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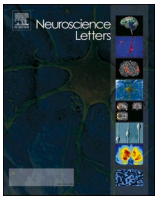

Inflammatory pain modifies reward preferences from larger delayed to smaller immediate rewards in male rats

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ABSTRACT

Self-control underlies goal-directed behavior in both humans and rodents. The ability to balance immediate and delayed gratification is essential for fine-tuning decision-making processes to achieve optimal rewards. Although delayed gratification has been extensively studied using human neuropsychological assessments, brain imaging techniques, and preclinical research, the impact of chronic pain on these processes remains poorly understood. In this study, we successfully trained male rats to perform a custom delayed gratification task (DGt) to evaluate time-reward gratification associations. The task required rats to choose between two levers associated with distinct schedules of reward delivery and magnitude. Behavioral performance was assessed within subjects following the induction of inflammatory chronic pain using the complete Freund's adjuvant (CFA) model. Our findings revealed that CFA-treated rats developed mechanical allodynia and demonstrated a strong preference for small and immediate rewards. In contrast, saline-treated control rats exhibited a more balanced choice profile, indicative of intact self-control. Collectively, these results offer novel insights into how chronic inflammatory pain disrupts time-reward preferences and impairs self-control mechanisms.

Significance

Chronic inflammatory pain disrupts time-reward decision making in male rats, shifting preference from larger delayed to smaller immediate rewards.

1. Introduction

Delayed gratification, the ability to forgo immediate rewards in favor of larger, delayed ones, is a critical aspect of self-control [1,2]. Studies indicate that, similarly to humans, rodents exhibit variability in their ability to delay gratification, with some consistently opting for immediate rewards, reflecting higher impulsivity [3–5]. This highlights the importance of understanding how individuals associate time intervals with rewards, critical to delay gratification. Rodents can also learn to anticipate rewards based on specific temporal cues [6,7], forming the foundation for tasks requiring the postponement of immediate rewards in favor of delayed ones. This includes temporal discrimination tasks, where rodents display variability in performance, that underscore individual differences in impulsivity [5,8].

Chronic pain has been shown to significantly impact cognitive functions and decision-making [9–11]. Persistent pain can lead to changes in mood [12], increased anxiety-like behaviors [13], and impairments in tasks requiring delayed gratification [2,14]. Rodents with chronic pain often exhibit a preference for immediate rewards over delayed ones, indicating a shift toward more impulsive decision-making [15]. This behavioral change is thought to result from pain-induced alterations in neural circuits involved in reward processing and executive function [4,16]. Here, we designed a novel delayed gratification task to examine the impact of inflammatory pain-induced neuroplasticity in behavioral performance and self-control temporal mechanisms of reward acquisition.

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2. Materials and methods

2.1. Rodent model and ethical statement

Experiments were conducted on adult male CD rats (275–325 g) (Charles River, France). The rats were housed under controlled standard laboratory conditions in an individual ventilated cage unit, with a simulated 12-hour light/dark cycle, constant temperature of 22 ± 2°C and relative humidity of 50 ± 5 %. Training sessions were approximately the same time each day (light cycle). Rats were food-deprived to 90–95 % of their *ad libitum* free-feeding body weights while having unrestricted access to water throughout experiments. All behavioral procedures were conducted in accordance with the European Union directive 2010/63/CE. The protocols received approval from the local Ethical Committee of the Faculty of Medicine of Porto and the Direção Geral de Alimentação e Veterinária board (Portugal) (Project 008,335 of 2019/04/11). Every effort was undertaken to adhere to the 3R's recommendations for animal experimentation, minimizing animal use and distress.

2.2. Inflammatory pain model

A monoarthritis inflammatory pain model [17] was induced by injecting 50 µl of complete Freund's adjuvant (CFA; 0.5 mg/mL, Sigma Aldrich, cat. No. F5881) under isoflurane anesthesia in the dorsal surface of the rat hindpaw (hereafter referred as CFA group). For control purposes, we applied the same volume of saline solution (NaCl 0.9 % w/v) (saline group). The sensory threshold for noxious stimulation was assessed 1 h after the end of the probe session by placing the rats individually in a circular chamber with a metal mesh floor and touching the plantar surface of the paw with von Frey filaments (Somedic, Sweden) for ~ 10 s until buckling was caused, as previously described [18].

2.3. Experimental design and behavioral procedures

To evaluate the impact of inflammatory pain on time-reward associations, rats were tested using a custom delay gratification task (DGt). The testing chamber (45x45x40 cm) contained two retractable levers, designated as the "immediate reward lever" and the "delayed reward lever", along with a food reward dispenser positioned in between (Fig. 1a). At the start of each trial, both levers are extended, signalling the rat to make a choice. Pressing the immediate reward lever delivered a small reward (1 pellet; Bioserv, F0023), while pressing the delayed lever delivered a larger reward (3 pellets). The immediate reward lever remained active for a time window of 5 to 25 s after the trial began, whereas the delayed lever is active only during the 10 to 25 s interval. Early responses (0—5 s) and late responses (25–30 s) were not rewarded. If the rat does not select either lever within a specified time window (30 s), the trial is recorded as an omission. Trials were separated by an interval (ITI) of 15 s. Intra-trial sound-cues were used to identify the trial start (1 s, 1 kHz), the beginning of immediate and delayed levers reward delivery period (1 s, 200 Hz), and the end of the reward delivery period (1 s, 6 kHz) (Fig. 1b). A schematic timeline of the experimental design is illustrated in Fig. 1c. The behavioral training phase consisted of multiple sessions for environment habituation and lever press training to receive rewards. Following this, a learning phase was implemented, where rats completed 50 trials per session for 10 consecutive days. During the learning phase both levers were set to an immediate reward delivery contingency. Only rats that achieved at least 75 % of completed trials during the final two sessions of the learning phase were selected to advance to the testing protocol. Finally, probe sessions were conducted 7 days after peripheral saline or CFA injection, each probe session consisting of 100 trials. To avoid possible bias, rats belonging to different experimental groups were tested alternately. The experimenter was blind to group treatment.

2.4. Data analysis and statistics

Custom MatLab scripts (R2024a, MathWorks, USA) were used to process behavioral data. Choice preference index was calculated by subtracting the number of delayed rewarded trials from the number of immediate rewarded trials, then dividing this difference by the total number of rewarded trials. To determine whether the trial-by-trial lever response tendency traces exhibited distinct distribution during the probe session, we employed the two-sample Kolmogorov-Smirnov goodness-of-fit hypothesis test (*kstest2*, $p < 0.05$). Statistical analysis was

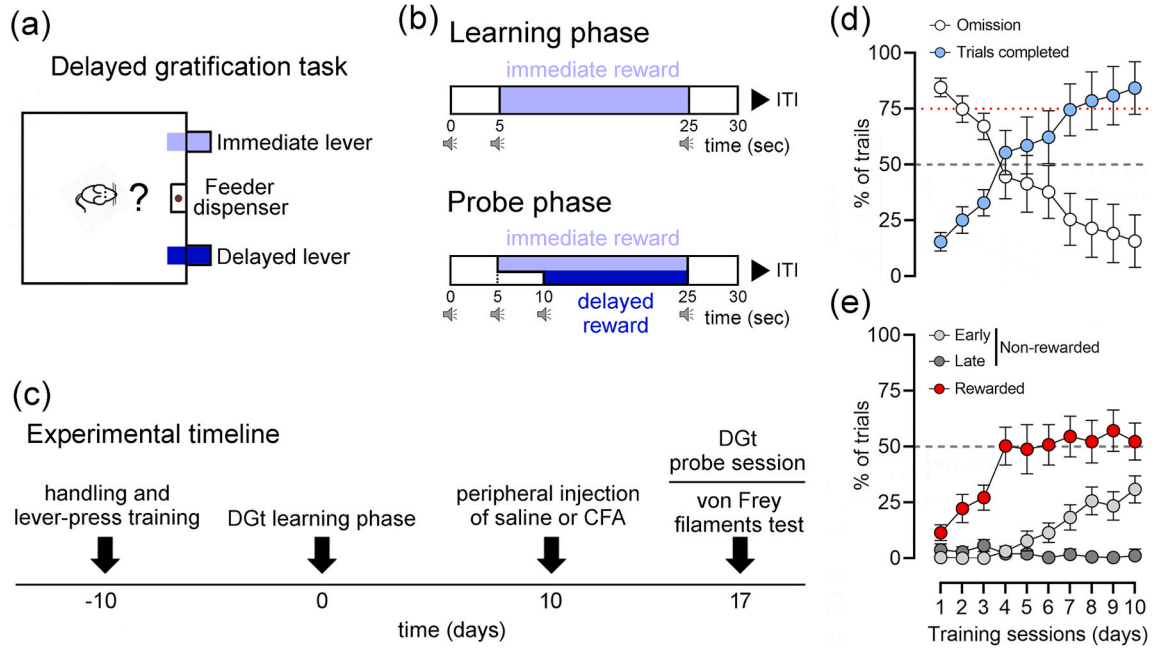

Fig. 1. Delay gratification task, protocol timeline, and learning phase. **(a)** Diagram of delay gratification task (DGt) used in this study. **(b)** Temporal structure of immediate and delayed trials. **(c)** Protocol timeline. **(d)** Percentage of trials completed and omissions during learning phase training sessions. **(e)** Percentage of trials rewarded and non-rewarded (early and late period) during learning phase training sessions.

conducted using GraphPad Prism version 9. All values were tested for normality using the one-sample Kolmogorov-Smirnov test (*kstest*) (with Dallal-Wilkinson-Lilliefors correct *p*-value). Parametric tests were used when *kstest* > 0.05. For single comparisons, we used a non-parametric unpaired Mann-Whitney test (*MW*) or unpaired parametric *t*-test; for multiple comparisons, we used a non-parametric Kruskal-Wallis test (*KW*) followed by the Dunn's *post hoc* test. The sample size was pre-estimated based on previously published research, pilot experiments conducted in the laboratory, and in-house expertise. Rats were randomly assigned to experimental groups, and each rat represented an analytical unit. All effects presented as statistically significant exceeded an α-threshold of 0.05. All independence tests were two-tailed. Data are presented as mean ± standard deviation (S. D.).

3. Results

3.1. Delayed gratification task and learning phase

We used a DGt to examine the impact of inflammatory pain on time-reward associations (Fig. 1a). Briefly, during the learning phase, rats were trained on the DGt with both levers set to deliver immediate rewards (Fig. 1b, learning phase). In the probe phase, one lever was set to deliver immediate rewards, while the other delivered delayed rewards (Fig. 1b, probe phase). All rats included in this study ($n = 12$) met the inclusion criteria after completing 10 daily learning sessions (Fig. 1d). As typically observed in goal-directed tasks, the learning process consisted of an initial phase of rapid improvement, followed by stabilization. Over the course of training, the percentage of completed trials progressively increased, which corresponded with a decline in the percentage of omissions (Fig. 1d). Additionally, the percentage of rewarded trials stabilized midway through the training period (Fig. 1e), while the percentage of non-rewarded early response trials also showed a similar upward trend. This effect was not observed in non-rewarded late response trials.

3.2. Inflammatory pain increases the preference for small and immediate rewards

To evaluate the role of inflammatory pain in behavioral responses, we administered a peripheral injection of CFA into one hindpaw and tested these animals in the DGt seven days later. Our results indicate that all CFA-treated rats developed mechanical allodynia, as evidenced by a significant decreased in the mechanical force required to evoke withdrawal of the hindpaw ipsilateral to the CFA injection ($MW = 0$, $p =$

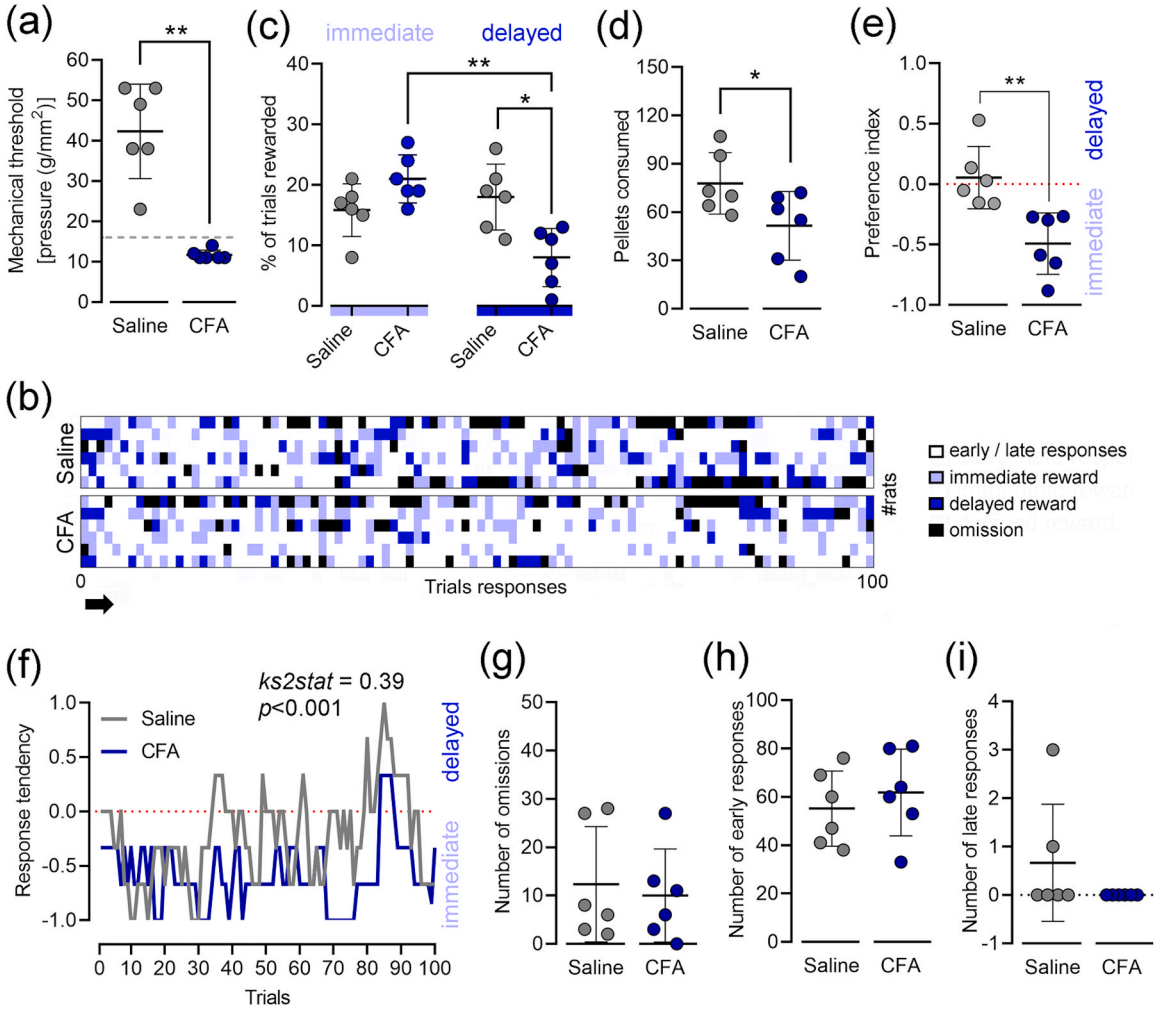

Fig. 2. Behavioral performance during probe sessions. **(a)** Level of mechanical sensitivity measured by withdrawal response to stimulation with von Frey filaments. **(b)** Map of responses of each DGt session. **(c)** Percentage of immediate and delayed trials rewarded for each experimental group. **(d)** Total number of pellets consumed per probe session. **(e)** Preference index. **(f)** Saline-treated rats demonstrated an initial tendency to prefer small and immediate rewards, which change to a preference pattern toward larger and delayed rewards during the course of the testing session. In opposite, CFA-treated rats maintain a constant preference for small and immediate rewards during the testing sessions. No significant differences were observed between experimental groups in the **(g)** number of omissions, and number of **(h)** early and **(i)** late non-rewarded responses. Significant results are indicated by *when $p < 0.05$, **when $p < 0.01$, and ***when $p < 0.01$.

0.0022; Fig. 2a). Fig. 2b illustrates the response map for all rats during the complete probe session. During the DGt probe sessions, saline-treated rats displayed a balanced percentage of immediate and delayed rewarded trials, whereas CFA-treated rats exhibited a clear preference for immediate rewards (Fig. 2c). Statistical analysis revealed a significant effect of experimental group and reward category ($KW = 12.81$, $p = 0.0051$). *Post hoc* test indicated that CFA-treated rats performed a higher percentage of immediate responses (immediate vs. delayed trials, $p < 0.01$), while saline-treated rats demonstrated a higher percentage of delayed trials compared to CFA-treated rats ($p < 0.05$). These differences were also translated in the higher number of pellets consumed by saline-treated rats versus CFA-treated rats (unpaired *t*-test, $t_{(10)} = 2.259$, $p = 0.0474$; Fig. 2d). Next, we evaluated the choice preference index across all rewarded trials (Fig. 2e). Statistical analysis indicated that CFA-treated rats exhibited a significant preference for immediate outcomes ($t_{(10)} = 3.701$, $p = 0.0041$). Trial-by-trial evolution of lever response tendencies across 100 trials revealed distinct profiles between the two experimental groups throughout the entire probe session ($kstest2 = 0.39$, $p < 0.0001$; Fig. 2f). Interestingly, saline-treated rats initially exhibited a tendency to prefer small and immediate rewards, but over the course of the session, they tended to shift their response pattern toward larger and delayed rewards. Finally, another interesting point to consider is that no significant effects were observed between experimental groups in the number of omissions performed ($t_{(10)} = 0.3725$, $p = 0.7173$; Fig. 2e), number of early responses ($t_{(10)} = 0.6869$, $p = 0.5077$; Fig. 2f), and number of later responses ($MW = 12$, $p = 0.2273$; Fig. 2g). Together, these results demonstrate a significant impact of inflammatory pain on time-reward associations, shaping the reward preference phenotype of these animals.

4. Discussion

Our study demonstrates that chronic inflammatory pain significantly alters decision-making in a delayed gratification task. Control rats initially preferred immediate rewards but gradually shifted toward larger, delayed rewards, whereas CFA-treated rats consistently chose immediate, smaller rewards. This finding suggests that chronic pain impairs cognitive flexibility and enhances impulsivity by disrupting the natural balance between immediate and delayed reward valuation.

These behavioral changes are consistent with previous reports that chronic pain impairs executive functions and decision-making [11,19–22]. The persistent preference for immediate rewards in pain-experiencing rats likely reflects a reduced capacity to integrate the benefits of delayed rewards, potentially due to pain-induced alterations in reward perception [23]. Although our behavioral data do not directly measure neural activity, they support the notion that neuroplastic changes in key regions, such as the ventral tegmental area (VTA), which plays a pivotal role in motivating behavior through its dopaminergic drive, may impair the encoding of reward prediction errors [24,25]. Such disruptions could underlie the inability to adjust choices over time, leading to a reliance on immediate gratification.

In summary, chronic inflammatory pain appears to drive maladaptive decision-making by favoring impulsive choices. These findings have significant implications for understanding how pain contributes to broader issues such as impulsivity, addiction, and compulsive disorders. Future studies employing direct neural recordings, such as those of VTA dopaminergic neurons, will be essential to further elucidate the underlying mechanisms and develop targeted interventions to restore cognitive flexibility in individuals affected by chronic pain.

5. Author's contributions

C.M. and H.C.-C. designed research; M.C.-N. and H.C.-C. performed research; H.C.-C. and V.G. analyzed data; M.C.-N., H.C.-C. and V.G. wrote the article; H.C.-C. and V.G. obtained the funding.

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CRediT authorship contribution statement

Mariana Cerqueira-Nunes: Writing – original draft, Investigation. **Clara Monteiro:** Writing – review & editing, Conceptualization. **Vasco Galhardo:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Data curation. **Helder Cardoso-Cruz:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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