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# Dentin hypersensitivity induces anxiety and increases corticosterone serum levels in rats

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ABSTRACT

*Aims:* Investigate the relationships between experimentally induced dentin hypersensitivity (DH) with behavioral, endocrine and dentin erosion data.

*Methods:* Male Wistar rats divided into four groups, two controls and two experimental, received tap water or isotonic solution (Gatorade®, lemon, pH 2.7) for 30 or 45 days. The DH test was performed by a cold water stimulus on molars. A score (0–3) was given to the rats' pain response. Anxiety was evaluated by the elevated plus maze model and by serum corticosterone levels. The dentin erosion was observed by scanning electron microscopy (SEM). Anatomopathological studies were performed on the stomach, adrenal, kidney, and liver.

*Results:* Relative to control groups, experimental rats showed: 1) increased hypersensitivity scores (control group, 0; experimental groups, 2 (limits 0.5–3) on the 30th day and 2 (limits 1–3) on the 45th day); 2) reduced percentage of time and entries in the open arms and in serum corticosterone levels; 3) totally exposed dentinal tubules on the 30th day in SEM analysis of the teeth; and 4) no alterations in the anatomopathological and histological evaluations.

*Conclusions:* The treatment with isotonic solution for 30 days was able to induce DH after erosive challenge and severe DH was observed after isotonic solution treatment for 45 days. The pain induced by cold stimuli was consistent with the grade of DH. The close relationships between dental erosion, response to pain, serum levels of corticosterone and the EPM behavior responses reveal the effects of DH at several levels.



## Introduction

Dentin hypersensitivity (DH) is defined as a response to the stimulation of vital dentin exposed to the oral environment, by thermal, volatile, tactile, osmotic, or chemical stimuli, and causes extreme discomfort to the patient. It is characterized by short-term, acute pain of variable intensity (Brannstrom and Astrom, 1972). Pain may be localized or generalized, affecting the surface of one or various teeth concomitantly, and generally ceases immediately after removal of the stimulus (Brannstrom and Astrom, 1972). The pain resulting from hypersensitivity is caused by the quick movement of dentinal fluid which, in turn, excites the mechanoreceptors in the periphery of the pulp (Hydrodynamic Theory) (Brannstrom and Astrom, 1972). The prevalence of DH among adults is reported to be as high as 57%, making it one of the most common clinical complaints of dental patients (Terry, 2011). Dentinal hypersensitivity can be caused by abrasion, abfraction, erosion, or associated factors. The regular consumption of acidic foods and isotonics appears to be increasing both in developed and developing countries and this may be associated with dental erosion (Barbour et al., 2011; Curro, 1990).

Tooth erosion is defined as a chemical process that involves the dissolution of enamel and dentin by acid not derived from bacteria, when the surrounding aqueous phase is undersaturated with tooth mineral (Terry, 2011; Hunter et al., 2000; Lussi et al., 2004). Erosion is classified, in its origin, as extrinsic (i.e., diet) or intrinsic (i.e., gastroesophageal) (Lussi et al., 2004; Larsen and Nyvad, 1999; Sgolastra et al., 2011) and is typically progressive, resulting in the wearing away of the exposed tooth surface (i.e., enamel, dentin, or root surface).

There is a range of treatments for DH proposed in the literature that do not always achieve the desired result, since they are restricted to obliterating tubules mechanically, without taking into account that the perception of pain is very subjective and dependent on variations in the patient's emotional state (Arteche and Cesero, 2001).

Positive emotions and motivated behavior can activate the system of central inhibition of pain in the body, which controls the painful stimulus from the periphery through the release of endorphins by the central nervous system (Arteche and Cesero, 2001). Therefore, a positive professional approach may contribute to successful treatment (Curro, 1990). On the other hand, anxiety, stress, and depression can contribute negatively to the resolution of pain.

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Although emotional factors can be considered, the literature does not provide evidence on these. There is only a suggestion that emotional behavioral factors might interfere in the resolution of pain (Terry, 2011).

Proof of the relationship between emotional factors and pain perception can lead to the proposal of a new protocol of care that addresses the patient holistically, technically, and psychologically and, consequently, contributes to the resolution of cases of DH. This validation will be part of our study that relates perception of pain due to HD with behavioral changes such as stress or depression. Justified by the high incidence of HD in dental clinics, whose resolution through obliteration of tubules techniques are not enough to result in pain resolution. In addition, high incidence of placebo effect is observed on dental offices.

Animal models, particularly rats, have been used for the elucidation of behavioral changes and their systemic interference (Sorvari et al., 1996). Thus, the purpose of this study was to validate a behavioral model to evaluate the responses of rats with DH after experimentally induced erosion. Therefore, the development of an animal model of hypersensitivity induced by erosion that correlates to behavioral and endocrine data may be a promising means for assessing the response to a number of variables involved in the clinical treatment of DH.

## Methods

### Animals

Thirty-six adult male Wistar rats were used. The rats were housed in polypropylene cages (38 × 32 × 16 cm, 5 rats/cage) at a controlled room temperature (22 ± 2 °C) with artificial lighting (12-hour light/12-hour dark cycle, lights on at 6:00 a.m.) with free access to Nuvilab rodent food (Nuvital Co., São Paulo, Brazil) and filtered water. Sterilized and residue-free wood shavings were used as animal bedding. The experiments were performed 1 week after the rats' arrival in our laboratory. The animals used in this study were maintained in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animal Resources of Paulista University, São Paulo, Brazil (protocol No. 051/11CEP/ICS/UNIP, October 13, 2011). These guidelines are similar to those of the National Research Council, USA.

### Procedures

#### Dental erosion treatment

Erosion was assessed by offering the rats an isotonic solution (Gatorade®, lemon flavor, pH 2.7) as drinking water for 30 or 45 days.

#### DH test

DH was assessed by cold water stimuli (jet of cold water 4 °C, 0.5 ml, assessed by a syringe provided with a metal cannula), applied for 5 s, on the labial surface of molars (the rearmost teeth in the mouth). Three days before the test, the rats were daily habituated to the test manipulation. The animal's response to pain was scored (0 = no response; 0.5 = slight contraction of the body; 1 = body contraction; 2 = strong body contraction and a short vocalization; 3 = strong body contraction and a prolonged vocalization). The scores were independently attributed by two observers and the mean score attributed by each one was employed in the DH evaluation. The score of each observer was used to perform the correlation test (0.97) and validate our method.

#### Body weight

Body weight was measured weekly and at the end of the experiments, and weight gain was calculated.

#### Elevated plus maze (EPM test)

The EPM, an apparatus first conceived by File and co-workers (Pellow et al., 1985) is commonly used to evaluate anxiety. The EPM was made of wood and had two open arms (50 × 10 cm) and two enclosed arms of the same size with 40 cm high walls, and was elevated 50 cm above the ground. Each rat was carefully placed in the center of the maze facing one of the closed arms. After 300 s of free exploration, it was put back in its home-cage. The following behavioral measures were scored by a trained observer: (1) frequency of arm entries, when the rat stepped with the fourth paw into an arm. Entries were recorded whether they occurred in open or closed arms; (2) time spent in each arm (in seconds); (3) grooming frequency: friction of any part of the body with the paws and/or the mouth; and (4) the number of the center crossings. The percentage of time in the open arms and the percentage of entries into the open arms were calculated. The apparatus was washed with a 5% ethanol solution before each behavioral test; control and experimental rats were intermixed, and the observations were made between 14:00 and 18:00.

#### Blood collection, anatomopathological and histological analysis

A blood aliquot (500 μL) of animals previously anesthetized with a 6/1 mixture of ketamine/xylazine was withdrawn from the abdominal aorta and centrifuged (10 min/1000 ×g).

Serum was removed, aliquoted in separate tubes (100 μL each), and stored at −80 °C. The levels of corticosterone were determined using the LUMINEX/Magpix system (RSH69K03, Millipore Corporation, Billerica, MA, USA). The range for the detection of the analyte was 1.6–400,000 pg/ml. The samples were analyzed in duplicate and the levels were estimated using a five-parameter polynomial curve (xPONENT® software, Millipore Corporation, Billerica, MA, USA). All results are expressed in pg/ml. Corticosterone is measured because it is one of the most important indicators to evaluate emotional changes in rats after chronic stress.

At the end of the experiments, the rats were euthanized in a $CO_2$ chamber, submitted to necropsy, and the adrenal weight was recorded. The livers and kidneys were fixed in 10% neutral-buffered formalin. After fixation, the tissues were embedded in paraffin and 5 μm sections were stained with hematoxylin and eosin (HE). The stomachs were removed, opened through the greater curvature, washed, and fixed on styrofoam plaques. They were freshly observed for the presence of gastric ulcer lesions. Scores were given as follows: (1) presence of superficial hemorrhagic points; (2) presence of moderate alterations, meaning the observation of submucosal hemorrhagic lesions with small erosions; or (3) presence of severe hemorrhagic erosion lesions and some invasive lesions (Suffredini et al., 1999).

#### SEM analysis

SEM qualitative analysis, a complement of the dental elements in the scanning electron microscope, is needed to prove that the isotonic solution administration was able to expose the dental tubules through erosion and this exposure will generate pain (HD). Thus, in this way, it is a validated model for HD.

After euthanasia, jaws were removed, cleaned free of soft tissues, and molars were isolated for SEM analysis. The specimens were stored in vials (Eppendorf, Hamburg, Germany) containing 5% chloramine. The pieces underwent processing in an acetone dehydration series (30% acetone for 15 min; 50% acetone for 15 min; 70% acetone for 10 min; 95% acetone for 20 min; 100% acetone for 10 min; and 100% acetone for 20 min) followed by coating with gold palladium at a thickness of ±15 nm (Suptter Coater MED-020, Bal-Tec). After these procedures, the specimens were observed under a scanning electron microscope (JEOL, JSM-6510, USA), in order to concomitantly analyze pain score, behavior, and structural condition of dentin.

### Experimental design

Thirty-six male Wistar rats were divided into four equal groups (n = 9): two control groups (C30 and C45) and two experimental groups (E30 and E45). Rats of E30 and E45 groups received for 30