The role of basal forebrain cholinergic and GABAergic neurons in normal and pathological aging

NKFIH KH 125294

Final report

Introduction

Axons of the basal forebrain (BF) project to the entire cerebral cortex and several subcortical areas, releasing acetylcholine, GABA, and glutamate neurotransmitters in target areas. Integrity of the BF is necessary for normal cognitive functions and the destruction of BF cholinergic neurons (BFCNs) leads to cognitive impairment. In the course of the reported project, we examined cholinergic, GABAergic, and glutamatergic cells of the basal forebrain in young, elderly, and Alzheimer's disease model mice.

We performed measurements at three different age groups: 2-6 months, 12-14 months and 18+ months. We published multiple studies from the experiments in young adult mice. Since obtaining aged mice through breeding took the larger part of the two-year grant period, our results from aged animals took shape only recently, and these will be foreseeably published in the near future. However, our results from both young and aged (wild type or 3xTg-AD model) mice are presented below.

Results

<u>1. Distinct synchronization, cortical coupling and behavioral function of two basal forebrain cholinergic neuron types</u>

Our measurements in young adult animals revealed that cholinergic cells could be classified into two basic types: burst-firing cells showed synchronous activity with each other and the cerebral cortex and strongly activated the sensory cerebral cortex, while regular cells showed asynchronous firing. Surprisingly, both cell types were able to generate the phasic responses characteristic of cholinergic cells in mice after reward or punishment (Figure 1). These results were published in Nature Neuroscience: Laszlovszky T, Schlingloff D, Hegedüs P, Freund TF, Gulyás A, Kepecs A, Ant B (2020) Distinct synchronization, cortical coupling and behavioral function of two basal forebrain cholinergic neuron types. Nat Neurosci, 23: 992-1003.

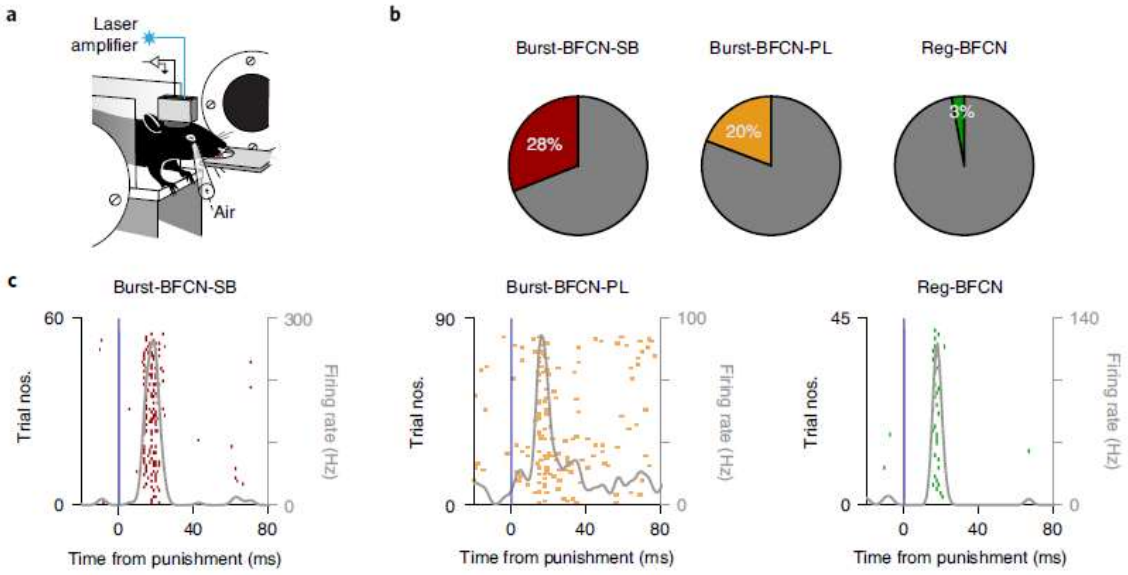

Figure 1. Two types of cholinergic neurons respond phasically to punishment. a, Head-fixed mice were trained on an auditory associative learning paradigm. b, Proportion of bursts in strongly bursting (SB), Poisson-like bursting (PL) and regular rhythmic (Reg) BFCNs.

2. Open Source Solution for Real-Time Peri-Event Time Histogram Based on Open Ephys

In our experiments, the activity of individual BF cells was recorded using movable tetrode electrodes implanted in the basal forebrain. We examined ChAT-Cre and PV-Cre mice injected with AAV-channelrhodopsin, in which cholinergic and PV-containing GABAergic neurons were photosensitive. For the identification of different cell types in extracellular recordings, the so-called optogenetic tagging method proved to be less efficient than expected, so we developed a new software that allows us to 'hunt' photosensitive neurons in real time while performing the experiment. This method was reported in Frontiers in Neuroinformatics: Széll A, Martínez-Bellver S, Hegedüs P, Hangya B (2020) OPETH: Open Source Solution for Real-time Peri-event Time Histogram Based on Open Ephys. Front Neuroinform, 14:21. Our software increased the efficiency of optogenetic tagging, as summarized in Figure 2.

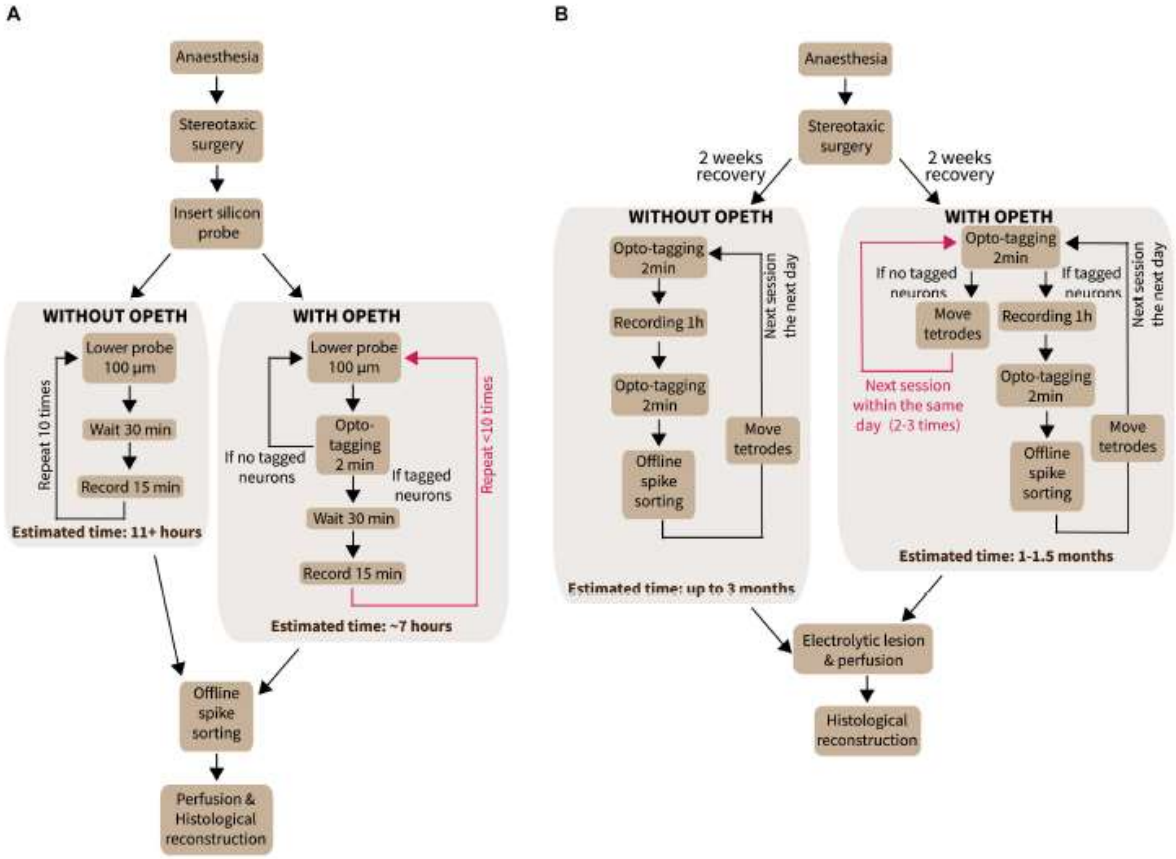

Figure 2. This figure summarizes the temporal gains of using the OPETH software in acute (A) and chronic (B) recording experiments.

3. In Vivo Localization of Chronically Implanted Electrodes and Optic Fibers in Mice

Accurate localization of brain implants was previously only possible after the experiment, using histological procedures. We have developed, in collaboration with Semmelweis University, a CT and MRI-

based technique (Figure 3) that allows implanted devices to be accurately localized immediately after surgery, thereby making experimentation faster and more efficient. This technique was published in Nature Communications: Király B, Balázsfi D, Horváth I, Solari N, Sviatkó K, Lengyel K, Birtalan E, Babos M, Bagaméry G, Máthé D, Szigeti K, Hangya B (2020) In Vivo Localization of Chronically Implanted Electrodes and Optic Fibers in Mice. Nat Comm, 11: 4686.

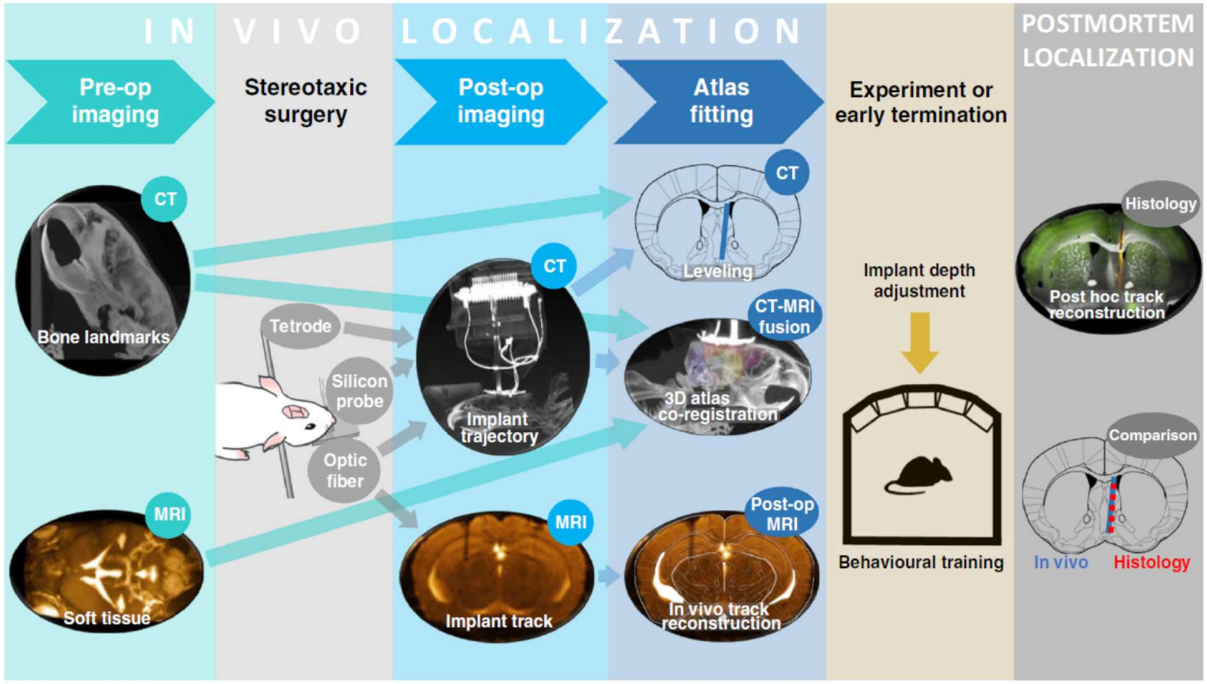

Figure 3. Workflow of in vivo implant localization based on CT-MRI fusion images.

4. Efficient training of mice on the 5-choice serial reaction time task in an automated rodent training system

Standardized behavioral protocols are needed to compare subtle behavioral variables in young and old animals. We have automated behavioral experiments in our lab so that mice can learn efficiently and in a stress-free environment without any human intervention. This method was published in Scientific Reports: Birtalan E, Bánhidi A, Sanders JI, Balázsfi D, Hangya B. (2020) Efficient training of mice on the 5-choice serial reaction time task in an automated rodent training system. Sci Rep, 10: 22362.

5. Huygens synchronization of medial septal pacemaker neurons generates hippocampal theta oscillation

We studied BF GABAergic cells and found that PV-containing GABAergic cells are important for the synchronization of the BF medial septum subregion through a specific frequency synchronization mechanism (Huygens synchronization; Figure 4). This synchronization is thought to result in the appearance of hippocampal theta oscillations, important for learning. In addition to PV-containing cells, other GABAergic cells were identified using VGAT-Cre transgenic mice. These results were published on the bioRxiv preprint server: Barnabás Kocsis, Sergio Martínez-Bellver, Richárd Fiáth, Andor Domonkos, Katalin Sviatkó, Péter Barthó, Tamás F. Freund, István Ulbert, Szabolcs Káli, Viktor Varga, Balázs Hangya (2021) Huygens synchronization of medial septal pacemaker neurons generates hippocampal theta

oscillation, bioRxiv 2021.01.22.427736; doi: https://doi.org/10.1101/2021.01.22.427736. We are in the process of publishing this paper in a peer-reviewed journal.

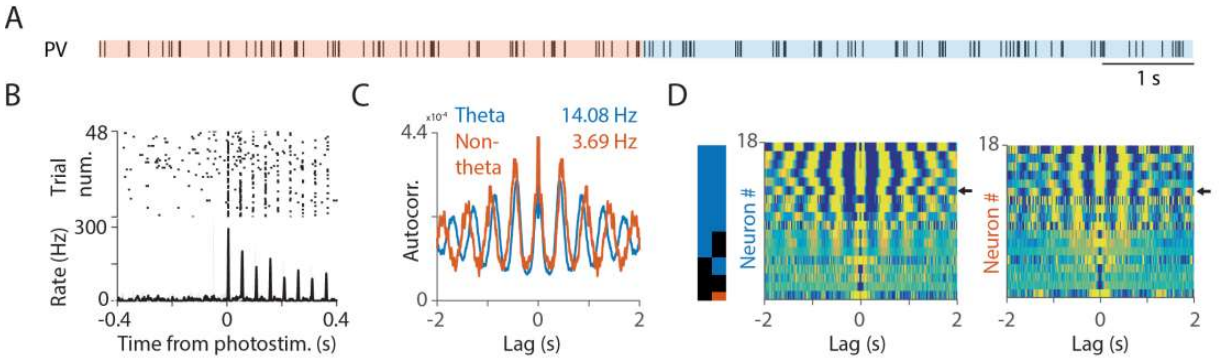

Figure 4. Optogenetically identified PV-expressing medial septal neurons show constitutive theta-bursting firing pattern. A, Raw spike raster. B, Raster plot and peri-stimulus time histogram aligned to photostimulation demonstrated optogenetic tagging. C, Autocorrelogram of an example PV-expressing neuron during hippocampal theta (blue) and non-theta state (orange). D, Color-coded autocorrelograms of n = 18 PV-expressing medial septal neurons during theta (left) and non-theta (right).

6. Cholinergic modulation of spatial learning, memory and navigation

Cholinergic cells in the basal forebrain play an important role in the regulation of spatial navigation and working memory. A review article on this topic was published in the European Journal of Neuroscience: Solari N, Hangya B (2018) Cholinergic modulation of spatial learning, memory and navigation. Eur J Neurosci, 48: 2199-2230.

7. Aging effects on the cholinergic basal forebrain

The study of elderly and Alzheimer's disease mice was hampered by several animal house infections as well as variability in phenotypic expression in the 3xTg-AD mouse line and derived crosses. Despite these, we managed to study cholinergic cells by optogenetic tagging in aged ChAT-Cre mice and by fiber photometry in both aged and Alzheimer's model mice. We have found that cholinergic responses to reward-predicting stimuli are drastically reduced during both healthy and pathological aging. Young animals (3-6 months of age) showed a cholinergic basal forebrain response after reward-predicting cues and feedback presentations (Figure 5A-C). We repeated the experiment in older animals (12-15 and 18-21 months of age) and observed that natural aging causes the BF reward-predicting response to decrease or even disappear (Figure 6A-D).

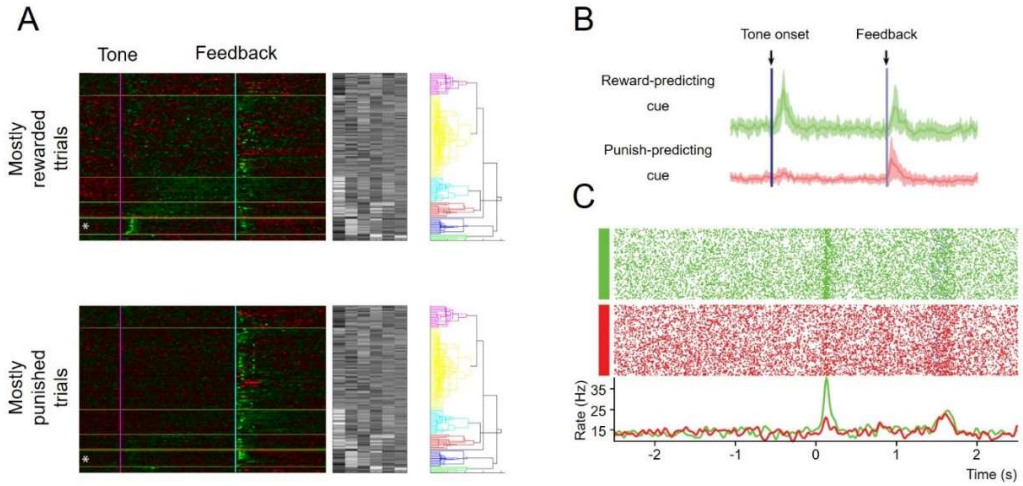

Figure 5. Young animals show cholinergic reward-prediction representation in the basal forebrain. A, Cluster analysis of over 500 individual neurons recorded from the BF of young animals. Green colors represent increased activity while red colors show activity suppression. Asterisk marks the cluster containing optogenetically identified cholinergic neurons. B, Average response of the BF cholinergic cluster in young (3-6 months) mice. C, Representative example of a cholinergic neuron of a young mouse; spike times were aligned to reward-predicting (green) and punishment-predicting (red) auditory cues (t=0).

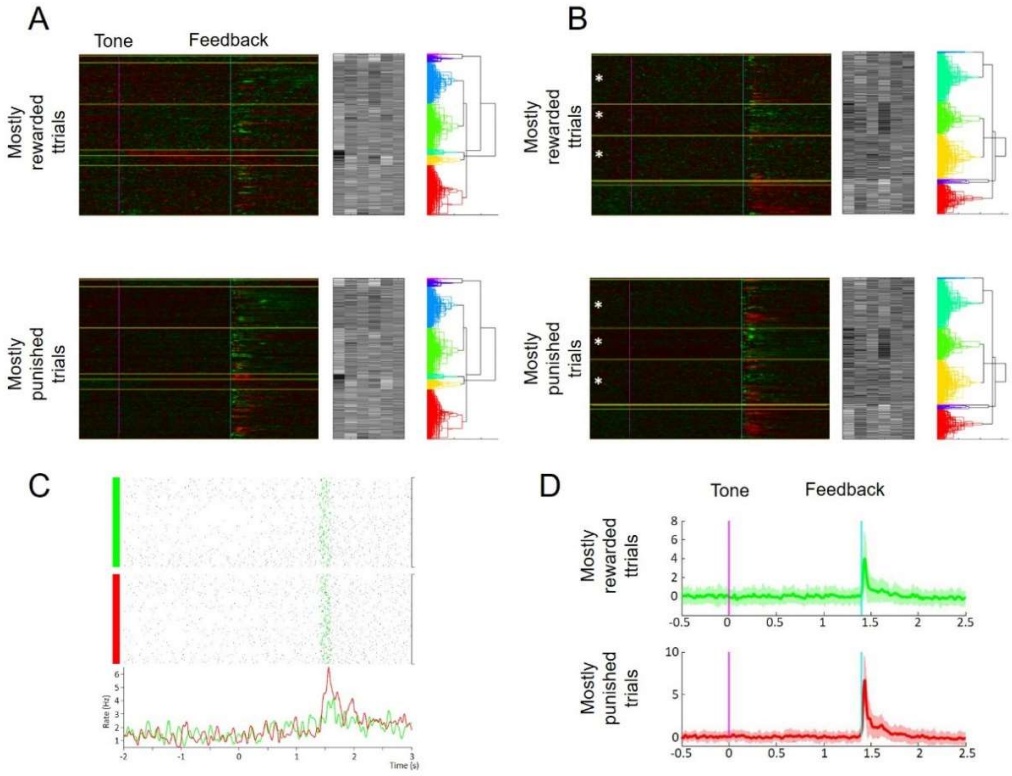

Figure 6. Reward-prediction responses fade with age. A, Cluster analysis of over 500 BF cells of aged animals (12-15 months of age) showing the lack of cue responses (after purple line). B, Cluster analysis of over 500 BF cells of elder animals (18-21 months of age) showing the lack of cue responses. Asterisks show clusters containing identified cholinergic neurons. C, Example neuronal response of a cholinergic cell (18-21 months group) aligned to cue onset (t=0). D, Average response of the cluster that contained most cholinergic units (18-21 months group).

Optogenetic tagging results were in good agreement with fiber photometry measurements of calcium responses from BF cholinergic cells and acetylcholine sensor-based fiber photometry measuring acetylcholine release in the amygdala and cerebral cortex. As observed with the cholinergic BF recordings, the release of acetylcholine in main cholinergic BF projection areas, basolateral amygdala and prefrontal cortex, showed a reward-predicting response in young animals (Figure 7A). Both natural and pathological aging caused the cue-related cholinergic responses to fade (Figure 7B-C), whereas feedback-related responses were not strongly affected.

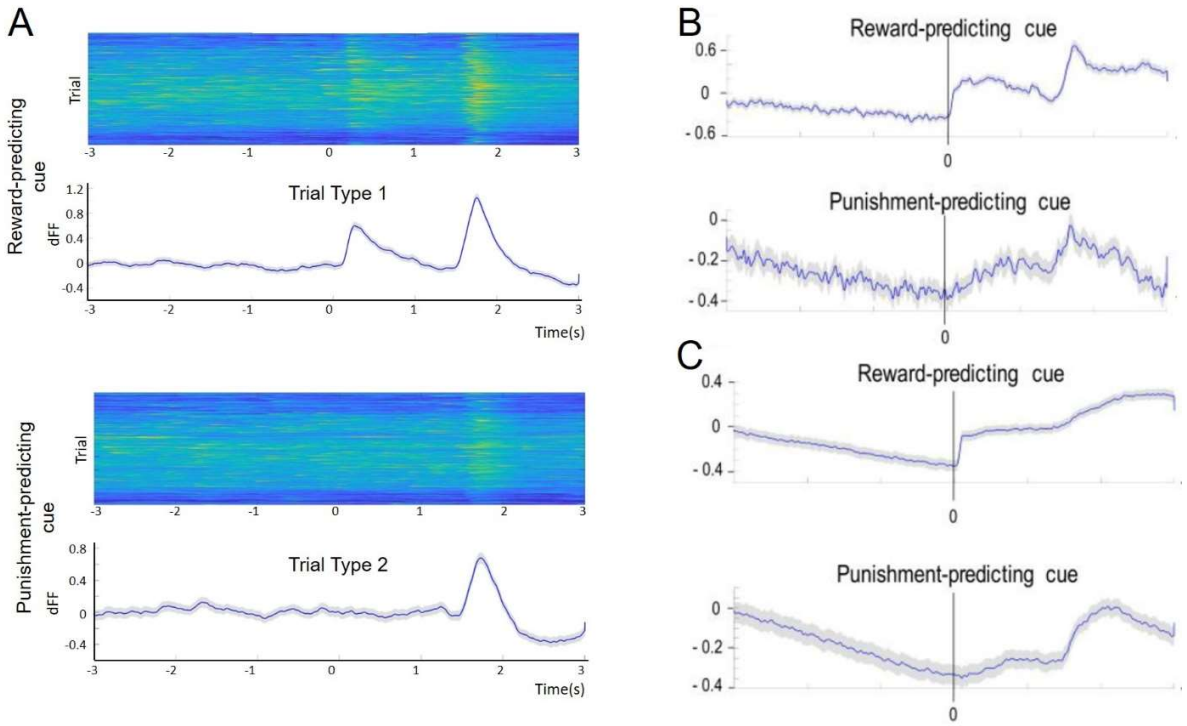

Figure 7. Acetylcholine is released in response to reward-predicting stimuli in the amygdala and prefrontal cortex. A, Fiber photometry measurements of acetylcholine release in the amygdala of a young mouse. An increase in release was observed after reward-predicting cues and feedback. B and C, Averaged amygdala (B) and prefrontal cortex (C) acetylcholine levels of an aged (12-15 months group) mouse. The cholinergic response to the reward-predicting cue is smaller than in young mice.

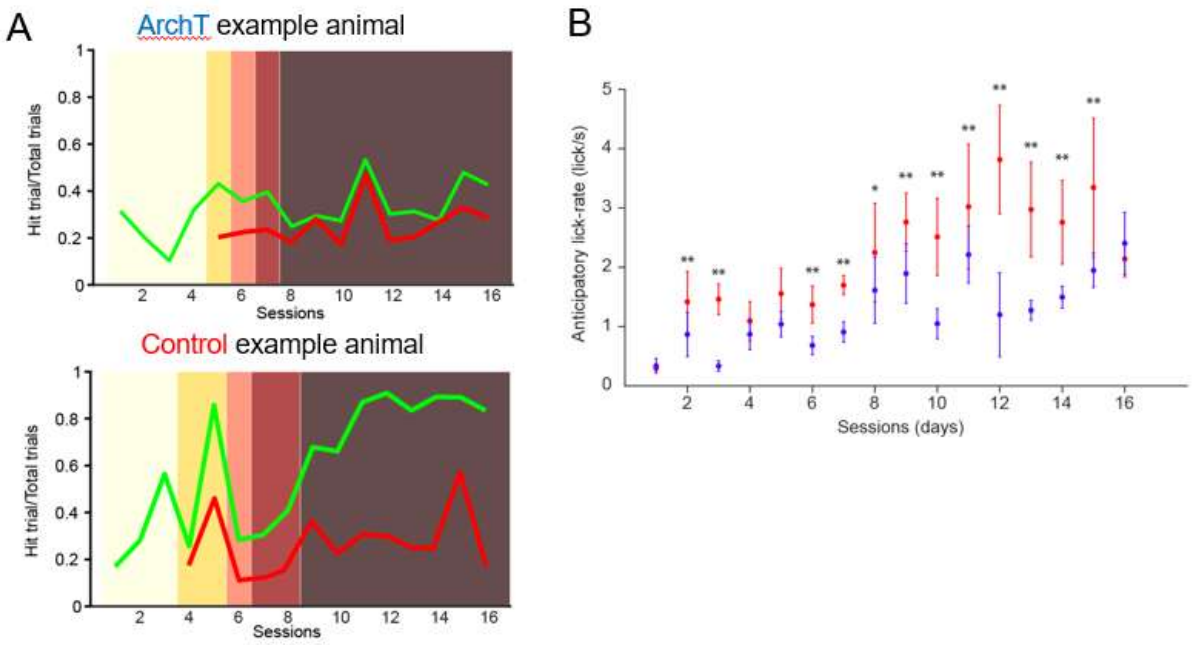

Figure 8. Optogenetic inhibition of cholinergic BF neurons impairs learning in young (3-5 months) animals. A, Representative learning curve of an inhibited animal (ArchT, top) and a control animal (Control, down). Reward-predicting cue discrimination rate in green and punishment-predicting cue discrimination rate in red. B, Group data showing significance differences between control (red) and inhibited (blue) groups in the anticipatory licking rate during the different training sessions.

We examined to what extent the cholinergic reward-predicting activity was related to the associative learning process during the Pavlovian conditioning. When these responses were specifically inhibited in young mice by optogenetically silencing BF cholinergic cells during the cue presentation, mice learned the task contingencies slower (Figure 8A-B), showing less anticipatory licks after the reward-predicting cues (Figure 8C).

We presented these results at the online FENS Forum in 2020. We plan to publish these results in a peer-reviewed journal in the near future.