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Neuronal Identity: The neuron types of a simple chordate sibling, the tadpole larva of *Ciona intestinalis*.

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Abstract

Neurons of the sparsely populated nervous system of the tadpole larva in the tunicate *Ciona intestinalis*, a chordate sibling, are known from sporadic previous studies but especially two recent reports that document the connectome of both the central and peripheral nervous systems at EM level. About 330 CNS cells comprise mostly ciliated ependymal cells, with ~180 neurons that constitute about 50 morphologically distinguishable types. The neurons reveal various chordate characters amid many features that are idiosyncratic. Most neurons are ciliated and lack dendrites, some even lack an axon. Synapses mostly form en passant between axons, and resemble those in basal invertebrates; some are dyads and all have heterogeneous synaptic vesicle populations. Each neuron has on average 49 synapses with other cells; these constitute a synaptic network of unpredicted complexity.

Introduction

Complete connectomes provide a substrate for hypothesis generation with respect to both circuits and cell identities. For many years, only the nervous system of the nematode *C. elegans* [1] was known at the level of its synaptic circuits, the sole connectome [2] having the synaptic connections of all its neurons, and the neurons themselves, compiled at electron microscopic (EM) level. The initial report from the adult hermaphrodite, derived from pre-digital technology [1,3], is now supplemented by a second connectome for the adult male [4].

Recently, a complete connectome has been reported for a second, quite different species, the tadpole larva of the ascidian *Ciona intestinalis* [5,6], often loosely considered a chordate *C. elegans* because of its comparable numerical complexity. But in fact, this simple member of the largest group of marine tunicates is far removed in its ancestry from *C. elegans*, an

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Conflict of interest statement

None of the authors of this work declares any conflict of interest.

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ectdysozoan more closely related to *Drosophila*. Instead *Ciona* is a member of the chordate phylum and is part of the sibling group of all vertebrates [7]. Like many members of its group, *Ciona* is a filter-feeding adult which liberates externally fertilized gametes that after fertilization each give rise to a tiny free-living, so-called tadpole larva [8,9]. The embryo producing this larva undergoes determinate, radial cleavages for about 18 h to produce the larva, with a dorsal central nervous system (CNS) [8]. The latter comprises in succession: a rostral brain ganglion, a caudal motor ganglion and a caudal nerve cord [6,10,11].

The larval connectome: a new resource

Three tadpole larvae of *Ciona*, the sibling progeny of a single fertilization, have been reported to exhibit a CNS complement of 331–339 cells [12]. A fourth unrelated larva had similar overall cell numbers, of which 177 are neurons and the remainder are ependymal cells. Ependymal cells abut and line the neural canal, have cilia that project into its lumen, and are non-neuronal. EM revealed these to comprise at least 25 different types and 52 subtypes [6]. Unlike more extensively characterized nervous systems most ascidian larval neurons are known only from morphological criteria. Some of the 52 subtypes are not entirely discrete, however, so as to be distinguishable by all morphological features. Even so, the number of classes is still considerable for this tiny constituency of neurons, and compares to the 118 classes reported among the 302 neurons in *C. elegans* [1]. As a result, with an arithmetic average of 2–3 cells per class in *Ciona*, the different classes of neurons range in size from 1 (for the single antenna- coronet relay neuron) to 13 coronet cells (see below) on the left-hand side and 23 Group I photoreceptors on the right (Figure 1; Table 1). The distribution of neuron numbers and types of these per class reflects the marked asymmetry of the brain vesicle [6], which will not be considered further here. Most numerous amongst neurons are the 37 photoreceptors, of three different types: 30 ocellus pigment-cup associated photoreceptors, 18–23 in group I and 8–11 in group II [6,13], with a further group of 7 Group III photoreceptors having outer segments that are less well organized [6].

Comprehensively exposed in the EM database, the cellular diversity of the simple *Ciona* larval CNS (Figure 2) may in fact be no less than in the more heavily populated brains of vertebrates. The connectome reveals features of ascidian larval neurons that in some cases are also unusual or unanticipated. Thus, the neuron terminal, which can be both pre- and postsynaptic, is simple and mostly unbranched, an axon is sometimes lacking, and most neurons are ciliated. Cilia from 81 such cells do not actually project into the lumen of the neural canal, however [6]. Unusual features include: some neurons such as three arborizing cells in the brain vesicle that each branch immediately into terminals and lack connecting axons; the coronet cells that lack both true axons and terminals; and neurons with bifid axons with long collaterals [6]. Most synapses form upon postsynaptic neurons, but others form onto the basal lamina that enwraps the CNS. Like their vertebrate counterparts ependymal cells conversely lack both synapses and an axon and in these ways resemble glia, which otherwise lack clear counterparts in the larval CNS of *Ciona*; myelin is also lacking [14].

Other features reflect the common ancestry of tunicates and chordates and are a more familiar read. Thus, photoreceptors in both groups are ciliary [15, and earlier literature cited therein], while coronet cells [6] are functionally enigmatic structural isomorphs of cells in the saccus vasculosus of the fish hypothalamus. Despite resembling saccus vasculosus neurons, ascidian coronet cells are likewise components of a morphologically homologous structure that is bilateral in vertebrates [16], but unilateral and unpaired in *Ciona* [6]. In vertebrates, the saccus vasculosus comprises coronet cells in addition to neurons contacting the cerebrospinal fluid. In *Ciona* newly reported ciliated coronet-associated neurons, having cilia that project into the neural canal toward the coronet cells' bulbous protrusions, are likewise similar to cells in the fish saccus vasculosus. The coronet cells alone, or in combination with these associated neurons have a unique and robust transcriptional identity [17]. In both vertebrate and ascidian cases, it is these neurons rather than the coronet cells that give rise to an axon [18].

Few cells and synapses

The ascidian larval CNS has many types of neurons, each type with few representatives (Figure 1; Figure 2; Table 1).

For *Ciona*'s entire larval nervous system old reports provide the following tally of cell populations. A single larva comprises about 2,600 larval cells [19] that include 40 notochord and 36 muscle cells but are otherwise mostly epidermal cells. These numbers may be essentially fixed, or eutelic, the product of fixed cell lineage in the embryo [20,21]. Cell numbers for the CNS come from data for three sibling larvae, which had 335 ± 4 CnS cells in total [12]. Of these, a single unrelated larva's CNS contained 177 neurons constituting 25 types with 72 subtypes, most identified uniquely, including 5 pairs of motor neurons [6] (Figure 2; Figure 3). Together these formed 6618 synapses, including 922 (14%) dyads - at which a single synaptic site incorporates two postsynaptic dendrites opposite a single presynaptic release site, and 1772 neuromuscular synapses [6]. In addition 1206 close membrane appositions were interpreted as gap junctions. Both synapses and presumed gap junctions can occur between neurons of the same cell type [6]. All data are from Ryan et al. [6]. The remaining cells are the non-neuronal ciliated ependymal cells that line, and project cilia into, the lumen of the neural canal.

Neurons are sparsely connected: on average each has 14 partners, and forms on average ~49 synapses of varied sizes, having a mean cumulative depth of $4.68 \mu\text{m}$ [6], that are each thus validated because they extend through several serial ultrathin sections. Neuromuscular junctions of the five pairs of motor neurons are larger than synapses between neurons; each pair forms a specific shape of endplate.

Peripheral neurons

The cells of the peripheral nervous system (PNS) are fairly well documented in older literature, and their inputs to the CNS recently identified and reviewed [11,22]. PNS input arises from scattered groups of epidermal neurons (Figure 2), 54 in total [23], of which 32 are reconstructed in serial EM [22]. Substrate preference and induction of early

metamorphosis in the presence of external chemical and physical stimuli suggest that PNS neurons, those that bear 9+2 cilia, can sense both chemical and mechanical cues [23,24]. The most anterior papillar neurons are designated touch chemoreceptors, although their mechanosensory capacity is untested while, among other classes of PNS neurons, all epidermal neurons are proposed mechanoreceptors, but their chemoreceptive capacity has not been evaluated [11,22]. The papillar neurons also group separately from remaining epidermal neurons in transcriptome analyses [17].

Neuronal identities: Many cell types

Despite their small number, CNS cells comprise many types, known almost entirely on morphological grounds (Table 1). Many remain anonymous among brain vesicle interneurons (Figure 2) and are classified according to their connections. Restricting the designation of these to neurons having a discriminable combination of anatomical characters, each type thus has few cells, many classes in fact having a sole representative (Figure 3). Additional criteria of neurotransmitter phenotype and electrical excitability are mostly not known, but may be expected to arbitrate additional cell types. Indeed, transcriptome analysis separates neurons with gene expression indicating a cholinergic phenotype from those indicating a GABAergic phenotype [17], but does not separate these neurons further; other neurotransmitters, and any further subcategories of neurons sharing neurotransmitter phenotype [25] await further transcriptome analyses. One value of a connectome is to enable a complete accounting of all cell types, including types that might be known in vertebrate brains but not from their numbers of representatives, or simply overlooked among the forest of others. For example, most CNS neurons -- 120 of 177 -- are ciliated, and all remaining cells appear to contain basal bodies, even if they may lack canonical ciliary extensions from the soma.

Neurons of the peripheral nervous system (PNS) are distributed in the epidermis and have relay neurons in the CNS [22]. Overall, many classes of sensory neurons have been identified, chiefly from their location over the epidermal field, and ciliary type.

Synapses

Ascidian larval neurons branch sparsely, and in most cases are axons with simple terminals arising from somata that mostly lack dendrites and deny easy morphological classification (Figure 4; Table 1). Given that the number and arborization of a neuron's dendrites have been interpreted as a function of the number and range of their synaptic inputs [26], the lack of dendrites in *Ciona* neurons can be interpreted to reflect the paucity of synaptic inputs. But this may not be so. Thus, although many of the larval neurons in *Ciona* with dendrites fall among those with the greatest overall number of synaptic inputs, others lacking dendrites fall among this population as well. In addition, in *Ciona* neurons many synaptic involvements fail to segregate to different regions of the cell surface, so that although some input neurons synapse upon the soma, and output neurons occupy the terminal, dendrites are lacking, most synapses are axo-axonic, forming en passant between their axon and the axons of neighbours, or between terminal regions [6]. These features are shared with the neurons of *C. elegans*, and given the phylogenetic gulf between the two species such similarities may

reflect more the compactness and similar neuron numbers of the nervous systems in the two species than deeper-seated similarities

The comprehensive matrix of synaptic contacts between identified neurons is a rich and mostly decisive resource for the identification and characterization of neuron types. Most of these synapses are simple and conventional, with a cumulus of presynaptic vesicles, either a sole tight cluster of small electron-lucent vesicles 30–60 nm in diameter, or accompanied at some synapses by larger (70–110 nm) electron-lucent vesicles, or dense-core vesicles 70–90 nm or 100–110 nm in diameter. Some synapses, especially those of antennal neurons, are dyads, having two postsynaptic elements opposite a single presynaptic site. Different functional configurations include reciprocal and serial synapses and bidirectional synapses at which pre- and postsynaptic structures sit opposite each other in partner neurons, like those reported in mollusc and cnidarian nervous systems [27].

Criteria for differentiating cell types

Neurons are so classified on the bases of being presynaptic to another cell at chemical synapses. Additionally, most have axons, although some, such as coronet cells, lack obvious axons or terminals. Axons are sometimes bifid, with collaterals. The simplicity of larval neuron morphology in *Ciona* also limits its use as a tool to categorize neurons, however. Sensory neurons are identified by their sensory structures, but otherwise neurons in this system are classified by their connections. Even motor neurons are so classified solely by their synaptic contacts onto muscle and diverse endplate morphologies, and interneurons are subdivided based upon the inputs from sensory classes. Individual neuron differences within larger classes do have morphological or ultrastructural criteria, however: depth of terminals within the CNS, projection of axons (Figure 3), expansions near the axon-hillock, bifurcation, terminal shapes (Figure 4). Postsynaptic sites are concentrated more densely near the axon hillock of many relay neurons. As in any nervous system, many of these distinguishing neuronal features are related to the locations of soma, axons, or targets. For example, large ventral interneurons are unimaginatively named because they are large and ventral, and their shape reflects their ventral location relative to the relay neuropil to the motor ganglion.

Recently reported single-cell transcript analyses [17] provide the prospect to validate and augment these anatomical categories, but also impose their own uncertainties. At present, transcriptome analysis has separated broad classes of neurons, but have not elucidated further cell type classifications. Cells in clusters other than the one containing photoreceptors express visual cycle genes; epidermal cells and brain vesicle cells are clustered together, possibly soliciting further subdivision; while glutamate transport gene expression is difficult to ascertain because of a poorly annotated gene model.

Cell types each have few constituent neurons

Most neuron classes have few constituents, many containing just a single cell (Figure 4); exceptions are photoreceptors, coronet cells, and local interneurons, which each comprise between 15 and 25 neurons. These are reported from multiple individuals for photoreceptors

[6,12,13, 28] and coronet cells [6,12,29,17]. Even among these, there may be subtypes however, such as those defined by the rows or outer segment arrangement in type I photoreceptors [5], or by their connections [30]. Insofar as most subtypes are defined by their connections, a single connectome can predict cell types, but the number of constituent types remains inconclusive until resolved in multiple individuals.

Network analysis endorses morphological cell types

Connectivity network analysis identifies communities or clusters that separate the identified cell types, providing computational support for their identities. Thus, even with subtle differences between individual neurons' contacts and networks, computational models group, for example, photoreceptor relay neurons together, and separate these from photoreceptor-AMG or photoreceptor-PN relay neurons [6,22]. This is not surprising given that many of these classes were determined based on their connections. Some also exhibit morphological characters, such as multipolar AMG neurons, and bifurcated terminals in many of the pr-AMG neurons, or positional clustering of somata or axons. In these cases, the questions of whether the cell lineage of these populations is perfectly fixed, and whether form determines function or function determines form is as yet unresolved.

Conclusions and prospects

The current analyses of the nervous system of the ascidian tadpole larva not only report the connectome of identified cell types, but yield a Pandora's box of further questions. High on the list of these is whether the cell types of a second larva would be identical to those now known from the first. A high second priority would be to identify the neurotransmitter system, cell by cell, and the neurotransmitter receptors expressed by postsynaptic neurons identified by the connectome, for which application of single-cell transcriptomic methods are now underway [17]. Technological and methodological advances will doubtless require further revisions in the lineages and cell types.

A cost of having a comprehensive connectome is that we can no longer cherry-pick cell homologies between *Ciona* and vertebrate neurons, without reference to many other types that until recently had been anonymous, nor can we simply assume that ascidian neurons are just simple versions of vertebrate neurons, when many cell types are demonstrably complex and their components unexplained, and yet others simply enigmatic. At best we see that many tunicate neurons have chordate features, some previously identified, but others with basal features that help to identify those in the nervous systems of forms ancestral to both urochordate and chordate brains.

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Figure 1.
Histogram of the rank-ordered numbers of cells per identified type, showing the many classes having only a single representative, and the distribution of those with more.

Figure 2.

A. Map of the sensory neurons and motor system of the larval nervous system of *Ciona*. Cell types named, labelled and colour-coded as in Table 1. After Ryan et al. [6].

B. As in A, but with the addition of interneurons in the brain vesicle to show their relative placement. These are shown enlarged in Figure 2. Redrawn after Ryan et al. [6,22].

C. Dorsal view of 3D reconstruction of cell bodies illustrated in B, note BVINs are yellow instead of pink. After Ryan et al. [22]

Figure 3.

Enlarged view of the brain vesicle interneurons named and colour-coded as in Table 1. Based on an enlarged view of Figure 2B.

Inset: Enlargement of the motor ganglion showing the locations of terminals from relay neuron types, relative to the locations of the motor ganglion somata.

Figure 4.

All reconstructed CNS larval neurons in *Ciona*, grouped by morphological type, showing representatives of different cell types/morphologies. Cells are arranged for greatest spacing efficiency, without reference to their placement in the larva. Most are presented from a left lateral view.

Table 1.

Colour-coded cell types of the larval nervous system in the ascidian tadpole of *Ciona*.

Cell Type	Alternative names	Abbreviation	Defining Characteristics	Reports in the literature	Putative Neurotransmitter	Subtypes	Number of cells	Cell IDs	Example: Morphology
Type I Photoreceptors	Pigmented ocellus Photoreceptors	PR-I	Outer segments into ocellus pigment	[31]; [12]; [32]; [33]; [34]; [13]; [28]; [6]	Glutam ate	outer segment projection: osa (anterior), osd (dorsal), osp (posterior); Layers I-v: I-i, I-ii, I-iii I-iv, I-v	23	pd-pr23
Group II Photoreceptors	Pigmented ocellus Photoreceptors; Type II Photoreceptors; Canal photoreceptors	PR-II	Outer segments directly into neural canal near ocellus pigment	[13]; [28]; [6]	GABA	outer segment projection: osa (anterior), osd (dorsal), osp (posterior), Layers i-ii: II-i, II-ii	7	pra-pig
Group III Photoreceptors	Non-pigmented ocellus photoreceptors, Type III photoreceptors	PR-III	Ventral vacuolated cells between Cor and lens cells with modified cilia into neural canal	[13]; [35]; [28] ; [6]		-	6	lens 6-7, 84, 101, 110, 114
Lens Cells		Lens	Large, vacuolated cells ventral to ocellus pigment	[36]; [37]; [12]; [38]		round, cup	3	lens4, lens5, lens8

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Cell Type	Alternative names	Abbreviation	Defining Characteristics	Reports in the literature	Putative Neurotransmitter	Subtypes	Number of cells	Cell IDs	Example: Morphology
Photoreceptor associated vacuolated neurons	photoreceptor associated neurons	vacIN	Anterior vacuolated neurons with short axons along photoreceptor axon tract	[6]	GABA	terminal depth: lens1, lens2	2	lens1, lens2
Photoreceptor tract interneuron		trIN	Large neuron dorsal to photoreceptors on right side, axon descending along photoreceptor tract, large expanded terminal in PBV	[13];[6]	Glutamate	-	1	36
Antenna neurons	Antenna neurons	Ant	Ventral neurons, contact otolith foot and oaces, looped axons, large terminals, invaginating terminal, many polyad synapses	[34]; [13]; [39] ; [6]	Glutamate	Ant1 (contacts both oaces) and Ant2 (contacts only oace2)	2	Ant1 (10), Ant2 (63)
otolith-associated ciliated cells		oaces	Ventral cells, contact otolith with modified cilia. Contact antenna cells. No neurites.	[6]		oace1 and oace 2	2	oace1, oace2

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Cell Type	Alternative names	Abbreviation	Defining Characteristics	Reports in the literature	Putative Neurotransmitter	Subtypes	Number of cells	Cell IDs	Example: Morphology
Otolith	Statocyst	Ot	Ventral cell, foot and large single pigment granule in neural canal, proposed gravity receptor	[36]; [31]	Glutamate	-	1	Otolith
Ocellus pigment	Ocellus pigment cup	Oc	Right cell, contains many pigment granules, receives outer segments of photoreceptors, ciliated	[36]		-	1	Ocellus
Coronet cells		Cor	Left side cells, each has single cilium modified to bulbous protrusion, each has short axon to left side along basement membrane, dense core vesicle synapses	[31]; [37]; [29]; [34]; [35]; [39] ; [6]	Dopamine	synaptic contacts: basal lamina only, neurons and basal lamina	16	Cor1-Cor16

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Cell Type	Alternative names	Abbreviation	Defining Characteristics	Reports in the literature	Putative Neurotransmitter	Subtypes	Number of cells	Cell IDs	Example: Morphology
anaxonal arborizing neurons		aalN	posterior BV neurons with large expanded branched terminal adjacent to their cell bodies	[6]		pr-ant-aalN (102, 115), ant-aalN (95)	3	95, 102, 115
bipolar brain vesicle interneurons	bipolar interneurons	BPIN	bipolar BV intrinsic interneurons	[6]		pr-BPIN and pr-ant-BPIN	2	90(pr-ant), 92(pr)
coronet associated ciliated brain vesicle interneurons		cor-ass BVIN	Ciliated BV intrinsic interneurons, cilia project to contact coronet bulbous protrusions, cells on left dorsal side	[35]; [6]		Varied sensory input, pr, ant, pr-ant, cor	14 [15]	1 (PNIN), 2(PNIN), 15, 23, 38(ant),55,59, 60(pr-ant), 62(cor),65 (PNIN) 68(pr-ant-cor), 70(pr-ant), 73(ant), 76(pr), 79(ant)
ciliated brain vesicle interneurons	BV intrinsic interneurons	BVIN	Ciliated neurons, dorsal left, fine axons	[35];[6]		Varied sensory input, pr, ant, pr-ant, cor	19	3, 13, 17, 18, 21, 22, 24, 20, 33, 41, 42, 43, 48, 50, 61, 65, 85, 88, 92
brain vesicle interneurons	BV intrinsic interneurons	BVIN	Cell bodies anterior to series	[12];[6]			2	ukn, ukn2
brain vesicle photoreceptor interneurons	brain vesicle interneurons	prIN	Photoreceptor input (ciliated except those in bold)	[6]		ciliated and non-ciliated, some receive additional sensory input	2 [10]	13, 16, 17, 22, 42, 50, 68, 70, 78, 138
brain vesicle antenna interneurons	brain vesicle interneurons	antIN	Antenna input (ciliated, Ant input only indicated by *)	[6]		some receive additional sensory input	[10]	16, 24*, 33*, 38, 60, 68, 70, 73*, 79, 138
anterior terminating rostral trunk epidermal neurons	RTENS I II III	pna/RTENS	anterior mechanosensory peripheral neuron (rostral trunk epidermal neuron) extending axon into the CNS BV	[41]; [42]; [43]; [44]; [45]; [39]; [23]; [46]; [14]; [22]	Glutamate	Left and Right	6	pns1(L), pns2(L), pns5(R), pns6(R), pns9(R), pns13(L)
posterior brain vesicle terminating rostral trunk epidermal neurons	RTENS I II III	pnb/RTENS	anterior mechanosensory peripheral neuron (rostral trunk epidermal neuron) extending axon into the CNS BV	[41]; [42]; [43]; [44]; [45]; [39]; [23]; [46]; [14]; [22]	Glutamate	Left and Right	6	pns3(L), pns4(L), pns7(R), pns11(R), pns12(L)

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Cell Type	Alternative names	Abbreviation	Defining Characteristics	Reports in the literature	Putative Neurotransmitter	Subtypes	Number of cells	Cell IDs	Example: Morphology
Papillar neurons	palp neurons	Pap	Anterior neurons of palps	[41]; [44]; [39]; [45]; [23]; [46]	GABA, Glutamate				
peripheral interneurons		PNIN	BV intrinsic neuron, postsynaptic to RTENs, clia do not enter	[22]	GABA	primary, secondary, ciliated, non ciliated	5 [10]	4, 6, 20, 25, 29, 30, 61, 65, 85, 88
posterior brain vesicle peripheral interneurons		PBV PNIN	BV intrinsic neuron, cell body in posterior BV, postsynaptic to RTENs	[22]		-	4	160, 162, 163, 164
photoreceptor relay neurons	large ventroposterior interneurons	prRN	BV neurons, receive photoreceptor input, axons to MG, terminate in MG	[6]	GABA, Glutamate, Acetylcholine?	pr exclusive , some antenna input; MGL, MGL/R*	6	80, 86*, 96*, 100*, 121, 126*
photoreceptor-ascending motor ganglion neuron relay neurons	photoreceptor-peripheral relay neurons	pr-AMG RN/pr-PN RN	BV neurons, receive photoreceptor, AMG, and BTN input, exclusive postsynaptic RN partners of PK II, axons to MG, terminate in MG, branched terminals	[6], [22], [47]	GABA, Glutamate, Acetylcholine?	to only right side MG , to bilateral MG	8	74, 94, 108, 116, 124, 127, 140, 157
photoreceptor-bipolar tail neurons	photoreceptor-peripheral neuron relay neurons	pr-BTN RN/pr-PN RN	BV neurons, receptor and Bipolar tail neuron input, axons to MG, terminate in MG	[6], [22], [47]	GABA, Glutamate, Acetylcholine?	-	2	123, 130
photoreceptor-coronet relay neurons	multimodal relay neurons	pr-cor RN	BV neurons, receive photoreceptor and coronet input, axons to MG, terminate in MG	[6]	GABA, Glutamate, Acetylcholine?	Presynaptic to MNI.2-4s , not presynaptic to MINS	3	105, 112, 119
antenna 1 relay neurons	antenna relay neurons	anti RN/antRN	BV neurons, receive antennal input, axons to MG, terminate in MG	[6]	GABA, Glutamate, Acetylcholine?	Presynaptic to right MG , presynaptic to left and right MG	3	147, 152, 161
antenna 2 relay neurons	antenna relay neurons	ant2 RN/antRN	BV neurons, receive antenna2 input, axons to MG, terminate in MG (left and right)	[6]	GABA, Glutamate, Acetylcholine?		3	135, 153, 159
antenna 1/2 relay neurons	antenna relay neurons	ant 1/2 RN/antRN	BV neurons, receive antennal 1 and antenna2 input, axons to MG, terminate in MG	[6]	GABA, Glutamate, Acetylcholine?	Ant-cor , ant-pr*, ant	5	120, 134*, 142, 152*
antenna-coronet relay neurons	antenna relay neuron	ant-cor RN/antRN	BV neuron, receives antenna 1 and 2 input and coronet input, axon terminates in MG	[6]	GABA, Glutamate, Acetylcholine?	-	[1]	120
secondary relay neurons	non-sensory relay neurons	non-sensory RN/2° RN	BV neurons, not postsynaptic to sensory neurons, axons to MG, terminate in MG	[6]	GABA, Glutamate, Acetylcholine?		5	93 (two single section Ant2 synapses), 103 (postsynaptic? To ATENa and presynaptic to prs), 106, 122 (postsynaptic to AMG7), 125
peripheral relay neuron		PNRN	BV neuron, postsynaptic to PNINs, PBV PNINs, BTNs, AMGk, pr-AMG, and presynaptic to Em1, terminates in MG	[6], [22]		-	1	131

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Cell Type	Alternative names	Abbreviation	Defining Characteristics	Reports in the literature	Putative Neurotransmitter	Subtypes	Number of cells	Cell IDs	Example: Morphology
eminens cells	peripheral relay neurons	Em	BV neurons, large, thick axon, receive input from pns neurons and their interneurons, terminate in rostral	[34] [44] [48] [49] [6], [22] [47]	GABA	Eminens1 and Eminens2	2	Em1 (109), Em2(99)	
neck neurons		NeckN	paired neck neurons, short axons, ventral, just anterior to MG	[6]		NeckNL and NeckNR	2	165, 166	
ascending motor ganglion peripheral interneurons	contrapelo cells, dorsal neurons, No. 4, 5, 6, 7, dorsal bipolar and multipolar neurons	AMG	Dorsal MG neurons, extend axons ventrally to MG then these turn and ascend to BV, interneurons of ATENp neurons	[34] ; [39] ; [6] ; [22] , [47]	GABA, Acetylcholine	Left, Right, Bilateral, multipolar to pns	7	AMG1-7	
descending decussating neurons	descending decussating neuron	ddN	Anteriomost paired MG neurons, axons decussate, terminate in CNC, postsynaptic to PNS interneurons	[39] ; [50] ; [6] ; [47]	Acetylcholine	Left and Right	2	ddNL (neuron1) and ddNR (count57)	
motor ganglion descending interneuron pair 1	Motor Ganglion paired descending interneuron	MGIN1	Paired ventral MG neurons, descending ipsilateral axons that are not presynaptic to muscle, terminate in anterior caudal nerve cord	[34] ; [51] ; [39] ; [50] ; [6] ; [47]	Acetylcholine	Left and Right	2	MGIN 1L, MGIN 1R	
motor ganglion descending interneuron pair 2	Motor Ganglion paired descending interneuron	MGIN 2	Second in series of paired ipsilateral MG interneurons, extend deep into caudal nerve cord	[34] ; [51] ; [39] ; [50] ; [6] ; [47]	Acetylcholine	Left and Right	2	MGIN 2L, MGIN 2R	
motor ganglion descending interneuron pair 3	Motor Ganglion paired descending interneuron	MGIN 3	Most dorsal cell body of MG ipsilateral interneurons, terminates in posterior MG	[34] ; [51] ; [39] ; [50] ; [6] ; [47]	Acetylcholine	Left and Right	2	MGIN 3L, MGIN 3R	
motor neuron pair 1	motor neuron 1L (left) and 1R (right); motoneuron 1; primary motor neurons	MN1	Paired ventral MG neurons, descending axons, frondose endplates presynaptic to dorsal and medial muscle, terminate in anterior CNC	[34] ; [51] ; [39] ; [50] ; [6] ; [47]	Acetylcholine	Left and Right	2	MN1L, MN1R	
motor neuron pair 2	motor neuron 2L (left) and 2R (right); motoneuron 2	MN2	Paired ventral MG neurons, descending axons with expansions along length like balls on string, presynaptic to muscle dorsal muscle, terminate deep in CNC	[34] ; [51] ; [39] ; [50] ; [6] ; [47]	Acetylcholine	Left and Right	2	MN2L, MN2R	
motor neuron pair 3	motor neuron 3L (left) and 3R (right); motoneuron 3	MN3	Paired ventral MG neurons, most dorsal of MNS, descending axons extend laterally then opposite dorsal muscle, presynaptic to muscle dorsal muscle, terminate in posterior MG/anterior CNC	[34] ; [51] ; [39] ; [50] ; [6] ; [47]	Acetylcholine	Left and Right	2	MN3L, MN3R	
motor neuron pair 4	motor neuron 4L (left) and 3R (right); motoneuron 4	MN4	Paired posterior MG/trunk tail border neurons, descending axons, presynaptic to dorsal muscle, terminate in posterior MG/anterior CNC	[51] ; [39] ; [6] ; [47]	Acetylcholine	Left and Right	2	MN4L, MN4R	
motor neuron pair 5	motor neuro n 5L (left) and 5R (right); motoneuron 5	MNS	Paired posterior MG/trunk tail border neurons, short descending axons, presynaptic to dorsal muscle , terminate in anterior CNC	[51] ; [39] ; [6] ; [47]	Acetylcholine	Left and Right	2	MNSL, MNSR	

Cell Type	Alternative names	Abbreviation	Defining Characteristics	Reports in the literature	Putative Neurotransmitter	Subtypes	Number of cells	Cell IDs	Example: Morphology
posterior motor ganglion interneurons	posterior MG interneurons	PMGIN	Posterior MG/trunk tail border neurons, unpaired, right side only, descending ipsilateral, mid-dorsal location	[6]	GABA	One longer with more synapses (1), one shorter with fewer synapses (2)	2	PMGN1 (tail8), PMGN2 (tail15)
ascending contralateral inhibitory neurons	Ascending commissural inhibitory neurons	ACIN	Posterior MG/trunk tail border neurons, ascending and decussating	[52]; [51]; [53]; [6]; [47]	Glycine	left and right, 1 and 2	3	ACINIL (109*) , ACIN2L (tail7), ACIN2R (tail10)
midtail motor neurons	midtail neurons, planate cells	MTN	CNC paired neurons, short axons descend along muscle	[34]; [51]; [39]; [6];	Acetylcholine	1,2,4, 7,	6	MTN1, 2,3,4,5,6
bipolar tail neurons	bipolar tail neuron, tail neurons	BTN	Neurons between epidermis and CNS in the tail, extend axons bilaterally into the CNS, extend along length of animal up to BV	[34]; [44]; [51]; [39]; [10]; [6]; [22]; [47]	GABA, Acetylcholine	2 left, 2 right; 1 bifid collateral axon 3 single axon	4	BTN1 (c2, Plan1), BTN2 (a4, Plan2), BTN3 (q3, Plan3), BTN4 (x4/x6, Plan4)
anterior apical trunk epidermal neurons		aATEN	Ciliated epidermal neurons dorsal to sensory neurons of CNS, short axons toward the epidermis opposite the CNS, terminate within epidermis, synapses onto basal lamina across from PNINs and Emine ns	[41]; [43]; [44]; [45]; [39]; [23]; [46]; [22]; [47]	Glutamate, GnRH		4	ATEN1-4
posterior apical trunk epidermal neurons		pATEN	Ciliated epidermal neurons dorsal to posterior BV (relay neurons), short axons along epidermis opposite the CNS to the caudal nerve cord, cross basal lamina to contact dorsal CNS AMG neurons, extend back into epidermis in tail and synapse with ascending axons from DCENs	[41]; [42]; [43]; [44];[45]; [39]; [23]; [46]; [22]; [47]	Glutamate		4	pna, pnk, pnc, pdf
dorsal caudal epidermal neurons		DCEN	Pairs of epidermal neurons with cilia to dorsal fin, axons along epidermis (PNS) opposite the CNS, overlap axon with next set of cell bodies	[41]; [42]; [43]; [44]; [45]; [39]; [23]; [46]; [22]	Glutamate			pnk, pna, pnx, pnz
ventral caudal epidermal neurons		VCEN	Pairs of epidermal neurons with cilia to ventral fin, axons along epidermis (PNS)	[41]; [42]; [43]; [44]; [45]; [39]; [23]; [46]; [22]	Glutamate				