The Journal of Experimental Biology 214, 1773-1782

doi:10.1242/jeb.054486

RESEARCH ARTICLE

Histaminergic signaling in the central nervous system of *Daphnia* and a role for it in the control of phototactic behavior

Matthew D. McCoole[1], Kevin N. Baer[1] and Andrew E. Christie[2,*]

[1]Department of Toxicology, College of Pharmacy, University of Louisiana at Monroe, 700 University Avenue, Monroe, LA 71209, USA and [2]Program in Neuroscience, John W. and Jean C. Boylan Center for Cellular and Molecular Physiology, Mount Desert Island Biological Laboratory, P.O. Box 35, Old Bar Harbor Road, Salisbury Cove, ME 04672, USA

*Author for correspondence (achristi@mdibl.org)

Accepted 14 February 2011

SUMMARY

***Daphnia magna* and *Daphnia pulex* are well-established model organisms in the fields of ecotoxicology and toxicogenomics. Among the many assays used for determining the effects of environmental and anthropogenic stressors on these animals is monitoring for changes in their phototactic behavior. In most arthropods, histamine has been shown to play a key role in the visual system. Currently, nothing is known about histaminergic signaling in either *D. magna* or *D. pulex*. Here, a combination of immunohistochemistry and genome mining was used to identify and characterize the histaminergic systems in these daphnids. In addition, a behavioral assay was used to assess the role of histamine in their phototactic response to ultraviolet (UV) light exposure. An extensive network of histaminergic somata, axons and neuropil was identified *via* immunohistochemistry within the central nervous system of both daphnids, including labeling of putative photoreceptors in the compound eye and projections from these cells to the brain. Mining of the *D. pulex* genome using known *Drosophila melanogaster* proteins identified a putative ortholog of histidine decarboxylase (the rate-limiting biosynthetic enzyme for histamine), as well as two putative histamine-gated chloride channels (hclA and hclB orthologs). Exposure of *D. magna* to cimetidine, an H2 receptor antagonist known to block both hclA and hclB in *D. melanogaster*, inhibited their negative phototactic response to UV exposure in a reversible, time-dependent manner. Taken collectively, our results show that an extensive histaminergic system is present in *Daphnia* species, including the visual system, and that this amine is involved in the control of phototaxis in these animals.**

Key words: *Daphnia magna*, *Daphnia pulex*, histamine, histidine decarboxylase, histamine-gated chloride channel, cimetidine, immunohistochemistry, genomics, phototaxis.

INTRODUCTION

Planktonic crustaceans function as keystone species in most aquatic ecosystems. In many freshwater habitats, cladoceran species predominate, serving both as the primary consumers of phytoplankton and as the major food source for larger invertebrates and vertebrates (e.g. de Bernardi et al., 1987; Hembre and Megard, 2005; Sarnelle, 2005). Cladocerans, particularly members of the genus *Daphnia*, are known to exhibit a remarkable ability to adapt morphologically, physiologically and/or behaviorally to environmental change (e.g. Grant and Bayly, 1981; Kreuger and Dodson, 1981; Hebert and Grewe, 1985; Ranta and Tjossem, 1987; Hembre and Megard, 2006; Hülsmann and Wagner, 2007; Vanoverbeke et al., 2007). This functional flexibility, in combination with their parthenogenetic reproduction and ease of laboratory culture, has resulted in their emergence as model organisms for many scientific fields, prime among them ecotoxicology and toxicogenomics (e.g. Iguchi et al., 2007; Poynton et al., 2007; Shaw et al., 2007; Soetaert et al., 2007; Tatarazako and Oda, 2007; Eads et al., 2008; Schaack, 2008).

Numerous morphological, physiological and behavioral traits have been used to assess the response of daphnids to environmental and anthropogenic stressors. One behavior that has been used extensively for assessing changes in behavior is vertical migration within a water column, which includes a phototactic component (Ringelberg, 1999). In a general sense, phototactic behavior can be described as an orientation reaction that is influenced by gradients in both light intensity and light direction. Phototaxis can be either positive or negative, with the animal moving towards or away from the light source, respectively. Under normal conditions *Daphnia* typically exhibit negative phototaxis in response to ultraviolet (UV) light exposure, moving away from the UV source towards the bottom of a water column (Poupa, 1948). This behavioral response is hypothesized to be adaptive in that it minimizes both UV damage and risk of predation (Lampert, 1993; Dodson et al., 1997; Van Gool and Ringelberg, 1998; Rhode et al., 2001). Previous studies in daphnids have shown that phototactic behavior is modulated by a variety of environmental factors, e.g. food abundance and quality, and the presence/absence of fish kairomones (e.g. Michels and De Meester, 1998; Cousyn et al., 2001; Kieu et al., 2001). Phototactic behavior in daphnids is also influenced by a variety of environmental pollutants (Michels et al., 2000; Semsari and Megateli, 2007; Brausch et al., 2011) and, given its relative ease to observe and quantify (Dojmi and Rotondo, 1988), has been used to monitor for these chemicals (e.g. Whitman and Miller, 1982; Martins et al., 2007). Regardless of origin, stressors that negatively impact phototaxis are likely to have a major influence on the fitness of individual daphnids, rendering them susceptible to both increased UV damage and increased predation (Lampert, 1993; Dodson et al.,

1997; Van Gool and Ringelberg, 1998; Rhode et al., 2001); changes in the fitness of many individuals simultaneously could have significant consequences for the ecosystem as a whole.

Although much is known about the behavioral ecology of daphnids, comparatively little is known about the control systems that mediate behavioral output in these animals. The nervous system (particularly the visual system) is undoubtedly a major contributor to the generation of phototactic behavior in *Daphnia*. Although several studies have focused on characterizing the structural organization of the visual system of these animals (Macagno et al., 1973; Lopresti et al., 1973; Sims and Macagno, 1985; Smith and Macagno, 1990), essentially nothing is known about the neurochemistry of this, or any other, portion of the daphnid nervous system. In most arthropods, histamine has been shown to play a major role in signaling within the visual system (e.g. Monastirioti, 1999; Nässel, 1999; Stuart, 1999; Homberg, 2002; Stuart et al., 2007), though exceptions to this rule appear to exist (e.g. Hartline and Christie, 2010). Here, a strategy combining immunohistochemistry and genome mining was used to identify and characterize the histaminergic systems in two daphnid species, *Daphnia magna* and *Daphnia pulex*. In addition, pharmacological manipulation utilizing the histamine antagonist cimetidine was used to assess whether this aminergic system plays a role in mediating the negative phototactic behavior seen in response to UV light exposure.

MATERIALS AND METHODS

Animals

Cultures of *Daphnia magna* Straus 1820 and *Daphnia pulex* (Linnaeus 1758) were purchased from Aquatic Research Organisms (Hampton, NH, USA). All animals were maintained at densities of approximately 100 animals per liter on a 12 h:12 h light:dark cycle in 0.5 or 1 l jars filled with room temperature (20–22°C) freshwater (see below). For anatomical studies (conducted at Mount Desert Island Biological Laboratory), animals were reared in filtered tap water and fed Roti-Rich Liquid Invertebrate Food (catalog no. DA-RR32; Florida Aqua Farms Inc., Dade City, FL, USA) twice weekly. For phototactic studies (conducted at the University of Louisiana, Monroe, LA, USA), animals were reared in high-hardness (HH)-COMBO medium, a defined freshwater culture medium (Baer and Goulden, 1998), and fed green algae, *Ankistrodesmus falcatus* ($250{,}000\ \text{cells ml}^{-1}$), three times weekly. It should be noted that water quality (e.g. temperature, pH, conductivity, dissolved oxygen and total hardness) was monitored continuously in all cultures and throughout the duration of all behavioral experiments; all water quality parameters were maintained within the acceptability criteria of the American Society for Testing Materials (ASTM, 2007).

Wholemount immunohistochemistry

Antibodies

A rabbit polyclonal antibody generated against a histamine–keyhole limpet hemocyanin conjugate (HA–KLH) (Panula et al., 1988) was used to map the distribution of histamine in the nervous systems of *D. magna* and *D. pulex*. This antibody was purchased commercially from ImmunoStar Corporation (catalog no. 22939; Hudson, WI, USA), and has been used previously to map the distribution of histamine in the nervous systems of a number of other crustacean species (e.g. Mulloney and Hall, 1991; Le Feuvre et al., 2001; Pulver et al., 2003; Christie et al., 2004; Fu et al., 2005; Hartline and Christie, 2010). Visualization of the histamine antibody was accomplished using an Alexa-Fluor-488-conjugated donkey anti-rabbit IgG (catalog no. A-21202; Invitrogen, Eugene, OR, USA).

Immunoprocessing

Immunoprocessing was conducted on whole animal preparations using a procedure modified from Hartline and Christie (Hartline and Christie, 2010). In brief, animals were placed into 1.5 ml microfuge tubes containing a solution of 4% 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC; catalog no. E7750; Sigma-Aldrich, St Louis, MO, USA) in $0.1\ \text{mol l}^{-1}$ sodium phosphate buffer (SPB; pH 7.4) and sonicated for approximately 20 min using a Blazer 4800 ultrasonic cleaner (Blazer, Farmingdale, NY, USA). Following sonication, animals were allowed to fix in the EDAC solution for approximately 24 h. After fixation, animals were rinsed five times at 1 h intervals in SPB containing 0.3% Triton X-100 (SPBT; catalog no. X100; Sigma-Aldrich), and then incubated for 72 h in a 1:500 dilution (in SPBT) of histamine antibody (see above). Following primary antibody incubation, animals were rinsed five times at 1 h intervals in SPBT, and then incubated overnight in a 1:300 dilution (in SPBT) of Alexa-Fluor-488-conjugated donkey anti-rabbit IgG (see above). After secondary antibody incubation, animals were rinsed five times at 1 h intervals in SPB and then mounted between glass microscope slides and cover slips using Vectashield Mounting Medium (catalog no. H1000; Vector Laboratories, Burlingham, CA, USA). Fixation, as well as incubation in both primary and secondary antibody, was done at 4°C whereas all rinses were conducted at room temperature (20–22°C). Secondary antibody incubation, as well as all subsequent processing, was conducted in the dark. All slides were stored in the dark at 4°C until examination.

Imaging

Data were collected and digital images were generated using a Zeiss Axiovert 200 epifluorescent microscope (Carl Zeiss MicroImaging Inc., Thornwood, NY, USA) or a Zeiss LSM 510 Meta confocal system. The Axiovert 200 was equipped with EC Plan-NEOFLUAR 10×/0.3, LD Plan-NEOFLUAR 20×/0.4 and LD Plan-NEOFLUAR 40×/0.6 dry objective lenses, an EXFO X-Cite Series 120 halide arc lamp (EXFO Photonic Solutions Inc., Mississauga, ON, Canada) and a standard Zeiss FITC filter set. The LSM 510 Meta confocal system consisted of a Zeiss Observer.Z1 inverted microscope, EC Plan-Neofluar 10/0.3 dry and Plan-Apochromat 20/0.8 dry objective lenses, and argon and HeNe lasers, as well as a manufacturer-supplied FITC filter set and manufacturer-supplied software.

Adsorption controls

To determine whether the histamine immunolabeling reported here is due to the presence of histamine, antibody adsorption controls were conducted. Specifically, the histamine antibody was incubated with $10^{-6}\ \text{mol l}^{-1}$ HA–KLH conjugate (Christie et al., 2004) or $10^{-6}\ \text{mol l}^{-1}$ KLH (catalog no. H5654; Sigma-Aldrich) alone for 2 h at room temperature prior to its application to tissue (Table 1). For comparison, some histamine antibody was held at room temperature for 2 h without peptide. The adsorbed and room temperature held unadsorbed antibodies were then used in immunohistochemical processing as described above.

Genome mining

For current descriptions of the preparation, sequencing and modeling of the *D. pulex* genome, readers are referred to the *Daphnia* Water Flea Genome Database (http://wfleabase.org/) (Colbourne et al., 2005), which is maintained by the Indiana University Genome Informatics Laboratory (Indiana University, Bloomington, IN, USA). Genome mining was accomplished using BLAST+ 2.2.23 software (downloadable from the National Center for Biotechnology

Table 1. Antibody adsorption controls for immunohistochemistry of *Daphnia magna* and *Daphnia pulex*

	Immunoreactivity present† (no. individuals)					
	D. magna replicates			*D. pulex* replicates		
Antibody treatment*	I	II	III	I	II	III
Unadsorbed	5/5	5/5	4/5	5/5	5/5	5/5
Adsorbed with 10^{-6} M HA–KLH conjugate	0/5	0/5	0/5	0/5	0/5	0/5
Adsorbed with 10^{-6} M KLH	5/5	5/5	5/5	5/5	5/5	5/5

HA, histamine; KLH, keyhole limpet hemocyanin.
*Adsorptions of HA–KLH and KLH alone were conducted for 2 h at room temperature prior to the antibody being applied to tissue; the unadsorbed antibody was also held at room temperature for 2 h prior to its use.
†Intensity of immunoreactivity, when present, was indistinguishable from that shown in Fig. 1.

Information, Bethesda, MD, USA; ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/) and the beta-release of the *Daphnia pulex* Genes 2010 frozen genome assembly (Indiana University Genome Informatics Laboratory, and Center for Genomics and Bioinformatics at Indiana University, Bloomington, IN, USA; http://wfleabase.org/). For all searches resulting in gene identifications, the BLAST score and BLAST-generated E-value for significant alignment are provided in Table 2. Alignments of the protein sequences were conducted using the online software program MAFFT (version 6.0; http://mafft.cbrc.jp/alignment/server/) (Katoh et al., 2002; Katoh and Toh, 2008). For all comparisons of *Daphnia* and *Drosophila melanogaster* proteins, amino acid identity was calculated as the number of identical amino acids divided by the total number of amino acids in the *D. melanogaster* sequence whereas amino acid similarity was calculated as number of identical and similar amino acids (the latter denoted by the ':' and '.' symbols in the protein alignments) divided by the total amino acids in the *D. melanogaster* sequence.

Behavioral assays

Behavioral assays were conducted in the presence or absence of the H2 receptor antagonist cimetidine (catalog no. C4522; Sigma-Aldrich) to assess the role of histamine in the phototactic response of *D. magna* to UV exposure; the design of the assay was based on a previous study conducted by Martins and colleagues (Martins et al., 2007). For all experiments, 3rd and 4th brood juveniles aged 7–8 days were used. The assay system consisted of five individuals placed into a glass test tube (20 cm height, 2.5 cm internal diameter) containing 70 ml of HH-COMBO medium or a solution of 2×10^{-3} mol l^{-1} cimetidine in HH-COMBO medium [a concentration below that of published 24 h half maximal effective concentration (EC_{50}) for *D. magna*] (Kümmerer, 2004). This system was exposed to UV light from above using a portable 120 V, 60 W UV lamp, and the phototactic behavior of the daphnids was quantified by assessing the location of the individuals within the tube: Compartment I comprised the uppermost 14 cm of the tube and Compartment II comprised the bottom 2.5 cm. During the UV exposure period (10 min), the number of animals within Compartment I was assessed at 1 min intervals. Changes in behavioral response to cimetidine were assessed in individuals that had been exposed to the antagonist for 1, 3, 5, 7 or 12 h, as well as an HH-COMBO control for each time point. Two replicates were conducted for each time point. The data are presented as an index calculated by dividing the number of animals present in Compartment I by the total number of individuals in the assay system; values therefore ranged between 0.0 (all individuals present at in Compartment II) and 1.0 (all individuals present in Compartment I). To evaluate the effects of cimetidine on the phototactic behavior of *D. magna*, one-way ANOVA ($P<0.05$) was used. Comparison of the means was accomplished using Dunnett's *post hoc* test. To determine differences between cimetidine-exposed groups, a Tukey's HSD *post hoc* test was performed. All statistical tests were performed using JMP IN software (SAS Institute, Inc., Cary, NC, USA).

To strengthen our confidence that the change in phototactic behavior seen in cimetidine was reversible, five sets of 5 h cimetidine-exposed animals were transferred to fresh HH-COMBO medium and assessed at 0, 3, 5, 7 and 12 h for their phototactic response to UV exposure (statistical comparisons were conducted as described above).

RESULTS

Histamine-like immunoreactivity is broadly distributed within the central nervous system of daphnids

Distribution of histamine-like immunolabeling

Wholemount immunohistochemistry was used to map the distribution of histamine in the nervous systems of both *D. magna* and *D. pulex* ($N>50$ individuals for each species). In each species, histamine labeling was broadly distributed within the central nervous system (CNS; defined here as the compound eye, optic ganglia, brain and thoracic nervous system), with little or no staining seen in peripheral structures. As the distribution of histamine-like immunoreactivity was identical in both daphnids, no distinction is made between species in the description that follows. Fig. 1 shows confocal micrographs of selected immunopositive structures, and

Table 2. Putative *Daphnia pulex* genes identified *via in silico* genome mining

Protein	Accession no. of query*	*D. pulex* gene name	*D. pulex* gene location/structure			Homology to query	
			Scaffold	Nucleotide start–stop	Exons†	Blast score	E-value
Histidine decarboxylase	AAF58823	*dappu-hdc*	29	563946–569316	16	847	0.0
Histamine-gated chloride channel A	AAF55691	*dappu-hcla*	65	670723–684477	11	517	6e-147
Histamine-gated chloride channel B	AAF54699	*dappu-hclb*	102	31360–34397	12	621	4e-178

*All mining was conducted using *Drosophila melanogaster* proteins as queries.
†Exon count based on the Genes 2010 gene prediction model.

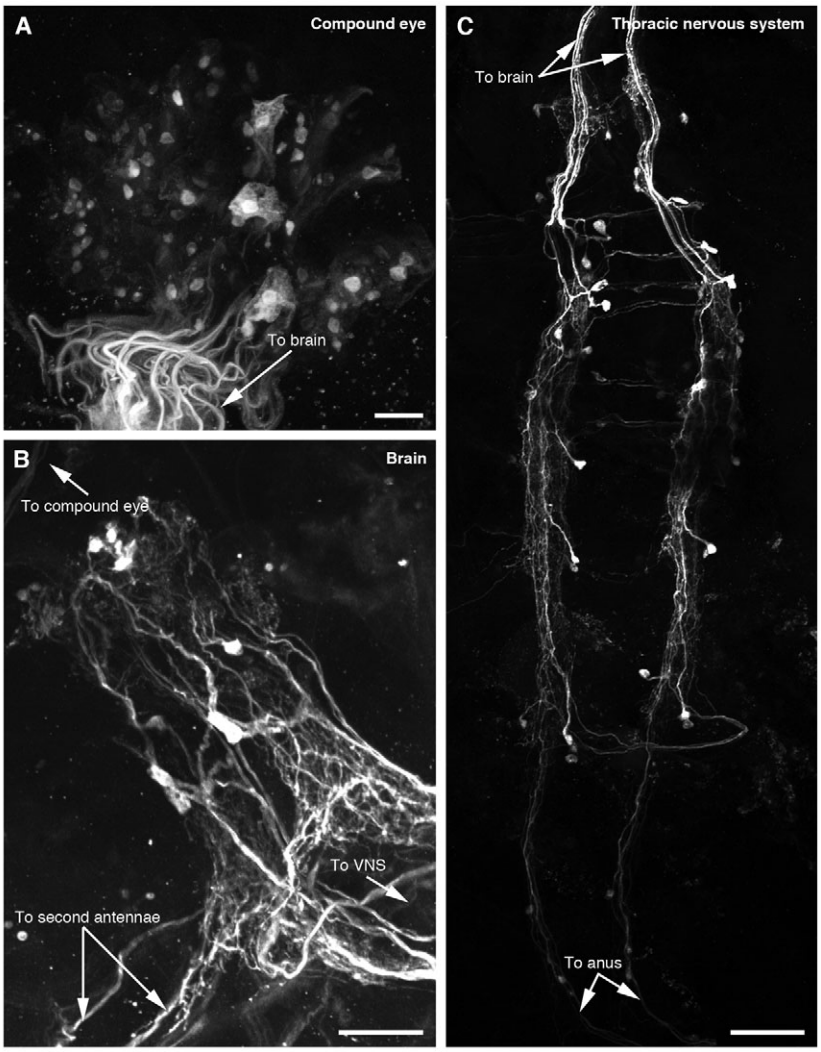

Fig. 1. Histamine-like labeling in the daphnid nervous system. (A) Histamine-like labeling in the compound eye. The confocal micrograph shown is of labeling in *Daphnia pulex* and is a brightest pixel projection of 56 optical sections collected at 0.9 μm intervals. (B) Histamine-like labeling in the supraoesophageal ganglion (brain). The confocal micrograph shown is of labeling in *Daphnia magna* and is a brightest pixel projection of 42 optical sections collected at 1.7 μm intervals. (C) Histamine-like labeling in the thoracic nervous system. The confocal micrograph shown is of labeling in *D. magna* and is a brightest pixel projection of 25 optical sections collected at 1.6 μm intervals. Scale bars, 50 μm. VNS, ventral nervous system.

Fig. 2 presents an artistic rendition of the distribution of histamine-like labeling consistently observed in the daphnid nervous system.

Extensive histamine-like immunoreactivity was present in the anterior portion of the daphnid nervous system (Fig. 1A,B and Fig. 2). In all preparations, cell bodies within the compound eye, likely photoreceptors, were labeled by the histamine antibody (Fig. 1A and Fig. 2). The number of immunopositive receptor cells within the compound eye was impossible to quantify, as its dark pigmentation often prevented clear imaging of some, or in a few cases most, of the labeling. In addition, histamine-like immunopositive axons derived from the putative photoreceptors were noted in most individuals, projecting from these cells into the supraoesophageal ganglion, commonly referred to as the brain (Fig. 1A and Fig. 2). Within the brain, approximately 20 histamine-like immunopositive somata were present, as was an extensive region of labeled neuropil (Fig. 1B and Fig. 2). Approximately 16 of the histaminergic somata resided in the anterior portion of the brain/optic ganglia, with the remaining cell bodies typically located near the brain's posterior margin. The anteriorly located somata tended to be slightly smaller and more weakly labeled than the more posteriorly located ones (~5 *vs* ~15 μm diameter, respectively), though significant variability in staining intensity was noted between preparations. Four or more histamine-labeled axons were typically present in each of the commissures that connect the brain with the thoracic nervous system; the somata that give rise to these fibers remain unidentified. In most preparations, a single histaminergic axon could be followed from the brain into each secondary antenna (Fig. 1B and Fig. 2).

Like the anterior nervous system, extensive histamine-like labeling was present in the posterior nervous systems of *D. magna* and *D. pulex* (Fig. 1C and Fig. 2). Within the thoracic portion of the nervous system, approximately 50 histamine-like immunopositive somata could routinely be visualized (Fig. 1C and Fig. 2). These somata were segmentally arranged, with two to eight cell bodies per segment (Fig. 1C and Fig. 2). Typically one soma pair per segment was slightly larger than the other (~5 *vs* ~10 μm diameter, respectively). As in the brain, the intensity of histamine-like labeling in the thoracic somata varied considerably between individuals; however, the larger cell bodies tended to show more intense labeling than the smaller ones. In all preparations, numerous histaminergic axons were present within the paired nerve cords, with several fibers labeled in each of the segmentally arranged commissures that connect them (Fig. 1C and Fig. 2). A single histaminergic axon was present in each nerve cord posterior to the thoracic ganglia (Fig. 1C and Fig. 2). In several preparations, these fibers could be followed unambiguously for a considerable distance and appeared to terminate on or near the anus (data not shown). In addition, in several preparations, a single, faintly labeled axon could be seen to project from each thoracic hemisegment toward the

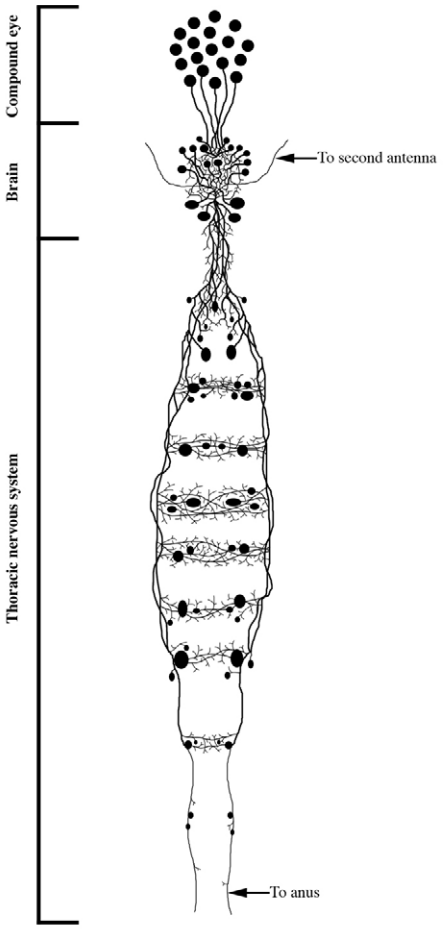

Fig. 2. Schematic representation of histamine-like immunoreactivity in the nervous system of *D. magna* and *D. pulex*. Filled circles represent immunopositive somata, thick lines within nerves represent immunopositive axons and tangles of thin lines represent regions of immunopositive neuropil.

periphery (data not shown). Given the weakness of the labeling in these axons, it was not possible to follow them for any appreciable distance, though in one individual they appeared to project toward the thoracic appendages (data not shown). No peripherally located somata showed any evidence of histamine-like labeling in the posterior portion of the nervous system.

Specificity controls

Although the histamine antibody used in our study has been employed for mapping the distribution of this amine in a variety of crustacean species (e.g. Mulloney and Hall, 1991; Le Feuvre et al., 2001; Pulver et al., 2003; Christie et al., 2004; Fu et al., 2005; Hartline and Christie, 2010), ours is its first use in daphnids. Thus, to increase our confidence in the specificity of the histamine-like immunoreactivity described above, antibody adsorption controls were conducted (Table 1). In support of the labeling being specific, incubation of the antibody with 10^{-6} mol l^{-1} HA–KLH conjugate for 2 h at room temperature prior to its application to tissue abolished all labeling within the CNS of both *D. magna* and *D. pulex* (N=15 individuals per species; Table 1). In contrast, when the histamine antibody was incubated with 10^{-6} mol l^{-1} KLH alone, labeling was unaffected (N=15 individuals per species; Table 1). Likewise, the unadsorbed antibody held at room temperature for 2 h produced normal labeling in all *D. pulex* samples (N=15 individuals; Table 1) and in 14 of the 15 *D. magna* preparations (in one *D. magna* replicate, one of the five individuals showed no labeling; Table 1).

Identification of histamine biosynthetic enzyme and channel proteins *via* mining of the *D. pulex* genome

With the recent public release of the genome of *D. pulex*, we became interested in determining whether orthologs of histidine decarboxylase (HDC), the rate-limiting biosynthetic enzyme for histamine, and histamine-gated chloride channels (hcls) could be identified in this species, and if found, how these proteins compared with those of *D. melanogaster* (the species used for all queries).

HDC

A single *D. pulex* gene (*dappu-hdc*) was identified as encoding a putative HDC protein *via* a query using *D. melanogaster* HDC (accession no. AAF58823). This gene is present on Scaffold 29 of the genome, with a predicted starting locus at nucleotide 563946 and ending locus at nucleotide 569316; the overall length of this gene is 5370 nucleotides. The Genes 2010 model predicts *dappu-hdc* to consist of 16 exons (Table 2).

Fig. 3 shows the alignment of the *D. pulex* HDC proteins deduced from the Genes 2010, Gnomon, SNAP, JGI and PASA gene models with that of the *D. melanogaster* query. As can be seen from this figure, the protein predicted by the SNAP model is the longest putative *D. pulex* HDC at 747 amino acids (labeled '*Daphnia* I' in Fig. 3); that predicted by both the Genes 2010 and Gnomon models is 688 amino acids in length (both identical in sequence; labeled '*Daphnia* II' in Fig. 3) and that predicted by the JGI and PASA models is 667 amino acids long (both identical in sequence; labeled '*Daphnia* III' in Fig. 3). Comparison of the sequences of these putative *D. pulex* HDCs with that of the *D. melanogaster* protein shows extensive amino acid identity among the proteins, with the only major variation occurring at the C terminus (where the *D. melanogaster* protein is extended relative to the putative *D. pulex* sequences) and three areas of internal insertion/deletion.

hclA

A single *D. pulex* gene (*dappu-hcla*) was identified as encoding a putative A-type hcl protein *via* a query using *D. melanogaster* hclA (accession no. AAF55691). This gene is present on Scaffold 65 of the genome, with a predicted starting locus at nucleotide 670723 and ending locus at nucleotide 684477; the overall length of this gene is 13754 nucleotides. The Genes 2010 model predicts *dappu-hcla* to contain 11 exons (Table 2).

Fig. 4A shows the alignment of the *D. pulex* hclA protein deduced from the Genes 2010, PASA and Gnomon gene models (all models predict an identical 398 amino acid protein) with that of the *D. melanogaster* query. As can be seen from Fig. 4A, the *Daphnia* and *D. melanogaster* proteins are 55% identical/71% similar in amino acid sequence, with the only major differences between the two proteins being a pair of insertions in the C-terminal portion of the *D. melanogaster* sequence.

Structural analysis of *D. melanogaster* hclA suggests that this protein contains two cysteine loops and four transmembrane domains (e.g. Zheng et al., 2002). In the alignment shown in Fig. 4A, the two cysteine loops are highlighted in yellow whereas four membrane-spanning domains are highlighted in red. Comparison of these regions with the corresponding portions of *D. pulex* hclA shows near identical conservation between predicted cysteine loops of the two species (loop I, 80% identity/93% similarity; loop II, 92% identity/100% similarity). Comparisons of the sequences of the putative membrane spanning domains show similar levels of conservation between the two proteins:

Histidine decarboxylase

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Drosophila      MDFKEYRQRGKEMVDYIADYLENIRERRVFPDVSPGYMRQLLPESAPIEGEPWPKIFSDV
Daphnia I       MDFDEYRQRGKEMVDYIADYLQNIRQRRVFPDVKPGYIRHLLPEQAPELGEDWDTIFADV
Daphnia II      MDFDEYRQRGKEMVDYIADYLQNIRQRRVFPDVKPGYIRHLLPEQAPELGEDWDTIFADV
Daphnia III     -----------MVDYIADYLQNIRQRRVFPDVKPGYIRHLLPEQAPELGEDWDTIFADV
                           *********:***:*******.***:*:****.**  ** * .**:**

Drosophila      ERIVMPGITHWQSPHMHAYFPALNSMPSLLGDMLADAINCLGFTWAS-------------
Daphnia I       ERVVMPGVTHWQSPYMHAYFPALNSFPSLLGDMLADAIGCLGFTWLRLLIMSRFVFSIYH
Daphnia II      ERVVMPGVTHWQSPYMHAYFPALNSFPSLLGDMLADAIGCLGFTWA--------------
Daphnia III     ERVVMPGVTHWQSPYMHAYFPALNSFPSLLGDMLADAIGCLGFTWAS-------------
                **:****:******:**********:************.******

Drosophila      ---------SPACTELEIIVMNWLGKMIGLPDAFLHLSSQSQGGGVLQTTASEATLVCLL
Daphnia I       WFAGPSQASSPACTELESLVMDWLGKMIGLPSEFLHARSDSLGGGVIQTTASESTFVALL
Daphnia II      --------SSPACTELESLVMDWLGKMIGLPSEFLHARSDSLGGGVIQTTASESTFVALL
Daphnia III     ---------SPACTELESLVMDWLGKMIGLPSEFLHARSDSLGGGVIQTTASESTFVALL
                        ******** :**:*********. ***  *:* ****:******:*:*.**

Drosophila      AGRTRAIQRFHERHPGYQDAEINARLVAYCSDQAHSSVEKAALIGLVRMRYIEADDDLAM
Daphnia I       AGRTEAIRRYKIQYPDLEDAEINSRLVGYCSDQAHSSVEKAGLIGLVKLRYIESDDELSM
Daphnia II      AGRTEAIRRYKIQYPDLEDAEINSRLVGYCSDQAHSSVEKAGLIGLVKLRYIESDDELSM
Daphnia III     AGRTEAIRRYKIQYPDLEDAEINSRLVGYCSDQAHSSVEKAGLIGLVKLRYIESDDELSM
                ****.**:*:: ::*. :*****:***.*************.*****::****:**:*:*

Drosophila      RGKLLREAIEDDIKQGLVPFWVCATLGTTGSCSFDNLEEIGIVCAEHHLWLHVDAAYAGS
Daphnia I       RGDTLATAIAQDREKGLIPFFVCATLGTTGACAFDHLREIGIVCRSEDIWLHVDAAYAGT
Daphnia II      RGDTLATAIAQDREKGLIPFFVCATLGTTGACAFDHLREIGIVCRSEDIWLHVDAAYAGT
Daphnia III     RGDTLATAIAQDREKGLIPFFVCATLGTTGACAFDHLREIGIVCKSDDIWLHVDAAYAGT
                **. *  ** :* ::**:**:*********:*:**:*.****** ...:**********:

Drosophila      AFICPEFRTWLRGIERADSIAFNPSKWLMVHFDATALWVRDSTAVHRTFNVEPLYLQHEN
Daphnia I       AFLCPEFRHWLDGIEFADSIAFNPSKWMMVHFDCTAMWVKNSGALHRTFNVEPLYLKHEN
Daphnia II      AFLCPEFRHWLDGIEFADSIAFNPSKWMMVHFDCTAMWVKNSGALHRTFNVEPLYLKHEN
Daphnia III     AFLCPEFRHWLDGIEFADSIAFNPSKWMMVHFDCTAMWVKNSGALHRTFNVEPLYLKHEN
                **:***** ** *** ***********:*****.**:**::* *:***********:***

Drosophila      SGVAVDFMHWQIPLSRRFRALKVWFVLRSYGIKGLQRHIREGVRLAQKFEALVLADHRFE
Daphnia I       SGMAIDYMHWQIPLSKRFRALKLWFVIRSYGLNGLQKHVRHGVRLAKEFENMVKSDGRFE
Daphnia II      SGMAIDYMHWQIPLSKRFRALKLWFVIRSYGLNGLQKHVRHGVRLAKEFENMVKSDGRFE
Daphnia III     SGMAIDYMHWQIPLSKRFRALKLWFVIRSYGLNGLQKHVRHGVRLAKEFENMVKSDGRFE
                **:*:*:********:******:***:****::***:*:*.*****::** :* :* ***

Drosophila      LPAKRHLGLVVFRIRGDNEITEKLLKRLNHRGNLHCIPSSLKGQYVIRFTITSTHTTLDD
Daphnia I       IPAARHLGMVVFRLKGPNDLTEALLKKINTSGKLHCVPAALKGNYVIRFTVTSSHTKLTD
Daphnia II      IPAARHLGMVVFRLKGPNDLTEALLKKINTSGKLHCVPAALKGNYVIRFTVTSSHTKLTD
Daphnia III     IPAARHLGMVVFRLKGPNDLTEALLKKINTSGKLHCVPAALKGNYVIRFTVTSSHTKLTD
                :** ****:****::* *::** ***::*  *:***:*::***:******:**:**.* *

Drosophila      IVKDWMEIRQVASTVLEEMNITISNRVYLKETK----------------------------
Daphnia I       IERDWEIIKSIAAIVIDACHSHHATNMAGDDQLEENGHSSGQETGDDEAVAGVPRSKTNK
Daphnia II      IERDWEIIKSIAAIVIDACHSHHATNMAGDDQLEENGHSSGQET----------------
Daphnia III     IERDWEIIKSIAAITGDDEAVAGVPRSKTNKMP----------------------------
                * :**  *:.:*: . :        .   ..

Drosophila      ------EKNEAFGSSLLLSNSPLSPKVVNGSFAAIFDAD---EFLAKTYAGVRIAHQESP
Daphnia I       MPLAETRKNKNFGTSLLLANRPMSPKIINGSFAAIFDNDDVLFDLARKISQFRYGDDDCP
Daphnia II      ----ETRKNKNFGTSLLLANRPMSPKIINGSFAAIFDNDDVLFDLARKISQFRYGDDDCP
Daphnia III     --LAETRKNKNFGTSLLLANRPMSPKIINGSFAAIFDNDDVLFDLARKISQFRYGDDDCP
                     .**: **:****:* *:***::********* *     **:. : .* ..::.*

Drosophila      SMRRRVRGILMSGKQFSLDSHMDVVVQTTLDAGNGATRTSTTNSYGHTTSAAQANSERQA
Daphnia I       ATQRRIRGMLMSGKQLSLDSRIDLVQRLVTDKDGSNPQIDESSECLATPSDIEEVPEEGS
Daphnia II      ATQRRIRGMLMSGKQLSLDSRIDLVQRLVTDKDGSNPQIDESSECLATPSDIEEVPEEGS
Daphnia III     ATQRRIRGMLMSGKQLSLDSRIDLVQRLVTDKDGSNPQIDESSECLATPSDIEEVPEEGS
                : :**:**:******:****::*:* : . * ... .: . :..   *.*  :  .*. :

Drosophila      SIQEDNEESPEETELLSLCRTSNVPSPEHAHSLSTPSRSCSSSSHSLIHSLTQSSPRSSP
Daphnia I       PCEEDERQRDR--------------------------SHSVDSSRQLSLSNSCTGSGGGG
Daphnia II      PCEEDERQRDR--------------------------SHSVDSSRQLSLSNSCTGSGGGG
Daphnia III     PCEEDERQRDR--------------------------SHSVDSSRQLSLSNSCTGSGGGG
                . :**:.:  .                          . .**:.*  * : :.. ..

Drosophila      VNQFRPITLCAVPSQSQLSMPLAMPLPNRNVTVSVDSLLNPVTTCNVYHGKRFLEPLENL
Daphnia I       CLKKKNVAFSERAGSIPEDGENAVNNPERELLRKRRILMLKDLETMLSTTSRS-------
Daphnia II      CLKKKNVAFSERAGSIPEDGENAVNNPEREVNQTKI------------------------
Daphnia III     CLKKKNVAFSERAGSIPEDGENAVNNPEREVNQTKI------------------------
                  : : :::.  ...   .   *:  *:*::  .

Drosophila      AQTSASFSSSIFRLPTPIATPTRESPEDPDWPAKTFSQLLLERYSSQSQSLGNNSSTESS
Daphnia I       ------------------------------------------------------------
Daphnia II      ------------------------------------------------------------
Daphnia III     ------------------------------------------------------------

Drosophila      SLSGGATPTPTPMSSLDELVTPLLLSFASPSQPMLSAHGIGEGQREQGSDSDATVCSTTS
Daphnia I       ------------------------------------------------------------
Daphnia II      ------------------------------------------------------------
Daphnia III     ------------------------------------------------------------

Drosophila      SMESL
Daphnia I       -----
Daphnia II      -----
Daphnia III     -----
```

Fig. 3. Alignment of the amino acid sequence of *Drosophila melanogaster* histidine decarboxylase (HDC) with its putative *Daphnia pulex* ortholog. The sequence labeled '*Daphnia* I' is predicted by the SNAP gene prediction model, that labeled '*Daphnia* II' is predicted by both the Genes 2010 and Gnomon gene prediction models and that labeled '*Daphnia* III' is predicted by both the JGI and PASA gene prediction models. In the line immediately below each sequence grouping, asterisks indicate amino acids that are identical between all sequences, colons indicate amino acids that are highly conserved and a single dot indicates amino acids that are conserved, but to a lesser degree than those denoted by colons.

domain I, 81% identity/100% similarity; domain II, 100% identity; domain III 86% identity/100% similarity; domain IV, 63% identity/79% similarity.

hclB

A single *D. pulex* gene (*dappu-hclb*) was identified as encoding a putative B-type hcl *via* a query using *D. melanogaster* hclB (accession no. AAF54699). This gene is present on Scaffold 102 of the genome, with a predicted starting locus at nucleotide 31360 and ending locus at nucleotide 34397; the overall length of this gene is 3037 nucleotides. The Genes 2010 model predicts *dappu-hclb* to contain 12 exons (Table 2).

Fig. 4B shows the alignment of the *D. pulex* hclB protein deduced from the Genes 2010, PASA and JGI gene models (all models predict an identical 505 amino acid protein) with that of the *D. melanogaster* query. As can be seen from Fig. 4B, the *Daphnia* and *D. melanogaster* proteins are 60% identical/74% similar in amino acid sequence; here, the major differences between the *D. pulex* protein

A Histamine-gated chloride channel A (hclA)

```
Drosophila          MVFQIIILVICTICMKHYAKGEFQQSLAITDILPEDIKRYDKMRPPKKEGQPTIVYFHVT
Daphnia             -----MAQIVALVISSLIGCGILVDAMELSDILPDRFN-YDKMRPPRLEGQPAKVFNHLT
                         :  ::. :  .  . * : ::: ::****: :: *******: ****: *: *:*

Drosophila          VMGLDSIDENSMTYVADVFFAQTWKDHRLRLPENMTQEYRLLEVDWLKNMWRPDSFFKNA
Daphnia             VMGLDSINENSMTYMADVFYVQSWRDHRLSLPENMTSEYRLLDVEWLKSIWRPDSYFKNA
                    *******:******:****:.*:*:**** ******.*****:*:***.:*****:****

Drosophila          KSVTFQTMTIPNHYMWLYKDKTILYMVKLTLKLSCIMNFAIYPHDTQECKLQMESLSHTT
Daphnia             KQVTFQTMTIPNHYLWLYRDKKILYVVKLTLTLSCSMNFALYPHDSQECKIQIESLSHTT
                    *.************:***:**.***:*****.*** ****:****:****:*:*******

Drosophila          DDLIFQWDPTTPLVVDENIELPQVALIRNETADCTQVYSTGNFTCLEVVFTLKRRLVYYV
Daphnia             DDLVFEWEEDVPLDVWKNIELPQLQLEGNYTNDCTQVYATGNFTCLEVVFVLKRRLGYYM
                    ***:*:*:  .** * :******: *  * * ******:***********.***** **:

Drosophila          FNTYIPTCMIVIMSWVSFWIKPEAAPARVTLGVTSLLTLSTQHARSQSSLPPVSYLKAVD
Daphnia             FHTYIPTCLIVVMSWVSFWIKPEVAPARVTLGVTSLLTLSTQHARSQAALPPVSYLKAVD
                    *:******:**:***********.**********************::***********

Drosophila          AFMSVCTVFVFMALMEYCLINIVLSDTPIPKPMAYPPKPVAGDGPKKEGEGAPPGGSNST
Daphnia             AFMSTCTIFVFMALMEYCLVNIILGDSDTPKTANEPPKPEK-------------------
                    ****.**:***********:**:*.*:  **.   ****

Drosophila          ASKQQATMLPLADEKIEKIEKIFDEMTKNRRIVTTTRRVVRPPLDADGPWIPRQESRIIL
Daphnia             ----------------------------------------------VFDLAAKENARMLM
                                                                  .::::*:::

Drosophila          TPTIAPPPPPPQPAAPEPELPKPKLTPAQERLKRAIYIDRSSRVLFPALFASLNGIYWCV
Daphnia             GQPPRPPGP----------------TPAQRARMRAIQVDRFSRFFFPFLFTVLNGSYWVV
                      .  ** *                ****.   *** :** **.:** **: *** ** *

Drosophila          FYEYL
Daphnia             FASYL
                    * .**
```

B Histamine-gated chloride channel B (hclB)

```
Drosophila          ------------------------MQSPTSKLVEFRCLIALAIYLHALEQSIQHCHCVHG
Daphnia             MTIYSKFHQDGADSNDTHLTLTYDVKYWLSELCNQRLTPCADFASIILESVLQMNDIENE
                                           ::   *:* : *   .  :    **. :*  .  :

Drosophila          YRNN---------------------------------------TESAELVSHYESSLSLP
Daphnia             GITNNLASGEMDSLEDASSQANAWCQQLGLGPGSRIVDVRALPLKSAGGYHREESSLVLG
                      .*                                      :**    : **** *

Drosophila          DILPIPSKTYDKNRAPKLLGQPTVVYFHVTVLSLDSINEESMTYVTDIFLAQSWRDPRLR
Daphnia             DILPRNSKSYDKNRAPKFFGQPTIVYFHVTVLSIDTINEESMTYVADIFLAQSWRDHRLR
                    ****  **:********:::****:*********:*:*********:********** ***

Drosophila          LPENMSEQYRILDVDWLHSIWRPDCFFKNAKKVTFHEMSIPNHYLWLYHDKTLLYMSKLT
Daphnia             LPEDMTEEYRILDVGWLQDIWRPDCFFKNAKKVTFHEMSVPNHYLWLYHDKTLIYMAKLT
                    ***:*:*:*******.**:.*********************:**************:**:***

Drosophila          LVLSCAMKFESYPHDTQICSMMIESLSHTVEDLVFIWNMTDPLVVNTEIELPQLDISNNY
Daphnia             LVLSCAMKFENYPHDTQVCSMQIESLSHTTHDLVFKWNFTDPLVTNPDIELPQLDIAKNT
                    **********.******:*** *******..**** **:*****.*.:********::*

Drosophila          TTDCTIEYSTGNFTCLAIVFNLRRRLGYHLFHTYIPSALIVVMSWISFWIKPEAIPARVT
Daphnia             TEDCTLEYSTGNFTCLAVVFNLRRRLGYHLFHTYIPSGLIVVMSWISFWIKPEAIPARAT
                    * ***:************:*******************.*********************.*

Drosophila          LGVTSLLTLATQNTQSQQSLPPVSYVKAIDVWMSSCSVFVFLSLMEFAVVNNFMGPVATK
Daphnia             LGVTSLLTLSTQSTQSQRSLPPVSYVKAIDVWMSSCTVFVFMSLMEFAVVNSFMGPVATK
                    *********:**.****:*****************:****:*********.********

Drosophila          AMKGYSDE---------------NISDLDDLKSALQHHRESIIEPQYDTFCHGHATAIYI
Daphnia             PMKGYSEEDLTIHRPSGYNGTSSSLRLRGKASSPARGPPPPPPGPQYVTFCNGREVALFI
                    .*****:*                .:   .. .*. :    .   *** ***:*: .*::*

Drosophila          DKFSRFFFPFSFFILNIVYWTTFL-
Daphnia             DQWSRLFFPLAFIILNVVYWTTFLH
                    *::**:***::*:***:*******
```

Fig. 4. Alignment of the amino acid sequences of (A) histamine-gated chloride channel A (hclA) and (B) histamine-gated chloride channel B (hclB) of *Drosophila melanogaster* and *Daphnia pulex*. The *D. pulex* hclA sequence shown in A is predicted by the Genes 2010, PASA and Gnomon gene models whereas the *D. pulex* hclB shown in B is predicted by the Genes 2010, PASA and JGI gene models. In both sets of alignments, cysteine loop regions are highlighted in yellow whereas membrane-spanning domains are highlighted in red [both as defined in Zheng et al. (Zheng et al., 2002)]. In the line immediately below each sequence grouping, asterisks indicates amino acids that are identical between the two sequences, colons indicate amino acids that are highly conserved and a single dot indicates amino acids that are conserved, but to a lesser degree than those denoted by colons.

and its *D. melanogaster* ortholog are that it is extended at its N terminus and contains an N-terminal insertion and a C-terminal deletion relative to that of *D. melanogaster* hclB.

In *D. melanogaster*, hclB, like hclA, is predicted to contain two cysteine loops and four transmembrane domains (e.g. Zheng et al., 2002). In the alignment shown in Fig. 4B, the two cysteine loops are highlighted in yellow whereas four membrane-spanning domains are highlighted in red. Comparison of the amino acids present in the cysteine loops of *D. melanogaster* with those present in the *Daphnia* ortholog show that these portions of the two proteins are nearly identical, exhibiting 87%/100% and 92%/100% identity/similarity, respectively. Likewise, comparisons of the amino acids that comprise the four membrane spanning domains present in the *D. melanogaster* protein with those of the corresponding region of *D. pulex* hclB show that these portions of the two proteins are nearly identically conserved: domain I, 95% identity/100% similarity; domain II, 88% identity/100% similarity; domain III, 90% identity/100% similarity; domain IV, 71% identity/100% similarity.

Cimetidine inhibition of the phototactic response to UV exposure in *D. magna*

As just described, two hcls were characterized *via* genome mining in *D. pulex*. These channels were identified *via* queries using *D. melanogaster* proteins, and show strong amino acid conservation with their *D. melanogaster* counterparts. In *D. melanogaster*, both hclA and hclB are blocked by the broad-spectrum H2 receptor antagonist cimetidine (Gisselmann et al., 2002), which has also been shown to block the effects of exogenously applied and neurally released histamine in several decapod crustaceans (e.g. Christie et al., 2004). Given the structural similarity between the *Daphnia* and *D. melanogaster* channels, and the fact that the distribution of histamine in daphnids strongly suggests a role for it in photoreception (see above), we became interested in determining what, if any, effect cimetidine might have on phototactic behavior in daphnids, specifically their phototactic response to UV light.

As Fig. 5 shows, when control animals (i.e. those maintained in HH-COMBO medium alone) were exposed to UV light, a strong

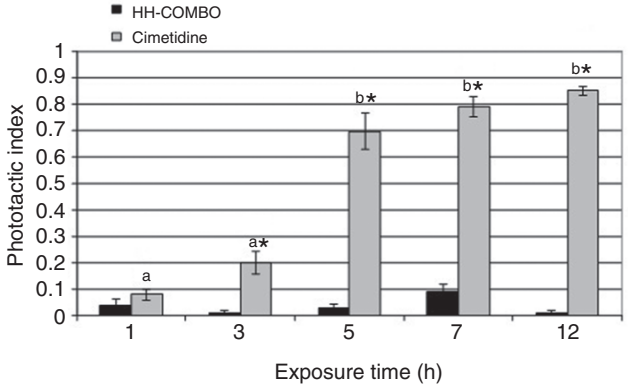

Fig. 5. Influence of cimetidine (2×10^{-3} mol l^{-1}) on the phototactic response of *Daphnia magna* to UV light. The phototactic index was calculated by dividing the number of animals in the upper compartment by the total number of animals. Values are means ± s.e.m. (N=10). *, Statistically significantly different from respective control (P<0.05). Lowercase letters denote treatments that are not significantly different from one another.

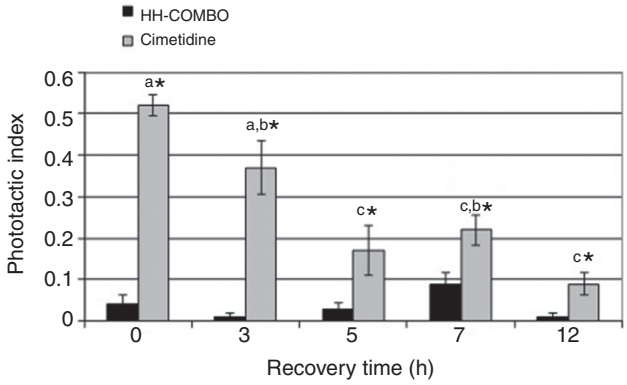

Fig. 6. Influence of cimetidine (2×10^{-3} mol l^{-1}; 5 h exposure) on the phototactic response of *Daphnia magna* to UV light following a recovery period. The phototactic index was calculated by dividing the number of animals in the upper compartment by the total number of animals. Values are means ± s.e.m. (N=10). *, Statistically significantly different from respective control (P<0.05). Lowercase letters denote treatments that are not significantly different from one another.

negative phototactic response was elicited, with most individuals in each trial moving to, and remaining in, the bottom portion (Compartment II) of the assay chamber (see Materials and methods). In contrast, this phototactic response was abolished in the presence of 2×10^{-3} mol l^{-1} cimetidine, with animals exposed to this H2 receptor antagonist exhibiting apparently 'normal' swimming behavior, but remaining in the top portion (Compartment I) of the assay chamber. Cimetidine's inhibition of the normal, negative phototactic response to UV exposure was time dependent, being statistically significant after 3 h of exposure to the blocker, and showed a maximal effect after approximately 5 h in cimetidine. As can be seen in Fig. 6, the effects of 2×10^{-3} mol l^{-1} cimetidine were largely reversible when animals were moved from the HH-COMBO medium containing the blocker to fresh, cimetidine-free HH-COMBO medium; cimetidine is cationic, and thus it is possible that the lack of a total reversal in its action is due to the drug being sequestered in some way by the animals.

DISCUSSION

The distribution of histamine-like labeling in daphnids suggests roles for histamine in photoreception and local neurotransmission

In our study, immunohistochemistry was used to map the distribution of histamine in the CNS of the daphnids *D. magna* and *D. pulex*. In both species, histaminergic somata, fiber tracts and neuropil were identified. These profiles were re-identifiable from individual to individual, with no notable differences seen between labeling in the two species. Histamine-like immunoreactivity was present throughout the CNS, including the compound eye, brain and thoracic portions of the nervous system.

Within the compound eye, presumptive receptor cells appeared histaminergic, with an extensive histamine-like immunopositive fiber tract projecting from them into the brain. The presence of histamine in these cells was not unexpected, as this amine is used as the transmitter in the photoreceptors of most members of the Arthropoda that have been investigated (e.g. Monastirioti, 1999; Nässel, 1999; Stuart, 1999; Homberg, 2002; Stuart et al., 2007). That said, there appear to be exceptions to this rule, e.g. little if any histamine-like labeling is present in the photoreceptor system of the copepod *Calanus finmarchicus* (Hartline and Christie, 2010). Thus, like most arthropods, our identification of histamine in the compound eye system of *D. magna* and *D. pulex* strongly suggests a role for this molecule in mediating phototransduction in these daphnids.

Approximately 50 re-identifiable histamine-like immunopositive somata were detected in the brain and thoracic regions of the daphnid CNS. These somata typically occur as bilaterally symmetric pairs, or small groups, within the ganglia that form these portions of the nervous system. No peripherally located somata were found to exhibit histamine-like labeling. Within the thoracic nervous system, the somata appeared segmentally arranged. Numerous fiber tracts and regions of central neuropil were present in the brain and thoracic nervous system; however, with two exceptions (see below), no peripherally projecting processes or neuroendocrine-like release areas were noted. Given its apparent restriction to central neuropil, it would appear that histamine functions as a neurotransmitter and/or locally released neuromodulator within the daphnid brain and thoracic nervous system, with little likelihood for it functioning as a circulating neurohormone.

Two sets of fiber tracts were the only peripherally located structures consistently labeled by the histamine antibody (in a few animals a single histaminergic axon was seen to project from each thoracic hemisegment towards the thoracic appendages). One set of these fibers projected from the brain into the second antenna whereas the other set projected from the thoracic nervous system to the anal region of the hindgut. As no histaminergic somata were present in the second antennae, nor were any seen near the gut, we believe these fibers originate from somata within the CNS. It is possible, however, that these axons are derived from sensory neurons whose cell bodies are present in the antenna and gut, but in which the concentration of amine is too low to be detected by our immunolabeling; histamine is a common transmitter/modulator used by sensory neurons (e.g. Nässel, 1999; Stuart, 1999). It has been noted by others that preloading tissue with histidine can enhance the histamine immunoreactivity in a variety of cells, including sensory neurons (e.g. Callaway and Stuart, 1999). Thus, it remains to be determined whether such manipulation would reveal soma labeling in the second antenna and/or gut of the daphnids investigated here, as well as in other regions of their nervous system (e.g. the thoracic appendages).

Genomic analyses of histaminergic signaling in daphnids

The recent release of the *D. pulex* genome provides a unique resource in crustacean biology, as thus far it is the only crustacean genome sequenced and available for public use. This resource has been used previously to glean information concerning the neurochemistry of *D. pulex*. Specifically, the peptides used by *D. pulex* as locally released neuromodulators and/or circulating neurohormones were deduced *via* genome mining and bioinformatics (Christie et al., 2011). Here, we have complemented our immunohistochemical mapping of histamine in the daphnid CNS with mining of the *D. pulex* genome for genes encoding key players in the histaminergic signaling pathway. Specifically, the genome was mined for orthologs of HDC, the rate-limiting biosynthetic enzyme of histamine, as well as for orthologs of two hcls; *D. melanogaster* sequences were used for this mining. Putative *D. pulex* genes for each of these proteins were identified. The predicted *D. pulex* HDC is highly similar in amino acid sequence (85%) to that of *D. melanogaster*. Similarly, the *D. pulex* protein orthologs of A- and B-type hcls show high levels of amino acid conservation with their *D. melanogaster* counterparts (89 and 74%, respectively). Although they are currently predictions, these putative *D. pulex* histaminergic pathway proteins are, to the best of our knowledge, the first HDC and hcls described from any crustacean. Moreover, the discovery of the genes encoding these molecules now allows for studies of their distribution in daphnids, as well as providing templates for searching the genomes and transcriptomes of other crustacean species for the genes/mRNAs encoding similar proteins (genes nearly identical in nucleotide sequence to those of *D. pulex* HDC, hclA and hclB are also present in an as of yet unreleased assembly of the *D. magna* genome (M.D.McC., A.E.C. and J. R. Shaw, unpublished). Likewise, the discovery of these *D. pulex* genes provide molecular targets for assessing whether specific environmental and or anthropogenic stressors might alter the expression of these proteins and hence influence histaminergic signaling in this important ecotoxicological model species.

Cimetidine influence on the phototactic response of *D. magna* suggests a role for H2 receptors in mediating this behavior

As discussed above, the two hcls described in our study show significant structural similarity to the hclA and hclB proteins of *D. melanogaster*. As both *D. melanogaster* channels are blocked by the broad-spectrum H2 receptor antagonist cimetidine (Gisselmann et al., 2002), and the distribution of histamine in daphnids suggests a role for it in phototransduction, we became interested in assessing the influence of cimetidine on *Daphnia*'s phototactic response to UV light. Our results show that placement of animals into cimetidine-laced culture medium suppresses their normal, negative phototactic response to UV exposure in a largely reversible, time-dependent fashion. The inhibition of *D. magna*'s normal response to UV exposure by cimetidine clearly strengthens the hypothesis that histamine plays a major role in the generation of this behavior.

In the wild, daphnids are hypothesized to rely on negative phototaxis to avoid both UV-induced cellular and/or genetic damage and predation (Lampert, 1993; Dodson et al., 1997; Van Gool and Ringelberg, 1998; Rhode et al., 2001). Likewise, photic information has been linked to many other physiological control systems and behaviors in these animals, e.g. the production of male progeny (Hobaek and Larsson, 1990; Kleiven et al., 1992). Given a role for histamine in the generation of *Daphnia*'s phototactic response (and likely photically mediated behaviors in a general sense), environmental and anthropogenic stressors that influence histaminergic signaling may well compromise the fitness of these animals. If the influence of these stressors is broad reaching, then the fitness of many individuals within a population could be impacted simultaneously. Because daphnids are often the major contributors to the zooplankton present in freshwater ecosystems, such large-scale challenges could lead to a potential crash in the ecosystem as a whole. Clearly additional study is needed to assess what, if any, influence environmental pollutants have on the histaminergic systems of daphnids; however, the present study provides a possible molecular framework for assessing chemical perturbations in this system.

LIST OF ABBREVIATIONS

CNS	central nervous system
EDAC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
HA	histamine
hcl	histamine-gated chloride channel
HDC	histidine decarboxylase
HH-COMBO	high hardness-COMBO medium
KLH	keyhole limpet hemocyanin
SPB	0.1 mol l^{-1} sodium phosphate buffer
SPBT	0.1 mol l^{-1} sodium phosphate buffer containing 0.3% Triton X-100
UV	ultraviolet

ACKNOWLEDGEMENTS

We thank Daniel Hartline and Monica Orcine (University of Hawaii at Manoa) for their help in the development of the immunohistochemical protocol used in this study; Joseph Shaw and John Colbourne (Indiana University) for providing access to the *Daphnia pulex* genome; Benjamin King and Sarah Harmon [Mount Desert Island Biological Laboratory (MDIBL)] for their help in the mining of this resource; Daniel Nolan (MDIBL) for his assistance with confocal imaging; and Petra Lenz (University of Hawaii at Manoa) for reading and commenting on early drafts of this manuscript. Financial support for this study was provided by NIH P20 RR046463-10 from the INBRE Program of the National Center for Research Resources (Patricia Hand, Ph.D., Principal Investigator) and through institutional funds provided by MDIBL (to A.E.C.). Travel funds (to M.D.McC.) were provided by the ULM College of Pharmacy. The authors acknowledge that the sequencing of the genome and portions of the analyses of it was performed at the DOE Joint Genome Institute under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Livermore National Laboratory (contract no. W-7405-Eng-48), Lawrence Berkeley National Laboratory (contract no. DE-AC02-05CH11231), Los Alamos National Laboratory (contract no. W-7405-ENG-36) and in collaboration with the *Daphnia* Genomics Consortium (DGC; http://daphnia.cgb.indiana.edu). Additional analyses were performed by wFleaBase, developed at the Genome Informatics Laboratory of Indiana University with support to Don Gilbert from the National Science Foundation and the National Institutes of Health. Coordination infrastructure for the DGC is provided by The Center for Genomics and Bioinformatics at Indiana University, which is supported in part by the METACyt Initiative of Indiana University, funded in part through a grant from the Lilly Endowment, Inc. Our work benefits from, and contributes to, the DGC. Deposited in PMC for release after 12 months.

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