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Normalization for Sparse Encoding of Odors by a Wide-Field Interneuron

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Summary

Sparse coding presents practical advantages for sensory representations and memory storage. In the insect olfactory system, the representation of general odors is dense in the antennal lobes but sparse in the mushroom bodies, only one synapse downstream. In locusts, this transformation relies on the oscillatory structure of antennal lobe output, feed-forward inhibitory circuits, intrinsic properties of mushroom body neurons, and connectivity between antennal lobe and mushroom bodies. Here we show the existence of a normalizing negative feedback loop within the mushroom body to maintain sparse output over a wide range of input conditions. This loop consists of an identifiable “giant” nonspiking inhibitory interneuron with ubiquitous connectivity and graded release properties.

Sparse coding, the properties and advantages of which have been known for decades (1-3) has recently found experimental support in a number of systems (4-8). In such representations, information is encoded by neurons that express rare, though not exclusive, responses. In some sparse encoding systems, such as the insect mushroom bodies (7, 9) or zebra finch song control nuclei (6), the responses of individual neurons are also very brief (one or two action potentials over a background of 0), making these representations difficult to discover, but the spikes produced extremely informative. In locust, the principal neurons of the mushroom bodies, called Kenyon cells (KCs), respond to odors with high specificity (7, 10) and can express concentration- (11) and category- (12) invariant properties. The baseline activity of KCs is close to 0 (7, 10, 13, 14), their responses to odors typically contain less than 3 action potentials, and the gain of their output synapses (the effectiveness of their rarely elicited spikes) is both high on average and modifiable by a Hebbian learning rule (15). Because each KC is, on average, connected to about half of its presynaptic population (the projection neurons or PNs) (16), small changes in the PN population's output could affect the reliability of the KCs' sparse output (SI 1), inconsistent with experimental observations (11).

Earlier anatomical studies (17) identified a single “giant GABAergic neuron” (GGN) in each mushroom body, with extensive overlap with Kenyon cell projections (Fig. 1Ai): the neurites of GGN in the peduncle and alpha lobe are fine and highly branched—consistent with dendrites—but varicose in the calyx—consistent with axonal projections (18) (Fig. 1Aii). (This neuron appears similar to neuron APL recently described in *Drosophila* (19)). Morphological data thus suggest that GGN is well suited to form a negative feedback loop

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with KCs. Using numerical simulations, we verified that an all-to-all feedback system between KCs and GGN could solve the normalization problem described above (SI 1). We show experimentally that GGN in fact fulfills this role.

All experiments were conducted *in vivo*, in immobilized, non-anesthetized animals. GGN was impaled from one (sometimes two) neurite(s) in the calyx or peduncle with a sharp microelectrode after blind search (SI 2). Our results are based on 80 such recordings in 55 animals. GGN, is a non-spiking neuron with a resting potential of -51 ± 5 mV. It responded to every odor tested (SI 3) with graded potentials composed of superimposed e- and i-psps (Fig. 1Bi). Overall, excitation dominated and depolarization grew with stimulus concentration (tested over a million-fold) with a peak depolarization of 15 - 20 mV above rest (Fig. 1B & 1Ci). The oscillatory power (15 - 30 Hz) of the mushroom body local field potential (LFP) increased with odor concentration (Fig. 1Biii & iv) (11). Simultaneously recorded LFP (power) and V_{GGN} ($\int V dt$) co-varied over this concentration range ($n = 364$ pairs, linear fit, $r = 0.93$, Fig. 1Cii). In addition, the instantaneous variations of V_{GGN} matched those of the LFP envelope (Fig. 1D). Hence, GGN output co-varies with the global drive provided to the mushroom body.

We next tested the synaptic connections between KCs and GGN. Paired intracellular recordings were made from randomly chosen KC somata and a neurite of GGN. Superimposed V_{GGN} sweeps ($n = 139$) triggered from the spikes of one KC are shown in Fig. 2Ai, together with their average (black). The spike-triggered averages for this and ten other pairs are shown in Fig. 2Aii. They all revealed waveforms typical of unitary EPSPs, with latencies consistent with monosynaptic connections after accounting for KC spike conduction delay ($n = 1,302$ events). Unitary EPSPs were 1 ± 0.50 mV ($n = 11$ KCs), with some nearing 2 mV. Using extracellular stimulation of KC somata, we could progressively recruit increasing numbers of KCs, and record increasingly large postsynaptic potentials in GGN, with a mean peak of 15 - 20mV (Figs 2B,C and SI 4). These compound potentials had non-monotonic falling phases, explained by an additional indirect inhibitory component (see below). We compared GGN responses evoked by odors—generated by periodic KC population input at the LFP frequency (about 20Hz) (red Fig. 2D)—to ones evoked by direct extracellular electrical stimulation of KCs at the same frequency (blue, Fig. 2D). This comparison revealed large unitary IPSPs, counteracting depolarizing summation, on the odor-evoked response (red trace, Fig. 2D). The discrete nature of these IPSPs suggested that they might originate from a single inhibitory interneuron. We found this putative interneuron (named IG, for “inhibitor of GGN”); its action potentials led with a consistent latency the IPSPs in GGN, whether at baseline or during responses to odors (Fig. 2Ei-iii). IG itself received phasic inhibitory inputs that each corresponded to phasic depolarizations (compound EPSPs) of GGN (Fig. 2Eiii,iv). The amplitudes of the e- and i-psps in the two neurons were positively correlated (Fig. 2Ev). We conclude that GGN receives direct input from the KC population, that GGN is an inhibitory neuron (consistent with its GABA-immunoreactivity (17)), that it releases neurotransmitter in a graded manner, and that GGN is itself reciprocally connected to a spiking inhibitory interneuron. During an odor response, GGN receives both excitatory input (from KCs) and inhibitory input from IG, itself driven by KCs and possibly also, antennal lobe projection neurons. Overall, the response of GGN to odors is depolarizing, but significantly less than the pure summation of KC-evoked EPSPs would suggest, due at least in part, to the action of IG on GGN. We now turn to the action of GGN on KCs.

Because GGN is a non-spiking neuron, we first performed double dendritic impalements (one for current injection, the other for voltage recording) and calibrated current injections to generate depolarizations commensurate with those evoked by odors (Figs 3A and SI 5). We could then assess the effect of depolarizing GGN on KC firing thresholds. A KC was

impaled in the soma, and a short 70-300pA pulse was injected to produce a few action potentials ($n = 9$ KCs, 85 trials) (Fig. 3B). This manipulation was subsequently combined with a depolarization of GGN, using increasing intensities (Fig. 3Bii). In every pair, GGN depolarization beyond 5mV reduced current-evoked firing of the recorded KC (Fig. 3B & Di). GGN thus exerts a direct, postsynaptic inhibitory effect on KCs. We then depolarized one KC (as in Fig. 3B) but depolarized GGN indirectly, by extracellular stimulation of other, unrelated KCs. A microelectrode was used to monitor GGN membrane potential (Fig. 3C). As above, GGN depolarization counteracted current-induced spiking of the KC (indeed activating GGN synaptically was nearly twice as effective as via direct current injection, Fig. 3C, Dii). Thus, GGN inhibits KCs post-synaptically in degrees correlated with membrane depolarization, itself a function of total KC population output.

We next sought to manipulate GGN during odor presentation. During these experiments, we monitored LFPs in the mushroom body calyx. These LFPs result mainly from synaptic currents caused (directly and indirectly) by PN input onto KCs, and are strongly oscillating in the 20Hz range during odor stimulation (13). Current-evoked depolarization of GGN during odor stimulation caused a strong and immediate reduction of the odor-evoked LFP oscillatory power (Figs 4Ai and SI 6). GGN hyperpolarization had a weaker but opposite effect (Fig. 4Aii, D). Replacing LFPs with intracellular KC recordings, we observed that odor-evoked KC membrane-potential oscillations were similarly affected by GGN polarization (Fig. 4B). One simple interpretation is that GABA released by GGN depolarization causes a conductance increase in KCs, shunting the odor-evoked synaptic currents (Fig. 4B) and the current loops responsible for the LFPs (Fig. 4A). It is possible, however, that GGN also affects KCs by presynaptic action on PN axons. Thus, while the results in Figs. 3 and 4 indicate a postsynaptic (shunting) action of GGN onto KCs, we cannot exclude the possibility that GGN also inhibits KCs presynaptically, by action onto PN axons.

Thus far, we have assessed the effects of GGN only on individual KCs. Because thousands of KCs converge on a small number of extrinsic neurons in the output lobes of the mushroom body (15, 20), we can use beta-lobe neurons (LNs) as assays of GGN action onto the KC population. We impaled LNs in a dendrite ($n = 10$ LNs) to monitor odor-evoked activity. Manipulating GGN membrane potential during the odor pulse changed the recorded LN's responses to the odor (Fig. 4C-E): a large GGN depolarization could silence the LN (Fig. 4Cii); conversely, hyperpolarizing GGN (moderately) increased LN firing rate (Fig. 4Civ, vii). The action of GGN on the LN was not direct, for GGN had no effect on LN firing evoked by current injection (SI 7). Increasing depolarization of GGN during an odor caused a progressive reduction of LN firing and LFP power (Fig. 4E), consistent with GGN's effect on current-induced firing of KCs (Fig. 3B-D). Hence, GGN affects LNs indirectly by its actions on the KC population output.

Using simultaneous intra-dendritic, intra-somatic and extracellular recordings *in vivo* and in non-anesthetized animals, we assessed directly and specifically the functional connectivity and actions of a single, identifiable wide-field interneuron (GGN) in a structure implicated in learning and memory in insects. This single neuron forms the negative arm of a feedback loop by KCs onto themselves thus regulating KC excitability adaptively, a function required to maintain the sparseness of odor representations by KCs (SI1). The effects of this neuron are such that it can, on its own, shut down entirely the output of the mushroom body. Conversely, its hyperpolarization can increase mushroom body output. Because GGN is also under the influence of at least one other inhibitory neuron (IG), however, the gain of the KC negative feedback loop can in principle itself be modulated. This attribute is highly desirable in a circuit involved in memory for it could allow the lowering or raising of KC firing

threshold and thereby increase the probability of—and degrees of refinement in—object recognition during recall.

GGN lacks action potentials, a property common in insect interneurons (21). While the implementation we described may be specific to invertebrate brains (see SI 8 for intracellular recordings from the *Drosophila* analog of GGN, for example), the underlying principles may be widespread among circuits with equivalent requirements for sparse representations. GGN acts as an integrator, similar in function to that of a population of spiking neurons: the membrane potential of GGN can be thought of as equivalent to the PSTH of a population of spiking interneurons, smoothed with an EPSP-like kernel. Just as functionally equivalent feed-forward inhibitory loops have been found in insect mushroom bodies (7) and in mammalian piriform cortex (22), we may find in mammalian olfactory cortex a global negative feedback loop comparable to the one we describe here.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Morphology and responses to odors of GGN

A. (i) GGN intracellular fill (5% biocytin) reveals extensive arbor in MB-calyx (c), α -lobe (α L), and in the lateral horn (LH). Left: pasted intracellularly-labeled KC image for comparison. p: pedunculus; -L: -lobe; s: soma. (ii) Higher magnification of GGN axonal (top) and dendritic (bottom) fields.

B. GGN membrane voltage (i. single traces; ii. 14 superimposed trials (average in black) and mushroom body LFP (iii. single trials; iv. spectral power in 10-30-Hz band; single trials: grey, average: black) recorded simultaneously in response to octanol concentration series (grey bar: odor).

C. (i) Cumulative integral of GGN voltage (mean and SD) in Bii against odor concentration. (ii) Scatter plot of LFP power vs. GGN intracellular voltage integral over response duration. Dots: single trials for each odor concentration (5 experiments); circles: averages for each concentration; light-to-dark grey or red: increasing odor concentration.

D. GGN intracellular voltage (single-trial, red) superimposed with simultaneous LFP (grey) and corresponding LFP negative envelope (black).

Fig. 2. Synaptic inputs to GGN

A. (i) Single (grey) and averaged (black) EPSPs caused by a single KC on GGN; spike-triggered sweeps and average (STA) from dual intracellular recording from KC soma and GGN neurite. KC spikes caused by direct current injection in KC soma. (ii) STA of 11 different KC-GGN pairs (grey), and their own average (black). Calibration as in (i).

B. (i) Compound GGN EPSP caused by extracellular stimulation of KCs (grey: single sweeps; blue: average). (ii) Same as (i), across stimulation intensities. Calibration as in (i).

C. Peak amplitude (i) and slope (ii) of compound EPSPs in B as function of KC stimulation amplitude.

D. Comparison of GGN responses to odor (red) and 20-Hz KC stimulation train (blue).

E. Simultaneous intradendritic recordings of GGN (red) and the source of its IPSPs (named IG, grey), indicating unique origin. (i) Spontaneous activity. (ii) Spike-triggered single (grey) and averaged (red) sweeps of GGN intracellular voltage, triggered on IG spikes. (iii) Response of simultaneously recorded GGN and IG to odor (grey bar), indicating antagonistic membrane potential fluctuations. Stippled lines indicate IG hyperpolarizing potentials coinciding with GGN EPSPs. (iv) Single sweeps of IG membrane potential (grey) triggered on GGN EPSPs, showing non-spiking, inhibitory synaptic transfer. (v) IG IPSP (absolute value) against GGN EPSP amplitude, showing positive correlation, indicating graded release. (vi) Schematic of inferred KC-GGN-IG interconnectivity.

Fig. 3. Action of GGN on KC responses to direct or synaptic depolarization

A. (i) Schematic of experiment in (ii): GGN is impaled simultaneously with two intra-neurite microelectrodes: one is used to inject direct current, the other to record resulting trans-membrane voltage. (ii) Calibration of intracellular current pulse amplitude needed to depolarize GGN membrane (blue series) to values comparable to odor-evoked response (red). Current injected in GGN: 1.5, 5.5, 13.5, 15.5, 17.5 and 19.5 nA.

B. (i) Schematic of experiment in (ii): one intracellular electrode is used to depolarize GGN (to values determined in Aii); another is used to record and depolarize a single test-KC above spike threshold. (ii) Pairing GGN and test-KC depolarizations reduces current-induced firing of KC, indicating graded postsynaptic inhibitory action of GGN onto KC. Current injected in GGN from left to right: 3.5, 11.5 and 19.5 nA

C. Same experiment as in B, but GGN direct depolarization has been replaced with electrical stimulation of many KCs, causing indirect GGN depolarization by synaptic excitation. Current-evoked firing of test-KC is again reduced by KC-induced GGN depolarization, in a graded manner (left to right). Stimulation intensity (from left to right): 10, 20, and 30 μ A. Downward deflections in KC traces (blue) are stimulation artifacts.

D. Quantification of relationship between KC firing rate reduction and GGN depolarization for experiments in B (Di, 5KCs) and C (Dii, 3KCs). See SI 5 for x -axis calibration in Di. For each experiment in Dii, stimulation strength is expressed as percent of the observed odor-induced depolarization in the same location.

Fig. 4. Nature of GGN action on KCs and consequences on mushroom body output

A. Action of GGN on odor-evoked LFP oscillations. (i) LFP traces (top) and spectral power in 10-30 Hz band (bottom) in control and GGN-depolarized conditions (20 interleaved trials; individual trials and averages are shown in lighter and darker colors respectively). Note massive reduction in LFP amplitude and power. (ii) Same as (i) but with intracellular hyperpolarization of GGN. Note enhancement of LFP, indicating graded release of GABA at rest.

B. Same as in A, but with single KC intracellular membrane potential replacing LFP. Note effect of GGN on KC membrane potential oscillations, indicative of postsynaptic shunt. (Bi: 18 trials, Bii: 8 trials.)

C. Intracellular recording of -lobe neuron responses to odors in control conditions (red: i, iii, v) and during GGN current injections (depolarizing, blue: ii; and hyperpolarizing, green: iv). (vi) Schematic of the experiment. (vii) Quantification of effect of GGN polarization on -lobe neuron instantaneous-firing-rate in response to odor.

D. Summary of all experiments in Fig. 4A-C, as well as control experiments evaluating the effect of positive (light grey) or negative (dark grey) current injection from 50-100 μm outside of GGN on LFP, after withdrawal of GGN micro-electrode.

E. Relationship and fit between LFP or -lobe neuron outputs and depolarizing current injected in GGN. Note the steeper action on -lobe neuron.