Nitric oxide metabolism controlled by formaldehyde dehydrogenase (fdh, homolog of mammalian GSNOR) plays a crucial role in visual pattern memory in Drosophila

Qinlong Hou a,c,1, Huoqing Jiang b,c,1, Xu Zhang a,c, Chao Guo b,c, Bo Huang a,c, Peng Wang a,c, Tiepeng Wang a,c, Kaiyuan Wu a, Jian Li a, Zhefeng Gong b, Libo Du d, Yang Liu d, Li Liu b,⇑, Chang Chen a,⇑

a National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China
b State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, China
c Graduate School of the Chinese Academy of Sciences, Beijing 100049, China
d State Key Laboratory for Structural Chemistry of Unstable and Stable Species, Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

ARTICLE INFO
Article history:
Received 2 April 2010
Revised 17 September 2010
Available online 12 October 2010

Keywords:
Nitric oxide
Visual pattern memory
Formaldehyde dehydrogenase
S-nitrosoglutathione reductase
Drosophila

ABSTRACT
Nitric oxide (NO) plays an important role in learning and memory which is essential for animals to adapt to the external environment. However, little is known about the role of NO metabolism in this process. S-nitrosoglutathione reductase (GSNOR) is a key protein in the control of NO metabolism and protein S-nitrosation. To study the relationship between NO metabolism and learning and memory, the expression of gene fdh which is homolog to mammalian GSNOR was modulated by the Gal4/UAS system in Drosophila. The over-expression of the fdh in the central nervous system significantly increased GSNOR activity and induced visual pattern memory defects of Drosophila. The role of fdh in learning and memory was independent of development and was neuron-specific: over-expression of the fdh in the fan-shaped body induced memory defect, while over-expression in the mushroom body did not. The visual pattern memory defect could be rescued by co-expression with exogenous cGMP-dependent protein kinase (PKG). Moreover, fdh over-expression resulted in denitrosation of multiple proteins functionally enriched in vesicle-mediated transport, which is important for learning and memory. These results showed that regulation of NO metabolism plays an important role in learning and memory, and the mechanism may involve both NO-cGMP-PKG signaling pathway and S-nitrosation modification.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Nitric oxide (NO) is a small molecule that plays a vital role in a multitude of biological processes [1,2]. As a neurotransmitter, NO participates versatility in learning and memory in the nervous system [3]. For example, NO acts as retrograde messenger to induce long-term potentiation (LTP), a prominent form of synaptic plasticity, which is associated with memory formation in the hippocampus [4,5]. The majority of NO bioactivity studies have focused on upstream regulation and NO synthesis controlled by nitric oxide synthases (NOSs) [6–9]. However, little is known regarding downstream regulation and the effect of NO metabolism on learning and memory.

In 1998, Jensen et al. reported that alcohol dehydrogenase class III (ADH III) blocks NO function by reducing S-nitrosoglutathione (GSNO) to NH3 [10], and for this reason, ADH III is also named S-nitrosoglutathione reductase (GSNOR). GSNOR is highly conserved from bacteria to humans and is extensively expressed in organisms [11]. GSNOR is the main reservoir for non-protein S-nitrosos thiols (SNOS) [12] and temporally and spatially extends functions of the fragile NO. GSNOR also induces S-nitrosation of protein cysteine thiols, post-translational modification (PTM) [13,14], to regulate protein functions such as enzyme activity, protein stability, and protein localization. GSNOR turnover significantly influences the level of whole-cell S-nitrosation [11,15–18]. Therefore, high specificity of GSNOR toward GSNO and regulation of S-nitrosation [11,16] have established a direct relationship between GSNOR and NO metabolism. In addition to the general functions of NO in an organism [1], GSNOR is involved in the cardiovascular system [16], immune system [16,19], and respiratory system [17,20] by regulating NO metabolism. However, the role of GSNOR and NO metabolism in the nervous system remains poorly understood. Results have been shown that nitric oxide synthase (NOS) inhibition results in learning and memory defects [21]. Therefore, the present study was designed to determine the

⇑ Corresponding authors. Addresses: Institute of Biophysics, Chinese Academy of Sciences, P.O. Box 46, 15 Datun Road, Chaoyang District, Beijing 100101, China. Fax: +86 10 64888474 (L. Liu), Institute of Biophysics, Chinese Academy of Sciences, P.O. Box 33, 15 Datun Road, Chaoyang District, Beijing 100101, China. Fax: +86 10 64888406 (C. Chen).
E-mail addresses: liulil@sunn5.ibp.ac.cn (L. Liu), changchen@moon.ibp.ac.cn (C. Chen).
1 These authors contributed equally to this work.

1089-8603/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.niox.2010.09.007
effect of GSNOR on learning and memory. GSNOR is the sole alcohol dehydrogenase isozyme in vertebrate brains, while the failure to detect any ethanol dehydrogenase activity makes its function in brains an interesting question [22].

The present study chose the fdh gene, the homolog of mammalian GSNOR, to generate UAS-fdh and fdh double-stranded RNA interference (ds-RNAi) transgenic Drosophila. These transgenic models were used to study the effect of GSNOR activity modulation on visual learning and memory and to explore the possible mechanisms of learning and memory mediated by NO metabolism.

Experimental procedures

Fly strains

All fruit flies were maintained at 25°C and were fed standard corn meal/molasses medium [23] in a 12-h light/12-h dark cycle at 60% humidity. Three- to six-day-old flies were used in behavioral experiments. The following fly strains were used:

- wild-type Canton-S (CS): wild type controls using in behavior test.
- w1118: white eyed background flies for constructing the transgenic flies and for controls using in screening the transgenic flies.
- tubulin-Gal80: temperature sensitive Gal80 driven by the tubulin promoter for temporally controlling gene expression.
- c205-Gal4: a Gal4 line for controlling fan-shaped body F5 neurons gene expression.
- UAS-forPl [24]: a UAS line for over-expression of forPl (coding for protein PKG).

All fly lines were back-crossed with w1118 for at least six generations to assure the same genomic background.

Plasmid construction and molecular biology

The cloning strategy of double-headed constructs for RNAi was performed according to previously described methods, with minor modifications [25]. To construct the UAS-fdh-RNAi lines, the BamHI site-flanked upstream primer (5'-CAAGattccCCTGCGGAAAAAG TCTGCT-3') and KpnI site-flanked downstream primer (5'- TGGctactCCATCCTGCGCCTTGC-3') were used to clone the fdh cDNA fragment from a wildtype CS fly cDNA library. The PCR products were digested with BamHI and KpnI and the resulting cDNA fragments were subcloned into BamHI/KpnI sites of a pHIBS vector. The plasmids were sequenced by Invitrogen (Shanghai). The PCR products were digested with SalI and KpnI and initially subcloned into Xhol/KpnI sites of pUAST, following digestion of the previous pHIBS plasmid with BamHI and EcoRI, and were subsequently subcloned into the EcoRI and BglII sites of the above-mentioned pUAST plasmid.

For constructing the UAS-fdh plasmid, the EcoRI site-flanked upstream primer (5'-AACACGatgATGCCTGACTCGAGGCAA-3') and Xhol site-flanked downstream primer (5'-GGCGGctgagTTA GTACTTAATGGATCGG-3') were used to clone the coding region of the fdh gene. The PCR product was subcloned into EcoRI/Xhol sites of pUAST.

UAS-fdh-RNAi and UAS-fdh plasmid DNA were then purified and subjected to germline transformation into w1118 embryos according to previously described protocols [26].

Two transformants of the UAS-fdh plasmid (UAS-fdh1 and UAS-fdh31) and one transformant of the UAS-fdh-dsRNAi plasmid (UAS-fdhr34) were used in this study.

Quantitative PCR

Quantitative PCR (qPCR) was used according to standard methods [24]. In brief, 0.5 μl cDNA, prepared from bodies of 3- to 5-day-old flies, as described previously [24], was used as templates and tested on a Chromo 4 system (M) Research/Bio-Rad). For each fly strain, cDNA was obtained from three independent RNA preparations. The relative difference in fdh mRNA expression levels was quantified by comparing expression levels with standard curves, which were constructed using the corresponding recombinant plasmids. Expression levels were normalized to expression levels of actin. A two-tailed t-test was used for statistical analysis of relative mRNA levels. The qPCR primers were as follows:

- qPCR-actin upper, 5'-CGTGACTAATCCGGAGCACA-3';
- qPCR-actin lower, 5'-GGAGGCTTGAGGAGGACA-3';
- qPCR-fdh upper, 5'-AGACGCGCAAGACGAGCT-3';
- qPCR-fdh lower, 5'-AGCAGGCGACTTTCTCCAG-3'.

GSNOR enzyme activity assay

Approximately, 100 3-day-old flies were collected and homogenized in 50 mM sodium phosphate buffer (pH 7.5)/1.1% isopropyl/1 mM PMSF on ice. The solution was then centrifuged at 13,000 rpm for 50 min at 4°C. The supernatent was filtered through a 0.22-μm filter to remove fat prior to GSNOR activity measurements. GSNO reductase activity was measured as described previously [15]. In brief, 0.85–1.0 mg/mL lysate was incubated with 200 μmol/L GSNO in reaction buffer (50 mM sodium phosphate buffer (pH 7.5)/1.1% isopropyl/1 mM PMSF) with 0 or 100 μmol/L NADH at 25°C for various times. The GSNO reductase activity was measured by GSNO-dependent NADH consumption using the change in absorbance at 340 nm per minute per mg protein. Protein concentrations were measured using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA) according to manufacturer’s instructions.

Visual pattern memory assays

Visual learning experiments were performed in a flight simulator described by Wolf and Heisenberg [27]. Briefly, the tethered fly, which was suspended by a torque meter, flew stationarily in the center of the arena, which was illuminated from behind and contained two upright and two inverted alternating T-shaped patterns on the wall. During training, the computer switched a heat beam on when the fly was headed toward a quadrant with, for example, an upright T, and switched the heat beam off when the fly oriented toward one of the other two quadrants. Therefore, half of all possible orientations in the arena are paired with heat, the other half with ambient temperature. During testing, the heat was switched off. Angular position was recorded every 50 ms and orientation preferences were calculated for nine consecutive 2-min periods (performance index, PI1–9). Pattern A was paired to ambient temperature during training, and pattern B to heat. The two patterns alternated between A and B and between flies. If tX represented time spent heading toward quadrants with pattern A, and tB represented time spent heading toward pattern B quadrants, the performance index was calculated as PI = (tX - tB)/(tX + tB). For
details of learning paradigms, procedures, and the definition of performance index (PI), see Ref. [27].

Identification of S-nitrosated proteins in Drosophila and data analysis

S-nitrosated proteins were purified using the biotin-switch method and were analyzed by mass spectrometry as previously described [28]. Briefly, approximately, 100 3- to 4-day-old whole fly bodies or dissected heads were homogenized in lysis buffer (20 mM Tris–Cl, pH 7.7/1 mM EDTA/0.1 mM neocuproine/protease inhibitor cocktail tablets/8 M urea/20 mM MMSTS) on ice. All steps were performed in the dark unless otherwise noted. Protein concentration was determined using a BCA protein assay kit and adjusted to 1 mg/mL following removal of MMSTS from the protein solution by TCA/acetone precipitation. Supernatant was collected following centrifugation at 13,200g at 4 °C for 30 min. Subsequently, urea-based biotin-switch method was performed [28]. Following tryptic digestion, peptide samples were purified using streptavidin agarose, alkylated with iodoacetamide, and diluted in 0.1% formic acid (FA) for liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis. Peptide samples were analyzed using a Thermo LTQ linear trap instrument equipped with a Thermo micro-electrospray source, a Thermo Surveyor pump, and an autosampler (Thermo Finnigan, San Jose, CA, USA). MS2 spectra, with a total ion current (TIC) greater than 104, were used to search for matches against a mouse protein sequence database from the National Center for Biotechnology Information (NCBI) (version: 2009.1, plus 180 sequences of the most common contaminants, e.g., keratins and proteases, provided by the Bioworks package) using a TurboSEQUEST algorithm. For detailed search parameters, see Ref. [28]. Gene ontology (GO) enrichment and analysis were performed via DAVID [29], and the preliminary analysis was restricted to a 179-term GO essential slim [30].

Statistical analysis

The two-independent sample t-test was used to compare experimental groups to controls. Error bars in the figures represent SEM (standard error mean), and asterisks indicate levels of significance (**p < 0.001; *p < 0.01; *p < 0.05).

Results

GSNOR activity in transgenic flies

The structure of the fdh gene in Drosophila melanogaster is shown in Fig. 1A. The genome length of fdh is approximately 1.3 kb, with three exons and two introns, which form a single transcript. The relatively small size and simple structure allows for convenient modulation of gene expression. For over-expression of fdh, the p[UAS-fdh] plasmid was constructed. Microinjection of recombinant P element plasmids [31] (Fig. 1B) yielded seven independent transformants carrying UAS-fdh, from which three different transgenic lines (UAS-fdh1, UAS-fdh22, and UAS-fdh31) with a single insertion on the first, second, and third chromosomes were selected for further testing. The p[UAS-fdhRNAi] plasmid was constructed for reducing GSNOR activity of fdh. Six independent transformants carrying UAS-fdhRNAi transgenes were generated on the second or third chromosomes, from which two different transgenic lines (UAS-fdhRNAi22 and UAS-fdhRNAi34), with a single insertion on the second and third chromosomes, respectively, were used for further experiments. All five transgenic lines were back-crossed for a minimum of six generations to a reference w1118 background to ensure genetic homogeneity. No visible developmental defects were observed in these transgenic flies (data not shown).

To assess effectiveness of the constructed transgenic fly strains, the constitutively expressed actin-Gal4 was selected to cross with the transgenic flies. Entire fly bodies were used to measure fdh mRNA expression by qPCR. Results showed that fdh mRNA expression was significantly greater in three over-expression lines (act>UAS-fdh1, act>UAS-fdh22, act>UAS-fdh31) compared with controls (act/+ or UAS-fdh22/+). One of the fdh RNAi lines (act>UAS-fdhRNAi34) revealed significantly reduced fdh mRNA expression compared with the control line (UAS-fdhRNAi34/+). However, there was no difference between fdh RNAi lines (act>UAS-fdhRNAi22) and the control (UAS-fdhRNAi22/) (Fig. 1C).

GSNOR activity of fdh in the transgenic flies was assessed by GSNOR-dependent NADH consumption. GSNOR activity in whole body homogenates increased by 3-fold in act>UAS-fdh1 flies compared with control flies (UAS-fdh1/+), and by 5-fold in act>UAS-fdh31 flies compared with control flies (UAS-fdh31+/+) (Fig. 1D). However, GSNOR activity was reduced by approximately 50% in act>UAS-fdhRNAi34 flies compared with control flies (UAS-fdhRNAi34/+), and there was no significant difference between act>UAS-fdhRNAi22 flies and control flies (UAS-fdhRNAi22+/+) (Fig. 1D). Subsequently, elav-Gal4 was utilized to regulate fdh expression in the nervous system and GSNOR activity in the brains of Drosophila was tested. Similar to the previous results, GSNOR activity in the two over-expression lines (elav>UAS-fdh1 and elav>UAS-fdh31) increased by 3-fold and 13-fold, respectively. However, GSNOR activity in the elav>UAS-fdhRNAi34 line was reduced by approximately 50% compared with the control line (UAS-fdhRNAi34+/+) (Fig. 1E). According to these results, two fdh over-expression lines (elav>UAS-fdh1 and elav>UAS-fdh31) and one fdh RNAi line (UAS-fdhRNAi34+) were used to modulate GSNOR activity in the Drosophila central nervous system and employed for further behavior experiments.

Over-expression of fdh blocks visual pattern memory in Drosophila

First, elav-Gal4 was crossed with two UAS-fdh flies (UAS-fdh1 and UAS-fdh31) to over-express fdh in pan neurons. The progeny of these flies were then tested for visual pattern memory in a flight simulator. Two over-expression lines (elav>UAS-fdh1 and elav>UAS-fdh31) presented with visual pattern memory defect compared with their controls UAS-fdh1/+ and UAS-fdh31/+, respectively (Fig. 2A). Following analysis of behavioral data, the effects of thermo-tolerance and pattern discrimination were excluded in fdh over-expressing flies that presented with visual pattern memory defect (see Supplementary Fig. 1). These results suggested that increased GSNOR activity, as a result of fdh over-expression in the nervous system, could damage visual pattern memory in Drosophila.

Subsequently, elav-Gal4 and UAS-fdhRNAi34 flies were crossed to reduce GSNOR activity in pan neurons. The progeny of these flies were tested for visual pattern memory. However, the partial reduction in GSNOR activity did not result in significantly different memory performance in elav>UAS-fdhRNAi34 flies compared with control flies (UAS-fdhRNAi34+/+) (Fig. 2A).

Spatial–temporal regulation of fdh expression in visual pattern memory

Previous results showed that the fan-shaped body (FB), rather than the mushroom body (MB), is necessary for visual pattern memory in Drosophila [32,33]. To further elucidate the effect of NO metabolism on learning and memory in Drosophila, the Gal4 line c205 (FB F5-labeled neurons) and the Gal4 line mb247 (MB-labeled and serving as negative control) were utilized to drive local expression of the fdh gene. As expected, fdh over-expression in the c205-driven FB resulted in visual pattern memory defect, whereas fdh over-expression in the mb247-driven MB
normal visual pattern memory (Fig. 2B). These results indicated that increased GSNO activity in the FB, but not in MB, resulted in visual pattern memory defect in Drosophila. These results were consistent with previous observations in rat and for Drosophila mutants [24,32].

To exclude possible developmental effects due to constitutive Gal4 activity, a temperature-sensitive protein, tubulin-Gal80ts, was used to suppress Gal4 activity at a permissive temperature (19 °C) and to recover Gal4 activity at a restrictive temperature (30 °C) for temporary fdh over-expression in the Drosophila brain [34]. The temperature shift from 19 °C to 30 °C did not alter fly visual pattern memory (Fig. 2C and Ref. [24]). Subsequently, the flies were housed at 19 °C and then transferred to a 30 °C environment in adulthood for 12 h prior to memory testing in the flight simulator. Flies from the same genotype served as the control group and were maintained at 19 °C until visual pattern memory testing. As shown in Fig. 2C, both elav> tubulin-Gal80ts; UAS-fdh31 and c205> tubulin-Gal80ts; UAS-fdh31 flies, which were maintained at 30 °C in adulthood, showed memory defect which is significantly different compared with the same fly strains constitutively maintained at 19 °C. Moreover, the tubulin-Gal80ts; UAS-fdh31 flies, which were maintained at 30 °C in adulthood, displayed normal visual pattern memory. These results excluded the possibility that tubulin-Gal80ts or 30 °C treatment induced memory defect. Collectively, these data suggested that temporally restricted expression of fdh during adulthood is sufficient to inhibit visual pattern memory in Drosophila.

**Regulation of protein S-nitrosation by fdh in transgenic Drosophila**

GSNO has been reported to be a key protein for the control of NO metabolism and protein S-nitrosation [35]. Therefore, the present study attempted to determine whether GSNO-regulated protein S-nitrosation is involved in learning and memory. The...
biotin-switch method was utilized to purify S-nitrosated proteins, and mass spectrometry (LC/MS/MS) was used to identify the targets. First, whole body homogenate protein SNOs were detected in over-expressing flies (act>UAS-fdh31), dsRNAi flies (act>UAS-fdhri34), and control flies (+/act). In fdh over-expressing flies, 61 S-nitrosated proteins were determined, whereas 139 and 122 were found in heterozygous act/+ control and act>UAS-fdhri34 dsRNAi flies, respectively (Fig. 3A). These results demonstrated that fdh over-expression reduced the level of protein S-nitrosation in Drosophila.

Moreover, the variation of protein S-nitrosation under adjusted expression levels of fdh in the nervous system of Drosophila was determined. Results of S-nitrosated proteins in the heads of flies (elav>UAS-fdh31), dsRNAi flies (elav>UAS-fdhri34), and control flies (+/elav) were shown in Fig. 3B and C. There were less S-nitrosated proteins (1st: 133; 2nd: 100) in fdh over-expressing flies than in control flies (1st: 176; 2nd: 136) or fdh RNAi flies (1st: 194; 2nd: 132), implying that the visual pattern memory defect in the fdh over-expression flies (elav>UAS-fdh31) may be related to the protein S-nitrosation, i.e., some S-nitrosated proteins in the control (+/elav) or fdh RNAi flies (elav>UAS-fdhri34) flies with normal visual pattern memory might be necessary for visual pattern memory. From the two independent experiments, we found that there was a total of 68 proteins de-nitrosated in over-expressing flies with the exception of duplications (see Table S1). Further analysis of these denitrosated proteins with gene ontology (GO) clustering [29] in respect of RNAi flies (1st: 194; 2nd: 132), implying that the visual pattern memory defect induced by fdh expression in visual pattern memory of Drosophila was then used to over-express PKG in an attempt to rescue the visual pattern memory defect induced by fdh.

The most prominent natural target of NO is soluble guanylyl cyclase (sGC), which produces cyclic GMP (cGMP) [38]. cGMP, in turn, activates cGMP-dependent protein kinase (PKG). A recent study demonstrated activation of the NO-cGMP pathway through the use of a GSNOR inhibitor [39]. Therefore, GSNOR may also function in learning and memory by initially regulating this pathway.
over-expression. As expected, elav-Gal4 driven pan-neuronal GSNOR and PKG co-expression flies restored normal memory performance as good as the genetic control (+/UAS-fdh31 and UAS-PKG/UAS-fdh31) (Fig. 4), indicating that the memory defect was rescued by co-expression of exogenous PKG. These results demonstrated that fdh might affect visual pattern memory by the NO-cGMP-PKG pathway.

Discussion

The present study investigated the relationship between NO metabolism and visual pattern memory in D. melanogaster. Results demonstrated that over-expression of fdh in adulthood resulted in memory defect in a visual learning paradigm and fdh participated in visual pattern memory in specific neurons. PKG over expression could rescue the memory defect caused by over-expression of fdh. Furthermore, flies with downregulated bioactivity of NOS displayed the same visual pattern memory defects as fdh over-expression flies (Fig. S1). Meanwhile, there were multiple proteins denitrosated in the memory-deficient flies. No specific phenotype was detected in the fdh down-regulated flies, which could be due to the compensatory effect of other enzymes that exhibit GSNO reductase activity [40,41]. Based on results from the present study and previously reported data [42], we proposed a model for the role of NO in visual pattern memory in Drosophila: the homeostasis of NO is doubly-controlled by NOS/GSNOR; GSNOR controls NO metabolism via GSNO regulation and NOS controls NO synthesis by using arginine as a substrate. Modulation of either GSNOR or NOS can regulate NO bioactivity. Over-expression of fdh resulted in NO imbalance, thereby affecting the NO-cGMP-PKG pathway and protein S-nitrosation and, ultimately, learning and memory function (Fig. 5). Our results demonstrated that NO metabolism regulated by fdh is crucial in visual pattern memory.

In this study, both the NO-cGMP-PKG pathway and protein S-nitrosation may be involved in fdh-regulated learning and memory. PKG deficiency has been shown to result in learning and memory defects [24], and GSNOR inhibitors increase the soluble guanylyl cyclase (sGC) activity and enhance signal transduction of the NO-cGMP pathway [39]. Therefore, over-expression of the fdh gene in the present model may block sGC activity. In turn, it could lead to decreased PKG activity via the NO-cGMP-PKG pathway, resulting in visual pattern memory defect. Moreover, because NO bioactivity was exerted through cGMP-dependent and cGMP-independent pathways, protein S-nitrosation may also play a role in learning and memory. It has been reported that S-nitrosation of the N-ethylmaleimide-sensitive factor (NSF) induces cerebellar LTP by modulating surface expression of AMPA

Fig. 4. PKG over-expression rescues visual pattern memory defect caused by fdh over-expression in flies. Over-expression of fdh gene driven by elav-Gal4 causes Drosophila visual pattern memory defect compared with control flies of UAS-fdh31 alone or UAS-fdh31 and UAS-PKG combined together. The behavioral defect was rescued by co-expressing of PKG. All graphs depict mean ± SEM; (n) number of flies tested in experiments.

Fig. 5. NOS–GSNOR double-gated control of NO homeostasis in learning and memory. GSNOR which controls NO metabolism together with NOS which controls the generation of NO constitute a double-gated control of NO homeostasis. GSNOR is involved in learning and memory through cGMP-PKG pathway and possibly through protein S-nitrosation modification simultaneously.
receptors [43] and S-nitrosation of cyclic nucleotide-gated (CNG) channels, which could affect learning and memory by affecting olfactory and visual transduction [44]. In the present study, multiple vesicle-meditated transport proteins were denitrosated, such as Arr1, Syt1, and Rop. Syt1 is functionally related to neurotransmitter secretion and synaptic vesicle endocytosis, which are important in learning and memory mechanisms. These results suggested that protein S-nitrosation/denitrosation modification could contribute to memory defect in $fh\text{d}$ over-expressing flies.

Results from the GO analysis revealed that some denitrosated proteins in $fh\text{d}$ over-expressing flies were related to the process of visual perception, which could interfere with visual learning and memory. Therefore, Fourier analysis was used to evaluate the ability of flies to discriminate between patterns [24]. As shown in Supplementary Fig. S2A, visual discrimination was not significantly different between $fh\text{d}$ over-expressing flies, $fh\text{d}$ dsRNAi flies, and the genetic control flies. A defect in thermo-tolerance can induce low memory performance in $fh\text{d}$ over-expressing flies. Therefore, the present study measured dwelling time of the transgenic flies during the first and last training [45]. As shown in Fig. S2B, there was no significant change in thermo-tolerance. These results excluded the effects of thermo-tolerance and pattern discrimination on visual pattern memory in $fh\text{d}$ over-expressing flies.

In addition, for the function of GSNO in brain, since GSNO is the sole alcohol dehydrogenase isozyme in brain, the failure to detect ethanol dehydrogenase activity poses a problem if it is assumed that this enzyme has evolved and developed as a protective mechanism for ethanol detoxification in that organ [46]. Our findings that GSNO participates in the regulation of learning and memory may provide an answer to this dilemma: control of NO metabolism and GSNO/SNO homeostasis as demonstrated in the present study suggested a novel theory for understanding the presence of GSNO in the brain.

In conclusion, the present study demonstrated that $fh\text{d}$-controlled NO metabolism plays a key role in visual pattern memory of Drosophila. This mechanism may be involved in protein S-nitrosation modification and the NO-cGMP-PKG signaling pathway. GSNO and NOS composed a double-control of NO bioactivity during synthesis and metabolism. GSNOR could be a potential way. GSNOR and NOS composed a double-control of NO bioactivity S-nitrosation modification and the NO-cGMP-PKG signaling pathway.

Acknowledgments

This work was supported by the ’973 Program’ (2006CB911001, 2005CB522804, 2011CB910900), the National Natural Sciences Foundation of China (31030023, 90606020, 30770512, 30625022, 30621004), and the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-YW-R-247). We acknowledge Haiyun Hou, Xuanfen Yang, and Jie He for technical support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.niox.2010.09.007.

References