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Multi-dimensional tuning in motor cortical neurons during active behavior

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ABSTRACT

1
2 A region within songbird cortex, AId (dorsal intermediate arcopallium), is functionally
3 analogous to motor cortex in mammals and has been implicated in song learning during
4 development. Non-vocal factors such as visual and social cues are known to mediate song
5 learning and performance, yet previous chronic-recording studies of regions important for song
6 behavior have focused exclusively on neural activity in relation to song production. Thus, we
7 have little understanding of the range of non-vocal information that single neurons may encode.
8 We made chronic recordings in AId of freely behaving juvenile zebra finches and evaluated
9 neural activity during diverse motor behaviors throughout entire recording sessions, including
10 song production as well as hopping, pecking, preening, fluff-ups, beak interactions, scratching,
11 and stretching. These movements are part of natural behavioral repertoires and are important
12 components of both song learning and courtship behavior. A large population of AId neurons
13 showed significant modulation of activity during singing. In addition, single neurons
14 demonstrated heterogeneous response patterns during multiple movements (including excitation
15 during one movement type and suppression during another), and some neurons showed
16 differential activity depending on the context in which movements occurred. Moreover, we
17 found evidence of neurons that did not respond during discrete movements but were nonetheless
18 modulated during active behavioral states compared to quiescence. Our results suggest that AId
19 neurons process both vocal and non-vocal information, highlighting the importance of
20 considering the variety of multi-modal factors that can contribute to vocal motor learning during
21 development.

SIGNIFICANCE STATEMENT

22
23 Motor cortex across taxa receives highly integrated, multi-modal information and has been
24 implicated in both execution and acquisition of complex motor skills, yet studies of motor cortex
25 typically employ restricted behavioral paradigms that target select movement parameters,
26 preventing wider assessment of the diverse sensorimotor factors that can affect motor cortical
27 activity. Recording in AId of freely behaving juvenile songbirds that are actively engaged in
28 sensorimotor learning offers unique advantages for elucidating the functional role of motor
29 cortical neurons. The results demonstrate that a diverse array of factors modulate motor cortical
30 activity and lay important groundwork for future investigations of how multi-modal information
31 is integrated in motor cortical regions to contribute to learning and execution of complex motor
32 skills.

33

INTRODUCTION

34 Goal-directed skill learning underlies our ability to acquire motor skills and flexibly perform
35 them in response to changing environmental contexts. Both learning and performance of motor
36 skills are highly sensorimotor processes: successful acquisition of motor behaviors entails
37 integration across internal and external sources of sensory feedback in order to guide accurate
38 refinement of motor output. Correspondingly, motor cortical neurons demonstrate multi-
39 dimensional tuning that reflects integration across a variety of inputs: motor cortical neurons
40 involved in performance of skilled behaviors have been shown to encode not only motor
41 parameters (e.g., movement force or direction) but also non-motor parameters such as
42 preparatory activity prior to movement execution or visual information specific to a target
43 object's location in object-directed reaching tasks (Tanji and Evarts, 1976; Evarts and Fromm,
44 1977; Murata et al., 1997; Shen and Alexander, 1997; Ferezou et al., 2007). In addition,
45 increasing evidence indicates that motor cortex serves not only as a driver of learned movements
46 but also as a central locus for the acquisition of new motor skills across a variety of movements
47 and training paradigms (Whishaw et al., 1991; Whishaw, 2000; Darling et al., 2011; Guo et al.,
48 2015; Kawai et al., 2015; Makino et al., 2016; Peters et al., 2017; Papale and Hooks, 2018;
49 Hwang et al., 2019). These findings suggest motor cortex as a dynamic substrate that actively
50 integrates diverse streams of information to contribute to sensorimotor learning and performance.
51 However, the potential influence of various multi-modal inputs on motor cortical activity during
52 behavior is difficult to assess in anesthetized and/or restrained experimental paradigms that focus
53 on a single motor task; recordings in freely behaving animals, especially in the context of
54 sensorimotor skill learning, afford opportunities for investigating sensorimotor integration in
55 motor cortical neurons (Ebbesen et al., 2017; Zhang et al., 2017; Mimica et al., 2018).

56 Vocal learning in juvenile songbirds entails integration of social cues as well as visual, auditory,
57 and somatosensory information to guide refinement of variable babbling into stereotyped song
58 (Price, 1979; West and King, 1988; Eales, 1989; Mann et al., 1991; Mann and Slater, 1995; King
59 et al., 2005; Derégnaucourt et al., 2013; Chen et al., 2016; Ljubičić et al., 2016; Carouso-Peck
60 and Goldstein, 2019). Moreover, song production occurs in the context of other social behaviors
61 such as hopping, beak interactions, and preening. For example, adult males must combine both
62 vocal and non-vocal elements in an integrated performance in order to successfully court females
63 (Morris, 1954; Williams, 2001; Cooper and Goller, 2004; Tomaszycski and Adkins-Regan, 2005;

64 Dalziell et al., 2013; Ota et al., 2015; Ullrich et al., 2016). Thus, songbirds offer a model system
65 for examining multiple diverse behaviors during acquisition and performance of a complex
66 motor skill. However, despite evidence for the importance of non-vocal factors in song learning
67 and performance, few studies of regions that process song-related information have examined
68 neural activity in relation to non-vocal behaviors.

69 In songbirds, the cortical region AId lies within an area that has been considered to be analogous
70 to motor cortex in mammals and has been implicated in both vocal learning and non-vocal motor
71 behavior (Feenders et al., 2008; Bottjer and Altenau, 2010; Karten, 2013). AId receives inputs
72 that process multi-modal sensory information via dNCL (dorsal caudolateral nidopallium) as
73 well as information from cortico-basal ganglia circuitry that mediates vocal learning (via
74 LMAN-SHELL), and in turn makes a variety of projections that give rise to feedforward and
75 feedback pathways through subcortical and brainstem regions (Fig. 1A) (Zeier and Karten, 1971;
76 Bottjer et al., 2000; Paterson and Bottjer, 2017). AId is thus well suited to integrate multiple
77 sources of external and internal sensory information to contribute to motor skill learning and
78 performance.

79 We made chronic recordings in AId of freely behaving juvenile songbirds during the
80 sensorimotor period of vocal learning and analyzed the activity of single neurons during singing
81 as well as during several discrete behaviors performed as part of the natural repertoire of zebra
82 finches. This novel approach enabled us to explore the idea that neural activity patterns in brain
83 regions that mediate vocal learning are not restricted to song production. Our results represent
84 an extensive assessment of motor cortical activity across a wide variety of natural behaviors,
85 thereby informing our understanding of how these neurons may contribute to motor skill learning
86 and production.

87

88

MATERIALS AND METHODS**89 Subjects**

90 All animal procedures were performed in accordance with the University of Southern California
91 animal care committee's regulations. Seven male juvenile zebra finches (43-58 days post hatch,
92 dph; mean age 46 dph on first day of recording) were used; this age range corresponds to the
93 early stages of the sensorimotor learning period, when birds have completed memorization of
94 vocal sounds from social tutors and are producing immature, variable vocalizations. Female
95 zebra finches do not produce song and were therefore excluded from this study. All birds were
96 raised in group aviaries until at least 33 dph, remaining with their natural parents and thereby
97 receiving normal auditory and social experience during the tutor memorization period
98 (Immelmann, 1969; Böhner, 1983, 1990; Eales, 1985; Mann and Slater, 1995; Roper and Zann,
99 2006). Juveniles were separated from group aviaries at 33-35 dph and housed in single cages
100 within the experimental rig. Each bird's tutor was placed in a separate cage within view of the
101 juvenile to help it acclimate to the experimental rig for 2-5 days prior to the start of recording.

102 Anatomy

103 We refer to our region of interest as AId (dorsal intermediate arcopallium), following the
104 terminology of Reiner, Perkel, Bruce, et al. (2004). AId extends laterally from the vocal motor
105 nucleus RA and is coterminous with RA. AId receives direct input from both LMAN-SHELL and
106 dNCL and projects to several downstream targets, including VTA (Fig. 1A; Bottjer et al., 2000).
107 Many sources of afferent input converge in the intermediate arcopallium and different arcopallial
108 subregions in turn make a variety of downstream projections, resulting in a complex and
109 heterogeneous area. It seems possible that AId as defined by afferent inputs from LMAN-SHELL
110 may overlap slightly with AIV, which was defined by Mandelblat-Cerf et al. (2014) as an area of
111 intermediate arcopallium that receives inputs from higher-level auditory cortical regions and
112 projects to VTA (cf. Gale et al., 2008). In addition, AIV clearly overlaps with or is identical to a
113 region known as "RA cup," which is a putative auditory area that is located primarily ventral and
114 anterior to RA (Kelley and Nottebohm, 1979; Vates et al., 1996; Mello et al., 1998; Yuan and
115 Bottjer, 2019). We discuss the high degree of anatomical complexity in the arcopallial regions
116 surrounding RA and AId in a Note to the paper by Mandelblat-Cerf et al.
117 (<https://hyp.is/LtYBegXREirqUf3clFvKw/elifesciences.org/articles/02152>, posted 31 October

118 2014). While such anatomical complexities remain to be clarified, we provide schematics of our
119 recording locations in Figure 1B to illustrate the region of arcopallium we refer to as AId.

120 Throughout the text, we refer to AId as analogous in function and connectivity to motor cortex,
121 using the term “cortex” in a generic sense as described by Reiner, Perkel, Bruce et al (2004, pg.
122 395) as including the part of telencephalon that is “pallial in nature and therefore homologous as
123 a field to the brain region of mammals that includes the neocortex, claustrum, and pallial
124 amygdala.”

125 **Electrophysiology**

126 At 35-40 dph, birds were anesthetized with 1.5% isoflurane (inhalation) and placed in a
127 stereotaxic instrument. An electrode assembly consisting of four tetrodes affixed to a movable
128 microdrive was fixed to the skull using C&B Metabond (Parkell), such that the tetrodes were
129 implanted 500 μm dorsal to AId. Each tetrode consisted of four twisted polyimide-coated
130 Nichrome wires (0.012 mm diameter Redi Ohm 800, Kanthal) routed through fused silica
131 capillary tubing and electroplated with non-cyanide gold plating solution (SIFCO 5355). One
132 day after surgery, the tetrode assembly was connected to a recording headstage (HS-16,
133 Neuralynx) with a flexible cable connected to a commutator (PSR, Neuralynx); 15 channels of
134 neural data were amplified, band passed between 300 and 5000 Hz (Lynx-8, Neuralynx), and
135 digitized at 32 kHz using Spike2 software (Power 1401 data acquisition interface, CED). Audio
136 and video were recorded coincident with neural activity – vocalizations were recorded with a
137 lavalier microphone (Sanken COS-11D) mounted in the cage; a USB-video camera (30 FPS,
138 ELP) was placed at the front of the cage to record video. Consecutive 30-minute recordings
139 were made from 7:00 AM to 6:00 PM each day. Tetrodes were manually advanced with the
140 microdrive when the cells being recorded were lost or had already been recorded for at least two
141 days, as indicated by stability and consistency of the extracellular signal. At the end of each
142 experiment, birds were perfused (0.7% saline followed by 10% formalin), and brains were
143 removed and post-fixed for 72 hours before being cryo-protected (30% sucrose solution) and
144 frozen-sectioned (50 μm thick). Sections were Nissl-stained with thionin to visualize tetrode
145 tracks and verify recording locations. Sites within 50 μm from the border of AId were
146 considered to be within AId if neural activity matched characteristic AId firing (intermittent
147 periods of high firing or a high rate of tonic activity during active behavior; Fig. 1C).

148 Movement artifact in neural recordings was correlated across recording channels and was
149 eliminated or reduced using offline common average referencing: for each recording channel, the
150 signal across ~8-14 remaining recording channels was averaged and subtracted from that channel
151 in order to remove movement artifact (Ludwig et al., 2009). Channels were visually inspected
152 after referencing to ensure that spiking activity was not distorted. After common average
153 reference subtraction, single units were sorted from multi-unit data by first automatically
154 clustering units with KlustaKwik (KD Harris, University College London). KlustaKwik clusters
155 were manually inspected across 18 different waveform features and further refined using MClust
156 (AD Redish, University of Minnesota). Clusters were considered for analysis only if the signal
157 to noise ratio was > 2 and less than 1% of spikes had an inter-spike interval < 2 ms.

158 **Behavioral scoring**

159 Sixteen 30-minute sessions were recorded across seven birds (median of 3 sessions per bird).
160 Videos from recording sessions were scored for movements and state periods using ELAN (The
161 Language Archive, Max Planck Institute for Psycholinguistics) (Movies 1-3). We scored each
162 single occurrence of pecks, hops, preening behavior, beak interactions (beak wipes and periods
163 when the bird's beak was in contact with perches, food cup edges, etc for longer than the
164 duration of a peck), fluff-ups, scratches, and stretches. Head and postural movements occurred
165 so frequently that it was impractical to score all of them for all cells. These movements did not
166 occur concurrently with any of the seven scored movements, but did occur during singing. To
167 test whether head movements contributed to singing activity, we scored head movements that
168 occurred during singing and during 30 seconds of non-singing before and after each singing
169 episode in a subset of 36 singing-responsive neurons. In addition, all head and postural
170 movements were scored for 12 neurons across two recording sessions in two birds.

171 In addition to scoring discrete movements, we developed a novel way of measuring behavior
172 throughout recording sessions: each session was segmented into contiguous time periods that
173 were classified into one of five behavioral "state" periods based on the bird's behavior: eating,
174 singing, active-movement, quiet-attentive, or quiescent; these state periods tiled the entire
175 duration of the recording session (Fig. 6A). Eating states were defined as periods during which
176 the bird was pecking at seed or grit, hulling or ingesting seeds, or pausing in between these
177 behaviors for at most one second. Although pecks occurred most often during eating, eating
178 states could also include other scored movements such as hops or preening, or unscored

179 movements such as head movements as long as they occurred within the brief (≤ 1 sec) pauses
180 that occurred while birds were actively eating. Singing states were defined by song behavior;
181 they began whenever the bird produced song and lasted as long as song syllables continued to
182 occur within one second of each other (inter-syllable interval ≤ 1 sec). Birds often made head
183 movements during singing and occasionally made scored movements such as hopping or pecking
184 in between bouts of singing. Active-movement states were defined as non-eating and non-
185 singing periods during which the bird made active movements with pauses of at most one second
186 in between movements; these periods could include any of the seven movements that were
187 scored as well as head and postural body movements that were not scored. Quiet-attentive states
188 were defined by times when the bird was not eating, singing, or moving around the cage for
189 more than one second; they continued as long as the bird made at most small head movements
190 and otherwise remained unmoving but alert. Quiescent periods were defined as periods during
191 which the bird was completely still and not obviously paying attention to any stimulus, with eyes
192 partially or fully closed. Quiescent state times were segmented into one-second intervals that
193 were used as baseline periods for analyses of scored movements (see below).

194 **Data Analysis**

195 To test for significant responses during scored movements, firing rates across occurrences of
196 each movement type were compared against quiescence. Quiescent baseline periods were
197 generated by dividing quiescent state periods (as described above) into one-second segments.
198 The firing rate during two one-second quiescent segments that occurred closest in time to each
199 movement occurrence was used as a corresponding baseline. Fourteen neurons were recorded
200 during sessions that lacked quiescent state periods. For these 14 neurons, one-second baseline
201 periods were taken from times within quiet-attentive state periods when the bird was verified to
202 be unmoving (though clearly alert, unlike in quiescent state). To compare movement responses
203 across neurons, standardized response strength was calculated for each movement type as:

$$\text{standardized response strength} = \frac{\overline{FR}_m - \overline{FR}_b}{\sqrt{\text{Var}(FR_m) + \text{Var}(FR_b) - 2 \times \text{Covar}(FR_m, FR_b)}}$$

204 where FR_m is the firing rate during movement occurrences and FR_b is the firing rate during
205 corresponding baseline periods. A positive value indicates an increase in firing rate during the

206 movement compared to quiescence whereas a negative value indicates a decrease in firing rate
207 during movement. This measure is referred to as response strength (RS) throughout the text.

208 Mean response strength values across neurons are reported as the mean \pm s.e.m.

209 To test for changes in activity around movement onsets, for each neuron we generated a 25 ms-
210 bin histogram of the spiking response across all occurrences of the movement; histogram
211 windows were one second long and centered on movement onsets. Spike times during each
212 movement repetition were shuffled to obtain a resulting histogram of shuffled spike data; this
213 was repeated 1000 times, resulting in 1000 histograms of shuffled data. Each bin of the actual
214 spike data histogram was considered significantly excited if the count in that bin was greater than
215 95% of maximum values from the shuffled data set; likewise, the bin was considered
216 significantly suppressed if the count was lower than 95% of minimum values from the shuffled
217 data set. Onset responses were defined as responses that contained at least two consecutive bins
218 (50 ms) of significant maxima or minima within 100 ms of movement onset.

219 We tested for significant offset-aligned responses using the same method and parameters as
220 onset-aligned responses, except that the one-second windows used for histograms of actual and
221 shuffled data were centered around movement offsets; offset responses were defined as
222 responses that contained at least two consecutive bins (50 ms) of significant maxima or minima
223 within 100 ms of movement offset. Due to the short duration of pecks and hops (mean hop
224 duration = .28 seconds; mean peck duration = .25 seconds), it was possible for the same maxima
225 or minima to be captured in both onset- and offset-aligned responses. To ensure that offset-
226 aligned activity could be accurately distinguished, for these movements we only counted excited
227 or suppressed offset responses that did not demonstrate significant changes in onset-aligned
228 activity. Preening movements were relatively long in duration (mean duration = 1.5 seconds), so
229 all preening-aligned offset responses were counted.

230 We tested for significant modulation of firing rate during states by dividing all state periods in
231 each recording session into one-second segments and calculating the average firing rate during
232 each segment for each neuron. We compared the distributions of average firing rates across
233 segments for each state against quiescence to determine whether activity was significantly
234 increased or decreased during non-quiescent states for each neuron. Fourteen neurons were

235 recorded during sessions that lacked quiescent state periods and were excluded from these
236 analyses.

237 As an additional means of characterizing firing rate modulation during states, we defined
238 “events” – brief periods of excitation and suppression – from histograms of spiking activity. We
239 segmented the instantaneous firing rate (IFR) across each recording session into 10-ms bins and
240 smoothed the IFR with a moving average filter (span = 3 bins). Excited events were defined as
241 periods during which the smoothed IFR across five or more 10-ms bins (50 ms or more)
242 exceeded the average firing rate across quiescent state periods by ≥ 3 standard deviations.
243 Suppressed events were defined as periods during which the smoothed IFR across five or more
244 50-ms bins fell below the average firing rate across quiescent state periods by ≤ 1.5 standard
245 deviations. To compare across neurons, the number of events in each state was normalized by
246 dividing the number of excited or suppressed events in each state by the total duration of that
247 state for each neuron.

248 **Statistics**

249 Movement responses were tested for significance against quiescent baselines (see above) using
250 Wilcoxon signed-rank tests; Benjamini-Hochberg post-hoc tests were used to apply corrections
251 for multiple comparisons (Benjamini and Hochberg, 1995). Neurons that demonstrated a
252 significant difference between movement and baseline for at least one scored movement were
253 considered movement-responsive. To test whether movement responses were context-selective,
254 response strengths during movements from one context versus another context were compared
255 using Mann-Whitney tests for each neuron (for example, comparing pecks during eating versus
256 non-eating periods, and comparing head movements during singing versus non-singing periods).
257 Mann-Whitney tests were also used to compare response strengths during singing periods with
258 versus without head movements in individual neurons. We were not able to use signed-rank tests
259 in these two cases due to different numbers of observations in the two comparisons. χ^2 tests
260 were run to compare proportions between more than two groups (for example, proportions of
261 neurons that were responsive during each movement type). In case of significance, Fisher’s
262 exact tests were used as a follow-up to make pairwise comparisons of proportions between
263 groups, and Benjamini-Hochberg post-hoc tests were used to apply a correction for multiple
264 comparisons. Binomial tests were used to judge whether the relative proportions of excited
265 versus suppressed responses among movement-responsive neurons were different from chance.

266 Comparisons of firing rate distributions between each state and quiescence (see above) were
267 made using Kolmogorov-Smirnov tests, with a Benjamini-Hochberg post-hoc test applied for
268 multiple comparisons. Measures between different state periods (inter-spike intervals,
269 normalized number of events, normalized firing rates among unresponsive neurons) were
270 compared using sets of pairwise linear contrasts based on trimmed means (20% trimming); this
271 linear contrast method has been shown to be robust to common assumption violations such as
272 non-normality and heteroscedasticity (Wilcox and Serang, 2017). For all tests, $p < .05$ was
273 considered significant.

274

275

RESULTS

276 We made extracellular recordings from 119 neurons in AId of freely behaving juvenile zebra
277 finches (43-58 dph) housed singly in a recording cage as they actively engaged in sensorimotor
278 vocal practice. Figure 1B illustrates locations of recording sites in AId. A typical 30-minute
279 recording period included various overt behaviors and periods of quiescence when the bird was
280 not moving. To investigate how neural activity in AId corresponds to different behaviors, we
281 scored seven different movements during each recording that could be identified reliably: pecks,
282 hops, preening episodes, beak interactions with objects in the recording cage (e.g., beak wipes or
283 non-peck interactions with cage bars), fluff-ups, stretches, and scratching episodes; we also
284 marked periods of singing (Movies 1-3). We developed a novel approach in which we examined
285 spiking patterns of single neurons throughout each recording period to investigate whether AId
286 neurons are selective for different movement types and/or singing behavior in juvenile birds.

287 *Responsivity of AId neurons during movements*

288 Patterns of spiking were highly variable across individual neurons, ranging from phasic to tonic
289 activity. In addition, each neuron's activity was highly modulated throughout a typical recording
290 session, showing either excitation and/or suppression during different movements. Figure 1C
291 illustrates two different neurons recorded in one bird while it was quiescent (no overt
292 movements, left columns) and while it hopped around the cage (right columns). The neuron in
293 the top panel fired intermittently in small bursts of at most three spikes during quiescence, while
294 the neuron in the bottom panel exhibited dense bursting activity. As the bird hopped around the

295 cage, the neuron in the top panel shifted to longer periods of high firing separated by relative
296 inactivity while the neuron in the bottom panel shifted to a high tonic rate of firing. To
297 investigate whether such modulations were related to specific movements, for each neuron we
298 compared firing rates during different movement types against baseline firing rates during
299 quiescent periods that were closest in time to each movement occurrence (see Materials and
300 Methods). To compare movement-related activity across neurons, we calculated the response
301 strength of each neuron during each movement type, defined as the standardized difference in
302 average firing rate during each movement type versus baseline periods (see Materials and
303 Methods).

304 The majority of AId neurons exhibited a significant change in firing rate during at least one
305 movement type compared to quiescent baseline periods and were thus classified as "movement
306 responsive" (101 out of 119 neurons, 85%). Among 101 movement-responsive neurons, 33
307 (33%) responded during only one scored movement whereas 68 (67%) responded during two or
308 more movements (Fig. 2A). Few cells responded during five or six movements, and no cells
309 responded during all seven movements. Figure 2B depicts the range of responsivity in these 101
310 neurons. For example, 10 neurons were either excited or suppressed during pecks but were
311 otherwise not responsive during any of the other six scored movements; likewise, two other
312 groups of 10 neurons each selectively modulated their firing rate only during preening or hops.
313 Neurons modulated during multiple movements showed heterogeneous responsivity: single
314 neurons could demonstrate excitation during some movement types and suppression during
315 others, and responsivity profiles across neurons included different subsets of movement types.

316 Higher proportions of neurons showed altered firing rates during pecks, preening, and/or hops
317 compared to other movements: 68 out of 107 neurons (64%) were significantly modulated during
318 pecks, 50 out of 93 (54%) during preening, and 60 out of 119 (50%) during hops (Fig. 2C).
319 These proportions did not differ (Fisher's exact test, Benjamini-Hochberg corrected, peck versus
320 preen $p = .23$, peck versus hop $p = .09$, preen versus hop $p = .71$), and were each greater than the
321 proportions of neurons that responded during fluff-ups (21/79, 27%), scratches (14/55, 25%), and
322 stretches (7/59, 12%) (Fig. 2C; Fisher's exact test, Benjamini-Hochberg corrected, $p < .05$ for
323 comparisons between pecks, hops, and preening against each of the other three movements; $p >$
324 $.05$ for comparisons among these latter three movements). In addition to the seven scored

325 movements, birds constantly made quick, saccade-like movements throughout recording periods,
326 resulting in over a thousand head or postural movements in a typical 30-minute session. As an
327 initial test of whether firing rate was modulated during these latter movements, we scored all
328 head and postural movements for a subset of 12 neurons and found that six neurons were excited
329 during these movements (50%) while four were suppressed (33%), demonstrating that AId
330 activity can be modulated during head and postural movements as well.

331 As indicated above, we observed both excited and suppressed responses within single neurons:
332 21 out of 68 neurons (31%) that responded during multiple movements exhibited excitation
333 during some movements and suppression during others (Fig. 2B). The overall proportions of
334 excited versus suppressed responses did not differ (56%, 138/247 excited; 44%, 109/247
335 suppressed; binomial test, $p = .07$), indicating a fairly even representation of excitation and
336 suppression across scored movements. In addition, the proportions of excited versus suppressed
337 responses during each movement type did not differ (Fig. 2C; binomial test, $p > .05$ in all cases).

338 *Temporal response patterns of AId neurons at movement onsets and offsets*

339 Some AId neurons demonstrated consistent temporal changes in firing rate at movement onsets
340 and/or offsets that could be masked by measures of average firing rate. For example, Figure 3A
341 shows rasters and histograms for a single neuron during preening (left) and peck (right)
342 responses. In both cases, mean firing rate during the movement was significantly excited relative
343 to quiescence (preening RS = 0.65, peck RS = 1.53, $p < 0.05$ in both cases). However, the
344 responses clearly contain periods of suppression that begin prior to movement onset.

345 To capture these firing rate modulations, we tested for significant excitation or suppression at
346 movement onsets and offsets. For each response, we compared histograms of spiking activity
347 centered on movement onsets or offsets to histograms of shuffled spike trains (25-ms bins) in
348 order to identify bins with significant firing rate changes (see Materials and Methods). Onset or
349 offset responses were defined as responses with two or more contiguous bins (50 ms) of
350 significant excitation or suppression occurring within 100 ms of movement onset or offset.

351 Figure 3 plots examples of onset-aligned (Fig. 3A, B; 3C, left) and offset-aligned (Fig. 3C, right)
352 responses; green and gray horizontal bars above each histogram mark excited and suppressed
353 bins, respectively.

354 We observed significant onset responses during pecking, hopping, and preening, but not other
355 movement types. The top of Table 1 lists onset responses for these three movements, classified
356 by whether neurons showed a significant response based on average firing rate. Eleven out of
357 107 neurons (10.3%) exhibited significant excitation (7/11) or suppression (4/11) at peck onsets.
358 Five out of 119 neurons demonstrated an onset response during hopping (4.2%; 4/5 excitation,
359 1/5 suppression), as did four out of 93 neurons during preening (4.3%; 3/4 excitation, 1/4
360 suppression). We also observed significant offset responses during pecking and preening: six out
361 of 107 neurons exhibited pecking offset responses (5.6%; 4/6 excitation, 2/6 suppression), and
362 three out of 93 neurons showed preening offset responses (3.2%; all excitation) (Table 1,
363 bottom).

364 13 of these 29 onset and offset responses did not match the average firing rate response (Table
365 1). For example, four responses (two pecking, two hopping) included consistent excitation at
366 movement onset even though the average firing rate during movement did not differ from
367 quiescent baseline (Table 1, top, “Not significant” row). Onset and offset responses could also
368 differ in sign (excitation or suppression) from the average firing rate response: for example,
369 while average firing rate during the hop response plotted in Figure 3B was suppressed relative to
370 baseline ($RS = -.76$), the raster and histogram reveal an excitatory peak starting just before hop
371 onset, indicating a complex temporal response with brief excitation followed by suppression.
372 These results suggest that single AId neurons can be modulated by multiple factors during
373 movements, resulting in excitation at movement onsets or offsets and suppression during the
374 movement itself, or vice versa.

375 *Context-dependency of pecking behavior*

376 Of the seven movements we scored, pecking behavior in particular tended to occur in different
377 contexts: birds always pecked while eating, but also frequently pecked at other objects such as
378 cage bars or perches. To investigate whether different contexts influenced responsivity, we
379 compared response strengths for pecks that occurred during eating versus non-eating.

380 Response strength differed for eating- versus non-eating pecks in 47 out of 97 neurons (48%;
381 Mann-Whitney test, $p < .05$). Figure 4A plots these context-sensitive cells according to whether
382 they exhibited greater absolute response strength during eating (29/47, 62%; left) or non-eating

383 (18/47, 38%; right). The left panel of Figure 4A shows 29 neurons that exhibited greater
384 absolute response strength during eating-related pecks. Most of these neurons showed higher
385 firing rates during eating-related pecks compared to non-eating pecks (21/29, 72%; gray lines).
386 The peck-aligned activity for one of these neurons (Fig. 4B) illustrates strong excitation during
387 pecks that occurred when the bird was eating (left) versus a weak response during non-eating
388 (right). The remaining neurons showed lower firing rates during eating-related pecks compared
389 to non-eating pecks (8/29, 28%; black lines). In contrast, the right panel of Figure 4A plots 18
390 neurons that showed greater absolute response strength during non-eating pecks. Eleven of these
391 neurons showed higher firing rates during non-eating pecks compared to eating pecks (61%, gray
392 lines); the remaining cells showed lower firing rates during non-eating pecks (7/18, 39%, black
393 lines).

394 These results indicate that pecking activity in many AId neurons was dependent on the context in
395 which the movement occurred and suggest that neurons can signal behavioral contexts with
396 either relative excitation or suppression. While peck duration did not differ between eating
397 versus non-eating (mean peck duration = $0.24 \pm .002$ versus $0.22 \pm .003$ seconds, respectively),
398 one possibility is that this context-dependent activity reflects differences in eating versus non-
399 eating pecking movements. Alternatively, these differential responses may reflect that these
400 neurons do not encode the physical movements of pecking behavior per se; for example, this
401 subpopulation may be involved in processing orofacial or external sensory information that is
402 present specifically in one context versus another.

403 *Singing-responsive neurons in AId*

404 One of AId's primary sources of afferents is from LMAN-SHELL, which contains neurons that are
405 active during singing behavior and have been implicated in guiding accurate imitation of the
406 tutor song during vocal learning (Fig. 1A) (Achiro and Bottjer, 2013; Achiro et al., 2017).
407 Moreover, lesions of AId in juvenile birds impair their ability to achieve an accurate imitation of
408 the adult tutor song without disrupting vocal motor output (Bottjer and Altenau, 2010; cf.
409 Mandelblat-Cerf et al., 2014, Materials and Methods). Given this evidence of a role for AId in
410 vocal learning, we hypothesized that the activity of AId neurons would be modulated as juvenile
411 birds engaged in singing behavior.

412 Firing rates were significantly modulated during singing relative to quiescence in the majority of
413 neurons (66/94, 70%), including 44 excited responses and 22 suppressed responses (mean RS =
414 $.76 \pm .09$ and $-.96 \pm .19$, respectively). Figure 5A illustrates the singing-aligned response of a
415 neuron that was excited during song renditions. Altered firing rates during vocal production in
416 songbirds have typically been interpreted as “singing-specific”. However, birds often make head
417 and postural body movements during singing, as well as beak-gape and gular-fluttering
418 movements that are specific to song production. This complexity raises the question of which
419 movements are an intrinsic part of singing behavior versus independent movements that are
420 performed simultaneously during song production. Given the range of movement responsivity
421 across AId neurons (Fig. 2), activity modulation in singing-responsive neurons may reflect
422 singing-specific actions as well as movements that are performed during both singing and non-
423 singing periods. As an initial test of this question, we compared neural activity during head
424 movements that occurred within singing periods versus adjacent non-singing periods in a subset
425 of 36 singing-responsive neurons. We then compared activity during singing periods with versus
426 without head movements to assess whether head movements contributed to the singing response
427 in these neurons (see Materials and Methods).

428 Response strength during head movements that occurred within singing versus non-singing
429 periods did not differ for most singing-responsive neurons (24/36, 67%; Mann-Whitney test, $p >$
430 $.05$ for each neuron) (Fig. 5B; 5C, left). In eight of these 24 neurons, response strength during
431 singing periods that contained head movements was significantly greater than during singing that
432 lacked head movements, indicating that activity during head movements contributed to the
433 singing response (7/8 excited, 1/8 suppressed; Table 2A, left). For the remaining 16 neurons,
434 response strength during singing that lacked head movements either did not differ from (14/24)
435 or was greater than (2/24) response strength during singing with head movements (Table 2A,
436 middle and right). In addition, most of these 24 neurons still showed significant changes in
437 firing rates during singing that lacked head movements (21/24, 88%). Thus, firing rate changes
438 in most of these singing-responsive neurons was not attributable to activity during head
439 movements.

440 Six neurons were singing-excited and showed greater response strength during head movements
441 that occurred within singing compared to non-singing (Fig. 5B; 5C, right, orange). In three of

442 these neurons, activity during singing periods with head movements was greater than during
443 singing without head movements, indicating that singing-specific head movements contributed to
444 excitation during song production (Table 2B, left). For the other three neurons, responses during
445 singing periods with versus without head movements were comparable, suggesting that discrete
446 head movements made little contribution to activity modulation during singing (Table 2B,
447 middle). Moreover, in all but one of these six neurons, activity during singing periods that
448 lacked head movements was still significantly greater than quiescence.

449 Three neurons were singing-suppressed and showed lower response strength during singing-
450 related head movements compared to non-singing (Fig. 5C, right, gray triangles). For two of
451 these neurons, decreased firing rates during head movements contributed to greater suppression
452 during singing (Table 2C, left, Suppressed). However, all three neurons were suppressed even
453 during singing periods that lacked head movements – in fact, for one of these neurons, this
454 suppression was significantly greater than during singing that contained head movements (Table
455 2C, right, Suppressed). Interestingly, three neurons showed lower response strength during
456 singing-related head movements but nevertheless showed significant excitation across singing
457 periods (Fig. 5C, right, gray circles; Excited columns in Table 2C), highlighting the presence of
458 multiple modulating factors during song behavior.

459 In summary, the singing-modulated activity of most neurons persisted in the absence of head
460 movements (32/36, 89%, Table 2). These results indicate that activity of many AId neurons
461 during song production may reflect singing-specific movements such as respiratory actions, beak
462 movements, or gular fluttering, or non-physical aspects of song production such as auditory-
463 vocal feedback. Interestingly, 12 neurons (33%) showed differential response strength during
464 head movements within singing versus non-singing periods (Figure 5B; 5C, right). One
465 interpretation is that these neurons integrate information about head movements and singing
466 behavior, such that changes in firing rate are enhanced specifically during head movements that
467 are performed concurrently with song. Developing associations between head or postural
468 movements and vocal behavior may be a crucial component of learning to produce female-
469 directed song and perform courtship dance movements during singing (Morris, 1954; Balaban,
470 1997; Williams, 2001; Tomaszyccki and Adkins-Regan, 2005). These results raise the possibility

471 that neural activity reflecting “non-singing” movements during song production may be a
472 ubiquitous feature of circuits involved with song learning and control.

473 *Additional sources of AId neuron modulation: behavioral states*

474 As indicated above, our goal was to assess the activity of AId neurons throughout entire sessions
475 of active behaviors. As part of this approach, we devised a novel way of characterizing each
476 recording session by classifying contiguous time periods across each session into one of five
477 different “state” periods based on the bird’s behavior: eating, singing, active-movement, quiet-
478 attentive, or quiescence (Fig. 6A; see Materials and Methods). Eating states were defined as
479 periods when the bird was engaged in eating behavior, including pecking at, hulling, and
480 ingesting seeds; although eating state periods were dominated by eating-related behaviors, other
481 movements such as head movements or hops could also occur. Similarly, singing states included
482 periods when birds were engaged in song production, as well as brief pauses in-between song
483 bouts during which birds occasionally hopped or pecked. During active-movement states, birds
484 could produce any of the seven movements we scored as well as head and/or postural body
485 movements that were not scored. The remaining two states characterized non-movement
486 periods: during quiet-attentive states, the bird was alert and could make small head movements
487 but was otherwise not moving; birds made no movements during quiescent states (quiescent
488 states included periods from which baseline intervals were sampled in the scored-movement
489 analyses above; see Materials and Methods).

490 For most neurons, firing rates during eating, singing, active-movement, and/or quiet-attentive
491 state periods differed from quiescence (99/109, 91%; Kolmogorov-Smirnov test, $p < .05$). Few
492 neurons showed changes in firing rate during only one state type; most neurons exhibited firing
493 rate modulations during two or more states (Fig. 6B). Figure 6C illustrates responsivity of single
494 neurons during each non-quiescent state type, categorized by the number of states during which
495 each neuron’s activity was modulated. AId neurons could show increased or decreased firing
496 rates during non-quiescent states, and many neurons were excited during one state type and
497 suppressed during another (29/109, 27%). However, whereas single neurons were equally likely
498 to be suppressed as excited during different discrete scored movements (Fig. 2B), modulation
499 across entire state periods tended to be excitatory: within each state type, the proportion of
500 neurons that were excited was significantly greater than the proportion that were suppressed

501 (binomial test, active-movement and eating states $p < .0001$, singing and quiet-attentive states p
502 $< .05$), and the overall proportion of excited state responses was greater than suppressed state
503 responses (binomial test, $p < .0001$).

504 In accord with this pattern of results, inter-spike intervals (ISIs) during non-quiescent states were
505 shorter than ISIs during quiescence (Fig. 6D, left, middle; pairwise linear contrasts, Benjamini-
506 Hochberg corrected, $p < .001$ in all cases). In addition, ISIs during active-movement and eating
507 states were shorter than quiet-attentive ISIs (Fig. 6D, left; $p < .001$ in both cases) and did not
508 differ from singing-state ISIs (Fig. 6D, right; $p > .05$ in both cases). These results indicate
509 greater increases in firing rate during periods of active behavior, particularly for active-
510 movement and eating states.

511 To capture dynamic changes in activity across state periods, we identified excited and suppressed
512 spiking "events" during each state type, defined as five or more contiguous 10-ms bins in which
513 the firing rate exceeded the average quiescence firing rate by ≥ 3 standard deviations (for excited
514 events) or fell below the average quiescence firing rate by ≤ 1.5 standard deviations (for
515 suppressed events). On average, more excited events occurred within active-movement and
516 eating states compared to quiet-attentive and quiescence states (Fig. 6E, left; pairwise linear
517 contrasts, Benjamini-Hochberg corrected, $p < .001$ in all cases). The frequency of excited events
518 during singing states was elevated relative to quiet-attentive and quiescence, but did not differ
519 significantly from any of the other four states (Fig. 6E, left; pairwise linear contrasts, Benjamini-
520 Hochberg corrected, $p > .05$ in all cases). The relatively modest incidence of excited events
521 during singing compared to active-movement and eating states may indicate that firing rate
522 modulation during singing states involves more uniform increases in tonic spike rate. The
523 number of suppressed events did not differ between state types (Fig. 6E, right; pairwise linear
524 contrasts, Benjamini-Hochberg corrected, $p > .05$ in all cases). Taken together, these results
525 indicate an increase in firing rate during non-quiescent states, with a greater degree of excitatory
526 modulation during active-movement, eating and singing states expressed as shorter ISIs as well
527 as an increase in discrete high-firing periods during active-movement and eating states.

528 *How does activity during scored movements contribute to behavioral states?*

529 Because eating and singing state periods were characterized primarily by one type of scored
530 movement (eating-related pecks and singing bouts, respectively; Fig. 7A), we wondered whether
531 excitation during each of these states was restricted to neurons that were excited during the
532 corresponding scored movements. If state responsivity of single neurons can be attributed to
533 their movement responsivity, then neurons excited during eating states should also be excited
534 during eating-related pecks, which accounted for 99% of scored movements during eating.
535 Similarly, neurons excited during singing state periods should also be excited during song bouts.
536 In contrast, if behavioral states include other sources of modulation, then we might expect to find
537 a more diverse pattern of movement-related responsivity among eating- and singing-state-excited
538 neurons; this latter outcome would be consistent with the heterogeneous pattern of movement
539 responsivity described above.

540 Figure 7B (left) illustrates the peck responsivity of 48 neurons that were excited during eating
541 states, grouped by their response during eating-related pecks. A majority of neurons that were
542 excited during eating states were also excited during discrete peck movements (31/48, 65%).
543 However, the remaining 35% of eating-state-excited neurons were suppressed or unresponsive
544 during eating-related pecks, indicating that the heightened firing rate of these neurons during
545 eating states did not relate to pecking behavior. Increased firing rates in these latter cells may be
546 related to unscored factors during eating such as head movements or hulling behavior, or
547 external sensory inputs. Likewise, most of the neurons that were excited during singing state
548 periods were excited during song bouts (26/37, 70%), but the remaining 30% were suppressed or
549 unresponsive during song bouts (Fig. 7C, left). Thus, excitation across singing states in these
550 latter neurons presumably reflects activity during unscored factors that occur in-between song
551 bouts.

552 Consistent with the fact that most neurons responded during multiple movements and states
553 (Figs. 2 and 6), neurons excited during eating and singing state periods were also responsive
554 during a variety of scored movements that did not occur within these states. For example, many
555 eating-state-excited neurons also responded during song bouts, preening, and fluff-ups, even
556 though these behaviors never occurred within eating states (Fig. 7A; 7B, right). Similarly, most
557 neurons excited during singing states were also responsive during scored movements that did not

558 occur within singing states, such as preening, beak interactions, and fluff-ups (Fig. 7A; 7C,
559 right).

560 These results highlight the complexity of information that AId neurons are processing. In many
561 instances, single neurons were excited during a given behavioral state, but not during the scored
562 movement that characterized that state. For example, some neurons were excited during eating
563 states but suppressed during discrete eating-related pecks, indicating that the overall excitation
564 seen across eating states was due in part to some other (non-pecking) influence. In addition,
565 many neurons that showed excitation during a behavioral state were also responsive during
566 multiple scored movements that were unrelated to that state. For example, an eating-state-
567 excited neuron could also respond during movements that occurred outside of eating states, such
568 as preening or fluff-ups. Thus, single neurons could demonstrate increased firing rates during
569 state periods that were unrelated to scored movements, while also exhibiting modulation during
570 specific movements that occur outside of that state type.

571 In addition, while the majority of neurons we recorded were responsive during one or more
572 scored movements (Fig. 2), 18 out of 119 cells (15%) were not significantly modulated during
573 any scored movement. However, firing rates of most of these “movement-unresponsive”
574 neurons were modulated during at least one state type compared to quiescence (15/18, 83%; Fig.
575 8). This result suggests that some of the neurons unresponsive during scored movements
576 nonetheless exhibit firing rate changes related to unscored factors as the juvenile is actively
577 behaving.

578

579

DISCUSSION

580 *Heterogeneous activity within AId reflects multi-dimensional tuning*

581 We found that most AId neurons were selective for single movements or for different
582 combinations of movements. Neurons responsive during different movements frequently
583 demonstrated excitation during one movement and suppression during another. Moreover,
584 individual responses could include transient excitation at movement onset and/or offset as well
585 as suppression of average firing rate during the movement itself, or vice versa. The diversity of
586 neural responses in AId is strikingly similar to the response profile of neurons in macaque motor
587 cortex, where single neurons demonstrate heterogeneous, multiphasic temporal patterns of
588 activity across reaching movements (Churchland and Shenoy, 2007). Such complex responses
589 may result from multiple inputs relating to different movements or aspects of movements onto
590 single AId neurons, as well as local transformation of afferent inputs. AId includes a local
591 inhibitory network, evidenced by the fact that blocking GABA-A receptors in AId of
592 anesthetized zebra finches elicits increased spontaneous firing rates, and parvalbumin expression
593 is higher in AId compared to surrounding motor cortex (Mello et al., 2019; Yuan and Bottjer,
594 2019). Similarly, mammalian motor cortex contains a substantial population of inhibitory
595 interneurons, which have been implicated in both regulating plasticity during motor skill learning
596 and coordinating activity across motor cortex during behavior (Jacobs and Donoghue, 1991;
597 Hess and Donoghue, 1994; Hess et al., 1996; Markram et al., 2004; Stagg et al., 2011; Donato et
598 al., 2013; Chen et al., 2015; Kida et al., 2016; Adler et al., 2019). Blocking inhibition may
599 unmask latent excitatory connections between spatially distant motor cortical neurons, providing
600 a mechanism by which dynamic modulation of inhibition could flexibly reorganize connectivity
601 and coordinate population activity across motor cortex (Jacobs and Donoghue, 1991; Spiro et al.,
602 1999; Schneider et al., 2002; Capaday, 2004). AId receives topographic input from parallel
603 circuits that process auditory, visual, and somatosensory information, so a similar mechanism to
604 link different neuronal subpopulations within AId would be advantageous for facilitating
605 sensorimotor integration across modalities (Zeier and Karten, 1971; Bottjer et al., 2000; Paterson
606 and Bottjer, 2017).

607 The heterogeneous response profile of motor cortical neurons across taxa raises interesting
608 questions about what factors contribute to the tuning of these neurons. Modulation of motor

609 cortical activity has been associated with a variety of behavioral parameters in arm-reaching
610 tasks, including movement direction, speed, trajectory, limb position, and joint angle (Evarts,
611 1968; Cheney and Fetz, 1980; Georgopoulos et al., 1982, 1988; Schwartz et al., 1988; Fu et al.,
612 1993; Schwartz and Moran, 2000; Reina et al., 2001; Paninski et al., 2004; Churchland and
613 Shenoy, 2007; Hatsopoulos et al., 2007). Increasing evidence indicates that multiple parameters
614 can be reflected in the activity of single neurons, suggesting that integrated multi-modal tuning
615 may be a fundamental feature of motor cortical activity. For instance, recordings from macaques
616 during unrestrained arm movements showed that parameters such as movement direction or end
617 position of the limb could account for only a portion of spiking patterns in single motor cortical
618 neurons, indicating that individual neurons may be tuned in a multidimensional space and that
619 testing neural activity relative to any single parameter may account only partially for
620 multidimensional tuning profiles (Ashe and Georgopoulos, 1994; Fu et al., 1995; Moran and
621 Schwartz, 1999; Aflalo and Graziano, 2006, 2007). Likewise, we found that single AId neurons
622 could be modulated both during diverse individual movements and during behavioral state
623 periods that did not include those movements, indicating that single neurons were modulated by
624 multiple factors.

625 Given convergent input from a diverse array of processing streams, the tuning profiles of
626 neurons in both avian and mammalian motor cortex are not limited to motor responsivity. For
627 instance, neurons in RA, which lies adjacent to AId in songbird motor cortex, drive vocal motor
628 output in singing birds and demonstrate robust responses to playback of song stimuli in
629 anesthetized or sleeping birds (Nottebohm et al., 1976; Doupe and Konishi, 1991; Vicario and
630 Yohay, 1993; Wild, 1993; Yu and Margoliash, 1996; Dave et al., 1998; Dave and Margoliash,
631 2000; Leonardo and Fee, 2005; Kojima and Doupe, 2007; Sober et al., 2008; Yuan and Bottjer,
632 2019). Recordings from macaque motor cortex have likewise demonstrated sensitivity to non-
633 motor stimuli: for instance, in visually-guided target-reaching paradigms, some motor cortical
634 neurons exhibit selective activity related to the visual target, regardless of the limb trajectory
635 used to reach that target (Tanji and Evarts, 1976; Evarts and Fromm, 1977; Murata et al., 1997;
636 Shen and Alexander, 1997). The activity of AId neurons may be similarly modulated by
637 integration of various factors to produce heterogeneous responses with complex temporal
638 patterning during diverse movements. In support of this idea, we found neurons whose activity

639 was modulated across behavioral state periods but not during any scored movements (Fig. 8).
640 Although this activity could be related to head movements, which we did not score
641 comprehensively, another possibility is that activity in these neurons relates to non-motor factors
642 such as visual processing, attention, or arousal (Knudsen et al., 1995; Rauske et al., 2003; Cardin
643 and Schmidt, 2004; Winkowski and Knudsen, 2007; Fernández et al., 2020).

644 ***Multi-modal integration provides behavioral context for voluntary movements***

645 We found that most AId neurons demonstrated altered response strength during movements
646 relative to quiescence; under the conditions of our recordings, firing rate modulations occurred
647 most often during pecks, preening, and/or hops. It is difficult to know whether movement-
648 related activity in AId neurons is pre-motor, modulatory, or reflective of movement feedback or
649 external sensory inputs. AId neurons project to several targets, including the striatum, a dorsal
650 thalamic zone, the lateral hypothalamus, a thalamic nucleus that relays to cerebellum (SpM,
651 medial spiriform nucleus), deep layers of the tectum, broad areas of the pontine and midbrain
652 reticular formation, and the ventral tegmental area (Fig. 1A) (Bottjer et al., 2000). The medial
653 pontine reticular formation contains premotor neurons that contribute to neck and locomotive
654 movements in other avian species (Steeves et al., 1987; Valenzuela et al., 1990; Dubbeldam,
655 1998; Wild and Krützfeldt, 2012); peck, preening, and hop-related activity may in part reflect
656 these projections to premotor centers. Previous studies have found evidence of increased
657 expression of the immediate early gene *egr-1* in AId specifically during hopping behavior
658 (Feenders et al., 2008), which could indicate motor-related activity.

659 While these studies may indicate a role for AId in motor execution, other lines of evidence
660 suggest that movement-related activity in many AId neurons is unlikely to reflect direct motor
661 drive of peck, preening, and/or hopping behavior per se. Importantly, lesions of AId in juvenile
662 birds do not disrupt song output or induce noticeable motor deficits, suggesting that AId neurons
663 are not driving voluntary pecking or hopping movements (Bottjer and Altenau, 2010; cf.
664 Mandelblat-Cerf et al., 2014, Materials and Methods). Moreover, the results presented here
665 demonstrate that single AId neurons do not respond consistently during one particular type of
666 movement. For instance, we found a substantial population of peck-responsive neurons that
667 modulated their firing rate during pecks when the bird was eating but not when the bird pecked
668 at other objects around the cage (or vice versa), even though pecking movements in these

669 different contexts would recruit many of the same muscle groups. Furthermore, behavioral and
670 functional experiments across avian species have implicated intermediate arcopallium in highly
671 integrative, complex behaviors that extend beyond pure motor control, including ingestive
672 behaviors, working memory processing, fear conditioning, and vocal learning (Lowndes and
673 Davies, 1994; Lowndes et al., 1994; Knudsen and Knudsen, 1996; Aoki et al., 2006; Campanella
674 et al., 2009; Saint-Dizier et al., 2009; Bottjer and Altenau, 2010; Achiro et al., 2017). One such
675 example is a region comprising caudal arco- and nidopallium, which partially overlaps with AId
676 and exhibited increased 2-deoxyglucose uptake when adult male zebra finches participated in
677 their first courtship experience following several weeks of isolation from females; the amount of
678 glucose consumption in this region correlated positively with isolation time but not with amount
679 of movement activity (Bischof and Herrmann, 1986).

680 Rather than generically driving motor behavior per se, an important function of motor cortical
681 circuitry is incorporating sensory information to appropriately direct motor output based on an
682 animal's environment and/or goals; this sensorimotor integration is necessary for voluntary
683 movements such as goal-directed motor behaviors (e.g., object-directed grasping) as well as
684 adaptive movements based on environmental perturbations. Avian and mammalian motor
685 cortices receive multi-modal inputs and target brainstem regions, making them ideally situated to
686 carry out sensorimotor integration during voluntary behaviors. In macaques, neurons in motor
687 cortical areas demonstrate a selective response when grasping at a particular object and
688 corresponding visual selectivity for the same object when the monkey fixates on the object
689 without grasping; inactivation of the same motor cortical region resulted in grasping deficits due
690 to disrupted preparatory hand shaping that was inappropriate for the target object, suggesting a
691 specific impairment in visuomotor transformations for targeted grasping rather than a gross
692 motor impairment of hand movements (Murata et al., 1997; Fogassi, 2001; Rizzolatti and
693 Luppino, 2001). Some AId neurons may serve a similar function in integrating sensory
694 information to provide appropriate context for motor output – for example, the “eating-peck”
695 responses observed here could represent an integrated response when the visual stimulus of seed
696 is present as the bird pecks; these neurons could link visual information about seed with
697 somatosensory information to contribute specifically to food-directed pecking behavior. In
698 contrast, neurons that showed excitation during pecks associated with non-eating behaviors may

699 process diverse inputs in the context of object exploration. Notably, some AId neurons could be
700 involved in sensory-motor integration and also play a role in motor execution; likewise, other
701 AId neurons could provide appropriate context for voluntary behaviors without directly driving
702 motor actions – for instance, rather than directly linking sensory information to premotor centers,
703 some neurons could instead feed multi-modal information back into ascending reticular or tectal
704 pathways to contribute to goal-directed behaviors, or differentially ascribe value to
705 environmental cues depending on the animal’s current state or needs (Burgess et al., 2018).

706 *AId is uniquely situated to mediate learning and performance of both vocal and non-vocal*
707 *elements of song behavior*

708 Although motor cortex is generally well-situated to integrate multi-modal information related to
709 a variety of goal-directed movements, AId’s unique connections suggest it may occupy a specific
710 role in vocal learning and behavior. LMAN-CORE neurons that drive vocal motor output in
711 juvenile birds make robust collateral projections into AId at 20-35 dph that substantially decline
712 by 45 dph (Miller-Sims and Bottjer, 2012; Chung and Bottjer, unpublished observations). While
713 our data set did not include ages young enough to test the functional role of this connection
714 between LMAN-CORE and AId, information from this developmentally regulated projection may
715 play a critical role during the earliest stages of vocal practice and influence patterns of
716 connectivity within AId that contribute to sensorimotor processing during subsequent learning.
717 In addition, AId projects to higher-order thalamic nuclei that are linked to vocal learning, DLM
718 (dorsolateral nucleus of the medial thalamus) and DMP (dorsomedial nucleus of the posterior
719 thalamus) (Fig. 1A) (Bottjer et al., 2000). DLM is required for normal song behavior and
720 projects to LMAN-SHELL, whereas DMP projects to MMAN (medial magnocellular nucleus of
721 anterior nidopallium); both LMAN and MMAN are required for development of an accurate
722 imitation of tutor song (Bottjer et al., 1984; Foster et al., 1997; Vates et al., 1997; Foster and
723 Bottjer, 2001; Aronov et al., 2008; Goldberg and Fee, 2011; Chen et al., 2014). AId also projects
724 to lateral hypothalamus, striatum, and the area of dopaminergic neurons in the ventral tegmental
725 area that projects to a nucleus in avian basal ganglia necessary for song learning (Fig. 1A)
726 (Bottjer et al., 2000); these limbic-related projections further suggest that AId neurons are well
727 situated to contribute to vocal learning and behavior.

728 One hypothesis to integrate these unique connections with the multi-modal integrative function
729 of motor cortex is that some AId neurons may be involved in mediating learning and
730 performance of movements in the context of song behavior. Song production in zebra finches is
731 a courtship behavior during which males vocalize while performing a dance-like sequence of
732 hopping movements oriented towards a female (Morris, 1954; Williams, 2001; Cooper and
733 Goller, 2004; Dalziell et al., 2013; Ota et al., 2015; Ullrich et al., 2016); the temporal patterning
734 of dance movements during song production is significantly correlated between father-son pairs
735 of zebra finches, suggesting that non-vocal behaviors that accompany singing may be learned as
736 well (Williams, 2001). Establishing a social context for courtship behavior likely involves
737 sensory cues. For instance, adult males can use visual cues to select between two female birds
738 shown in a silent video feed, but the addition of auditory cues induces stronger courtship
739 responses (Galoch and Bischof, 2006, 2007). AId neurons are ideally positioned to integrate
740 environmental context cues when females are present to guide learning and performance of
741 movements that accompany singing.

742 In this framework, the relatively high proportions of peck, preening, and hopping-related
743 responses observed here may reflect the fact that beak movements and hopping are important
744 components of courtship behavior. In quail-chick chimeras, chicks that received transplants of
745 lower brainstem somites from quails retained chick-like call structures but adopted quail-like
746 patterns of head movements specifically during vocalizations; similar head movements made
747 outside of vocalization periods were not affected, indicating the presence of specialized circuitry
748 that mediates movements in the context of vocal behavior (Balaban, 1997). Circuitry processing
749 non-vocal elements of singing in zebra finches may be similarly specialized; our results suggest
750 that investigating how activity patterns during singing correspond to movements of different
751 peripheral targets would benefit hypotheses for mechanisms of song production: for instance, we
752 found a subpopulation of AId neurons that exhibited differential response strength during head
753 movements that occurred during singing versus non-singing periods. These responses may
754 indicate integration between non-vocal and vocal elements of song behavior, such that neural
755 activity is enhanced specifically during head movements that accompany singing. This
756 hypothesis raises an interesting prediction: although AId lesions in juvenile birds do not induce
757 any gross motor deficits, it is possible that hopping and/or head movements performed during

758 song production would be disrupted. Such a result would be consistent with previous studies in
759 which c-Fos expression in AId was increased after adult male zebra finches performed non-
760 singing courtship behaviors directed towards a live female (Kimpo and Doupe, 1997).
761 Involvement of AId in courtship-related movements would draw an interesting parallel to
762 mammalian studies that have suggested that motor cortex is parceled into “action zones” that
763 each process information for different ethologically relevant categories of movement (Graziano,
764 2006; Graziano and Aflalo, 2007). For instance, stimulation of one region of macaque motor
765 cortex results in the animal closing its hand in a grip while bringing it to its mouth and opening
766 its mouth, as if eating an object, while stimulation of another region results in the monkey raising
767 its arm and turning its head sharply to one side as if in defense (Graziano et al., 2002). In this
768 context, adjacent motor cortical regions RA and AId could serve as an “action zone” that
769 mediates the vocal and non-vocal movements that comprise song behavior.

770 While brainstem projections may mediate sensorimotor processing during vocal motor
771 performance, the thalamic and midbrain projections of AId that give rise to recurrent feedback
772 loops through cortico-basal ganglia circuitry may integrate multi-modal information to facilitate
773 song learning. Although AId does not drive song output (Bottjer et al., 2000; Bottjer and
774 Altenau, 2010), we found a substantial population of singing-responsive neurons. For a large
775 proportion of these neurons, the changes in firing rate during singing could not be attributed to
776 any of the movements that we scored (including head movements). These firing rate
777 modulations may instead be related to singing-specific movements such as beak movements or
778 gular fluttering, or sensory activity such as auditory or proprioceptive feedback (Goller et al.,
779 2004; Ohms et al., 2010; Bottjer and To, 2012; Riede et al., 2013). Alternatively, singing-related
780 activity could reflect active evaluations of the juvenile’s vocal behavior during sensorimotor
781 practice – iterative evaluations between self-generated output and the goal tutor song are
782 essential for guiding accurate refinement of the juvenile’s song, and evidence of neural activity
783 processing these comparisons has been reported in LMAN-SHELL, which projects directly to AId
784 (Achiro et al., 2017). Importantly, successful song learning also requires multiple factors beyond
785 simply matching vocal output to an auditory goal – for instance, vocal learning in juvenile zebra
786 finches that are tutored with only passive playback of the tutor song is severely impaired,
787 whereas pairing auditory tutoring with a visual model of an adult zebra finch enhances learning

788 (Derégnaucourt et al., 2013; Ljubičić et al., 2016). Moreover, visual cues provided during
789 singing, such as wing strokes or fluff-ups from adult females, provide feedback that can
790 influence juvenile vocal learning (West and King, 1988; Morrison and Nottebohm, 1993; King et
791 al., 2005; Carouso-Peck and Goldstein, 2019). Multi-modal inputs from dNCL and singing-
792 related inputs from LMAN may converge in AId, integrating important non-vocal and vocal
793 elements of courtship song behavior that must be learned during a sensitive period of
794 development.

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REFERENCES

- 797 Achiro JM, Bottjer SW (2013) Neural representation of a target auditory memory in a cortico-
798 basal ganglia pathway. *J Neurosci* 33:14475–14488.
- 799 Achiro JM, Shen J, Bottjer SW (2017) Neural activity in cortico-basal ganglia circuits of juvenile
800 songbirds encodes performance during goal-directed learning. *Elife* 6:e26973.
- 801 Adler A, Zhao R, Shin ME, Yasuda R, Gan WB (2019) Somatostatin-expressing interneurons
802 enable and maintain learning-dependent sequential activation of pyramidal neurons. *Neuron*
803 102:202–216.e7.
- 804 Afalo TN, Graziano MSA (2006) Partial tuning of motor cortex neurons to final posture in a
805 free-moving paradigm. *Proc Natl Acad Sci U S A* 103:2909–2914.
- 806 Afalo TN, Graziano MSA (2007) Relationship between unconstrained arm movements and
807 single-neuron firing in the macaque motor cortex. *J Neurosci* 27:2760–2780.
- 808 Aoki N, Csillag A, Matsushima T (2006) Localized lesions of arcopallium intermedium of the
809 lateral forebrain caused a handling-cost aversion in the domestic chick performing a binary
810 choice task. *Eur J Neurosci* 24:2314–2326.
- 811 Aronov D, Andalman AS, Fee MS (2008) A specialized forebrain circuit for vocal babbling in
812 the juvenile songbird. *Science* 320:630–634.
- 813 Ashe J, Georgopoulos AP (1994) Movement parameters and neural activity in motor cortex and
814 Area 5. *Cereb Cortex* 4:590–600.
- 815 Balaban E (1997) Changes in multiple brain regions underlie species differences in a complex,
816 congenital behavior. *Proc Natl Acad Sci U S A* 94:2001–2006.
- 817 Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful
818 approach to multiple testing. *J R Stat Soc* 57:289–300.
- 819 Bischof HJ, Herrmann K (1986) Arousal enhances [14C]2-deoxyglucose uptake in four forebrain
820 areas of the zebra finch. *Behav Brain Res* 21:215–221.
- 821 Böhner J (1983) Song learning in the zebra finch (*taeniopygia guttata*): Selectivity in the choice
822 of a tutor and accuracy of song copies. *Anim Behav* 31:231–237.

- 823 Böhner J (1990) Early acquisition of song in the zebra finch, *Taeniopygia guttata*. *Anim Behav*
824 39:369–374.
- 825 Bottjer SW, Altenau B (2010) Parallel pathways for vocal learning in basal ganglia of songbirds.
826 *Nat Neurosci* 13:153–155.
- 827 Bottjer SW, Brady JD, Cribbs B (2000) Connections of a motor cortical region in zebra finches:
828 relation to pathways for vocal learning. *J Comp Neurol* 260:244–260.
- 829 Bottjer SW, Miesner EA, Arnold AP (1984) Forebrain lesions disrupt development but not
830 maintenance of song in passerine birds. *Science* 224:901–903.
- 831 Bottjer SW, To M (2012) Afferents from Vocal Motor and Respiratory Effectors Are Recruited
832 during Vocal Production in Juvenile Songbirds. *J Neurosci* 32:10895–10906.
- 833 Burgess CR, Livneh Y, Ramesh RN, Andermann ML (2018) Gating of visual processing by
834 physiological need. *Curr Opin Neurobiol* 49:16–23.
- 835 Campanella LCA, Silva AA da, Gellert DS, Parreira C, Ramos MC, Paschoalini MA, Marino-
836 Neto J (2009) Tonic serotonergic control of ingestive behaviours in the pigeon (*Columba*
837 *livia*): The role of the arcopallium. *Behav Brain Res* 205:396–405.
- 838 Capaday C (2004) The integrated nature of motor cortical function. *Neuroscientist* 10:207–220.
- 839 Cardin JA, Schmidt MF (2004) Auditory responses in multiple sensorimotor song system nuclei
840 are co-modulated by behavioral state. *J Neurophysiol* 91:2148–2163.
- 841 Carouso-Peck S, Goldstein MH (2019) Female Social Feedback Reveals Non-imitative
842 Mechanisms of Vocal Learning in Zebra Finches. *Curr Biol* 29:631–636.e3.
- 843 Chen JR, Stepanek L, Doupe AJ (2014) Differential contributions of basal ganglia and thalamus
844 to song initiation, tempo, and structure. *J Neurophysiol* 111:248–257.
- 845 Chen SX, Kim AN, Peters AJ, Komiyama T (2015) Subtype-specific plasticity of inhibitory
846 circuits in motor cortex during motor learning. *Nat Neurosci* 18:1109–1115.
- 847 Chen Y, Matheson LE, Sakata JT (2016) Mechanisms underlying the social enhancement of
848 vocal learning in songbirds. *Proc Natl Acad Sci U S A* 113:6641–6646.

- 849 Cheney PD, Fetz EE (1980) Functional classes of primate corticomotoneuronal cells and their
850 relation to active force. *J Neurophysiol* 44:773–791.
- 851 Churchland MM, Shenoy K V. (2007) Temporal complexity and heterogeneity of single-neuron
852 activity in premotor and motor cortex. *J Neurophysiol* 97:4235–4257.
- 853 Cooper BG, Goller F (2004) Multimodal Signals: Enhancement and Constraint of Song Motor
854 Patterns by Visual Display. *Science* 303:544–546.
- 855 Dalziell AH, Peters RA, Cockburn A, Dorland AD, Maisey AC, Magrath RD (2013) Dance
856 choreography is coordinated with song repertoire in a complex avian display. *Curr Biol*
857 23:1132–1135.
- 858 Darling WG, Pizzimenti MA, Morecraft RJ (2011) Functional recovery following motor cortex
859 lesions in non-human primates: Experimental implications for human stroke patients. *J*
860 *Integr Neurosci* 10:353–384.
- 861 Dave AS, Margoliash D (2000) Song Replay During Sleep and Computational Rules for
862 Sensorimotor Vocal Learning. *Science* 290:812–816.
- 863 Dave AS, Yu AC, Margoliash D (1998) Behavioral state modulation of auditory activity in a
864 vocal motor system. *Science* 282:2250–2254.
- 865 Derégnaucourt S, Poirier C, Kant A Van der, Linden A Van der, Gahr M (2013) Comparisons of
866 different methods to train a young zebra finch (*Taeniopygia guttata*) to learn a song. *J*
867 *Physiol* 107:210–218.
- 868 Donato F, Rompani SB, Caroni P (2013) Parvalbumin-expressing basket-cell network plasticity
869 induced by experience regulates adult learning. *Nature* 504:272–276.
- 870 Doupe AJ, Konishi M (1991) Song-selective auditory circuits in the vocal control system of the
871 zebra finch. *Proc Natl Acad Sci U S A* 88:11339–11343.
- 872 Dubbeldam JL (1998) The neural substrate for “learned” and “nonlearned” activities in birds: A
873 discussion of the organization of bulbar reticular premotor systems with side-lights on the
874 mammalian situation. *Acta Anatomica*, pp 157–172.
- 875 Eales LA (1985) Song learning in zebra finches: some effects of song model availability on what

- 876 is learnt and when. *Anim Behav* 33:1293–1300.
- 877 Eales LA (1989) The influences of visual and vocal interaction on song learning in Zebra
878 finches. *Anim Behav* 37:507–508.
- 879 Ebbesen CL, Doron G, Lenschow C, Brecht M (2017) Vibrissa motor cortex activity suppresses
880 contralateral whisking behavior. *Nat Neurosci* 20:82–89.
- 881 Evarts E V. (1968) Relation of pyramidal tract activity to force exerted during voluntary
882 movement. *J Neurophysiol* 31:14–27.
- 883 Evarts E V., Fromm C (1977) Sensory responses in motor cortex neurons during precise motor
884 control. *Neurosci Lett* 5:267–272.
- 885 Feenders G, Liedvogel M, Rivas M, Zapka M, Horita H, Hara E, Wada K, Mouritsen H, Jarvis
886 ED (2008) Molecular mapping of movement-associated areas in the avian brain: A motor
887 theory for vocal learning origin. *PLoS One* 3(3):e1768.
- 888 Ferezou I, Haiss F, Gentet LJ, Aronoff R, Weber B, Petersen CCH (2007) Spatiotemporal
889 Dynamics of Cortical Sensorimotor Integration in Behaving Mice. *Neuron* 56:907–923.
- 890 Fernández M, Morales C, Durán E, Fernández-Colleman S, Sentis E, Mpodozis J, Karten HJ,
891 Marín GJ (2020) Parallel organization of the avian sensorimotor arcopallium: Tectofugal
892 visual pathway in the pigeon (*Columba livia*). *J Comp Neurol* 528:597–623.
- 893 Fogassi L (2001) Cortical mechanism for the visual guidance of hand grasping movements in the
894 monkey: A reversible inactivation study. *Brain* 124:571–586.
- 895 Foster EF, Bottjer SW (2001) Lesions of a telencephalic nucleus in male zebra finches:
896 Influences on vocal behavior in juveniles and adults. *J Neurobiol* 46:142–165.
- 897 Foster EF, Mehta RP, Bottjer SW (1997) Axonal connections of the medial magnocellular
898 nucleus of the anterior neostriatum in zebra finches. *J Comp Neurol* 382:364–381.
- 899 Fu QG, Flament D, Coltz JD, Ebner TJ (1995) Temporal encoding of movement kinematics in
900 the discharge of primate primary motor and premotor neurons. *J Neurophysiol* 73:836–854.
- 901 Fu QG, Suarez JI, Ebner TJ (1993) Neuronal specification of direction and distance during
902 reaching movements in the superior precentral premotor area and primary motor cortex of

- 903 monkeys. *J Neurophysiol* 70:2097–2116.
- 904 Gale SD, Person AL, Perkel ADJ (2008) A novel basal ganglia pathway forms a loop linking a
905 vocal learning circuit with its dopaminergic input. *J Comp Neurol* 508:824–839.
- 906 Galoch Z, Bischof HJ (2006) Zebra Finches actively choose between live images of conspecifics.
907 *Ornithol Sci* 5:57–64.
- 908 Galoch Z, Bischof HJ (2007) Behavioural responses to video playbacks by zebra finch males.
909 *Behav Processes* 74:21–26.
- 910 Georgopoulos AP, Kalaska JF, Caminiti R, Massey JT (1982) On the relations between the
911 direction of two-dimensional arm movements and cell discharge in primate motor cortex. *J*
912 *Neurosci* 2:1527–1537.
- 913 Georgopoulos AP, Kettner RE, Schwartz AB (1988) Primate motor cortex and free arm
914 movements to visual targets in three-dimensional space. II. Coding of the direction of
915 movement by a neuronal population. *J Neurosci* 8:2928–2937.
- 916 Goldberg JH, Fee MS (2011) Vocal babbling in songbirds requires the basal ganglia-recipient
917 motor thalamus but not the basal ganglia. *J Neurophysiol* 105:2729–2739.
- 918 Goller F, Mallinckrodt MJ, Torti SD (2004) Beak gape dynamics during song in the zebra finch.
919 *J Neurobiol* 59:289–303.
- 920 Graziano M (2006) The organization of behavioral repertoire in motor cortex. *Annu Rev*
921 *Neurosci* 29:105–134.
- 922 Graziano MS., Taylor CS., Moore T (2002) Complex movements evoked by microstimulation of
923 precentral cortex. *Neuron* 34:841–851.
- 924 Graziano MSA, Aflalo TN (2007) Mapping behavioral repertoire onto the cortex. *Neuron*
925 56:239–251.
- 926 Guo JZ, Graves AR, Guo WW, Zheng J, Lee A, Rodríguez-González J, Li N, Macklin JJ,
927 Phillips JW, Mensh BD, Branson K, Hantman AW (2015) Cortex commands the
928 performance of skilled movement. *Elife* 4:e10774.
- 929 Hatsopoulos NG, Xu Q, Amit Y (2007) Encoding of movement fragments in the motor cortex. *J*

- 930 *Neurosci* 27:5105–5114.
- 931 Hess G, Aizenman CD, Donoghue JP (1996) Conditions for the induction of long-term
932 potentiation in layer II/III horizontal connections of the rat motor cortex. *J Neurophysiol*
933 75:1765–1778.
- 934 Hess G, Donoghue JP (1994) Long-term potentiation of horizontal connections provides a
935 mechanism to reorganize cortical motor maps. *J Neurophysiol* 71:2543–2547.
- 936 Hwang EJ, Dahlen JE, Hu YY, Aguilar K, Yu B, Mukundan M, Mitani A, Komiyama T (2019)
937 Disengagement of motor cortex from movement control during long-term learning. *Sci Adv*
938 5:eaay0001.
- 939 Immelmann K (1969) Song development in the zebra finch and other estrildid finches. In: Bird
940 Vocalizations (Hinde RA, ed), pp 61–74. Cambridge University Press.
- 941 Jacobs K, Donoghue J (1991) Reshaping the cortical motor map by unmasking latent
942 intracortical connections. *Science* 251:944–947.
- 943 Karten HJ (2013) Neocortical evolution: Neuronal circuits arise independently of lamination.
944 *Curr Biol* 23:R12–R15.
- 945 Kawai R, Markman T, Poddar R, Ko R, Fantana AL, Dhawale AK, Kampff AR, Ölveczky BP
946 (2015) Motor cortex is required for learning but not for executing a motor skill. *Neuron*
947 86:800–812.
- 948 Kelley DB, Nottebohm F (1979) Projections of a telencephalic auditory nucleus-field L-in the
949 canary. *J Comp Neurol* 183:455–469.
- 950 Kida H, Tsuda Y, Ito N, Yamamoto Y, Owada Y, Kamiya Y, Mitsushima D (2016) Motor
951 training promotes both synaptic and intrinsic plasticity of layer II/III pyramidal neurons in
952 the primary motor cortex. *Cereb Cortex* 26:3494–3507.
- 953 Kimpo RR, Doupe AJ (1997) FOS is induced by singing in distinct neuronal populations in a
954 motor network. *Neuron* 18:315–325.
- 955 King AP, West MJ, Goldstein MH (2005) Non-vocal shaping of avian song development:
956 Parallels to human speech development. *Ethology* 111:101–117.

- 957 Knudsen EI, Cohen YE, Masino T (1995) Characterization of a forebrain gaze field in the
958 archistriatum of the barn owl: Microstimulation and anatomical connections. *J Neurosci*
959 15:5139–5151.
- 960 Knudsen EI, Knudsen PF (1996) Disruption of auditory spatial working memory by inactivation
961 of the forebrain archistriatum in barn owls. *Nature* 383:428–431.
- 962 Kojima S, Doupe AJ (2007) Song selectivity in the pallial-basal ganglia song circuit of zebra
963 finches raised without tutor song exposure. *J Neurophysiol* 98:2099–2109.
- 964 Leonardo A, Fee MS (2005) Ensemble coding of vocal control in birdsong. *J Neurosci* 25:652–
965 661.
- 966 Ljubičić I, Hyland Bruno J, Tchernichovski O (2016) Social influences on song learning. *Curr*
967 *Opin Behav Sci* 7:101–107.
- 968 Lowndes M, Davies DC (1994) The Effects of Archistriatal Lesions on One-trial Passive
969 Avoidance Learning in the Chick. *Eur J Neurosci* 6:525–530.
- 970 Lowndes M, Davies DC, Johnson MH (1994) Archistriatal Lesions Impair the Acquisition of
971 Filial Preferences During Imprinting in the Domestic Chick. *Eur J Neurosci* 6:1143–1148.
- 972 Ludwig KA, Miriani RM, Langhals NB, Joseph MD, Anderson DJ, Kipke DR (2009) Using a
973 common average reference to improve cortical neuron recordings from microelectrode
974 arrays. *J Neurophysiol* 101:1679–1689.
- 975 Makino H, Hwang EJ, Hedrick NG, Komiyama T (2016) Circuit Mechanisms of Sensorimotor
976 Learning. *Neuron* 92:705–721.
- 977 Mandelblat-Cerf Y, Las L, Denisenko N, Fee MS (2014) A role for descending auditory cortical
978 projections in songbird vocal learning. *Elife* 3:e02152.
- 979 Mann NI, Slater PJB (1995) Song tutor choice by zebra finches in aviaries. *Anim Behav* 49:811–
980 820.
- 981 Mann NI, Slater PJB, Eales LA, Richards C (1991) The influence of visual stimuli on song tutor
982 choice in the zebra finch, *Taeniopygia guttata*. *Anim Behav* 42:285–293.
- 983 Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C (2004) Interneurons

- 984 of the neocortical inhibitory system. *Nat Rev Neurosci* 5:793–807.
- 985 Mello C V, Kaser T, Buckner AA, Wirthlin M, Lovell P V (2019) Molecular architecture of the
986 zebra finch arcopallium. *J Comp Neurol* 527:2512–2556.
- 987 Mello C V, Vates GE, Okuhata S, Nottebohm F (1998) Descending auditory pathways in the
988 adult male zebra finch (*Taeniopygia guttata*). *J Comp Neurol* 160:137–160.
- 989 Miller-Sims VC, Bottjer SW (2012) Auditory experience refines cortico-basal ganglia inputs to
990 motor cortex via remapping of single axons during vocal learning in zebra finches. *J*
991 *Neurophysiol* 107:1142–1156.
- 992 Mimica B, Dunn BA, Tombaz T, Srikanth Bojja VPTN, Whitlock JR (2018) Efficient cortical
993 coding of 3D posture in freely behaving rats. *Science* 362:584–589.
- 994 Moran DW, Schwartz AB (1999) Motor cortical representation of speed and direction during
995 reaching. *J Neurophysiol* 82:2676–2692.
- 996 Morris D (1954) The Reproductive Behaviour of the Zebra Finch (*Poephila Guttata*), With
997 Special Reference To Pseudofemale Behaviour and Displacement Activities. *Behaviour*
998 6:271–322.
- 999 Morrison RG, Nottebohm F (1993) Role of a telencephalic nucleus in the delayed song learning
1000 of socially isolated zebra finches. *J Neurobiol* 24:1045–1064.
- 1001 Murata A, Fadiga L, Fogassi L, Gallese V, Raos V, Rizzolatti G (1997) Object Representation in
1002 the Ventral Premotor Cortex (Area F5) of the Monkey. *J Neurophysiol* 78:2226–2230.
- 1003 Nottebohm F, Stokes TM, Leonard CM (1976) Central control of song in the canary, *Serinus*
1004 *canarius*. *J Comp Neurol* 165:457–486.
- 1005 Ohms VR, Snelderwaard PC, ten Cate C, Beckers GJL (2010) Vocal tract articulation in zebra
1006 finches Reby D, ed. *PLoS One* 5:e11923.
- 1007 Ota N, Gahr M, Soma M (2015) Tap dancing birds: The multimodal mutual courtship display of
1008 males and females in a socially monogamous songbird. *Sci Rep* 5:6–11.
- 1009 Paninski L, Fellows MR, Hatsopoulos NG, Donoghue JP (2004) Spatiotemporal tuning of motor
1010 cortical neurons for hand position and velocity. *J Neurophysiol* 91:515–532.

- 1011 Papale AE, Hooks BM (2018) Circuit changes in motor cortex during motor skill learning.
1012 *Neuroscience* 368:283–297.
- 1013 Paterson AK, Bottjer SW (2017) Cortical inter-hemispheric circuits for multimodal vocal
1014 learning in songbirds. *J Comp Neurol* 525(15):3312–3340.
- 1015 Peters AJ, Liu H, Komiyama T (2017) Learning in the Rodent Motor Cortex. *Annu Rev Neurosci*
1016 40:77–97.
- 1017 Price PH (1979) Developmental determinants of structure in zebra finch song. *J Comp Physiol*
1018 *Psychol* 93:260–277.
- 1019 Rauske PL, Shea SD, Margoliash D (2003) State and neuronal class-dependent reconfiguration
1020 in the avian song system. *J Neurophysiol* 89:1688–1701.
- 1021 Reina GA, Moran DW, Schwartz AB (2001) On the relationship between joint angular velocity
1022 and motor cortical discharge during reaching. *J Neurophysiol* 85:2576–2589.
- 1023 Riede T, Schilling N, Goller F (2013) The acoustic effect of vocal tract adjustments in zebra
1024 finches. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 199:57–69.
- 1025 Rizzolatti G, Luppino G (2001) The cortical motor system. *Neuron* 31:889–901.
- 1026 Roper A, Zann R (2006) The Onset of Song Learning and Song Tutor Selection in Fledgling
1027 Zebra Finches. *Ethology* 112:458–470.
- 1028 Saint-Dizier H, Constantin P, Davies DC, Leterrier C, Lévy F, Richard S (2009) Subdivisions of
1029 the arcopallium/posterior pallial amygdala complex are differentially involved in the control
1030 of fear behaviour in the Japanese quail. *Brain Res Bull* 79:288–295.
- 1031 Schneider C, Devanne H, Lavoie BA, Capaday C (2002) Neural mechanisms involved in the
1032 functional linking of motor cortical points. *Exp Brain Res* 146:86–94.
- 1033 Schwartz AB, Kettner RE, Georgopoulos AP (1988) Primate motor cortex and free arm
1034 movements to visual targets in three-dimensional space. I. Relations between single cell
1035 discharge and direction of movement. *J Neurosci* 8:2913–2927.
- 1036 Schwartz AB, Moran DW (2000) Arm trajectory and representation of movement processing in
1037 motor cortical activity. *Eur J Neurosci* 12:1851–1856.

- 1038 Shen L, Alexander GE (1997) Neural correlates of a spatial sensory-to-motor transformation in
1039 primary motor cortex. *J Neurophysiol* 77:1171–1194.
- 1040 Sober SJ, Wohlgenuth MJ, Brainard MS (2008) Central contributions to acoustic variation in
1041 birdsong. *J Neurosci* 28:10370–10379.
- 1042 Spiro J, Dalva M, Mooney R (1999) Long-range inhibition within the zebra finch song nucleus
1043 RA can coordinate the firing of multiple projection neurons. *J Neurophysiol* 81:3007–3020.
- 1044 Stagg CJ, Bachtiar V, Johansen-Berg H (2011) The role of GABA in human motor learning.
1045 *Curr Biol* 21:480–484.
- 1046 Steeves JD, Sholomenko GN, Webster DMS (1987) Stimulation of the pontomedullary reticular
1047 formation initiates locomotion in decerebrate birds. *Brain Res* 401:205–212.
- 1048 Tanji J, Evarts E V. (1976) Anticipatory activity of motor cortex neurons in relation to direction
1049 of an intended movement. *J Neurophysiol* 39:1062–1068.
- 1050 Tomaszycski ML, Adkins-Regan E (2005) Experimental alteration of male song quality and
1051 output affects female mate choice and pair bond formation in zebra finches. *Anim Behav*
1052 70:785–794.
- 1053 Ullrich R, Norton P, Scharff C (2016) Waltzing Taeniopygia: Integration of courtship song and
1054 dance in the domesticated Australian zebra finch. *Anim Behav* 112:285–300.
- 1055 Valenzuela JI, Hasan SJ, Steeves JD (1990) Stimulation of the brainstem reticular formation
1056 evokes locomotor activity in embryonic chicken (in ovo). *Brain Res Dev Brain Res* 56:13–
1057 18.
- 1058 Vates GE, Broome BM, Mello C V, Nottebohm F (1996) Auditory pathways of caudal
1059 telencephalon and their relation to the song system of adult male zebra finches (*Taeniopygia*
1060 *guttata*). *J Comp Neurol* 366(4):613–42.
- 1061 Vates GE, Vicario DS, Nottebohm F (1997) Reafferent thalamo-’cortical’ loops in the song
1062 system of oscine songbirds. *J Comp Neurol* 380:275–290.
- 1063 Vicario DS, Yohay KH (1993) Song-selective auditory input to a forebrain vocal control nucleus
1064 in the zebra finch. *J Neurobiol* 24:488–505.

- 1065 West MJ, King AP (1988) Female visual displays affect the development of male song in the
1066 cowbird. *Nature* 334:244–246.
- 1067 Whishaw IQ (2000) Loss of the innate cortical engram for action patterns used in skilled
1068 reaching and the development of behavioral compensation following motor cortex lesions in
1069 the rat. *Neuropharmacology* 39:788–805.
- 1070 Whishaw IQ, Pellis SM, Gorny BP, Pellis VC (1991) The impairments in reaching and the
1071 movements of compensation in rats with motor cortex lesions: an endpoint, videorecording,
1072 and movement notation analysis. *Behav Brain Res* 42:77–91.
- 1073 Wilcox RR, Serang S (2017) Hypothesis Testing, p Values, Confidence Intervals, Measures of
1074 Effect Size, and Bayesian Methods in Light of Modern Robust Techniques. *Educ Psychol*
1075 *Meas* 77:673–689.
- 1076 Wild JM (1993) Descending projections of the songbird nucleus robustus archistriatalis. *J Comp*
1077 *Neurol* 338:225–241.
- 1078 Wild JM, Krützfeldt NEO (2012) Trigeminal and telencephalic projections to jaw and other
1079 upper vocal tract premotor neurons in songbirds: Sensorimotor circuitry for beak
1080 movements during singing. *J Comp Neurol* 520:590–605.
- 1081 Williams H (2001) Choreography of song, dance and beak movements in the zebra finch
1082 (*Taeniopygia guttata*). *J Exp Biol* 204:3497–3506.
- 1083 Winkowski DE, Knudsen EI (2007) Top-down control of multimodal sensitivity in the barn owl
1084 optic tectum. *J Neurosci* 27:13279–13291.
- 1085 Yu AC, Margoliash D (1996) Temporal hierarchical control of singing in birds. *Science*
1086 273:1871–1875.
- 1087 Yuan RC, Bottjer SW (2019) Differential developmental changes in cortical representations of
1088 auditory-vocal stimuli in songbirds. *J Neurophysiol* 121:530–548.
- 1089 Zeier H, Karten HJ (1971) The archistriatum of the pigeon: Organization of afferent and efferent
1090 connections. *Brain Res* 31:313–326.
- 1091 Zhang Q, Yao J, Guang Y, Liang S, Guan J, Qin H, Liao X, Jin W, Zhang J, Pan J, Jia H, Yan J,

1092 Feng Z, Li W, Chen X (2017) Locomotion-related population cortical Ca²⁺ transients in
1093 freely behaving mice. *Front Neural Circuits* 11.
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TABLES

1096 **Table 1.** Proportions of onset and offset responses across all neurons for different movement
1097 types

	Peck-onset responses (± 100 ms from peck onset)		Hop-onset responses (± 100 ms from hop onset)		Preening-onset responses (± 100 ms from preening onset)	
Response based on average firing rate	Excited (7)	Suppressed (4)	Excited (4)	Suppressed (1)	Excited (3)	Suppressed (1)
Excited	.05 (5/107)	.04 (4/107)	.01 (1/119)	0	.03 (3/93)	.01 (1/93)
Not significant	.02 (2/107)	0	.02 (2/119)	0	0	0
Suppressed	0	0	.01 (1/119)	.01 (1/119)	0	0

1098

	Peck-offset responses (± 100 ms from peck offset)		Preening-offset responses (± 100 ms from preening offset)	
Response based on average firing rate	Excited (4)	Suppressed (2)	Excited (3)	Suppressed (0)
Excited	.04 (4/107)	.01 (1/107)	.01 (1/93)	0
Not significant	0	0	.01 (1/93)	0
Suppressed	0	.01 (1/107)	.01 (1/93)	0

1099

1100 Onset responses (top) and offset responses (bottom) are shown separately (total n = 29
1101 responses), categorized based on whether average firing rate during the movement showed
1102 significant excitation (Excited), suppression (Suppressed), or no response (Not significant).

1103 **Table 2.** Mean standardized response strengths during singing periods with versus without head movements (n =
 1104 36)

1105 A. 24 singing-responsive neurons that showed comparable firing rates during head movements that occurred within
 1106 singing and non-singing periods. (Blue lines in Fig. 5C, left, depict head movement responses.)

	Singing w/ head movements > singing w/out head movements (n = 8/24)		Singing w/ head movements = singing w/out head movements (n = 14/24)		Singing w/ head movements < singing w/out head movements (n = 2/24)	
	Excited (7*)	Suppressed (1)	Excited (12)	Suppressed (2)	Excited (1)	Suppressed (1)
Singing w/ movements	0.47 ± 0.04	-1.75	1.08 ± 0.22	-0.67 ± 0.10	0.24	-0.74
Singing w/out movements	0.29 ± 0.03	-0.69	0.62 ± 0.10	-0.41 ± 0.17	0.49	-0.87

1107 * 3/7 neurons were significantly excited during singing periods that contained head movements but not during
 1108 singing periods that lacked head movements.

1109 B. Six singing-responsive neurons that showed greater response strength during head movements that occurred
 1110 within singing compared to non-singing periods. (Orange lines in Fig. 5C, right, depict head movement responses.)

	Singing w/ head movements > singing w/out head movements (n = 3/6)		Singing w/ head movements = singing w/out head movements (n = 3/6)		Singing w/ head movements < singing w/out head movements (n = 0/6)	
	Excited (3*)	Suppressed (0)	Excited (3)	Suppressed (0)	Excited (0)	Suppressed (0)
Singing w/ movements	0.84 ± 0.44		0.49 ± 0.08			
Singing w/out movements	0.47 ± 0.20		0.32 ± 0.09			

1111 * 1/3 neurons was significantly excited during singing periods that contained head movements but not during
 1112 singing periods that lacked head movements.

1114 C. Six singing-responsive neurons that showed lower response strength during head movements that occurred
 1115 within singing compared to non-singing periods. (Gray lines in Fig. 5C, right, depict head movement responses.)

	Singing w/ head movements > singing w/out head movements (n = 3/6)		Singing w/ head movements = singing w/out head movements (n = 1/6)		Singing w/ head movements < singing w/out head movements (n = 2/6)	
	Excited (1)	Suppressed (2)	Excited (1)	Suppressed (0)	Excited (1)	Suppressed (1)
Singing w/ movements	.35	-0.91 ± 0.70	.44		.44	-2.75
Singing w/out movements	.10	-0.36 ± 0.34	.24		.74	-3.88

1116 For each table, neurons are categorized by whether response strength during singing that included head movements
 1117 was greater than (left), equal to (middle), or lower than (right) response strength during singing that lacked head
 1118 movements. For all neurons, firing rates were significantly modulated during singing periods both with and
 1119 without head movements unless otherwise noted (*).
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1121

LEGENDS

1122 **Figure 1.** AId neurons are well situated to integrate multi-modal inputs and distribute
1123 information across various cortical-subcortical circuits. *A*, AId receives inputs from upstream
1124 cortical regions LMAN-SHELL and dNCL. LMAN-SHELL is part of a cortico-basal ganglia loop
1125 that mediates vocal learning, whereas dNCL receives inputs from LMAN-SHELL as well as
1126 multiple pathways processing somatosensory, visual, and auditory information. AId of juvenile
1127 birds also receives inputs from LMAN-CORE via axon collaterals of LMAN-CORE→RA neurons
1128 that drive vocal output; robust collaterals are present in juvenile birds prior to ~40-45 dph but are
1129 not present in older juvenile or adult birds. Projections from AId to striatum and several
1130 midbrain and thalamic regions give rise to both feedback and feed-forward pathways, creating
1131 several opportunities for information transfer between cortical and subcortical regions.
1132 Abbreviations: AId, dorsal intermediate arcopallium; dNCL, dorsal caudolateral nidopallium;
1133 DTZ, dorsal thalamic zone (includes both DLM, dorsolateral nucleus of the medial thalamus, and
1134 DMP, dorsomedial nucleus of the posterior thalamus); LMAN, lateral magnocellular nucleus of
1135 the anterior nidopallium; RA, robust nucleus of the arcopallium; SpM, medial spiriform nucleus;
1136 VTA, ventral tegmental area. *B*, Top to bottom, caudal-to-rostral series of coronal sections 250
1137 μm apart mapping locations of recordings made in AId. Different colored circles represent sites
1138 recorded from different birds ($n = 7$). *C*, Raw traces of extracellular activity simultaneously
1139 recorded at two different sites within AId of a juvenile bird (44 dph) while the bird was resting
1140 (left column; “quiescent”) versus hopping around the recording cage (right column; “actively
1141 behaving”). Vertical lines above each raw activity trace indicate spikes from a single neuron
1142 sorted from the extracellular activity.

1143 **Figure 2.** AId neurons respond during different scored movements with excitation and/or
1144 suppression. *A*, Proportions of single AId neurons that responded during different numbers of
1145 movements. 33/101 movement-responsive neurons responded during one movement, 25/101
1146 during two, 20/101 during three, 14/101 during four, 6/101 during five, and 3/101 during six
1147 movements. *B*, Each row of each chart indicates movements during which each neuron was
1148 excited (green), suppressed (dark gray), or not responsive (light gray). Unfilled boxes indicate
1149 that no data during that movement was recorded for that neuron. Charts are grouped according
1150 to colors in *A*, based on the number of movements during which neurons responded. *C*,

1151 Proportions of AId neurons that were significantly excited (green) or suppressed (dark gray)
1152 during each movement type. Table below indicates the number of neurons recorded during each
1153 movement type and the corresponding excited and suppressed response strengths (mean
1154 standardized response strength \pm s.e.m.). $**p<.005$, $***p<.0001$.

1155 **Figure 3.** AId neurons show a variety of temporal response patterns during different scored
1156 movements. **A**, Rasters and histograms illustrating the response of a single AId neuron during
1157 preening (left) and pecks (right). **B**, Raster and histogram illustrating the response of a single
1158 AId neuron during hops. **C**, Rasters and histograms illustrating the onset- (left) and offset-
1159 aligned (right) preening response of an example AId neuron. Rows are sorted by movement
1160 duration. Blue vertical lines mark movement onsets; red lines mark movement offsets. Green
1161 and gray horizontal bars above histograms denote periods of excitation or suppression,
1162 respectively (see Materials and Methods). RS, average standardized response strength over the
1163 entire duration of each movement type.

1164 **Figure 4.** AId neurons exhibit context-sensitive peck responses. **A**, Mean standardized response
1165 strengths of neurons during pecks that occurred during eating versus non-eating periods, grouped
1166 by neurons that showed greater absolute response strength during eating (left) and non-eating
1167 (right). Left: Gray and black lines represent neurons that showed positive or negative response
1168 strength, respectively, during eating-pecks. Right: Gray and black lines represent neurons that
1169 showed positive or negative response strength, respectively, during non-eating pecks. Horizontal
1170 bars represent medians. Response strengths during eating-related pecks were significantly
1171 different from non-eating pecks for all plotted neurons (Mann-Whitney tests). **B**, Rasters and
1172 histograms of an example neuron's response during pecks that occurred during eating (left)
1173 versus non-eating (right). Peck response strength of this neuron is indicated by the cross-marked
1174 plot point in **A**, left. Rows are sorted by peck duration. Blue vertical lines mark peck onsets; red
1175 lines mark peck offsets.

1176 **Figure 5.** A substantial population of AId neurons are responsive during singing. **A**, Raster and
1177 histogram illustrating activity of an example singing-excited AId neuron during singing episodes.
1178 Rows are sorted by duration of each singing episode. Blue vertical line marks onset of each
1179 singing episode; red lines mark ends of singing episodes. **B**, Proportions of 36 singing-

1180 responsive neurons for which response strength during head movements within singing periods
1181 was greater than (orange), less than (gray), or not different from (blue) head movements within
1182 non-singing periods. **C**, Mean standardized response strengths during head movements that
1183 occurred within singing versus non-singing periods. Left: neurons that showed comparable
1184 response strength during head movements that occurred within singing versus non-singing
1185 periods. Right: neurons that showed higher response strength during head movements that
1186 occurred within singing (orange) or non-singing (gray) periods. Lines connect data points from
1187 single neurons. Horizontal bars represent medians. Circles versus triangles represent neurons
1188 that showed an increase or decrease, respectively, in average firing rate across singing episodes
1189 relative to quiescence (see Table 2).

1190 **Figure 6.** AId neurons are differentially modulated during different behavioral states.
1191 **A**, Schematics of example eating, singing, active-movement, quiet-attentive, and quiescent states.
1192 Text boxes represent example scored and unscored (starred) movements that typically occurred
1193 within that state type, though other behaviors could also occur (see Materials and Methods). **B**,
1194 Proportions of single AId neurons that were modulated during different numbers of state types.
1195 8/99 state-responsive neurons were modulated during one state type, 26/99 during two, 42/99
1196 during three, and 23/99 during four states. **C**, Each row of each chart indicates the states during
1197 which each neuron was excited (green), suppressed (dark gray), or not responsive (light gray).
1198 Unfilled boxes indicate that no data during that state was recorded for that neuron. Charts are
1199 grouped according to colors in **B**, based on the number of states during which activity of neurons
1200 was modulated. **D**, Histograms comparing distributions of inter-spike intervals (ISIs) during
1201 active-movement, eating, quiet-attentive, and quiescent states (left); singing, quiet-attentive, and
1202 quiescent states (middle); active-movement, eating, and singing states (right). Horizontal lines
1203 indicate distributions that had significantly different means; dotted lines indicate means of the
1204 respective distributions. $p < .001$ for all significant differences. **E**, Number of excited (left) and
1205 suppressed (right) events that occurred during each state type, normalized by the total duration of
1206 each state type in a given recording session. Box-and-whisker plots indicate medians and first
1207 and third quartiles; whiskers indicate data points not considered outliers; circles represent data
1208 points from individual neurons. *** $p < .001$.

1209 **Figure 7.** Movement-excited AI_d neurons are modulated by scored and unscored factors during
 1210 different state periods. *A*, Relative proportions of scored behaviors that occurred during each
 1211 state type. Proportion totals include all occurrences of each of the seven scored movements as
 1212 well as all song bouts. *B*, Left: peck-responsivity of 48 neurons that were significantly excited
 1213 during eating state periods. Right: proportions of eating-excited neurons that were responsive
 1214 during scored behaviors, categorized by peck-responsivity: eating-excited neurons that were
 1215 peck-excited (left), peck-suppressed (middle), and unresponsive during pecks (right). “No other
 1216 movement” represents neurons that did not respond during any other scored behavior. *C*, Left:
 1217 song-bout-responsivity of 37 neurons that were significantly excited during singing state periods.
 1218 Right: proportions of singing state-excited neurons that were responsive during scored
 1219 movements, categorized by song-responsivity: singing state-excited neurons that were song-
 1220 excited (left), song-suppressed (middle), and unresponsive during song bouts (right). “No other
 1221 movement” represents neurons that did not respond during any other scored behavior. Italicized
 1222 numbers indicate number of neurons within each song-responsivity grouping.

1223 **Figure 8.** AI_d neurons that lack responsivity during scored movements are nonetheless
 1224 modulated during different states. *A*, Proportions of non-responsive AI_d neurons that were
 1225 modulated during different numbers of state types. 3/18 neurons were not modulated during any
 1226 states; 1/18 was modulated during one state type, 5/18 during two, 6/18 during three, and 3/18
 1227 during four state types. *B*, Proportions of non-responsive neurons that were significantly excited
 1228 (green) or suppressed (dark gray) during each state type. Italicized numbers indicate number of
 1229 non-responsive neurons recorded during each state type.

1230 **Table 1.** Proportions of onset and offset responses across all neurons for different movement
 1231 types. Onset responses (top) and offset responses (bottom) are shown separately (total $n = 29$
 1232 responses), categorized based on whether average firing rate during the movement showed
 1233 significant excitation (Excited), suppression (Suppressed), or no response (Not significant).

1234 **Table 2.** Mean standardized response strengths during singing periods with versus without head
 1235 movements ($n = 36$). *A*, 24 singing-responsive neurons that showed comparable firing rates
 1236 during head movements that occurred within singing and non-singing periods. (Blue lines in Fig.
 1237 5C, left, depict head movement responses). *B*, Six singing-responsive neurons that showed

1238 greater response strength during head movements that occurred within singing compared to non-
1239 singing periods. (Orange lines in Fig. 5C, right, depict head movement responses.) **C**, Six
1240 singing-responsive neurons that showed lower response strength during head movements that
1241 occurred within singing compared to non-singing periods. (Gray lines in Fig. 5C, right, depict
1242 head movement responses.) For each table, neurons are categorized by whether response
1243 strength during singing that included head movements was greater than (left), equal to (middle),
1244 or lower than (right) response strength during singing that lacked head movements. For all
1245 neurons, firing rates were significantly modulated during singing periods both with and without
1246 head movements unless otherwise noted (*).

1247 **Movie 1.** Example video of a juvenile zebra finch demonstrating the seven scored movements:
1248 pecks, beak interactions, preening, hopping, stretching, scratching, and fluff-ups. See Video 2
1249 for examples of pecking movements during eating versus non-eating periods.

1250 **Movie 2.** Example video of a juvenile zebra finch during periods of active behavior, singing,
1251 and eating.

1252 **Movie 3.** Example video of neural activity recorded on a single channel while the juvenile zebra
1253 finch hopped around the cage, demonstrating firing rate increases whenever the bird hopped
1254 towards the left side of the cage. This example suggests the possibility that hopping-related
1255 activity was context- or location-dependent, but we did not have enough examples to test this
1256 idea. Vertical lines above the raw activity trace indicate spikes from a single neuron sorted from
1257 the extracellular activity.

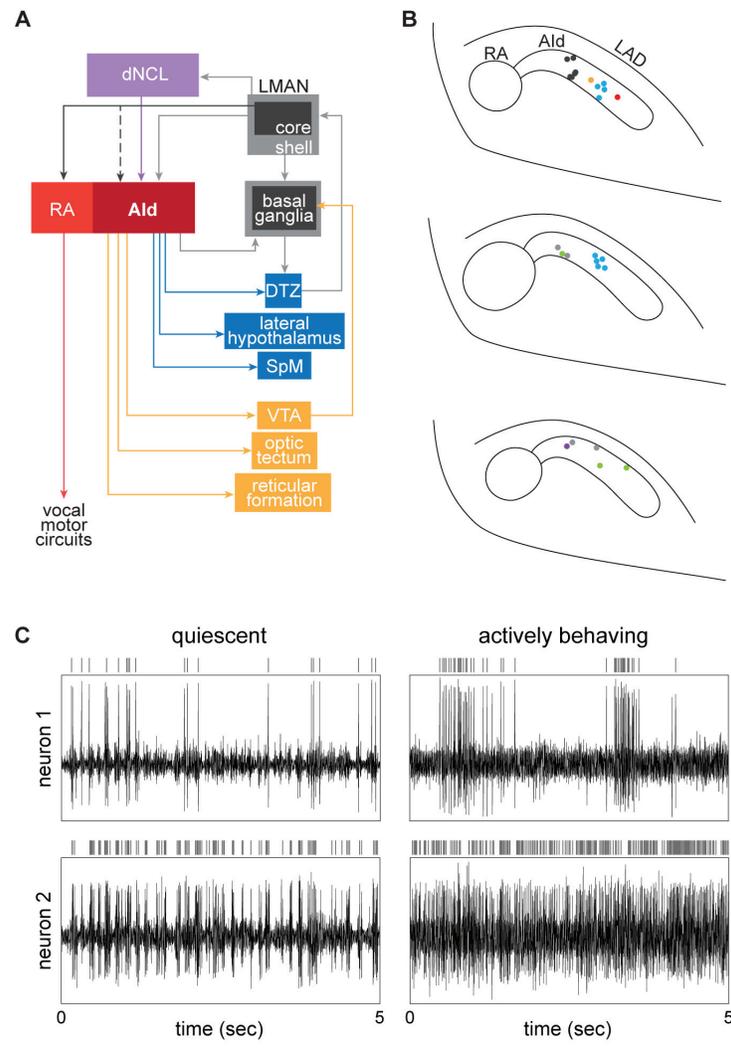


Figure 1

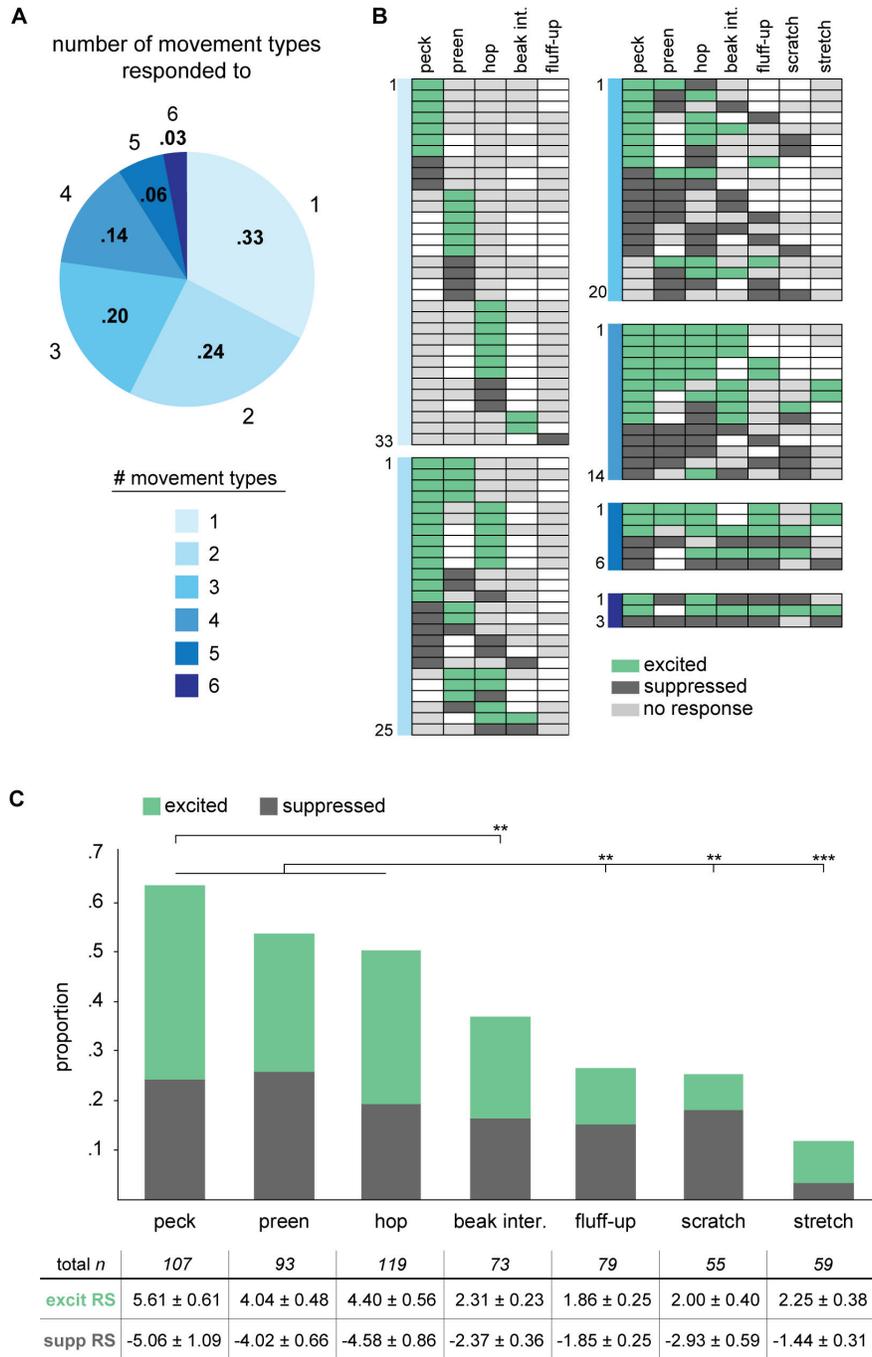


Figure 2

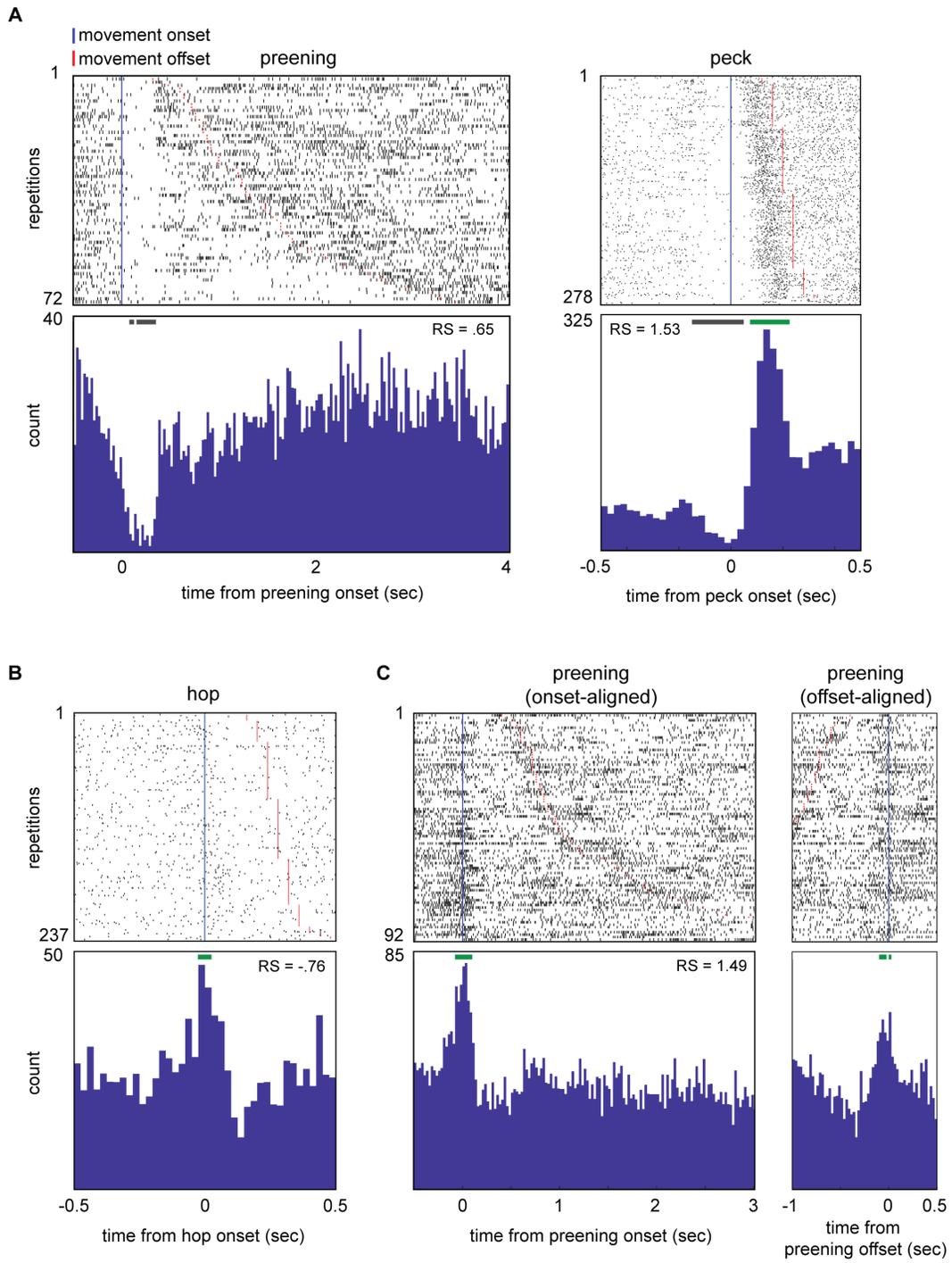


Figure 3

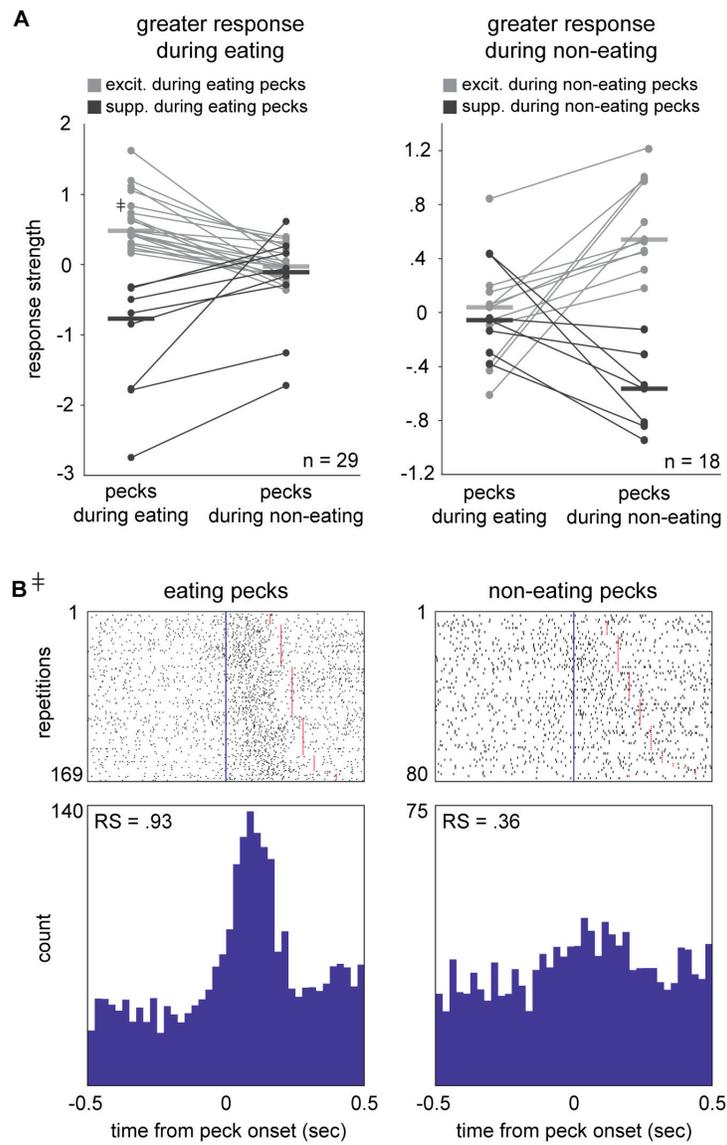


Figure 4

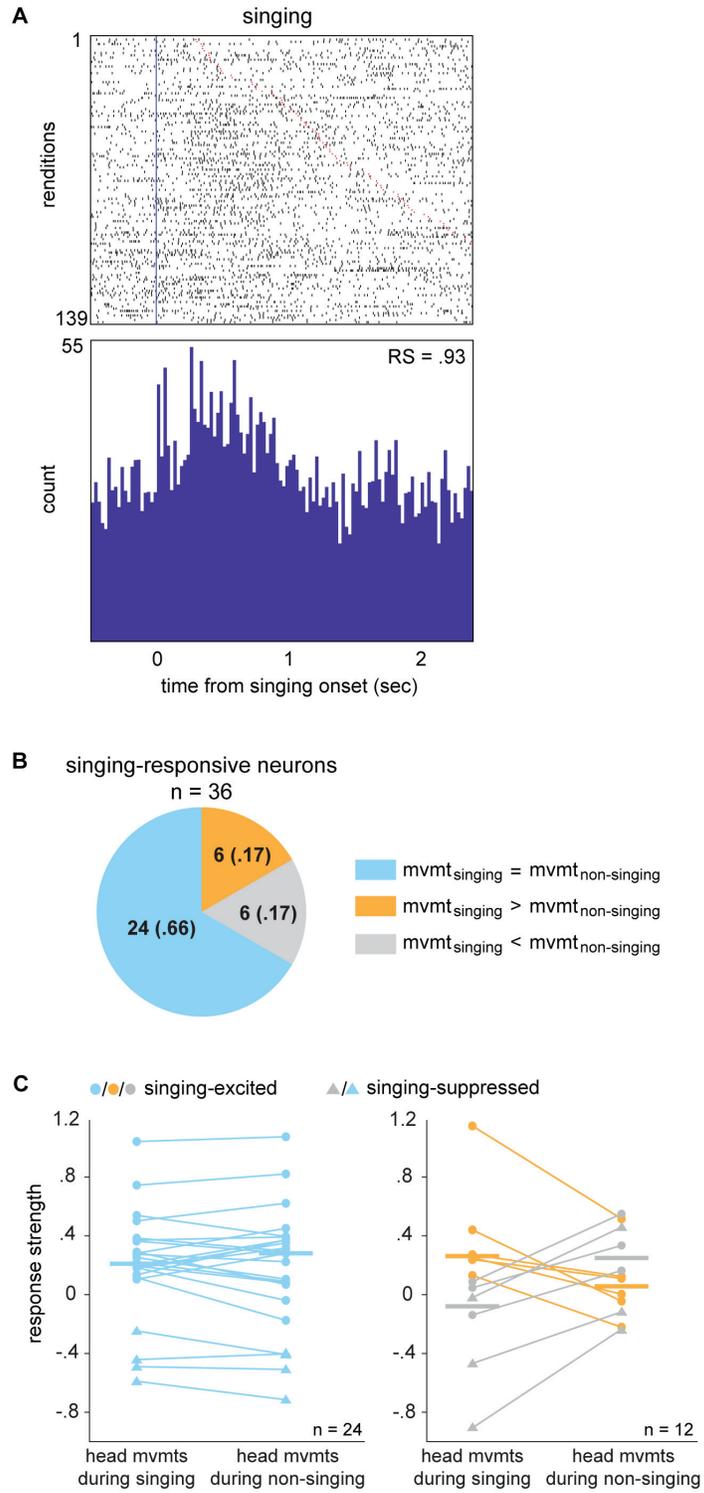


Figure 5

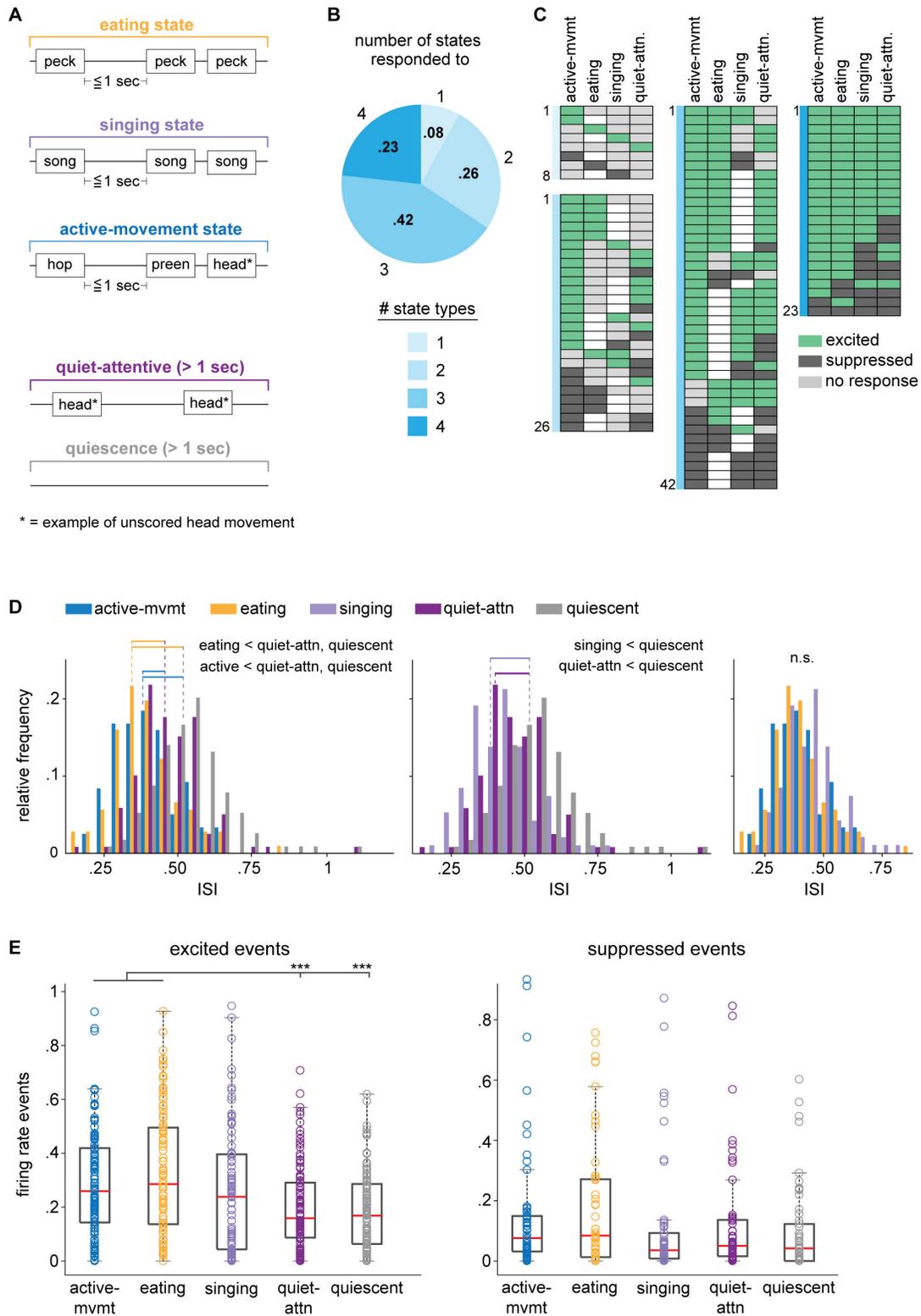


Figure 6

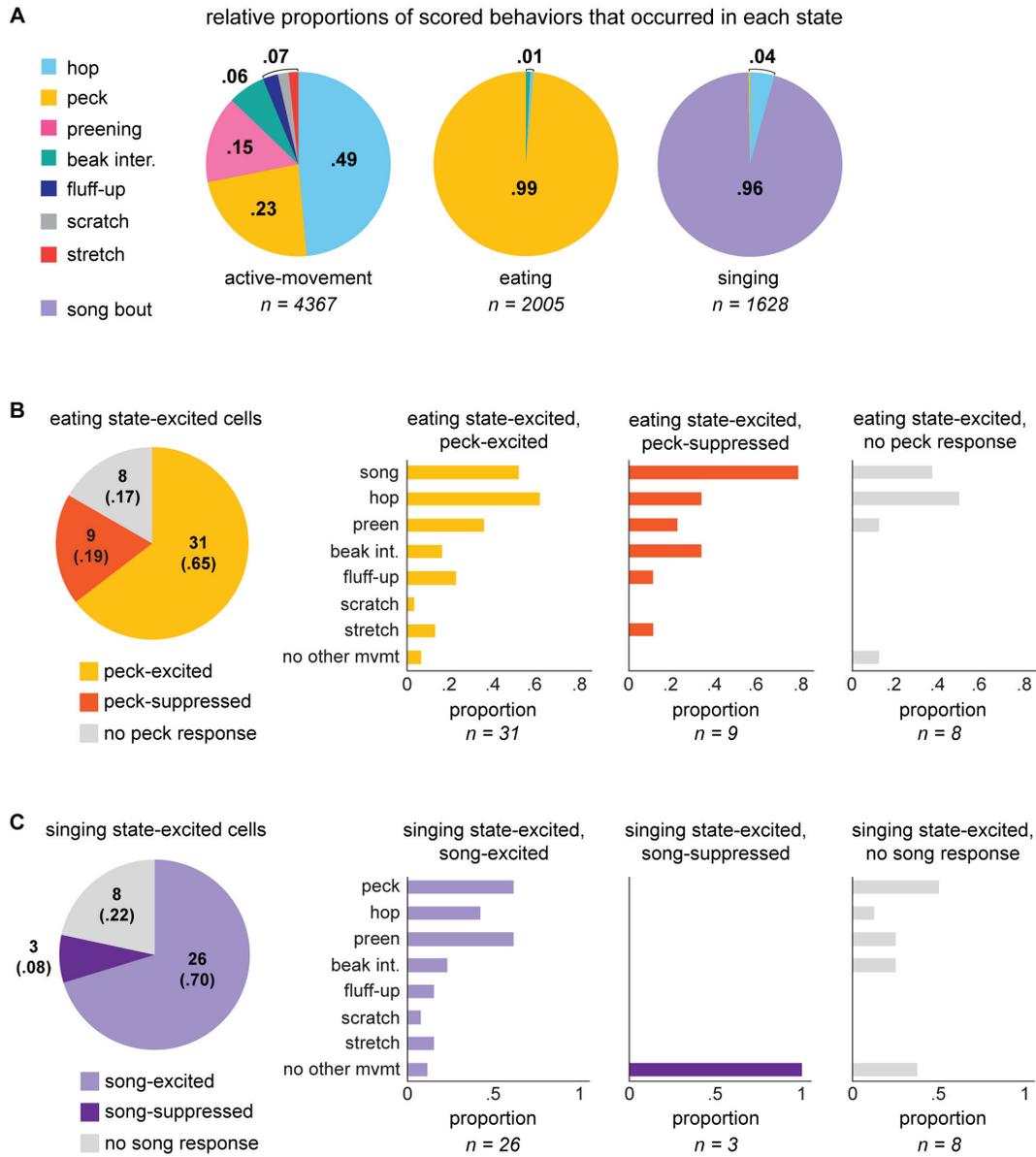


Figure 7

A neurons with no significant response during scored movements

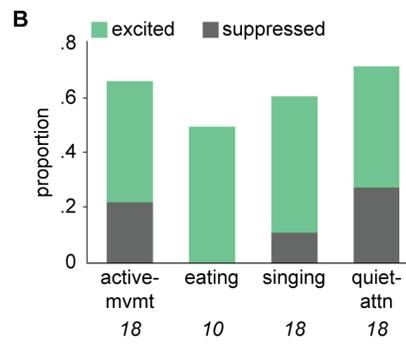
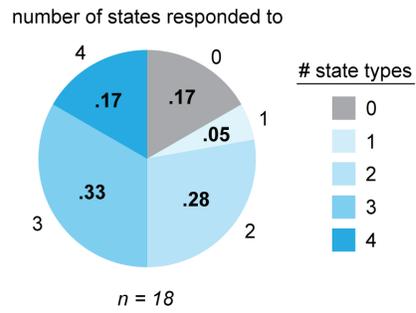


Figure 8