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Identification and structural characterization of two genes encoding glutamate transporter homologues differently expressed in the nervous system of *Drosophila melanogaster*

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Abstract In vertebrates, excitatory amino acid transporters (EAATs) are believed to mediate the removal of glutamate released at excitatory synapses and to maintain extracellular concentrations of this neurotransmitter below excitotoxic levels. Glutamate is also used in insects as an excitatory neurotransmitter at the neuromuscular junction and probably in the central nervous system where its role remains to be established. We report the molecular characterization and developmental expression pattern of two *Drosophila* cDNAs: dEAAT1, which has recently been identified as a high affinity glutamate transporter [1], and dEAAT2, a novel protein sharing strong homology to dEAAT1 and to the mammalian EAAT protein family. The developmental expression pattern of the two *Drosophila* EAAT genes has been compared by Northern blot analysis and whole-mount in situ hybridizations. The two transporters are transcribed in distinct cell types of the nervous system and are strongly expressed in the adult visual system.

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Key words: Glutamate transporter; Central nervous system; Visual system; *Drosophila*

1. Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). It plays an essential role in brain development [2] and in the neural networks controlling memory [3], learning and motivity [4]. The termination of glutamatergic synaptic transmission is controlled by the high affinity excitatory amino acid transporters (EAATs), which are expressed in neurons and glial cells [5]. These transporters are believed to capture the neurotransmitter released in the synaptic cleft and prevent glutamate-induced neuronal death (or excitotoxicity) by maintaining a low extracellular concentration of this neurotransmitter [6]. They also play a role in developmental processes in the brain [7]. Increased levels of extracellular glutamate are observed in several neurodegenerative processes in humans such as Alzheimer's, Huntington's, Parkinson's diseases and amyotrophic lateral sclerosis [8–11], but the mechanisms by which glutamate is increased are unknown. A defect in glutamate transport could be in part responsible for the neurological disorders in amyotrophic lateral sclerosis and ischemia [12].

By now, five glutamate transporters have been identified in human: hEAAT1 or GLAST, hEAAT2 or GLT, hEAAT3 or EAAC, hEAAT4 and hEAAT5 [13–16]. Homologous genes were found both in vertebrates and in invertebrates. All these proteins form a family with functional and structural homologies [17].

In insects, glutamate is the excitatory neurotransmitter at the neuromuscular junction [18,19] and it is also used in the CNS where its role remains to be established [20]. Several glutamate receptors have been characterized which are expressed in the *Drosophila* CNS [21–24] or in somatic muscles [25,26]. However, little is known about the physiology of the glutamatergic synapse and the occurrence of excitotoxicity in insects.

We report here the isolation and molecular characterization of two *Drosophila* cDNAs that encode proteins belonging to the family of high affinity glutamate transporters, named dEAAT1 and dEAAT2. While this work was in progress, a cDNA encoding dEAAT1 was reported and the corresponding protein shown to be a high affinity glutamate transporter [1]. We have extended this work by analyzing the developmental expression profile of dEAAT1 and its cellular expression pattern from embryo to adult in the *Drosophila* CNS. In addition, we have identified and characterized dEAAT2, a novel protein which is distinct from dEAAT1 but equally related to glutamate transporters. These two transporters are expressed most strongly in the adult fly heads. Whole-mount in situ hybridizations show that the two genes are expressed in different cellular populations of the nervous system from embryo to adult. Our results suggest that glutamate plays a significant role in the *Drosophila* visual system as is the case for vertebrates.

2. Materials and methods

2.1. Reverse transcription linked to polymerase chain reaction (RT-PCR)

The procedures used for RNA isolation and RT-PCR have been described previously [27]. Total RNA was extracted from larval CNS and adult heads of *Drosophila melanogaster* Oregon R strains. Reverse transcription was performed with SuperScript II reverse transcriptase (Gibco-BRL). Typically, for PCR reactions, cycles of 1 min at 94°C, 1 min at 60°C and 30 s at 72°C were repeated 35 times, followed by a final step of 10 min at 72°C.

2.2. Isolation of a dEAAT1 PCR probe

To isolate a *Drosophila* glutamate transporter homologue, the following EAAT degenerate primers were used: for the reverse transcription step, 5′-(G,C)(A,T,C)(A,G)TC(A,T)CC(A,C,G)A(A,G)(A,C,G,T)AC(A,G)TT (antisense, corresponding to amino acids 439–435 in the dEAAT1 sequence), for the subsequent PCR, 5′-GC(A,T,C)-

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[1]These authors contributed equally to this work.

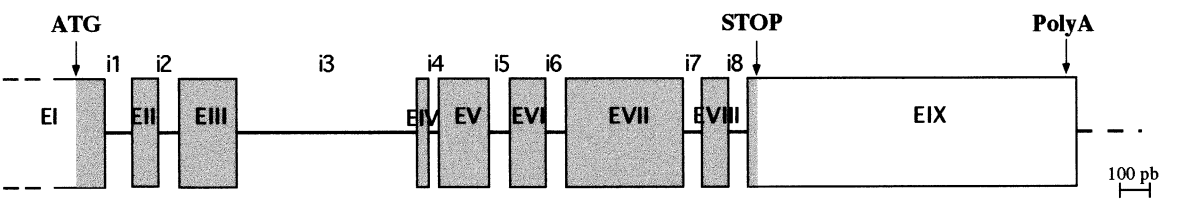
Abbreviations: BDGP, Berkeley *Drosophila* Genome Project; CNS, central nervous system; EAAT, excitatory amino acid transporter; PCR, polymerase chain reaction; RT-PCR, reverse transcription linked to polymerase chain reaction; RACE, rapid amplification of cDNA ends

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   1       M  T  R  P  K  Q  D  G  G  K  F  K  A  F  M  Q  E  N  V  L  T  M  A  T  V  I  G  V  F  V  G  G  L  I  G  F  I  I  K  N  S  T
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D

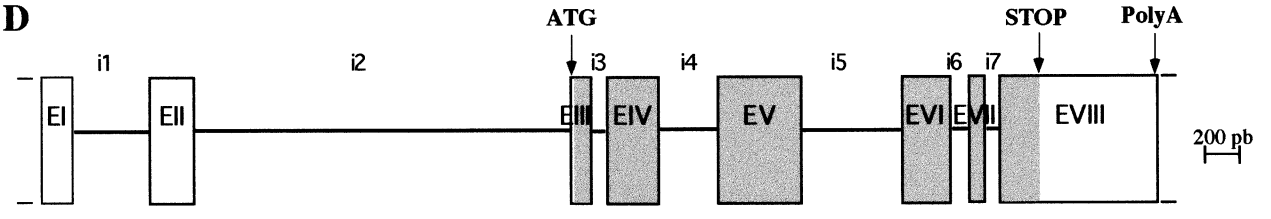


Fig. 1. Nucleotide sequences of the *Drosophila* EAAT cDNAs and schematic organization of their genomic regions. A, B: dEAAT1. C, D: dEAAT2. A: Sequences of the isolated cDNA clone and 3′ and 5′ RACE-PCR products were combined to construct the complete dEAAT1 cDNA sequence. 5′ RACE-PCR yielded only a few more bases upstream of the clone insert. C: The sequence of the longest dEAAT2 cDNA clone is presented. The deduced amino acid sequences are indicated below the nucleotide sequences. Nucleotide and amino acid positions are numbered in the left margins. The 5′ and 3′ untranslated regions are in lower case and the coding sequences in upper case. The translation start sites and the termination codons are boxed. The polyadenylation signals are in bold letters. Putative sites for *N*-linked glycosylation are circled. Consensus sites for phosphorylation by protein kinase C are in squared circles. Positions of the intron/exon boundaries are indicated by the ϕ symbols. For both genes, the untranslated sequence is not complete since the size of the transcripts estimated on Northern blot is larger than the size of the cDNAs. B, D: The exon/intron structure of the dEAAT genes was deduced by comparing the cDNA sequences with the genomic sequences available from the BDGP database. The exons (E) are represented by boxes and the introns (i) by a solid line. The coding part of the exons is in gray.
←

AC(G,T,C)(A,G)T(C,T)A(A,C)(A,C)ATGGA(C,T)GG (sense, corresponding to amino acids 347–353 in dEAAT1) and 5′-C(G,T)(A,G)TC(A,C)AG(A,G,C)A(A,G)CCA(A,G)TC(A,G,C)AC (antisense, corresponding to amino acids 429–422 in dEAAT1). The 248 bp RT-PCR product obtained was sequenced and specific oligonucleotides were synthesized for amplification of cDNA ends. 3′ RACE was performed as described [27] and 5′ RACE with the Gibco-BRL kit (5′ RACE System, Version 2.0). After gel separation, the specific bands amplified were purified, cloned and sequenced.

2.3. Isolation of a dEAAT2 PCR probe

By comparison with the vertebrate glutamate transporters, a homologous *Drosophila* genomic sequence has been found by performing a BLAST search on the Berkeley *Drosophila* Genome Project (BDGP) database (see Section 3.1). Specific sense and antisense oligodeoxynucleotides (respectively 5′-ATGCGCGTACTCAAGCTGATG, starting 219 nucleotides after the ATG in the dEAAT2 cDNA, and 5′-TCGCACTGTAGGACTTGTCCG, starting 1357 nucleotides after the ATG in dEAAT2) were synthesized and used for PCR amplification. The template was a single-stranded cDNA obtained by reverse transcription of *Drosophila* adult head RNA and oligo-dT priming.

2.4. Screening of Drosophila head cDNA library

The probes were radiolabeled with [α-^{32}P]dCTP (New England Nuclear) by random priming and used to screen a cDNA library from *Drosophila* adult heads in λZap II (generous gift of Y.N. Jan, UCSF, kindly provided by Y. Grau). Hybridizations were carried out at 42°C in 50% formamide, 5×SSC, 2.5×Denhardt's, 0.05 M sodium phos-

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dEAAT2  RSGTNTLGIVFFCLVFGTFLGTIGQKGQVVVDFFAAIFEVIMKMVTCVMWLIPVGISSVIAGKILSVGDLGLVMSQLMWFIVIVAIGVFIYQFVVMQAIYFVVVRRNPFKFYAGLIQAML
hEAAT2  KDGMNVLGLIGFFIAFGIAMGKMGDQAKLMVDFFNILNEIVMKLVIMIMWYSPLGIACLICGKIIAIKDLEVVARQLGMYMVTVIIGLIIHGGIFLPLIYFVVTRKNPFSLFAGIFQAWI

dEAAT1  TAFGTGSSSAIMPLTIKCLDN-MGIDPRVTRFVIPVGATINMDGTALYEAVAAIFIAQYREMSYSFGTIVAVSITATAASIGAAGIPQAGLVTMVMVLDTVGLEPKDVSLIIAVDWLLDR
dEAAT2  TAFATASTAAALPITFRCMDEKLKVDPRITRFVLPIGCNINMDGTALYIAVASIFIAQMSGMVLGFGELLTVLLISTAASMSSASVPSAALVLLLVVLTAIDAPVQDVTLLFAVDWFVDR
hEAAT2  TALGTASSAGTLPVTFRCLEENLGIDKRVTRFVLPVGATINMDGTALYEAVAAIFIAQMNGVVLDGGQIVTVSLTATLASVGAASIPSAGLVTMLLILTAVGLPTEDISLLVAVDWLLDR

dEAAT1  FRTTINVMCDALGTILVNHLSKNDLASVDRLN--AEPHELLELGPNGHEMKE----------------------------------------------
dEAAT2  IRTTNNMLGDCYTAAVVEELSRKELMALDAASVNYQDMPAGTPNGHGHGHEDGGLLEGQTELETSSKCVMTMTDSVVVDISAVMNNVNLQQEHCNRRV
hEAAT2  MRTSVNVVGDSFGAGIVYHLSKSELDTIDSQHRVHEDIEMTKTQSIYDDMKNHRESNSNQCVYAAHNSVIVDECKVTLAANGKSADCSVEEEPWKREK
```

B

	hEAAT1	hEAAT2	hEAAT3	hEAAT4	hEAAT5	Ceglut-1	TrnEAAT1
dEAAT1	41	35	42	37	37	41	59
dEAAT2	39	40	42	36	36	45	39

Fig. 2. Homology of the *Drosophila* EAAT proteins with previously identified glutamate transporters. A: Amino acid sequence alignment of dEAAT1 and dEAAT2 with human EAAT2 (hEAAT2). Identical residues are shaded and conserved residues are boxed. Comparable alignments were obtained with the other EAAT proteins. B: Amino acid identity score of human and invertebrate glutamate transporters with the dEAAT proteins. The sequence of each EAAT protein was aligned with dEAAT1 and dEAAT2 respectively to determine the percent identity. Ceglut-1: *Caenorhabditis elegans* glutamate transporter [45]. TrnEAAT1: *Trichoplusia ni* glutamate transporter [32]. All alignments were generated with the Clustal W software [46].

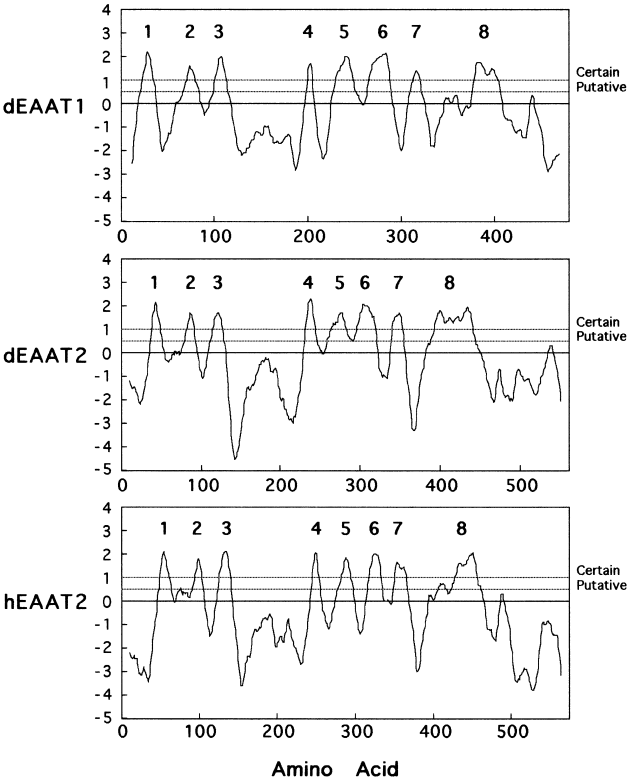


Fig. 3. Hydrophobicity profiles of dEAAT and hEAAT2 proteins. Hydropathy analysis was performed with the TopPred II software [47] using the Goldman, Engleman and Steitz algorithm [48]. The hydrophobic peaks that qualify as certain transmembrane domains (above 1.0 GES units) are numbered.

phate buffer pH 6.8, 125 μg/ml heat-denatured herring sperm DNA, 10% dextran sulfate, 0.1% *N*-lauroyl sarcosine, 0.02% SDS. The filters were washed at 65°C with 0.2×SSC-0.1% SDS as a final wash. Autoradiography was performed at −70°C using BioMax (Kodak) film and screen. After the third round of screening, isolated positive clones were characterized with regard to insert size. The largest *Eco*RI insert was recovered by in vivo plasmid excision. The complete sequence of the selected clones was performed and compared to the corresponding genomic sequences available on the BDGP database.

2.5. Cytological localization of the dEAAT genes

A biotin-labeled dEAAT1 probe was used to map the localization of the gene by hybridization to polytene chromosomes of salivary glands as described in [28]. The result was confirmed by hybridization on a *Drosophila* P1 clone array filter (Genome Systems) and by the recent sequencing of the genomic region by the BDGP. Localization of dEAAT2 was deduced from mapping of a P1 clone containing this gene by the BDGP.

2.6. Northern blot analysis

Total RNA was isolated from time-staged embryos, larvae and adult heads and bodies. Ten micrograms of RNA were fractionated by electrophoresis on denaturing formaldehyde-1% agarose gels and blotted onto a positively charged nylon membrane (ICN) by capillary transfer. RNA blots were hybridized as described above for the cDNA library screening, with a final 0.1×SSC-0.1% SDS wash at 65°C. The blots were successively probed with the radiolabeled inserts of the dEAAT1 and dEAAT2 cDNA clones, and a ribosomal DNA probe to control loading. Blots were stripped by two 0.01×SSC-0.1% SDS, 1 mM EDTA washes at 100°C between each probe. Sizes of the mRNA were deduced by comparison with the migration of standard RNAs (Perfect RNA markers, Novagen).

2.7. Whole-mount in situ hybridization

In situ hybridization was carried out essentially as described in [29]. *Drosophila* embryos were treated for 8 min in 4 μg/ml activated proteinase K (Amresco) at room temperature with agitation [30]. Third instar larval CNS and adult brains were dissected at 4°C in PBS and fixed for 20 min in 4% paraformaldehyde in PBS. After four washes, they were treated for 4 min only in 4 μg/ml activated proteinase K. Single-stranded digoxigenin-labeled RNA probes were synthesized from linearized cDNA plasmids using the Boehringer-Mannheim DIG RNA labeling kit. After the labeling reaction, the RNA was size-reduced by mild alkali hydrolysis with 2 volumes of 100 mM carbonate buffer pH 10.2 for 20 min at 65°C. The final pellet was resuspended in 70 μl of hybridization buffer (50% formamide, 5×SSC, 100 μg/ml heparin, 100 μg/ml heat-denatured herring sperm DNA, 0.1% Tween-20) and 5 μl were used for each labeling reaction.

3. Results and discussion

3.1. Isolation and characterization of dEAAT1 and dEAAT2 cDNA

The C-terminal part of high affinity glutamate transporters presents the highest level of amino acid identity [17]. Degenerate oligonucleotides were chosen in this region for RT-PCR experiments, leading to the isolation of a 248 bp cDNA fragment homologous to glutamate transporters from *Drosophila* larval CNS. Using this sequence, a larger 1.1 kb cDNA fragment was subsequently amplified from *Drosophila* adult head RNAs by 3′ RACE. This 1.1 kb fragment was used as a probe to screen a *Drosophila* head cDNA library, yielding several positives cDNA clones from the same gene that was called dEAAT1. The largest cDNA insert obtained (2.4 kb) contains a 1437 bp open reading frame but lacks the polyadenylation signal, which was identified by sequencing the 3′ RACE fragment. The deduced amino acid sequence of dEAAT1 predicts a protein of 479 residues (Fig. 1A). The dEAAT1 nucleotide sequence presents some base discrepancies in the coding region with the sequence reported recently [1] but the predicted amino acid sequences are identical. In situ hybridization to polytene chromosomes indicates that the gene is located at position 30A7–8 on the left arm of the second chromosome. The dEAAT1 gene is included in two P1 clones, DS06478 and DS03809, which were mapped by the BDGP at the same chromosomal locus.

To isolate other glutamate transporter candidates from *Drosophila*, a BLAST search was performed on the BDGP

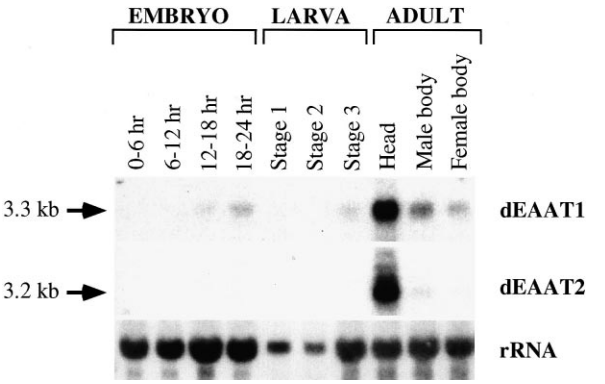


Fig. 4. Developmental pattern of the dEAAT transcripts determined by Northern blot analysis. The same developmental Northern blot was probed successively with radiolabeled dEAAT1 cDNA, dEAAT2 cDNA, and finally ribosomal DNA to quantify RNA loading. The dEAAT probes corresponded to the entire cDNA sequences. The size of transporter mRNAs is indicated on the left (arrows).

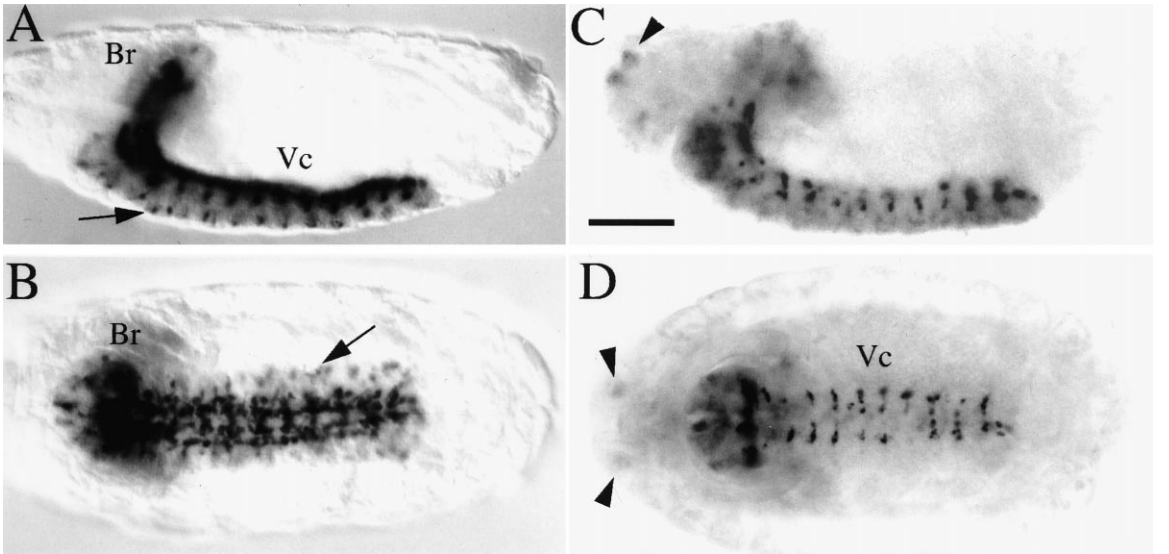


Fig. 5. Whole-mount in situ hybridization on stage 15–16 *Drosophila* embryos. A, B: Lateral and ventral views of embryos stained with an antisense dEAAT1 probe. No signal is detected out of the CNS. Arrows indicate a population of ventrally located cells. C, D: Lateral and ventral views of embryo stained with an antisense dEAAT2 probe. Small clusters of cells are labeled in the frontal part of the embryos (arrowheads), which are probably in the larval photoreceptor organs. Anterior part of embryos is on the left and dorsal on top. Br: brain; Vc: ventral cord. Bar = 100 μm.

sequence database. A single sequence presenting a significant homology to glutamate transporters was detected. This sequence is included in the P1 clone DS02649 that maps to the 21D chromosomal region. Oligonucleotides were designed from this sequence to amplify a 1 kb cDNA fragment from adult fly head RNA by RT-PCR. This fragment was used as a probe to screen the *Drosophila* head cDNA library, resulting in the isolation of several clones. The cDNA clone with the larger insert size (3.1 kb) contains an open reading frame encoding a protein of 561 amino acids, named dEAAT2 since it is the second member of this family characterized in *Drosophila* (Fig. 1C). A polyadenylation signal is located 16 nucleotides upstream of the cDNA end. The translation start site at nucleotide 531 matches for the *Drosophila* consensus sequence [31].

The exon/intron structure of the dEAAT genes was determined by comparing the cDNA sequences with the corresponding genomic sequences recently available from the

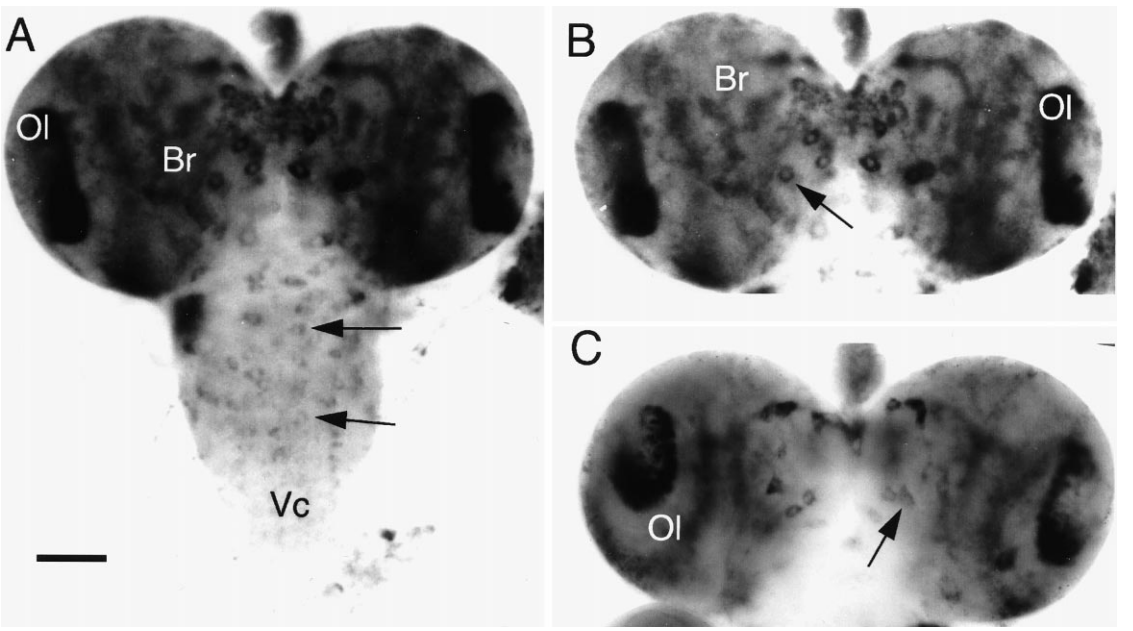


Fig. 6. Localization of the dEAAT1 mRNA in *Drosophila* larval CNS. A: Third instar larval CNS stained with the antisense dEAAT1 probe. Large cells are labeled in the ventral chain (arrows) but the staining is more intense in the brain and optic lobes. B: Same CNS as in A but less exposed to show the large cell bodies that are stained in the brain (arrow). C: Deeper focus plan of the same CNS showing the characteristic half-ring structure labeled in the optic lobes and more cell bodies (arrow). Anterior is on top. Br: brain; Ol: optic lobes; Vc: ventral cord. Bar = 50 μm.

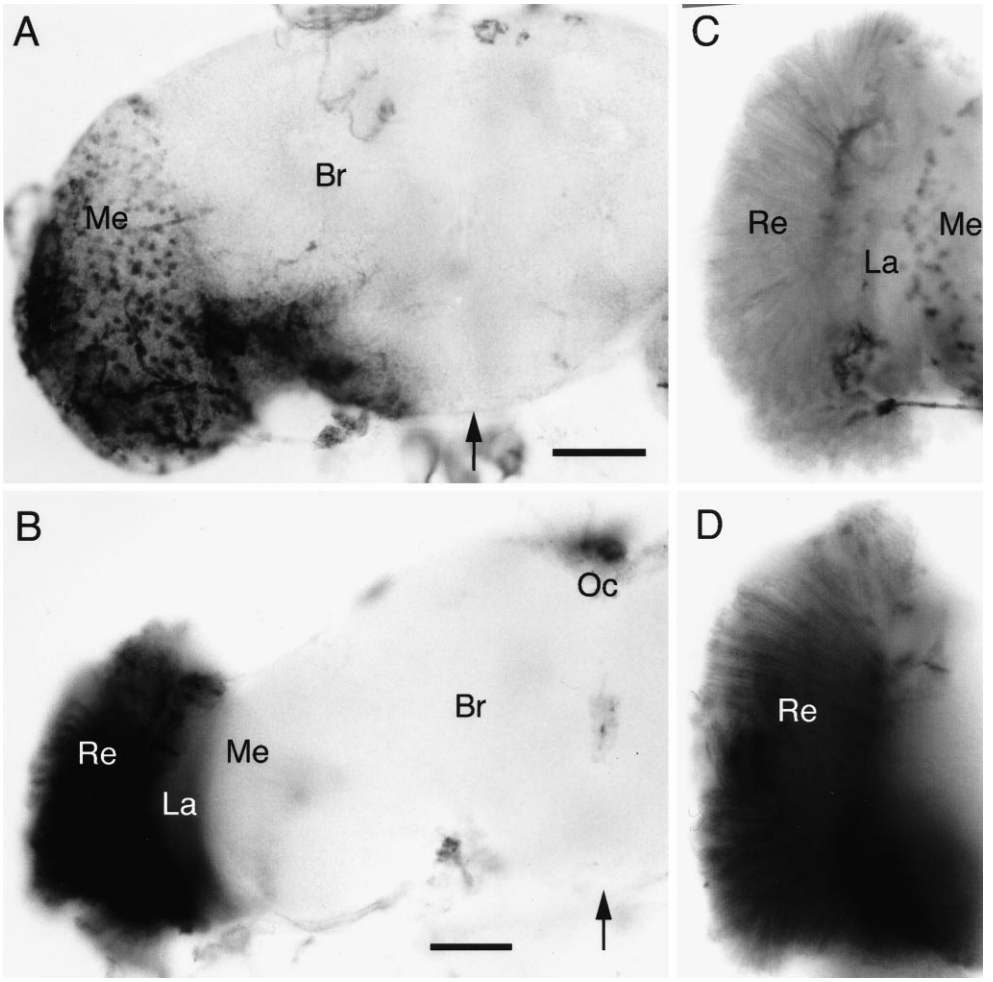


Fig. 7. Whole-mount in situ hybridization on adult *Drosophila* brain and visual system. A: Localization of dEAAT1 transcripts in adult brain. dEAAT1 is mostly detected in large cortical cells of the medulla. Note that retina and lamina have been peeled off this brain. B: Localization of dEAAT2 transcripts in adult brain, ocelli and retina. C: dEAAT1 mRNA is not detected in the retina or lamina. D: dEAAT2 is strongly expressed in the retina. Apparent differences in staining intensity come from the unequal thickness of the preparation and wrapping of the whole-mounted brain by the retina in the lower half of the figure. Br: brain; La: lamina; Me: medulla; Oc: ocelli; Re: retina. Arrows indicate the midline of the brains. Bar = 50 μm (same magnification for A, C and D).

BDGP. dEAAT1 contains nine exons and eight introns (Fig. 1B) and dEAAT2 eight exons and seven introns (Fig. 1D). Both genes may contain one additional exon in 5′ which would comprise the transcription start site. Southern analysis shows that the dEAAT genes are not redundant in the *Drosophila* genome (not shown).

3.2. Sequence analysis of dEAAT proteins

BLAST searches revealed that dEAAT1 and dEAAT2 are more homologous to the family of high affinity glutamate transporters than to any other known proteins. This is illustrated by the alignment of dEAATs with a human glutamate transporter (hEAAT2) (Fig. 2A). The identity scores of several human and invertebrate glutamate transporters with dEAAT1 and dEAAT2 are presented on Fig. 2B. It can be seen that both *Drosophila* proteins have similar high levels of amino acid identity (35–45%) with previously identified EAAT sequences. Similarly, dEAAT1 and dEAAT2 are 36% identical. An exception is the recently isolated lepidopteran *Trichoplusia* glutamate transporter (TrnEAAT1) [32], which is specifically more homologous to dEAAT1 (59% identity). A less significant homology (31–33%) is observed between dEAATs and the vertebrate sodium-dependent neutral amino acid (ASC) transporters, which are related to the glutamate transporter family [33,34].

As shown in Fig. 3, the dEAAT proteins display almost identical hydrophobicity profiles with the glutamate transporters: seven conserved distinct hydrophobic peaks corresponding to transmembrane α-helical domains and a long and rather hydrophobic region in the C-terminal half of the sequences. This region is the most highly conserved between the different EAAT transporters [17], but the number of membrane-spanning domains is not well defined in this region. The N-terminal parts of the two proteins are hydrophilic suggesting a cytoplasmic localization of their N-terminus.

For dEAAT2, two sites of *N*-glycosylation are present in the large loop between the transmembrane segments 3 and 4, which must be extracellular. Such glycosylation sites are located in the same domain in other glutamate transporters [35]. Protein kinase C phosphorylation sites are also found in dEAAT2 (Fig. 1C) like in vertebrate glutamate transporters. As shown in Fig. 1A, dEAAT1 presents the same pattern of putative sites for post-translational modifications. Therefore, sequence analysis shows that the two *Drosophila* proteins

share the general structural features and topology of glutamate transporters, strongly suggesting that they belong to the EAAT protein family.

3.3. Developmental expression profiles of dEAAT1 and dEAAT2

dEAAT1 and dEAAT2 mRNAs are most strongly expressed in adult fly heads in the course of development, as shown by Northern analysis (Fig. 4). They are also expressed in late embryos, larval stages and adult bodies but the signal is much weaker for dEAAT1 and hardly detectable for dEAAT2 on Northern blots. The dEAAT1 mRNA migrates as a single 3.3 kb band. The dEAAT2 mRNA is apparently more complex, with a major 3.2 kb transcript and three larger minor transcripts (3.8, 4.3, and 4.8 kb) that could result from differential RNA processing (Fig. 4). A similar mRNA polymorphism has been observed for hEAAT2 [36,37].

3.4. Cellular localization of dEAAT1 and dEAAT2 mRNAs by whole-mount in situ hybridization

The pattern of dEAAT expression was first examined by in situ hybridization with antisense RNA probes to 9–17 h old *Drosophila* embryos. This developmental stage coincides with differentiation of the central and peripheral nervous system. At stages 15–16, the dEAAT1 transcript is strongly expressed in the brain and ventral nerve cord but no signal is detected in the peripheral nervous system or in other tissues (Fig. 5A,B). Control embryos hybridized with a sense RNA probe had no detectable staining at any embryonic stage (not shown). In the ventral cord, several populations of cells express dEAAT1. The ventral view shows a segmentally repeated pattern of four large clustered cells in each abdominal neuromere at the level of the longitudinal connectives (Fig. 5B). The location of these cells appears similar to that of neurons RP1, 3, 4, 5 [38]. Other cells are stained near the longitudinal connectives. A distinct population is found more ventrally (Fig. 5A,B, arrows): these ventro-lateral cells are smaller than the others and not clustered. At the same embryonic stages, dEAAT2 is expressed in the ventral nerve cord in four to six cells per abdominal neuromere (Fig. 5C,D). In the anterior part of the embryos, three symmetrical clusters of cells also express dEAAT2 that could correspond to the Bolwig's organ, the larval photoreceptor organ [39] (Fig. 5C,D, arrowheads). Therefore, in the embryonic CNS, dEAAT1 is expressed in several cell populations that probably include motoneurons whereas dEAAT2 is expressed in a smaller number of apparently distinct nerve cells, which could be interneurons or glial cells.

The expression profile of dEAAT mRNAs was then examined in isolated CNS of third instar larvae. Fig. 6 shows localization of the dEAAT1 transcript. This gene is strongly expressed in a characteristic half-ring structure in the optic lobe (Fig. 6C). This structure could be part of the proliferative centers that give rise to the adult optic lobes [40]. In addition, numerous large cell bodies are stained in the brain and ventral ganglia (arrows). The morphology and location of these cells suggest that they are motoneurons in the ventral ganglia and interneurons in the brain. For dEAAT2, no signal could be demonstrated in third instar larval CNS by in situ hybridization, suggesting that expression of this transporter is down regulated at this stage. However, expression of dEAAT2 can be detected by RT-PCR from larval CNS RNA (not shown).

In the adult brain, in situ hybridization reveals that dEAAT1 is strongly expressed in cells of the medulla cortex in the optic lobe (Fig. 7A). For dEAAT2, an intense labeling is observed in the retina and the three dorsal ocelli (Fig. 7B,D). No specific staining was obtained in the retina with a sense dEAAT2 probe (not shown) or the antisense dEAAT1 probe (Fig. 7C). Thus, the two dEAAT genes are expressed in distinct regions of the visual system in adult *Drosophila*. These results suggest that the neurotransmitter glutamate plays a significant role in the fly visual system as is the case for vertebrates.

In conclusion, dEAAT1 and dEAAT2 are two *Drosophila* proteins highly homologous to glutamate transporters which seem to be expressed in distinct cellular populations from embryo to adult. Until now, we have not found other members of this protein family in *Drosophila* either by searching the genome databases or by RT-PCR with degenerate primers. The strong expression of dEAAT1 and dEAAT2 in the nervous system, particularly in adult fly heads, argues for a role of these transporters in neurotransmission in *Drosophila*. The dEAAT1 transcript is present in large cell bodies that probably include motoneurons. This protein could therefore play a role at the neuromuscular junction. The two transporters are expressed in the adult visual system, but in different cells. dEAAT1 is present in large neuronal cell bodies of the medulla cortex whereas dEAAT2 is in the retina and ocelli. Several glutamate transporters are similarly expressed in the vertebrate retina [41–44], where they contribute to the regulation of the extracellular glutamate concentration. In embryos, the expression pattern of the two dEAAT genes is clearly different from the glial cell pattern. In addition, the size and shape of the dEAAT expressing cells suggest that they are neuronal. However, these transporters could also be expressed at a lower level in other cell types of the CNS. This point will be clarified once antibodies are available to detect these molecules. The specific functions of dEAAT1 and dEAAT2 and the role of glutamate in the insect brain and visual system can now be addressed by a genetic analysis in *Drosophila*.

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