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Inflammatory pain disrupts the orbitofrontal neuronal activity and risk-assessment performance in a rodent decision-making task

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ABSTRACT

It has been recently described that disruption of the neural mechanisms of emotion-based decision making occurs in both chronic pain patients and in animal models of pain; moreover, it also has been shown that chronic pain causes morphological and functional changes in the prefrontal cortex that may be crucial for this decision-making dysfunction. However, it is not known whether pain alone is capable of altering the neuronal encoding of decision exhibited by prefrontal neurons. We have previously shown that naïve animals have risk-averse performance in the rodent gambling task, whereas chronic pain animals reverse their choice preference and become risk prone. Using this paradigm, we chronically implanted arrays of multielectrodes and recorded from neuronal ensembles in the orbitofrontal cortex of freely moving animals performing 4 sessions of the rodent gambling task: 2 in control conditions and 2 after the onset of inflammatory pain induced by complete Freund's adjuvant injection. Our results show that the instantaneous neuronal firing rate was correlated with the probability of choosing a specific lever in 62.5% of the neurons; however, although in the control sessions 61% of the neurons encoded the reward magnitude, after the pain onset only 16% of the neurons differentiated small from large rewards. Moreover, we found that the fraction of risk-sensitive neurons recorded in each session predicted the overall risk bias of the animal. Our data suggest that orbitofrontal cortex encoding of risk preference is compromised in chronic pain animals.

1. Introduction

Decision making under uncertainty depends on the ability to integrate information from current and past experiences to estimate the value of alternative choices. The orbitofrontal cortex (OFC) has an important role in the update of value and probability of rewards in humans, primates, and rodents [8,43,45]. Several studies have shown that neuronal activity in the OFC encodes certainty about reward estimation [31,33,34]. In rats, the OFC has been described as having an important role in discounting value tasks [77], while reversal learning deficits are associated with the inability of OFC neurons to specifically develop response-outcome associations before and after the preferred choice is made [59,66].

An increasing number of studies supports the possibility that decision making mechanisms are disrupted during chronic pain conditions in both humans and animal models [4,25,32,40,47,70,71]. The possibility of an impairment in the ability to evaluate choices and outcomes is supported by previous findings showing that chronic pain animals have decreased levels of dopamine in the OFC [47], because it is known that this change in dopaminergic content alters the breakpoint of progressive ratio tasks [15] or decision-making tasks [64,69,79]. Moreover, emotional decision-making is known to be highly dependent on normal prefrontal functioning, and several studies have shown that chronic pain induces morphological and physiological alterations in prefrontal areas [28,32,36,38,62].

We have recently developed a rodent gambling task (RGT) [45] that reproduces the trial-by-trial progressive strategic learning typical of the decision-making under uncertainty of the Iowa gambling task [6]. Moreover, we have demonstrated that similar to the Iowa gambling task, performance in the RGT is also sensitive to both OFC lesioning and stressful chronic pain onset [45,47]. Because the onset of prolonged noxious conditions alters RGT performance by shifting the individual normal risk-aversion

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behavior to a state of risk-prone behavior, we were for the first time able to study the orbitofrontal neural activity in lesion-free animals with both risk-averse and risk-prone biases. Our hypothesis was that OFC cells would develop differential activity for choices and outcomes during the task, reflecting different sensitivities to the task's contingencies.

To test this hypothesis, we chronically implanted multielectrodes in the OFC of rats and repeatedly performed the RGT in the control period and pain period conditions in each animal. Using this approach, we were able to test whether the behavioral profile of choice observed in both conditions reflected the gradual development of a choice bias, or if it reflected impulsive choices determined by the outcome of the preceding trial. Moreover, we were able to test whether the choice and reward encoding properties of OFC neurons are important to the overall performance in decision making under uncertainty.

2. Materials and methods

2.1. Animals

Sprague-Dawley male rats (n = 6) with weights between 300 and 320 g were used in all experiments (Charles-River, Barcelona, Spain). Animals received water ad libitum but were food deprived to 85% of age-matched body weight by limiting their access to food. All experiments were conducted in accordance with the local ethics committee on animal research, the Policies on the Use of Animals in Neuroscience Research of the Society for Neuroscience, as well as with the guidelines of the International Association for the Study of Pain on the use of awake animals [83].

2.2. Chronic pain model

Persistent monoarthritic inflammatory pain was induced by injecting complete Freund's adjuvant (CFA) in the tibiotarsal joint of the left hindpaw [11]. CFA was prepared by mixing 60 mg desiccated *Mycobacterium butyricum* (Difco Laboratories, Oxford, England) with paraffin oil (6 mL), saline (4 mL), and Tween 80 (1 mL), and injected under brief halothane anesthesia. Sensory threshold for noxious stimulation was measured using von Frey filaments (Somedic, Horby, Sweden) as previously described [16].

2.3. Electrode implantation

Anesthesia was induced with an intramuscular injection of xylazine and ketamine (10 and 60 mg/kg mixture, respectively), and the rat was positioned in the stereotaxic apparatus. Animals were implanted with a bundle of 8 tungsten Isonel filaments in the right OFC according to the following coordinates: LM −2.4, DV −4.5, RC +3.5. More detailed description of the electrodes and of the neurophysiology recording setup may be found in our previous articles [12,13].

At the end of the behavioral experiments, animals were anesthetized and perfused with phosphate-buffered saline (0.1 M) followed by 4% formaldehyde in phosphate-buffered saline. The frontal region of the brain was removed and serially sectioned in 90-μm slices. Sections were stained with thionin, and electrode sites were identified and compared with standard coronal diagrams of an atlas of rat brain [48].

2.4. RGT

The RGT of risk assessment has been described in detail elsewhere [45]. Briefly, it consisted of a custom-built 45-cm octagonal hub connected to a 10 × 30-cm runway that functioned as a waiting chamber; the hub was partially divided into 2 adjacent chambers by a 15-cm protruding opaque Plexiglas panel, and entrance from the waiting chamber to the hub was blocked by an automatically operated door. Both the hub and the runway had 45° angled walls to prevent accidental bumping of the tethered head stage.

Each half of the hub contained 1 nonretractable lever and 1 food cup connected to an automated pellet dispenser (Coulbourn Instruments, Allentown, PA, USA). Video tracking of animal position and control of feeders and levers was done using OpenControl [1]. After a brief tone, the door of the waiting chamber opened and the animal entered the hub and had to choose between the left and right side of the hub and press the lever to receive a food reward (45 mg chocolate flavour sucrose pellets, Research Diets Inc., New Brunswick, NJ, USA). After receiving the reward, the animals were placed back in the waiting chamber and had to wait 5 seconds for the start of the following trial. After lever press training criteria was accomplished, the animals started the behavioral protocol (see further details later). Briefly, the animal had 6 sessions (1 session per day, with a random duration of 60 to 120 trials) of the nongambling phase in which both levers had the same associated reward of 1 food pellet per trial. However, rewards were given only in 8 of every 10 trials regardless of the hub side chosen by the animal. After these 6 sessions, animals were tested in the gambling phase: a single session of 90 trials in which the levers now corresponded to food rewards of distinct value and probability: one lever remained, as in the nongambling phase, the low-risk lever, returning 1 food pellet in every 8 of 10 visits, whereas the other returned 3 food pellets, but only in 3 of every 10 visits, the high-risk lever.

Results of the behavioral task were obtained from the analysis of the individual patterns of choice during the RGT. Four parameters were calculated for each animal: preference index, final preference, incomplete trials, and total gain. The preference index was defined as the proportion of choices between both levers and calculated for each 10 consecutive trials as in the Iowa gambling task: (low-risk lever choices) − (high-risk lever choices)/number of completed trials. Hence, the preference index resulted in a sequence of 9 values ranging from −1 to +1 whether the animal always preferred the high-risk reward or the low-risk lever, respectively. Final preference was defined as the preference index for the final 30 trials (trials 61 to 90) of the probe session. Incomplete trials were those in which the 20-second trial limit was reached without the animal pressing any of the levers. Total gain was calculated as the number of pellets obtained during the probe session.

2.5. Behavioral protocol

The behavioral protocol timeline consisted of 2 phases (Fig. 1) and comprised 4 gambling probe sessions per animal: 2 sessions performed before the CFA injection (control period) and 2 performed after CFA injection (pain period). In the control period, all animals were first trained to press the 2 levers in the nongambling contingencies (both levers set at low-risk values dispensing 1 food pellet in 8 of every 10 visits). After reaching the criteria for lever performance, electrodes were implanted in the OFC and animals were allowed 1 week to recover from surgery. After recovery, animals were retrained on nongambling lever pressing for 2 days, and on the third day they were tested in the first gambling probe session of the RGT (RGT1) with the left lever set as the high-risk lever. Over the next 2 days, animals were retrained in lever press with both levers set at the nongambling contingency, and on the third day after RGT1 they were again tested in a gambling probe session (RGT2), now with reversed probabilities because the left lever was set as the low-risk lever. On the next day, animals were injected with CFA (see description of chronic pain model earlier) and daily training in the nongambling lever press was resumed the following day. On the fifth day after injection, a new gambling probe session,

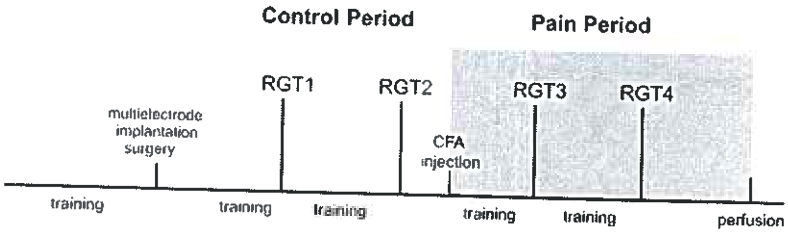

Fig. 1. Timeline of experimental protocol. Each animal was introduced to the task arena and trained for lever pressing to retrieve food pellets. After successful training of lever press and reward delivery, animals were exposed daily to the non-gambling phase of the rodent gambling task with the exception of the 4 days in which they were exposed to the gambling probe sessions (rodent gambling task 1 to 4). Further details about protocol scheduling are found in the Methods section.

RGT3, was performed with the left lever again set as the high-risk lever. Finally, animals were retrained for another 2 days in nongambling lever press, and 8 days after CFA injection were tested in the last probe session, RGT4, with the left lever set as the low-risk option. Sensory testing with the von Frey filaments was applied 30 minutes before each RGT.

2.6. Single-unit recording

Before each gambling or nongambling session, the animals were placed in the RGT arena, connected to the preamplifier, and allowed to freely explore the arena for 10 minutes; levers were removed during this period. Signals from electrodes were carried through a head stage and then preamplified before being recorded in a 16-channel MAP system (Plexon Inc., Dallas, TX, USA). Waveforms that were >2.5:1 signal-to-noise were recorded to disk. Cluster analysis for separation of action potentials from different units was made with Offline Sorter (Plexon Inc.). Peristimulus histograms and firing rates were calculated using Neuroexplorer (Plexon Inc.).

2.7. Statistical analysis

Values of final preference, total gain, number of incomplete trials, and sensory threshold were compared with paired t tests or Wilcoxon signed rank tests (when a significant P value was found in the Kolmogorov-Smirnov test). Comparison of average firing rates of the overall population of cells for each lever was made using a 2-factor analysis of variance (ANOVA) with experimental condition (control period or pain period) as a between-subjects factor and lever (high- or low-risk) as a within-subjects factor. Post hoc analyses were made with a Bonferroni test. Comparison of firing frequencies between choices, presence or absence of reward, and reward sizes were made with Student t tests, paired samples t tests, or nonparametric equivalent (when normal distribution of data could not be assumed) using 5 seconds of activity in bins of 0.1 seconds. The significance level was adjusted for multiple comparisons. Comparison of proportions was made with the χ^2 test when more than 5 elements per group occurred; otherwise a Fisher exact test was used. Statistical tests were considered statistically significant for $\alpha = 0.05$. Data analysis was performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) and Matlab version 7.0 (Mathworks, Natick, MA, USA).

3. Results

First we verified that CFA injection did reduce the mechanical threshold for noxious perception. Tactile painful sensitivity was significantly decreased after CFA injection immediately before pain period RGT3 and RGT4 sessions, when compared with control period RGT1 and RGT2 sessions (control: 3.60 ± 0.30; pain: 0.031 ± 0.001; Wilcoxon signed ranks: $W = 36.00$; $P = .0078$). Left/right levels of individual choice during all of the nongambling phases between probe sessions of the task showed no specific preference for one side of the arena (average of ratio of left/right choices as measured by the final preference index of the last session of 60 trials before each probe session) (repeated-measures ANOVA: $F_{3,9} = 0.76$, $P = .76$, not significant [NS]; values of training sessions before RGT1: 0.01 ± 0.05, before RGT2: -0.07 ± 0.10; before RGT3: -0.01 ± 0.05; before RGT4: -0.03 ± 0.09).

The preference index of the probe sessions resulted in a characteristic pattern with animals obtaining positive values in control

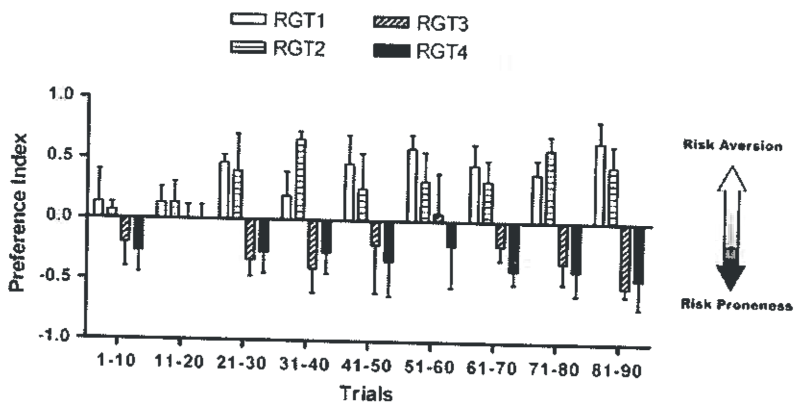

Fig. 2. Choice preference in the rodent gambling task. The graphic tracks the progress in task performance using the preference index: the number of choices at the high-risk lever divided by the number of completed trials in each block of 10 consecutive trials. Note that in the first 20 trials of all rodent gambling task probe sessions the animals do not display an explicit risk preference; on the other hand, it is noticeable in the final blocks of all sessions that despite having a clear preference for a particular lever, the task animals display a high exploratory behavior because the preference index never reaches maximal positive or negative values.

period sessions (RGT1 and RGT2 sessions) that were reversed to negative values in the pain period sessions after CFA injection (RGT3 and RGT4 sessions) (Fig. 2). Final preference in the last 30 trials of each probe session showed significant differences between control period sessions and the pain period sessions (control: 0.49 ± 0.07, pain: −0.43 ± 0.04, paired samples t test: t7 = 8.753, P < .0001), but no differences were found within the 2 control period sessions (RGT1: 0.51 ± 0.10; RGT2: 0.47 ± 0.13; paired samples t test: t3 = 0.20, P = .86, NS) or the 2 pain period sessions (RGT3: −0.42 ± 0.08; RGT4: −0.4375 ± 0.05; paired samples t test: t3 = 0.12, P = .92, NS). Total gain, the number of pellets earned in each probe session, was similar between pain period and control period conditions (control: 72.67 ± 3.62; pain: 77.5 ± 1.31; Wilcoxon signed ranks: W = −10.00, P = .1875) and within each condition (RGT3: 77.0 ± 2.65, RGT4: 78.00 ± 1.15; paired samples t test: t3 = 0. 28, P = .80, NS; RGT1: 68.67 ± 6.98, RGT2: 76.67 ± 0.88; paired samples t test: t3 = 1.14, P = .37, NS). The number of incomplete trials was similar between control period and pain period sessions (control: 2.83 ± 2.83; pain: 0.71 ± 0.71, t5 = 0.64, P = .5484).

3.1. Descriptive model of choice transition

The raw results from the RGT are sequences of choices with underlying associated probabilities. We defined a matrix of Markov decision process (MDP) for all probabilities of transition between task states (namely, pressing the low-risk lever and getting no reward, pressing the high-risk lever and getting a small reward, pressing the high-risk lever and getting no reward, and pressing the high-risk lever and getting a large reward). Each entry in this MDP transition matrix represents the probability of making a specific lever choice conditioned to the RGT state the animal was in the previous trial. The only approximation made in this calculation is to assume, as in a Markov chain, that the transition probabilities depend exclusively on the previous state. The diagram of the descriptive model is represented in Fig. 3A, in which A stands for the random variable representing the lever choice that can be either the low-risk lever, L, or the high-risk lever, H. The random variable R represents the reward from the previous choice which can be 0, 1, or 3 pellets. All transition probabilities are represented using conventional notation. For example, $p(A^{(t+1)} = L|R_L^{(t)} = 0)$ represents the probability of choosing the low-risk lever in trial $t + 1$, given that the rat received no reward from the low-risk lever at the current trial t. Because there is a causality factor implicit in these conditional probabilities, the trial superscripts are redundant and

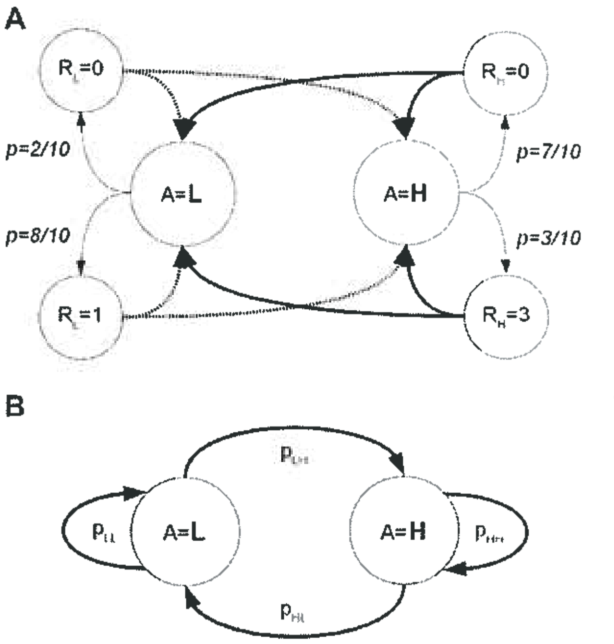

Fig. 3. The descriptive model of choice as a Markov decision process. (A) Full model comprising all state transitions between states, in which A stands for the lever (high or low risk) and R stands for reward (0, 1, or 3 food pellets). (B) The results from this full Markov decision process model, presented in Table 1, showed that a simpler matrix with only 1 free parameter was able to describe the state transitions in the rodent gambling task; this simplified model will be used in the calculations presented in Fig. 4, 7, and 8A.

will not be shown. Notice that all reward probabilities (thin arrows) are fixed and predefined in the task.

When the actual results of the experiments were introduced in this transition matrix, the results showed that for both control and pain period sessions the instantaneous probability of choosing the same lever again is not affected by having received or not received a reward in the previous trial (for example, receiving a large reward in the high-risk lever changes only slightly from 0.34 to 0.39 the probability of returning to the high-risk lever in the next trial) (Table 1). This result allows the simplification of the transition matrix and the definition of a new description model with fewer free parameters and using only the previous lever choice (Fig. 3B). The conditional probability notation was

Table 1
Probabilities of lever choice according to MDP matrixes of state transitions.

Control period		Pain period	
Full MDP model			
P (A = L | RL = 0) = .75	P (A = L | RL = 1) = .71	P (A = L | RL = 0) = .32	P (A = L | RL = 1) = .38
P (A = H | RL = 0) = .25	P (A = H | RL = 1) = .29	P (A = H | RL = 0) = .68	P (A = H | RL = 1) = .62
P (A = L | RH = 0) = .66	P (A = L | RH = 3) = .61	P (A = L | RH = 0) = .33	P (A = L | RH = 3) = .35
P (A = H | RH = 0) = .34	P (A = H | RH = 3) = .39	P (A = H | RH = 0) = .67	P (A = H | RH = 3) = .65
P (A = L) = .69	P (A = H) = .31	P (A = L) = .35	P (A = H) = .65
Simplified MDP model			
P LL = .72			P LL = .36
P LH = .64			P LH = .34
P HL = .28			P HL = .64
P HH = .36			P HH = .66
P L = .69			P L = .35

Values of probability were calculated for all of the rodent gambling task probe sessions using the full MDP matrix of state transitions (Fig. 3A) and the simplified MDP matrix (Fig. 3B).
HH = consecutive high-risk choices, LL = consecutive low-risk choices, HL = high-risk choice before low-risk choice, LH = low-risk choice before high-risk choice, RH = number of reward pellets at high-risk lever, LH = number of reward pellets at low-risk lever, MDP = Markov decision process.

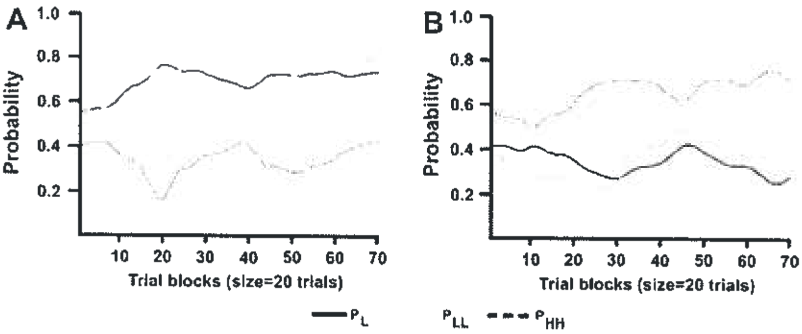

Fig. 4. Probability curves generated by the Markov decision process descriptive model of choice. The results show the calculated transition probabilities for contiguous blocks of 20 trials for all sessions of all animals. (A) Population averages of control period sessions. (B) Population averages of pain period sessions. Block 1 incorporates trials 1 to 20, block 71 incorporates trials 71 to 90, and the probability curves are filtered using a moving average kernel of 5 trials.

simplified using $p(A^{(t+1)} = X|A^{(t)} = Y) = p_{YX}$, where X and Y stand for either L or H lever. The results for the simplified transition matrix are presented in Table 1.

To track the evolution of the choice probabilities as a learning process, we calculated the transition probabilities for consecutive blocks of trials in each probe session. In Fig. 4 we present the matrix transition probabilities for moving data blocks of 20 contiguous trials (the first data point incorporates the results from trials 1 to 20, the second data point the results from trials 2 to 21, and so forth). The probability curves are filtered using a moving average kernel of 5 trials.

3.2. Single-unit recordings

Neural recordings were obtained from 96 OFC neurons in 16 probe sessions in 4 rats: 46 neurons were recorded in the RGT1 and RGT2 control period sessions, and 50 neurons were recorded in the RGT3 and RGT4 pain period sessions. The average number of cells recorded per wire was 1.56 ± 0.6 across all sessions. Average firing frequencies for the entire duration of each probe session were not different between all probe sessions (RGT1 1.95 ± 0.23; RGT2 2.13 ± 0.27; RGT3 1.89 ± 0.36; RGT4 2.96 ± 0.82, Kruskal-Wallis test statistic = 2.324; P = .5080) or between control period and pain period conditions (control: 2.04 ± 0.26; pain: 2.32 ± 0.35; Mann-Whitney U = 1118.0; P = .8173).

3.3. OFC encoding of lever choice and reward size is lost after pain onset

We first compared whether OFC cells in each ensemble presented different firing frequencies for each lever choice. ANOVA indicated significant effects of risk condition (F1,916 = 5.193, P = .024), lever (F1,916 = 44.00, P < .0001), and interaction (F1,916 = 34.777, P < .0001); post hoc comparisons showed significant differences during control period sessions (control: low-risk lever 0.446 ± 0.006 spikes/s; high-risk lever 0.366 ± 0.007 spikes/s; t_{49}! = 8.38, P < .0001), but not during pain period sessions (pain: low-risk lever 0.423 ± 0.006; high-risk lever 0.419 ± 0.006; t_{49} = 0.605, P = .548) (Fig. 5E) (see also individual examples in Fig. 5A to D). A higher proportion of cells with different firing frequencies for high- and low-risk lever press was found in control period sessions (control: 32 of 46 = 69.57% cells; pain: 25 of 50 = 50.00%; χ^2 = 3.802; df = 1; P = .05) (Table 2).

We also calculated to which lever these neurons presented higher firing rates and found that in both the control period and the pain period sessions the majority of these neurons presented higher firing frequencies before pressing the low-risk lever, with an increased proportion in control period sessions (control: 30 of 32 = 93.75%; pain: 17 of 25 = 68.00%, χ^2 = 6.707, df = 1, P = .01) (Fig. 6). Because each animal was tested twice with the lever's reward probability reversed between sessions, this similar distribution of neurons across all sessions shows that this increase of firing rate in anticipation of low-risk lever press is not due to side bias or due to a different number of events during the task (i.e., number of visits to a specific lever).

On the other hand, comparison of the number of cells that fired differently to rewarded and nonrewarded trials (independently of referring to high-risk or low-risk lever) showed no difference between control period and pain period sessions (control: 22 of 46 = 47.83%; pain: 26 of 50 = 52.00%; χ^2 test = 0.167, df = 1, P = .683, NS) (Table 2). This result suggests that in both conditions OFC cells were able to process information regarding the reward delivery. No clear difference was found in the proportion of cells that differentially signaled rewarded and nonrewarded trials after pressing the high-risk lever (control: 17 of 46 = 36.96%; pain: 28 of 50 = 56.00%; χ^2 test = 3.489, df = 1, P = .06, NS) (Table 2), but during control period sessions there was a lower proportion of cells with different firing rates for outcomes after pressing the low-risk lever (control: 14 of 46 = 30.43%; pain: 27 of 50 = 78.00%; χ^2 test = 5.437, df = 1, P = 0.02) (Fig. 6, Table 3). The proportion of cells that presented differential firing rates after rewarded trials in each lever (i.e., 1 pellet vs 3 pellets) was significantly different in control period and pain period sessions (control: 21 of 46 = 45.65%; pain: 8 of 50 = 16.00%; χ^2 test = 9.992, df = 1, P = .002) (Table 2). From the total of cells that presented differential firing rates for the large and small rewards, the proportion of cells that presented increased firing rates for small rewards was similar (control: 19 of 21 = 90.48%; pain: 6 of 8 = 75.00%; Fisher exact test, P = .3).

We asked whether the same cells that presented differential firing rates for levers would also present differential firing rates for rewards, or if these were separate encoding conditions. This comparison showed that most lever-encoding cells also encoded for different rewards in the control period sessions, whereas during pain period sessions only a small proportion of cells presented differential firing rates for levers and rewards (control: 14 of 32 = 43.75%; pain: 4 of 25 = 16.00%; χ^2 test = 5.002, df = 1, P = .025). This shows that the firing frequencies encoding choice and reward are most likely not independent functions processed by distinct groups of cells, but that evaluative encoding of reward magnitude is somehow lost after the induction of risk-proneness conditions.

3.4. OFC encoding changes during trial progression

The differences found in the event-related firing rates of cells suggested that their activity could be coding some form of expectation related to choices and outcomes. As the animals start the

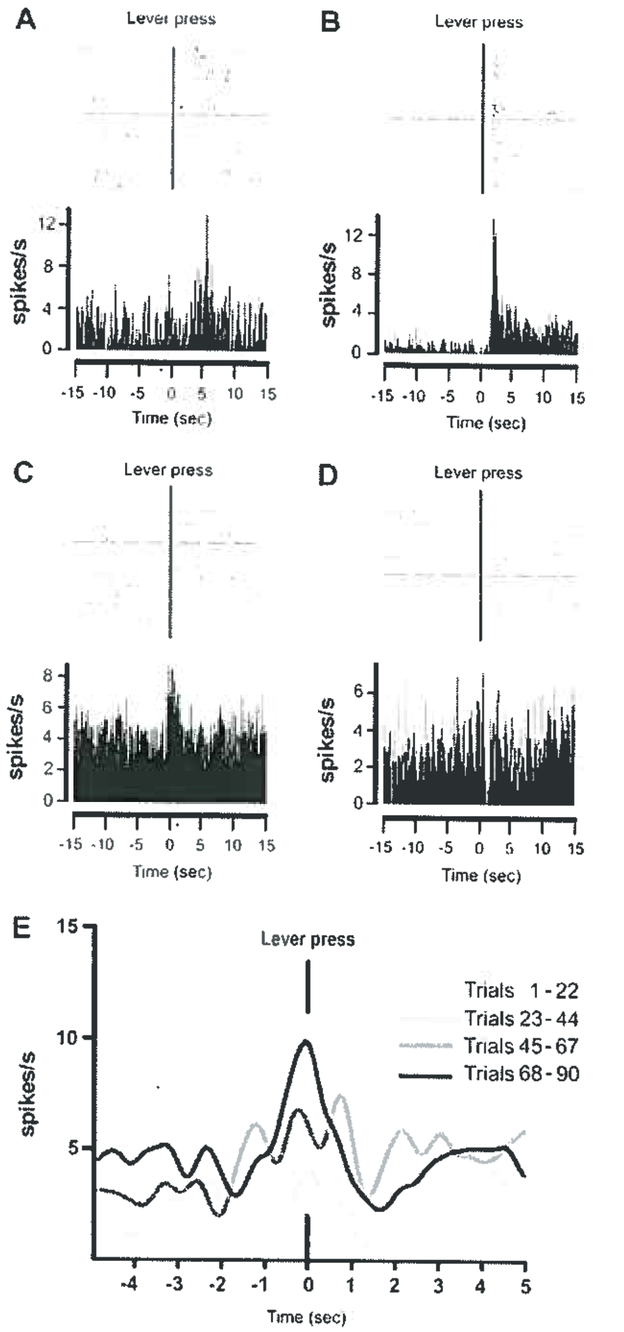

Fig. 5. Examples of task-related units recorded during the rodent gambling task sessions. (A–D) Raster plots and histograms of the lever press perievent activity of orbitofrontal cortex single cells show that although the majority of the recorded neurons presented task-related activity, their responses differed. The red vertical line indicates the moment of lever press, and the horizontal blue dashed line indicates trial 45, i.e., the halfway moment of the rodent gambling task session. (A) Neuron that responded only to rewarded trials in the low-risk choice during the first half of the sessions. (B) Neuron that responded with an increased firing rate approximately 2 seconds after a lever press, independently of the outcome of each trial. (C) Neuron with increased firing frequency locked with the lever press moment. (D) Neuron with a temporary decrease immediately after a lever press. (E) Histogram of lever press perievent activity of a neuron that gradually tuned for the exact moment of the lever press.

probe session naïve to the contingencies, expectations are formed as the task progresses in a truly inferential way. Hence, we decided to compare the proportion of cells that differentially fired for levers or for rewards during the first and second half of each session (i.e., trials 1 to 45 vs trials 46 to 90). The comparison of the proportion of cells that was able to encode lever preference in the first half of the session showed an increased proportion of cells in control period sessions (control: 29 of 46 = 63.04%; pain: 21 of 50 = 42.00%; χ^2 test = 4.251, df = 1, P = .039), but no differences were found in the proportion of cells that differentially fired for small vs large rewards (control: 18 of 46 = 39.13%; pain: 22 of 50 = 44.00%; χ^2 test = 0.234, df = 1, P = .6288, NS). During the second half of the task no differences were found in the proportion of cells that differentially fired for levers (control: 24 of 46 = 52.17%; pain: 23 of 50 = 46.00%; χ^2 test = 0.365, df = 1, P = .545, NS), but there was a difference in the proportion of cells that differentially fired for each reward (control: 35 of 46 = 76.09%; pain: 14 of 50 = 28.00%; χ^2 test = 22.170, df = 1, P < .0001) (Table 3). These results show that in the control period sessions, animals presented cells that differentially fire for levers during the whole task and that most of these cells developed differential firing frequencies for each reward in the second part of the task.

Table 2
Proportion of recorded orbitofrontal cortex neurons that encode rodent gambling task contingencies; comparison between control period and pain period sessions.

	Control period sessions (%)	Pain period sessions (%)	χ^2 (df = 1)	Significance
High-risk lever vs low-risk lever	69.57	50.00	3.802	P = .05
Reward vs no reward (both levers)	47.83	52.00	0.167	P = .68
Reward vs no reward (low-risk lever)	30.43	78.00	5.437	P = .02
Reward vs no reward (high-risk lever)	36.96	56.00	3.489	P = .06
Reward high-risk vs reward low-risk	45.65	16.00	9.992	P = .002

Because the animals during pain period sessions presented a smaller proportion of cells that differentially fired for each lever during the first 45 trials we asked if the behavioral deficit found in these animals could be related to an inability of OFC cells to represent choices as the task progresses. We tested whether the firing frequencies of cells would be associated with the probabilities of choosing a specific lever in the following trial. For this we calculated each cell's firing frequency for the period of 5 seconds before a lever press and correlated this firing frequency with the calculated probabilities of choice according to the MDP matrix of state transition probabilities. In Fig. 7 we present examples of 4 neurons in which this correlation between lever press instantaneous firing frequency and the probability of choosing each lever is evident (note that the probability of choosing the high-risk lever is the exact inverse, and so the absolute correlation value is the same for both levers). The correlation values for entire population of recorded neurons showed that in both the control period and the pain period conditions there was a similar proportion of neurons in which the firing frequency was correlated with the descriptive MDP model of choice probability (control: 27 of 46 = 58.70%; pain: 33 of 50 = 66.00%; χ^2 = 0.545, df = 1, P = .46). These correlation results support the idea that choice biases may be represented in OFC instantaneous cell's frequencies, and that this encoding of preference may be altered but not disrupted by the shift from risk aversion to risk proneness.

3.5. *Pain onset alters the overall encoding of choice in OFC*

Taking into account these results, we hypothesized that if the modulation of firing rates before a lever press was reflecting some

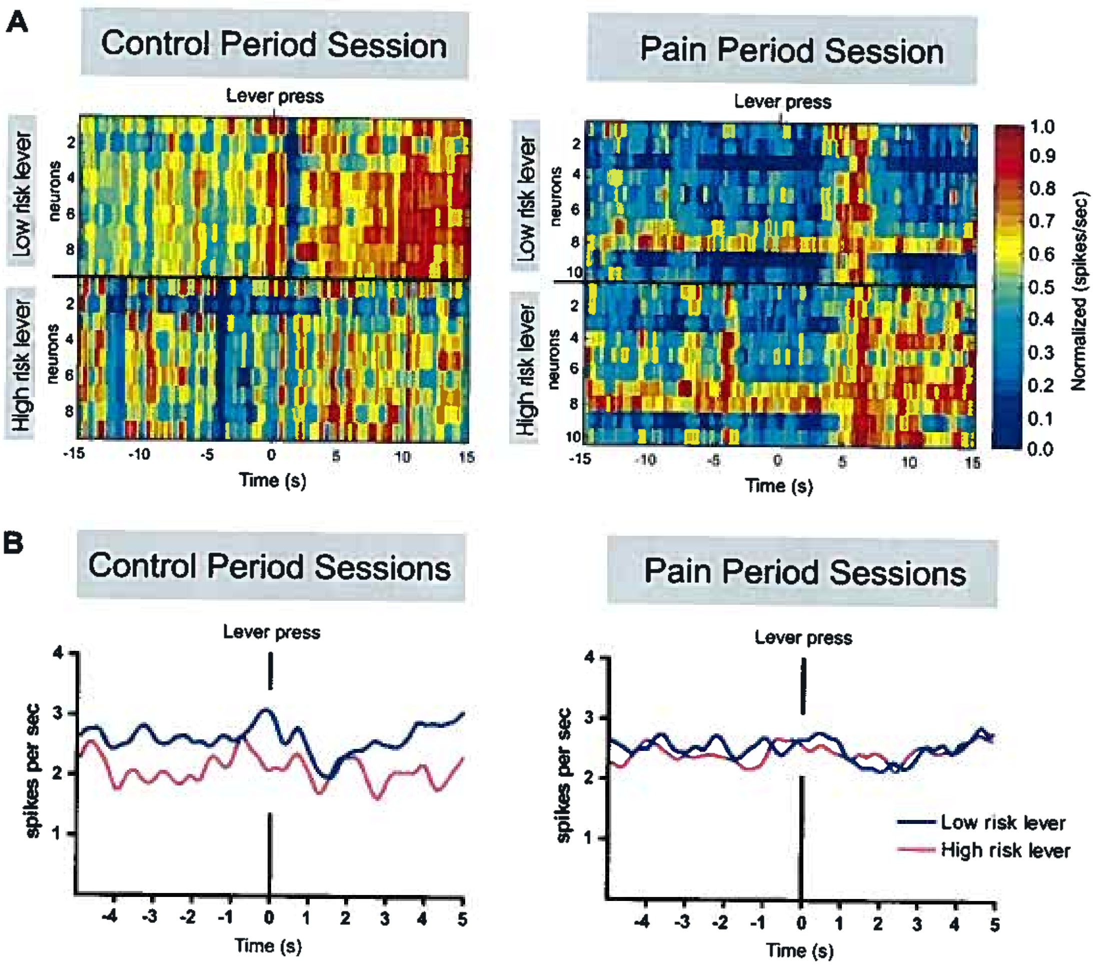

Fig. 6. Comparison of lever press perievent neuronal activity between control period and pain period conditions. (A) Example of ensemble activity in the same animal of the 9 recorded orbitofrontal cortex neurons in the control period rodent gambling task 1 session and of the 10 recorded neurons in the pain period rodent gambling task 3 session. In each panel we separated the perievent activity of the trials in which the animal chose the low-risk lever choice from the trials in which the animal chose the high-risk lever. This example shows that in the control period session the neuronal activity differs according to lever choice, whereas that difference is lost after induction of risk-proneness by the complete Freund's adjuvant injection. (B) Comparison of the lever press perievent activity for all recorded cells shows that in the control period sessions different firing frequencies encode different choices, whereas no difference was found in the pain period population.

Table 3
Proportion of recorded orbitofrontal cortex neurons that encode rodent gambling task contingencies; progression of encoding from the first 45 trials to the last 45 trials.

		Control period (%)	Pain period (%)	χ^2 (df = 1)	Significance
Levers	First half (trials 1 to 45)	63.04	42.00	4.251	P = .039
	Second half (trials 46 to 90)	52.17	46.00	0.365	P = .545
Rewards	First half (trials 1 to 45)	39.13	44.00	0.234	P = .629
	Second half (trials 46 to 90)	76.09	28.00	22.170	P < .0001

form of reward expectancy then the number of cells that presented higher activity for a specific lever could be related to the actual decision making during the RGT. To study this possibility, we subdivided control period and pain period sessions according to the fraction of cells that presented increased frequencies for a specific lever. We use the term high-risk cell if a cell presented increased firing frequencies in the period before the high-risk choice and low-risk cell if a cell presented increased firing frequencies before the low-risk lever press. In control period sessions, 2 groups were considered: the 4 sessions in which only low-risk cells were recorded, and the 2 sessions in which we recorded both low- and high-risk cells. In the pain period sessions, 2 groups were considered: first, the 3 sessions with low-risk or high- and low-risk cells (in 1 session only low-risk cells were found), and in the second group, we grouped the 3 sessions with only high-risk cells recorded. In another 4 sessions there were no neurons with differential firing rates for levers, and these sessions were not included in this analysis. Despite the small number of recorded neurons per session that by no means are able to represent the populational OFC activity, the observed differences are nonetheless surprising: sessions in which we recorded only low-risk neurons were the most control period sessions, and sessions with only high-risk neurons were the most risk-prone sessions (Fig. 8A). These results suggested that the proportion of neurons encoding each lever choice may be crucial for the behavior observed during the task. We further tested this possibility by comparing the proportion of neurons encoding the

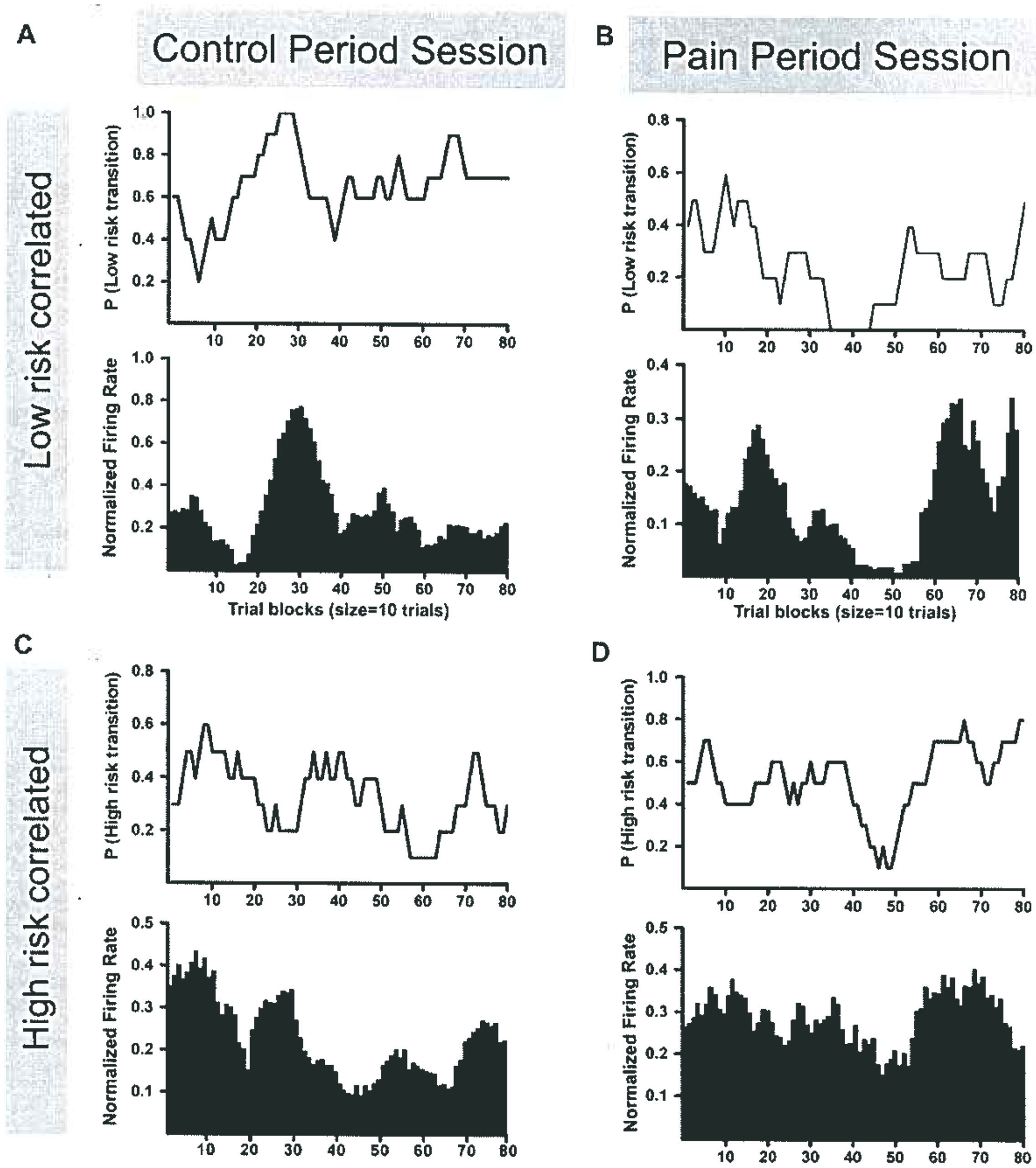

Fig. 7. Correlation between instantaneous firing rate and probability of lever choice. Examples of 4 distinct neurons in which the trial firing frequency preceding each lever choice is correlated with the Markov decision process descriptive model of choice. In each subpanel the top line graph represents the Markov decision process probability of choosing the low risk in that trial (with a moving average of 10 trials), whereas each bin in the bottom histogram presents the firing rate of the 5 seconds preceding consecutive lever presses. Although the absolute correlation is independent of the preferred lever (note that the instantaneous probability of choosing the low-risk lever is the inverse of the probability of choosing the high-risk lever), some cells presented firing rates with the low-risk choice (A, B), whereas others presented correlation for the high-risk choice (C, D).

low-risk lever with the behavioral patterns of choices. The fraction of low-risk cells was calculated as follows: (low-risk cells)/(low-risk cells + high-risk cells). This value was calculated for each recording session and compared with the probability of choosing the low-risk lever for the whole session. The results showed a significant correlation between these 2 measures ($F_{1,10}$ = 18.49; P = .0016, R^2 = 0.649), suggesting that the fraction of cells that specifically signal each choice might have an important role in overall decision making and expression of preference (Fig. 8B).

4. Discussion

In this work we monitored single-neuron activity in the OFC of rats performing a decision-making task under uncertainty before and after the onset of prolonged inflammatory pain conditions. Neuronal activity during the control period sessions was characterized by the development of differential firing rates for each specific choice and its associated reward. On the other hand, the behavioral pattern of risk proneness observed in chronic pain sessions was

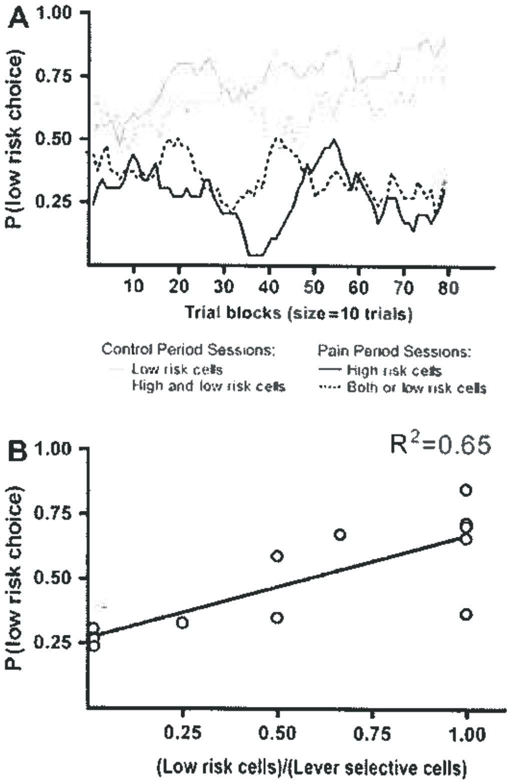

Fig. 8. Correlation between individual neuronal encoding of lever choice and behavioral expression of overall choice preference. (A) Average values of choice probability calculated for each session that contained similar distributions of low-risk and high-risk choice encoding neurons, during control period sessions (solid and dashed gray lines) and pain period sessions (solid and dashed black lines). The terms high-risk cells and low-risk cells were used to refer to cells with statistically different firing rates before each choice (increased firing frequencies before a high- or low-risk choice, respectively). In control period and pain period sessions, the probability of choosing the low-risk lever is increased when animals have low-risk cells. (B) Correlation between the fraction of low-risk lever encoding neurons recorded in each session (open circles) and the overall behavioral expression of lever preference. Note that the sessions in which only low-risk encoding neurons were recorded (rightmost points in the graph) are also the sessions with the highest probability of preference for the low-risk lever, whereas the opposite is true for the leftmost points, which correspond to sessions in which only high-risk encoding neurons were recorded.

associated with a reduced proportion of cells that were able to encode reward magnitude. Moreover, similar proportions of cells were correlated with the actual probabilities of lever choice, showing that choices are still represented in the OFC activity after the onset of chronic pain. However, the fraction of cells that presented higher firing frequencies for each lever differed between conditions and were correlated with the overall RGT behavior and with the presence or absence of pain. Altogether, these results suggest that the behavioral pattern of risk proneness observed in the chronic pain animals could be related to an inability of cells to encode choices and outcomes, leading to impairment in the estimate of reward frequency or magnitude. This evidence of higher firing rates for specific choices is in agreement with previous results that show that OFC is crucial for the association between spatial locations and values for actions [22,37,52], and for risk aversion processing [10,17,44,67]. However, our results cannot demonstrate a direct causal link between the changes in OFC encoding of reward and the shift in risk aversiveness, because this may ultimately be a complex behavior dependent on many brain areas.

The RGT [45] used in this study presents features that make it unique [18]. From these features we emphasize the fact that there is no absolute "correct" strategy in this task because both the risk-averse behavior of control animals and the risk-prone patterns of choice of the chronic pain animals lead to overall similar gains; therefore, it measures choice preference rather than optimal performance, which is a novel feature in the field of rodent neuroeconomical studies. Moreover, animals start the task unaware of the lever contingencies and both have either a 0.2 or a 0.7 probability of no reward; the animals are not able to anticipate when they will or will not be rewarded and need to explore both choices to develop inferential estimates of reward probability and magnitude during the session.

The involvement of OFC in the emotional assessment of painful stimuli is well established in human imaging studies [2,27], in which it has been shown to play a crucial role in the empathy of pain and placebo responses [19,49,65,73,84]; moreover, a few studies have shown that OFC in chronic pain patients presents structural and functional abnormalities [39,56,63]. However, most of those animal studies have only looked at the effect over acute painful stimulation, and demonstrated that activation of OFC is crucial for descending opioid-mediated antinociception [80–82]. Very little is known about the effects that pain has over prefrontal areas, apart from the description that inflammatory pain leads to an increase in prefrontal *N-methyl-D-aspartate* currents [78] and that neuropathic pain leads to an increase in prefrontal interleukin-1 expression and neuronal activation after light touch [3,20].

Previous studies have shown increased firing rates in the OFC cells for the animal's preferred choice and expected outcome in other decision-making tests [51,57,66]. However, in all cases these decision tasks were performed after extensive training and following cues for correct choices. In contrast, during our current task the animals are not overtrained, are not presented with choice cues, and have a Bayesian-like inferential approach to learn the lever contingencies. The results presented here show that the encoding of choices and outcomes may reflect the development of specific estimates of reward values as the task progressed. It thus can be considered that the choice behavior in the final part of the task is more related to risk aversion than to outcome ambiguity. To some extent, the encoding of preferences and outcomes in the OFC described here seems to support the notion that different degrees of uncertainty are processed by the same neural substrate [31,61].

A recent study has shown that OFC encoding of reward size is crucial for the establishment of risk preference behaviors in rats [53]. In our study, the induction of pain led to an increase in the proportion of OFC neurons that fire differently to the rewarded events, while at the same time there was a huge decrease in the proportion of neurons able to encode the size of the delivered reward (only 16% of the recorded cells) (Table 2). This almost complete absence of cells that are able to simultaneously encode lever and reward magnitude after the onset of chronic pain could be the basis of an inability to generate an adequate estimator of value and consequently to predict outcomes [54,57,66]. However, we must stress that our results show a dramatic change in neuronal encoding properties that mirrors the existing shift in risk preference. For example, the comparison of the encoding properties between the first and the final 45 trials (Table 3) shows that in the control period the neurons adapt their encoding as the task progresses, suggesting that reward-based learning mechanisms about the contingencies of the task are disrupted in the chronic pain animals; this inability to learn ambiguous rules is well documented in the OFC [35] and it has been proposed to be the cause of reduced risk-aversion behavior in pathological gambling or drug addiction [7,26,42].

It remains to be elucidated whether the pain-induced risk proneness in this task results from a reduced aversiveness to the succession of unrewarded trials or from an enhanced attraction to the larger and immediate rewards, or simply from an inability to distinguish between choices. Our results seem to suggest the first hypothesis based on 2 observations: first, the neuronal encoding of the magnitude of the rewards (which reasonably would occur in a condition of reward craving) was smaller in the pain vs the control sessions, and second, the neuronal encoding in the pain sessions did not adapt as the task progressed, which reasonably would occur if the accumulation of unrewarded trials would affect the preference. This is also in agreement with recent human and animal studies that have shown that the OFC is critical for the integration of the ongoing information within a broader context of outcomes across trials [68,72] and that it is important for encoding the overall states relevant for reward rather than encoding the rewards themselves [60]. This hypothesis is in good agreement with our observation that only in the control period the OFC was encoding the preferred lever.

Another possibility is that the animals have reduced attentional performance or that the change in performance is caused by a shift in attention from the task to the ongoing pain, which are well-known effects of chronic pain in both human and animal models [9,21,24,30,46,75]. However, when attention is experimentally compromised this commonly reflects in a disruption of preference, i.e., the pattern of choice between 2 distinct options tend to occur at chance levels [41,50,76], which is in disagreement with the behavior displayed in our task, in which there is a shift in the preference between the 2 levers from the control to the pain period, but never an indifference between the options.

Despite clear differences in the OFC activity, the results found in this work do not exclude the highly probable possibility that these physiological and behavioral deficits could be attributable to functional changes in other brain areas relevant to both outcome-related information and chronic pain. The OFC is connected with several regions, such as the nucleus accumbens, amygdala, cingulate cortex, and hippocampus [14,29], that have also been described to be altered during chronic pain and to be involved in decision making in humans [4,5] and in rodents [23,74]. For example, it has been previously described in rats performing the RGT that temporary inactivation of the amygdala leads to high-risk choices and that monoarthritic animals injected with a CRF1 antagonist in the amygdala presented patterns of low-risk choices [32]. It also has been shown that encoding the predictive value in the OFC requires the basolateral amygdala [58] and that associative encoding between a response and an outcome in the amygdala also requires an intact OFC [55].

The results presented in this work show that the OFC plays an important role in the encoding of choice and reward information, and that the onset of pain disrupts this prefrontal-based important neuroeconomical processing. Future studies will need to address whether these alterations are on the origin of risk proneness under the RGT contingencies, or if they are instead a downstream consequence of pain-induced behavioral shift in risk-aversion.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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References

[1] Aguiar P, Mendonça L, Galhardo V. OpenControl: a free opensource software for video tracking and automated control of behavioral mazes. J Neurosci Methods 2007;166:66–72.
[2] Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. Pain 2011;152:S49–64.
[3] Apkarian AV, Lavarello S, Randolf A, Berra HH, Chialvo DR, Besedovsky HO, del Rey A. Expression of IL-1beta in supraspinal brain regions in rats with neuropathic pain. Neurosci Lett 2006;407:176–81.
[4] Apkarian AV, Sosa Y, Krauss BR, Thomas PS, Fredrickson BE, Levy RE, Harden RN, Chialvo DR. Chronic pain patients are impaired on an emotional decision-making task. Pain 2004;108:129–36.
[5] Bechara A. The role of emotion in decision-making: evidence from neurological patients with orbitofrontal damage. Brain Cogn 2004;55:30–40.
[6] Bechara A, Damasio AR, Damasio H, Anderson SW. Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 1994;50:7–15.
[7] Bechara A, Dolan S, Denburg N, Hindes A, Anderson SW, Nathan PE. Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. Neuropsychologia 2001;39:376–89.
[8] Bechara A, Tranel D, Damasio H, Damasio AR. Failure to respond autonomically to anticipated future outcomes following damage to prefrontal cortex. Cereb Cortex 1996;6:215–25.
[9] Boyette-Davis JA, Thompson CD, Fuchs PN. Alterations in attentional mechanisms in response to acute inflammatory pain and morphine administration. Neuroscience 2008;151:558–63.
[10] Burke CJ, Tobler PN. Coding of reward probability and risk by single neurons in animals. Front Neurosci 2011;5:121.
[11] Butler SH, Godefroy F, Besson JM, Weil-Fugazza J. A limited arthritic model for chronic pain studies in the rat. Pain 1992;48:73–81.
[12] Cardoso-Cruz H, Lima D, Galhardo V. Instability of spatial encoding by CA1 hippocampal place cells after peripheral nerve injury. Eur J Neurosci 2011;33:2255–64.
[13] Cardoso-Cruz H, Sameshima K, Lima D, Galhardo V. Dynamics of circadian thalamocortical flow of information during a peripheral neuropathic pain condition. Front Integr Neurosci 2011;5:43.
[14] Carmichael ST, Price JL. Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. J Comp Neurol 1995;363:615–41.
[15] Cetin T, Freudenberg F, Fuchtemeier M, Koch M. Dopamine in the orbitofrontal cortex regulates operant responding under a progressive ratio of reinforcement in rats. Neurosci Lett 2004;370:114–7.
[16] Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.
[17] Christopoulos GI, Tobler PN, Bossaerts P, Dolan RJ, Schultz W. Neural correlates of value, risk, and risk aversion contributing to decision making under risk. J Neurosci 2009;29:12574–83.
[18] de Visser L, Homberg JR, Mitsogiannis M, Zeeb FD, Rivalan M, Fitoussi A, Galhardo V, van den Bos R, Winstanley CA, Dellu-Hagedorn F. Rodent versions of the Iowa gambling task: opportunities and challenges for the understanding of decision-making. Front Neurosci 2011;5:109.
[19] Decety J. Empathy, sympathy and the perception of pain. Pain 2009;145:365–6.
[20] Devoize L, Alvarez P, Monconduit L, Dallel R. Representation of dynamic mechanical allodynia in the ventral medial prefrontal cortex of trigeminal neuropathic rats. Eur J Pain 2011;15:676–82.
[21] Dick BD, Rashiq S. Disruption of attention and working memory traces in individuals with chronic pain. Anesth Analg 2007;104:1223–9.
[22] Feierstein CE, Quirk MC, Uchida N, Sosulski DL, Mainen ZF. Representation of spatial goals in rat orbitofrontal cortex. Neuron 2006;51:495–507.
[23] Floresco SB, Onge St JR, Ghods-Sharifi S, Winstanley CA. Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. Cogn Affect Behav Neurosci 2008;8:375–89.
[24] Ford GK, Moriarty O, McGuire BE, Finn DP. Investigating the effects of distracting stimuli on nociceptive behaviour and associated alterations in brain monoamines in rats. Eur J Pain 2008;12:970–9.
[25] Fu Y, Han J, Ishola T, Scerbo M, Adwanikar H, Ramsey C, Neugebauer V. PKA and ERK, but not PKC, in the amygdala contribute to pain-related synaptic plasticity and behavior. Mol Pain 2008;4:26.
[26] Goudriaan AE, Oosterlaan J, de Beurs E, Van den BW. Decision making in pathological gambling: a comparison between pathological gamblers, alcohol dependents, persons with Tourette syndrome, and normal controls. Brain Res Cogn Brain Res 2005;23:137–51.
[27] Grabenhorst F, Rolls ET. Value, pleasure and choice in the ventral prefrontal cortex. Trends Cogn Sci 2011;15:56–67.
[28] Grachev ID, Fredrickson BE, Apkarian AV. Abnormal brain chemistry in chronic back pain: an in vivo proton magnetic resonance spectroscopy study. Pain 2000;89:7–18.
[29] Haber SN, Kunishio K, Mizobuchi M, Lynd-Balta E. The orbital and medial prefrontal circuit through the primate basal ganglia. J Neurosci 1995;15:4851–67.
[30] Hauck M, Lorenz J, Engel AK. Attention to painful stimulation enhances gamma-band activity and synchronization in human sensorimotor cortex. J Neurosci 2007;27:9270–7.

[31] Hsu M, Bhatt M, Adolphs R, Tranel D, Camerer CF. Neural systems responding to degrees of uncertainty in human decision-making. Science 2005;310:1680–3.
[32] Ji G, Sun H, Fu Y, Li Z, Pais-Vieira M, Galhardo V, Neugebauer V. Cognitive impairment in pain through amygdala-driven prefrontal cortical deactivation. J Neurosci 2010;30:5451–64.
[33] Kepecs A, Uchida N, Zariwala HA, Mainen ZF. Neural correlates, computation and behavioural impact of decision confidence. Nature 2008;455:227–31.
[34] Kiani R, Shadlen MN. Representation of confidence associated with a decision by neurons in the parietal cortex. Science 2009;324:759–64.
[35] Krain AL, Wilson AM, Arbuckle R, Castellanos FX, Milham MP. Distinct neural mechanisms of risk and ambiguity: a meta-analysis of decision-making. Neuroimage 2006;32:477–84.
[36] Luerding R, Weigand T, Bogdahn U, Schmidt-Wilcke T. Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: structural correlates of pain-cognition interaction. Brain 2008;131:3222–31.
[37] Mainen ZF, Kepecs A. Neural representation of behavioral outcomes in the orbitofrontal cortex. Curr Opin Neurobiol 2009;19:84–91.
[38] Metz AE, Yau HJ, Centeno MV, Apkarian AV, Martina M. Morphological and functional reorganization of rat medial prefrontal cortex in neuropathic pain. Proc Natl Acad Sci USA 2009;106:2423–8.
[39] Moayedi M, Weissman-Fogel I, Crawley AP, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD. Contribution of chronic pain and neuroticism to abnormal forebrain gray matter in patients with temporomandibular disorder. Neuroimage 2011;55:277–86.
[40] Mongini F, Keller R, Deregibus A, Barbalonga E, Mongini T. Frontal lobe dysfunction in patients with chronic migraine: a clinical-neuropsychological study. Psychiatry Res 2005;133:101–6.
[41] Murphy ER, Dalley JW, Robbins TW. Local glutamate receptor antagonism in the rat prefrontal cortex disrupts response inhibition in a visuospatial attentional task. Psychopharmacology (Berl) 2005;179:99–107.
[42] Noel X, Van Der LM, d'Acremont M, Colmant M, Hanak C, Pelc I, Verbanck P, Bechara A. Cognitive biases toward alcohol-related words and executive deficits in polysubstance abusers with alcoholism. Addiction 2005;100:1302–9.
[43] O'Doherty J, Kringelbach ML, Rolls ET, Hornak J, Andrews C. Abstract reward and punishment representations in the human orbitofrontal cortex. Nat Neurosci 2001;4:95–102.
[44] O'Neill M, Schultz W. Coding of reward risk by orbitofrontal neurons is mostly distinct from coding of reward value. Neuron 2010;68:789–800.
[45] Pais-Vieira M, Lima D, Galhardo V. Orbitofrontal cortex lesions disrupt risk assessment in a novel serial decision-making task for rats. Neuroscience 2007;145:225–31.
[46] Pais-Vieira M, Lima D, Galhardo V. Sustained attention deficits in rats with chronic inflammatory pain. Neurosci Lett 2009;463:98–102.
[47] Pais-Vieira M, Mendes-Pinto MM, Lima D, Galhardo V. Cognitive impairment of prefrontal-dependent decision-making in rats after the onset of chronic pain. Neuroscience 2009;161:671–9.
[48] Paxinos G, Watson C. The rat brain in stereotaxic coordinates, 4. San Diego: Academic Press; 1998.
[49] Petrovic P, Kalso E, Petersson KM, Andersson J, Fransson P, Ingvar M. A prefrontal non-opioid mechanism in placebo analgesia. Pain 2010;150:59–65.
[50] Pezze MA, Dalley JW, Robbins TW. Differential roles of dopamine D1 and D2 receptors in the nucleus accumbens in attentional performance on the five-choice serial reaction time task. Neuropsychopharmacology 2007;32:273–83.
[51] Roesch MR, Stalnaker TA, Schoenbaum G. Associative encoding in anterior piriform cortex vs orbitofrontal cortex during odor discrimination and reversal learning. Cereb Cortex 2007;17:643–52.
[52] Roesch MR, Taylor AR, Schoenbaum G. Encoding of time-discounted rewards in orbitofrontal cortex is independent of value representation. Neuron 2006;51:509–20.
[53] Roitman JD, Roitman MF. Risk-preference differentiates orbitofrontal cortex responses to freely chosen reward outcomes. Eur J Neurosci 2010;31:1492–500.
[54] Rolls ET, Grabenhorst F. The orbitofrontal cortex and beyond: from affect to decision-making. Prog Neurobiol 2008;86:216–44.
[55] Saddoris MP, Gallagher M, Schoenbaum G. Rapid associative encoding in basolateral amygdala depends on connections with orbitofrontal cortex. Neuron 2005;46:321–31.
[56] Schmidt-Wilcke T, Luerding R, Weigand T, Jurgens T, Schuierer G, Leinisch E, Bogdahn U. Striatal grey matter increase in patients suffering from fibromyalgia—a voxel-based morphometry study. Pain 2007;132(Suppl. 1):S109–16.
[57] Schoenbaum G, Roesch M. Orbitofrontal cortex, associative learning, and expectancies. Neuron 2005;47:633–6.
[58] Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron 2003;39:855–67.
[59] Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. Encoding changes in orbitofrontal cortex in reversal-impaired aged rats. J Neurophysiol 2006;95:1509–17.
[60] Schoenbaum G, Takahashi Y, Liu TL, McDannald MA. Does the orbitofrontal cortex signal value? Ann N Y Acad Sci 2011;1239:87–99.
[61] Schultz W, Preuschoff K, Camerer C, Hsu M, Fiorillo CD, Tobler PN, Bossaerts P. Explicit neural signals reflecting reward uncertainty. Philos Trans R Soc Lond B Biol Sci 2008;363:3801–11.
[62] Seifert F, Bschorer K, De Col R, Filitz J, Peltz E, Koppert W, Maihöfner C. Medial prefrontal cortex activity is predictive for hyperalgesia and pharmacological antihyperalgesia. J Neurosci 2009;29:6167–75.
[63] Seminowicz DA, Labus JS, Bueller JA, Tillisch K, Naliboff BD, Bushnell MC, Mayer EA. Regional gray matter density changes in brains of patients with irritable bowel syndrome. Gastroenterology 2010;139(48–57):e42.
[64] Simon NW, Montgomery KS, Beas BS, Mitchell MR, LaSarge CL, Mendez IA, Bañuelos C, Vokes CM, Taylor AB, Haberman RP, Bizon JL, Setlow B. Dopaminergic modulation of risky decision-making. J Neurosci 2011;31:17460–70.
[65] Singer T, Seymour B, O'Doherty J, Kaube H, Dolan RJ, Frith CD. Empathy for pain involves the affective but not sensory components of pain. Science 2004;303:1157–62.
[66] Stalnaker TA, Roesch MR, Franz TM, Burke KA, Schoenbaum G. Abnormal associative encoding in orbitofrontal neurons in cocaine-experienced rats during decision-making. Eur J Neurosci 2006;24:2643–53.
[67] Tobler PN, O'Doherty JP, Dolan RJ, Schultz W. Reward value coding distinct from risk attitude-related uncertainty coding in human reward systems. J Neurophysiol 2007;97:1621–32.
[68] Tsuchida A, Doll BB, Fellows LK. Beyond reversal: a critical role for human orbitofrontal cortex in flexible learning from probabilistic feedback. J Neurosci 2010;30:16868–75.
[69] van den Bos R, Homberg J, Gijsbers E, Den HE, Cuppen E. The effect of COMT Val158 Met genotype on decision-making and preliminary findings on its interaction with the 5-HTTLPR in healthy females. Neuropharmacology 2009;56:493–8.
[70] Verdejo-García A, López-Torrecillas F, Calandre EP, Delgado-Rodríguez A, Bechara A. Executive function and decision-making in women with fibromyalgia. Arch Clin Neuropsychol 2009;24:113–22.
[71] Walteros C, Sánchez-Navarro JP, Muñoz MA, Martínez-Selva JM, Chialvo D, Montoya P. Altered associative learning and emotional decision making in fibromyalgia. J Psychosom Res 2011;70:294–301.
[72] Walton ME, Behrens TE, Buckley MJ, Rudebeck PH, Rushworth MF. Separable learning systems in the macaque brain and the role of orbitofrontal cortex in contingent learning. Neuron 2010;65:927–39.
[73] Watson A, El-Deredy W, Iannetti GD, Lloyd D, Tracey I, Vogt BA, Nadeau V, Jones AK. Placebo conditioning and placebo analgesia modulate a common brain network during pain anticipation and perception. Pain 2009;145:24–30.
[74] Westlund KN, Vera-Portocarrero LP, Zhang L, Wei J, Quast MJ, Cleeland CS. FMRI of supraspinal areas after morphine and one week pancreatic inflammation in rats. Neuroimage 2009;44:23–34.
[75] Wiech K, Seymour B, Kalisch R, Stephan KE, Koltzenburg M, Driver J, Dolan RJ. Modulation of pain processing in hyperalgesia by cognitive demand. Neuroimage 2005;27:59–69.
[76] Winstanley CA, Chudasama Y, Dalley JW, Theobald DE, Glennon JC, Robbins TW. Intra-prefrontal 8-OH-DPAT and M100907 improve visuospatial attention and decrease impulsivity on the five-choice serial reaction time task in rats. Psychopharmacology (Berl) 2003;167:304–14.
[77] Winstanley CA, Theobald DE, Cardinal RN, Robbins TW. Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. J Neurosci 2004;24:4718–22.
[78] Wu LJ, Toyoda H, Zhao MG, Lee YS, Tang J, Ko SW, Jia YH, Shum FW, Zerbinatti CV, Bu G, Wei F, Xu TL, Muglia LJ, Chen ZF, Auberson YP, Kaang BK, Zhuo M. Upregulation of forebrain NMDA NR2B receptors contributes to behavioral sensitization after inflammation. J Neurosci 2005;25:11107–16.
[79] Zeeb FD, Robbins TW, Winstanley CA. Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task. Neuropsychopharmacology 2009;34:2329–43.
[80] Zhang S, Tang JS, Yuan B, Jia H. Involvement of the frontal ventrolateral orbital cortex in descending inhibition of nociception mediated by the periaqueductal gray in rats. Neurosci Lett 1997;224:142–6.
[81] Zhang S, Tang JS, Yuan B, Jia H. Inhibitory effects of electrical stimulation of ventrolateral orbital cortex on the rat jaw-opening reflex. Brain Res 1998;813:359–66.
[82] Zhao M, Wang JY, Jia H, Tang JS. Mu- but not delta- and kappa-opioid receptors in the ventrolateral orbital cortex mediate opioid-induced antiallodynia in a rat neuropathic pain model. Brain Res 2006;1076:68–77.
[83] Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.
[84] Zubieta JK, Stohler CS. Neurobiological mechanisms of placebo responses. Ann N Y Acad Sci 2009;1156:198–210.