Optogenetic manipulation of interneurons in the premotor nucleus HVC alters song in zebra finches

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Premotor neurons (PN) in HVC burst during specific time points throughout song production of adult zebra finches[1]. Inhibitory HVC interneurons (IN) on the other hand exhibit elevated spiking activity with silent gaps during song production[2]. This interplay between excitation and inhibition leads to the hypothesis that INs stabilise learnt song in adult birds[2]. In order to test this, we virally transduced GABAergic IN in HVC of adult zebra finches[4] and stimulated them optogenetically during song production.

Interplay between excitation and inhibition in HVC of singing zebra finches

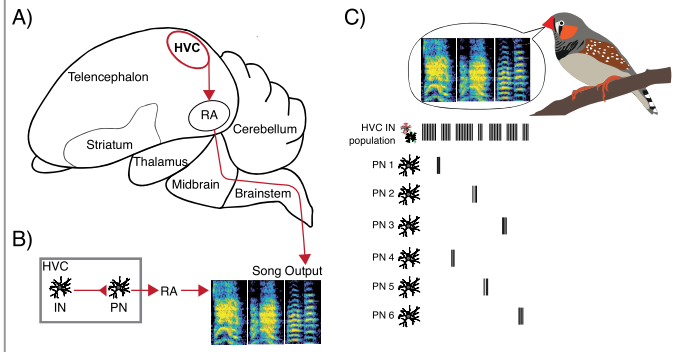

Figure 1: Simplified model of a zebra finch brain (A). HVC INs inhibitit PNs projecting to RA which ultimately generate song timing (B). During singing, INs leave silent gaps to allow PN bursting only during production of specific elements (C).

Optogenetic manipulation of stereotyped song patterns

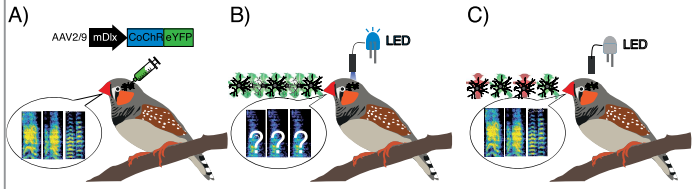

Figure 2: Viral transduction of HVC INs in adult zebra finches (A). Optogenetic stimulation of HVC INs during song production leads to persistent IN activity and altered song properties (B). During controls where no light stimulation occurs, INs return to active or silent periods and normal song production is reestablished (C)

Optogenetic stimulation of INs shortens song bouts

Optogenetic manipulation of INs in HVC modifies song parameters in highly stereotyped song of adult zebra finches. Future experiments with localised and precise depolarisation of INs during elements of premature song will help us to determine time-locked effects of INs on song learning in zebra finches. Additionally, we will hyperpolarise INs in adult birds to lift inhibition and reintroduce song plasticity.

Extracellular recordings during local light stimulation in HVC of anaesthetised birds reveals time-locked multi-unit and single cell excitation or suppression.

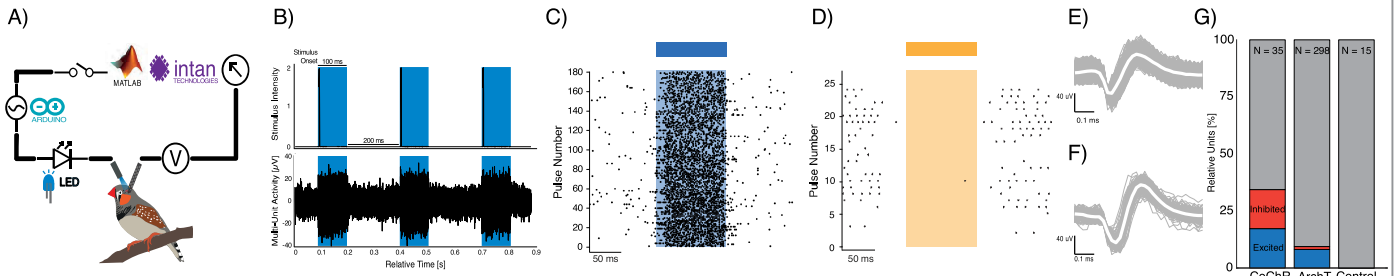

Figure 4: Multi-unit activity in HVC of anaesthetised birds during local light stimulation (A). Single channel activity reveals precise and time locked increase of spiking activity during light stimulation (B). Single units in HVC of anaesthetised birds transduced with depolarising CoChR (C) or hyperpolarising ArchT (D) and their waveforms (E, F). Excited, inhibited or unaltered units across multiple birds and hemispheres for CoChR and ArchT (G).

Altered song parameters in birds transduced with depolarising Channelproteins

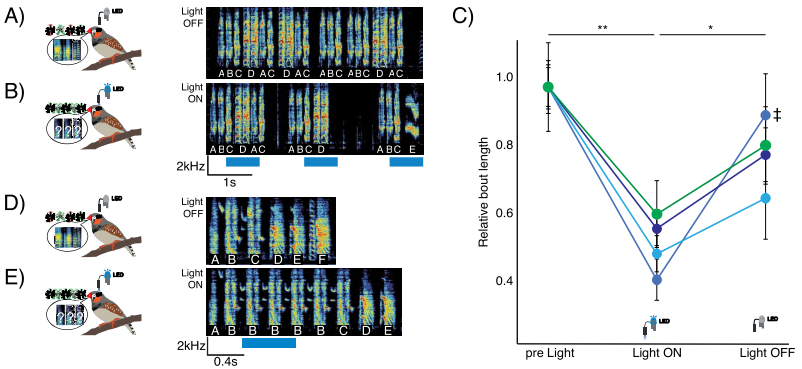

Figure 5: Depolarisation of inhibitory INs in HVC leads to faster bout ends by increased gap duration between motifs (A, B). Bout length recovers after light stimulation in birds transduced with CoChR (blue) or CsChRimson (green, C). Stuttering occured in one bird during light stimulation (D,E).

Interpreting partially modified song production during optogenetic stimulation of HVC INs

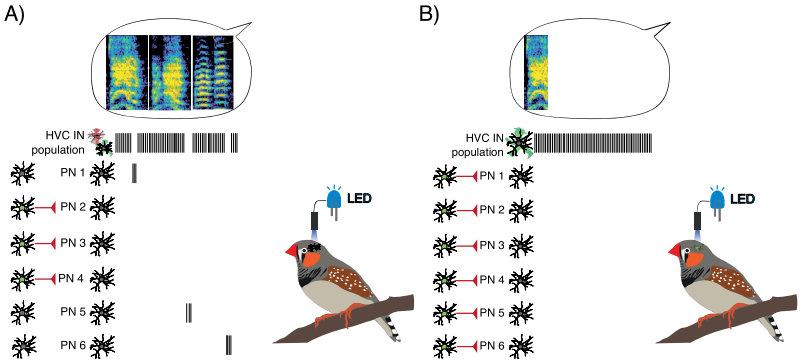

Figure 7: Scheme of optogenetically altered IN activity resulting in altered song properites. Optogenetic IN excitation during song production abolishes silent gaps in IN activity that would enable PN bursts during specific time points of song. This leads to an interrupted sequence of PN activity and pauses between motifs (A) if not all INs are transduced and assuming a systemic redundancy. Abrupt breaks during a motif might be achieved by complete transduction of HVC INs and unlimited light protrusion (B).

Immunohistochemistry of recording sites and HVC of transduced birds

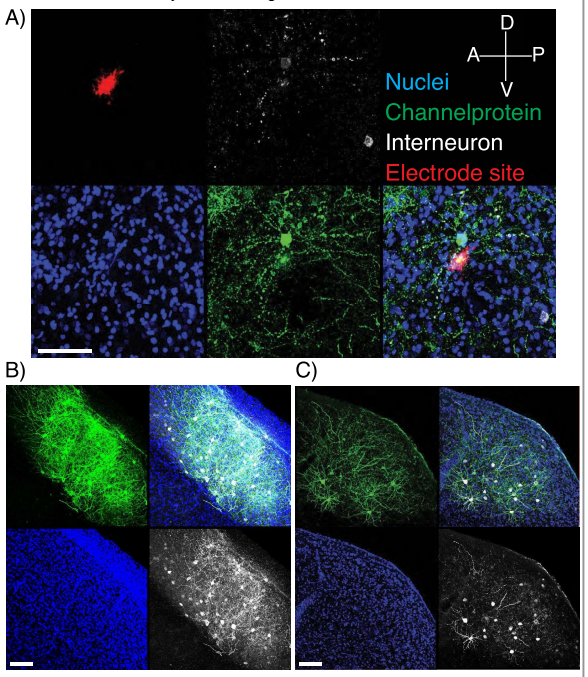

Figure 6: Immunohistochemical stainings of HVC slices of virally transduced birds. DiI staining (red) indicates recording site in HVC next to a transduced IN expressing ArchT (A). Green CoChR expression in left HVC of a transduced bird (B), corresponding behaviour is shown in Fig. 5C (‡). Green ArchT expression in right HVC of another adult bird (C). Scale bars: 50 μm.

References:

[1]Hahnloser *et al.* 2002 Nature

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[3]Vallentin *et al.* 2016 Science

[4]Dimidschstein *et al.* 2016 Nat Neurosci

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