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Decoding the effect of expectation in pain from mice's facial expressions using visual machine analysis

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Abstract

Objectives
An environmental context can modulate pain perception by generating expectations based on previous experiences. A significant issue has been the limitations in objectively evaluating pain and its modulation in rodents. This work aimed to utilize machine vision to analyze the characteristics of facial pain-like expressions and their modulation in C57BL/6 mice.
Methods
We employed a conditioned sensitivity model and subsequently generated an expectation induced by the environmental context.
Results
Our findings indicate that machine vision can detect facial pain-like expressions following thermal nociceptive stimulation, resulting in an exacerbated facial response upon re-exposure to a conditioning context, even in the absence of thermal stimulation. Female mice exhibited a stronger facial expression in response to thermal stimulation compared to males. Conversely, by analyzing facial responses, these results affirm male-specific conditioned pain and female-conditioned responses in a non-context-dependent manner.
Conclusion
Thus, we propose that analyzing facial expressions through machine vision is a novel and useful tool for studying pain and its modulation in mice.

Highlights

Machine vision detects facial pain expressions from thermal stimuli

Machine vision detects pain modulation through facial expressions

Facial code analysis confirms male conditioning of pain response

Our results show a female facial response that is not context-dependent

1. Introduction

Pain is a multidimensional experience that includes biological, psychological, and social aspects. It also has a subjective component that makes it particular for every individual. [1, 2] Moreover, cognitive processes such as previous experiences, learning, and memory can broadly modulate perception. [3, 4].

Attention, expectation, and constant reevaluation of the environment influence the cognitive modulation of pain. As such, previous painful experiences under specific conditions could affect future pain perception by associating certain environmental cues with the anticipation of pain [5].

Expectation is a neurobiological mechanism that estimates the probability of an event's occurrence and prepares an individual to modulate sensory, cognitive, and motor systems to execute appropriate

behaviors in response. [6].

The association of a painful event with a specific environmental context is enough to generate a negative expectation, known as pain hypersensitivity, that consists of the worsening in response to a painful stimulus due to the anticipation of pain elicited by predictive cues [4, 7]. It has long been shown that expectancy can modulate the experience of pain relief. [8, 9] Recent research showed that expectation can modulate painful experiences in humans, and this modulation is produced by facial expressions in the socially transmitted placebo effect. This contributes to the behavioral importance of health care providers treating pain and affecting clinical interactions. [10]

This type of associative learning is called “classical conditioning,” which is defined as an unconscious process by which a conditioned response is associated with a specific stimulus (CS+), like environmental cues. On the other hand, an unconditioned stimulus (US) does not induce a behavioral response. [11–13]

Initially, some aspects of the environmental context are neutral and do not provoke any physiological response. Over time, when we associate those aspects with personal, painful experiences, these neutral stimuli become conditioned, and the association of elements in the environmental context can elicit a prototypical facial response based on expectancy from prior experiences. We posit that this must be reflected in facial expressions as a modulation process of the nociceptive experience. The phenomenon of anticipation of pain is an adaptive behavior that would allow animals to escape to avoid future physical harm, and from which the associated neural substrate has been proposed. [14]

Despite significant advances, quantifying the subjective experience of pain and the role of expectation in the perceived intensity has been challenging in animal models and human studies. [1, 2, 15]. To date, sub-spinal-mediated responses have been assessed by estimating the latency time to paw withdrawal. [4] However, this assessment does not represent the multidimensionality of pain (sensory-emotional). It only evaluates the reflex process of the response to nociceptive stimulation. It requires a greater number of animals per group and a longer time of nociceptive stimulation, which gives it ethical disadvantages.

Recent alternatives for evaluating pain based on facial expression analysis using artificial vision [15–18] A grimace scale has been proposed to be determined in rodents. [19] This work aims to evaluate whether the expectation of environmental context (CS+) modulates facial expression associated with nociceptive thermal stimulation (CS-) in rodents using machine vision analysis. We assessed facial expression coding using artificial vision in a mouse model of conditioned hypersensitivity in male and female mice. [4]

Our results demonstrate that evaluating facial expressions as pain-like signs is a reliable, valuable method for assessing the modulation of pain by exposure to different environmental contexts in mice.

2. Material and methods

2.1. Animals

Twenty C57BL/6 mice (ten female and ten male, age 16 weeks, weight< 22g) were used for this study. Animals were placed in individual polycarbonate boxes in a room at 25°C, with food and water *ad libitum* during all tests. Weight was recorded throughout the study to verify the rodents' health status. All procedures were approved by the ethics committee of the Faculty of Higher Studies Iztacala, National Autonomous University of Mexico (Oficio: CE/FESI/092020/1369).

2.2 Head-fixation device implantation

Animals were anesthetized with isoflurane (3% for induction, 1% for maintenance in 1l/min oxygen). The scalp hair was removed, and a 1 cm long incision was made parallel to the cranial sagittal suture. The skin was retracted to both sides to expose the skull. The attachment for the head-fixation system was made using a 3D printer (polylactic acid filament) and cemented to the skull with dual resin. Finally, the tissues were repositioned with cyanoacrylate, preventing skull exposure, and mice were returned to their cages for recovery (7 days).

2.3 Behavioral Set-Up (Head-Fixation System)

All procedures were performed in a room isolated from external sound and light. The virtual reality system consisted of a spherical treadmill with forward and backward movement placed in front of 3 screens (Fig. 1a), as well as a holder for the head-fixation device to maintain the mice in the same position throughout the evaluations.

Context I and II consisted of projections of a white background with parallel vertical black lines and a white background with black dots, respectively. Additionally, the treadmill surface presented different textures for each Context (Fig. 1b).

2.4 Nociceptive Baseline Thermal Stimuli Assessment

Before evaluating each Context, animals were habituated to be placed on the head-fixation system. [17]. First, mice were placed in "Context I" to evaluate the pain-like facial response elicited by a nociceptive thermal stimulus that consisted of an aluminum thermode at 45°C gently pressed on the tail. [16, 20] This session consisted of video recording the orofacial region for 2 seconds during the application of 6 nociceptive thermal stimuli, with a period of inter-stimulation of 3 minutes (see Fig. 1b and Fig. 2, Habituation phase).

2.5 Conditioned Sensitivity to the "Context I"

We differentiated contexts through two main elements. On three screens, one of two options was presented: bars or dots, as previously proposed, that rodents can perceive [21] and that functioned as the walls of the contextual environment (see Fig. 1) as a virtual reality. [22] We also used two types of texture on the walking surface. Both elements were used to configure Context I or II in a

counterbalanced manner. Animals subjected to the same context in the test trial were exposed to the same environmental elements, and these were changed in animals exposed to a different context. [4]

After a baseline assessment of the facial response to a nociceptive thermal stimulus, mice were immediately removed from the head fixation system to administer an intraperitoneal injection of 0.9% acetic acid to cause abdominal constriction discomfort. Immediately after the injection, mice were returned to "context I" for 30 minutes to generate an association between the painful experience and "context I," as shown in Fig. 1b and Fig. 2 in the conditioning phase. At the end of this period, mice were removed from the system and placed back in the home cage.

2.6 Orofacial video recording

Rodents' facial expressions were video recorded (FHD 1920x1080p, 60fps) during all the sessions. To facilitate the homogeneity of the facial position, the camera was placed parallel to the right orofacial region, and the mouse head was fixed-positioned in the system. Image processing was performed in MATLAB R2020a. First, each video was further aligned to visualize the face in the same size and position compared to a single frame from the first recorded video as the standard. For this, we used a geometric transformation with 6 pairs of controlled points: 2 for the corner of the eyes, 1 at the top and bottom of the face (centered to the eye), 1 at the tip of the nose, and 1 in the base of the ear. Once aligned, the image was cropped and rescaled to 1540x875p with the same parameters for all the frames to specifically show the mice face-centered, and where the background represents < 25% of the total of pixels. Finally, each image was processed to obtain the descriptors, denominated "Histograms of Oriented Gradients" (HOGs), by dividing each frame by blocks and cells with the following parameters: 8 histogram orientation blocks, square cells with a height of 32 pixels, and one cell per block. The extracted descriptors were 10368 HOGs per image (Fig. 1a and Fig. 3a). This way, the characteristics of the facial response in each frame could be numerically represented to detect subtle changes in the facial expressions of the mice and perform a quantitative analysis (Dolensek et al., 2020).

2.7 Creation of the nociceptive prototype

After conducting baseline assessments, the videos recorded during the nociceptive stimulations were converted to HOG descriptors, as previously described. All facial descriptors were pairwise compared with Pearson correlation. The frames were hierarchically clustered using a dendrogram to group the frames with higher similarity between them. Posteriorly, each frame was labeled with its corresponding period (Pre-stimulation or stimulation). We performed a Principal Component Analysis of the HOGs and then plotted the bidimensional distribution of the 2 principal components of t-SNE dimensional reduction. The 25 descriptors from the stimulation period (corresponding to the nociceptive thermal stimuli) with more difference from the baseline period were extracted and averaged. This vector is considered the nociceptive prototype. In each subsequent analysis, the correlation score was obtained, comparing each frame to this prototype. As such, the similarity score obtained represents a nociceptive expression when closer to 1 and a more neutral expression when closer to 0.

2.8 Test

After conditioning sensitivity, the test phase evaluated the facial response elicited by applying a thermal nociceptive stimulus (Fig. 2 Test phase). Female and male mice were divided into the same and different contexts (Fig. 1b). The first two groups (associated with the pain-like experience) were evaluated in Context I using the same conditions during the baseline test. In contrast, the different context groups were placed in the novel Context II. This way, the effect of expectation induced by environmental Context on nociceptive perception was evaluated.

2.9 Statistical analysis

Statistical analyses were performed using MATLAB R2020a and GraphPad Prism 8; for the comparison between the 2 groups, paired Student's t-test and Mann-Whitney U test were used. Differences were considered statistically significant if $p < 0.05$ (Table 1).

3. Results

3.1 Nociceptive thermal stimulation evokes facial pain-like expressions detected by machine vision.

First, to ensure that the mice did not exhibit anxiety-like behaviors, we performed the elevated plus maze test one day before and after the last session of conditioning to the head-fixation system. We found that animals travelled less distances at the maze on the second test (Fig. 4a, $t_{(5)} = 3.194$, $p = 0.0242$). Meanwhile, there were no significant differences in the index of time spent at the open arms (Fig. 4b, $t_{(5)} = 0.3061$, $p = 0.7719$), and in the total entries to both open arms (Fig. 4c, $t_{(5)} = 1.865$, $p = 0.1212$) and closed arms (Fig. 4d, $t_{(5)} = 1.431$, $p = 2117$) compared with the baseline test.

To generate a pain-like prototype of facial response in mice, we first determined if the nociceptive thermal stimulation applied on the mice's tails would evoke changes in facial expression. To answer this, we performed a Pearson's correlation analysis of the frames of each video pre-stimulation period and nociceptive thermal stimulation (2s per period). The frames were hierarchically clustered to group the most similar images at each corner. The heat map in Fig. 5a shows an example of the similarity matrix of thermal stimulation, the correlation between each pair of frames, and how they are grouped according to their correlation with each other. To further determine if this clustering represented the changes in facial expression evoked by the thermal stimulation, we added the frame origin at the border; as such, the labeling organization shows that the images from the baseline and thermal stimulation were clustered separately. The dendrogram above the heat map shows the grouping of the 2 periods (pre-stimulation and nociceptive thermal stimulation), which agrees with the periods clustered at the origin frame and the heat map. The HOGs were dimensionally reduced to be plotted in a bi-dimensional way to understand further how similar they were to each other. In Fig. 5b we observed how the frames were grouped according to their origin, indicating that the thermal stimuli affected the facial expression. This data

shows that the algorithm was sensitive to the changes in the facial features and could group images with similar characteristics. Finally, the 25 frames more different from the baseline period were used to calculate the nociceptive prototype.

3.2 Machine vision analysis detects expectation effect observed during exposure to Context only on test day in male and female mice

Once we generated the nociceptive prototype, we evaluated its similarity to it for each period. A simple exposure to the Context for the first time (day 1) does not elicit any facial expression of pain (see 3.1 and Fig. 8a). However, re-exposure to the Context without stimulation elicited an exacerbated facial response in a non-dependent context (Same Context, $p < 0.0001$; Different Context, $p = 0.0004$) (Fig. 6a, Table 1). Figure 6b shows differences between day 1 and the test in the stimulation period in a non-dependent context (Same Context $p = 0.0003$; Different Context $p < 0.0001$, Table 1).

To elucidate differences between male and female mice, we analyzed data separately. We performed a Mann-Whitney U statistical analysis (significance, $p < 0.01$) to compare the similarity to the nociceptive prototype at the exposure to Context only and stimulation period. Figure 7a shows no differences between female and male mice the first time they were exposed only to the Context ($p = 0.0923$, Table 1). Interestingly, female mice had an exacerbated facial pain-like expression in the baseline of the thermal nociceptive stimulation period on day 1 ($p < 0.0001$, Table 1). Figure 7b shows that female mice respond more than males to re-exposure only to the Context ($p = 0.0002$, Table 1) and exacerbated responses to stimulation on Test Day ($p = 0.0001$, Table 1).

3.3 Facial coding elucidates male-specific conditioning to context pain hypersensitivity and female non-context dependent responses.

To make a more detailed observation, we divided the analyses into groups: females same Context (FSC), females in the different Context (FDC), males same Context (MSC), and males different Context (MDC). We performed a Mann-Whitney U statistical analysis to evaluate the similarity to the nociceptive prototype during the stimulation period ($p < 0.01$). Figure 8a confirms that first-time exposure to any environmental Context alone did not induce a facial pain response in either group. In all cases, the rodents responded only to nociceptive stimulation: FSC ($p < 0.0001$), FDC ($p < 0.0001$), MSC ($p < 0.0001$), and MDC ($p = 0.0038$) (Table 1).

Interestingly, both groups of female mice increase their facial response during re-exposure to any context: FSC($p < 0.0001$) and FDC ($p < 0.0001$) (Table 1). Male mice show a context-dependent modulation of face-like pain expression; MSC ($p < 0.0001$, Table 1); the MDC group did not show this increasing facial response ($p = 0.1589$, Table 1).

On Test Day, during stimulation, we found that there was a significant decrease in similarity to the nociceptive prototype for most of the groups compared with baseline: FSC ($p = 0.0021$), FDC ($p < 0.0001$), MDC ($p < 0.0001$) (Table 1), but it remained similar for the MSC group ($p = 0.1022$, Table 1), as shown in Fig. 8b.

4. Discussion

The results of this work demonstrate, for the first time, that facial expressions of pain were elicited by expectancy related to environmental context, which is detected by machine vision. Briefly, we propose a facial pain-like expression evaluation via artificial vision to study cognitive pain modulation in mice.

We adapted a previously published protocol of conditioning sensibilization [4] to facial pain-like coding evaluation by machine vision algorithms [16] in head-fixed male and female mice [23] receiving noxious thermal stimuli.

At first, we wondered if the machine vision system could detect facial pain-like responses when a non-intense painful stimulus was applied. Surprisingly, the facial expression coding in mice shows changes related to thermal stimulation on mice tails. This could indicate that most mice perceived and responded to nociceptive thermal stimulation reflected in their pain-like facial responses, just like in previous experiments in which mice were electroshocked. [16]. This result confirms earlier findings that the mouse could perceive our experiment temperature as harmful (45°C) [24].

Previous results also showed that machine vision analysis is sensitive to mechanical stimulation. We conducted sophisticated experiments to measure the facial response associated with mechanical hypersensitivity in mice with chronic pain conditions. [17]. Altogether, we can conclude that mice facial pain coding can be evoked from nociceptive different modalities of stimuli of lower intensity and less duration than electric shock but detected by machine vision.

Multiple efforts in developing artificial intelligence analysis technologies aim to develop tools for automated pain assessment (APA) that can be applied to research or clinical practice to accurately identify pain events. [25].

Our results confirm that the algorithm proposed by Dolensek et al. in 2020 can effectively assess pain in rodents. Regarding ethical considerations, it reduces the number of individuals used in the experiments and the intensity and time of exposure to the noxious stimulus in future experiments. It also works to study both nociceptive and chronic pain in mice.

The analysis of the facial expression for pain consists of generating a pain prototype and determining the degree of similarity with photograms from a particular time window during a short experiment in which the rodent is stimulated. [16] For the first time, we demonstrate that both electroshock and short thermal stimulation at 45°C are equally effective in generating a prototype of the facial expression of pain.

We used a protocol in which expectations were related to Context by conditioning sensitization induced by 9% of acetic acid in a particular environment called Context I [4]. To measure the degree of nociception, they used the paw withdrawal latency test for nociceptive stimulation, which ranges from approximately 5 to 30 seconds, implying long periods of stimulation. The assessment of nociceptive perception has been limited to spinal reflex responses; therefore, the need to evaluate supraspinal responses that reflect qualitative elements of the nociceptive experience arises. From an ethical point of view, analyzing facial expressions by machine vision is more effective, with less time for stimulation (2-s), fewer animals by group, and less experimentation time.

A recent article proposed an automated paw-tracking system to study pain-like responses at the millisecond level, which could be a novel alternative. [26] However, it has the disadvantage of assessing a reflexive response, and the facial expression may include sensory-emotional processes.

The next question we posed was whether the machine vision system's facial analysis was sensitive to pain modulation. Our results showed that facial expressions reflect changes in pain modulation induced by expectations induced by environmental Contexts, like early findings from withdrawal paw latency. [4]. This result suggests that facial expressions can be modulated by cognitive elements such as expectancy associated with cues from an environmental context.

We also analyzed the effect of expectation in females and males separately since previous results determined a male-specific pain memory mechanism in mice and humans in experiments that use expectancy to modulate pain perception. [4, 27]. It was attractive to validate if the result would be the same when we evaluated the facial expression compared to the paw withdrawal response.

Interestingly, in the case of females, both groups, FSC FDC, showed a greater facial response to thermal stimulation from the first exposure. These results are consistent with experiments in which it was observed that females showed greater facial expression to thermal pain, even when they received a lower intensity, indicating a lower thermal threshold and a greater emotional response reflected in better facial communication of pain in an intensity-discriminative manner. [28] These FSC and FDC groups presented an increase in similarity to the nociceptive prototype, which could indicate that the anticipation of nociceptive stimulus in females occurs independently of the Context or that possibly females can relate other elements that we did not contemplate in the environmental Context that conditioned their response in both groups.

The phenomenon of anticipation of nociceptive stimulation has been studied, and neuronal substrates that underline this phenomenon have been proposed. [14] Interestingly, the neuronal response in regions associated with anticipation increases when there is the expectation of pain. [29] Anticipatory responses reflect a behavioral adaptive phenomenon to escape and avoid damage that underlies the important role of cortical and subcortical regions. [30] This anticipation phenomenon may also activate brain sites that mediate nociceptive experience, which change the response to painful experiences in chronic pain situations and which could be proposed as therapeutic targets for pain. [31]

On the other hand, anticipation is also important for generating the placebo effect and the brain processes involved in it. [32] It has also been proposed that there are specific modulatory neural circuits that modulate the perception of a noxious stimulus when pain intensity expectancy is generated. [33] Cortical regions associated with the processing and perception of cues that predict nociceptive stimuli and modulate the nociceptive experience have been proposed. [34] All this evidence supports the importance of studying anticipation and its influence on the nociceptive experience. Hence, these findings open the door for further studies.

For males, this increase in similarity occurred exclusively in the MSC group, which was associated with the painful experience, and it did not happen for the MDC group. These shreds of evidence indicate that the anticipation of nociceptive stimulus in males depends on the Context, corroborating previous results and validating the use of facial expressions and machine vision in pain modulation studies.

5. Conclusion

This study demonstrates that machine vision analysis can detect the pain-like facial expression associated with stimulation and the exacerbated facial response elicited by re-exposure to a conditioning context in males but in any context in female mice. Therefore, the coding of facial expressions by machine vision is a novel and useful tool for studying pain and its modulation in rodents.

Declarations

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8. Sample CRediT author statement

Perez-Martinez IO: Conceptualization, Methodology, Software, Formal analysis Resources, Writing – Original Draft, Writing - Review & Editing, Visualization, Funding acquisition, Garcia-Gama JE: Methodology, Investigation, Software, Visualization, Formal analysis, Writing – Original Draft, Writing - Review & Editing; Perrusquia Hernandez E: Methodology, Software, Visualization, Writing - Review & Editing; Andrade-Gonzalez RD: Methodology, Software, Visualization, Writing - Review & Editing; Montes-Ángeles CD: Conceptualization, Writing - Review & Editing; Miranda Herrera F: Conceptualization, Writing - Review & Editing;

The authors declare no conflicts of interest.

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Tables

Table 1 is available in the Supplementary Files section.

Figures

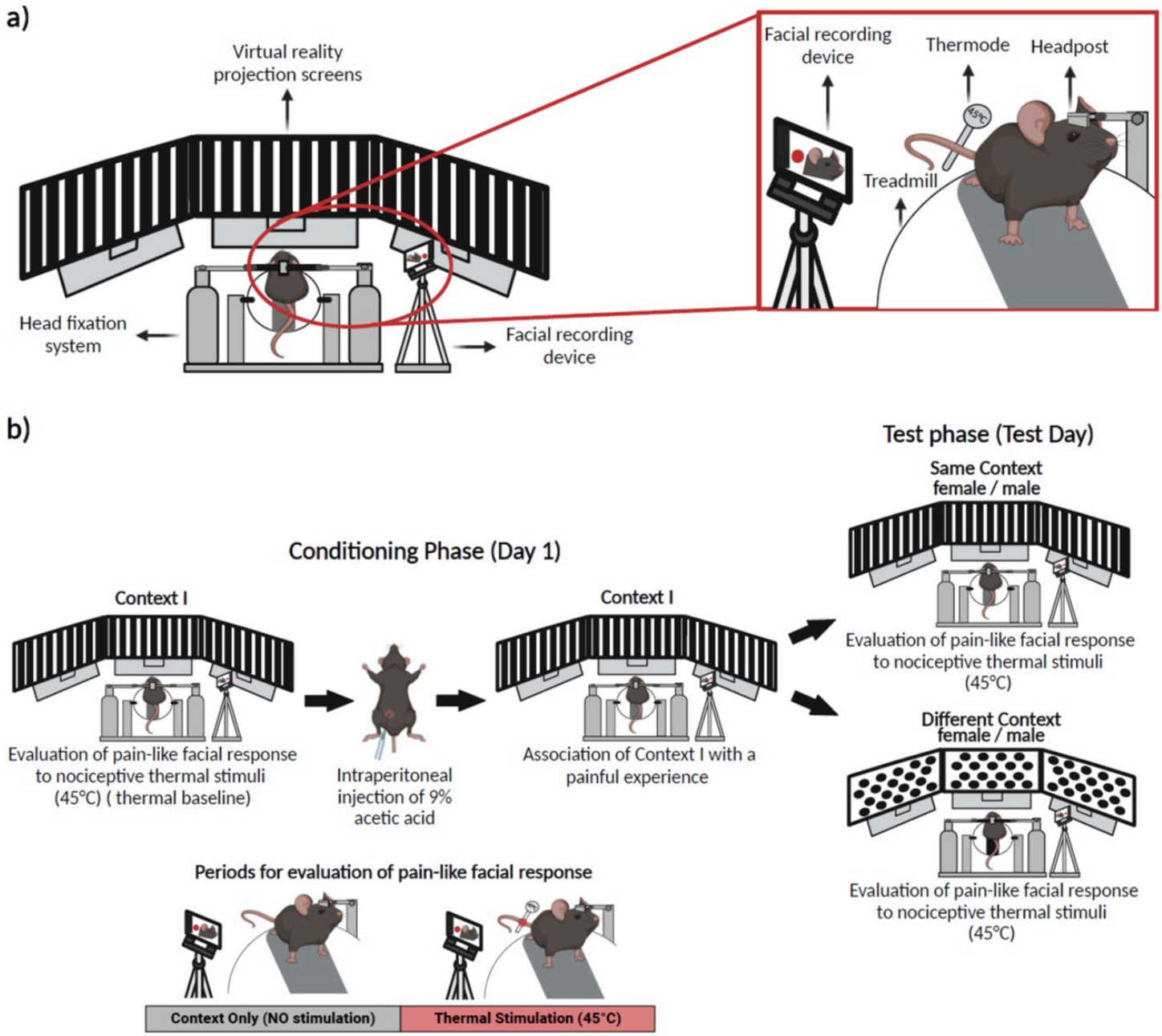


Figure 1

Fig. 1 Behavioral set-up and conditioned sensitivity model. a) The figure displays a mouse in the fixed head system (virtual reality screens, treadmill, recording camera, and thermode for stimulation) placed in Context I. **b)** The first phase is the induction of conditioned sensitivity or conditioning phase, in which the mouse was placed in Context I, and a baseline evaluation of perception of nociceptive thermal stimuli was performed. Afterward, an intraperitoneal injection of 9% acetic acid was administered, and the mouse was placed back in Context I for 30 minutes to associate it with a painful experience. Afterward, an intraperitoneal injection of 9% acetic acid was administered, and the mouse was placed back in Context I for 30 minutes to associate it with a painful experience. The testing phase is shown on the right, where the perception of nociceptive thermal stimuli was re-evaluated. Still, the mice were separated into four groups: females were evaluated in the same Context (Context I). Males were

assessed in the same Context (Context I), females were evaluated in a different context (Context II), and males were assessed in a different context (Context II).

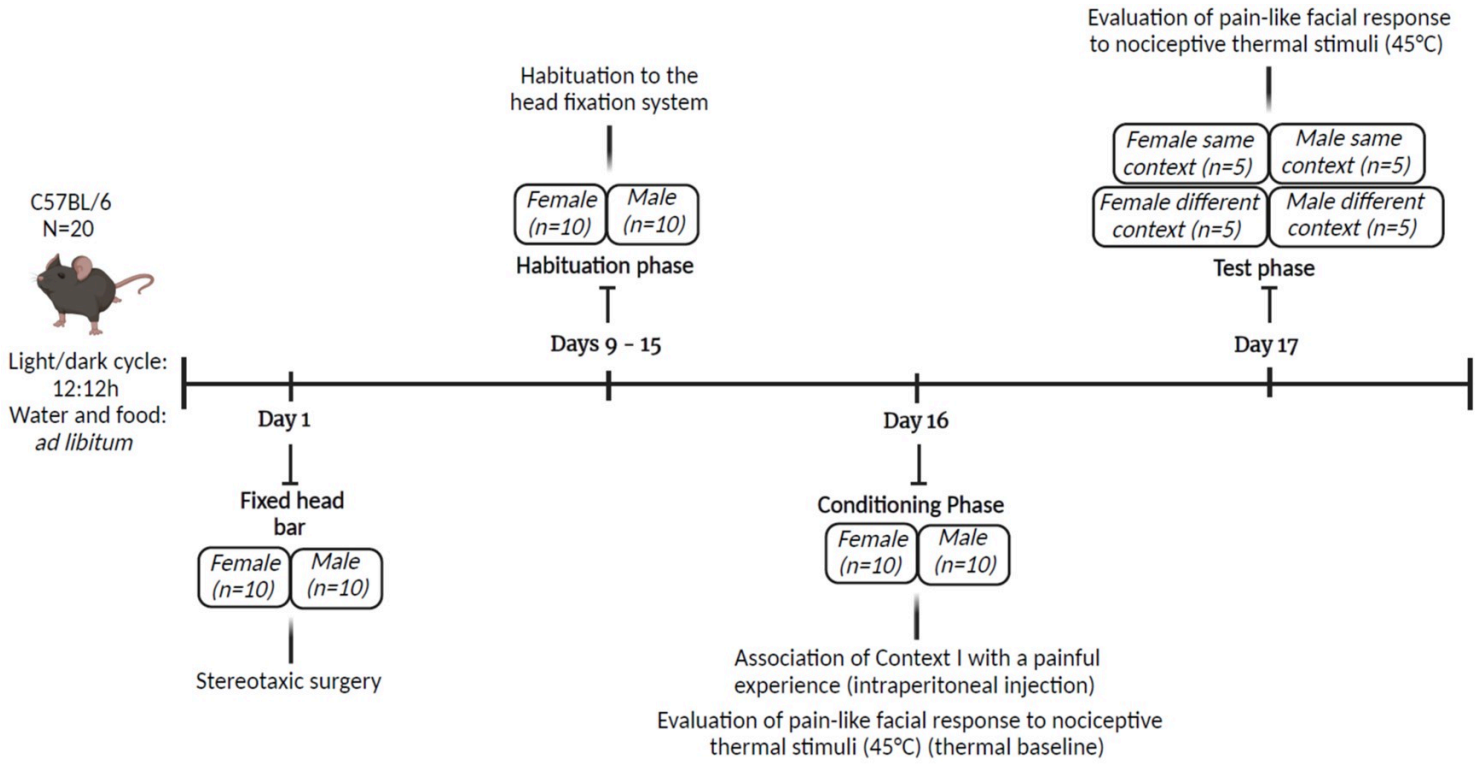


Figure 2

Fig. 2 Experimental Design. All mice underwent stereotaxic surgery to place the attachment for the fixed head system. After seven days of rest, the habituation phase began, during which all mice were placed in the system for 15 minutes per day for seven days. After the habituation phase, the conditioned sensitivity model began. This protocol started with the induction day of conditioned sensitivity, during which all mice underwent a baseline evaluation of perception to nociceptive thermal stimuli, which were associated with Context I and a painful experience. The next day, a testing phase was conducted. The mice were divided into four groups: females evaluated in the same Context, males assessed in the same Context, females evaluated in a different context, and males evaluated in the same Context. The evaluation was performed using nociceptive thermal stimuli.

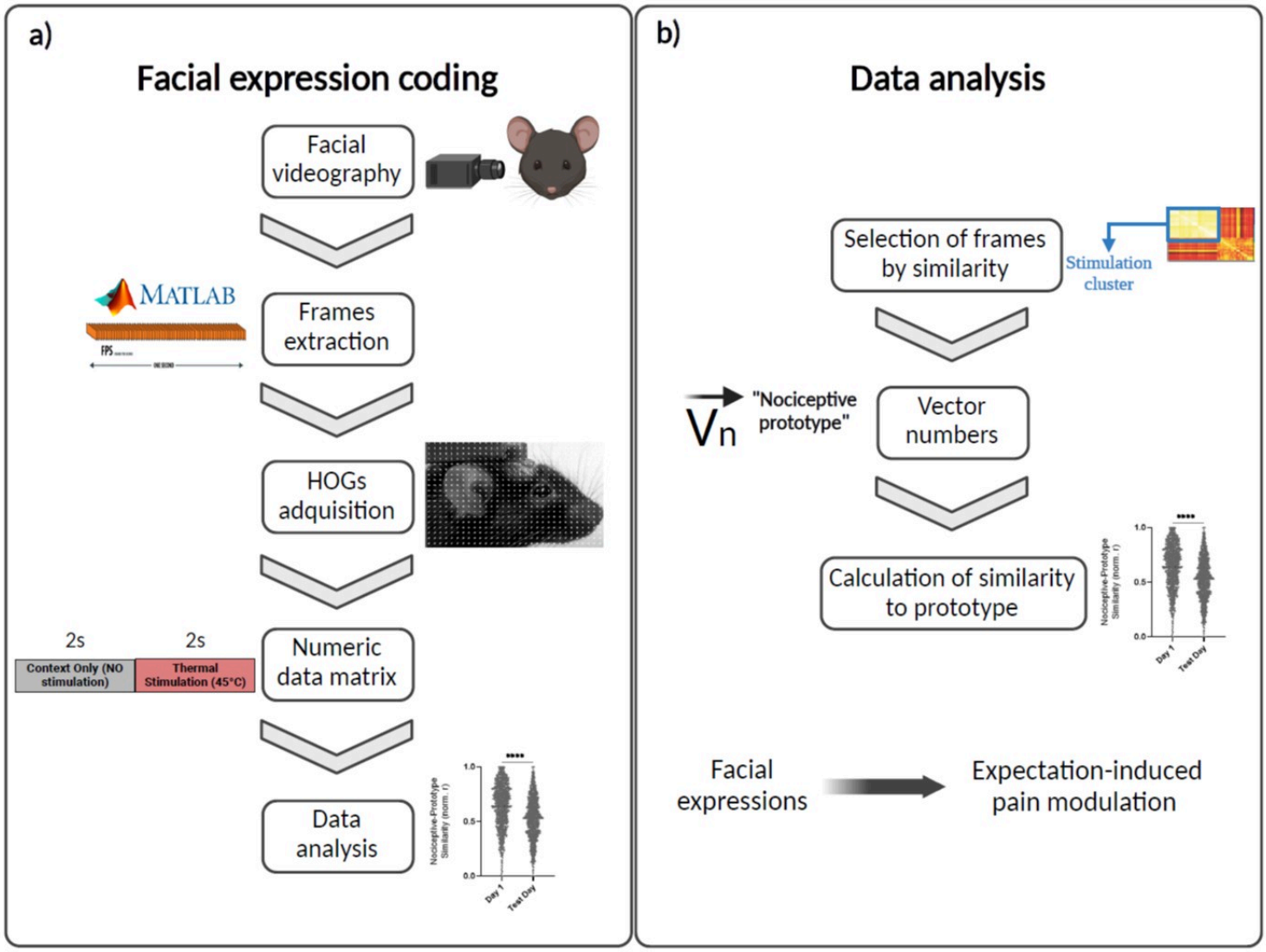


Figure 3

Fig. 3 The analysis of facial expression coding. a) First, videos of the mice's orofacial region were recorded, and then the obtained videos were digitized. For digitization, each video was first converted into a sequence of frames. Each frame was then edited to homogenize its characteristics. Once the edited images were obtained, the Histogram of Oriented Gradients (HOG) was calculated for each frame. This process transformed each video into a numerical data matrix, which is then used for data analysis. **b)** For the study, the nociceptive prototype was first obtained. Facial expressions were then selected based on similarity during the 2 seconds of stimulation. This vector of numbers represents the nociceptive prototype, which was used to calculate the similarity of all frames against the prototype.

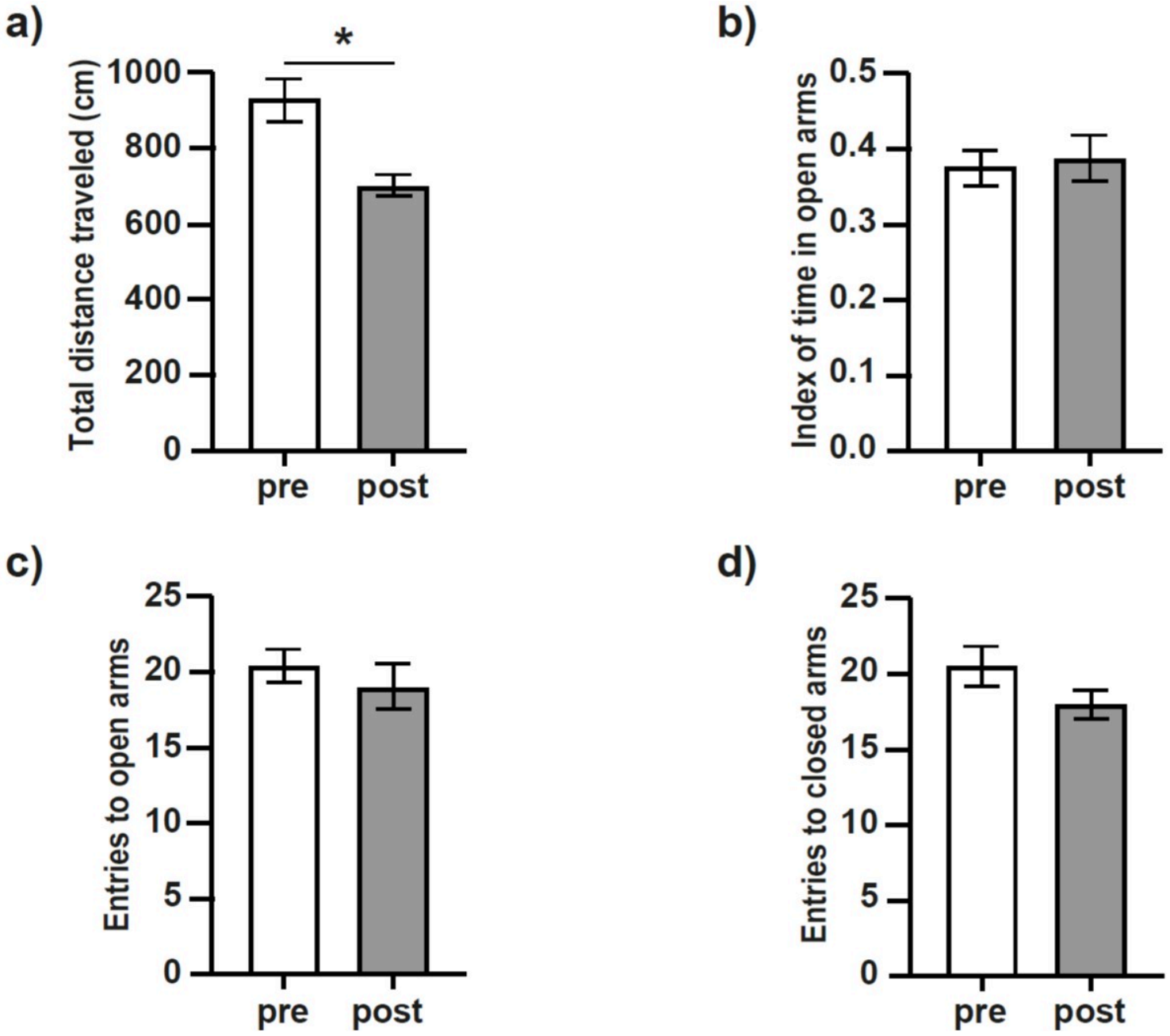


Figure 4

Figure 4. Evaluation of anxiety-like behavior before and after habituation phase to the head-fixation system. This figure shows comparisons between mice (before and after habituation phase) of **a)** total distance travelled at the maze. **b)** index of time spent in the open arms, **c)** total entries to the open arms during the test, and **d)** total entries to the closed arm during the test. The statistical analysis of Student's t-test was performed to analyze this data ($p<0.05$) *$p=0.024$. The graphs show the mean ± SEM.

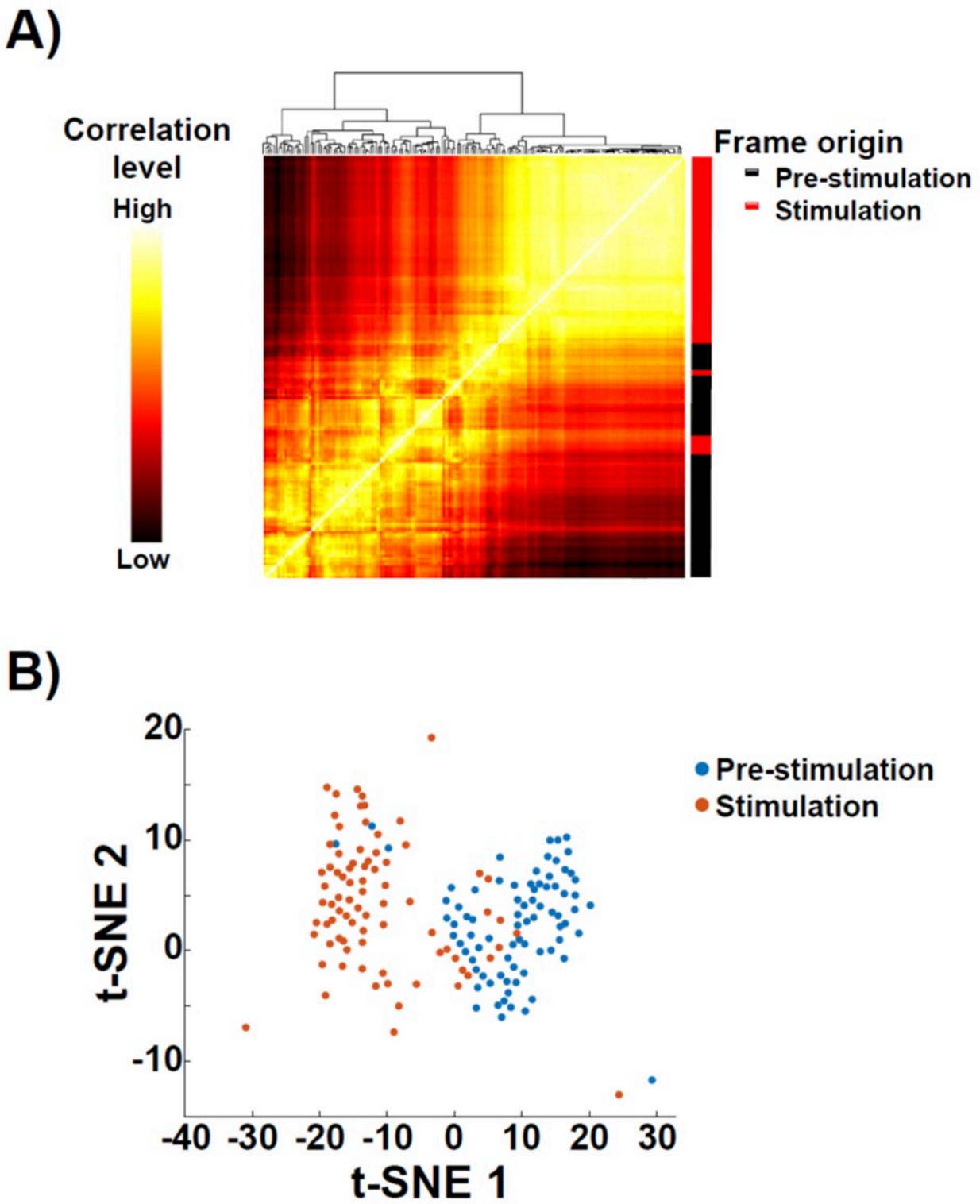


Figure 5

Figure 5. The similarity matrix of facial expression during thermal nociceptive stimulus. a) Heatmap of the pairwise correlation between each pair of images before and during the application of thermal stimulation (45°C) (2 seconds each). The color scale indicates the level of correlation of the similarity matrices (high correlation r=0.9 and low correlation r=0.5). The dendrograms at the top of the similarity matrices display the hierarchical grouping of the frames based on their similarity. The origin of each

frame is indicated at the right border. **b)** Scatter plot of the distribution of the frames according to their first two components.

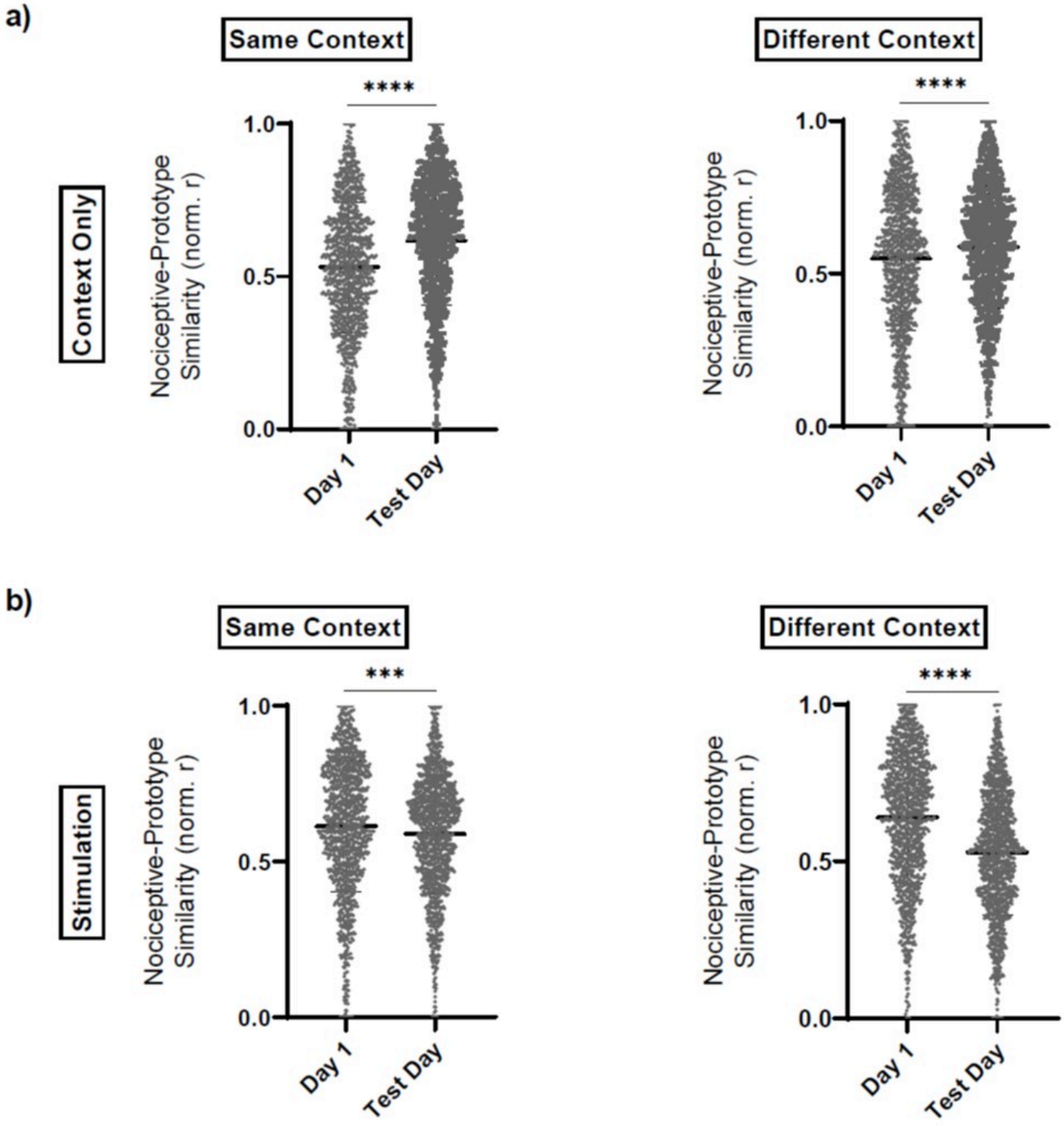


Figure 6

Figure 6. The similarity to the nociceptive prototype on mice evaluated in the same or a different Context. This figure compares the similarity of the frames (represented by each point in the graphs) to

the nociceptive prototype. **a)**shows the comparison between Day 1 (conditioning phase) and Test Day (test phase), during context only (NO stimulation) period, in mice evaluated in the same or different Context. **b)** compares Day 1 (conditioning phase) and Test Day (test phase), during the stimulation, in mice evaluated in the same or different Context. The statistical analysis of the Mann Whiney U test was performed to analyze this data ($p<0.05$) ***$p<0.0005$ ****$p<0.0001$ (Table 1). The graphs show the mean ± SEM.

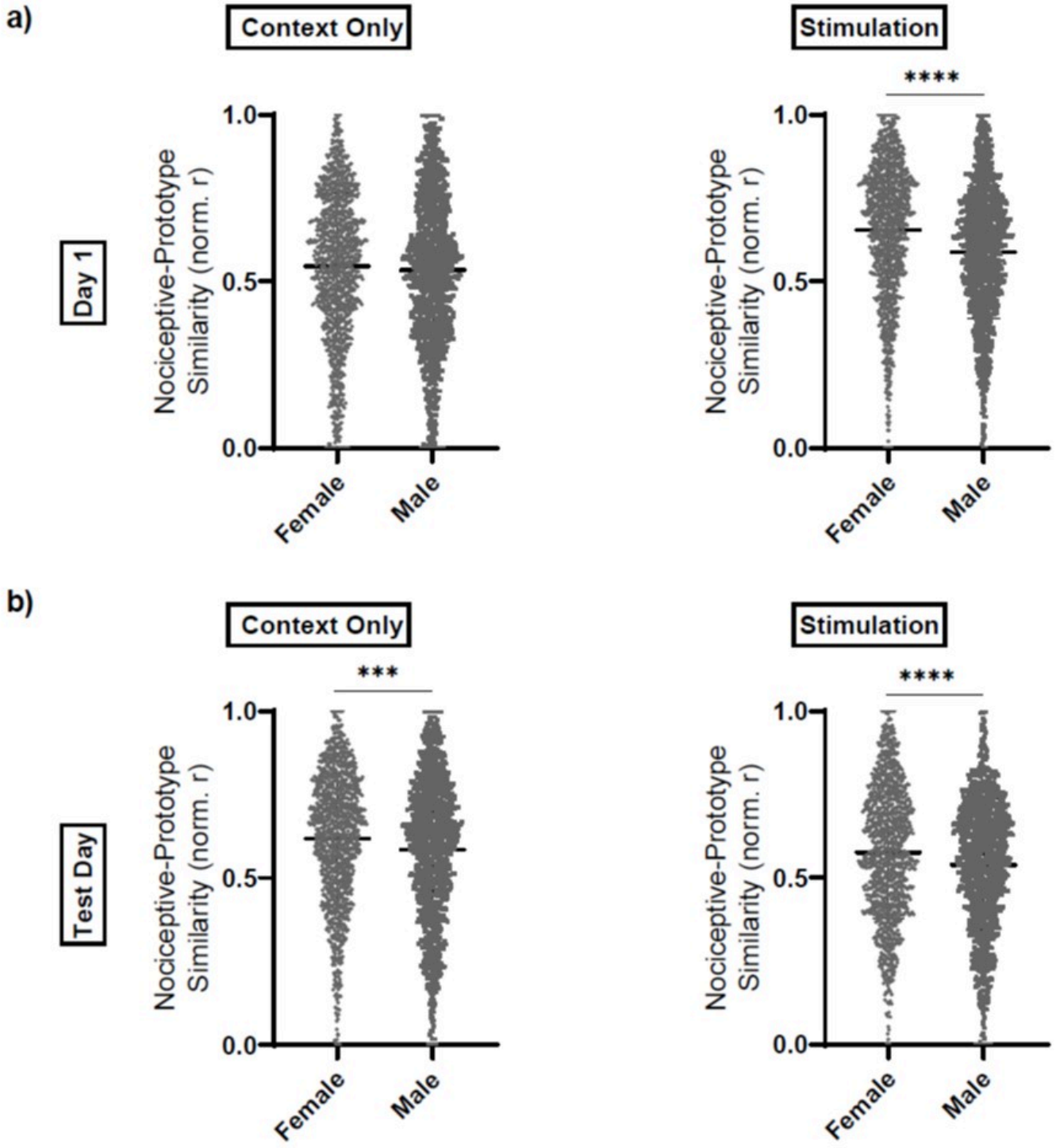


Figure 7

Figure 7. The nociceptive prototype similarity during Day 1 and Test Day in male and female mice. This figure compares the similarity of the frames (represented by each point in the graphs) to the nociceptive prototype. **a)** compares female and male mice during Context only (NO stimulation) and thermal stimulation, specifically on Day 1 (conditioning phase). **b)** compares female and male mice during Context only (NO stimulation) and thermal stimulation, specifically on Test Day. The statistical analysis of the Mann Whiney U test was performed to analyze this data ($p<0.05$) ***$p=0.0002$ ****$p=0.0001$ (Table 1). The graphs show the mean ± SEM.

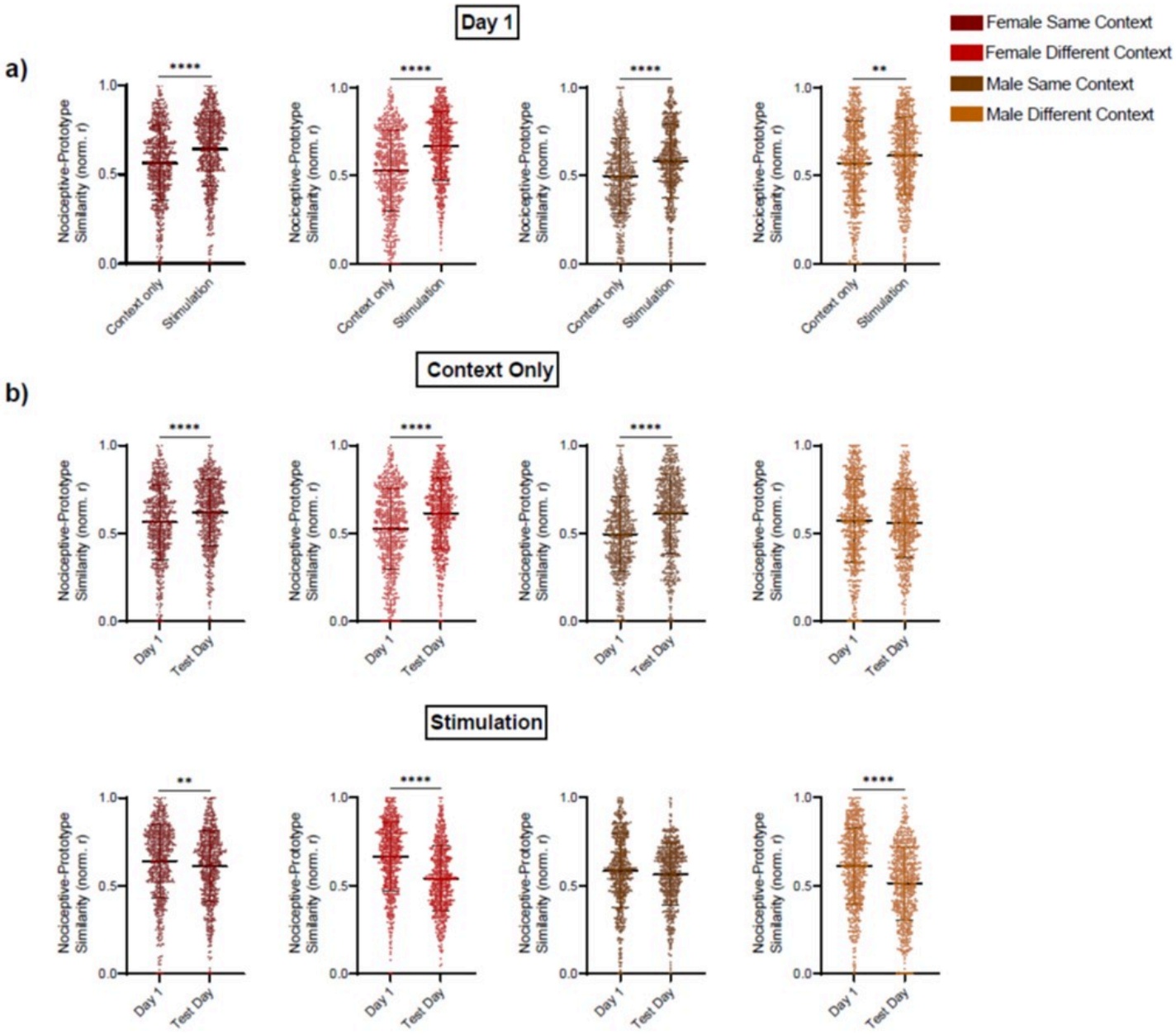


Figure 8

Figure 8. Similarity to the nociceptive prototype in separated groups. a) The comparison between stimulation period and exposure to Context for each group: females in the same Context (FSC) (dark red), females in a different context (FDC) (light red), males in the same Context (MSC) (dark brown), and males in a different context (MDC) (light brown). In all groups n= 600, and N=5, **b)** The comparison of similarity to the nociceptive prototype between the test phase and baseline (day 1) is shown for the period of exposure to Context (above) and during the period of nociceptive thermal stimulation (45°C) (below). FSC (dark red), FDC (light red), MSC (dark brown), and MDC (light brown). The Mann-Whitney U test indicated statistically significant differences ($p<0.05$) **$p<0.004$ ****$p<0.0001$ (Table 1). The graphs show the mean ± SEM.

Supplementary Files

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- table1.docx
- Graphicalabstract.jpg