

# Characterizing Animal Behavior through Audio and Video Signal Processing

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Quantifying animal behavior in neuroscience research is essential for properly interpreting results, ensuring reproducibility of experiments, and providing a more complete picture of the genotype–phenotype relationship in the nervous system context. Increasingly, multimedia systems are becoming a vital part of the behavioral neuroscientist’s tool kit.

Research in the neurosciences typically focuses on one of several organization levels: the cellular level, which involves cellular processes such as enzyme kinematics and gene transcription; the network level, which involves neuroanatomical organization and electrical communication within neural circuits; and the behavioral level, or the observable outcome of activity at the cellular and network levels in a living organism.

One of the major challenges in neuroscience today (and all of biology, for that matter) is developing a functional relationship between an organism’s genotype, or genetic makeup, and its phenotype—that is, its observed physical and behavioral characteristics. Applied to a neural system, such a relationship would bridge these organization levels and allow unprecedented insight into the nervous system’s functioning and development. With the sequencing of many genomes now complete, researchers have impressive control over the genotype. However, the phenotype

remains sparsely characterized, especially when it comes to quantitatively describing behavior.

The study of animal behavior has a long history—arguably beginning with early humans’ need to become successful hunters. However, the birth of the independent discipline of ethology—the scientific study of animal behavior—didn’t occur until the early part of the last century.<sup>1</sup> Some 80 years later, it’s hard to overemphasize ethology’s influence on neuroscience. To wit, behavior is often regarded as the output of the brain, as the design of many neuroscientific experiments suggests—that is, modify a certain gene, inject a certain drug, or stimulate a certain neural circuit and assess the resulting behavioral change. Such has also been the standpoint in modern psychology, which has relaxed the definition of behavior to include cognitive processes.

Unfortunately, quantification of behavior in neuroscience has taken a backseat to the advances occurring in molecular neuroscience. Recent years, however, have seen an upsurge of interest in developing quantitative measures (which might be recognized as the birth of quantitative ethology). Multimedia systems and signal-processing techniques are proving invaluable to this effect. Although researchers have used signal-processing methods quite effectively at the cellular level (most notably in microscopy) and at the network level (exploring the nature of neural signals), they’re only beginning to exploit such techniques in behavioral neuroscience.

This article presents two instances in which multimedia systems and processing have elucidated animal behavior and have been central in developing quantitative descriptions. These examples demonstrate multimedia systems’ utility and necessity in developing a complete phenotypic description. We hope that this article will spur interest in this subject in the multimedia community, so more advanced processing techniques will enter the field of quantitative neuroethology.

## Video processing: Locomotor activity and exploratory behavior

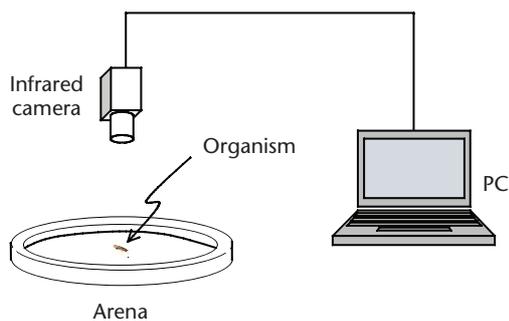
Many behavioral characterizations in neuroscience are founded on descriptions of locomotor activity and exploratory behavior. Neuroscientists define notions of fear, anxiety, motivation, and other anthropomorphisms by assaying the extent to which an animal does or doesn’t move given some set of circumstances.<sup>2</sup> In addition, this

behavior underlies many studies of metabehavior, such as learning and memory, attention, and decision making. For example, fear conditioning in rodents relies on a measure of the amount of time that the animals remain frozen when reintroduced to an environment that they previously associated with a harmful stimulus.<sup>3</sup> As another example, measuring olfactory memory in the fruit fly relies on a ratio of how many flies move toward or away from an odor to which they were aversively conditioned.<sup>4</sup>

Such measures are relatively coarse-grained in the time domain, averaging over the organism's fine-scale movements. Because a dynamic description of how behavior unfolds over time is arguably more descriptive than a static endpoint measurement, we can hardly overemphasize the utility of extracting relevant information from a video of the process. From a signal-processing standpoint, these videos can present extra time series, and analysis of the time series—using feature extraction and proper postprocessing—can uncover large-scale dynamic phenomena. Thus, by using image-processing techniques, video analysis can add to the quantitative toolbox of phenotypic descriptions.

One of the more ethologically relevant and insightful setups for phenotyping this behavior is the study of organisms in the open field (see Figure 1). Researchers introduce an organism (in this case, a fly) into an environment and videotape it. In this setup, video acquisition and analysis is vital. Using a camera (and a proper acquisition system) allows minimal experimenter intrusion and examination of the organism over exceedingly long time periods, thus creating a situation that approximates behavior in a natural setting. Of interest in this paradigm is the trajectory the animal takes while exploring the environment: its position and velocity, as well as the body axis' general orientation. These variables putatively contain all of the information necessary to quantify this type of locomotor behavior, at least at the resolution where individual body part movements can't be assessed. Because of this, researchers must take care in measuring these variables directly from the video. This process should include choosing a frame rate appropriate for capturing the organism's fast movements. In some cases (such as that of a walking fly), the appropriate frame rate can exceed 40 Hz.

Having obtained a video of the behavior, we track the animal's position, either in real time or at postacquisition. Real-time tracking is ideal,



*Figure 1. An example of the experimental setup for behavior in the open field. The figure shows a fly in the open field arena. Arenas can vary in size and are useful for examining organisms such as insects, mammals, and bacteria.*

because it frees us from having to store entire video frames. Sessions can last as long as 10 hours per animal, which presents file storage problems even when using compression. In many cases, the tracking algorithms used are relatively rudimentary. As an example of one such algorithm, we've implemented an offline version to track a single fruit fly in a circular open field arena.

### Tracking

Figure 2 (next page) illustrates our tracking algorithm. In the videos acquired with the setup in Figure 1, the fly is a single ellipsoidal dot moving on a relatively constant background. Therefore, it suffices to find the fly in a frame obtained by subtracting the current frame  $I[n]$  from an averaged background  $I_B[n]$  and squaring the result

$$I_{\Delta}[n] = (I[n] - I_B[n])^2$$

where  $n$  is the current frame index. Squaring increases the signal-to-noise ratio. We calculate the background image from a running average, following the rule

$$I_B[n+1] = \alpha I_B[n] + (1-\alpha)I[n]$$

Here,  $0.9 < \alpha < 1$  is a weighting parameter. This parameter ensures that any unexpected and large (but transient) changes to the background won't significantly affect the update and reduce tracking reliability. To further reduce false tracks, we don't include the area around the fly in the update. This prevents a motionless fly from becoming part of the background.

To determine the fly's location, we find the pixel of maximum intensity in each frame, and extract a subset image around this pixel. We calculate the subset image's center of intensity and use this to calculate the object's  $x$  and  $y$  locations:

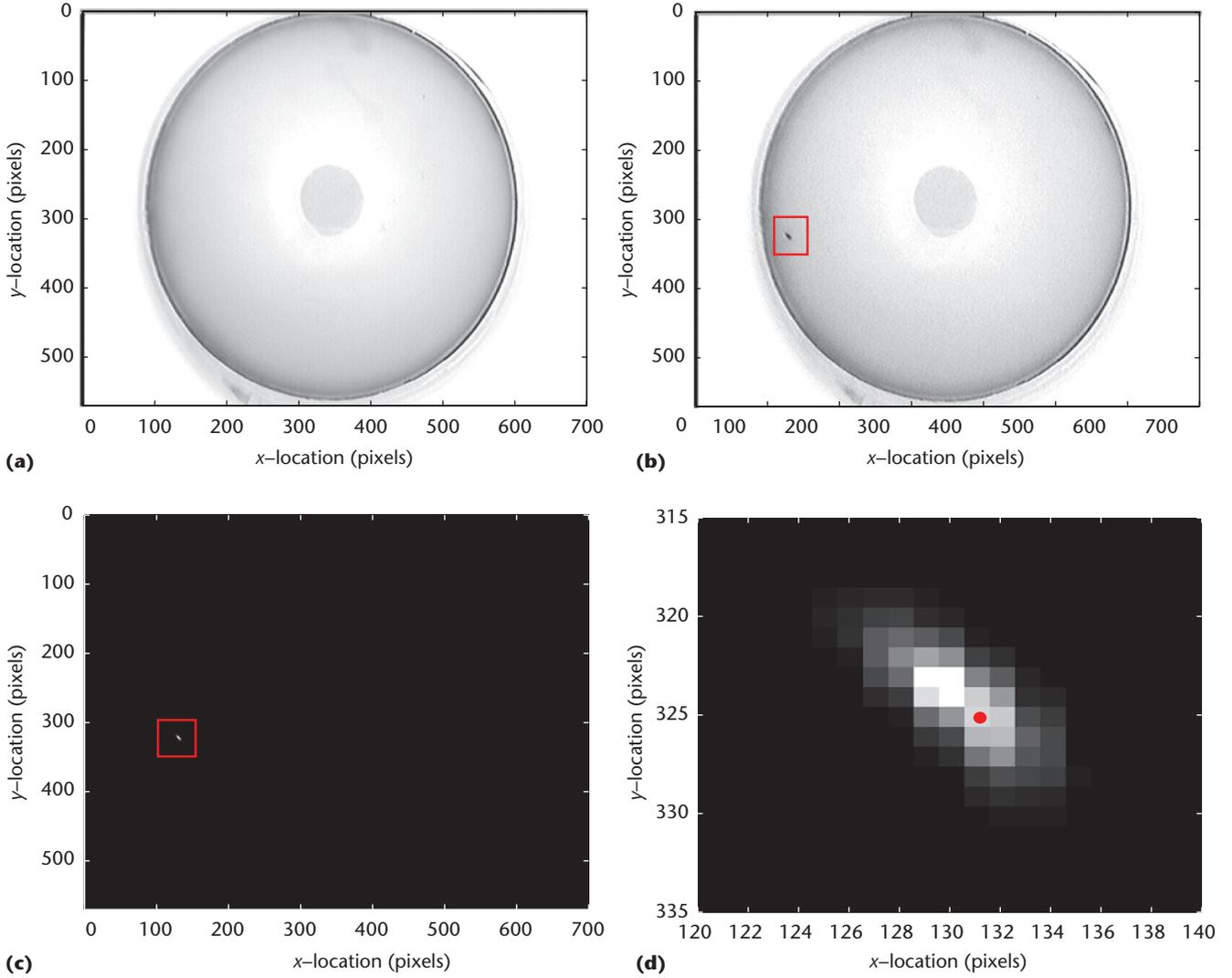


Figure 2. Our algorithm for tracking a fruit fly in a circular open field arena. (a) An example background image,  $I_B$ , calculated after 699 frames. (b) An example frame,  $I$  (frame 700). We've drawn a red box around the fly's location. (c) The image obtained after subtracting the panel in (a) from the panel in (b) and squaring the result,  $I_\Delta$ . Bright pixels represent large differences between the current image and the background. (d) The subset image  $I_{\Delta sub}$  extracted around the pixel of maximum intensity in panel (c). A red dot indicates the subset image's intensity center, and is assumed to be the fly's location in this frame.

$$x[n] = \frac{\sum_{j=1}^{L_x} j \mathbf{I}_{\Delta sub}[n]}{\sum_{i,j} \mathbf{I}_{\Delta sub}[n]} + (\mathbf{I}_\Delta^{\max}[n])_x - L_x$$

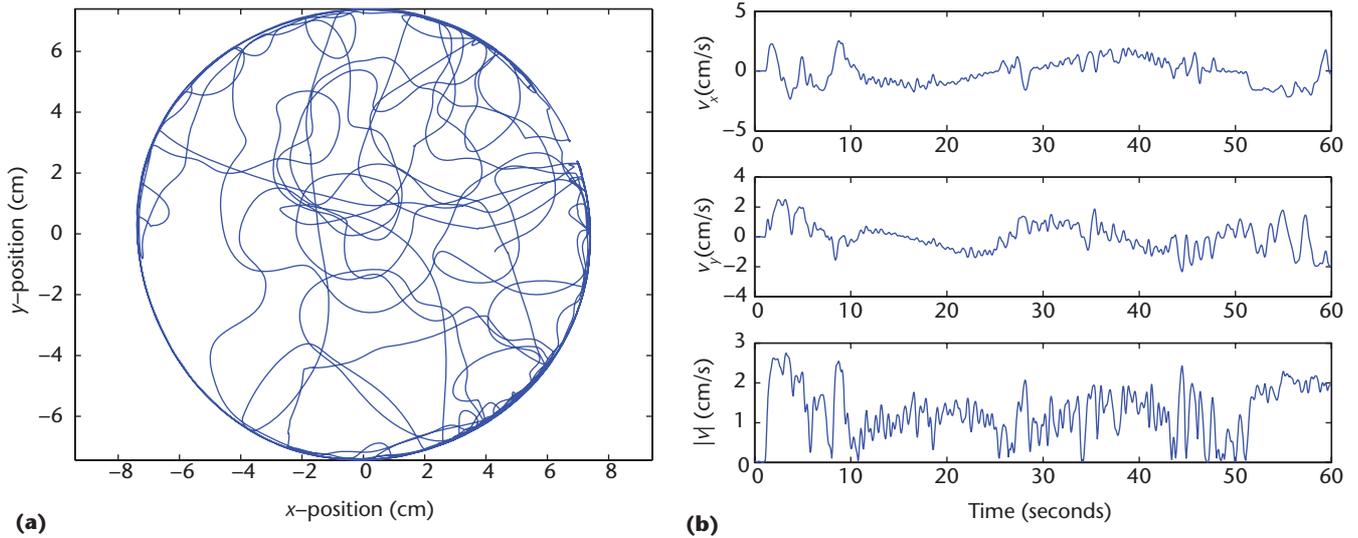
$$y[n] = \frac{\sum_{i=1}^{L_y} i \mathbf{I}_{\Delta sub}[n]}{\sum_{i,j} \mathbf{I}_{\Delta sub}[n]} + (\mathbf{I}_\Delta^{\max}[n])_y - L_y$$

Here,  $i$  and  $j$  correspond to row and column indices;  $L_x$  and  $L_y$  are the subset image's dimen-

sions (in pixels); and  $\mathbf{I}_\Delta^{\max}[n]$  is the pixel of maximum intensity. To initially find the object ( $n = 1$ ), we calculate a static background from a small number of initial frames. We use principal components analysis to find the body axis' orientation. Figure 3 shows trajectory data resulting from 10 minutes of observation of a fly in the open field, tracked using our tracking algorithm.

### Smoothing and segmentation

After we've obtained the trajectory, we must properly smooth and segment the time series. In many animals, it's possible to segment the locomotor activity into two (or more) major classifi-



**Figure 3.** (a) The trajectory obtained from tracking a fly during a 10-minute session using our tracking algorithm. (b) The  $x$ -velocity,  $y$ -velocity, and speed obtained after smoothing the trajectory for the first minute of tracking. The stop-start motion is typical of animal locomotor behavior.

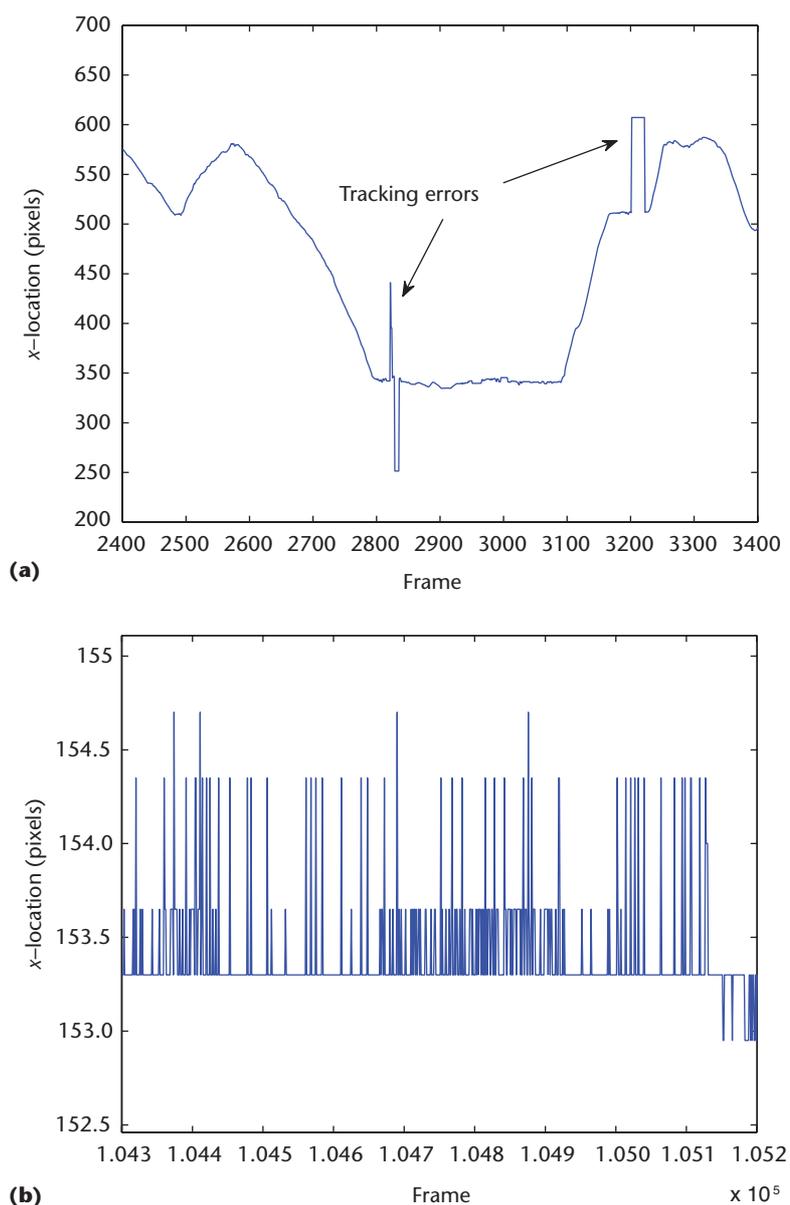
cations: lingering (slow velocity) and progression (high velocity) episodes.<sup>5-8</sup> Although this classification might seem obvious, its quantification is anything but trivial. The quantification begins necessarily with a smooth estimate of the trajectory data, because the results from any tracking algorithm suffer from precision-level noise, noise from individual body part movements, or other tracking errors.<sup>7</sup> In our tracking algorithm, the most common errors occur when you don't remove the area around the fly from the image before updating the background, causing a ghost image of the fly to appear in the background. Subtracting the image greatly reduces the signal-to-noise ratio, and the pixel of maximum intensity doesn't always lie on the fly. Other errors can occur if the fly becomes obstructed by an object in the arena (even the arena walls) or if lighting conditions change. Lastly, a single wing's extension in any given direction can shift the image's center of intensity from a body-based location, which is desired, to a location as much as a millimeter away from the fly's body. Any of these errors manifest themselves as noise in the unsmoothed trajectory (see Figure 4, next page).

Because neuroethologists typically use the organism's velocity or velocity distribution to segment the motion, smoothing is essential to accurately estimate the velocity. As a consequence, a proper smoothing algorithm must neglect any outliers in the data that have arisen due to tracking errors. These outliers can cause drastic and unphysical velocity estimates. In addition, complete arrests of the organism must not be smoothed to the extent that a stop becomes a slow walk. These requirements often

cause researchers to use a combination of algorithms to accurately characterize the movement.

One particularly robust smoothing method that we've used for both rats and flies is the locally weighted sum of squares algorithm.<sup>7,9</sup> The Lowess algorithm is an extension of the local polynomials algorithm, which fits a polynomial to the data in a window centered at time  $t$ , and moves this window across the entire length of data. An advantage to Lowess is that it uses the residuals from the polynomial fit to calculate weights for each point in the window. It then uses these weights in a second iteration, where a local polynomial is fit to the weighted data. It can repeat this process of calculating residuals and weighting the data iteratively until it shows no significant smoothing improvement. This process makes Lowess robust to outliers in the data—it gives points with large residuals a zero weight. In addition, because the data is fit to a polynomial in each window, it obtains smooth estimates of velocity at each time point. (More details regarding the algorithm are available elsewhere.<sup>9</sup>)

In conjunction with this smoothing algorithm, we can use a repeated running median to identify the location of true stops.<sup>7</sup> RRM is another smoothing algorithm robust to outliers. It calculates the median of the data in a given window (again, the window moves along the data record). RRM assigns the window's median to the data point at  $t$ . Repeating this procedure with windows of different sizes can yield a smooth estimate of the data. The disadvantage of RRM as a smoothing algorithm, however, is in the case of repeated values. Here, the estimate becomes fixed at the repeated value, and the subsequent estimates



**Figure 4. The need for trajectory smoothing.** (a) Tracking errors that have occurred when a stationary fly wasn't removed from the image before the background update. (b) Jitter in the tracking algorithm while the fly is stationary. Small variations in lighting or the fly's slight body movements can cause this effect.

aren't very smooth. Fortunately, its failure as a smoother is exactly what we need to properly calculate the length of an arrest, which is a repeated series of zero-velocity points. So, the RRM can identify record sections where the velocity should be set to zero and identified as a full stop.

Other smoothing methods are possible. RRM is but one example, and has proven useful in video analysis of mammal exploratory behavior. In choosing any algorithm, the experimenter must ensure that the smoothed position and velocity estimates are accurate and physically plausible. Algorithms can produce visually smooth trajectories that aren't representative of the organism's true motion, so choosing algo-

rithm parameters takes considerable effort. This decision typically involves a visual inspection of videos to ensure that parameters are set correctly. For example, if the outliers in Figure 4a weren't neglected, you would conclude that the fly jumped suddenly and quickly. Inspection of the video revealed that this wasn't the case—that these points were, in fact, tracking errors. This is where more automation could be extremely beneficial. However, once parameters are chosen, they aren't changed from video to video (unless, of course, you're examining different species).

Once we've obtained a smooth velocity, we examine a density estimate of the velocity distribution to separate the lingering episodes (those of small or no motion) from progression episodes (those in which the organism is moving considerably).<sup>8</sup> Here, we could set a hard velocity threshold or we could use statistical estimates of the distribution using expectation maximization or other techniques to determine the animal's repertoire of available movements (in terms of speeds). Figure 5 illustrates how we can segment motion into lingering and progression episodes using expectation maximization to estimate the velocity distribution as a mixture of two Gaussian distributions.

We can extract other variables from properly smoothed and segmented trajectory data to expand the phenotype quantitatively. Distributions of distance between episodes, angular orientation after lingering, excursions from a wall or a home base, and number or length of runs can all effectively characterize behavior in organisms ranging from rats to nematodes to bacteria.<sup>6,7</sup> This type of analysis has greatly elucidated the neural mechanisms at work in locomotor behavior, and the measurements' quantitative nature has let investigators compare data across laboratories, eliminating subjective bias and any lab-specific interactions. This analysis would have been difficult, tedious, and time-consuming without automated video systems and processing techniques. These systems have allowed fine temporal scale observation of behavior, which is essential to developing an outside-in description of neural dynamics. On the horizon are more advanced techniques, such as edge detection, multiobject tracking, and feature extraction, which will certainly let researchers add to these phenotypic descriptions and will present a slew of time series on which researchers can perform complete behavioral analyses.

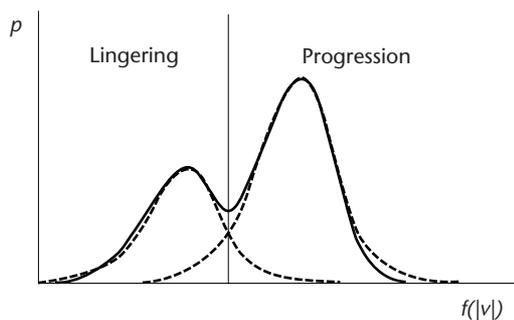
## Audio processing: Song learning in the zebra finch

Video analysis is only one part of deciphering behavior's neural basis—watching animals can only take you so far. In fact, much animal behavior is associated with vocal communication between species members, and understanding how and why these animals vocalize can greatly elucidate gene–environment interactions in neural processes. Accurately characterizing such acoustic-based behaviors can yield more descriptive phenotypes. Birdsong is one behavior that lends itself to audio processing and analysis.

Although the basic neural circuitry required for singing is innate in birds, all true songbirds (order Passeriformes, suborder Oscines) develop their songs through a process of vocal learning.<sup>10</sup> Young males both improvise songs and imitate older males, comparing their own vocalizations with stored representations of these model songs.<sup>11,12</sup> The bird thus uses auditory feedback to refine its song. An objective and quantitative analysis of birdsongs is essential to phenotyping this behavior, especially because researchers are increasingly using songbirds as a model system for studying the neurological basis of learning and memory.

The procedure for quantifying song learning is not entirely different from that for locomotor behavior. We acquire songs by microphone, store, segment, and process them, and then extract descriptive quantitative parameters from the resulting time series. Because song learning is a dynamic process that develops over the bird's life, it's important to record its entire vocal ontogeny. As with video processing methods, for a complete characterization, long time series are ideal. This, again, presents storage issues. Recording every song a single bird sings over its lifetime can require more than 40 Gbytes of storage for 100 to 150 hours of song at a 44.1-kHz sample rate and 16 bits per sample with no compression. With current storage technologies, however, this is becoming less of a limitation. Instead, automating the analysis, organization, and curation of such a large database is one of the major technical challenges we faced in this research.

In contrast to the open field setup, however, we've fully automated the acquisition stage, as well as some preliminary analysis and storage of relevant features.<sup>13</sup> During training, the bird can choose to hear model songs by pecking at a key placed in the cage. Delivery of song playbacks in response to key-pecking introduces interactions between the pupil and the tutor. Such interactions



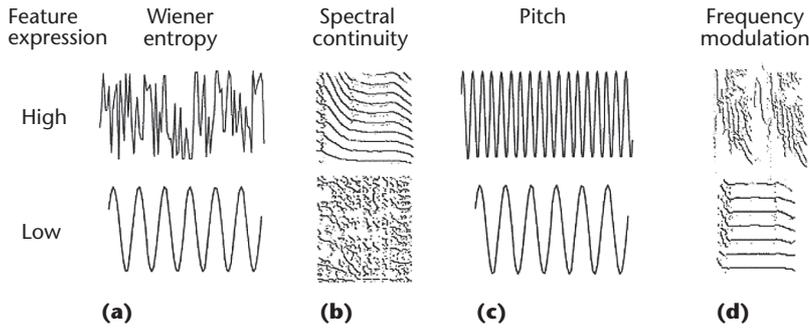
mimic the interactions between a pupil and a live tutor bird and improve the zebra finch's song-learning performance. The bird then attempts to imitate the model song, which the acquisition system automatically detects. The system continuously records, discarding noise and isolated calls. After acquisition, the system segments songs into syllables (continuous sounds that are preceded and followed by silent intervals or abrupt frequency changes) just as we can segment locomotor behavior into progression and lingering episodes. A spectral decomposition of the data lets the system perform this segmentation easily, and is the natural domain in which to work if seeking to characterize birdsong.

### Characterizing a song

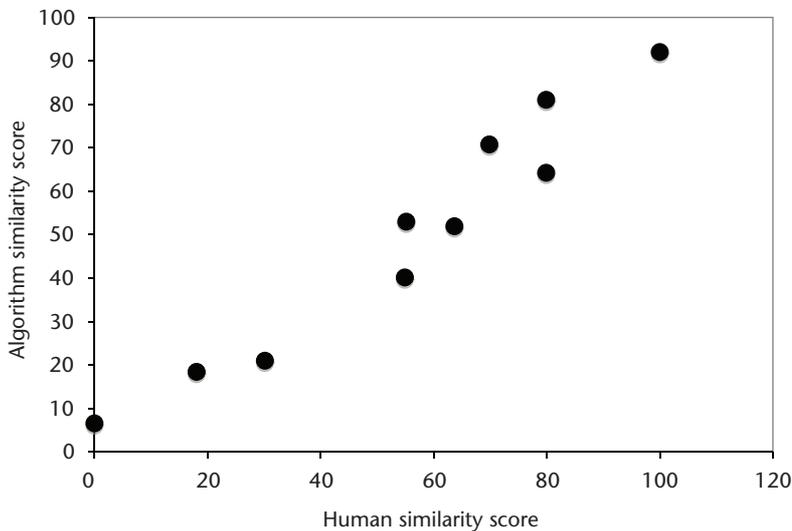
Traditionally, visual inspection of spectrograms (time-frequency plots) was the primary means for characterizing birdsong.<sup>14</sup> Although this method was insightful and yielded many important findings, it isn't entirely objective and doesn't scale well to high-throughput experiments. We circumvent these problems using time and frequency derivatives of the spectrogram, which can accurately detect events (such as peaks) in the time-frequency plane. Furthermore, we calculate the spectrograms using multitaper spectral methods, which produce superior estimates to the traditional spectrogram.<sup>15</sup> These methods let us estimate the time-frequency derivatives robustly, and can remove slowly varying noise sources. We use zero crossing of these derivatives to identify the time-dependent positions of peaks in the spectrogram.

To automate song analysis, we must reduce the spectrogram's high dimensionality to a tractable number of degrees of freedom. We do this by introducing a small set of features that we extract from the spectrogram. We choose these features to better capture the relationship between the sound and the apparatus (that is, the vocal fold) producing it. The most obvious features of an oscillating membrane's motion are the oscillation's period and regularity (entropy). They're reflected in sound as

*Figure 5. Segmenting the animal's motion into lingering and progression episodes. Here, an example density estimate of the (smoothed) speed distribution is shown. We applied a transformation  $f(v)$  to the speeds to facilitate the peak separation. We fit Gaussian distributions (dotted lines) to each peak, and used the intersection of these distributions as the threshold that defines a lingering episode and a progression episode.*



**Figure 6.** The four features characterizing birdsong. (a) Wiener entropy is low for pure tone and reaches its maximum for white noise. (b) Spectral continuity is high when the frequency contour is continuous (spanning multiple time windows), and low if the contour is broken. (c) Pitch measures the period. (d) The frequency modulation is high when rapid changes take place in the song's frequency content.<sup>16</sup> (Used with permission.)



**Figure 7.** Comparison of the automated similarity score to the score assigned by a human observer. The short timescale similarity matrix provides a score similar to that of human pattern matching, so we can consider the automation of similarity calculation as accurate.

pitch and Wiener entropy, respectively. Pitch is the median difference between consecutive harmonics. Wiener entropy is the ratio of the spectrum's geometric mean to its arithmetic mean (or the spectrogram within a time window),

$$W = \frac{\exp\left[\int df \log S(f)\right]}{\int df S(f)}$$

Because songs progress with time, it also makes sense to measure how pitch changes with

time by frequency modulation—that is, the angle of the spectrogram's maximum directional derivative. Finally, another metric of sound's progression in time, spectral continuity, estimates the continuity of frequency contours across time windows. Consequently, a feature vector of the four components represents each time window of the spectrogram (see Figure 6).<sup>16</sup> We normalize and combine the features using appropriate weights. When combined with a robust spectral analysis, they simplify the objective study of vocal learning.

### Tests for song similarity

Because young males learn their songs from older males, a natural quantitative measure is the extent to which a pupil's song is similar to its tutor's song. Early studies compared this similarity visually. Following segmentation of the song, researchers identified the best-matched note of the tutor's song for each of the pupil's song syllables, and assigned a numerical similarity score for the match.<sup>17</sup> Such a measure was idiosyncratic (albeit accurate), and didn't facilitate cross comparison between results obtained by different laboratories. Automating feature extraction from spectrograms lets us more quantitatively test for similarity.

Consider the short timescale similarity matrix of two songs, a matrix of values defined by taking pair-wise Euclidean distances between features corresponding to different time slices in the songs. We accept the hypothesis of match between two windows when a critical similarity threshold is met. To get the similarity score, we calculate the probability that the match's goodness would have occurred by chance. Thus, we obtain a well-defined metric for song comparison, in contrast to visual inspection of song spectrograms. Figure 7 compares this automated similarity test to that of human visual inspection. The algorithm performs on par with a human observer's pattern recognition.

### Dynamics of vocal imitation

We based the similarity matrix on a feature vector obtained by sliding a window along the data. To preserve a song's temporal structure, we could instead segment the spectrogram into syllables. The basic idea behind the segmentation algorithm is that syllables are characterized both by a high power value in a particular frequency band and by this value's approximate constancy over a certain time. The syllable's length, or dura-

tion, is a component in its feature vector, in addition to the four features discussed earlier. This method emphasizes a birdsong's syntax.

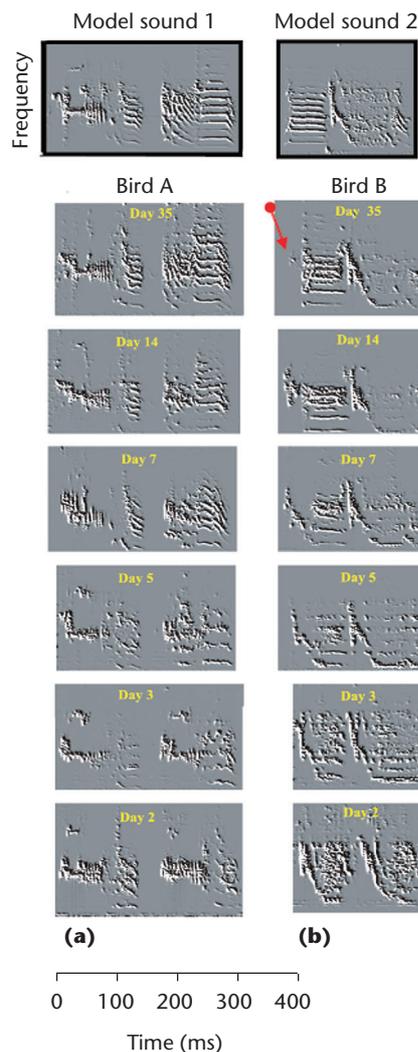
This feature vector lets us easily trace the syllable's origin and transformations in song development history. This imitation trajectory exhibits interesting dynamics, at least in the case of the zebra finch vocal-imitation process shown in Figure 8.<sup>18</sup> In Figure 8a, the two syllables of song produced by bird A on day 35 originate from repetitions of the same prototype sound on day 2. Although the first syllable remains approximately unchanged, the second syllable drastically transforms. However, the two sounds' relative temporal positions remain intact—a phenomenon called *sound differentiation in situ*. In the imitation trajectory of a syllable from bird B (shown in Figure 8b), we can trace the first and second parts of the syllable back to two back-to-back renditions of the same prototype sound. Again, sounds were differentiated in situ.

Young zebra finches tend to produce back-to-back repetitions of similar sounds, much like human infants' reduplicated (canonical) babbling. The in situ differentiation of two back-to-back renditions of a same prototype sound provides a transition mechanism from the primitive, repetitive state to a mature state in which sequences of dissimilar sounds are produced in a fixed order. This is clearly a common mechanism, even if it isn't responsible for all transitions. Such vocal imitation processes indicate the presence of interesting dynamics in the development of the underlying neural networks. Characterizing this process wouldn't have been possible without the automated audio-acquisition system and processing techniques.

### Future directions in birdsong learning

The features we've discussed are closely related to the articulatory variables involved in sound production. When combined with a robust spectral analysis, they simplify the objective study of vocal learning. In the near future, researchers will use new features derived from a deeper understanding of the sound-production apparatus and statistical analysis of the songs to characterize birdsong, thus quantitatively expanding the phenotype.

Given the vocal-learning process's complexity, performing integrated data analysis at multiple levels of the song-production system has great value. The incorporation of neural and peripheral recordings as well as molecular-level



**Figure 8. Sound differentiation in situ.** (a) Two similar prototype sounds transform into two different syllables with the temporal relation unchanged. (b) Two successive similar prototype sounds transform into the two markedly different parts of a single syllable with their temporal relation intact.<sup>18</sup> (Used with permission.)

data should provide us with further insights into the process, paving the way for identifying the molecular, cellular, and circuit mechanisms underlying the vocal learning. A multimedia analysis of the process will undoubtedly facilitate such an understanding.

### Conclusion

You might have noticed that in our two examples, there was nothing very multimodal about the media techniques used. Both of these systems are transparently unimodal. This speaks to the limited crossover between the multimedia community and the behavioral neuroscientists (or neuroethologists). These examples did show, however, that the neuroscientific community can benefit greatly from incorporating multimedia techniques into their experiments and data analysis. As the walls between these disciplines

begin to fall, experimental setups that are truly multimedia will likely appear. Such systems will allow complete phenotypic descriptions of animals in ethologically relevant settings, along with methods for analyzing, manipulating, annotating, and storing the resulting data. Combining these phenotypic descriptions with the corresponding genetic and neural network properties will facilitate the connection of these organization levels and lead to a more thorough understanding of brain functioning. **MM**

## Acknowledgments

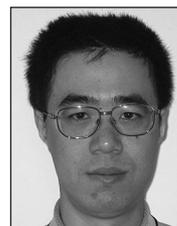
An award from Dart Neuroscience, LLC (for fly locomotor studies) and US National Institutes of Health grants R01MH071744 and R01NS0436 to Ofer Tchernichovski and Partha Mitra (for the birdsong studies) partly supported the research discussed in this article.

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