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Supplementary Material for

Two Dimensions of Value: Dopamine Neurons Represent Reward But Not Aversiveness

Christopher D. Fiorillo*

*E-mail: fiorillo@kaist.ac.kr

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Materials and Methods

The present data is a subset of data that has been described previously, and thus the methods described below are mostly identical to previous descriptions (*16,17,24*).

Animals

Two rhesus macaques (*Macaca mulatta)* were studied, one female (monkey O; 10.5 kg) and one male (monkey F; 5.5 kg). These same two monkeys contributed data on dopamine neurons to several previous studies (*16,17,24*), and monkey O contributed data to a third study (*29*). Procedures complied with guidelines established by the National Institutes of Health, and were overseen locally by the Stanford University Animal Care and Use Committee. Liquid intake was restricted to insure the motivation of monkeys to participate in experiments.

Eye tracking and data acquisition

A monkey's head was fixed in place in front of a computer monitor in a sound-insulated room. Eye position was monitored with an infrared eye tracking system (Eyelink II from SR Research of Toronto, Canada), except for initial experiments performed in monkey O, in which a scleral search coil was surgically implanted and eye position was monitored within a magnetic field. Expo software (written by Peter Lennie and modified by Julian Brown) was used to deliver stimuli and to collect all data.

Stimuli

Juice, saline and bitter solutions were delivered directly into the mouth by a computer-controlled solenoid valve (Parker Hanafin part 002-0010-900) under the force of gravity. The solenoids were located about 0.7 m from the monkeys head, and their opening and closing emitted a click of 72 dB as measured at the location of the monkey's head. One delivery system (reservoir, solenoid valve, polyethylene tube, and metal spout) was used to deliver juice, and a second system was used to deliver saline or bitter solutions. Thus appetitive and aversive solutions could be delivered together or separately on each trial.

Juice was two-thirds apple juice and one-third water. In the experiments of figures 1A, 2A, and 3, juice was 240 µL delivered over 250 ms, 130 µL over 150 ms, and 180 µL over 200 ms, respectively. The intensities of aversive stimuli that were delivered during neuronal recordings were selected based on a choice task (*2*). "Saline" was an aqueous solution of 8% NaCL delivered over 60 ms (30 µL) in monkey O, and 4% NaCL for 30 ms (10 µL) in monkey F (seawater typically has a salinity of 3.5%). In initial choice experiments, concentrations of 0.5%, 1%, 4%,

and 8% were tested in monkey O, but only 4% was tested in monkey F. Bitter solution was 1 or 10 mM denatonium for 80 ms (40 μL), and was only tested in monkey O. The neutral "unconditioned" stimulus (Fig. 1B-D) was a sound of 72 dB ("glass," available in Mac OSX 10.4).

Air was delivered by a computer-controlled solenoid valve (Parker Hanafin part 009-0339-900) connected to a pressurized gas tank. The gas tank and solenoid valve were located in an adjacent room. Air was delivered from a tube having an inner diameter and length of approximately 3 mm and 3 m, respectively. Air was directed, parallel to the ground, at the left nostril from the left side, exiting the tube at a distance of about 1 cm from the nostril. During all neuronal recordings, the air pressure (measured at the gas tank) was 35 and 20 pounds per square inch (PSI) in monkeys O and F, and the solenoid valve was open for 200 ms. The sound of the air exiting the tube had an intensity of 82 dB at the location of the monkey's head.

The Pavlovian conditioned stimuli that predicted juice were purely visual (Figs. 1A, 2A, and 3). The data of figure 1A and 1B was the subject of a previous study on the sensitivity of dopamine neurons to reward risk (uncertainty) (*24*). The CSs that predicted aversive outcomes (Figs. 1B-D, 2B-D) had both visual and auditory components. The visual stimuli were circles or squares of 4 degrees of visual angle in diameter presented in the center of the monitor. The icons shown in figures were not the actual stimuli used for experiments. A single visual stimulus was conditioned to predict only one outcome (US) in each monkey, and the same stimuli were used in the other monkey but predicted different outcomes. In all experiments with audiovisual Pavlovian stimuli, the same sound of 72 dB was presented ("ping," available in Mac OSX 10.4), whereas the particular features of the visual stimulus differed across experiments. Onset of both auditory and visual components of the CS was synchronous.

Pavlovian Experimental Design

Conditioning with Pavlovian stimuli was performed for several hundred trials over 2-4 days prior to the start of neuronal recordings. The Pavlovian designs are illustrated in figure S1. Each US was delivered 1.0 s after CS onset. Visual CS offset was synchronous with US offset ("delay conditioning"), However, in the case of audiovisual CSs (Figs. 1B-D, 2B-D), the offset of the auditory component of the CS was synchronous with the onset of the US (which had its own sound). Inter-trials intervals were 2 - 6 s. "Unpredicted" juice (Fig. 2A) was delivered with a randomly distributed inter-trial interval of 2-16 s, in a block of trials with no other stimuli.

To minimize generalization between stimuli, minimal numbers of stimuli were included within a single block of trials (Fig. S1). In the experiments of figures 1 and 2, aversive stimuli were delivered in a block of trials in which no juice was delivered. If dopamine neurons were sensitive to aversiveness, this should maximize their sensitivity to the aversiveness of the stimuli [because their sensitivity will be focused on small aversive values rather than being spread over a large range of aversive and appetitive values, as would be expected based on studies of how dopamine neurons efficiently represent the magnitude of reward (*18*)]. A block of trials included about 40 trials of each trial type. In the experiments of figure 1, in which a single CS predicted an aversive stimulus with a probability of 0.5, about 80 total trials were run for each neuron, 40 with the aversive outcome and 40 with a neutral outcome. The order of trials was pseudorandom, with each 10 consecutive trials including 5 of each type.

Details of the chronology of all experiments, over a period of months, has been presented previously (*16*).

Choice tasks to measure aversiveness

Choice tasks were used to quantify the aversiveness of stimuli, as previously described (*16*). Although none of the choice data is presented in the present work, it is critical to the interpretation of the present results. The aversiveness of air, saline, bitter, and loud sound was estimated by measuring how much juice a monkey would sacrifice to avoid the stimulus. Several weeks of choice experiments were performed before each set of neuronal recordings, and a few days of choice behavior were performed during and after the period of neuronal recordings to insure that aversiveness had not changed. In initial choice experiments, aversiveness was measured repeatedly after adjusting stimulus intensity (air pressure and location, or saline or bitter concentration or volume) in order to find an intensity that was equal and opposite to a small volume of juice (at least 70 μL). Once this target level of aversiveness was reached, and proven to be stable over at least several days of choice behavior, neuronal recordings were performed and responses to that same stimulus intensity were measured. Neuronal recordings were not performed during the choice task.

Recording and localization of dopamine neurons

Glass-insulated tungsten electrodes (Alpha-Omega, Nazareth, Israel) were plated with gold and platinum (*24*). The region of dopamine cell bodies in the ventral midbrain was localized with the aid of physiologically identified landmarks. Dopamine neurons were distinguished from other neurons in the region by the

characteristics of their extracellularly recorded impulses, including long, multiphasic waveforms and slow, fairly regular basal firing rates (*17*), consistent with previous criteria for electrophysiological identification of dopamine neurons (e.g. *21*). Neurons were localized with the aid of physiologically identified landmarks, particularly the somatosensory representation of the face in ventroposteromedial thalamus, and the oculomotor nucleus near the midline of ventral midbrain. The location of recorded neurons was then estimated in atlas coordinates (*17*), using methods described previously (*24*).

Data analyses

Data were analyzed using Matlab (Natick, MA). Firing rates were measured during the time periods illustrated in the peri-stimulus time histograms of figures 1-3. To quantify the statistical significance of these firing rates relative to baseline firing rate (during the ITI) in a single neuron (as shown in Fig. 1D), or relative to firing rates in the same time period on other trials (as shown in Fig. 2D), firing rates (spike counts) for all individual trials were compared between the two conditions using an unpaired t-test; $p < 0.05$ was taken to be significant, without any correction for the fact that the same test was performed separately on all neurons. For comparisons across the whole population of recorded neurons, the mean firing rate across trials was calculated for each condition in each neuron, and these single neuron mean firing rates were then compared between conditions across the population of neurons using paired t-tests.

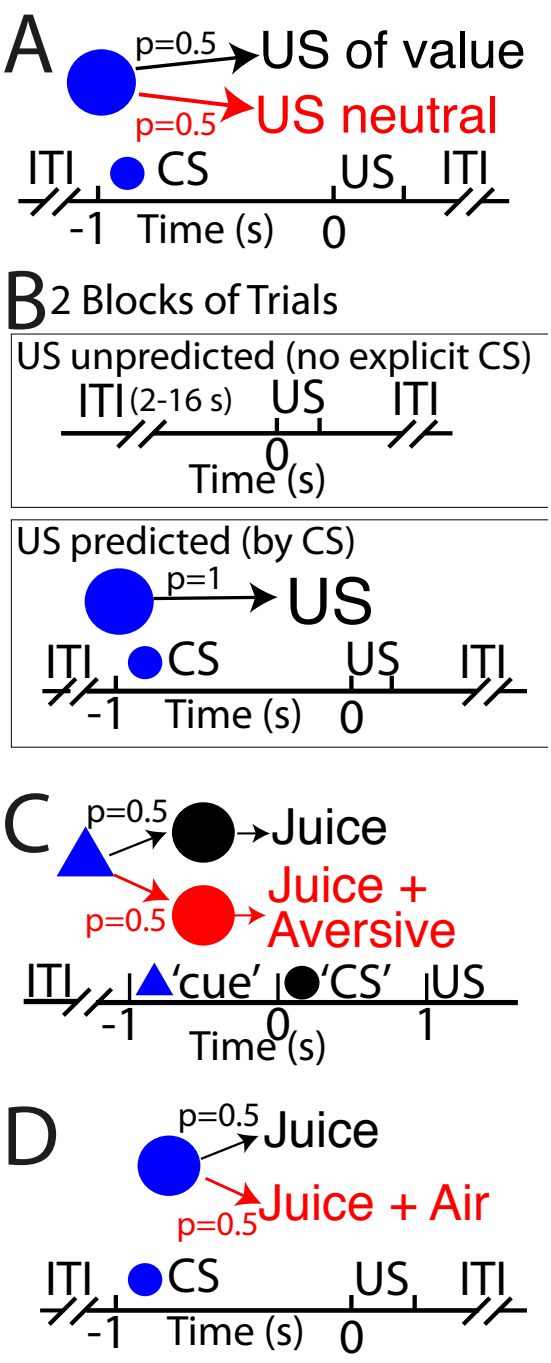

Fig. S1. Design of experiments (Figs. 1-3, S2) with Pavlovian conditioned stimuli (CSs). The visual icons shown here are for illustration only; a diversity of distinct images were used in the experiments. **(A)** Design of experiments shown in figure 1 in which a CS was followed (with probability of 0.5) by either an "unconditioned" stimulus (US) of value (appetitive or aversive) or a neutral sound. As shown on the timeline, CS onset was 1.0 s before US onset, which was at time "0" in figure 1. The inter-trial interval (ITI) varied randomly between 2 and 6 s. **(B)** Design of experiments shown in figure 2. One block of trials consisted of repeated delivery of a US with a long and variable ITI (2-16 s), in the absence of any explicit CS. In a second block of trials, the same US was preceded 1.0 s earlier by onset of a CS. **(C)** Design of experiments shown in figures 3A,B and S2A-C. Each trial started with a "cue" which was followed 1.0 s later by onset of either a CS predicting juice alone, or a CS predicting juice plus an aversive stimulus. The use of the cue made the onset of the CSs more predictable, which in principle should facilitate the ability of dopamine neurons to discriminate between the values of the two CSs (*18*). The experiments of figure 3C were analogous but no cue was presented prior to CS onset. **(D)** In the experiment of figure 3D, one of the three CSs (shown in figure 3C) was followed by air on 50% of trials, and the peri-stimulus time histogram is aligned to US onset at time '0' (juice or juice plus air).

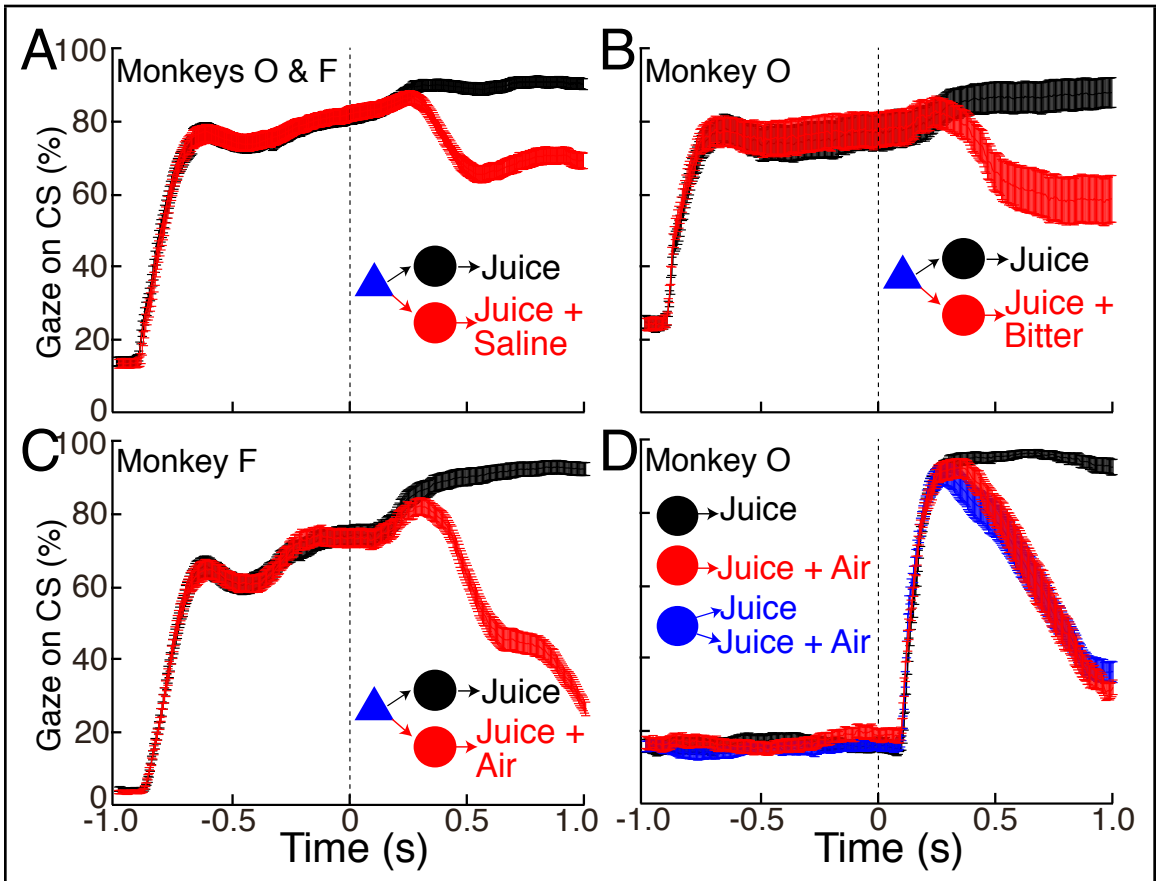

Fig. S2. Monkeys averted their gaze from stimuli predicting aversive outcomes. The fraction of all trials with gaze on the visual CS is shown as a function of time (mean ±sem in 5 ms bins; sampling rate of 200 Hz). The design of these same experiments is shown in figure S1C,D and neuronal responses are shown in figure 3. The average net value of juice plus aversive stimuli was positive (appetitive) based on the choice task (*16*). (**A)** A cue of 1 s duration was presented at -1.0 s, followed by appearance of one of two CSs at the same location on the monitor at time "0" and a US at time 1.0 s. Gaze was averted in response to the CS that predicted delivery of saline (red), but maintained on the CS the predicted juice alone (black). All data from monkeys O and F has been averaged together. (**B)** The same as in (A) but for bitter in monkey O. (**C)** The same as in (A) but for air in monkey F. (**D)** Analogous to A-C, except that three CSs were used in the absence of any cue. The greater aversion of gaze in anticipation of air, relative to anticipation of saline or bitter solutions, is likely to be due to the additional motivation to avoid stimulation of the eyes by air (although the air was directed at the nose).

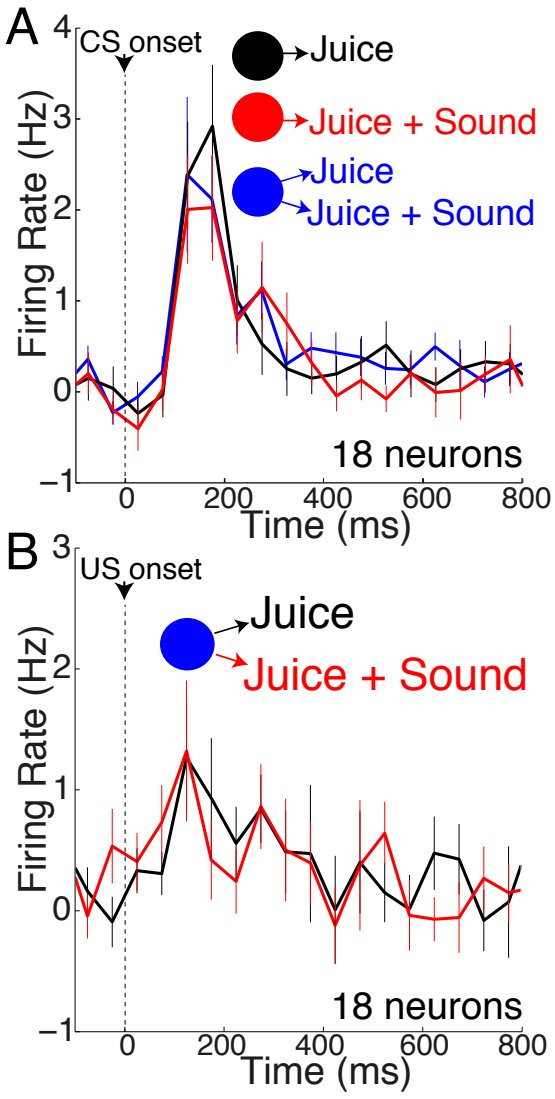

Fig. S3. In the context of juice, dopamine neurons are insensitive to a loud but neutral sound. The experimental design is fully analogous to that illustrated in figure S1C,D, for which neuronal responses are shown in figure 3C,D, except that a loud (90 dB) but neutral sound was substituted for air puff. The neutrality of the sound was demonstrated through choice experiments (*16*). This experiment was only performed in monkey O, and it serves to partially control for the high sensory intensity of the aversive air puff (82 dB with tactile stimulation of the nose). **(A)** Prediction of loud sound does not alter responses to CSs. Each CS predicted the same volume of juice, but one predicted sound (red), a second predicted no sound (black), and a third predicted a 50% chance of sound (blue). Baseline firing rates have been subtracted. **(B)** Responses to juice alone and juice with sound when the probability of sound was 0.5 following the CS shown in blue in (A). Delivery of the same loud sound (or air), by itself, caused a large activation at short latency (peak at ~80 ms) when it was unpredicted (*16*).

References and Notes

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<foot>22. Materials and methods are available as supplementary materials on *Science* Online.</foot>

<foot>23. Of 195 dopamine neurons, 134 were from monkey O, and 61 were from monkey F. It was estimated that 81, 13, and 6% were in substantia nigra, ventral tegmental area, and retrorubral field, respectively, with 45% of all neurons being in the “ventral tier” (ventral substantia nigra compancta and reticulata) and the other 55% being in the “dorsal tier” (*17*).</foot>

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