

37 **Abstract**

38 The cochlear nucleus (CN) transforms the spike trains of spiral ganglion cells into a new set of
39 sensory representations that are essential for auditory discriminations and perception. These
40 transformations require the coordinated activity of different classes of neurons that are
41 embryologically derived from distinct sets of precursors. Decades of investigation have shown
42 that the neurons of the CN are differentiated by their ion channel expression and intrinsic
43 excitability. In the present study we have used linear discriminant analysis (LDA) to perform an
44 unbiased analysis of measures of the responses of CN neurons to current injections to
45 mathematically separate cells on the basis of both morphology and physiology. Recordings
46 were made from cells in brain slices from CBA mice and a transgenic mouse line, NF107,
47 crossed against the Ai32 line. For each cell, responses to current injections were analyzed for
48 spike rate, spike shape (action potential height, afterhyperpolarization depth, first spike half-
49 width), input resistance, resting membrane potential, membrane time constant,
50 hyperpolarization-activated sag and time constant. Cells were filled with dye for morphological
51 classification, and visually classified according to published accounts. The different
52 morphological classes of cells were separated with the LDA. Ventral cochlear nucleus (VCN)
53 bushy cells, planar multipolar (T-stellate) cells, and radiate multipolar (D-stellate) cells were in
54 separate clusters, and were also separated from all of the neurons from the dorsal cochlear
55 nucleus (DCN). Within the DCN, the pyramidal cells and tuberculoventral cells were largely
56 separated from a distinct clusters of cartwheel cells. DCN cells fell largely within a plane in the
57 first 3 principal axes, whereas VCN cells were in 3 clouds approximately orthogonal to this
58 plane. VCN neurons from the two mouse strains were slightly separated, indicating either a
59 strain dependence or the differences in slice preparation methods. We conclude that cochlear
60 nucleus neurons can be objectively distinguished based on their intrinsic electrical properties,
61 but that such distinctions are still best aided by morphological identification.

62

63 **Introduction**

64 Neurons of the mammalian cochlear nucleus exhibit a variety of responses to
65 intracellular current injection, reflecting the distinct expression of collections of ion
66 channels amongst different classes. However, even within a class, such as bushy cells,
67 individual cells may express specific conductances at different magnitudes [1–3],
68 leading to diversity in excitability features such as action potential threshold, action
69 potential height, and rheobase. In spite of this variability, cells of a given morphological
70 class appear to possess common properties that have been used to identify cells on the
71 basis of their electrical signatures alone [4–12].

72

73 Quantitative methods for identifying cell classes have been explored in the context of
74 the myriad interneuronal populations in cortex [13], within the olfactory bulb [14] and
75 across neuronal populations throughout the brain [15]. These methods rely on
76 systematic measurement of distinct features of intrinsic excitability such as action
77 potential shape, firing rates, passive membrane measures, and responses to
78 hyperpolarization, and have used principal components analysis (PCA), support vector
79 machine model, or stepwise linear regressions. Within the cochlear nucleus, application
80 of an hierarchical clustering analysis to *in vivo* single unit data provided evidence for
81 partial separation of unit response types in the gerbil AVCN [16], although further
82 analysis (using PCA) suggested that there was extensive overlap between cell classes.

83

84 Here we apply linear discriminant analysis [17] to the problem of separating cell classes
85 in the cochlear nucleus based on intrinsic excitability. Whereas PCA separates classes

86 by finding the axes that maximize the variance within a data set, and does not rely on
87 labels, LDA maximizes the separation between classes, utilizing label (e.g., class)
88 information. We find that LDA is an effective tool for segregating the cell classes based
89 on their excitability, while also suggesting that there is either overlap between the
90 properties of some of the classes, or that they may not be entirely morphologically
91 distinguishable. Such a classification tool should be useful in future studies of the
92 excitability of cochlear nucleus neurons following hearing loss as a way of objectively
93 assessing how the excitability of neurons changes.

94

95 **Materials and Methods**

96 Whole cell tight-seal recordings were made in brain slices from adult CBA (P28-69) and
97 NF107::Ai32 (P31-166) mice. The NF107::Ai32 mice are the F1 cross of the NF107
98 mouse line, originally from the GENSAT Consortium [18], and the channel rhodopsin
99 (ChR2) expressing line Ai32 [19], and so are on a mixed CD-1, C57Bl/6J and FVB
100 background. The ChR2 was not activated during these experiments. CBA mice were of
101 either sex, whereas the NF107::Ai32 mice were only males, as the Cre driver is carried
102 on the Y chromosome. The data from the CBA mice were taken from a previous series
103 of studies [11,20]. Data from the NF107::Ai32 mice were taken from unpublished work
104 (Kasten, Ropp and Manis, in preparation). The CBA slices were prepared following
105 anesthesia (100 mg/kg ketamine and 10 mg/kg xylazine), and decapitation, with slicing
106 in warm ACSF. The NF107::Ai32 slices were prepared using the same anesthesia
107 followed by transcardial perfusion with an NMDG-based solution [21]. Electrodes
108 contained 126 K-gluconate, 6 KCl, 2 NaCl, 10 HEPES, 0.2 EGTA, 4 Mg-ATP, 0.3 Tris-

109 GTP, and 10 Tris-phosphocreatine, with pH adjusted to 7.2 with KOH and recordings
110 were made with a MultiClamp 700B (Molecular Devices) amplifier, low-pass filtered at
111 6kHz, and digitized at 10-20 kHz with 16-bit A-D converters (National Instruments).
112 Stimulus presentation and acquisition were controlled by either a custom Matlab®
113 program or by *acq4* [22]. All animal procedures were approved by the University of
114 North Carolina Institutional and Animal Care Committee (protocols 12-253, 15-253 and
115 18-160).

116 For each cell, responses to current injections (100-500 msec duration, ranging from -1
117 to 4 nA) were analyzed. Data from either acquisition program were converted to a
118 common format for analysis by Python (V3.6) scripts. Passive measures included input
119 resistance (from the slope of the current-voltage relationship just below rest), resting
120 membrane potential, membrane time constant (measured from responses to small
121 hyperpolarizing current steps that produced 2-10 mV voltage deflections), the
122 magnitude of the hyperpolarization sag [23] and the time constant for the sag measured
123 near -80 mV. Active measures included action potential height (measured from rest to
124 action potential peak), first spike half-width (measured at half the action potential height
125 from rest), afterhyperpolarization depth (measured from rest to the first
126 afterhyperpolarization), an adaptation index measured near firing threshold (see below),
127 the number of rebound spikes after hyperpolarizing steps, the coefficient of variation of
128 interspike intervals, and the slope of the firing rate versus current curve for the first 3
129 current levels above threshold. Cells were filled with dyes (AlexaFluor 488 for CBA
130 mice; tetramethylrhodamine biocytin for the NF107: Ai32 mice) for morphological
131 classification, and visually classified according to published accounts, based on digital

132 images and image stacks collected at low (4X) and high (40-63X) power either during or
133 immediately after each cell was recorded.

134 Adaptation was measured for the lowest two levels of current that elicited spikes as:

$$\frac{-2}{N} \sum_{i=0}^{i=N} \left(\frac{t_i}{t_d} - 0.5 \right)$$

135 Where t_i is the time of the i^{th} spike in the trace, t_d is the trace duration, and N is the
136 number of spikes. This measure ranges from -1 to 1. Neurons that fire regularly without
137 adaptation throughout the trace will have an index of 0. Neurons that fire preferentially
138 only at the onset of the trace will have an index of 1, whereas those that fire near the
139 end of the trace will have an index of -1. Thus, bushy cells will have an index of 1,
140 stellate cells and tuberculoventral cells will usually have an index near 0, and pyramidal
141 cells may have a negative index, depending on the delay to the first spike. Note that this
142 measure depends on the current level that is used relative to the spike threshold, as
143 well as the current duration. The adaptation measured at the threshold current was
144 found to be uninformative in preliminary analyses, and so the only adaptation computed
145 from the next higher current that evoked spikes was used.

146

147 All absolute voltage measurements are corrected for a -11 mV junction potential for the
148 K-gluconate electrodes. All other voltage measurements are differential (action potential
149 height from peak to the minimum of the following AHP) and are independent of the
150 junction potential.

151

152 Computed measures were then analyzed using LDA and PCA using standard libraries
153 in Python (scikit-learn v0.20, Python 3.6), and in R (3.5, using the packages DisplayR
154 and flipMultivariates).

155

156

157 **Results**

158

159 The discharge patterns of cochlear nucleus neurons have been reported in a series of
160 studies over the years from multiple laboratories using similar, but not identical
161 recording conditions. Fig 1 shows the intrinsic physiology of example cells from six
162 major morphological classes as recorded in our dataset. Briefly, bushy cells (Fig 1A)
163 fire 1-3 action potentials at the onset of depolarizing current injections, and are silent
164 thereafter [4,5]. At higher current levels, oscillatory membrane responses, which may
165 represent axonally initiated action potentials, are sometimes visible. In response to
166 hyperpolarizing pulses, bushy cells can show a slow sag in membrane potential, and
167 following the hyperpolarizing step can generate an anodal break spike. The planar
168 multipolar cells (Fig 1B) fire regularly in response to depolarizing current injections, and
169 also show a slow sag in response to hyperpolarizing current steps; they can also show
170 anodal break spikes [11,23]. Radiate multipolar cells also fire regularly in response to
171 depolarization, sometimes exhibiting an adapting spike train. They show a rapid sag in
172 response to hyperpolarization, and frequently have anodal break spikes. As noted
173 previously [11], radiate multipolar cells also may fire only at the onset of a weak
174 depolarizing current pulse. Pyramidal (fusiform) cells of the dorsal cochlear nucleus fire

175 regularly [9,24,25], and may have a long delay to the first spike or a long first interspike
176 interval [25–27]. In the adult mice studies here, these cells do not show a prominent
177 sag, but do show a rapidly activating rectification in response to hyperpolarizing steps,
178 which is likely generated by Kir channels [28]. Cartwheel cells show mixed single-
179 spiking regular firing and burst firing [7,10]. The tuberculoventral neurons show regular
180 firing, and often have trains of rebound spikes after hyperpolarization [12,29]. The
181 principal cell database included 18 bushy cells, 31 planar multipolar cells, 32 radiate
182 multipolar cells, 38 pyramidal cells, 12 cartwheel cells, and 31 tuberculoventral cells.
183 Additional cell classes (all from the DCN) had too few cells for effective classification.
184 These included 1 “Type-B” cell [30], 1 chestnut cell [31], 7 giant cells, 2 “horizontal
185 bipolar” cells (small neurons in the pyramidal cell layer of the DCN with a bipolar
186 morphology where the moderately spiny dendrites reside mainly within the pyramidal
187 cell layer), 2 molecular layer stellate cells [32], 3 unipolar brush cells [31,33], and 3 cells
188 that could not clearly be identified on comparison with the literature. These were not
189 included in the analyses.

190

191 **Fig 1. Examples of discharge patterns and passive responses, for different levels**
192 **of current injection for 6 classes of cochlear nucleus neurons. A. Bushy; B. planar**
193 **multipolar; C. radiate multipolar; D. pyramidal; E. cartwheel; F. tuberculoventral.. Red**
194 **dots indicate spikes evoked by current injection.**

195

196 In order to classify the principal cell types, we extracted a set of measurements from the
197 current-voltage and spiking responses (N = 162 cells). These are illustrated in Figs 2

198 and 3. Fig 2 summarize the passive properties of the cells (columns) against the cell
199 types (rows). From this, differences in the resting membrane potential, input resistances
200 and time constants can be appreciated between the groups. In addition, measurements
201 of the time constant of the hyperpolarizing sag, and a previously-used measure [23], the
202 B/A sag ratio, also show clear differences between the cells, with neurons from the DCN
203 generally showing weaker I_h .

204

205 **Fig 2. Summary of passive measurements from responses to hyperpolarizing**
206 **current pulses across the population of CN neurons.** RMP: resting membrane
207 potential; R_{in} : input resistance; τ_m : membrane time constant; τ_h : time constant of
208 repolarizing sag from traces near -80 mV; B/A: steady-state over peak voltage
209 deflection for hyperpolarizing sag (from Fujino and Oertel, 2001). The ordinate indicates
210 the population density based on the kernel density estimate (blue line). The histograms
211 shows the distribution of values from the population cells for each type and measure.

212

213 Fig 3 shows measures of action potential shape and firing properties. Again, population-
214 based distinctions are evident, such as the relatively small and wide action potentials of
215 bushy cells, and the tendency of tuberculoventral and some cartwheel cells to show
216 rebound responses, and the wide coefficient of variation of firing of the cartwheel cells.
217 The firing rate slope measured near threshold also was lowest for bushy, pyramidal and
218 cartwheel cells, and highest for the planar and radiate multipolar cells, and
219 tuberculoventral cells.

220

221 **Fig 3. Summary of action potential shape and firing patterns across the**
222 **population of CN neurons.** AP Peak: action potential peak potential; APHW: action
223 potential half-width; AHP: action potential afterhyperpolarization; Adaptation: adaptation
224 calculated from the response to a suprathreshold current injection; Rebound: count of
225 rebound action potential after the end of the hyperpolarizing current injection; CV:
226 coefficient of variation of interspike intervals; Irate: slope of the current-firing relationship
227 for current levels just above spike threshold. The ordinate indicates the population
228 density based on the kernel density estimate (blue line). The histograms show the
229 distribution of values from the population cells for each type and measure.

230
231 Next, we submitted the data to a LDA, using all of the parameters measured in Figs 2
232 and 3. Data were first standardized for each measure before being submitted to the
233 LDA. The standardization rescaled the individual measurements for each measurement
234 type so that it had a zero mean and a unit standard deviation. Fig 4 illustrates the first 3
235 components of the LDA, with each cell colored by its classified type, in 3 views (Fig 4A,
236 B, C). The LDA effectively separated the different types of cells into distinct spaces. The
237 bushy and cartwheel cells were the most separated from the remainder of the regular
238 firing cells. Interestingly these two cell groups did not form tight clusters, suggesting
239 some diversity in their properties. The pyramidal and tuberculoventral cells were
240 clustered next to each other, although with minimal overlap. The radiate and planar
241 multipolar cells formed two slightly overlapping clusters that were largely separate from
242 all other cell classes. Note that although most of the bushy, planar and radiate
243 multipolar cells were recorded in CBA mice, those cells recorded from the NF107::Ai32

244 mice (FVB and C57Bl/6 backgrounds; solid symbols) were close to the measures of the
245 CBA populations, although they were slightly separated in one of the first 3 axes, as
246 more clearly seen in Fig 4D, where only cells from the VCN are shown. Cross-validation
247 of the LDA yielded an estimated accuracy of 0.79 (+/- 0.31).

248

249 **Fig 4. LDA with supervised clustering by cell morphology.** A. The first 3 (largest)
250 LDA components are shown in a perspective view. The axes represent the projections
251 of each cell on to the 3 largest components from the standardized data (zero mean, unit
252 standard deviation; therefore there are no units). Note the clear separation of the bushy
253 cell and cartwheel cells populations from the rest of the CN neurons. Although the other
254 populations are close together, they are also separated as can be appreciated by
255 comparing the different views. Cells from CBA mice are shown with open symbols; cells
256 from the NF107 mice are shown with closed symbols. B, C. Two other perspective
257 views (rotated) of the same data as in A. D. A view of the data for the VCN cells only
258 (bushy, planar multipolar and radiate multipolar) for clarity. This is the same perspective
259 view as panel C.

260

261 We also submitted the data set to a standard principal components analysis, following
262 the same standardization across cells for each measure (Fig 5). In this case, the
263 supervisory classifier (cell morphology) was not used in the initial classification. The
264 PCA resulted in clusters of cells from the same morphological class, but these had
265 greater overlap than with the LDA. Cross-validation of the PCA data yielded a low
266 accuracy of 0.17 (+/- 0.086).

267

268 **Fig 5. Principal components analysis (PCA) on the same data set as Fig 4.** The
269 PCA method is not supervised by cell type, and so the results depend only on factors
270 that maximize the variance. The PCA successfully separated the cell classes, but did
271 not have good accuracy with cross-validation. The view shown is the same format as
272 Fig 4A.

273

274 In order to determine which measures provided the most information in the LDA
275 classification, we performed the LDA using combinations of measures, from individual
276 measures through all available measures, and estimated the accuracy of the
277 classification across all cells by dividing the data into training and testing sets. The
278 accuracy as a function of the number of combined measures is shown in Fig 6. As
279 expected, the accuracy improves as new measures are added, up until about 6
280 parameters, at which point the accuracy plateaus. However, the overall *worst* accuracy
281 continues to improve as more measures are added. The black line indicates the mean
282 of the best 5 combinations of measures. From this we conclude that some of the
283 measures are possibly redundant and that some measures may be non-informative.
284 With 7 or 8 combined measures (where the largest number of combinations was
285 tested), AP height, R_{in} , RMP, τ_h and I_{rate} occurred together in each of the 5 most
286 accurate runs. Similar, but not identical distributions were present for 8 combined
287 measures. Note that the accuracy of each point includes a standard deviation estimate
288 (not shown) as it is the result of multiple runs with different subsamples of training and
289 test cells, so the best measures can vary with an arbitrary threshold, and there is no

290 single “optimal” set. With the number of cells in the sample and the large number of
291 parameters, the SD can be 15-20% of the mean value.

292

293 **Fig 6. Accuracy of separation for different number of combined measures, using**
294 **the LDA separation score for each set of measures.** All 12 measures were
295 considered in all possible combinations in groups from 1 to 12 (the number of
296 combinations are shown above the data), and each combination is plotted as a point
297 along the ordinate representing the number of combinations. The mean score is
298 indicated by the red line. The average of the best 5 scores for each combination set are
299 plotted as a black line. In general, including more measures improves the accuracy of
300 the separation of groups.

301

302 To further investigate those factors that drove the prediction accuracy, we performed
303 the same analysis using the R package `flipMultivariates`. Table 1 summarizes the
304 prediction accuracy by cell type, and provides mean measures for each of the
305 parameters. Although all parameters provide a significant contribution (r^2) to the
306 separation, the five that accounted for the largest proportions of the variance ($r^2 > 0.50$)
307 were the AP height, AP half width, the adaptation measure, the coefficient of variation of
308 interspike intervals, and the firing rate slope (I_{rate}). However, all of the measures showed
309 a significant contribution.

310

311

312 **Table 1**

Measure	Bushy	Cartwheel	Radiate Multipolar	Pyramidal	Planar multipolar	Tuberculo-ventral	R ²	p
RMP (mV)	-62.5	-75.2	-63.1	-68.5	-62.7	-67.9	0.41	<0.001
τ_m (ms)	1.29	5.25	2.83	8.04	4.90	12.71	0.41	<0.001
R _{in} (M Ω)	27.5	79.6	60.1	68.8	87.9	155.6	0.39	<0.001
τ_h (ms)	27.5	56.9	12.1	51.0	40.0	74.4	0.32	<0.001
B/A	0.50	0.95	0.39	0.59	0.63	0.71	0.28	<0.001
AP Height (mV)	-27.6	2.9	5.9	2.2	6.7	-6.9	0.63	<0.001
AP Width (ms)	1.32	0.83	0.28	0.40	0.26	0.35	0.73	<0.001
AP AHP (mV)	-57.7	-53.8	-68.3	-64.6	-69.2	-62.1	0.31	<0.001
AP Adaptation	0.96	0.22	0.21	0.10	0.05	0.13	0.62	<0.001
AP Rebound (N)	0.33	1.37	1.17	1.37	1.10	11.41	0.26	<0.001
CV	N/A	0.71	0.07	0.10	0.05	0.14	0.65	<0.001
I _{rate} (spikes/nA)	5.8	768	1158	412	1232	1148	0.51	<0.001
Correct Predictions (%)	94.4	83.3	81.3	92.1	83.9	90.3		

313

314 **Table 1.** Relations between the predictors and each cell class, indicating the number of
 315 correct predictions overall, and for each class. The top predicting variables (largest R²
 316 values) are highlighted in bold. N = 162 cases used in estimation. Null hypotheses: two-
 317 sided; multiple comparisons correction: False Discovery Rate correction applied
 318 simultaneously to entire table. N/A: not applicable. See Methods and Materials for
 319 details of measurements.

320

321 Fig 7 plots the overall prediction accuracy against the observed classifiers. Although the
 322 overall accuracy was fairly high (87.1%), there were several confounds. The most

323 common of these was radiate vs. planar multipolar, which occurred in about 25% of the
324 cells in these groups. The next most common confound was misclassifying planar
325 multipolar and tuberculoventral cells as pyramidal cells, followed by pyramidal cells
326 being misclassified as tuberculoventral and planar multipolar cells. As these cells all fire
327 regularly, and have similar measures on other properties, this is not surprising.

328

329 **Fig 7. Prediction accuracy table (confusion matrix) by cell type.** The most frequent
330 mis-classifications occurred amongst cells with similar general firing patterns, such as
331 planar and radiate multipolar cells, and pyramidal and tuberculoventral cells. This table
332 was generated in R using data from all 12 measures. The numbers in each box indicate
333 the proportion of correct classifications for each cell type.

334

335

336 **Discussion**

337

338 We find that cells throughout the cochlear nucleus can be classified by firing patterns,
339 action potential shapes, and responses to hyperpolarizing steps, as well as their
340 morphology. Although not surprising given the long history of the identification of various
341 electrophysiological features as identifying characteristics of different morphological cell
342 classes, the extension of these comparisons across classes that fire regularly, and the
343 consistency of these measures in a population of cells taken from adult mice extends
344 the previous observations made in younger mice. In addition, these results suggest that

345 cells can be reasonably classified, at least coarsely, according to their
346 electrophysiological signatures.

347

348 We found that the linear discriminant analysis can be used to classify cells based on 12
349 measured electrophysiological parameters with ~80% success, although there was little
350 improvement when using more than 7 parameters. This requires that the LDA
351 coefficients be estimated from a standardized (training) set of cells before application to
352 an unidentified population. The selection of the training cell, and the quality of that data
353 set, is critical to the success of the technique. Cell morphology should be positively
354 identified, and ambiguous cases discarded, although it is essential to include a full
355 range of cell properties in each class. The measurements should be complete and
356 precise for each cell. Our data set has three limitations in part because it was collected
357 as part of a different experiments. The first is that due to different levels of
358 hyperpolarizing current injection, the voltages reached with hyperpolarizing pulses were
359 not consistent across cells, so that the estimates of the magnitude of the
360 hyperpolarizing sag are influenced by the variability of the voltages reached. The
361 second is that for depolarizing pulses, not all cells reached saturation of their firing
362 rates. For this reason, we did not include maximal firing rates as a measure, but rather
363 focused on the discharge patterns closer to spike threshold. A third limitation is that the
364 current steps near spike threshold were, in some cells, too coarse to precisely define a
365 threshold current. These limitations partly reflect the different input resistances of
366 neurons, as an example, hyperpolarizing pulses strong enough to damage
367 tuberculoventral cells often fail to hyperpolarize pyramidal cells to -80 mV.

368

369 Classification errors principally occurred between cell classes with similar firing patterns,
370 such as planar and radiate multipolar cells, and between pyramidal and tuberculoventral
371 cells. In addition, the TV and bushy cells show significant dispersion in the first 3
372 dimensions of the LDA. This may indicate variability in the intrinsic excitability of these
373 cell classes as noted before [12,34,35], or possibly the existence of distinct subclasses.
374 This dispersion was also evident in the unsupervised PCA analysis. Substantially larger
375 datasets of identified cells from individual strains would be needed to clarify the
376 existence of such subclasses in the electrophysiology. An improvement in the
377 classification would be expected to result from the inclusion of additional parameters
378 such as maximal firing rates and the time courses of synaptic events. In addition, in our
379 data set there was some overlap between the planar and radiate multipolar cells. This
380 may in part reflect the limitations of classification for these two similarly firing cell types,
381 but may also indicate the limitations of our morphological classification method, which
382 was qualitative and relied on fluorescent images of the cells collected during the
383 experiments. The qualitative morphological classification of DCN neurons is much
384 easier, so the overlap between the pyramidal and tuberculoventral cells probably
385 represents the limitations of the measurement parameters used, although it could also
386 reflect a true confluence of intrinsic excitability.

387

388 Part of the dispersion in the VCN cell classes may reflect strain or preparation
389 differences, as the cells from CBA mice were slightly offset from those from the
390 NF107::Ai32 mice along the second LDA component. The strain difference is

391 reminiscent of the differences in HCN channels seen in bushy, planar multipolar and
392 octopus cells between ICR and a knockout on a hybrid 129S and C57Bl/6 background
393 [3]. This raises a cautionary flag that the LDA should be trained on data acquired from
394 cells recorded from animals of the same genetic background (and age and preparation
395 techniques) if it is to be used to categorize cells from a novel data set.

396

397 **Acknowledgements**

398 This research was supported by the National Institute on Deafness and other
399 Communicative Disorders of the National Institutes of Health grants R01 DC004551 to
400 PBM and R03 DC013396 to RX

401 **References**

402

- 403 1. Rothman JS, Manis PB. Differential expression of three distinct potassium
404 currents in the ventral cochlear nucleus. *J Neurophysiol.* 2003;89: 3070–3082.
405 doi:10.1152/jn.00125.2002
- 406 2. Rothman JS, Manis PB. The roles potassium currents play in regulating the
407 electrical activity of ventral cochlear nucleus neurons. *J Neurophysiol.* 2003;89:
408 3097–3113. doi:10.1152/jn.00127.2002
- 409 3. Cao X-J, Oertel D. The magnitudes of hyperpolarization-activated (I_h) and low-
410 voltage-activated potassium (I_{KL}) currents co-vary in neurons of the ventral
411 cochlear nucleus. *J Neurophysiol.* 2011;106: 630–640. doi:10.1152/jn.00015.2010
- 412 4. Oertel D. Synaptic responses and electrical properties of cells in brain slices of
413 the mouse anteroventral cochlear nucleus. *J Neurosci.* 1983;3: 2043–2053.
414 doi:10.1523/JNEUROSCI.03-10-02043.1983
- 415 5. Wu SH, Oertel D. Intracellular injection with horseradish peroxidase of
416 physiologically characterized stellate and bushy cells in slices of mouse
417 anteroventral cochlear nucleus. *J Neurosci.* 1984;4: 1577–1588.
418 doi:10.1523/JNEUROSCI.04-06-01577.1984
- 419 6. Oertel D, Wu SH, Garb MW, Dizack C. Morphology and physiology of cells in slice
420 preparations of the posteroventral cochlear nucleus of mice. *J Comp Neurol.*
421 1990;295: 136–154. doi:10.1002/cne.902950112
- 422 7. Zhang S, Oertel D. Cartwheel and superficial stellate cells of the dorsal cochlear
423 nucleus of mice: intracellular recordings in slices. *J Neurophysiol.* 1993;69: 1384–
424 1397. doi:10.1152/jn.1993.69.5.1384

- 425 8. Zhang S, Oertel D. Giant cells of the dorsal cochlear nucleus of mice: intracellular
426 recordings in slices. *J Neurophysiol.* 1993;69: 1384–1397.
427 doi:10.1152/jn.1993.69.5.1398
- 428 9. Zhang S, Oertel D. Neuronal circuits associated with the output of the dorsal
429 cochlear nucleus through fusiform cells. *J Neurophysiol.* 1994;71: 914–930.
430 doi:10.1152/jn.1994.71.3.914
- 431 10. Manis PB, Spirou GA, Wright DD, Paydar S, Ryugo DK. Physiology and
432 morphology of complex spiking neurons in the guinea pig dorsal cochlear nucleus.
433 *J Comp Neurol.* 1994;348: 261–276. doi:10.1002/cne.903480208
- 434 11. Xie R, Manis PB. Radiate and Planar Multipolar Neurons of the Mouse
435 Anteroventral Cochlear Nucleus: Intrinsic Excitability and Characterization of their
436 Auditory Nerve Input. *Front Neural Circuits.* 2017;11: 77.
437 doi:10.3389/fncir.2017.00077
- 438 12. Kuo SP, Lu H-W, Trussell LO. Intrinsic and synaptic properties of vertical cells of
439 the mouse dorsal cochlear nucleus. *J Neurophysiol.* 2012;108: 1186–1198.
440 doi:10.1152/jn.00778.2011
- 441 13. Druckmann S, Hill S, Schurmann F, Markram H, Segev I. A hierarchical structure
442 of cortical interneuron electrical diversity revealed by automated statistical
443 analysis. *Cereb Cortex.* 2012/09/20. 2013;23: 2994–3006.
444 doi:10.1093/cercor/bhs290
- 445 14. Tavakoli A, Schmaltz A, Schwarz D, Margrie TW, Schaefer AT, Kollo M.
446 Quantitative Association of Anatomical and Functional Classes of Olfactory Bulb
447 Neurons. *J Neurosci.* 2018;38: 7204–7220. doi:10.1523/JNEUROSCI.0303-

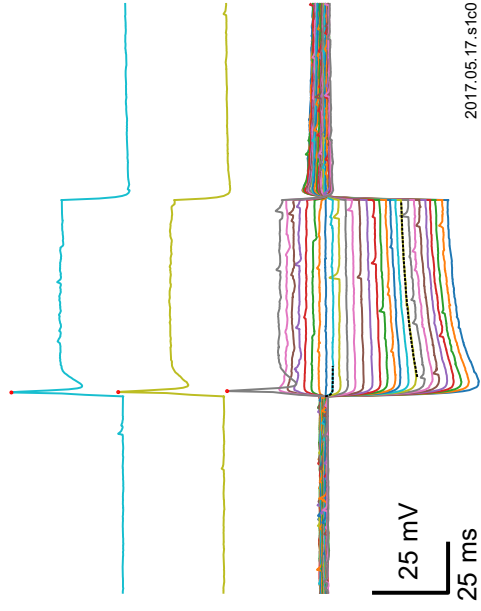
- 448 18.2018
- 449 15. Tripathy SJ, Burton SD, Geramita M, Gerkin RC, Urban NN. Brain-wide analysis
450 of electrophysiological diversity yields novel categorization of mammalian neuron
451 types. *J Neurophysiol.* 2015;113: 3474–3489. doi:10.1152/jn.00237.2015
- 452 16. Typlt M, Englitz B, Sonntag M, Dehmel S, Kopp-Scheinflug C, Ruebsamen R.
453 Multidimensional characterization and differentiation of neurons in the
454 anteroventral cochlear nucleus. *PLoS One.* 2012;7: e29965.
455 doi:10.1371/journal.pone.0029965
- 456 17. Rao CR. The Utilization of Multiple Measurements in Problems of Biological
457 Classification. *J R Stat Soc Ser B.* 1948;10: 159–203. doi:10.2307/2983775
- 458 18. Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, et al. A
459 gene expression atlas of the central nervous system based on bacterial artificial
460 chromosomes. *Nature.* 2003;425: 917–925. doi:10.1038/nature02033
- 461 19. Madisen L, Mao T, Koch H, Zhuo J, Berenyi A, Fujisawa S, et al. A toolbox of Cre-
462 dependent optogenetic transgenic mice for light-induced activation and silencing.
463 *Nat Neurosci.* 2012;15: 793–802. doi:10.1038/nn.3078
- 464 20. Xie R, Manis PB. Target-Specific IPSC Kinetics Promote Temporal Processing in
465 Auditory Parallel Pathways. *J Neurosci.* 2013;33: 1598–1614.
466 doi:10.1523/JNEUROSCI.2541-12.2013
- 467 21. Ting JT, Daigle TL, Chen Q, Feng G. Acute Brain Slice Methods for Adult and
468 Aging Animals: Application of Targeted Patch Clamp Analysis and Optogenetics.
469 *Methods Mol Biol.* 2014;1183: 221–242. doi:10.1007/978-1-4939-1096-0_14
- 470 22. Campagnola L, Kratz MB, Manis PB. ACQ4: an open-source software platform for

- 471 data acquisition and analysis in neurophysiology research. *Front Neuroinform.*
472 2014;8: 3. doi:10.3389/fninf.2014.00003
- 473 23. Fujino K, Oertel D. Cholinergic modulation of stellate cells in the mammalian
474 ventral cochlear nucleus. *J Neurosci.* 2001;21: 7372–7383.
475 doi:10.1523/JNEUROSCI.21-18-07372.2001
- 476 24. Hirsch JA, Oertel D. Intrinsic properties of neurones in the dorsal cochlear
477 nucleus of mice, in vitro. *J Physiol.* 1988;396: 535–548.
478 doi:10.1113/jphysiol.1988.sp016976
- 479 25. Manis PB. Membrane properties and discharge characteristics of guinea pig
480 dorsal cochlear nucleus neurons studied in vitro. *J Neurosci.* 1990;10: 2338–
481 2351. doi:10.1523/JNEUROSCI.10-07-02338.1990
- 482 26. Kanold PO, Manis PB. Transient potassium currents regulate the discharge
483 patterns of dorsal cochlear nucleus pyramidal cells. *J Neurosci.* 1999;19: 2195–
484 2208. doi:10.1523/JNEUROSCI.19-06-02195.1999
- 485 27. Street SE, Manis PB. Action potential timing precision in dorsal cochlear nucleus
486 pyramidal cells. *J Neurophysiol.* 2007;97: 4162–4172. doi:10.1152/jn.00469.2006
- 487 28. Leao RM, Li S, Doiron B, Tzounopoulos T. Diverse levels of an inwardly rectifying
488 potassium conductance generate heterogeneous neuronal behavior in a
489 population of dorsal cochlear nucleus pyramidal neurons. *J Neurophysiol.*
490 2012/03/02. 2012;107: 3008–3019. doi:10.1152/jn.00660.2011
- 491 29. Zhang S, Oertel D. Tuberculoventral cells of the dorsal cochlear nucleus of mice:
492 intracellular recordings in slices. *J Neurophysiol.* 1993;69: 1409–1421.
493 doi:8389823

- 494 30. Lorente de No R. The Primary Acoustic Nuclei. NY: Raven Press; 1981.
- 495 31. Weedman DL, Pongstaporn T, Ryugo DK. Ultrastructural study of the granule cell
496 domain of the cochlear nucleus in rats: mossy fiber endings and their targets. J
497 Comp Neurol. 1996;369: 345–360. doi:10.1002/(SICI)1096-
498 9861(19960603)369:3<345::AID-CNE2>3.0.CO;2-5
- 499 32. Apostolides PF, Trussell LO. Superficial stellate cells of the dorsal cochlear
500 nucleus. Front Neural Circuits. 2014;8: 1–9. doi:10.3389/fncir.2014.00063
- 501 33. Mugnaini E, Sekerková G, Martina M. The unipolar brush cell: A remarkable
502 neuron finally receiving deserved attention. Brain Res Rev. 2011;66: 220–245.
503 doi:10.1016/j.brainresrev.2010.10.001
- 504 34. Francis HW, Manis PB. Effects of deafferentation on the electrophysiology of
505 ventral cochlear nucleus neurons. Hear Res. 2000;149: 91–105.
506 doi:10.1016/S0378-5955(00)00165-9
- 507 35. Cao X-J, Shatadal S, Oertel D. Voltage-sensitive conductances of bushy cells of
508 the Mammalian ventral cochlear nucleus. J Neurophysiol. 2007;97: 3961–3975.
509 doi:10.1152/jn.00052.2007
- 510

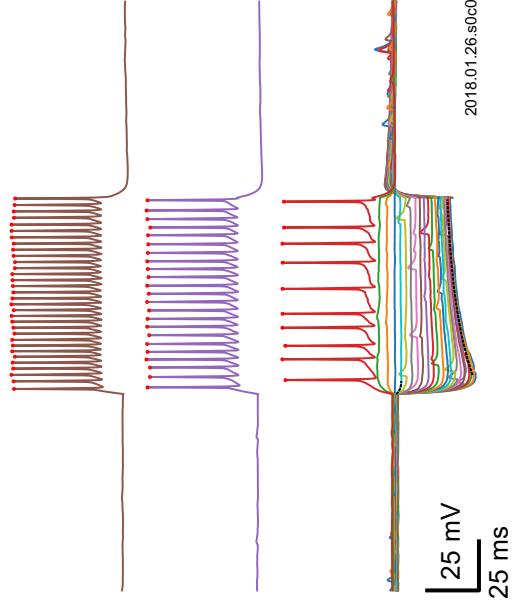
A

bushy



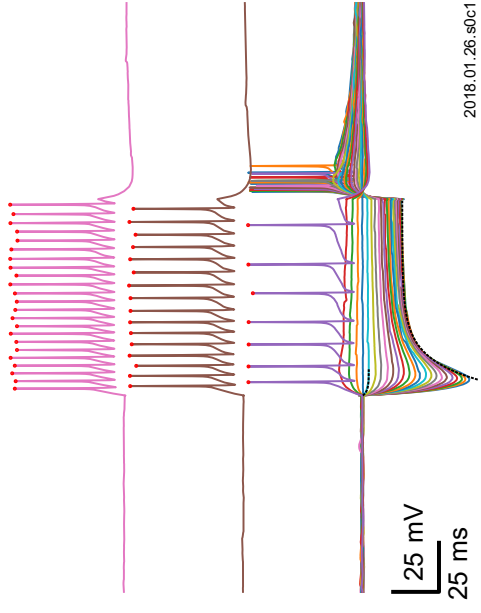
B

planar multipolar



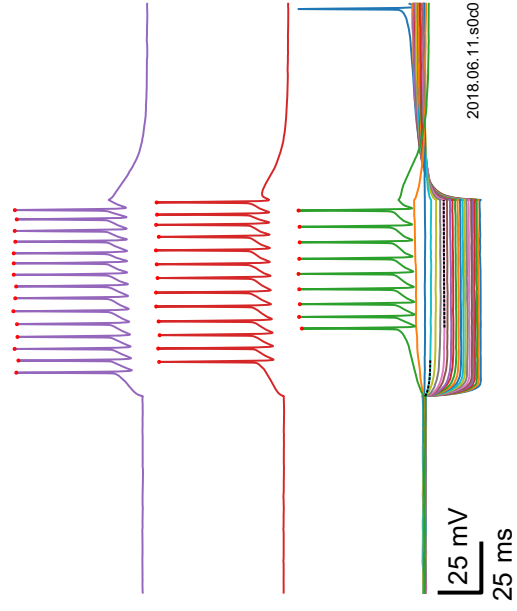
C

radiate multipolar



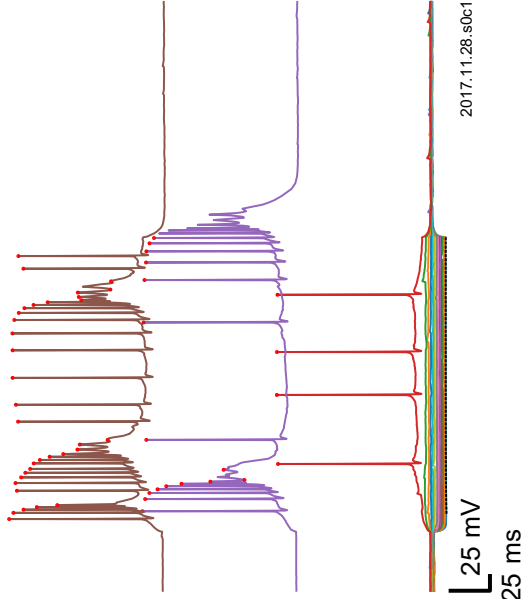
D

pyramidal



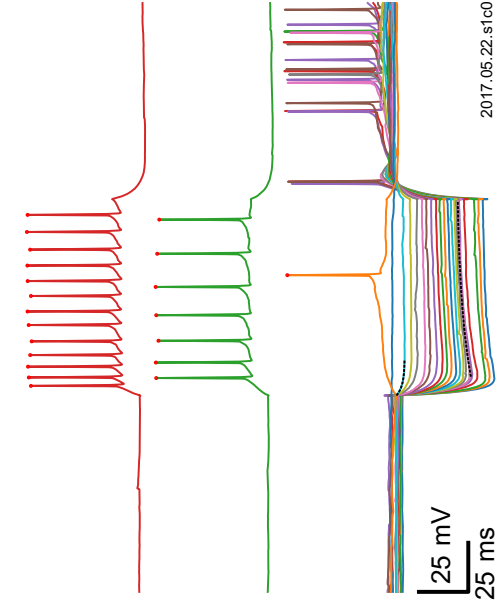
E

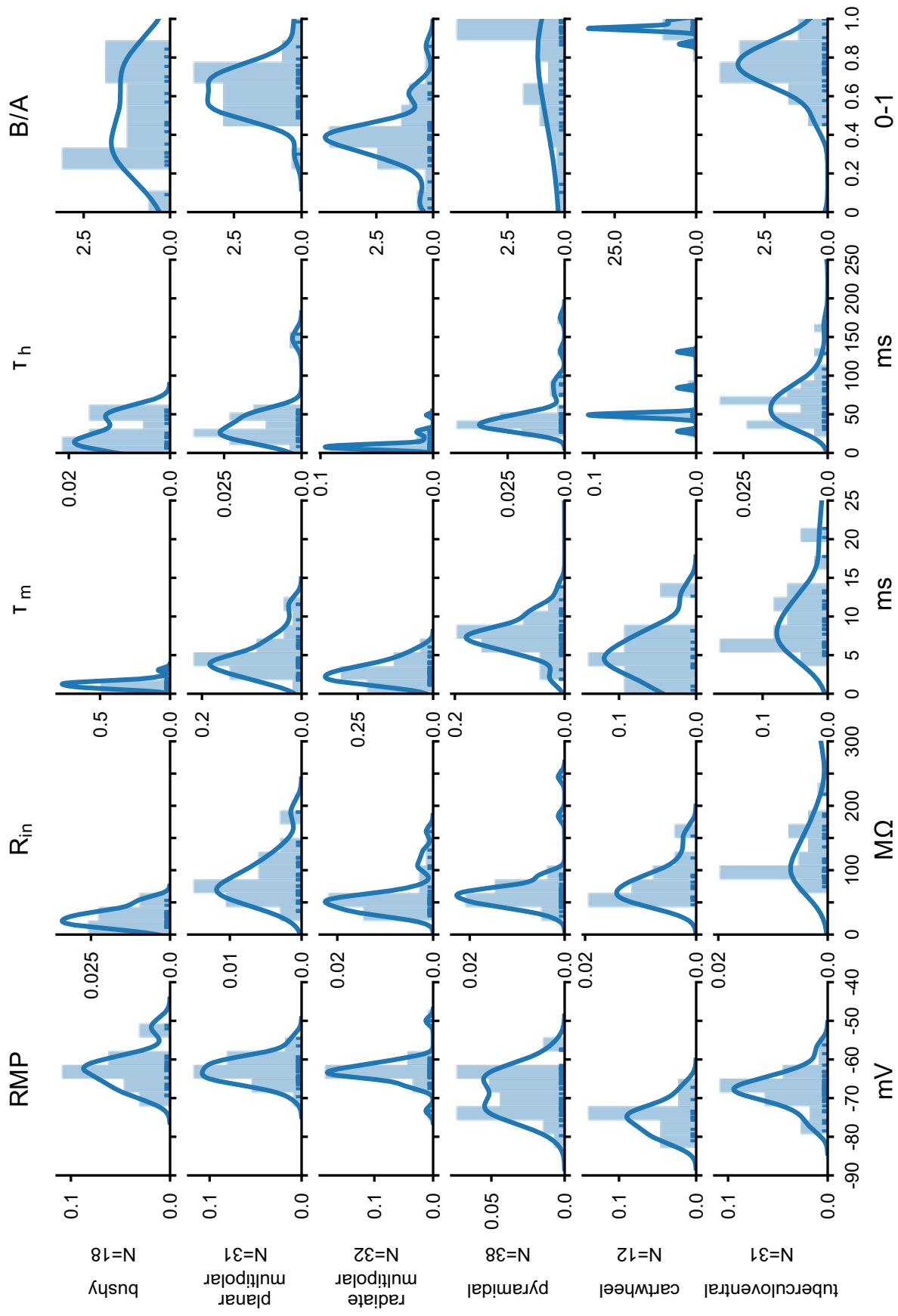
cartwheel

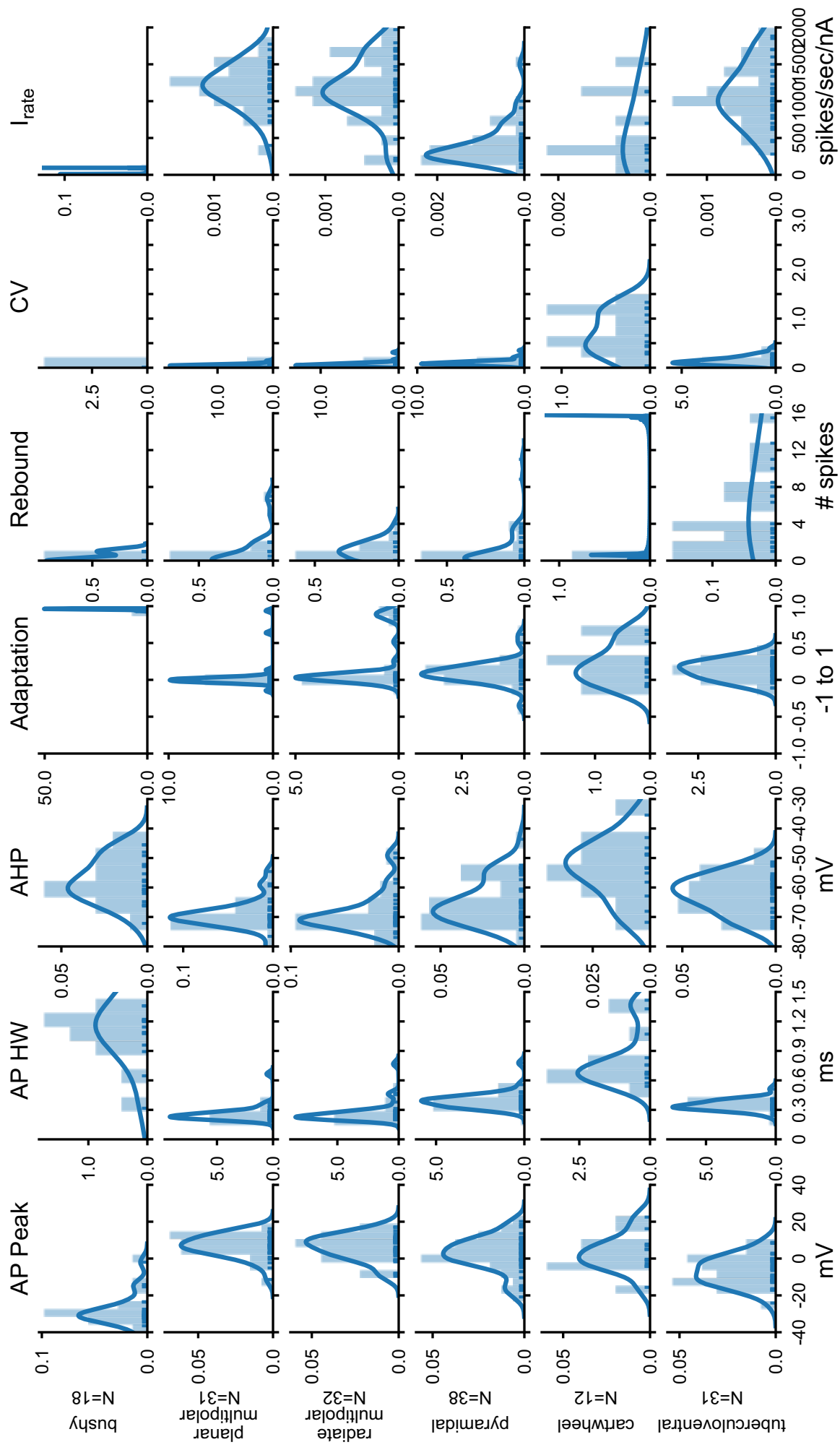


F

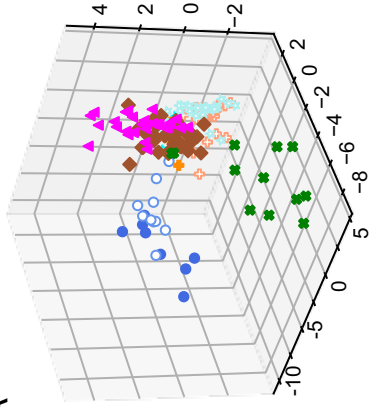
tuberculoventral



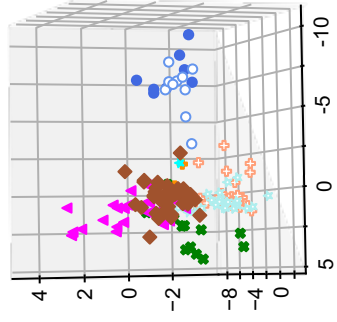




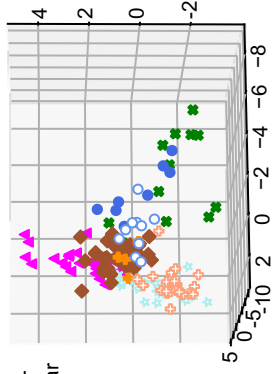
A



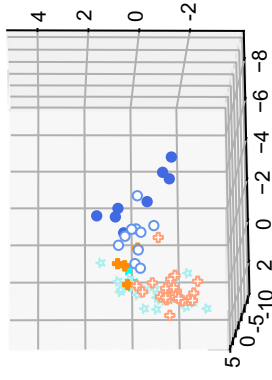
B



C



D



- bushy
- ★ planar multipolar
- ✦ radiate multipolar
- ◆ pyramidal
- ⬢ cartwheel
- ✱ tuberculoventral

