Abstract

We discovered a new type of dendritic spine. It is found on space-specific neurons in the barn owl inferior colliculus, a site of experience-dependent plasticity. Connectomic analysis revealed dendritic protrusions of unprecedented morphology including topological holes, hence termed 'toric' spines (n = 76). More significantly, presynaptic terminals converging onto individual toric spines displayed numerous active zones (up to 49) derived from multiple axons (up to 11) with incoming trajectories distributed widely throughout 3D space. This arrangement is suited to integrate input sources. Dense reconstruction of two toric spines revealed that they were unconnected with the majority (~84%) of intertwined axons, implying a high capacity for information storage. We developed an *ex vivo* slice preparation and provide the first published data on space-specific neuron intrinsic properties, including cellular subtypes with and without toric-like spines. We propose that toric spines are a cellular locus of sensory integration and behavioral learning.

15 **Significance statement**

16 The majority of excitatory synapses in the brain are formed onto dendritic spines, which
17 typically act to isolate the action of individual synapses. We discovered a new type of
18 spine which in contrast receives convergent input from many different axons. These
19 'toric' spines, named for their topological holes, are found on space-specific neurons in
20 the barn owl auditory system. We used a combination of *in vivo* electrophysiology,
21 super-resolution optical microscopy and serial block electron microscopy to characterize
22 the ultrastructure and wiring of toric spines, and *ex vivo* patch clamp recording to
23 elucidate the cells' electrical properties. These data lead us to propose that toric spines
24 serve as microanatomical hubs for neuronal computation, plasticity and learning.

25 **INTRODUCTION**

26 Neuronal computations are shaped by postsynaptic morphology (Rall, 1974). Across
27 circuits and species, dendritic spines receive the majority of excitatory synaptic input
28 (Hering and Sheng, 2001). Most spines have narrow necks that serve to
29 compartmentalize, electrically (Yuste, 2013; Kwon et al., 2017) and biochemically
30 (Muller and Connor, 1991; Sabatini et al., 2001), a small number of synaptic inputs -
31 usually one. In this arrangement the dendritic branch performs the first stage of
32 integration of inputs coming from multiple sources (Poirazi et al., 2003). Here, we
33 identify an alternative motif involving first stage integration via a new type of dendritic
34 spine.
35
36 Space-specific neurons (SSNs) in the barn owl inferior colliculus Knudsen and Konishi,
37 1978) compute sound source direction via integration of binaural cues, interaural time
38 difference (ITD) and interaural level difference (ILD), and the elimination of phase
39 ambiguity by convergence across frequency channels (Wagner et al., 1987; Olsen et
40 al., 1989; Brainard et al., 1992; Konishi, 2003; Singheiser et al., 2012), computations
41 known to involve multiplication and non-linear thresholding, respectively (Pena and
42 Konishi, 2000, 2001, 2002, 2004). In addition, space-specific neuron responses can be
43 modulated by visual input (Bergan and Knudsen, 2009) and attention (Winkowski and
44 Knudsen, 2006; Mysore and Knudsen, 2014). Thus, space-specific neurons act as
45 pattern detectors via the integration of complex sensory inputs, and make response
46 adjustments based on behavioral context.
47
48 Space-specific neurons provide an opportunity to understand how neuronal
49 computations are shaped by microanatomical patterns of synaptic convergence, but
50 their ultrastructure is unknown. To fill this gap we used serial block-face scanning
51 electron microscopy (SBEM) (Denk and Horstmann, 2004) and stimulated emission
52 depletion (STED) microscopy (Hell, 2003). Space-specific neurons in young adult owls
53 were labeled *in vivo* by focal injection of retrograde tracer at functionally defined map
54 locations (Rodriguez-Contreras et al., 2005). STED imaging revealed two cellular
55 subtypes, one studded with typical spines and the other with a previously unreported

56 type of spine with unusually complex and variable morphology, termed 'toric' spines.
57 SBEM imaging was used to reconstruct all 76 toric spines found on the soma and
58 proximal dendrites of one space-specific neuron, along with their associated presynaptic
59 terminals. In length, cytoplasmic volume and innervation density, toric spines resemble
60 thorny excrescences (TEs) found on pyramidal cells in mammalian hippocampus and
61 amygdala (Amaral and Dent, 1981). Thorny excrescences receive numerous active
62 zones (Chicurel and Harris, 1992) coming from one or at most two input sources (Wilke
63 et al., 2013) (e.g. individual mossy fibers in the hippocampus), forming the so-called
64 detonator synapse (Urban et al., 2001). We found that toric spines receive a
65 comparable number of active zones, but in contrast, these come from up to 11 different
66 inputs. The latter arrangement is suited for integration, not detonation nor
67 compartmentalization.
68
69 To date, knowledge of space-specific neuron function derives from extracellular
70 recordings (numerous studies) and three reports using intracellular sharp electrodes
71 (Pena and Konishi, 2001, 2002, 2004). Neither technique provides information on
72 intrinsic electrical properties or is capable of resolving individual synaptic events. We
73 performed *ex vivo* patch-clamp recordings from putative space-specific neurons in brain
74 slices from juvenile owls. Many of these cells exhibited large, atypical spines including
75 toric-like structures, the apparent developmental precursors to mature toric spines. In
76 total, these findings and newly established methods provide a path to investigate the
77 causal connections between microanatomical structure and the neuronal computations
78 that underlie high-level pattern detection.

79 **MATERIALS AND METHODS**

80 *Animals.* 12 barn owls (Tyto alba) of unidentified sexes were used in this study. Adult
81 animals (>250d old) were group-housed in large flight aviaries while juveniles (35-40d)
82 remained in isolated nest boxes with parents and siblings. All husbandry and
83 experimental methods were approved by the University 1's Institutional Animal Care and
84 Use Committee.

85

86 *Microelectrode recording and retrograde labeling* in vivo

87 Four adult owls were used for *in vivo* retrograde labeling. Owls were anesthetized using
88 2% isofluorane, a mixture of nitrous oxide and oxygen (1:1) and wrapped in soft cloth.
89 Craniotomies were opened above the tectal lobes and the owl was secured in a
90 stereotax located inside a soundproof recording room. Insulated tungsten recording
91 electrodes ~6 MOhms impedance (FHC Bowdoin, ME) were lowered through the
92 forebrain to the optic tectum (OT) based initially on stereotaxic coordinates (17 mm
93 rostral to the neck muscle insertion, 6 mm lateral to the midline) and then refined on the
94 basis of response criteria. Visual receptive fields were measured by computer-controlled
95 projection of static or moving dots onto a calibrated screen and the responses were
96 used to navigate to the deep layers of OT (in 5/6 injections) based on characteristic
97 latencies, preference for negative contrast stimuli, and absence of bursting behavior.
98 Sites representing frontal space near 0^0 azimuth were targeted. ITD tuning was
99 determined via dichotic stimulation delivered through speakers (ED-21913-000,
100 Knowles Electronics) positioned 5 mm from the tympanic membrane. Tungsten
101 electrodes were removed and replaced with glass electrodes in the same location (1.5
102 mm borosilicate glass, 10-20 μm tip), containing 10% biotinylated dextran-conjugated
103 tetramethylrhodamine 3000 MW (microruby, #D-7162; Thermo Fisher, Walthman, MA)
104 or 10% lysine fixable dextran 3000 MW (Texas-red, #D3328; Thermo Fisher, Walthman,
105 MA) in 1% potassium chloride solution. Chloridized silver wires were used to record
106 physiological responses to confirm injection location. Ionophoretic injections were
107 performed using 7 second on/off cycles of 3 μA for 15 minutes. Glass electrodes were
108 withdrawn and the procedure was repeated on the opposite tectal lobe. In 1/6 injections,
109 the external nucleus of the inferior colliculus (ICX) was directly targeted (Table 1).

110 Following injections, craniotomies were disinfected with 1% chloroptic ointment. After 3-
111 7 day survival time, owls were anesthetized using 5% isofluorane + 1:1 nitrous
112 oxide/oxygen. Heparin (300 U) was injected into the left cardiac ventricle and the owl
113 was perfused with 0.1 M phosphate buffer (P04, pH 7.4), followed by 4%
114 paraformaldehyde in PO4 buffer (for STED imaging; see below for tissue preparation for
115 SBEM imaging). Brains were removed and placed in 30% sucrose and 0.1 M PB for 24
116 hours. Tectal lobes were isolated, sectioned at 50 μm on a vibratome (Leica, Wetzlar,
117 Germany), and mounted with an antifade agent (Prolong gold, P36930; Thermo Fisher,
118 Walthman, MA).
119
120 *STED imaging and morphometric analysis*
121 Imaging was performed on a Leica TCS SP8 STED 3x confocal microscope (Leica
122 Microsystems, Wetzlar, Germany), using a 100x oil-immersion objective (HC PL APO
123 CS2, 1.4 NA; Leica Microsystems, Wetzlar, Germany). Parameters for STED imaging
124 were white light laser, line 551 nm for stimulating, and 660 nm depletion laser, both
125 between 20-40%. Image frame size was 4800 X 4800. Step size for z-stacks was 17.44
126 μm, resulting in pixel/voxel dimensions of 0.024, 0.024, and 0.18 μm (*x*, *y*, and *z*,
127 respectively). Huygens Professional SVI software (Hilversum, Netherlands) was used
128 for deconvolution of all images, using a theoretical point spread function (PSF). All well-
129 labeled neurons in the rostrolateral aspects of IC were imaged (n = 39 cells in perfusion-
130 fixed tissue from three adult owls; n = 16 cells in immersion-fixed tissue from five
131 juvenile owls as described later).
132
133 STED images were analyzed using Imaris 8.1 (Bitplane AG, Zurich, Switzerland).
134 Surface creation was used to measure surface area and volume of the soma and
135 primary dendrites. To isolate the primary dendrite, the slice tool was used to separate
136 the base of the dendrite from the soma at the point where the dendritic caliber first
137 became uniform (i.e. avoiding sharp curvature at the interface). The cross-sectional
138 surface of the ensuing dendritic face was used to measure thickness of primary
139 dendrites.
140

141 Cell classification was accomplished using Imaris' 3D view. For adult tissue, cells
142 containing toric spines were classified as Type I, while cells lacking toric spines were
143 classified as Type II. For juvenile tissue the cell population was more heterogeneous.
144 Cells were not classified as Type I or Type II but scored for the presence or absence of
145 toric-like spines.
146
147 *Serial Block Electron Microscopy (SBEM)*
148 In one owl, microelectrode recordings were made as described above followed by
149 multiple tracer injections in deep OT to increase the likelihood of generating a
150 completely filled neuron. The owl was perfused as before but with 1% glutaraldehyde in
151 cacodylate buffer (Wilke et al., 2013). 150 μm Vibratome sections were collected and
152 shipped to University 2 for further processing and imaging. Sections were freeze-
153 fractured, reacted *en bloc* with streptavidin-HRP (which binds to the biotin moiety on
154 microruby) and detected using diaminobenzidine (DAB). Light microscope image (LM)
155 of the section at this point was obtained. Following osmication and staining with heavy
156 metals the tissue was extremely opaque to visible light, obscuring internal structure. In
157 order to target the labeled cell for SBEM volume imaging, a Zeiss Versa 510 X-ray
158 microscope (XRM) was used to generate 3D tomographic volumes relative to tissue
159 landmarks with 0.7 μm pixel resolution. Based on the XRM volume, a 45 μm x 75 μm x
160 75 μm tissue block centered on the labeled cell was targeted and imaged with a Gatan
161 3View unit on a Zeiss Merlin SEM at 2.5 kV, with a magnification of 2800X, 15k x 15k
162 raster size, and 1 μs pixel dwell time. The final pixel size was 5 nm and the Z step size
163 was 70 nm. The imaging parameters yield sufficient resolution to identify synaptic
164 contacts while maximizing the rate of data collection. The SBEM image volume was
165 post-processed for proper alignment at University 2 and annotated at University 1.
166
167
168 *SBEM analysis*
169 A team of eight annotators used the open source platform IMOD (Kremer, Mastronarde,
170 and McIntosh, 1996) to analyze the SBEM image volume. It contained 54 cell nuclei
171 representing six distinct cell types (neuronal and glial somatic profiles), four putative

172 Space-specific neurons, one of which was well labeled throughout entire cytosolic
173 compartment including spines, an estimated 100s of myelinated axons, 1000s of
174 unmyelinated axons and 10000s of synapses. The labeled SSN was traced through all
175 sections, meshed and rendered in 3D. This reconstruction includes the entire soma and
176 proximal dendrites but not distal dendrites as they extended outside of the volume (SSN
177 dendritic fields can be ~200 μm in diameter). Each toric spine was numbered and
178 isolated for further analysis. The surface areas and contour volumes were determined
179 using the *imodinfo* command, which calculates surface area by adding the areas of the
180 triangles making up the mesh and the volume by summing the areas of each contour
181 times the Z distance connecting the contours. The number of holes was assessed by
182 visual inspection of the 3D model. Because the cytosolic label was dense, in most
183 cases it was not clear whether these tori were cytoplasmically contiguous or interrupted
184 by tight junctions, although at least one clear example of each was found (data not
185 shown).
186
187 Twenty-seven toric spines representing the full spectrum of complexities were selected
188 for further analysis. All unlabeled axons innervating these 27 spines were traced
189 outwards from their points of synaptic contact until the axonal process became
190 ambiguous or reached the end of the volume.
191
192 *Synapse identification in SBEM*
193 Synapses were identified by the presence and distribution of presynaptic vesicles.
194 Active zones were identified using the following criteria: (1) A cluster of five or more pre-
195 synaptic vesicles, (2) Vesicle cluster apposed to the pre-synaptic membrane, (3)
196 Parallel pre- and post-synaptic membranes (no gaps), (4) These features present in at
197 least two consecutive sections. In some cases, the postsynaptic density (PSD) could be
198 discerned but the presence of the dark DAB label made this an unworkable
199 requirement.
200
201 *Axonal trajectory analysis*

202 Seven spines whose axons had been extended for longer distances without ambiguity
203 were selected for trajectory analysis. The length, number of boutons and outgoing
204 trajectories at each axonal end were measured. Length was measured by manual
205 skeletonization. Boutons were defined as axonal swellings containing presynaptic
206 vesicles. Trajectories were determined by marking the XYZ coordinates of the skeleton
207 endpoint as well as a second internal skeletal point within 2 μm of the skeleton
208 endpoint. A small number of axons branched and were split into segments. If the axon
209 had a clear main segment with a smaller side branch it was classified as a "T" branch
210 and the axon was split into two segments; the main segment and side branch. The
211 length and number of boutons were measured separately for each segment. For
212 trajectory analysis, only the two ends of the main segment of these axons were
213 included. If the axon branched into two pieces that were comparable and a "main
214 segment" could not be distinguished, it was classified as a "Y" branch and split into 3
215 segments at the branch point. End 1 of each segment was defined as the open end,
216 and end 2 was the branch point. Only one analyzed axon was classified as a Y-branch.
217 For this axon, the trajectory was measured at all three open ends.
218
219 Axonal trajectories were analyzed by custom scripts written in MatLab (Mathworks). The
220 two points at each end were converted to a 3D vector with the outside point as the
221 vector head and the inside point as the vector tail. All Z-coordinates were scaled by a Z
222 ratio of 13.78 pixels in X-Y / Z section. Each end vector was scaled to a length of 1 and
223 plotted in 3D with its tail at the origin. End vectors were grouped together by spine, with
224 the two vectors from the same axon paired together. To determine whether input
225 trajectories were clustered, the distance between vectors was measured as the
226 minimum angle of arc drawn from the head of one vector to the head of the other.
227 Vector distance (θ) was calculated from the dot product of the Euclidean vectors:
228 $$\cos\emptyset = \frac{\vec{u}\cdot\vec{v}}{\|\vec{u}\| * \|\vec{v}\|}$$
229
230 Distances were calculated pairwise between each end vector and every other end
231 vector on the same spine, except the other end of the same axon. The distance

232 between the two end vectors from the same axon was called “angle of separation” to
233 differentiate from other vector distances. This is a proxy measure for curvature of the
234 axon. A perfectly straight axon yields an angle of separation of 180°. The single Y-
235 branched axon on TS24 was included in the vector plots but excluded from the distance
236 calculations for simplicity.
237
238 *Connection fraction*
239 Potential connectivity for TS1 and TS7 was assessed by partial reconstruction of all
240 unmyelinated axons that passed within 2 μm of the spine. Connection fraction was
241 calculated as (the number of axons that synapse with the spine / the total number of
242 axons within 2 μm). To determine the probability envelope of observing the actual
243 distribution of active zones across each potential axonal input, a bootstrap analysis was
244 constructed based on the simple assumption of equal access. The simulation was run
245 10,000 times for each spine to determine mean values and +/- 2SD.
246
247 In vitro *slice preparation*
248 Six juvenile owls (35-40d old) were used for current clamp experiments to determine
249 intrinsic properties and 2 juveniles (40-50d old) were used for voltage-clamp
250 experiments to measure EPSCs. Juvenile owls were anesthetized with isoflurane and
251 perfused transcardially with heparin-containing (300 units/L) artificial CSF (aCSF; in mM:
252 124 NaCl, 1.3 KCl, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 1.3 $MgCl_2$, 2.5 $CaCl_2$, and 11 glucose).
253 Brains were rapidly removed, blocked, and placed in ice-cold modified aCSF (in mM:
254 248 sucrose, 11 glucose, 1.3 KCl, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 1.3 $MgCl_2$, and 2.5
255 $CaCl_2$). Horizontal midbrain sections (250 μM) containing ICX and ICCls were cut on a
256 vibratome (Leica VT1200S) and transferred to an incubation chamber containing aCSF
257 at 32°C for 30 min before moving to aCSF at room temperature until used for
258 recordings. Recordings were made in a submersion chamber perfused with aCSF (2
259 ml/min) at room temperature. All solutions were bubbled with 95% O_2-5% CO_2
260 continuously.
261
262 *Patch-clamp recordings*

263 Whole-cell patch-clamp recordings were made from visually identified cells in the ICX
264 region using borosilicate glass pipettes (2-4 MΩ). For current-clamp experiments,
265 pipettes were filled with K-methanesulfonate-based internal solution (in mM: 135
266 CH3KO3S, 10 HEPES, 1 EGTA, 4 Mg-ATP, 0.4 Na-GTP, 10 phosphocreatine; 310
267 mOsm) containing 0.5% biocytin (Thermo Fisher). For voltage-clamp experiments, a
268 Cs-methanesulfonate-based internal (in mM: 120 CH3CsO3S, 15 CsCl, 8 NaCl, 10
269 TEA-Cl, 10 HEPES, 0.5 EGTA, 2 QX314, 4 Mg-ATP, 0.3 Na-GTP; 310 mOsm) was
270 used. All chemicals were from Sigma. Recordings were acquired using a Multiclamp
271 700B amplifier (Molecular Devices), digitized at 20 kHz with a Digidata 1550 digitizer
272 (Molecular Devices), and low-pass filtered at 8 kHz. Evoked EPSCs were elicited in
273 cells voltage-clamped at -70 mV in response to a brief electrical stimulus (0.2 ms, 20-50
274 μA) delivered through a concentric bipolar stimulating electrode (FHC). The stimulating
275 electrode was placed locally in the ICC lateral shell. Synaptic blockers (bicuculine: 20
276 μM; NBQX: 10 μM; both from Sigma) were washed in during recordings, as indicated.
277 Slices were immersion-fixed in 4% paraformaldehyde and stained with SA-488 for
278 STED imaging.
279
280 *Data analysis*
281 Resting membrane potential (in mV) was measured while injecting no current (I = 0)
282 immediately after breaking into a cell (average of first 500 ms). Membrane capacitance
283 was estimated using a single-exponential fit to the first 200 ms of the response to a
284 0.5 s injection of hyperpolarizing current. Membrane resistance was measured from the
285 linear portion of a current clamp input-output curve as the slope of the line fitted to the
286 voltages recorded at increasing current injections. Action potentials were elicited by
287 increasing depolarizing steps (0.1-3 nA, 0.5 s) delivered every 15 s. Spikes were
288 identified using a 20 mV/ms threshold of the first-order derivative of membrane
289 potential. Results were identical when a more conservative 50 mV/ms detection
290 threshold was applied. Spike onset was determined using the 2nd-order derivative
291 (maximum acceleration) of membrane potential within a 2-ms time window before and
292 after threshold crossing. Spike peak voltage (V_P) was defined as the maximal V within a
293 2-ms time window from threshold crossing and was used to calculate spike amplitude

294 (peak V minus V at spike onset). First spike latency was defined as the time between
295 stimulus onset and first spike onset, and inter-spike interval as the time between
296 consecutive spike onsets. All data were analyzed with custom scripts written in Matlab.

297 **RESULTS**

298 The strategy for labeling space-specific neurons is shown in Figure 1. Tungsten and
299 glass electrodes were used to record multi-unit activity in the tectal lobes, which include
300 the inferior colliculus (IC) and optic tectum (OT). The lobes are shown in a magnetic
301 resonance image of an intact owl brain at mid-transverse plane (Fig. 1A). Space-
302 specific neurons found in the external nucleus of the inferior colliculus (ICX) are tuned
303 for distinct values of interaural time difference, as illustrated in Fig. 1B. Their auditory
304 spatial receptive fields are constructed from convergent inputs which originate in the
305 lateral shell of the central nucleus of the inferior colliculus (ICCls) and other structures
306 not shown (see Discussion). Space-specific neurons were labeled by injection of the
307 tracer microruby or Texas-red in the deep layers of OT (dOT) (5/6 injections), which
308 receive monosynaptic input from space-specific neurons. Injection sites were confined
309 to the deep layers with minimal rostrocaudal spread (Fig. 1C, D). Interaural time
310 difference and visual spatial tuning at the injection sites (Fig. 1E) represented frontal
311 space; as expected, the retrogradely labeled neurons were found within the region of
312 the inferior colliculus representing frontal space, shown by the white circle in Figure 1C.
313 This region includes the full mediolateral extent of ICX as well as lateral aspects of
314 ICCls. Because no cytoarchitectonic marker that delineates the border between ICCls
315 and ICX (Takahashi et al., 1987; Wagner et al., 2003) was compatible with the three
316 tissue preparation methods used in this study (glutaraldehyde perfusion, 150 μm
317 section; paraformaldehyde perfusion, 50 μm section; *in vitro* slice, 250 μm section),
318 neurons were not assigned to one or the other structure. In one case the injection was
319 directly targeted to ICX; labeled neurons resulting from this injection were
320 indistinguishable in both location and morphology from those labeled by the five dOT
321 injections and therefore included in the analysis below. No neurons outside of the ICCls-
322 ICX microcircuit were analyzed.
323
324 The tracer often produced complete cell-fills, revealing fine aspects of dendritic
325 structure, similar to previous reports using *in vivo* labeling (Pena and Konishi, 2001) or
326 immunostaining for CaMKII (Rodriguez-Contreras et al., 2005; Niederleitner and
327 Luksch, 2012). From these studies it was known that space-specific neurons are large

328 multipolar cells with dendritic protrusions that often included typical spines. Unlike
329 previous studies, we used high-resolution methods to reveal, for the first time, their
330 ultrastructure. Stimulated emission depletion (STED) microscopy of 39 well-labeled
331 space-specific neurons derived from five injections in three owls revealed two cell
332 classes within a spectrum of overlapping morphological features. The most striking
333 difference was the presence of highly atypical dendritic spines on a subset of cells,
334 defined here as Type I space-specific neurons. These large structures exhibited tubular
335 build, tended to lack spine heads and were often riddled with holes (Fig. 2A). The
336 morphological diversity of toric spines was so large that no single feature distinguishing
337 them from typical spines was present in all. Type I space-specific neurons were studded
338 with toric spines and a paucity of typical spines, sometimes none. (Fig. 2A). Type II
339 space-specific neurons, defined here, were studded with a high density of typical spines
340 and devoid of toric spines (Fig. 2B). In addition, Type I space-specific neurons tended to
341 have larger somas (Fig. 2C, F, G) and thicker primary dendrites (Fig. 2D, H). The
342 number of primary dendrites was not significantly different (Fig. 2E, I).
343
344 While the resolution of STED was sufficient to identify toric spines when they presented
345 in the right orientation, high anisotropy (~40nm lateral, ~600nm axial resolution)
346 introduced unacceptable distortions to the apparent 3D structure of spines presenting in
347 other orientations (the majority of spines). Combined with high morphological diversity
348 and narrow tubular structure, volumetric measurement (toric vs typical) were not
349 informative for the population of spines as a whole. We therefore turned to serial block
350 electron microscopy (SBEM). Space-specific neurons were labeled as before, this time
351 with multiple injections in the deep OT to increase the chance of detection, the brain
352 perfused with glutaraldehyde and reacted *en bloc* with DAB to produce a durable
353 reaction product that survived all subsequent processing. One well-labeled space-
354 specific neuron was found on the ICCls-ICX border (Fig. 3 LM panel). After osmication
355 the labeled SSN was isolated using X-ray microscopy (XRM panel) and a 75 x 75 x 45
356 μm volume (253,125 μm^3) imaged at 5 nm lateral resolution.
357 The DAB reaction product completely filled the neuron, which received profuse synaptic
358 contacts from many unlabeled afferents (Fig. 3 SBEM panel). Volumetric reconstruction

359 revealed a large multipolar neuron (Fig. 4), bearing strong resemblance to Type I
360 space-specific neurons identified by STED. In particular, the soma and its six thick
361 primary dendrites were studded with 76 toric spines (TS1 – TS76) and devoid of typical
362 spines. Comparison of representative “zoos” of toric spines identified by SBEM or STED
363 (Fig. 5) indicated that these populations are qualitatively indistinguishable. A thin
364 process resembling an axon emerged from near the base of one primary dendrite. No
365 somatic axon was found.
366
367 To map synaptic input, all active zones were identified by a team of annotators using
368 criteria defined in the Methods. Active zones occurring on the soma, dendrites and toric
369 spines appeared similar as a relatively homogenous population of chemical synapses
370 with clusters of synaptic vesicles apposed to the postsynaptic membrane (Figure 6).
371 Bifurcated synapses with two or more active zones belonging to the same axonal
372 bouton were occasionally observed on all compartments. 622 active zones were found
373 on toric spines and 754 on the soma and dendrites, indicating that toric spines are a
374 major input hub (Figure 7A-C). The innervation density (# active zones per surface
375 area) of toric spines was on average higher than that observed on the six primary
376 dendrites, soma, or thin process (Figure 7D).
377
378 Quantification of the ultrastructural features of toric spines is shown in Figure 8.
379 Volumes (mean = 1.99×10^9 nm^3) and surface areas (mean = 8.20×10^7 nm^2) varied
380 over a wide range and were strongly correlated, the latter consistent with structures built
381 largely of narrow tubes (Fig. 8A). Holes (up to seven on a single spine) were found on
382 37% of spines and were more prevalent in larger spines (Fig. 8B). Some holes were
383 formed by tight junctions between two membrane-bound filopodial extensions while
384 others appeared as cytoplasmic continuities (Fig. 9). The number of active zones per
385 spine ranged from 1-49 and was strongly correlated with size (Fig. 8C) and number of
386 holes (Fig. 8D). These data demonstrate large morphological diversity within the toric
387 spine population.
388

389 To determine patterns of synaptic convergence, 27 spines representing the
390 morphological spectrum were selected for reconstruction of axonal inputs. Three
391 examples are shown in Figure 10. In each case, the active zones derived from multiple
392 axons: 10 axons provided 25 AZs to TS1; 3 axons provided 6 AZ to TS2 and 10 axons
393 provided 23 AZ to TS7. In fact, the majority of toric spines (all except three of the 27
394 representative spines) received more than one axonal input. The number of axonal
395 inputs strongly correlated with the number of AZs, and this was true for spines located
396 on the soma or dendrites; spines on the soma tended to receive more inputs than those
397 on the dendrites or thin process (Fig. 11A), consistent with their tendency to be larger.
398
399 To determine the degree to which inputs to a single spine were balanced or unbalanced
400 in anatomical strength we measured the input fraction, defined as the number of active
401 zones made by a single input divided by the total number of active zones received
402 (metric range = 0 to 1). A low fraction indicates the input has a weak contribution to the
403 overall innervation of the spine, while a high fraction indicates the input is a dominant
404 driver. The distribution of input fractions was skewed low (mean = 0.20, SD = 0.19, n =
405 138) indicating the most common arrangement was integration of many individually
406 weak inputs - a situation of anatomical balance (Fig. 11B). Large input fractions were
407 mostly limited to spines that received few inputs (Fig. 11C) and active zones (Fig. 11D),
408 both of which set a high lower limit on the possible values of input fraction. Overall, the
409 common motif was anatomically balanced integration.
410
411 To determine whether multiple inputs derived from the same parent axon, seven spines
412 whose axons had been extended for considerable distance without ambiguity were
413 selected for trajectory analysis (mean length = 20.6 μm; see Methods and Fig. 12A, B).
414 None of these 40 axons joined together. Further, the population of trajectories was
415 distributed near randomly in 3D (Fig. 12C), and this divergent profile (Fig. 12D and Fig.
416 13) was evident at individual spines (with one exception) including all of the most
417 complex spines. The axonal segments tended to exhibit minimal curvature between
418 incoming and outgoing ends (Fig. 14). In total, wide divergence of axons with linear

419 trajectories strongly suggests that the axons originate from diverse input sources (see
420 Discussion).
421
422 To investigate the potential for toric spines to form synapses with novel inputs we
423 investigated the “connection fraction” of two complex spines, TS1 and TS7. Prior work
424 defined “filling fraction” as the number of synapses formed on a postsynaptic target (in
425 that case, a dendrite or neuron) divided by the total number of synapses available within
426 a local volume of surrounding neuropil extending 2 μm in all directions away from the
427 postsynaptic target (Stepanyants et al., 2002). This is the typical range of filopodial
428 extensions that occur across circuits and species in healthy tissue undergoing
429 experience-dependent synaptic plasticity (Grutzendler et al., 2002; Trachtenberg et al.,
430 2002). We define a closely related concept, “connection fraction” (CF), which is the
431 number of axons connected to the spine divided by the total number of axons that could
432 be connected by virtue of proximity and lack of intervening myelin (Mishchenko et al.,
433 2010). To determine CF, all unmyelinated axons within two μm of the target spine were
434 reconstructed (Fig. 15A). Infiltrating TS1 were 61 unmyelinated axons, 10 of which were
435 synaptically connected to it (CF = 0.164). For TS7 the numbers were 64 axons, 10
436 connected, CF = 0.156. These low CFs are on par with prior reports of filling fractions
437 for circuits in mammalian tissue e.g. 0.18 and 0.19 for two densely reconstructed
438 hippocampal dendrites (Mishchenko et al., 2010).
439
440 Synaptic connectivity of the infiltrating axons could in principle be random or structured.
441 To test this, we constructed a bootstrap analysis based on the assumption that each
442 axon has equal ability to form an active zone with the target spine during a
443 hypothesized epoch of microstructural plasticity. We determined the probability
444 envelope for distributing the total number of AZs received (25 for TS1, 23 for TS7)
445 across each potential axonal input (61 for TS1, 64 for TS7). The actual data fell well
446 outside the probability envelope based on random access (Fig. 15C). This indicates that
447 connectivity is structured such that certain axons have, or develop, preferential access.
448 Such non-random connectivity is also found in mammalian cortex (Kasthuri et al., 2015)
449 and thalamus (Morgan et al., 2016) suggesting a common principle for circuit

450 development.To open a path for linking microanatomical patterns of synaptic
451 convergence with functional measurement of neuronal computation, we developed an
452 *ex vivo* slice preparation to record from space-specific neurons. Recording electrodes
453 were targeted to large somata in ICX under visual guidance (Fig. 16A) and used to
454 record membrane potential responses using a current-clamp protocol (Fig. 16B).
455 Neurons (n = 20 from 5 owlets) exhibited a spectrum of intrinsic membrane properties
456 (Fig. 16C, D and Table 2). Some neurons (n = 16) were filled with biocytin for
457 retrospective imaging to determine cell type. In this population of neurons in juvenile
458 owls, large atypical spines were prevalent including toric-like structures of narrow
459 tubular build with multiple arms whose ends often infolded upon themselves, but which
460 infrequently presented as closed holes (Fig. 17D). The morphological diversity was
461 greater than in the adult SSN population, with many juvenile cells expressing some
462 mixture of atypical and typical spines (Fig. 17A, B), while a minority (n = 5) lacked a high
463 density of spines (Fig. 14C). Therefore, juvenile cells were not classified as Type I or
464 Type II, but instead scored for the presence (n = 7) or absence (n = 9) of toric-like
465 spines. There was a trend for juvenile cells with toric-like spines to have larger soma
466 and thicker primary dendrites than those lacking them (Fig. 17E-G), although these
467 differences were not statistically significant, and both juvenile and adult cells had on
468 average the same number of primary dendrites (mean = 4.5 for both), a comparative
469 profile similar to the differences between Type I and Type II adult cells (Fig. 2). In total,
470 the juvenile cells appeared as developmental precursors to toric space-specific
471 neurons. Finally, in separate voltage-clamp experiments, electrical stimulation of the
472 known input source to ICX, the ICCls, evoked typical AMPA-mediated EPSCs (Fig. 16E,
473 F; n = 2), consistent with all prior studies of this monosynaptic connection.

474 **DISCUSSION**
475 We describe the morphology and synaptic innervation patterns of a novel brain
476 structure, toric spines. Across circuits and species, toric spine morphology appears
477 unique. In contrast, their high integrative capacity and low connection fractions,
478 qualitatively similar to sparse reports of complex spine types found in mammalian
479 thalamus, cortex, hippocampus and cerebellum, suggest that toric spines drive cellular
480 computations common across the animal kingdom. . The discussion below critically
481 analyzes toric spine morphology, integrative capacity, implications for cellular
482 computation, potential role in plasticity, and concludes by outlining testable hypotheses
483 to probe more deeply into their structure and function.
484
485 Both STED and SBEM imaging revealed a broad spectrum of spine morphologies.
486 Features that varied markedly across the population include size, number of holes and
487 degree of branching. Features that tend to be shared include narrow tubular build,
488 paucity of bulbous heads, and wide necks at the process interface. This profile
489 distinguishes toric spines from all previously documented spine types including typical
490 thin, stubby, mushroom or branched spines (Bourne and Harris, 2008), and atypical
491 dendritic structures including thorny excrescences, dendritic appendages or glomerular
492 excrescences (Jones and Powell, 1969; Spacek and Lieberman, 1974; Rapisardi and
493 Miles, 1984; Erisir et al., 1997; Morgan et al., 2016). Perhaps most striking, toric spines
494 received up to 49 active zones contributed by up to 11 axonal inputs - a stark contrast to
495 typical spines that compartmentalize single inputs or thorny excrescences which
496 detonate their postsynaptic target (Table 3). Given their size and integrative capacity
497 (discussed below, toric spines could be classified as atypical dendrites or proto-
498 dendrites. We call them spines because, like typical spines, they are substantially
499 smaller in caliber than the parent process, a size differential that exceeds the difference
500 between higher and lower order dendritic branches.
501
502 The narrow tubular build of toric spines should produce a short length constant due to
503 high axial resistance, and therefore predicts that single synaptic inputs made onto one
504 spine are isolated from those onto another (Rall, 1974). At the same time neighboring

505 inputs onto the same spine should be subject to summation. Compartmental simulations
506 of the reconstructed space-specific neuron to test this intuition have so far been
507 unsuccessful due computational limitations of available modeling platforms. In
508 comparison, simulations of neocortical pyramidal cells have shown that neuronal
509 computation can be represented by a two-stage model in which individual dendritic
510 branches perform the first-stage processing of summation of EPSPs (Poirazi et al.,
511 2003). Active properties (voltage-gated ion channels) modify the rules of dendritic
512 integration providing a range of operations from supra-linear to sub-linear. The second
513 stage is linear summation of dendritic branch output at the soma. The multipolar
514 structure of space-specific neurons is well-suited to emulate this processing scheme
515 with the significant addition of a earlier-stage operation at toric spines.
516
517 Direct tests of these computational strategies requires whole-cell recording in
518 combination with targeted synaptic stimulation. We demonstrate, for the first time to our
519 knowledge, the ability to make high quality patch-clamp recordings from ICX neurons.
520 Development of this ex vivo preparation opens a path for understanding the casual
521 connections between microanatomical convergence and cellular computation.
522
523 All large toric spines whose convergence patterns were analyzed (24) received synaptic
524 input from multiple axons, indicating that they are structured to act as integrators.
525 Synaptic convergence onto branched spines and other atypical dendritic protrusions
526 has been suggested by earlier studies. Pioneering work using serial EM to reconstruct
527 rat cerebellar Purkinje cells demonstrated a complex spine type with up to 5 branches.
528 Independent axonal boutons could be found on distal branches of the same spine,
529 several microns apart, raising the possibility that branched spines receive multiple
530 axonal inputs (Harris and Stevens, 1988). The prevalence of branches spines on
531 Purkinje cells has been estimated between 2% and ~6% (4/64) (Harris and Stevens,
532 1988; Lee et al., 2004). It is well established that the large branched spines of rat
533 hippocampal CA3 cells (i.e. thorny excrescences) receive numerous active zones from
534 single mossy fiber boutons. In addition, early reports indicated that multiple boutons can
535 contact a single excrescence, though concluded “it is not known if these were from the

536 same axon" (Chicurel and Harris, 1992). Synaptic glomeruli in mammalian thalamus are
537 known to receive convergent input from anatomically and neurochemically diverse
538 sources (Jones and Powell, 1969; Spacek and Lieberman, 1974; Erisir et al., 1997). In a
539 recent connectomics study, two densely reconstructed glomeruli received input from 15
540 different thalamocortical afferents (Morgan et al., 2016), a level of convergence similar
541 to that observed in our dataset. The central postsynaptic compartment of glomeruli are
542 dendritic shafts from which multiple tiny excrescences emerge, distinct electrotonically
543 from toric spines. Nonetheless, the above results in combination with our findings raise
544 the possibility that synaptic convergence onto complex spine types is more common
545 than currently appreciated.
546 The conclusion that toric spines are innervated by multiple input sources is supported
547 by analysis of axonal convergence onto 27 spines and trajectory analysis for seven. Not
548 one shared branchpoint was found (40 axons, mean length = 20.6 μm). Until the axons
549 are traced back to their parent cell bodies, a single source explanation cannot be
550 logically eliminated. We think it is highly unlikely because it would require parent axons
551 to branch, diverge at least 10s to 100s of μm, then re-converge and terminate within an
552 extremely small volume of tissue. There is no evidence for such an arrangement. Owls
553 are known for unusual axonal specializations – delay lines (Carr and Konishi, 1988) –
554 yet these are found in the projection from nucleus magnocellularis to nucleus laminaris
555 (Carr and Konishi, 1990), distant brainstem structures, not ICX. In fact, ICCls axons,
556 which provide the bulk of input to ICX, exhibit typical terminal fields in which axonal
557 branches diverge, not converge (DeBello et al., 2001; Rodriguez-Contreras et al., 2005;
558 McBride et al., 2008; McBride and DeBello, 2015). In principle, short branchlets with
559 terminal boutons could be within range of a toric spine – but because those
560 branchpoints are mere microns away from the parent axon, such arbors could not have
561 contributed to the divergent trajectories of much longer axonal segments observed in
562 our dataset.
563
564 The fact that individual ICCls axons cannot account for the convergence results does
565 not rule out ICCls axons as a potential input source to toric spines. In fact, space-
566 specific neurons are known to receive input from multiple ICCls neurons, especially

567 ones encoding different sound frequencies. Integration across frequency is required to
568 eliminate phase ambiguity and ultimately provide the owl with an accurate estimation of
569 sound source azimuth. At the level of spiking output, frequency convergence is a non-
570 linear operation. Thus, one hypothesis is that axons originating from multiple ICCls
571 neurons encoding the same interaural time difference but different frequencies
572 converge onto individual toric spines where their EPSPs are combined and thresholded
573 (Pena and Konishi, 2000, 2002). These predictions are testable using dual anterograde
574 injections placed at different dorsoventral laminae, and whole-cell recordings,
575 respectively.
576 A second possibility is that toric spines integrate interaural time difference (ITD) and
577 interaural level difference (ILD), a multiplicative operation. Binaural integration first
578 occurs in the ICCls and is largely inherited by space-specific neurons in ICX. Yet the
579 precise anatomical and functional boundaries between ICCls and ICX are unclear.
580 Several cytoarchitectonic markers delineate the lateral border of ICX, and separately,
581 the border of the core of the central nucleus. But on close inspection of commonly used
582 markers calbindin, calretinin and CaMKII, the ICCls-ICX border appears as a gradient.
583 Microelectrode surveys also support the idea of functional heterogeneity along this
584 border. Moreover, while cytoarchitectonic markers were not routinely employed in our
585 study (see Methods), estimates based on comparison across qualitatively similar
586 sections indicate that at least some Type I space-specific neurons, including the one in
587 the SBEM volume, were located in ICCls. This is a minor revision to the notion that IC-
588 OT projections neurons are strictly confined to ICX. More importantly, it motivates the
589 second hypothesis - that toric spines in ICCls receive convergent input encoding
590 behaviorally relevant ITD-ILD pairs, from contralateral ICC (for ITD) and contralateral
591 dorsal lateral lemniscal nucleus pars posterior (for ILD), and perform a multiplicative
592 operation (Pena and Konishi, 2001) (Pena and Konishi, 2004) via a different
593 complement of ion channels. A third possibility is that both hypotheses are true: toric
594 spines are multipurpose integrators whose computation can be customized to cellular
595 need.
596

597 Other potential sources of input to toric spines include the Arcopallial Gaze Field (Cohen
598 et al., 1998), which provides top-down attentional modulation and the intermediate/deep
599 layers of OT, which provides a visually-based instructive signal to guide calibration of
600 the auditory map (Hyde and Knudsen, 2000; Luksch et al., 2000). Both are anatomically
601 sparse and unlikely to be dominant contributors. Space-specific neurons do receive
602 lateral connections from GABAergic neurons within ICX. The inputs to toric spines we
603 observed, however, more closely resemble asymmetric glutamatergic synapses.
604

605 To our knowledge this is the first report of a toric postsynaptic structure. Presynaptic
606 perforations described extensively in the literature are cytoplasmically contiguous (Sorra
607 et al., 1998). In contrast, at least some tori in our SBEM volume were interrupted by
608 plasma membranes that appeared as sites of contact between two ‘arms’ of the same
609 spine (data not shown). The simplest interpretation is these represent enduring
610 adhesions between filopodial extensions emanating from the same base structure.
611 These could be maintained or later converted to cytoplasmic continuities via
612 anastomosis. Regardless of the developmental mechanics, the relative prevalence and
613 functional properties of contact sites vs cytoplasmic continuities is expected to
614 significantly impact current flow during synaptic stimulation.
615

616 The morphologies of ICX neurons from 35-40d old juveniles labeled *ex vivo* and adult
617 space-specific neurons labeled *in vivo* were qualitatively similar with a few clear
618 differences: (a) juvenile cells often expressed a mix of atypical (branched and toric-like)
619 and typical spines whereas adult cells were dominated by either toric (Type I) or typical
620 (Type II) spines, but never both; (b) juvenile cells appeared smaller, and; (c) juvenile
621 cells sometimes appeared ciliated. We consider three interpretations: *ex vivo* artifact,
622 unrelated cell type or developmental age.
623

624 Preparation of acute brain slices can induce changes in dendritic morphology in
625 comparison to the *in vivo* condition as reflected in perfusion-fixed tissue (Fiala et al.,
626 2003). In this scenario, toric-like spines would represent the slice-induced disassembly
627 of fully-formed toric spines. This is highly unlikely in our view due to the number of

628 neuropil elements involved in such a disassembly, specifically the high density of
629 intertwining axons. Whole cell recordings in juvenile slices were targeted under visual
630 guidance to the largest somatic profiles in ICX, the exact location of retrogradely labeled
631 toric space-specific neurons in adults. That the measured size of juvenile cells was
632 smaller is expected from the differential impact of immersion vs perfusion fixation.
633 Specifically, measurements of three neuron types in primate hippocampus indicate that
634 apparent soma volume is 1.36 – 2.7 times larger in perfusion-fixed than immersion-fixed
635 tissue (Lavenex et al., 2009). On average, our adult cells (perfused brain) were 2.42
636 (volume) and 1.81 (surface area) times as large as our juvenile cells (immersed slices).
637 Further, the one morphological attribute not subject to differential shrinkage – number of
638 primary dendrites – was the same in juveniles (4.5) and adults (4.7). In summary, the
639 most straightforward interpretation is that the observed juvenile cell types represent
640 developmental precursors of adult cell types.
641
642 Lesion reconstruction (Brainard and Knudsen, 1993), anatomical (Feldman and
643 Knudsen, 1997; DeBello et al., 2001) and pharmacological (Feldman et al., 1996; Zheng
644 and Knudsen, 1999) experiments pinpoint space-specific neurons as the cellular locus
645 of learning (Knudsen, 2002). This raises the question of what role, if any, toric spines
646 have in experience-dependent synaptic plasticity (DeBello et al., 2014). We found that
647 toric spines are conspicuously well-structured to be hubs for microstructural plasticity: of
648 all unmyelinated axons passing within 2 microns of TS1 and TS7 only ~16% were
649 synaptically connected. Consequently, the number of different wiring diagrams that
650 could be achieved by biologically plausible, small-scale dendritic extensions is very
651 large (Stepanyants et al., 2002; Stepanyants and Chklovskii, 2005). We propose a
652 model in which toric spines actively search for axonal inputs in their local environment,
653 make "tester" synapses, then stabilize or eliminate these on the basis of efficacy in firing
654 the postsynaptic cell (DeBello, 2008). This would provide a mechanism for pattern
655 detection within input sources carrying a complex array of information, and the means to
656 adapt connectivity to changing experiential needs. The high degree of cytoskeletal
657 dynamics implicit in this model could also explain the broad spectrum of observed

658 morphologies, with each spine stabilized not by genetic programs but by chance
659 encounters of its extensions within a local, yet information diverse, axonal milieu.

660 **FIGURE LEGENDS**

661 **Figure 1 *In vivo* labeling of space-specific neurons (SSNs)**

662 ***A,*** MRI image of owl brain with tectal lobes indicated by asterisks. ***B,*** Diagram of
663 horizontal section through R tectal lobe. ICC = central nucleus of the inferior colliculus;
664 ICX = external nucleus of the inferior colliculus; OT = optic tectum. ITD tuning of SSNs
665 in ICX is indicated in microseconds left-ear leading. Visual receptive field (Vrf) location
666 is indicated in degrees azimuth SSNs project to the deep layers of the OT where the
667 auditory map aligns and integrates with visual space map indicated in degrees azimuth.
668 Tracer injection at 0 μs / 0° in the deep layers of OT (solid red) retrogradely labels SSNs
669 at the cognate map locations in the inferior colliculus. ***C,*** Representative horizontal
670 section (50 μm thick) shows location of injection site (arrow) and location of retrogradely
671 labeled neurons in the inferior colliculus (circle). Green label is immunostaining for
672 CaMKII which is highly expressed in the lateral aspects of ICX. The border between the
673 lateral shell of the ICC (ICCls) and ICX is not well delineated. ***D,*** Higher magnification
674 view of a representative injection (red) in the deep layers of OT. Blue label is DAPI
675 staining. ***E,*** Raster plots displaying the auditory (left) and visual (right) tuning recorded
676 at the site of injection.

677

678 **Figure 2 Type I and Type II space-specific neurons (SSNs)**

679 STED images of representative examples of Type I (***A***) and Type II (***B***) SSNs. N = 39
680 SSNs total, 23 Type I (solid circles in ***C-E***) and 16 Type II (open circles in ***C-E***). ***C,***
681 Soma volume vs surface area across both types: $r = 0.968$, $r^2 = 0.936$, $p <0.00001$, ***D,***
682 Mean cross sectional surface area of primary dendrites vs surface area of the soma: $r =$
683 0.728, $r^2 = .530$, $p < 0.00001$. ***E,*** Mean number of primary dendrites vs surface area of
684 the soma: $r = 0.243$, $r^2 = 0.059$, $p = 0.1356$. ***F***, Mean somatic surface area (Type I =
685 1,895 μm^2 vs Type II = 1,008 μm^2; $p < 0.00001$). ***G***, Mean somatic volume (Type I =
686 4,648 μm^3 vs Type II = 1,909 μm^3; $p = 0.00006$). ***H***, Mean cross sectional surface area
687 of primary dendrites (Type I = 50.7 μm^2 vs Type II = 25.0 μm^2; $p = 0.00148$). ***I***, Mean
688 number of primary dendrites (Type I = 4.96; Type II = 4.44; $p = 0.271$). Comparisons for
689 **F-I** were based on Mann-Whitney U-test.

690 **Figure 3 SBEM pipeline and SSN reconstruction**

691 ***LM,*** Light microscopy of 150 μm thick section reacted en bloc for DAB, prior to
692 osmication. The labeled SSN is shown in the box and appears near the border of ICCls
693 and ICX. ***XRM,*** X-ray microscopy was used to locate the SSN after osmication. ***SBEM,***
694 Serial block electron microscopy was used to image a volume 75 x 75 x 45 μm at 5 nm
695 resolution.
696
697 **Figure 4 Reconstructed Type I space-specific neuron laden with toric spines.**
698 Full reconstruction of all labeled processes contained within the volume.
699 Somatodendritic architecture is rendered in mesh green; toric spines in solid white.
700
701 **Figure 5 Toric spine 'zoo'.** ***A,*** SBEM-based reconstructions of ten example toric
702 spines, selected to cover the range of diversity in size, shape, and complexity. Holes
703 were present in 37% of spines (29/78 spines). Note the relatively uniform process
704 diameter and relative lack of spine heads. ***B,*** STED-based reconstructions of ten
705 example toric spines, selected as above, for comparison. The apparent ultrathin
706 features result from thresholding a locally variable concentration of intracellular tracer as
707 opposed to volumetric reconstruction based on plasma membrane tracing in SBEM.
708 Otherwise, the population of spine morphologies derived from each imaging method
709 appear indistinguishable. Scale bar is 5 μms and applies to both panels.
710
711 **Figure 6 Synapse identification.** Three representative synapses are shown onto the
712 soma (***A***), dendrite (***B***) and toric spine (***C***) of the labeled SSN. For each synapse two
713 successive EM sections are shown. The SSN is darkly labeled and the presynaptic
714 compartment has been traced (light orange fill). The synapse on the soma is bifurcated,
715 with two separate active zones from the same bouton. Bifurcated synapses were also
716 common on the dendrites and toric spines. The axon shown in the bottom images
717 makes synapses onto TS1 (larger spine) and TS62 (smaller spine; synapse shown in
718 EM images), as well as the soma (not shown). See the methods for the criteria used to
719 identify synapses in SBEM.
720 **Figure 7 Distribution of synaptic input onto the labeled SSN.** A team of five
721 annotators independently marked all active zones on the labeled SSN using criteria

722 described in the methods. ***A,*** Labeled SSN without active zones. Scale bar is 5 microns
723 and applies to panels A-C. ***B,*** 754 active zones were found on the soma (bright yellow;
724 228) and five primary dendrites (multiple colors; 526 total). ***C,*** 622 active zones were
725 found on 76 toric spines (pale orange spheres). ***D,*** Innervation density indicated by
726 active zones per surface area (y axis) or active zones per volume (x axis) was on
727 average largest for toric spines (open diamonds; mean = 0.81 and 12.6 for AZ/μm^2 and
728 AZ/μm^3, respectively) than dendrites (open squares; mean = 0.177 and 0.32), soma
729 (filled circle; 0.091 and 0.056) or thin process (filled triangle; 0.279 and 1.41).
730

731 **Figure 8 Morphometric analysis of the toric spine population.** Volume, surface
732 area, number of holes and number of active zones were quantified for each toric spine,
733 (n = 76) with 52 located on dendrites (open squares), 21 on the soma (filled circles) and
734 3 on the thin process (filled triangles). ***A***, Volume vs surface area. Data were marginally
735 better fit (dashed line) by a power law (chi-squared = 8.15 x 10^{14}) than linear regression
736 (chi-squared = 9.28 x 10^{14}; $r^2 = .97$, $p < 0.00001$. ***B***, Holes vs surface area: marginally
737 better fit (dashed line) by power law (chi-squared = 90.5) than linear regression (chi-
738 squared 91.1; $r^2 = .42$, $p < 0.00001$. ***C***, Active zones vs surface area: marginally better
739 fit (dashed line) by linear regression (chi-squared = 1254; $r^2 = .73$, $p < 0.00001$) than
740 power law (chi-squared = 1282) ***D***, Active zones vs holes: best fit by linear regression
741 ($r^2 = .50$, $p < 0.00001$). There was a weak tendency for spines located on the soma to
742 be larger than those located on dendrites, in both volume (mean values 3.13 vs 1.62 x
743 10^9 nm^3; p = 0.026 Mann-Whitney U-test) and surface area (mean values 2.75 vs 1.50 x
744 10^7 nm^2; p = 0.016 Mann-Whitney U-test). Spines on the thin process tended to be
745 smaller though there were not enough data points to make a statistical comparison.
746

747 **Figure 9 Examples of tight junction and cytoplasmic continuities. *A and B,***
748 consecutive EM sections showing of a torus formed by a tight junction (white arrows). ***C,***
749 Location of the tight junction shown above on TS1, indicated by white line. A separate
750 torus exhibited cytoplasmic continuity across all sections (pale loop).
751

752 **Figure 10 Toric spines receive multiple presynaptic inputs.** Representative
753 examples of axonal convergence onto three toric spines for TS 1, TS 2 and TS 7. Top
754 panels depict the spines (light grey) with all of its active zones (yellow circles). The
755 lower panels show one, two and all axonal inputs (10, 3 and 10, respectively) added
756 sequentially in color.
757
758 **Figure 11 Synaptic convergence onto toric spines**. Quantification of synaptic
759 convergence for 27 representative toric spines: 13 located on dendrites (open squares),
760 11 on the soma (filled circles) and 3 on the thin process (filled triangles). ***A***, The number
761 of axonal inputs strongly correlated with the number of active zones (linear regression:
762 r^2 = .813, p = < 0.00001, chi-squared = 49.4) and the data was better fit by an
763 exponential function (chi-squared = 14.59; dotted line). This was also true for both
764 subpopulations of toric spines on the dendrites (linear regression: r^2 = 0.895, p = <
765 0.00001, chi-squared = 8.88; exponential fit chi-squared = 6.177) and on the soma (r =
766 .837, r^2 = .700, p = 0.00125, chi-squared = 25.46; exponential fit chi-squared = 6.65).
767 There was a weak tendency for spines located on the soma to have more inputs (mean
768 values 7.1 vs 4.3; p = 0.03 Mann-Whitney U-test) and active zones (17.9 vs 8.9; p =
769 0.026 Mann-Whitney U-test) than those on the dendrites. ***B***, Histogram of the fraction of
770 total synaptic input to each spine (see Methods) contributed by each axonal input (n =
771 138). ***C***, Input fraction decreased exponentially (dotted line) as the number of inputs
772 increased. ***D***, Input fraction decreased exponentially (dotted line) as the number of
773 active zones increased. For both ***C*** and ***D***, the x-coordinate was randomly shuffled by
774 +/- 0.5 for display purposes only.
775
776 **Figure 12 Trajectory analysis of axonal inputs. *A,*** Representative example trajectory
777 analysis. Axons providing input (in this case to TS1) were extended to the edge of the
778 volume or until a reconstruction ambiguity was encountered. ***B,*** For a total of 40 axons
779 synapsing onto 7 toric spines, the length (red line), number of boutons (blue dots), and
780 the outgoing trajectories at each end of the axon (small yellow dots) were measured
781 (see Methods). ***C,*** Axonal trajectories were represented as vectors through the two end
782 points, scaled to a length of one and plotted in three dimensions with their tails at the

783 origin (n = 81). ***D,*** In plots 1-7, the vectors are grouped by spine and the ends of the
784 same axon are paired visually by color within each plot.
785
786 **Figure 13 Angular distances between the input trajectories onto toric spines.**
787 Histograms of the angular distance between each trajectory vector and every other
788 vector for each spine, excluding the vector from the other end of same axon. Distances
789 were calculated with the dot product (see Methods). Mean across all spines = 89.7°; SD
790 = 40; N = 78 vectors, 976 pairings; bin width = 5. A population of random trajectories
791 predict a Gaussian distribution of distances with a mean of 90°, as observed from 6/7
792 spines and the overall population, while a population of bundled trajectories predicts a
793 bimodal distribution with a mean of 90°, as observed for TS76.
794
795 **Figure 14 Angle of separation between paired ends.** Histograms of the angular
796 distance between the paired ends of each axon. Mean = 132.9°; SD = 31.5; N = 40.
797 Linear trajectories predict a clustered distribution near 180°, as observed for the overall
798 population, while highly curved trajectories predict a Gaussian distribution with a mean
799 near 90°.
800
801 **Figure 15 Potential connectivity of toric spines.** To assess potential connectivity of
802 TS1 and TS7, all unmyelinated axons that passed within 2 μm of the spine were
803 reconstructed. ***A***, SBEM section showing four annotated axons (shaded in color)
804 synapsing onto TS1 (dark purple). Arrows point to nearby axons which do not make any
805 synapses onto TS1. ***B***, All 61 unmyelinated axons within 2 μm of TS1 (solid grey).
806 There were also 5 myelinated long-range axons that came within 2 μm of TS 1 (not
807 shown and excluded from this analysis; see text for explanation). In all, 10 unmyelinated
808 axons synapsed onto TS 1 (solid axons) and 51 did not (meshed axons). Thus, the
809 connection fraction for TS 1 is 0.164. The connection fraction of TS7 (not shown) is
810 0.156 (10 connected axons, 54 unconnected axons. ***C***, Bootstrap analysis of potential
811 connectivity of TS1 (left panel) and TS7 (right panel). Histograms indicate the relative
812 frequency of observing axons connected with the specified number of active zones to
813 TS1 in the real data (solid circles) and 10,000 simulations (grey line; light grey envelope

814 indicates +/- 2 SD). In both spines, four of the ten data points lie outside the envelope of
815 expectation based on random connectivity. Insets display the same data over a
816 magnified range.
817
818 **Figure 16 Patch clamp recordings.** ***A,*** Mosaic image of a 300 μm thick acute
819 horizontal section through the tectal lobe. Photobleached squares reveal locations of
820 recorded neurons in ICX. ***B,*** Example of current step protocol used to measure intrinsic
821 membrane properties (n = 20 cells, 5 slices, 6 owlets age 35-40d). ***C,*** Resistance vs
822 capacitance for 13 cells. ***D,*** action potential (AP) threshold vs resting membrane
823 potential for 20 cells. For both ***C*** and ***D***, filled circles = cells with toric, atypical and
824 typical spines (n = 5); filled triangles = cells with atypical and typical spines but not toric
825 spines (n = 3); open squares = cells with no spines (n = 1); open circles = not
826 determined (n = 11). ***E,*** electrical stimulation experiment with glass recording electrode
827 in ICX and bipolar stimulating electrode in ICCls. ***F,*** Example of electrically-evoked
828 EPSC (n = 2) that was completely abolished by bath application of AMPA receptor
829 antagonist NBQX.
830
831 **Figure 17 Morphologies of putative space-specific neurons in juvenile owls**. ***A-C,***
832 Examples of the morphological diversity of ICX cells in juvenile owls. Cells studded with
833 a variety of spine types - atypical, branched, toric-like, and typical – were common and
834 resembled Type I adult space-specific neurons in multipolar structure (***A, B***). A minority
835 of juvenile cells exhibited relatively few spines (***C***). Toric-like structures with
836 incompletely closed holes (***D***) were found on 7/16 juvenile cells (filled circles in **E, F, G**).
837 Scale bar is 30 μm for ***A-C*** and 5 μm for ***D***. ***E,*** Soma volume vs surface area across
838 both types: r = 0.962, r^2 = 0.925, p <0.00001, ***F,*** Mean cross sectional surface area of
839 primary dendrites vs surface area of the soma: r = 0.676, r^2 = 0.457, p = 0.004. ***G,*** Mean
840 number of primary dendrites vs surface area of the soma: r = 0.323, r^2 = 0.104, p =
841 .222.
842

843 **TABLE LEGENDS**

844 **Table 1 Owls**

845 Owls and procedures.

846

847 **Table 2 Patch-clamp recordings**

848 Intrinsic membrane properties recorded from 20 cells across 5 juvenile owls age 35-
849 40d. All reported data are from cells that passed multiple quality controls.

850

851 **Table 3 Comparison of Spine Types.**

852 The morphologies, dimensions, patterns of synaptic convergence and known or
853 hypothesized functions are shown for typical thin spines, thorny excrescences and toric
854 spines.

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