

1 **Separating overlapping bat calls with a bi-directional long short-term**
2 **memory network**

3 **Using deep neural network to separate overlapping bat calls**

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15

16 **Abstract**

17 Acquiring clear and usable audio recordings is critical for acoustic analysis of

18 animal vocalizations. Bioacoustics studies commonly face the problem of overlapping

19 signals, but the issue is often ignored, as there is currently no satisfactory solution.

20 This study presents a bi-directional long short-term memory (BLSTM) network to

21 separate overlapping bat calls and reconstruct waveform audio sounds. The separation

22 quality was evaluated using seven temporal-spectrum parameters. The applicability of
23 this method for bat calls was assessed using six different species. In addition,
24 clustering analysis was conducted with separated echolocation calls from each
25 population. Results showed that all syllables in the overlapping calls were separated
26 with high robustness across species. A comparison between the seven
27 temporal-spectrum parameters showed no significant difference and negligible
28 deviation between the extracted and original calls, indicating high separation quality.
29 Clustering analysis of the separated echolocation calls also produced an accuracy of
30 93.8%, suggesting the reconstructed waveform sounds could be reliably used. These
31 results suggest the proposed technique is a convenient and automated approach for
32 separating overlapping calls using a BLSTM network. This powerful deep neural
33 network approach has the potential to solve complex problems in bioacoustics.

34 **Author summary**

35 In recent years, the development of recording techniques and devices in animal
36 acoustic experiment and population monitoring has led to a sharp increase in the
37 volume of sound data. However, the collected sound would be overlapped because of
38 the existence of multiple individuals, which laid restrictions on taking full advantage
39 of experiment data. Besides, more convenient and automatic methods are needed to
40 cope with the large datasets in animal acoustics. The echolocation calls and
41 communication calls of bats are variable and often overlapped with each other both in
42 the recordings from field and laboratory, which provides an excellent template for
43 research on animal sound separation. Here, we firstly solved the problem of

44 overlapping calls in bats successfully based on deep neural network. We built a
45 network to separate the overlapping calls of six bat species. All the syllables in
46 overlapping calls were separated and we found no significant difference between the
47 separated syllables with non-overlapping syllables. We also demonstrated an instance
48 of applying our method on species classification. Our study provides a useful and
49 efficient model for sound data processing in acoustic research and the proposed
50 method has the potential to be generalized to other animal species.

51 **Introduction**

52 The structural identification of vocal units is essential in animal acoustic studies for
53 sound feature analysis, sound emitter recognition, and species identification and
54 monitoring. However, wild animal monitoring, both in the field and in the laboratory,
55 often involves problems caused by the overlapping of different vocal units in time and
56 frequency space, which prevents the components from being suitable for parameter
57 analysis. As a result, the separation of overlapping sounds is an important task in
58 bioacoustic signal processing. However, existing analysis software often struggles to
59 process overlapping calls and previous research on the acoustic identification of
60 animals primarily focuses on extracting target signals from background noise for
61 species classification or population monitoring [1-4]. The process of separating
62 overlapping calls from mixed sounds has received little attention to date and
63 researchers conventionally abandon sounds that overlap in both time and frequency,
64 requiring an extension of the experimental period to obtain sufficient non-overlapping
65 recordings [5, 6]. As such, an effective method for successfully and automatically

66 separating overlapping calls would be of significant interest and benefit to animal
67 researchers.

68 Previous studies using deep neural networks have produced promising results for
69 automated sound recognition in complex acoustic environments for animal species
70 recognition and classification [6-8]. However, in this study, we consider the more
71 difficult task of separating different types of syllables from overlapping calls and
72 reconstructing sound waves from these separated signals. Existing techniques used for
73 animal sound separation often require prohibitive quantities of labelled data. For
74 example, multiple-instance machine learning (MIML) algorithms were proposed for
75 use in sound feature extraction and species identification in birds [1]. However, this
76 technique requires a cropped mask of a signal segment (without overlap) in order to
77 extract each syllable.

78 Deep learning networks have been applied to bioacoustic studies but have primarily
79 been used for classification. For instance, convolutional bidirectional recurrent neural
80 networks (CBRNNs) have been used to identify the presence of bird calls in audio
81 samples [4]. Acoustic features were learned by the network (a classifier) and the
82 presence or absence of a bird call was output as an indicator. Convolutional neural
83 networks (CNNs) have been used to predict the presence of a search-phase bat
84 echolocation call in spectrograms. This binary classification problem was used to
85 detect the presence of bats [2]. To our knowledge, the use of deep learning techniques
86 to separate animal calls that overlap in both time and frequency space has yet to be
87 reported.

88 Multiple studies have been conducted using deep learning-based supervised speech
89 separation with humans. Early systems included shallow models that performed a
90 linear transformation of given mixture features during the prediction time interval.
91 This has included Gaussian mixture models [9], support vector machines [10], and
92 non-negative matrix factorization [11]. However, in real-world scenarios, the mapping
93 relationship between mixture signals and sources is typically a nonlinear
94 transformation. Nonlinear models, such as deep neural networks (DNNs), are
95 therefore highly applicable because of their ability to identify nonlinear structures in
96 audio signals [12-14]. Additionally, recurrent neural networks (RNNs) that exhibit the
97 temporal behavior of a time sequence can be trained to predict time-frequency masks
98 for target signals and separate sources from a mixed waveform [15]. Specifically, long
99 short-term memory (LSTM) networks, a variation of RNN models that exhibit strong
100 learning capabilities and simple construction, have been widely used for word and
101 continuous speech recognition [16-18]. By concatenating two separate LSTM
102 networks, bidirectional LSTMs (BLSTMs) can predict each element of a sequence
103 based on past and future context and can naturally account for the temporal dynamics
104 of speech. These models are typically faster and more accurate than standard RNNs in
105 frame-by-frame phoneme classification [19]. In addition, the BLSTM network can
106 compensate for exploding and vanishing gradient issues that can occur during the
107 training of standard RNN models [20]. At present, BLSTMs have achieved
108 state-of-the-art performance for speech recognition [14, 21], natural language
109 processing [22, 23], and speaker-independent speech separation [24]. As such, a

110 BLSTM model was selected in this study for overlapping bat call separation.

111 Echolocating bats have two vocal repertoires, stereotypical echolocation calls for
112 orientation and a variety of communication calls for social activities [25-27].
113 Recordings from both field and laboratory studies indicate that utterances from
114 individual bats often overlap in both time and frequency, which provides an excellent
115 template for research on overlapping sound separation in animals. The primary
116 objective of this study is to develop a technique for separating two target signals
117 (echolocation and socialization calls) from mixtures of acoustic sounds. Although
118 deep learning has been employed in the acoustic classification of multiple species,
119 including nonhuman primates [28], birds [4], whales [5], and bats [2, 3], the goal of
120 the present study is distinct from these previous cases in which deep neural networks
121 were primarily used as classifiers.

122 Both overlapping and non-overlapping calls (of both echolocation and
123 communication types) were recorded from each of the collected bat species studied in
124 our previous work. We developed a BLSTM network and used the recorded
125 non-overlapping calls to train the model. Recorded overlapping calls were input to the
126 trained model and separated. Independent sound files were then reconstructed for each
127 separated signal. The correctness of these separated signals was measured by
128 comparing the temporal-spectrum parameters between separated calls and the initially
129 recorded (non-overlapping) calls from each species. Finally, clustering analysis was
130 conducted to classify the bats using separated echolocation calls, which provided a
131 practical application of the proposed technique.

132 Results

133 The proposed algorithm performed well and achieved high accuracy in separating
134 overlapping calls for each of the six species. The BLSTM model was iteratively
135 trained until the training and validation losses reached a minimum. Loss is a
136 summation of errors made with each sample in the training or validation sets and
137 measures how well the model adapts during optimization. Training loss for this model
138 decreased significantly in the first epoch. The validation loss function tended toward
139 an asymptotic value, indicating the training algorithm had converged (S2 Fig). The
140 BLSTM model converged slightly faster when training with CF bat samples (as
141 opposed to FM samples).

142 All echolocation and communication calls in the overlapping signals were
143 correctly extracted during the separation procedure, regardless of their pulse duration
144 or energy characteristics (see Table 1 and Fig 1). In addition, low-intensity FM
145 components in echolocation pulses were successfully extracted from three CF bat
146 species (Figs 1d, 1e, and 1f).

147 **Table 1. Separation results.**

Species	Call type	Number of syllable types	Number of syllables in overlapping calls	Number of overlapping syllables	Number of separated syllables
<i>Rhinolophus ferrumequinum</i>	Echolocation	1	14	14	14
	Communication	4	8	8	8
<i>Vespertilio sinensis</i>	Echolocation	1	21	13	13
	Communication	4	8	8	8
<i>Hipposideros armiger</i>	Echolocation	1	28	19	19
	Communication	6	13	13	13
<i>Myotis</i>	Echolocation	1	54	36	36

<i>macrodactylus</i>	Communication	6	15	15	15
<i>Rhinolophus</i>	Echolocation	1	42	30	30
<i>pusillus</i>	Communication	6	10	10	10
<i>Ia io</i>	Echolocation	1	26	16	16
	Communication	4	11	11	11

148

149 **Fig 1. Spectrograms from original recordings of overlapping calls and calls separated by**
150 **the BLSTM network.** The first graph represents each line of the original overlapping calls
151 and the second and third graphs show the separated echolocation and communication calls,
152 respectively.

153

154 A comparison of seven temporal-spectrum parameters from the separated calls
155 and the original recorded non-overlapping calls showed no significant differences (Fig
156 2 and S3 Table). In addition, parameter deviations in separated calls and original
157 non-overlapping calls showed minimal RMSE values for both echolocation and
158 communication signals (Fig 3 and Fig 4). Clustering analysis performed with
159 separated echolocation calls produced an accuracy of 93.8% across species (Fig 5).

160

161 **Fig 2. Comparisons between the separated and original calls.** Two principle
162 components extracted from seven temporal-spectral parameters were used in the study.
163 Results for echolocation and communication calls are shown in (A-F) and (G-L),
164 respectively.

165 **Fig 3. A comparison of deviations for separated and original echolocation calls.**

166 The RMSE value is shown under each plot. The vertical axis represents values for

167 each parameter and the horizontal axis represents the number of syllables measured.
168 The red triangles represent separated calls and the blue dots represent original calls.
169 Abbreviations include duration (duration), Fstart (starting frequency), Fend (ending
170 frequency), Fpeak (peak frequency), Fmin (minimum frequency), Fmax (maximum
171 frequency), and bandw (bandwidth).

172 **Fig 4. A comparison of deviations in separated and original communication calls.**

173 The RMSE value is shown under each plot. The vertical axes and abbreviations are
174 the same in Fig 3.

175 **Fig 5. Clustering analysis for six bat species based on their separated**
176 **echolocation calls.** Overlapping echolocation signals cannot be used for species
177 identification until after separation.

178

179 **Discussion**

180 The BLSTM network used in the present study achieved high accuracy in
181 separating overlapping echolocation and communication calls from bats. The training
182 and validation loss for the model also exhibited fast convergence and high robustness
183 for bat vocalizations. In particular, the separated calls extracted by the proposed
184 algorithm were reconstructed as waveform files with nearly the same quality as the
185 non-overlapping calls, suggesting BLSTM networks to be useful tools for separating
186 signals in future bioacoustic research, such as sound analysis, acoustic identification,
187 species classification, and wild animal monitoring.

188 It was difficult to compare the performance of this algorithm with that of previous

189 studies, primarily because of differences in the experimental procedure. However, a
190 comparison of temporal-spectrum parameters between separated calls and
191 non-overlapping calls was included as an evaluation metric. The seven parameters
192 used in this study are commonly used in bat studies to describe the temporal-spectral
193 features of syllables [26, 29]. Statistical results for this comparison showed no
194 significant differences and small deviations in parameters between separated calls and
195 original recordings, indicating the system was able to separate calls without affecting
196 syllable quality. In addition, clustering analysis conducted with reconstructed
197 echolocation calls was highly accurate (93.8%) for species classification, indicating
198 that calls separated from overlapping signals could be used to synthesize initial data.

199 The BLSTM network exhibited good performance across all six bat species using
200 both narrow and broad time-frequency calls. It also successfully separated different
201 syllable types from both overlapping echolocation and communication calls (Table 1,
202 Fig 1). No species-specific *a priori* knowledge or particular acoustic sensor was
203 directly encoded into the system, making it generalizable to other animal populations
204 with additional training data. Although the dichotomy between communication and
205 echolocation calls is relatively drastic, the proposed separation system has potential
206 applications for other species, as such mixtures are very common in bats. In the future,
207 more complex emitter-independent separation could be conducted using the proposed
208 system, such as combinations of echolocation or social calls from other animals.

209 While deep learning models generally perform better when provided with more data,
210 training with bat calls requires fewer samples than human speech separation, in which

211 available training sets can exceed hundreds of hours [13]. One possible reason for this
212 may be the high signal-to-noise ratio (SNR) of bat sounds recorded with high-quality
213 ultrasound devices. Previous studies have indicated that a high SNR can improve
214 separation accuracy [30] and our results suggest this model was suitable for use with
215 small, high-quality datasets. Although the sound data in this study were sampled in
216 controlled lab conditions, producing recordings that were essentially free of
217 background noise, acoustic analysis software could potentially optimize the separation
218 further by excluding any background noise that was present in the signal.

219 Future studies will also assess the performance of this network for other animal
220 species. Stereotypical patterns and clearly classifiable syllables have been observed in
221 the vocalizations of birds, non-human primates, whales, dolphins, and several other
222 species [31-33]. Features used in the proposed BLSTM were log spectral magnitudes,
223 which can be acquired from any vocal sound. This could potentially lead to robust
224 software that is not specific to a certain species or task. The model could also be
225 generalized to other animals, though limitations may exist. In addition to the quality
226 and quantity of training samples, hyper-parameters must be tuned in accordance with
227 the data [34, 35].

228 **Conclusion**

229 A sound separation model was proposed for extracting bat calls, achieving
230 excellent results. This is the first experimental evidence that the BLSTM model is
231 suitable for separating overlapping bioacoustic signals. These results provided a new
232 source for sound data analysis in animal acoustics research, which may contribute to

233 sample sizes and improve efficiency. This study also demonstrates the potential of
234 deep neural networks for applications to animal vocalization research, including
235 species classification and speech separation.

236 **Materials and Methods**

237 **Sound recording and data preparation**

238 **Species selection and sound sources.** Echolocation calls from bats are primarily
239 composed of constant frequency (CF) components and frequency modulated (FM)
240 components. Social calls are composed of CF, FM, and noise-burst (NB) components.
241 FM calls have short pulse durations and wide bandwidths. As such, they overlap with
242 social calls less in time but more in frequency. In contrast, CF calls have long pulse
243 durations and narrow bandwidths. They overlap with social calls more in time but less
244 in frequency. In consideration of the varied overlapping patterns found in bat calls, we
245 selected both CF bats (*Rhinolophus ferrumequinum*, *Hipposideros armiger*, and
246 *Rhinolophus pusillus*) and FM bats (*Vespertilio sinensis*, *Myotis macrodactylus*, and
247 *Ia io*) to test the separation capabilities of the proposed network, including six
248 different species to test method generalizability.

249 Source sound files from *V. sinensis*, *M. macrodactylus*, *R. ferrumequinum*, *R.*
250 *pusillus*, and *H. armiger* were collected from previous studies in our lab (S1 Table).
251 Sound files for *Ia io* were selected from unpublished data as follows. Bats captured
252 from the field were housed in a husbandry room with abundant food and fresh water.
253 During each sound recording experiment, 4–5 bats were transferred to a temporary
254 cage. Sound recordings were collected using the Avisoft UltraSoundGate 116H

255 (Avisoft Bioacoustics, Berlin, Germany) and a condenser ultrasound microphone
256 (CM16/CMPA, Avisoft Bioacoustics). The sampling frequency was set to 375 kHz at
257 16 bits. The recording experiment lasted five days in order to acquire a sufficient
258 number of recordings, beginning at 18:00 and finishing at 6:00 the following morning.
259 S1 Table shows sample numbers and locations for the bats, as well as the total
260 duration of sound files selected for the study. All experimental procedures complied
261 with the ABS/ASAB guidelines for the Use of Animals in Research and were
262 approved by the Committee on the Use and Care of Animals at the Northeast Normal
263 University (approval number: NENU-W-2010–101).

264 **Sound analysis.** The total duration of recorded sound files (i.e., original recording
265 files) used for each bat species is shown in S1 Table. We employed Avisoft-SASLab
266 Pro (Version 5.2.12, Avisoft Bioacoustics, Berlin, Germany) to identify
267 non-overlapping and overlapping syllables in echolocation and communication calls.
268 These syllables and calls were described and classified following the nomenclature
269 developed by Kanwal, Matsumura (36) and Ma, Kobayasi (37). The recorded
270 non-overlapping calls were used for preparing training files of each call type and the
271 recorded overlapping calls were used for separation.

272 **Data preparation.** Supervised machine learning algorithms use training samples to
273 “learn” the steps required for completing a task. The training phase in this study
274 involved preparing clear and non-overlapping echolocation and communication calls,
275 selected from original recording sounds. In this process, the BLSTM network learned
276 features found in both call types.

277 Training samples consisted of randomly selected non-overlapping syllables in
278 echolocation and communication calls from each bat species (in the original
279 recordings), with signal-to-noise ratios (SNRs) above -20 dB. The echolocation
280 training files contained 1,300–6,240 pulses and the communication training files
281 contained 780–1,800 syllables (S1 Table). Although the quantity of selected syllables
282 varied between studies, the data was sufficient for model training. Efforts were made
283 to include roughly equivalent quantities of each syllable type. Time intervals between
284 syllables in the training files were consistent with those of the original recordings. The
285 lengths of training files for echolocation and communication calls were the same for
286 each bat species (S1 Table).

287 **Model training and call separation**

288 **Model structure and training stage.** We developed a network with four BLSTM
289 layers, followed by one feedforward layer (Fig 6). Each BLSTM layer included one
290 forward and one backward basic LSTM layer, both of which were added with dropout
291 functions (`tensorflow.nn.rnn_cell.DropoutWrapper`). Each BLSTM layer contained
292 300 hidden cells and the feedforward layer corresponded to the embedding dimension
293 (i.e., a 3D matrix with depth $N=40$ in this experiment). Stochastic gradient descent
294 with a momentum of 0.9 and a fixed learning rate of 10^{-3} was used for training. The
295 tanh activation function and the Adam optimizer were adopted to support adaptive
296 learning rates and faster convergence. The structure and hyper-parameters for the
297 model were designed based on the work of Hershey, Chen (21).

298 **Fig 6. The BLSTM model architecture and workflow graph.**

299 The model was trained using the files for one bat species in each trial.
300 Echolocation and communication call training files were loaded using the librosa
301 (version 0.6.2) Python package. Frames from the two sound files were read and added
302 together to create sound mixtures. Sound features used for training (log spectral
303 magnitudes) were extracted from this mixture. The extraction process was completed
304 using a short-time Fourier transform (STFT) with a Hamming window (length of 512
305 and shift of 256).

306 The mixture from each bat species was then segmented into 100-frame samples,
307 all of which were divided into a training set and a validation set using a ratio of 2:1
308 (see S1 Table for detailed sample quantities). The training set, validation set, and
309 indicator labels were combined and input to the model. The validation set was used to
310 optimize tuning parameters and evaluate call separation performance. Indicator labels
311 were set to 0 or 1, representing the two types of calls in the mixture. Ideal binary
312 masks were used to train the network and gradients were calculated using shuffled
313 mini-batches (batch size of 128) from larger segments.

314 The output of this model was a set of embeddings that included learned features
315 for both echolocation and communication calls. In this framework, the deep network
316 assigned embedding vectors to each time-frequency bin in the spectrogram. The
317 network then minimized the distance between embeddings dominated by the same
318 call type in each bin while maximizing the distance between embeddings dominated
319 by different call types. The output was then compared with the validation set and
320 indicator labels to calculate loss, which was back propagated from the output to the

321 input through each layer. Model weights and parameters were then updated based on
322 the calculated loss and training was completed after sufficient iteration epochs.

323 **Separation stage.** In this stage, overlapping echolocation and communication calls
324 were randomly selected from the original recordings to create a sound file of test sets,
325 used for separation. The log spectral magnitudes of the overlapping calls were then
326 extracted, combined into samples, and input to the trained model. The phases of calls
327 extracted from the sound files were also saved for use in sound reconstruction. The
328 trained model then output embeddings for each segment (100 frames) in a process
329 similar to the training stage. Embeddings were clustered using the k-means method
330 from Scikit-learn (Version 0.20.0) to produce time-frequency masks. The number of
331 clusters corresponded to the number of call types in the mixture (2 - echolocation and
332 communication). These masks and the clustering method were then used to determine
333 which parts of each segment in the overlapped calls would be preserved or neglected
334 based on their correspondence to each call type. For example, if the maximum
335 magnitudes were more likely to belong to echolocation calls, the related mask values
336 were set to 1 and the others were set to 0, allowing the echolocation calls to be separated
337 correctly. Finally, output calls were reconstructed using the inverse fast Fourier
338 transform (IFFT) function `numpy.fft.ifft` in NumPy (Version 1.15.1). The IFFT
339 transformed the magnitude into a wave using phase information saved at the beginning
340 of the separation stage. The model produced two waveform files, each containing one
341 call type. Additional detail concerning the sound separation algorithms can be found in
342 the work of Hershey (2016).

343 **Model evaluation**

344 The quality of reconstructed echolocation and communication calls was assessed
345 by comparing their temporal-spectrum parameters to the non-overlapping calls
346 selected from the original recording files (excluding training data). Avisoft-SASLab
347 Pro was used for automatic parameter measurements of duration, bandwidth, peak
348 frequency, minimum frequency, maximum frequency, starting frequency, and ending
349 frequency. A t-SNE (t-distributed stochastic neighbor embedding - R3.6.1 package)
350 analysis was adopted for dimensionality reduction. Two dimensions were extracted
351 from these seven parameters for original and separated syllables and compared with
352 one-way ANOVA (aov in R3.6.1) or two-sided Wilcoxon signed-rank tests
353 (wilcox.test in R3.6.1), depending on their fit to a normal Gaussian distribution. The
354 significance level was set to 0.05 for all tests. We adopted the root mean square error
355 (RMSE) to measure and avoid obscuring individual variations between reconstructed
356 and original calls. Clustering analysis was conducted using the reconstructed
357 echolocation calls from the six bat species, to assess whether the separated calls could
358 be further used in species classification.

359 **Acknowledgements**

360 We are grateful to Dr. Yanhong Xiao of the Experimental Center of the School of
361 Environment at Northeast Normal University, for her assistance in acquiring the
362 experimental materials. We thank LetPub (www.letpub.com) for its linguistic
363 assistance during the preparation of this manuscript.

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- 374 **Writing – review & editing:** Ying Liu, Walter Metzner.
- 375 **Competing interests**
- 376 The authors have declared that no competing interests exist.

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476

477 **Supporting information**

478 **S1 Table. A summary of calls used for model training.**

479 **S2 Fig. Training loss and validation loss during model training.**

480 **S3 Table. Statistical comparisons of principle components extracted from seven**

481 **parameters.** No significant differences were observed between parameters for

482 separated and original syllables. A one-way ANOVA was used to test the normal

483 distributed data and a two-sided Wilcoxon signed-rank test was used to assess the data

484 that did not conform well to a normal distribution.













