing hormones, gastric distension, and external cues that signal danger. Therefore, we tested the hypothesis that activity in these neurons is modulated by exposure to aversive, attractive, and neutral odorants. Using fiber photometry in mice, we found that $PSTN^{Tac1}$ neurons increase activity in response to the aversive predator odorants 2-methyl-2-thiazoline (2MT) and 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), but not to neutral or attractive odorants. This activation correlates with a reduction in food intake and an increase in the latency to initiate feeding. Furthermore, chemogenetic inhibition of $PSTN^{Tac1}$ neurons blocks the suppression of feeding caused by 2MT and TMT. These findings highlight the specificity of $PSTN^{Tac1}$ neurons in processing aversive olfactory signals and their critical role in integrating external threat cues with internal signals that regulate appetite.

1. Introduction

Aversive and attractive odorants play a crucial role in regulating feeding behavior by signaling the presence of danger or potential food sources in the environment. For example, predator odors are potent environmental cues that trigger innate defensive behaviors in prey species, including mice. These odorants, such as 2-methyl-2-thiazoline (2MT) and 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), have been widely studied for their ability to elicit fear responses, immobility, and changes in autonomic function [1–3]. Elucidating the neural mechanisms by which the brain integrates aversive and attractive olfactory signals to modulate feeding behavior is essential for understanding the complex sensory dynamics underlying feeding.

In recent years, tachykinin-1-expressing neurons in the parasubthalamic nucleus (PSTN), a population of neurons located in the posterolateral hypothalamus, have been identified as key regulators of feeding behavior caused by both internal cues [4–6] and by fear-inducing environmental stimuli [7–9]. These $PSTN^{Tac1}$ neurons co-express pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide known to suppress feeding and promote avoidance behaviors [8]. $PSTN^{Tac1}$ neurons increase their activity in response to various appetite-suppressing stimuli including stomach distension [10], administration of appetite-suppressing hormones (e.g., amylin, CCK, PYY, and GLP-1) [4,10,11], or lipopolysaccharide, which mimics bacterial infections and induces hypophagia [5]. They also respond robustly to aversive predator odorants, such as TMT [7]. Notably, inhibiting $PSTN^{Tac1}$ neurons blocks the appetite suppression normally induced by these hormones [4,5,11], as well as the fear and avoidance behaviors triggered by TMT exposure [7]. Moreover, inhibition of these neurons has been shown to prevent fear-induced hypothermia caused by exposure to 2MT [9]. However, the specific role of $PSTN^{Tac1}$ neurons in mediating changes in feeding behavior following exposure to 2MT and TMT remains unexplored.

Given the ability of $PSTN^{Tac1}$ neurons to respond to both appetite-suppressing signals and aversive predator odorants, these neurons may be integral in mediating odorant-induced changes in feeding behavior. Additionally, it remains unknown whether other aversive or attractive odorants influence $PSTN^{Tac1}$ neuron activity and whether such changes affect feeding. Therefore, we aimed to determine whether exposure to a variety of aversive, attractive, or neutral odorants alters $PSTN^{Tac1}$ neural activity and whether these changes correlate with variations in food intake behavior. We also investigated whether the activity of $PSTN^{Tac1}$

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neurons is necessary for mediating odorant-induced changes in appetite, providing insight into their role as integrators of external environmental cues and internal appetite regulation.

2. Materials and methods

2.1. Animals

All experiments were approved by the Institutional Animal Care and Use Committee at Williams College and were performed in accordance with the guidelines described in the U.S. National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. We used *Tac1*$^{Cre/+}$ mice [12] bred on a C57Bl/6 background. Each experimental group was composed of a randomized selection of mice with identical sex ratios and ages. To comply with NIH guidelines for using both sexes of animals [13], we used an equal number of male and female mice across data sets. All mice were 7–9 weeks old at the time of surgery and no more than 16–20 weeks old at the cessation of experiments. During experimental procedures, mice were housed in individual cages with a 12 h/12 h light/dark cycle at 22 °C.

2.2. Virus preparation

Cre-inducible recombinant adeno-associated virus (AAV) vectors carrying GCaMP6s (AAV9-CAG-Flex-GCaMP6s, #100,842), mCherry (AAV9-hSyn-DIO-mCherry, #50,459), and hM4Di-mCherry (AAV9-hSyn-DIO-hM4Di-mCherry, #44,362) were obtained from Addgene. Viral aliquots were stored at –80 °C before stereotaxic injection.

2.3. Odorant preparation

The following odorants were used for experiments: 2-methyl-2-thiazoline (2MT), 97 % (Thermo Scientific Chemicals, #AAL0132922); 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), 90 % (BioSRQ, #1G-TMT-90); trimethylamine solution, 25 wt.% in water (Sigma Aldrich, #W324108), 2-phenylethylamine (PEA), 99 % (Sigma Aldrich, #807,334); cinnamaldehyde, 95 % (Sigma Aldrich, #W228613), peanut butter solution, 10 % in water; 0.1 M sucrose solution, and water. To prepare the 10 % peanut butter solution, 5.45 g of creamy peanut butter (Jif) was boiled in 45 ml distilled water until completely dissolved. 2MT, TMT, trimethylamine, PEA, and Cinnamaldehyde are all liquids at room temperature and no further dilutions were made.

2.4. Stereotaxic surgery

Mice were anaesthetized with 4 % isoflurane (Henry Schein Animal Health) and placed on a stereotaxic frame (David Kopf Instruments). Once on the frame and throughout the remainder of surgical procedures, mice received 1–2 % isoflurane trans-nasally. The skull was exposed and leveled in the horizontal plane. AAV was stereotaxically injected unilaterally or bilaterally, as described in the text, into the PSTN [anteroposterior (AP), –2.4 mm; mediolateral (ML), ±1.1 mm; dorsoventral (DV), –5.25 mm]. A total of 0.5 μl of virus was injected at a rate of 0.1 μl/min and was allowed 8–10 min to diffuse before the injection needle was removed. Following viral injection, mice used for fiber photometry experiments received unilateral surgical implantation of a mono fiber-optic cannula (Doric Lenses) above the PSTN (AP, –2.4 mm; ML, 1.1 mm; DV, –4.85 mm). The cannulae were fixed onto the skull with C&B Metabond (Parkell) and dental acrylic.

All mice were allowed at least 14 days to recover from surgery before the start of experimental procedures. Following behavioral experiments, brain sections containing the PSTN were examined for expression of virus and proper implantation of fiber-optic cannulae. Animals that did not show viral expression (mCherry or GCaMP fluorescence) or proper cannulae placement were not included in subsequent data analysis.

2.5. Fiber photometry

All fiber photometry experiments (Fig. 1) were performed in clear circular chambers (Pinnacle Technologies, #8228-SD) placed under a fume hood with fresh bedding for each trial. Mice were fed ad libitum before recording sessions, and all recordings took place during the middle four hours of the light (inactive) cycle. For three consecutive pre-trial days, mice were attached to optical patch cords (400 μm core, 0.48 NA, 1 m long; Doric Lenses) via zirconia connectors (Doric Lenses, Sleev_ZR_2.5_BK) and acclimated for 30 mins in circular chambers with two ~1.75-inch diameter cotton pads (CareOne Basic Cotton Rounds) under the fume hood (Fig. 1B). On the subsequent recording day, mice were again attached to a patch cord and placed in circular chambers with cotton pads for a 30-min habituation period before recordings took place. After a 20-min recording to measure baseline GCaMP signal, a third cotton pad with 20 μl of odorant was added for an additional 20 min recording period. Following each recording session, the experimental room and fume hood were cleared of all mice and odorants for at least 30 min before a new session began.

After each recording session, mice repeated a cycle of three acclimation days in which no odors were present, followed by a new recording session with a new odorant. To account for the possibility that the order of odorant presentation affects PSTN^{Tac1} neural activity, each mouse was assigned a random schedule of odor exposure. Each mouse was exposed to each odor only once, with a total of eight recording sessions for each of the eight odorants.

During each trial, a GCaMP excitation wavelength of 465 nm blue light modulated at 566 Hz was delivered through the patchcord. A control wavelength of 405 nm violet light modulated at 211 Hz was also delivered to detect calcium-independent GCaMP fluorescence or photobleaching. Delivered frequencies were offset to mitigate contamination or interference from electrical noise in the testing room. Excitation and control lights were generated from light emitting diodes (LEDs; Tucker-Davis Technologies, CLED_465 and CLED_405) and processed through a real-time amplifier (Tucker-Davis Technologies, RZ5P). Fluorescence signals were detected by a visible femtowatt photoreceiver (Tucker-Davis Technologies, Model 2151) with gain set to DC low. The light was then converted to electrical signals and demodulated by a real-time processor (Tucker-Davis Technologies, RZ5P). Data were recorded using Synapse software (Tucker-Davis Technologies).

Data were analyzed using custom MATLAB (MathWorks) scripts [14]. The 465 and 405 signals were independently downsampled to 1 Hz and normalized to baseline signals to determine ΔF/F, in which $\Delta F/F = (F - F_{baseline}) / F_{baseline}$, and $F_{baseline}$ is the median of 30 s baseline recording prior to time zero. No isosbestic normalization was introduced. To eliminate movement and bleaching artifacts, animals producing a recording with >25 % change in the 405 nm signal in any of the sessions were removed from the study. Plots representing mean ± standard error ΔF/F signals for each experiment and heatmaps representing ΔF/F for each trial were generated in MATLAB. Quantitative data analysis was performed in MATLAB and Prism 8.0 (GraphPad). The area under the curve was defined as the integral of ΔF/F from $t = 0$ until the time when ΔF/F returned to 0. The maximum ΔF/F was defined as the maximum ΔF/F signal intensity from $t = 0$ to $t = 300$ s.

2.6. Food intake measurements

For feeding assays (Fig. 2), mice were food deprived for 18 h prior to each trial. During experimental procedures, mice were habituated to a circular chamber with two cotton pads under a fume hood for 10 min. Next, one piece of standard mouse chow was added to the chamber simultaneously with a third cotton pad containing 20 μl of an odorant. Trials lasted for 5 mins, and food intake was measured as the change in chow mass after each trial. Additionally, the latency from the addition of food to the first bite of food was recorded. After each trial, mice were provided with 3 d ad libitum food access before the next trial began.

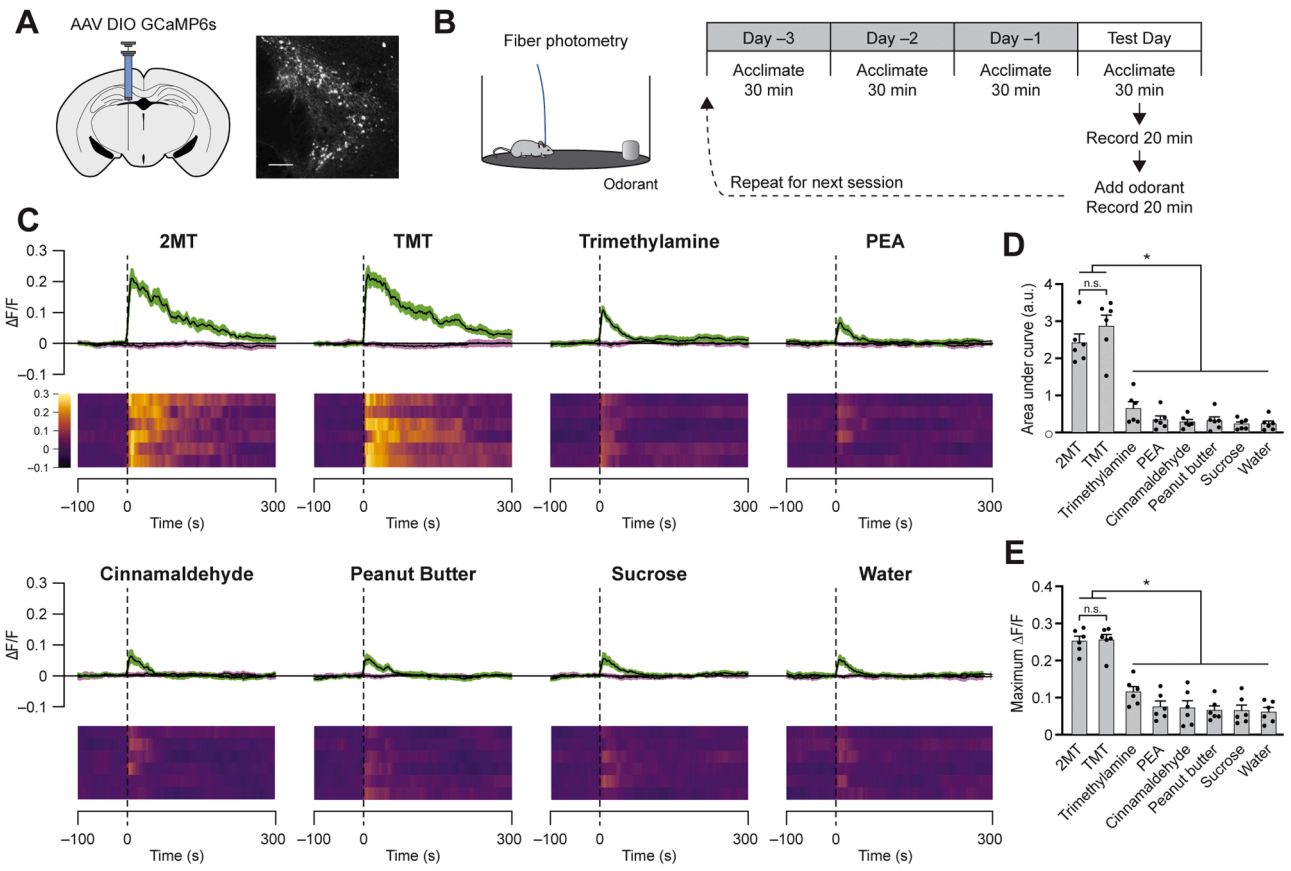

Fig. 1. Aversive predator odorants, but not other odorants, activate PSTN^{Tac1} neurons. **(A)** Diagram showing viral injection strategy to unilaterally target PSTN^{Tac1} neurons with GCaMP6s, along with representative photomicrograph showing GCaMP6s expression in PSTN^{Tac1} neurons. Scale bar, 100 μm. **(B)** Diagram depicting fiber photometry recording conditions in a single-housing chamber and timeline of acclimatization and recordings. **(C)** Top graphs, fiber photometry traces in PSTN^{Tac1} neurons following exposure to various odorants. Data represent the mean ± standard error. Vertical dashed lines depict time of odorant exposure. Bottom, heat maps depicting changes in fluorescence intensity in individual animals. **(D)** Quantification of area under the curve of fluorescence intensity among conditions. **(E)** Quantification of maximum values of fluorescence intensity among conditions. N = 6 mice in all experiments. Data represent mean ± standard error. Dots represent individual experimental animals. Post hoc comparisons: $^{*}p < 0.01$. See Supplementary File 1 for additional statistical information.

Each mouse was exposed to each odor only once, with a total of eight recording sessions for each of the eight odorants.

For chemogenetic experiments, food intake procedures were repeated in a new cohort of *Tac1*$^{Cre/+}$ mice in which PSTN neurons were transduced with either mCherry or hM4Di-mCherry. All mice received intraperitoneal injection of clozapine-N-oxide (CNO; 0.3 mg/kg, Sigma-Aldrich, #C0832) 20 min prior to the 10-min habituation period and subsequent 5-min odorant exposure and recording session. As before, mice were provided with 3 d ad libitum food access before the next trial began, and each mouse was exposed to each odor only once for a total of eight recording sessions.

2.7. Perfusions, sectioning, and microscopy

Mice were anesthetized with intraperitoneal injection of 2, 2, 2-tribromoethanol (Sigma-Aldrich, #48,402) dissolved in Tert-amyl alcohol and sterile 0.9 % saline. Mice were then transcardially perfused with cold 0.01 M phosphate buffered saline (PBS), pH 7.4, followed by 4 % paraformaldehyde in PBS. The brains were extracted, allowed to postfix overnight in 4 % paraformaldehyde at 4 °C, and cryoprotected in 30 % sucrose dissolved in PBS for an additional 24 h at 4 °C. Each brain was sectioned coronally at 30 μm on a microtome (Leica Microsystems) and collected in cold PBS. Brain sections were mounted onto SuperFrost Plus glass slides (VWR, #48,311–703) and either immediately used for in situ hybridization experiments, immunohistochemistry experiments, or coverslipped with DAPI Fluoromount-G (Southern Biotech, #0100–20) and stored in the dark at 4 °C.

Slides were examined using an Eclipse 80i epifluorescent microscope (Nikon) and images were captured using a Retiga 2000R digital camera (QImaging). The resulting images were minimally processed using Photoshop (Adobe) to enhance the brightness and contrast for optimal representation of the data.

2.8. Experimental design and statistical analysis

We used a within-subjects design for fiber photometry and food intake experiments and a between-subjects experimental design for chemogenetic experiments. To determine an effective sample size for statistical comparisons, we used an online power and sample size calculator (https://clincalc.com/stats/samplesize.aspx). Assuming a significance level of 0.05, this calculator shows that with at least four mice per group, we had an 80 % confidence level of achieving statistical significance between means of 1.1-fold. We excluded an animal from data analysis if flagged by an animal care technician for health reasons during the experimental period or if post hoc histological analysis showed no viral transduction as indicated by an absence of mCherry or GCaMP fluorescence.

Data were analyzed using Prism 9.0 (GraphPad Software). Statistical tests included one-way ANOVA with repeated measures (Figs. 1D and E; Figs. 2B and C); two-way ANOVA with repeated measures (Figs. 2G and H); and linear regression analysis (Figs. 2D and E), as described in the text and Supplementary File 1. Graphs were exported from Prism 9.0 to

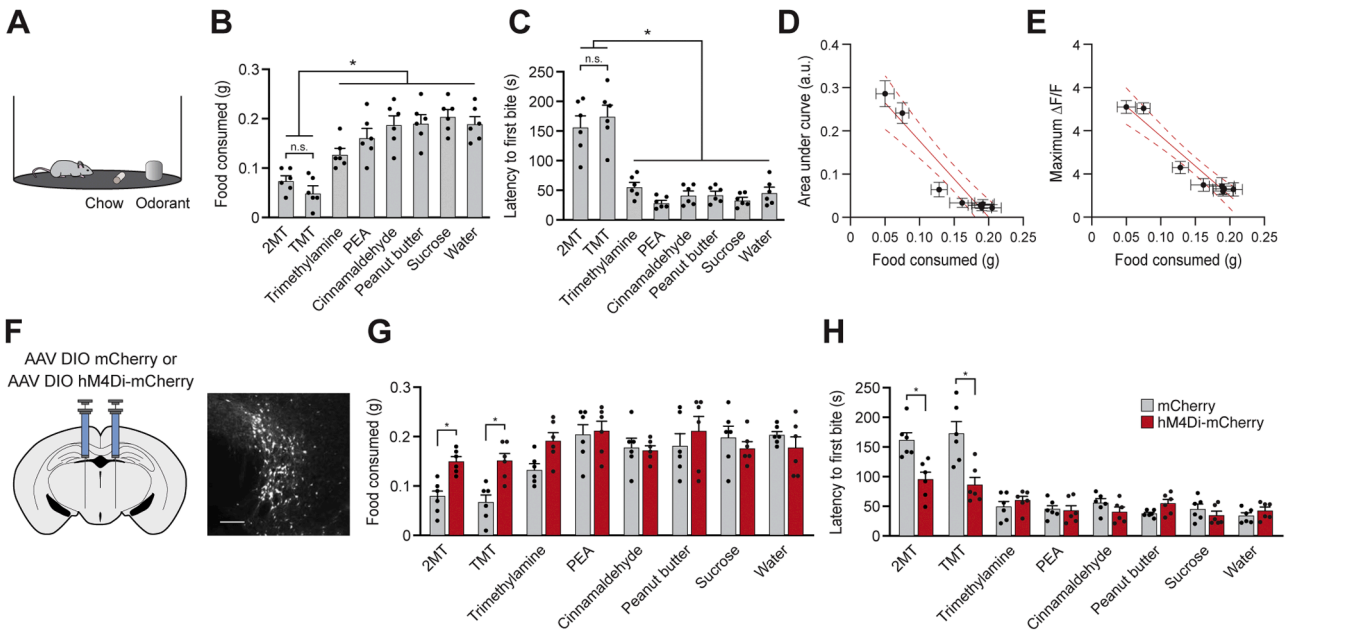

Fig. 2. Chemogenetic inhibition of PSTN^{Tac1} neurons attenuates the effects of aversive predator odorants on feeding behavior. **(A)** Diagram depicting 18-h food-deprived mouse exposed to mouse chow and odorant. **(B)** Food consumption and **(C)** latency to first bite of food following exposure to chow and odorants. **(D)** Correlation between the area under the curve of fluorescence intensity or **(E)** maximum value of fluorescence intensity from fiber photometry experiments vs food consumed following exposure to chow and odorants. **(F)** Diagram showing viral injection strategy to bilaterally target PSTN^{Tac1} neurons with hM4Di-mCherry or mCherry, along with representative photomicrograph showing hM4Di-mCherry expression in PSTN^{Tac1} neurons. Scale bar, 100 μm. **(G)** Food consumption and **(H)** latency to first bite of food following administration of CNO and exposure to chow and odorants. N = 6 mice in all experiments. Data represent mean ± standard error. Figures B-C, G-H, dots represent individual experimental animals; Figures D-E, dots represent fiber photometry parameters vs feeding parameters for a specific odorant. Dashed lines represent 95 % confidence intervals. Post hoc comparisons: *$p < 0.01$. See Supplementary File 1 for additional statistical information.

Illustrator (Adobe) for preparation of figures.

3. Results

To determine the effect of various odorants on acute activity patterns in PSTN^{Tac1} neurons, we transduced PSTN neurons in *Tac1*$^{Cre/+}$mice with the GCaMP6s calcium indicator and measured real-time fluorescence intensity using fiber photometry (Fig. 1A and B). For each odorant, mice were first acclimated to fiber optic cables under a hood with two cotton pads for 30 min for three consecutive days; on recording days, mice were acclimated to fiber optic cables for 30 min, and a baseline recording was made for 20 min before a third cotton pad with odorant was added to the cage (Fig. 1B). Aversive odorants included 2MT, TMT, and 2-phenylethylamine (PEA), all chemicals found in predator urine [1–3,15]. Attractive odorants included trimethylamine, found in mouse urine [16], and peanut butter and sucrose solutions. We also tested the effects of a neutral odorant, cinnamaldehyde, that does not elicit an innate emotional response in mice [9,17], as well as water as a negative control.

Fiber photometry recordings demonstrated individual differences in PSTN^{Tac1} neurons between the different odorants (Fig. 1C). Interestingly, addition of all odorants caused a minimal, transient increase in fluorescent intensity, including a neutral water stimulus—these changes likely represent brief PSTN^{Tac1} neuron responses to the addition of an additional cotton pad. However, the area under the curve measurements (Fig. 1D) and maximum fluorescence intensity (Fig. 1E) were significantly greater for 2MT and TMT relative to all other odorants. Therefore, PSTN^{Tac1} neurons increase activity in response to 2MT and TMT, but not the other odorants presented.

To measure the effects of these odorants on food intake, we food deprived mice for 18 h and subsequently measured feeding parameters during a 5-min exposure to mouse chow concomitant with addition of a cotton pad with individual odorant (Fig. 2A). Across all mice, food consumption was significantly decreased (Fig. 2B) and the latency to the first bite was significantly increased (Fig. 2C) during exposure to 2MT and TMT relative to all other odorants. The amount of food consumed in response to each odorant was inversely correlated with the degree of activation of PSTN^{Tac1} neurons (Fig. 2D and E), indicating that PSTN^{Tac1} neural activity correlates with both presentation of specific odors and the degree to which those odors decrease food intake.

To determine if PSTN^{Tac1} neural activity is necessary for the reduction in food intake following exposure to 2MT and TMT, we bilaterally injected AAV carrying either cre-inducible hM4Di-mCherry or mCherry transgenes into the PSTN of *Tac1*$^{Cre/+}$mice (Fig. 2F). Similar to previous experiments, we food deprived mice for 18 h. Mice were intraperitoneally administered clozapine N-oxide (CNO) 20 mins before exposure to mouse chow and a cotton pad with odorant. Administration of CNO attenuated the reduction in food intake (Fig. 2G) and increase in latency to first bite (Fig. 2H) caused by exposure to 2MT and TMT, but did not affect feeding parameters during exposure to the other odorants. Therefore, activity in PSTN^{Tac1} neurons is necessary for the reduction in food intake following exposure to 2MT and TMT.

4. Discussion

Taken together, we found that PSTN^{Tac1} neurons increase neural activity in response to the aversive predator odorants 2MT and TMT, but not the other attractive or neural odorants tested. The degree of activation of PSTN^{Tac1} neurons correlates with a reduction in food consumption and increased latency to initiate feeding. Notably, activity in PSTN^{Tac1} neurons is necessary for odorant-induced suppression of feeding. The heightened activity of PSTN^{Tac1} neurons in response to 2MT and TMT, along with their necessity in mediating odorant-induced appetite suppression, underscores the PSTN's critical role in integrating internal and external signals to regulate behavior.

Our novel finding that neutral and attractive odorants do not affect activity in PSTN^{Tac1} neurons suggests a high degree of specificity of this neural population in detecting and responding to olfactory cues

associated with danger. This specificity suggests that PSTN^{Tac1} neurons are highly tuned to prioritize immediate survival signals, such as predator odors, over non-threatening stimuli. Such selective responsiveness aligns with the PSTN's established role as a critical hub for integrating interoceptive information about physiological states, such as food intake, with emotionally salient environmental cues [6]. The preferential activation of these neurons by aversive stimuli may reflect their connectivity with broader neural circuits, including the central nucleus of the amygdala (CeA) and the lateral parabrachical nucleus (lPBN), both of which are central to mediating stress-induced autonomic and behavioral responses [4,6,8,11,18].The reciprocal connections between the PSTN, CeA, and lPBN allow for bidirectional communication, facilitating rapid and coordinated response to threats, such as defensive behaviors and physiological ailments. In contrast, the processing of attractive odors likely involves distinct neural circuits, such as those engaging hypothalamic populations or the mesolimbic dopamine system, which are essential for appetitive and reward-driven behaviors. The lack of response to neural odorants further underscores that PSTN^{Tac1} neurons are not involved in general olfactory processing but are selectively tuned to detect biologically salient, aversive cues.

The specificity of PSTN^{Tac1} neurons in responding to predator odorants raises the intriguing question of how other aversive odorants, such as those associated with spoiled food (e.g., butyric acid) or irritants like acetic acid, might engage distinct neural circuits. While these compounds are known to evoke strong aversive responses in rodents, they do not typically elicit the survival-critical defensive behaviors characteristic of predator odors or the lethargy and hunched-back posture observed during gastrointestinal malaise [19–21]. Notably, brain regions activated by predator odorants like TMT, including the CeA and PBN, appear to differ from those engaged by butyric acid [22]. This divergence suggests a functional specialization in the neural encoding of aversive stimuli, with CeA, PBN, and potentially PSTN^{Tac1} neurons dedicated to processing danger cues that require immediate defensive action, whereas other circuits may mediate the avoidance of unpleasant but non-threatening odors. Exploring the divergence of these pathways could provide deeper insights into the neural mechanisms underlying aversion.

Interestingly, all stimuli (even water) seemingly caused a transient activation of PSTN^{Tac1} neurons (Fig. 1C). Upon closer inspection, transient responses to PEA, cinnamaldehyde, peanut butter, sucrose, and water occurred only in a subset of trials (as evidenced in the heatmaps). Importantly, our experimental design involved a single trial per mouse per odorant, and not all mice exhibited transient responses to all odorants. In fact, observed responses tended to occur during the initial trials for a given mouse, suggesting that the brief activation of PSTN^{Tac1} neurons to non-predator odorants may reflect the novelty of introducing the cotton pad with odorant into the cage, which diminishes with repeated exposure as the mice habituate to the experimental context. The transient nature of their activation to addition of a novel object in the testing chamber may reflect a broader function of PSTN neurons in detecting potentially threatening environmental changes [8].

The effect of trimethylamine on PSTN^{Tac1} neurons, while not statistically significant, appears slightly larger than that of other neutral or positive odorants (Fig. 1C). This subtle difference raises the possibility that trimethylamine engages additional receptor systems beyond trace amine-associated receptors (TAARS), potentially contributing to weak PSTN activation. While trimethylamine primarily binds to TAARs, evidence suggests it may also interact with non-TAAR olfactory receptors, thereby influencing broader neural pathways [23]. This promiscuity in receptor binding could account for its deviation from other neutral or positive odorants. Moreover, the slight activation observed could reflect a lower engagement threshold for PSTN^{Tac1} neurons that is insufficient to elicit robust behavioral responses like food intake suppression.

It is interesting that the appetitive odorants peanut butter and sucrose did not cause increases in PSTN^{Tac1} neural activity, as such increases are observed when mice actively consume peanut butter [4]. This result suggests that the PSTN responds only to palatable food consumption and not the sensory detection of palatable food by the olfactory system. It is important to note, however, that the fiber photometry experiments performed here were conducted in mice fed ad libitum, while the effects of peanut butter consumption were observed in 18-h food-deprived mice. It is therefore possible that the response of PSTN^{Tac1} neurons to appetitive odorants may be dependent on hunger. Additionally, hunger may also modulate responses to predator odorants, as competing survival needs could influence the neural salience of threat versus food-related cues. Future studies could explore whether food deprivation amplifies or suppresses PSTN^{Tac1} neural responses to these biologically salient stimuli.

The ability of 2MT and TMT to evoke fear and defense responses depends on the expression of transient receptor potential ankyrin 1 (TRPA1) chemoreceptors in the vagal and trigeminal nerves [3]. Likewise, the behavioral effects of 2MT on eliciting fear-induced hypothermia and tail temperature increases via the PSTN is dependent on functional TRPA1 receptors [9]. Interestingly, cinnamaldehyde, the main odorant found in cinnamon, also binds to TRPA1 receptors but does not have the same fear-inducing or appetite-suppressing effects as the predator odorants 2MT and TMT, perhaps due to different kinetics of calcium influx in trigeminal neurons compared to 2MT [17]. Furthermore, a different predator odorant found in the urine of several carnivorous species, PEA, also does not cause PSTN^{Tac1} activation or suppress food intake. Because we did not dilute PEA and used a dose similar to previous studies [15], we do not believe the lack of effect is due to a low concentration. PEA, as well as the conspecific chemical trimethylamine, binds to TAARs, suggesting that the PSTN does not respond to odorants that activate other receptor types [15]. Thus, these findings indicate that the PSTN is specifically activated by predator odorants that bind to TRPA1 receptors, and TRPA1-binding odorants acutely suppress food intake via the PSTN.

While PEA is a predator-derived odorant found in terrestrial carnivores and binds to TAARs, the lack of significant effects on food intake or latency to eat in our data suggests that not all predator-associated odorants robustly engage PSTN^{Tac1} circuitry responsible for feeding suppression. One potential explanation is that the PSTN is specifically tuned to respond to predator-derived odorants that reliably signal acute and immediate threats, such as those mimicking active predation or direct proximity to predators, as opposed to cues that may represent more generalized predator presence. The lack of feeding suppression in response to PEA might also reflect a contextual distinction in how predator-derived odorants are encoded. While PEA is a predator-derived signal, it may lack the immediate and salient sensory features required to fully activate PSTN-mediated survival responses. The selective activation of PSTN^{Tac1} neurons by TRPA1 ligands, such as TMT and 2MT, suggests that these neurons are highly specialized to detect olfactory cues with a strong association to immediate danger.

Overall, our study highlights the critical role of PSTN^{Tac1} neurons in modulating feeding behavior in response to aversive odorants and suggests that these neurons serve as a central hub for integrating external threat signals with internal physiological states.

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CRediT authorship contribution statement

Zoe E. Kaegi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Matthew E. Carter:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None declared.

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Supplementary materials

Supplementary File 1. This file contains detailed statistical information for all data analyzed throughout this study.

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2025.114836.

Data availability

Data will be made available on request.

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