

# Investigating neural mechanisms of intrinsic motivation for non-courtship singing in zebra finches.

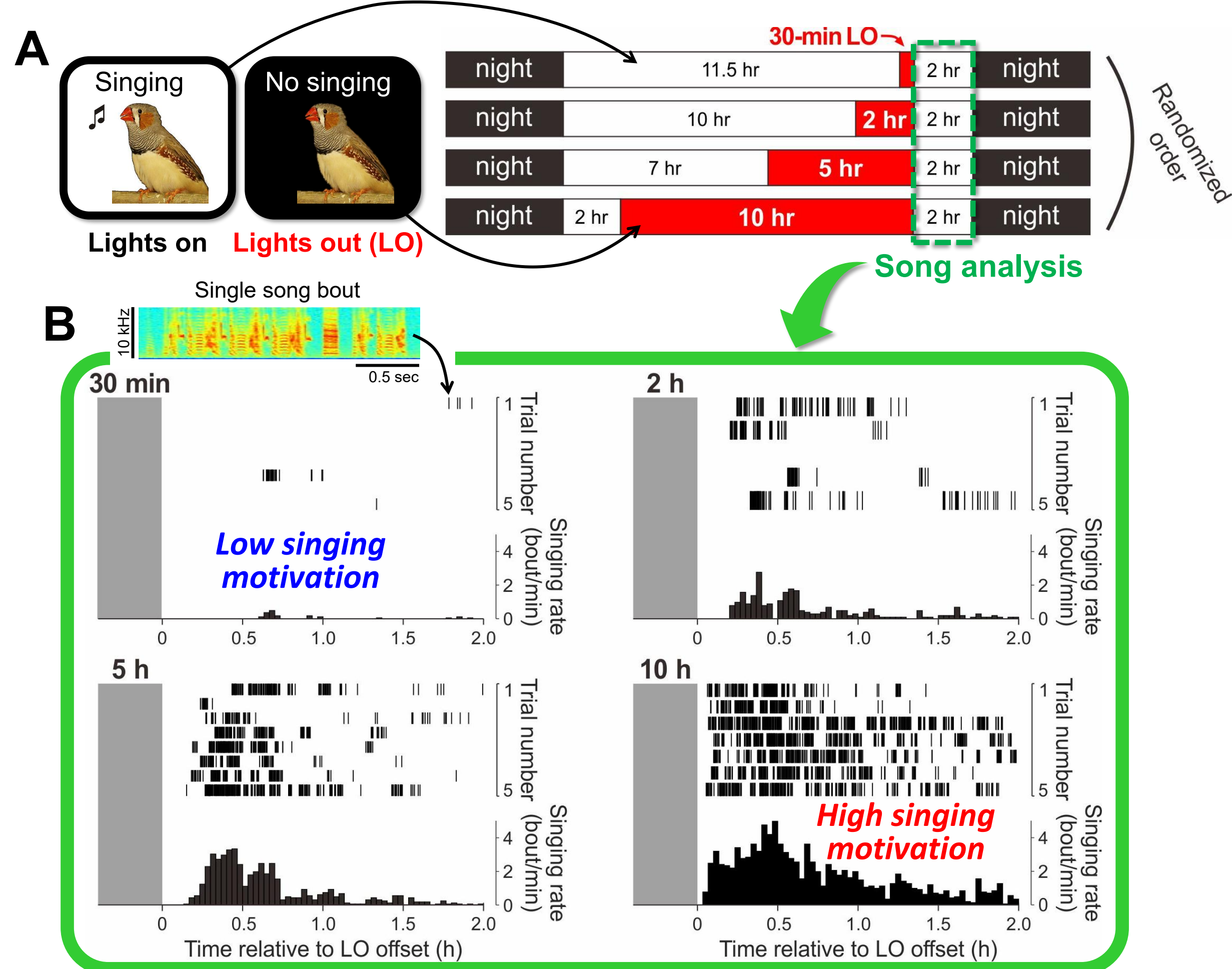
Ok-Yi Jeong<sup>1,2</sup>, Chihiro Mori<sup>3</sup>, Yunbok Kim<sup>1</sup>, Satoshi Kojima<sup>1</sup>

<sup>1</sup>Sensory&Motor Systems Research Group, Korea Brain Research Institute (KBRI), <sup>2</sup>Department of Brain Science, Daegu Gyeongbuk Institute of Science and Technology (DGIST), <sup>3</sup>Department of Molecular Biology, Faculty of Pharmaceutical Sciences

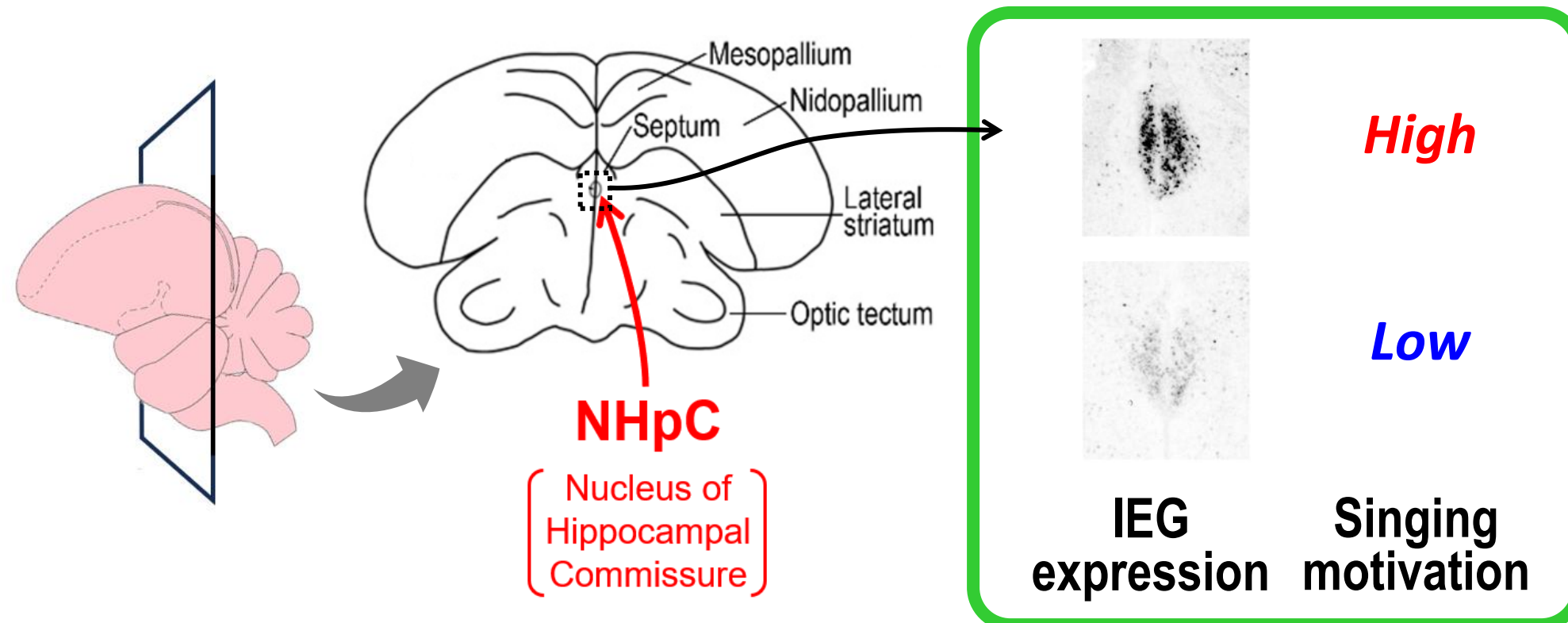


## Introduction

- Intrinsic motivation drives various voluntary behaviors independently of external stimuli such as rewards or punishments, and plays a critical role in the development and maintenance of physical, cognitive, and social functions (Ryan and Deci, 2000).
- Zebra finches (songbirds) offer a unique opportunity to study the neural substrates of intrinsic motivation, because they spontaneously produce many renditions of non-courtship songs for vocal practice (undirected singing, US), even in the absence of apparent recipients.

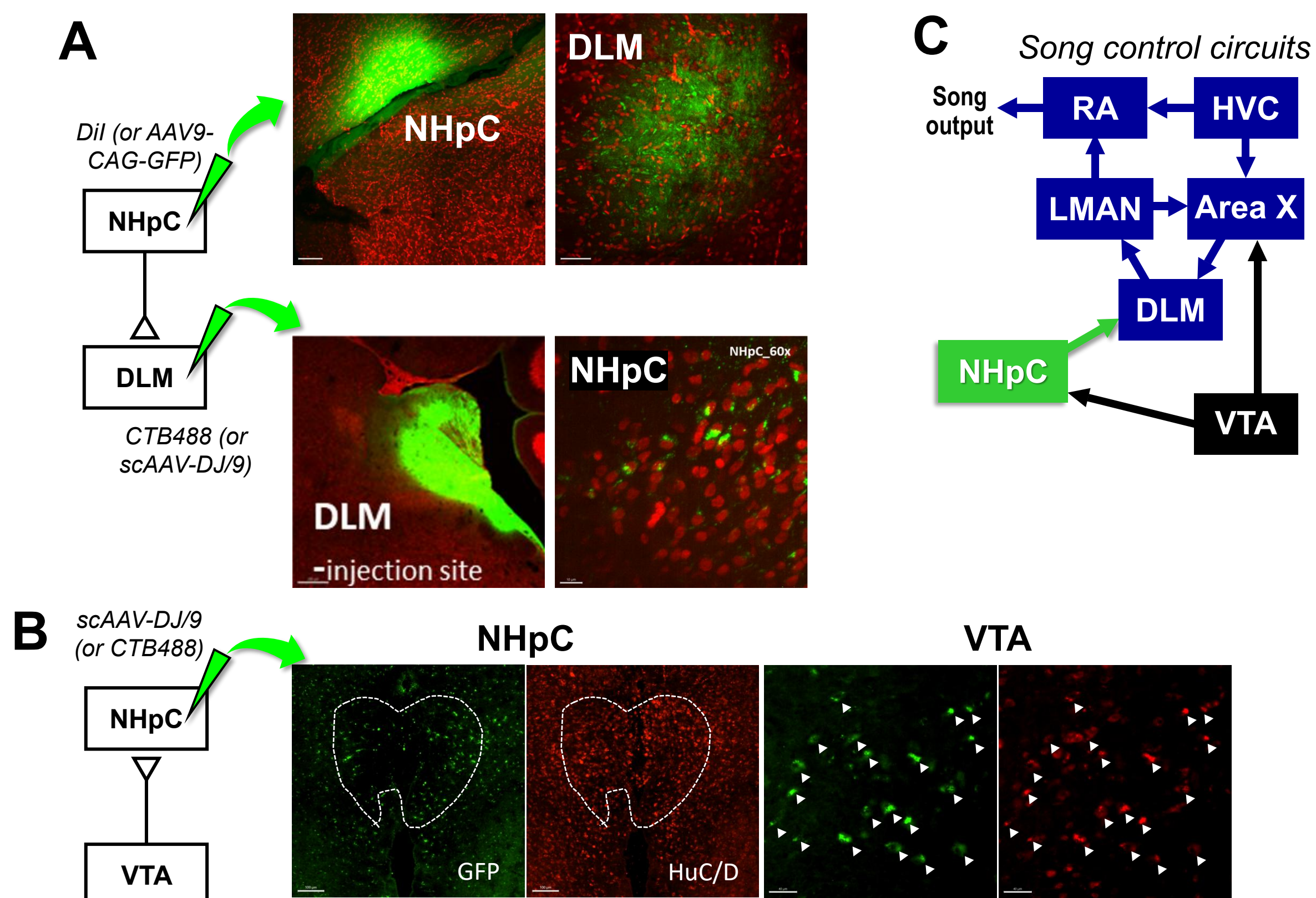


**Intrinsic motivation for undirected singing (US) is enhanced by long-term US suppression.** (A) Daily schedule of lights-out (LO) periods with four different durations. (B) Raster plots of song bouts produced after LO periods with variable durations (top) and corresponding singing rate histograms (bottom) in a representative bird. Adapted from Kim et al. 2021, *Sci. Rep.*



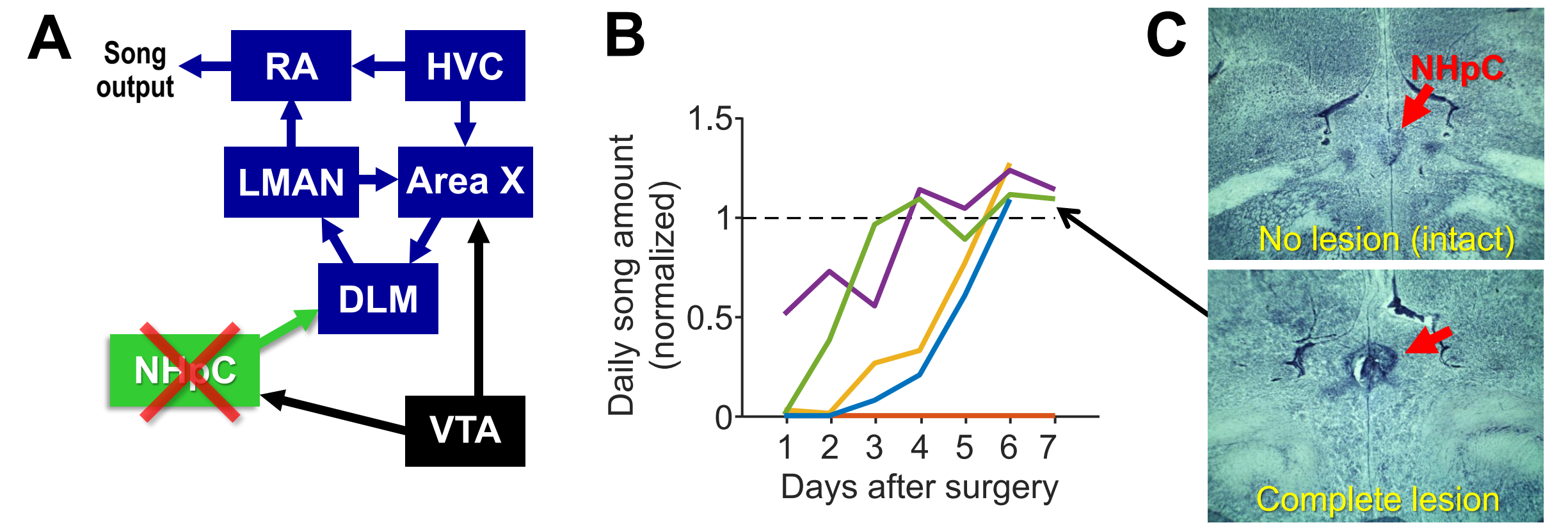
**A septal area NHpC is activated in parallel with intrinsic US motivation.** Birds with relatively high singing motivation exhibit increased expression levels of an immediate early gene (IEG) Arc in a septal area, the nucleus of the hippocampal commissure (NHpC). Adapted from Mori et al. 2025, *Sci. Rep.*

## Results 1. Neuroanatomical tracing of NHpC connectivity.



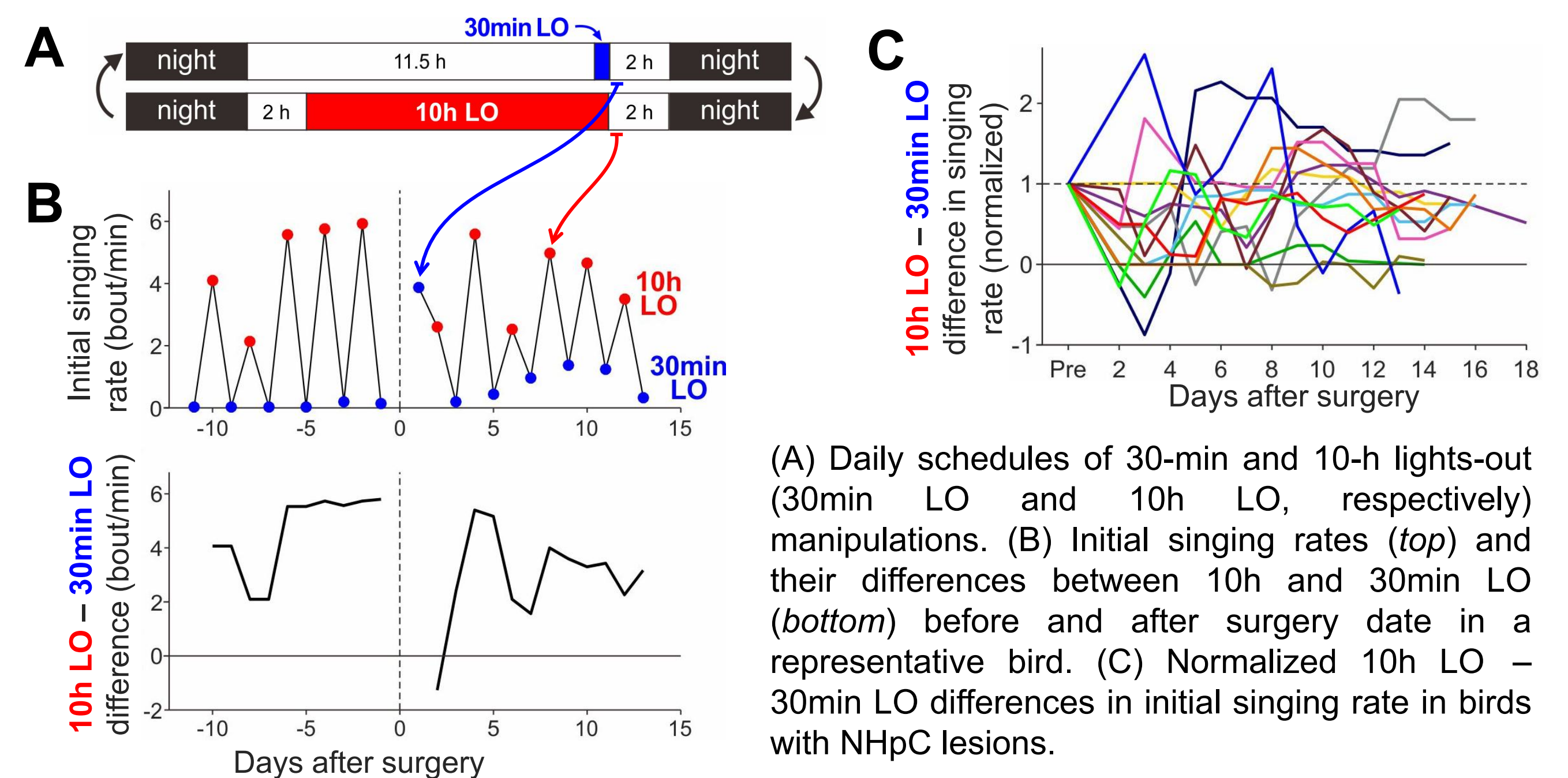
(A) Anterograde (top) and retrograde (bottom) tracing of NHpC-to-DLM projections. For anterograde tracing, Dil particle (green) was implanted in NHpC, and fluorescent labeled fibers were observed in DLM. For retrograde tracing, CTB488 (green) was injected into DLM, and fluorescent labeled neurons were observed in NHpC. Red, DAPI staining. (B) Retrograde tracing of VTA-to-NHpC projections. A retrograde virus was injected into NHpC. GFP-labeled neurons were observed in VTA. (C) Schematic diagram of major song control brain areas and the neural connections of NHpC revealed by our study.

## Results 2. Lesions of NHpC do not block US production.



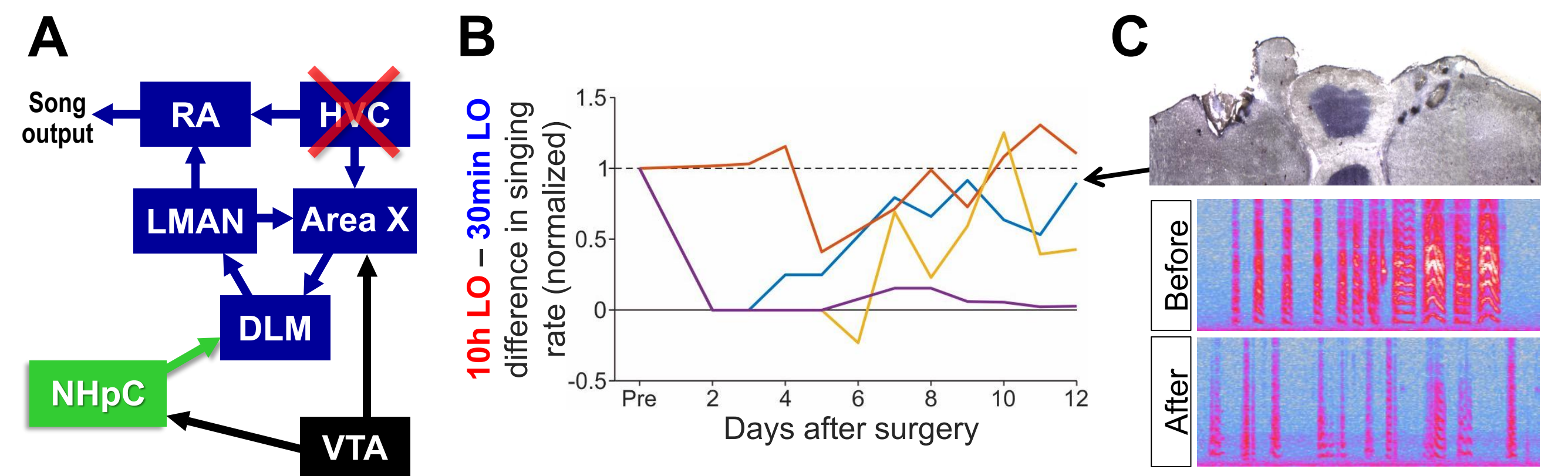
(A) Schematic diagram showing NHpC lesions. (B) Daily song amount (# of bout/day) after NHpC lesion surgery, normalized to that before surgery. Lines indicate individual birds. (C) Example brain sections with no (top) and almost complete (bottom) lesions in NHpC.

## Results 3. Lesions of NHpC do not block the enhancement of US motivation.



(A) Daily schedules of 30-min and 10-h lights-out (LO) manipulations. (B) Initial singing rates (top) and their differences between 10h and 30min LO (bottom) before and after surgery date in a representative bird. (C) Normalized 10h LO - 30min LO differences in initial singing rate in birds with NHpC lesions.

## Results 4. Birds exhibit the enhancement of intrinsic motivation for HVC-independent (AFP-driven) song.



(A) Schematic diagram showing HVC lesions. (B) Normalized 10h LO - 30min LO differences in singing rate in birds with HVC lesions. (C) Example coronal brain section showing HVC lesions (top) and spectrograms of US motifs before and after HVC lesions (bottom) in a representative bird.

## Summary and future plans

### Summary

- A septal area NHpC is activated when intrinsic US motivation is enhanced by long-term US suppression.
- NHpC receives neural projections from VTA and send projections to DLM (preliminary data).
- NHpC is not required for US production or the enhancement of intrinsic US motivation.
- Intrinsic motivation for HVC-independent (AFP-driven) song is enhanced by long-term US suppression.

### Future plans

- Examine whether lesions of both HVC and NHpC impair US production or US motivation enhancement.
- Record and manipulate neural activity in NHpC to further examine its functional roles in US motivation.
- Search for other brain areas that directly regulate intrinsic motivation for HVC-dependent songs.

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