Supporting Online Material for

BOLD Responses Reflecting Dopaminergic Signals in the Human Ventral Tegmental Area

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Supporting Online Material

Materials and Methods

The Princeton University Institutional Review Board approved this experiment for human participants and participants were recruited from the University community. In the first experiment, 20 participants were imaged and 2 were excluded for excessive head motion (motion greater than 1.5 mm in any direction). The remaining 18 participants (7 males) varied in age from 18 to 28 years with an average age of 20.9 years, and all were right-handed. In the second experiment, 23 participants were imaged and again 2 were excluded for excessive head motion (motion greater than 1.5 mm in any direction). The remaining 21 participants (10 males) varied in age from 20 to 37 years with an average age of 25.3 years, and 15 were right handed. Written consent was obtained from all participants.

Stimulus paradigm

In both experiments, participants were told they were participating in a task designed to study reward processing.

Experiment 1: Participants were instructed to watch the visual display while in the scanner and swallow squirts of juice or water as they were delivered. Juice and water were selected as rewards because they both have been shown to affect neuronal responses in reward processing areas such as midbrain dopaminergic areas and the ventral striatum

in non-human primates (*S1*). Additionally, delivery of juice and water to humans has been shown to robustly elicit a BOLD response in reward processing areas (*S2,S3*).

Our task consisted of two types of trials: control and catch. Water or juice squirts were delivered for both types of trials. In control trials, a visual cue (a red circle for juice or a blue circle for water) was followed by a squirt, either juice or water depending on the cue color, after 6 s had passed since display of the cue (Fig S1). In catch trials, the squirt of juice or water arrived at 12, 14, or 16 s after the cue. In all trials, the cue was presented for 1 s. Water and juice trials were randomized throughout all scanning runs.

In total, the task consisted of five scanning runs, each of which was approximately 8 minutes in duration. The first scanning run contained an equal number of juice and water control trials (30 trials total: 15 juice control and 15 water control trials). The inter-trial-interval (ITI) between control trials varied between 6 and 10 s (in 2 s increments). The subsequent four scanning runs consisted of a total of 25 trials each: 20 control trials and 5 catch trials. Juice and water trials were randomized across the final four scanning runs and the order in which catch trials and control trials were presented was also determined randomly. As in the first scanning run, the ITI varied between 6 and 10 s (in 2 s increments) for all trials in the last four scanning runs.

Experiment 2: Participants were instructed to watch the visual display while in the scanner. At the start of a trial, the visual display consisted of a number on the left side of the screen (range 0 to 10) and a white box on the right side of the screen (Fig S2). Participants were further instructed to press a button on an MR compatible button-box to indicate whether a second number, hidden by the white box, was higher or lower than the

first number. The second number also ranged from 0 to 10 and all numbers were generated randomly but restricted so that the first number and second number were never equal. The first number was displayed for 2 s and was followed by the addition of two yellow triangles, one pointing up (corresponding to a button press for the second number being higher) and one pointing down (corresponding to a button press for the second number being lower). Participants had 2 s to enter a response or they would automatically lose $1 for the trial. Once the participant pressed a button, his/her choice was indicated by the triangle turning red; this screen remained visible for a variable time period ranging between 6 s and 10 s until the trial outcome was revealed. If the participant guessed correctly, they won $1 and if they guessed incorrectly, they lost $1. Trial outcome was displayed in words below the two numbers. The outcome was displayed for 4 s and was followed by a black screen for 4 s to 14 s comprising the ITI.

MR image acquisition

The same imaging protocols were used for both experiments. All images were acquired using a 3 T Siemens Allegra head-only MRI system using a circularly polarized head volume coil. The visual stimuli were displayed on a rear projection screen and viewed by participants through a mirror attached to the head coil. High-density foam padding was used to stabilize the participant's head to minimize head motion during the experiment.

High-resolution (1 mm^3 voxels) T1-weighted structural images were acquired with an MP-RAGE pulse sequence at the beginning of the scanning session.

All functional data were acquired using a high-resolution echo-planar imaging pulse sequence (128 x 128 matrix, 1.5 x 1.5 mm^2 in-plane voxels, 1.9 mm thick slices, TE 41 ms, FA 60 degrees) and that was cardiac gated. The participant's pulse, monitored using a finger pulse-oximeter that interfaces with the scanner, was used to trigger the scanner during functional imaging. The pulse-oximeter was placed on the left middle finger of each participant. Pulse-oximetry was used to trigger the scanner because the pulse transit time from the heart to the finger is within approximately 50 - 70 ms of the pulse transit time from the heart to the upper neck region, where the brainstem is located (*S4,S5*).

The midbrain was identified in the central sagittal slice of the high-resolution structural, and a slab comprising axial/coronal slices (each slice 1.9 mm thick) was centered on the midbrain and tilted to include as much of the SN and VStr as possible (Fig 1C in main text). The number of slices in the axial/coronal slab was determined based on the participant's heart rate. In addition, the volume acquisition time (in non-cardiac gated imaging, this corresponds to the repetition time, TR) as well as the maximum length of the acquisition window were also determined based on the participant's heart rate. The acquisition window corresponds to the amount of time during which the scanner will wait for a heartbeat to trigger the next image acquisition. For a participant with a heart rate of 60 beats per minute (or 1000 ms per beat), 9 slices would be used with a volume acquisition time of 895 ms and an acquisition window of 950 ms. The volume acquisition time was always set to be as fast as possible depending on the number of slices used. The maximum length of the acquisition window was always set to be between 50 and 70 ms longer than the volume acquisition time to allow

for the pulse transit time between the heart and neck region. For the first experiment, the mean number of slices was 8.22 ± 0.92 (s.d.) with a maximum of 10 slices and a minimum of 7 slices across participants. The mean volume acquisition time was 818.89 ± 101.13 (s.d.) ms and the mean acquisition window was 880.55 ± 99.34 (s.d.) ms. For the second experiment, the mean number of slices was 7.82 ± 1.47 (s.d.) with a maximum of 11 slices and a minimum of 6 slices. The mean volume acquisition time was 777.64 ± 147.33 (s.d.) ms and the mean acquisition window was 832.53 ± 146.68 (s.d.) ms. All scanner trigger times during functional scanning were recorded and used in data analysis.

After functional scanning, a non-cardiac-gated whole-brain functional image (TR/TE 2500/41, 25 slices, 6 mm thick, 30% gap, FA 60 degrees, 4 volumes) with the same center and orientation as the functional image slices was acquired solely to facilitate registration of the whole-brain structural image to the functional data. Finally, a proton-density weighted image (TR/TE 6000/16 ms, FA 149 degrees, echo spacing 15.6 ms, 0.75 x 0.75 mm^2 voxels in-plane) was acquired using the same slices (orientation and thickness) as the functional images for localization of the SN and VTA in the midbrain (*S6*).

Liquid Delivery (Experiment 1)

Juice and water were delivered using two computer-controlled syringe pumps. Plastic tubes (9 m in length) were attached to the syringe pumps in the control room and run to the participant in the scanner room. Pacifiers were used to stabilize the tubes in the participant's mouth while in the scanner. Squirts of juice and water were 1 s in duration and had a volume of 1.0 mL. The amount of time it took a squirt to reach a participant

was measured to be approximately 1 s; this time delay was incorporated into generation of regressors.

Data Analysis

Before any preprocessing was carried out, data were corrected for T1 variations that occur with cardiac-gated fMRI (*S7*) using software written in MATLAB (The MathWorks, Natick, MA). Corrected data were then preprocessed and analyzed using AFNI (*S8*). To account for the variable time between image acquisitions, regressors were calculated at high temporal resolution and then resampled at the image acquisition times. Functional images were corrected for slice-timing effects using Fourier interpolation and motion corrected to the fifth volume of the training run also using Fourier interpolation (*S9*). The motion correction parameters were used to determine if participant head movement exceeded 1.5 mm in any direction. Participants with head motion in excess of 1.5 mm were not included in the analysis. In addition, the motion correction parameters were used as regressors of non-interest in a multiple linear regression analysis. Data were next spatially smoothed with a 3 mm FWHM Gaussian kernel and mean subtracted. For all participants, the most superior and inferior slices were excluded from analysis as a precaution against those slices shifting into previously non-excited brain areas due to head motion.

The T1-weighted whole-brain structural image was aligned to the functional data and then transformed into Talairach space. All T1-weighted whole brain structural images were also brainstem normalized (*S10*). The transform to Talairach space and to brainstem normalized space was then applied to the functional data.

Experiment 1: general linear model analysis

For each participant, design matrices were created in which each experimental event was considered as an impulse stimulus that generates a hemodynamic response function of unknown amplitude. The set of modeled experimental events included the presentation of juice and water cues, delivery of liquid in control trials, delivery of liquid in catch trials, and the absence of expected liquid at 6 s during catch trials. Additionally, regressors of non-interest that modeled baseline drift (scanning run mean, linear and quadratic trends) were included in the model. The full model was regressed to the data, giving the best-fitting amplitude values for each experimental event.

To determine what the BOLD response in the SN and VTA encodes, statistical maps for each participant were generated for the following experimental events: (i) presentation of the visual cue signaling reward expectation, (ii) liquid delivery in control trials signaling reward delivery, (iii) liquid delivery in catch trials signaling both reward delivery and a positive reward prediction error, (iv) nothing received in catch trials compared to nothing expected (modeled as baseline) signaling a negative reward prediction error, and (v) dV/dt. The dV/dt regressor was modeled as a positive response at the time of the cue, a negative response at the time of reward delivery (unless the trial was a catch trial), and a negative response at the time when a squirt was expected during catch trials. All statistical maps were thresholded ($p < 0.05$, two-sample t-test corrected for multiple comparisons) to identify brain areas where the regression coefficients (called beta values) for the modeled events were significantly different from zero. The thresholded statistical maps that were generated for the presentation of the visual cue and

for dV/dt did not show significant results for any participant and will not be discussed further.

Experiment 2: general linear model analysis

For each participant, design matrices were created where each experimental event was considered as an impulse stimulus that generates a hemodynamic response function of unknown amplitude. Experimental events modeled included the display of the first number, the onset of the choice indicators, the button press indicating the participant's decision, and the trial outcome. To determine how the SN and VTA respond to rewarding and non-rewarding events, trial outcomes were grouped into wins and losses. Since we also wanted to test whether the measured BOLD response varied according to the probability of a trial outcome, we generated regressors where the BOLD response to rewarding and non-rewarding outcomes parametrically varied with the probability of winning. As in experiment 1, regressors of non-interest that modeled baseline drift (scanning run mean, linear and quadratic trends) were included in the model. The full model was regressed to the data, giving the best-fitting amplitude values for each experimental event.

Group results (Experiments 1 and 2):

To identify brain areas where the beta coefficients from individual participants' GLM analyses, described above, are significantly different from zero, two-sample t-tests were performed on the statistical maps that were generated for each modeled experimental event. The significance of the results was determined using the AFNI

program AlphaSim. AlphaSim implements the cluster-size threshold procedure as a protection against Type I error (*S11*). Based on AlphaSim results, we determined that a corrected p value of 0.05 is achieved with a minimum cluster size of 18 contiguous voxels each significant at $p < 0.001$ (two-sample t-test). However, the size of the VTA is approximately 60 mm^3 (*S12*), which corresponds to 14 voxels at 1.5 x 1.5 x 1.9 mm resolution. Because the VTA is smaller than the minimum cluster size according to AlphaSim, we determined the significance of results in the VTA using a Bonferroni correction. The corrected threshold is $p < 0.003$ ($p < 0.05 / 14$ voxels = $p < 0.003$, two-sample t-test). We reported results where all voxels are each significant at $p < 0.001$, but they were unchanged at $p < 0.003$. The VTA regions reported at $p < 0.05$ (two-sample t-test corrected for multiple comparisons) consist of at least 9 contiguous voxels. Outside the midbrain, all results were displayed for brain areas having at least 18 or more contiguous voxels each with a significance value of $p < 0.001$ (two-sample t-test).

All group results for each contrast were calculated in both Talairach space and brainstem normalized space (*S10*). Active regions in the brainstem were visualized on brainstem normalized anatomical images while active regions outside the brainstem were shown on anatomical images that had been transformed into Talairach space.

Event-related time courses were generated by running a deconvolution analysis. Onset times of events of interest were written to a vector and the best fitting impulse response function for a given time interval was estimated using AFNI.

In the first experiment, BOLD time courses were generated for the response to the cue during control trials through the duration of the trial (6 s until liquid reward delivered). Because the ITI varied from 6 s to 10 s, the time course for the BOLD

response to delivery of the expected reward was limited to 6 s in duration to eliminate any contamination from presentation of cues for subsequent trials. For catch trials, time courses generated for the BOLD response to the cue were 11 s in duration because the earliest time a catch squirt (unexpected reward) could be delivered is 12 s after a catch cue. Time courses for the BOLD response to delivery of the catch squirt were 6 s in duration to eliminate any contamination from presentation of cues for subsequent trials. Average event-related BOLD time courses were generated for the VTA (Fig S3) and VStr (Fig S4).

In the second experiment, average BOLD time courses were generated for the VTA (Fig S5). Time courses are collapsed across the probability of an outcome because there were too few trials for each probability of winning to generate time courses separately.

Fig S1. Task design for the first experiment. (A) Control trials. Participants were trained to expect a liquid reward 6 s after the display of a visual cue. For juice trials, the visual cue was a red circle and for water trials the visual cue was a blue circle. In all trials, the cue was presented for 1 s. The first scanning run (training) contained an equal amount of juice and water control trials (30 trials total: 15 juice control and 15 water control trials). The ITI varied between 6 and 10 s (in 2 s increments). Shown are two example control trials, one for water reward and one for juice reward. Water and juice trials were randomized throughout training and subsequent testing runs. (B) Catch trials. Shown are two example catch trials, one for water reward and one for juice reward. In catch trials, the squirt of juice or water arrived at 12, 14, or 16 s after the cue. Like control trials, the visual cue was presented for 1 s. Inclusion of catch trials in the experimental design allowed testing for both positive and negative reward prediction error signals. The contrast between delivery of a catch squirt (unexpected reward) versus the delivery of a control squirt (expected reward) illustrates a positive reward prediction error. Likewise, the contrast between the absence of any reward expectation (during baseline periods) versus the absence of an expected reward (6 s after a cue during a catch trial) illustrates a negative reward prediction error. The subsequent four scanning runs consisted of a total of 25 trials each: 20 control trials and 5 catch trials. Water and juice trials were randomized throughout all scanning runs. The order in which catch trials and control trials were presented was also determined randomly. As in the first scanning run, the ITI varied between 6 and 10 s (in 2 s increments) for the last four runs.

Fig S2. Task design for the second experiment. Participants were shown a number on the left side of the screen and a white box on the right side of the screen. Participants were instructed press a button indicating whether a second number, hidden by the white box, was higher or lower then the first number. All numbers ranged from 0 to 10, were generated randomly and were never equal. The first number was displayed for 2 s and was followed by the addition of two yellow triangles, one pointing up (corresponding to a button press for the second number being higher) and one pointing down (corresponding to a button press for the second number being lower). Participants had 2 s to enter a response or they would automatically lose $1 for the trial. Once the participant pressed a button, his/her choice was indicated by the triangle turning red; this screen remained visible for a variable time period ranging between 6 - 10 s until the trial outcome was revealed. If the participant guessed correctly, they won $1, and if they guessed incorrectly, they lost $1. The outcome was displayed for 4 s and was followed by a black screen for 4 -14 s (the ITI).

Fig S3. VTA BOLD time courses to delivery of primary rewards. (A) Average BOLD event-related time courses were generated from the VTA voxels indicated in Fig 2A in the main text. The BOLD response to the delivery of an unexpected reward (catch trial; green line) is greater than the BOLD response to the delivery of an expected reward (control trial; orange line). Reward delivery occurs at time t = 0. Error bars represent standard error of the mean across participants. (B) The VTA BOLD response does not show a negative reward prediction error. The BOLD response at the time when a reward was expected is not significantly different from baseline, when nothing is expected (x-

axis). Presentation of the visual cue occurs at time t = 0 and reward delivery was expected at time t = 6 s. Error bars represent standard error of the mean across participants.

Fig S4. VStr BOLD time courses to delivery to primary rewards. (A) Average event-related VStr BOLD time courses were generated from voxels shown in Fig 3A of the main text. The striatal BOLD response to the delivery of an unexpected reward (catch trial; green line) is larger than the BOLD response to the delivery of an expected reward (control trial; orange line). Reward delivery occurs at time t = 0. Error bars represent standard error of the mean across participants. (B) The striatal BOLD response to the absence of an expected reward (catch trial; green line) is less than baseline, when nothing is expected (x-axis). Presentation of the visual cue occurs at time t = 0 and reward delivery was expected at time t = 6 s. Error bars are standard error of the mean across participants.

Fig S5. VTA BOLD time courses to monetary gains and losses. (A) Average event-related time course for the VTA BOLD response to trial outcome when the participant guessed correctly (and received $1). Voxels shown in Fig 4A of the main text were used to generate BOLD time courses. Time course shown is the average for all trials when the participant won, collapsed across the probability of winning. Display of the outcome occurs at time t = 0. Error bars represent standard error of the mean across participants. (B) Average event-related time course for the BOLD response to trial outcome when the participant guessed incorrectly (and lost $1). Because no midbrain regions were found to

correlate significantly with trial outcome when the participant guessed incorrectly and lost $1, the same voxels shown in Fig 4A were used to generate the BOLD time course. Time course shown is the average for all trials when the participant lost, collapsed across the probability of winning. Display of outcome occurs at time t=0. Error bars represent standard error of the mean across participants.

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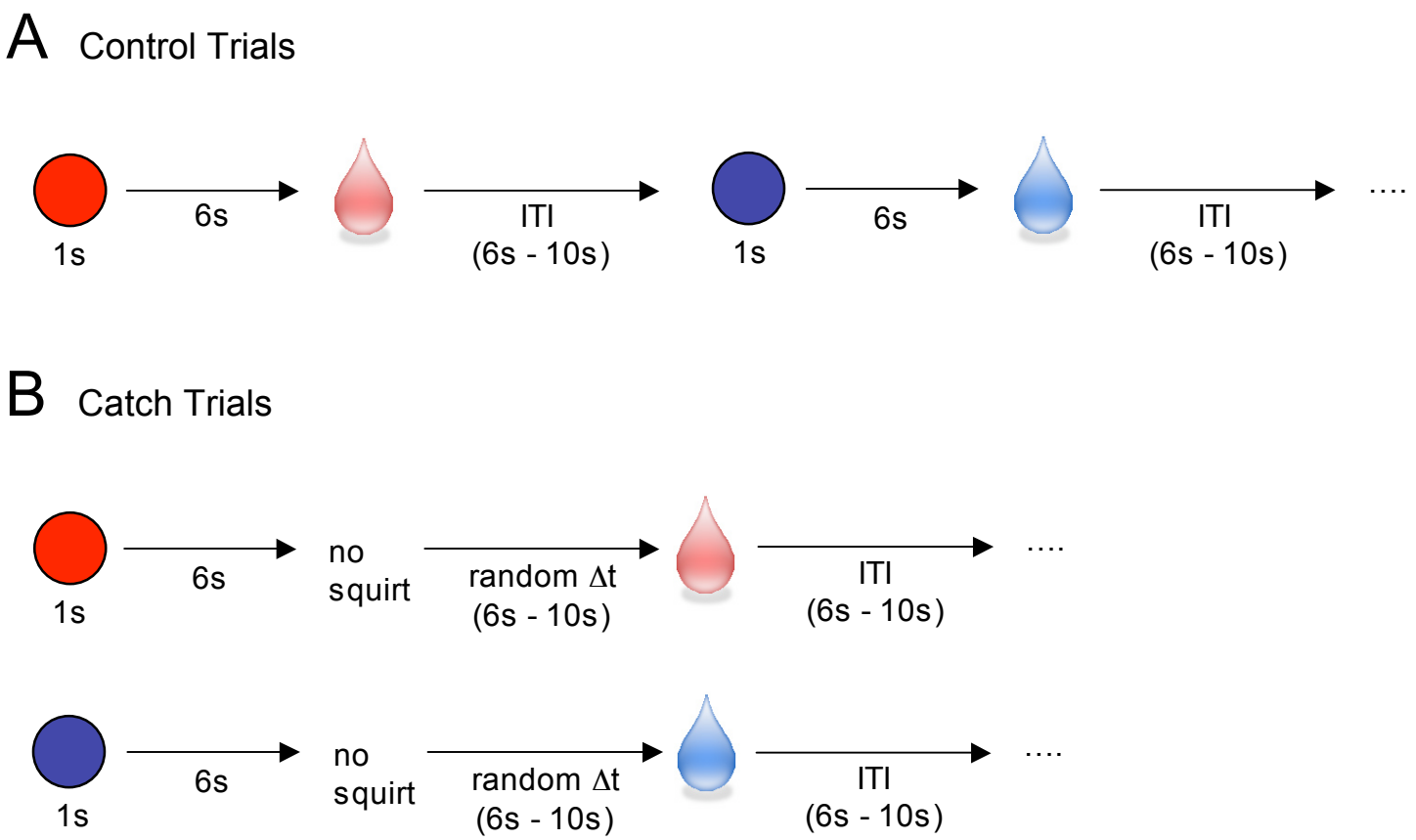
Fig S1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(6s - 10s)
1s
6s
ITI
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6s
no
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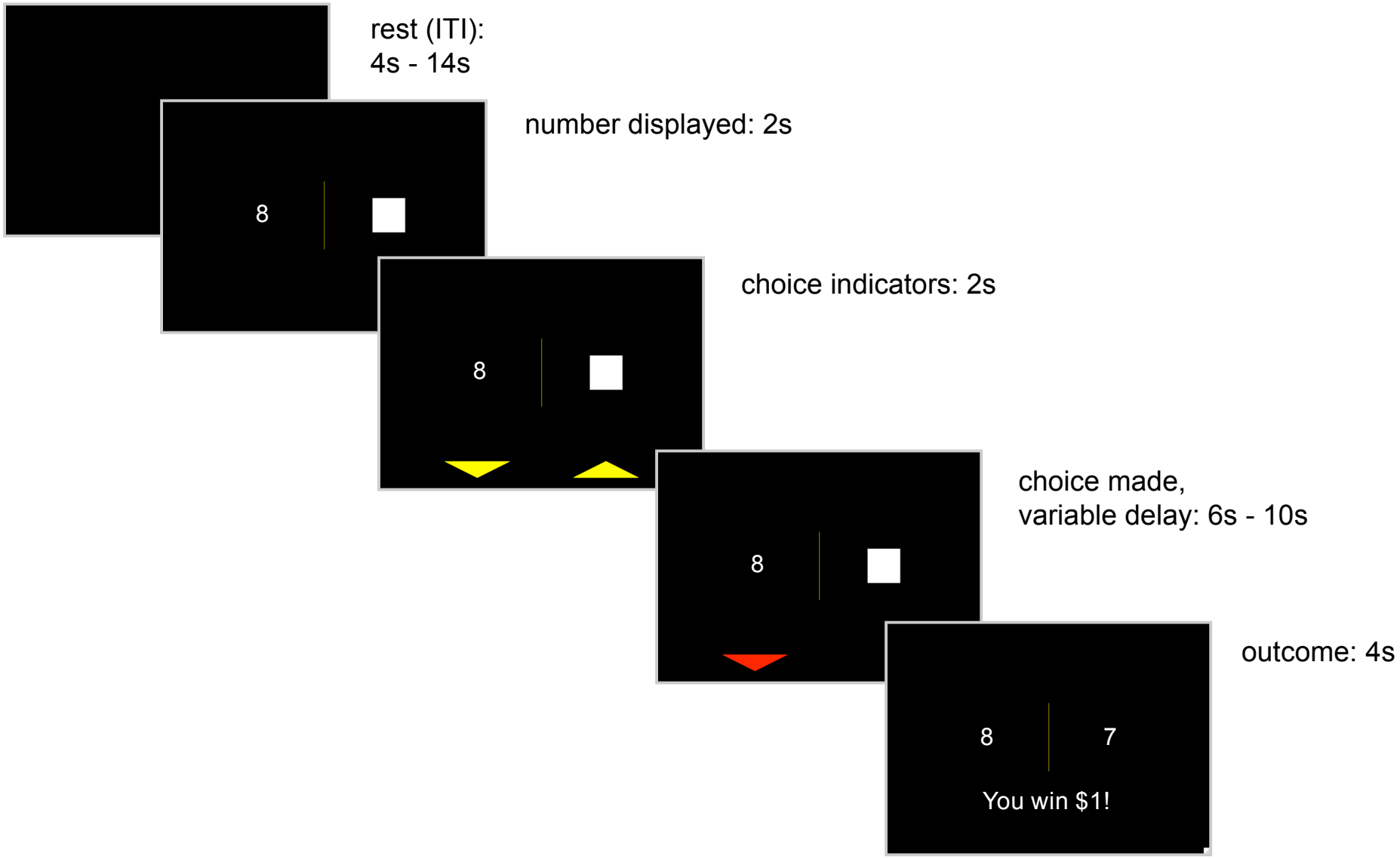
Fig S2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4s - 14s
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choice indicators: 2s
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8
7
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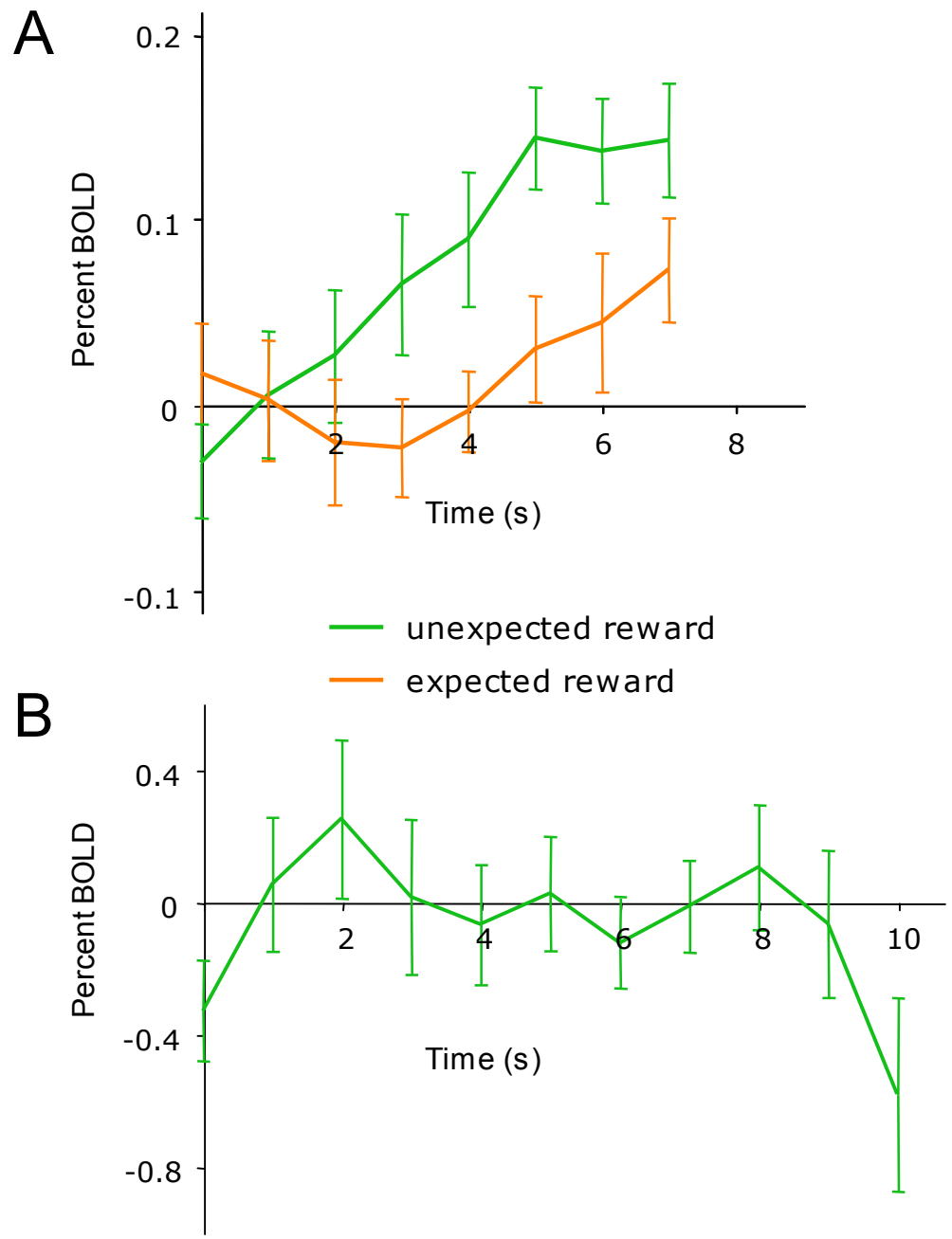
Fig S3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0.1
0
-0.1
Percent BOLD
2
4
6
8
Time (s)
unexpected reward
expected reward
B
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0
-0.4
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Percent BOLD
2
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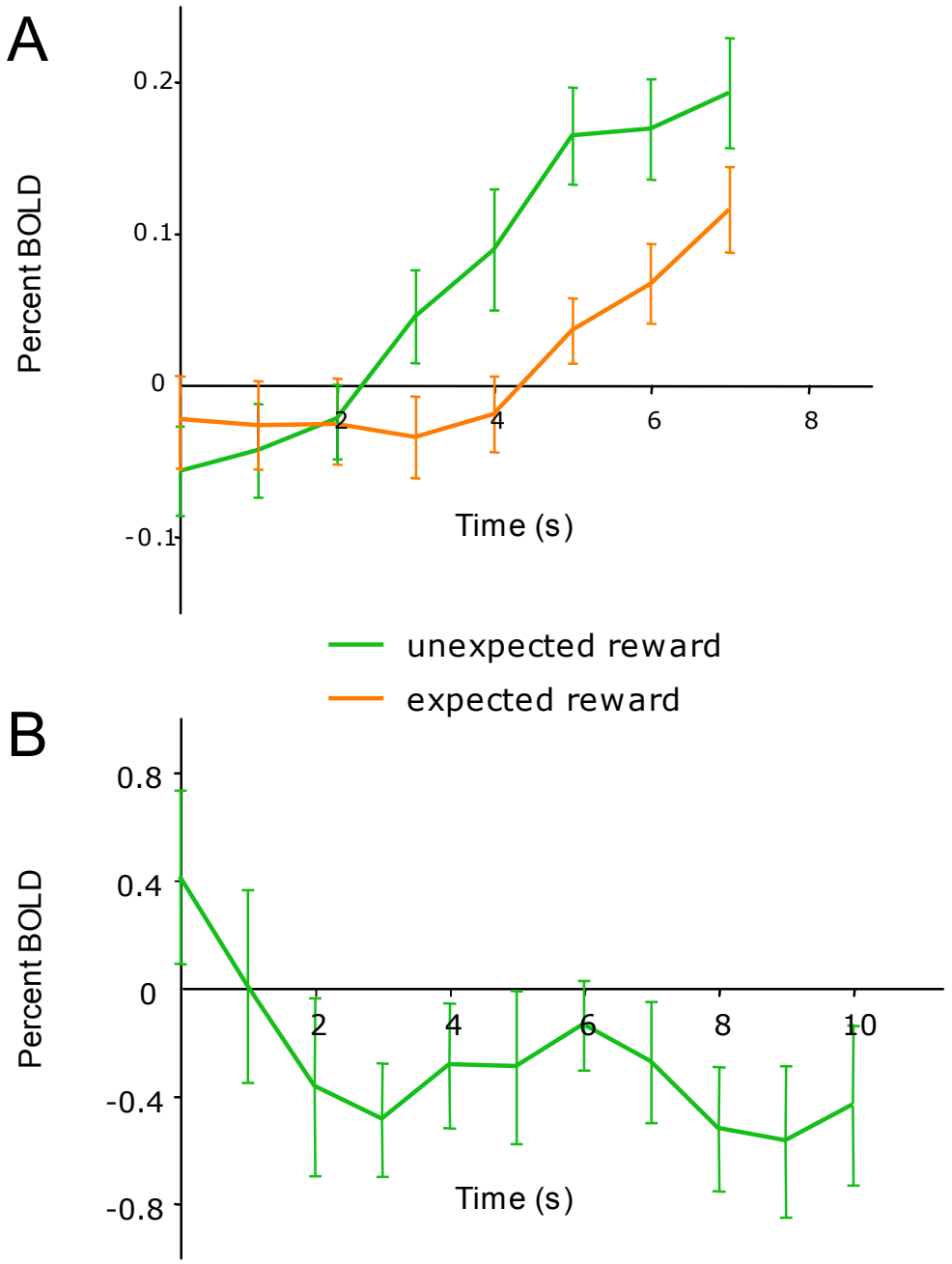
6
8
10
Time (s)

Fig S4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0.1
0
-0.1
Percent BOLD
2
4
6
8
Time (s)
unexpected reward
expected reward
B
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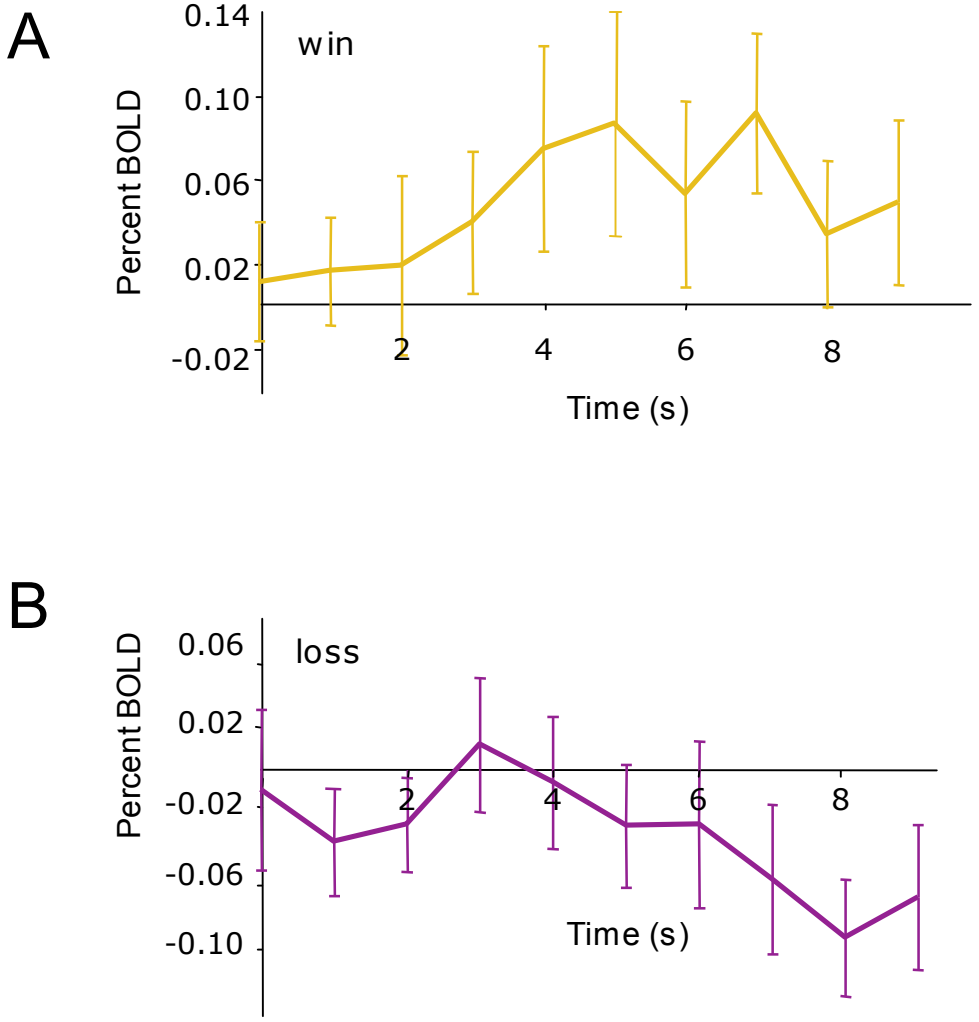
0.4
0
-0.4
-0.8
Percent BOLD
2
4
6
8
10
Time (s)

Fig S5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0.02
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Percent BOLD
2
4
6
8
Time (s)
B
loss
0.06
0.02
-0.02
-0.06
-0.10
Percent BOLD
2
4
6
8
Time (s)