Serotonergic amplification of odor-evoked neural responses maps flexibly onto behavioral outcomes

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Abstract
Behavioral responses to many odorants are not fixed but vary based on organismal needs. How such variations arise and the role of various neuromodulators in achieving flexible neural-to-behavioral mapping is not fully understood. In this study, we examined how serotonin modulates the neural and behavioral responses to odorants in locusts (*Schistocerca americana*). Our results indicated that serotonin can increase or decrease appetitive behavior in an odor-specific manner. On the other hand, in the antennal lobe, serotonergic modulation non-selectively enhanced odor-evoked response strength but left the temporal features and the combinatorial response profiles unperturbed. This result suggests that serotonin facilitates sensitive and robust recognition of odorants. Nevertheless, the uniform neural response amplification appeared to be at odds with the observed stimulus-specific behavioral modulation. We show that a simple linear model with neural ensembles segregated based on behavioral relevance is sufficient to explain the serotonin-mediated flexible mapping between neural and behavioral responses.

Introduction
Often the same sensory stimulus can trigger different behavioral responses. For example, appetitive odorants that are attractive while hungry may not drive the same behavioral response after feeding to satiety.[1] How neural circuits process sensory stimuli to flexibly drive varying outcomes is not fully understood. It is hypothesized that different neuromodulators should be involved in mediating state-dependent changes in neural and behavioral responses.[2,3] However, whether neuromodulation globally changes responses to sensory cues (i.e. non-specific increases or decreases), or mediates selective alterations in sensory input-driven behavior, and how this is achieved, is also not clear.[4]

In insects, serotonin is a key neuromodulator that is linked with the regulation of many behaviors, including feeding[5], socialization[6–8], aggression[9,10], and mating[11,12]. Food intake is known to be highly dependent on the serotonin levels in the brain of an organism. Elevated levels of serotonin have been shown to decrease the time spent feeding and the amount of consumed food in blowflies and flesh flies.[13,14] In *Drosophila* larvae and adults, inhibition of neural serotonin synthesis or global increase of serotonin levels, increases or suppresses food intake, respectively.[15–17] In locusts, serotonin is linked to triggering phenotypical plasticity. A spike in serotonin levels in the locust brain is highly correlated with the solitary stage,[18] whereas, high levels of serotonin in the locust thoracic ganglion are known to trigger the transformation of solitary animals into gregarious ones.[7,8] Serotonin modulation has also been linked with increased activity and aggression in crickets and flies,[10,19] as well as reduction of courtship behaviors, like male wing extension towards female and coupling in flies.[12]

How serotonin alters sensory processing to drive these behavioral changes is yet to be completely resolved. In olfaction, serotonin is known to modify the processing of odor-driven neural signals right from the periphery. In both vertebrates and invertebrates, the serotonergic

release is known to the gate and reduce olfactory sensory neuron output to the following circuit through enhancement in pre-synaptic GABAergic inhibition.[20–22] In contrast, exogenous serotonin is known to increase the odor-evoked responses of second-order neurons in the insect antennal lobe and vertebrate olfactory bulb.[20,23] The serotonin-mediated increase in odor-evoked responses has been reported to be odor-specific and hypothesized to enhance the sensitivity to odorants in a state-dependent manner.[24]

How are odor-evoked neural responses modulated to produce flexible, odor-specific changes in behavioral outcomes? In this study, we examined this question in the locust olfactory system. We show that serotonin can increase or decrease innate appetitive behavioral responses in an odor-specific manner. In contrast, exogenous serotonin increased the strength of odor-evoked neural responses for all odorants without altering the temporal processing features or the ensemble response fidelity. We present a simple model to map the serotonin-mediated non-selective amplification of neural responses onto odor-specific changes in behavioral outcomes. Finally, we examine the relevance of these findings for modulating hunger-state-dependent modulation of appetitive responses in locusts. In sum, our results provide a more systems view of how a specific neuromodulator (serotonin) alters neural circuits to produce flexible behavioral outcomes.

Results

Serotonin modulates appetitive behavior in an odor-specific manner

We began by examining how serotonin modulates odor-driven innate behavioral responses in locusts (*S. americana*). In this assay, starved locusts opened their sensory appendages close to the mouth, called maxillary palps, when encountering certain food-related odorants.[25–28] We examined the palp-opening responses (POR) for an odor panel including four odorants: hexanol (HEX), benzaldehyde (BZA), linalool (LOOL), and ammonium (AMN). Note that hexanol (green) is a green-leaf volatile[29], and benzaldehyde (blue) is a putative locust aggregation pheromone[30]. Whereas, linalool (red) is used in many pesticides.[31] Hence, this panel included odorants that have diverse behavioral preferences.[28] Each odorant was delivered at 1% v/v concentration and presented for ten trials or repetitions in a pseudorandomized order (see Methods).

We used a binary metric to categorize the presence or absence of PORs **(Figure 1A)**. The response matrix across locusts (rows) and trials (columns) is summarized in **Figure 1B**. It is worth noting that *HEX* evoked supra-median POR responses, *BZA* and *LOOL* has a response close to the mean POR response level (mean POR = 0.43 across odorants and locusts). In contrast, *AMN* elicited sub-median POR responses across locusts. For every locust, we also examined the PORs before and after serotonin (5HT) injection (see Methods). Notably, we found that the probability of PORs (**Figure 1C**) changed after 5HT injection but only for a subset of odorants (HEX and BZA). Intriguingly, the PORs to LOOL reduced after 5HT injection. Injection of saline or merely repeating the same set of odorants after a three-hour time window (similar time frame as before and after 5HT injection) did not produce any significant change in the PORs (**Supplementary Figs. 1 and 2**). These results suggest that 5HT altered appetitive behavioral responses in an odor-specific manner.

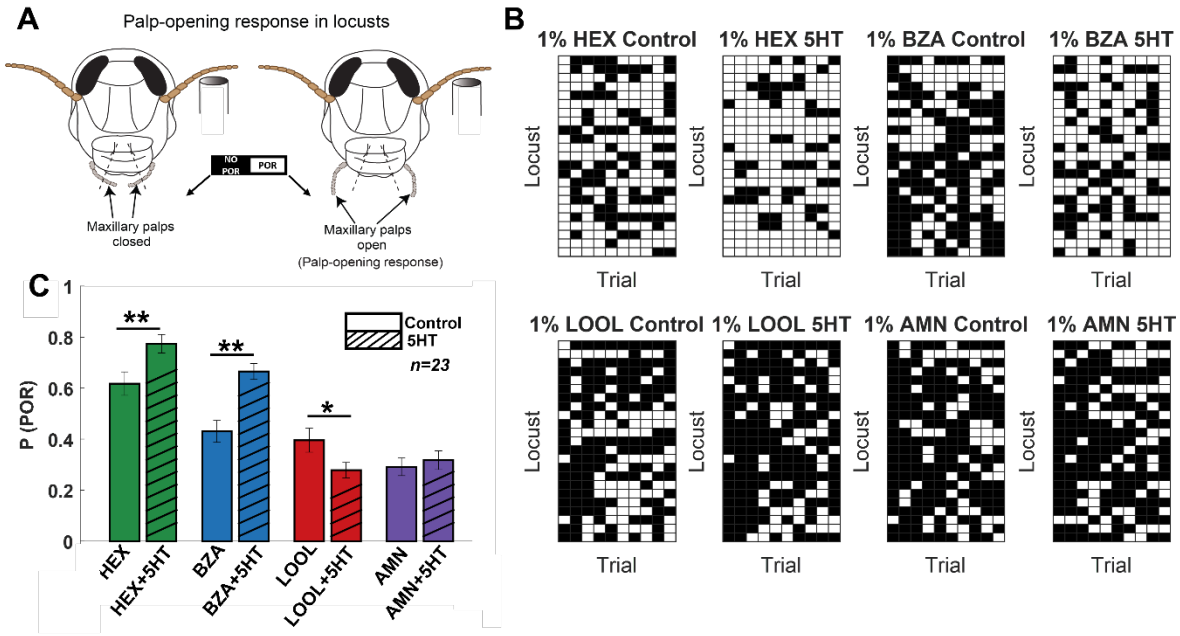

Figure 1. **Serotonin modulates innate appetitive behavioral responses in an odor-specific manner.**
(A) *A schematic of the locust palp opening response (POR) is shown. Starved locusts (> 24 hours) were presented with a panel of four odorants (hexanol, (HEX); benzaldehyde, (BZA); linalool, (LOOL); ammonium, (AMN)) at 1% v/v dilution. Each locust was presented with ten trials of each odorant. The odor pulse duration was 4 seconds and the time period between two consecutive odor exposures (inter-trial interval; ITI) was 56 seconds. The movement of the palps during the odor presentation was identified as a positive POR. The presence or absence of a POR was noted for each trial.*
(B) *A summary of trial-by-trial PORs for each locust is shown. Each trial was categorized by the presence (white box) or absence (black box) of a POR. Each row represents the PORs recorded from a single locust, and each column indicates a specific trial. PORs of twenty-three locusts were recorded and summarized as a response matrix. The POR response matrix for the same set of locusts before and after 5HT injection is shown to allow comparison.*
(C) *The PORs before and after (5HT) serotonin injection are summarized and shown as a bar plot for all four odorants in the panel. Striped bars signify the data collected after 5HT injection. Significant differences are identified in the plot (one-tailed paired-sample t-test; (*p<0.05; **p<0.01; standard paired sample t-test).*

Serotonin enhances behavioral sensitivity to odorants

Next, we wondered whether serotonin introduction altered the behavioral dose-response relationship. To examine this, we recorded PORs to the same four odorants at widely different concentrations **(Figure 2**; spanning four log units of concentration). First, even without serotonin application, it can be noted that PORs to the odorants tended to increase as a function of odor intensity. Further, the increase was more significant for HEX and BZA, two odorants that generally elicited more PORs. However, note that there was a detectable decrease in PORs at the highest concentration of HEX and BZA.

Injection of serotonin increased the POR level of all concentrations of HEX and BZA. In contrast, changes in PORs elicited by LOOL and AMN were only modestly modified. Hence, these findings imply that serotonin enhanced appetitive behavioral responses over a wide range of concentrations but only for a subset of odorants (HEX and BZA). Furthermore, the reductions in behavioral responses at the highest intensity of HEX and BZA were no longer detectable or significant after 5HT treatment. In sum, these results indicate that the overall sensitivity to appetitive odorants is enhanced after serotonin application, and increases in behavioral response are maintained over a wide range of concentrations.

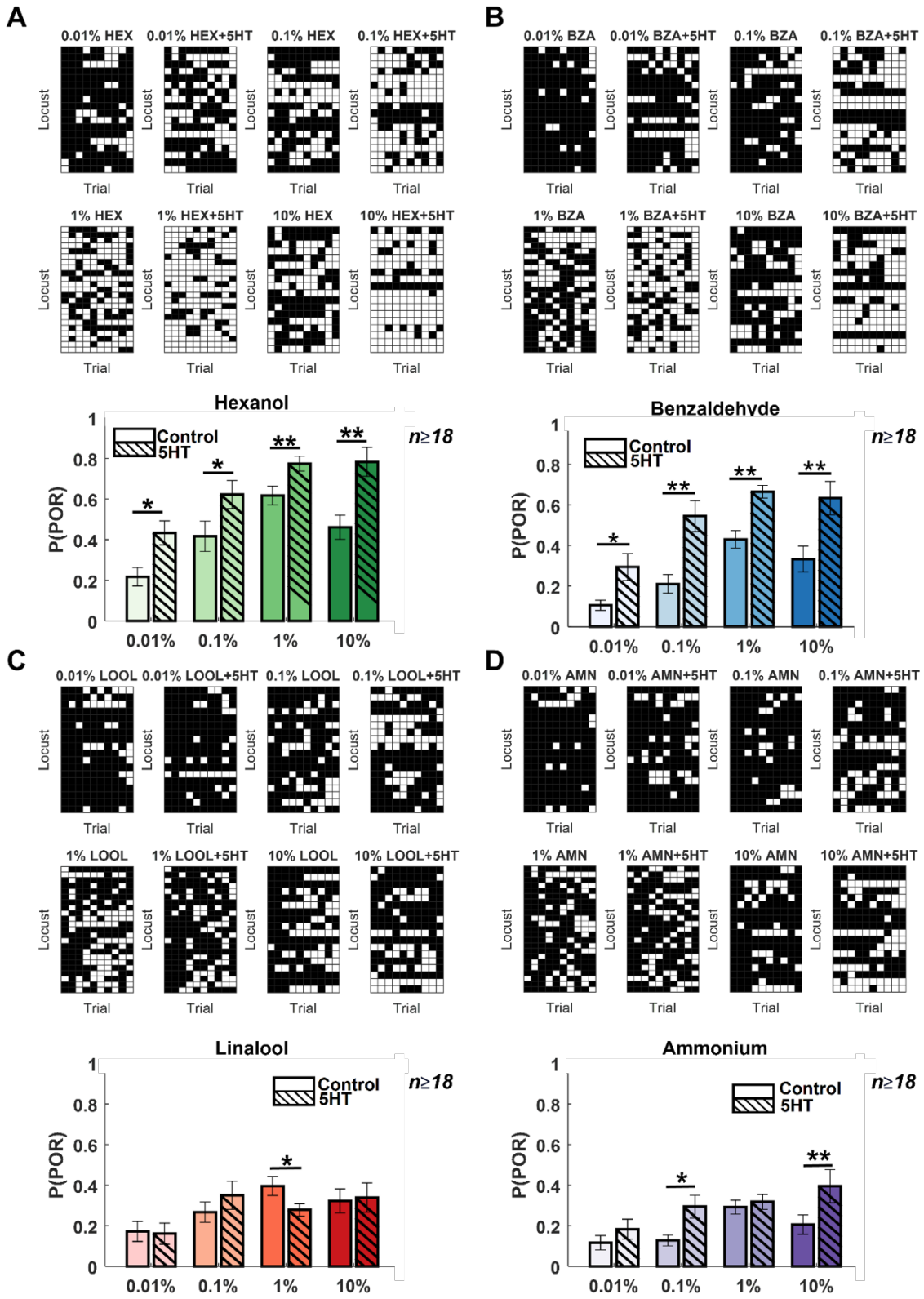
A
0.01% HEX
0.01% HEX+5HT
0.1% HEX
0.1% HEX+5HT
1% HEX
1% HEX+5HT
10% HEX
10% HEX+5HT
Locust
Trial
Hexanol
Control
5HT
n≥18
P(POR)
0.01%
0.1%
1%
10%
B
0.01% BZA
0.01% BZA+5HT
0.1% BZA
0.1% BZA+5HT
1% BZA
1% BZA+5HT
10% BZA
10% BZA+5HT
Benzaldehyde
C
0.01% LOOL
0.01% LOOL+5HT
0.1% LOOL
0.1% LOOL+5HT
1% LOOL
1% LOOL+5HT
10% LOOL
10% LOOL+5HT
Linalool
D
0.01% AMN
0.01% AMN+5HT
0.1% AMN
0.1% AMN+5HT
1% AMN
1% AMN+5HT
10% AMN
10% AMN+5HT
Ammonium

Figure 2. **Serotonin alters the dose-response relationship for select odorants.**
(A-D) *POR matrix for four odorants at four different concentration levels: 0.01%, 0.1%, 1%, 10% v/v dilution are shown. The same color conventions as Figure 1 (black – no POR and white – POR) are used. Bar plots represent the probability of POR for each concentration and odor (p(POR)).* ***Left*** *solid bar shows the p(POR) for each odorant as a function of concentration before serotonin injection.* ***Right*** *striped bar summarizes p(POR) after serotonin injection for the same set of locusts (*p<0.05; ** p<0.01 standard one-tailed paired sample t-test).*

Serotonin alters the spontaneous activity of projection neurons in the antennal lobe
Next, we investigated the neural basis of the observed serotonergic modulation of behavioral outcomes. To assess this, we intracellularly monitored the spiking activity of projection neurons *(PNs)* in the antennal lobe. We first compared the spontaneous spiking activities of the same neuron before and after *(5-HT)* serotonin treatment (**Figure 3A vs. 3B**). Note that the baseline spontaneous firing patterns in individual projection neurons changed after bath application of serotonin. The PNs fired spontaneous bursts of spikes after serotonin exposure (**Figure 3A** (before 5HT) vs. **3B** (after 5HT). To quantify these differences, we computed the inter-spike interval (ISI) distributions across all PNs (**Figure 3 C-E;** *n = 82 PNs*). The ISI distributions after the 5HT application showed a left shift indicating that pairs of consecutive spikes occurred in quick succession (**Figure 3C**). Consistent with these results, the median values for the ISI distribution computed for each PN showed a significant reduction (**Figure 3D**). Finally, we plotted the median ISI before serotonin (control; along the x-axis) against median ISI values after serotonin application (y-axis) for each PN (**Figure 3E**). As can be noted, the point represents a PN. Clustering closer to the X-axis indicates that serotonin altered the excitability of PNs and induced bursts of spikes.

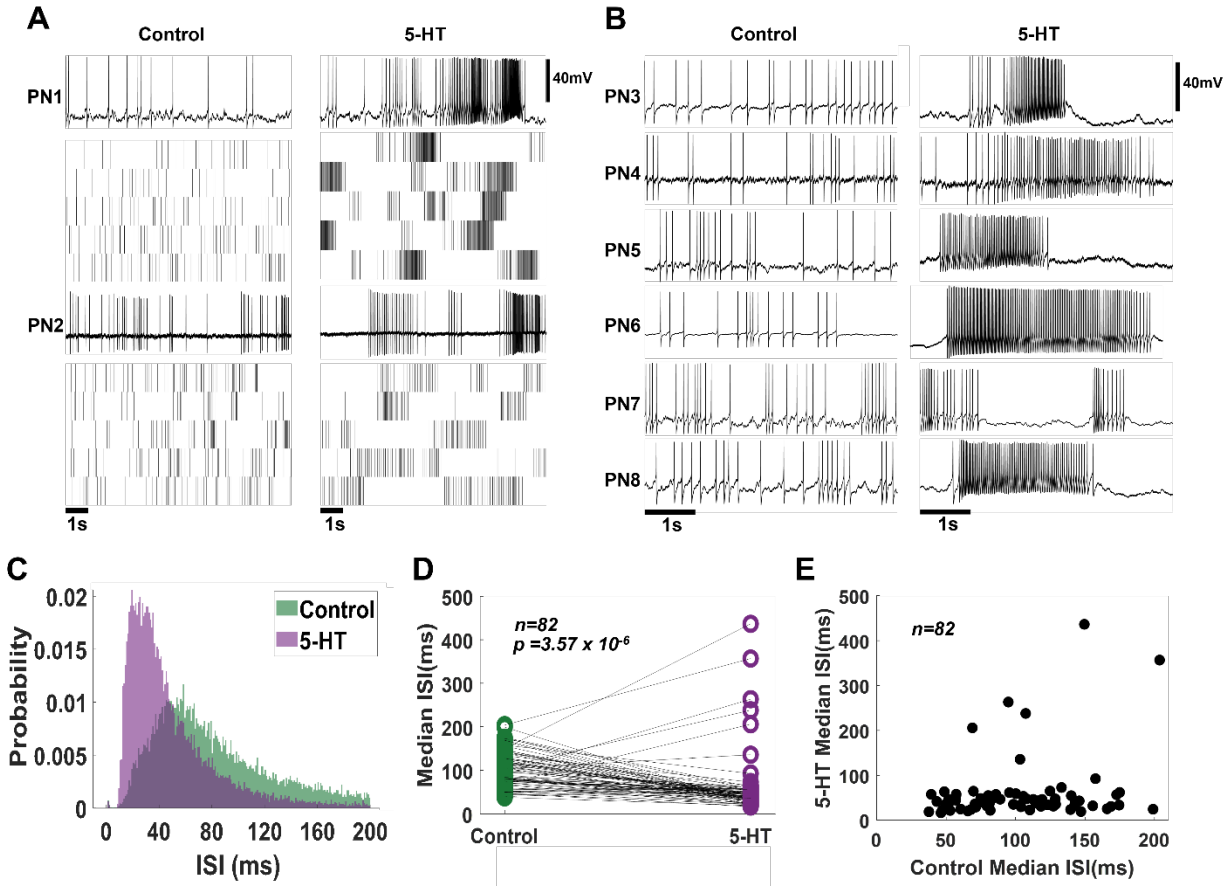

Figure 3. ***Exogenous serotonin induces bursting behavior in antennal lobe projection neurons.***
(A)*Representative intracellular recordings showing membrane potential fluctuations as a function of time for two separate projection neurons (PNs) in the locust antennal lobe. A ten-second window when no odorant was presented is shown. Raw voltage traces are shown during the first trial, and spiking rasters are shown for the subsequent trials. Firing patterns before (left) and after (right) serotonin application are shown for comparison. Note, the spiking activity becomes more bursty after the 5HT application.*
(B) *Firing patterns for a larger set of PNs are compared before (control) and after (5-HT) serotonin. Changes in PN excitability are observed in all recorded PNs.*
(C) *The distribution of Inter-spike intervals (ISI) across PNs is shown before serotonin application (control; in green), and after serotonin application (purple). Note, the purple histogram is taller and shifted to the left indicating shorter gaps between consecutive action potentials.*
(D) *Comparison of the median ISIs for each individual PN before (control) and after (5HT) serotonin application. The black line connects median ISI values for a single PN in control and 5HT conditions. Note, the majority of the black lines are tilted downwards indicating a reduction in the gap between spikes.*
(E) *Median ISI values before and after the 5HT application are plotted for each PN.*

Serotonin modulates odor-evoked response intensity but not timing

As noted, serotonin modulated the excitability of all individual PNs we recorded. How are the odor-evoked responses modulated? And does serotonergic modulation confound the information about odor identity? To examine this, we analyzed the odor-evoked responses of eighty-two PN-odor combinations. In addition to the four odorants examined in behavioral experiments, the odor panel used included three additional odorants (4-vinyl anisole, 1-nonanol, and octanoic acid). Consistent with previous observations, projections neurons responded to odorants by increasing their spiking activity either during the odor presentation (ON response) or after the stimulus termination (OFF response) (**Figure 4A**). These odor-evoked response patterns were consistent across trials.

We examined whether the odor-evoked response timing (ON vs OFF responses) was preserved after the serotonin application (**Figure 4A**). As can be noted, the stimulus-evoked ON and OFF responses in these four representative PNs remained intact after the 5HT application. We found that for a majority of the PNs in our dataset, that exhibited either ON or OFF response, the response timing was maintained after 5HT application **(Figure 4A–C).** Notably, ON responses tended to be more robustly maintained after the 5HT application compared to the OFF responses (**Figure 4C, F**). Furthermore, most of the non-responsive PNs continued to remain inactivated during the odorant presentation after serotonin introduction.

What variations in spiking patterns were observed after the 5HT application? Our results indicate that the total number of spikes (i.e. response magnitude) evoked by an odorant increased after 5HT application during both ON and OFF time periods (**Figure 4D, F).** Note that the mere introduction of saline or the solvent in which serotonin was diluted (HCl) did not alter the PN responses (**Supplementary Figure 2**). In sum, these results indicate that serotonin modulates the excitability of individual PNs but only those PNs that are activated by an odorant tended to increase their response magnitude. Further, the response timing (ON vs OFF periods) was robustly maintained across PNs.

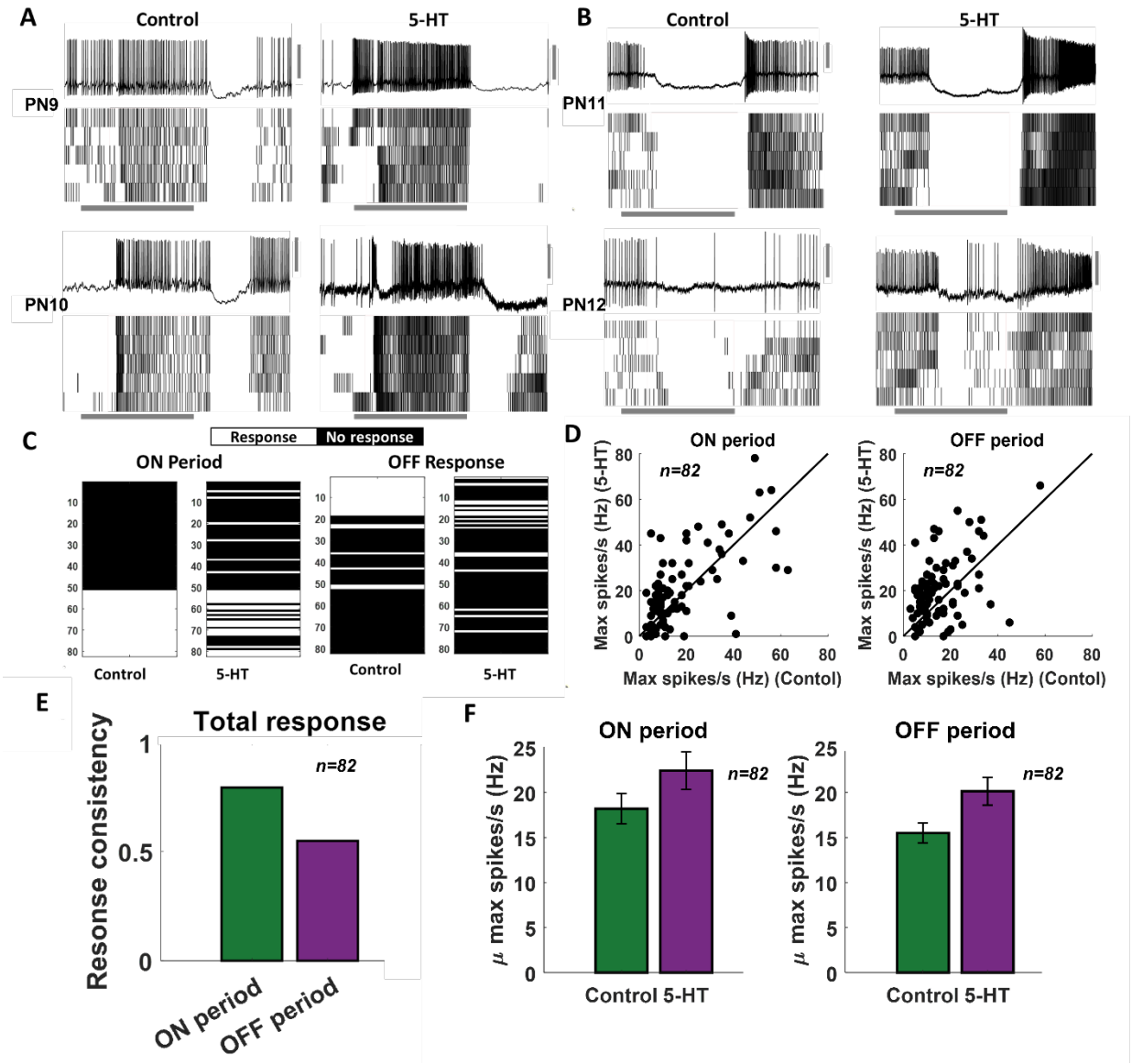

Figure 4. **Serotonin alters the magnitude of odor-evoked spiking activity but not its timing.**
(A-B) *Representative plot of the odor-evoked ON responses (PN9-10) and OFF responses (PN11-12) for five trials are shown. The first trial is shown as a raw voltage trace. Spiking activities during all five trials are shown as a raster plot. The horizontal grey bar indicates the four second odor delivery period. The vertical grey scale bar identifies 40mV.*
(C) *A binary plot categorizing PN activity as responsive or non-responsive during odor presentation (ON responses) or after odor termination (OFF responses). Response categorization before and after %HT application are shown for each PN to examine response robustness.*
(D) *Left panel: Peak spiking activity for each PN during odor presentation in the control condition and after 5HT application. Right panel: Comparing peak spiking activity observed during the OFF period.*
(E) *Fraction of PNs that maintain their response or lack of response to an odorant before and after 5HT application are quantified during ON and OFF periods.*
(F) *Left panel: Mean odor-evoked spiking activity across PNs during odor presentation in the control condition and after 5HT application is compared. Error bars indicate SEM. Right panel: Similar plot comparing mean spiking activity across cells during the OFF period.*

Robust encoding of odor identity

Is the identity of the odorant robustly encoded by the ensemble-level odor-evoked responses? To understand this, we first visualized the overall PN responses across all neuron-odor combinations in our dataset (**Figure 5A**). Consistent with the individual PN analyses, the changes in spontaneous activities and stability of spiking responses during the odor presentation period can be readily observed. To qualitatively analyze this, we visualized the neural activity using principal component analysis dimensionality reduction. The 82-dimensional PN spike counts in 50ms time-bins were projected onto the top two eigenvectors of the response covariance matrix and connected in the order of occurrence to generate closed-loop trajectories (**Figure 5B**). Neural response trajectories during (ON) and after (OFF) odor presentation periods activated different subsets of PNs and therefore did not have much overlap during these time periods. We also plotted the neural response trajectories before and after the 5HT application to allow comparison. Our results indicate that the ON response trajectories before and after 5HT overlapped with each other indicating that the ensemble activity across the eighty-two PNs was similar before and after serotonin application. Similar results were also observed during the OFF-response window.

To further support the qualitative dimensionality reduction analysis, we performed a quantitative correlation analysis using high-dimensional PN activity vectors (**Figure 5C**). As can be noted, the spontaneous activity structure before and after serotonin changed, and as a result, the correlation between them became negative. Consistent with the results from the PCA analysis, we found that the odor-evoked responses before and after the 5HT application were highly correlated.

Taken together, these results indicate that the identity of the odorant is robustly maintained by ensemble neural activity. Both ON and OFF responses continue to maintain the information they carry about the odorant.

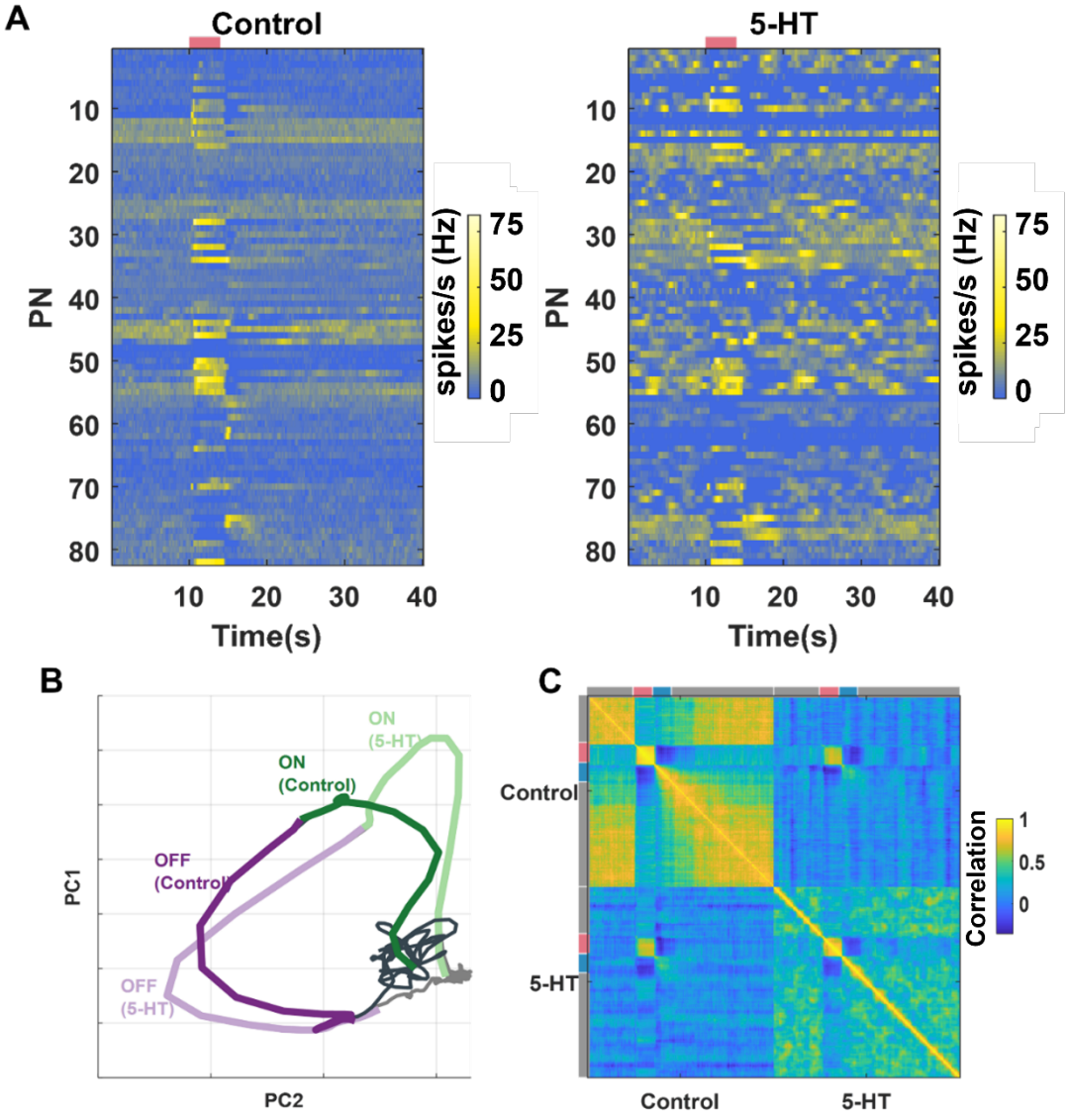

Figure 5. **Ensemble-level odor-evoked response patterns robustly maintain odor identity after 5-HT treatment.**
(A) *Trial-averaged spiking activity as a function of time is shown for eight-two neurons. The hotter color identifies the higher average firing rates per bin (200 ms). Each row represents one PN, each column represents a one-time bin. The red bar identifies the odor presentation time period. The heatmap on the left shows the PN activity matrix before the 5HT application, and the heatmap on the right shows the PN activity matrix (neurons sorted in the same order) after(5-HT) exposure.*
(B) *PN odor-evoked responses (n=82 projection neuron-odor combinations) are visualized after dimensionality reduction using Principal Component Analysis (PCA). The neural responses were binned in 50 ms windows and projected onto the top two eigenvectors of the response covariance matrix and connected in the order of occurrence to generate the response trajectory shown. Neural response trajectories evoked during the OFF period are shown in purple, and ON response trajectories are shown in green. Darker colors indicate response trajectories before the 5-HT treatment, and lighter shades show neural trajectories after the 5-HT treatment.*
(C) *Correlation matrix summarizing the similarity between each 82-dimensional PN activity vector with all other response vectors is shown. Different time segments (spontaneous (grey), odor ON (red), and odor OFF (blue)) are indicated along the X and Y axis. Hotter color indicates a higher correlation.*

A simple linear model explains serotonergic modulation of neural-behavioral mapping

Our results indicate that serotonin modulated behavioral responses in an odor-specific manner. However, neural responses elicited by all odorants increased (**Figure 6A**). These results seem to be at odds with each other. Hence, to gain mechanistic insights regarding how 5HT uniformly amplifies neural responses and yet generates odor-specific changes in behavioral outcomes, we performed a simple linear regression (**Figure 6B**). We used a previously published extracellular recording dataset[28] consisting of odor-evoked responses of 89 PNs to HEX, BZA, and LOOL to build this model. The output of the model was the amount of increase or decrease in PORs compared to the overall mean response levels across all odorants used in our behavioral experiments (see Methods). Note that this model is equivalent to assigning a weight to each projection neuron and using the weighted sum of projection neuron responses to generate the observed POR output.

We hypothesized that the antennal lobe projection neurons can be divided into two non-overlapping ensembles: Encoding Ensemble 1 is assigned mostly positive weights and Encoding Ensemble 2 is assigned negative weights (**Figure 6C**). Odorants that evoke supra-median PORs should activate more neurons in Encoding Ensemble 1, and those that produce sub-median level PORs are expected to activate neurons in Encoding Ensemble 2 more. It is worth noting that this architecture is similar to having 'neuron – anti-neuron' pairs where one decoding neuron weighs the positive contribution to generate PORs, and the second decoding neuron collects the negative contributions and suppresses the same behavioral output. Such 'neuron – anti-neuron' pairs have been utilized for predicting overall motor outputs[32–34], and are highly consistent with the emerging view from other insect models that have shown mushroom body output neurons form segregated channels to drive opposing behaviors[35,36].

Our results indicate that this simple linear regression-based model was sufficient to robustly predict the observed PORs (**Figure 6D**). Furthermore, as expected HEX and LOOL activated highly distinct neural ensembles. HEX-activated projection neurons received mostly positive weights and LOOL-activated neurons received negative weights (**Figure 6E**). Any increase in positively-weighted PN responses (HEX response after 5HT) should increase the overall POR, whereas an increase in negatively-weighted PN responses (LOOL after 5HT) should similarly decrease the behavioral output. Segregating odor encoding into behavior-specific channels in the antennal lobe would allow serotonin to amplify neural responses to all odorants, and yet produce odor-specific increase or decrease.

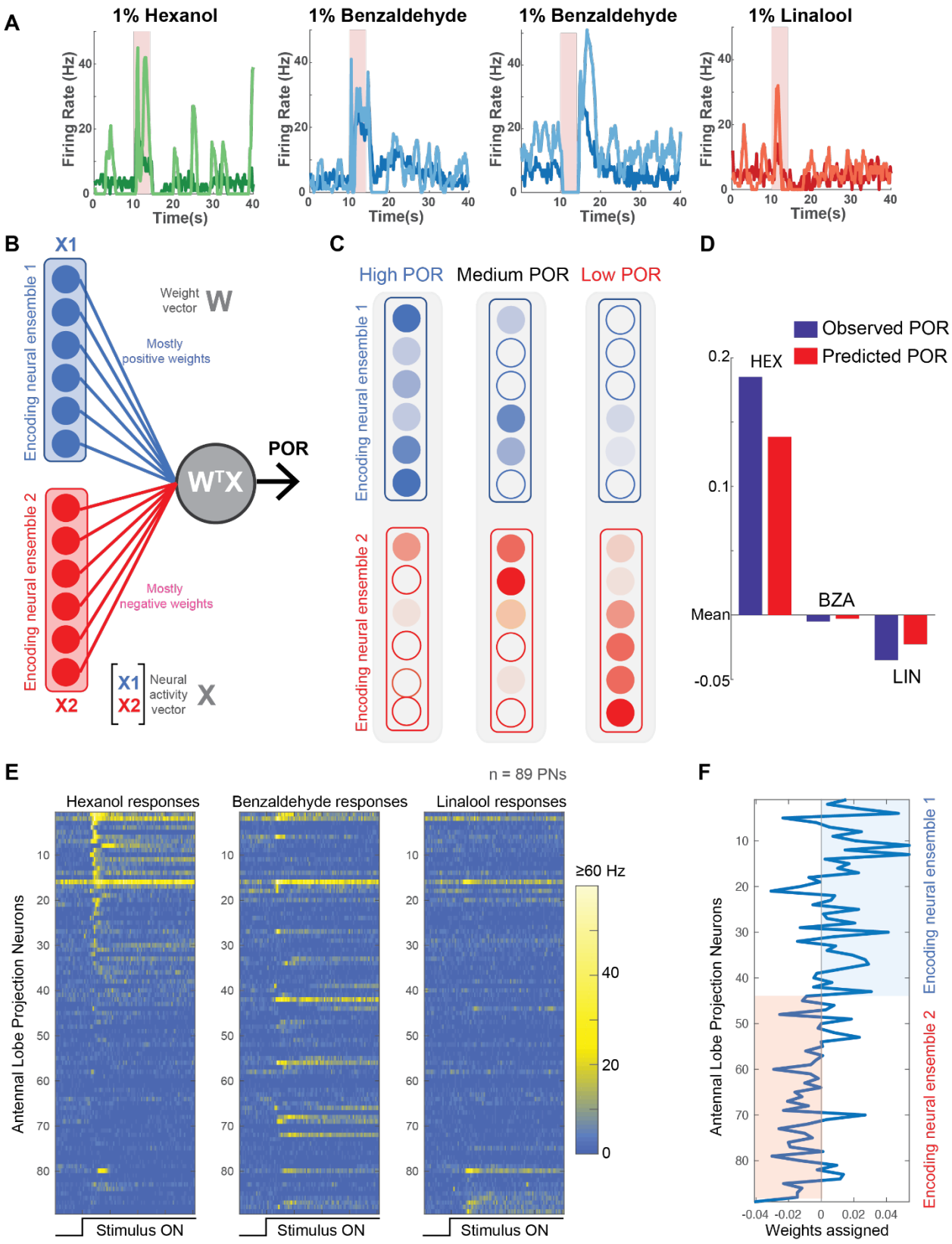
A
1% Hexanol
1% Benzaldehyde
1% Benzaldehyde
1% Linalool
Firing Rate (Hz)
Time(s)
B
X1
Encoding neural ensemble 1
Weight vector W
Mostly positive weights
POR
W^TX
Encoding neural ensemble 2
Mostly negative weights
X2
X1 X2 Neural activity vector X
C
High POR
Medium POR
Low POR
Encoding neural ensemble 1
Encoding neural ensemble 2
D
Observed POR
Predicted POR
HEX
BZA
LIN
Mean
E
n = 89 PNs
Hexanol responses
Benzaldehyde responses
Linalool responses
Antennal Lobe Projection Neurons
Stimulus ON
≥60 Hz
F
Antennal Lobe Projection Neurons
Weights assigned
Encoding neural ensemble 1
Encoding neural ensemble 2

Figure 6: **Neural data maps onto the behavioral results.**
(A) *PSTHs of four representative PNs are shown before (darker shade color) and after 5HT (lighter shade color) application. Note that 5HT increased overall response amplitudes to all odorants in the panel.*
(B) *A schematic of the linear model used for predicting PORs given neural data. Each neuron is assigned a weight. The weighted sum of PN activity is fit to PORs values for HEX, BZA, and LOOL (see Methods). The neurons were split into two ensembles based on their assigned weights.*
(C) *Odorants that evoke stronger PORs are expected to activate more PNs that receive positive weights. In contrast, odorants that reduce POR output compared to the mean response are expected to activate more PNs that received negative weights.*
(D) *Comparison of observed versus predicted POR values across locusts for the three odorants used in the model.*
(E) *Odor-evoked responses of 89 PNs to HEX, BZA, and LOOL are shown. The PNs are ordered based on the difference in peak responses to HEX and LOOL (i.e. HEX activated PNs are at the top and LOOL-activated neurons are at the bottom).*
(F) *Weights assigned to each PN are shown. PNs are ordered in the same as in* ***panel E****.*

Hunger-state dependent serotonergic modulation of appetitive behavioral responses. Serotonin is regarded as one of the neuromodulators associated with feeding behaviors.[5] Therefore, we wondered whether serotonin modulates behavioral appetitive responses of locusts in a hunger-state-dependent manner. To understand this, we first examined the PORs in locusts that were fed grass blades before the experiments (satiated). **(Figure 7)** Compared to hungry locusts **(Figure 1)**, the fed locusts responded less to HEX and BZA. However, consistent with results from hungry locusts, the introduction of serotonin increased the appetitive response to HEX and BZA. Intriguingly, the appetitive responses of fed locusts treated with 5HT were comparable to or slightly higher than the responses of hungry locusts to the same set of odorants. It is worth noting that responses to LOOL and AMN, non-food related odorants with weaker PORs, remained unchanged in fed locusts treated with 5HT. Therefore, we conclude that, like in many species, serotonin influences food-driven behaviors in locusts.

In sum, our results reveal a clear mapping between serotonergic modulation of odor-driven neural responses and how it regulates innate appetitive behavioral outcomes.

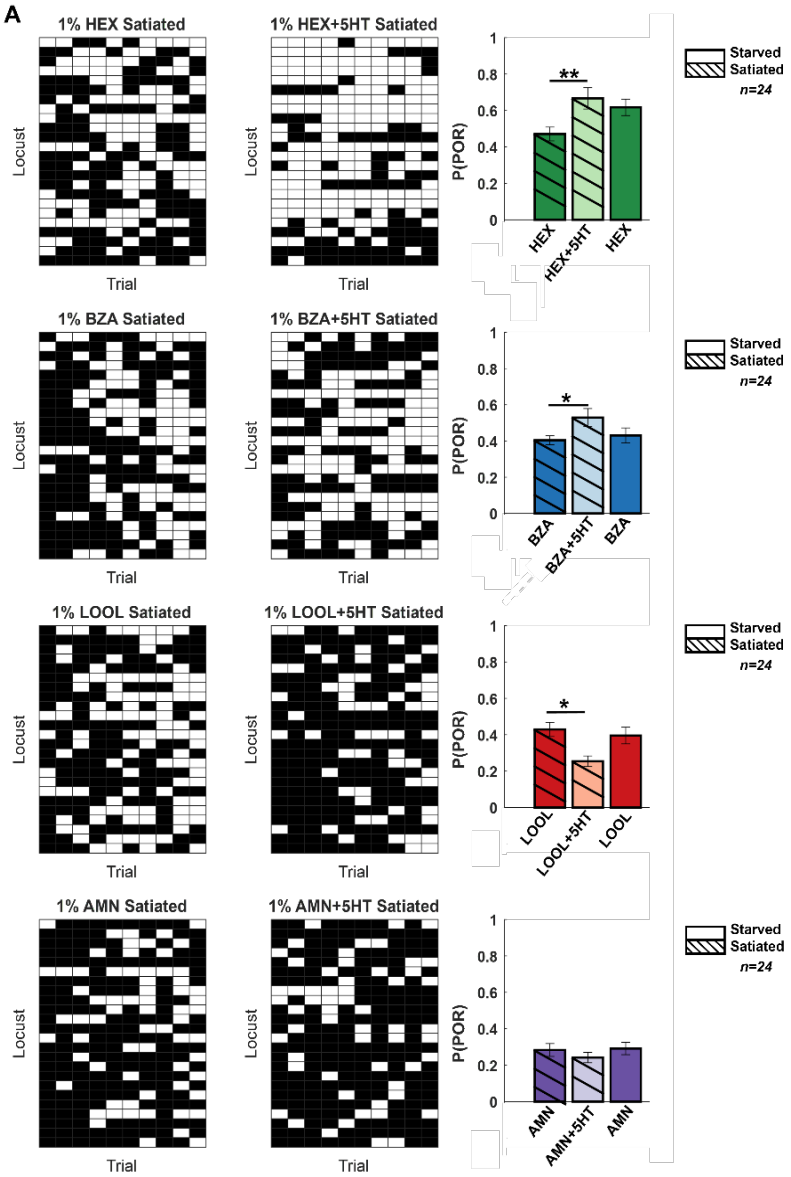

Figure 7. **Hunger-state dependent serotonergic modulation of appetitive behavioral responses.**
Left: *A summary of trial-by-trial PORs to the same four odorants used in* ***Figure 1****. The same convention was used in Figure 1 (POR – white; no POR – black). Each row represents PORs recorded across a single locust in ten trials. Twenty-four locusts were used. Each column represents a trial. Each odorant was presented at the 1% v/v concentration. The POR matrix for the same set of locusts before and after 5HT injection is shown to allow comparison.* ***Right:*** *PORs are summarized and shown as a bar plot for all four odors for satiated locust (highlighted with lines), before (dark shade), and after 5HT injection (lighter shade). To allow comparison with POR in starved locust, results from* ***Figure 1*** *are re-plotted in solid bars without stripes Significant differences are identified in the plot (one-tailed paired-sample t-test; (*p<0.05; ** p<0.01; standard paired sample t-test).*

Discussion

We examined how serotonin modulates odor-evoked neural and behavioral responses in locusts to a small but diverse panel of odorants. Our behavioral results revealed that serotonin increased innate appetitive responses to a subset of odorants (HEX and BZA), and in general, increased overall sensitivity to these odorants. In contrast, responses to aversive or neutral odorants like LOOL[31] or AMN[37] decreased or had no significant change in their response levels. In contrast, for all odorants examined, we found that the strength of the stimulus-driven responses increased in several PNs in the antennal lobe. However, the overall combination of neurons activated, and their temporal patterns of activation (ON vs. OFF responses), remained consistent. As a direct consequence, the identity of the odorant could be robustly maintained after exogenous serotonin introduction. Finally, fed locusts injected with serotonin generated similar appetitive responses to food-related odorants as starved locusts indicating the role of serotonin in hunger state-dependent modulation of odor-evoked responses.

Prior results from a number of invertebrate and vertebrate models have shown similar changes in odor-evoked neural responses in the antennal lobe.[20,24,38–40] The increase in the spiking activity of second-order neurons seems inconsistent with the serotonergic gating of sensory input through pre-synaptic inhibition.[21,22] Our results indicate that serotonin also modified the overall excitability of individual PNs and made them fire action potentials in bursts. Hence, it is possible that the increased neural sensitivity could compensate for the decreased input from sensory neurons. The change in input-output mapping as a result of serotonin introduction increased the behavioral response to different concentrations of HEX and BZA.

In contrast to the changes in the response strength, our results indicate that the timing of odor-evoked spiking activity was robustly maintained. As a result, the combination of neurons activated during and immediately after the presentation of the odorant remained similar before and after the introduction of serotonin. Hence, serotonergic modulation altered sensitivity to some odorants without altering the identity of the odorant. These results are consistent with prior imaging studies in flies that reported odor-specific changes in glomerular activity.[24] Further, this interpretation of our physiological results is consistent with the behavioral observation that serotonin altered response levels in an odor-specific manner.

However, behavioral responses could both increase or decrease depending on the odor identity. In contrast, our neural data indicated an enhancement for all odorants examined. This apparent mismatch between neural and behavioral responses could be resolved using a very simple linear regression model. In the model, a subset of neurons that were activated strongly by odorants with stronger PORs received positive weights. In contrast, the subset of neurons that responded to odorants that evoked fewer PORs received negative weights. Notably, the segregation of neural subsets based on the behavioral relevance and the opposing weights assigned to them was sufficient to produce odor-specific increase or decrease in behavioral PORs as observed during serotonergic modulation.

While serotonin is implicated in a range of behaviors, the modulation of feeding behavior is widely conserved across invertebrates and vertebrates. Hence, we finally explored whether this role is conserved in locusts as well. We found that the appetitive response evoked by a food-related odorant (HEX; green leaf volatile[29]) was reduced in animals that were fed. Notably, we found that serotonin application in locusts that were fed recovered back their appetitive response to HEX indicating a putative role of this neuromodulator in this organism. Earlier studies have shown that serotonergic neurons could mediate hunger-state dependent switch in behavioral response preference (attraction vs. repulsion) to the same food-related odorant in fruit fly larvae[1]. Our studies complement these findings and show how serotonin could modulate behavioral responses to different odorants in a stimulus-specific manner.

Data availability
All data presented in this paper will be publicly available in Figshare upon publication of this manuscript.

Code Availability
The custom code used to generate figures in this paper will be publicly available along with the datasets in Figshare after publication.

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Author contributions
YB and BR conceived the study and designed the experiments/analyses. YB performed all the behavioral and electrophysiology experiments and analyzed the data. BR developed the model. YB and BR wrote the paper. BR obtained the funds and supervised all aspects of the work.

Competing Interest Statement
The authors declare no competing interests.

Methods

Animals

We used adult *Schistocerca americana* of both sexes from a crowded colony for our electrophysiology and behavioral experiments. Sixth-instar locusts were identified by the developed wings and soft cuticle in the neck area.

Odor Stimulation

Odor stimulus was delivered using a standard procedure.[26,28,41–43] Briefly, all odorants were diluted to their 0.01-10% concentration by volume (v/v) in mineral oil. Ammonium alone was diluted in distilled water. 20 ml of diluted odor solution was kept in 60 ml sealed bottles. During stimulus presentations, a constant volume (0.1 L per minute) from the odor bottle headspace was displaced and injected into the carrier stream using a pneumatic pico-pump (WPI Inc., PV-820). A vacuum funnel placed right behind the locust antenna ensured the removal of odor vapors.

Behavior experiments

Sixth instar locusts of either sex were starved for 24-48 hours before the experiment or taken straight from the colony and fed blades of grass for the satiated condition. Locusts were immobilized by placing them in the plastic tube and securing their body with black electric tape. Each locust was presented with one concentration of four odorants (hexanol, benzaldehyde, linalool, and ammonium) in a pseudorandomized order. The odor pulse was 4 s pulses in duration and the inter-pulse interval was set to 60 s. The experiments were recorded using a web camera (Microsoft). The camera was fully automated with the custom MATLAB script to start recording 2 seconds before the odor pulse and end recording at odor termination. An LED was used to track the stimulus onset/offset. The POR responses were scored offline. Responses to each odorant were scored a 0 or 1 depending on if the palps remain closed or opened (**Figure 1A**). A positive POR was defined as a movement of the maxillary palps during the odor presentation time window as shown on the locust schematic (**Figure 1A**).

Serotonin treatment

After the initial set of POR experiments, a 0.1 M serotonin solution was injected directly into the locust's head. The needle of a U-100syringe was inserted slightly under the cuticle of the locust head, ~ 1mm above the median ocellus. 1ul of the solution was injected and the opening was sealed with a small amount of melted dental wax. The locust was let to stabilize for a time period of 3 hours after the injection and before the second set of behavioral experiments.

Electrophysiological Experiments

Surgery

Sixth instar locusts of either sex were used for these experiments. The legs and wings were removed and the locust was immobilized on a platform. The head was fixed with wax and a cup was built around the head to hold saline solution. The locust antennae were held in place using clear tubing and allowed to pass through the wax cup. The cuticle between the antenna was removed and the air sacs/trachea was removed to expose the brain. Additionally, the gut was removed and a metal wire platform was placed underneath the brain to lift and stabilize it. Finally, the transparent sheath was removed by careful pinch, using very sharp tweezers. Locust brains were super-fused with artificial saline buffer. A visual demonstration of this entire protocol is available online[43].

Electrophysiology
Intracellular recordings were performed using glass electrodes (resistance 8-15 MΩ) filled with intracellular saline (130mM L-aspartic acid potassium salt, 2mM MgCl2, 1mM CaCl2, 10mM HEPES, 10mM EGTA, 2mM Na2ATP, 3mM D-glucose, 0.1mM cAMP; osmolarity ~315mmol/kg; pH 7.0). Glass electrodes were pulled using micropipette puller (Sutter Instrument, Novato, CA). Spontaneous firing, as well as real-time odor-evoked responses, were recorded in the current-clamp configuration. Each set of experiments consisted of 5 consecutive 40-second trials, with 20 seconds intervals in between for each odorant/odor concentration. Odor stimulation was performed at the 10th second of the trial for 4 seconds. Voltage signals were amplified (Axoclamp-2B, Molecular Devices) and saved using a custom MATLAB script.

After monitoring the responses to the odor panel, a serotonin solution was applied directly into the bath using a thin nozzle pipette. The serotonin solution (0.01M serotonin hydrochloride in locust saline buffer) was made fresh before every experiment due to its light sensitivity. The same set of recordings and odor panel was repeated 5-10 minutes following serotonin application.

Analysis
Probability of POR calculation
POR responses were scored in a binary fashion. POR responses of locusts were summed across ten trials and across all locusts. The probability for each odorant was calculated as follows:

$$p(POR) = \frac{Total\ Score_{odor}}{Total\ \#\ of\ locusts\ X\ 10} \quad (1)$$

Significance in differences between the POR responses observed before and after serotonin application was calculated using a single-tailed paired-sample *t*-test (‘*ttest’* built-in function in MATLAB).

Electrophysiology data
In total, electrophysiological data from 82 odor-neuron combinations was obtained intracellularly and used for analyses. Each recording was preprocessed and converted into a response matrix. MATLAB's built-in function “findpeaks” was used for identifying action potentials.

PN response classification
We defined 4 s of odor presentation as an ON period, and the 4 s immediately following odor termination as an OFF period. For each PN, we used the mean baseline response during the pre-stimulus time period + 6.5 standard deviations of baseline activity as the threshold that needs to be exceeded to be classified as a response.

Dimensionality reduction analysis
We used Principal Component Analysis (PCA) to visualize ensemble PN activity. The spiking activity for each PN during 4 s of odor presentation was averaged across all 5 trials and binned in 200ms non-overlapping time bins. In this manner, we obtained a 164 PN x 200 time-bin matrix for all odorants. The first 82 rows included PN responses before serotonin introduction and the other 82 rows included the responses of the same set of neurons after exogenous serotonin application. The response covariance matrix was calculated and the data was projected onto the top two eigenvectors corresponding to the largest eigenvalues.

Correlation analysis

Similar to the PCA analysis, the data matrix used for this analysis was 164 PN x 200 time bins (40 s; 200 ms time bins). This included the 82-dimensional PN spike count vectors before, during, and after odor presentations. Spike counts were averaged across five trials.

The correlation analysis was done time-bin-by-time-bin. Each pixel or matrix element in time-bin-by-time-bin correlation plots (**Figure 5C**) indicates the correlation value between neural activity vectors observed in the ith and j^{th} time bins. All time-bin-by-time-bin correlation analyses were computed using high-dimensional response vectors. Correlations were calculated as:

$$C_{ij} = \frac{cov(X_i, X_j)}{\sigma_i \cdot \sigma_j} \quad (2)$$

Here, *i* and *j* represent time bins, X_i, *and* X_j represents the population activity vector in the ith and j^{th} time bin respectively, σ_i, σ_j are standard deviations of spiking activities during the ith and j^{th} time bins respectively.

<u>Linear Regression Model</u>

We used a recently published dataset[28] of extracellular PN responses to HEX, BZA, and LOOL to build our linear regression model. The input to the model was the spiking activity across the eighty-nine neurons (89 PNs x 40 s; 89 x 800 response matrix for each odorant; each time bin was 50 ms in duration). The response matrix for the three odorants was concatenated (**X**; 89 x 2400). The PN weights were determined as follows:

$$W = (XX^T)^{-1}XY \quad (3)$$

X denotes the concatenated matrix of neural activity. Each column of **X** represents trial-averaged firing activity across 89 PNs in a 50 ms time bin. Neural responses before, during, and after the termination of all three odorants (hex, bza, and lool) were included. **Y** is a row vector with values set to mean subtracted p(POR) only during those time bins when HEX, BZA, and LOOL were presented (zeros otherwise).

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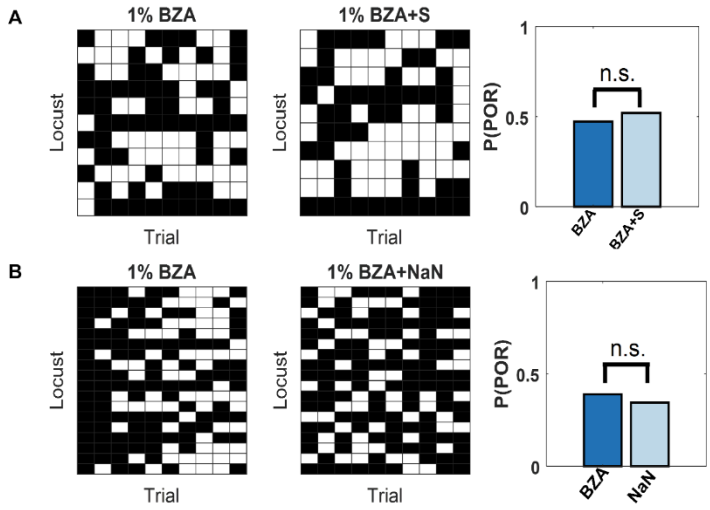
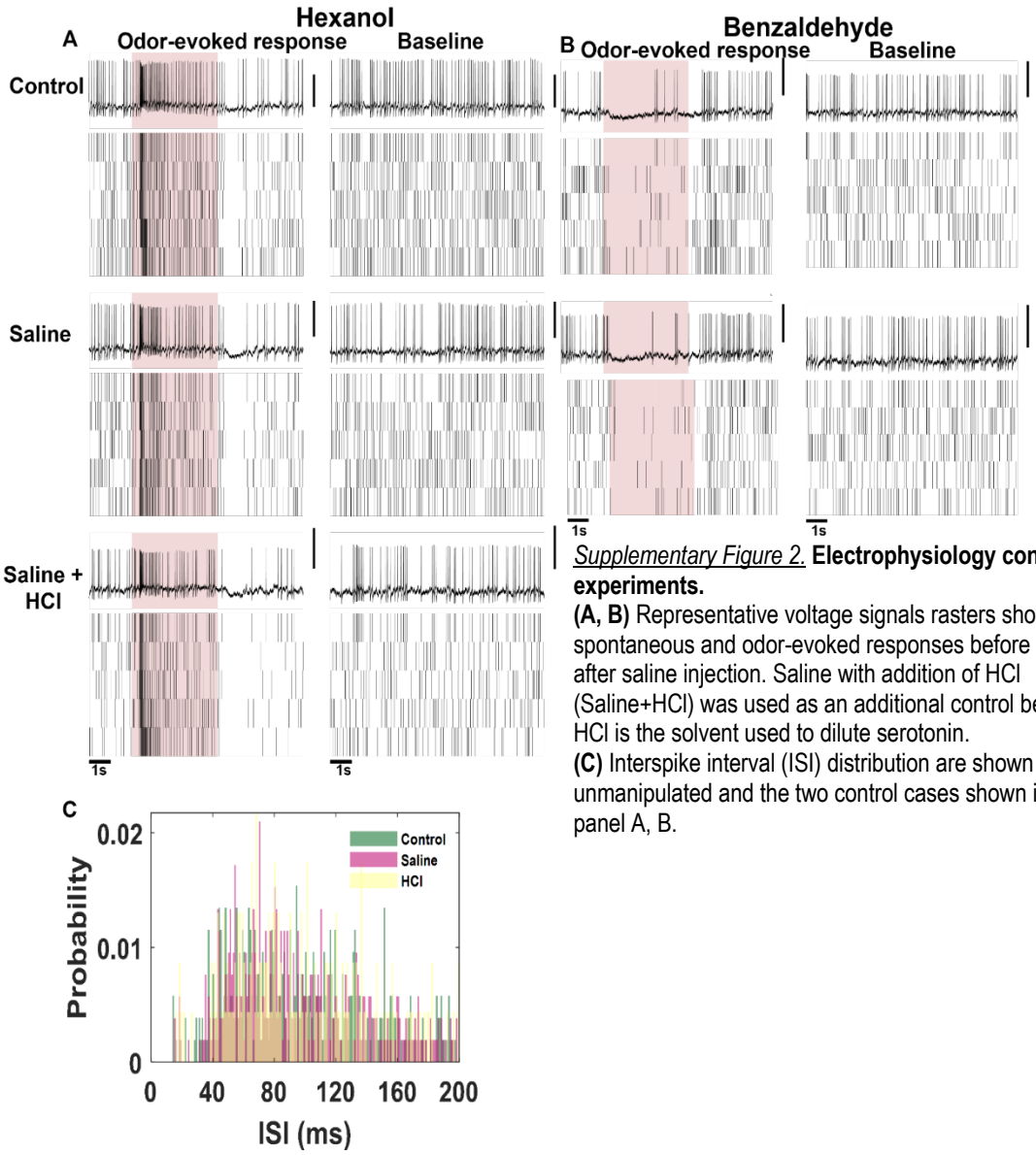
Supplementary figures.



Supplementary Figure 1. **Saline injection control experiments.**
The panel presents results from a series of control experiments. Odor Benzaldehyde at 1% concentration by volume (v/v) was used in this study. On the left, a plot identifying the POR response matrix is shown. Same color convention as Figure 1. The probability of POR represented as a bar plot is shown on the right side. Each row represents PORs recorded from a single locust (n=11 for saline injection (top panel) and n = 18 locusts when the injection was absent (bottom panel)) over 10 consecutive trials (columns). The results before and after the injection of locust saline are presented in ***panel A. Panel B*** *shows the results from a second control experiment. We compared POR responses to BZA before and after 3-hour wait (without any injection). This time period is comparable to the time lag between before and after 5HT injections. Significance in POR differences tested using standard paired-sample t-test. (**indicates p<0.05,n.s. – not significant)*



Supplementary Figure 2. **Electrophysiology control experiments.**
(A, B) Representative voltage signals rasters showing spontaneous and odor-evoked responses before and after saline injection. Saline with addition of HCl (Saline+HCl) was used as an additional control because HCl is the solvent used to dilute serotonin.
(C) Interspike interval (ISI) distribution are shown for the unmanipulated and the two control cases shown in panel A, B.