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Report

Sensitivity to Temporal Reward Structure in Amygdala Neurons

Maria A. Bermudez,[1] Carl Göbel,[1,2] and Wolfram Schultz[1,*]
[1]Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK
[2]Department of Neurology, University Hospital Cologne, 50924 Cologne, Germany

Summary

The time of reward and the temporal structure of reward occurrence fundamentally influence behavioral reinforcement and decision processes [1–11]. However, despite knowledge about timing in sensory and motor systems [12–17], we know little about temporal mechanisms of neuronal reward processing. In this experiment, visual stimuli predicted different instantaneous probabilities of reward occurrence that resulted in specific temporal reward structures. Licking behavior demonstrated that the animals had developed expectations for the time of reward that reflected the instantaneous reward probabilities. Neurons in the amygdala, a major component of the brain's reward system [18–29], showed two types of reward signal, both of which were sensitive to the expected time of reward. First, the time courses of anticipatory activity preceding reward delivery followed the specific instantaneous reward probabilities and thus paralleled the temporal reward structures. Second, the magnitudes of responses following reward delivery covaried with the instantaneous reward probabilities, reflecting the influence of temporal reward structures at the moment of reward delivery. In being sensitive to temporal reward structure, the reward signals of amygdala neurons reflected the temporally specific expectations of reward. The data demonstrate an active involvement of amygdala neurons in timing processes that are crucial for reward function.

Results and Discussion

Experimental Design

We studied the activity of single amygdala neurons in two rhesus monkeys using a Pavlovian reward prediction task superimposed on an ocular fixation task. We varied the instantaneous temporal reward probability, defined as the probability of reward occurring in the next time interval given that task progression is in the current interval. The instantaneous probability is the behaviorally relevant probability of obtaining a reward in the next time interval and thus determines the prediction of reward from moment to moment. The fact that the Pavlovian task did not allow the animal control over reward occurrence precluded confounds by operant behavioral responses.

We varied instantaneous reward probability across four trial types. In one trial type, a singular reward occurred at the end of a specific 2.0 s visual stimulus (A; Figure 1A, top) with a probability of 1.0. Thus, instantaneous reward probability was zero (0) at all times during the stimulus and p = 1.0 at its end (Singular reward, Figure 1B, blue). In a second trial type, a different visual stimulus (B; Figure 1A, middle) predicted that reward would occur with an instantaneous probability of 0.025 in each interval of 50 ms during the entire stimulus duration of 2.0 s, but not in the absence of the stimulus. Thus, at any 50 ms interval during the stimulus, the probability that a reward would occur in the next 50 ms interval was 0.025. This probabilistic schedule allowed no reward or multiple rewards to occur during a single stimulus, which maintained the same flat instantaneous reward probability with flat reward rate during the entire stimulus duration (Figure 1B, red). In a third trial type, another visual stimulus (C) served as control without predicting any reward (Figure 1A, bottom). In a fourth trial type, no stimulus appeared, and reward occurred with a flat instantaneous probability of 0.025 in each 50 ms interval throughout 5.0 s of the 6.0 s trial cycle.

Behavior

Both animals maintained key touch and central eye fixation in >95% of all trials with stimuli A–C throughout neuronal recordings. Error rates (erroneous key release) varied insignificantly between the rewarded trials but were significantly higher in the explicit no reward trial type (means of 8%–16% versus 27%–36% errors; $p < 0.0001$, one-way ANOVA; $p < 0.01$ for any rewarded trial type versus no reward trial type, Fisher's PLSD test; n = 86, 25, and 15 trial blocks during neuronal recordings of prereward and postreward activations). Anticipatory prestimulus licking was low (0 ms median lick duration across trials, in all three trial types) (Figure 1C). Careful adjustment of the licking spout with same distance between spout and mouth of the head-fixed animal resulted in similar licking in the two animals.

Stimulus A, which produced a singular reward at the fixed time of 2.0 s after stimulus onset, elicited licking in two periods, as observed before [30]. One peak occurred around 500 ms after stimulus onset as behavioral response to the stimulus, and a second peak occurred around 300 ms in anticipation of the time of the reward (Figure 1C, top; median lick duration/2 s, 473 ms). By contrast, stimulus B, which produced reward with flat instantaneous probability, elicited a lower, more tonic rate of licking during stimulus presentation compatible with the more spread-out reward occurrence (Figure 1C, middle; rewarded trials excluded from analysis; median lick duration/2 s, 395 ms). Very little licking occurred in the explicit no reward trials with stimulus C (Figure 1C, bottom; median lick duration/2 s, 0 ms).

Lick durations differed significantly between prestimulus and stimulus periods ($p < 0.0001$; $F(1,208) = 193$; two-way ANOVA) and among the three trial types ($p < 0.0001$; $F(2, 208) = 94$). Post hoc analysis identified longer licking durations during both rewarded stimuli compared to the no reward stimulus (both $p < 0.0001$, Fisher's PLSD test). Although the two rewarded stimuli elicited different temporal licking patterns, the overall amount of licking during the entire periods of the two stimuli differed insignificantly ($p = 0.34$).

These data demonstrate that the animals distinguished between the different temporal structures of reward predictions. Their licking followed the temporal profiles of reward

*Correspondence: ws234@cam.ac.uk

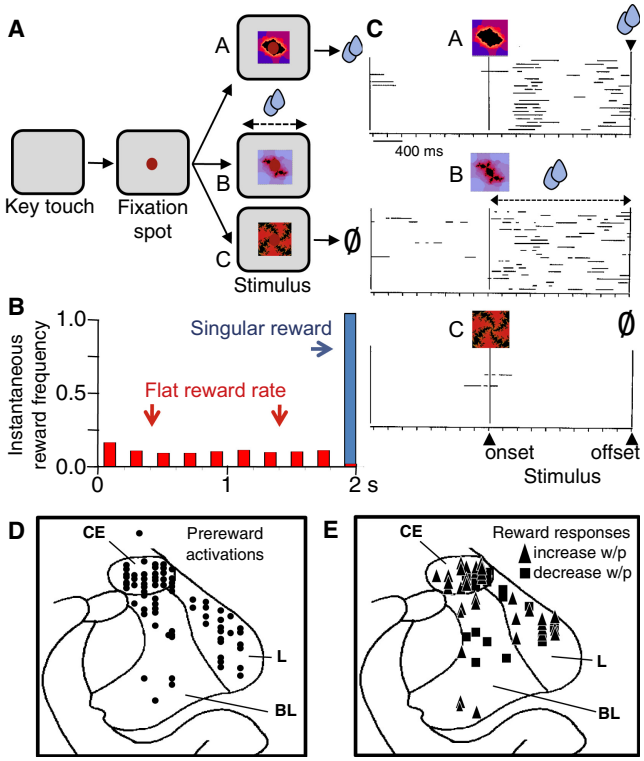

Figure 1. Task, Behavior and Recording Sites

(A) Sequence of task events involving stimuli. Three pseudorandomly alternating stimuli predicted a singular reward with a probability of 1.0 at stimulus end (top), reward with a flat instantaneous reward probability of 0.025/50 ms interval during stimulus (middle), and no reward (bottom). (A fourth trial type, not shown, involved a flat instantaneous reward probability of 0.025/50 ms during the trial but without any stimulus.)

(B) Measured instantaneous frequency of reward occurrence at stimulus end (blue, n = 1,045 trials) or with flat instantaneous probability (p = 0.025/50 ms) during stimulus (red, n = 646) (each vertical bar shows average from four intervals of 50 ms). Note that multiple rewards could occur during a single stimulus, thus producing flat moment-to-moment reward probability (analogous to "rate of occurrence of failure" for repairable systems rather than "hazard rate"). 0, stimulus onset.

(C) Licking behavior in the three trial types shown in (A). Horizontal lines indicate photo beam interruptions by tongue at liquid spout. Each line shows one trial; trial sequence is from top to bottom. In middle graph, rewarded trials were excluded from analysis.

(D and E) Histological reconstruction of recording sites in animal A, with approximate positions for animal B superimposed. (D) Location of neurons with prereward activations (n = 86 neurons). (E) Location of neurons with reward responses modulated by instantaneous reward probability. Triangles indicate higher responses with higher instantaneous reward probability (n = 36); squares show lower responses (n = 22). CE, central nucleus; L, lateral nucleus; BL, basolateral nucleus.

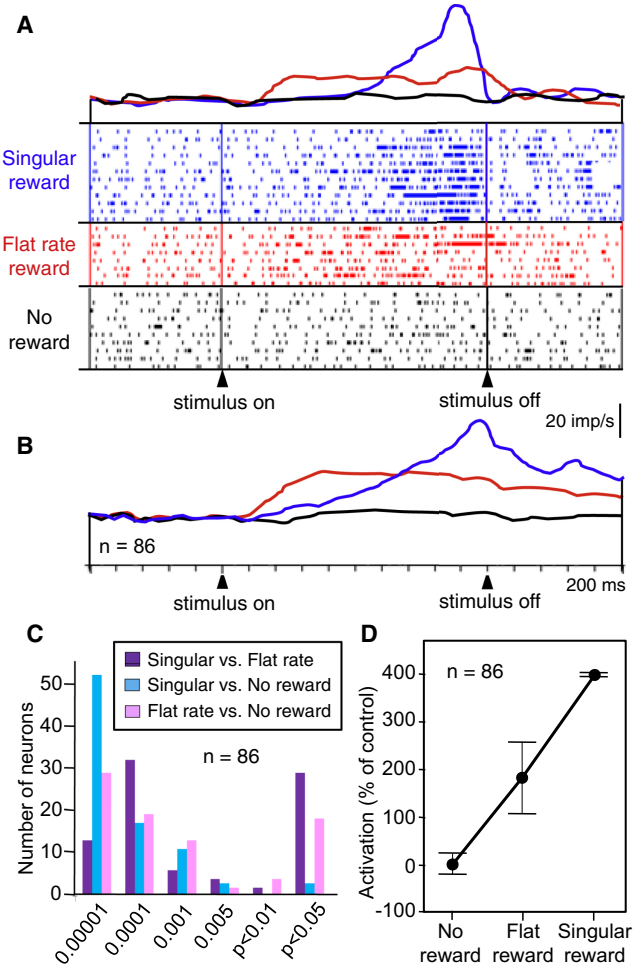

Figure 2. Modulation of Temporal Profiles of Neuronal Prereward Activity by Different Instantaneous Reward Probabilities

(A) Single neuron. imp, impulse.

(B) Population density functions of averaged activity elicited by the three stimuli predicting different instantaneous reward probabilities (blue, singular reward; red, flat reward rate during stimulus, rewarded trials excluded from analysis; black, no reward) (n = 86 neurons). Same bin width (10 ms) and impulses/s calibration bar apply to (A) and (B).

(C) Distribution of neuronal p values from Fisher's PLSD post hoc two-sample comparisons following one-way ANOVA on prereward activations between singular, flat rate, and no reward trials.

(D) Median activation strengths with different instantaneous reward probabilities (±95% confidence intervals).

occurrence, which suggests temporally differentiated reward expectations. The singular reward with stimulus A induced an expectation at stimulus end, whereas the reward occurring with flat instantaneous probability during stimulus B was associated with longer and more tonic reward expectations.

Activity Preceding Reward

We tested 312 amygdala neurons in the two animals (204 and 108 neurons, respectively) with the singular reward delivered at the end of the 2.0 s stimulus A. Of these, 86 (28%) showed significant prereward activations during stimulus A ($p < 0.05$, Wilcoxon test against prestimulus control period). We tested the 86 neurons with the three stimuli (A–C) in pseudorandom alternation but skipped the fourth trial type, which lacked the required prestimulus control period. The 86 neurons were located in the central nucleus (47 of 144 tested neurons), basolateral nucleus (17 of 90 tested neurons), and lateral nucleus (22 of 78 tested neurons) of amygdala (Figure 1D). The activated neurons were insignificantly distributed among these three amygdala nuclei ($p > 0.05$, chi-square test).

The prereward activations in all 86 amygdala neurons were sensitive to reward timing. The activation of a typical neuron preceding the singular reward at the end of stimulus A is shown in Figure 2A (blue). It increased gradually after stimulus onset and dropped immediately when the reward was delivered after the fixed 2.0 s period at stimulus end. The average population activity shown in Figure 2B (blue) displayed

a similar time course. The increase became significant against baseline at a mean of 1,200 ms (±54 ms SEM) after stimulus onset. By contrast, with flat instantaneous reward probability during stimulus B, activity increased earlier after stimulus onset compared to singular reward (mean latency, 350 ± 27 ms; $p < 0.0001$; $n = 86$; t test) and exceeded early activations with singular reward during 600–1,000 ms after stimulus onset [$p < 0.0001$, $F(2,255) = 25.03$, one-way ANOVA; $p < 0.005$, Fisher's PLSD on flat rate versus singular reward]. Activity remained tonically elevated during the remainder of the stimulus period (Figures 2A and 2B, red). (Note that rewarded trials were excluded from the main analysis and displays because of occasional confounding responses to the reward themselves; see below.) The activations failed to ramp up further during the stimulus and did not reach a clear peak (Figures 2A and 2B), despite the continuing increase in the sum of future reward. This temporal profile occurred also in the few trials in which the pseudorandom schedule produced several rewards. The differences in time courses of neuronal activity between singular reward and flat reward rate paralleled well the differences of the behavioral licking responses (compare Figures 2A and 2B with 1B, blue versus red).

Prereward activations were highest during the 400 ms window immediately preceding stimulus offset and significantly exceeded activations with flat instantaneous reward probability in the neuron of Figure 2A [$p < 0.0001$, $F(2,27) = 12.64$, one-way ANOVA; $p = 0.0172$, Fisher's PLSD on singular versus flat rate reward], in all 86 neurons analyzed individually, and in the population activity of the 86 neurons shown in Figure 2B [$p < 0.0001$, $F(2,255) = 58.115$, one-way ANOVA; $p < 0.0001$, Fisher's PLSD on singular versus flat rate reward and all other comparisons; Figure 2C]. During this 400 ms window, a Spearman correlation coefficient of rho = 0.615 and a comparison of activation strengths (Figure 2D) independently confirmed the neuronal sensitivity to instantaneous reward probability across explicit no reward, flat rate, and singular reward trials. Amygdala neurons showed bursts of impulses that increased toward the singular reward but were scattered throughout the occurrence of flat rate reward (Figure 2A, top and middle rasters). Defining bursts as greater than five impulses/100 ms, the overall burst rate varied insignificantly between singular and flat rate reward (means of 14.3 ± 4.8 SEM and 13.8 ± 3.9 bursts/ten trials, respectively; $p > 0.5$, t test). Bursts ended significantly later with singular compared to flat rate reward (472 ± 24.1 and 850 ± 47.3 ms before stimulus offset, respectively; $p < 0.0001$), which is compatible with the different temporal patterns of impulse rate with the two instantaneous reward rates. All 86 neurons failed to change activity during the no reward stimulus ($p > 0.05$, Wilcoxon test; Figures 2A and 2B, black).

Taken together, instantaneous reward probability had a remarkable influence on the temporal profiles of all prereward activations of the tested amygdala neurons. Whereas the neuronal activations preceding singular reward started late and reached high peaks, the activations with flat instantaneous reward probability started earlier and maintained a modest plateau until reward probability dropped with stimulus offset. The absence of ramping with flat reward rate during the stimulus suggested predictive coding of the instantaneous reward rate rather than coding of the increasing sum of future reward. Thus, the different temporal profiles reflected the different occurrences of predicted reward and constituted a typical characteristic of instantaneous reward expectation.

Responses to Reward Delivery

Of the 312 amygdala neurons tested, 219 (70%) responded significantly to the reward delivered at the end of the 2.0 s stimulus ($p < 0.05$, Wilcoxon test against prereward control period, with exceptions stated below). We tested 169 of these neurons with all three rewarded trial types, and a subset of them with all four trial types (see below). Responses in 58 of the 169 neurons (34%) were sensitive to the instantaneous reward probability at the time of reward.

Reward responses in 36 of the 169 neurons (21%) increased with increasing instantaneous reward probability. The 36 responding neurons were insignificantly distributed among the central, basolateral, and lateral amygdala nuclei (19, 8, and 9 of 95, 33, and 41 tested neurons, respectively; $p > 0.05$, chi-square test; Figure 1E, triangles). The responses were highest to the singular reward occurring after the fixed delay (Figure 3A, blue), lower with flat reward rate during the stimulus (red), lowest with flat reward rate during the trial (dotted), and absent in explicit no reward trials. The graded increases were seen in the neuron of Figure 3A [$p < 0.0001$, $F(3,29) = 27.24$, one-way ANOVA; $p = 0.0075$ singular versus flat rate during stimulus, $p < 0.0301$ flat rate during stimulus versus flat rate during trial, $p < 0.0001$ singular versus flat rate during trial; Fisher's PLSD test] and in all 36 neurons analyzed individually. The responses to flat rate reward during the stimulus varied only insignificantly between reward delivered during the first and the second half of stimulus duration (see Figure S1A available online; $p > 0.1$, t test). We tested 25 of these 36 neurons also in explicit no reward trials and found similar significant differences in the population activity of these neurons [Figure 3B; $p < 0.0001$, $F(3,96) = 21.58$, one-way ANOVA; $p < 0.09$ singular versus flat rate during stimulus, $p < 0.001$ flat rate during stimulus versus flat rate during trial, $p < 0.0001$ singular versus flat rate during trial, Fisher's PLSD test; Figure 3C]. Similar relationships were found in an additional 12 of 23 neurons that showed reward responses in addition to their prereward activations (the 23 neurons belonged to the group of 86 prereward neurons described above and were tested with a prestimulus control period).

Taken together, activity in these 36 amygdala neurons was positively modulated by instantaneous reward probability. The more likely the reward was to occur at any given moment, the higher was the neuronal response. Thus, the modulations varied positively with the temporal predictability of reward. The similarity of responses to flat rate reward during early and late stimulus periods suggested dependence on the predicted instantaneous reward rate rather than the predicted increasing sum of future reward. The observation that responses with flat reward rate without stimulus were lowest among all rewarded trial types would suggest that the stimulus had increased the predictability of the otherwise pseudorandomly timed reward. These activations might function to maintain established, temporally specific reward predictions after reinforcement learning. The positive relationship to instantaneous reward probability resembles the attentional modulation seen in monkey visual cortex V4, which parallels the hazard function of stimulus change during individual trials [12]. Our data demonstrate that such temporal modulations are not restricted to attentional processes but occur also with reward.

By contrast, reward responses in 22 of the 169 neurons (13%) decreased with increasing instantaneous reward probability. The 22 neurons were located in the central, basolateral, and lateral nuclei of amygdala (11, 5, and 6 of 95, 33, and 41

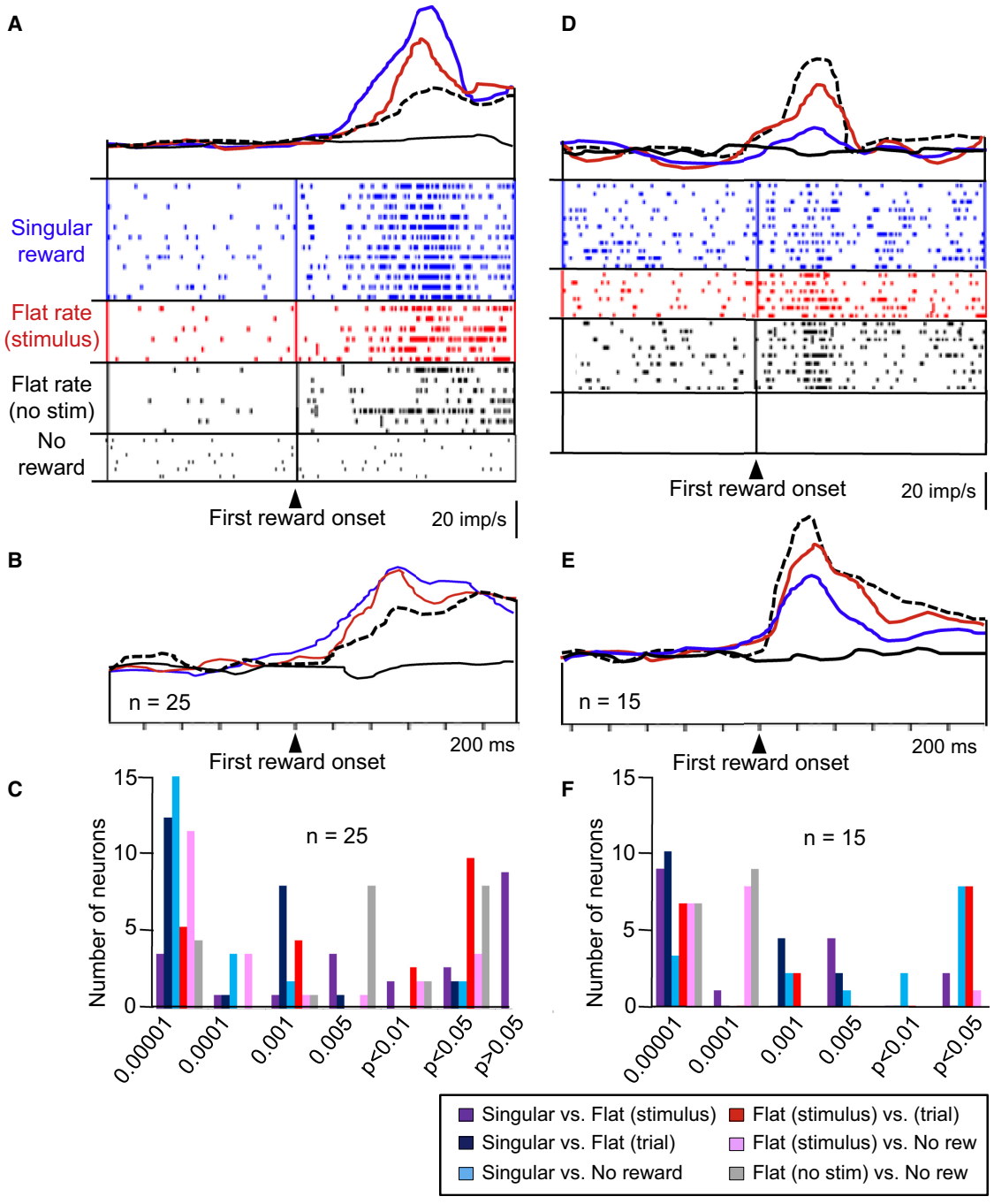

Figure 3. Increases of Neuronal Reward Responses to Onset of First Reward with Increasing Instantaneous Reward Probability

(A) Single neuron.

(B) Averaged population responses (n = 25 neurons). Same bin width (10 ms) and impulses/s calibration apply to (A) and (B). Color code in (A) and (B) same as for Figures 2A and 2B; dotted line indicates flat reward rate during entire trial without stimulus.

(C) Distribution of p values from Fisher's PLSD post hoc test following one-way ANOVA.

(D–F) The same as (A)–(C) but for decreases of neuronal reward responses with increasing instantaneous reward probability (n = 15 neurons).

tested neurons, respectively; $p > 0.05$, chi-square test; Figure 1E, squares). The responses were absent in explicit no reward trials, lowest to singular reward (Figure 3D, blue), higher with flat reward rate during stimulus B (red), and highest with flat reward rate during the trial (dotted). This relationship was observed in the neuron of Figure 3D ($p < 0.0001$, $F(3,30) = 17.78$, one-way ANOVA; $p = 0.0463$ singular versus flat rate during stimulus, $p = 0.0121$ flat rate during stimulus versus flat rate during trial, $p < 0.0001$ singular versus flat rate during trial, Fisher's PLSD test) and in all 22 neurons analyzed individually. The responses to flat rate reward during the stimulus varied only insignificantly between rewards delivered during the first and the second half of stimulus duration (Figure S1B; $p > 0.4$, t test). We tested 15 of the 22 neurons

also in explicit no reward trials and found similar significant differences in the average population activity of these neurons [Figure 3E; $p < 0.0001$, $F(3,56) = 18.005$, one-way ANOVA; $p < 0.02$ singular versus flat rate during stimulus, $p < 0.02$ flat rate during stimulus versus flat rate during trial, $p < 0.0001$ singular versus flat rate during trial, Fisher's PLSD test; Figure 3F]. The inverse relationship to instantaneous reward probability contrasted clearly with the positive relationship in the other neuronal group (Figure S2A). The responses in the two neuronal groups differed without overlap (Figure S2B).

Taken together, these 22 amygdala neurons showed an analogous but opposite instantaneous reward sensitivity to the 36 neurons described above. The less likely the reward was to occur at a given moment, the higher was the neuronal response, suggesting a relationship to temporal reward surprise. The similarity of responses to flat rate reward in the two stimulus periods suggested dependence on instantaneous reward rate rather than summed future reward. Such responses may reflect coding of positive temporal reward prediction errors, as observed previously [31, 32]. Further work may elucidate the nature and extent of time-sensitive prediction error responses of amygdala neurons.

Conclusions

These data show that the temporal structure of reward occurrence influenced the activity of amygdala neurons. Although there was no explicit requirement to monitor reward timing, the animals' behavior reflected well the experimentally imposed instantaneous reward probabilities. The temporal statistics of reward occurrence modulated two forms of reward signal in different groups of amygdala neurons. Prereward activity paralleled the behaviorally expressed reward expectation. It ramped up to a singular reward at stimulus end but stayed at a lower, tonic level with reward dispersed over the whole stimulus period. These temporal profiles reveal an internal anticipatory process rather than simple build up of sensory responses over time and thus reflect a fundamental characteristic of reward expectation. Responses following the reward were enhanced when a reward was either more likely or more surprising to occur. Both types of activity reflected predictability of instantaneous reward rate rather than overall sum of future reward. These modulations suggest that amygdala neurons have access to an internal clock that processes the time of future reward occurrence. In being sensitive to reward timing, amygdala neurons process a fundamental characteristic of reward function and thus may play a more profound role in reward than hitherto known. Neuronal reward signals sensitive to the expected time of reward occurrence may be involved in a wide range of behavioral functions, including allocation of behavioral resources to specifically timed rewards, planning of sequential steps of goal-directed acts, choices between temporally distinct rewards, and assignment of credit to specifically timed reward during novel reward learning, value updating, and economic decision making, as conceptualized by animal learning and economic decision theories.

Experimental Procedures

Animals and Behavioral Task

Two adult male *Macaca mulatta* monkeys (4.4 and 6.7 kg) used before [28] served for the experiment. All procedures conformed to US National Institutes of Health Guidelines and were approved by the Ethical Review Committee of the University of Cambridge and the Home Office of the United Kingdom. Each trial started when the animal contacted a touch-sensitive key. Three trial types used visual stimuli A–C and eye fixation. A 1.3° ocular fixation spot appeared after key touch at the center of a computer monitor placed 450 mm in front of the animals. At 1,150 ms plus mean of 500 ms (truncated exponential distribution) after fixation spot onset, a single central 7° fractal visual stimulus appeared with the fixation spot superimposed (Figure 1A). An infrared optical system tracked eye position with 5 ms resolution (ISCAN). Stimulus and fixation spot extinguished together at 2.0 s after stimulus onset. Key release or fixation break during fixation spot presentation constituted an error and led to trial abortion and trial repetition. Intertrial periods lasted 4.0 s (from stimulus offset to onset of next stimulus). Thus, cycle time was 6.0 s (stimulus plus intertrial). The fourth trial type required key touch, did not use the fixation spot and specific stimuli, and had the same cycle time of 6.0 s.

The instantaneous reward probability states the probability with which a reward will be delivered in the next interval from the perspective of the current interval (see Supplemental Information for details). With stimulus A, a singular reward occurred with $p = 1.0$ at the fixed time of stimulus offset (Figures 1A and 1B), resulting in increases of instantaneous reward probability toward stimulus end. With stimulus B, instantaneous reward probability immediately increased after stimulus onset and subsequently was flat at $p = 0.025/50$ ms for the rest of the 2.0 s stimulus period. Stimulus C was not followed by any reward. These three trial types alternated pseudorandomly, sequences being limited to three consecutive same-trial types. In a fourth trial type, reward occurred with a flat instantaneous probability of 0.025/50 ms throughout 5.0 s of the 6.0 s trial cycle and without any stimulus (reward delivery stopped during 1.0 s for data storage and preparation of next trial, unannounced to the animal). To make this trial type detectable in the absence of any stimuli, it was run in separate blocks from the other three trial types. Because none, one, or several rewards could occur with the flat probability schedules, reward occurrence corresponded to the "rate of occurrence of failure" for repairable systems in reliability engineering [33]. An electromagnetic, computer-controlled liquid solenoid valve delivered identical magnitudes of individual reward (Ribena juice) in all rewarded trial types and emitted a noticeable, low-intensity click.

Data Acquisition and Analysis

We recorded the activity of single neurons with single moveable microelectrodes and standard electrophysiological techniques during task performance while monitoring licking movements (see Supplemental Information for details). We used the paired Wilcoxon test on neuronal activity in each trial against control activity to assess onset and significance of prereward activations and postreward responses. We subsequently determined the influence of temporal reward structure on neuronal activity by comparing the Wilcoxon-identified activations between the different trial types with one-way ANOVA followed by Fisher's post hoc test and, independently, Spearman correlation (see Supplemental Information for details).

Supplemental Information

Supplemental Information includes two figures, Supplemental Introduction, Supplemental Results, Supplemental Experimental Procedures, and Supplemental Discussion and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.07.062.

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Supplemental Information

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Supplemental Inventory

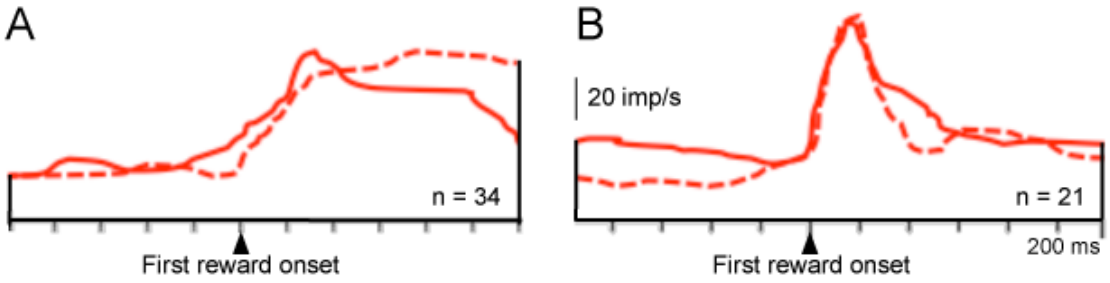

Figure S1. Lack of Dependence of Responses to Reward Delivery on Predicted Sum of Future Rewards

(**A**) Averaged population response in 34 neurons (1 trial per neuron), showing increased responses with increasing instantaneous reward probability. The responses failed to vary between first and last seconds of stimulus presentation (dotted and solid lines, respectively).

(**B**) Same as A, but for 22 neurons showing decreased responses with increasing instantaneous reward probability.

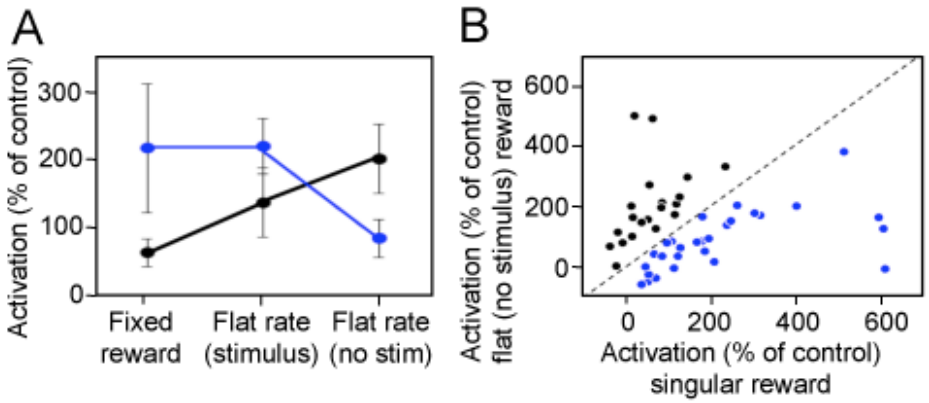

Figure S2. Quantitative Comparisons of Modulations of Neuronal Reward Responses by Instantaneous Reward Probability

(A) Median response strength modulated by instantaneous reward probability. Blue: responses with positive relationships; N=36 neurons. Black: negative relationships; N=22 neurons (±95 confidence intervals).

(B) Scatter plot demonstrating the effects of instantaneous reward probabilities. Blue: higher responses with singular reward than with flat reward rate during trial; N=34 neurons. Black: higher responses with flat reward rate during trial than with singular reward; N=20 neurons. Four neurons are not shown because of outlier (>750%) activations.

Supplemental Introduction

Time is of fundamental importance for behavioral reward processes. Humans and animals estimate time intervals with great accuracy [1, 2], which decays proportionally with delay but is timescale-invariant for constant delay:mean ratios (Weber-type, scalar expectancy theory; [3-5]). Reward intervals and rates are of crucial importance for conditioning and affect key learning variables such as contingency [5]. Theoretical reinforcement models are particularly efficient when taking time into account [6]. Conditioning requires optimal stimulus-reward intervals and becomes less efficient with shorter or longer intervals [7, 8]. Changes in reward timing induce learning and unblocking [9]. Subjective reward value is discounted with increasing delays to reward [10-12]. Thus, the crucial reward functions are highly sensitive to timing.

Despite the importance of timing and temporal structure for reward functions, we know little about underlying neuronal processes. Changes in dopamine neurotransmission following lesions or receptor binding drugs affect timing behavior [13-15], possibly reflecting an enabling function of dopamine. Neuronal responses in rat primary visual cortex vary with predicted reward delays [16]. Responses to reward predicting stimuli in cortical and subcortical reward neurons decrease with increasing delays [17-20], reflecting temporal discounting of reward value rather than specific temporal structure in reward predictions. Neurons in the striatum and several cortical areas show anticipatory activity that varies with instantaneous temporal probabilities of visual or somatosensory stimuli or movements [21-26]. However, it is unclear how these phenomena apply to reward processing. Anticipatory activity in thalamic, striatal and orbitofrontal neurons ramps up to the expected time of reward and is displaced by temporal shifts of reward [27-29]. Although suggesting sensitivity to reward timing, the displacements could result from delayed or slowed onsets of stimulus driven recruitment of neuronal activity. The only suggestion for sensitivity to temporal structure of reward predictions derives from the dopamine prediction error response elicited by temporally shifted rewards [30, 31], although this effect may be specific for error responses. Taken together, there is surprisingly little study of sensitivity to specific temporal reward structures.

The amygdala is a major component of the brain's reward system. Amygdala lesions disrupt behavioral reward processes and associated brain activations in humans [32-34] and impair several components of reward related behavior and reward learning in animals [35-39]. Amygdala neurons respond to reward predicting stimuli and reward delivery [40-45] and show slower activations preceding behavioral responses and reward [41, 46, 47].

Despite the importance of time in behavioral reward processes, and despite the demonstrated involvement of the amygdala in several reward processes, the temporal sensitivity of amygdala reward signals is unknown. The current study varied the instantaneous probability of reward occurrence in order to investigate the neuronal sensitivity to temporal reward structure. We tested the two principal and representative forms of amygdala reward signals, namely anticipatory activity preceding reward and responses to reward delivery. Our data suggest that both types of amygdala reward signal are sensitive to reward timing.

Supplemental Results

Responses to Reward Delivery

The responses to reward delivery with flat instantaneous reward rate during the stimulus varied only insignificantly between rewards delivered during the first second and the last second of the 2 s stimulus duration. This result refers to both activations increasing with

increasing instantaneous reward probability (Fig. S1A; P>0.1, t-test) and to activations decreasing with increasing instantaneous reward probability (Fig. S1B).

The differences in modulation by instantaneous reward probability in the two groups of amygdala neurons were confirmed by further quantitative comparisons. Both types of modulation showed high Spearman rank correlation coefficients (rho=0.725 and 0.851 for positive and negative relationships to instantaneous probability, respectively), but their slopes were opposite to each other (Fig. S2A blue vs. black). The separation of the two neuronal groups was also visible when plotting reward responses for flat reward rate against singular reward (Fig. S2B). Thus, instantaneous reward probability affected the responses to reward delivery in two distinct manners in the tested amygdala neurons.

Supplemental Experimental Procedures

Instantaneous Reward Probability

To achieve flat instantaneous reward probability during stimulus B, the computer advanced through the 2.0 s stimulus period in time steps of 50 ms and chose at every step an equally probable random number between 1 and 40; it marked that step when number 1 occurred. It delivered one unit of reward at every marked step. Thus no, one or several rewards could occur during a single stimulus. To avoid large variations, we recalculated reward occurrences for any 2.0 s stimulus period that contained more than 3 rewards. The delivered rewards summed to 10 within 10 trials. Thus, in any 50 ms interval during the 2 s stimulus period, the probability of receiving a reward in the next interval was p=0.025, which summed to p=1.0 in the 40 time steps during the stimulus. Through this procedure, the stimulus produced an extended, uniform, flat and constant instantaneous reward probability (Fig. 1B). In the fourth trial type without any stimulus, flat instantaneous reward probability was achieved in a similar manner, but the probabilty of p=0.025 applied to 5.0 s of the 6.0 s trial cycle.

Neuronal Recordings

After 5-7 months of behavioral training, a head holder and a recording chamber were fixed to the skull under general anesthesia and aseptic conditions. Recordings using tungsten microelectrodes and standard electrophysiological techniques and served to visualize impulses on oscilloscopes and transform them by threshold discrimination into binary electrical signals for 2 kHz sampling. We estimated the position of the amygdala from bone marks on frontal and lateral radiographs taken with an electrode guide cannula inserted at known coordinates relative to the stereotaxically implanted chamber [48]. Electrode positions were reconstructed in one animal from small electrolytic lesions (15-20 µA x 20-60 s) on 50 µm thick, cresyl violet-stained histological brain sections. As histological reconstruction was not available for the second animal for reasons of ongoing recordings, we reconstructed recording positions approximately from radiographic images. We collapsed recording sites from both monkeys spanning 3 mm in the anterior-posterior dimension onto the same coronal outline (Fig. 1D, E).

Data Acquisition and Analysis

Animals performed at least eight trials of each type for data acquisition (mean n=15 trials). We monitored licking movements by tongue interruptions of an infrared light beam at the liquid spout (STM Sensor Technology; 0.5 ms resolution). We assessed anticipatory licking as total durations of tongue interruptions during 2.0 s immediately preceding the stimuli and during the 2.0 s stimulus period and compared them between trial types (two-way Anova). To

avoid capturing reward reactions with flat instantaneous reward probability, we assessed licking only in trials in which the probabilistic schedule produced no reward.

We identified prereward activations in individual neurons by comparing activity increases between a fixed 400 ms time window immediately preceding reward onset and a standard 400 ms control period immediately preceding stimulus onset in the same trials with the nonparametric, one-tailed, signed-rank, matched-pairs Wilcoxon test ($P<0.05$). We expressed neuronal activations as percent of control period activity. We then compared the Wilcoxon-identified prereward activations with one-way Anova ($P<0.05$) across trial types using stimuli A-C. Fisher's PLSD posthoc test served to locate the activity differences ($P<0.05$). We used Spearman's rank correlation coefficient rho for additional, independent assessment of graded activity differences between trial types with differently timed rewards.

We used two measures to compare the speed of onset of the Wilcoxon-identified prereward activations between the different trial types. In the first measure we determined the latency of significant activations with a sliding time window procedure [49] which applied a Wilcoxon test ($P<0.01$) between a 100 ms time window and the constant 400 ms prestimulus control period. The window was moved in steps of 100 ms from stimulus onset through the stimulus period. We defined onset of activation at the center of the first 100 ms time window showing significant activation. In the second measure we compared activation strength in the interval of 600-1,000 ms after stimulus onset, during which amygdala neurons showed substantial activations with flat rate reward (Fig. 2A, B), using one-way Anova with Fisher's PLSD posthoc test. When analyzing data from trials with flat instantaneous reward probability, we considered only those trials in which the probabilistic reward schedule produced no rewards, to avoid contamination by potential reward responses.

We identified responses to reward delivery in individual neurons by comparing activity between a fixed 400 ms time window immediately following reward onset and the standard prestimulus 400 ms control window in the same trials with the Wilcoxon test ($P<0.05$). In trials using flat instantaneous reward probability without any stimulus, we aligned neuronal responses to the first reward after an initial period of 400 ms in each trial. We used the 400 ms prereward period as control period in these trials. We normalized the Wilcoxon-identified reward responses to control period activity and compared them with one-way Anova ($P<0.05$; followed by Fisher PLSD test) across all four trial types.

Control for Mouth Movements

We discarded two neurons whose activity showed temporal relationships to licking. These responses were closely related to tongue extension and retraction, resembling previously reported mouth movement-related activity in striatal neurons [50].

Supplemental Discussion

Task and Behaviour

The instantaneous reward probabilities imposed specific temporal reward structures and thus determined the temporal reward prediction during specific task epochs. Our instantaneous rate of occurrence of reward is analogous to the 'rate of occurrence of failure' (ROCOF) for repairable systems in reliability engineering [51] and reflects a discrete renewal process [52]. As with quickly repairable failures, our schedule allowed several rewards to occur during a stimulus or trial and thus defined reward prediction over the whole stimulus duration (stimulus B) or during the whole trial (fourth trial type). ROCOF differs from failure rate or hazard rate which refers to the conditional probability of event occurrence given that the

event has not yet occurred. Thus, failure or hazard rate would apply only to the first reward during the stimuli or trial and drop to zero afterwards, different from the current schedule.

Delivery of the singular reward at stimulus end is the usual way of reward delivery in most experiments. This temporal structure elicited licking during two periods. An early peak of licking followed the stimulus and constituted a simple conditioned response. A second phase of licking anticipated the time of reward and likely reflected the animals' reward expectation. By contrast, the flat instantaneous reward probability during the stimulus induced rather tonic anticipatory licking. Thus, the different licking patterns corresponded to the different temporal reward structures and likely reflected the animals' temporal reward expectations.

The current licking data corroborate earlier results suggesting behavioral sensitivity to temporal structure. During temporal discounting, anticipatory licking in monkeys peaks later with longer delays [19, 31]. During the temporal bisection procedure, monkeys distinguish well between two different, subsecond stimulus durations [23]. With movement triggering stimuli, ocular reaction times anticipate stimulus occurrence [26]. Thus, the current results are in line with previously reported behavioral sensitivity to temporal reward structure in monkeys.

Activity Preceding Reward

The experimentally imposed temporal structure affected the prereward activations of amygdala neurons in parallel with the behavioral licking responses. With singular reward, neuronal activity increased towards stimulus end, during a period in which anticipatory licking before the reward was highest. With flat instantaneous reward probability, the earlier, longer, more tonic and smaller increase of neuronal activity during most of the stimulus period paralleled the flat and low instantaneous reward probability and the prolonged anticipatory licking. The anticipatory activities did not just reflect anticipation of reward as such but, importantly, indicated when it would occur and at which rate. In the flat rate reward trials, the instantaneous rate of reward stayed constant during the stimulus whereas the sum of future rewards increased due to the probabilistic schedule. Thus, the flat neuronal activations during these trials seemed to reflect the prediction of the flat instantaneous reward rate rather than prediction of the sum of future rewards. These data suggest that these reward signals in the amygdala neurons have access to internal representations of temporal reward structure evoked by the specific reward predicting stimuli. In being sensitive to time, they process one of the most fundamental properties of reward expectation.

Our use of different temporal reward structures helps to resolve alternative interpretations. First, the different activities might simply reflect differences between the specific sensory stimuli predicting the two temporal structures. However, both the time courses and magnitudes covaried in a systematic and consistent fashion with the different imposed temporal structures and the resulting behavioral licking responses. None of the neurons showed a reverse relationship, namely sustained low activation with singular reward at stimulus end and ramping activity with flat instantaneous reward probability. These observations make a simple sensory relationship unlikely. Second, the gradual increase in neuronal activity toward the singular reward at stimulus end might result from a slow build up of neuronal activity induced by the stimulus. However, neuronal activity with flat reward rate paralleled the flat instantaneous reward probability and the corresponding licking behavior. These differences in time course argue against a neuronal build up induced by the stimulus. Third, reward rate was identical between the singular and flat reward schedules, thus making a simple relationship to value unlikely. Taken together, the observed temporal sensitivity of prereward activity reflected a typical characteristic of reward expectation.

Neuronal responses in monkey parietal cortex are sensitive to differently delayed discriminatory cues during temporal bisection [23] and show time dependent anticipatory activity that reflected the hazard rates of stimuli triggering eye movements [26]. Interestingly, time sensitive reward anticipatory activity occurred also in primary visual cortex [16]. Our data demonstrate temporal sensitivity of anticipatory activity in a typical reward structure. The combined evidence suggests that neurons may code the anticipated time for a range of behavioral events, such as sensory discrimination, movement and reward. However, it remains to be investigated whether these temporal influences derive from a central processor that operates independent of sensory discrimination, movement and reward. Alternatively, these functions may rely on their own timing devices.

Responses to Reward Delivery

The observed neuronal responses to reward delivery showed striking sensitivities to instantaneous reward probability in parallel with behavioral licking. The temporal modulations of these straightforward responses reflected the time varying strength of reward prediction at the moment of its delivery. The effects of reward predictability reflected the instantaneous reward rate, rather than sum of future rewards, but incorporated overall predictability, as shown by the differences in responses to flat rate rewards between presence and absence of stimulus. Together with the anticipatory coding of temporal reward expectation by prereward activations, the two major types of reward signals in the amygdala are highly sensitive to time.

Reward responses in one group of amygdala neurons were lowest to rewards delivered at flat instantaneous reward probability of p=0.025/50 ms over the entire trial, and highest to singular reward delivered with probability of p=1.0 at stimulus end. Responses with flat reward rate during the stimulus were intermediate between the two temporal profiles, possibly reflecting greater focus of reward expectation onto the stimulus period. Thus the modulations varied positively with the temporal predictability of reward. They might function to maintain established, temporally specific reward predictions after reinforcement learning. The positive relationship to instantaneous reward probability resembled the attentional modulation seen in monkey visual cortex V4 which paralleled the hazard function of stimulus change during individual trials [21]. Our data demonstrate that such temporal modulations are not restricted to attentional processes but occur also with rewards.

Reward responses in the second group of amygdala neurons showed the inverse relationship, increasing with lower instantaneous reward probability. They were lowest to the singular reward delivered with probability of p=1.0 at stimulus end, higher with rewards delivered with lower instantaneous reward probability during the stimulus, and highest with low probability rewards distributed over the entire trial. Such responses may reflect coding of positive temporal reward prediction errors, as observed previously [53, 54]. Although these studies did not address temporal relationships and our study did not test reward omission, the combined evidence suggests that time might influence the limited reward prediction error coding by amygdala neurons. The temporal influence may derive from dopamine projections [55] involved in interval timing [13, 15] and coding bidirectional reward prediction errors in a time sensitive manner [19, 30, 31]. The time specific reward responses are broadly analogous to the movement specific reward responses in dorsolateral and orbital prefrontal cortex [56], suggesting that reward signals in general may distinguish also between behavioural variables other than value and risk. Further work may elucidate the nature and extent of time sensitive prediction error responses of amygdala neurons.

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