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Lesions of a higher auditory brain area during a sensorimotor period do not impair birdsong learning

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Abstract: One important function of the nervous system is to form and retrieve memories to direct behavior. A prime example of memorization occurs in songbirds when they imitate the songs of another bird heard early in life. Although many brain areas required for song learning have been identified, their separate roles in memory formation and retrieval remain unclear. In juvenile male zebra finches, we test the role of a higher auditory cortical area in memory retrieval. The Caudal Medial Nidopallium (NCM) has previously been shown to be necessary for song memory acquisition and is thus a likely location for holding the memory of tutor song necessary for learning. We extensively exposed young birds to tutor song after which we performed large bilateral lesions in NCM using injection of ibotenic acid. We observe successful song copying in lesioned animals, comparable to song behavior in untreated control birds. Our results show that intact NCM is not required to guide vocal learning based on a previously formed song memory.

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Lesions of a higher auditory brain area during a sensorimotor period do not impair birdsong learning

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One important function of the nervous system is to form and retrieve memories to direct behavior. A prime example of memorization occurs in songbirds when they imitate the songs of another bird heard early in life. Although many brain areas required for song learning have been identified, their separate roles in memory formation and retrieval remain unclear. In juvenile male zebra finches, we test the role of a higher auditory cortical area in memory retrieval. The Caudal Medial Nidopallium (NCM) has previously been shown to be necessary for song memory acquisition and is thus a likely location for holding the memory of tutor song necessary for learning. We extensively exposed young birds to tutor song after which we performed large bilateral lesions in NCM using injection of ibotenic acid. We observe successful song copying in lesioned animals, comparable to song behavior in untreated control birds. Our results show that intact NCM is not required to guide vocal learning based on a previously formed song memory.

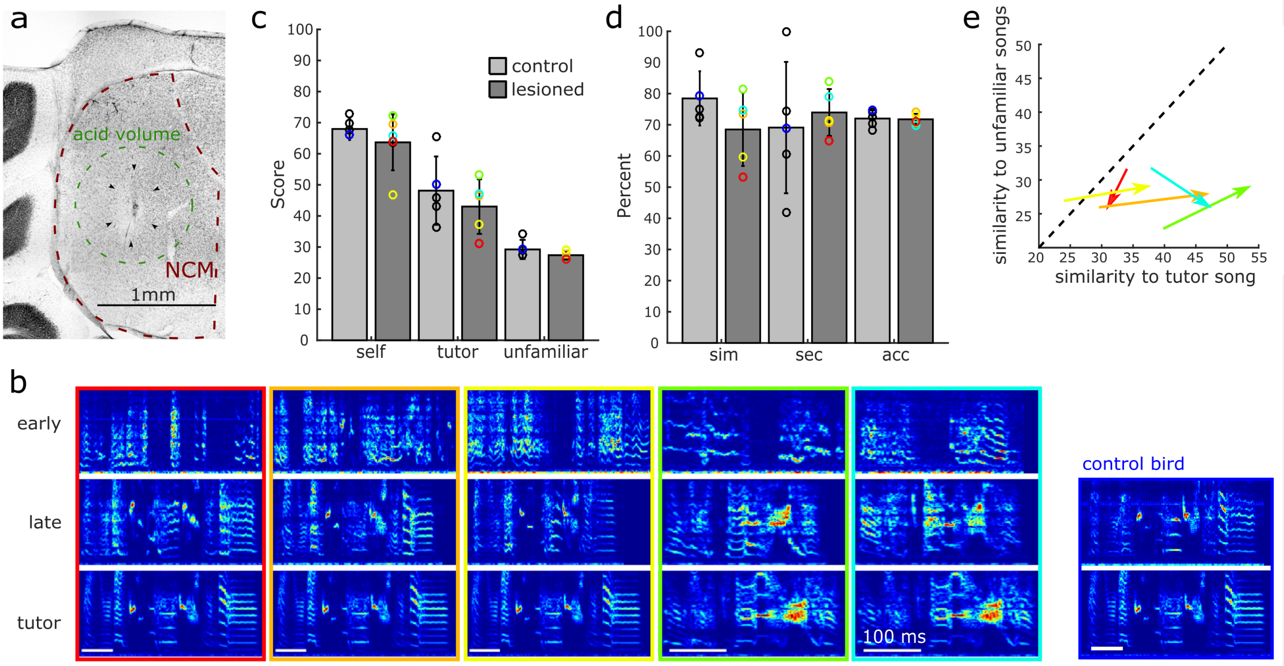
Objective

We investigate NCM's role in recalling of a tutor song memory. We study the effects of very large irreversible NCM lesions in about 40 dph old juvenile zebra finches that had been extensively exposed to a tutor song prior to the lesions.

Introduction

We study the organization of memory in a behavior of imitation learning based on observing and copying a conspecifics' performance. We focus on birdsong development, which similarly to speech development involves perception and memorization of a sensory stimulus within a critical sensory period (**Fromkin 1974**[1]) (**Kuhl 2010**[2]). Juvenile zebra finches start to sing subsong (soft vocalizations comparable to the babbling of human infants) typically at an age of 30 days post hatch (dph). Gradually they transform their subsong into a good imitation of the father's song, even if separated from the latter as early as 40 dph (**Immelmann K 1969**[3])(**Böhner 1990**[4]) (**Funabiki 2003**[5]).

A key brain area that is important for tutor song memorization is the caudal medial nidopallium (NCM). Good tutor song copying correlates with increased NCM neural activation, assessed via either immediate early genes (IEGs) (**Mello 1995**[6]) (**Bolhuis 2001**[7]) (**Bolhuis 2000**[8]) or spiking responses (**Chew 1995**[9]) (**Miller Sims_2014**[10]). There is evidence that NCM holds a song memory in adult birds: tutor song recognition and recall of learned song are both impaired after irreversible lesions in NCM (**Gobes 2007**[11])(**Canopoli 2014**[12]), the impairment being memory-specific because neither motor behavior nor auditory perception are significantly affected by the lesions. Evidence for a necessary role of NCM in tutor memory acquisition in juveniles comes from pharmacological manipulations: IEG suppression specifically during tutoring severely impairs tutor song learning (**London 2008**[13]), paralleling the IEG requirements for memory formation in rodents (**Steward 1998**[14]) (**Steward 2001**[15]) (**Korb 2011**[16]).



a: Sample sagittal Nissl-stained brain section from an NCM lesioned bird, confirming the lesion placement (black arrowheads) within NCM (dashed line). Dorsal is up, rostral is right. The green dashed line indicates the volume of the injected acid, providing a lower bound to the initial lesion extent.

b: Example log-power song spectrograms (red: high sound intensity, blue: low sound intensity) from five NCM-lesioned birds (left) and from one non-lesioned control bird (right, same bird marked in blue in panels c and d). Initially, spectrograms in 43 days post hatch (dph) old juveniles (early, top) look different from spectrograms of tutor song (bottom). However, at 77 dph (late, middle), there is a good visual correspondence between juvenile and tutor song spectrograms. The spectrogram associated with the bird in a is outlined in red.

c: Average scores of juvenile songs tested for similarity with themselves (self), with tutor songs (tutor), and with unfamiliar songs (unfamiliar). On average, all three similarity scores are indistinguishable between control birds (light grey bars) and NCM-lesioned birds (dark grey bars, mean ± 1 standard deviation, t-test, $p>0.05$), which reveals that NCM lesions do not impair song development. The circles represent scores in individual birds, the colors correspond to the colors of outlines delimiting the spectrograms in b.

d: The songs in 77 dph NCM-lesioned birds and of age-matched control birds exhibit similar averages of percent similarity, of sequential match, and of accuracy relative to their tutors' songs (i.e., the 3 measures that define the similarity score in c): all three comparisons yield $p>0.05$ (t-test).

e: Between 43 and 77 dph, the songs in NCM-lesioned birds typically change (arrows) in the direction of higher similarity with their tutors' songs (horizontal) rather than with songs in unfamiliar birds (vertical).

Results & Discussion

Birds were normally raised with their parents until about 40 days post hatch (dph) after which they were housed individually with no further exposure to tutor song. Hence, we make use of the fact that song learning is based on a memory of the father's song which had already been acquired before 40 dph. We investigate the role of NCM in recalling this memory to guide song learning during the sensorimotor period of birdsong development. If NCM stores the tutor song memory used as a template for learning, then the developed songs of NCM-lesioned birds should be impaired compared to the developed songs in control animals.

At 42 dph the juvenile birds received large bilateral lesions in NCM. Before the lesions, juveniles produced highly variable vocalizations (mean self-similarity score 38.0±6.7, range 26.1 to 41.7, N=5 birds) with little resemblance to the tutor song (mean similarity score 33.0±6.4, range 24.0 to 39.8, N=5 birds). At the end of the experiments, birds (76-78 dph) produced highly stereotyped song motifs (mean self-similarity score 63.7±10.0, range 46.7 to 72.4, N=5 birds) comparable to song stereotypy in control birds that had not received any treatment (68.0±3.5, range 63.6 to 72.8, N=5, p=0.34, t-test, Figure 1c). Most importantly, motifs of lesioned birds were good copies of their tutors' motifs: The mean similarity score in NCM-lesioned birds at 75 dph was 43.0±8.8 (range 31.1 to 53.2, N=5 birds) comparable to the mean similarity score in age-matched controls (48.1±11.0, range 36.2 to 65.6, N=5 birds, p=0.44, t-test, example in Figure 1b, summary in Figure 1c). None of the individual similarity measures (% similarity, sequential match, and accuracy) significantly discriminated by itself between lesioned and non-lesioned birds (p=0.17, 0.64 and 0.86, t-test, Figure 1d).

We tested whether the observed increase in motif similarity between NCM-lesioned birds and their tutors could be explained simply by pupils' songs becoming more similar to wild-type zebra finch song rather than specifically to the tutors' songs. We calculated similarity scores between pupils' motifs and those of 14 unfamiliar adult birds, both at the beginning and at the end of the experiment. At 43 dph, the average similarity scores with unfamiliar songs was 27.81±3.86 (range 22.79 to 31.76, N=5 birds), and at the end of the experiments (76-78 dph) the average score was similarly low, 27.62±1.40 (range 26.15 to 29.02, N=5 birds, p=0.92, t-test, Figure 1e), significantly lower than the average similarity score with tutor song (p=0.002, t-test). In comparison, the similarity between the controls' songs and the songs of unfamiliar adults was also significantly lower (29.2±3.0, range 26.1 to 34.2, N=5 birds) than the similarity between the controls' songs and tutor songs (Figure 1c, p=0.006, t-test). This persistence of low similarity scores with unfamiliar songs demonstrates that songs in NCM-lesioned birds developed specifically towards their tutors' songs rather than nonspecifically towards generic zebra finch songs. In summary, despite the very large lesions made, birds successfully developed a good copy of their tutors' songs.

Conclusions

The observed post-lesion convergence of pupils' songs towards their tutors' songs reveals that NCM is necessary neither for retrieving a tutor song memory nor for long-term storage of that memory. Additionally, successful song learning in NCM-lesioned birds indicates that NCM is not necessary for any of the sensorimotor functions required for song learning. Given the reported involvement of NCM in tutor song memorization **(London 2008[13])**, we conclude that NCM's role in song memorization is restricted to the process of memory acquisition, after which the memory may be consolidated elsewhere in the song control system. At the very least, there must exist another song memory outside of NCM which birds can use to shape their developing songs.

Limitations

In **(Canopoli 2014[12])** it was reported that the apparent lesion size visible after Nissl staining shrinks with time. We found a similar effect of the visible lesions at 75 dph (Figure 1a) being much smaller than the injected acid volumes, which limited our ability to verify the extent of the initial lesion.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201603000018.

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Ethics Statement

All the experimental procedures were in accordance with the Veterinary Office of the Canton of Zurich.

Citations

[1] Victoria Fromkin et al. "The development of language in genie: a case of language acquisition beyond the "critical period"". In: *Brain and Language* 1.1 (Jan. 1974), pp. 81–107. DOI: 10.1016/0093-934x(74)90027-3. URL: http://dx.doi.org/10.1016/0093-934x(74)90027-3.

[2] Patricia K. Kuhl. "Brain Mechanisms in Early Language Acquisition". In: *Neuron* 67.5 (Sept. 2010), pp. 713–727. DOI: 10.1016/j.neuron.2010.08.038. URL: http://dx.doi.org/10.1016/j.neuron.2010.08.038.

[3] Immelmann K. "Song development in the zebra finch and other estrildid finches". In: *Bird vocalizations* (1969), pp. 61–74.

[4] Böhner J. "Early acquisition of song in the zebra finch, Taeniopygia guttata". In: *Animal Behaviour* 39 (1990), pp. 369–374.

[5] Funabiki Y and Konishi M. "Long memory in song learning by zebra finches". In: *The Journal of Neuroscience* 23 (2003), pp. 6928–35.

[6] Mello C, Nottebohm F, and Clayton D. "Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon". In: *The Journal of neuroscience : the official journal of the Society for Neuroscience* 15 (1995), pp. 6919–25.

[7] Johan J. Bolhuis et al. "Localized immediate early gene expression related to the strength of song learning in socially reared zebra finches". In: *European Journal of Neuroscience* 13.11 (June 2001), pp. 2165–2170. DOI: 10.1046/j.0953-816x.2001.01588.x. URL: http://dx.doi.org/10.1046/j.0953-816x.2001.01588.x.

[8] J. J. Bolhuis et al. "Localized neuronal activation in the zebra finch brain is related to the strength of song learning". In: *Proceedings of the National Academy of Sciences* 97.5 (Feb. 2000), pp. 2282–2285. DOI: 10.1073/pnas.030539097. URL: http://dx.doi.org/10.1073/pnas.030539097.

[9] S. J. Chew et al. "Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain." In: *Proceedings of the National Academy of Sciences* 92.8 (Apr. 1995), pp. 3406–3410. DOI: 10.1073/pnas.92.8.3406. URL: http://dx.doi.org/10.1073/pnas.92.8.3406.

[10] V. C. Miller-Sims and S. W. Bottjer. "Development of neural responsivity to vocal sounds in higher level auditory cortex of songbirds". In: *Journal of Neurophysiology* 112.1 (Apr. 2014), pp. 81–94. DOI: 10.1152/jn.00484.2013. URL: http://dx.doi.org/10.1152/jn.00484.2013.

[11] Sharon M.H. Gobes and Johan J. Bolhuis. "Birdsong Memory: A Neural Dissociation between Song Recognition and Production". In: *Current Biology* 17.9 (May 2007), pp. 789–793. DOI: 10.1016/j.cub.2007.03.059. URL: http://dx.doi.org/10.1016/j.cub.2007.03.059.

[12] A. Canopoli, J. A. Herbst, and R. H. R. Hahnloser. "A Higher Sensory Brain Region Is Involved in Reversing Reinforcement-Induced Vocal Changes in a Songbird". In: *Journal of Neuroscience* 34.20 (May 2014), pp. 7018–7026. DOI: 10.1523/jneurosci.0266-14.2014. URL: http://dx.doi.org/10.1523/jneurosci.0266-14.2014.

[13] Sarah E London and David F Clayton. "Functional identification of sensory mechanisms required for developmental song learning". In: *Nature Neuroscience* 11.5 (Apr. 2008), pp. 579–586. DOI: 10.1038/nn.2103. URL: http://dx.doi.org/10.1038/nn.2103.

[14] Oswald Steward et al. "Synaptic Activation Causes the mRNA for the IEG Arc to Localize Selectively near Activated Postsynaptic Sites on Dendrites". In: *Neuron* 21.4 (Oct. 1998), pp. 741–751. DOI: 10.1016/s0896-6273(00)80591-7. URL: http://dx.doi.org/10.1016/s0896-6273(00)80591-7.

[15] Oswald Steward and Paul F Worley. "Selective Targeting of Newly Synthesized Arc mRNA to Active Synapses Requires NMDA Receptor Activation". In: *Neuron* 30.1 (Apr. 2001), pp. 227–240. DOI: 10.1016/s0896-6273(01)00275-6. URL: http://dx.doi.org/10.1016/s0896-6273(01)00275-6.

[16] Erica Korb and Steven Finkbeiner. "Arc in synaptic plasticity: from gene to behavior". In: *Trends in Neurosciences* 34.11 (Nov. 2011), pp. 591–598. DOI: 10.1016/j.tins.2011.08.007. URL: http://dx.doi.org/10.1016/j.tins.2011.08.007.

[17] Ofer Tchernichovski et al. "A procedure for an automated measurement of song similarity". In: *Animal Behaviour* 59.6 (June 2000), pp. 1167–1176. DOI: 10.1006/anbe.1999.1416. URL: http://dx.doi.org/10.1006/anbe.1999.1416.

[18] M. L. Phan, C. L. Pytte, and D. S. Vicario. "Early auditory experience generates long-lasting memories that may subserve vocal learning in songbirds". In: *Proceedings of the National Academy of Sciences* 103.4 (Jan. 2006), pp. 1088–1093. DOI: 10.1073/pnas.0510136103. URL: http://dx.doi.org/10.1073/pnas.0510136103.

[19] Todd F Roberts et al. "Motor circuits are required to encode a sensory model for imitative learning". In: *Nature Neuroscience* 15.10 (Sept. 2012), pp. 1454–1459. DOI: `10.1038/nn.3206`. URL: `http://dx.doi.org/10.1038/nn.3206`.

[20] Falk Dittrich et al. "Maximized song learning of juvenile male zebra finches following BDNF expression in the HVC". In: *European Journal of Neuroscience* 38.9 (Aug. 2013), pp. 3338–3344. DOI: `10.1111/ejn.12329`. URL: `http://dx.doi.org/10.1111/ejn.12329`.