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Research Article

Morphology and Ultrastructure of Brain Tissue and Fat Body from the Flesh Fly, *Sarcophaga bullata* Parker (Diptera: Sarcophagidae), Envenomated by the Ectoparasitic Wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae)

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This study tested the hypothesis that venom from the ectoparasitic wasp *Nasonia vitripennis* targets brain tissue and fat body from its flesh fly host, *Sarcophaga bullata*. By 1 h postenvenomation, some brain neurons began to show irregularities in nuclear shape, and though they were predominately euchromatic, there was evidence of heterochromatin formation. Irregularity in the nuclear envelope became more prominent by 3 h after envenomation, as did the condensation of heterochromatin. The severity of ultrastructural changes continued to increase until at least 24 h after parasitoid attack. At this point, cellular swelling and extensive heterochromatic inclusions were evident, multivesicular bodies occurred in the cytoplasm of some cells, and the rough endoplasmic reticulum was dilated in many of the cells. Immunohistochemical staining revealed significant apoptosis in neurons located in brain tissues. By contrast, there was no evidence of any morphological or ultrastructural disturbances in fat body tissues up to 24 h after envenomation, nor did any of the cells display signs of cell death.

1. Introduction

Ectoparasitic wasps typically subdue their insect hosts by induction of some type of halt or delay in development [1, 2]. Host arrest often is the result of venom-induced paralysis [3, 4]. In most cases, paralysis is sustained until host death and the venom constituents operate at neuromuscular junctions and/or block synaptic transmission [5–7]. The paralyzed host essentially becomes nothing more than a fixed or finite resource for feeding parasitoid progeny. In fact, there is little evidence available to suggest that ectoparasitic wasps relying on paralytic venoms physiologically manipulate their hosts, or even have a need to, beyond triggering permanent paralysis.

The situation can be quite different with ectoparasitic wasps that use nonparalytic venoms. Host developmental suppression is associated with more than just immobilization of the host: a series of physiological and biochemical alterations occur in the host that resemble those changes evoked by koinobiotic endoparasitoids [8, 9]. One example is the pupal parasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). This wasp injects a complex proteinacious venom [10] into fly hosts during oviposition that elicits a developmental arrest sustained until death [11]. The arrestment is characterized by a reduction in respiratory metabolism [12], followed by tissue-specific increases in lipid content [13] that are essential to the wasp's offspring successfully completing development [14]. Most features of pharate adult development fail to occur in envenomated pupae and pharate adults of the flesh fly, *Sarcophaga bullata* Parker (Diptera: Sarcophagidae). However, depending on the stage of host development at the onset of parasitism, eye pigment deposition and body bristle formation in fly hosts still occur, albeit as intermediates of wild-type phenotypes

and greatly delayed in terms of onset by comparison to normal pharate adult development [2, 11]. The fact that envenomation triggers developmental arrest in the host and that fly development is not completely retarded suggests that wasp venom targets brain tissue [15].

Venom-induced manipulations of host physiology and development appear to depend on signaling pathways involving G-protein sensitive receptors [15, 16]. *In vitro* assays suggest that disruption of these signaling pathways leads to an imbalance in calcium homeostasis that culminates in cell death. Ultrastructural and morphological evidence using cultured cells (BTI-TN-5B1-4 cells) indicates that venom-induced death shares features consistent with apoptosis, non-apoptotic programmed cell death, and oncosis [17]. Genome mining and proteomic analyses of venom glands from *N. vitripennis* have led to the identification of multiple venom proteins with defined roles in programmed cell death [10, 18]. However, no functional studies have been performed with isolated venom proteins to determine their roles in the host-parasitoid system, nor have any investigations examined the type of cell death evoked in host tissues *in vivo*.

In this study, we attempted to address the hypothesis that venom from *N. vitripennis* targets brain tissue and fat body from the fly host, *S. bullata*. We specifically examined the morphological and pathological changes that occur in select fly tissues (brain and fat body) following envenomation by *N. vitripennis*.

2. Materials and Methods

2.1. Insect Rearing. *N. vitripennis* was maintained as a laboratory colony on pupae and pharate adults of *S. bullata* as described previously [11]. Adults and larvae were reared under a light-dark cycle of light 15 h: dark 9 hours at 25°C. Twenty to thirty females (3–7 days after emergence from host puparia) were placed in a Petri dish (15 × 100 mm) with 40–60 nondiapausing pupae (4 days after pupariation at 25°C) of *S. bullata* and a 50% (v/v) honey-water solution. After 24 h, the adult wasps were removed and parasitized pupae maintained at 25°C, LD 15 : 9 h. Under these conditions, *N. vitripennis* completes development from egg to adult (emergence) in 12 days.

A colony of *S. bullata* was maintained as described by Denlinger [19]. Larvae were fed beef liver throughout development at 25°C with a photoperiodic cycle of LD 15 : 9 h. Adults were allowed to feed *ad libitum* on beef liver, sugar cubes, and water at 25°C with a photoperiodic cycle of LD 15 : 9 h. To synchronize fly development for assessing host age, third stage larvae that had begun to wander from food but prior to crop emptying were placed in a vented glass jar with 1-2 mL tap water. Larvae were held under these conditions for 3 days at 25°C with frequent (3–5 times/d) water changes. This "wet" treatment temporarily inhibits the release of ecdysteroids until the larvae are placed in dry conditions, thereby synchronizing initiation of pupariation [20].

2.2. Exposure of Parasitoids to Hosts. Nondiapausing pharate adults (5 days after pupariation at 25°C) of *S. bullata* were exposed singly to individual females of *N. vitripennis* as described previously [11]. Oviposition was restricted to the posterior 1/3 of the fly puparia (individual puparia were wrapped in aluminum foil) [11] to facilitate parasitoid removal. After host exposure, the adult wasps were discarded, the posterior cap of each puparium was opened, and the parasite's eggs were removed. Each pharate adult was then kept separately in glass culture tubes (13 × 100 mm) capped with cotton plugs and maintained at 25°C, LD 15 : 9 h until brains or fat body were excised.

2.3. Preparation of Brain Tissue. Brains were removed from envenomated and nonenvenomated pharate adults of *S. bullata* by dissection under a stereo dissecting microscope (Zeiss Stemi 2000, Germany). The head of each fly was severed from the thorax using iris scissors and placed in phosphate buffered saline (25 mM, pH 7.4). The head integument was then cut longitudinally along the dorsal surface to bisect between the optic lobes and expose the brain. Iris scissors were used to gently cut all tracheal and neural connections to the brain so that the brain could be transferred to cold Karnovsky's fixative (4% paraformaldehyde and 5% glutaraldehyde in 0.1 M phosphate buffer (PB) (pH 7.2) for 8–18 h [21]. After rinses in cold PB, tissues were postfixed in 1% osmium tetroxide for 2 h, rinsed again in PB, and stained with 2% uranyl acetate for 3 h. Tissues were rapidly dehydrated in an ethanol series (50%, 70%, 90%, and 100%) for 10 min each, infiltrated with propylene oxide: plastic, and stored in desiccated plastic overnight. Tissue pieces were placed in molds and polymerized overnight in an oven preset at 60°C. Brains were excised from pharate adults at 0, 0.5, 1, 3, 6, and 24 h postenvenomation, with tissues from 2–4 hosts examined by transmission electron microscopy (TEM) at each time point.

Thick sections (1 μm) were cut on an LKB Astrodome 8800 ultramicrotome and collected on glass microscope slides or glass cover slips for light microscopic observations and for immunocytochemical staining. Thin sections were then cut and collected on 200-mesh copper grids, stained with uranyl acetate and lead citrate. All electron microscopic observations were made on a JEOL 100S electron microscope at 80 kV. Section orientations were determined by using optic lobes as reference points. The percentage of cells that displayed formation of plasma membrane blebs, irregularities (i.e., convolutions) of the nuclear envelope, or heterochromatin formation in nuclei were determined from captured images. Image analyses were performed using brain thin sections from three hosts, counting a minimum of 500 cells/treatment/time point. To ensure the same cells were not counted multiple times, sequential thin sections from the same host were not used for cell counts.

2.4. Preparation of Fat Body Tissue. Fat body tissues were removed from envenomated and nonenvenomated pharate adults of *S. bullata* by dissection under a stereo dissecting microscope essentially as described by Rivers and Denlinger [13]. Lobes of fat body were collected from the head and anterior thoracic regions using fine forceps. Iris scissors were used to gently cut all tracheal and neural connections so that isolated fat body were transferred to cold Karnovsky's

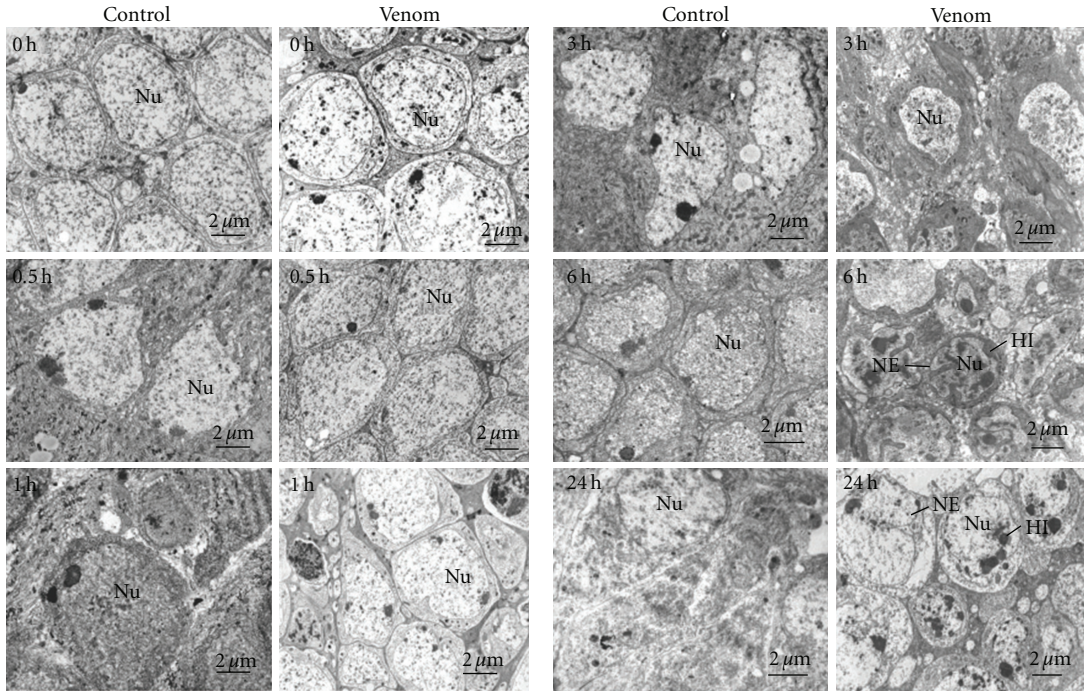

Figure 1: Ultrastructure of brains from young pharate adults of *S. bullata* following envenomation by *N. vitripennis*. At 0 and 0.5 h postenvenomation, neuronal cells in brain tissues from healthy and envenomated flies appeared virtually identical in appearance. Neuronal nuclei (Nu) were euchromatic with a regular oval shape. By 1 h and 3 h, neuronal nuclei displayed irregularity of shape with indentations in nuclear envelope (NE) and the cytoplasm became more electron dense. At 6 h after envenomation, large heterochromatic inclusions (HI) were evident within nuclei of many of the cells and the nuclei became very irregular in shape. By 24 h, large heterochromatic inclusions were present in nuclei of cells from envenomated flies. Nuclear envelopes remained intact but were irregular in shape.

fixative and then subjected to the same fixation, staining, and sectioning procedures described for brain tissue. Fat bodies were excised from pharate adults at 0, 0.5, 1, 3, 6, and 24 h postenvenomation, with tissues from 2–4 hosts examined by TEM at each time point.

2.5. Toluidine Blue Staining. In order to analyze the basic architecture of fat body tissues following envenomation, thick sections were stained with toluidine blue. Toluidine blue stains cytoplasm and, when present, cytoplasmic inclusions or granules [22]. Tissue sections (1 μm) were transferred to glass slides and then stained with 1% toluidine blue in PB, followed by mounting in Permount mounting media (Fisher Scientific Supply, Hanover, Ill, USA) and with a glass cover slip placed over the media. Sections were examined by light microscopy and images captured using an insight 4 SPOT RT fire wire digital camera (14.1 Monochrome with IR filter, Diagnostic Instruments. Inc, Sterling Heights, Mich), mounted on a compound microscope (Nikon), and connected to a Macintosh Power Mac G5 computer (Apple). Images were analyzed using SPOT (v. 4.5) and Adobe Photoshop software (Creative Suite 2, Photoshop v. 9.0).

2.6. Immunocytochemical Staining. Detection of cell death in thick sections of brain tissue isolated from envenomated flies was performed using *in situ* labeling based on the TUNEL assay (Apoptag Peroxidase *in situ* Apoptosis Detection Kit, Chemicon International). Tissue sections were washed in three changes of xylene (5 min/wash), followed by two washes (5 min each) in absolute ethanol and a single wash (3 min) each in 95% and 70% ethanol, respectively, prior to following the manufacturer's instructions with the kit. Apoptotic cell detection relied on a peroxidase reporter molecule with diaminobenzidine serving as the enzymatic substrate. Tissue sections were counterstained in 0.5% (w/v) methyl green in 0.1 M sodium acetate (pH 4.0) to visualize nuclei. Stained sections were mounted in Permount, and then a glass cover slip was placed over the media. Dried specimens were examined by light microscopy and images captured and sanalyzed as described for thin sections. Apoptosis was determined from captured images of brain thick sections from three hosts, counting a minimum of 500 cells/treatment/time point. To ensure the same cells were not counted multiple times, sequential thick sections from the same host were not used for cell counts.

2.7. Statistical Analyses. Means were compared using one- and two-way analyses of variance (ANOVA) and Student Newman-Keuls multiple comparisons tests using GraphPad statistical software (InStat v. 3.00, $\alpha = 0.05$). Percentage data was arcsine transformed prior to analysis.

3. Results

3.1. Brain Organization. Brains from young pharate adults of *S. bullata* resemble the structural organization of other

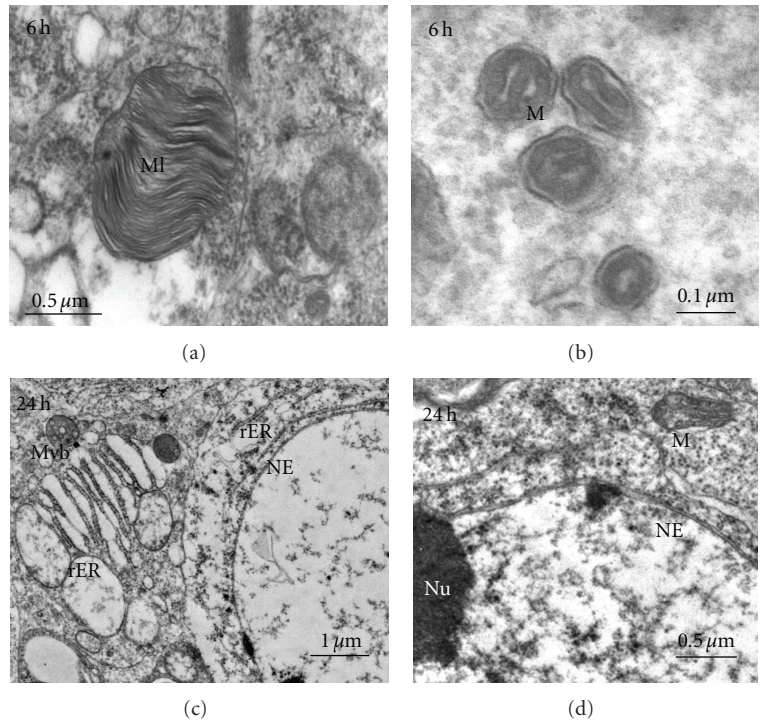

Figure 2: Venom-induced changes in neuronal ultrastructure in brain tissue from young pharate adults of *S. bullata* envenomated by *N. vitripennis*. Following venom injection, some host brain cells (a) displayed prominent multilamellar bodies (Ml) by 6 hr postenvenomation. The mitochondria (M) in the 6 hr (b) and 24 hr (d) cells displayed similar structure with no evidence of swelling of the intermembrane space. By 24 h postenvenomation, some cells (c) contained multivesicular bodies (Mvb) and very swollen rough endoplasmic reticulum (rER), while others (adjacent cells in (c)) appeared to contain healthy rER. NE: nuclear envelope.

Table 1: Cellular responses of brain tissue from *S. bullata* to envenomation by *N. vitripennis*.

			Cell responses ($X \pm$ SEM) %		
Time (hours)	Treatment	n	Bleb formation	Irregular nucleus	Heterochromatin
0	Control	3278	0a	0a	0a
	Venom	2743	0a	0a	0a
0.5	Control	3701	0a	0a	0a
	Venom	3929	0a	0a	0a
1	Control	3362	0a	0a	0a
	Venom	3578	0a	17.9 ± 2.1b	24.6 ± 1.7b
3	Control	2954	0a	1.6 ± 0.8c	0a
	Venom	2881	0a	31.6 ± 2.4d	30.8 ± 3.1c
6	Control	3460	0a	0.8 ± 0.1c	0a
	Venom	4012	0a	46.1 ± 3.6e	53.2 ± 3.8d
24	Control	3055	0a	0a	0a
	Venom	2896	23.1 ± 2.4b	68.4 ± 3.2f	63.8 ± 4.3e

The percentage of cells that displayed formation of plasma membrane blebs, irregularities (i.e., convolutions) of the nuclear envelope, or heterochromatin formation in nuclei were determined from captured images and analyzed using SPOT (v. 4.5) and Adobe Photoshop software (Creative Suite 2, Photoshop v. 9.0). Image analyses were performed using brain thin sections from three hosts, counting a minimum of 500 cells/treatment/time point. Values in the same column followed by the same letter do not differ significantly from each other at $P < 0.05$.

cyclorrhaphous flies: the brain represents the fusion of supra and subesophageal ganglia, with two distinct optic lobes extending from the protocerebrum [23, 24]. Like in *Drosophila*, the protocerebrum appeared to be composed of closely arranged neurons and neuroglia. Differentiation during the cryptocephalic to phanerocephalic stage metamorphosis leads to the apoptotically controlled degeneration of the ring gland [25] and the complete formation of two optic lobes in *S. bullata* [26]. In this study, the cellular organization of phanerocephalic brain tissue observed from

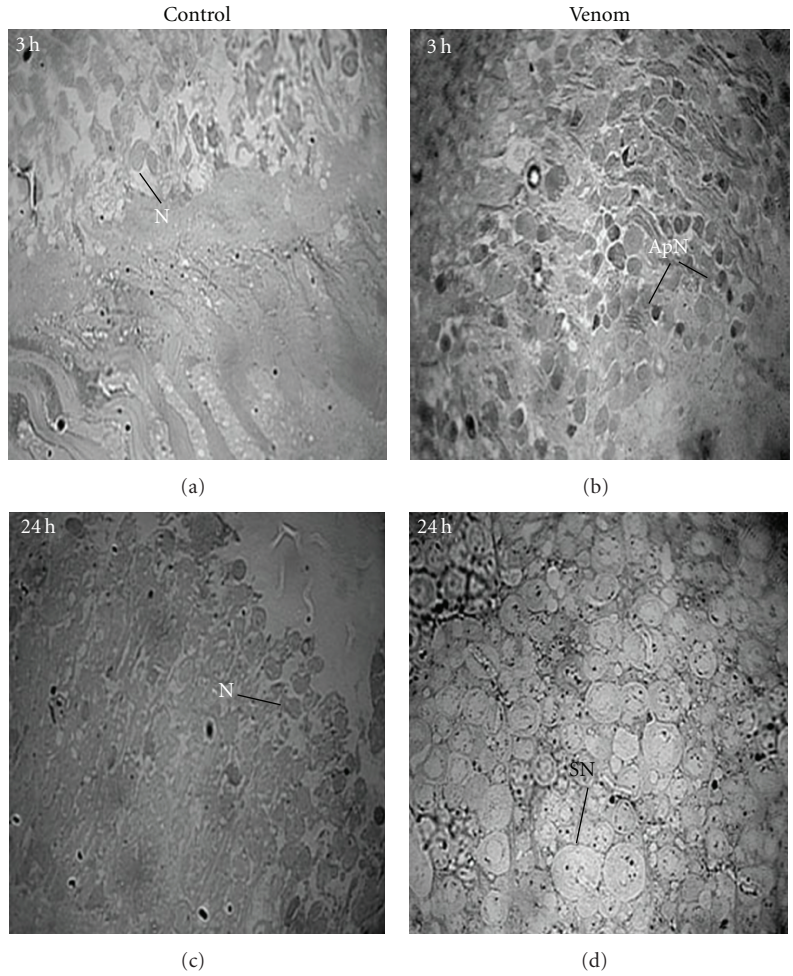

Figure 3: Light micrographs showing apoptosis in brain tissue of *S. bullata* following envenomation by *N. vitripennis*. Detection of apoptotic (ApN) or nonapoptotic neurons (N) relied on *in situ* labeling based on the TUNEL assay. Apoptotic cells are evident as darkly stained cells (peroxidase) in each panel. Several swollen neurons (SNs) were evident by 24 postenvenomation. Brain tissue extracted from nonparasitized flies served as controls. Magnification is 750x.

unparasitized *S. bullata* closely resembled that described for *Drosophila* [27].

3.2. Venom-Induced Ultrastructural Changes in Fly Brains. Following parasitism by *N. vitripennis*, wasp eggs were removed from young pharate adults of *S. bullata* so that the impact of venom on brain ultrastructure could be examined. Brain sections from unparasitized flies revealed no irregularities in neuronal cell structure: cell bodies and their nuclei mostly appeared oval in shape, nuclei were euchromatic, and the cells were devoid of obvious vacuoles and blebs (Figure 1(a)). Similarly, brain tissue removed from flies 30 min after envenomation appeared essentially identical to controls, with no detectable changes in cell ultrastructure (Figure 1(a); Table 1). By 1 h postenvenomation, some irregularities in nuclear shape were observed, although the nuclei did not appear convoluted. In these cells, the nuclei were predominantly euchromatic (>70%, Table 1), but there was some evidence of heterochromatin formation (Figure 1(a)). Convolutions in the nuclear envelope of neuronal cells became more prominent by 3 h after envenomation, as did heterochromatin in nuclei and increased

Table 2: Incidence of apoptosis in brain tissue of *S. bullata* envenomated by *N. vitripennis*.

Time (hours)	Treatment	n	Apoptotic cells ($X \pm$ SEM) %
0	Control	1745	4.7 ± 0.3a
	Venom	2011	5.9 ± 0.2a
3	Control	1540	3.6 ± 0.4a
	Venom	1687	13.5 ± 0.9b
6	Control	2431	6.2 ± 0.1a
	Venom	1938	46.6 ± 2.6c
24	Control	1867	4.3 ± 0.4a
	Venom	1805	59.7 ± 3.0d

Apoptosis was determined from captured images and analyzed using SPOT (v. 4.5) and Adobe Photoshop software (Creative Suite 2, Photoshop v. 9.0). Image analyses were performed using brain sections from three hosts, counting a minimum of 500 cells/treatment/time point. Values in the same column followed by the same letter do not differ significantly from each other at $P < 0.05$.

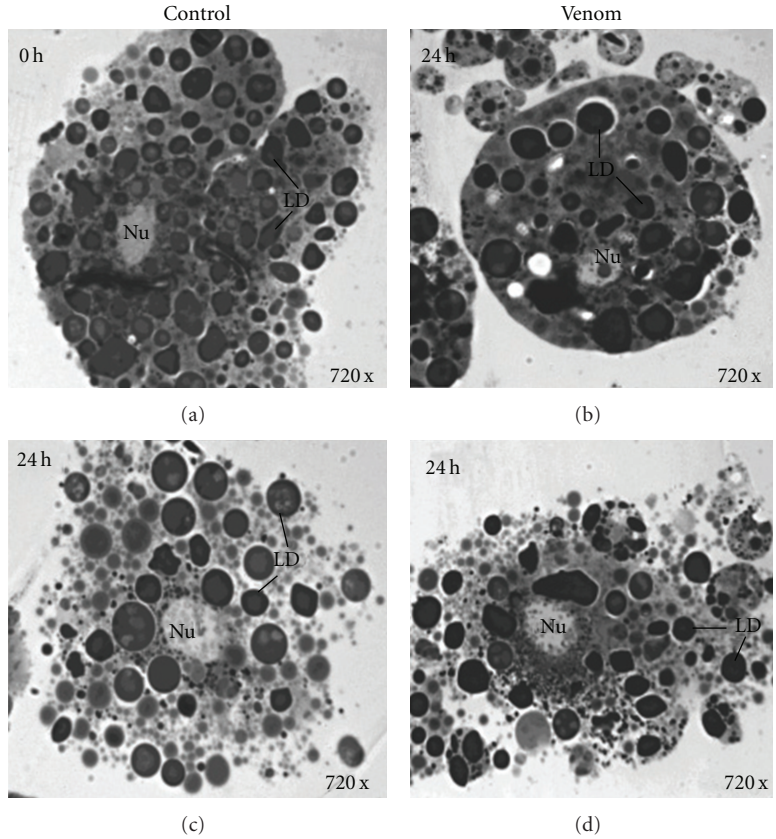

Figure 4: Light micrographs showing toluidine blue stained fat body tissue from young pharate adults of *S. bullata* following envenomation by *N. vitripennis*. Fat body examined in control and envenomated flies displayed similar cytology at 0 and 24 h posttreatment: fat body cells were large with prominent nuclei (Nu) and numerous lipid droplets (LDs) evident throughout the cytoplasm. Magnification is 720x.

electron density of the cytoplasm (Figure 1(b)). Significant ultrastructural changes were evident within 6 h following parasite attack as evidenced by convolutions of the nuclear envelope (Table 1), enlarged and darkened nucleoli, and extensive heterochromatic inclusions (Figure 1(b)). Multilamellar bodies were also evident at this time point (Figure 2(a)). These changes were even more pronounced by 24 h postenvenomation (Table 1). In addition to extensive convolutions of the nuclear envelope and heterochromatic inclusions (Figure 1(b)), multivesicular bodies occurred in the cytoplasm of some cells (Figure 2(c)), and the rough endoplasmic reticulum was dilated in many of the brain neurons (Figure 2(c)). By contrast, mitochondria in brain tissue did not appear to be altered by venom for at least 24 h after envenomation (Figures 2(b) and 2(d)).

3.3. Venom-Induced Death in Fly Brains. The cellular distortions detected in brain tissue following envenomation by *N. vitripennis* resulted in widespread, but not indiscriminate, cell death. Induction of apoptotic cell death was monitored using an *in situ* labeling kit based on the TUNEL assay and that relied on a peroxidase reporter molecule. Apoptotic cells were readily distinguished as cells developed blue-purple color intermediates, while living (prior to being embedded), and oncotic cells were unstained. Consistent with venom-induced ultrastructural changes, the number of apoptotic cells detected in brain tissue increased with the length of time after envenomation (Figure 3, Table 2). Few neuronal cells appeared dead due to apoptosis in brain tissue from unparasitized flies or from tissue extracted from envenomated flies up until 3 h postvenom injection (<14%, $n = 1482$, Table 2). However, the vast majority of dead cells observed in brain tissue 6 h after parasitism stained positively for apoptosis (Table 2). This pattern of staining was not as evident in 24 h brains, presumably because the cells were irreversibly injured and the nuclear DNA severely degraded due to endonuclease activity. The latter would prevent detection of apoptotic cells since the TUNEL assay relies on *in situ* labeling of nucleotides.

Apoptosis did not appear to be the only form of cell death induced by venom in brain tissue. Some of the cells were observed at 24 h postenvenomation (Figure 3), and to a lesser extent at 6 h, to be swollen. Cellular swelling is consistent with oncosis, which typically results in lysis. However, there was little evidence that cytolysis occurred in brain tissue at any time point examined.

3.4. Venom-Induced Morphological and Ultrastructural Changes in Fat Body. Light microscopic examination of fat body sections stained with toluidine blue revealed no obvious morphological changes in these tissues at any time point up to 24 h postenvenomation (Figure 4). All fat body cells

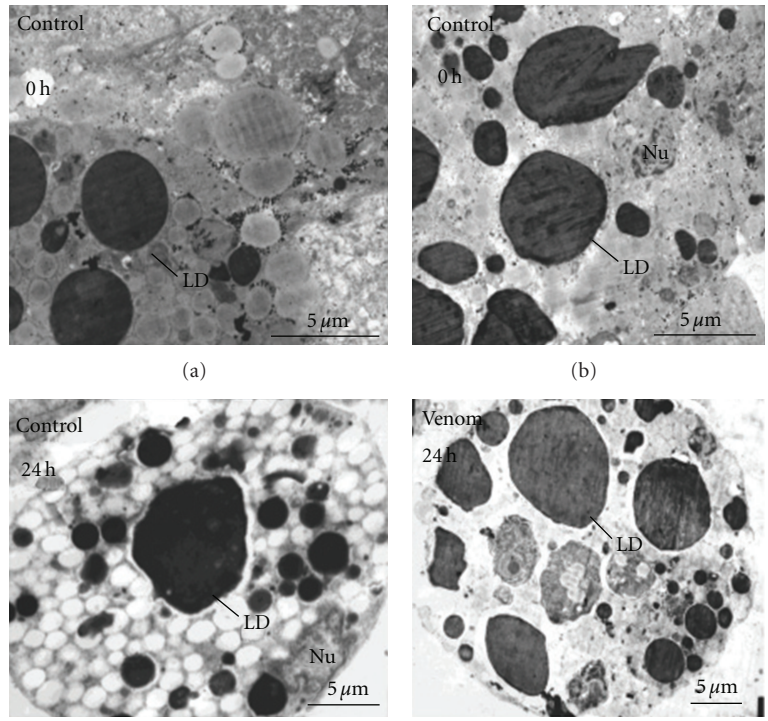

Figure 5: Ultrastructure of fat body from young pharate adults of *S. bullata* following envenomation by *N. vitripennis*. At 0 h, numerous lipid droplets (LD) of varying sizes and densities were evident throughout the cytoplasm. Large, prominent nuclei (Nu) were also typical of fat body cells. By 24 h posttreatment, fat body cells from controls and envenomated hosts appeared nearly identical: numerous lipid droplets were present, yet few inclusions appeared in the cytoplasm of either cell type.

displayed a large, centrally located nucleus, several prominent vacuoles, and the presence of lipid droplets distributed throughout the cytosol (Figure 4). Similarly, transmission electron micrographs revealed nearly indistinguishable ultrastructure of nuclei, vacuoles, and lipid droplets in fat body excised from unparasitized and envenomated pharate adult of *S. bullata* at all time intervals examined up to 24 h (Figure 5 only shows 0 and 24 h). There was also no morphological or ultrastructural evidence for induction of apoptosis or any other form of cell death in fat body cells following envenomation by *N. vitripennis* (Figure 5).

4. Discussion

Parasitic wasp venoms contain a wealth of regulatory agents that are capable of modifying growth and development of host insects to suit the needs of the parasitoid's progeny [8, 10]. Despite increasing efforts to characterize wasp venoms, including cloning and sequencing of some venom proteins [18, 28–30], very little has been revealed regarding the mechanism of action of any of these venoms [5]. Venom from *N. vitripennis* has been the subject of several recent modes of action studies [16, 31, 32], yet insufficient information is available to determine precisely which tissues are targeted in the fly host and how those tissues are injured to alter normal functions. Venom assays exploiting pupariation, extrication, and posteclosion behaviors of a preferred host *S. bullata* suggest that *N. vitripennis* venom alters neurons of the central nervous system (CNS) presumed to reside within the brain [33, 34]. However, the stages of host development (larval and imago) used in those studies are not attacked in nature by adult females of *N. vitripennis*, so implication of the brain as a target of venom based on these behavioral assays is circumstantial at best. This study has provided the first evidence that venom from *N. vitripennis* directly targets the brain in natural hosts. The fact that neurons in brain tissue displayed susceptibility to wasp venom, that the onset of significant cell death in neuronal tissue did not occur until several hours after envenomation, and that the predominant form of cell death induced by venom was apoptosis argues that *N. vitripennis* venom targets specific regions of the brain to manipulate, rather than kill, the host. These observations are also consistent with our recent findings that show *in vitro* this venom induces multiple forms of cell death and that the dominant mechanism of death triggered by *N. vitripennis* is apoptosis [17].

The dominant host response to envenomation by *N. vitripennis* is in fact not death, but instead, the induction of a developmental arrest [11, 14]. The halt in fly development begins a dynamic set of changes in fly physiology characterized by a suppression of respiratory metabolism [12], elevations in lipid synthesis [12, 13], depression of immune responses [35], and altered protein expression [15]. Disrupted protein expression has been detected in several host tissues, but changes in brain heat shock protein (hsp) synthesis appear to be some of the most dramatic deviations

from "normal" protein expression. The function of hsps during parasitism has yet to be deciphered, but these proteins may be required to arrest host development via apoptotic pathways [4, 36]. Evidence from *Drosophila melanogaster* indicates that expression of hsp70 during nonstress conditions leads to slowed development [37] and the cell cycle in cultured cells from *D. melanogaster* can be arrested in the presence of small hsps [38]. Further investigation is needed to elucidate whether venom-mediated apoptosis in brain tissues and/or altered hsp expression are keys to induction and/or maintenance of developmental arrest following venom injection by *N. vitripennis*.

The injured neurons in flies envenomated by *N. vitripennis* appeared morphologically identical to brain neurons in adult *D. melanogaster* exposed to either high-LET krypton or argon ions [39, 40]. In the case of irradiated flies, the swollen cells eventually lysed and fragmented, whereas there was little evidence of lysis in brain tissue of *S. bullata*. Similarly, both radiation treatment and envenomation initially displayed no little impact on neuroglia [40]. However, in contrast to *D. melanogaster* [40], neurons in envenomated *S. bullata* displayed convoluted nuclear membranes with condensed heterochromatin by 6 h postenvenomation, and, by 24 h, these cells were enlarged due to swelling. The differences evoked by these two types of toxic insults, high energy radiation, and envenomation may be consistent with the earlier prediction of Rinehart et al. [15] that though venom from *N. vitripennis* injures cells of the host, it does not appear to turn on a typical general stress response in the host [41]. Instead, venom appears to be targeting specific cells in brain tissue of the host to induce developmental arrest and redirect the physiology of the fly for the benefit of its progeny.

The morphology and ultrastructure of fat body tissue did not appear to be affected by wasp envenomation, at least not during the first 24 h following parasitoid attack. This was unexpected since earlier studies have shown sharp elevations in hemolymph and fat body lipid levels in *S. bullata* following parasitism, envenomation, and artificial venom injections [12, 13]. If venom elicits *de novo* synthesis of lipid in host fat body, then increased lipid droplet content would be expected in envenomated hosts. However, there were no differences between unparasitized and envenomated fat body detected by light microscopy or transmission electron microscopy. Alternatively, venom may function to liberate accumulated lipids from fat body by inducing cell death, such as occurs with *Meteorus pulchricornis* and *Cotesia kariyai* [42, 43]. Indeed, lipases and hydrolases have been identified in venom by genomic mining and proteomic analyses [10, 18], and these enzymes have been predicted to function in fat body digestion [8, 10]. However, there was no evidence in this study that venom from *N. vitripennis* induced cell death in fat body cells. This suggests that either the cellular events associated with venom-induced elevations in host lipids occur later than 24 h postenvenomation or that the primary changes in host lipids are independent of fat body tissues.

Conflict of Interests

The authors declare that they have no conflict of interests.

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References

[1] Y. Nakamatsu and T. Tanaka, "Venom of ectoparasitoid, *Euplectrus* sp. near plathypenae (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource," *Journal of Insect Physiology*, vol. 49, no. 2, pp. 149–159, 2003.

[2] D. B. Rivers, "Evaluation of host responses to envenomation as a means to assess ectoparasitic pteromalid wasp's potential for controlling manure-breeding flies," *Biological Control*, vol. 30, no. 2, pp. 181–192, 2004.

[3] D. L. J. Quicke, *Parasitic Wasps*, Chapman & Hall, London, UK, 1997.

[4] D. B. Rivers, F. Uçkan, and E. Ergin, "Characterization and biochemical analyses of venom from the ectoparasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae)," *Archives of Insect Biochemistry and Physiology*, vol. 61, no. 1, pp. 24–41, 2006.

[5] S. J. M. Moreau and S. Guillot, "Advances and prospects on biosynthesis, structures and functions of venom proteins from parasitic wasps," *Insect Biochemistry and Molecular Biology*, vol. 35, no. 11, pp. 1209–1223, 2005.

[6] C. Walther and W. Rathmayer, "The effect of *Habrobracon* venom on excitatory neuromuscular transmission in insects," *Journal of Comparative Physiology*, vol. 89, no. 1, pp. 23–28, 1974.

[7] C. Walther and M. Reinecke, "Block of synaptic vesicle exocytosis without block of Ca^{2+}-influx. An ultrastructural analysis of the paralysing action of *Habrobracon* venom on locust motor nerve terminals," *Neuroscience*, vol. 9, no. 1, pp. 213–224, 1983.

[8] S. Asgari and D. B. Rivers, "Venom proteins from endoparasitoid wasps and their role in host-parasite interactions," *Annual Review of Entomology*, vol. 56, pp. 313–335, 2011.

[9] M. A. Jervis and J. W. Copeland, "The life cycle," in *Insect Natural Enemies: Practical Approaches to Their Study and Evaluation*, M. A. Jervis and N. A. C. Kidd, Eds., pp. 63–161, Chapman & Hall, London, UK, 1996.

[10] E. L. Danneels, D. B. Rivers, and D. C. de Graaf, "Venom proteins of the parasitoid wasp *Nasonia vitripennis*: recent discovery of an untapped pharmacopee," *Toxins*, vol. 2, pp. 494–516, 2010.

[11] D. B. Rivers and D. L. Denlinger, "Developmental fate of the flesh fly, *Sarcophaga bullata* (Diptera: Sarcophagidae), envenomated by the pupalextoparasitioid, *Nasonia vitripennis* (Hymenoptera: Pteromalidae)," *Journal of Insect Physiology*, vol. 40, pp. 121–127, 1994.

[12] D. B. Rivers and D. L. Denlinger, "Redirection of metabolism in the flesh fly, *Sarcophaga bullata*, following envenomation by the ectoparasitoid *Nasonia vitripennis* and correlation of metabolic effects with the diapause status of the host," *Journal of Insect Physiology*, vol. 40, no. 3, pp. 207–215, 1994.

[13] D. B. Rivers and D. L. Denlinger, "Venom-induced alterations in fly lipid metabolism and its impact on larval development of the ectoparasitoid *Nasonia vitripennis* (walker)

(Hymenoptera: Pteromalidae)," *Journal of Invertebrate Pathology*, vol. 66, no. 2, pp. 104–110, 1995.

[14] D. B. Rivers, M. A. Pagnotta, and E. R. Huntington, "Reproductive strategies of 3 species of ectoparasitic wasps are modulated by the response of the fly host *Sarcophaga bullata* (Diptera: Sarcophagidae) to parasitism," *Annals of the Entomological Society of America*, vol. 91, no. 4, pp. 458–465, 1998.

[15] J. P. Rinehart, D. B. Rivers, and D. L. Denlinger, "Upregulation of transcripts encoding select heat shock proteins in the flesh fly *Sarcophaga crassipalpis* in response to venom from the ectoparasitoid wasp *Nasonia vitripennis*," *Journal of Invertebrate Pathology*, vol. 79, no. 1, pp. 62–63, 2002.

[16] D. B. Rivers, T. Crawley, and H. Bauser, "Localization of intracellular calcium release in cells injured by venom from the ectoparasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) and dependence of calcium mobilization on G-protein activation," *Journal of Insect Physiology*, vol. 51, no. 2, pp. 149–160, 2005.

[17] D. B. Rivers, F. Uçkan, E. Ergin, and D. A. Keefer, "Pathological and ultrastructural changes in cultured cells induced by venom from the ectoparasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae)," *Journal of Insect Physiology*, vol. 56, no. 12, pp. 1935–1948, 2010.

[18] D. C. De Graaf, M. Aerts, M. Brunain et al., "Insights into the venom composition of the ectoparasitoid wasp *Nasonia vitripennis* from bioinformatic and proteomic studies," *Insect Molecular Biology*, vol. 19, no. 1, pp. 11–26, 2010.

[19] D. L. Denlinger, "Induction and termination of pupaldiapause in *Sarcophaga* (Diptera: Sarcophagidae)," *Biological Bulletin*, vol. 142, pp. 11–24, 1972.

[20] T. Ohtaki, "On the delayed pupation of the flesh fly, *Sarcophaga peregrina* Robineau-Desvoiday," *Japanese Journal of Medicinal Science and Biology*, vol. 19, pp. 97–104, 1966.

[21] M. J. Karnvosky, "A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy," *Journal of Cell Biology*, vol. 27, pp. 137A–138A, 1965.

[22] H. A. Davenport, *Histological and Histochemical Techniques*, WB Saunders, Philadelphia, Pa, USA, 1960.

[23] C. Chi and S. D. Carlson, "Membrane specializations in the first optic neuropil of the housefly, *Musca domestica* L. II. Junctions between glial cells," *Journal of Neurocytology*, vol. 9, no. 4, pp. 451–469, 1980.

[24] M. M. Herman, J. Miquel, and M. Johnson, "Insect brain as a model for the study of aging—age-related changes in *Drosophila melanogaster*," *Acta Neuropathologica*, vol. 19, no. 3, pp. 167–183, 1971.

[25] K. H. Joplin, D. L. Stetson, J. G. Diaz, and D. L. Denlinger, "Cellular differences in ring glands of flesh fly pupae as a consequence of diapause programming," *Tissue and Cell*, vol. 25, no. 2, pp. 245–257, 1993.

[26] G. Fraenkel and C. Hsiao, "Manifestations of a pupal diapause in two species of flies, *Sarcophaga argyrostoma* and *S. bullata*," *Journal of Insect Physiology*, vol. 14, no. 5, pp. 689–705, 1968.

[27] J. Miquel, M. M. Herman, E. V. Benton, and G. Welch, "Effects of high LET particles (^{40}A) on the brain of *Drosophila melanogaster*," *International Journal of Radiation Biology*, vol. 29, no. 2, pp. 101–124, 1976.

[28] N. M. Parkinson, C. M. Conyers, J. N. Keen et al., "Towards a comprehensive view of the primary structure of venom proteins from the parasitoid wasp *Pimpla hypochondriaca*," *Insect Biochemistry and Molecular Biology*, vol. 34, no. 6, pp. 565–571, 2004.

[29] N. M. Parkinson, I. Smith, R. Weaver, and J. P. Edwards, "A new form of arthropod phenoloxidase is abundant in venom of the parasitoid wasp *Pimpla hypochondriaca*," *Insect Biochemistry and Molecular Biology*, vol. 31, no. 1, pp. 57–63, 2001.

[30] E. H. Richards and M. P. Dani, "Biochemical isolation of an insect haemocyte anti-aggregation protein from the venom of the endoparasitic wasp, *Pimpla hypochondriaca*, and identification of its gene," *Journal of Insect Physiology*, vol. 54, no. 6, pp. 1041–1049, 2008.

[31] M. Abt and D. B. Rivers, "Characterization of phenoloxidase activity in venom from the ectoparasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae)," *Journal of Invertebrate Pathology*, vol. 94, no. 2, pp. 108–118, 2007.

[32] D. B. Rivers and A. Brogan, "Venom glands from the ectoparasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) produce a calreticulin-like protein that functions in developmental arrest and cell death in the flesh fly host, *Sarcophaga bullata* Parker (Diptera: Sarcophagidae)," in *Insect Physiology: New Research*, R. Maes, Ed., pp. 259–278, NOVA Scientific Publisher, New York, NY, USA, 2008.

[33] D. B. Rivers, J. Zdarek, and D. L. Denlinger, "Disruption of pupariation and eclosion behavior in the flesh fly, *Sarcophaga bullata* parker (Diptera: Sarcophagidae), by venom from the ectoparasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae)," *Archives of Insect Biochemistry and Physiology*, vol. 57, no. 2, pp. 78–91, 2004.

[34] J. Zdarek, G. Fraenkel, and S. Friedman, "Pupariation in flies: a tool for monitoring effects of drugs, venoms, and other neurotoxic compounds," *Archives of Insect Biochemistry and Physiology*, vol. 4, pp. 29–46, 1987.

[35] D. B. Rivers, M. M. Rocco, and A. R. Frayha, "Venom from the ectoparasitic wasp *Nasonia vitripennis* increases Na^+ influx and activates phospholipase C and phospholipase A2 dependent signal transduction pathways in cultured insect cells," *Toxicon*, vol. 40, no. 1, pp. 9–21, 2002.

[36] H. Hisaeda and K. Himeno, "The role of host-derived heat-shock protein in immunity against *Toxoplasma gondii* infection," *Parasitology Today*, vol. 13, no. 12, pp. 465–468, 1997.

[37] R. A. Krebs and M. E. Feder, "Deleterious consequences of Hsp70 overexpression in *Drosophila melanogaster* larvae," *Cell Stress and Chaperones*, vol. 2, no. 1, pp. 60–71, 1997.

[38] E. M. Berger and M. P. Woodward, "Small heat shock proteins in *Drosophila* may confer thermal tolerance," *Experimental Cell Research*, vol. 147, no. 2, pp. 437–442, 1983.

[39] F. E. D'Amelio, L. M. Kraft, E. V. Benton, and J. Miquel, "An electron-microscopic study of the brain of the fruit fly, *Drosophila melanogaster*, exposed to high-LET krypton (^{84}Kr) particle radiation," *Acta Neuropathologica*, vol. 57, no. 1, pp. 37–44, 1982.

[40] F. E. D'Amelio, L. M. Kraft, E. D'Antoni-D'Amelio, E. Benton, and J. Miquel, "Ultrastructural findings in the brain of fruit flies (*Drosophila melanogaster*) and mice exposed to high-energy particle radiation," *Scanning Electron Microscopy*, vol. 11, no. 2, pp. 801–812, 1984.

[41] M. E. Feder, "Ecological and evolutionary physiology of stress proteins and the stress response: the *Drosophila melanogaster* model," in *Animals and Temperature: Phenotypic and Evolutionary Adaptations*, I. A. Johnston and A. F. Bennett, Eds., pp. 79–102, Cambridge University Press, Cambridge, UK, 1996.

[42] Y. Nakamatsu, M. Suzuki, J. A. Harvey, and T. Tanaka, "Regulation of the host nutritional milieu by ecto- and endoparasitoid venoms," in *Recent Advances in the Biochemistry, Toxicity, and Mode of Action of Parasitic Wasp Venoms*, D. B. Rivers and J. A. Yoder, Eds., pp. 37–56, Research Signposts, Kerala, India, 2007.

[43] M. Suzuki and T. Tanaka, "Virus-like particles in venom of *Meteorus pulchricornis* induce host hemocyte apoptosis," *Journal of Insect Physiology*, vol. 52, no. 6, pp. 602–613, 2006.