1

2

3

4

5

6

7     Differential immediate early gene activity revealed by playback of male and female incomplete

8     *chick-a-dee* calls

9

10    Scully, E.N.[a], Montenegro, C.[a], & Sturdy, C.B.[ab]

11    Department of Psychology[a], University of Alberta

12    Neuroscience and Mental Health Institute[b], University of Alberta

13

14

15

16

17

18

19    Correspondence should be addressed to Christopher Sturdy, Department of Psychology,

20    University of Alberta, 11455 Saskatchewan Drive, Edmonton, Alberta, T6G 2E9, Canada.

21    Email: csturdy@ualberta.ca

22    Phone: (780) 492-7843

23    Fax: (780) 492-1768
24

**Abstract**

25

26     In both humans and animals, biological differences between males and females has long

27 been a topic of research. In songbirds, sexual dimorphisms can be seen in many species'

28 plumage and heard in some species' songs. However, not all songbirds have such overt

29 phenotypic sexual differences, leading to the question: are all vocalizations dimorphic? One of

30 the most used and versatile vocalizations of the black-capped chickadee (*Poecile atricapillus*) is

31 their namesake *chick-a-dee* call, that is produced by both sexes. This call is composed of four

32 note types: A, B, C (together chick-a), and D (dee). Previous research has found that A notes

33 contain information regarding the sex of the caller. However, chickadees do not categorize full

34 *chick-a-dee* calls, or altered *chick-a* calls, based on the sex of the caller. Here we presented both

35 male and female chickadees with altered *chick-a* calls (*dee* portion removed) of both sexes and

36 measured the number of ZENK labeled cells in auditory nuclei. We found that calls produced by

37 males and females had more ZENK labeled cells than the control condition; however, there was

38 no significant difference in ZENK labeled cells between male and female listeners.. Overall, our

39 results suggest that black-capped chickadees do not perceive sexual differences in the production

40 of chick-a calls.

41

42

43

44

45 *Keywords*: black-capped chickadee; ZENK; chick-a-dee call; songbird; chick-a;

46 immunohistochemistry

**Introduction**

47

48          One of the oldest and most persistent research questions is whether there are

49    neurobiological differences, and if there are of what type, between the sexes. Investigations of

50    sexual differences extends to the animal kingdom, and questions how animals perceive signals

51    produced by males versus females. Many songbird species have sexually dimorphic plumage

52    (e.g., northern cardinal (*Cardinalis cardinalis*), superb fairy-wren (*Malurus cyaneus*), and the

53    amethyst starling (*Cinnyricinclus leucogaster*)), a visual signal that can be used as a tool for mate

54    attraction and choice. Even without these visual differences, most songbird species can have

55    some degree of vocal dimorphism. In most songbird species, songs are acoustically complex and

56    used for mate attraction and territory defence (Catchpole & Slater, 2008). Due to the main

57    functions of songs, it was thought that only males produced songs; however, more species are

58    being found to have female singers as well (e.g. tufted titmouse (*Baeolophus bicolor*), yellow

59    warbler (*Setophaga petechia*), and black-capped chickadee (*Poecile atricapillus*)). While we

60    know of these sex differences in production of a signal, is there a difference in the perception of

61    these signals as well?

62          In songbirds, calls tend to be simpler acoustically than songs and are produced for

63    multiple reasons including food location, predator alarm, and individual recognition (Catchpole

64    & Slater, 2008). Like most songbirds, the black-capped chickadee relies on vocal

65    communication. The black-capped chickadee is a small North American songbird known for

66    producing two main types of vocalizations, the *fee-bee* song and the *chick-a-dee* call (Smith,

67    1991). Unlike the songs and calls typical of other songbirds, the *fee-bee* song is an acoustically

68    simple two-note song while the *chick-a-dee* call is more complex, consisting of four note-types.

69    Both of these vocalizations are produced, and perceived, by both sexes. Potential sex differences

70  in auditory perception has been studied in the fee-bee song (i.e. Hahn et al., 2013; Hahn et al.,

71  2015), but sex differences in the chick-a-dee call remain largely unexplored. In order to

72  investigate any sex differences in the namesake call, we must first understand some of the

73  previous research on the structure and function of the call.

74      The black-capped chickadee chick-a-dee call consists of four note types: A, B, C, and D

75  (Smith, 1991). Like most songbird songs, including the black-capped chickadee fee-bee song, the

76  *chick-a-dee* call is learned by listening to adult conspecifics. However, there is some evidence to

77  suggest that the A note can be produced properly without learning (Hughes et al., 1998).

78  Chickadees have also been shown to change the composition of their *chick-a-dee* call depending

79  on the context (Mammen & Nowicki, 1981; Templeton et al., 2005). While the composition of

80  the call can change, the four note types always occur in a fixed order (A→B→C→D), with note

81  types being repeated or omitted (Ficken, Ficken, & Witkin, 1978). For example, when recruiting

82  other birds to mob a predator, chickadees increase the number of D notes to represent the level of

83  threat (Templeton et al., 2005).

84      Many studies, both behavioural and activity-dependent gene response studies, have

85  focused on the importance of the *dee* portion of the *chick-a-dee* call (made up of just the D note;

86  e.g., Dawson et al., 2006; Bloomfield, Farrel, & Sturdy, 2008; Avey et al., 2014). Expanding on

87  the work of Templeton and colleagues (2005) measuring vocalizations in response to visual

88  predators, Avey and colleagues (2011) conducted an activity-dependent gene response study

89  measuring the protein product ZENK expression in response to both predator calls and mobbing

90  calls made in response to predators of differing threat levels. They found that mobbing calls and

91  predator calls of the same threat level resulted in similar ZENK levels, and greater ZENK

92  labeling with high threat over low threat predators (Avey et al., 2011). This is just one example

93    of how behavioural and activity-dependent gene response  studies have complemented each

94    other.

95         Here, we will be focusing on the *chick-a* portion (made up of A, B, and C notes) of the

96    *chick-a-dee* call. As the presence and number of D notes contain vital information, we believe

97    focusing on just the first three notes will allow us to understand separate information contained

98    within the *chick-a* portion. A recent study once more expanded on the results of Templeton and

99    colleagues (2005), Billings, Green, and Jensen (2015) found that in response to hearing high-

100   threat predators, chickadees produced not just more *chick-a-dee* calls, but also more *chick-a* calls

101   during and after playback. While we do not fully understand the function of a *chick-a* call (as

102   opposed to the *chick-a-dee* call in its entirety), these findings suggest that *chick-a* calls may play

103   a role in predator alarm. Additionally, Campbell and colleagues (2016) conducted a bioacoustic

104   analysis of all *chick-a-dee* call note types and found that A notes contained information

105   regarding the caller's sex more so than B, C, or D notes, thus providing further suggestions on

106   how chickadees may use the *chick-a* portion of their call. An important factor that may be

107   driving this discrepancy between A notes and the other is the fact that this is the only note type

108   thought to be innate (Hughes et al., 1998).

109        However, subsequent studies investigating the role of sex identification in the *chick-a-dee*

110   call using an operant go/no-go task, (Campbell et al., 2020) found chickadees did not categorize

111   calls by the sex of the caller. When birds were first trained to respond to either male or female

112   *chick-a-dee* calls, they found no difference in responding between groups or sexes. When new

113   birds were trained using only the *chick-a* portion (i.e. with the *dee* portion removed) of the same

114   calls, the group trained to respond to female *chick-a calls* did transfer their training to untrained

115   female stimuli; however, there still was no effect of sex, nor was there a difference between

116  groups in responding to untrained male stimuli (Campbell et al., 2020). These findings suggest

117  that while the A note contains information that differs depending on the sex of the caller, birds

118  are not using this information, at least in the context of solving an operant discrimination task.

119      Due to the findings of Campbell and colleagues (2020), we questioned whether there

120  would be any differences in ZENK expression as a response to the *chick-a* portion of the black-

121  capped chickadee *chick-a-dee* call in males and female black-capped chickadees. We chose to

122  focus on the chick-a portion of the call as the A notes are thought to contain the sex information,

123  eliminating any overshadowing as a result of the D notes. We conducted a playback experiment

124  using both male and female produced *chick-a* calls and measured the number of ZENK positive

125  cells in three auditory areas (caudomedial mesopallium; CMM, caudomedial nidopallium dorsal;

126  NCMd, and caudomedial nidopallium ventral; NCMv) to visualize neural reactivity in order to

127  identify any possible sex differences in auditory perception. We predicted that there would be a

128  difference in how male and female chickadees responded to same or different sex calls, as

129  supported by the bioacoustic findings of sex differences in the A notes (Campbell et al., 2016).

130  **Methods**

131  **Subjects**

132      Fourteen black-capped chickadees (7 males and 7 females) caught from two sites in

133  Edmonton, Alberta, Canada (North Saskatchewan River Valley, 53.53N, 113.53W; Mill Creek

134  Ravine, 53.52N, 113.47W; Stony Plain) were used in this study. All birds were captured between

135  7 February, 2015 and 23 January, 2018, and were at least one year of age when captured. Birds

136  were housed indoors in individual Jupiter Parakeet cages (30 x 40 x 40 cm; Rolf C. Hagen Inc,

137  Montreal, QB, Canada) that enabled visual and auditory, but not physical, contact with other

138  male and female black-capped chickadees. Colony rooms were kept on the natural light cycle of

139 Edmonton, and maintained at 20 ℃. Subjects were given *ad libitum* access to food (Mazuri

140 Small Bird Maintenance Diet; Mazuri, St. Louis, MO, U.S.A), water, grit, cuttlebone, and

141 various environmental enrichment materials (perches, separators, houses). A mixture of egg and

142 spinach or parsley, worms, and water supplements (Prime Vitamin Supplement; Hagen, Inc.)

143 were given on alternating days and three to five sunflower seeds daily.

144 **Playback Stimuli**

145 Black-capped chickadees *chick-a-dee* calls were recorded from six males and six females

146 in individual soundproof chambers (1.7m x 0.84m x 0.58m; Industrial Acoustics Corporation,

147 Bronx, New York, USA). One *chick-a-dee* call was randomly selected from each individual, and

148 the *dee* portion was selected and removed using SIGNAL 5.10.24 software (Engineering Design,

149 Berkeley, CA, USA). One *chick-a* call from each of two different individuals of the same sex

150 were combined and separated by three seconds of silence to create one stimulus, for a total of

151 three male and three female stimuli.

152 All stimuli were bandpass filtered (400 Hz-13,000 Hz) outside the frequency range of

153 each vocalization type using GoldWave version 5.58 (GoldWave, Inc., St. John's, NL, Canada)

154 to reduce any background noise. For each manufactured stimulus, 5 ms of silence was added to

155 the leading and trailing portion of the vocalization. The first 5 ms of the stimuli were tapered to

156 remove transients, then the amplitude was equalized using SIGNAL 5.10.24 software

157 (Engineering Design, Berkeley, CA, USA). Stimuli were presented at approximately 75 dB as

158 measured by a Brüel & Kjær Type 2239 (Brüel &amp; Kjær Sound & Vibration Measurement

159 A/S, Nærum, Denmark; A-weighting, slow response) decibel meter.

160 **Playback procedure and equipment**

161    Throughout playback, birds were kept in modified cages (Jupiter Parakeet), with free

162    access to food and water. Birds were housed in individual soundproof chambers (1.7m x 0.84m x

163    0.58m; Industrial Acoustics Corporation, Bronx, New York, USA) for approximately 24 hours

164    before playback. All birds were first exposed to 30 min of pre-playback silence, followed by 30

165    min of playback. Birds were exposed to another hour of silence with the lights extinguished and

166    then perfused immediately to ensure maximum quantity and quality of ZENK preservation. A

167    lethal dose of 0.04 ml of 100 mg/ml ketamine and 20 mg/ml xylazine (1:1) was administered

168    intramuscularly to each subject. Birds were perfused via the left ventricle using heparinized 0.1

169    M phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). The brain of each

170    individual black-capped chickadee was then extracted and placed in a solution of PFA for 24

171    hours, followed by a 30% sucrose PBS solution for 48 hours. The brains were then fast frozen

172    and stored at -80°C until sectioned.

173    **Histology**

174    After being sectioned sagittally from the midline, 40μm sections of each hemisphere

175    were collected and stored in 0.1 M PBS. The protocol used follows the findings of Scully et al.

176    (2019a) that showed the efficacy of the primary antibody used following the discontinuation of

177    the previously standard ZENK antibody. In order to visualize ZENK, sections were first washed

178    twice in 0.1 M PBS for a minimum of five minutes, transferred to a 0.5% $H_2O_2$ solution, and

179    incubated for 15 minutes. Incubation was followed by three 5 min washes in 0.1 M PBS. A

180    second incubation in 10% normal goat serum for 20 hours at room temperature followed.

181    Sections were then transferred into the primary antibody (erg-1, Abcam Monoclonal Egr-1

182    ab133695; Abcam Inc, Toronto, ON, Canada) for 24 hours at a concentration of 1:5,000 in

183    Triton X-100 (PSB/T), then washed three times in PBS/T before being incubated in 1:200

184    biotinylated goat-anti-rabbit antibody (Vector Labs, Burlington, ON, Canada) in PBS/T for one

185    hour. After 3 more washes in PBS/T, sections were incubated in avidin-biotin horseradish

186    peroxidase (ABC Vectastain Elite Kit; Vector Labs, Burlington, ON, Canada) for one hour,

187    followed by three washes in 0.1M PBS. Sections were then processed with 3,3'-

188    diaminobenzidine tetrachloride (Sigma FastDAB, D4418, Sigma-Aldrich, Santa Fe Springs, CA,

189    USA) to visualize expression of ZENK, followed by three washes with 0.1M PBS to remove any

190    excess visualizing agents.

191    **Imaging**

192         Eight sections were mounted per slide and coverslipped. Three neuroanatomical regions

193    (CMM,NCMd, and NCMv) were subsequently imaged using a Leica microscope (DM5500B;

194    Wetzlar, Germany) to analyze ZENK expression. Eight images of each region were taken per

195    hemisphere, for a total of 48 images per subject (Figure 1). Images were taken using a 40x

196    objective lens, a Retiga Exi camera (Qimaging, Surrey, BC, Canada), and Openlab 5.1 on a

197    Macintosh OS X (Version 10.4.11). Overlap in the ventral and dorsal regions of the NCM was

198    carefully avoided. ImageJ version. 1.46v (Image Processing and Analysis in Java; publish) was

199    then used to quantify immunopositive ZENK cells (Figure 2).

200    **Statistical Analysis**

201         We conducted a repeated measures analysis of variance (ANOVA) using SPSS (IBM

202    SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) with brain region (CMM,

203    NCMd, and NCMv), hemisphere (left vs. right) and section number (1-8) as within subject

204    factors and playback condition (Male Chick-a, Female Chick-a, and Pink noise) and sex as

205    between subject factors. We then conducted Tukey HSD-corrected pairwise comparisons on the

206    playback condition with an alpha level set at 0.05.

207 **Results**

208        As expected from previous studies (e.g. Avey et al., 2011; Scully et al., 2019b), there was

209 a significant main effect of brain region ($F_{(2, 16)}$ = 7.363, $p$ = 0.005) and hemisphere ($F_{(1, 8)}$ =

210 11.157, $p$ = 0.100).

211        There was a significant main effect of playback condition ($F_{(2, 8)}$ = 8.259, $p$ = 0.011). The

212 Tukey-HSD corrected pairwise comparison found that the Pink noise condition was significantly

213 different from the Male *Chick-a* ($p$ = 0.009) playback group; however Pink noise was found to

214 be not significantly different from the Female *Chick-a* ($p$ = 0.066) playback group, we believe

215 this difference could still be biologically meaningful (Figure 3a). We also found that the Male

216 *chick-a* and Female *chick-a* groups were not significantly different from each other ($p$ = 0.193).

217 We did not find any significant effects of Sex ($F_{(1, 8)}$ = 0.000, $p$ = 0.991) or any significant

218 interactions (Figure 3b).

219 **Discussion**

220        Our results showed both male and female chickadees respond similarly, in terms of their

221 ZENK protein response, to both male and female *chick-a* calls. This suggests that there is no bias

222 towards calls of either sex, at least in the *chick-a* portion of the *chick-a-dee* call. While we only

223 found a significant difference in protein expression between the Male *chick-a* group and the Pink

224 noise control group, the Female *chick-a* group was close to significance when compared with the

225 Pink noise group and should still be considered meaningful. As the Pink noise group was

226 comprised of only one bird of each sex, for a total *n* of 2, it is possible that the limited sample

227 size restricted the results.

228        Initial research examining the function of the black-capped chickadee *chick-a-dee* call

229 examined the bioacoustics (i.e., characterized the acoustic properties) of each note type. After

230   first understanding the semantics of the call, Hailman, Ficken, and Ficken (1987) expanded on

231   the importance of the D notes, finding that  D notes  are acoustically different from the other note

232   types, thus suggesting that they may encode for separate information relative to the rest of the

233   call (Hailman et al., 1987). Charrier, Bloomfield, and Sturdy (2004) conducted an extensive

234   bioacoustic analysis of each note type measuring 10 features of non-D notes and found that 9 out

235   of 10 of these acoustic features differed significantly between all note types. Descending

236   duration was the only acoustic feature that was not different among note types and no sex

237   differences were detected for any note type (Charrier et al., 2004). However, a recent and more

238   extensive study revealed that in fact A notes do contain some sex specific information (Campbell

239   et al., 2016). Interestingly, sex specific information appears only in A notes, as this note type is

240   also thought to be the only unlearned note in the *chick-a-dee* call (Hughes et al., 1998). Since our

241   stimuli used randomly selected vocalizations, it is possible that not all of our stimuli contained

242   equal numbers of each note type, making some stimuli easier to obtain sex information than

243   others.

244        Operant go/no-go experiments have also been used to examine how chickadees perceive

245   the call note types and whole calls. An important first step by Sturdy and colleagues (2000)

246   showed that black-capped chickadees do categorize the four *chick-a-dee* call note types as

247   separate open-ended categories. Since then, many studies have used full *chick-a-dee* calls to

248   show how the call contains information regarding species (Bloomfield et al., 2003) and threat

249   level (Templeton et al., 2005). Arguably just as important, studies have also shown how

250   chickadees do not seem to use the *chick-a-dee* call, the calls does not vary between seasons

251   (Scully et al., 2019) nor is it used to distinguish sex (Campbell et al., 2020). Our findings support

252   the behavioural results of Campbell et al. (2020,) that suggests while birds are able to learn to

253    categorize both full *chick-a-dee* calls and just the *chick-a* portion of calls by sex, they do not

254    generalize this learning to untrained calls, suggesting that discrimination of caller sex is not a

255    natural function of the *chick-a-dee* call.

256        At a neural level, *chick-a-dee* calls are processed in the same auditory areas as all other

257    vocalizations, the NCM (dorsal and ventral) and CMM. An important study on the effects of sex

258    found that both male and female chickadees had more ZENK labeled cells in the CMM and

259    NCMd in response to hearing male *chick-a-dee* calls compared to female calls (Avey et al.,

260    2008). This suggests that birds are using an acoustic feature within the call to identify the sex of

261    the caller. While previous studies have examined how these brain areas respond to full *chick-a-*

262    *dee* calls (e.g., Avey et al., 2011; Scully, 2018), few have examined individual aspects of the call.

263    Avey and colleagues (2014) used just the D notes to investigate the effect of conspecific versus

264    heterospecific simple calls on neural expression. By using only the D note, the call was more

265    acoustically similar to the calls of heterospecifics and demonstrated that there was no difference

266    in the amount of ZENK expression induced by conspecific or heterospecific calls (Avey et al.,

267    2014). In combination with a previous behavioural study that found chickadees use the *chick-a-*

268    *dee* call for species discrimination (Bloomfield et al., 2003), Avey and colleagues (2014) ZENK

269    study then suggests that some component in the *chick-a* portion of the call is responsible for

270    driving this categorization.

271        Since previous studies have focused on either the full *chick-a-dee* call or D notes, here we

272    instead narrowed in on the beginning of the call. The stimuli used in the current study, as well as

273    in Campbell et al.'s (2020) study were created by manually removing the *dee* portion of a full

274    *chick-a-dee* call, therefore, we are not able to generalize our findings to naturally produced

275    *chick-a* calls. As chickadees have been shown to produce just *chick-a* calls, our altered stimuli

276    may not contain the same information that these calls are used for in the wild. Thus, the next step

277    to understanding the function of *chick-a* calls must then be to use naturally produced calls.

278    **Conclusions**

279         Overall, our results showed no activity-dependent gene response differences in the

280    auditory perception of male and female *chick-a* calls in the auditory forebrain. Although A notes

281    contain some information regarding the sex of the caller (Campbell et al., 2016), this information

282    is not attended to in the context of a *chick-a* call. Mirroring the findings of a behavioural study,

283    we found that male and female chickadees react similarly to calls of both the same and different

284    sex as listeners. It is possible that while information regarding an individual's identity is located

285    within the *chick-a-dee* call, the function of the call does not require knowing an individual's sex.

286    Future studies examining sex differences should focus on identifying functional difference

287    resulting from or causing sexual dimorphisms.

288

289

290
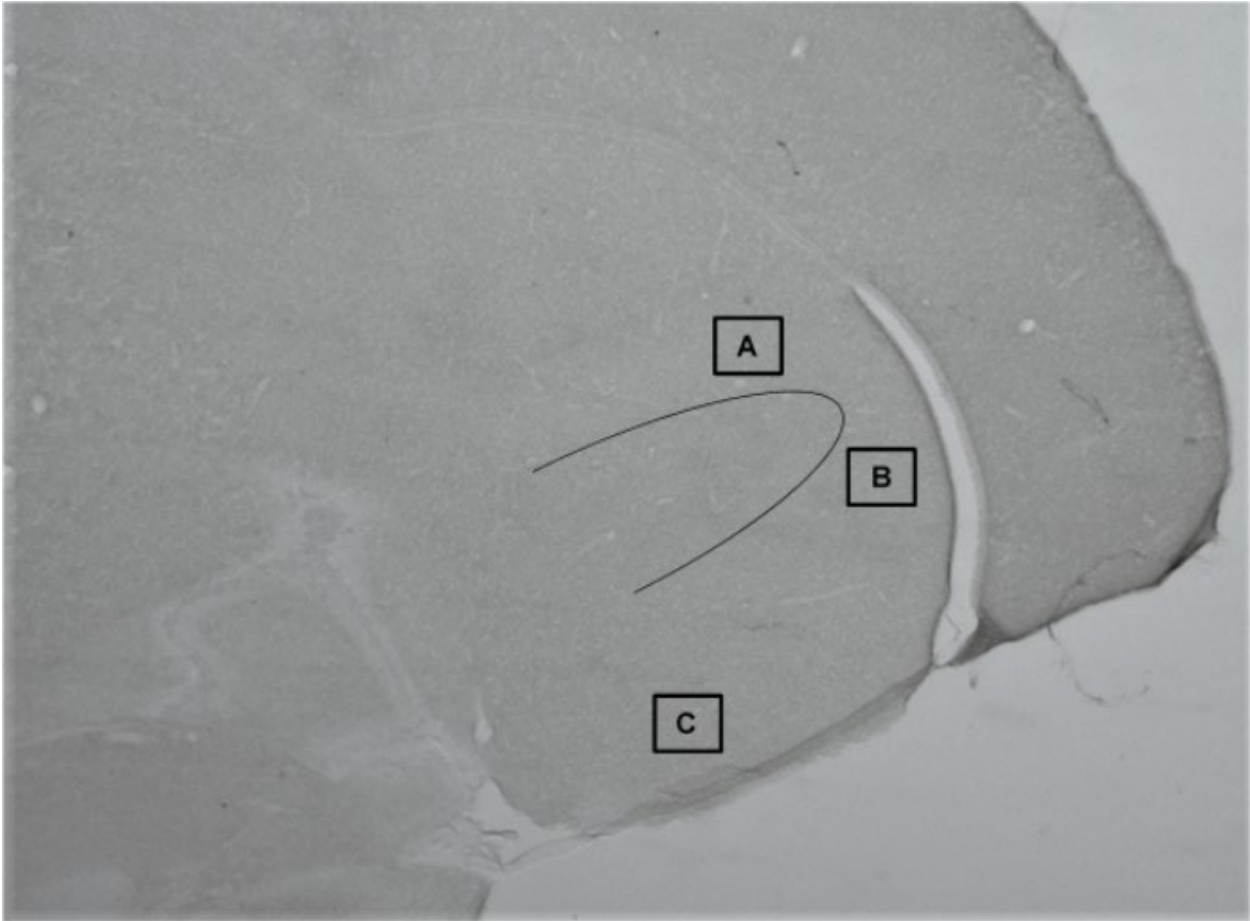
**Figure 1: Telencephalon with areas imaged marked.** Each picture was taken in the same spot

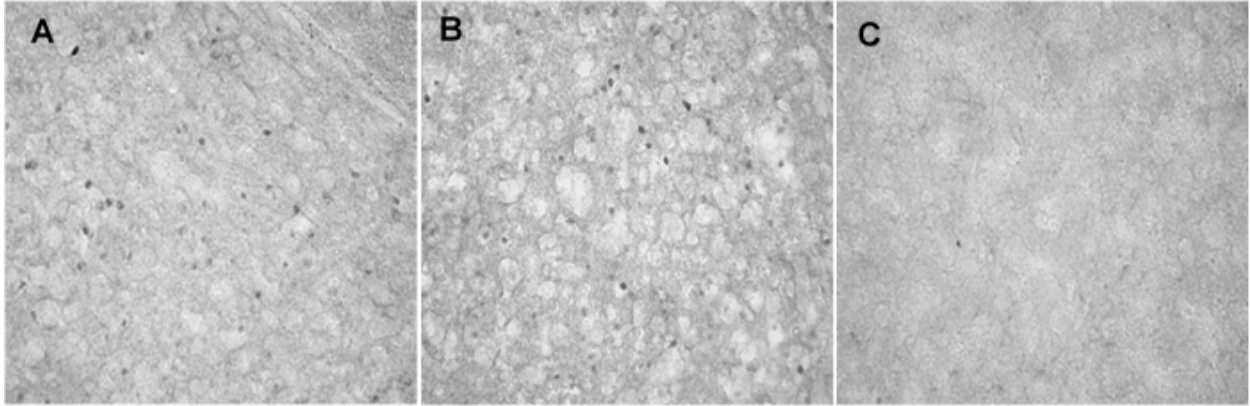in regards to Field L (boundary represented by the arc). A) represents the sample area for CMM.

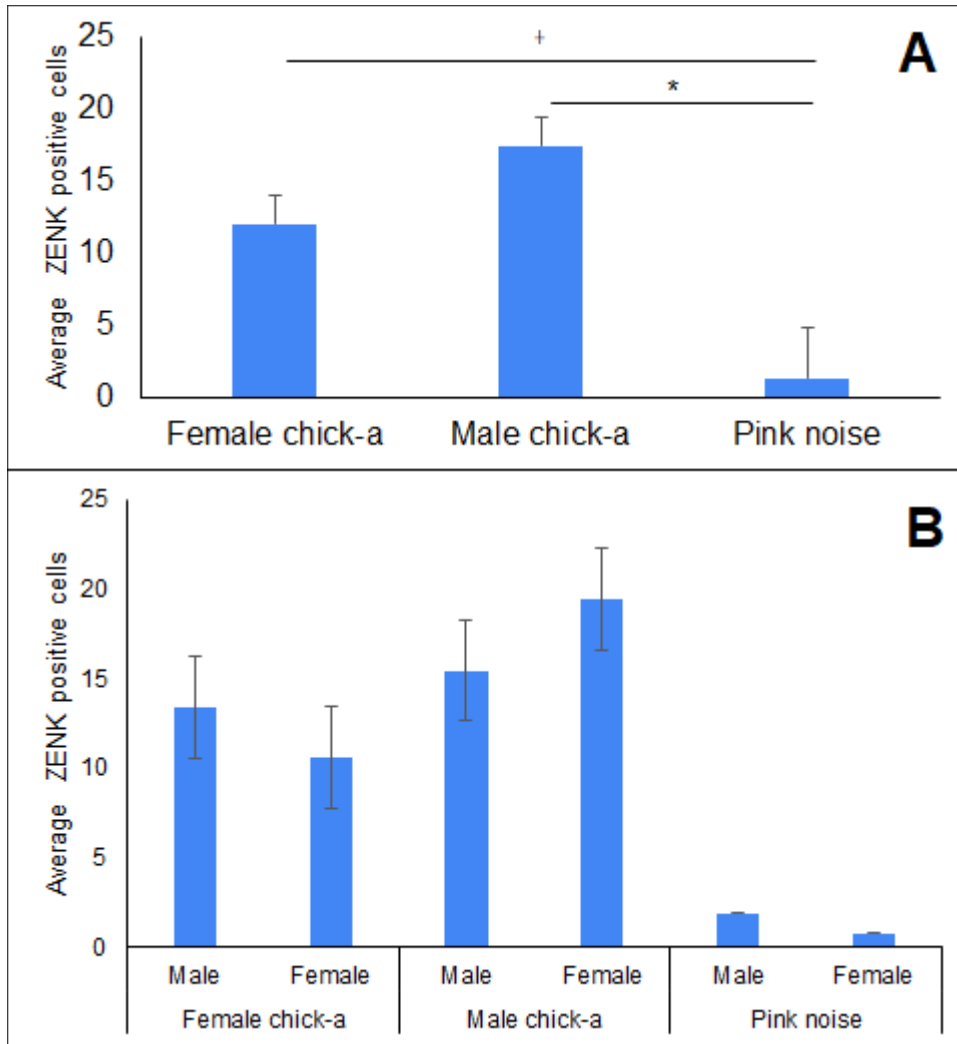B) represents the sample area for NCM, and C) represents the sample area for NCMv.

298

**Figure 2:** ZENK labeling in the CMM across groups. A) a female that heard male *chick-a*, B) a

female that heard female *chick-a*, and C) a female that heard pink noise.

301

302

303

304

**Figure 3: Neural expression per group.** A) Average ZENK positive cells labeled per playback group. * indicates a significant difference (p < 0.050) between the Male *chick-a* and Pink noise groups. ┼ indicates a meaningful difference (p = 0.066) between the Female chick-a and Pink noise groups. B) Breakdown of playback group by sex of the listener.

322        **References**

323    Avey, M.T., Bloomfield, L. L., Elie, J. E., Freeberg, T. M., Guillette, L. M., Hoeschele, M., Lee,

324        H., Moscicki, M. K., Owens, J. L., Sturdy, C. B. (2014). ZENK activation in the

325        nidopallium of black-capped chickadees in response to both conspecific and heterospecific

326        calls. PLoS ONE, *9*: e100927. DOI: 10.1371/journal.pone.0100927

327    Avey M. T., Hoeschele, M., Moscicki, M. K., Bloomfield, L. L., & Sturdy, C. B. (2011). Neural

328        correlates of threat perception: Neural equivalence of conspecific and heterospecific

329        mobbing calls is learned. *PLoS ONE*, *6*: e23844. DOI: 10.1371/journal.pone.0023844

330    Avey, M. T., Kanyo, R. A., Irwin, E. L., & Sturdy, C. B. (2008). Differential effects of

331        vocalization type, singer and listener on Zenk immediate early gene response in black-

332        capped chickadees (*Poecile atricapillus*). *Behavioral Brain Research*, *188*, 201-208. DOI:

333        http://dx.doi.org/10.1016/j.bbr.2007.10.034

334    Billings, A. C., Greene, E., & Jensen, S. M. D. (2015). Are chickadees good listeners?

335        Antipredator responses to raptor vocalizations. *Animal Behaviour*, *110*, 1-8.

336        DOI: https://doi.org/10.1016/j.anbehav.2015.09.004

337    Bloomfield, L. L., Farrell, T. M., & Sturdy, C. B. (2008). All "*chick-a-dee*" calls are not created

338        equally. Part II: Mechanisms for discriminations by sympatric and allopatric chickadees.

339        *Behavioural Processes*, *77*, 87-99. DOI: http://dx.doi.org/10.1016/j.beproc.2007.06.008

340    Bloomfield, L. L., Sturdy, C. B., Phillmore, L. S., & Weisman, R. G. (2003). Open-ended

341        categorization of *chick-a-dee* calls by black-capped chickadees (*Poecile atricapilla*).

342        *Journal of Comparative Psychology*, *117*, 290-301. DOI: 10.1037/0735-7036.117.3.290

343    Campbell, K. A., Hahn, A. H., Congdon, J. V., Scully, E. N., & Sturdy, C. B. (submitted

344        December 2019). Perception of sex differences in black-capped chickadee (*Poecile*

345       *atricapillus*) *chick-a-dee* calls. Manuscript submitted for publication in Animal Behavior

346       and Cognition.

347    Campbell, K.A., Hahn, A.H., Congdon, J.V., & Sturdy, C.B. (2016). Sex differences in black-

348       capped chickadee (*Poecile atricapillus*) *chick-a-dee* calls. *Journal of the Acoustical Society*

349       *of America*, *140*, 1598–1608. DOI: http://dx.doi.org/10.1121/1.4962281

350    Catchpole, C. K. & Slater, P. J. B. (2008). *Bird Song: Biological Themes and Variations.*

351       Cambridge University Press, Cambridge, UK.

352    Charrier, I., Bloomfield, L .L., & Sturdy, C. B. (2004). Note types and coding in parid

353       vocalizations. I: The *chick-a-dee* call of the black-caooed chickadee (*Poecile*

354       *atricapillus*). *Canadian Journal of Zoology*, *82*, 769-779.

355    Dawson, M. R. W., Bloomfield, L. L., Charrier, I., & Sturdy, C. B. (2006). Statistical

356       classification of black-capped (*Poecile atricapillus*) and mountain chickadee (*P. gambeli*)

357       call notes. *Journal of Comparative Psychology*, *120*, 147-153. DOI: 10.1037/0735-

358       7036.120.2.147

359    Ficken, M. S., Ficken, R. W., & Witken, S. R. (1978). Vocal repertoire of the black-capped

360       chickadee. *The Auk*, *95*(1), 34-48.

361    Hahn, A. H., Krysler, A., & Sturdy, C. B. (2013). Female song in black-capped chickadees

362       (*Poecile atricapillus*): Acoustic song features that contain individual identity information

363       and sex differences. Behavioural Processes, 98, 98-105.

364       https://doi.org/10.1016/j.beproc.2013.05.006

365    Hahn, A. H., Hoang, J., McMillan, N., Campbell, K., Congdon, J., & Sturdy, C. B. (2015).

366       Biological salience influences performance and acoustic mechanisms for the

367       discrimination of male and female songs. Animal Behaviour, 104, 213-228.

Hailman, J. P., Ficken, M. S., & Ficken, R. W. (1987). Constraints on the structure of

combinatorial "Chick-a-dee" calls. *Ethology*, *75*, 62-80.

Hughes, M., Nowicki, S., & Lohr, B. (1998). Call learning in black-capped chickadees (*Parus

atricapillus*): The role of experience in the development of '*chick-a-dee*' calls. *Ethology*,

*104*, 232-249.

Mammen, D. L., & Nowicki, S. (1981). Individual differences and within-flock convergence in

chickadee calls. *Behavioral Ecology and Sociobiology*, *9*, 179-186.

Scully, E. N., Campbell, K. A., Congdon, J. V., & Sturdy, C. B. (2020). Discrimination of black-

capped chickadee (*Poecile atricapillus*) *chick-a-dee* calls produced across seasons. *Animal

Behaviour and Cognition*, *7*, 247-256.

Scully, E. N., Montoya Sanchez, J., & Sturdy, C. B. (2019a). Abcam Monoclonal Egr-1

ab133695 is an effective primary antibody replacement for Santa Cruz sc-189 polyclonal

Egr-1 in songbirds. Heliyon, e02938. DOI: https://doi.org/10.1016/j.heliyon.2019.e02938

Scully, E. N., Schuldahaus, B. C., Congdon, C. V., Hahn, A. H., Campbell, K. A., Wilson, D. R.,

& Sturdy, C. B. (2019b). ZENK expression in the auditory pathway of black-capped

chickadees (Poecile atricapillus) as a function of D note number and duty cycle of chick-a-

dee calls. Behavioral Brain Research, 356, 490-494.

Smith, S. M. (1991). *The black-capped chickadee: Behavioral ecology and natural history*.

Ithaca, NY: Cornell University Press.

Sturdy, C. B., Phillmore, L. S., & Weisman, R. G. (2000). Call-note discriminations in black-

capped chickadees (*Poecile atricapillus*). J*ournal of Comparative Psychology*, *114*, 357-

364. DOI: 10.1037/0735-7036.114.4.357

390    Templeton, C. N., Greene, E., & Davis, K. (2005). Allometry of alarm calls: black-capped

391        chickadees encode information about predator size. *Science*, *308*, 1934-1937.

392