3-Hydroxykynurenine as a Potential Ligand for Hsp70 Proteins and Its Effects on Drosophila Memory After Heat Shock

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Abstract
Kynurenine products of tryptophan metabolism are modifiers of the nervous activity and oxidative processes in mammals and invertebrates. 3-Hydroxykynurenine (3HOK) in moderate concentrations is a lipid peroxidation inhibitor. However, its accumulation and oxidative auto-dimerization lead to oxidative stress development manifested in age-related neurodegenerative diseases (NDD) and neurological disorders provoked by acute stress. Different forms of stress, the mostly studied being heat shock response, rely on functioning of heat shock proteins of the Hsp70 superfamily. Since kynurenines are called “kids of stress,” we performed computational estimation of affinity of 3HOK and other kynurenines binding to predicted ATP site of Drosophila melanogaster Hsp cognate 71 protein (Dhsp71) using AutoDock Vina. The binding energy of 3HOK dimer is −9.4 kcal/mol; its orientation within the active site is close to that of ATP. This might be a new mechanism of producing a competitive inhibitor of Hsp70 chaperones that decreases organism ability to adapt to heat shock. We also showed that the Drosophila cardinal (cd1) mutant with 3HOK excess, serving as a model for Huntington’s disease (HD), manifests severe defects of short-term memory after heat shock applied either in adults or at the prepupal stage.

Keywords Drosophila cardinal · 3-Hydroxykynurenine · Heat shock · Hsp70 · Short-term memory

Abbreviations
AD Alzheimer’s diseases
AIF Apoptosis-inducing factor
CCSP Conditioned courtship suppression paradigm
CI Courtship index
DAN Dopaminergic neurons
Dhsp71 Drosophila Heat shock protein cognate 71
3HAA 3-Hydroxyanthranilic acid
3HOK 3-Hydroxykynurenine
HD Huntington’s disease
HS Heat shock
HSP Heat shock proteins
HSF1 Heat shock transcription factor 1
KP Kynurenine pathway
KYN L-kynurenine
KYNA Kynurenic acid
LI Learning index
LTM Long-term memory
MB Mushroom bodies
NDD Neurodegenerative disorders
PD Parkinson’s disease
PHS Phenoxazinone synthase
QUIN Quinolinic acid
RMSD Root-mean-square deviation
ROS Reactive oxygen species
STM Short-term memory
XAN Xanthommatin

Introduction
Kynurenine pathway (KP) is a main route of tryptophan catabolism in different animal species and phyla. KP intermediates, collectively called kynurenines, possess a broad spectrum of neurotropic activities [1, 2]. The pivotal role of kynurenines is widely discussed in scholarly literature in connection to age-dependent neurodegenerative diseases
(NDD) manifested as progressive memory loss and neuroinflammation [3, 4].

The major prerequisite to NDD development is the concentration ratio of different kynurenines. L-Kynurenine (KYN), 3-hydroxykynurenine (3HOK), and quinolinic acid (QUIN) exert either neuroprotective or neurodegenerative effects. As to 3HOK, its high concentration is a critical factor for the development of many NDD. In moderate concentrations, 3HOK and 3-hydroxyanthranilic acid (3HAA) are inhibitors of lipid peroxidation that can ameliorate the toxic action of reactive oxygen species (ROS) [5, 6]. However, 3HOK accumulation and auto-dimerization lead to oxidative stress development, as manifested in NDD and under acute stress [7–9]. 3HOK and QUIN generate hydrogen peroxide, which induces hyperproduction of toxic free radicals within a cell [10]. On the contrary, kynurenic acid (KYNA) is an endogenous protector against neurotoxicity [11, 12].

Contrary to vertebrates, the terminal stage of insect KP is 3HOK dimerization to brown eye pigment xanthommatin (XAN) [13]. The activity of phenoxazinone synthase (PHS) catalyzing transamination of 3HOK to xanthurenic acid is decreased in Drosophila cardinal (cd') mutant [14]. The 2.9-fold rise in the free 3HOK level is pertinent to this strain [15]. In cd', XAN is formed non-enzymatically due to 3HOK oxidative dimerization. Total antioxidant capacity in cd' is lower than in the wild-type strain Canton-S (CS) [16].

In mammals, KP is induced under stress conditions [2]. The key enzyme of hepatic KP tryptophan-2,3-dioxygenase is activated by corticosterone leading to kynurenines rise and depressive behavior [17]. The brain-specific indole-2,3-dioxygenase is activated by proinflammatory cytokines [18]. Different types of stress induce the universal response originally described in Drosophila — massive production of heat shock proteins (HSP), the stress response proteins having chaperone properties and involved in adaptation [19, 20].

The main diagnostic manifestation of human NDD is the progressive memory loss. This is the first trait evident in humans not only to physicians, but also to relatives. This presents an ever-growing home and hospital problem. Problem solving lies in dissecting the triangle: learning/memory — HSP — kynurenines.

If 3HOK is an oxidative stress activator [7], it is reasonable to assess the age-dependent dynamics of learning acquisition and memory retention (learning/memory) in Drosophila model, using KP mutants. Conditioned courtship suppression paradigm (CCSP) or a “rejection” paradigm is rather convenient for such an analysis in fly [21–25]. The essence is that naïve Drosophila male that has been repeatedly rejected by mated female either during 30 min training for testing STM or for 5 h for testing long-term memory (LTM) stop to court another mated female [22].

STM decay and age-dependent memory loss, synaptic pathology, and altered brain plasticity were registered beginning from the 13th day of adult life in cd' (high 3HOK/KYNA index) but not in cn mutant strain with KYNA excess (low 3HOK/KYNA index) or in the wild-type CS [26]. Heat shock (HS) treatment of young flies (1 h exposure to 37 °C) did not lead to apoptosis only in cn, indicating the neuroprotective role of KYNA, but induced it in normal and more intensively in cd' males. Moreover, HS exacerbated the toxic 3HOK action on Drosophila causing courtship song defects [27]. Spontaneous locomotor activity is suppressed in aged 40-day-old cd' males, whereas running speed is increased in the middle-age mutant [28]. High 3HOK/KYNA index is also a crucial factor for neurodegeneration in htt, a Drosophila model for Huntington’s disease (HD) [29, 30].

The most recent disclosure of HSP and memory link is that of Zatsepina et al. [31]. It proves the assumption that memory processes and the ancient stress–response systems are closely intermingled: any reinforcement used in different learning paradigms, either punishment or reward, utilizes stress reactions. While using Drosophila strains with different copy numbers of chaperone Hsp70, in particular strains having a deletion of all six hsp70 genes, the authors have demonstrated that a low constitutive level of Hsp70 pertinent to Drosophila is sufficient for learning and formation of STM and LTM. Moreover, they have revealed major pathways participating in memory formation and consolidation that depend on the presence of hsp70 in the genome. The roles of heat shock transcription factor 1 (HSF1) and Hsp70 in memory formation and neuroprotection were established [32].

If so, can kynurenines, the “kids of stress” [2], directly affect HSP functions via binding to any of their important sites? Possibly, the activity of Hsp70 might be suppressed in cd', making it vulnerable to HS. Therefore, cd' STM not impaired until day 21 under normal conditions might suffer after HS already in young flies at day 5. To estimate the affinity of kynurenines binding to the Hsp70 superfamily proteins, we performed a computer docking of L-KYN and its derivatives to the model structure of Drosophila Hsp cognate 71 (Dhs71), a homologue of Escherichia coli DNAK and mammalian Hsp70. Dhs71, or Hsc5, is a constitutively expressed member of the Hsp70 superfamily with predicted mitochondrial localization [33]. In addition, we used CCSP to reveal putative effects of HS on learning acquisition and memory retention in cd', which might be caused by 3HOK-dependent inactivation of Hsp70.

**Material and Methods**

**Protein Modeling and Computer Docking**

Hsp70 structure is characterized by the N-end ATP binding domain where ATP hydrolysis occurs, intermediate linker,
and C-end with substrate binding domain [34]. Experimental protein structure of E. coli Hsp70 (DNAK) in complex with ATP and Mg$^{2+}$ was taken from RCSB Protein Data Bank (ID: 4jn4, subunit A). D. melanogaster ATP binding domain of Hsp cognate 71 protein (Dhsp71) was constructed with the help of MODELLER software [35] using Hsp cognate 71 protein sequence (GenBank ID: AAA28628.1) and 4jn4_A in complex with ATP as a 3D template. The model with the highest negative potential energy was selected for docking. The optimal ligand conformations, including that of 3HOK dimer D2', were published in Zhuravlev et al. [16].

Flexible ligand and rigid protein structures were prepared for docking using AutoDockTools4 [36]. Gasteiger charges were used; Mg charge in the complex with ATP was set as 0, so that ATP-Mg complex has a total charge of $-2$ [37]. Docking was performed using AutoDock Vina [38] with the following parameters: grid box size, 40 Å, centered on ligand (the experimental ATP position); maximum number of binding modes, 100; exhaustiveness of the global search, 20; and other parameters, default. The structures with the highest negative free energies of binding (ΔG) were selected, except for 3HOK where two different ligand orientations were considered (see Results section). The dissociation constants (Kd) were calculated as follows: Kd = exp(ΔG/RT), ΔG being expressed in J/mol, T = 298.15 K. All figures were prepared using VMD [39].

The structures of Dhsp71 and the docked ligands can be found in Supplementary Data, S1 Docking coordinates file.

**Drosophila Strains**

Fly strains were taken from the Research Center “Biocollection of Pavlov Institute of Physiology RAS for the study of integrative mechanisms of the nervous and visceral systems.”

The following strains were used:

1. Canton-S (CS), the wild-type strain
2. cardinal (cd$^I$) with a mutation in phenoazinone synthase gene leading to 3HOK increase. cd$^I$ was outcrossed to CS for 20 generations.

Flies were raised on standard yeast–raisin medium with 8 a.m. to 8 p.m. daily illumination cycle at 25 ± 0.5 °C.

**Heat Shock Treatment**

HS treatment was applied at one of the following stages: (1) HS1, 1st instar larval stage, the mushroom bodies development; (2) HS2, prepupal stage, the central complex development; and (3) HS3, 5-day-old adult males [40]. Flies were subjected to HS in empty glass vials in a water bath for 30 min at 37 °C. After HS3, flies were kept for 1 h before experiment in food-containing vials.

**Learning and Memory**

Learning and 3 h STM were assayed in Drosophila males in CCSP using retraining test with a fertilized female [22]. Males were kept individually from eclosion until the experiment. Virgin CS females were kept in small groups. Before the experiment, CS females were fertilized overnight. All experiments were performed in 5-day-old males and females at 25 °C in the first half of the day.

For training, a naive male (without courtship experience) was placed together with a fertilized CS female in a Plexiglas experimental chamber (15 mm in diameter, 5 mm high) for 30 min. The retraining test was performed immediately after training (for learning acquisition) or 3 h after training (for STM retention). A trained male was tested in a fresh chamber with another fertilized female. After 45 s of adaptation, the male courtship behavioral elements were recorded for 300 s using a specially designed program [22]. The following courtship elements were scored: orientation/pursuit, wing vibration, licking, and attempted copulation. The non-courtship elements were activity (running), preening, and rest. For each group (naive control, learning acquisition, STM), 20 males were tested.

The courtship index (CI), a percentage of time spent in courtship over a 300 s period [41], was calculated for each male. The learning index (LI) was calculated as follows [42]:

$$LI = \left( \frac{\text{CI}_{na} - \text{CI}_{tr}}{\text{CI}_{na}} \right) \times 100 = (1 - \text{CI}_{tr}/\text{CI}_{na}) \times 100$$

where CI$\text{na}$ and CI$\text{tr}$ are the mean courtship indices for independent samples of naive and trained males, respectively.

Statistical analysis was performed using a two-sided randomization test [43]. This test is distribution-free and more sensitive than the Wilcoxon W (J) and the Mann–Whitney U rank-based tests and appeared to be the best one for CI and LI comparison using a specifically designed software [22]. Ten thousand permutations were used. The null hypothesis was rejected at αR < 0.05.

**Results**

**Computer Docking**

ATP docking to DNAK yields a ligand orientation nearly matching to that in the experimental DNAK–ATP complex: the root-mean-square deviation (RMSD) is 0.256 Å for non-hydrogen atoms. Approximately the same orientation was obtained for Dhsp71 (RMSD 0.502 Å). ATP binding sites
are described in Table 1. Mg-triphosphate group is localized above the salt bridge formed by the amino group of Lys 70 and carboxylic group of Glu 171/172. The oxygen atoms of triphosphate group contact Thr 11 to Asn 13 and Ala/Thr 199, while one negatively charged oxygen forms an ionic bond with Lys 70. The ribose group forms hydrogen bonds with Lys 270/266 and Glu 267/263. The adenine group is surrounded by several non-polar groups, such as the hydrophobic part of Arg 345/341 and Ile 271/Cys 267, and also forms a hydrogen bond with Ser 274/270.

The binding energy predicted by Autodock Vina is $-12.3$ kcal/mol for DNAK and $-11.7$ kcal/mol for Dhsp71 (Table 2). The reported experimental constant of ATP binding to Hsp70 is $4.4E-06$ M [44] or $7.3E-06$ M (for DNAK) [45]. This is a significantly lower affinity compared to predicted Kd values, hence docking seems to overestimate the ATP binding energy. The somewhat lower absolute computational ATP binding energy for Dhsp71 can be caused by a lower quality of the model compared to the experimental structure. Some minor differences between the active sites also can affect ATP binding, such as change of polar Gln 343 to hydrophobic Met 339 contacting the adenine group.

For kynurenines, the absolute binding energy is significantly lower (6.9–8.7 kcal/mol). 3HAA has the lowest affinity for DNAK and Dhsp71. KYNA that brings a negatively charged group forming an ionic bond with Lys 70 has the highest affinity for both proteins (see Supplementary Data, Table S1, for the full description of ligand-receptor interactions). The affinity of L-KYN and 3HOK for Hsp70 active sites is nearly the same, demonstrating the minor impact of the aromatic OH group. For 3HOK, two different orientations were found with nearly equal binding energies, one of which preferably occurs in complex with DNAK and the other in complex with Dhsp71. Being smaller than ATP and having a zwitterionic or anionic group, kynurenines tend to interact with only one part of the ATP site and the salt pair Lys 70–Glu 171/172. Hence, they do not contact Hsp70 residues that bind ATP ribose group (Ser 274/270) or adenine group (Lys 270/266, Glu 267/263). Some other residues beyond ATP site, such as Pro 367/363 and Asp 368/364, can also be involved in kynurenines' binding.

D2', a non-enzymatically formed 3HOK dimer with the size comparable to ATP, can interact with the both parts of

| Table 1 | ATP sites of E. coli and D. melanogaster HSP70 proteins |
| DNAK | Dhsp71 | Ligand–protein bonds |
| --- | --- | --- |
| Thr 11 | Thr 11 | NH.. O1G(3P): HB |
| Thr 12 | Thr 12 | NH.. O2B(3P): HB |
| Asn 13 | Asn 13 | NH.. O2B(3P), HD22.. OA1(3P): 2 HB |
| Lys 70 | Lys 70 | NH3.. O1G(3P): IB |
| Ala199 | Thr 199 | NH.. O2G(3P): HB |
| Glu 267 | Glu 263 | COO.. H2'O2'(Rib): HB |
| Lys 270 | Lys 266 | NH.. O2'(Rib): HB, Lys.. Ade: 2 NP |
| Ile 271 | Cys 267 | Ile/Cys.. (Ade): NP |
| Ser 274 | Ser 270 | OH.. N1(Ade): HB |
| Gly 342 | Gly 338 | NH.. O2A(Rib), O5'(Rib): 2 HB |
| Gln 343 | Met 339 | Gln/Met.. (Rib): NP |
| Arg 345 | Arg 341 | Arg.. (Ade): NP |
| Mg 1001 | Mg 604 | Mg.. O1B, O3G (3P): 2 IB |

Outside ATP site

| DNAK | Dhsp71 |
| --- | --- |
| Glu 171 | Glu 172 |
| Asp 194 | Asp 194 |
| Pro 367 | Pro 363 |
| Asp 368 | Asp 364 |

The residues in Dhsp71 that differ from DNAK are marked in italics. Some residues beyond the ATP site important for 3HOK binding are shown: Ade, adenine group; ar, the side chain of 3HOK aromatic group; 3P, triphosphate group; and Rib, ribose group. Bond is described as follows: active site residue atom group; ligand atom group (ligand part), the number and type of bonds; HB, hydrogen bond; IB, ionic bond; and NP, non-polar (van der Waals or hydrophobic) bond.

| Table 2 | Free energies and dissociation constants of kynurenines docking to Hsp70 proteins |
| --- | --- | --- |
| DNAK | Dhsp71 |
| ΔG (kcal/mol) | Kd (M) | ΔG (kcal/mol) | Kd (M) |
| --- | --- | --- | --- |
| ATP-Mg | -12.3 | 9.6E-10 | -11.7 | 2.6E-09 |
| 3HOK | -7.8 | 1.9E-06 | -7.8/-7.6* | 1.9E-06/2.7E-06* |
| L-3HOKzi-D2' | -7.7 | 2.3E-06 | -7.6 | 2.7E-06 |
| L-KYN | -7.3 | 4.4E-06 | -7.1 | 6.2E-06 |
| 3HAA | -6.9 | 8.7E-06 | -8.7 | 4.1E-07 |
| KYNA | -8.1 | 1.1E-06 | -7.3 | 4.4E-07 |
| QUIN | -7.1 | 6.2E-06 | |

Ligands description: ATP-Mg, ATP the complex with Mg; 3HOK, 3-hydroxykynurenine; L-3HOKzi-D2', dimer of 3HOK (D2') in zwitterionic form; 3HAA, 3-hydroxyanthranilic acid; L-KYN, L-kynurenine; KYNA, kynurenic acid; QUIN, quinolinic acid. The values for the second optimal 3HOK–Dhsp71 complex are show by asterisk.
the binding site (Fig. 1). In contrast to 3HOK, D2’ forms a hydrogen bond with Glu 267 in DNAK or ionic bond with Lys 267 in Dhsp71. D2’ binding can occur in two opposite orientations. In complex with DNAK, it interacts with Lys 70–Glu 171 by the amino acid moiety of the monomer (2) lacking the aromatic NH2 group. For Dhsp71, the other D2’ monomer (1) contacts the same residues. The second orientation lets D2’ to form more bonds with higher affinity to Dhsp71 (Kd 9.4 kcal/mol) which is the median between the respective values for ATP and monomeric kynurenines. However, as Hsp70 structure is rather conserved, we cannot exclude the ability of D2’ to bind to DNAK and other Hsp70 proteins in the same orientation. Thus, among the studied compounds, D2’ is the most potent competitive inhibitor of ATP binding and, therefore, Hsp70 activity.

**Learning and Short-Term Memory in cd1 After Heat Shock**

Figure 2 presents the analysis of learning acquisition and STM retention in cd1 and the wild-type CS males. A strong CCSP expressed as courtship index (CI) and learning index (LI) stays for 3 h (memory retention) at the level reached immediately after training (learning acquisition) in both strains. HS applied at the 1st instar larval stage (HS1), prepupal stage (HS2), or before the experiment (HS3) does not affect learning/memory in CS. Thus, 5-day-old CS males are able to form STM both under normal conditions and after HS applied at different stages of ontogenesis, as shown in [46].

As to cd1, its learning/memory performance does not differ from that of CS both under normal conditions [26] and following HS1. However, 3 h LI sharply decrease both after HS2 and HS3, being significantly different from the intact control and CS. Thus, 5-day-old cd1 demonstrates drastic STM disturbances: there is a fivefold LI reduction after HS applied either at prepupal stage or immediately before the experiment in adults.

**Discussion**

At organism level, the expression and content of stress proteins, particularly of Hsp70, depend on the type of stress and can change both in different organs and over time. HSP interact with many cell proteins, performing multiple functions — from the regeneration of partly broken protein structure and their protection from proteolysis to defense functions and escort for other proteins.

The most recent and comprehensive reviews on linking the ancient and interconnected systems of stress defense and learning acquisition are those of Zatsepina and coauthors [31, 32]. The mutant flies without hsp70 genes showed a fourfold lower 3 h learning and memory scores than control CS flies. This clearly pinpointed the involvement of Hsp70 in the learning process, namely, in the formation of STM. Also, hsp70− males, both intact and treated with heat shock, demonstrated a drastic, 70-fold LTM decrease. The presence of one copy of the hsp70 gene restored learning, STM, and LTM formation. The authors have emphasized the protective role of Hsp70 chaperones in several Parkinson’s (PD) and Alzheimer’s diseases (AD) models [32]. NDD, PD, AD, and HD are accompanied by imbalance of KP metabolites content [29, 47]. The Drosophila AD model with the overexpressed human amyloid precursor protein shows 30-fold increase in 3HOK and high ROS level, as well as the impaired learning and memory [48]. Thus, 3HOK is a promising early disease biomarker in human AD.

**Fig. 1** Ligand docking into the ATP site of Dhsp71 model structure. a The complex with ATP; b the complex with 3HOK dimer D2’ (L-3HOKz1_D2’). Hydrogen bonds with ≥ 155° angle and ≤ 3.2 Å distance between the heavy atoms are shown by dotted lines. See Table 1 and Table S1 for the whole pattern of ligand–protein bonds.
Fig. 2 Learning and memory abilities in *Drosophila* strains under normal conditions and heat shock. CI: box and whisker plots, the median is shown as an orange line. X axis: N, naïve; 0 h, learning; 3 h, STM. Y axis: percentage. Statistical differences: $ from CI (naive), # from LI (CS), & from LI (control); two-sided randomization test, $\alpha_R < 0.05, N=20$
cd¹ mutant is an excellent model to study the neurotoxic effects of endogenously accumulated 3HOK. In mammals, 3HOK is further metabolized to QUIN, which accumulation in the brain leads to excitotoxicity and epileptogenic effects, being blocked by KYNA [11]. QUIN is not synthesized in Drosophila; hence the neurotoxic effects in cd¹ are caused by 3HOK or products of its oxidative dimerization. As HS exacerbate neurodegeneration and behavioral defects in cd¹, the additional mechanism of 3HOK action on Drosophila nervous system may be its inhibitory interaction with HSP.

A rather strong binding of Hsp70 to different proteins under certain conditions can affect their functions. At the same time, issues related to possible binding of HSP to tryptophan metabolites have never been discussed in literature. Hsp70 proteins have a nucleotide binding domain consisting of two lobes with a deep cleft between them, binding ATP and ADP at the bottom [49]. Using AutoDock Vina software, we have estimated the affinity of both 3HOK and other kynurenines binding to predicted ATP site of Dhs7p71. There were two reasons to choose Dhsp71 as a target. Firstly, among Drosophila Hsp70 proteins, Dhsp71 has the highest similarity to DNAK, a well-described E. coli Hsp70, which was used as a template in comparative modeling. Secondly, Dhsp71 is predicted to localize to mitochondria, the organelles highly susceptible to oxidative stress. It should be noted, however, that the Hsp70 superfamily is quite conserved: the nucleotide sequences of human and Drosophila Hsp70 genes are 72% identical [50]. Seventy-five percent of DNAK and Dhsp71 ATP binding site residues are the same, and the rest are functionally similar. Hence, the affinity of kynurenines and their derivatives to different Hsp70-like proteins should be more or less the same, though some minor differences were observed for DNAK and Dhs7p71.

3HOK is capable of auto-dimerization [16]. In this study, we demonstrate that 3HOK dimer binds to Dhsp71 protein with a rather high energy (−9.4 kcal/mol), both to the active side part that binds ATP phosphate group, and to the part that binds the adenine group, whereas the monomer binds only to the first part of the site. In liver tissue of healthy people, Hsp70 level is about 180 ng/g [51]; in serum, its level is about 6 ng/ml [52]. The average level of 3HOK in cd¹ tissues is about 396 ug/g [15], being significantly higher compared to mammals [53]. The protein-bound form of 3HOK (PB-3HOK) is present in Drosophila head capsule containing pigment cells where XAN is synthesized from 3HOK. PB-3HOK was also found in the brain, though in less quantity [28]. Hence, 3HOK is able to penetrate the brain. This all makes 3HOK a potent competitive inhibitor of Dhsp71 and structurally similar proteins of the Hsp70 superfamily, affecting the nervous processes in a stress-dependent manner.

We observed the negative effect of HS on cd¹ STM, starting from prepupal stage, when 3HOK accumulation begins. Under normal conditions, synaptic pathology of the calyces of the mushroom bodies (MB) is observed in aged cd¹ relative to CS. MB is a crucial structure for Drosophila associative Pavlovian learning [54]. However, MB does not seem to participate in courtship learning. γd neurons of the ventral accessory calyces, which send axons to γ5 MB compartment, regulate STM formation. α/β lobes of MB, as well as dopaminergic neurons (DAN) innervating α1 and γ5 area, are important for the courtship STM [55, 56]. In the Drosophila PD model scarlet, the increased 3HOK and ROS level result in age-dependent progressive loss of PPL1 DAN and locomotor defects [57]. DAN regulating STM retrieval may also suffer from 3HOK accumulation in cd¹, leading to memory defects in this strain.

Why is STM intact in 5-day-old cd¹ without HS but impaired after HS exposure, similar to that in 21 day-old flies? The reason seems to be the different rate of neurotoxicity development, provoked by 3HOK accumulation and accelerating under HS [27]. Cell survival rate under stress is related to an increase in HSP content [58]. HSP27 and HSP70 can block apoptosis, enhancing cell survival [59]. HSP70 inhibits the activity of mitochondrial apoptosis-inducing factor (AIF) [60]. Hydrogen peroxide can also impair human HSP40/HSP70 induction, inhibiting HS response and refolding activity under heat stress, which may enhance cell death [61]. The non-enzymatic autoxidation of 3HOK produces hydrogen peroxide that induces apoptosis in neuronal cultures [7]. Hence, 3HOK oxidative products may decrease both HSP induction and functional activity. Over-expression of Hsp70 in Drosophila hemocytes significantly increases flies’ survival during hypoxia and oxidants action, reducing ROS in the whole body [62]. Thus, the 3HOK-dependent decrease of Hsp70 activity may further aggravate the toxic effects of 3HOK oxidative products on Drosophila neurophysiological functions.

In summary, a synergistic effect of HS and 3HOK accumulation has been revealed in cd¹ model of memory disturbances. This strengthens the findings of Zatsepina et al. [31] on the close interconnection of the two ancient systems, KP and HSP, of HS response and learning/memory. Drosophila Hsp70 homologue is among the putative targets of 3HOK, as its dimer was predicted to inhibit ATP binding to the protein active site. As both KP and HSP are conserved among different animal phyla, we can expect similar effects in human disorders provoked by 3HOK excess, such as HD. Thus, our finding helps to reveal the molecular mechanisms of stress-dependent exacerbation of neurophysiological and cognitive disorders, associated with kynurenines’ misbalance in the brain.

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Code Availability Non-applicable.

Declarations

Ethics Approval Non-applicable.

Consent to Participate Non-applicable.

Consent for Publication Non-applicable.

Conflict of Interest The authors declare no competing interests.

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