

Trait anxiety affects the orofacial nociceptive response in rats

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ABSTRACT

The aims of the present study were to assess the influence of: a) trait anxiety on orofacial pain; and b) orofacial pain on state anxiety. Forty-four rats were initially exposed to the free-exploratory paradigm for the evaluation of their anxiety profiles. In accordance to the parameter “Percentage of time in the novel side”, the animals were considered as presenting high or low levels of trait anxiety when presenting values below the 1st quartile, or above the 3rd quartile, respectively. A week later, formalin-1.5% was injected into the upper lip of each animal. The behavioural nociceptive response, characterized by increased orofacial rubbing (OR), was quantified for 30 minutes, as follows: Total time OR (0-30 minutes: total pain), 1st phase OR (0-6 minutes: neurogenic pain), and 2nd phase OR (12-30 minutes: inflammatory pain). Immediately after this test, but still under the effect of formalin, the rats were submitted to the Elevated Plus-maze test (EPM). The results showed that the high trait anxiety individuals presented higher frequency of OR than the low trait anxiety ones, except during the neurogenic pain period. However, no correlation was found between OR frequency and levels of state anxiety presented on the EPM. In conclusion, the animals presenting higher anxiety profiles were the most susceptible to orofacial pain, nevertheless, orofacial pain did not influence state anxiety.

Keywords: trait anxiety; formalin test; orofacial nociception; state anxiety.

INTRODUCTION

The relationship between anxiety and pain is a common experience in both preclinical (Quintero et al., 2000; Torres et al., 2001; Gameiro et al., 2006; Wilson et al., 2007) and clinical studies (Brown, 1990; Grachev et al., 2001; Dersh et al., 2002; McWilliams et al., 2003; Bonjardim et al., 2005). Preclinical investigations have confirmed the enhancing effect of anxiety on nociception for different components and measures, including nociception intensity (Al Absi and Rokke, 1991), nociception threshold (Rhudy and Meagher, 2000), and nociception discrimination (Schumacher and Velden, 1984). Also, in clinical settings, it has been shown that anxiety levels predict pain severity and pain behavior in acute and chronic pain patients (Kain et al., 2000; Van Den Hout et al., 2001), while anxiety reduction techniques and anxiolytic drugs are successful in ameliorating medical procedure associated pain (Suls and Wan, 1989; Dellelmin and Fields, 1994).

So far, the general approach of preclinical studies to assess the relationship between anxiety and nociception has been to expose an animal to a stressor and examine the impact of this stressor on a nociception measure, or to evaluate alterations in anxiety processing following exposure to nociceptive stimuli (Takahashi and Kaneto 1999; Quintero et al., 2000; Torres et al., 2001; Gameiro et al., 2006; Boccalon et al., 2006). To the best of our knowledge, until now, only two studies have been aimed at evaluating the influence of baseline levels of anxiety

(high and low) on pain processing (Ramos et al., 2002; Wilson et al., 2007). However, in the first study, the anxiety levels were genetically determined, i.e., Lewis (more anxious) and SHR (less anxious) rat strains were submitted to the paw formalin test. Despite the fact that different levels on pain sensitivity were found between the strains, this result could be due to genetic differences in pain processing, not necessarily related to anxiety. In the second study, the anxiety levels were evaluated by the Elevated Plus-maze Test, which confronts the animals with an anxiety provoking situation, modeling the so-called state anxiety.

State anxiety is the anxiety a subject experiences at a particular moment in time, it is transitory and may be influenced by external stimuli; whereas trait anxiety is considered to be an enduring feature of an individual and is relatively stable over time (Spielberger et al., 1970; Lister, 1990). Therefore it would be of great scientific interest to investigate the relationship of trait anxiety and nociception in the same rat strain.

At present, the only test that has been proposed as an animal model of trait anxiety is the Free-exploratory Paradigm (FEP) (Griebel et al., 1993; Teixeira-Silva et al., 2009), which presents no correlation to the Elevated Plus-maze Test (EPM) (Goes et al., 2009).

With all this in mind, the aim of the present study was to explore the influence of trait anxiety (FEP) on orofacial nociception, and of orofacial nociception on state anxiety (EPM).

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ANIMALS

Forty four (2-3 months) male Wistar rats were obtained from the animal facility of the Universidade Federal de Sergipe (UFS). The animals were kept four to five per cage (41 x 34 x 18 cm), in a temperature (22-24°C) and light (12h/12h light/dark cycle, lights on at 06:00 a.m.) controlled room, with water and food *ad libitum*. All procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and conformed to the guidelines of the International Association for the Study of Pain (IASP) (Zimmermann, 1983). Moreover, the Federal University of Sergipe Ethics Committee approved all the procedures for animal research in accordance with the guideline of the Brazilian Council for Animal Experimentation.

Free-exploratory paradigm (FEP – Figure 1)

FEP was set up as described in a previous study (Teixeira-Silva et al., 2009). The apparatus consisted of a wooden box, divided into two compartments, with each of these further subdivided into three exploratory units (20 x 20 cm), interconnected by small openings.

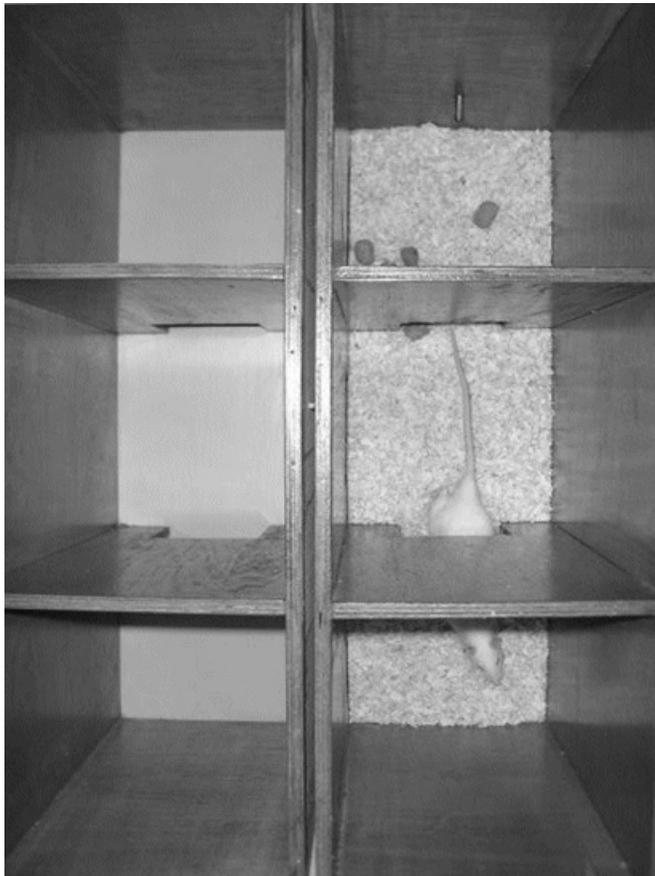


Fig. 1. Free-exploratory paradigm apparatus.

The two compartments were separated by a removable partition. The box was placed on a stand in the rat room. Approximately 24h before testing, the partition was installed and an animal was put into one half of the apparatus in order to become familiarized with it. This familiar half had zeocel (Zoocel®, Celta Brasil, SC, Brazil) covering the floor and the animal had free access to food and water. On the test day the partition, between the familiar and the novel compartments was removed and the animal was observed for 15 minutes, under infra-red light. During this period, the time spent in each compartment was measured, then the percentage of time in the novel side (%TNS) was calculated and used as a parameter of anxiety. This parameter was measured using a computerized system for animal tracking (Anymaze®, Stoelting Co., Wood Dale, Illinois, USA).

Orofacial Formalin Test (OFT – Figure 2)

For the OFT, each animal was placed in a test chamber (30 x 30 x 30 cm, mirrored-wood chamber with a glass at the front side) for a 15-min habituation period to acclimatize to the new environment. Following this period, formalin 1.5% (50 µl) was injected into the right upper lip of the animal (Clavelou et al., 1995). Immediately after this, the rat was returned to the test chamber for an observation period of 30 min. During this period, the time the animal presented the orofacial rubbing behavior was measured using a chronometer. Such behavior is defined as the animal rubbing the orofacial region with the ipsilateral fore or hindpaw (Clavelou et al., 1989). Subsequently, the data was grouped as follows: (1) Total time rubbing (Total Time - 30 min.); (2) Time rubbing from 0 to 6 minutes (1st Phase – Neurogenic Pain) (Tjølsen et al., 1992); and (3) Time rubbing from 12 to 30 minutes (2nd Phase – Inflammatory Pain) (Tjølsen et al., 1992). Keeping in mind that rubbing the orofacial region is part of grooming, the enhancement of this behavior was quantified by the application of a nociceptive index for each animal: $[(\text{Test}/\text{min} - \text{Basal}/\text{min}) / \text{Basal}/\text{min}] \times 100$, where Test is the amount of time the animal spent rubbing the orofacial region under the formalin effect (Total Time, 1st Phase or 2nd Phase), and Basal is the amount of time the animal spent rubbing during the habituation period.

The analyses of the orofacial rubbing behavior were performed by an investigator who was unaware of the animals' levels of anxiety.

Elevated plus-maze (EPM – Figure 3)

The EPM apparatus consisted of a wooden maze with two closed arms (50 x 10 x 40 cm) and two open arms (50 x 10 cm) connected by an open central area (10 x 10 cm). The arms were arranged such that those of the same type were opposite each other. The maze was positioned 50 cm above the floor and was used under artificial white lighting (Pellow et al., 1985).

Animals were individually put into the centre of the maze and allowed to explore the apparatus for five minutes. Time spent in each type of arm was evaluated. Subsequently, the percentage of time spent in the open arms (%TOA) was calculated.

These parameters were measured using a computerized system for animal tracking (Anymaze®, Stoelting Co., Wood Dale, Illinois, USA).



Fig. 2. Orofacial formalin test chamber.

Procedure

The animals were first tested on FEP. The obtained results were used to classify them according to the %TNS, as presenting high (<1st quartile), medium (>1st quartile and <3rd quartile) or low (>3rd quartile) levels of anxiety. A week later, rats with high (n=11) and low anxiety (n=11) were submitted to the OFT immediately followed by the EPM.

FEP was performed in the dark phase of the light cycle, between 6:00 and 7:00 p.m., while OFT and EPM were performed in light phase, between 8:00 and 12:00 a.m.

Statistical Analyses

The statistical analyses were performed with non-parametric methods. The influence of trait anxiety on nociceptive responses was analyzed by comparing the nociceptive index between the high and low trait anxiety groups, through Mann-Whitney U test. The influence of orofacial nociception on state anxiety was evaluated through a correlational study between the nociceptive index and the %TOA, using the Spearman correlation coefficient. All significance tests were performed at the 5% significance level.

RESULTS

The data from FEP presented as follows: median = 69.83%; 1st quartile = 47.15%; 3rd quartile = 81.09%. Therefore, animals with values below the 1st quartile (47.15%; n=11) were classified as presenting high levels of trait anxiety, while animals with values above the 3rd quartile (81.09%; n=11) were classified as presenting low levels of trait anxiety.

The Mann-Whitney U test revealed a significant difference on the nociceptive index between animals of high and low anxiety (Table 1) for the parameter Total Time (U= 27, p= 0.028). A similar result was found for the 2nd Phase of OFT (U= 28, p= 0.033), but no significant difference was observed for the 1st Phase (U= 53, p= 0.622). The Spearman correlation coefficient found no significant correlation between the nociceptive index and %TOA (EPM) (Figure 4) for any group of data (Total Time: r= -0.098, p= 0.663; 1st Phase: r= -0.069, p= 0.759; 2nd Phase: r= -0.147, p= 0.514).

DISCUSSION

The aims of the present study were to verify (a) the influence of trait anxiety on nociceptive behavior response evoked by injection of formalin into right upper lip region of rats and (b) the influence of orofacial nociception on state anxiety.

The trait anxiety was evaluated by FEP that, so far, is the only animal model proposed as a model of trait anxiety (Griebel et al., 1993; Teixeira-Silva et al., 2009; Goes et al., 2009). While orofacial nociception was assessed using the OFT (Clavelou et al., 1995; Clavelou et al., 1989), and the state anxiety was evaluated through the EPM – the most used animal model of anxiety (Carobrez, 2005).



Fig. 3. Elevated plus-maze test apparatus.

TABLE 1
Effects of level of anxiety on the Orofacial Formalin Test (OFT)

Anxiety level	Nociceptive index (OFT)		
	Total Time (%)	1 st Phase (%)	2 nd Phase (%)
Low	150.3 (92.6 - 200.5)	180.2 (128.7 - 340.0)	198.4 (156.8 - 227.4)
High	326.1 (265.7 - 489.0)*	288.3 (285.2 - 531.2)	427.8 (311.3 - 635.1)*

Data are presented as: median (inter-quartile interval).

* Significantly different from Low ($p < 0.05$)

The results showed significant differences between animals with high and low trait anxiety levels, in that the more anxious rats exhibited greater pain sensitivity than the less anxious ones. However, looking at the different nociceptive phases (neurogenic and inflammatory) separately, this difference could only be observed on the latter. Such findings suggest that the descending pain modulation system does not work differently in animals with high or low anxiety profile. It could be argued that the formalin concentration used here did not generate neurogenic pain intense enough to demonstrate differences between groups. However, previous studies, which evaluated the role of stress on pain sensitivity, found similar results using higher formalin concentrations (Brown, 1990; Ramos et al., 2002; Andre et al., 2005).

Both Brown's and Andre's studies verified that the application of a stressful stimulus induced hyperalgesia only on the 2nd phase of the paw formalin test, yet, the baseline anxiety levels were not considered. Ramos et al. (2002), observing genetically selected rat strains for high and low anxiety levels, also verified anxiety-induced hyperalgesia only on the 2nd phase of the paw formalin test (Ramos et al., 2002). Conversely, in another study, significant differences were not found between high and low anxiety groups of rats, categorized by the EPM, on the degree of mechanical hypersensitivity induced by paw carrageenan injection, suggesting that individual baseline anxiety levels do not modulate pain processing (Wilson et al., 2007). It is worth mentioning, though, that EPM is a model of state anxiety that is not stable over time (Andreatini and Bacellar, 2000).

Therefore, this is not a very satisfactory method of animal classification, as it is based on a short lasting reaction and not on an enduring feature.

The influence of trait anxiety on pain processing could be explained by: 1) possible differences on opioidergic and serotonergic activities between animals with high and low anxiety levels (Quintero et al., 2000; Torres et al., 2003; Gameiro et al., 2006); 2) possible differences in the levels of prostaglandins released during the inflammatory response between animals with high and low anxiety levels (Morimoto et al., 1991); 3) possible glucocorticoid-resistance in animals with high anxiety levels (Miller et al., 2002); 4) possible differences on NK1 receptor expression between animals with high and low levels of anxiety (Ramos et al., 2002).

In opposition to what was observed for trait anxiety, state anxiety did not seem to be related to orofacial pain, as no significant correlation was found between orofacial rubbing and %TOA. This means that acute orofacial pain does not present an anxiogenic effect, contrary to what has been shown for chronic pain by Narita et al., 2006. These researchers suggested that the anxiogenic effect of chronic pain is associated with changes in amygdala opioidergic function. This being the case, a single nociceptive stimulus would probably not be able to cause such changes, explaining the absence of the anxiogenic effect of pain seen in the present study. Here it is worth mentioning that the categorization of rats, according to their anxiety profiles was not considered for the evaluation of state anxiety as a recent study in our laboratory showed absence of correlation between FEP and EPM (Goes et al., 2009).

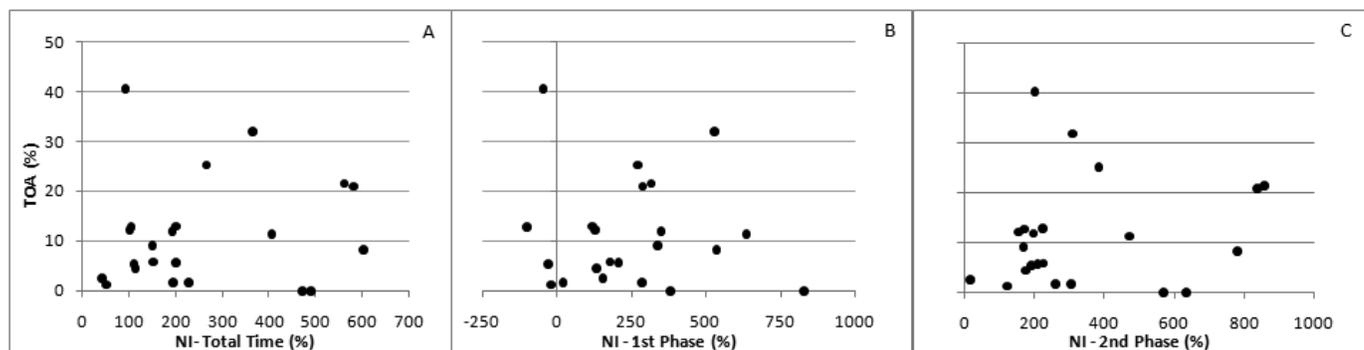


Fig. 4. Correlation between the percentage of time spent in the open arms (TOA) of the elevated plus-maze and the nociceptive index (NI), during the whole formalin test (A); during the first phase of the test (B); and during the second phase of the test (C).

Taking all this into consideration, it is possible to say that the present investigation is the first to evaluate the influence of anxiety as a personality trait on the painful sensitivity of rats of the same strain, and its results lead us to conclude that while anxiety as a state is not influenced by an acute nociceptive stimulus, anxiety as a constitutional trait is able to increase acute orofacial nociception.

ACKNOWLEDGEMENTS

Tiago Costa Goes received grant from Programa Institucional de Bolsas de Iniciação Científica (PIBIC/CNPq).

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