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Research Report

Environmentally induced antinociception and hyperalgesia in rats and mice

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ABSTRACT

Stress can enhance and inhibit nociception depending on the situation. Thus, simply shifting the context from the elevated plus maze (EPM) which has been shown to produce stress-induced antinociception to a different environment could produce drastic and rapid changes in nociception. The present experiment tested this hypothesis by assessing nociception in rats and mice during and immediately after removal from the maze. Experiment 1 found hyperalgesia in female and male rats tested on the hot plate immediately after exposure to the elevated plus maze. This hyperalgesia occurred with or without the added stress of a hind paw formalin injection and regardless of whether rats were exposed to an EPM with open (oEPM) or enclosed (eEPM) arms despite a clear antinociceptive effect while on the oEPM. Experiment 2 showed a similar shift from antinociception to nociception on the formalin test in mice immediately after removing them from the EPM. These data demonstrate that a mild stressor such as the EPM can produce both antinociception and hyperalgesia depending on the context. This shift from antinociception to hyperalgesia occurs rapidly and is evident in mice, male and female rats, and with the hot plate and formalin tests.

1. Introduction

Exposure to dangerous conditions (commonly referred to as stressors) result in a group of organized behavioral, autonomic and hormonal responses that increase the chance of survival (Van de Kar and Blair, 1999). In general, these reactions are accompanied by pain inhibition (Fanselow, 1991; Fardin et al., 1984; Kelly, 1986; Terman et al., 1984; Watkins and Mayer, 1982). For instance, rats exposed to a cat display species-specific defensive behaviors and antinociception (Fanselow, 1991; Lester and Fanselow, 1985). Environmentally induced antinociception also has been shown to occur following

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Abbreviations: oEPM, Open elevated plus maze; eEPM, Enclosed elevated plus maze; EPM, elevated plus maze; G-G, group exposed to the glass box from 25–55 min; oEPM-G, group exposed to the open elevated plus maze for 10 min (25–35 min) and returned to the glass box until the end of the test; oEPM-oEPM, group exposed to the open elevated plus maze until the end of the test

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conspecific confrontations or exposure to dangerous stimuli (e.g., Canto-de-Souza et al., 1997; Costa et al., 2005; Kelly, 1982; Miczek et al., 1982; Rodgers et al., 1990; Rodgers and Hendrie, 1983; Rodgers and Randall, 1986; Rodgers and Shepherd, 1989; Terman et al., 1984).

Subtle aversive stimuli such as exposure to the elevated plus-maze (EPM) have also been shown to produce antinociception (e.g., Lee and Rodgers, 1990, 1991; Nunes-de-Souza et al., 2000; Rodgers et al., 1992; Taukulis and Goggin, 1990). The EPM (Pellow et al., 1985) or elevated X maze (Handley and Mithani, 1984) was originally validated to study anxiety-like behaviors in rats and mice (Lister, 1987; Stephens et al., 1986). This test is based on the natural aversion of rodents to open spaces and has been used to study anxiety and its modulation by drug treatment. The maze has recently been modified to show that antinociception is caused by exposure to the open (oEPM; an elevated platform with four open arms), not the enclosed arms (Cornélio and Nunes-de-Souza, 2009; Mendes-Gomes et al., 2011; Mendes-Gomes and Nunes-de-Souza, 2005, 2009). The EPM is a much more natural stimulus than footshock and seems particularly well suited to assess the rapid changes in nociception that probably occurs with subtle changes in natural aversive situations.

Although numerous studies have shown that threatening situations induce pain inhibition in a variety of animal models (Canto-de-Souza et al., 1997; Fanselow, 1991; Lester and Fanselow, 1985; Miczek et al., 1982; Rodgers et al., 1990; Rodgers and Hendrie, 1983; Rodgers and Randall, 1986, Rodgers and Shepherd, 1989), conflicting pre-clinical and clinical results involving the interaction of fear/anxiety and nociceptive responses suggest that this relationship may not be so simple. For example, it has been shown that anxiety can exacerbate pain symptoms (Kain et al., 2000; Rhudy and Meagher, 2000). A significant number of animal studies have shown that stressful stimuli induce hyperalgesia. Rats exposed to relatively mild non-noxious stressors, such as vibration (Devall et al., 2009), a novel environment (Vidal and Jacob, 1986), gently holding by the nape of the neck (Vidal and Jacob, 1986) or exposing rats to repeated stressful stimuli can produce hyperalgesia (Quintero et al., 2000). Although exposure to the EPM seems like a relatively mild stressor, clear antinociceptive effects occur while in the maze (Cornélio and Nunes-de-Souza, 2009; Mendes-Gomes et al., 2011; Mendes-Gomes and Nunes-de-Souza, 2005, 2009). Whether those antinociceptive effects persist when rodents are removed from the maze is not known. The objective of the present study was to test the hypothesis that antinociception will disappear as soon as the animal is removed from the EPM exposure. Moreover, given that the responsiveness to noxious stimuli and to opioid drugs varies with sex and strain (Craft, 2003b; Mogil et al., 2000), we also investigated whether exposure to the EPM produces differential nociceptive responses in male and female rats.

2. Results

2.1. *Experiment 1: effect of EPM exposure on hot plate latency*

The primary objective of this experiment was to determine the duration of an antinociceptive response following the exposure of a rat to the EPM. This was assessed in rats with and without formalin administration into the hind paw and in male and female rats. A number of studies report sex-related differences in nociceptive responses in humans and animals (for review see Craft, 2003a, 2007; Hurley and Adams, 2008; Wiesenfeld-Hallin, 2005), but relatively few studies have assessed the effects of sex in environmentally induced antinociception.

Rats were exposed to the enclosed or open EPM for 10 min and then removed to test nociception using the hot plate test. A decrease in hot plate latency was evident immediately after removing rats from the EPM compared to control rats not exposed to the EPM (Fig. 1). This hyperalgesia was evident in both male and female rats and independent of whether they were exposed to the enclosed or open EPM. Two-way ANOVA revealed significant effects for place of exposure [F (2, 43) = 9.02, P < 0.05], but not for sex [F (1, 43) = 0.02, P > 0.05] or an interaction between these factors [F (2, 43) = 0.08, P > 0.05]. Newman Keuls test indicated that eEPM and oEPM groups had lower response latencies than rats kept in the home cage (p < 0.05).

Previous experiments used the formalin test to assess antinociception while rats were in the EPM (Cornélio and Nunes-de-Souza, 2009). Thus, a few rats were tested as described above except that formalin was injected in the right hind paw prior to exposure to the EPM. Administration of formalin caused comparable nociception in all rats prior to exposure to the EPM as indicated by a non-significant difference in the amount of time licking the hind paw [F (2, 10) = 0.6, P > 0.05]. Rats placed in the oEPM during the second phase of the formalin test spent almost no time licking the paw compared to rats not placed in the EPM (Fig. 2). The time spent licking the formalin injected paw in rats exposed to the eEPM was intermediate between these two groups as has been reported previously (Cornélio and Nunes-de-Souza, 2009; Mendes-Gomes et al., 2011; Mendes-Gomes and Nunes-de-Souza, 2005, 2009). This

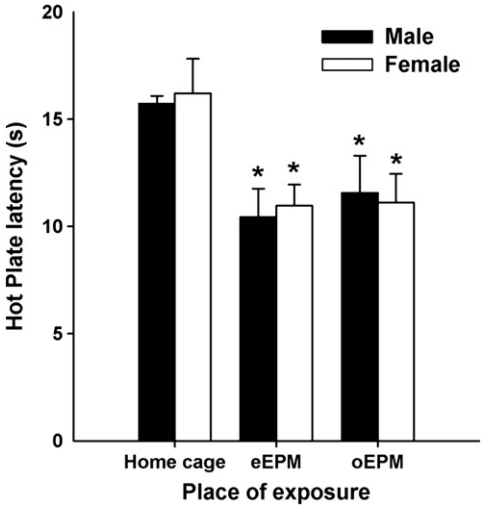

Fig. 1 – Hyperalgesia in male and female rats following exposure to the EPM. Hot plate latency (n = 8–9) was reduced in rats previously exposed to the eEPM or oEPM for 10 min compared to rats remaining in their home cage. Columns represent means (±S.E.M). *p < 0.05 vs home cage.

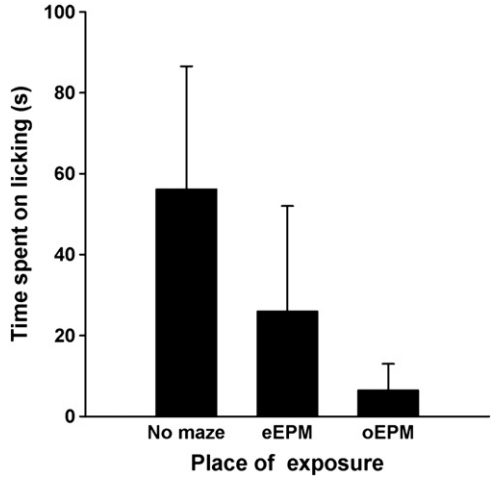

Fig. 2 – Antinociception occurs during exposure to the oEPM. Time spent licking the formalin (formaldehyde 0.925%) injected paw during the second phase of formalin test in rats (n = 4–5/group) kept in the Plexiglas box (no maze) or exposed to the eEPM or oEPM for 10 min. Columns represent means (± S.E.M).

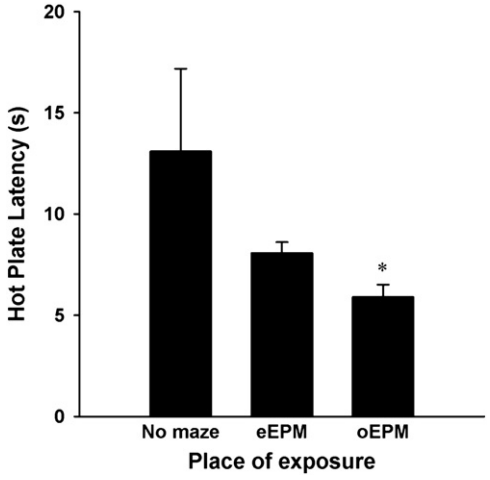

Fig. 3 – Formalin administration does not alter post-EPM hyperalgesia. Hot plate latency (n = 4–5) of rats previously injected with formalin (formaldehyde 0.925%) into the right hind paw and kept in the Plexiglas box (no maze) or exposed to the eEPM or oEPM for 10 min. Hot plate latency was assessed immediately after the EPM exposure. Columns represent means (± S.E.M). *$p < 0.05$ vs No maze and eEPM.

antinociceptive effect when rats were on the oEPM turned to hyperalgesia when they were placed on the hot plate immediately after EPM exposure ($H = 5.51$, $P = 0.05$). Post hoc comparisons revealed that oEPM-exposed rats displayed lower hot plate latency than No Maze- and eEPM-exposed rats (Fig. 3).

2.2. *Experiment 2: assessment of formalin nociception in mice following EPM exposure*

The objective of this experiment was to determine whether EPM-induced hyperalgesia generalizes across species (rat to mouse) and nociceptive test (hot plate to formalin test). Injection of formalin into the mouse hind paw produced a comparable time licking the paw (18–23 s) during the first phase (0–5 min) of the test [F (4, 38) = 1.28, $P > 0.05$]. This lack of effect confirmed the sample homogeneity of mice kept in the glass box.

The amount of time spent licking the hind paw during the second phase of the formalin test was determined by the environment. Control mice remaining in the glass box spent much more time licking the hind paw than mice in the oEPM (Fig. 4). This antinociception was reversed immediately in mice removed from the oEPM to the glass box compared to mice remaining in the oEPM. Two-way ANOVA for repeated measures revealed significant effects for place of exposure [F (4, 38) = 12.63, $P < 0.05$], time [F (2, 76) = 6.68, $P < 0.05$] and the interaction between these two factors [F (8, 76) = 11.54, $P < 0.05$]. For the interval 25–35 min, one way ANOVA followed by Duncan's test revealed that mice in the oEPM spent significantly less time licking the injected paw than the control group in the glass box ($p < 0.05$). Antinociception was maintained by mice remaining in the oEPM during the interval 35–45 min, but was instantly reversed in mice moved from the oEPM to the glass box. This shift to the glass box led to hyperalgesia during the 45–55 min interval compared to mice maintained in the glass box throughout ($p < 0.05$).

3. Discussion

The present data demonstrate that the antinociception produced by exposure to the EPM is specific to the stressful environment and shifts to hyperalgesia upon removal. This shift from antinociception to hyperalgesia is immediate,

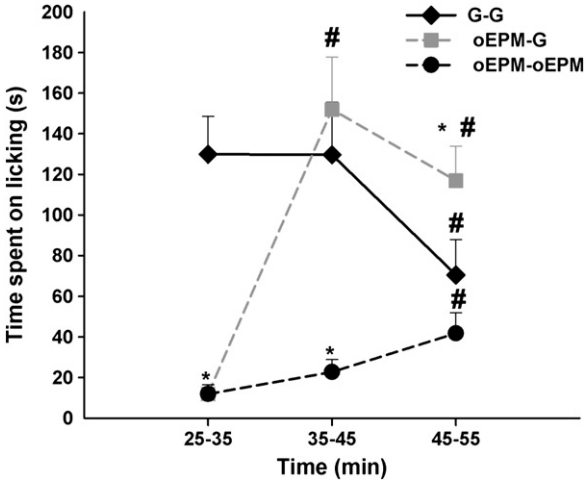

Fig. 4 – Antinociception is limited to EPM exposure in mice. Time spent licking the right hind paw during the second phase (25–55 min) of the formalin test in mice (n = 9). G-G: glass box exposure until the end of the test; oEPM-G: open EPM exposure for 10 min (25–35 min) and returned to the glass cage until the end of the test (55 min); oEPM-oEPM: open EPM exposure until the end of the test. *$p < 0.05$ vs G-G. #$p < 0.05$ vs 25–35 min.

occurs in both rats and mice, males and females, and whether nociception is assessed with the hot plate or formalin tests.

Post-maze hyperalgesia contrasts with the pronounced antinociception in rats (Cornélio and Nunes-de-Souza, 2009) and mice (Mendes-Gomes et al., 2011; Mendes-Gomes and Nunes-de-Souza, 2005, 2009) exposed to the oEPM. In those studies nociception was assessed by measuring the time spent licking the paw injected with formalin during, rather than after maze exposure. In contrast, the antinociception produced by footshock persists well beyond the period of exposure to the environment in which the shock was administered (Terman et al., 1984). Thus, both severe and mild stress produce antinociception, but the duration of that antinociception depends on the stressor. Exposure to other mild non-noxious stressors such as inescapable holding, novel environment or vibration evokes an immediate and transient hyperalgesia (Devall et al., 2009; Vidal and Jacob, 1982). However, despite studies showing that threatening situations induce pain inhibition in a variety of animal models (Canto-de-Souza et al., 1997; Fanselow, 1991; Lester and Fanselow, 1985; Miczek et al., 1982; Rodgers et al., 1990; Rodgers and Hendrie, 1983; Rodgers and Randall, 1986, Rodgers and Shepherd, 1989), conflicting pre-clinical and clinical results involving the interaction of fear/anxiety and nociceptive responses suggest that anxiety can exacerbate pain symptoms (Kain et al., 2000; Rhudy and Meagher, 2000). In this context it is necessary to highlight that the results obtained in this study cannot be generalized to other ethological animal models of anxiety/fear since currently there is separation of anxiety disorders in different diagnostic categories. Then, different animal models of anxiety/fear could model a different subtype of anxiety disorder implicating that this hyperalgesia caused by changing the context at the end of the test may not be valid for all tests. More studies are necessary to investigate this question.

Although the antinociceptive effects of the oEPM are much greater than that produced by exposure to the eEPM (Cornélio and Nunes-de-Souza, 2009; Mendes-Gomes et al., 2011; Mendes-Gomes and Nunes-de-Souza, 2005, 2009), hyperalgesia was evident following exposure to either maze. In this context, it seems that exposure to the eEPM may be a form of novelty stress. Supporting this assumption are the results showing that mice exposed to the eEPM display higher plasma corticosterone levels (a biological marker of stress) than mice from control groups not exposed to the EPM (Mendes-Gomes et al., 2009). It has been shown that rats exposed to a new environment for 5 min display hyperalgesia as assessed by tail shock in addition to other signs of novelty stress such as exploration, rearing, grooming and defecation (Vidal and Jacob, 1982).

Whether a stressor induces antinociception or hyperalgesia depends on many factors such as the type of stressor, the pain test (Vidal and Jacob, 1986), repetition and/or intensity of the stimulus, or the time elapsed post-stress (Imbe et al., 2006; Langford et al., 2011). For example, acute exposure to emotionally arousing non-noxious stress, such as inescapable holding, novel environments or vibration produces an immediate and transient hyperalgesia (Vidal and Jacob, 1982, 1986). Hyperalgesia also occurs as a result of chronic stress induced by repeated exposure to a cold environment (Omiya et al., 2000), restraint (Gamaro et al., 1998) or forced swim (Quintero et al., 2000, Quintero et al., 2003). Chronic stress-induced hyperalgesia generally lasts longer than that induced by acute stress. The short-lived hyperalgesia in the present study suggests that hyperalgesia is caused by residual stress resulting from the acute exposure of rats to non-noxious stress (eEPM or oEPM).

Regarding sex-related differences, several studies with humans have reported sex-related differences in pain perception, from laboratory experiments to epidemiological studies of clinical conditions (for review see Craft, 2007). Clinical studies reveal that women report more severe pain, more frequent bouts of pain, more anatomically diffuse and longer-lasting pain than males with similar disease processes (Hurley and Adams, 2008). Animal research showing that female rodents display a lower threshold in experimental models of thermal, chemical, inflammatory and mechanical nociception is consistent with the clinical data (for review see Hurley and Adams, 2008). In contrast, stress-induced hyperalgesia was comparable in male and female rats when removed from the EPM. Female and male rats submitted to vibration stress also display comparable hyperalgesia (Devall et al., 2009).

Hyperalgesia was evident after the EPM whether rats were tested with or without formalin injected into the paw. Rats exposed to the eEPM or oEPM without prior administration of formalin exhibited hyperalgesia on the hot plate test compared to control rats kept in their home cage. Rats injected with formalin also showed hyperalgesia on the hot plate test following exposure to the oEPM. Experiment 2 extended these findings to mice and demonstrated post-EPM hyperalgesia with the formalin test. These findings indicate that it is the environment and not the noxious effect of the formalin injection that causes both the antinociception while on the EPM and the hyperalgesia upon removal.

As shown in Fig. 4, oEPM-exposed mice exhibited antinociception when compared to those exposed to the glass box during the second phase (25–35 min after formalin) of the formalin test. These results corroborate previous findings showing that mice injected with formalin into the right hind paw and exposed to the oEPM display antinociception (Mendes-Gomes et al., 2011; Mendes-Gomes and Nunes-de-Souza, 2005, 2009). However, during the 35–45-min interval only mice kept in the oEPM (i.e., the oEPM-oEPM group) displayed antinociception when compared to the control group. The magnitude of the antinociception begins to decrease after approximately 20 min of exposure to the oEPM although this is difficult to measure given that the nociception associated with the formalin test is greatly reduced at this time. In contrast, moving mice from the oEPM to the glass box caused an immediate loss of antinociception. These results suggest that oEPM-induced antinociception is closely associated with the aversive environment.

In conclusion, the present study shows rapid changes in nociception depending on the environment. The antinociception produced by the EPM disappears immediately after removal. The shift occurs in both female and male rats, rats and mice, and whether nociception is assessed with the hot plate or formalin tests.

4. Experimental procedures

4.1. Subjects

Subjects were adult male (210–285 g) and female (160–220 g) Sprague–Dawley rats (Harlan, Kent WA, USA) and adult male Swiss mice (25–35 g, Univ. Estadual Paulista-UNESP, SP, Brazil). The rats were maintained under a reverse light/dark schedule (lights off: 7:00 am) in a temperature controlled environment. They were handled for 3–5 min for 3 consecutive days prior to testing. Mice were maintained under a 12 h light cycle (lights on at 7:00 am) in a temperature (23±1 °C) and humidity (55±5%) controlled environment. In all experiments, animals were transported to the experimental room and left undisturbed for at least 1 h prior to testing.

All procedures were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize the number and potential suffering of subjects. All experimental protocols carried out with mice were analyzed and approved by the Sao Paulo State University Research Ethics Committee (CEP/FCF/Car. protocol 43/2008). The studies using rats were approved by the Animal Care and Use Committee at Washington State University.

4.2. Apparatus and nociceptive tests

4.2.1. Enclosed and open elevated plus-mazes

The enclosed (eEPM) and the open (oEPM) elevated plus mazes are modifications of the previously described standard elevated plus maze (Lister, 1987; Pellow et al., 1985). They comprise four enclosed (eEPM) or open arms (oEPM) connected to a common central platform. The dimensions of these apparatuses vary for rats and mice and are described in Experiments 1 and 2, below. The maze was thoroughly cleaned with ethanol 20% between tests.

4.2.2. Hot plate test

The hot plate test was used in Experiment 1 to assess nociception, as previously described (Morgan et al., 2006). It consists of placing a rat on an enclosed square plate (25.4×25.4 cm) heated to 52.5 °C. The latency for hind paw licking, a supraspinally integrated response, was measured. Rats were removed from the hot plate if no response occurred within 50 s. Between subjects, the hot plate was thoroughly cleaned with ethanol 20%.

4.2.3. Formalin test

Nociception during exposure to the EPM was assessed using the formalin test. This test causes a biphasic nociceptive response (Dubuisson and Dennis, 1977). The first phase begins instantly after formalin injection and lasts approximately 5 min. It results from the direct stimulation of nociceptors (Dubuisson and Dennis, 1977; McCall et al., 1996). This phase was assessed in a glass (mice) or Plexiglas (rats) box. The second phase begins approximately 20 min after formalin injection, lasts approximately 40 min (Bon et al., 2002) and involves C fiber activation (McCall et al., 1996; Tjolsen et al., 1992). It is related to a period of sensitization during which an inflammatory process occurs (e.g., Le Bars et al., 2001; Tjolsen et al., 1992). Rats and mice were injected with 50 μl of formalin (formaldehyde 2.5% for mice and 0.925% for rats) into the dorsal (for mice) or plantar surface (for rats) of the right hind paw. The animal was placed in a glass (30×20×25 cm) or Plexiglas (32×32×46) box to record the duration of hind paw licking for the first 5 min following administration of formalin. The second phase of the formalin test was evaluated during 10 (Experiment 1: 25–35 min after the formalin injection) or 30 min (Experiment 2: 25–55 min after formalin injection) of exposure to the eEPM, oEPM or glass box.

4.3. Procedure

4.3.1. Experiment 1: effect of EPM exposure on hot plate latency

Enclosed (50×10×40 cm) and open (50×10×0.25 cm) elevated plus mazes differed only in the height of the Plexiglas walls. The four arms were connected to a common central platform (10×10 cm) covered by a gray Plexiglas floor. The maze was elevated 50 cm above floor level.

Naïve male (n=25, 8–9 per group) and female rats (n=24, 8 per group) were exposed to the eEPM or oEPM for 10 min and then removed and immediately placed on the hot plate to assess nociception. Non-EPM exposed control rats were moved directly from their home cage to the hot plate apparatus for testing. A few additional male rats were injected with formalin (0.925%) into the plantar surface of the right hind paw to insure that antinociception occurred during exposure to the oEPM as reported previously (Cornélio and Nunes-de-Souza, 2009; Mendes-Gomes et al., 2011; Mendes-Gomes and Nunes-de-Souza, 2005, 2009). Immediately after the injection rats were placed in a Plexiglas box and nociception assessed for 5 min. Twenty-five minutes later, during the second phase of the formalin test, rats either remained in the Plexiglas box (control group; n=5) or were placed in the eEPM (n=4) or oEPM (n=4) for 10 min. Time spent (in seconds) licking the formalin injected paw was recorded. At the end of this 10 min of exposure the rat was placed on the hot plate to assess nociception.

4.3.2. Experiment 2: assessment of formalin nociception in mice following EPM exposure

Enclosed (30×5×15 cm) and open (30×5×0.25 cm) elevated plus mazes for mice were used. The EPM consists of four arms connected to a common central platform (5×5 cm) elevated 38.5 cm above floor level. The maze was constructed with wood floors and transparent glass walls.

Male mice (n=9/group) were injected with formalin 2.5% into the dorsal right hind paw and placed in a glass box (30×20×25 cm; control situation) to record the time spent licking the paw for 5 min (first phase of the formalin test). Twenty-five minutes after the formalin injection, the second phase of this nociceptive test was carried out in three different conditions: the control group was exposed to the glass box from 25 to 55 min. A second group was exposed to the oEPM for 10 min (25–35 min) and returned to the glass box until the end of the test (55 min). The final group was exposed to the oEPM for the entire 25 to 55 min.

4.4. *Statistics*

All results were initially submitted to Levene's test for homogeneity of variance. Non-homogeneous data were transformed to square root and then submitted to analysis of variance (ANOVA).

In Experiment 1, the hot plate latency data were analyzed by two-way ANOVA (Factor 1: place of exposure; Factor 2: sex) or Kruskal–Wallis ANOVA for non-parametrically distributed data. Where indicated by significant *F* values, group differences were identified by Newman Keuls test (parametric) or by Mann–Whitney U-tests (non-parametric).

In Experiment 2, results obtained during the first and second phase of the formalin test were submitted to one-way or two-way ANOVA for repeated measures (factor 1: place of exposure; factor 2: interval, as dependent factor), respectively. One-way ANOVA followed by Duncan test were used for between-group comparisons. Planned comparisons were used for within-group comparisons. In all cases, a *P* value of 0.05 or less was required for significance.

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