Ten-a Affects the Fusion of Central Complex Primordia in Drosophila

Xuebo Cheng1,2, Huoqing Jiang1, Weizhe Li1, Hailong Lv1, Zhefeng Gong1,3*, Li Liu1,4*

1 State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing, People’s Republic of China, 2 University of the Chinese Academy of Sciences, Beijing, People’s Republic of China, 3 School of Medicine, Zhejiang University, Hangzhou, Zhejiang, People’s Republic of China, 4 Key Laboratory of Mental Health, Chinese Academy of Sciences, Beijing, People’s Republic of China

Abstract

The central complex of Drosophila melanogaster plays important functions in various behaviors, such as visual and olfactory memory, visual orientation, sleep, and movement control. However little is known about the genes regulating the development of the central complex. Here we report that a mutant gene affecting central complex morphology, ceb (central brain defect), was mapped to ten-a, a type II trans-membrane protein coding gene. Down-regulation of ten-a in pan-neural cells contributed to abnormal morphology of central complex. Over-expression of ten-a by C767-Gal4 was able to partially restore the abnormal central complex morphology in the ceb mutant. Tracking the development of FB primordia revealed that C767-Gal4 labeled interhemispheric junction that separated fan-shaped body precursors at larval stage withdrew to allow the fusion of the precursors. While the C767-Gal4 labeled structure did not withdraw properly and detached from FB primordia, the two fan-shaped body precursors failed to fuse in the ceb mutant. We propose that the withdrawal of C767-Gal4 labeled structure is related to the formation of the fan-shaped body. Our result revealed the function of ten-a in central brain development, and possible cellular mechanism underlying Drosophila fan-shaped body formation.

Citation: Cheng X, Jiang H, Li W, Lv H, Gong Z, et al. (2013) Ten-a Affects the Fusion of Central Complex Primordia in Drosophila. PLoS ONE 8(2): e57129. doi:10.1371/journal.pone.0057129

Editor: Troy Zars, University of Missouri, United States of America

Received August 21, 2012; Accepted January 17, 2013; Published February 20, 2013

Copyright: © 2013 Cheng et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by the ‘973 Program’ (2009CB918702, 2012CB825504), the National Natural Sciences Foundation of China (31030037, 30921064 and 31070944), and the External Cooperation Program of the Chinese Academy of Sciences (GJHZ1005). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: zfgong@gmail.com (ZG); liuli@sun5.ibp.ac.cn (LL)

Introduction

The central complex is an interconnected neuropil structure across and along the sagittal mid-section of the fly brain and includes the protocerebral bridge (PB), the fan-shaped body (FB), the paired nodule (NO), and the ellipsoid body (EB). It is involved in multi-modal behavioral control, such as locomotion [1–3], visual pattern memory [4,5] and spatial orientation [6,7]. The development of the central complex can be traced back to the larval stage [8,9]. Lineage analysis has revealed the neurons that contribute to the central complex [8,9,10], but the molecular and cellular mechanism of central complex formation is not fully understood.

In the 1980s, Martin Heisenberg and coworkers generated a series of structural mutants, in which the morphology of adult central brain structures like mushroom bodies and the central complex were destroyed [1,11,12]. Among these mutants, mbm (mushroom body miniature), ceb (central brain deranged) and nob (no-bridge) have been identified. mbm was found to be a transcription factor, a nucleic acid-binding zinc finger protein [13], while ceb was reported to encode Neuroglian, a cell adhesion molecule that is crucial for axonal development, synapse formation and female receptivity [14–17]. As to nob, it interacted with det at the interhemispheric junction to affect the formation of protocerebral bridge [18]. Another mutant type is central body defect (cbd), of which the most typical phenotype is that the fan-shaped body and the ellipsoid body are fragmented in the middle, or some fusion of the fan-shaped body and the ellipsoid body. So far, the molecular basis of most structural mutants is unclear.

Ten-a belongs to a large protein family, Teneurin, which contains an N-terminal intracellular domain, a single transmembrane domain, eight EGF-like domains, a 6-blade β-propeller TolB-like domain, and 26 YD repeats [19,20]. From invertebrates to vertebrates, Teneurins function as signaling molecules at the cell surface as type II transmembrane receptors, while the intracellular domain cleaved from membrane works as a transcription regulator [21–23] and carboxyl terminus functions as a bioactive peptide [24–27]. The Teneurin family members are thought to be important for establishment and maintenance of neuronal connections, neurite outgrowth and axon guidance [20]. Recent reports showed that two Drosophila Teneurin members, Ten-a and Ten-m, are crucial for proper synaptic matching and the maintenance of neuromuscular junction [28,29]. Although Teneurin may play a role in mammalian brain function [20,30], detailed study is still largely lacking.

Here, we report that the Drosophila structural mutant gene cebd, the most typical phenotype of which is the fragmented fan-shaped body and ellipsoid body, is ten-a. The cebd mutation disrupts the formation of the FB, by preventing the merging of the two FB parts. This defect was rescued by over-expression of ten-a in a C767-Gal4 labeled structure which separated the FB parts but later disappeared to allow the merging of the two FB primordia. Our results might reveal the molecular and cellular mechanism of Drosophila central complex development.

PLOS ONE | www.plosone.org 1 February 2013 | Volume 8 | Issue 2 | e57129
**Materials and Methods**

**Fly strains**

Flies were cultured on standard cornmeal food at 25°C with a 12 h light : 12 h dark cycle at 60% humidity [31]. Wild-type flies Berlin (WTB) and 

**DNA Sequencing**

To determine whether there was a mutation in genes between 11A3 and 11A7, we sequenced the coding regions of CG1924, CG32653, and ten-a in wild-type flies (WTB) and cbd flies. The genome fragments were PCR amplified using Phu polymerase (Promega). The gene-specific primers were designed according to the FlyBase Release 5.45 genome sequence (www.flybase.org). The primers for amplification also were used for sequencing for genes ten-a and CG2653. For gene CG1924, besides primer30 for amplifying and sequencing, other sequencing primers (S1, S2) were used.

**Construction of transgenic flies UAS-ten-a**

The first strand cDNA of ten-a was synthesized from fly head mRNA using the SuperScript III first-strand synthesis system (Invitrogen). PCR reactions were performed by Phu polymerase (Promega) with 5' primer 5'-GAGTTGCTGATGCTGAGA-3' and 3' primer 5'-ATTACCTGCGTTCGAGG-3' and 3' primer 5'-ATTACCTGCGTTCGAGG-3'. The purified construct was introduced into FlyC31 strains.

**Immunohistochemistry**

Fly strains were allowed to lay eggs on standard fly food for 30 min and brain dissection was performed at different time points of development: larvae, 48–72 h, 72–96 h and 96–120 h after egg laying; pupa, 0–2 h and 8–9 h after puparium formation; adult fly, 3–5 days after elusion. Immunostaining was performed in a dish covered with cold PBS (phosphate-buffered saline, pH 7.4). The tissues were fixed in freshly prepared paraformaldehyde (4% in PBS) for 3 h on ice. Brains were then washed in PBT (PBS+0.5% Triton X-100) for 5–15 min, followed incubation for 1 h in PNT (PB+10% normal goat serum) at room temperature. Subsequently, rat monoclonal antibody to DNB-cadherin (DSHB, DN-EX #8, dilution 1:100) was used to track the development of the central complex in the larval and pupal stage. Ne80 antibody (DSHB; mAb ne82, dilution 1:100) against Brp protein was the primary antibody used to stain the neuropil in adult flies. Rabbit anti-GFP antibody (Invitrogen Inc, dilution 1:1000) was used to check the expression pattern of Gal4 lines. After incubating overnight at 4°C...
in primary antibody, samples were washed in PBT 3 times and then incubated overnight in secondary antibody. Goat anti-Rat antibody (TRITC-conjugated, Jackson Laboratories, dilution 1:200), goat anti-Mouse antibody (TRITC-conjugated, Jackson Laboratories, dilution 1:200), and goat anti-rabbit antibodies (FITC-conjugated, Invitrogen Inc, dilution 1:200) were used as secondary antibodies. In the following day, after being rinsed in PBT 3 times, brains were mounted in Vectashield Fluorescent Mounting Media (Vector Laboratories, Burlingame) and observed.

Imaging
Mounted brains were scanned with a confocal microscope (Leica TCS SP5). Each brain stack z-resolution was 0.8 μm or 1.0 μm for adult fly brains and 0.5 μm for larval or pupal brains, at pixel resolution of 1024×1024. The images were then processed with ImageJ (National Institutes of Health, rsbweb.nih.gov/ij/).

Statistics
A two-tailed Fisher exact test was used to evaluate the efficiency of rescue experiments, and two sample t-test was carried out for the numbers of F1 neurons or F5 neurons. Statistical significance was assigned, *P<0.05, **P<0.01, ***P<0.001.

Results

**cbd** is mapped to ten-a

Although most structural mutants generated by Heisenberg and colleagues are still uncharacterized, some of them have been preliminarily mapped [13,15,18,32]. The structural mutant gene **cbd**, which contributed to the disrupted fan-shaped body and ellipsoid body, was localized to 11A1–A7 on the X chromosome [1,32]. To further localize **cbd**, we adopted a traditional method of deficiency mapping using complementation tests (Fig. 1). **cbd KS171**, a representative **cbd** mutant, was crossed with a series of deficiency lines around the 11A region and the brain morphology of trans-heterozygous female offspring was checked. Three large deficiencies, Df(1)ED7161, Df(1)ED7153, Df(1)KA10, which respectively remove 10D1-11B14, 11A1-11B1 and 11A1–11A7, failed to restore the deranged central complex in **cbd KS171** mutants, confirming that the **cbd** gene lies within 11A1–11A7 (Fig. 1B–I).

Next we used deficiencies with a breakpoint between 11A1 and 11A7 for fine mapping. Both deficiency lines Df(1)RC29 and Df(1)ED7147, that remove 11A1–11A5 and 10D6-11A1 respectively, could complement the **cbd KS171** mutation, suggesting that **cbd** lies outside of 11A1–11A5 and locates in 11A5–11A7 (Fig. 1J–M). The complementation tests for another two **cbd** mutants, **cbd KS96** and **cbd 2254**, confirmed that **cbd** was located in 11A5–11A7 (Fig. 1N–Y).

We then checked the annotated genes in 11A5–11A7. According to FlyBase annotation release 5.45, there are three genes in this region: CG1924, CG32655 and CG42338. We sequenced the coding DNA region of these three genes in the three **cbd** mutant lines **cbd KS171**, **cbd 2254** and **cbd KS96**, and found no
sequence change in CG1924 and CG32655 that could lead to changes in amino acids or splicing sites. Interestingly, we found significant DNA sequence changes in the coding region of CG42358, also named ten-a (Fig. 2A). For convenience of illustration, the mutation points were assigned to the transcript RE, which encodes the largest protein isoform (3378 aa) according to the FlyBase annotation. In cdb KS171, a TGA codon (Tryptophan, 1562) was changed to TGA (a premature stop codon). In cdb KS171, a GGC codon was changed to AGC (Glycine in 1723 changed to Serine). In cdb KS96, the CGT codon was changed to TGT (Arginine 2846 changed to Cysteine) (Fig. 2B, S1). It is worth noting that all three amino acid changes occur in the conserved domains of the Ten-a protein (Fig. S2). Besides these three mutations, there are several amino acid residues that are same in the three cdb lines but different from control flies (WTB), and all are in non-conserved domain of Tenacurin (Fig. S3). All of these data strongly hint that cdb is ten-a.


ten-a is required for normal central complex morphology

Since cdb is likely ten-a, we wondered if manipulating ten-a gene expression would affect morphology of the fly central complex, as cdb mutations do. We first made use of the ten-a deficiency line ten-a^900 which was generated by P element imprecise excision in ten-a. In ten-a^900, a 2219 bp region covering two ten-a exons was deleted (Fig. 2A). ten-a^900 is homozygous viable, but the central complex morphology is disrupted (Fig. 3A, B). Complementation tests between ten-a^900 and cdb mutants, cdb KS171, cdb KS96 and cdb KS594 showed that ten-a^900 was unable to complement the cdb mutation based on brain morphology (Fig. 3C–H). Thus, ten-a^900 is another cdb mutation allele. However, the question still remained that there might exist some other common mutation outside of ten-a in these mutant lines that generated the abnormal brain phenotype. We then specifically down-regulated ten-a expression by driving expression of UAS-ten-a^900 with tub-Gal4. We noticed that morphology of both FB and EB was destroyed comparing with control flies (Fig. 3I–L). We further asked where ten-a functioned to affect fly brain morphology. By driving UAS-ten-a^900 with pan-neuronal elav-Gal4 and pan-glial repo-Gal4, we found distinct results: down-regulation of ten-a in neurons produced strong brain derangement, while down-regulation of ten-a in glial cells had no effect on fly brain morphology (Fig. 3M–P). It suggests that ten-a is required in neurons for normal central complex morphology.

Over-expression of ten-a restored normal brain morphology in cdb mutant

Next, we undertook fine mapping of the cells in which Ten-a functions to affect the central complex morphology by attempting to rescue the cdb mutant phenotype via over-expressing ten-a with screening various Gal4 lines. During the screening, pan-neuronal Gal4 line, glia-specific Gal4 line, and some region specific Gal4 lines were chosen for rescue experiments. Finally only C767-Gal4, which labeled the EB as well as the median bundle in adult central brain, significantly rescued the cdb mutant phenotype (Fig. 4). Since the phenotype is the cleavage of FB, it is reasonable to postulate that it is the C767-Gal4 labeled structure at midline but not at other regions that affected the FB morphology. Interestingly, the percentage of rescue was a little higher when flies were cultured at a constant temperature of 18°C comparing with flies cultured at a constant temperature of 25°C (Fig. 4H). Thus, Ten-a in C767-Gal4 labeled cells contributed to normal central complex formation.

Defective FB in cdb mutants is caused by the failure of FB merging

We then asked how ten-a mutation led to the deranged central complex morphology. Theoretically, the deranged FB in cdb mutants could result from either abnormal development, or from degeneration of a normal mature FB. We tracked the whole developmental process of the FB in both wild-type and cdb mutants from 2rd instar larva to pupa (Fig. 5), when the FB is supposed to form [8]. We could see several thin commissural axon tracts which was supposed as supraesophageal commissure [34] in 2nd and early 3rd instar larval brains in both wild-type and cdb mutants (Fig. 5A–D), which suggests that ten-a mutation doesn’t affect the midline crossing of this structure, at least in these stages. In late 3rd instar larval brains, we found immature FB stained by DN-cadherin anterior to the supraesophageal commissure on the two sides of the midline (Fig. 5E, F). The FB precursors expand and merge at the midline by 8–9 h after pupa formation (APF) in wild-type flies (Fig. 5G, I). In cdb KS171 flies, the similar event happens until 0–2 h APF. However, in cdb mutants the two strong DN-cadherin labeled precursors were unable to merge at 8–9 h APF (Fig. 5H, J), even at later time points that we have checked (data not shown). Thus, the broken FB in cdb mutants was likely caused by a developmental defect in FB formation, but not by neuronal degeneration of the formed FB.

C767-Gal4 labeled structure at interhemispheric junction might be required for FB formation

We then asked exactly how ten-a affected the process of FB development. As cdb mutant phenotype can be partially rescued by over-expressing ten-a in C767-Gal4 labeled structure, we supposed that the labeled cells are closely related to the event. The expression pattern of C767-Gal4 in the region of larval FB precursors was tracked during the process of FB formation in both wild-type flies and cdb mutants. At the 2nd instar larval stage, C767-Gal4 labeled precursors were separated by a C767-Gal4 labeled structure at interhemispheric junction (Fig. 6G, J, M). Later in the 3rd instar larval stage,
the interhemispheric junction was narrowed while the FB precursors expanded and invaded medially. In wild type background, the interhemispheric junction and the FB precursors are still tightly connected as if the withdrawal of C767-Gal4 labeled structure at interhemispheric junction was accompanied with the extending of the DN-cadherin signal (Fig. 6G). By the pupal stage, the C767-Gal4 labeled midline structure further shrank and disappeared completely to allow complete merging of the two FB primordium parts (Fig. 6J, M). Judging from the concurrency between the morphological changes in C767-Gal4 stained structure and DN-cadherin labeled FB primordia, we postulated that the merging of FB primordia was influenced by the C767-Gal4 labeled midline junction.

To confirm this hypothesis, we checked the development of C767-Gal4 labeled cells and FB morphology in cbd KS171 mutant (Fig. 7). In cbd KS171 mutant, the C767-Gal4 labeled midline structure that separated the two FB patches persisted during 0–2 h APF. But the structure is detached from the precursors and failed

Thecbdmutant phenotype could be significantly rescued when ten-a was driven by C767-Gal4. (A, B) WTB showed normal FB and EB. (C–F) Both UAS-ten-a and C767-Gal4 in the cbd KS171 background showed defect in FB and EB. (G, H) FB and EB were restored to normal when UAS-ten-a was driven by C767-Gal4. (I) Percentage of normal FB and EB in controls (5.6% for cbd KS171;UAS-ten-a, n = 36; 10% for cbd KS171,C767-Gal4/+; UAS-ten-a, n = 30) and in flies with UAS-ten-a driven by C767-Gal4 (36%, n = 25). Two tailed Fisher exact test. **P<0.05, ***P<0.001. When ten-a over-expressing flies were kept at 18°C, 47% (n = 32) of flies showed a normal FB and EB, much higher than control flies (12.5% for cbd KS171;UAS-ten-a, n = 24; 11.1% for cbd KS171;C767-Gal4/+; UAS-ten-a, n = 18). Scale bar, 25 μm. Arrows indicate the central complex defect.

doi:10.1371/journal.pone.0057129.g004
to guide the merging of FB parts and consequently the formation of a normal FB (Fig. 7). Based on this morphological observation, as well as the rescue results, we concluded that the Ten-a molecule in the C767-Gal4 labeled structure affected merging of FB primordia.

Defective FB in *cbd* mutants is not caused by abnormal axonal projections

Since the deficiency is not due to the degeneration, we also wondered if the failure in FB part merging in *cbd* mutants was due to improper generation or specification of FB neurons. We counted the neurons labeled by NP6310-Gal4 and C205-Gal4 in both *cbd* K5171 mutant and wild type adult flies. The somata of F1 neurons were located anterior to the antennal lobes and the somata of F5 neurons were around the calyces of mushroom bodies. The numbers of neurons varied even in each hemisphere in the same brain and there was no significant difference between wild type and *cbd* K5171 flies (Fig. 8A, C, E, G, Fig. S4). We also wondered if the abnormal morphology of FB was caused by abnormal axonal projections of FB neurons. The morphology of F1 neurons labeled by NP6310-Gal4 and F5 neurons labeled by C205-Gal4 were checked in adult *cbd* mutant flies. As shown in Figure 8, FB in *cbd* K5171 mutant were fragmented into pieces (Fig. 8D, H), but the projection tracts of both F1 neurons and F5 neurons appeared to be normal in *cbd* K5171 mutants (Fig. 8B, F). Thus, the mutant FB phenotype in *cbd* mutants is not caused by

Figure 5. The development of the FB in both WTB and *cbd* K5171 from 2nd instar larva to pupa. (A–D) DN-cadherin signal in the commissure in 2nd instar larval brain of WTB (A) or *cbd* K5171 mutant (B), and in early 3rd instar larval brain of WTB (C) or *cbd* K5171 mutant (D). Scale bar in (D) equals 25 μm and applies to (A–D). (E–J) FB precursors strongly labeled by DN-cadherin in the commissure in a late 3rd instar larval brain from WTB (E) or a *cbd* K5171 mutant (F), and in a 0–2 h APF pupal brain from WTB (G) or a *cbd* K5171 mutant (H), and in an 8–9 h APF pupal brain from WTB (I) or a *cbd* K5171 mutant (J). Scale bar in (J) equals 25 μm and applies to (E–J).

doi:10.1371/journal.pone.0057129.g005
abnormalities in neuronal projection. Rather, the FB defect in \textit{cbd} mutants was caused by abnormalities in growth of the FB midline-crossing arborizations, but terminal arborizations in the non-cleft region could still form in \textit{cbd} mutants.

**Discussion**

In this study, we found that the \textit{Drosophila} central brain morphological mutant \textit{cbd} is actually \textit{ten-a}, a member of the teneurin family. Ten-a is required for fusion of the fan-shaped body precursors, before the formation of the complete normal FB. Mutation in \textit{ten-a} leads to the failure of the two FB precursors to merge and consequently to the deranged fan-shaped body in adult flies.

Aside from the FB morphological defect itself, \textit{ten-a} mutation might cause other abnormalities that contribute to the morphological defect. For example, Ten-a might affect the projections and contra-lateral crossing of FB neurons resulting from lineages of FBP1 and FBP2, which contribute to two staves of the fan-shaped body [10], consequently led to a cleaved fan-shaped body. Nevertheless, the generation and projection of large field ExF1 neurons labeled by \textit{NP6510-Gal4} or \textit{C203-Gal4} are not affected when \textit{ten-a} mutated (Fig. 8), which suggested that \textit{ten-a} mainly produce the morphological defect by exerting its effect on FBP1 and FBP2 neuron arborizations. Actually, based on the morpho-
logical observation in *cbd* *Ks171* flies and the rescue results, we postulated that the interhemispheric structure *C767*-Gal4 labeled was related to FB primordial fusion. But it seemed to have no effect on axonal projections and terminal arborization of F1 and F5 neurons.

*ten-a* knockdown results showed that the neuronal *ten-a* was required for the central complex formation. Further rescue experiments suggested that neither neurons nor glial cells alone were sufficient for normal central complex formation. After screening, one Gal4 line was found finally. *C767*-Gal4 could be used to rescue the *cbd* mutant phenotype significantly. To identify the cell types labeled by *C767*-Gal4, we used neuron specific marker ELAV or glial cell specific marker REPO to co-stain with *C767*-Gal4 labeling cells. The results showed that nlsGFP driven by *C767*-Gal4 was co-localized with both neuronal and glial markers from larval to early pupal stages (Fig. S5). Since previous studies showed some adhesion molecules were expressed both in neurons and glia for mediating the fasciculation of axon bundles, axon guidance or targeting [35], we suggested that the rescue results by *C767*-Gal4 might just attribute to that the Gal4 expressed both in neurons and glial cells. That is to say, only when *ten-a* functions in certain neurons and glial cells together, the FB precursors could merge normally. However, we could not identify which neurons and glial cells were required for the partial rescue from current results. To solve this problem, more Gal4 lines which can rescue the *cbd* mutant phenotype are needed. Then, dependent on the expression patterns of these Gal4 lines, the neurons and glial cells which *ten-a* functions in may be identified.

If Ten-a functions in *C767*-Gal4 labeled cells to influence the merging of FB primordia, what is its working partner for the

Figure 7. The expression pattern of *C767*-Gal4 and DN-cadherin signal during FB formation in *cbd* *Ks171* mutants. (A–C) 2nd instar larval brain. (D–F) early 3rd instar larval brain. (G–I) late 3rd instar larval brain. (J–L) pupal brain 0–2 h APF. (M–O) pupal brain 8–9 h APF. Scale bar in (O) equals 25 μm and applies to (G–O).

doi:10.1371/journal.pone.0057129.g007
arborization of FB neurons? As a Drosophila homolog of vertebrate Teneurin, Ten-a has been reported to be involved in embryo development, especially in the central nervous system. Ten-a, as well as its homologue Ten-m, was recently found to be required for synaptic matching between olfactory receptor neurons and corresponding projection neurons [28]. Ten-a and Ten-m were also important for establishing the correct connection in the larval neuromuscular junction [29,36]. In our work, lack of normal Ten-a function led to failure in merging of FB precursors. It is possible to assume that Ten-a itself mediates homophilic interaction between neurons and glial cells to regulate the fusion of the central complex, such as Nrg, which is expressed on both neurons and glial cells and interacts to control axonal sprouting and dendrite branching [37]. Meanwhile, Ten-a may interact with other molecules such as Ten-m, or other membrane proteins that function in heterophilic way at the cell surface. Further molecular and cellular experiments are needed to elaborate this important issue.

Vertebrate Teneurins have been suggested to be related to mental diseases and our discovery of Ten-a function in Drosophila brain development seems to support the hypothesis. Neuroglian (Nrg), whose vertebrate homologue L1-CAM has been implicated in neurological disorders [38,39], is also required for development of normal brain morphology in Drosophila [40,41]. Considering that both Nrg and Ten-a are type-II transmembrane proteins with extracellular EGF repeats and also function in glial cells for brain development [40], it is possible that Teneurins in vertebrates also affect brain development, and probably synapse formation, as vertebrate Nrg does.

In summary, our work elucidates the function of ten-a in development of the Drosophila central brain, and the cellular mechanism underlying FB formation.

### Supporting Information

**Figure S1** Sequence traces show the mutation in cbd lines. (A) Sequence trace shows the nucleotide change of G in control flies to A in cbd2254 leading to the nonsense mutation (W1562*). (B) Sequence trace in cbdKS171 shows G to A nucleotide change, leading to missense mutation (G1723S). (C) Sequence trace in cbdKS96 shows C to T nucleotide change, leading to missense mutation (R2846C). The underlines indicate the base substitution position.

**Figure S2** Conservation analysis of amino acids which were mutated in cbd2254, cbdKS171, and cbdKS96, respectively. Multiple-sequence alignment for Teneurin homologues surrounding the coding changes (boxed) was done by MegAlign program.
We found the three regions all are with high conservation (Cyath), especially the changed sites, W1562, G1723, R2046, which can be found in all 20 homologues. (TIF)

Figure S3 Conservation analysis of amino acids that were seen in all three cd3 lines, but not in control flies (WTB). A Schematic drawing of sites that are different between cd3 and control cd2 flies. At position 168, P in WTB was changed to A in all three cd3 lines. D328 was changed to G328. I691 was changed to M691. L3372 was changed to F3372. B Conservation analysis of the sites. From the alignment results, we can see these sites are in a region with low conservation (Cyath) and the changed sites (boxed) are not appeared in other homologues. (TIF)

Figure S4 Average numbers of F1 and F5 neurons in control flies (C205-Gal4>UAS-GFP and NP6510-Ga4>UAS-GFP) and cd2 K5727 mutant flies. No significant difference of neuron numbers was observed between control flies (light grey) and cd2 K5727 (dark grey), either for C205-Gal4 labeled F1 neurons (left) or for C205-Gal4 labeled F5 neurons (right). Two sample t-test, error bars represent the s.e.m.; n.s., not significant. (TIF)

Figure S5 C767-Gal4 labels both neurons and glial cells from the 3rd instar larval to early pupal stage. For easy illustration, multiple middle z-axis slices were stacked. GFP-labeled cell bodies (green) driven by C767-Gal4 co-localized with neurons (arrowheads) with a neural specific marker, ELAV, stained by anti-ELAV antibody (magenta) in 3rd instar larval brain (A), pupal brain 0−2 h APF (B), and pupal brain 8−9 h APF (C). GFP-labeled cell bodies driven by C767-Gal4 co-localized with glial cells (arrowheads) with a glial specific marker, REPO, stained by anti-REPO antibody (blue) in 3rd instar larval brain (D), pupal brain 0−2 h APF (E), and pupal brain 8−9 h APF (F). Schematics of distributions of neurons and glial cells in whole brains of 3rd instar larva (G), pupa 0−2 h APF (H), and pupa 8−9 h APF (I). Scale bars, 25 μm. (TIF)

Acknowledgments
We are grateful to Martin Heisenberg and Ron Wides for providing the flies, Konrad Basler for providing pUASTattB. We thank Haiyun Gong, Mo Han, and Jianwen Wu for technical assistance. We also thank the Bloomington Drosophila Stock Centers, the Vienna Drosophila RNAi Center, and Drosophila Genetic Resource Center at Kyoto Institute of Technology for fly stocks.

Author Contributions
Conceived and designed the experiments: XC ZG LL. Performed the experiments: XC HJ WL. Analyzed the data: XC ZG LL. Contributed reagents/materials/analysis tools: XC ZG LL. Wrote the paper: XC ZG LL.

References
1. Strauss R, Heisenberg M (1995) A higher center of locomotor behavior in the Drosophila brain. J Neurosci 15: 1852−1861.
2. Martin J R, Raabe T, Heisenberg M (1999) Central complex substructures are required for the maintenance of locomotor activity in Drosophila melanogaster. J Comp Physiol A 185: 277−288.
3. Strauss R (2002) The central complex and the genetic dissection of locomotor behaviour. Curr Opin Neurobiol 12: 653−638.
4. Liu G, Seiler H, Wen A, Zars T, Ito K, et al. (2006) Distinct memory traces for two visual features in the Drosophila brain. Nature 439: 531−536.
5. Pan Y, Zhou Y, Guo C, Gong H, Gong Z, et al. (2006) Distinct memory traces for visual features in the Drosophila brain. J Neurosci 13: 1852−1861.
6. Neuser K, Tripathan T, Mrone M, Pocek B, Strauss R (2008) Analysis of a spatial memory network in Drosophila. Nature 453: 1244−1247.
7. Xiong Y, Lu H, Gong Z, Liu L (2010) Fixation and locomotor activity are required for the maintenance of locomotor activity in Drosophila central complex in icebox mutants caused by a mutation in the L1-tenascin, shows selective transcript localization. Mech Dev 40: 165−176.
8. Xu S M, Ferralli J, Choi K, Brown-Luedi M, Minet A D, et al. (2005) The intracellular domain of teneurin-1 interacts with MBD1 and CAP/ponsin resulting in subcellular codistribution and translocation to the nuclear matrix. Exp Cell Res 305: 122−136.
9. Qian X, Barry-lovey-Wong D, Wang L, Chewboy B, Gantam N, et al. (2004) Cloning and characterization of teneurin C-terminus associated peptide (TCAP-3) from the hypothalamus of an adult rainbow trout (Oncorhynchus mykiss). Gen Comp Endocrinol 137: 205−216.
10. Wang L, Rotzinger S, Al Chavaf A, Elias C F, Barry-lovey-D, et al. (2005) Teneurin proteins possess a carboxy terminal sequence with neuromodulatory activity. Brain Res Mol Brain Res 133: 233−265.
11. Carhan A, Allen F, Armstrong J D, Horsch M, Goodwin S F, et al. (2005) Receptor activation phenotype of ic5 mu mutants caused by a mutation in the l1-receptor cell adhesion molecule. Genes Brain Behav 4: 449−465.
12. Godenschwege T A, Kristiansen L V, Uthaman S B, Horsch M, Murphey R K (2006) A conserved role for Drosophila Neuroglian and human L1-CAM in central-synaptic formation. Curr Biol 16: 12−23.
13. Gossens T, Kang Y Y, Waytes G, Zimmermann P, Callaerts-Vegh Z, et al. (2011) The Drosophila L1-CAM homolog Neuroglian signals through distinct pathways to control different aspects of mushroom body axon development. Development 138: 1395−1405.
32. Hanisch U (1987) Der Zentralkomplex von *Drosophila melanogaster*. Ph. D. Thesis, Universiaet Wuerzburg, Germany.

33. Li W, Pan Y, Wang Z, Gong H, Gong Z, et al. (2009) Morphological characterization of single fan-shaped body neurons in *Drosophila melanogaster*. Cell Tissue Res 336: 509–519.

34. Nassif C, Noveen A, Hartenstein V (2003) Early development of the *Drosophila* brain. III. The pattern of neuropile founder tracts during the larval period. J Comp Neurol 455: 417–434.

35. Silies M, Klambt C (2011) Adhesion and signaling between neurons and glial cells in *Drosophila*. Curr Opin Neurobiol 21: 11–16.

36. Kurusu M, Cording A, Taniguchi M, Menon K, Suzuki E, et al. (2008) A screen of cell-surface molecules identifies leucine-rich repeat proteins as key mediators of synaptic target selection. Neuron 59: 972–985.

37. Yamamoto M, Ueda R, Takahashi K, Saigo K, Uemura T (2006) Control of axonal sprouting and dendrite branching by the Nrg-Ank complex at the neuron-glia interface. Curr Biol 16: 1678–1683.

38. Egle E C (2010) Human genetic disorders of axon guidance. Cold Spring Harb Perspect Biol 2: a001794.

39. Kurumaji A, Nomoto H, Okano T, Toru M (2001) An association study between polymorphism of LICAM gene and schizophrenia in a Japanese sample. Am J Med Genet 103: 99–104.

40. Chen W, Hang H (2008) The L1-CAM, Neuroglian, functions in glial cells for *Drosophila* antennal lobe development. Dev Neurobiol 68: 1029–45.

41. Godenschwege T A, Murphey R K (2008) Genetic interaction of Neuroglian and Semaphorin1a during guidance and synapse formation. J Neurogenet 23: 147–55.