Decomposition of brain calcium signals in a Pavlovian learning task

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Abstract

Learning tasks, even the simplest ones, involve multiple brain regions (involved in sensory processing, evaluation of outcomes and generation of movement). Experimental protocols combined with modern techniques (e.g., fiber photometry) allow for the simultaneous recording of these observed values (or predictors, e.g., movement, sensory signals, etc.) and the activities of multiple brain regions (e.g., cerebellum, substantia nigra pars compacta) during a learning task. However decomposing the neural signals into components that encode different predictors and the neural signal they produce during the learning process remains challenging. Existing methods (e.g., generalized linear models) that allow for the identification of the neural signals produced by each predictor and the estimation of the contribution of each predictor to the total signal during the learning process fail to capture the signal kinetics associated with each predictor. In this work we address this issue. We use optimization tools to decompose the neural signals into the respective time-dependent contributions (kernels) of each predictor under certain modeling assumptions. We used this kernels and their defining properties to examine how the associated signals change on a session-by-session basis and established the extent to which the cerebellum contributes to dopaminergic signaling in the process of conditioned learning.

Keywords: Learning; neural circuits; cerebellum; basal ganglia

Introduction

Even the simplest learning tasks involve multiple brain regions that are involved in sensory processing, evaluation of outcomes and generation of movement. Experimental protocols combined with modern techniques such as fiber photometry allow for the simultaneous recording of these observed values (or predictors, e.g., movement, sensory signals, etc.) and the activities of multiple brain regions (such as those involved in this project; see below) during a learning task. However decomposing the neural signals into components that encode different predictors and the neural signal they produce during the learning process remains challenging. Methods such as GLM can provide link functions that estimate the contribution of each predictor to the total signal, but do not estimate the signal kinetics associated with each predictor.

In this work, we use optimization tools to decompose the neural signals into the respective time-dependent contributions (kernels) of each predictor based on a priori assumptions (such as linear decomposition) in order to estimate how these kernels change during the learning process. These kernels provide an estimate of both the amplitudes and the kinetics of the signal components contributed by each predictor. Because the time-dependent kernels we use are the output of dynamical systems (solutions to ordinary differential equations in response to inputs) they are interpretable in terms of the systems building blocks.

We recently characterized functional monosynaptic projections (Cb-SNc) from the cerebellum to the substantia nigra (SNc) midbrain dopaminergic nucleus (Washburn et al., 2024). Recordings and optogenetic stimulations in mice

showed that the Cb-SNc pathway is active during movement and induces SNc-mediated dopamine release in the dorsal striatum, suggesting a possible role for these projections in movement modulation. Additionally, in a simple Pavlovian task, the Cb-SNc activity is highly responsive to reward (water) consumption and to reward value (sweet vs regular water), indicating a possible role for this pathway in modulating reward-based functions in the basal ganglia. Both the basal ganglia and the cerebellum are involved in movement control and learning. The functions of both systems are mostly understood in terms of their respective reciprocal interactions with the cortex. The cerebellum ensures that movements are performed smoothly and efficiently, whereas the basal ganglia are important in proper movement initiation and the control of movement speed (vigor), functions that depend crucially on dopamine modulation. Dopamine is released by the midbrain nucleus substantia nigra pars compacta (SNc) in the striatum, the primary input nucleus of the basal ganglia and its actions are known to be important for movement initiation and speed as well as reward-based learning. The cerebellum is also known to be essential for motor learning, but recent studies have shown the cerebellum may also participate in reward-based functions. Here, we describe the methodology of decomposing these signals into components correlated with the different predictors and we use these components to track how these signals change through the learning process.

Methods and Results

This work is based on a simple Pavlovian task in which animals (mice) learned to associate an auditory cue with a reward (water or sweet water) which they consume by licking. The animals were head fixed and signals were acquired with embedded optic fibers. To record the activity of the cerebellar projections to the SNc, GCamp7 was expressed in the deep cerebellar nuclei (DCNs; the output nuclei of the cerebellum) using a viral vector and to record the activity of the SNc dopaminergic neurons, we used a mouse line (DAT-Cre) that expressed Cre under the dopamine transporter (DAT) and expressed Cre-dependent red calcium indicator (jRGECO1) with a viral vector. The two-color fluorescent indicators allowed us to simultaneously record the activities of both cerebellar axons and the SNc dopamine neuron somata using the same fibers embedded (bilaterally) in the SNc. In some experiments, we also expressed the dopamine sensor dLight1.1 in the dorsolateral striatum and recorded dopamine release with fibers embedded in this region.

Here we describe one set of experiments. Other experiments followed a similar protocol but sometimes with variation (e.g., addition of sweet water High reward). Animals were water restricted on experiment days. The experiments included at least 2 days of habituation followed by several days of the experimental protocol for the Pavlovian task. Although the protocol was done every day, fiber photometry recording was done at most on alternate days to allow the genetically encoded indicators to recover from photobleaching. In the experiments described below, recordings were done only on days 1, 3, 5, 10, 15 and 20. The protocol was as follows: in each trial, following a period of at least 3 s that the animal did not perform licking, a 0.3 s sound cue was presented, followed by a drop of water (Reward) presented after exactly 1 s. In 20 % of trials chosen at random, a cue was presented but no reward was given (Omission trials). Each session (day) consisted of 200-250 trials. Recordings for each trial included a time interval of 10 s before to 10 s after the cue. The animals movements (including licking) were recorded with a high-speed video camera and analyzed post hoc using DeepLabCut.

To obtain a first-order (linear) decomposition of each signal into its components (kernels), we made a set of a priori simplifying assumptions, listed below. These assumptions were relaxed or varied in further iterations of the analysis, depending on how well the first-order decomposition approximated the recorded data.

1. The recorded signal correlates with one of the three factors: the cue (C), motor movement (licking; M), or the reward (R).
2. Signal kernels of each type (C, m or R) are identical in each session and sum linearly. Therefore, in all trials within each session:
   (a) Each individual lick produces a signal of a fixed amplitude and kinetics (the motor kernel m, which is used to calculate the motor signal M).
   (b) The cue produces a signal of fixed amplitude and kinetics (the cue kernel C).
   (c) The reward produces a signal of fixed amplitude and kinetics (the reward kernel R). In experiments that include High reward, the High reward signal is a fixed scaling of the Regular reward.
3. All signals are non-negative. In the first pass, we used a rectified difference-of-exponentials function to estimate each signal component (This assumption may be relaxed for the dopamine signal, since omission of reward could result in a negative signal.)

Given these assumptions, we used standard optimization methods to decompose the calcium signals into distinct components as described below.

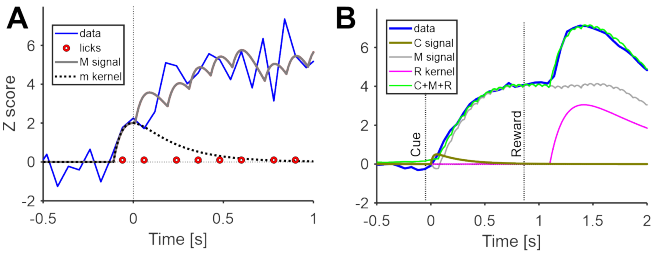

Figure 1: **A.** Estimate of a single trial signal with a motor kernel. **B.** Estimate of the mean Rewarded signal

To obtain the cue kernel, we took advantage of the trials in each session where the animal did not start to lick the water

spout immediately after the cue. These late-lick trials occurred more in the early sessions because in later sessions, as the animal learned to associate the cue with the reward delivery, it started to lick earlier in response to the cue. However, even in the late sessions there were a few late-lick trials especially later in the session when the animal was not as thirsty. The cue signal was obtained by fitting the average signal across these trials with

$$C(t) = C_{amp}\left[e^{-t/t_{fall,c}} - e^{-t/t_{rise,c}}\right]^{+},$$

where $t = 0$ is the cue time, $t_{fall,c}$ / $t_{rise,c}$ are the decay / rise times and $X^{+} = \max(X, 0)$.

After obtaining the cue kernel, this component was subtracted from the total signal in each trial and the remaining signal was used for additional analysis. To obtain the motor kernel, we used the Omission trials only after the cue kernel was subtracted from each trial. We assumed that each lick produced a unitary signal:

$$m(t) = m_{amp}\left[e^{-(t-t_m)/t_{fall,m}} - e^{-(t-t_m)/t_{rise,m}}\right]^{+}.$$

The motor kernel was added across all lick times in each Omission trial. If the licks in each trial are given by $L(t)$ which is 1 when $t = t_k$ and 0 otherwise, this results in a total motor signal of

$$M(t) = (m \otimes L)(t) = \sum_k L(t - t_k)\, m(t)$$

where $\otimes$ is the convolution operator. These signals were then averaged across all trials, and then compared to the average Omission signal from the biological data to optimize the parameters. To obtain the reward kernel, we subtracted the cue kernel and the motor signal from the total signal in each Rewarded trial and fit the remaining signal, averaged across trials to obtain

$$R(t) = r_{amp}\left[e^{-t/t_{fall,r}} - e^{-t/t_{rise,r}}\right]^{+},$$

where $t = 0$ is the reward time. An example of the full process is summarized in Fig. 1.

Conclusion

We examined the trial-by-trial simultaneous fiber photometry signals recorded from Cb-SNc projections and SNc DA neurons, and decomposed these signals into components that correlate with sensory cue, movement, and reward. Using these components, we examined how these signals change on a session-by-session basis and established the extent to which the cerebellum contributes to dopaminergic signaling in the process of conditioned learning.

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