Animal Behaviour 83 (2012) 1411–1420

Contents lists available at SciVerse ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Sensory constraints on birdsong syntax: neural responses to swamp sparrow songs with accelerated trill rates

J. F. Prather [a], S. Peters [b], R. Mooney [c], S. Nowicki [b,c,*]

[a] Department of Zoology and Physiology, University of Wyoming, Laramie, WY, U.S.A.
[b] Department of Biology, Duke University, Durham, NC, U.S.A.
[c] Department of Neurobiology, Duke University Medical Center, Durham, NC, U.S.A.

ARTICLE INFO

Article history:
Received 29 August 2011
Initial acceptance 4 November 2011
Final acceptance 29 February 2012
Available online 17 April 2012
MS. number: A11-00683R

Keywords:
auditory
birdsong
development
HVC
Melospiza georgiana
sensorimotor
swamp sparrow
syntax
vocal learning

Both sensory and motor mechanisms can constrain behavioural performance. Sensory mechanisms may be especially important for constraining behaviours that depend on experience, such as learned birdsongs. Swamp sparrows, *Melospiza georgiana*, learn to sing by imitating the song of a tutor, but they fail to accurately imitate artificial tutor songs with abnormally accelerated trills, instead singing brief and rapid trills interrupted by silent gaps. This ‘broken syntax’ has been proposed to arise from vocal-motor limitations. Here we consider whether sensory limitations exist that could also contribute to broken syntax. We tested this idea by recording auditory-evoked activity of sensorimotor neurons in the swamp sparrow’s brain that are known to be important for the learning, performance and perception of song. In freely behaving adult sparrows that sang songs with normal syntax, neurons were detected that exhibited precisely time-locked activity to each repetition of the syllable in a trill when presented at a natural rate. Those cells failed to faithfully follow syllables presented at an accelerated rate, however, and their failure to respond to consecutive syllables increased as a function of trill rate. This ‘flickering’ auditory representation in animals performing normal syntax reveals a central constraint on the sensory processing of rapid trills. Furthermore, because these neurons are implicated in both song learning and perception, and because auditory flickering began to occur at accelerated trill rates previously associated with the emergence of broken song syntax, these sensory constraints may contribute to the emergence of broken syntax.

Behavioural performance can be constrained by a variety of factors. Some behaviours may be shaped by the physical properties of the peripheral motor system, or by physiological limitations of the central motor networks that pattern the behaviour (Lauder & Liem 1989). Other behaviours, especially those that are learned, depend heavily on sensory experience and sensorimotor integration. Therefore, the constraints that shape the performance of learned behaviours could also arise from a limited ability of the central nervous system to accurately encode sensory experience relevant to learning. Juvenile songbirds learn to sing by copying the song of an adult tutor in a process that depends heavily on auditory experience and vocal rehearsal (reviewed in Mooney et al. 2008). Despite the songbird’s remarkable capacity for vocal learning, even the best singers are constrained in their imitative ability (Ryan & Brenowitz 1985; Nowicki et al. 1992). When tutored with songs containing features exceeding species-typical norms, birds are often unable to accurately copy those songs (Podos 1996; Podos et al. 1999). A possible explanation for this inaccurate copying is that the properties of the vocal and respiratory musculature, or of the central circuits important to song patterning, limit the bird’s ability to reproduce an otherwise accurately encoded auditory representation of the abnormal model (Podos 1996). Alternatively, learning could be constrained by the brain’s inability to accurately encode an auditory representation of a song model with abnormal acoustic features (Marler 1976). In this study, we recorded the auditory responses of neurons in freely behaving adult swamp sparrows, *Melospiza georgiana*, to ask whether sensory limitations constrain the representation of abnormal song models that are difficult for young sparrows to copy.

Swamp sparrow songs consist of a multinote syllable trilled continuously at a steady rate. Young swamp sparrows tutored with manipulated songs composed of artificially accelerated trills often fail to copy those songs accurately, producing instead versions of those songs that have been modified in ways that make them apparently easier to produce (Podos 1996; Podos et al. 1999). Sparrows tutored with song models containing highly accelerated trill rates subsequently sing songs in which they accurately

---

[*] Correspondence: S. Nowicki, Department of Biology, Duke University, Box 90338, Durham, NC 27708-0338, U.S.A.
E-mail address: snowicki@duke.edu (S. Nowicki).

0003-3472/$38.00 © 2012 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.anbehav.2012.03.012

reproduce both the acoustic features of the song syllable and match the accelerated trill rate, but only in short bursts that are separated by silent gaps, resulting in what is referred to as ‘broken song syntax’. Broken song syntax is highly atypical of normal swamp sparrow songs, and it remains unclear what constrains the copying of accelerated trills during the song-learning and development process in such a way as to produce this outcome. Because birds can accurately copy the accelerated model in brief epochs, Podos and colleagues concluded that the central nervous system must contain an accurate auditory representation of the song, and speculated that broken syntax arises because of peripheral motor constraints (Podos 1996). Here we consider the possibility that the sparrow’s brain is limited in its ability to accurately encode a sensory representation of songs with artificially accelerated trill rates.

If the production of broken song syntax is influenced by the inability of the central nervous system to accurately encode tutor songs with accelerated trills, then this encoding constraint may be most clearly evident at the point where auditory information about song models is integrated with vocal motor commands (Nottebohm et al. 1976; Hahnloser et al. 2002; Nick & Konishi 2005; Prather et al. 2008, 2010; Roberts et al. 2010). In that light, we recorded the activity of individual neurons in the swamp sparrow’s HVC (used here as proper name), a sensorimotor brain structure where individual neurons are active during both singing and listening to song (McCasland & Konishi 1981; Prather et al. 2008), and where auditory-evoked activity has been linked to song learning and perception (Nick & Konishi 2005; Prather et al. 2009; Roberts et al. 2010). Specifically, we sampled the activity of HVC neurons that project to the striatal Area X (i.e. HVC_X neurons), which in swamp sparrows exhibit auditory-evoked action potential activity that is precisely time-locked to each syllable in a natural song trill (Prather et al. 2008, 2009). If the properties that constrain the production of broken syntax are exclusively motor-related, then HVC_X auditory responses should accurately encode continuous syllables in an accelerated trill. Alternatively, if the emergence of broken syntax also reflects auditory-processing constraints, then HVC_X cells may be limited in their ability to follow accelerated trills. We compared the responses of HVC_X neurons to auditory presentation of natural swamp sparrow songs versus variants of those songs in which the trill rate had been accelerated. Our previous studies have shown that these cells respond in a precisely time-locked fashion to each syllable presented at a normal trill rate (Prather et al. 2008, 2009). In contrast, HVC_X neurons failed to faithfully follow syllables presented at an accelerated rate, and failure to respond to consecutive syllables increased as a function of the trill rate. Notably, gaps in neural responses began to form at accelerated trill rates associated with the emergence of broken song syntax in previous behavioural studies. These data were recorded in birds that were naïve to accelerated trills and that were not themselves performing broken syntax, eliminating motor performance of broken trills as a possible influence on this failure to respond faithfully to songs with accelerated trills. Together, these data reveal a previously unknown limitation on the central representation of accelerated trills that could limit the bird’s ability to learn these songs.

METHODS

All procedures were in compliance with recommendations of Duke University Animal Care and Use Committee and state and federal regulations governing the capture and use of wild birds.

Animal Collection and Song Stimulus Preparation

Swamp sparrows were caught with mist nets as adults (age >1 year) on either their winter grounds in Orange County, NC, U.S.A., or their summer breeding grounds in Crawford County, PA, U.S.A. Birds were housed in cages (46 × 22 × 26 cm) individually throughout their time in the laboratory, both before and during experimentation, and were provided seed and water ad libitum and given a regular supplement of mealworms. Males ($N = 6$) were identified either by external morphology (breeding season) or by molecular marker techniques (out of season), and females were released (methods described in Prather et al. 2008). Prior to implantation of the stimulus and recording devices, birds were subjected to gradually lengthening photoperiod (1 h/week, 9:15 h to 15:9 h light:dark cycle) meant to simulate the onset of the spring breeding season, when swamp sparrows sing most robustly. This change in photoperiod, combined with a subcutaneous implant of testosterone, T (20 mm length of silastic tubing, inner diameter 1.47 mm, outer diameter 1.96 mm, packed with crystalline T; see Nowicki & Ball 1989), was sufficient to induce the birds to sing. Birds were recorded in a semianechoic chamber (Industrial Acoustics Model AC-1, Sony TCM 5000 EV recorder and Shure SM57 microphone), and many examples of song (typically >100) were recorded from each bird to ensure that the bird’s full repertoire was sampled (two to five song types). Although the exact age of birds we used for these experiments was unknown, all songs were performed in the ‘crystallized’ song syntax that typifies adult swamp sparrow song (Marler & Peters 1982), indicating birds were at least 1 year old. Exemplars of each song type were digitized (25 kHz) and saved onto a computer hard drive (Signal, Engineering Design, Belmont, MA, U.S.A., LabView, National Instruments, Austin, TX, U.S.A.) to be used as stimulus songs.

Song stimuli consisted of natural song types and synthetic variants of those song types from the experimental subject and conspecific birds. Natural song types (unaltered from the original recordings) were used to assess the auditory selectivity of each neuron. Following methods used to generate accelerated trills in previous studies, songs were digitally edited to create synthetic variants of the natural song type (Podos et al. 1999). Individual notes were not altered in this process, but the silent intervals between notes and syllables were compressed to 50%, 25% or 0% of their respective durations in the natural song type, resulting in a slight compression of the syllable duration and an acceleration of the overall trill rate (Fig. 1, see Supplementary Fig. S1 for WAV files of songs). Individual synthetic syllables were concatenated to generate trilled stimuli with total song duration like that of natural song types (typically ~2 s).

Microdrive Implantation Surgery

Neurons were sampled using a miniaturized micromanipulation device in awake and freely behaving birds (Fig. 2a) (Fee & Leonardo 2001; Prather et al. 2008, 2009). Several days prior to implantation, birds were transferred from their housing cage to a cage within the recording chamber, a sound-attenuating box (Industrial Acoustics, Bronx, NY, U.S.A., Model AC-1) where they would reside throughout experimentation. During implantation, adult male swamp sparrows were anaesthetized using isoflurane (inhalation, 1–3% in 100% O_2) and placed in a stereotaxic device. A small incision was made in the skin overlying the skull, and the outer leaflet of bone was removed over HVC and the sites of its projections into the avian striatum (Area X) and the robust nucleus of the arcopallium (RA) (Reiner et al. 2004) (HVC is typically 0.3 mm anterior and 2.3 mm lateral of the bifurcation of the midsagittal sinus, Area X is typically 5.7 mm anterior and 1.9 mm lateral, RA is typically 0.7 mm posterior and 2.3 mm lateral, head angle 30° below zero in all cases). A small craniotomy (approximately 300 × 300 μm) was made in the inner leaflet over Area X, and a small custom-made bipolar stimulus electrode