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COGNITIVE NEUROSCIENCE

Risk-preference differentiates orbitofrontal cortex responses to freely chosen reward outcomes

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Abstract

To successfully evaluate potential courses of action and choose the most favorable, we must consider the outcomes that may result. Many choices involve risk, our assessment of which may lead us to success or failure in matters financial, legal or health-related. The orbitofrontal cortex (OFC) has been implicated as critical for evaluating choices based on risk. To measure how outcomes of risky decisions are represented in the OFC, we recorded the electrophysiological activity of single neurons while rats made behavioral responses to obtain rewards under conditions of either certainty or risk. Rats exhibited different risk-preferences when given the opportunity to choose. In risk-preferring rats, OFC responses were enhanced following the delivery of large rewards obtained under risk compared with smaller, certain rewards and reward omission. However, in risk-neutral rats, neurons showed similarly enhanced responses to both large rewards obtained under risk and smaller, certain rewards compared with reward omission. Thus, the responses of OFC neurons reflected the subjective evaluation of outcomes in individuals with different risk-preferences. Such enhanced neural responding to risky rewards may serve to bias individuals towards risk-preference in decision-making.

Introduction

Adaptive decision-making requires us to weigh many factors – reward magnitude, probability and risk – which ultimately establish the value of a potential course of action. The factor of risk takes into account the impact of potential loss or gain for an uncertain outcome. Patients with orbitofrontal cortex (OFC) damage are insensitive to differing conditions of risk in decision-making (Bechara *et al.*, 2000; Hsu *et al.*, 2005). Neuroimaging studies in humans also implicate the OFC as a critical component of the circuitry responsible for judgments to obtain rewards. Activity in the OFC has been shown to encode expectations about reward outcome (Breiter *et al.*, 2001), risk of obtaining reward (Tobler *et al.*, 2008) and ambiguity during decision-making (Hsu *et al.*, 2005). Elevated responses in the OFC to risky or unusual outcomes may reflect affective encoding of highly salient information, updating the value of potential courses of action (Bechara *et al.*, 2000; Kringelbach, 2005). Overvaluation of an outcome obtained under risk, as represented in the OFC, may reinforce future preference for that risky alternative.

Animal models complement human imaging studies by directly measuring activity at the level of single neurons in circuitry related to reward processing and decision-making. In non-human primates, OFC neurons are modulated by the expected value of upcoming reward (Roesch & Olson, 2004, 2007). In rats, OFC responses are stronger when they receive immediate or large rewards over delayed or small rewards, suggesting that the value of the outcome is encoded. Although rats with OFC lesions fail to evaluate the outcomes of risky choices in the same manner as intact subjects (Pais-Vieira *et al.*, 2007), it is not known how certain and risky outcomes may be differentially encoded by single OFC neurons. Such findings could offer a mechanism for the differences observed in imaging studies.

The goal of the current study was to compare OFC responses to the receipt of freely chosen risky reward outcomes to those same outcomes obtained without choice. We recorded the activity of single neurons while rats pressed one of two levers – the 'certain', which resulted in the delivery of two sucrose pellets on every trial; or the 'risky', which provided either four sucrose pellets or reward omission. Rats first performed a block of 'forced response' trials in which the certain and risky levers were presented alone, in alternation. Subsequently, rats performed a block of 'free choice' trials, in which both levers were presented simultaneously and rats were able to choose between them. When provided with the opportunity to choose, rats could opt to receive certain reward on every trial, thus the outcome from the risky lever on any given trial yielded a relative loss (reward omission) or gain (large reward). Thus, we measured OFC responses to the same reward outcomes (0, 2 or 4 sucrose pellets) in different contexts of risk. We hypothesized that OFC responses would be modulated by reward outcome, and that modulation would be enhanced under conditions of free choice.

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Materials and methods

Subjects

Fourteen male Sprague–Dawley rats (300–350 g) were used for behavioral testing in the risk task. Six of these animals were implanted with recording electrodes in the OFC (see below). The remaining eight rats were implanted with recording electrodes in the nucleus accumbens (NAc), and are not discussed in the electrophysiological results sections here. All rats were individually housed with access to a minimum of 20 g of chow per day and *ad libitum* water, with a 12 : 12 h light : dark cycle (lights on at 07:00 h). Experiments were conducted in the light phase between 10:00 and 15:00 h. All procedures were approved by the University of Illinois at Chicago Animal Care Committee in accordance with the ethical guidelines set by the National Institutes of Health.

Surgical procedures

Custom-designed (Micro Probe, Gaithersburg, MD, USA) electrode arrays, organized into two columns of four microwires (50 μm diameter; tip separation 0.25 mm spanning 1 mm) were stereotaxically guided into the OFC in six rats under ketamine (100 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.) anesthesia. Bilateral arrays were centered at AP +3.0, ML ± 3.2 relative to bregma and −4.0 relative to the surface (Paxinos & Watson, 2007). Ground wires for each array were inserted into the brain remote to the electrode arrays. Connectors for the microwire arrays are anchored to the skull via stainless steel screws and dental acrylic. Electrode arrays were implanted 1 week prior to behavioral training, and typically 2 weeks before the first recording.

Behavioral test chamber

Recording sessions were conducted in standard operant chambers (30.5 × 24.1 × 21.0 cm; Med Associates, St Albans, VT, USA). One side of the chamber contained two retractable levers with cue lights positioned directly above them. Separating the levers is a food receptacle port where sucrose pellets (45 mg sucrose; BioServ, Frenchtown, NJ, USA) were delivered. Head entry into the food receptacle was measured with a photobeam. Events were controlled (cue light illumination, lever extension, etc.) and monitored (lever press, head entry, etc.) by a PC computer using a commercially available software program (MED-PC; Med Associates). Events coincided with TTL outputs that were time-stamped to enable temporal alignment of electrophysiological activity.

Risk task

Each behavioral session consisted of two blocks of trials: 'forced response' and 'free choice'.

Forced response

During the first 20 trials, one of two possible lever/cue light combinations was presented on each trial. For each subject, one lever was designated as 'certain' and the other 'risky', with the designation counterbalanced across rats. On the first trial, the certain lever was extended and a cue light illuminated above the lever. When pressed, the lever retracted, the cue light was extinguished and two 45-mg sucrose pellets were dispensed into the food dish. The first pellet was delivered 0.4 s following the press and the second 0.65 s later. Head entries into the pellet receptacle were continuously monitored by a photobeam, and the times of beam-breaks were recorded. A 30–40-s variable interval separated consecutive trials. On the second trial, the risky lever was extended and a cue light illuminated above it. When pressed, the lever retracted, the cue light was extinguished, and either zero or four 45-mg sucrose pellets were dispensed. When four pellets were administered, the first was delivered 0.4 s following the press, and the remaining three at 0.65-s intervals (last pellet at 2.35 s following press). The reward outcome following uncertain presses was determined randomly, with replacement, on each trial to ensure unpredictable outcomes across a series of trials. For the remaining trials, each lever was presented in alternation. The 'forced response' block ensured that subjects had equal experience with both levers and their associated outcomes at the start of each session. Although rats received uncertain outcomes on half of the trials, they did not have to decide which behavioral response to make.

Free choice

Immediately following the initial block of 20 trials, subjects had an additional 40 'free choice' trials. On each trial, both levers were extended simultaneously and both cue lights were illuminated above them. Both levers remained available until one was pressed by the rat. Upon the behavioral response, the levers were retracted, both cue lights extinguished and sucrose pellets (if any) were dispensed into the pellet receptacle. For certain choices the rat received two sucrose pellets, and for risky choices he received either zero or four sucrose pellets, assigned randomly with replacement, so that subjects could not anticipate or track outcome across the session. The alternative would have been to set a maximum of 20 large rewards that could be obtained during the 40 free choice trials. This was not employed so that rats could not track the cumulative number of rewarded risky trials (and prior probability of receiving the large reward) as the session progressed. It is important to note that in the free choice block, rats had the option to receive two pellets on every trial. Receiving four pellets following a risky press was therefore a relative gain for those trials, while reward omission was a relative loss compared with the certain outcome.

Electrophysiological recordings

Before the start of the recording session, the rat was connected to a flexible recording cable (Plexon, Dallas, TX, USA) attached to a commutator (Crist Instrument Company, Hagerstown, MD, USA) that allowed virtually unrestrained movement within the chamber. The headstage of each recording cable contained 16 miniature unity-gain field effect transistors. The activity of single neurons was recorded differentially between each active and an inactive (reference) electrode from the permanently implanted microwire arrays. The inactive electrode was examined before the start of the session to verify the absence of neuronal spike activity and served as the differential electrode for other electrodes with cell activity. Online isolation and discrimination of neuronal activity was accomplished using a commercially available neurophysiological system (multichannel acquisition processor; MAP System, Plexon). Another computer controlled behavioral events of the experiment (Med Associates) and sent digital outputs corresponding to each event to the MAP system to be time-stamped along with the neural data. Typically one or two neurons were recorded per active microwire (Roitman *et al.*, 2005). Principal component analysis (PCA) of continuously recorded waveforms was performed prior to each session and aided in the separation

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of multiple neuronal signals from the same electrode. During the recording session, waveforms that matched the templates generated by PCA were collected as the same neuron. Cell recognition and sorting was finalized after the experiment using the OFFLINE SORTER program (Plexon), which assessed neuronal data based on PCA of the waveforms, cell firing characteristics and inter-spike intervals. Data were exported to MATLAB (Mathworks, Natick, MA, USA) and STATISTICA (StatSoft, Tulsa, OK, USA) for statistical analyses. Electrode arrays were implanted bilaterally, so that we were able to record neurons contralateral and ipsilateral to both the certain and risky levers.

Data analysis

For each trial, 'baseline' activity was calculated as the firing rate (spikes/s) in the 5 s preceding lever presentation. Variability of the inter-trial interval prevented rats from anticipating lever presentation on each trial. Thus, this interval was selected to be most distant in time from the preceding lever press, but not affected by activity related to anticipation of a predictable event. To examine neural responses to 'reward evaluation', we calculated the average firing rate during the epoch 2–4 s following the lever press for every trial in the session. This time window was selected to include the time that reward outcome of the trial was known (see 'Risk task' above) without introducing a pre-selection bias for units with sustained activity. Results of subsequent analyses of activity during reward evaluation did not depend critically on using this specific epoch, as screening with other epochs following the initial post-press period did not alter the results. We used two-sided *t*-tests to compare responses during the epoch with baseline activity. Supporting information, Fig. S1, shows the correlation between the change in firing rate for forced response and free choice blocks in the neurons that showed significantly different responses from baseline 2–4 s following the lever press. Because individual neurons had different baseline levels of activity (supporting Fig. S2), averages for a population of neurons were calculated based on normalized firing rates. To normalize each neuron's activity, we divided the firing rate across the trial by the average baseline firing rate across all trials of that neuron. Normalization by converting firing rates to *z*-scores did not alter the observed results. Neurons that showed significant increases or decreases in firing rate following lever presses were further analysed with weighted means ANOVA to detect differences in response due to reward outcome, availability of choice and risk-preference, and to determine interactions between these factors. ANOVAs were conducted on three non-overlapping time windows: 2–5, 6–10 and 11–15 s following lever press. Main effects were tested with Unequal N HSD *post hoc* tests.

Individual neurons were also tested for transient changes in activity during the intervals immediately following lever presentation and lever press. These analyses and results are discussed in the supporting Appendix S1.

Histological verification of recording sites

Rats were deeply anesthetized with a lethal dose of sodium pentobarbital (100 mg/kg; i.p.). To mark the placement of electrode tips, a 100-μA current was passed through each electrode of the microelectrode array for 4 s. Transcardial perfusions were then performed using physiological saline followed by 3% potassium ferrocyanide in a 10% formalin solution. After post-fixing and freezing, 50-μm coronal brain sections were taken using a cryostat and mounted. The potassium ferrocyanide reacts with deposited iron in the electrodes to reveal a blue reaction product corresponding with the location of an electrode tip. The specific position of individual electrodes was assessed by visual examination of successive coronal sections. Figure S3 shows the extent of placements of electrode tips within the OFC as determined by examining the relative position of observable reaction product to visual landmarks represented in a stereotaxic atlas (Paxinos & Watson, 2007).

Results

Rats show consistent risk-preferences

When allowed to freely choose between levers yielding certain and risky payoffs, rats most often preferred the risky option. We measured risk-preference as the proportion of risky lever presses on free choice trials. Each line in Fig. 1A represents risk-preference for a single rat across three behavioral sessions. Performance differed across rats (ANOVA, $F = 6.866$, $P = 0.00001$), but not across days ($F = 0.701$, $P = 0.502$), suggesting that individual rats exhibited stable risk-preferences. This preference was not affected by the actual rewards rats received for risky choices. As outcome was determined randomly on each risky trial, the proportion of large payoffs varied from session to session. We observed no significant correlation between the frequency of receiving the large payoff and risk-preference (Fig. 1B; $r^2 = 0.03$, $P = 0.30$). The line in Fig. 1B is the best fit to the data by linear regression, with a slope not different from 0 (slope = −0.38; CI:

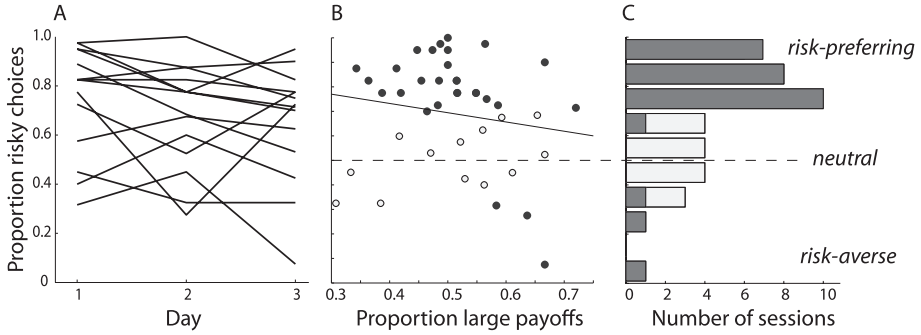

FIG. 1. Behavioral performance on risk task. (A) Proportion of risky choices across three behavioral test sessions. Each line represents a single rat. (B) For each session, the proportion of risky choices is plotted as a function of the proportion of risky choices resulting in the large payoff, which was determined at random, with replacement. Filled symbols indicate sessions in which the proportion of choices differed from chance. The line shows best fit to data by linear regression. (C) Histogram summarizing the total number of sessions grouped by proportion of risky choices. Shading indicates sessions in which there was a significant proportion of choices favoring one lever over the other.

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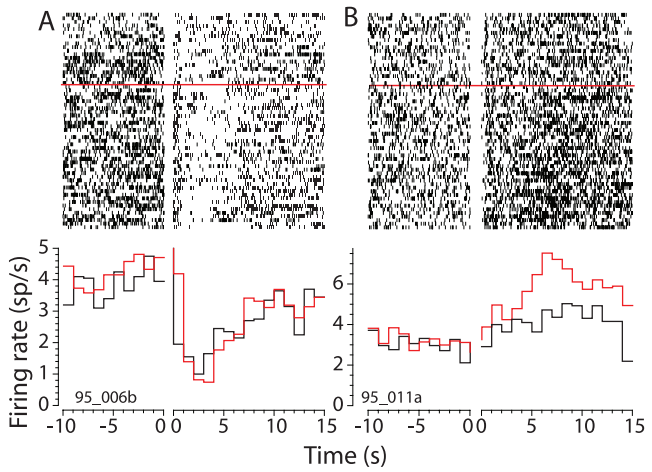

FIG. 2. Examples of single OFC units with phasic decreasing (A) or increasing (B) activity following lever press. Rasters (top) show 10 s of neural activity before lever presentation (−10 to 0 s) before the break in the *x*-axis, and 15 s of activity aligned to time of press (0–15 s). The horizontal red line separates the rasters of data obtained during forced response trials (above) from free choice trials (below). Histograms (bottom) show the average firing rate (in 1-s time bins) for forced response (black line) or free choice (red line) trials separately. Activity during the variable interval between lever extension and press is not shown.

−1.10 to 0.35), suggesting that rats did not adjust their behavior according to its consequences in individual sessions. In 29 of the 42 sessions shown in Fig. 1B, rats reliably preferred one lever over the other (filled circles, difference from 0.50 at $P < 0.05$). Risk-preference across all sessions is summarized in Fig. 1C. In the majority of sessions where one lever was preferred (26/29), rats were significantly 'risk-preferring', consistently choosing the risky lever when given a choice. In 13 of the sessions, rats were 'neutral' and did not show a significant preference for either lever (open symbols/bars). In the remaining three sessions, rats were 'risk-averse', significantly preferring the certain lever. Thus, on free choice trials, rats showed consistent individual differences in risk-preference across days that did not closely track trial-to-trial consequences.

Response times (RTs) to press the lever on each trial also reflected the animals' behavioral preferences. The average RT fluctuated between animals (range: 0.83–2.48s, ANOVA, $F = 14.07$, $P = 0.00001$), we therefore compared normalized RT by dividing RT on each trial by that subject's average RT. In the forced response block, we observed different patterns of RT, with risk-neutral rats making slower responses to the risky lever than risk-preferring rats (ANOVA, pref × lever, $F = 4.93$, $P = 0.029$, *post hoc* LSD for risky press RT, $P = 0.037$). During the free choice block, RT was overall faster than for forced responses ($F = 27.49$, $P = 0.00001$), with no differences between groups of rats or lever selected (supporting Fig. S4). Faster performance on choice trials may reflect increased general arousal or attentiveness during this block (Robbins, 2002).

OFC neurons respond during reward evaluation

We recorded the activity of multiple single neurons in the OFC during behavioral sessions using previously established techniques (Roitman *et al.*, 2005). For each rat, we analysed the neural data from one session to examine how neural responses during reward evaluation were affected by reward size, condition of choice and risk-preference. Sessions were analysed for risk-neutral and -preferring rats, but not risk-averse, as there were not sufficient neural data. To determine whether neurons were modulated by task events, we defined baseline activity as the firing rate in the 5 s preceding lever presentation for each of 90 neurons recorded from six rats. Consistent with previous reports (Roesch *et al.*, 2006), the baseline firing rate of OFC neurons was 3.12 spikes/s (SEM = 0.22). There was a strong correlation between baseline firing rate in the forced response and free choice blocks (supporting Fig. S2; $r^2 = 0.80$, $P = 0.0001$). Linear regression indicated that baseline activity in choice block was approximately 20% lower than in the forced response block (slope = 0.79; CI: 0.71–0.87). A subset of neurons showed transient responses to the presentation of the lever (see supporting Figs S5 and S6).

OFC neurons responded with changes in activity during the period following reward delivery. We observed 28 neurons (31%) that 'decreased' activity during reward evaluation, and 24 (27%) that 'increased' activity. Figure 2 shows examples of single neurons with

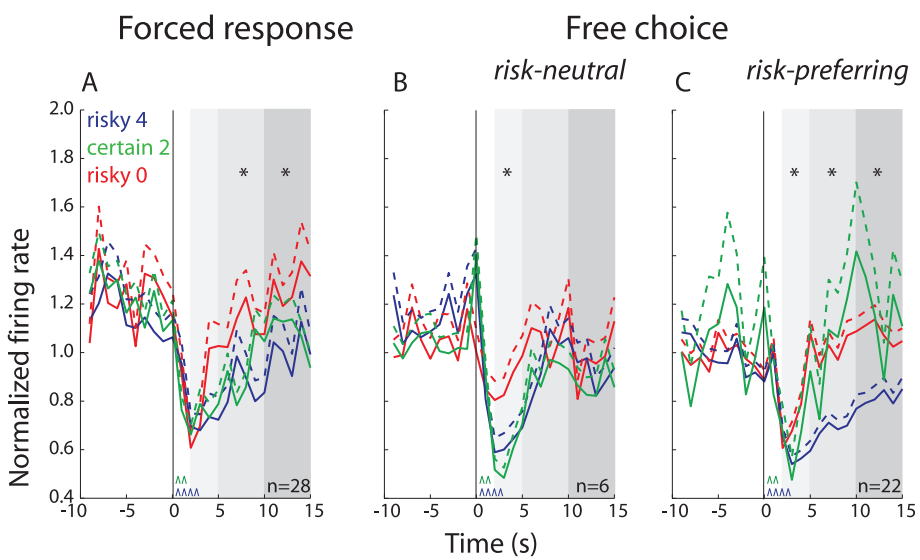

FIG. 3. (A–C) Average responses for 28 decreasing OFC neurons, aligned to time of lever press. The color of the line indicates reward outcome (0, 2 or 4 sucrose pellets) following lever press. The dashed line shows +1 standard error of average. Green and blue carets mark the time of pellet delivery for certain and risky-high payoff trials. The shading of the background denotes three non-overlapping epochs of analysis, 2–5 s, 6–10 s, 11–15 s. Asterisks mark epochs of significant modulation by outcome.

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decreasing or increasing activity. The rasters in the top half of each panel are divided by the red line into forced response (above) and free choice (below) trials. Below the rasters, peri-event histograms show the average response to lever extension for forced response (black lines) and free choice (red lines) trials. Decreases in activity, as shown in Fig. 2A, are similar to those reported in recordings of NAc neurons in response to primary rewards (Roitman *et al.*, 2005; Taha & Fields, 2006). Increases in OFC activity following the lever press, such as those shown in Fig. 2B, are consistent with prior reports (Schoenbaum *et al.*, 2006). There was no anatomical gradient that explained the observance of increasing and decreasing neurons, as they were recorded from neighboring wires of electrode arrays or, in some cases, from the same wire. Both increasing and decreasing responses were recorded from every rat.

Further analysis of the single neuron examples in Fig. 2 revealed that changes in activity were modulated by aspects of the task. The decreasing neuron (Fig. 2A) had responses that differed by outcome 2–5 s following the lever press (not shown, $F = 6.83$, $P = 0.002$). Although responses were reduced to a greater degree during this epoch, the difference did not reach significance ($F = 1.99$, $P = 0.16$). Activity of the increasing neuron (Fig. 2B) was modulated by reward outcome 6–10 and 11–15 s following the lever press (not shown, 6–10 s, $F = 13.58$, $P = 0.0001$; 11–15 s, $F = 4.31$, $P = 0.018$), and there was a larger increase for free choice trials in both epochs (6–10 s, $F = 17.34$, $P = 0.00001$; 11–15 s, $F = 9.08$, $P = 0.004$). We hypothesized that the magnitude of increases and decreases in OFC during reward evaluation would be modulated by multiple aspects of the task, including trial outcome (i.e. number of sugar pellets received), as well as whether the outcome was obtained under conditions of choice. We also examined whether differences in neural responses correlated with risk-preference.

Outcome, choice and risk-preference modulate changes in activity

The OFC has been proposed to update the value of stimuli and actions as we acquire new information about them (Schoenbaum & Roesch, 2005), thereby contributing to decision-making based on reward outcome evaluation (Schoenbaum *et al.*, 2006). Because we observed differences in risk-preference across rats (Fig. 1), we were able to analyse increasing and decreasing responses to reward outcome in rats with different behavioral biases. In the subjects from which we obtained OFC recordings, risk-neutral rats chose the risky lever on an average of 51% of trials and risk-preferring rats chose the risky lever on an average of 92% of trials.

The population of neurons with decreasing activity to reward outcome showed reductions in activity that persisted beyond the delivery of the sucrose pellets. These reductions in activity were modulated by reward outcome, and depended on whether rats were free to choose and their risk-preferences. Of these 28 neurons, 14 were recorded from the hemisphere contralateral to the risky lever and 14 ipsilateral. Prior to lever extension, there were no differences in baseline that correlated with the outcome of the trial (baseline: Fig. 3A; $F = 0.04$, $P = 0.96$; Fig. 3B; $F = 1.25$, $P = 0.29$; Fig. 3C; $F = 0.13$, $P = 0.88$). During forced response trials, decreases in OFC activity differed according to reward outcome 6–10 and 11–15 s following the lever press (Fig. 3A; outcome: 2–5 s, $F = 1.90$, $P = 0.15$; 6–10 s, $F = 4.59$, $P = 0.011$; 11–15 s, $F = 4.86$, $P = 0.008$). The decreases were larger on trials in which the largest reward was received compared with trials resulting in reward omission. The differences in firing rate to reward outcome were not affected by risk-preference (outcome × risk-pref: 2–5 s, $F = 0.74$, $P = 0.14$; 6–10 s, $F = 0.92$, $P = 0.40$; 11–15 s, $F = 0.42$, $P = 0.65$), therefore we averaged them together in Fig. 3A.

During free choice trials, responses of decreasing OFC neurons were qualitatively different depending on the risk-preferences of the animals. Across all subjects, responses were modulated by reward outcome over the intervals spanning 2–15 s following the lever press (outcome: 2–5 s, $F = 14.85$, $P = 0.00001$; 6–10 s, $F = 9.07$, $P = 0.0001$; 11–15 s, $F = 4.98$, $P = 0.007$). Unlike forced response trials, modulation by reward outcome also depended on risk-preference from 6–10 s and 11–15 s following lever press (outcome × risk-pref, 2–5 s, $F = 1.99$, $P = 0.14$; 6–10 s, $F = 7.50$, $P = 0.0006$; 11–15 s, $F = 4.67$, $P = 0.001$). In risk-neutral animals, activity differed with outcome only in the early (2–5 s) epoch following the lever press (Fig. 3B; outcome, 2–5 s, $F = 14.93$, $P = 0.00001$; 6–10 s, $F = 1.95$, $P = 0.14$; 11–15 s, $F = 1.12$, $P = 0.33$). In contrast, modulation by outcome persisted for a longer duration from 2 to 15 s following the lever press in risk-preferring animals (Fig. 3C; outcome: 2–5 s, $F = 15.78$, $P = 0.00001$; 6–10 s, $F = 34.39$, $P = 0.00001$; 11–15 s, $F = 20.30$, $P = 0.00001$).

Not only did decreases in activity persist longer in risk-preferring animals, but we found different patterns of activity compared with risk-neutral animals. During epochs in which there was a significant effect of outcome for decreasing neurons, we used a *post hoc* test to examine the pattern of responses. In both risk-neutral and risk-preferring animals, neural activity differentiated large payoffs from reward omission on risky choices with a larger decrease for the large reward. In risk-neutral animals, the decrease in firing rate following certain choices was equivalent to that of risky choices yielding the large payoff 2–5 s following lever press. Only when risky choices resulted in reward omission did neural activity show a weaker reduction (Fig. 4A; *post hoc*, 0 vs. 2, $P = 0.00002$; 0 vs. 4, $P = 0.0001$). In risk-preferring rats, neural activity persisted at a level lower than baseline only for risky choices yielding a large payoff, and quickly returned to baseline level for certain choices and risky choices resulting in reward omission (Fig. 4B; *post hoc*, 2–5 s, 4 vs. 0, $P = 0.00002$; 6–10 and 11–15 s, 4 vs. 0, $P = 0.00002$; 4 vs. 2, $P = 0.00003$). Thus, for risk-neutral rats, OFC responses to certain trials were equivalent to having received a large reward, while in risk-preferring rats neural responses to certain trials were equivalent to having received no reward.

Increasing OFC neurons also differed qualitatively between forced response and free choice trials. Of the 24 increasing neurons, 13 were recorded from the hemisphere ipsilateral to the risky lever, and 11 contralateral. In all rats, neurons from both hemispheres were modulated by the task. Prior to lever extension, there were no differences in baseline that correlated with the outcome of the trial (baseline: Fig. 5A; $F = 0.11$, $P = 0.90$; Fig. 5B; $F = 0.89$, $P = 0.41$; Fig. 5C; $F = 1.83$, $P = 0.16$). During the forced response block of trials, increasing neurons showed significantly higher firing rate during reward evaluation, but the increase was not significantly modulated by reward outcome (Fig. 5A; outcome: 2–5 s, $F = 2.57$, $P = 0.078$; 6–10 s, $F = 2.44$, $P = 0.088$; 11–15 s, $F = 0.52$, $P = 0.60$), nor did it depend on risk-preference (outcome × risk-pref: 2–5 s: $F = 0.27$, $P = 0.76$; 6–10 s, $F = 0.05$, $P = 0.96$; 11–15 s, $F = 0.17$, $P = 0.83$).

In contrast to the pattern of neural activity during forced responses, firing rate was strongly modulated by reward outcome during the free choice block. Increases in activity were affected by reward outcome across all free choice trials during the intervals 6–15 s following lever press (outcome: 2–5 s, $F = 2.47$, $P = 0.09$; 6–10 s, $F = 19.86$, $P = 0.00001$; 11–15 s, $F = 7.56$, $P = 0.001$), and depended on risk-preference during the same intervals (outcome × risk-pref: 2–5 s,

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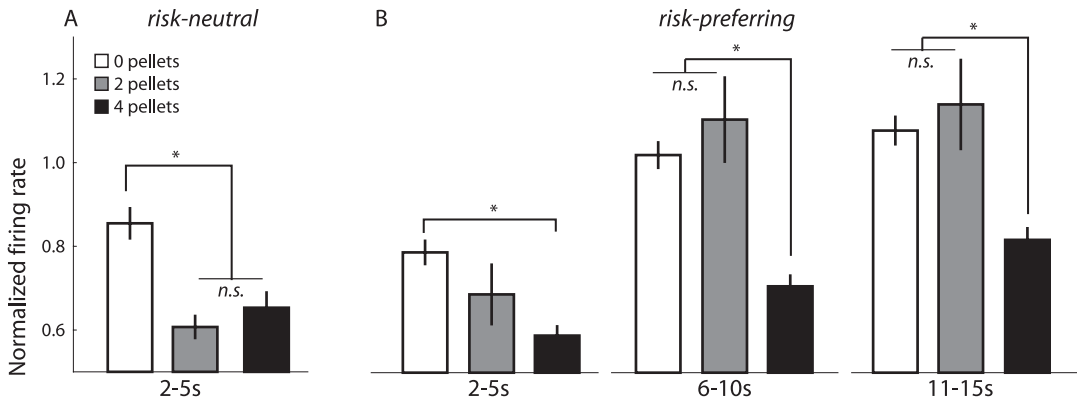

FIG. 4. Average normalized firing rate (spikes/s, ±1 SEM) for decreasing neurons as a function of outcome in risk-neutral (A) and risk-preferring (B) rats. Each panel shows one epoch during which responses were significantly modulated. Asterisk marks outcomes that result in significantly different levels of activity. Black bar and N.S. marks conditions that did not significantly differ.

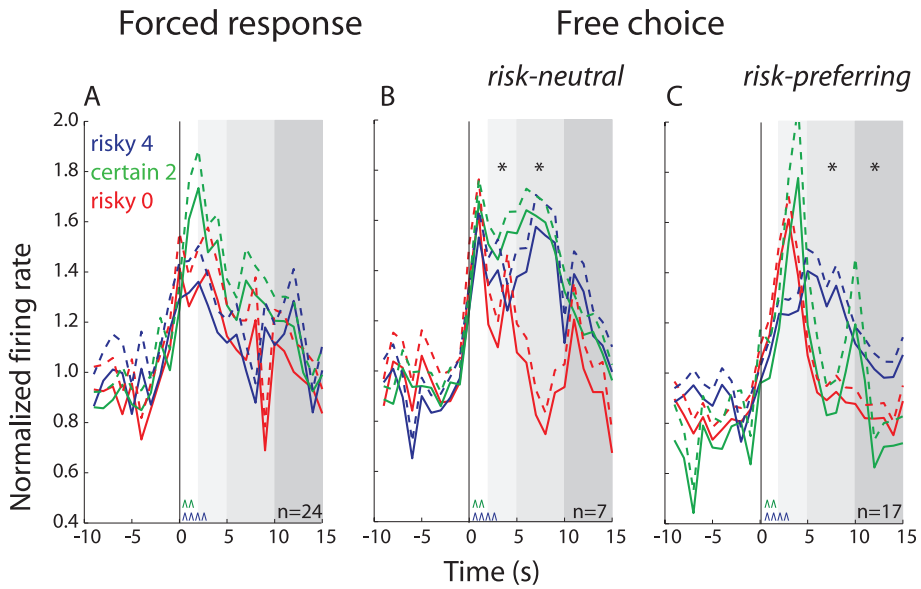

FIG. 5. Average responses for 24 increasing OFC neurons, aligned to time of lever press. Same conventions as Fig. 3.

$F = 1.82$, $P = 0.16$; 6–10 s, $F = 5.88$, $P = 0.003$; 11–15 s, $F = 2.30$, $P = 0.03$). OFC neurons from risk-neutral animals were modulated earlier (2–10 s) during the reward evaluation period (Fig 5B; 2–5 s, $F = 5.85$, $P = 0.003$; 6–10 s, $F = 23.75$, $P = 0.00001$; 11–15 s, $F = 2.41$, $P = 0.10$). Neurons from risk-preferring animals responded differently to outcome later (6–15 s following press; Fig. 5C; 2–5 s, $F = 1.15$, $P = 0.31$; 6–10 s, $F = 15.8$, $P = 0.00001$; 11–15 s, $F = 8.20$, $P = 0.0003$).

Increasing OFC neurons showed similar patterns of response properties to decreasing neurons. Responses of increasing neurons to certain reward delivery depended on risk-preferences of the rats during the free choice block. In risk-neutral rats, responses increased for both smaller, certain and large, risky rewards, but not for reward omission following risky choices (Fig. 6A; *post hoc*, 2–5 s: 0 vs. 2, $P = 0.003$; 2 vs. 4, $P = 0.31$; 6–10 s: 0 vs. 2 and 4, $P = 0.00002$). In risk-preferring rats, firing rate was significantly elevated only for the large payoff following risky choices, but not for smaller, certain reward or reward omission on risky trials (Fig. 6B; *post hoc*, 6–10 s: 4 vs. 0, $P = 0.0002$; 4 vs. 2, $P = 0.048$; 11–15 s: 4 vs. 0, $P = 0.0008$; 4 vs. 2, $P = 0.02$).

The differences in OFC responses in risk-preferring and -neutral rats observed here could not be explained as simply due to differences in the motor behavior of the animals. Following lever presses, we measured the times of head entries into the pellet dispenser during reward delivery and through the inter-trial interval. This potential confound is discussed more thoroughly in supplemental Results (see supporting Appendix S1), but we did not find differences in RT (supporting Fig. S7) or head entry frequency (supporting Figs S8 and S9) that accounted for differences in OFC responses (supporting Fig. S10). Further, the patterns of neural responses were not altered when aligned to the time of the first head entry rather than lever press (supporting Figs S11 and S12).

Discussion

The OFC is thought to play a critical role in processing reward information for the purpose of organizing and directing behavior (Wallis, 2007). Adaptive behavior requires that we appropriately map environmental cues and context with the outcomes of our actions.

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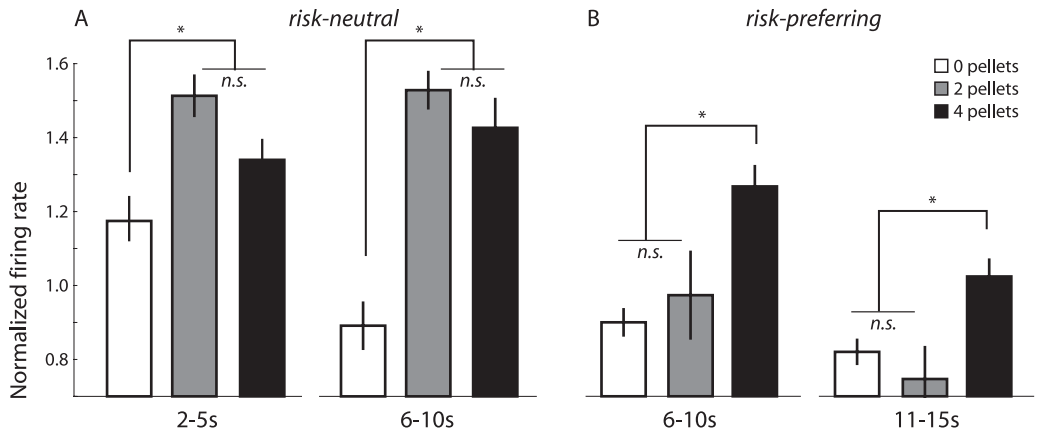

FIG. 6. Average normalized firing rate (spikes/s, ±1SEM) for increasing neurons as a function of outcome in risk-neutral (A) and risk-preferring (B) rats. Same conventions as Fig. 4.

OFC is thought to update the value of stimuli and actions as we acquire new information about them (Schoenbaum & Roesch, 2005). Clinical populations that have difficulty controlling impulsive actions, learning to reverse previously established reward contingencies, and assessing risk show reduced activity in OFC (Berlin *et al.*, 2004; Remijnse *et al.*, 2006). Using a task in which rats were trained to associate rewards of different size and probability with two different behavioral options presented alone or simultaneously, we tested whether responses of single OFC neurons would be affected by the opportunity to choose between options and by the rewards received as a consequence.

We found that OFC representation during reward evaluation was affected by both availability of choice and reward size. Most strikingly, the impact of choice and reward outcome on OFC activity was moderated by risk-preference. During forced response trials, neural activity did not differ according to the risk-preferences of the animals. Decreasing neurons had larger reductions in firing rate when the largest reward was received, and modulations of increasing neurons by reward outcome did not reach statistical significance. It is possible that rats experienced reduced general arousal during forced response trials due to the blocked design, which may have attenuated potential differences in neural responses according to reward outcome. Faster latencies to lever press during the free choice block are consistent with this potential difference in arousal (supporting Fig. S4).

During free choice trials, we observed patterns of responses that not only differed from the forced response block, but were also distinguished by the rats' risk-preferences. While the two populations of neurons had responses opposite in direction, decreasing and increasing, they were remarkably similar in how they were modulated by choice and reward outcome. For all animals, the changes in firing rate from baseline were greatest following large, risky payoffs and weakest following reward omission, such that the risky 'good' and 'bad' outcomes served to anchor neural responses. In risk-neutral animals, the neural responses to certain rewards aligned with large, risky payoff. Conversely, in risk-preferring animals, responses to the certain reward aligned with reward omission, and only neural responses to the large, risky payoff showed stronger changes in firing rate. Neural responses to certain trials were therefore indistinguishable from large, risky reward in risk-neutral rats and reward omission in risk-preferring rats. If differentiation of OFC responses to large, risky payoffs and reward omission reflects rats' evaluations of outcome, the pattern of responses observed here suggests that risk-neutral rats find certain rewards as valuable as large, risky rewards, and risk-preferring rats find certain outcomes as devalued as reward omission.

Previous studies have reported OFC encoding of outcomes based on value and uncertainty. In a delay discounting paradigm, most OFC neurons responded with stronger activity for preferred immediate rewards over non-preferred delayed rewards (Roesch *et al.*, 2006). The magnitude of OFC responses correlated with preference for the immediate reward, suggesting that these neurons reflected the subjective value of the outcome. Although we report similar increases in activity related to value, we also found neurons with transient decreases. These reductions are similar to the observation of a subset of neurons recorded in the delay discounting task with diminished activations for immediate rewards compared with delayed (Roesch *et al.*, 2006). In addition, the decreases reported here are comparable to decreases in NAc activity upon delivery of primary rewards (Roitman *et al.*, 2005; Taha & Fields, 2006). Our results also differ from previous work that did not show differences in neural activity between forced response and free choice trials (Roesch *et al.*, 2006). In the current study, we observe greater modulation when rats are able to choose between options. Varying the level of certainty may contribute to this observed difference. In the previous studies, rats' behavior showed that they valued immediate/large rewards over delayed/small rewards, but outcome was certain on every trial. It is possible that requiring animals to factor risk into action selection results in enhanced neural activity, but only when they need to choose. Indeed, in an olfactory discrimination spanning psychophysical threshold, OFC neurons showed graded responses that reflected the difficulty of the decision, which directly correlated with uncertainty about correctly choosing a response to earn a reward (Kepecs *et al.*, 2008). During this olfactory discrimination, two types of responses were observed that were similar in pattern, but opposite in sign. One population of OFC neurons responded maximally when rats discriminated the most uncertain stimuli, and decreased in activity with increasing certainty. A separate population had minimal activity when rats discriminated the least certain stimuli, which increased with certainty.

During the free choice block, we also observed that responses to outcome were more persistent in risk-preferring animals. All neurons began to increase or decrease activity within seconds of the lever press. Neurons recorded from risk-neutral animals showed short-lived differences related to outcome following the lever press. However, in risk-preferring animals, modulation by trial outcome emerged slightly later

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and persisted longer into the inter-trial interval. The potentiated response to large, risky payoffs in risk-preferring animals could result from the integration of multiple sources of information. To perform this task, animals must process information about reward size and probability and risk of loss. Other regions interconnected with the OFC, such as the NAc, amygdala and cingulate cortex, are also involved in encoding reward and emotion (Kolb, 1984; McDonald, 1991; Morgane *et al.*, 2005). These regions are likely to contribute different types of information relevant for these processes.

OFC connections with the cingulate cortex and amygdala have been proposed to be important for conveying information about risk. Human imaging studies have shown changes in activation throughout the cingulate cortex in response to monetary gains and losses (Fujiwara *et al.*, 2009). Elevated activity in the anterior cingulate cortex (ACC) was associated with subjects making high-risk decisions for reward (Hewig *et al.*, 2009; Rao *et al.*, 2008; Smith *et al.*, 2009). In monkeys, neurons in the posterior cingulate cortex showed activity that increased with risk when choosing between certain and risky outcomes (McCoy & Platt, 2005), and predicted the switch from risky to safe alternatives (Hayden *et al.*, 2008). In rats, disconnecting the ACC from the NAc resulted in a failure to make normal choices between differently valued rewards (Hauber & Sommer, 2009). Basolateral amygdala (BLA) may also influence risk-preference, as BLA inactivation resulted in rats showing greater risk-aversion in choices with greater uncertainty (Ghods-Sharifi *et al.*, 2009). The connection between BLA and OFC has been shown to be critical for rats to flexibly associate behavioral responses with their outcomes (Stalnaker *et al.*, 2007).

The ventral striatum/NAc and its dopaminergic (DA) input likely contribute to outcome evaluation by providing information about reward that is relevant for judgments. Humans performing tasks that require decisions based on risky monetary outcomes typically show qualitatively different activity in the OFC and the striatum, with striatal activity proportional to the expected value of the outcome and OFC modulated by risk (Breiter *et al.*, 2001; Tobler *et al.*, 2009). This striatal signal, reflecting outcome, could be integrated with risk signals to reflect subjective value. The NAc receives a dense DA projection from the midbrain, which has been hypothesized to signal the occurrence of salient events crucial for forming learned associations about outcome (Schultz, 2007). In rats, DA signaling is critical for mediating increased risk-preference induced by amphetamine administration (St Onge & Floresco, 2009). DA neurons also send projections to the prefrontal cortex to signal information about reward (Schultz, 2007). This input may serve to mediate the persistent changes in neural activity during free choice (Lavin *et al.*, 2005).

Our results suggest that OFC responses represent the relative value of certain and risky outcomes according to rats' risk-preferences. However, they do not determine the specific role OFC plays in relation with other structures carrying relevant information in risk-preference. OFC responses to large, risky payoffs could result from the integration of striatal activity representing reward with ACC responses signaling the condition of risk. Alternatively, overvaluation of an outcome obtained in a context with risk by OFC could set a disproportionate reward expectation for risky choices, thus perpetuating risk-preference (Schoenbaum & Roesch, 2005). Enhanced activity in OFC could lead to a strengthening of OFC–NAc synapses, therefore inappropriately biasing behavior, much like that observed in addiction (Jones & Bonci, 2005; Gao & Wolf, 2008). Further study will be needed to characterize how different components of the circuit represent different aspects of risky decisions and how their activity correlates with individual biases. By varying reward parameters or physiological state to steer behavior toward different degrees of risk-preference, future studies will correlate how potentially maladaptive decisions are represented across multiple neural structures. Biases in OFC responses to risky outcomes such as those observed here may serve to potentiate the selection of maladaptive actions that ultimately do not serve the organism well.

Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Neural responses following lever press decreased or increased similarly during forced response and free choice block.

Fig. S2. For each OFC neuron recorded, average baseline activity for the free choice block plotted as a function of baseline activity during forced response block.

Fig. S3. For each animal, extent of cortex sampled according to histological markers in Figure 11 of Paxinos & Watson (2007).

Fig. S4. Normalized response time for forced response and free choice blocks of trials.

Fig. S5. Examples of single OFC units with phasic decreasing or increasing activity to lever presentation.

Fig. S6. Average responses for neurons that responded to lever presentation.

Fig. S7. Normalized response time of head entry into the pellet receptacle following lever press for forced response and free choice blocks of trials.

Fig. S8. Frequency of head entries into pellet receptacle following lever press.

Fig. S9. Frequency of head entries following lever press as a function of outcome for forced response and free choice trials.

Fig. S10. A comparison of behavioral and neural responses to certain outcome (2 pellets) in risk-preferring and risk-neutral rats during the free choice block of trials.

Fig. S11. Normalized firing rate of 28 decreasing neurons, aligned to time of first head entry into pellet dispenser following lever press.

Fig. S12. Normalized firing rate of 24 increasing neurons, aligned to time of first head entry into pellet dispenser following lever press.

Appendix S1. Supplemental methods.

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Abbreviations

ACC, anterior cingulate cortex; BLA, basolateral amygdala; DA, dopamine; NAc, nucleus accumbens; OFC, orbitofrontal cortex; PCA, principal component analysis; RT, response time.

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