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- 1 A three-dimensional immunohdorescence atlas of the brain of the hackled-orb weaver spider,
2 Uloborus diversus.
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6 ¹Department of Biology, Johns Hopkins University, Baltimore, MD Gregory Artiushin⁻
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- 2 Dioborus diversus.
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4 Gregory Artiushin¹,
5 ¹Department of Bio 4 5 6 7 Gregory Artiushin*, Abel Corver^{2,9,4}, Andrew Gordus^{2,4}
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4 Abstract 8 Solomon H. Snyder Department of Neuroscience, John
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2 Spider orb-web building is a captivating, rare example o ⁴Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, MD
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- 9 ⁴Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, MD
10
10 Abstract
5 Spider orb-web building is a captivating, rare example of animal construction, whose neural
13 Inderpinnings remai 11
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15 11 Abstract
12 Spider or
13 underpin
14 foundatic
15 diversus,
17 syngangli
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- 13 underpinnings remain undiscovered. An essential step in understanding the basis of this beh
14 foundational mapping of the spider's neuroanatomy, which has thus far been primarily studi
15 non-web building species. We c 14 foundational mapping of the spider's neuroanatomy, which has thus far been primarily studied using
15 non-web building species. We created a three-dimensional atlas for the hackled orb-weaver, *Uloborus*
16 *diversus*,
-
-
- non-web building species. We created a three-dimensional atlas for the hackled orb-weaver, *Uloboru*.
16 *diversus*, based on immunostaining for the presynaptic component, synapsin, in whole-mounted spiderynganglia. Aligne 15 non-web building species. We created a three-dimensional atlas for the hackled orb-weaver, orborids
16 diversus, based on immunostaining for the presynaptic component, synapsin, in whole-mounted spider
17 synganglia. Al 17 synapsin, based on immunostanting for the presynaptic component, synapsin, in whole-mounted spider
18 synganglia. Aligned to this volume, we examined the expression patterns of neuronal populations
18 representing many 18 representing many of the classical neurotransmitter and neuromodulators, as well as a subset of
19 neuropeptides – detailing immunoreactivity in an unbiased fashion throughout the synganglion,
20 revealing co-expression 18 representing many of the classical neurotransmitted material ship in mean operator and neuropeptides – detailing immunoreactivity in an unbiased fashion throughout the synganglion,

20 revealing co-expression in known s 19 neuropeptides – detailing immunoreactivity in an unbiased fashion throughout the synganglion,
- 21 This optically-sliced, whole-mount atlas is the first of its kind for spiders, representing a substantive
22 addition to knowledge of brain anatomy and neurotransmitter expression patterns for an orb-weaving
24 **htroduc**

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25 **Introduc**
26 Brain atl
27 annotati
28 connecti 25
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- 22 addition to knowledge of brain anatomy and neurotransmitter expression patterns for an orb-weavi
23 species.
24 Introduction
26 Brain atlases are essential tools for neuroscience in model organisms ranging from neurop 25 Introduction
26 Brain atlases
27 annotations (
28 connectivity r
29 spiders (8, 9).
- 22 addition to knowledge of brain analysis, and neurotransmitter expression patterns for an orb-weaving
26 addition
26 Brain atlases are essential tools for neuroscience in model organisms ranging from neuropil
27 annota 27 annotations (1), to neuronal subtype and transcriptional expression pattern atlases (2), to ultr
28 connectivity maps (3, 4). In recent years, three-dimensional atlases of major neuropil structur
29 also been created fo
-
- 27 annotations (1), to neuronal subtype and transcriptional expression pattern atlases (2), to ultrastructural
28 connectivity maps (3, 4). In recent years, three-dimensional atlases of major neuropil structures have
29 a 28 connectivity maps (3, 4). In recent years, three dimensional atlases or major neuroph structures have
29 also been created for non-canonical arthropod study species, including a number of insects (5–7) and
30 spiders (8
-
- 29 also been created for non-canonical arthropod study species, including a number of insects (5–7) and

30 spiders (8, 9).

31 The hackled orb-weaver spider, *Uloborus diversus* (10), is an emerging model system for the s
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- 30 spiders (8, 9).
31 The hackled o
32 orb-web build
33 the majority o
36 capture (13). \
36 (9, 14–21), the 31 The hackled orb weaver spider, *bibborus* diversus (10), is an emerging model system for the study or
32 orb-web building in spiders (11, 12), whose central nervous system has yet to be investigated. To date
34 model sp 33 orb-web building in spiders (11, 12), whose central nervous system has yet to be investigated. To date,
33 the majority of studies of the spider central nervous system have been performed in one, *de facto*
34 model sp 33 the majority of studies of the spider central nervous system have been performed in one, de judio
33 model species, *Cupiennius salei* (*C. salei*), a cursorial spider which hunts without building webs for p
35 capture 35 capture (13). While isolated anatomical treatments exist for orb-weavers and other web-based spiders (9, 14–21), the preponderance of *C. salei* literature is even starker when considering examinations beyond general n 35 capture (13). While isolated anatomical treatments exist for orb-weavers and other web-based spiders
36 (9, 14–21), the preponderance of C. salei literature is even starker when considering examinations
37 beyond gen
- 37 beyond general neuronal stains, where *C. salei* is essentially the only spider species in which the beyond general neuronal stains, where *C. salei* is essentially the only spider species in which the 37 beyond general neuronal stains, where C. salei is essentially the only spider species in which the

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- 23 expression pattern of more than a single neurotransmitter has been mapped (14, 22–32). Furthermore,
39 the current understanding of spider brain anatomy is almost exclusively based on tissue slice analysis,
40 which can 39 The current understanding of spider at almost the disadvantage of being often limited in completeness
39 the planes which authors chose to exhibit.
39 Given that the substantial behavioral adaptation of web-building may 41 by the planes which authors chose to exhibit.
42 Given that the substantial behavioral adaptation of web-building may be reflected in the presence of
43 necessary brain structures or their proportionality, and certainly 42 Given that the substantial behavioral adaptatines
43 Given that the substantial behavioral adaptatines
44 an important step in understanding the basis
45 nervous system which generates it. We create
46 neurotransmitter 42 Given that the substantial behavioral adaptation of web-building may be reflected in the presence of

43 necessary brain structures or their proportionality, and certainly in distinct underlying neuronal circuitry,

44 an important step in understanding the basis of this behavior is to have a foundational architecture of a

45 nervous system which generates it. We created a three-dimensional immunofluorescence atlas of major

46 neurotra 144 and international memberstanding the basis of the basis of the basis of this behavior is to the dimensional immunofluorescence atlas of major
146 and international immunostaning a formulation is to have a foundation of 146 neurotransmitter and neuromodulator populations for *U. diversus*, using whole-mounted synganglia.

147 Using immunostaining against the presynaptic marker, synapsin, we assembled a standard, full volume

148 of *U. di* Hotel is interesting a mean of populations for U. diversus, daily whole-moduled synganglia.

47 Using immunostaining against the presynaptic marker, synapsin, we assembled a standard, full volum

48 of U. diversus syngangl 47 Using immunostanting against the presping protections of U. diversus synganglion onto which specific neurosignaling molecule expression patterns were
49 aligned. These include markers for classical neurotransmitters (GA 49 of U. diversus synganglion onto which specific neurosignaling molecule expression patterns were
aligned. These include markers for classical neurotransmitters (GABA, acetylcholine), neuromodu
for dopamine, serotonin, oc 49 (dopamine, serotonin, octopamine/tyramine) and several neuropeptides (AllatostatinA, Proctolin, CCAF
49 FMRFamide). These volumes provide comprehensive and comparable detail throughout the
49 synganglion, in both undiff FMRFamile, These volumes provide comprehensive and comparable detail throughout the
52 synganglion, in both undifferentiated and established regions – such as the arcuate body, whose layers
53 become distinguishable throug
- 52 synganglion, in both undifferentiated and established regions such as the arcuate body, wh
53 become distinguishable through the use of neurosignaling molecule co-stains. We further ide
54 several previously undescrib
- 53 become distinguishable through the use of neurosignaling molecule co-stains. We further identify
54 several previously undescribed neuropils in the supraesophageal ganglion, and the neuronal subtype
55 populations whose Sample through through the use of neurosymmatry of the use of the suprassion demarcates them.

Sample populations whose specific expression demarcates them.

Sample 2013 and the
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- 54 Several previously and estimated neuropin in the suprases program, and the neuronal subtype
55 separations whose specific expression demarcates them.
56 Separations in the neuronal subtype in the subtext of the neuronal
- 56
57 **Results**
58 The central nervous system of spiders is distinctive among
59 Residing within the prosoma, the synganglion is a fusion o
60 esophageal passage running between them the subeson 57
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60
61
- 59 Sophageal passage running between them the subesophageal ganglion, comprised primarily of motor
51 and sensory interneurons and comparable to the ventral nerve cord in insects, and the supraesophageal
52 ganglion, con
- and sensory interneurons and comparable to the ventral nerve cord in insects, and the supraesophageal
62 ganglion, considered the brain proper, containing the higher-order integration centers (Fig. 1a).
63 Consistent with
- 57 Results
58 The cent
59 Residing
60 esophag
61 and sent
62 ganglior
- 59 Residing within the prosoma, the synganglion is a fusion of two major ganglia, named in reference
60 esophageal passage running between them the subesophageal ganglion, comprised primarily of
61 and sensory interneuro Consistent with general arthropod nervous system morphology, the neuronal somata are arrang
64 superficially around the synganglion (Fig. 1b,c), while all the internal tissue is neuropil. An average
65 volume of anti-synap
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- Examplion, considered the brain proper, containing the higher-order integration centers (Fig. 1a).

63 Consistent with general arthropod nervous system morphology, the neuronal somata are arranged

64 superficially around Sa superficially around the synganglion (Fig. 1b,c), while all the internal tissue is neuropil. An averaged

Sa volume of anti-synapsin immunostaining (for neuropil) and DAPI stain (for nuclei), reveals that a

substantial For the substantial proportion of somata are found on the ventral side of the subesophageal ganglion, with
66 substantial proportion of somata are found on the ventral side of the subesophageal ganglion, with
67 some nucl For anti-synapsim immuniterating (for neuropil) and of the subesophageal ganglion, w
some nuclei found laterally, but little to any on the dorsal surface of the subesophageal ganglion
patch is also present on the posterior
- some nuclei found laterally, but little to any on the dorsal surface of the subesophageal ganglion. A

patch is also present on the posterior aspect adjoining the opisthosomal neuromere. Nuclei are four

completely through
-
- be patch is also present on the posterior aspect adjoining the opisthosomal neuromere. Nuclei are found

completely throughout the ventral-dorsal plane of the anterior side of the synganglion, with populations

also seen o
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- For the present on the present on the present on the anterior side of the synganglion, with populatio

10 also seen on the lateral sides (Fig. 1b,d).

11 In the supraesophageal ganglion as well, nuclei are not present on t For also seen on the lateral sides (Fig. 1b,d).

19 complete a cap of DAPI-positive staining envelse the posterior side, except for at the

19 dorsal-most end, where a cap of DAPI-positive staining envelse the posterior, a The suppression in the lateral sides (Fig. 1b, 1
71 In the supraesophageal ganglion as well,
72 aspects of the supraesophageal ganglion
74 1b,c).
75 Within the subesophageal ganglion there
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-
-
- 22 In the suppressive ganglion as the supraes are not present on the present only suppressed are different on the supraesophageal ganglion, beginning approximately at the level of arcuate body (Fig 1b,c).

23 Ib,c).

25 Wi 24 aspects.
74 as pectrum of the subesophageal ganglion there are a limited number of conspicuous neuropils, which are
76 evident from the exterior, and have been previously described in other species (*8, 9, 13*). Most v 75 Withir
76 Withir
76 evider
- 22 dorsal-most end, where a cap per centre per cannong entermediate posterior, and action, and action
32 dorsal-most end, beginning approximately at the level of arcuate body (Fig
25 Within the subesophageal ganglion there 76 evident from the exterior, and have been previously described in other species (8, 9, 13). Most ventopils, which are a limited number of constant $\frac{1}{2}$. 76 evident from the exterior, and have been previously described in other species (8, 9, 13). Most ventrally,

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- Supplying these neuropils, as well as others and found

28 Supplying these neuropils, as well as others and found

28 Supplically-negative circular openings assumed to be tr

28 Supplically-negative circular openings assum
- The subsection is the subsection in the subsection is gainged (or neuromeres) per hemigeal ganglion corresponding to the eight legs of the spider (Fig. 2a-e).

The subplying these neuropils, as well as others and founding
-
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- 79 Supprementation in both the method in solon major ganglia, are a series of major nested fiber tracts, having a stacked organization in both the metal and ventro-dorsal planes. A chiasm structure is visible at the midlin
- 82 synaptically-negative circular openings assumed to be tracheal passageways. Throughout these
83 planes, the pedipalpal neuropil appears anteriorly (Fig. 2b-e).
84 Further dorsally (Fig. 2f,g), the tract pattern takes on be pedipalpal neuropil appears anteriorly (Fig. 2b-e).

84 Further dorsally (Fig. 2f,g), the tract pattern takes on a ladder appearance, which correspond to an

85 arcade of finer commissures, not visible in this represent France, the pedipalpal neuropil procepts anteriorly (Fig. 2nd Further dorsally (Fig. 2f, g), the tract pattern takes on a ladder
arcade of finer commissures, not visible in this representation
neuropils of the subesophagea Further dorsally (Fig. 2f,g), the tract pattern takes on another depthenesial particle in and
a reade of finer commissures, not visible in this representation. These commissures connect all majo
neuropils of the subesophag
- 86 meuropils of the subesophageal ganglion. The courses of these tracts are most comprehensible when
-
-
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- metals of the subesophageal ganglion. The courses of these tracts are most comprehensible when

87 followed by studying individual neurosignaling molecule stains, as exemplified by anti-tyrosine

88 hydroxylase immunofluor followed by studying individual neurosignaling molecule stains, as exemplified by anti-tyrosine

88 hydroxylase immunofluorescence, discussed below.

89 The opisthosomal neuromere, supplying the hind compartment of the spi 1888 hydroxylase immunofluorescence, discussed below.

88 followed by starts to emerge by the poisthosomal neuromere, supplying the hind compartment of the spider body, starts to emerged (Fig. 2f, g), and will reach its fu
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- 89 The opisthosomal neuromere, supplying the hind correlation (Fig. 2f, g), and will reach its full width in a shared plane diminishing more dorsally after the esophageal passaneuropil, a ladder-like appearance of medio-la 90 (Fig. 2f, g), and will reach its full width in a shared plane with the esophageal passage (Fig. 2h,i) befor
91 diminishing more dorsally after the esophageal passage closes (Fig. 2j, k). Within the opisthosomal
92 neur diminishing more dorsally after the esophageal passage closes (Fig. 2j, k). Within the opisthosomal

92 neuropil, a ladder-like appearance of medio-laterally running tracts can also be appreciated (Fig. 2g-i).

93 Posteri 92 methods after the esophageal passage of medio-laterally running tracts can also be appreciated (Fig. 2_j, Posteriorly travelling tracts also diverge laterally to follow the circumference of the opisthosomal neuropil (F Posteriorly travelling tracts also diverge laterally to follow the circumference of the opisthosomal
1941 neuropil (Fig. 2i).
1951 At the level of the esophageal passage, an anterior-lateral neuropil begins to form, wrappi
-
-
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- 94 neuropil (Fig. 2i).
95 At the level of the esophageal passage, an anterior-lateral neuropil begins to form, wrapping med
96 to form the cheliceral neuropil (Fig. 2g-i), as medially the esophageal passage begins to close
-
- 95 At the level of the
96 to form the chelic
97 esophageal passa
98 A bridge structure
99 flanking. Within tl
00 axis, and appears 1956 to form the cheliceral neuropil (Fig. 2g-i), as medially the esophageal passage begins to close. The esophageal passage is bridged at the anterior side by a region named the stomodeal bridge (Fig. 2j) (8)
A bridge str 97 esophageal passage is bridged at the anterior side by a region named the stomodeal bridge (Fig. 2
98 A bridge structure also exists at the posterior end, where additional undifferentiated synaptic den
99 flanking. With 97 esophageal passage is bridged at the anterior side by a region named the stomodeal bridge (Fig. 2j) (0).
98 A bridge structure also exists at the posterior end, where additional undifferentiated synaptic density is
99 f meta and the position of the protocerebral tract is essentially parallel to the ventro-dorsal
axis, and appears as twin, dense nodes rising in the central burgeoning supraesophageal ganglion.
01 **Subesophageal ganglion fea**
-

- axis, and appears as twin, dense nodes rising in the central burgeoning supraesophageal ganglion.

99 Subesophageal ganglion features and expression patterns:

99 Explorations of neurosignaling population innervation in th
- 101
101 **Subesophageal ganglion features and expression patterns:**
103 Explorations of neurosignaling population innervation in the subesophageal ganglion have general
104 been less detailed than within the supraesophageal ---
102
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104
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106 Explorations of neurosignaling population innervation in the
103 Explorations of neurosignaling population innervation in the
105 briefly shown to be immunoreactive (such as AllatostatinA (8
106 subesophageal ganglion (e.g Explorations of neurosignaling population innervation in the subesophageal ganglion have generally

104 been less detailed than within the supraesophageal ganglion. Certain neuropeptides were either only

105 briefly sho
-
- 105 briefly shown to be immunoreactive (such as AllatostatinA (8)) or not presented on in the

106 subesophageal ganglion (e.g. CCAP (28)). We find that all neuropeptidergic antisera, as we

107 others, examined in this
- 105 briefly shown to be immunoreactive (such as AllatostatinA (8)) or not presented on in the

106 subesophageal ganglion (e.g. CCAP (28)). We find that all neuropeptidergic antisera, as well as the

107 others, examined i
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- 107 subesophageal ganglion (e.g. CCAP (28)). We find that all neuropeptidergic antisera, as well as the others, examined in this study have robust expression throughout the subesophageal ganglion. On observation which do 108 observation which does not appear to be previously noted is that there is a roughly equal

109 anterior/posterior division in the leg neuromeres. Whereas some immunostains reveal equal

110 innervation of the halves 109 anterior/posterior division in the leg neuromeres. Whereas some immunostains reveal eq
110 innervation of the halves (α-TH), others show divergent patterns (α-TDC2), or predominan
111 only one compartment (α-AstA). B 110 innervation of the halves (α-TH), others show divergent patterns (α-TDC2), or predominant examples only one compartment (α-AstA). Based on select examples where the origin of innervation is discernable, the posterior 111 only one compartment (α -AstA). Based on select examples where the origin of innervation is
112 discernable, the posterior and anterior compartments of the leg neuropils may be supplied by neurites
113 from differen 112 discernable, the posterior and anterior compartments of the leg neuropils may be supplied b
113 from different tracts within the interior of the subesophageal ganglion.
114 The opisthosomal neuropil is a section of th
-
- 113 from different tracts within the interior of the subesophageal ganglion.
114 The opisthosomal neuropil is a section of the subesophageal ganglion which has received relatively less
115 attention. The preeminent referen 114 The opisthosomal neuropil is a section of the subesophageal ganglion w
115 attention. The preeminent reference for major tracts within the spider s
116 salei (13), but despite a detailed annotation throughout the synga
- 115 attention. The preeminent reference for major tracts within the spider synganglion is the treatment in C
116 salei (13), but despite a detailed annotation throughout the synganglion, the trajectories within the
116 sa
- 115 attention. The preeminent reference for major tracts within the spider synganglion is the treatment in C.

116 salei (13), but despite a detailed annotation throughout the synganglion, the trajectories within the 116 salei (13), but despite a detailed annotation throughout the synganglion, the trajectories within the

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- 118 further cursorial as well as web-based species of spiders (9) likewise did not comment on the
119 opisthosomal neuropil. A depiction from Hanström (33), shows that longitudinal tracts run parallel to
120 the midline, a 119 opisthosomal neuropil. A depiction from Hanström (33), shows that longitudinal tracts run pa
120 the midline, as well as more laterally, and that there are crossing branches between them, for
121 ladder-like architectu
-
-
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- 119 opisitiosomal neuropil. A depiction from Hanström (33), shows that longitudinal tracts run parallel to

120 the midline, as well as more laterally, and that there are crossing branches between them, forming a

121 dive 121 ladder-like architecture. This bears a resemblance to the pattern revealed by specific antisera in *U.*

122 *diversus*, confirming the central tracts, perimeter defining tracts, as well as crossing fibers within the
 121 ladder like architecture. This bears a resemblance to the pattern revealed by specific antisera in b.

122 diversus, confirming the central tracts, perimeter defining tracts, as well as crossing fibers within the

123 2122 diversus, comming the entire rates, permeter defining tracts, as well as elossing fibers within the
2123 opisthosomal ganglion – though whether they cross completely from midline to periphery was not
2124 apparent. In
-
- 124 apparent. In certain cases we observed a ladder structure as well as a ring-like central structure wit
125 neurites projecting like spokes. Immunoreactivity within the opisthosomal ganglion was variable
126 between tar 125 neurites projecting like spokes. Immunoreactivity within the opisthosomal ganglion was variable
126 between target neurosignaling molecules. One additional subesophageal feature previously identified
127 *C. salei* is
-
- 127 C. salei is the Blumenthal neuropil (34), which is innervated by afferents from the thermoreceptive and

128 hygroreceptive tarsal organ. Although we also see a paired, synapsin-density close to the midline in the

129
-
- 126 between target neurosignaling molecules. One additional subesophageal feature previously iden
127 *C. salei* is the Blumenthal neuropil (34), which is innervated by afferents from the thermoreceptiv
128 hygroreceptive
-
-
- 127 C. sale is the Blumenthal neuropin (34), which is innervated by afferents from the thermoreceptive and
128 hygroreceptive tarsal organ. Although we also see a paired, synapsin-density close to the midline in the
130 th
- hygroreceptive tarsal organ. Although we also see a paired, synapsin-density close to the midline in the
approximate anterio-ventral subesophageal location as described for *C. salei*, we cannot be confident
that this is t approximate anterio-ventral subesophageal location as described for C. sulei, we cannot be confident
that this is the same structure – a question which will benefit from tracing techniques.
131 Acetylcholine:
132 In order 131 Acetylcholine:

132 In order to visualize acetylcholinergic populations and their expression patterns, we em

133 for choline acetyltransferase (ChAT). To our knowledge, the only previous study of chol

134 in the spid 131 Acetylemente.

132 In order to vist

133 for choline ace

134 in the spider C

135 Beginning vent

136 from the leg ne 133 for choline acetyltransferase (ChAT). To our knowledge, the only previous study of cholinergic neurons

134 in the spider CNS was done in the wandering spider, *Cupiennius salei* (30).

135 Beginning ventrally, numerou
-
-
- 134 in the spider CNS was done in the wandering spider, *Cupiennius salei* (30).

135 Beginning ventrally, numerous ChAT+ somata are seen in the dense field of neurons located medially

136 from the leg neuromeres along th 134 In the spider CNS was done in the wandering spider, cupientius saler (30).
135 Beginning ventrally, numerous ChAT+ somata are seen in the dense field of
136 In the leg neuromeres along the midline of the hemiganglia (F 136 from the leg neuromeres along the midline of the hemiganglia (Fig. S1a). Cholinergic neurons are also
137 present between leg neuromeres in the anterior-posterior direction, and for both cases, there is a
138 diversity
-
-
- 137 present between leg neuromeres in the anterior-posterior direction, and for both cases, there is a

138 diversity of both size and staining intensity. The interspersed presence of more intensely ChAT-

139 immunoreacti diversity of both size and staining intensity. The interspersed presence of more intensely ChAT-

139 immunoreactive neurons within the subesophageal ganglion was also observed in *C. salei* (30). At

140 approximately the
- minimized the level of the pedipalp ganglia (Fig. S1b, arrows) there are 3-4 relatively smaller,
141 strongly immunoreactive somata.
142 GABA:
143 GABA:
-
-
- 143 diversity of both size and state and state and state of more intersection of more intersection of more intensely α

2014 approximately the level of the pedipalp ganglia (Fig. S1b, arrows) there are 3-4 relatively smaller,
141 strongly immunoreactive somata.
142 GABA:
144 GABAergic neurons can be identified with antisera to γ-aminobutyr 142
143 GABA:
144 GABAergic neurons can be identifi
145 in the CNS in *C. salei* (26, 29) as we
146 *tepidariorum (also known as Paras* 143
144
145
146
147 143 GABA:
144 GABAe
145 in the (
146 *tepidar*
148 surface
149 opisthe 146 *tepidariorum* (also known as *Parasteatoda tepidariorum* (17). GABAergic neurons are the most populous
147 subtype that we have visualized in *U. diversus*, with a large portion of these cells residing on the ventral
 145 in the CNS in C. salei (26, 25) as well as the barn spider, Araneas cavalicas (15) and Achdeminea
146 *tepidariorum* (also known as *Parasteatoda tepidariorum* (17). GABAergic neurons are the most po
148 surface in the 147 subtype that we have visualized in *U. diversus*, with a large portion of these cells residing on the ventral
148 surface in the subesophageal ganglion (Fig. S2a-b), with presence posteriorly as well, ventral to the
14

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- 147 subtype that we have visualized in *D. diversus*, which along portion of these cells residing on the ventral
148 surface in the subesophageal ganglion (Fig. S2a-b), with presence posteriorly as well, ventral to the
150 149 opisthosomal neuropil (Fig. S2c). While used coincidentally with other successful antibodies by our standard preparation, GAD antisera unfortunately exhibited poor signal penetration in the interior of the tissue, limi
-
- 150 standard preparation, GAD antisera unfortunately exhibited poor signal penetration in the interior
151 the tissue, limiting our analysis to the presence of GAD+ somata, as well as a number of neuropil
152 features whic 151 the tissue, limiting our analysis to the presence of GAD+ somata, as well as a number of neuropil
152 features which happened to be closer to the surface of the tissue, such as the opisthosomal neuropil
153 (Fig. S2d). 152 features which happened to be closer to the surface of the tissue, such as the opisthosomal neur
153 (Fig. S2d).
154 Dopamine:
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-
- 153 (Fig. S2d).
154
155 Dopamine: 154
155 Dopamine
155 Dopamine 155
|
| 155 Dopamine:

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- 157 been investigated in C. salei, but rather in the wolf spider, *Hogna lenta*, and the jumping spider,
158 *Phidippus regius*, where it was interrogated using antisera to core synthesizing enzyme, tyrosine
159 hydroxylas Deen investigated in C. surer, but rather in the worr spider, *nogha lenta*, and the jumping spider,
158 *hydroxylase (35)*.
160 We found this antibody to be effective in *U. diversus*, staining both cell bodies and projec 159 Philoppus regius, where it was interrogated using antisera to core synthesizing enzyme, tyrosine
169 We found this antibody to be effective in *U. diversus*, staining both cell bodies and projections with
161 enough cl 159 hydroxylase (35).
160 We found this ant
161 enough clarity to
162 with each of the k
163 variable, but it ap
164 arrowheads), typi
165 larger neurons wh 160 We found this antibody to be effective in D. diversus, starling both cell bodies and projections with

161 enough clarity to follow the innervation patterns of many individual dopaminergic neurons. Associa

162 with ea
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-
-
-
-
- with each of the leg neuromeres are 7-8 TH+ neurons (Fig. S3a). The positioning of these is somewhat
163 variable, but it appears that they form two subgroups a cluster of 5-6 smaller neurons (Fig. S3a –
164 arrowheads), 163 variable, but it appears that they form two subgroups – a cluster of 5-6 smaller neurons (Fig. S3a –
164 arrowheads), typically with a couple being less intensely immunoreactive to TH, and the remaining 1-2
165 larger
- 164 arrowheads), typically with a couple being less intensely immunoreactive to TH, and the remaining
165 larger neurons which are spaced further from the rest (Fig. S3a arrows). Dopaminergic projection
166 clearly trace 165 larger neurons which are spaced further from the rest (Fig. S3a – arrows). Dopaminergic projections

166 clearly trace each of the 4 leg neuromere commissures, as well as two anterior commissures (Fig. S3e).

167 The 166 clearly trace each of the 4 leg neuromere commissures, as well as two anterior commissures (Fig. S34
167 The smaller subset appears to give rise to the leg neuromere commissures, as well as supplying some
168 innervati The smaller subset appears to give rise to the leg neuromere commissures, as well as supplying some

innervation within the neuromere. The projections of the more populous cluster are more difficult to

169 follow, but pre
-
-
- innervation within the neuromere. The projections of the more populous cluster are more difficult to
169 follow, but presumably contribute to the neuromere pattern. Each leg neuromere is evenly filled by a
170 mesh network 169 follow, but presumably contribute to the neuromere pattern. Each leg neuromere is evenly filled by a
170 mesh network of dopaminergic varicosities (Fig. S3b).
171 Medio-ventral to the pedipalp neuromere are a cluster o 170 mesh network of dopaminergic varicosities (Fig. S3b).
171 Medio-ventral to the pedipalp neuromere are a cluster of 2-3 TH+ neurons per hemiganglia in the
172 anterior field of somata (Fig. S3c, d - arrows). The project 171 Medio-ventral to the pedipalp neuromere are a cluste
172 anterior field of somata (Fig. S3c, d - arrows). The proj
173 the pedipalp and cheliceral commissures, suggesting t
175 the midline. Posterior to these neurons i 171 Medio-ventral to the pedipalp neuromere are a cluster of 2-3 TH+ neurons per hemiganglia in the
172 anterior field of somata (Fig. S3c, d - arrows). The projections of these neurons can be traced through
173 the pedipa
- 173 the pedipalp and cheliceral commissures, suggesting that they are supplying both of the respective
174 neuropils. The posterior of these commissures (pedipalp) is subdivided into two tracts which mingle at
175 the midl
-
- neuropils. The posterior of these commissures (pedipalp) is subdivided into two tracts which mingle

175 the midline. Posterior to these neurons is an area of denser immunoreactivity continuous with the

176 strongly label
- 175 the midline. Posterior to these neurons is an area of denser immunoreactivity continuous with the
176 strongly labelled anterior-most arching commissure of the dorsal-tract (as referred to by Auletta et al
177 (35), fo 176 strongly labelled anterior-most arching commissure of the dorsal-tract (as referred to by Auletta et
177 (35), for the same antibody).
179 Serotonin:
180 To visualize serotonergic populations we used an antibody raised
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- 177 (35), for the same antibody).
178
179 Serotonin:
180 To visualize serotonergic populations we used an antibody raised directly against serotonin. The
181 patterning of serotonergic innervation has been studied througho 177 (35), for the same antibody).
178
179 Serotonin:
180 To visualize serotonergic pop
181 patterning of serotonergic in
182 although only a literal descrip ---
179
180
181
183
184 179 Serotomin:
180 To visualize
181 patterning
182 although o
184 serotonin-
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-
- 180 To visualize serotonergic populations we used an antibody raised directly against serotonin. The

181 patterning of serotonergic innervation has been studied throughout the synganglion in C. salei –

182 although only patterning of serotonergic innervation has been studied throughout the synganghomme. Saler

182 although only a literal description has been accessible to us (22) – and briefly shown for the arcua

183 body in the wolf spi although only a literal description has been accessible to us (22) – and briefly shown for the arcuate
body in the wolf spider, *Pardosa* (36). Matching what has been reported for *C. salei* (22), a cluster of
184 seroton
-
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- 183 body in the worl spider, Parlabsa (36). Mateming what has been reported for C. saler (22), a cluster of ~5
184 serotonin-positive cells are evident adjacent to each leg neuromere (Fig. S4a).
185 Most notably in the neu 185 Most notably in the neuromeres of Legs I (anterior), the serotonergic innervation in the limb
186 neuroarchitecture appears to be supplied in two roughly equal halves, filling the periphery and leaving
187 an area dark
-
-
-
-
- 188 to be supplied from the medial branch of the "dorsal-most tract" (as referenced by Auletta et al. (35)).
189 Several 5-HT+ neurons are seen ventral to the pedipalp neuropil (Fig. S4c arrows), which has
190 serotonerg 187 an area dark of immunoreactivity within (Fig. S4b – brace). The anterior half of the innervation appears
188 to be supplied from the medial branch of the "dorsal-most tract" (as referenced by Auletta et al. (35)).
189 188 to be supplied from the medial branch of the "dorsal-most tract" (as referenced by Auletta et al. (35)).
189 Several 5-HT+ neurons are seen ventral to the pedipalp neuropil (Fig. S4c – arrows), which has
190 serotonerg 189 Several 5-HT+ neurons are seen ventral to the pedipalp neuropil (Fig. S4c – arrows), which has
190 serotonergic immunoreactivity on the medial portions flanking the midline. Ventral to the opisthosoma
191 neuromere are 190 Serotonergic immunoreactivity on the medial portions flanking the midline. Ventral to the opis
191 neuromere are clusters of serotonergic somata which project into a robust tract travelling med
192 S4d – arrows).
193 O 191 neuromere are clusters of serotonergic somata which project into a robust tract travelling medially (Fig.
192 S4d – arrows).
193 Octopamine/Tyramine: 192 S4d – arrows).
193
194 *Octopamine/Tyramine*:
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- .
193
194 Octopamine/T₎
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|
| 194 Octopamine/Tyramine:

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- 196 found to be effective. Tyrosine decarboxylase 2 (TDC2) is an enzyme the catalyzes the conversion of
197 tyrosine to tyramine, which is subsequently necessary for octopamine metabolism, meaning that TDC2
198 is present
-
-
-
-
- 197 tyrosine to tyramine, which is subsequently necessary for octopamine metabolism, meaning that TD
198 is present in both neuronal subtypes in invertebrates. We found a *Drosophila melanogaster* antibody
199 TDC2 to be e 198 is present in both neuronal subtypes in invertebrates. We found a *Drosophila melanogaster* antibody to
199 TDC2 to be effective in *U. diversus.*
200 Given that TDC2-immunoreacitivty should include both octopaminergic 198 is present in both neuronal subtypes in invertebrates. We found a *Drosophila inclunegaster* antibody to
199 TDC2 to be effective in *U. diversus.*
200 Given that TDC2-immunoreacitivty should include both octopaminergi 200 Given that TDC2-immunoreacitivty should include both octopaminergic and tyraminergic neurons, we

201 might expect that potentially more positive somata would be seen in *U. diversus* than in *C. salei*, where

202 oct
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- 199 TDC2 to be effective in D. alversus.

200 Given that TDC2-immunoreacitivty

201 might expect that potentially more

202 octopamine was stained for directly

203 Instead in the subesophageal gangl

204 S5a), which is fe
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- 201 might expect that potentially more positive somata would be seen in D. *thersus* than in C. salei, where
202 octopamine was stained for directly (24), assuming the relative population sizes in the species are equal
203 202 octopamine was stained for directly (24), assuming the relative population sizes in the species are equal.
203 Instead in the subesophageal ganglion we find a cluster of 5-6 TDC2+ somata per leg neuromere (Fig.
204 S5a 204 S5a), which is fewer than for C. salei (24). Unlike the uniform mesh-like innervation of each leg

205 neuromere produced by dopaminergic neurons, or the more or less symmetrical pattern for serotonin

206 the pattern 204 S5a), which is fewer than for C. salei (24). Unlike the uniform mesh-like infervation of each reg-
205 neuromere produced by dopaminergic neurons, or the more or less symmetrical pattern for set
206 the pattern in TDC2 206 the pattern in TDC2 staining is notably different. The anterior side of each neuromere contains a patch
207 of continuous, diffuse, and more lightly stained immunoreactivity, while on each posterior side there is
208 s 207 of continuous, diffuse, and more lightly stained immunoreactivity, while on each posterior side there is
208 swath of brightly reactive, sparse puncta (Fig S5b).
209 All subesophageal ganglion tracts and commissures wh
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- 208 swath of brightly reactive, sparse puncta (Fig S5b).
209 All subesophageal ganglion tracts and commissures which were revealed by fine dopaminergic
210 projections are likewise labelled with TDC2-immunoreactivity. We a 209 All subesophageal ganglion tracts and commissures
210 projections are likewise labelled with TDC2-immun
211 opisthosomal neuromere (Fig. S5c – arrows), but di
212 this vicinity in C. salei (24). There is substantial TD 210 projections are likewise labelled with TDC2-immunoreactivity. We also observed somata ventra
211 opisthosomal neuromere (Fig. S5c – arrows), but did not see any such gargantuan cell bodies as
212 this vicinity in C. sa 211 opisthosomal neuromere (Fig. S5c – arrows), but did not see any such gargantuan cell bodies as seen in
212 this vicinity in C. salei (24). There is substantial TDC2-immunoreactivity in the pedipalpal (Fig. S5c) and
213
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- 212 this vicinity in C. *salei* (24). There is substantial TDC2-immunoreactivity in the pedipalpal (Fig. S5c) and
213 cheliceral neuromeres (Fig. S5d).
214 TDC2-immunoreactivity displays an intricate pattern within the opi 212 This vieling in C. saler (24). There is substantial TDC2 immunoreactivity in the pedipalpal (Fig. 35c) and
213 TDC2-immunoreactivity displays an intricate pattern within the opisthosomal neuromere. At the ventra
215 an 214 TDC2-immunoreactivity displays a
215 anterior end two triangular forma
216 varicosities on each lateral side, v
217 the opisthosomal neuromere (Fig
218 triangles are found with their ape
219 the interior of the opistho varicosities on each lateral side, which then becomes heavier and continues to outline the bour
217 the opisthosomal neuromere (Fig. S5d – arrow). An approximately mirrored pair of immunorear
218 triangles are found with t
-
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- 215 anterior end two triangular formations of puncta (Fig. S5d brace) abut the input of a string of
216 varicosities on each lateral side, which then becomes heavier and continues to outline the boundary of
217 the opist 217 the opisthosomal neuromere (Fig. 55d – arrow). An approximately mirrored pair of immunoreactive
218 triangles are found with their apex pointing posteriorly, at the posterior end of this neuromere. Within
219 the inter 217 the opisthosomal neuromere (Fig. S5d – arrow). An approximately mirrored pair of immunoreactive

218 triangles are found with their apex pointing posteriorly, at the posterior end of this neuromere. Within

219 the int 219 the interior of the opisthosomal neuromere, fibers resembling spokes emanate to a ring-like midline
220 where there is a small chiasm, and a thicker bridge structure joining lateral segments which travel in the
221 ant where there is a small chiasm, and a thicker bridge structure joining lateral segments which travel in
221 anterior-posterior direction.
222 AstA:
224 The earlier work in *C. salei* (28) did not comment on AstA-immunoreact
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- 224 The earlier work in *C. salei* (28) did not comment on AstA-immunoreactivity outside of the dorsal
225 supraesophageal ganglion, but an image from the jumping spider, *Marpissa muscosa*, confirms that 222
223 AstA:
224 The earlier work in *C. salei* (2
225 supraesophageal ganglion, bi
226 AstA-immunoreactive expres 223
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227 224 The ea

225 suprair

225 AstA-i

227 the su

228 -4 lar

229 smalle 224 The earlier work in C. saler (28) did not comment on AstA-immanoreactivity outside of the dorsar
225 supraesophageal ganglion, but an image from the jumping spider, *Marpissa muscosa*, confirms the
226 AstA-immunoreact 225 supraesophageal ganghori, but an image from the jumping spider, Marphssa muscosa, commins that
226 AstA-immunoreactive expression is present throughout the synganglion (8). In the far ventral portio
227 - 4 large AstA+ 227 Asta-immunoreactive expression is present throughout the synganghon (8). In the far ventral portion of
227 the subesophageal ganglion where there is a complete covering of somata, there are paired clusters of 3
228 -4 228 -4 large AstA+ somata located on the posterior side (Fig. S6a – arrow). In a similar plane, there are two
229 smaller somata located along the midline (Fig. S6a). AstA-immunoreactivity has a distinctive pattern
230 wit 229 Smaller somata located along the midline (Fig. S6a). AstA-immunoreactivity has a distinctive pattern
230 within the leg neuromeres, showing robust varicosities but only the posterior portion of neuromere (Fig
231 S6b,c within the leg neuromeres, showing robust varicosities but only the posterior portion of neuromere
231 S6b,c). This innervation appears to be supplied from the lateral branches of the centro-lateral tract.
232 Similar to w 231 S6b,c). This innervation appears to be supplied from the lateral branches of the centro-lateral tract.
232
233 Similar to what has been described for *M. muscosa* as the stomodeal bridge, the area adjacent to the
234 e 232
232 Similar to what has been described for *M. muscosa* as the stomodeal bridge, the area adjacent to the supplied from the anterior side of the subesophageal ganglion is prominently immunoreactive to allatostatin (Fig
-
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233
234
235 233 Similar to what has been described for *M. masebsa as* the stomodeal bridge, the area adjacent to the esophagus on the anterior side of the subesophageal ganglion is prominently immunoreactive to allatostatin (Fig. S6
- 235 allatostatin (Fig. S6d brace), although the actual bridge which crosses the midline is more mode
allatostatin (Fig. S6d brace), although the actual bridge which crosses the midline is more mode 235 allatostatin (Fig. S6d – brace), although the actual bridge which crosses the midline is more modest than

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in other stains, having only a few neurites, and thin representation in the posterior commissure (Fig. S6e

237 – arrow). Faint somata are also seen closely anterior to this region.

238 Proctolin:

240 Proctolin expressio 238
238 – Proctolin:
240 – Proctolin expression patterns were previously explored in C. salei b
241 – well as in a focused manner in the protocerebrum, as a means to re
242 – al., 2011). Beginning in the subesophageal gang 239
240
241
242
243
243 239 Proctolin:
240 Proctolin e
242 al., 2011).
243 clusters of
244 other wea
245 associated 240 Proctom expression patterns were previously explored in e. stilen both throughout the CNS (26, 32), as
241 well as in a focused manner in the protocerebrum, as a means to reveal arcuate body layering (Loesel e
242 al., al., 2011). Beginning in the subesophageal ganglion, proctolin-immunoreactive somata were found in
243 clusters of multiple somata along each neuromere ((37) citing Duncker et al., 1992), as well as many
244 other weakly l 243 clusters of multiple somata along each neuromere ((37) citing Duncker et al., 1992), as well as many
244 other weakly labelled Proc+ cells (32). Curiously, in *U. diversus* we see a single bright Proc+ soma
245 associa 243 clusters of multiple somata along each neuromere ((37) clung Duncker et al., 1992), as well as many
244 other weakly labelled Proc+ cells (32). Curiously, in *U. diversus* we see a single bright Proc+ soma
245 associat 244 other weakly labelled Procet cells (32). Curiously, in 0. *diversus we see a single bright Procet somal* associated with each of the 8 leg neuromeres in the subesophageal ganglion (Fig. S7a). These net are found approx 246 are found approximately at the same area as clusters for other populations, such as the aforementione
247 monoamines. They are generally posterior and medial to the bulk of the respective leg neuromere.
248 Smaller and are found approximately at the same area as clusters for other populations, such as the aforementioned

247 monoamines. They are generally posterior and medial to the bulk of the respective leg neuromere.

248 Smaller and 248 Smaller and faintly immunoreactive Proc- neurons are also seen in the vicinity and it is possible that
249 sensitivity to weakly-labelled somata is lesser than in stained slices.
250 Medial to the emerging pedipalp neu 249 Smaller and faint and faint and faint of the vicinity and the vicinity of the constant in the vicinity to weakly-labelled somata is lesser than in stained slices.
250 Medial to the emerging pedipalp neuropils are 2-3 P

250
251 Medial to the emerging pedipalp neuropils are 2-3 Proctolin+ somat
252 strongly staining anterior zone, also highlighted by serotonergic inne
253 in this plane, densely labeled somata are present in the field ventr

251
252
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257

252 strongly staining anterior zone, also highlighted by serotonergic innervation (Fig. $S7b$ – arrow). Like
253 in this plane, densely labeled somata are present in the field ventral to the opisthosomal neurome
254 $S7b$ 253 in this plane, densely labeled somata are present in the field ventral to the opisthosomal neuromere (Fig. 37b – arrowhead). A circular form of saturated proctolin-immunoreactivity is seen at the posterior end can oval 254 S7b – arrowhead). A circular form of saturated proctolin-immunoreactivity is seen at the posterior end of
255 an oval shaped synapsin-density (Fig. S7c – arrow), suggesting that it is a subset of a major tract bundle.
 254 S7b – arrowhead). A circular form of saturated proctolin-immunoreactivity is seen at the posterior end o
255 an oval shaped synapsin-density (Fig. S7c – arrow), suggesting that it is a subset of a major tract bundle.
2 256 In dorsal planes this immonreactivity morphs into lateral moving strands of varicosities becoming
257 difficult to trace. Such an appearance is not found in the other neurosignaling molecule stains, even
258 those with 257 difficult to trace. Such an appearance is not found in the other neurosignaling molecule stains, even
258 those with profound subesophageal expression.
259
260 The fine neurites projecting to the center of the opisthos

258 those with profound subesophageal expression.
259
260 The fine neurites projecting to the center of the opisthosomal neuropil as seen for TDC2 are also
261 apparent for proctolin-immunoreactivity (Fig. S7d).
262 CCAP:

259
260 The fine neurites projecting to the center of the
261 apparent for proctolin-immunoreactivity (Fig. S7
262
263 CCAP:
264 Despite its name alluding to function in the bear ---
260
261
262
263
264

261 The fine neuropide immunoreactivity (Fig. S7d).
262 The fine neuropil immunoreactivity (Fig. S7d).
263 CCAP:
264 Despite its name alluding to function in the heart, CCAP has considerable immunoreactivity throut
265 The 262
262
263 *CCAP*:
264 Despite its name alluding to function in the heart, C
265 the sub- and supraesophageal ganglia in *U. diversus*
266 presented CCAP expression patterns only for the bra
267 immunoreactive neurons is 263
264
265
266
267
268 263 CCAP:
264 Despit
265 the sul
266 presen
268 associa
269 presen

- 265 the sub- and supraesophageal ganglia in *U. diversus*. The one prior investigation of CCAP in *C. salei*
266 presented CCAP expression patterns only for the brain (28). In our volumes, a cluster of ~5 intensely
267 imm 263 the sub- and supraesophagear ganglia in 0. diversus. The one prior investigation of CCAP in C. such
266 presented CCAP expression patterns only for the brain (28). In our volumes, a cluster of ~5 intensel
267 immunorea 266 presented CCAP expression patterns only for the brain (28). In our volumes, a cluster of ~5 intensely immunoreactive neurons is seen around the Leg IV neuromere (Fig. S8a), and positive somata are als associated with t 268 associated with the opisthosomal neuromere (Fig. S8c – arrow). CCAP-immunoreactive neurons are
269 present in a more dispersed fashion within the ventral subesophageal ganglion (Fig. S8b).
270 Immunoreactivity within t examples as a specifical matrix of the ventral subesophageal ganglion (Fig. S8b).

269 by present in a more dispersed fashion within the ventral subesophageal ganglion (Fig. S8b).

271 are evenly distributed (Fig. S8b).

2 more dispersent in a more dispersent in a more dispersed in a more dispersed are evenly distributed (Fig. S8b).
271 are evenly distributed (Fig. S8b).
272 *FMRFamide*:
274 At the ventral end of the synganglion, FMRFamide+
-
-
-

271 are evenly distributed (Fig. S8b).
272
273 FMRFamide:
274 At the ventral end of the synganglion, FMRFamide+ neurons are numerous and dispersed throughout the
275 width of the ventral field of somata (Fig. S9a). Unlike 272
273 FMRFamide:
274 At the ventral end of the syngang
275 width of the ventral field of some
276 immunoreactive somata cannot l
277 neuromeres. A concentration of l ---
273
274
275
277
277 273 FMM dimetered:
274 At the ventra
275 width of the ventra
276 immunoreact
278 opisthosomal 275 width of the ventral field of somata (Fig. S9a). Unlike other neuropeptides and monoamines, these
276 immunoreactive somata cannot be readily attributed to clusters corresponding to individual
277 neuromeres. A concen

277 neuromeres. A concentration of FMRFamide neurons are present in the somata field ventral
278 opisthosomal neuromere (Fig. S9b – arrows), which was also found for *C. salei* (*30*). Ample FI
78 opisthosomal neuromere (F

276 immunoreactive somata cannot be readily attributed to clusters corresponding to individual
277 neuromeres. A concentration of FMRFamide neurons are present in the somata field ventral to the
278 opisthosomal neuromere 278 opisthosomal neuromere (Fig. S9b – arrows), which was also found for *C. salei* (30). Ample FMRFam
278 opisthosomal neuromere (Fig. S9b – arrows), which was also found for *C. salei* (30). Ample FMRFam 278 opisthosomal neuromere (Fig. S9b – arrows), which was also found for C. salei (30). Ample FMRFamide

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- 279 Signal is the cheliceral neuromeres in the area of the stomodeal bridge (Fig. S9d arrows).
281
282 Supraesophageal ganglion features:
283 Within the supraesophageal ganglion reside a number of dense neuropil regions
- 281
282 **Supraesophageal ganglion features:**
283 Within the supraesophageal ganglion reside a number of dense neuropil regions which are
284 from their surroundings. These include major recognizable structures such as the 282
283
284
285
286
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- 282 Supraesophageal ganglion reatures:
283 Within the supraesophageal ganglion
284 from their surroundings. These include
285 2m-o) and arcuate body (Fig. 2q-t), as
286 the present image volumes. 284 from their surroundings. These include major recognizable structures such as the mushroom bodies (Fi
285 2m-o) and arcuate body (Fig. 2q-t), as well as some previously undescribed structures, made evident by
286 the pr 285 a 2m-o) and arcuate body (Fig. 2q-t), as well as some previously undescribed structures, made evident by
286 the present image volumes.
287 The protocerebral tract can be followed further dorsally (Fig. 2j-l). The prot 286 the present image volumes.

287 The protocerebral tract can be followed further dorsally (Fig. 2j-I). The protocerebral tract dissipates,

288 and the protocerebral commissure (PCC) appears centrally (Fig. 2l). In this 287 The protocerebral tract can I
288 and the protocerebral comm
289 structures are the hafts, the
290 anterior to the PCC, paired a
291 as the 'hagstone' neuropil, g
292 the bulk of the mushroom b
-
-
- 288 and the protocerebral commissure (PCC) appears centrally (Fig. 2l). In this plane, the brightest lateral
289 structures are the hafts, the ventral-most reaches of the mushroom bodies. The neuropil directly
290 anterior 289 structures are the hafts, the ventral-most reaches of the mushroom bodies. The neuropil directly
290 anterior to the PCC, paired and adjacent to the midline, forms a distinct landmark, which we refer to i
291 as the 'h
-
-
-
- anterior to the PCC, paired and adjacent to the midline, forms a distinct landmark, which we refer

as the 'hagstone' neuropil, given its pendular and pierced form (Fig. 2m). Continuing dorsally (Fig.

292 the bulk of the
- 291 as the 'hagstone' neuropil, given its pendular and pierced form (Fig. 2m). Continuing dorsally (Fig. 2n),

292 the bulk of the mushroom bodies is present, the hagstone neuropil persists, and a faintly arching,

293 um 292 as the bulk of the mushroom bodies is present, the hagstone neuropil persists, and a faintly arching, umbrella-like density is visible at the posterior side of the supraesophageal ganglion. The mushroom body bridge and 292 the bulk of the mushroom bodies is present, the hagstone neuropil persists, and a faintly arching,
293 umbrella-like density is visible at the posterior side of the supraesophageal ganglion. The mushroom
294 body bridg 294 body bridge and head is found dorsally (Fig. 20), and centrally, an ovoid neuropil coalesces (Fig. 2p-r),
295 which has not been apparent in previous anatomical investigations (tonsillar neuropil, Fig. 2q). The
296 arc 295 which has not been apparent in previous anatomical investigations (tonsillar neuropil, Fig. 2q). The
296 arcuate body lobes are present on the posterior side of the dorsal supraesophageal ganglion (Fig. 2 q-
297 while
- 296 arcuate body lobes are present on the posterior side of the dorsal supraesophageal ganglion (Fig. 2
297 while anterio-laterally a previously uncharacterized banded neuropil structure is visible (protocerek
299 Mushroom
-

- while anterio-laterally a previously uncharacterized banded neuropil structure is visible (protocerebral
298 bridge (PCB) neuropil, Fig. 2S-t).
299 **Mushroom bodies**
301 The mushroom bodies (MBs) (or corpora pendunculata) 298 bridge (PCB) neuropil, Fig. 2S-t).
299
300 **Mushroom bodies**
301 The mushroom bodies (MBs) (or corpora pendunculata) are a paired neuropil structure whose size,
302 shape and mere presence are substantially variable ac
-
- 299
200 **Mushroom bodies**
201 The mushroom bodies (MBs) (or
202 shape and mere presence are su
2S4
- 300
301
302
303
304
305 300 Mushroom bodies
301 The mushroom bod
302 shape and mere pre
303 general. Their fund:
305 model species, and
305 model species, and
306 relationship of inse 302 shape and mere presence are substantially variable across not only chelicerates, but arthropods in general. Their fundamental morphological attributes are a stalk and head region reflecting their namesake structure, an 303 general. Their fundamental morphological attributes are a stalk and head region reflecting their
304 namesake structure, and their mirrored distribution in the hemiganglia. Best characterized in insect
305 model specie 304 namesake structure, and their mirrored distribution in the hemiganglia. Best characterized in ins
305 model species, and while sharing in anatomical and molecular characteristics (38), the evolution
306 relationship of
-
- 305 model species, and while sharing in anatomical and molecular characteristics (38), the evolutionary
306 relationship of insect MBs to those of chelicerates and other arthropods, and particularly spiders, ha
307 been a model species, and while sharing in anatomical and molecular entineteensties (30), the evolutionary

relationship of insect MBs to those of chelicerates and other arthropods, and particularly spiders, ha

307 been a contin
-
-
- 307 been a continuing debate (9, 38–40).
308 The mushroom bodies of *U. diversus* (
309 immunoreactivity of all structures in t
310 projection), indicating a great degree
311 are present in *U. diversus* and retain t
313 i 309 Internushroom bodies of *O. diversus* (Fig. 3a,b) tend to show the most robust synpasin-
309 Immunoreactivity of all structures in the supraesophageal ganglion (Fig. 3c, maximum in
310 projection), indicating a great d
- projection), indicating a great degree of synaptic density. While web-building species have been
311 imported to have simplified (9) or even entirely absent mushroom bodies (9, 20, 33), these structures are present in *U.*
- 307 been a continuing debate (9, 38–40).

308 The mushroom bodies of *U. diversus* (Fig. 3a, b) tend to show the most robust synpasin-

309 immunoreactivity of all structures in the supraesophageal ganglion (Fig. 3c, maxi 1311 reported to have simplified (9) or even entirely absent mushroom bodies (9, 20, 33), these struct
312 are present in *U. diversus* and retain the complete form seen in more visually-reliant species (8, 1
313 if they a
- 312 reported to have simplified (9) or even entirely absent mushroom bodies (9, 20, 39), these situaties
312 are present in *U. diversus* and retain the complete form seen in more visually-reliant species (8, 9), ev
313 i
-
-
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-
- 313 are present in 0. alternals and retain the complete form seen in more visually reliant species (0, 9), even
313 if they are smaller relative to the supraesophageal ganglion as a whole (Fig. 3a,b,c).
314 U. diversus MB 313 if they are smaller relative to the supraesophageal ganglion as a whole (Fig. 3a,b,c).
314 *U. diversus* MBs display a haft, body and head region, with the two hemiganglion pairs connected by a
315 bridge (Fig. 3a-c).
-
- 315 bridge (Fig. 3a-c). Synapsin-immunoreactivity is modest within the bridge region, whose true thickness
316 better visualized with staining for βTubulin3 (Fig. 3d). Despite the strong synapsin-immunoreactivity in
317 t 316 better visualized with staining for β Tubulin3 (Fig. 3d). Despite the strong synapsin-immunoreactivity in
317 the MBs, we surprisingly did not see co-expression with most of our specific neurosignaling molecule
318 317 the MBs, we surprisingly did not see co-expression with most of our specific neurosignaling molecule
318 antibodies. This pattern is also reflected in the extant spider literature, with a single study showing
319 immu 318 antibodies. This pattern is also reflected in the extant spider literature, with a single study showing
319 immunoreactivity in the mushroom bodies of *C. salei* for anti-GAD and anti-proctolin staining (26). In 319 immunoreactivity in the mushroom bodies of C. *salei* for anti-GAD and anti-proctolin staining (26). I
319 immunoreactivity in the mushroom bodies of C. *salei* for anti-GAD and anti-proctolin staining (26). immunoreactivity in the mushroom bodies of C. saler for anti-GAD and anti-proctoin staming (26). In our

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- ands, only anti-AllatostatinA staining showed co-immunoreactivity throughout the mushroom body
321 (Fig. 3e). Although difficult to trace the source far, it appears the hafts are innervated from the posterior
322 side (Fi 322 side (Fig. 3f). By βTubulin3-immunoreactivity, we observe two tracts which straddle the MB hafts as they
323 descend from the dorsal somata layer (Fig. 3g). Finer neurites are not distinguishable in the βTub3-
324 imm
-
-
- descend from the dorsal somata layer (Fig. 3g). Finer neurites are not distinguishable in the βTub3-
324 immunoreactivity, but it seems plausible that the AstA+ neurites entering the MB hafts might stem from
325 the media 324 immunoreactivity, but it seems plausible that the AstA+ neurites entering the MB hafts might stem
325 the medial of these two tracts.
326 Babu and Barth (1984) described the protocerebro-dorsal tract as providing inpu 325 the medial of these two tracts.
326 Babu and Barth (1984) described the protocerebro-dorsal tract as providing input to the hafts of the
327 mushroom bodies. The connection of this tract to the MB hafts is not apparen 326 Babu and Barth (1984) describe
327 mushroom bodies. The connect
328 U. diversus, which was likewise
329 and P. tepidariorum (9).
330 The antero-dorsal input to the l 327 mushroom bodies. The connection of this tract to the MB hafts is not apparent by our synapsin stain:
328 U. diversus, which was likewise the case with silver staining for P. amentata, M. muscosa, A. bruennic
329 and P.
- 238 U. diversus, which was likewise the case with silver staining for P. amentata, M. muscosa, A. bruennichi,
329 and P. tepidariorum (9).
330 The antero-dorsal input to the MB heads, representing the secondary eye pathway
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- 329 U. diversus, which was likewise the case with silver staining for F. amentata, M. museosa, A. brachment,
329 and P. tepidariorum (9).
330 The antero-dorsal input to the MB heads, representing the secondary eye pathway 329 and P. tepidariorum (9).
330 The antero-dorsal input
331 conspicuous and has rec
332 are sometimes referred
333 which give this structure 331 Conspicuous and has received considerable treatment within the literature. The mushroom body heads
332 are sometimes referred as the third-order visual neuropil in this pathway, with the ample parallel fibers
333 which
-
-
- are sometimes referred as the third-order visual neuropil in this pathway, with the ample parallel fibers
which give this structure its shape arising from globuli cells which cap the mushroom body head.
The globuli cells a 333 which give this structure its shape arising from globuli cells which cap the mushroom body head.
334 The globuli cells are not distinguishable from the surrounding nuclei by DAPI signal, but can potentially
335 be disc The globuli cells are not distinguishable from the surrounding nuclei by DAPI signal, but can poter
be discerned through specific neurosignaling molecule immunostains. We find the cluster of cells
associated with the MB he
-
- 335 be discerned through specific neurosignaling molecule immunostains. We find the cluster of cells closel
336 associated with the MB heads are revealed by ChAT-immunoreactivity, and to a lesser extent by GAD-
337 immuno 336 associated with the MB heads are revealed by ChAT-immunoreactivity, and to a lesser extent by GAD-
337 immunoreactivity, suggesting they represent cholinergic and GABAergic populations, respectively (see
338 Acetylchol
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- 338 Acetylcholine and GABA subsections, below). Globuli cells in C. salei have previously been shown to be

ChAT+ (30). By βTubulin3 staining, we also observed a trident of tracts feeding into the dorsal aspect of

341 Vis 2338 Acetylcholine and GABA subsections, below). Globuli cells in C. suler have previously been shown to be
339 ChAT+ (30). By βTubulin3 staining, we also observed a trident of tracts feeding into the dorsal aspect of
340
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-

$\frac{3}{5}$ s associated by ChAT-immunoreactivity, and to a lesser extent by GAD-immunoreactivity, and to a lesser extent by GAD-immunoreactivity, and to a lesser extent by GAD-immunoreactivity, and to a lesser extent by GAD

- 3339 ChAT+ (30). By βTubulin3 staining, we also observed a trident of tracts feeding into the dorsal aspect of
340 the mushroom body head (Fig. 3g).
343 U. diversus, like many orb-weavers, builds its web in the night and 341
342 **Visual System**
343 *U. diversus,* like many orb-weavers,
344 darkness in laboratory conditions, s
345 behavioral repertoire (*41*). Web-bui
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- 342
343
344
345
346 342 Visual System
343 *U. diversus,* like
345 behavioral repo
345 which depend
347 pathways (9, 2 343 U. diversus, like many orb-weavers, balled its web in the night and can do so in essentially complete
344 darkness in laboratory conditions, suggesting that vision is expendable to much of the spider's
345 behavioral r
-
- 345 behavioral repertoire (41). Web-building spiders are considered to have poorer vision than spiders
346 which depend on sight to capture prey, which is reflected in their diminished optic neuropils and tra
347 pathwa
- darkness in laboratory conditions, suggesting that vision is expendable to much of the spider's

345 behavioral repertoire (41). Web-building spiders are considered to have poorer vision than spiders

346 which depend on
- 349 containing the first and second-order optic neuropils are considerably thinner and not as extensively
350 fused with the continuous neuropil of the supraesophageal ganglion, and are prone to separating during
351 disse
-
- Barry Bathways (9, 21).

348 Relative to cursor

349 containing the firs

350 fused with the contains dissection. Consection of the ant

352 enough in the ant

353 nevertheless thes SHOT KICRUM SPECIS (8, 9, 19), in D. diversus the difference extensions of the protocerebrum

containing the first and second-order optic neuropils are considerably thinner and not as extensive

star fused with the continu 350 fused with the continuous neuropil of the supraesophageal ganglion, and are prone to separating dural dissection. Consequently, neither the primary or secondary visual pathway neuropils appear reliably
351 dissection. dissection. Consequently, neither the primary or secondary visual pathway neuropils appear reliably

enough in the anti-synapsin volumes to be apparent in the averaged standard brain representation, but

nevertheless these enough in the anti-synapsin volumes to be apparent in the averaged standard brain representation, in the averaged standard brain representation, is
353 nevertheless these structures are exhibited in various individual prep
-
- 353 nevertheless these structures are exhibited in various individual preparations. The optic neuropils in *U.*
354 *diversus* tended to show weaker synapins-immuoreactivity, but were clearly seen with antisera to HRP
355 353 Intertateless these structures are exhibited in various individual preparations. The optic neuropils in U.
354 *diversus* tended to show weaker synapins-immuoreactivity, but were clearly seen with antisera to HRP
355 (
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-
- 3534 diversus tended to show weaker synaphis-immuoreactivity, but were elearly seen with antisera to HRP
355 (Fig. 4a).
356 As in other species, the secondary pathway is larger (Fig. 4b), lifting away anteriodorsally to th 356 As in other
357 the MB h
358 projectio
359 bulbous s 357 the MB heads. This continuity can be inferred from the sliced three-dimensional maximum intensity
358 projection of synapsin (Fig. 4b). The primary pathway is diminutive in *U. diversus*, and emerges as a
359 bulbous
- 358 projection of synapsin (Fig. 4b). The primary pathway is diminutive in *U. diversus*, and emerges as a
359 bulbous shape at the dorsal-most end of the brain through a field of somata (Fig. 4a).
- Brojection of synapsin (Fig. 4b). The primary pathway is diminutive in D. diversus, and emerges as a
bulbous shape at the dorsal-most end of the brain through a field of somata (Fig. 4a). 359 bulbous shape at the dorsal-most end of the brain through a field of somata (Fig. 4a).

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- 360 Previous reports have used GABA (26), histamine (25), dopamine (35), CCAP (26), and FMRFamide (26) to reveal the successive neuropils of the visual pathways. As noted above, the only features within the optic pathway f
- 362 optic pathway for which we observed neurosignaling molecule immunoreactivity were the globuli cells
363 with GAD and ChAT staining. It is possible that targets for which we could not acquire an effective
364 antisera,
- with GAD and ChAT staining. It is possible that targets for which we could not acquire an effective

364 antisera, such as histamine, could be revelatory of the optic lamellae and other visual pathway

365 structures, as t
- 364 antisera, such as histamine, could be revelatory of the optic lamellae and other visual pathway
365 structures, as they have been for *C. salei* (14, 23). Specific compartments of the pathways, such as
366 medulla or l
- 365 structures, as they have been for *C. salei* (14, 23). Specific compartments of the pathways, such
366 medulla or lamellae could not be discerned with any preparation.
367 **Arcuate body**:
369 The arcuate body is a prom
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- 363 structures, as they have been for C. salei (14, 23). Specific compartments of the pathways, such as the
366 medulla or lamellae could not be discerned with any preparation.
367 The arcuate body is a prominent neuropil
- 367
368 **Arcuate body**:
368 **Arcuate body**:
369 The arcuate body is a prominent neuropil structure found in all spi
370 system anatomy has been examined closely to date. Residing in the
371 esophageal ganglion, this solita
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368
369
370
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372 369 The arcuate body:
369 The arcuate bo
370 system anatom
371 esophageal gar
372 broad divisions
373 Additional laye 369 The arcuate body is a prominent neuropil structure found in all spider species whose central nervous
370 system anatomy has been examined closely to date. Residing in the dorso-posterior aspect of the supra-
371 esopha
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	-
- 372 broad divisions, the ventral and dorsal lobes $(8, 28, 33)$.

373

374 Additional layers have been noted by synapsin staining in the dorsal arcuate body (8) . The precise

375 number of layers varies within the liter 372 broad divisions, the ventral and dorsal lobes (8, 28, 33).
373 Additional layers have been noted by synapsin staining in
375 number of layers varies within the literature, and it is un
376 between the gross lobes of th
- 377 the fact that slices are not always made in a consistent orientation. In absence of a whole-mounted
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- 374
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375
378
379
380 Example of layers have been noted by synapsin standing in the dorsal arcuate body (8). The precise
above not always varies within the literature, and it is unclear to what extent authors distinguish
between the gross lobes 376 between the gross lobes of the AB, and sublayers which may be found within. This is also complid
377 the fact that slices are not always made in a consistent orientation. In absence of a whole-mount
378 example or a se
-
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- 377 the fact that slices are not always made in a consistent orientation. In absence of a whole-mounted
378 example or a seamless stack of slices, an oblique slice may over- or underestimate the size of a layer,
379 depend example or a seamless stack of slices, an oblique slice may over- or underestimate the size of a layer
379 depending on the angle taken. Additionally, the degree of layering may also reflect a true difference
380 between s 379 depending on the angle taken. Additionally, the degree of layering may also reflect a true difference
380 between species, independent of methodology.
381 In *U. diversus*, at the grossest level, we likewise observe tw 380 between species, independent of methodology.
381 In *U. diversus*, at the grossest level, we likewise observe two lobes of the arcuate body, which we wild
383 In *U. diversus*, at the grossest level, we likewise observ 381
382 between species, in U. diversus, at the grossest level, we likewise
383 between species and the ventral (ABV) and dorsal (ABd) (Fig.
385 between the ventral direction and lingering posteriorly or
386 arcuate body p 382
383
384
385
386
387 383 In U. diversus, at the grossest level, we likewise observe two lobes of the arcuate body, which we will
383 In the control axis, the ventral arcuate body somewhat envelopes the dorsal arcuate lobe, hence appearing firs
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- 384 axis, the ventral arcuate body somewhat envelopes the dorsal arcuate lobe, hence appearing first from
385 the ventral direction and lingering posteriorly on the dorsal side, with only a smaller part of the dorsal
386 a 385 the ventral direction and lingering posteriorly on the dorsal side, with only a smaller part of the dorsal
386 arcuate body protruding independently beyond the ABv at the dorsal end (Fig. 5b).
387 Each lobe (ABv and AB 386 arcuate body protruding independently beyond the ABv at the dorsal end (Fig. 5b).
387 Each lobe (ABv and ABd) can be further subdivided into two sub-lobes or layers – a posterior (posABv
388 and posABd) and anterior (a Each lobe (ABv and ABd) can be further subdivided into two sub-lobes or layers – a
388 and posABd) and anterior (antABv and antABd) section, making a total of four units
389 sublayers of the arcuate body lobes are distingu
- 388 and posABd) and anterior (antABv and antABd) section, making a total of four units (Fig. 5c). The
389 sublayers of the arcuate body lobes are distinguishable by immunostaining for specific neuronal
390 subpopulations w
- sublayers of the arcuate body lobes are distinguishable by immunostaining for specific neuronal
390 subpopulations which differentially innervate the layers (Fig. 5d, Fig. 6). By examining these expre
391 patterns, another
- subpopulations which differentially innervate the layers (Fig. 5d, Fig. 6). By examining these expr

patterns, another tier of complexity can be appreciated, as each of these sublayers (posABv, ant

posABd, antABd) can be
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- 2391 patterns, another tier of complexity can be appreciated, as each of these sublayers (posABv, antAbv, posABd, antABd) can be further subdivided into 2 or even 3 aspects, depending on the antisera used.
393 There is a d posABd, antABd) can be further subdivided into 2 or even 3 aspects, depending on the antisera used.
393
394 There is a diversity of layering patterns (Fig. 6), but some basic motifs emerge. Innervation can be
395 partial, 393
393 There is a diversity of layering patterns (Fig. 6), but some basic motifs emerge. Innervation can be
395 partial, as in filling a single sublayer (anterior or posterior) of a lobe, or complete throughout the lobe
3 394
395
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396
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399 395 partial, as in filling a single sublayer (anterior or posterior) of a lobe, or complete throughout the k
396 taking on a saturated appearance, a meshwork of neurites, or a sparse field of puncta. The space
397 betwee
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- 397 between marked sublayers may at times have finer neurite connections which have been describing palisade-like (28). Most commonly at the dorsal end of the ventral arcuate body (ABv), heavy garlike varicosities may for
- 396 taking on a saturated appearance, a meshwork of neurites, or a sparse field of puncta. The space
397 between marked sublayers may at times have finer neurite connections which have been described as
398 palisade-like basis palisade-like (28). Most commonly at the dorsal end of the ventral arcuate body (ABv), heavy garland-
399 like varicosities may form, in certain examples (α -Proctolin, α -TDC2, α -FMRFamide, Fig. 6) appearing
-
- 398 palisade-like (28). Most commonly at the dorsal end of the ventral arcuate body (ABv), heavy garland-
399 like varicosities may form, in certain examples (α -Proctolin, α -TDC2, α -FMRFamide, Fig. 6) appearing a 399 disjointed units, suggestive of an undergirding column. More prevalently in the dorsal arcuate, a robust
401 disjointed units, suggestive of an undergirding column. More prevalently in the dorsal arcuate, a robust
401 1000 disjointed units, suggestive of an undergiviling column more prevalently, in the dorsal arcuate, a robust
1000 distribution of thicker immunoreactive fibers weave between roughly trapezoidal signal-negative areas
1000 40% networking of thicker immunoreactive fibers weaver between roughly trapezoidal signal-negative areas α

- 402 (α-5-HT, α-TDC2, Fig. 6), resembling a flagstone pathway. Detailed descriptions of arcuate body layer
403 projection patterns (Fig. 6) and comparisons to other spider species are found below in the respective
404 sub
- 404 subsections for each neurosignaling molecule.
405 The innervation pattern of a given neuronal subpopulation in a layer of the arcuate body is not a gener
406 delineation of the structure of that layer, as different tra 405 The innervation pattern of a given neuronal su
406 delineation of the structure of that layer, as dif
407 expression patterns within the same layer. An
408 immunoreactivity shows a prominent columna
409 of fine puncta
-
-
- delineation of the structure of that layer, as different transmitter populations can display distinct

407 expression patterns within the same layer. An example is the dorsal arcuate body (ABd), where TDC2-

408 immunoreac
-
-
- Expression patterns within the same layer. An example is the dorsal arcuate body (ABd), where TI
408 immunoreactivity shows a prominent columnar, flagstone innervation, while proctolin has a spars
409 of fine puncta in th immunoreactivity shows a prominent columnar, flagstone innervation, while proctolin has a sparse fie
of fine puncta in the same layer (Fig. 5f).
410 Posterior to the arcuate body is crest of somata which has been previousl of fine puncta in the same layer (Fig. 5f).
409 of fine puncta in the same layer (Fig. 5f).
410 Posterior to the arcuate body is crest of somata which has been previously referred to as the posterior
411 cell layer (PCL) Frame puncta in the same layer (Fig. 34).
410 Posterior to the arcuate body is crest of s
411 cell layer (PCL) (30). Neurons of the PCL s
413 successively run medially as one progress
414 being thicker than others. Hill no example arcuate to the PCL send their projections anteriorly through the ventral arcuate

data body is crest of the PCL send their projections anteriorly through the ventral arcuate

data body is as revealed by immunostain
- 411 cerrify (PCL) (30). Neurons of the PCL send their projections anteriorly through the ventral arcuate
412 layers, as revealed by immunostaining for βTubulin3 in conjunction with synapsin (Fig. 5e). The fibers
413 succe
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- All a successively run medially as one progresses further dorsally in the arcuate lobes, with certain tracts

being thicker than others. Hill noted the presence of tracts running through the arcuate body to join t

PCDt i being thicker than others. Hill noted the presence of tracts running through the arcuate body to join

415 PCDt in jumping spider, *P. johnsoni* (42).

416 Acetylcholine:

417 In the arcuate body, cholinergic signal is pr 415 PCDt in jumping spider, P. johnsom (42).
416 Acetylcholine:
417 In the arcuate body, cholinergic signal is
418 α -ChAT, ventral, Fig. S1 h,i). Within the v
419 which completely fill the anterior sub-lay
420 immunore 416 Acetylcholine:
417 In the arcuate
418 α -ChAT, ventra
419 which complet
421 patterns joinin
422 innervated on
-
- being thicker than others. Hill noted the presence of tracts running through the arcuate body to join the

415 PCDt in jumping spider, *P. johnsoni* (42).

416 *Acetylcholine:*

417 In the arcuate body, cholinergic signal 418 α -ChAT, ventral, Fig. S1 h,i). Within the ventral side of this lobe, cholinergic signal forms fine puncta
419 which completely fill the anterior sub-layer of this lobe. Toward the dorsal end of this lobe, the punc
- 419 which completely fill the anterior sub-layer of this lobe. Toward the dorsal end of this lobe, the punction
420 immunoreactivity forms heavier beaded varicosities. Midway there are faint column-like expression
421 pat
-
-
- 1419 immunoreactivity forms heavier beaded varicosities. Midway there are faint column-like expression patterns joining from a thin layer within the posterior ventral AB (pABv). A single layer is sparsely innervated on th Action is a minimum of the anti-time intervention with the posterior ventral AB (pABv). A single layer is sparsely

422 innervated on the anterior side of the dorsal lobe (Fig. 6) (Fig. 6 - α-ChAT, dorsal). Cholinergic

4 innervated on the anterior side of the dorsal lobe (Fig. 6) (Fig. 6 - α -ChAT, dorsal). Cholinergic
423 innervation within the layers of the arcuate body has yet to be described for any other spider spears
424 GABA:
125 innervation within the layers of the arcuate body has yet to be described for any other spider
424 GABA:
425 In the ventral lobe of the arcuate body are several layers of faint GAD-immunoreactivity (Fig. 1
426 α -GAD).
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-
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- 424 GABA:
425 In the ventral lobe of the arcuate body are several layers of faint GAD-immunoreactivity (Fig. S2g, Fig. 4
426 α -GAD). At the edge of the posterior layer, GAD+ somata of the adjacent posterior cell layer 424 GABA:
425 In the v
426 α-GAD)
427 anterio
429 Iayer w
430 immun anterior to which there is wider layer fine signal (Fig. 6 - α -GAD, ventral). Moving anteriorly, this is
428 followed by a very thin layer of puncta which may be connected through minute projections to the ne
429 layer
- 428 followed by a very thin layer of puncta which may be connected through minute projections to the
429 layer which is as thick as the first. The anterior-most layer of the ventral lobe appears empty of
430 immunoreacti
-
- α -GAD). At the edge of the posterior layer, GAD+ somata of the adjacent posterior cell layer are seen,

anterior to which there is wider layer fine signal (Fig. 6 α -GAD, ventral). Moving anteriorly, this is

428 f However which is as thick as the first. The anterior-most layer of the ventral lobe appears empty of

430 immunoreactivity. Apart from a haze which is difficult to disentangle from bleedthrough or background,

431 the sam 430 immunoreactivity. Apart from a haze which is difficult to disentangle from bleedthrough or back
431 the same can be said of the dorsal arcuate body lobe. However, in the dorsal arcuate body lobe
432 clear illustration
- the same can be said of the dorsal arcuate body lobe. However, in the dorsal arcuate body lobe we see a
dear illustration of how neurites stemming from the somata of the posterior cell layer extend through
the arcuate bod
- dear illustration of how neurites stemming from the somata of the posterior cell layer extend through
the arcuate body layers (Fig. 6 α -GAD, ventral). The pattern in *C. salei* (26, 29) is similar for the first
layer
-
- 433 the arcuate body layers (Fig. 6 α -GAD, ventral). The pattern in *C. salei* (26, 29) is similar for the first
434 layers beginning from the posterior side, but diverges at the anterior-most arcuate body section, w 433 the arcuate body layers (Fig. 6 - α-GAD, ventral). The pattern in C. sure (26, 29) is similar for the first
434 layers beginning from the posterior side, but diverges at the anterior-most arcuate body section, where
43
- 435 the thickest and most densely stained layer appears to be in what would be the anterior dorsal arcuate
436 body layer, where we see little to no signal.
437 Dopamine:
438 Within the arcuate body, TH-immunoreactivity o
-
-
- 437 *Dopamine:*
438 Within the arcuate body, TH-immunoreactive
439 dorsal lobe (Fig. 6 α -TH, dorsal), supplied b
440 located tracts (Fig. S3k). This single layer of p
441 consistent with both *H. lenta* and *P. regiu* 437 Dopumine:
438 Within the a
439 dorsal lobe
440 located trace
441 consistent
-
- 436 body layer, where we see little to no signal.
437 Dopamine:
438 Within the arcuate body, TH-immunoreactivity occupies a single layer in the posterior aspect of the
439 dorsal lobe (Fig. 6 α-TH, dorsal), supplied by dorsal lobe (Fig. 6 - α -TH, dorsal), supplied by thin and sparse neurites stretching from the anteriorly
located tracts (Fig. S3k). This single layer of punctate terminals with anteriorly branching projections
consiste 440 located tracts (Fig. S3k). This single layer of punctate terminals with anteriorly branching projections
441 consistent with both *H. lenta* and *P. regius* (35), but otherwise the *U. diversus* dopaminergic arcuate
4
- 441 consistent with both *H. lenta* and *P. regius* (35), but otherwise the *U. diversus* dopaminergic arcuate terminals with and projections is the *U. diversus* dopaminergic arcuate 441 consistent with both H. lenta and P. regius (35), but otherwise the U. diversus dopaminergic arcuate

443

442 body immunoreactivity in anterior layers as in *H. lento.*

443 additional wispy immunoreactivity in anterior layers as in *H. lento.*

444 For the wolf spider, *H. lento*, TH labelling reveals densely stained fi

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- For the wolf spider, *H. lenta*, TH labelling reveals densely stained fit
444 For the wolf spider, *H. lenta*, TH labelling reveals densely stained fit
445 (35). In contrast we see a stark lack of immunoreactivity in ante Example 2018 Contain the contained transformation
448 Immunoreactivity in the arcuate body; a wide difference dense puncta on each side, with columnar-like examples and adjacent layers, this pattern is remarkably similar 448 Immunosta
449 Immunorea
450 dense puno
451 adjacent la
452 serotonerg
453 ventral act
454 dorsal arcu
455 hinting at a
456 seen for TL
457 *Octopamin*
458 Both anter
459 side showil
460 side showil
461 varicosities immunoreactivity in the arcuate body, a wide diffuse layer of puncta, and a thinner layer bordered
dense puncta on each side, with columnar-like expression in between (36). Taken together as two
adjacent layers, this patt dense puncta on each side, with columnar-like expression in between (36). Taken together as two

adjacent layers, this pattern is remarkably similar to that seen for our model species. In U. diversus,

serotonergic-immuno 450 dense pulling and the side of this spatial series of the acts of the series of the series of the series of the series in U. diversus serotonergic-immunoreactivity shows a faint layer in the posterior ventral arcuate l
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- (35). In contrast we see a stark lack of immunoreactivity in anterior regions which would be expected to
contain the comparable neuropils in *U. diversus.*
444 Serotonin:
448 Immunostaining against 5-HT in the social hunt 446 contain the comparable neuropils in *U. diversus.*
447 *Serotonin:*
448 Immunostaining against 5-HT in the social huntsman, *Delana cancerides* shows two gross levels of
450 immunoreactivity in the arcuate body; a wid 452 serotonergic-immunoreactivity shows a faint layer in the posterior ventral arcuate lobe, and an ante
453 ventral actuate sublayer broadly flush with minutely fine fibers (Fig. 6 - α -5-HT, ventral, Fig. S4k). The
45 453 ventral actuate sublayer broadly flush with minutely fine fibers (Fig. 6 - α-5-HT, ventral, Fig. S4k). The
454 dorsal arcuate lobe displays a robust and wide immunoreactive pattern resembling flagstone-pavement,
455 454 dorsal arcuate lobe displays a robust and wide immunoreactive pattern resembling flagstone-paveme
455 hinting at a columnar structure (Fig. 6 - α-5-HT, dorsal). This layers innervation greatly resembles that
456 seen but the same of the same lobe (Fig. 6 - α -5-HT, dorsal). This layers innervation greatly resembles that
456 seen for TDC2 in the same lobe (Fig. 6 - α -TDC2, dorsal).
457 Octopamine / Tyramine:
868 Both anterior and 456 seen for TDC2 in the same lobe (Fig. 6 - α-TDC2, dorsal).
457 Octopamine / Tyramine:
458 Both anterior and posterior sublayers of the ventral arcuate body exhibit TDC2 immunoreactivity (Fig. α-TDC2, Fig. S5I,m). The 457 *Octopamine / Tyramine:*
458 Both anterior and posterior sublayers of the ventral arcu
459 α-TDC2, Fig. S5I,m). The posterior layer of this sublayer is
460 side showing faint minute columnar arrangement. The a
461 var 458 Both anterior and poster
459 α -TDC2, Fig. S5I,m). The p
460 side showing faint minute
461 varicosities. In the dorsal
462 sublayer (aABd), where it
463 keystone-shaped column
464 body (labelled 'central bother
465
- α -TDC2, Fig. S5l,m). The posterior layer of this sublayer is saturated with diffuse puncta with the anterior
460 side showing faint minute columnar arrangement. The anterior sublayer has denser, garland-like
461 varico
-
-
- varicosities. In the dorsal arcuate body lobe, TDC2 immunoreactivity appears only in the anterior sublayer (aABd), where it fully fills the span of this layer with robust staining resembling a series columnar arrangements. sublayer (aABd), where it fully fills the span of this layer with robust staining resembling a series c

462 sublayer (aABd), where it fully fills the span of this layer with robust staining resembling a series c

463 keys
- side showing faint minute columnar arrangement. The anterior sublayer has denser, garland-like
varicosities. In the dorsal arcuate body lobe, TDC2 immunoreactivity appears only in the anterior
sublayer (aABd), where it ful keystone-shaped columnar-like elements. Octopaminergic expression has been reported in the arce body (labelled 'central body' in source) (24) of C. salei, where a parasagittal section shows strong immunoreactivity in the v
-
-
- horizontal view, but it would appear by the gaps in immunoreactivity that a dorsal horizontal slice in 1

467 salei should show three general layers of AB staining, which is essentially what we see from a dorsal

468 plane
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-
-
- body (labelled 'central body' in source) (24) of C. salei, where a parasagittal section shows strong

465 immunoreactivity in the ventral portion of both arcuate body lobes. We must imagine the respective

466 horizontal v Affects and the second of the general data view, the gap secondary which is essentially what we see from a dorsal

plane in U. diversus.

AllatostatinA:

The pattern of arcuate body innervation by AstA+ neurons is in gener
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-
- 468 plane in U. diversus.
468 plane in U. diversus.
469 AllatostatinA:
470 The pattern of arcuate body innervation by AstA+ neurons is in general agreement with findings from
471 salei and M. muscosa (8, 28) where signal i 469 AllatostatinA:

470 The pattern of arcuat

471 salei and M. muscosa

472 staining in the dorsal

473 immunoreactivity is :

474 encompasses the ant

475 discernible units of in
-
- 471 salei and M. muscosa (8, 28) where signal is prominent in the ventral arcuate lobe (ABv), with little to no
472 staining in the dorsal arcuate (ABd). Concerning the sublayers of the ventral arcuate lobe, AstA-
473 imm
- 470 The pattern of
471 salei and M. m
472 staining in the
473 immunoreacti
474 encompasses
475 discernible uni
476 which is prese
477 *Proctolin:*
479 Proctolin imm
480 S7l,m). In the v
481 diffuse puncta
482 in the poster
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- 478
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482

465 immunoreactivity in the ventral portion of both arcuate body lobes. We must imagine the respect
466 horizontal view, but it would appear by the gaps in immunoreactivity that a dorsal horizontal slice
467 salei should 472 staining in the dorsal arcuate (ABd). Concerning the sublayers of the ventral arcuate lobe, AstA-

immunoreactivity is seen on the anterior aspect of the posterior ventral arcuate (posABv), and fully

476 encompasses 474 encompasses the anterior ventral arcuate (antABv) (Fig. 6 - α-AstA). In a given sample, a series of
475 discernible units of immunoreactivity are seen in the posABv layer, suggesting the columnar organizal
476 which 475 discernible units of immunoreactivity are seen in the posABv layer, suggesting the columnar organ
475 discernible units of immunoreactivity are seen in the posABv layer, suggesting the columnar organ
476 which is pre which is present, but generally obscured by the density of staining (Fig. S6k – arrowheads).

477

478 Proctolin:

479 Proctolin immunoreactivity is evident in all lobes and layers of the arcuate body (Fig. 6 - α-Proctoli 477
478 Proctolin:
479 Proctolin immunoreactivity is evident in all lobes and layers of the arcuate body (Fig. 6 - α-
480 S7l,m). In the ventral arcuate body (ABv), at the posterior ABV a line of intense terminals, u
481 479 Proctolin i
480 S7l,m). In
481 diffuse pu
482 in the post

- 480 S7l,m). In the ventral arcuate body (ABv), at the posterior ABV a line of intense terminals, underlayed by
481 diffuse puncta. Towards the dorsal end of the ventral arcuate body (ABv), the proctolin-immunoreactivity
- 479 S7l,m). In the ventral arcuate body (ABv), at the posterior ABV a line of intense terminals, underlayed by
480 S7l,m). In the ventral arcuate body (ABv), at the posterior ABV a line of intense terminals, underlayed by 481 diffuse puncta. Towards the dorsal end of the ventral arcuate body (ABv), the proctolin-immunoreactivity in the posterior-most layer transforms into heavy garland-like columnar varicosities extending at an 482 in th
- 482 in the posterior-most layer transforms into heavy garland-like columnar varicosities extending at an
dorsal end of the ventral arcuate body (ABV), the process of the process of the process of the process of the p
 $\frac{1$ $\frac{4}{3}$

(28). In the anterior ventral accuate (antABv), the anterior and posterior sublayers take on an intricate

485 mesh-like form, also with smaller flagstone formations. Between these two layers are fine palisade

483 ceurit 485

1485 mesh-like form, also with smaller flagstone formations. Between these two layers are fine palisade

486 neurites. Both sublayers of the dorsal arcuate (ABd) are also filled, but with a sparse field of fine punct meurites. Both sublayers of the dorsal arcuate (ABd) are also filled, but with a sparse field of fine pu

487

488 CCAP:

488 CCAP:

490 CCAP expression is strong in the posterior ventral AB layer with a fine mesh, puncta CCAP:

488 CCAP:

489 CCAP expression is strong in the posterior ventral AB layer with a fine mesh, punctate appearance which

490 seemingly contours the columnar structures on the anterior and posterior boundaries of thi 488
489
490
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493
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495 489 CCAP
490 seemin
491 anteric
492 area berges
493 expres
494 singula
495 sub-la₁
495 layer h
497 the po
498 C. sale
498 C. sale
498 C. sale
500 immur
500 FMRFc
501 FMRFc
503 From k 490 seemingly contours the columnar structures on the anterior and posterior boundaries of this layer. The anterior and posterior sublayers of the ventral AB are highlighted, with a decrease in staining within the area b anterior and posterior sublayers of the ventral AB are highlighted, with a decrease in staining within the area between the sublayers (Fig. 6 - α -CCAP, ventral, Fig. S8h). In the anterior ventral AB, the anti-CCAP expr area between the sublayers (Fig. 6 - α -CCAP, ventral, Fig. S8h). In the anterior ventral AB, the anti-CCAP expression is slightly finer and more punctate than in the preceding description. The staining appears singular expression is slightly finer and more punctate than in the preceding description. The staining appears
singular unlike in the posterior ventral AB – this might be reflective of expression in the area between
sub-layers w 494 singular unlike in the posterior ventral AB – this might be reflective of expression in the area between
495 sub-layers within anterior ventral AB. Within the dorsal AB (Fig. 6 - α -CCAP, dorsal), only the posterior 495 sub-layers within anterior ventral AB. Within the dorsal AB (Fig. 6 - α -CCAP, dorsal), only the posterior layer has appreciable expression, showing a single, finely innervated but moderately thick layer huggir the aby-layer has appreciable expression, showing a single, finely innervated but moderately thick layer hugging the posterior boundary of the dorsal AB. CCAP-immunoreactive layers in U. diversus are comparable to C. salei (28 the posterior boundary of the dorsal AB. CCAP-immunoreactive layers in U. diversus are comparable to
498 C. salei (28), as for both species the thickest staining layer is the most posterior one (ventral arcuate
499 body lo C. salei (28), as for both species the thickest staining layer is the most posterior one (ventral arcuate

499 body lobe), followed anterio-dorsally by a lesser layer, and with a thinner strand of intensely

500 immunoreac 499 body lobe), followed anterio-dorsally by a lesser layer, and with a thinner strand of intensely

500 immunoreactive boutons running through the more anteriorly located dorsal arcuate body lobe.

502 FMRFamide:

502 F

499 600 immunoreactive boutons running through the more anteriorly located dorsal arcuate body lot and the more anteriorly located dorsal arcuate body lot and the more anteriorly located dorsal arcuate body lot and the str FONT FONTIFIES THE MATTEN CONTROLLED INTERNATION INTERNATION CONTROLLED FOR THE MATTED TO FORD THOM THOS THE MA

FONT FONT FONT FONT FONTIFIES THE MORE SPIDENT THAT IS A SIGNATION OF THE MORE THAT THE MORE THAT THE MORE TH

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509 503 From both C.

504 FMRFamider

505 immunoreact

506 arrangement

507 immunoreact

508 we see in U.

609 volume (Fig.

510 lt appears that

511 lt appears that

512 innervation p

513 interior. This

515 the dorsal arc
 FOM FATH Example For a sharp strander for the entire dorsal arcuate body lobe is suffused immunoreactivity, there is a sharp strand of garland-like varicosities giving way to the typical columoreactivity, there is a shar EXECT FIRMFAMIDER IN the standard of garland-like varicosities giving way to the typical columnar

strangement in the posterior dorsal arcuate body layer (posABd), and more diffuse, punctate

immunoreactivity in the anter For a strangement in the posterior dorsal arcuate body layer (posABd), and more diffuse, punctate

507 immunoreactivity in the anterior dorsal arcuate body layer (antABd). This pattern is approximately wha

508 we see in 507 immunoreactivity in the anterior dorsal arcuate body layer (antABd). This pattern is approximing we see in *U. diversus*, with additional details made clear by access to a continuous stacked imachood volume (Fig. 6 -

510
511 It appears that the saturated sig
512 Innervation pattern which is stre
513 Interior. This can be seen from s
515 Ithe dorsal arcuate body, the imr
516 Varicosities at the ventral aspect
517 Relative to other exami 511
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518 Figure 2.12 Intervalsion pattern which is stronger in the wall of each tubular-like sublayer, and weaker in the viel interior. This can be seen from several specific planes which slice longitudinally through both reveali

513 interior. This can be seen from several specific planes which slice longitudinally through both sub
514 revealing four layers, each being the boundary of one of the sublayers (Fig. 6 - α-FMRFamide, ver
515 the dorsal

So the sead in U. diversus, with additional details made clear by access to a continuous stacked image

so the anterior (Fig. 6 - α -FMRFamide).

S10 It appears that the saturated signal within the ventral arcuate lobe 514 revealing four layers, each being the boundary of one of the sublayers (Fig. 6 - α-FMRFamide, ventral). In
515 the dorsal arcuate body, the immunoreactivity is primarily in the posterior sublayer, having the heavy
51

1515 the dorsal arcuate body, the immunoreactivity is primarily in the posterior sublayer, having the heavy

515 the dorsal arcuate body, the immunoreactivity is primarily in the posterior sublayer, having the heavy

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509 volume (Fig. 6 - α-FMRFamide).

510

511 tappears that the saturated signal within the ventral arcuate lobe is actually the result of an

511 trappears that the saturated signal within the ventral arcuate lobe is act 516 varicosities at the ventral aspect, and keystone column pattern more dorsally (Fig. 6 - α-FMRFamide).
517 Relative to other examined spiders, the punctate pattern in the anterior sublayer is weakly present. The arcua Fig. 8 at the varies at the varies at the ventral aspects at the ventral aspects. The ventral strength are at the ventral strength in the anterior sublayer is weakly present. The ventral strength as the ventral strength in Figure 12 The Latin Comparison of FMRFamile immunoreactivity is similar to that of CCAP.

S18 arcuate body layering pattern of FMRFamile immunoreactivity is similar to that of CCAP.

S20 **Tonsillar neuropil**

S21 Within th For a measure body and the pattern of the simulation in the similar of FMRFamily is similar to that of CMRFA
520 **Tonsillar neuropil**
521 Within the historically non-descript central supraesophageal ganglion, we observed a 520 Tonsillar neuropil
521 Within the historic
522 neuropil structure
523 structure is positic
524 perimeter of the s

For the historical process. Beginning in the planes dorsal to the mushroom bodies, this paired
structure is positioned directly on either side of the midline, and is centrally located, being medial to the
perimeter of the 523 structure is positioned directly on either side of the midline, and is centrally located, being medial to
524 perimeter of the supraesophageal ganglion from both the lateral as well as anterior and posterior lim
524 pe

524 perimeter of the supraesophageal ganglion from both the lateral as well as anterior and posterior limits. 524 perimeter of the supraesophageal ganglion from both the lateral as well as anterior and posterior limits.

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- Figure 2.1 The half in each posterior aspect. Between the two halves, at the midline, is a furrow which is

527 negative for synapsin-immunoreactivity, giving this neuropil, in conjunction with the synapsin-negative

528 For end, while the posterior presentativity, giving this neuropil, in conjunction with the synapsin-negative

state zone, a likeness to tonsils when viewed from the horizontal optical planes (Fig. 7a, c – α-synapsin).

I 528 zone, a likeness to tonsils when viewed from the horizontal optical planes (Fig. 7a, c – α-synapsin).
529 In individual anti-synapsin stains, a fiber tract traveling laterally adjoins this neuropil in the more dorsal Fig. 1993 In individual anti-synapsin stains, a fiber tract traveling laterally adjoins this neuropil in the more do
530 posterior portions. By tubulin-immunoreactivity, it appears to bifurcate the structure below the bri
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- in the dorsal portion (Fig. 7b). As evidenced by at least octopaminergic/tyraminergic co-staining, this

stract may be supplying input from yet another hitherto undescribed neuropil, the protocerebral bridge,

to be discus Fig. 2013 In the dorsal population (Fig. 7b). As the discussed below.

532 tract may be supplying input from yet another hitherto undescribed neuropil, the protocerebral bridg

533 their immunoreactivity is circumscribed b The subset of antisera for specific neuronal populations are instrumental in confirming this neuropil, as

their immunoreactivity is circumscribed by its boundaries, with little neighboring signal to obscure the

distincti 534 A subset of antisera for
535 their immunoreactivity
536 distinction (Fig. 7c). Mo
537 varicosities which neat
538 tronger on the periphe
540 compartments, most no
540 compartments, most no their immunoreactivity is circumscribed by its boundaries, with little neighboring signal to obscure the
distinction (Fig. 7c). Most representative among these is serotonergic-immunoreactivity, exhibiting fir
varicosities distinction (Fig. 7c). Most representative among these is serotonergic-immunoreactivity, exhibiting fine
varicosities which neatly fill the area. TDC2+ signal, indicating innervation from octopaminergic and
tyraminergic ne
- 537 varicosities which neatly fill the area. TDC2+ signal, indicating innervation from octopaminergic and
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- varicosities which neatly fill the area. TDC2+ signal, indicating innervation from octopaminergic and

styraminergic neurons, are also prominent in this neuropil. The relatively heavier terminals appear

stronger on the p 538 tyraminergic neurons, are also prominent in this neuropil. The relatively heavier terminals appear
539 stronger on the periphery, and when viewed in alignment with the 5-HT channel, resemble a division
540 compartmen 539 stronger on the periphery, and when viewed in alignment with the 5-HT channel, resemble a divisity compartments, most notably in the ovoid regions where serotonin is found in an internal, core pat with octopaminergic Example in the periphery, but the void regions where serotonin is found in an internal, core pattern,

540 compartments, most notably in the ovoid regions where serotonin is found in an internal, core pattern,

541 with The comparison of the comparison of the anterior-posterior dimension as AllatostatinA-immunoreactivity is

542 There may also be a division in the anterior-posterior dimension as AllatostatinA-immunoreactivity is

5543 mo There may also be a division in the anterior-posterior dimension as AllatostatinA-immunore

543 There may also be a division in the anterior-posterior dimension as AllatostatinA-immunore

543 There pronounced in the poste 543 more pronounced in the posterior bridging region, and sparsely punctated in the anterior ovoid zones
544 (Fig. 7c – α -AstA), while proctolin-immunoreactivity is limited to the posterior region (Fig. 7c – α -
545
-
- 544 (Fig. 7c α -AstA), while proctolin-immunoreactivity is limited to the posterior region (Fig. 7c α -
545 Proctolin). FMRFamide immunostaining is diffusely present, particularly in the anterior-dorsal portions
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- 550 Originating anterio-laterally and progressing posterior-medially through the ascending dorsal planes of 551
551 the supraesophageal ganglion is a banded neuropil structure which we will designate as the of the tonsillar neuropil, but this is amidst broadly saturated signal from this antibody throughout the
547 supraesophageal ganglion.
548 Protocerebral bridge
550 Originating anterio-laterally and progressing posterior-me
- Example
548 **Protocerebral bridge**
550 Originating anterio-laterally
551 the supraesophageal gangli
552 protocerebral bridge. Wide
553 thinnest, midline-crossing c 549
555
5552
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- supraesophageal ganglion.
548 **Protocerebral bridge**
550 Originating anterio-laterally and progressing posterior-medially through the ascending dorsal planes of
551 the supraesophageal ganglion is a banded neuropil structu
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- 549 **Protocerebral bridge**
550 Originating anterio-la
551 the supraesophageal
552 protocerebral bridge.
553 thinnest, midline-cros
555 dense cap of somata
555 dense cap of somata 551 the supraesophageal ganglion is a banded neuropil structure which we will designate as the
552 protocerebral bridge. Wider in the lateral aspect, the structure tapers towards the medial end with the
553 thinnest, midli For protocerebral bridge. Wider in the lateral aspect, the structure tapers towards the medial er

thinnest, midline-crossing component only being apparent in specific neuronal subpopulatio

This is the dorsal-most neuro thinnest, midline-crossing component only being apparent in specific neuronal subpopulation stains.

554 This is the dorsal-most neuropil seen in the interior of the supraesophageal ganglion before reaching th

555 dense c This is the dorsal-most neuropil seen in the interior of the supraesophageal ganglion before reaching

dense cap of somata (Fig. 8a,b).

As with the previous neuropil, only a subset of antisera show immunoreactivity withi 555 dense cap of somata (Fig. 8a,b).

556 As with the previous neuropil, only a subset of antisera show immunoreactivity within this neuropil. The

557 most filling is GABAergic-immunoreactivity (by anti-GAD stain) which 556 As with the previous neuropil, or
557 most filling is GABAergic-immun
558 the neuropil with dense signal (F
559 reveals that the protocerebral bi
560 throughout the length of the process of the bridge (Fig. 8b - α -
-
-
- 559 reveals that the protocerebral bridge has a layered structure. TDC2-immunoreactivity is pronounced throughout the length of the protocerebral bridge, displaying heavy chains of puncta on the posteried ge of the bridg
- 550 throughout the length of the protocerebral bridge, displaying heavy chains of puncta on the posterio edge of the bridge (Fig. 8b α-TDC2). Proctolin-immunoreactivity forms a tight, thinner band, primari at the media
- Example of the bridge (Fig. 8b α-TDC2). Proctolin-immunoreactivity forms a tight, thinner band, primarily at the medial end of the neuropil, comprised of fine puncta and is centrally located among the layers (Fig. 8b -
-
- 557 most filling is GABAergic-immunoreactivity (by anti-GAD stain) which defines a nearly complete swath of
558 the neuropil with dense signal (Fig. 8b α -GAD). Comparing further neuronal-subtype preparations
559 reve 558 the neuropil with dense signal (Fig. 8b - α -GAD). Comparing further neuronal-subtype preparations
559 reveals that the protocerebral bridge has a layered structure. TDC2-immunoreactivity is pronounced
560 throughou 562 at the medial end of the neuropil, comprised of fine puncta and is centrally located among the layers
563 (Fig. 8b - α-Proctolin). The acetylcholinergic pattern is a distinct thin layer on the anterior and posterior
5 563 (Fig. 8b - α -Proctolin). The acetylcholinergic pattern is a distinct thin layer on the anterior and posterional puncta and is a distinct thin layer on the anterior and posterional puncta and puncta and puncta and p 563 (Fig. 8b - α-Proctolin). The acetylcholinergic pattern is a distinct thin layer on the anterior and posterior

565 of AstA-immunoreactivity was also seen (Fig. 8b - α-AstA).
565 of AstA-immunoreactivity was also seen (Fig. 8b - α-AstA).
566 Posterior and ventral to the protocerebral bridge is an arching string of varicosities whi 566 Posterior and ventral to the protocerebral bridge is an arch
567 apex just before the appearance of the dorsal arcuate bod
568 protocerebral commissure (dPCC) (Fig. 8b – arrows) and the
569 protocerebral bridge expre S67 apex just before the appearance of the dorsal arcuate body layer. We refer to this as the dorsal
568 protocerebral commissure (dPCC) (Fig. 8b – arrows) and the neuronal subtype populations which sho
569 protocerebral 568 protocerebral commissure (dPCC) (Fig. 8b – arrows) and the neuronal subtype populations whic
569 protocerebral bridge expression tend to also innervate this commissure. The strongest of these
570 octopaminergic/tyram For protocerebral bridge expression tend to also innervate this commissure. The strongest of these are octopaminergic/tyraminergic neurons (anti-TDC2) and proctolinergic neurons (anti-Proctolin) (Fig. 8b - α-TDC2, α-Proc 570 octopaminergic/tyraminergic neurons (anti-TDC2) and proctolinergic neurons (anti-Proctolin) (Fig. 8
571 α-TDC2, α-Proctolin). This pathway separates from the posterior-lateral contour of the protocerebra
572 bridge, **EXECUTE AND THE SET ALL AND SET AND SET AND THE PROCESSION OF T** bridge, where there is an approximately triangular expansion of immunoreactivity before travelling
573 bridge, where there is an approximately triangular expansion of immunoreactivity before travelling
573 medially, and p medially, and passing just posterior to the edge of the tonsillar neuropil. A similar pattern, though o

relatively lesser staining intensity is also seen for allatostatinA (Fig. 8b - α-AstA). Cholinergic-innerva

is als For metally and passing intensity is also seen for allatostatinA (Fig. 8b - α -AstA). Cholinergic-innervations is also apparent in the posterior arch, but is more subtle and in a single layer in the anterior domain, bei 575 is also apparent in the posterior arch, but is more subtle and in a single layer in the anterior domain,
576 being less comparable to that of TDC2, Proctolin and AllatostatinA.
577
580 Acetylcholine:
581 Acetylcholine:

576 being less comparable to that of TDC2, Proctolin and AllatostatinA.
577
578 **Additional supraesophageal observations:**
582 The most strongly ChAT-immunoreactive neurons are found in a 6-7 neuron cluster in the anterior 577
577 **Additional supraesophageal observations:**
579 *Acetylcholine*:
581 The most strongly ChAT-immunoreactive neurons are found in a 6-7
582 The most strongly ChAT-immunoreactive neurons are found in a 6-7
583 umn, med 578
579
580
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585 578 **Additional supraesophageal observations:**
579 *Acetylcholine*:
581 The most strongly ChAT-immunoreactive no
583 umn, medial to the cheliceral ganglia and ju
584 al bridge (Fig. S1d).
585 The nature of this antibody, a 580
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583 985
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Examining (Fig. 1997)
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585 The nature of this a
587 scribe the contribut
588 neuropil. ChAT-imm
590 immunoreactivity the specific structures.
592 The aforementione 586
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591 specific structures.

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582 The most stror

583 umn, medial to

584 al bridge (Fig. 9

585

586 The nature of 1

587 scribe the cont

588 neuropil. ChAT

589 subesophagea

591 specific structure

592 The aforement

594 sal still to thes 595 medial cluster are more typically sized (Fig. S1h - small arrow), while the lateral pair are the largest meuropil. ChAT-immunoreactivity forms dense puncta abundant throughout the supra- and

589 subesophageal ganglia (Fig. S1b-f). Compared to other immunostains in our study, the relatively uni

590 immunoreactivity throughou 589 subesophageal ganglia (Fig. S1b-f). Compared to other immunostains in our study, the relation-
590 immunoreactivity throughout the neuropil, apart from a couple exceptions, does not clearly
591 specific structures.
59 590 immunoreactivity throughout the neuropil, apart from a couple exceptions, does not clearly highlight
591 specific structures.
592 The aforementioned globuli cells are visible anterio-dorsally to the level of the MB hea specific structures.

591 specific structures.

592 The aforementioned globuli cells are visible anterio-dorsally to the level of the MB heads (Fig. S1g). Do

594 sal still to these appear two bright clusters, each contain 592

593 The aforementione

594 sal still to these app

595 medial cluster are r

596 ChAT+ neurons we

597 somata (~16-20) are

598 small arrowheads),

599 Two strings of ChAT 593
594
595
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601 595 medial cluster are more typically sized (Fig. S1h - small arrow), while the lateral pair are the largest

ChAT+ neurons we observed in *U. diversus* (Fig. S1h - large arrow). A concentrated line of cholinergic

somata 596 ChAT+ neurons we observed in *U. diversus* (Fig. S1h - large arrow). A concentrated line of cholinerg somata (~16-20) are present on the thinner band of cell bodies posterior to the arcuate body (Fig. S
593 small arrow 597 somata (~16-20) are present on the thinner band of cell bodies posterior to the arcuate body (Fig. S11)
598 small arrowheads), and at the far dorsal end a roughly equal amount are dispersed medially (Fig. S1i)
599 ChAT Space 598 small arrowheads), and at the far dorsal end a roughly equal amount are dispersed medially (Fig. S1i).

599 Two strings of ChAT+ varicosities arc with their zenith at the posterior midline, before the arcuate bod

The stringent of the fart arrowship are the far dorsation of the far dorsal end of the far dorsal end of the posterior arc has stronger immunoreactive puncta and travels ventrally to the dorsal arcuate body (ABd). The wide $\begin{array}{r} -0.600 \\ 600 \\ 601 \\ 602 \\ 603 \\ 604 \\ 605 \\ 606 \\ 607 \\ 608 \end{array}$

- 602 (ABd). The wider anterior arc is made of fine parallel fibers and appears to comprise a part of the
-

From the posterior arc has stronger immunoreactive puncta and travels ventrally to the dorsal arcuate body

(ABd). The wider anterior arc is made of fine parallel fibers and appears to comprise a part of the

foos (ABd). T (ABd). The wider anterior arc is made of fine parallel fibers and appears to comprise a part of the

protocerebral bridge-like neuropil (Fig. 8b, Fig. S1i – arrows).

604

605 GABA:

606

607 GAD-immunoreactive neurons ar For protocerebral bridge-like neuropil (Fig. 8b, Fig. S1i – arrows).

604

605 GABA:

606

607 GAD-immunoreactive neurons are also found abundantly throughout the anterior side of the brai

608 within the deep furrow of s 604
605 *GABA*:
606 GAD-immunoreactive neurons are also found abundantly throws).
608 within the deep furrow of somata (Fig. S2d,f). One feature when we will be seen furrow of somata (Fig. S2d,f). One feature when - - - -
605
606
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608 606
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607 GAD-in
608 within 607
608

608 within the deep furrow of somata (Fig. S2d,f). One feature which is visible with this antibody is the $\overline{}$ $\mathcal{C}(\mathcal{G})$ with the deep further with the deep function is visible with this visible with this antibody is the

610 with the full breadth of synapsin-immunoreactivity corresponding to this neuropil (Fig. S2g). A bulbous shape is outlined by GAD-immunoreactivity, and overlays with the apex of the mushroom body heads in the standard b 611 shape is outlined by GAD-immunoreactivity, and overlays with the apex of the mushroom body heads it the standard brain volume (Fig. S2e - arrow), suggesting that the globuli cells contain GABAergic innervition. Anterio

612 the standard brain volume (Fig. S2e - arrow), suggesting that the globuli cells contain GABAergic innervation. Anterior to the tonsillar neuropil there is a sharp band of GABAergic cell bodies arranged in the medio-la

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621 Continuing dorsally, the next clusters of TH+ neurons are at the level of and dorsal to the closure of the medio-lateral direction (Fig. S2f). Further in the dorsal subpraesophageal ganglion, there is a distinction.
615 ascending column of GAD+ somata on each of the hemiganglia which stand out due to not being imm
616 diately f 615 ascending column of GAD+ somata on each of the hemiganglia which stand out due to not being immediately flanked by other GABAergic neurons to each side (Fig. S2g). Such a grouping also seems present in C. salei (37) a 617 in C. salei (37) and P. tepidariorum (43).
618 Dopamine:
620 Continuing dorsally, the next clusters of TH+ neurons are at the level of and dorsal to the closure of the
622 Continuing dorsally, the next clusters of TH+ - 19
619
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627 - 621
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624 stomodeal bridge (STb, as per (44), Fig. S3f - asterisk). These tandem neurons match well to the posi-

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618

619 Dopamine:

620

621 Continuing dorsally, the next clusters of

622 esophagus. The dorsal cluster is a tandel

623 which are seen a band of neurites and a

624 stomodeal bridge (STb, as per (44), Fig. 5

625 tioni 620

621 Continuing

622 eophagus.

623 which are s

624 stomodeal I

625 tioning of "

626 se neurons

627 ageal bridge

628 The opistho

630 the lateral k

631 while the in

632 dorsal to th

633 (Fig. S3g – a

634 more 622 esophagus. The dorsal cluster is a tandem pair of two neurons each (Fig. S3f – arrows), posterior to
623 which are seen a band of neurites and adjoining immunoreactivity on each side, representing the
624 stomodeal br which are seen a band of neurites and adjoining immunoreactivity on each side, representing the

stomodeal bridge (STb, as per (44), Fig. S3f – asterisk). These tandem neurons match well to the pos

tioning of "Group 3" n 524 stomodeal bridge (STb, as per (44), Fig. S3f – asterisk). These tandem neurons match well to the p

525 tioning of "Group 3" neurons in the wolf spider, *H. lenta* (45). Similarly, to the "Group 3" member:

525 se neu 628
629 The opisthose
630 the lateral bo
631 while the inte
632 dorsal to the
633 (Fig. S3g – arr
634 more numero
635 the supraesor
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635 the supraesophageal ganglion (45) .

630 the lateral borders of this structure are highlighted by intensely staining tracts of TH-immunoreactivity, while the interior has a mesh of varicosities, including its own fine commissures (Fig. S3f - brace). Just dor

(Fig. S3g – arrows). These appear to be a similar population to "Group 2" neurons in *H. lenta*, which a
formore numerous, but share the description of projecting posteriorly and slightly dorsally to the edge of
formore n more numerous, but share the description of projecting posteriorly and slightly dorsally to the edge of

635 the supraesophageal ganglion (45).

636 The final cluster of the ventral end of the supraesophageal ganglion appe while the interior has a mesh of varicosities, including its own fine commissures (Fig. S3f - brace). Just dorsal to the neurons in the vicinity of the bridge, are the second cluster comprised of 5 TH+ somata (Fig. S3g – dorsal to the neurons in the vicinity of the bridge, are the second cluster comprised of 5 TH+ somata

(Fig. S3g – arrows). These appear to be a similar population to "Group 2" neurons in *H. lenta*, which ar

more numero Fig. S3g – arrows). These appear to be a similar population to "Group 2" neurons in *H. lenta*, which a

more numerous, but share the description of projecting posteriorly and slightly dorsally to the edge of

the supraes 635 the supraesophageal ganglion (45).
636 The final cluster of the ventral end of the supraesophageal ganglion appear laterally as 4 neurons per
638 homiganglia (Fig. S3g, h – arrow). This is approximately at the level w - 537
638
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645 637 The final cluster of the ventral end of the supraesophageal ganglion appear laterally as 4 neurons per
638 hemiganglia (Fig. S3g, h – arrow). This is approximately at the level where the "ventral-most TH-ir tract"
639

639 (using terminology in (45)) joins in an arch above the esophagus (seen clearer medially in Fig. S3g), the
640 third and most posterior bridging of that channel. These neurons are likely the counterpart to those la-
64

634 more numerous, but share the description of projecting posteriorly and slightly dorsally to the edge of
635 the supraesophageal ganglion (45).
637 The final cluster of the ventral end of the supraesophageal ganglion a 636

637 The final cluster of the ventral end c

638 hemiganglia (Fig. S3g, h – arrow). The sum (45) ioins in a

640 third and most posterior bridging of

641 the supraes of the suppression of the sum of the sum of the su 640 third and most posterior bridging of that channel. These neurons are likely the counterpart to those la-
belled "Group 1" in *H. lento* (45). As for *H. lento*, these neurons contribute heavily to the tracts running
b 649 About the level of the arcuate body there are a doublet and singlet (Fig. S3i, j – arrowhead and arrow, 642 briefly posteriorly, and then medially to course through the protocerebro-dorsal tract (PCDt), ventral to the arcuate body. Interestingly, a subset (potentially 2 of the 4) of these lateral subpraesophageal neurons al the arcuate body. Interestingly, a subset (potentially 2 of the 4) of these lateral subpraesophageal neurons also produce descending projections (Fig. S3f – arrowhead), which join the "intermediate TH-ir tract" (as define fig. tract" (as defined in (45)). In short, the TH+ somata of the ventral supraesophageal ganglion are rem
646 ably similar in organization and projection patterns to those reported in the wolf spider, with the on
647 dis discrepancy being fewer neurons found for each cluster in *U. diversus.*
648 About the level of the arcuate body there are a doublet and singlet (Fig. S3i,j – arrowhead and arrow, respectively) of TH+ neurons, totaling th Moreopency being for the arcuster body there are a doublet and singlet (Fig
648
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6649 About the level of the arcuste body there are a doublet and singlet (Fig
655 These neurons, and particularly the lone medial ones (Fi 649
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For the metallong is a stating three per side, which are present on anterior facing somata field
These neurons, and particularly the lone medial ones (Fig. S3i, j – arrow), form extensive projections
within this sector of These neurons, and particularly the lone medial ones (Fig. S3i, j – arrow), form extensive projections

within this sector of the supraesophageal ganglion. The doublet population forms a wide arc laterally

and ventrally,

652 within this sector of the supraesophageal ganglion. The doublet population forms a wide arc laterally

within this sector of the supraesophageal ganglion. The doublet population forms a wide arc laterally and ventrally, which melds into the PCDt, wherein its individual fibers can no longer be discerned. The single neurons p and ventrally, which melds into the PCDt, wherein its individual fibers can no longer be discerned. The single neurons project slightly dorsally, also contributing to the PCDt, the looped and crossed portion visible at the

654 single neurons project slightly dorsally, also contributing to the PCDt, the looped and crossed portion
655 visible at the posterior midline (Fig. S3i), and appear to also be the source of innervation to the arcuate
6 654 single neurons project slightly dorsally, also contributing to the PCDt, the looped and crossed portion
655 visible at the posterior midline (Fig. S3i), and appear to also be the source of innervation to the arcuate
6

655 visible at the posterior midline (Fig. S3i), and appear to also be the source of innervation to the arcuat
656 body layer appearing dorsally (Fig. S3k). In *H. lenta*, a 'triad' of neurons is described at this area, w 656 body layer appearing dorsally (Fig. S3k). In *H. lenta*, a 'triad' of neurons is described at this area, with a
be the source of inner to the source of innervation to the source of independent of the arcuate of the ar essay. In the same appearing dorsally (Fig. S3k). In H. lenta, a 'triad' of neurons is described at this area,
In H. lenta, a 'triad' of neurons is described at the same at the same at the same at the same at this area, wi

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668 subset provides are evident in a reac projection and number, down to the division of targets match our findings for the described neurons in *U. diversus.*

659 our findings for the described neurons in *U. diversus.* 659 our findings for the described neurons in *U. diversus.*
660 A final, dorsal-most cluster of 2 – 3 neurons appears medially (Fig. S3k), anterior to the arcuate body.
662 Despite very close confirmation of the preceedi 660

661 A final, dorsal-most cluster of $2 - 3$ neurons appears r

662 Despite very close confirmation of the preceeding sor

663 *lenta* or *P. regius* (45), and therefore may be particula

664 are more difficult to foll 661
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668 bespite very close confirmation of the preceeding somata, these neurons were not reported in either *lenta* or *P. regius* (45), and therefore may be particular to *U. diversus*. The projections of these neuror are more di For the protocons of the method in the protocons of the protocons of the projections of the properties are more difficult to follow due to the mass of neurites which they overlap through just ventrally, but it are more dif For a memorial or the proton in the posterior of the mass of neurites which they overlap through just ventrally, out it
supposes that at least the most prominent of their neurites actually continue to descend ventrally, cr appears that at least the most prominent of their neurites actually continue to descend ventrally, crossing below the PCDt in the posterior direction before turning sharply to continue into the ventral supraesophageal and suppraesophageal and potentially even the subesophageal ganglion. At this same dorsal plane as

somata, densely fine varicosities are evident in an area positioned correctly to potentially overla

the protocerebral bridge For somata, densely fine varicosities are evident in an area positioned correctly to potentially overlap with
the protocerebral bridge neuropil (Fig. S3k), but this has not been confirmed with a reliable alignment.
670
671 methed with a reliable alignment.

669 the protocerebral bridge neuropil (Fig. S3k), but this has not been confirmed with a reliable alignment.

670 Serotonin:

671 Serotonin:

672 S-HT-immunoreactivity is prominent in the 670
671 Serotonin:
672 5-HT-immunoreactivity is prominent in the posterior bridging area dorsal to the esophageal passage, as
674 well as the laterally adjacent tissue (Fig. S4e), and not as apparent in anterior stomodeal 671
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674 well as the laterally adjacent tissue (Fig. S4e), and not as apparent in anterior stomodeal bridge. Sero-

well as the laterally adjacent tissue (Fig. S4e), and not as apparent in anterior stomodeal bridge. Sero-
formulas the laterally adjacent tissue (Fig. S4e), and not as apparent in anterior stomodeal bridge. Sero-
tonergic

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673 5-HT-immu

674 well as the

675 tonergic fik

676 glion (Fig. 9

677 at several p

679 bending m

680 (Fig. S4h).

681 Continuing

682 Continuing

683 pierced by

684 synapsin-n

685 A pair of st

686

687 A 683 pierced by a circular spot lacking synapsin-immunoreactivity (Fig. $S4i - brace$) – with such internal 684 synapsin-negative areas assumed to be tracheal passageways or potentially glia. It is difficult to ascertain at several points surrounding the intensely synapsin-immunoreactive protocerebral tract, suggesting
678 that serotonergic projections are running among this fiber bundle (Fig. S4f). The semi-circular tracts
679 bending med that serotonergic projections are running among this fiber bundle (Fig. S4f). The semi-circular tracts

bending medially (Fig. S4g), before a chiasm of seemingly all three directions is seen immediately dor:

(Fig. S4h). O bending medially (Fig. S4g), before a chiasm of seemingly all three directions is seen immediately do
679 bending medially (Fig. S4g), before a chiasm of seemingly all three directions is seen immediately do
680 (Fig. S4h) (Fig. S4h). On the posterior end is a diffuse umbrella-like band of varicosities (Fig. S4h, i).

680 (Fig. S4h). On the posterior end is a diffuse umbrella-like band of varicosities (Fig. S4h, i).

681 Continuing dorsally 681

681 Continuing dorsally from this point, the innervation spreads to fill a kidney shaped structure

683 Continuing dorsally from this point, the innervation spreads to fill a kidney shaped structure

684 synapsin-nega 682
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689 pierced by a circular spot lacking synapsin-immunoreactivity (Fig. S4i – brace) – with such internal
684 synapsin-negative areas assumed to be tracheal passageways or potentially glia. It is difficult to ascer
685 to what

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synapsin-negative areas assumed to be tracheal passageways or potentially glia. It is difficult to as
685 to what degree the innervation in this region is continuous with that of dorsally located features.
686 A pair of st to what degree the innervation in this region is continuous with that of dorsally located features.

685 A pair of strongly immunoreactive 5-HT neurons (one for each hemiganglion) (Fig. S4j – arrows) are

687 A pair of str 686

687 A pair of strongly immunoreactive 5-HT neurons (one for each hemiganglion) (Fig. S4j – arrows) an

688 found medially, at the plane of the MB heads, and appear to send a neurite into a varicose-filled r

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for strongly in the plane of the MB heads, and appear to send a neurite into a varicose-filled regular poinned in by the MB bridge to the posterior and lateral sides, and the cell body furrow anteriorly (Fig. 54j). Ventral pinned in by the MB bridge to the posterior and lateral sides, and the cell body furrow anteriorly (Fig.

690 S4j). Ventrally this region leads to the aforementioned hagstone structure. Dorsally, the

691 immunoreactivity 690 S4j). Ventrally this region leads to the aforementioned hagstone structure. Dorsally, the
691 immunoreactivity can be followed until the very strikingly defined contours of the tonsillar neuropil,
692 which has been d 691 immunoreactivity can be followed until the very strikingly defined contours of the tonsill
which has been described (Fig. 7).
693 For *C. salei* (46), additional clusters of serotonergic neurons were reported in the d 692 which has been described (Fig. 7).
693 For *C. salei* (46), additional clusters of serotonergic neurons were reported in the dorsal
695 supraesophageal ganglion, but apart from a given cluster (Fig. 54k – arrows) we h 693
694 For *C. salei* (46), additional clusters
695 supraesophageal ganglion, but apa
696 reliably distinguish groupings withi
697 *Octopamine/Tyramine:*
699 In the anterior wall of somata spar
701 fusion of the esophagea 694
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702 695 supraesophageal ganglion, but apart from a given cluster (Fig. S4k – arrows) we have not
696 reliably distinguish groupings within the dorsal somata.
697 *Octopamine/Tyramine:*
698 *Octopamine/Tyramine:*
698 *Octopamin*

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- 698
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- Franchillary of the dorsal somata.

696 Filiably distinguish groupings within the dorsal somata.

697 Octopamine/Tyramine:

699 In the anterior wall of somata spanning from the frontal plane at the level of cheliceral neur 697
698 *Octopamine/Tyramine:*
699
700 In the anterior wall of somata spanning from the frontal
701 fusion of the esophageal bridge are atleast 15-20 TDC2+
702 (Fig. S5f,g). This number corresponds closely to the cour
703 699
700 In the anterior wall of so
701 fusion of the esophagea
702 (Fig. S5f,g). This number
703 immunoreactivity is pro 700
701
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703 700 In the anterior of the esophageal bridge are atleast 15-20 TDC2+ neurons, of varying size and staining intensity

702 (Fig. S5f, g). This number corresponds closely to the counts for *C. salei* in the same region (47, 702 (Fig. S5f, g). This number corresponds closely to the counts for *C. salel* in the same region (47, 48). TDC2 immunoreactivity is prominent in the stomodeal bridge and adjacent areas, and at this plane two laterations Fig. 35 (Fig. 25). The straight in the stomodeal bridge and adjacent areas, and at this plane two lateral immunoreactivity is prominent in the stomodeal bridge and adjacent areas, and at this plane two lateral in the stomo 703 immunoreactivity is prominent in the stomodeal bridge and adjacent areas, and at this plane two lateral

channel.

705 channel.

706 channel.

707 A variant of the subesophageal tract arrangement is reprised in the opisthosomal neuropil, where in

708 tense boutons line a tract running parallel to the midline between hemigang 706

707 A variant

708 tense boi

709 posterior

710 structure

711 A diagrar

712 a frontal

713 "protoce

714 areas wit 707
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715 probable posterior end (Fig. S5f, g – arrow), while also giving rise dorsal and laterally to a concentric ladder-like

710 structure with tracts laying in the anterior-posterior direction (Fig. S5h – brace).

711 A diagram

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- 716 hagstone neuropil. Dorsally, the signal remains strong within the interior, and prominent in an anterio-
- of the magnetic state in the materior-posterior direction (Fig. S5h brace).

The diagram from C. salei points to octopaminergic expression in the interior supraesophageal ganglion,

The diagram from C. salei points to oc 711 A diagram from C. salei points to octopaminergic expression in the interior supra
712 a frontal slice indicates two areas of octopaminergic-immunoreactivity, both refer-
713 "protocerebral neuropil" – suggesting the us 212 a frontal slice indicates two areas of octopaminergic-immunoreactivity, both referred to as

213 "protocerebral neuropil" – suggesting the use of this term to be a general placeholder for non-descript

214 areas within "Transmuthment individually was extinged anglian (48). We find TDC2-immunoreactivity strongly in a reas within the supraesophageal ganglion (48). We find TDC2-immunoreactivity strongly in la-like posterior region also inne areas within the supraesophageal ganglion (48). We find TDC2-immunoreactivity strongly in the umbre la-like posterior region also innervated by 5-HT (Fig. S5i), and sparser puncta within the bounds of the hagstone neuropil
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716 hagstone neuropil. Dorsally, the signal remains strong within the interior, and prominent in an anterio medial stretch hemmed in by the mushroom bodies, as well as thin strands which run along the latera periphery (Fig periphery (Fig. S5j). TDC2-immunoreactivity is also found in the tonsillar central neuropil, where it is

719 substantial throughout, but particularly strong in a peripheral type of shell pattern, especially when

720 alig Fig. 21

219 substantial throughout, but particularly strong in a peripheral type of shell pattern, especially when

210 aligned to 5-HT staining which respectively forms the core (Fig. S5k).

221 We have described above h aligned to 5-HT staining which respectively forms the core (Fig. S5k).

720 aligned to 5-HT staining which respectively forms the core (Fig. S5k).

721 We have described above how TDC2-immunoreactivity heavily marks the pr 721
722 We have described above how TDC2-immunoreactivity heavily marks
723 as a commissure connecting posterio-dorsally (Fig. 8, Fig. S5I). Seyfar
724 octopamine-immunoreactivity revealing "fine varicose fibers in proto
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725 a single cropped micrograph, it is difficult to orient and draw a comparison with confidence to the

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- as a commissure connecting posterio-dorsally (Fig. 8, Fig. S5I). Seyfarth and colleagues (48) report

724 octopamine-immunoreactivity revealing "fine varicose fibers in protocerebral bridge". As this reference

725 a singl

223 as a commissure connecting posterio-dorsally (Fig. 8, Fig. S5I). Seyfarth and colleagues (48) report

224 octopamine-immunoreactivity revealing "fine varicose fibers in protocerebral bridge". As this references

225 a 223 as a commine-immunore activity revealing "fine varicose fibers in protocerebral bridge". As this reference a single cropped micrograph, it is difficult to orient and draw a comparison with confidence to the neuropil w 725 a single cropped micrograph, it is difficult to orient and draw a comparison with confidence to the

725 a single cropped micrograph, it is difficult to orient and draw a comparison with confidence to the

727 of punc neuropil which we are describing as the protocerebral bridge. A final detail of interest concerns a sof puncta which extend from the protocerebral bridge to the tonsillar neuropil (Fig. S5l and inset) -
T27 of puncta which 727 of puncta which extend from the protocerebral bridge to the tonsillar neuropil (Fig. S5I and inset) –
728 which could correspond to tract highlighted by tubulin-immunoreactivity (Fig. 7b) – revealing a putative
729 pa mann means the puller shows the puller shows the puller shows the put and the put put pathway between these neuropils.

The puncta which could correspond to tract highlighted by tubulin-immunoreactivity (Fig. 7b) – reveal pathway between these neuropils.

730 Interestingly, earlier reports indicated no octopaminergically immunoreactive somata within the

732 Interestingly, earlier reports indicated no octopaminergically immunoreactive soma 730

731 Interestingly, earlier reports indicat

732 protocerebrum in C. salei ((47) repr

733 neuronal cell bodies are visible in the

734 supraesophageal ganglion, with sor

735 terior to the arcuate body (Fig. S5m

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protocerebrum in C. salei ((47) reprinting table from Dunker 1992, (49)). In U. diversus, a series of
neuronal cell bodies are visible in the anterior half of the far dorsal cap of somata covering the
supraesophageal gang

9734 supraesophageal ganglion, with some TDC2+ somata also being present in the thinner layer of

735 terior to the arcuate body (Fig. S5m – arrow). Given that TDC2 also should be present in tyrami

736 neurons which can b

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732 protocol sales are visible in the anterior half of the far dorsal cap of somata covering the

1732 suprassophageal ganglion, with some TDC2+ somata also being present in the thinner layer of cells pos-

1735 terior to 743 closes, where a commissure is also seen crossing (Fig. S6f). Moving dorsally, this gives way to even more meurons which can be octopamine-negative, these findings may be consistent with the picture for C.

1737 salei, or alternatively may reveal that U. diversus has octopaminergic populations which are lacking in

1738 the wan 37 salei, or alternatively may reveal that *U. diversus* has octopaminergic populations which are lacking in

38 the wandering spider.

38 the wandering spider.

38 the wandering spider.

39 closes, where a commissure is 738 the wandering spider.

738 the wandering spider.

739

740 AllatostatinA:

741 In U. diversus, strong AstA-immunoreactivity is present on the posterior side of where the esophagus

743 closes, where a commissure is al 739
740 AllatostatinA:
741 In U. diversus, strong A
743 closes, where a commi
744 synaptically dense area
745 tions, and forming an u
746 sibly illuminated by an
747 740
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742 In *U. diversus,*

743 closes, where

744 synaptically de

745 tions, and forn

746 sibly illuminate

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748 Heavy AstA-im

749 names of distil

750 a coronal slice

751 immunoreacti^l - 742
743 445 647
744 748 749
747 747 75 743 coses, where a commissure is also seen crossing (Fig. S6f). Moving dorsally, this gives way to even more sympatically dense areas, eventually highlighting the circular structure circumnavigated by thin projections, and 9744 synaptically dense areas, eventually highlighting the circular structure circumnavigated by thin projections, and forming an umbrella-like structure at the posterior side (Fig. S6g)– which is more comprehensibly illu

747
748 Heavy AstA-immunoreactivity was noted in the c
749 mames of distinct regions and neuropils have bee
750 a coronal slice, making a direct alignment to our
751 immunoreactivity within the interior of the supra 748
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The mand of distinct regions and neuropils have been lacking (50). The best view of staining in this regional successor and the central game in the central supersymptom of the supraesophageal ganglion, is consistent in *U* 750 a coronal slice, making a direct alignment to our images challenging, but the fact of substantial AstA-
751 immunoreactivity within the interior of the supraesophageal ganglion is consistent in *U. diversus* (Fig.
751

751 immunoreactivity within the interior of the supraesophageal ganglion is consistent in U. diversus (Fig.

-
- TS3 are present in the anterio-medial channel (Fig. 56h arrows).

753 are present in the anterio-medial channel (Fig. 56h arrows).

755 One structure that we identify are the tonsillar neuropils, the form of which AstA 754

755 One structure that we identify are the tonsillar neuropils, the

755 One structure that we identify are the tonsillar neuropils, the

757 large, intensely stained AstA+ neurons are present alongside t

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- 755
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763 abundantly fills out, resembling similar patterns as seen with 5-HT and TDC2 (Fig. S6i, Fig. 7c). A pair

1757 large, intensely stained AstA+ neurons are present alongside the neuropil, deep and medial within the

1758 fur 1757 large, intensely stained AstA+ neurons are present alongside the neuropil, deep and medial within the furrow of somata, and whose neurites enter the anterior aspect of the adjoining tonsillar neuropil (Fig. 759 S6i). Figure 1.1 The most dorsal AstA+ somata are a pair found laterally once the adjoining tonsillar neuropil (Fig.
759 S6i). The most dorsal AstA+ somata are a pair found laterally once the arcuate body emerges (Fig. S6j).
760 958 (Fig. 56). The most dorsal AstA+ somata are a pair found laterally once the arcuate body emerges (Fig. 56j).

759 S6i). The most dorsal AstA+ somata are a pair found laterally once the arcuate body emerges (Fig. 56j).
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761 Contrary to the jumping spider (51), we did not see AstA-immunoreactive somata in the posterior cell

762 layer adjacent to the arcuate body. This region is prone to damage during preparation, but nevertheles

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769 AstA+ neurons were not seen here in any of our samples.

763 AstA+ neurons were not seen here in any of our samples.

764 Proctolin:

766 On the posterior edge of the STb there is a thin Proc+ commissure while the anterior
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- 764
765 *Proctolin:*
766 *Proctolin:*
767 On the posterior edge of the STb there is a thin Proc+ com
768 highlighted by a bolder vein of varicosities (Fig. S7e). Med
769 which the protocerebral tract rises, there is a band
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- 1762

The poster adjacent to the arcuate body. This region is prone to damage during preparation, but neverthele

261 AstA+ neurons were not seen here in any of our samples.

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263 Archive in the posterior edge of 765
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767 88
769 77
777 772
777 77 766

767 On the position

768 highlighte

770 space that

771

772 A cluster c

773 (Fig. S7g),

774 - arrow). I

775 of the MB

776 Here there

777 as well as

7879 Just dorsa

780 brace) trav

781 other post

781 other p - 767
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769 70
777 773
777 777 1768 highlighted by a bolder vein of varicosities (Fig. S7e). Medial to the synapsin-negative channel through

1768 which the protocerebral tract rises, there is a band of proctolin-immunoreactivity which occupies a

170 s which the protocerebral tract rises, there is a band of proctolin-immunoreactivity which occupies a

170 space that is not thoroughly labelled by any other target of this study (Fig. S7f).

171 A cluster of small, brightly 970 space that is not thoroughly labelled by any other target of this study (Fig. S7f).

771 A cluster of small, brightly immunoreactive proctolin+ neurons are evident at the level of the MB ha

773 (Fig. S7g), with less i 771

772 A cluster of small, brightly immunoreactive proctolin+ neurons are evident at th

773 (Fig. S7g), with less immunoreactive but larger somata appearing dorsally aroun

774 – arrow). Posterior to the cup-shaped syna - 772
773 4 5
777 777 778
777 778
780 (Fig. S7g), with less immunoreactive but larger somata appearing dorsally around the MB heads (Fig. S7

773 (Fig. S7g), with less immunoreactive but larger somata appearing dorsally around the MB heads (Fig. S7

774 - arro -arrow). Posterior to the cup-shaped synaptic-density formed by the MB hafts continuing with the rest

774 -arrow). Posterior to the cup-shaped synaptic-density formed by the MB hafts continuing with the rest

775 of the M 775 of the MB is a crescent of Proc-immunoreactivity (Fig. S7h), which also appears present in C. salei (52).
775 of the MB is a crescent of Proc-immunoreactivity (Fig. S7h), which also appears present in C. salei (52).
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776 Here there is signal in the posterior, midline-spanning umbrella structure observed for 5-HT and TDC2,

777 as well as fine varicosities in the hagstone neuropil.

778 Uust dorsally past the level of the MB heads, a st as well as fine varicosities in the hagstone neuropil.

778

178 Just dorsally past the level of the MB heads, a strand of varicosities forms anteriolaterally (Fig. S7j –

179 Just dorsally past the level of the MB heads, 778

779 Just dorsally past the level of the MB heads, a strance

780 brace) travelling into the center and splitting into a

781 other posteriorly (Fig. S7k). Dorsal still, this strand d

782 ing varicosities which will 779
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Frace) travelling into the center and splitting into a delta with one branch pointing medially, while the other posteriorly (Fig. S7k). Dorsal still, this strand disappears and is overlayed at the delta by the are ing va 781 other posteriorly (Fig. S7k). Dorsal still, this strand disappears and is overlayed at the delta by the arching varicosities which will form the dorsal posterior protocerebral commissure (Fig. S7l), as has been de sc

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790 In general, CCAP-immunoreactivity resembles anti-ChAT staining in the sense that CCAP signal in the 784
785 At the plane of
786 near the midlin
787 so clearly Proct
788 CCAP:
790 In general, CCA
791 supraesophage
792 as, but generall 785
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792 TREPT THE meant of the protocolin, dorsal). Dorsal to this neuropil in the cap of somata, another 10

1787 In so clearly Proctolin-immunoreactive somata are dispersed centrally and laterally (Fig. S7m).

1787 In general, 587 so clearly Proctolin-immunoreactive somata are dispersed centrally and laterally (Fig. S7m).

788 *CCAP*:

789 *CCAP*:

1790 In general, CCAP-immunoreactivity resembles anti-ChAT staining in the sense that CCAP signal

791 supraesophageal ganglion is composed of intense but isolated puncta, showing expression in many are-

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TRET COLORE THE USE COLORET COLORET COLORET COLORET COLORET THE STAT USE OF THE STAT USE OF THE STAT USE OF TH
THE SUPRESS SUPERENT ARE SUPPLY AT STAT SUPPLIES A proving expression in
The ventral are dispersed central in a 789
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797 The Term
T90 In genes
T92 as, but
T92 as, but
T93 CCAP+
T95 CCAP+
T96 orly as
T97 gularly
T98 (Fig. S8 suppraesophageal ganglion is composed of intense but isolated puncta, showing expression in many and as, but generally lacking concentration in any given area (Fig. S8d,e). CCAP-immunoreactivity highligh the ventral trajec the ventral trajectory of the PCDt more prominently than other immunostains (Fig. S8f – arrow).

794

795 CCAP+ somata are numerous in the dorsal supraesophageal ganglion. They are found clustered posteri-

795 CCAP+ somat 794

795 CCAP+ somata are numerous in the dorsal supraesophageal ganglion. They are found clustered p

796 orly as well as directly dorsal to the ventral AB. A number of other CCAP+ somata which are space

793 gularly apar 795
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- 798 (Fig. S8h). While CCAP expression has been identified in pre-optic neuropil (50), we could not discern
799 optic pathway expression reliably above background.
799 optic pathway expression reliably above background.
- optic pathway expression reliably above background.

The could not discussed in pre-optic neuropide in pre-optic neuropide in pre-optic neuropide in pre-optic not

The could not discussed in pre-optic neuropide in pre-opti 799 optic pathway expression reliably above background.

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803 FMRFamide-i

804 boundaries o

805 appearance v

806 ventral to the

807 band (Fig. S9₁

808 other immun

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810 FMRFamide+

811 supraesopha_i

812 layer, as well

813 fainter FMRF

814 number and (815
 803
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811 boundaries of individual features difficult to ascertain (Fig 17F, G). A similarly immunoreactively-d
appearance was presented from slice work in *C. salei* (52). At the apex of the protocerebral comm
ventral to the dorsal appearance was presented from slice work in C. salei (52). At the apex of the protocerebral commissure

805 appearance was presented from slice work in C. salei (52). At the apex of the protocerebral commissure

806 ventra band (Fig. S9g – brace), representing a pattern of immunoreactivity which was not salient for any of our other immunostained targets.
809
810 FMRFamide+ neurons are numerous and fairly evenly dispersed at the dorsal cap of
-
-
- 808 other immunostained targets.
809
810 FMRFamide+ neurons are numerous and fairly evenly dispersed at the dorsal cap of the
811 supraesophageal ganglion (Fig. S9h). They are found in the area posterior and dorsal to the 809
810 FMRFamide+ neurons are num
811 supraesophageal ganglion (Fig.
812 layer, as well as somewhat larg
813 fainter FMRFamide+ neurons r
814 number and distribution repor
815
816 **Discussion**: 810
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818 811 supraesophageal ganglion (Fig. S9h). They are found in the area posterior and dorsal to t
812 supraesophageal ganglion (Fig. S9h). They are found in the area posterior and dorsal to t
812 layer, as well as somewhat lar
- 812 layer, as well as somewhat larger somata present in the anterior portion of the tissue. Again, while the
813 fainter FMRFamide+ neurons may not be fully apparent to us, the bright ones are comparable to the
814 number
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- entral to the dorsal arcuate body (ABd), is an approximately rectangular FMRFamide-immunoreactive

807 band (Fig. S9g brace), representing a pattern of immunoreactivity which was not salient for any of our

809 of the im Frank State Town and distribution reported for C. salei (53).

813 fainter FMRFamide+ neurons may not be fully apparent to us, the bright ones are comparable to the

814 number and distribution reported for C. salei (53).
 814 number and distribution reported for *C. salei* (53).
815
813 **Discussion:**
813 Almost the entirety of spider CNS literature has been studied from tissue slices, with few examples of
820 whole-mounts (8, 9, 23, 35). O 814 number and distribution reported for C. salei (53). - 16
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824 817 **Discussion:**
818 Almost the
820 Mhole-mou
821 innervation
822 Furthermor
823 clarifying fo
824 While nine
825 individual n 819
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825 820 whole-mounts (8, 9, 23, 35). Our ability to observe novel structures and make comparisons between
821 innervation patterns was aided by whole-mount preparation and averaged brain alignment.
822 Furthermore, imaging an
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- Furthermore, imaging and alignment of many neurosignaling molecule stains in a single spec

823 clarifying for the identification of novel structures, as a subset of stains crystalized putative k

824 While nine spider spe clarifying for the identification of novel structures, as a subset of stains crystalized putative boundari

While nine spider species have been the subject of examination for the expression pattern of an

individual neuro While nine spider species have been the subject of examination for the expression pattern of an

825 individual neurosignaling molecules (8, 14, 15, 17, 35, 36) the wandering spider, C. salei, is essentially

826 the only
- 825 individual neurosignaling molecules (*8*, 14, 15, 17, 35, 36) the wandering spider, *C. salei*, is essent
826 the only species prior to the current work to have had multiple targets annotated. Given the utili
827 spec
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- 821 innervation patterns was aided by whole-mount preparation and averaged brain alignment.

822 innervation patterns was aided by whole-mount preparation and averaged brain alignment.

822 clarifying for the identificati specific stains for understanding of neuropil structures, tracts and other features, this atlas provides a

828 rich source for comparative anatomy in an orb-weaving spider, *U. diversus*, while also extending

829 knowled Fich source for comparative anatomy in an orb-weaving spider, *U. diversus*, while also extending

829 knowledge of a number of different neurosignaling pathways for spiders at large.

830 **Mushroom bodies**

832 As evident 830
831 **Mushroom bodies**
832 As evident from synapsin volumes, the mushroom bodies of *U. diversus* are the m
833 the central supraesophageal ganglion. The *U. diversus* MBs have a complete appear
834 attached haft regio
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- 829

829 knowledge of a number of different neurosignaling pathways for spiders at large.

829 As evident from synapsin volumes, the mushroom bodies of *U. diversus* are the most salient feat

831 Mushroom bodies

832 As e 831
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837 831 **Mushroom bodies**
832 As evident from sy
833 the central supraes
834 attached haft regio
835 innervation, albeit
836 third-order visual r
837 the subject of a sul
838 optic neuropils of *l*
839 mechanosensation 835 innervation, albeit from an unknown origin. Historically, the MBs have at times been referred to third-order visual neuropil, and have been discussed in the context of the visual pathways, which
the subject of a subst
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- 825 individual neurosignaling molecules (8, 14, 15, 17, 35, 36) the wandering spider, C. salei, is essentially 833 the central supraesophageal ganglion. The *U. diversus* MBs have a complete appearance, exhibiting an attached haft region similar to visually-dependent spiders (9), and to which we find evidence of sather more order 834 attached haft region similar to visually-dependent spiders (9), and to which we find evidence of
835 innervation, albeit from an unknown origin. Historically, the MBs have at times been referred to as the
835 third-or 836 third-order visual neuropil, and have been discussed in the context of the visual pathways, which form
837 the subject of a substantial portion of the spider nervous system literature (8, 9, 21, 39, 42, 54). The
838 o the subject of a substantial portion of the spider nervous system literature $(8, 9, 21, 39, 42, 54)$. The optic neuropils of *U. diversus* are diminutive, which is consistent with hunting through mechanosensation on a we 838 optic neuropils of *U. diversus* are diminutive, which is consistent with hunting through

839 mechanosensation on a web. While we employed several neurotransmitter stains which have identif

840 upstream optic pathway mechanosensation on a web. While we employed several neurotransmitter stains whice
840 optice neurotic pathway elements (e.g. medulla, lamellae) in other species, these first structures were not evident even in preparation
-
- upstream optic pathway elements (e.g. medulla, lamellae) in other species, these first and second-order
structures were not evident even in preparations where the labile tissue of the secondary pathway was
intact. The dimi state is the structures were not evident even in preparations where the labile tissue of the secondary pathway was
842 intact. The diminished nature of the optic pathways, but simultaneous presence of a distinct mushroom
8
- intact. The diminished nature of the optic pathways, but simultaneous presence of a distinct mushroom
body structure in *U*. diversus raises an incongruence concerning the role of the mushroom body. A
growing literature is
- 843 body structure in *U*. diversus raises an incongruence concerning the role of the mushroom body. A growing literature is suggestive of a deeper complexity, as examples of both cursorial and web-based growing literatur 844 growing literature is suggestive of a deeper complexity, as examples of both cursorial and web-base
growing the must relate the must
struc

 8444 growing literature or a galaxies of a deeper complexity, as examples of both cursorial and web-based α

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- state of the mushroom bodies may differ between species. The mushroom bodies of insects, most granularly understood in *Drosophila melanogaster*, were originally considered to be olfactory integration centers, and while re
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- most granularly understood in *Drosophila melanogaster*, were originally considered to be olfactory

integration centers, and while remaining the most apparent input, subsequent studies have shown this

center to also proc
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- Found which either species whose visual capacities seem all but irrelevant to their lifestyle indicates the
season primary input to the mushroom bodies may differ between species. The mushroom bodies of insects, a
spinor g center to also process multiple sensory modalities and influence behaviors not directly related to
851 offaction (55). Evolutionary pressures on certain species may also force a 'modality switch', as evidence
852 by the wh 851 olfaction (55). Evolutionary pressures on certain species may also force a 'modality switch', as evidenty by the whirlygig beetle, *Dineutus sublineatus*, which has lost antennal lobes and instead have muss bodies supp Salternative methods are the that mushem of the that mushem of the that may also necess

1855 sensory information, such as mechanosensation, relevant for web activities – which may also necess

1854 learning and memory pro
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- 865 learning and memory processes. Closer identification and annotation of the innervation patterns of non-
1857 visual sensory streams leading to the MBs would strengthen such a viewpoint.
1858
1860 Arcuate body
1860 The
- integration centers, and while remaining the most apparent input, subsequent studies have shown

standard to different of also process multiple sensory modalities and influence behaviors not directly related to

851 of int of the whilry gighe betel, *Dinet* on Certain species may also force a 'modality switch', as evidenced
by the whilry gigheetle, *Dineutus sublineatus*, which has lost antennal lobes and instead have mushroom
853 bodies sup 853 bodies supplied by the optic lobe, displaying a transition from olfactory to visual processing (56). An alternative hypothesis would be that mushroom bodies in web-building species may integrate other sensory informati alternative hypothesis would be that mushroom bodies in web-building species may integrate other
Sets sensory information, such as mechanosensation, relevant for web activities – which may also necess
alterning and memory 865 these patterns overlap. In U. diversus, we confirmed two broad lobular divisions, which each contain an 858
859 Arcuate body
860 The arcuate body, being unmistakable and consistently present among species,
862 detailed structure in the spider brain, particularly in regards to innervation by r
863 populations. By aligning vo 859
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865 859 **Arcuate body**
860 The arcuate b
862 detailed struc
863 populations. F
864 disambiguatic
865 these pattern.
866 additional two
867 innervation pa - 361
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868 detailed structure in the spider brain, particularly in regards to innervation by neurotransmitter sub
populations. By aligning volumes to a common reference, the present methodology allowed for
disambiguation of the layer 863 populations. By aligning volumes to a common reference, the present methodology allowed for disambiguation of the layers innervated by specific signaling molecules and understanding of where these patterns overlap. In 864 disambiguation of the layers innervated by specific signaling molecules and understanding of wh
865 these patterns overlap. In *U. diversus*, we confirmed two broad lobular divisions, which each cor
866 additional two
- these patterns overlap. In *U. diversus*, we confirmed two broad lobular divisions, which each contain additional two major layers, supporting a number of structural motifs. Generalizing for the arcuate b innervation patte
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- Solutional terms in *U. diversus* of specific neuronal populations, as compared to *C. salei* and a few other species, one can conclude that there is a great degree of similarity, in the relative arrangement of the gross the gross layers, and even in certain fine structural details. In comparative studies, the arcuate body has
869 the gross layers, and even in certain fine structural details. In comparative studies, the arcuate body has
87 been found to compose a roughly proportionate percentage of the brain across the species examined—

871 be they web-builders or visually-based hunters (19, 57). It is thus assuredly involved in various spider

872 behavior been they web-builders or visually-based hunters (19, 57). It is thus assuredly involved in various spider

872 behaviors, and it will be illuminating to unravel how this conserved circuitry is harnessed for different

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- 866 additional two major layers, supporting a number of structural motifs. Generalizing for the arcuate body

867 innervation patterns in *U. diversus* of specific neuronal populations, as compared to *C. salei* and a few
 868 other species, one can conclude that there is a great degree of similarity, in the relative arrangement c

869 other species, one can conclude that there is a great degree of similarity, in the relative arrangement c
 872 behaviors, and it will be illuminating to unravel how this conserved circuitry is harnessed for different
873 ethological needs, The arcuate body lobes have been previously compared to the two nested neuropi
875 known ethological needs. The arcuate body lobes have been previously compared to the two nested neuropil

873 ethological needs. The arcuate body lobes have been previously compared to the two nested neuropil

874 known generall known generally in insects as the upper and lower central bodies (28, 36, 58) and the architecture of *U.*
875 diversus supports these observations, showing obvious layering intersected by perpendicular neurites
876 and co diversus supports these observations, showing obvious layering intersected by perpendicular neurites
and columnar-like patterns.
876
879 Structures which are conspicuous in our orb-building model spider but potentially not 876 and columnar-like patterns.

8778 Novel neuropils

878 Structures which are conspicuous in our orb-building model spider but potentially not in hitherto

881 structures which are conspicuous in our orb-building model s 877

878 **Novel neuropils**

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880 Structures which are conspic

881 studied cursorial species ma

882 it is not currently clear whet

883 apparent by prior technique

884 within the interior of the sup 878
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879 Structures which
881 studied cursoria
882 it is not currentl
883 apparent by pric
884 within the interi
885 refer to a "centr
886 supraesophagea 880
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885 studied cursorial species may be indicative of areas which are important for web-building. Nevert

882 it is not currently clear whether similar neuropils are absent in other species, or if they were simp

883 apparent by it is not currently clear whether similar neuropils are absent in other species, or if they were simply not
apparent by prior techniques. Apart from the mushroom bodies and arcuate body, neuropil structures
within the inte above approach the mushroom bodies and arcuate body, neuropil structures

1884 within the interior of the supraesophageal ganglion have not been well distinguished. Multiple works

1885 refer to a "central" or "protocerebr 1884 within the interior of the supraesophageal ganglion have not been well distinguished. Multiple works
1885 refer to a "central" or "protocerebral neuropil" seemingly in regards to the undifferentiated mass of th
1886 s efer to a "central" or "protocerebral neuropil" seemingly in regards to the undifferentiated mass of t
supraesophageal ganglion as a whole. The image volume produced by aligning whole-mounted
synganglia immunostained again supraesophageal ganglion as a whole. The image volume produced by aligning whole-mounted
887 synganglia immunostained against synapsin instead reveals an intricacy of structures, beyond those
888 described here. Two of the synganglia immunostained against synapsin instead reveals an intricacy of structures, beyond the described here. Two of the most conspicuous neuropils found in the dorsal supraesophageal ga are the protocerebral bridge and 888 described here. Two of the most conspicuous neuropils found in the dorsal supraesophageal ganglio
889 are the protocerebral bridge and the tonsillar (central) neuropils.
- 889 are the protocerebral bridge and the tonsillar (central) neuropils.
 $\frac{1}{2}$ suppression in the dorsal supervisor of the dorsal suppression in the dot of the dot 889 are the protocerebral bridge and the tonsillar (central) neuropils.

891 demonstration of such a structure in the spider to date. The use of this name has a preced
892 spider literature (24), although whether the referent structure in C. salei is the same as in c
893 species will require a spider literature (24), although whether the referent structure in C. salei is the same as in our model
species will require additional clarification. Whether or not the authors chose this name in order to draw
a parallel spacies will require additional clarification. Whether or not the authors chose this name in order to department of the insect central complex (59), but demonstrations in non-insect arthropods are constituent of the insec a parallel to the insect protocerebral bridge is likewise ambiguous. The protocerebral bridge is a core
constituent of the insect central complex (59), but demonstrations in non-insect arthropods are scarcer.
Examples hav 895 constituent of the insect central complex (59), but demonstrations in non-insect arthropods are scarced Examples have been found in crustaceans, such as the crayfish *Cherax destructor* (60), as well as rock slater *L* 896 Examples have been found in crustaceans, such as the crayfish *Cherox destructor* (60), as well as rock
897 slater *Ligia occidentalis* and sidestriped shrimp *Pandalopsis dispar*, the latter of which shows widely
899 Search is a significant in the total is and sidestriped shrimp *Pandalopsis dispar*, the latter of which shows widely

arching, layered structure, stopping short of the midline (61). We find such an anterior midline struc 901 cockroaches and moths (insectbraindb.org). A columnar pattern is not as of now forthcoming in
902 diversus protocerebral bridge, which may be a consequence of density, as columnar structures c
903 difficult to see by i

898 arching, layered structure, stopping short of the midline (61). We find such an anterior midline structure in *U. diversus,* possessing layers as revealed by antisera to neurotransmitter populations, and having the ho 899 in *U. diversus*, possessing layers as revealed by antisera to neurotransmitter populations, and having a

800 thinning (to absent) midline crossing, reminiscent of disjointed PCBs in certain insects including

800 co 890 thinning (to absent) midline crossing, reminiscent of disjointed PCBs in certain insects including

8901 cockroaches and moths (insectbraindb.org). A columnar pattern is not as of now forthcoming in the *U.*

899 diver diversus protocerebral bridge, which may be a consequence of density, as columnar structures can be difficult to see by immunohistochemistry (59), demonstrated by the fact that the PCB is no more evidently columnar in cock 903 difficult to see by immunohistochemistry (59), demonstrated by the fact that the PCB is no more

904 evidently columnar in cockroach than in the sidestriped shrimp when visualizing with the same antiser

906 to TRP (6 evidently columnar in cockroach than in the sidestriped shrimp when visualizing with the same a

905 to TRP (61).

906 different to see by immuno the superator of the suprase opplagal ganglia was the central

903 dicated, to TRP (61).

905 to TRP (61).

906 A final undescribed neuropil which was apparent in the supraesophageal ganglia was the centrally

908 located, tonsillar neuropil. Based on the ovoid form, paired appearance close to the 906

907 A final undes

908 located, tons

909 proximity to

910 general rese

911 (59). To our

912 arthropods.

913 architecture

915 division. Not

916 (59).

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918 A spider cen

919 Based on gro

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915 located, tonsillar neuropil. Based on the ovoid form, paired appearance close to the midline, and c
proximity to the unpaired midline neuropil(s) (arcuate body-ABv and Abd), the tonsillar neuropil b
general resemblance to proximity to the unpaired midline neuropil(s) (arcuate body-ABv and Abd), the tonsillar neuropil bears
910 general resemblance to the noduli, a smaller constituent of the central complex of pterygote insects
911 (59). To o general resemblance to the noduli, a smaller constituent of the central complex of pterygote insects

911 (59). To our knowledge, an analogous structure to this region has not been documented in non-insect

912 architectur 911 (59). To our knowledge, an analogous structure to this region has not been documented in non-insections arthropods. Unlike the arcuate body and protocerebral bridge, neither a columnar nor layered architecture is appar architecture is apparent in the tonsillar neuropil, although specific neurosignaling molecule statement the concentrate in certain domains, including a potential core and shell, as well as an anterior/post division. Noduli examples are concentrate in certain domains, including a potential core and shell, as well as an anterior/posterior

915 division. Noduli in insects also contain compartments, and the presence of layering is species-depe

and thropods. Unlike the arcuate body and protocerebral bridge, neither a columnar nor layered
architecture is apparent in the tonsillar neuropil, although specific neurosignaling molecule stains
oncentrate in certain doma division. Noduli in insects also contain compartments, and the presence of layering is species-depenent (59).
917
A spider central complex?
919 Based on gross morphology, it is tempting to speculate that these novel neur 916 (59).
917 **A spider central complex?**
919 Based on gross morphology, it is tempting to speculate that these novel neuropils, when considered
920 along with each individual lobe of the arcuate body may form an equivale 917

918 A spi

919 Based

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921 *diver*:

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931 and la 918
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923 918 **A spider central complex?**
919 Based on gross morpholog
920 along with each individual
921 *diversus* (Fig. 9). The centra
922 columnar and pontine neu
923 neuropils across species (6
924 bridge also appear to inner
9 along with each individual lobe of the arcuate body may form an equivalent to a central complex in *L diversus* (Fig. 9). The central complex of insects is innervated and interconnected by tangential, columnar and pontin diversus (Fig. 9). The central complex of insects is innervated and interconnected by tangential,

columnar and pontine neurons in insects, forming a consistently identifiable relationship between

neuropils across species 922 columnar and pontine neurons in insects, forming a consistently identifiable relationship betwere neuropils across species (62). Apart from the crayfish (60), where neurons supplying the protocol bridge also appear to France of the insect of the insect protocerebral bridge in poperato lack columnar organizations of which has gross species (62). Apart from the crayfish (60), where neurons supplying the protocere bridge also appear to inn 924 bridge also appear to innervate the central body, knowledge of intra-complex connectivity is lacking in
925 non-insect arthropods. A detailed study of the Onychophoran (velvet worm, sister to arthropods) brain
928 reve 1925 hon-insect arthropods. A detailed study of the Onychophoran (velvet worm, sister to arthropods) brain
1926 hon-insect arthropods. A detailed study of the Onychophoran (velvet worm, sister to arthropods) brain
1927 (63 revealed several brain structures that appeared anatomically similar to those observed in arthropods
927 (63). However, whether these ganglia are functionally homologous is a matter of debate. Mushroom
928 body anatomy var (63). However, whether these ganglia are functionally homologous is a matter of debate. Mushroom

body anatomy varies greatly across arthropods (38). While the Onychophoran central body is thought

be truly homologous to t 927 body anatomy varies greatly across arthropods (38). While the Onychophoran central body is though
929 be truly homologous to the insect central body (and arcuate body in chelicerates), the frontal body
930 (which has g 929 be truly homologous to the insect central body (and arcuate body in chelicerates), the frontal body (which has gross similarities to the insect protocerebral bridge) appears to lack columnar organization and lacks an o 930 (which has gross similarities to the insect protocerebral bridge) appears to lack columnar organizational diacks an obvious connection to the central body. No noduli were observed in the Onychophorar brain, nor have th 931 and lacks an obvious connection to the central body. No noduli were observed in the Onychophoran
932 brain, nor have they been observed in arthropod brains outside of insects. The tonsillar neuropils we
933 observe app brain, nor have they been observed in arthropod brains outside of insects. The tonsillar neuropils we

933 observe appear to share connectivity with the protocerebral bridge, but no clear connectivity with the arcuate body 933 observe appear to share connectivity with the protocerebral bridge, but no clear connectivity with the arthropods (59), are arthropods (59), the anatomy of the stores. The topologies we have neuropide of the topologies arcuate body. Since noduli have not been observed in non-insect arthropods (59), the anatomy of the arcuate body. Since noduli have not been observed in non-insect arthropods (59), the anatomy of the 934 arcuate body. Since noduli have not been observed in non-insect arthropods (59), the anatomy of the

execution of the antisera used in this study do not consistently trace neurites, the connectivity

935 Given that many of the antisera used in this study do not consistently trace neurites, the connectivity

938 patterns b 937 Given that many of the patterns between the
938 patterns between the
939 investigations employ
940 within the context of t
942 comparable to better
943 variety of information
944 memory of a heading 938 patterns between the neuropils of *U. diversus* supraesophageal ganglion require clarification. Future
939 investigations employing techniques capable of isolating the ramification patterns of individual neuro
940 wit 939 investigations employing techniques capable of isolating the ramification patterns of individual neuro within the context of the present neuropils in *U. diversus* will be essential to defining whether these currently within the context of the present neuropils in *U. diversus* will be essential to defining whether these

eurrently disparate structures are truly members of a complex, and to what extent the connectivity is

comparable to 941 currently disparate structures are truly members of a complex, and to what extent the connectivity is

942 comparable to better studied arthropods. As a unit, the modules of the central complex integrate a

943 variet Figure 2012 comparable to better studied arthropods. As a unit, the modules of the central complex integrate a

942 comparable to better studied arthropods. As a unit, the modules of the central complex integrate a

944 we variety of information including present orientation with respect to a salient environmental feature,

944 variety of information including present orientation with respect to a salient environmental feature,

944 memory o 944 memory of a heading goal, and speed – which can accomplish tasks such as path integration, migration and other goal-directed movements relevant to particular species (64). While occurring in a much movements outation and other goal-directed movements relevant to particular species (64). While occurring in a much more spatially constrained context, these informational components could likewise be vital for organizing movements during t 946 spatially constrained context, these informational components could likewise be vital for organizing
movements during the process of web-building, as well as maintaining a conception of the 360-degree
web space as the 947 movements during the process of web-building, as well as maintaining a conception of the 360-degr
948 web space as the spider strikes out to capture prey and subsequently return to the resting position a
949 the hub. web space as the spider strikes out to capture prey and subsequently return to the resting position at
949 web space as the spider strikes out to capture prey and subsequently return to the resting position at
950 likely b the hub. In such a scenario for *U. diversus* and other orb-weavers, updates to present heading would
1949 the hub. In such a scenario for *U. diversus* and other orb-weavers, updates to present heading would
1950 likely b 950 likely be provided by mechanosensation, rather than optic flow, which has been shown to contribute
951 eentral bodies maintain a representation of the flies orientation within the environment in regards to
952 central 951 even in insects which otherwise predominantly employ vision (65). The columnar segments of the
952 central bodies maintain a representation of the flies orientation within the environment in regards to
953 given featur 952 central bodies maintain a representation of the flies orientation within the environment in regards

951 central bodies maintain a representation of the flies orientation within the environment in regards

953 given fe given feature (66). Although the exact number of columnar elements in the spider arcuate body lobes

has not been established, they are numerous (with some suggestions in the thousands (58)), which

could support a much mo 953 has not been established, they are numerous (with some suggestions in the thousands (58)), which
955 could support a much more refined representation of the animal's radial self-made realm, underlying
956 the often-stu 955 could support a much more refined representation of the animal's radial self-made realm, underlyir
956 the often-stunning speed and precision with which the spider builds and navigates.
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968 956 the often-stunning speed and precision with which the spider builds and navigates.
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976 Materials and Methods:
977 Animals
978 Adult female *Uloborus di*
979 housed freely in a green
980 dark cycles.
981 **Immunohistochemistry**
982 Spiders were anesthetize 977 **Animals**
978 Adult fer
979 housed 1
980 dark cyc
981 I**mmuno**
982 Spiders 1
983 with 0.1 979 housed freely in a green house, or as $1 - 4$ individuals in acrylic habitats within the lab, under 12:12 lig

980 ank cycles.

981 Immunohistochemistry

982 Spiders were anesthetized with carbon dioxide, and rapidly d

dark cycles.
981 **Immunohistochemistry**
982 Spiders were anesthetized with carbon dioxide, and rapidly dissected in HEPES-buffered saline (HBS)
983 with 0.1% TritonX, and prepared for immunostaining following the methodolo 981 Immunohis
982 Spiders wer
983 with 0.1% T
984 (2008). Sam
985 10 minutes
986 for 1 hour a
987 in 90%, 70% 981 Immunohistochemistry
982 Spiders were anesthetiz
983 with 0.1% TritonX, and p
984 (2008). Samples were fi:
985 10 minutes in HBS + 0.1
987 in 90%, 70%, 50%, 30%,
988 normal goat serum, 1%
989 were incubated for 3-5. with 0.1% TritonX, and prepared for immunostaining following the methodology described by Ott (2008). Samples were fixed overnight in ZnFA (2%) at 4° C. The following day samples were washed 3 10 minutes in HBS + 0.1% Tri 984 (2008). Samples were fixed overnight in ZnFA (2%) at 4° C. The following day samples were washed 10 minutes in HBS + 0.1% TritonX on a nutator. Samples were dehydrated in 80% methanol/20% D
685 for 1 hour and 30 minut 985 10 minutes in HBS + 0.1% TritonX on a nutator. Samples were dehydrated in 80% methanol/20% DMSC
986 for 1 hour and 30 minutes, followed by 30 minutes in 100% methanol. A series of 5 minute incubations
987 in 90%, 70%,

987 in 90%, 70%, 50%, 30%, and 0% methanol in 0.1 M Tris was applied and the samples were blocked in 5%
988 normal goat serum, 1% DMSO, in PBS with 0.1% Triton (PBST) for at least 1 hour. Primary antibodies
989 were incub

10 993 1986 10 minutes, followed by 30 minutes in 100% methanol. A series of 5 minute incubations

987 in 90%, 70%, 50%, 30%, and 0% methanol in 0.1 M Tris was applied and the samples were blocked in 5%

988 normal goat se normal goat serum, 1% DMSO, in PBS with 0.1% Triton (PBST) for at least 1 hour. Primary antibodies
were incubated for 3-5 days on a nutator at 4° C, before being washed with PBST for 3 x 15 minutes.
Secondary antibodies we

were incubated for 3-5 days on a nutator at 4° C, before being washed with PBST for 3 x 15 minutes.
990 secondary antibodies were applied in blocking solution and incubated for 2-3 days on a nutator at 4°
991 Se 990 Secondary antibodies were applied in blocking solution and incubated for 2-3 days on a nutator at 4° Secondary antibodies were washed off with 3 x 15 minutes washes with PBST, including DAPI (1:1000 one of the wash st 991 Secondary antibodies were washed off with 3 x 15 minutes washes with PBST, including DAPI (1:1000) in
992 one of the wash steps. The sample was dehydrated for mounting through a glycerol series of 2%, 4%,
993 8%, 15%, 992 one of the wash steps. The sample was dehydrated for mounting through a glycerol series of 2%, 4%, 8%, 15%, 30%, 50%, 70%, and 80% glycerol in 0.1 M Tris for 20 minutes each. Nutation was performed for 2% through 15%, 893 8%, 15%, 30%, 50%, 70%, and 80% glycerol in 0.1 M Tris for 20 minutes each. Nutation was performed for 2% through 15%, but only occasional hand agitation for the remaining steps. The sample was protected from light. Fo For 2013 and the sample was underlayed with methyl salicylate, and allowed to sink, where it stored in the dark at room temperature until mounting.

995 pipetted off and the sample was underlayed with methyl salicylate, an provided off and the sample was underlayed with methyl salicylate, and allowed to sink, where it was stored in the dark at room temperature until mounting.

998 For anti-TH staining, samples were dissected in Millonig's bu

1994 for 2% through 15%, but only occasional hand agitation for the remaining steps. The sample was

1995 protected from light. Following 30 minutes of washing with 100% ethanol, most of the ethanol was

1996 pipetted off 997 stored in the dark at room temperature until mounting.

998 For anti-TH staining, samples were dissected in Millonig's buffer with 0.1% TritonX, and fixed in 4% PF

999 in PBS for 45 minutes at room temperature while n

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1009 25x/0.8
1010 M27 75r
1011 **Volume**

or slide using cyanoacrylate glue. A coverslip was also adhered to the top of the outer washer. Samples
1008 were imaged using a Zeiss LSM700 or LSM880 confocal microscope, with a LD LCI Plan-Apochromat
1009 25x/0.8 Imm Co

1008 were imaged using a Zeiss LSM700 or LSM880 confocal microscope, with a LD LCI Plan-Apochromat

1009 25x/0.8 Imm Corr DIC M27objective (set to oil immersion), or a W Plan-Apochromat 20x/1.0 DIC D=0.17

1010 M27 75mm wa 25x/0.8 Imm Corr DIC M27objective (set to oil immersion), or a W Plan-Apochromat 20x/1.0 DIC D=
1010 M27 75mm water immersion objective, respectively.
1011 **Volume alignment**
1012 Alignment of confocal image volumes was pe

1010 1027 75mm water immersion objective, respectively.

1011 1012 1010 Volume alignment

1012 1013 1014). Registration was performed first by a rigid method using Elastix 5.0.1 (Klein et al., 2010, Shamonin et

1013 10.2

1006 well of methyl salicylate. Wells were constructed by adhering nested metal washers to a glass coverslip
1007 or slide using cyanoacrylate glue. A coverslip was also adhered to the top of the outer washer. Samples
1008 1011 Volume alignment

1012 Alignment of confocal image volumes was performed

1013 al., 2014). Registration was performed first by a rigid

1014 stochastic gradient descent optimizer for 20000 iterat

1015 levels. This wa 1011 **Volume alignment**
1012 Alignment of confo
1013 al., 2014). Registrat
1014 stochastic gradient
1015 levels. This was foll
1016 descent optimizer i
1017 AdvancedMattesM
1018 transform with a st 1013 al., 2014). Registration was performed first by a rigid method using an affine transform with an adaptive
1014 stochastic gradient descent optimizer for 20000 iterations, with 40000 spatial samples at 5 resolution
101 1014 stochastic gradient descent optimizer for 20000 iterations, with 40000 spatial samples at 5 resolution

1015 levels. This was followed by a non-rigid registration using a bspline transform with a standard gradient

10 1015 levels. This was followed by a non-rigid registration using a bspline transform with a standard gradient
1016 descent optimizer for 200000 iterations at 5 resolution levels and using the
1017 AdvancedMattesMutualInfor 1016 descent optimizer for 200000 iterations at 5 resolution levels and using the

1017 AdvancedMattesMutualInformation metric. This was followed by a non-rigid registration using B-spline

1017 AdvancedMattesMutualInforma 1017 Advanced Mattes Mutual Information metric. This was followed by a non-rigious transform with a standard gradient descent optimizer for 200000 iterations at 5 resolution levels and using the Advanced Mattes Mutual Info

1018 transform with a standard gradient descent optimizer for 200000 iterations with 40000 spatial samples
1019 at 5 resolution levels and using the AdvancedMattesMutualInformation metric. Transformation matrice
1020 were

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- 1019 at 5 resolution levels and using the AdvancedMattesMutualInformation metric. Transformation matrice

1020 were established using the anti-synapsin stain as a registration channel. A preliminary subset of synapsi

1021 1020 were established using the anti-synapsin stain as a registration channel. A preliminary subset of synapsin

1021 volumes were mutually transformed onto each other, and the brain sample for which the most

1022 satisfa
- 1021 wolumes were mutually transformed onto each other, and the brain sample for which the most

1022 satisfactorily aligned pairings resulted was selected as the reference brain, onto which all other

1023 subsequent imag Figure 2022 satisfactorily aligned pairings resulted was selected as the reference brain, onto which all other
1022 satisfactorily aligned pairings resulted was selected as the reference brain, onto which all other
1023 su 1023 subsequent image volumes were aligned. The standard brain depicted in the figures above is an averaged composite of 6 aligned synapsin volumes. The final transformation matrix generated k
1025 registration of the syna 1024 averaged composite of 6 aligned synapsin volumes. The final transformation matrix generated b
1025 registration of the synapsin channel, was then applied to other channels present for each sample
1026 volume (the neur 1025 registration of the synapsin channel, was then applied to other channels present for each sample
1026 volume (the neurosignaling target immunostains).
1027 In limited cases, no satisfactory image volume alignment coul 1026 volume (the neurosignaling target immunostains).

1027 In limited cases, no satisfactory image volume alignment could be obtained based on the Elastix

1028 parameters specified previously. In these cases, we manually 1027 In limited cases, no satisfactory image volume align
1028 parameters specified previously. In these cases, we
- 1028 parameters specified previously. In these cases, we manually applied a small correction to the El
1028 parameters specified previously. In these cases, we manually applied a small correction to the El 1028 parameters specified previously. In the elastic correction to the Elastic correction to

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- 1030 correspondences were manually annotated in the reference and moving image. An additional N^3
1031 regularly spaced location correspondences were automatically created where no manual annotati
1032 was present within a 1031 regularly spaced location correspondences were automatically created where no manual annotation

1032 was present within a 100-pixel distance, with N=ceil[Image Axis Length / 100]. The moving image

1033 coordinates was present within a 100-pixel distance, with N=ceil[Image Axis Length / 100]. The moving image

1033 coordinates were subsequently transformed using RBF interpolation with a thin plate spline kernel.

1034 Visualization

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- 1033 coordinates were subsequently transformed using RBF interpolation with a thin plate spline kerne
1034 Visualization
1035 Annotations of neuropils were drawn using Imagel and Napari (napari.org), and 3D renderings cre
 1034 Visualization
1035 Annotations of neuropils were drawn using ImageJ and Napari (napari.org), and 3D renderings creat
1036 using Imaris 10.1 (Oxford Instruments). Renderings of z-planes on to the 3D synapsin volume wer 1034 Visualization
1035 Annotations
1036 using Imaris
1037 created using
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1039 1036 using Imaris 10.1 (Oxford Instruments). Renderings of z-planes on to the 3D synapsin volume were
1037 created using VisPy (vispy.org)
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1243 evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materi
1244 Raw data files can 1244 Raw data files can be provided by A.G. Requests for files should be submitted to: a <u>gordus@jhu.edu</u>
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1267 **Figure 1: Synganglion of** *Uloborus diversus***.**

1268 (A.) 3D rendering of *U. diversus* (female) syn,

1269 posterior-lateral (left) and oblique anterio-la

1270 and DAPI stained (blue) synganglion, posterio

1271

1269 posterior-lateral (left) and oblique anterio-lateral (right) views (**B**.) 3D rendering of α-synapsin (green)
1270 and DAPI stained (blue) synganglion, posterior, lateral and anterior views (**C**.) Sequence of horizont

1270 and DAPI stained (blue) synganglion, posterior, lateral and anterior views (C.) Sequence of horizontal

1271 optical slices from averaged α-synapsin (gray) volume with averaged DAPI stains (blue), from ventral

1272

(A.) 3D rendering of *U. diversus* (female) synganglion from averaged α-synapsin volume, oblique

2126 posterior-lateral (left) and oblique anterio-lateral (right) views (**B**.) 3D rendering of α-synapsin (g

2127 and DAPI 1272 subesophageal ganglion (left) to dorsal end of supraesophageal ganglion (right). Compass abbreviation
1273 A = anterior, P = posterior, D = dorsal, V = ventral, L = lateral, M = medial.
1274 **Figure 2: Overview of a** 1273 A = anterior, P = posterior, D = dorsal, V = ventral, L = lateral, M = medial.

1274

1275 **Figure 2: Overview of averaged α -synapsin immunoreactivity in whole-mount synganglion.**

1276 Sequence of optical horizont 1274

1275 **Figure 2: Overview of averaged α -synapsin immunoreactivity in whole-**

1275 **Sequence of optical horizontal sections from averaged α-synapsin volume**

1277 position of respective slice in a 3D full volume re 1275
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1280
1281 1275 **Figure 2: Overview of averaged α -synapsin immunoreactivity in whole-mount synganglion.**

1276 Sequence of optical horizontal sections from averaged α-synapsin volume, with top-right inset

1277 position of respect

1277 position of respective slice in a 3D full volume rendering (A. – I.) Subesophageal ganglion, beginning

1278 ventrally (A.) and progressing dorsally until (I.). Notable features include the leg neuromeres (LN1-4, for 1277 position of respective slice in a 3D full volume rendering (A. – I.) Subesophageal ganglion, beginning
1278 ventrally (A.) and progressing dorsally until (I.). Notable features include the leg neuromeres (LN1-4,
1279 1278 ventrally (A.) and progressing dorsally until (I.). Notable features include the leg neuromeres (LN1-4, for
1279 respective legs 1-4), pedipalpal neuropil (PdN), cheliceral neuropil (ChN), opisthosomal neuropil (OpN,

1280 which is still visible until (L.)), and the esophageal passage. (J. – T.) Supraesophageal ganglion, with

1281 marked features including the stomodeal bridge (STb), protocerebral tract, protocerebral commissure

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1281 marked features including the stomodeal bridge (STb), protocerebral tract, protocerebral commissi

1282 (PCC), hagstone neuropil (HsN), mushroom body (haft, body, and head), tonsillar neuropil, arcuate

1283 (ventra 1282 (PCC), hagstone neuropil (HsN), mushroom body (haft, body, and head), tonsillar neuropil, arcuate bod
1283 (ventral and dorsal lobes, ABv and ABd, respectively), and protocereral bridge (PCB).
1284 **Figure 3: Mushroo** 1283 (ventral and dorsal lobes, ABv and ABd, respectively), and protocereral bridge (PCB).

1284
 1285 Figure 3: Mushroom bodies.
 1286 (A.) 3D rendering of mushroom body neuropil as annotated from averaged α-synapsi 1284

1284 **Figure 3: Mushroom bodies.**

1285 **Figure 3: Mushroom bodies.**

1286 **(A.)** 3D rendering of mushroom body neuropil as annotated from averaged α -synapsi

1287 (top) and oblique posterior (bottom) **(B.)** Maxi 1285
1285
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1291 1285 **Figure 3: Mushroom bodies.**

1286 (A.) 3D rendering of mushroo

1287 (top) and oblique posterior (k

1288 showing the mushroom bodie

1299 supraesophageal ganglion (C.

1290 synapsin volume (ventral (top

1291 label

1289 supraesophageal ganglion (C.) Optical sections of the supraesophageal ganglion from an
1290 synapsin volume (ventral (top) to dorsal (bottom). The haft, body and head regions of th
1291 labelled (D.) α-βTubulin3 (ma

1286 (A.) 3D rendering of mushroom body neuropil as annotated from averaged α-synapsin volume, dorsal
1287 (top) and oblique posterior (bottom) (B.) Maximum intensity projection of averaged α-synapsin volum
1288 showing 1287 (top) and oblique posterior (bottom) (**B.**) Maximum intensity projection of averaged α-synapsin volume,
1288 showing the mushroom bodies to be the most strongly immunoreactive structure in the
1289 supraesophageal g 1289 supraesophageal ganglion (C.) Optical sections of the supraesophageal ganglion from an averaged α-
1290 synapsin volume (ventral (top) to dorsal (bottom). The haft, body and head regions of the MB are
1291 labelled 1291 labelled (**D**.) α-βTubulin3 (magenta) immunoreactivity aligned with α-Synapsin volume (gray) (com
1292 to bottom portion of previous subfigure) showing the arching form of the mid-line spanning mush
1293 body bridge

1291 labelled (**D.**) α-βTubulin3 (magenta) immunoreactivity aligned with α-Synapsin volume (gray) (compare
1292 to bottom portion of previous subfigure) showing the arching form of the mid-line spanning mushroom
1293 body

1293 body bridge (E.) AllatostatinA immunoreactivity (α-AstA, green) present in the MB haft (pink dotted line

1294 marking location of α-synapsin immunoreactivity) with arrows pointing to innervation from the

1295 post

1295 posterior side (F.) α-βTubulin3 (magenta) and α-Synapsin (green) immunoreactivity in the

1296 supraesophageal ganglion at the plane where the mushroom body hafts appear (round, intense

1297 immunoreactive). Arrows 1295 posterior side (F.) α-βTubulin3 (magenta) and α-Synapsin (green) immunoreactivity in the
1296 supraesophageal ganglion at the plane where the mushroom body hafts appear (round, in
1297 immunoreactive). Arrows mark a

- 1293 body bridge (E.) AllatostatinA immunoreactivity (α-AstA, green) present in the MB haft (pink dotted line
1294 marking location of α-synapsin immunoreactivity) with arrows pointing to innervation from the
1295 poster
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1297 immunoreactive). Arrows mark a fiber tract flanking the haft which could be the origin of the

1298 innervation in the preceding subfigure (G.) Tripart tract entering at the mushroom body head to fu

1299 with the tr 1298 innervation in the preceding subfigure (G.) Tripart tract entering at the mushroom body head

1299 with the tract descending through the MB.

1300 **Figure 4: Visual pathways.**

1302 (A.) Immunostaining for α -HRP (innervation in the preceding subfigure (G.) Tripart tract entering at the mushroom body head to fuse
1299 with the tract descending through the MB.
1300 **Figure 4: Visual pathways.**
1302 **(A.)** Immunostaining for α-HRP (ma 1300

1301 Figure 4: Visual pathways.

1302 (A.) Immunostaining for α -HRP (magenta) f

1303 primary ($\boxed{2}$) and secondary ($\boxed{2}$) visual pathw

1304 (B.) 3D renderings of synpasin-immunoreac

1305 the primary ($\boxed{$ ----
1301
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1305 1301 **Figure 4: Visual pathways.**

1302 **(A.)** Immunostaining for α -

1303 primary ($\boxed{2}$) and secondary **(1304 (B.)** 3D renderings of synpare

1305 the primary ($\boxed{2}$) and secondary

1302 (A.) Immunostaining for α-HRP (magenta) for neuropil and use of DAPI (blue) for nuclei, arrows show the
1303 primary (@) and secondary (@@) visual pathway extensions from the bulk of the supraesophageal tissue
1304 (

1303 primary (図) and secondary (図) visual pathway extensions from the bulk of the supraesophageal tissue (B.) 3D renderings of synpasin-immunoreactivity in the dorsal supraesophageal ganglion, with tissue of the primary (1304 (B.) 3D renderings of synpasin-immunoreactivity in the dorsal supraesophageal ganglion, with tissue of
1305 the primary (圍) and secondary (圍) visual pathway visible.
1305 the primary (囘) and secondary (道) visual pathw

1305 the primary (図) and secondary (図2) visual pathway visible.
The primary (이 and secondary (이 pathway visible.
The pathway visible.

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1312
1313 1307 **Figure 5: Arcuate body.**
1308 (A.) 3D rendering of arcu
1309 oblique, posterior, and are
1310 the ventral arcuate body
1311 magenta envelope repres
1312 row images are dorsal vie
1313 synapsin immunoreactivi
1314 the (A.) 3D rendering of arcuate body neuropil as annotated from averaged α-synapsin volume, posterior
1309 oblique, posterior, and anterior oblique views, left to right, respectively (B.) Individual 3D rendering of
1310 the 1309 oblique, posterior, and anterior oblique views, left to right, respectively (**B.**) Individual 3D rendering of
1310 the ventral arcuate body lobe (ABv, dark green) and dorsal arcuate body lobe (ABd, light green), wit 1311 magenta envelope representing space which would be occupied by the missing lobe in each image. To

1312 row images are dorsal views, bottom row are oblique posterior (C.) Optical horizontal slices of α-

1313 synapsi 1312 row images are dorsal views, bottom row are oblique posterior (C.) Optical horizontal slices of α-
1313 synapsin immunoreactivity from the dorsal supraesophageal ganglion. Top image is relatively ventral to
1314 the 1312 row images are dorsal views, bottom row are oblique posterior (C.) Optical horizontal slices of α-
1313 synapsin immunoreactivity from the dorsal supraesophageal ganglion. Top image is relatively ver
1314 the bottom 1314 the bottom, and shows the ventral arcuate body lobe (ABv), while the bottom image features both the ventral and dorsal arcuate body lobe (ABd). Each lobe contains an anterior (ant.) and posterior (pos.) section, mar 1315 ventral and dorsal arcuate body lobe (ABd). Each lobe contains an anterior (ant.) and posterior (pos.)

1316 section, marked with yellow dashed lines (**D.**) Ventral (top) and dorsal (bottom) views showing aligned

1 1316 section, marked with yellow dashed lines (D.) Ventral (top) and dorsal (bottom) views showing aligne

1317 image volumes of Proctolin (α -Proctolin, yellow), Crustacean Cardioactive Peptide (α -CCAP, cyan) and

1 1316 section, marked with yellow dashed lines (D.) Ventral (top) and dorsal (bottom) views showing aligned

1317 image volumes of Proctolin (α-Proctolin, yellow), Crustacean Cardioactive Peptide (α-CCAP, cyan) and

1318 1318 FMRFamide (α-FMRFamide, red) immunoreactivity, demonstrating distinct structures as well as

overlapping innervation of the arcuate body layers (**E.**) α-βTubulin3 (magenta) and α-Synapsin (green)

immunoreactivity i

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- 1319 overlapping innervation of the arcuate body layers (**E**.) α-βTubulin3 (magenta) and α-Synapsin (ϵ immunoreactivity in the arcuate body (ventral to dorsal as top to bottom, respectively), with arriver marking wher 1319 overlapping innervation of the arcuate body layers (**E**.) α-βTubulin3 (magenta) and α-Synapsin (green)
1320 immunoreactivity in the arcuate body (ventral to dorsal as top to bottom, respectively), with arrows
1321 ma 1321 marking where pronounced fiber tracts pass through the arcuate body layers (F.) Dorsal view of arcuate body showing layering in ABv and ABd (brace), for Proctolin (α -Proctolin, yellow) and
1323 Octopaminergic/Tyra
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- 1321 marking where pronounced fiber tracts pass through the arcuate body layers (F.) Dorsal view of arcuate

1322 body showing layering in ABv and ABd (brace), for Proctolin (α-Proctolin, yellow) and

1323 Octopaminergic/ 1323 botopaminergic/Tyraminergic (α-TDC2, magenta) immunoreactivity which have over
1324 innervation patterns in the anterior ABd.
1325 **Figure 6: Arcuate body layers revealed by staining for specific neurosignaling popul** 1324 innervation patterns in the anterior ABd.

1325 **Figure 6: Arcuate body layers revealed by staining for specific neurosignaling populations.**

1327 Ventral (left column) and dorsal (right column) horizontal optical s 1325

1326 **Figure 6: Arcuate body layers revealed b**

1327 Ventral (left column) and dorsal (right col

1328 (perimeter of whole arcuate body marked

1329 Dopaminergic (α -TH), Serotonergic (α -5-

1330 AstA), Proc 1326
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1331 1326 **Figure 6: Arcuate body layers revealed by staining for specific neurosignaling populations.**

1327 Ventral (left column) and dorsal (right column) horizontal optical section views of the arcuate

1328 (perimeter of
- 1328 (perimeter of whole arcuate body marked by dashed line) for GABAergic (α -GAD), Cholinergic (α -Ch
1329 Dopaminergic (α -TH), Serotonergic (α -5-HT), Octopaminergic/Tyraminergic (α -TDC2), AllatostatinA
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1329 Dopaminergic (α -TH), Serotonergic (α -5-HT), Octopaminergic/Tyraminergic (α-TDC2), AllatostatinA (α-

1330 AstA), Proctolin (α-Proctolin), Crustacean Cardioactive Peptide (α-CCAP), and FMRFamide (α-

1331 FIRE TO i 1332

1332 **Figure 7: Centrally-located, ton**

1334 **(A.)** 3D rendering of tonsillar net

1335 posterior oblique, anterior obliq

1336 of supraesophageal ganglion wit

1337 tonsillar neuropil is seen central

1338 *(C.)* V 1333
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1339 Figure 7: Centrally-located, tonsillar neuropil.

1334 (A.) 3D rendering of tonsillar neuropil as annot

1335 posterior oblique, anterior oblique, and dorsal

1336 of supraesophageal ganglion with α-Synapsin (

1337 tons 1334 (A.) 3D rendering of tonsillar neuropil as annotated from averaged synapsin immunovolume with

1335 posterior oblique, anterior oblique, and dorsal views, left to right (B.) Oblique horizontal optical se

1336 of su

1335 posterior oblique, anterior oblique, and dorsal views, left to right (**B.**) Oblique horizontal optical section
1336 of supraesophageal ganglion with α -Synapsin (green) and α -BTubulin3 (magenta) immunoreactivity

-
- 1337 tonsillar neuropil is seen centrally, with the arrow denoting a fiber tract which passes medially across it 1335 posterior oblique, anterior oblique, and dorsal views, left to right (B.) Oblique horizontal optical section
1336 of supraesophageal ganglion with α-Synapsin (green) and α-βTubulin3 (magenta) immunoreactivity. The
1 1337 tonsillar neuropil is seen centrally, with the arrow denoting a fiber tract which passes medially across it

1338 (C.) Ventral and dorsal views of the tonsillar neuropil, as demarcated by dotted lines. Synapsin (gra
- 1338 (C.) Ventral and dorsal views of the tonsillar neuropil, as demarcated by dotted lines. Synapsin (gray),

1339 Serotonergic (α-5-HT, green), Octopaminergic/Tyraminergic (α-TDC2, magenta), Proctolin (α-Proctolin,

13 1338 (C.) Ventral and dorsal views of the tonsillar neuropil, as demarcated by dotted lines. Synapsin (gray),
1339 Serotonergic (α-5-HT, green), Octopaminergic/Tyraminergic (α-TDC2, magenta), Proctolin (α-Proctolin
1340
- 1340 Server, Green, Serotonergic (α-15-15, στραπολία), στραπολίας (α-15-15, magenta), Processing, Versional, A
1340 Serotonergic (α-AstA, green) and FMRFamide (α-FMRFamide, red) immunoreactivity.
1341 **Figure 8: Protocere**

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1342 Figure 8: Protocerebral bridge neuropil.
Allatostation and Figure 8: Protocerebral bridge neuropil. 1342
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| 1342 Figure 8: Protocerebral bridge neuropil.

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- 1343 (A.) 3D rendering of protocerebral bridge neuropil as annotated from averaged synapsin
1344 immunovolume. (B.) Ventral and dorsal views of the PCB, as demarcated by dotted lines.
1345 (gray), GABAergic (α-GAD, red),
- 1344 immunovolume. (**B.**) Ventral and dorsal views of the PCB, as demarcated by dotted lines. Synapsin (gray), GABAergic (α-GAD, red), Octopaminergic/Tyraminergic (α-TDC2, magenta), Proctolin (α-Pro
1346 yellow), Choline
- 1346 (gray), Cholinergic (α-ChAT, cyan) and AllatostatinA (α-AstA, green) immunoreactivity. Arrows point to
1347 (posterior protocerebral commissure.
1348 (a.) 3D renderings of averaged U. diversus synganglion with annot
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- 1347 posterior protocerebral commissure.

1348 Figure 9: A potential central complex in *U. diversus*

1350 (A.) 3D renderings of averaged U. diversus synganglion with annotations of potential central complex

1350 (A.) 3 1348

1349 **Figure 9: A potential central complex**

1350 (A.) 3D renderings of averaged U. dive

1351 constituents in shades of green (proto

1352 showing the mushroom body (purple)

1353 complex as found in the insects *R* 1349
1350
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1355 1349 Figure 9: A potential central complex in *U. diversus*
1350 (A.) 3D renderings of averaged U. diversus syngangli
1351 constituents in shades of green (protocerebral bridg
1352 showing the mushroom body (purple) (**B.**) (A.) 3D renderings of averaged U. diversus synganglion with annotations of potential central complex

1351 constituents in shades of green (protocerebral bridge, arcuate body lobes, tonsillar neuropil), also

1352 showing
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- 1352 showing the mushroom body (purple) (**B.**) 3D neuropil renderings from of neuropils of the central

1353 complex as found in the insects *Rhyparabia maderae*, *Scarabaeus lamarcki*, and *Manduca sexta* (in

1354 from 2352 showing the mushroom body (purple) (**B.**) 3D neuropil renderings from of neuropils of the central

2353 complex as found in the insects *Rhyparabia maderae*, *Scarabaeus lamarcki*, and *Manduca sexta* (in

2354 from
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- 1354 from insectbraindb.org)

1355 **Fig. S1: Cholinergic population expression pattern (α-ChAT immunoreactivity)**

1357 α-ChAT (ryan) and α-synapsin (gray) immunoreactivity across the synganglion, top part of image is

13 1355
1356 **Fig. S1: Cholinergic popt**
1357 α -ChAT (cyan) and α -syn
1358 posterior and bottom is
1359 somata of various sizes a
1360 dorsal slice in the subesc 1356
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1361
1362 1356 **Fig. S1: Cholinergic population expression pattern (α-ChAT immunoreactivity)**

1357 α-ChAT (cyan) and α-synapsin (gray) immunoreactivity across the synganglion, t

1358 posterior and bottom is anterior (A.) ventral posterior and bottom is anterior (A.) ventral subesophageal ganglion displaying medially located C
1359 somata of various sizes and staining intensity, as well as somata between leg neuromeres (B.) furth
1360 dorsal slice 1358 posterior and bottom is anterior (A.) ventral subesophageal ganglion displaying medially located ChAT+
1359 somata of various sizes and staining intensity, as well as somata between leg neuromeres (B.) further
1360 do somata of various sizes and staining intensity, as well as somata between leg neuromeres (B.) further
1360 dorsal slice in the subesophageal ganglion showing abundant staining throughout, arrows mark small
1361 intensely C
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- 1361 intensely ChAT+ somata just ventral to the pedipalpal neuropil (C.) overlay with synapsin-
1362 immunoreactivity in the subesophageal ganglion (D.) anteriorly located cluster of the most intensely
1363 ChAT+ somata in
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- 1361 intensely ChAT+ somata just ventral to the pedipalpal neuropil (C.) overlay with synapsin-
1362 immunoreactivity in the subesophageal ganglion (D.) anteriorly located cluster of the mos
1363 ChAT+ somata in proximity 1362 immunoreactivity in the subesophageal ganglion (D.) anteriorly located cluster of the most intensely

1363 ChAT+ somata in proximity to the esophageal passage closure (E.) plane dorsal to esophageal closure

1364 with ChAT+ somata in proximity to the esophageal passage closure (E.) plane dorsal to esophageal closure,
1364 with immunoreactivity in the stomodeal bridge, opisthosomal neuropul and protocerebral tract (F.)
1365 supraesophage with immunoreactivity in the stomodeal bridge, opisthosomal neuropul and protocerebral tract (F.)
1365 supraesophageal ganglion expression at the plane of the mushroom bodies (G.) plane just dorsal to
1366 mushroom body he supraesophageal ganglion expression at the plane of the mushroom bodies (G.) plane just dorsal to the
1366 mushroom body heads, showing putative globuli cells (arrows) within the protrusion of the secondary
1367 visual pat 1367 visual pathway, DAPI stain (red) (H.) further dorsal supraesophageal slice, large arrow marks a couple of very large, strongly stained ChAT+ neurons, smaller arrow shows medially located smaller ChAT+ somata, and ar visual pathway, DAPI stain (red) (H.) further dorsal supraesophageal slice, large arrow marks a couple of
1368 very large, strongly stained ChAT+ neurons, smaller arrow shows medially located smaller ChAT+
1369 somata, an
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- 1369 somata, and arrowheads point to string of ChAT+ somata in the posterior cell layer (I.) far dorsal supraesophageal ganglion showing dispersed ChAT+ somata in the dorsal cap. Arrows indicate are
1370 supraesophageal g
- 1369 somata, and arrowheads point to string of ChAT+ somata in the posterior cell layer (I.) far dorsal end of
1370 supraesophageal ganglion showing dispersed ChAT+ somata in the dorsal cap. Arrows indicate arcs of
1371 i 1371 immunoreactivity part of the protocerebral bridge.

1372 **Fig. S2: GABAergic population expression pattern (** α **-GAD immunoreactivity).**

1373 **Fig. S2: GABAergic population expression pattern (** α **-GAD immunoreactiv** 1372

1373 **Fig. S2: GABAergic population expression pattern**

1374 α -GAD (red) and α -synapsin (gray) immunoreactivity

1375 posterior and bottom is anterior. Lack of signal in in

1376 antibody (A.) GAD-immunoreact
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- 1373
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1379 1373 **Fig. S2: GABAergic population expression pattern (α-GAD immunoreactivity).**

1374 α-GAD (red) and α-synapsin (gray) immunoreactivity across the synganglion, top

1375 posterior and bottom is anterior. Lack of signa
- 1375 posterior and bottom is anterior. Lack of signal in interior of tissue is due to poor penetrance of t
1376 antibody (A.) GAD-immunoreactive somata in the far ventral subesophageal ganglion (B.) in a mo
1377 dorsal pl 1376 antibody (A.) GAD-immunoreactive somata in the far ventral subesophageal ganglion (B.) in a more
1377 dorsal plane (C.) GAD+ somata ventral to the opisthosomal neuromere (D.) GAD-immunoreactivity a
1378 the level of antibody (A.) GAD-immunoreactive somata in the far ventral subesophageal ganglion (B.) in a more
1377 dorsal plane (C.) GAD+ somata ventral to the opisthosomal neuromere (D.) GAD-immunoreactivity a
1378 the level of the st 1377 dorsal plane (C.) GAD+ somata ventral to the opisthosomal neuromere (D.) GAD-immunoreactivity at
1378 the level of the stomodeal bridge, showing ample somata anteriorly, and innervation of the
1379 opisthosomal neurop 1379 opisthosomal neuropil (E.) split views of GAD and synapsin immunoreactivity at the level of
1380 body heads, with arrow indicating a (F.) supraesophageal ganglion view at the level of the to
1381 neuropil showing a gr
- 1379 opisthosomal neuropil (**E.**) split views of GAD and synapsin immunoreactivity at the level of mushroom
1380 body heads, with arrow indicating a (F.) supraesophageal ganglion view at the level of the tonsillar
1381 neu
- 1380 body heads, with arrow indicating a (F.) supraesophageal ganglion view at the level of the tonsillar
1381 neuropil showing a grouping of GAD+ somata appearing in the medio-lateral axis (G.) dorsal
1382 supraesophageal
- 1381 neuropil showing a grouping of GAD+ somata appearing in the medio-lateral axis (**G**.) dorsal
1382 supraesophageal ganglion view revealing columns of GAD+ somata on the anterior side as w
with a supraesophageal ganglio 1382 supraesophageal ganglion view revealing columns of GAD+ somata on the anterior side as well as

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1384 faintly visible neurites crossing perpendicular to the arcuate body layers.

1385 **Fig. S3: Dopaminergic population expression pattern (α-TH immunoreactivity).**

1386 **Fig. S3: Dopaminergic population expression pat** 1385
 1385 Fig. S3: Dopaminergic population expression pattern (α **-TH immunoreadors)**
 1387 α -TH (green) immunoreactivity across the synganglion, top part of image
 1388 anterior (**A**.) TH+ somata in the ventr 1386
1387
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1390
1391
1392 1386 **Fig. S3: Dopaminergic population expression pattern (α-TH immunoreactivity).**

1387 α-TH (green) immunoreactivity across the synganglion, top part of image is poste

1388 anterior (**A**.) TH+ somata in the ventral s anterior (A.) TH+ somata in the ventral subesophageal ganglion, with brightened view on right. The
1389 approximate boundary of the tissue marked by the dotted line. Each leg neuropil is associated with
1390 cluster of som anterior (A.) TH+ somata in the ventral subesophageal ganglion, with brightened view on right. The

1389 approximate boundary of the tissue marked by the dotted line. Each leg neuropil is associated with

1390 cluster of s 1390 cluster of somata made of a smaller, more numerous population (arrowheads), and 1-2 larger neuron

1391 (arrows) (B.) maximum projection focus on the mesh-like filling of leg neuropils by TH varicosities,

1392 dotted 1391 (arrows) (**B.**) maximum projection focus on the mesh-like filling of leg neuropils by TH varicosities,

1392 dotted line showing perimeter of leg neuropil 2, as an example (**C.**) Fibers of the ventral-most tract

139 1391 (arrows) (**B.**) maximum projection focus on the mesh-like filling of leg neuropils by TH varicosities,

1392 dotted line showing perimeter of leg neuropil 2, as an example (C.) Fibers of the ventral-most tract

1393 dotted line showing perimeter of leg neuropil 2, as an example (C.) Fibers of the ventral-most tract
1393 travelling parallel to the midline and showing commissures. Arrows mark a cluster of somata ventra
1394 the pedipalp 1394 the pedipalpal neuropil which project to the pedipalpal and cheliceral commissures (D.) further dorsal
1395 view of the subesophageal ganglion, the thicker medial tracts running in the anterio-posterial axis are
1396 1394 the pedipalpal neuropil which project to the pedipalpal and cheliceral commissures (D.) further dorsal
1395 view of the subesophageal ganglion, the thicker medial tracts running in the anterio-posterial axis are
1396 1396 part of the intermediate-tracts (as defined by Auletta et al., 2019(4)), the thinner lateral tract (left side

1397 is part of the ventral-most tract (E.) fully visible intermediate-tract, containing a chiasm is seen 1397 is part of the ventral-most tract (E.) fully visible intermediate-tract, containing a chiasm is seen medially

1398 the ventral tract fibers are lateral and also give rise to 6 major midline crossing commissures,

13 1397 is part of the ventral-most tract (E.) fully visible intermediate-tract, containing a chiasm is seen medially,

1398 the ventral tract fibers are lateral and also give rise to 6 major midline crossing commissures,

13 1399 representing the 4 leg neuropils and pedipalpal and cheliceral neuropils. Somata ventral to the opisthosomal neuropil are seen posteriorly (F.) Tandem clusters of two pairs of TH+ somata (a adjacent to the closure of 1400 opisthosomal neuropil are seen posteriorly (F.) Tandem clusters of two pairs of TH+ somata (armow and alay
1401 adjacent to the closure of the esophageal passage, with immunoreactivity visible in the stomod
1402 bridg opisthosomal neuropil are seen posteriorly (F.) Tandem clusters of two pairs of TH+ somata (arrows)

adjacent to the closure of the esophageal passage, with immunoreactivity visible in the stomodeal

bridge (asterisk) just indele (asterisk) just posteriorly. Arrowhead marks the descending projection of the 4 lateral neuro presented in the next two subfigures. Opisthosomal neuropil immunoreactivity (brace) shows thick tracts on the perimeter, 1403 presented in the next two subfigures. Opisthosomal neuropil immunoreactivity (brace) shows thick

1404 tracts on the perimeter, and crossing fibers internally, as well as somata on the lateral aspect. (G.)

1405 maxi 1404 tracts on the perimeter, and crossing fibers internally, as well as somata on the lateral aspect. (G.)

1405 maximum projection of ventral supraesophageal, arrows marking an additional cluster of 5 TH+ son

1406 dors 1404 tracts on the perimeter, and crossing fibers internally, as well as somata on the lateral aspect. (G.)
1405 maximum projection of ventral supraesophageal, arrows marking an additional cluster of 5 TH+ sor
1406 dorsal 1406 dorsal to the preceeding subfigure. (H.) Four neuron lateral cluster (arrow) giving rise to projections

1407 joining within the protocerebral dorsal tract as well as a subset descending to the intermediate-tract of
 1406 dorsal to the preceeding subfigure. (**H.**) Four neuron lateral cluster (arrow) giving rise to projections
1407 joining within the protocerebral dorsal tract as well as a subset descending to the intermediate-tract
140 1408 the subesophageal ganglion (**l. - J.**) Max projection views of the dorsal supraesophageal ganglion where

1409 a single (arrows) and doublet (arrowheads) contribute substantially to the TH immunoreactivity in this

1 1408 the subesophageal ganglion (I. – J.) Max projection views of the dorsal supraesophageal ganglion where

1409 a single (arrows) and doublet (arrowheads) contribute substantially to the TH immunoreactivity in this

141 1410 region, with the doublet population arching laterally to join the PCDT, and single medial neuron also

1411 contributing, while innervating the arcuate body layer seen in (K.) where a cluster of 2 or 3 TH+ somata

14 1411 contributing, while innervating the arcuate body layer seen in (K.) where a cluster of 2 or 3 TH+ some

1412 are found centrally, which do not have a counterpart in previously examined species.

1413 **Fig. S4: Seroto**

1411 contributing, while innervating the arcuate body layer seen in (**K**.) where a cluster of 2 or 3 TH+ somata
1412 are found centrally, which do not have a counterpart in previously examined species.
1413 **Fig. 54: Ser** 1413

1414 **Fig. S4: Serotoninergic population expression pattern (** α **-5-HT immunoreactivity).**

1415 α -5-HT (green) and α -synapsin (gray) immunoreactivity across the synganglion, top pa

1416 posterior and bottom 1414
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1420 1414 **Fig. S4: Serotoninergic population expression pattern (α-5-HT immunoreactivity).**

1415 α -5-HT (green) and α -synapsin (gray) immunoreactivity across the synganglion, top

1416 posterior and bottom is anterior 1416 posterior and bottom is anterior (A.) ventral subesophageal ganglion where clusters of ~5 somata

1417 positive for 5-HT are seen at the medial aspect of the leg neuropils (B.) further dorsal subesophagea

1418 gangl 1416 posterior and bottom is anterior (A.) ventral subesophageal ganglion where clusters of ~5 somata

1417 positive for 5-HT are seen at the medial aspect of the leg neuropils (**B.**) further dorsal subesophage

1418 gang 1417 positive for 5-HT are seen at the medial aspect of the leg neuropils (B.) further dorsal subesophageal
1418 ganglion plane showing pattern of neuropil innervation (brace) comprised of a posterior and anterior
1419 hal be half, leaving a dearth of signal in the center of the neuropil (C.) 5-HT+ somata present anteriorly
1420 (arrows), ventral to the pedipalpal neuropil, pathways of the ventral-tract appear internally (D.) 5-HT
1421 immun 1419 half, leaving a dearth of signal in the center of the neuropil (C.) 5-HT+ somata present anteriorly (arrows), ventral to the pedipalpal neuropil, pathways of the ventral-tract appear internally (D.)!
1421 immunoreacti 1420 (arrows), ventral to the pedipalpal neuropil, pathways of the ventral-tract appear internally (**D.**) 5-HT immunoreactive somata (arrows) with thick neurites found ventral to the opisthosomal neuropil (**E.)**
1422 begin 1421 immunoreactive somata (arrows) with thick neurites found ventral to the opisthosomal neuropil (**E.)**
1422 beginning planes of the supraesophageal ganglion showing a bridging commissure on the posterior si
1423 with pr 1423 beginning planes of the suppression ganglion showing a bringing commission showing present the posterior show
1423 with pronounced immunoreactivity in the adjacent region (F.) multiple strong 5-HT+ puncta adjoin the 1423 with pronounced immunoreactivity in the adjacent region (F.) multiple strong 5-HT+ puncta adjoin the

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- 1425 structure forms (**G**.) through arches of innervation travelling medially to midline varicosities (**H**.) which
1426 all intersect, beginning innervation just anteriorly of the hagstone neuropil. In the posterior
1427 s suppraesophageal ganglion at this plane an umbrella-like structure of fine varicosities appear.

1428 Continuation of the umbrella-like structure found posteriorly, with expanding immunoreacti

1429 hagstone neuropil (brac 1429 hagstone neuropil (brace) found aside the midline (J.) strongly immunoreactive 5-HT neurons near the
1430 plan of the mushroom body head, whose neurites innervate the area found laterally to the somata. (K.
1431 fain
- 1425 structure forms (**G**.) through arches of innervation travelling medially to midline varicosities (**H**.) which
1426 all intersect, beginning innervation just anteriorly of the hagstone neuropil. In the posterior
1427 s
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-

1427 supraesophageal ganglion at this plane an umbrella-like structure of fine varicosities appears (I.)
1428 Continuation of the umbrella-like structure found posteriorly, with expanding immunoreactivity in
1429 hagstone

1429 hagstone neuropil (brace) found aside the midline (J.) strongly immunoreactive 5-HT neurons near the
1430 plan of the mushroom body head, whose neurites innervate the area found laterally to the somata. (K.
1431 fai 1430 plan of the mushroom body head, whose neurites innervate the area found laterally to the somata. (K.)
1431 faint evidence of 5-HT+ populations in the far dorsal supraesophageal ganglion, 5-HT immunoreactivity
1432 i 1432 in layers of arcuate body seen posteriorly.

1432 in layers of arcuate body seen posteriorly.

1433 **Fig. S5: Octopaminergic/Tyraminergic population expression pattern (α-TDC2 immunoreactivity).**

1435 **α-TDC2 (mage** 1433
1433 **Fig. S5: Octopaminergic/Tyraminergic pop**
1435 **α-TDC2 (magenta) and α-synapsin (gray) in**
1436 **posterior and bottom is anterior (A.) ventrals of ventral subesophageal ganglion demons
1438 of ventral subesoph** ----
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1440 1434 **Fig. S5: Octopaminergic/Tyraminergic population expression pattern (α-TDC2 immunoreactivity).**

1435 α-TDC2 (magenta) and α-synapsin (gray) immunoreactivity across the synganglion, top part of imag

1436 posterior 1436 posterior and bottom is anterior (A.) ventral subesophageal ganglion horizontal optical slice showing
1437 medial clusters of TDC2+ somata corresponding to each leg neuropil (B.) maximum intensity projection
1438 of 1436 posterior and bottom is anterior (A.) ventral subesophageal ganglion horizontal optical slice showing
1437 medial clusters of TDC2+ somata corresponding to each leg neuropil (B.) maximum intensity projectic
1438 of ve medial clusters of TDC2+ somata corresponding to each leg neuropil (B.) maximum intensity projection
1438 of ventral subesophageal ganglion demonstrating an anterior/posterior division in innervation pattern
1439 within ea 1439 within each leg neuropil, with sparse heavy puncta posteriorly and denser but diffuse patterning

1439 within each leg neuropil, with sparse heavy puncta posteriorly and denser but diffuse patterning

1440 anteriorly anteriorly (C.) Bright TDC2+ somata (arrows) ventral to the opisthosomal neuropil, TDC2+

1440 anteriorly (C.) Bright TDC2+ somata (arrows) ventral to the opisthosomal neuropil, TDC2+

1441 immunoreactivity in the medial f 1440 anteriorly (C.) Bright TDC2+ somata (arrows) ventral to the opisthosomal neuropil, TDC2+

1441 immunoreactivity in the medial fiber tracts and pedipalpal neuropil (D.) horizontal optical

1442 opisthosomal neuromere 1441 immunoreactivity in the medial fiber tracts and pedipalpal neuropil (D.) horizontal optical slice showing

1442 opisthosomal neuromere posteriorly, and anteriorly the region ventral to the closure of the esophageal
 1443 passage. Anteriolaterally, TDC2+ immunoreactivity is seen in the cheliceral neuropil. A triangular

1444 strucuture (brace) is formed as strings of puncta travel posteriorly to become heavier on the lateral

1445 per 1444 strucuture (brace) is formed as strings of puncta travel posteriorly to become heavier on the late
1445 perimeters of the opisthosomal neuropil. Interiorly there is a ring-like structure and chiasm with
1446 spoke neu perimeters of the opisthosomal neuropil. Interiorly there is a ring-like structure and chiasm with fine
1446 spoke neurites connecting to it (**E**.) the same view as preceeding but overlayed onto α-synapsin
1447 immunoreac 1446 spoke neurites connecting to it (E.) the same view as preceeding but overlayed onto α -synapsin
1447 immunoreactivity (F.) maximum intensity projection encompassing a span from the level of the
1448 esophageal pass 1446 spoke neurites connecting to it (**E**.) the same view as preceeding but overlayed onto α-synapsin
1447 immunoreactivity (**F**.) maximum intensity projection encompassing a span from the level of the
1448 esophageal pa immunoreactivity (F.) maximum intensity projection encompassing a span from the level of the
1448 esophageal passage to the appearance of the bridge, where a cluster of 15-20 TDC2+ somata ar
1449 (arrows). The opisthosmal 1449 (arrows). The opisthosmal neuropil displays strings of immunoreactivity along the borders, roughly

1450 parallel to the midline, as well as travelling laterally across the halves of the neuropil (G.) TDC2+

1451 imm 1450 parallel to the midline, as well as travelling laterally across the halves of the neuropil (G.) TDC2+
1451 immunoreactivity is present in the stomodeal bridge, seen immediately posterior to the somata clu
1452 In the parallel to the midline, as well as travelling laterally across the halves of the neuropil (G.) TDC2+
1451 immunoreactivity is present in the stomodeal bridge, seen immediately posterior to the somata
1452 In the opisthoso 1452 In the opisthosomal neuropil, pronounced tracts run along the length of the midline, with a fine arching
1453 commissure at the posterior end (arrow). (H.) maximum intensity projection of planes just dorsal to the
145 1453 commissure at the posterior end (arrow). (H.) maximum intensity projection of planes just dorsal to the
1454 preceeding figure demonstrate a lateral nested pathway of longitudinal puncta, and an additional strance
14 1453 commissure at the posterior end (arrow). (H.) maximum intensity projection of planes just dorsal to the
1454 preceeding figure demonstrate a lateral nested pathway of longitudinal puncta, and an additional stranc
14 1455 positioned in the medial-lateral direction (brace) (I.) supraesophageal ganglion plane at the level of the
1456 MB hafts displays ample TDC2+ immunoreactivity, with presence in the umbrella-like structure at the
145 1455 positioned in the medial-lateral direction (brace) (I.) supraesophageal ganglion plane at the level of the
1456 MB hafts displays ample TDC2+ immunoreactivity, with presence in the umbrella-like structure at the
145 1457 posterior side, sparser puncta within the bounds of the hagstone neuropil, and signal found anterio-
1458 mushroom body heads, where strands of immunoreactivity also follow the contours of the lateral edg
1459 mushro 1465 intensity projection from supraesophageal ganglion, with TDC2+ somata (arrow), and innervation
1466 patterns of the ventral and dorsal arcuate body lobes visible posteriorly. 1458 medially to the mushroom body (J.) which continues in these areas dorsally to the level of the
1459 mushroom body heads, where strands of immunoreactivity also follow the contours of the late
1460 of the supraesophag 1460 of the supraesophageal ganglion (K.) α -TDC2 (magenta) and α -5-HT (green) immunoreactivity overlaps
1461 in the centrally located tonsillar neuropil, showing TDC2+ signal in a peripheral pattern, with more 5-H 1460 of the supraesophageal ganglion (K.) α -TDC2 (magenta) and α -5-HT (green) immunoreactivity overlaps
1461 in the centrally located tonsillar neuropil, showing TDC2+ signal in a peripheral pattern, with more 5-HT+
14 1462 immunoreactivity at the center of the neuropil. (L.) subesophageal ganglion plane at the level of the arcuate body and protocerebral bridge, with magnification focusing on a series of puncta (arrows) which might be in 1462 immunoreactivity at the center of the neuropil. (L.) subesophageal ganglion plane at the level of the
1463 arcuate body and protocerebral bridge, with magnification focusing on a series of puncta (arrows) w
1464 might 1464 might be indicative of innervation to or passage by the tonsillar neuropil (M.) Further dorsal maximum
1465 intensity projection from supraesophageal ganglion, with TDC2+ somata (arrow), and innervation
1466 patterns 1464 might be indicative of innervation to or passage by the tonsillar neuropil (**M.**) Further dorsal maximum
1465 intensity projection from supraesophageal ganglion, with TDC2+ somata (arrow), and innervation
1466 pattern 1466 patterns of the ventral and dorsal arcuate body lobes visible posteriorly. 1466 patterns of the ventral and dorsal arcuate body lobes visible posteriorly.

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1474 1468 **Fig. S6: AllatostatinA population expression pattern (α-AstA immunoreactivity).**

1469 α-AstA (green) and α-synapsin (gray) immunoreactivity across the synganglion, to

1470 posterior and bottom is anterior (A.) ve 1470 posterior and bottom is anterior (A.) ventral subesophageal ganglion slice with clusters (arrow) and
1471 individual AstA+ somata (B.) maximum intensity projection of planes in the subesophageal ganglion
1472 reveali 1470 posterior and bottom is anterior (A.) ventral subesophageal ganglion slice with clusters (arrow) and
1471 individual AstA+ somata (B.) maximum intensity projection of planes in the subesophageal ganglion
1472 revealin 1471 individual AstA+ somata (B.) maximum intensity projection of planes in the subesophageal ganglion
1472 revealing AstA+ varicosities in the posterior halves of the leg neuropils (C.) AstA+ immunoreactivity i
1473 later 1472 revealing AstA+ varicosities in the posterior halves of the leg neuropils (C.) AstA+ immunoreactivity in
1473 lateral branches of the centro-lateral tract, supplying the leg neuropils (D.) a section of AstA+
1474 immu lateral branches of the centro-lateral tract, supplying the leg neuropils (D.) a section of AstA+
1474 immunoreactivity is visible adjacent to the esophagus (brace) with (E.) thin neurites at the cro
1475 the stomodeal bri 1474 immunoreactivity is visible adjacent to the esophagus (brace) with (E.) thin neurites at the crossing of
1475 the stomodeal bridge (arrow). Paired longitudinal strands of puncta are seen extending into the
1476 opisth 1476 opisthosomal neuromere, posteriorly (F.) a more robust commissure and appreciable immunore
1477 is seen on the posterior side of the ventral supraesophageal ganglion (G.) Medially-arching circu
1478 pattern of AstA+ i 1476 opisthosomal neuromere, posteriorly (F.) a more robust commissure and appreciable immunoreactivity

1477 is seen on the posterior side of the ventral supraesophageal ganglion (G.) Medially-arching circular

1478 patt 1477 is seen on the posterior side of the ventral supraesophageal ganglion (G.) Medially-arching circular
1478 pattern of AstA+ immunoreactivity in the posterior supraesophageal ganglion, similar to 5-HT signa
1479 the sam 1479 the same region (H.) Plane of supraesophageal ganglion at the level of the mushroom body hafts,

1480 showing strong expression on the posterior side, AstA+ immunoreactivity encompassing the umbrella-

1481 like form 1479 the same region (H.) Plane of supraesophageal ganglion at the level of the mushroom body hafts,
1480 showing strong expression on the posterior side, AstA+ immunoreactivity encompassing the umbr
1481 like form seen in 1480 showing strong expression on the posterior side, AstA+ immunoreactivity encompassing the umbrella-1482 (arrows) (I.) a pair of large, intensely AstA+ somata are present deep within the furrow of the anterior
1483 (arrows) (I.) a pair of large, intensely AstA+ somata are present deep within the furrow of the anterior
14 1482 (arrows) (I.) a pair of large, intensely AstA+ somata are present deep within the furrow of the anterior
1483 somata field, sending neurites into the immediately posterior tonsillar neuropil, whose shape is
1484 disti 1484 distinguishable (J.) Just dorsally, the centrally located tonsillar neuropil is still visible, as the arch

1485 posterior protocerebral commissure is visible laterally and posteriorly. A pair of AstA+ somata are

1 1484 distinguishable (J.) Just dorsally, the centrally located tonsillar neuropil is still visible, as the arching
1485 posterior protocerebral commissure is visible laterally and posteriorly. A pair of AstA+ somata are
1 1486 present laterally. (K.) AstA+ innervation of the posterior side of the ventral arcuate body (ABv), with

1487 circular units of immunoreactivity visible on the posterior edge (arrowheads), suggestive of colum

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1486 present laterally. (**K.**) AstA+ innervation of the posterior side of the ventral arcuate body (ABv), with
1487 circular units of immunoreactivity visible on the posterior edge (arrowheads), suggestive of columna
148 1488 structure.

1489 **Fig. S7: Proctolin population expression pattern (** α **-Proctolin immunoreactivity).**

1491 α -Proctolin (yellow) and α -synapsin (gray) immunoreactivity across the synganglion, top part of image 1489

1489 **Fig. S7: Pre**

1491 **α-Proctolin**

1492 posterior a

1493 showing a

1494 Proctolin+ ----
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1495
1496 1490 **Fig. S7: Proctolin population expression pattern (α-Proctolin immunoreactivity).**

1491 α-Proctolin (yellow) and α-synapsin (gray) immunoreactivity across the synganglic

1492 posterior and bottom is anterior (A.) 1492 posterior and bottom is anterior (A.) maximum intensity projection of ventral subesophageal ganglion

1493 showing a single brightly Proctolin+ neuronal cell body per each leg neuropil. More faintly labelled

1494 Pr posterior and bottom is anterior (A.) maximum intensity projection of ventral subesophageal ganglion
1493 showing a single brightly Proctolin+ neuronal cell body per each leg neuropil. More faintly labelled
1494 Proctolin+ Proctolin+ somata are also visible (**B**.) Optical plane in the subesophageal ganglion at the level of the perchapal perchapal neuropil, showing a cluster of Proctolin+ somata (arrow) and a concentration of signal in immedi Proctolin+ somata are also visible (B.) Optical plane in the subesophageal ganglion at the level of the
1495 pedipalpal neuropil, showing a cluster of Proctolin+ somata (arrow) and a concentration of signal in t
1496 immed 1496 immediately posterior-medial vicinity. Small Proctolin+ somata are also seen in the field ventral to the
1497 opisthosomal neuropil (arrowheads). (C.) further dorsal view of the subesophageal ganglion at the level
149 1497 opisthosomal neuropil (arrowheads). (C.) further dorsal view of the subesophageal ganglion at the leve
1498 of commissures of the major dorsal tract. Densely-immunoreactive pair of roughly circular shapes
1499 (arrow) 1497 opisthosomal neuropil (arrowheads). (C.) further dorsal view of the subesophageal ganglion at the level
1498 of commissures of the major dorsal tract. Densely-immunoreactive pair of roughly circular shapes
1499 (arrow (arrow) represent a tract which is rising directly dorsally (D.) Proctolin+ immunoreactivity is preser
1500 the opisthosomal neuropil, covering similar trajectories as other immunostains (e.g. TDC2), but in
1501 more fragm (arrow) represent a tract which is rising directly dorsally (D.) Proctolin+ immunoreactivity is present in
1500 the opisthosomal neuropil, covering similar trajectories as other immunostains (e.g. TDC2), but in a
1501 mmun 1501 more fragmentary manner (E.) Optical section at the level of the stomodeal bridge, featuring Procto

1502 immunoreactivity crossing the midline on the anterior and posterior bounds of the bridge. Proctolin

1503 immun 1501 more fragmentary manner (E.) Optical section at the level of the stomodeal bridge, featuring Proctolin
1502 immunoreactivity crossing the midline on the anterior and posterior bounds of the bridge. Proctolin
1503 immu immunoreactivty is also seen concentrated adjacent to the midline on the posterior side of the emer
1504 supraesophageal ganglion, which is seen further dorsally (F.) in addition to immunoreactivity in a pat
1505 medial to 1504 supraesophageal ganglion, which is seen further dorsally (F.) in addition to immunoreactivity in a patch
1505 medial to the synapsin-negative channel through which the protocerebral tract travels, a zone not
1506 obvi 1504 supraesophageal ganglion, which is seen further dorsally (F.) in addition to immunoreactivity in a patch
1505 medial to the synapsin-negative channel through which the protocerebral tract travels, a zone not
1506 obvi 1506 obviously present with other immunostains. (G.) Proctolin+ somata become visible in the anterior
1507 somata field beginning at the level of the mushroom body hafts, where Proctolin immunoreactivity
1508 concentrated 1506 obviously present with other immunostains. (**G.**) Proctolin+ somata become visible in the anterior
1507 somata field beginning at the level of the mushroom body hafts, where Proctolin immunoreactivity
1508 concentrate 1508 concentrated posteriorly about the midline, and the hagstone neuropil is also highlighted. (H.) Further
1508 concentrated posteriorly about the midline, and the hagstone neuropil is also highlighted. (H.) Further 1508 concentrated posteriorly about the midline, and the hagstone neuropil is also highlighted. (H.) Further

1510 cup-shape structure formed by the mushroom body hafts and body, which is also a distinctive feature of α -Proctolin staining. Somata continue in the anterior furrow, likewise (I.) further dorsally where faint Proc 1511 a-Proctolin staining. Somata continue in the anterior furrow, likewise (I.) further dorsally where faint
1512 Proctolin+ somata (arrow) are present at the level of the mushroom body heads (J.) At this level too, as
15 1511 a-Proctolin staining. Somata continue in the anterior furrow, likewise (I.) further dorsally where faint

1512 Proctolin+ somata (arrow) are present at the level of the mushroom body heads (J.) At this level too, a
 1512 Proctolin+ somata (arrow) are present at the level of the mushroom body heads (J.) At this level too, as
1513 shown in a maximum intensity projection of the neighboring planes, a strongly immunoreactive strand
1514 of 1514 of varicosities begins anterio-laterally (brace) (K.) continuing posterior-medially, to bifurcate into a
1515 medial and posterior facing branch (brace). Proctolin immunoreactivity is seen centrally in the posterior
1 1514 of varicosities begins anterio-laterally (brace) (K.) continuing posterior-medially, to bifurcate into a
1515 medial and posterior facing branch (brace). Proctolin immunoreactivity is seen centrally in the post
1516 a 1516 aspect of the tonsillar neuropil. (L.) maximum intensity projection spanning planes in the previous
1517 subfigure as well as dorsal ones overlays a dorsal strand of immunoreactivity which we describe as a
1518 dorsal 1516 aspect of the tonsillar neuropil. (L.) maximum intensity projection spanning planes in the previous
1517 subfigure as well as dorsal ones overlays a dorsal strand of immunoreactivity which we describe as
1518 dorsal 1518 dorsal posterior protocerebral commissure, crossing the midline just anterior to the arcuate body (M
1519 maximum intensity projection of planes of far dorsal supraesophageal ganglion showing Proctolin+
1520 somata d 1518 dorsal posterior protocerebral commissure, crossing the midline just anterior to the arcuate body (M.)
1519 maximum intensity projection of planes of far dorsal supraesophageal ganglion showing Proctolin+
1520 is al

1520 somata distributed centrally and laterally, layering pattern of the ventral and dorsal arcuate body lo

1521 is also visible posteriorly.

1522 **Fig. S8: Crustacean cardioactive peptide population expression pattern** 1521 is also visible posteriorly.

1522 **Fig. S8: Crustacean cardioactive peptide population expression pattern (** α **-CCAP immunoreactivity).**

1524 α -CCAP (red) and α -synapsin (gray) immunoreactivity across the syn 1522

1523 **Fig. S8: Crustacean cardic**

1524 α -CCAP (red) and α -synap

1525 posterior and bottom is a

1526 showing clustering of CCA

1527 as well as the immunorea 1523
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1529 1523 **Fig. S8: Crustacean cardioactive peptide population expression pattern (α-CCAP immunoreactivity).**

1524 α-CCAP (red) and α-synapsin (gray) immunoreactivity across the synganglion, top part of image is

1525 poster 1525 posterior and bottom is anterior (A.) maximum intensity projection of ventral supraesophageal ga
1526 showing clustering of CCAP+ somata (B.) further dorsal plane showing sparsely located CCAP+ son
1527 as well as th 1525 posterior and bottom is anterior (A.) maximum intensity projection of ventral supraesophageal ganglion
1526 showing clustering of CCAP+ somata (B.) further dorsal plane showing sparsely located CCAP+ somata,
1527 as w 1526 showing clustering of CCAP+ somata (**B**.) further dorsal plane showing sparsely located CCAP+ somata,
1527 as well as the immunoreactivity pattern within the leg neuropils made of a evenly-spaced distribution
1528 bri 1528 bright puncta but only in the posterior portion of each neuropil (C.) CCAP immunoreactivity is visible
1529 anteriorly around the pedipalpal neuropil, and faint CCAP+ somata are also seen in the area ventral to
1530 t bright puncta but only in the posterior portion of each neuropil (C.) CCAP immunoreactivity is visible
1529 anteriorly around the pedipalpal neuropil, and faint CCAP+ somata are also seen in the area ventral to
1530 the op 1530 the opisthosomal neuropil (arrow). (**D.**) Horizontal optical slice at the plane of the stomodeal bridge
1531 showing where CCAP immunoreactivity is present. Dense staining is also apparent in the opisthosomal
1532 neu the opisthosomal neuropil (arrow). (**D.**) Horizontal optical slice at the plane of the stomodeal bridge
1531 showing where CCAP immunoreactivity is present. Dense staining is also apparent in the opisthosom
1532 neuropil (neuropil (E.) Supraesophageal ganglion plane at the level of the mushroom bodies where CCAP
immunoreactivity is punctate broadly across the tissue, with some concentrations in the posterior
1533 immunoreactivity is punctat neuropil (E.) Supraesophageal ganglion plane at the level of the mushroom bodies where CCAP
1533 immunoreactivity is punctate broadly across the tissue, with some concentrations in the posteri
1534 umbrella-like structure 1534 umbrella-like structure and the anterior bounds of the hagstone neuropil (F.) Supraesophageal gar
1535 plane at the emergence of the ventral arcuate body lobe, with arrow marking the ventral trajector
1536 the PCDt (G 1534 umbrella-like structure and the anterior bounds of the hagstone neuropil (F.) Supraesophageal ganglion
1535 plane at the emergence of the ventral arcuate body lobe, with arrow marking the ventral trajectory of
1536 1536 the PCDt (G.) maximum intensity projection of planes in the vicinity of the ventral arcuate body lobe,

1537 showing clustering of CCAP+ somata deep and medial in the anterior furrow of neuronal cell bodies (H

1538 1536 the PCDt (G.) maximum intensity projection of planes in the vicinity of the ventral arcuate body lobe,

1537 showing clustering of CCAP+ somata deep and medial in the anterior furrow of neuronal cell bodies (1

1538 1537 showing clustering of CCAP+ somata deep and medial in the anterior furrow of neuronal cell bodies (H.)
1538 dispersed CCAP+ somata at the dorsal end of the supraesophageal ganglion, with abundant CCAP
1539 innervatio

1540
1541 **Fig. S9: FMRFamide population expression pattern (** α **-FMRFamide** α **-FMRFamide (red) and** α **-synapsin (gray) immunoreactivity a
1543 posterior and bottom is anterior (A.) ventral subesophageal g
1544 FMRFamid**

1539 innervation of all layers of the arcuate body seen posteriorly.

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 1541 Fig. S9: FMRFamide population expression pattern (α **-FMRFamide immunoreactivity).**

1542 α -FMRFamide (red) and α -synapsin (gray) im

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1547 1541 **Fig. S9: FMRFamide population expression pattern (α-FMRFamide immunoreactivity).**

1542 α-FMRFamide (red) and α-synapsin (gray) immunoreactivity across the synganglion, top

1543 posterior and bottom is anterior (1543 posterior and bottom is anterior (A.) ventral subesophageal ganglion showing distribution of
1544 FMRFamide+ somata (B.) FMRFamide immunoreactivity in the leg neuropils and somata pres
1545 the cell bodies ventral to
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- 1543 posterior and bottom is anterior (A.) ventral subesophageal ganglion showing distribution of
1543 posterior and bottom is anterior (A.) ventral subesophageal ganglion showing distribution of
1545 FMRFamide+ somata (B 1544 FMRFamide+ somata (**B.**) FMRFamide immunoreactivity in the leg neuropils and somata present among
1545 the cell bodies ventral to the opisthosomal neuropil (arrows) (C.) a dorsal subesophageal plane at the
1546 level 1545 the cell bodies ventral to the opisthosomal neuropil (arrows) (C.) a dorsal subesophageal plane at the
1546 level of the major neuropil commissures showing FMRFamide immunoreactivity (D.) FMRFamide+
1547 somata in the 1546 level of the major neuropil commissures showing FMRFamide immunoreactivity (D.) FMRFamide+
1547 somata in the anterior cell body wall (arrows), with immunoreactivity around the cheliceral neurop
1548 FMRFamide innerva 1548 FMRFamide innervation of the opisthosomal neuropil is also apparent (E.) continuing dorsally, at the plane of the stomodeal bridge (F.) FMRFamide immunoreactivity is extensive across the plane of the stomodeal bridge 1548 FMRFamide innervation of the opisthosomal neuropil is also apparent (**E.**) continuing dorsally, at the
1549 plane of the stomodeal bridge (**F.**) FMRFamide immunoreactivity is extensive across the
1549 plane of the sto
- 1549 plane of the stomodeal bridge (F.) FMRFamide immunoreactivity is extensive across the

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- 1550 supraesophageal ganglion, as seen for the plane of the mushroom body (G.) as well as further dorsally
1551 where the arcuate body emerges. An approximately rectangular pattern of immunoreactivity (brace) is
1552 seen
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- 1552 seen posterior to the tonsillar neuropil, which is distinctive to FMRFamide immunoreactivty. FMRFamid
1553 signal is seen posteriorly in both sublayers of the ventral arcuate body (H.) while a pronounced layer of
1554 1553 signal is seen posteriorly in both sublayers of the ventral arcuate body (H.) while a pronounced layer of
1554 FMRFamide immunoreactivity appears in the posterior aspect of the dorsal arcuate body layer.
1555 FMRFamid
-
- 1553 signal is seen posteriorly in both sublayers of the ventral arcuate body (**H.**) while a pronounced layer of
1554 FMRFamide immunoreactivity appears in the posterior aspect of the dorsal arcuate body layer.
1555 FMRFam 1555 FMRFamide + somata are abundantly distributed across the dorsal end of the supraesophageal ϵ .
1556 1556 FMRFamilie- somata are abundantly distributed across the dorsal end of the supraesophageal ganglion. The supraesophageal ganglion \mathcal{L}

Scarabaeus lamarcki (Scarab beetle)

Rhyparabia maderae (Cockroach)

Manduca sexta (Moth) insectbraindb.org

Central body (lower) Noduli

Protocerebral bridge Central body (upper)