Biochemical and Biophysical Research Communications 436 (2013) 35–40

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The GABA system regulates the sparse coding of odors in the mushroom bodies of *Drosophila*

Zhengchang Lei [a,b], Ke Chen [a,b], Hao Li [a,b], He Liu [a,b], Aike Guo [a,c,*]

[a] Institute of Neuroscience and State Key Laboratory of Neuroscience, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

ARTICLE INFO

Article history:
Received 26 April 2013
Available online 21 May 2013

Keywords:
Olfactory system
Sparse coding
GABA receptor
Mushroom body
APL neuron
Calcium imaging

ABSTRACT

In the mushroom bodies (MBs) of *Drosophila*, an analogue of the mammalian olfactory cortex, olfactory stimuli are sparsely encoded by Kenyon cells (KCs) that exhibit a high level of odor selectivity. Sparse coding of olfactory stimuli has significant advantages for maximizing the discrimination power and storage capacity of MBs. The inhibitory gamma-aminobutyric acid (GABA) system is important for regulating information processing in MBs, but its specific role in the sparse coding of odors is unclear. In this study, we investigated the role of the GABA system in the sparse coding of odors using an *in vivo* calcium imaging strategy, which allowed us to measure the activity of the KC population at single cell resolution while the components of the GABA system were genetically manipulated. We found that the down-regulation of $GABA_A$ but not $GABA_B$ receptors in KCs reduced the sparseness of odor representations in the MB, as shown by an increase in the population response probability and decrease in the odor selectivity of single KCs. Furthermore, the down-regulation of GABA synthesis in a pair of large GABAergic neurons innervating the entire MB reduced the sparseness of odor representations in KCs. In conclusion, the sparse coding of odors in MBs is regulated by a pair of GABAergic neurons through the $GABA_A$ receptors on KCs, thus demonstrating a specific role of the inhibitory GABA system on information processing in the MB.

1. Introduction

Sparse coding of sensory inputs has been observed to be a ubiquitous strategy employed in the nervous system of many species [1–3]. In these systems, only a small fraction of the neuronal population responds to any specific stimulus, and each neuron exhibits a high level of selectivity toward sensory stimuli. This strategy significantly contributes to information coding by maximizing the storage capacity and discrimination power of neural systems [4].

Mushroom bodies (MBs) in *Drosophila* are a pair of brain structures that are generally regarded as the third layer in the olfactory pathway [5]. Olfactory stimuli are sparsely encoded by the activity of Kenyon cells (KCs), the major intrinsic neurons in MBs [6,7]. Taking advantage of the sophisticated tools for genetic manipulation and neural activity recording in *Drosophila*, we used the MB as a model system for the dissection of molecular and circuit mechanisms underlying sparse coding.

Previous experiments have shown that the gamma-aminobutyric acid (GABA) system is important in the regulation of information processing in MBs [8–10]. GABA is the major inhibitory neurotransmitter in the central nervous system of *Drosophila*, and was expressed in a pair of anterior paired lateral (APL) neurons that innervated the entire MB [11,12]. Electrophysiological studies have shown that a pair of neurons in the locust that are analogous to the APL neurons provides feedback inhibition to the MB [13]. However, whether and how the GABA system modulates sparse coding in the MB is not clearly understood.

Here we addressed this problem by performing *in vivo* calcium imaging on a large population of KCs while manipulating the components of the GABA system genetically. By sampling approximately one-third of the KCs in a MB (typically more than 600 out of ~2000 total KCs), the population response pattern to odors could be comprehensively mapped and compared between flies of different genotypes. When the $GABA_A$ but not $GABA_B$ receptors were down-regulated in the KCs, we observed an increase in the response probability and decrease in odor selectivity, which resulted in a decreased sparseness in the odor representations. Similar results were observed when the GABA level was down-regulated in the APL neurons. Together, these results suggested

* Corresponding author. Address: Institute of Neuroscience and State Key Laboratory of Neuroscience, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, No. 320 Yue Yang Road, Shanghai 200031, China.
E-mail address: akguo@ion.ac.cn (A. Guo).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.05.036

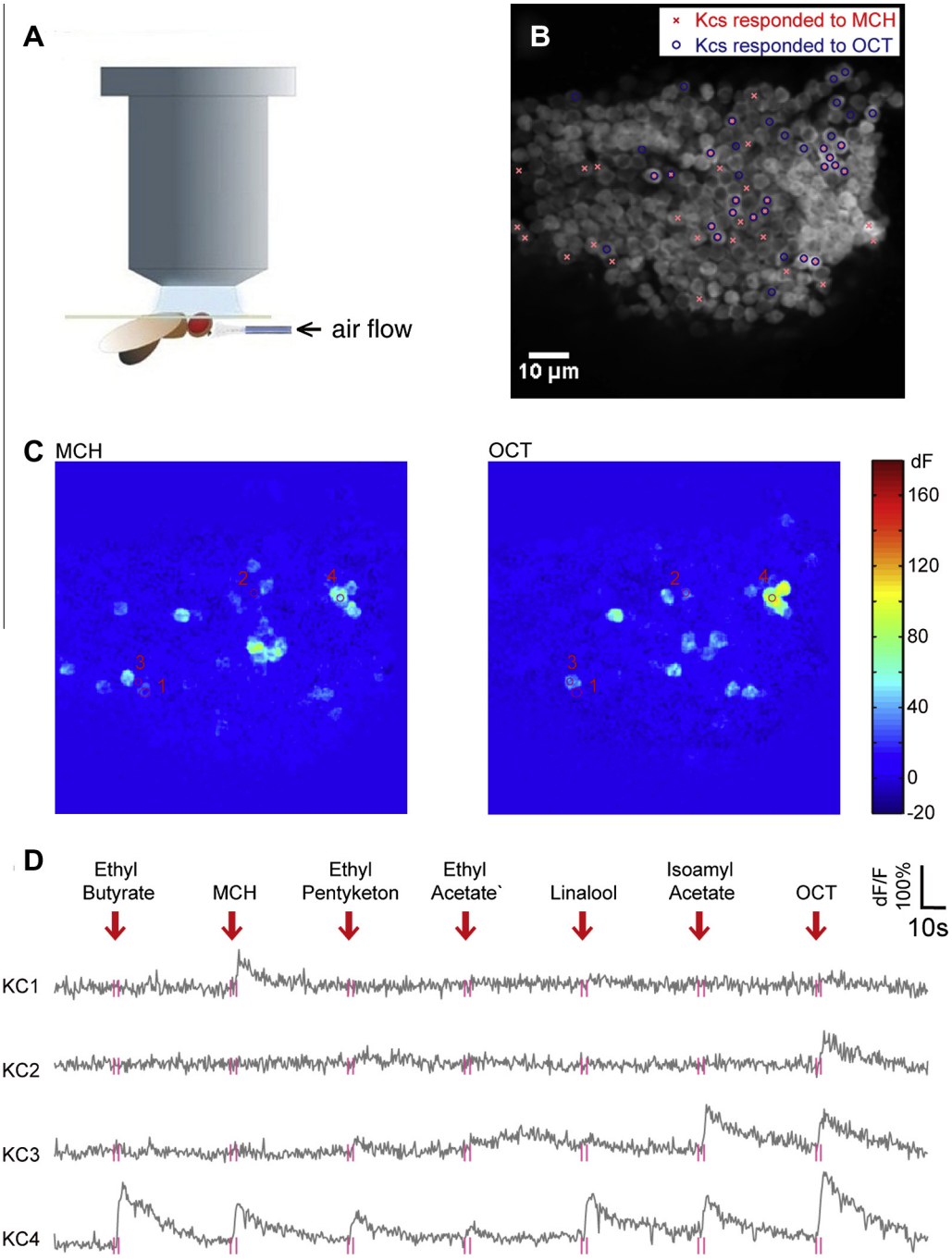

Fig. 1. The sparse coding of odors in the MB of *Drosophila*. (A) The experimental setup for the *in vivo* calcium imaging. (B) The KC somas sampled from an optical section under two-photon microscopy. The red crosses and blue circles mark two groups of KCs responding to the odorants MCH and OCT, respectively. (C) The response patterns of the KCs to MCH and OCT from the same KCs shown in (B). Four KCs with significant responses to MCH or OCT are indicated with circles. (D) Example response traces from the four KCs marked in (C). The red vertical lines indicate the time of odor stimulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that the sparse coding of odors in the MB was regulated by a pair of GABAergic neurons through the $GABA_A$ receptors on KCs.

2. Materials and methods

2.1. Fly stock and rearing

The flies were reared at 25 °C and ~60% humidity on a standard medium under a 12:12 h light:dark cycle [14]. The following transgenic flies were used: *UAS-G-CaMP3* (III) [15], *OK107-GAL4* (IV) [16], *UAS-Rdl$_{RNAi}$8–10j* (III) [10], *GH146-GAL4* (II) [17], *UAS-GBR$_{RNAi}$* (III) (VDRC v1784) and *UAS-GAD$_{RNAi}$* (III) (VDRC v32344) [18], *247-LexA* and *LexAop-G-CaMP3* (III) [19,20].

The fly genotype for Fig. 1 was *UAS-G-CaMP3;OK107-GAL4*. The fly genotypes for Fig. 2 were *UAS-G-CaMP3;OK107-GAL4* (control), *UAS-G-CaMP3/+;UAS-GBR$_{RNAi}$/+;OK107-GAL4/+* (*GBR^{RNAi}*) and *UAS-G-CaMP3/+;UAS-Rdl$_{RNAi}$8-10j/+;OK107-GAL4/+* (*Rdl^{RNAi}*). The fly genotypes for Fig. 3 were *Bl/CyO;247-LexA,LexAop-G-CaMP3/TM2* (control) and GH146-*GAL4/CyO;247-LexA,LexAop-G-CaMP3/UAS-GAD$_{RNAi}$* (*GAD^{RNAi}*). The fly genotypes for Supplementary Fig. 1 were

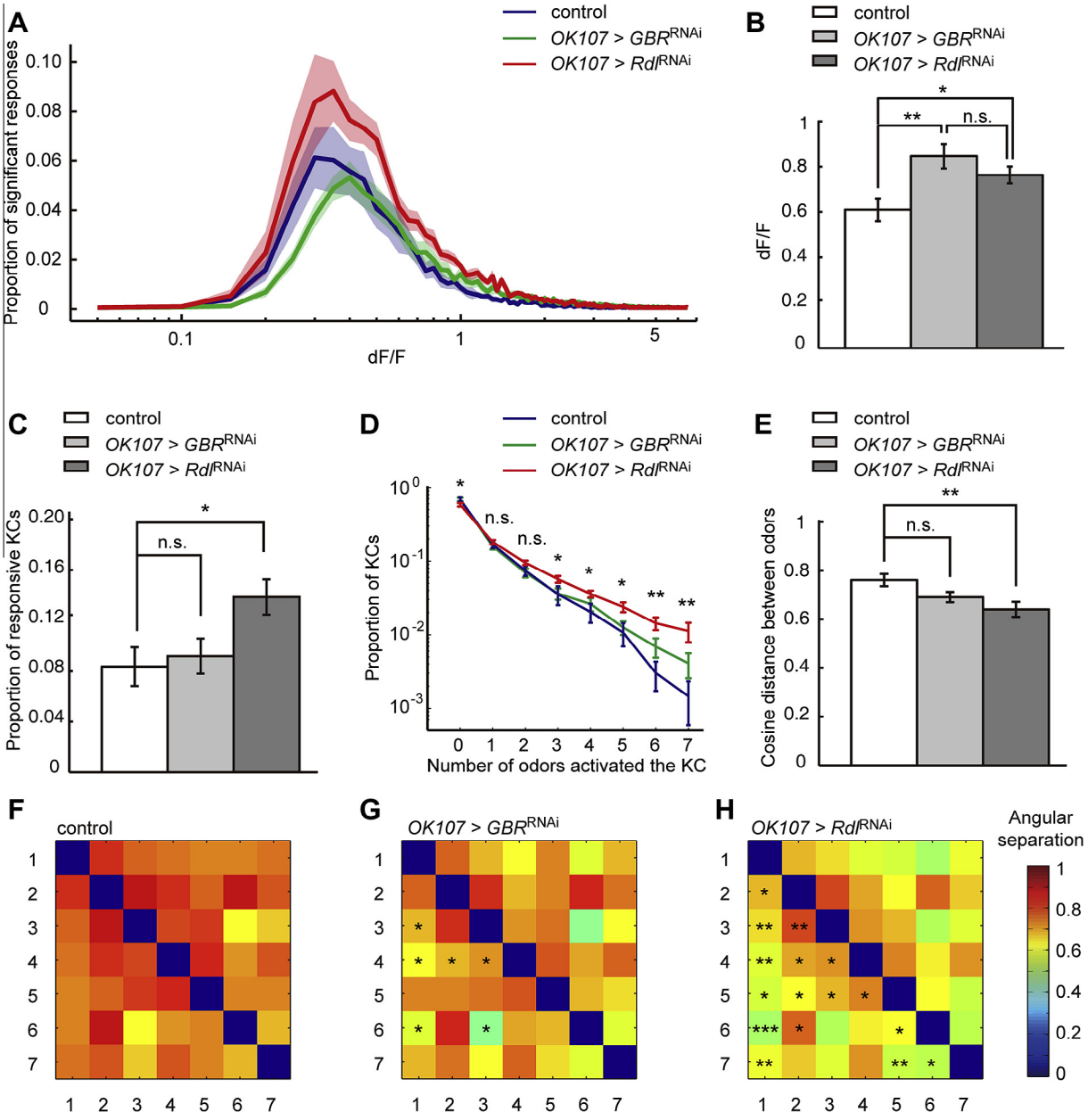

Fig. 2. The down-regulation of RDL but not GBR reduced the sparseness of odor representations in MBs. (A) The distribution of the population response amplitudes of KCs to odors. The colored regions around the lines indicate means ± s.e.m. (B) The mean response amplitude was higher in the Rdl^{RNAi} and GBR^{RNAi} flies. (C) The proportion of odor responsive KCs was higher in Rdl^{RNAi} but not GBR^{RNAi} flies. (D) The number of odors that single KCs responded to was generally higher in Rdl^{RNAi} but not GBR^{RNAi} flies. (E) The angular separation between pairs of odors was lower in Rdl^{RNAi} but not GBR^{RNAi} flies. (F–H) The angular separation between seven odors including: 1, ethyl butyrate; 2, 4-methylcyclohexanol (MCH); 3, ethyl pentylketon; 4, ethyl acetate; 5, linalool; 6, isoamyl acetate; and 7, 3-octanol (OCT). The asterisk indicates a significant difference from the control. The data are shown as means ± s.e.m, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Bl/CyO;247-LexA,LexAop-G-CaMP3/TM2 (LexAop/LexA) and *UAS-G-CaMP3;OK107-GAL4* (UAS/GAL4).

2.2. Fly preparation

Females 2–7 days old were used in the experiments. The fly was immobilized on ice, transferred to a cold plate, and then gently inserted into a hole in a custom recording chamber. The position of the fly was carefully adjusted to ensure that the dorsal part of the head was exposed above the chamber, whereas the antenna remained below the chamber for odor presentation. UV curing adhesive (Loctite) was then applied around the hole and hardened by 20 s of UV irradiation to immobilize the fly in the chamber. With the dorsal section of the head bathed in saline, a rectangular cuticle between the eyes was removed to expose the MB. The air sacs and fat tissues were removed with tweezers. To ensure that the brain remained stable during imaging, the no. 16 muscles were cut to disable the frontal pulsate organ [21]. The esophagus was also cut and carefully removed via the neck.

2.3. Odor stimuli

In each trial, a total of seven odors were presented to the fly sequentially. Each odor was presented for 2 s followed by an interval of 40 s. The odorants used included ethyl butyrate, 4-methylcyclohexanol (MCH), ethyl pentylketon, ethyl acetate, linalool,

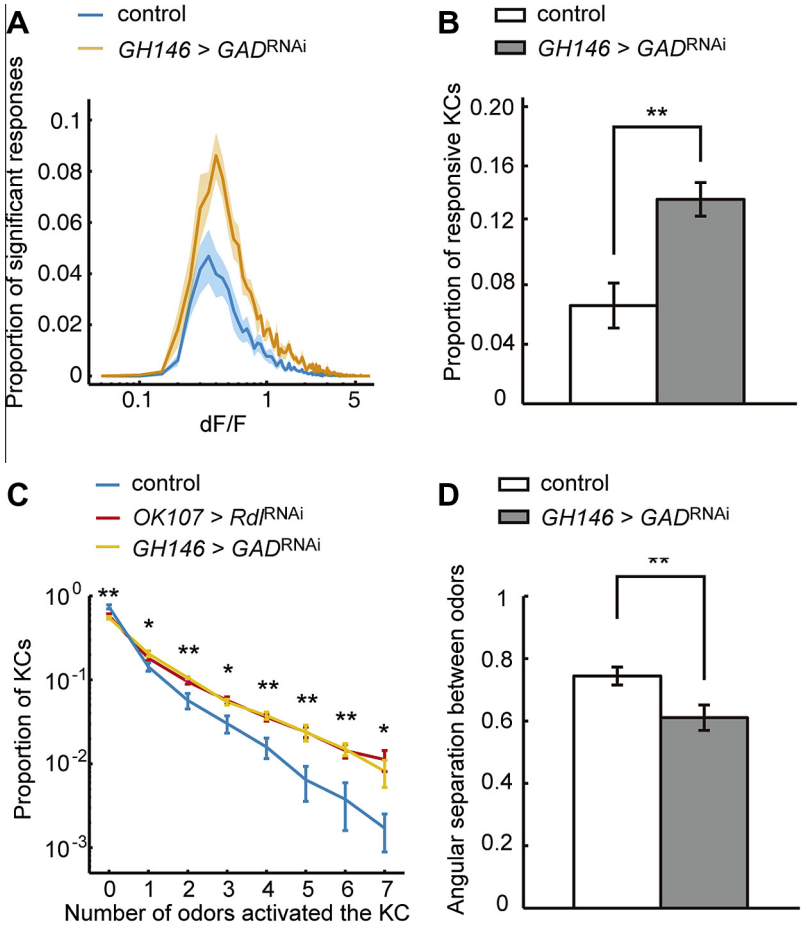

Fig. 3. The down-regulation of the GABA level in the APL neurons reduced the sparseness of odor representations in MBs. (A) The distribution of the population response amplitudes of KCs to odors. The colored regions around the lines indicate means ± s.e.m. (B) The proportion of odor responsive KCs was higher in GAD^{RNAi} flies. (C) The number of odors that single KCs responded to was generally higher in GAD^{RNAi} flies, which was similar to the Rdl^{RNAi} flies. (D) The angular separation between pairs of odors was lower in the GAD^{RNAi} flies. The asterisk indicates a significant difference from the control. The data are shown as means ± s.e.m, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

isoamyl acetate and 3-octanol (OCT). All odorants were purchased from Sigma at the highest purity possible. The odorants were diluted 100-fold in mineral oil in glass bottles. The saturated vapor from each bottle was further diluted ~10-fold by mixing it with a purified air stream.

2.4. Odor delivery

All the odor channels could be independently controlled in our system. For each of the channels, the path of a 50 ml/min air stream could be switched using a three-way solenoid valve between a bottle containing diluted odorant and a bottle only containing mineral oil. The streams from all the channels were then mixed together and combined with a purified air stream, which resulted in a final stream of 500 ml/min. The mixed stream was presented to the fly through a 2 mm wide opening placed ~1 cm from the antenna. The sequence and timing of odor delivery were recorded by a computer equipped with a data acquisition card (National Instruments, PCI-6220). The temporal accuracy of the odor delivery system was calibrated with a photo-ionization detector system (miniPID, Aurora Scientific Inc.).

2.5. Calcium imaging

Two photon calcium imaging was performed using a Prairie Ultima IV microscope equipped with an Olympus LUMPlan FI/RI 40x/0.8 objective and 525/70 nm emission filter. The excitation laser was tuned to 910 nm, and the beam strength on the specimen was adjusted to 0.3–0.6 mW. The acquired images were 256×256 pixels with each pixel covering $0.387 \times 0.387\ \mu m^2$. The pixel dwelling time was adjusted to ensure an acquisition speed of ~3 frames per second. Three optical sections with an interval of ~7 μm were imaged for each MB, which ensured that the same KC soma would not be sampled twice.

2.6. Image analysis

The raw images were initially aligned with the ImageJ plugin Turboreg [22]. Image stacks with excessive movement artifacts after registration were discarded. The images were then analyzed with custom programs written with MATLAB.

The somas of single KCs could be easily recognized from the images. The regions of interest (ROIs) that corresponded to single KCs were selected based on a reference image averaged from the entire stack. To visualize the odor responsive KCs, images acquired during the 10 s before odor onset were averaged and subtracted from the averaged images during the 5 s after odor onset. Based on these images, the size and position of the ROIs were carefully adjusted. The response traces of single KCs were then calculated by averaging the fluorescence intensity within the corresponding ROIs.

For an objective criterion of whether a given KC responded to an odor, the mean F_0 and the standard deviation (SD) of the response trace during the 10 s before odor onset were calculated as measures of baseline activity. The peak fluorescence level (F_{max}) during the 5 s after odor onset must have exceeded $F_0 + 3$ SD for the re-

sponse to be judged as statistically significant. If a KC showed a statistically significant response to an odor, its response amplitude was calculated as $dF/F = (F_{max} - F_0)/F_0$. Otherwise, the response amplitude (dF/F) was set to zero.

Considering the response amplitudes of all KCs to a single odor as a vector, the difference of two odor representations could be measured by the angle θ between the two response vectors. The angular separation of two odor representations was defined by $1 - \cos\theta$ [23], which increases from 0 for identical vectors to 1 for orthogonal vectors. The average separation of 21 odor pairs from seven odors was used as a measure of the separation of odor representation in the MB of a fly.

3. Results and discussion

3.1. Sparse representation of odors in the MB

To investigate how odors are represented by the neural activity of KCs, we used the UAS/GAL4 system to express a calcium-sensitive fluorescent protein, G-CaMP3, in nearly all KCs of *UAS-G-CaMP3*; *OK107-GAL4* flies [15,16]. By imaging the fluorescence intensity of KCs during the odor presentations *in vivo*, the response patterns of KCs could be recorded at the population level (Fig. 1A). Under our experimental settings, 600–800 KC somas could be recorded from a total of ~2000 KCs (Fig. 1B). We found that only a small fraction of the recorded KCs (8.4 ± 1.5%, mean ± s.e.m, $n = 9$ flies, seven odors tested for each fly) showed a significant response to a single odor stimulus (Fig. 1B,C), which was consistent with previous studies showing that odors are encoded sparsely in MBs [7,23]. Different odors were represented by the activity of non-identical sets of KCs, with only a few KCs responding to multiple odors (Fig. 1B–D).

3.2. $GABA_A$ receptors regulated the sparseness of odor representation in KCs

We first examined whether the down-regulation of GABA receptors on KCs affected the sparse coding of odors in the MB. In *Drosophila*, *resistance to dieldrin* (*Rdl*) is the best-characterized gene that encodes a $GABA_A$ receptor highly expressed in all substructures of KCs except the somas [9,10,24]. By contrast, a metabotropic $GABA_B$ receptor, *$GABA_B$-R2* (GBR) subunit, is highly expressed only in the calyx region of the KCs [25]. We used an RNA interference (RNAi) strategy to down-regulate the expression of GABA receptors on KCs (*UAS-Rdl^{RNAi}8-10j* and *UAS-GBR^{RNAi}*), and the efficiency of this strategy was well documented in previous studies [8–10]. By driving the expression of these RNAi alleles together with *UAS-G-CaMP3* using *OK107-GAL4*, whether and how the down-regulation of GABA receptors in KCs affected sparse coding could be examined.

We compared the population distribution of KC response amplitudes (dF/F) of the GABA receptor down-regulated flies with the control flies that only expressed *UAS-G-CaMP3*. We observed that the down-regulation of RDL increased the proportion of KCs showing odor responses in general, thus amplifying the distribution without an obvious change in shape. By contrast, the down-regulation of GBR increased the proportion of KCs showing larger odor responses and decreased the proportion of KCs showing smaller odor responses, which effectively shifted the distribution rightward (Fig. 2A). The mean KC response amplitudes of both GBR and RDL down-regulated flies (GBR^{RNAi} and Rdl^{RNAi} flies) were significantly higher compared to the control flies (Fig. 2B, Student's *t*-test, control flies vs. GBR^{RNAi} flies, $P = 0.0053$, $n = 10$ flies; control flies vs. Rdl^{RNAi} flies, $P = 0.023$, $n = 10$ flies). There was no significant difference observed between GBR and RDL down-regulated flies (Fig. 2B, $P = 0.15$, $n = 10$ flies). These results suggested that GBR and RDL play different roles in the modulation of odor representation in MBs.

Two complementary and independent properties constitute a sparse code: a low population response probability, which implies that a small proportion of the available neuronal population responds to any particular stimulus, and a narrow tuning curve, which implies a high response selectivity of single neurons to different stimuli. We therefore examined how these two properties were affected in the GABA receptor down-regulated flies. The population response probability of the KCs was significantly higher in Rdl^{RNAi} (Fig. 2C, Student's *t*-test, $P = 0.017$, $n = 10$ flies) but not GBR^{RNAi} flies (Fig. 2C, Student's *t*-test, $P = 0.69$, $n = 10$ flies). When the response selectivity of KCs was measured by calculating the number of odors that single KCs responded to, we observed a significantly higher proportion of KCs that responded to multiple odors in Rdl^{RNAi} (Fig. 2D, Student's *t*-test, $P < 0.05$, $n = 10$ flies) but not GBR^{RNAi} flies (Fig. 2D, Student's *t*-test, $P > 0.05$ for all data points, $n = 10$ flies).

Because the sparseness of odor representation in Rdl^{RNAi} flies was reduced compared to that of the control flies, we predicted that the separation between the odor representations would also be reduced. We used the angular separation as a measure of the separation between the responses of the KC population to different odors (see Materials and methods for details). The angular separation was significantly reduced in the Rdl^{RNAi} (Student's *t*-test, $P = 0.0019$, $n = 10$ flies) but not GBR^{RNAi} flies (Student's *t*-test, $P = 0.1$, $n = 10$ flies) (Fig. 2E). Of the 21 odor pairs, the angular separations of 16 pairs were reduced in the Rdl^{RNAi} flies (Student's *t*-test, one-tailed, $P < 0.05$, $n = 10$ flies), whereas the angular separation of six pairs were significantly reduced in GBR^{RNAi} flies (Student's *t*-test, one-tailed, $P < 0.05$, $n = 10$ flies) (Fig. 2F–H).

Therefore, the down-regulation of RDL, but not GBR, in the KCs significantly reduced the sparseness of odor representations in MBs, as was shown by an increase in the population response probability and decrease in odor selectivity of single KCs. Consequently, the down-regulation of RDL reduced the odor discrimination power in the MBs. The down-regulation of GBR also slightly decreased the odor separations, which was possibly because of its effect on the mean population response amplitudes of the KCs.

3.3. The down-regulation of GABA synthesis in APL neurons reduced the sparseness of odor representation in MBs

We then examined the identity of the inhibitory GABAergic neurons that regulate sparse coding through the $GABA_A$ receptors on KCs. A pair of well-known GABAergic neurons, the anterior paired lateral (APL) neurons, intensely innervate the entire MB in *Drosophila* [12]. The APL neuronal analogues in the locust, the giant GABAergic neurons (GGN), were found to provide direct feedback inhibition to the KCs [13], which prompted us to examined the role of the APL neurons in the regulation of sparse coding in KCs. Using the RNAi allele *UAS-GAD_{RNAi}* [18], we down-regulated the expression of a crucial enzyme for GABA synthesis, the glutamatic acid decarboxylase (GAD), in the APL neurons with *GH146-GAL4*. Although the *GH146-GAL4* driver labels both the projection neurons in the antennal lobe and the APL neurons, only the APL neurons are GABAergic [12,26], which ensured that the GABA level was specifically down-regulated in the APL neurons. To simultaneously monitor the KCs activity, we used another independent binary expression system, the LexAop/LexA system, to express G-CaMP3 in KCs by driving the expression of *LexAop-G-CaMP3* with *247-LexA* [18,21,27,28]. To control for any possible difference in the KC response recorded using different systems, we compared the odor responses recorded with the LexAop/LexA system with those recorded with the UAS/GAL4 system. We observed no

significant difference in either the response probability (Student's *t*-test, $P = 0.50$, $n = 7$ flies) or selectivity (Student's *t*-test, $P > 0.05$ at all data points, $n = 7$ flies) of KCs (Supplementary Fig. 1).

In the GAD down-regulated flies (GAD^{RNAi} flies), the distribution of the population response amplitude of KCs showed that the down-regulation of the GABA level in the APL neurons increased the proportion of all response amplitudes (Fig. 3A). The proportion of KCs responding to odors significantly increased (Fig. 3B, Student's *t*-test, $P = 0.0036$, $n = 7$ flies), and the response selectivity of KCs was significantly lower (Fig. 3C, Student's *t*-test, $P < 0.05$ at all data points on the curve, $n = 7$ flies) with a similar effect as that of the RDL down-regulation in KCs. Consistently, the separation of odor representations were also significantly lower compared to those of the control flies (Fig. 3D, Student's *t*-test, $P = 0.0099$, $n = 7$ flies). Together, these results suggested that the APL neurons regulated sparse coding in the MB through the $GABA_A$ receptor RDL.

4. Discussion

In this study, we investigated whether and how the GABA system regulates the sparse coding of odors in the MB. We observed that the down-regulation of the $GABA_A$ receptor RDL in KCs increased the population response probability and decreased the odor selectivity of KCs, thus resulting in a reduced sparseness of odor representations. Furthermore, the down-regulation of the GABA level in a pair of GABAergic neurons innervating the entire MB produced similar effects, which suggested that these neurons regulate the sparse coding in MBs through the $GABA_A$ receptors in KCs.

Because the APL neurons are believed to provide direct feedback inhibition to the KCs [14,17], down-regulation of $GABA_A$ receptors in KCs or the GABA level in the APL neurons should reduce the level of feedback inhibition. Our results suggest that an optimal level of feedback inhibition is essential for the maintenance of odor representation sparseness in the MB. A lower level of feedback inhibition might reduce the amount of excitation required to exceed the firing threshold in KCs, which would result in an increased population response probability and decreased odor selectivity in KCs. One advantage of using feedback inhibition to regulate sparse coding is that its strength is proportional to the strength of the total excitatory inputs, thus resulting in a balanced state that may help to maintain sparse coding over a wide range of input strengths. Therefore, our study revealed a specific role of feedback inhibition from the GABA system in the regulation of sparse coding in the MB.

Financial interest

The authors declare no competing financial interests.

Acknowledgments

We thank Q. Hu, Q. Ma and Y. Yang of the Optical Imaging Facility at the Institute of Neuroscience (ION) for support with the optical imaging, M. Zhang for support with electronics and machining, L. Looger for the *UAS-G-CaMP3* flies, S. Waddell for the *247-LexA* flies, G. Rubin for the *LexAop-G-CaMP3* flies, R. Davis for the *UAS-Rdl^{RNAi}8–10j* flies, and the Vienna *Drosophila* RNAi Center and Bloomington Stock Center for providing the flies. This study was supported by grants from the 973 Program (2011CBA00400 to A.G.), the Natural Science Foundation of China (30921064, 90820008 and 31130027 to A.G.) and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB02040100 to A.G.). The funders had no role in study design, data collection and analysis, the decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.05.036.

References

[1] T. Hromadka, M.R. Deweese, A.M. Zador, Sparse representation of sounds in the unanesthetized auditory cortex, PLoS Biol. 6 (2008) e16.
[2] W.E. Vinje, J.L. Gallant, Sparse coding and decorrelation in primary visual cortex during natural vision, Science 287 (2000) 1273–1276.
[3] M.P. Young, S. Yamane, Sparse population coding of faces in the inferotemporal cortex, Science 256 (1992) 1327–1331.
[4] B.A. Olshausen, D.J. Field, Sparse coding of sensory inputs, Curr. Opin. Neurobiol. 14 (2004) 481–487.
[5] N.Y. Masse, G.C. Turner, G.S. Jefferis, Olfactory information processing in *Drosophila*, Curr. Biol. 19 (2009) R700–713.
[6] Y. Wang, H.F. Guo, T.A. Pologruto, F. Hannan, I. Hakker, K. Svoboda, Y. Zhong, Stereotyped odor-evoked activity in the mushroom body of *Drosophila* revealed by green fluorescent protein-based Ca^{2+} imaging, J. Neurosci. 24 (2004) 6507–6514.
[7] K.S. Honegger, R.A. Campbell, G.C. Turner, Cellular-resolution population imaging reveals robust sparse coding in the *Drosophila* mushroom body, J. Neurosci. 31 (2011) 11772–11785.
[8] Y. Wu, Q. Ren, H. Li, A. Guo, The GABAergic anterior paired lateral neurons facilitate olfactory reversal learning in *Drosophila*, Learn Mem. 19 (2012) 478–486.
[9] Q. Ren, H. Li, Y. Wu, J. Ren, A. Guo, A GABAergic inhibitory neural circuit regulates visual reversal learning in *Drosophila*, J. Neurosci. 32 (2012) 11524–11538.
[10] X. Liu, W.C. Krause, R.L. Davis, GABAA receptor RDL inhibits *Drosophila* olfactory associative learning, Neuron 56 (2007) 1090–1102.
[11] D. Lee, H. Su, D.K. O'Dowd, GABA receptors containing Rdl subunits mediate fast inhibitory synaptic transmission in *Drosophila* neurons, J. Neurosci. 23 (2003) 4625–4634.
[12] X. Liu, R.L. Davis, The GABAergic anterior paired lateral neuron suppresses and is suppressed by olfactory learning, Nat. Neurosci. 12 (2009) 53–59.
[13] M. Papadopoulou, S. Cassenaer, T. Nowotny, G. Laurent, Normalization for sparse encoding of odors by a wide-field interneuron, Science 332 (2011) 721–725.
[14] A. Guo, L. Li, S.Z. Xia, C.H. Feng, R. Wolf, M. Heisenberg, Conditioned visual flight orientation in *Drosophila*: dependence on age, practice, and diet, Learn Mem. 3 (1996) 49–59.
[15] L. Tian, S.A. Hires, T. Mao, D. Huber, M.E. Chiappe, S.H. Chalasani, L. Petreanu, J. Akerboom, S.A. McKinney, E.R. Schreiter, C.I. Bargmann, V. Jayaraman, K. Svoboda, L.L. Looger, Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators, Nat. methods 6 (2009) 875–881.
[16] J.B. Connolly, I.J. Roberts, J.D. Armstrong, K. Kaiser, M. Forte, T. Tully, C.J. O'Kane, Associative learning disrupted by impaired Gs signaling in *Drosophila* mushroom bodies, Science 274 (1996) 2104–2107.
[17] R.F. Stocker, G. Heimbeck, N. Gendre, J.S. de Belle, Neuroblast ablation in *Drosophila* P[GAL4] lines reveals origins of olfactory interneurons, J. Neurobiol. 32 (1997) 443–456.
[18] G. Dietzl, D. Chen, F. Schnorrer, K.C. Su, Y. Barinova, M. Fellner, B. Gasser, K. Kinsey, S. Oppel, S. Scheiblauer, A. Couto, V. Marra, K. Keleman, B.J. Dickson, A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*, Nature 448 (2007) 151–156.
[19] J.L. Pitman, W. Huetteroth, C.J. Burke, M.J. Krashes, S.L. Lai, T. Lee, S. Waddell, A pair of inhibitory neurons are required to sustain labile memory in the *Drosophila* mushroom body, Curr. Biol. 21 (2011) 855–861.
[20] C.M. Root, K. Masuyama, D.S. Green, L.E. Enell, D.R. Nassel, C.H. Lee, J.W. Wang, A presynaptic gain control mechanism fine-tunes olfactory behavior, Neuron 59 (2008) 311–321.
[21] M. Demerec, Biology of *Drosophila*, Wiley, New York, 1950.
[22] P. Thevenaz, U.E. Ruttimann, M. Unser, A pyramid approach to subpixel registration based on intensity, IEEE Trans. Image Process 7 (1998) 27–41.
[23] G.C. Turner, M. Bazhenov, G. Laurent, Olfactory representations by *Drosophila* mushroom body neurons, J. Neurophysiol. 99 (2008) 734–746.
[24] S.D. Buckingham, P.C. Biggin, B.M. Sattelle, L.A. Brown, D.B. Sattelle, Insect GABA receptors: splicing, editing, and targeting by antiparasitics and insecticides, Mol. Pharmacol. 68 (2005) 942–951.
[25] L. Enell, Y. Hamasaka, A. Kolodziejczyk, D.R. Nassel, Gamma-Aminobutyric acid (GABA) signaling components in Drosophila: immunocytochemical localization of GABA(B) receptors in relation to the GABA(A) receptor subunit RDL and a vesicular GABA transporter, J. Comp. Neurol. 505 (2007) 18–31.
[26] N.K. Tanaka, H. Tanimoto, K. Ito, Neuronal assemblies of the *Drosophila* mushroom body, J. Comp. Neurol. 508 (2008) 711–755.
[27] A.H. Brand, N. Perrimon, Targeted gene expression as a means of altering cell fates and generating dominant phenotypes, Development 118 (1993) 401–415.
[28] S.L. Lai, T. Lee, Genetic mosaic with dual binary transcriptional systems in *Drosophila*, Nat. Neurosci. 9 (2006) 703–709.