

***Ocimum basilicum* leaf essential oil and (-)-linalool reduce orofacial nociception in rodents: a behavioral and electrophysiological approach**

Antônio M. Venâncio,¹ Murilo Marchioro,¹ Charles S. Estavam,¹ Mônica S. Melo,¹ Marília T. Santana,¹ Alexandre S.C. Onofre,¹ Adriana G. Guimarães,¹ Makson G. B. Oliveira,¹ Péricles B Alves,² Hugo de Carvalho Pimentel,¹ Lucindo J. Quintans-Júnior^{*,1}

¹Departamento de Fisiologia, Universidade Federal de Sergipe, Brazil,

²Departamento de Química, Universidade Federal de Sergipe, Brazil.

Abstract: The present study investigated the antinociceptive effects of *Ocimum basilicum* L. (Lamiaceae) leaf essential oil (LEO) and (-)-linalool (LIN) in formalin (2%), glutamate (25 μ M)- and capsaicin (2.5 μ g)- induced orofacial nociception models in mice. The involvement of these substances was further evaluated on the neuronal excitability of the hippocampal dentate gyrus. Male mice (n=8/group) were pretreated separately with LEO and by LIN (50, 100, and 200 mg/kg, *i.p.*), morphine (5 mg/kg, *i.p.*) and vehicle (saline + Tween 80 0.2%), before injection of nociceptive agent into the right upper lip (perinasal area). The LEO and LIN reduced the nociceptive face-rubbing behaviour in both phases on formalin test. LEO and LIN, at high doses, produced significantly antinociceptive effect in the capsaicin and glutamate tests. In hippocampal slices, LEO inhibited the population spike generated by stimulation of the hylus (antidromic stimulation), with an IC₅₀ of 0.1 \pm 0.05 mg/mL. This response was reversibly blocked by lidocaine (0.5 mg/mL), a known voltage-dependent sodium channel antagonist and by LIN (0.5 mg/mL). Our results suggest that LEO and LIN modulate neurogenic and inflammatory pain in the tests of orofacial nociception induced by formalin, capsaicin and glutamate. Part of these effects may be associated with decreased peripheral and central neuronal excitability.

Introduction

The orofacial region is one of the most densely innervated (by the trigeminal nerve) areas of the body, which focuses some of the most common acute pains, *i.e.* those accompanying the pathological states of the teeth and the related structures. It is also the site of frequent chronic (post-herpetic neuralgia, migraine) and referred pains (Raboisson & Dallel, 2004). Furthermore, many of the difficulties in the management of acute and chronic orofacial pain conditions stem from a lack of recognition and understanding of orofacial pain mechanisms (Miranda et al., 2009).

Pain is a complex integrative phenomenon resulted from the integration of three components: sensory-discriminative, affective-emotional and cognitive-evaluative (Liu & Chen, 2009). When pain becomes chronic, the sensory dysfunction is accompanied by several brain disorders, such as anxiety, amnesia and depression (Ling et al., 2007; Narita et

al., 2006). Cumulative behavioral, electrophysiological and molecular data suggested important roles for the hippocampal formation (HF) in pain process (Cecarelli et al., 1999; Liu & Chen, 2009). The main afferent input to the HF is the entorhinal cortex (EC), via the so called perforant path, which terminates on the dendrites of the dentate gyrus (DG). From there, the sensory information enters the trisynaptic hippocampal pathway to be processed. It is believed that this process is involved in the motivational dimension of pain (Melzak, 2008).

The management of pain continues being a major challenge for medicine. Opioids, anticonvulsants and non-steroidal anti-inflammatory drugs (NSAIDs) are the main agents used to relieve acute and chronic pain (Pace et al., 2006). However, numerous therapeutic approaches are being used to better control the widespread clinical problem that affect a significant proportion of the human population (Lu et al., 2009). An actual approach is to develop new biological compound

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that inhibits orofacial pain from natural products, such as medicinal plants or its secondary metabolites, with enhanced efficacy and minimal side-effects (Holanda-Pinto et al., 2008; Quintans-Júnior et al., 2010; Siqueira et al., 2010).

Essential oils are natural products generally obtained from medicinal plants that exhibit a variety of biological properties, such as analgesic (Almeida et al., 2001), anticonvulsant (De Sousa et al., 2007; Quintans-Júnior et al., 2008a), anxiolytic and hypnotic (Almeida et al., 2009). Monoterpenes are the main components of these essential oils and the pharmacological properties of many medicinal plants have been attributed to them (Peana et al., 2004; De Sousa et al., 2006; De Sousa et al., 2007; Melo et al., 2010).

Several *Ocimum* species (Lamiaceae) are used to treat central nervous system (CNS) disorders in various parts of the world and its anticonvulsant activity is frequently reported (Quintans-Júnior et al., 2008b; Oliveira et al., 2009). *Ocimum basilicum* L. essential oil is rich in monoterpenes, such as δ -cadinol (10.2%), estragole (22.6%), and linalool (47.3%) (Mazutti et al., 2006). However, the major chemical constituent of *O. basilicum* essential oil, named "Maria Bonita" [a new variety of *Ocimum* derived from the accession PI 197442, from the Germplasm Bank North Central Regional PI Station (USA)] is linalool (76.93%) (Blank et al., 2007). (-)-Linalool is one natural enantiomer monoterpene compound of many essential oils, which is known to exhibit several biological activities such as CNS depressant, antinociceptive, anxiolytic and anticonvulsant (Peana et al., 2002; Peana et al., 2006; Kamatou & Viljoen, 2008; Batista et al., 2008). Until now, no data exists about the possible orofacial antinociception effect of (-)-linalool and *O. basilicum* leaf essential oil (LEO).

The aim of this work was to evaluate the effect of LEO and LIN in formalin-, glutamate- and capsaicin-induced orofacial nociception on mice and to investigate if these substances could also interfere with the hippocampal neuronal excitability.

Material and Methods

Animals

The experiments were conducted using male Swiss mice (25-32 g) and male Wistar rats (50-80 g), housed at 22±2 °C under a 12-h light/12-h dark cycle (lights on at 06:00) and with access to food and water *ad libitum*. The animals were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the experiments. The experiments were performed after gaining approval of the protocol by the Animal Care and Use Committee (CEPA/UFS

Nº 07/08) at the Federal University of Sergipe and followed the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). The numbers of animals (eight per group) and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments. All nociception tests were carried out by the same visual observer.

Drug and reagents

For all *in vivo* experiments the following agents were used: (-)-linalool (97% purity, Sigma, USA), morphine hydrochloride (União Química, Brazil), 37% formaldehyde (Vetec, Brazil), Tween 80 (polyoxyethylene-sorbitan monolate), glutamate and capsaicin (Sigma, USA). Vehicle was Tween 80 0.2% dissolved in 0.9% saline solution and used to dilute the test drugs. In those protocols the agents were injected intraperitoneally (*i.p.*) at a dose volume of 0.1 mL/10 g, with exception of algogen agents, such as formalin, glutamate and capsaicin, which were injected subcutaneously (*s.c.*) into the right upper lip. For the electrophysiological experiments the following agents were used: (-)-linalool, lidocaine hydrochloride (Hipolabor, Brazil) (0.5mg/mL) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 2.5µg/mL) (Sigma, USA). The LEO and LIN were dissolved with dimethyl sulphoxide (DMSO) (Vetec, Brazil) and absolute ethyl alcohol (Vetec, Brazil) at a ratio 1:10 (v/v), and, then, diluted at 100 mL in artificial cerebrospinal fluid (ACSF). The DMSO and absolute ethyl alcohol at the concentrations used had no effect on the cell membrane current (Ohkubo & Kitamura, 1997; Ardjmand et al., 2006). Lidocaine was directly dissolved in ACSF.

Plant material and essential oil extraction

Leaves were collected from the cultivation of the *Ocimum basilicum* L. (Lamiaceae) (named "Maria Bonita") obtained at the Research Station "Campus Rural da UFS" of the Federal University of Sergipe, Brazil. *O. basilicum* named "Maria Bonita" was derived from the accession PI 197442 of the Germplasm Bank "North Central Regional PI Station", USA. It is a basil cultivar with a rounded canopy, rose petals and purple sepals. It is cultivated at Brazilian northeast region (Blank et al., 2007). The leaves of *O. basilicum* were dried in an oven with air renewal and circulation (model MA-037/18) at 40 °C until complete dehydration has been achieved. The essential oil was obtained by hydrodistillation in a Clevenger-type apparatus using 100 g of dried leaves. The *O. basilicum* leaf essential oil (LEO) obtained was dried over anhydrous sodium sulphate, producing yields of 4.75 mL (v/w). Gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID)

analysis were realized to recognize the compounds of the LEO.

Orofacial nociception tests

Formalin test

Orofacial nociception was induced in mice by *s.c.* injection of 20 μ L of 2% formalin into the right upper lip (perinasal area), using a 27-gauge needle (Clavelou et al., 1995; Luccarini et al., 2006). This volume and percentage concentration of formalin was selected from our pilot study that revealed a nociception-related biphasic behavioural response (face-rubbing) of great intensity at periods of 0-5 min (first phase) and 15-40 min (second phase). Nociception was quantified at those periods by measuring the time (s) that the animals spent face-rubbing in the injected area with its fore- or hindpaws (Luccarini et al., 2006). To assess the effects of test drugs, groups of mice (n=8, each group) were pretreated systemically with vehicle (tween 80 0.2% in distilled water), LEO or LIN (50, 100, and 200 mg/kg, *i.p.*), 0.5 h before the local injection of formalin. Morphine (MOR, 5 mg/kg, *i.p.*), administered 0.5 h before the algogen, was included as positive control.

Glutamate-induced nociception

In an attempt to provide more direct evidence concerning the interaction of the LEO or LIN with the glutamatergic system, we separately investigated whether or not the LEO or LIN was able to antagonize glutamate-induced orofacial nociception in mice. The procedure used was similar to that previously described by Beirith et al. (2002) with some alterations, including the local of application according to Quintans-Júnior et al. (2010). A volume of 20 μ L of glutamate (25 μ M/paw prepared in phosphate buffered saline) was injected in the right upper lip (perinasal area), using a 27-gauge needle. Animals were observed individually for 15 min following glutamate injection. Nociception quantification was performed at this period measuring the time (s) that the animals spent face-rubbing the injected area with fore- or hindpaws. Mice (n=8, per group) were treated with the LEO or LIN (50, 100, and 200 mg/kg, *i.p.*), morphine (MOR, 5 mg/kg, *i.p.*) and vehicle 0.5 h before glutamate injection.

Capsaicin test

The orofacial nociception was induced by capsaicin in rodents as described earlier (Pellisier et al., 2002). Mice (n=8, each group) were injected with capsaicin (20 μ L, 2.5 μ g) subcutaneously into the right upper lip (perinasal area), using a 27- gauge needle.

Capsaicin was dissolved in ethanol, dimethyl sulfoxide and distilled water (1:1:8). In a pilot study, rodents manifested nociception-related face-rubbing behaviour following the injection of capsaicin with a high intensity at 10-20 min period. Therefore, nociception quantification was performed at this period measuring the time (s) that the animals spent face-rubbing the injected area with fore- or hindpaws. LEO or LIN (50, 100, and 200 mg/kg, *i.p.*) or vehicle were given to animals as described for formalin test, 0.5 h before the local injection of capsaicin. MOR (5 mg/kg, *i.p.*), administered 0.5 h before the algogen, was included as a positive control. An additional group received a similar volume of capsaicin vehicle.

Electrophysiology

Hippocampal slice preparation

Animals were anesthetized with ether, their brains were quickly removed and transferred to ACSF as ice cold, which was composed of (mM): NaCl 96.5; KC1 2.6; CaCl₂ 2; Mg SO₄ 2; NaHCO₃ 31.5; glucose 10, constantly bubbled with O₂ (95%) and CO₂ (5%). The brains were glued on the base of a vibroslicer (WPI, USA) and cut horizontally until finding the hippocampi, which were sliced transversally at 400 μ m due to their anatomical position. After one hour of resting, the slices were transferred to the recording chamber (Warner, USA).

Field potential recordings

The field potentials were recorded on the cellular layer of the hippocampal dentate gyrus, using microelectrodes made from borosilicate glass (od: 1.5 mm, AM-System, USA), which has resistances from 1-5 M Ω when filled with ACSF. Stimulation (0.1 ms, 0.1-0.3 mA, 0.05 Hz) was applied in the hylus (antidromic) and in the perforant path (ortodromic) with tungsten bipolar electrodes connected to an isolated unit, the Isostim A320 (WPI, USA). Field potentials were amplified by 100X (Axopatch1-D, Molecular Devices, USA), filtered at 2KHz, digitalized at 10KHz, at the Digidata 3220 machine (Molecular Devices, USA) and recorded by the WCP software (Dempster, Univ. of Strathclyde, Scotland).

Pharmacological studies

As lidocaine (0.5 mg/mL) is a dependent of voltage sodium channel blocker, we evaluated this effect using LEO (0.01, 0.1, 0.5, 1 mg/mL) and LIN (0.5 mg/mL) in comparison with it. These responses were recorded (at 0.05 Hz) for ten min or until its

stabilization. Then, the test substance was perfused for ten minutes before the field potentials were recorded. Unless full recovery of the control response was achieved, only one substance and one dose were tested for each slice. For the dose-response curve of the LEO, 3-4 slices were used for each tested dose.

Statistical analysis

For the *in vivo* tests, the data obtained were expressed as the mean \pm SEM and the differences were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test. The percent of inhibition by an antinociceptive agent was determined using the following formula (Reanmongkol et al., 1994):

$$\text{Inhibition \%} = 100 \cdot (\text{control} - \text{experiment}) / \text{control}$$

For the electrophysiological data, the analysis was performed off line with the WCP software. The amplitude (mV) of the field potentials were measured between the most negative to the most positive peak. For each experiment, the mean amplitude of ten responses before and ten minutes after the perfusion of the tested substances were used. Data were expressed as the mean \pm SEM. For the LEO dose-response curve, data were fitted by a non-linear regression sigmoidal curve, using the Graph Pad Prism (v 4.00) software.

Results

GC-MS and GC-FID analysis of the essential oil resulted in the identification of 13 compounds, constituting 99.96 % of the total oil. Furthermore, 1,8-cineole, linalool, geraniol and neryl acetate were the main components, comprising 96.89% of the oil. Linalool was the major component (76.93%) of leaves oil (Table 1).

Intraperitoneal administration of LEO or LIN produced a reduction in face rubbing behavior in formalin test (Table 2). All tested doses of LIN produced significantly antinociceptive effect in the first and second phase compared to control group (vehicle). LEO demonstrates, only in high dose, significantly antinociceptive effect in both phases. Morphine was able to reduce nociceptive behaviour in both phases.

The results of the orofacial nociception induced by glutamate and capsaicin tests are represented in Table 3. Pretreatment with LIN or LEO reduced the neurogenic inflammatory nociception induced by capsaicin injection into the right upper lip (perinasal area). This effect was evidenced by suppression of the face rubbing behaviour compared with control group (vehicle). High doses produced a similar effect to morphine (5 mg/kg). Nociceptive behavior was

not found in the control group. At the glutamate test, the administration of LEO or LIN (100 and 200 mg/kg, *i.p.*) decreased significantly the face rubbing behaviour compared with control group (vehicle).

Table 1. Volatile composition of essential oil of *Ocimum basilicum*.

Peak	RT (min)	Compounds	GC/MS (%)	GC-FID (%)	RI ^a
1	6.967	α -tujeno	0.08	-	932
2	8.225	sabineno	0.17	-	971
3	8.392	β -pineno	0.45	0.61	976
4	10.292	1,8-cineol	6.66	6.25	1031
5	12.817	linalol	76.13	76.93	1096
6	16.333	α -terpineol	0.49	0.65	1188
7 and 8	18.342	geraniol*	11.16	11.13	1252
9	19.567	acetato de isobornila	0.15	-	1284
10	22.842	acetato de geranila	3.03	2.58	1437
11	24.675	α -trans-bergamoteno	0.96	0.88	1432
12	27.308	γ -cadineno	0.17	0.33	1512
13	31.300	<i>epi</i> - α -cadinol	0.55	0.60	1642

RT: Time retention; *RI: Retention indices (Adams, 2007); *Coelution; the process whereby two or more chemical compounds elute from a chromatographic column at the same time, making separation and identification difficult.

The stimulation of the hilar region of the dentate gyrus (antidromic stimulation) generated a field potential response in the granular layer, which is characterized by a major negative component (population spike) followed by a small positive phase (Figure 1). Since this response is a consequence of activation of voltage-dependent sodium channels in the axons of the granular cells (Andersen et al., 1971), antagonists of these channels were expected to block the response. As expected, lidocaine (0.5 mg/mL) reversibly inhibited (about 92%) the population spike generated by stimulation of the hilus (Figure 1A). Similar effects were seen for LEO (0.5 mg/mL) (82.7 \pm 2.6 %; Figure 1B) and LIN (0.5 mg/mL) (63.3 \pm 0.8 %; Figure 1C). The effect of LEO could not be completely reversed in our experiments.

The IC₅₀ of LEO for this response was 0.1 \pm 0.05 mg/mL as determined by the fitting with a sigmoidal equation of the dose-response curve (Figure 2).

A similar field potential could be generated in the cellular layer by stimulation of the perforant path of the hippocampal slice (orthodromic stimulation). However, in this case the response is a consequence of glutamate release and glutamate receptor activation in the molecular layer. As shown in Figure 3A, DNQX (2.5 μ g/mL), a non-NMDA glutamate receptor antagonist inhibited the response. Similar results were seen for LEO (0.5 mg/mL) (Figure 3B) and linalool (0.5 mg/mL) (Figure 3C). In both cases the inhibitory effect was reversible.

Table 2. Effect of LEO, LIN or morphine on formalin-induced the orofacial pain in mice.

Treatment	Dose (mg/kg)	Face rubbing (s)			
		0-5 min		15-40 min	
		Score of pain ^a	% inhibition	Score of pain ^a	% inhibition
Vehicle	-	80.1±4.4	-	87.7±15.4	-
LEO	50	78.6±9.6	1.9	86.0±5.2	1.9
LEO	100	57.4±5.79 ^b	28.3	33.1±13.5 ^d	62.2
LEO	200	26.6±4.9 ^d	66.8	1.7±1.4 ^d	98.1
LIN	50	16.7±3.5 ^d	73.9	37.7±6.8 ^c	57.0
LIN	100	11.3±3.0 ^d	85.9	18.6±5.1 ^d	78.8
LIN	200	0.7±0.4 ^d	99.1	1.7±0.4 ^d	98.1
Morphine	5	1.7±0.5 ^d	97.9	0.6±0.2 ^d	99.3

n=8; ^aValues represent mean±SEM; ^bp<0.05, ^cp<0.01, ^dp<0.001 (one-way ANOVA and Tukey's test), significantly different from control.

Table 3. Effect of LEO, LIN or morphine on glutamate- and capsaicin- induced the orofacial pain in mice.

Treatment	Dose (mg/kg)	Glutamate		Capsaicin	
		Face rubbing (s)		Face rubbing (s)	
		Score of pain ^a	% inhibition	Score of pain ^a	% inhibition
Vehicle	-	53.1±6.5	-	116.3±10.3	-
LEO	50	40.1±5.6	24.5	73.0±9.6 ^b	37.2
LEO	100	17.6±3.3 ^c	66.9	27.1±8.3 ^d	76.7
LEO	200	2.3±1.1 ^d	95.7	8.3±6.7 ^d	92.8
LIN	50	34.6±10.8	34.8	69.4±8.6 ^c	40.3
LIN	100	25.0±5.1 ^b	52.9	45.1±12.4 ^d	61.2
LIN	200	17.4±5.1 ^c	67.2	7.1±3.5 ^d	93.9
Morphine	5	1.6±0.6 ^d	97.0	1.4±0.5 ^d	98.8

n=8; ^aValues represent mean±SEM; ^bp<0.05, ^cp<0.01, ^dp<0.001 (one-way ANOVA and Tukey's test), significantly different from control.

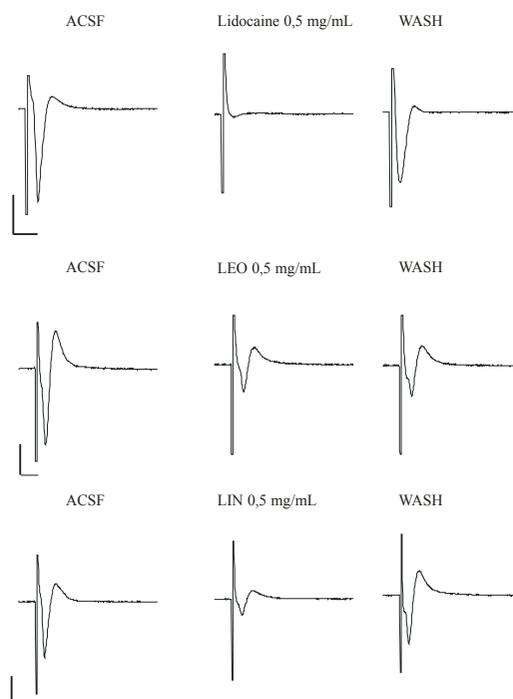


Figure 1. The LEO and linalool block the population spike on dentate gyrus. Representative samples of field potential recordings on the cellular layer of the hippocampal dentate gyrus in response to stimulation of the hylus (antidromic stimulation). Lidocaine (0.5 mg/mL)(A), the LEO (0.5 mg/mL)(B) and linalool (0.5 mg/mL)(C) blocked the population spike of the field responses. The effect was reversed for lidocaine and linalool but not for the LEO. Scale bars: 5 mV and 10 ms for A and C and 3 mV and 10 ms for B.

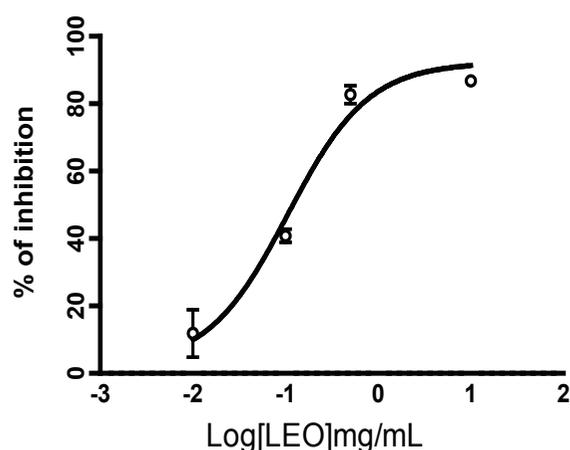


Figure 2. The LEO blocks the population spike on dentate gyrus. Dose-response curve for the LEO on the negative phase (population spike) of the field potential response evoked by the hylus stimulation (antidromic stimulation). Each point on the graph represents the mean \pm SEM of four slices. The curve is a non-linear regression with estimated IC₅₀ of 0.1 mg/mL.

Discussion

Considerable efforts have recently been made to discover new analgesic agents with increased efficacy and improved side effect profiles. A high number of secondary metabolites obtained from medicinal plants, such as monoterpenes, have been extensively studied, with relevant results (Calixto et al., 2000; Almeida et al., 2001; Melo et al., 2010; Quintans-Júnior et al., 2010). In the present study, LEO and LIN demonstrated to play an antinociceptive effect using orofacial nociception tests and to inhibit hippocampal excitability.

There are relatively few behavioral models in laboratory animals dedicated to study orofacial pain. Indeed, Raboisson & Dallel (2004) demonstrated that orofacial formalin test in rodents is a well-established pre-clinical model to investigate the efficacy of analgesic compounds in pain of the facial district. The test is based on a chemical stimulus (formalin) and induces a tissue damage that mimics acute post-injury pain in humans.

The orofacial formalin test is a very useful method for to evaluate antinociceptive drugs, and to elucidate its action mechanism (Luccarini et al., 2006). During the test, two phases can at least partially distinct mechanisms of nociception. The first phase is associated to direct stimulation of C-nociceptors, whereas the second phase reflects integration between peripheral (nociceptors) and central (spinal/ brainstem) signaling (Dallel et al., 1995; Capuano et al., 2009). Furthermore, it has been reported that the development of hyperalgesia due to injection of formalin involves glutamatergic system, such as *N*-methyl-D-aspartate (NMDA) receptors (Beirith et al., 2002;

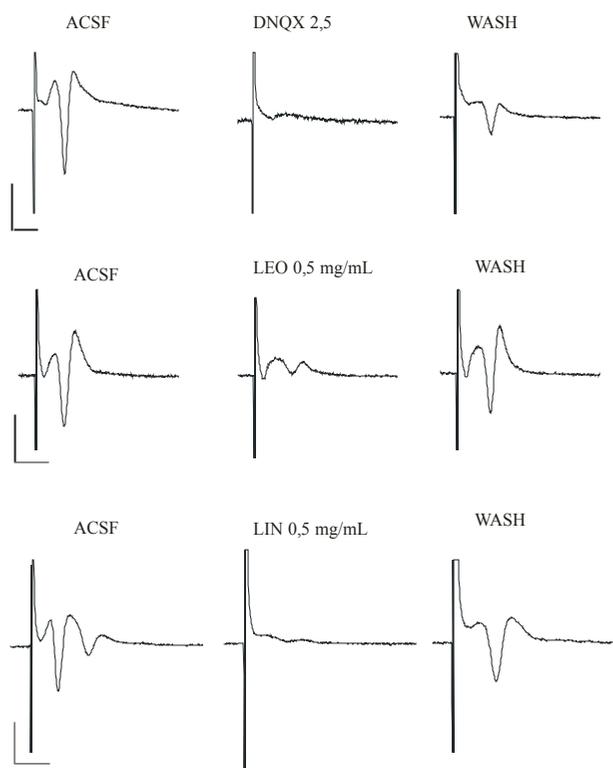


Figure 3. The LEO and linalool block the synaptic-evoked field response on dentate gyrus. Representative samples of field recordings on the cellular layer of the hippocampal dentate gyrus in response to perforant path stimulation (ortodromic stimulation). DNQX (2.5 μ g/mL) (A), the LEO (0.5 mg/mL) and linalool (0.5 mg/mL) blocked the field responses. The effect was reversed for the LEO and linalool. Scale bars: 3 mV and 10 ms for A and C and 2 mV and 10 ms for B.

Luccarini et al., 2006). Pretreatment with LEO or LIN were able to block both phases of the formalin response. The effect was more prominent to LIN.

LIN revealed antinociceptive activity in acetic acid-induced visceral pain in mice; effect that involves activation of both opioidergic and cholinergic neurotransmission (Peana et al., 2003). It is well established that glutamate is involved in transmission of nociceptive signals from peripheral nervous system to the dorsal horn of the spinal cord. It has been reported that the glutamate injection elicited marked nociceptive responses, which is mediated by neuropeptides (such as substance P) released from sensory fibers and due to activation of glutamate receptors (*i.e.* NMDA). NMDA can stimulate the production of a variety of intracellular second messengers, such as nitric oxide (NO) (Carlton et al., 1998). Beirith et al. (2002) described that the nociceptive response induced by glutamate appears to involve peripheral, spinal and supraspinal sites of action and it is greatly mediated by both NMDA and non-NMDA receptors. In this regard, Batista et al. (2008) demonstrated that antinociceptive effect of LIN

may have relationship with glutamate receptors, namely α -amino-3-hydroxy-5-methyl-4-isoxasolepropionic acid (AMPA), NMDA and kainate. Our results confirmed this hypothesis, since pretreatment with LIN significantly protected, at doses 100 and 200 mg/kg (*i.p.*), against orofacial formalin test. A similar result was obtained by acute administration of LEO.

AMPA receptors mediate most fast synapses, and are responsible for responses to glutamate in the synapses. Its activation opens the channels for sodium ions, resulting in neuronal membrane for polarization (Sanacora et al., 2008; Zarate & Manji, 2008). AMPA receptors play an integral role in brain function. Its dysregulation has been implicated in many neurological diseases. There are evidences that AMPA receptors dysfunction may be one of the first manifestations of the synaptic dysfunction that underlies Alzheimer's disease (Shepherd & Huganir, 2007). Glutamatergic AMPA receptors are considered targets to suppress epileptic crisis as they have the property to modulate transmission induced by glutamate (Porto et al., 2007). The kainate receptors are associated with channels voltage-dependent actions and mediate excitatory direct and indirect modulation (Sanacora et al., 2008). NMDA receptors are normally blocked under resting conditions by the obstructing effects of Mg^{2+} ions. Since the membrane is depolarized, these receptors can be activated by the combined action of two molecules of glutamate and two molecules of glycine or D-serine. Thus, NMDA receptor activation serves as a functional marker of converging excitatory input and produces excitation over longer periods of time. Synaptic NMDA receptors activate mitogen-activated protein kinase (MAPK) and the transcription factor cyclic AMP- Ca^{2+} response element-binding protein (CREB); induce expression of the gene that encodes brain-derived neurotrophic factor (BDNF); and promote neuronal survival (Sanacora et al., 2008).

NMDA receptors, located in extrasynaptic space, act in an opposite manner, propagating signals that promote cell death (Sanacora et al., 2008). Glutamatergic system changes have been reported in plasma, serum, cerebrospinal fluid and brain tissue of affected individuals with mood disorders (Sanacora et al., 2008). Several classes of antidepressants alter the release of glutamatergic substances in several brain regions. Stoll et al. (2007) described antidepressant activity involving the glutamatergic system.

LEO or LIN inhibits the neurogenic inflammatory pain induced by capsaicin injection into the right upper lip (perinasal area). Capsaicin applied to skin, muscle, and other tissues has been shown to produce inflammation, to activate and to sensitize trigeminal and spinal small-diameter nociceptive afferents as well as dorsal horn neurons. It also evokes nociceptive behavior in animals and intense pain, hyperalgesia and referred pain in humans (Hu et al., 2005; Lam et al., 2009).

The inhibitory effect observed with LEO or LIN on capsaicin-, and in the second phase of formalin-induced face rubbing behaviour may be a result of its possible inhibition on substance P release or due to a direct blocking action on its receptor neurokinin-1 (NK-1) (Holanda-Pinto et al., 2008). In this context, previous studies provided evidence for tonic activation of NK-1 receptors, through NK-1 receptor antagonist SR14033 administration, which blocked the second phase of the orofacial formalin test in rat (Henry et al., 1999; Luccarini et al., 2003). Waning et al. (2007) demonstrated that capsaicin-sensitive transient receptor potential vanilloid 1 (TRPV1) is one of the Ca^{+2} influx channels involved in cell migration, which plays an important role in pain transduction. Moreover, Honda et al. (2008) suggest that TRPV1 receptor mechanisms in rat facial skin influence nociceptive responses to noxious cutaneous thermal and mechanical stimuli by inducing neuroplastic changes in subnucleus caudalis (Vc) and C1-C2 neurons. Genetic approaches in worms, flies and mice have demonstrated the involvement of transient receptor potential (TRP) in a variety of sensory processes that includes thermosensation, mechanosensation, and pain perception (Vogt-Eisele et al., 2007). Besides, microscopic studies have revealed the expression of immunoreactivity for TRPV1 in the trigeminal ganglion (Ichikawa & Sugimoto, 2001; Hou et al., 2002).

The behavioral experiments suggest that the most of the analgesic effects of LEO could be attributed to LIN, its major component, as revealed by the GC-MS and GC-FID analysis. We gathered further support for this hypothesis in our electrophysiological results, performed in the hippocampal slice preparation (Figures 1, 2 and 3). LEO and LIN inhibited the field potentials activated by the antidromic stimulation of the hylus. These results suggest that these substances interfere with the activation of the voltage-dependent sodium channels present in the granular neurons of the hippocampal dentate gyrus (Andersen et al., 1971). Other electrophysiological data have demonstrated the involvement of the hippocampal formation in pain processing. Khanna & Sinclair (1992) showed that noxious heat stimulation cause depression of hippocampal neurons in the area CA1. A similar effect was demonstrated with the formalin-induced pain model by electrophysiological methods (Khanna & Zeng, 1999). Besides blocking action potential generation, LEO and LIN could interfere with pain process in the hippocampus as has been demonstrated by Khanna & Zheng (1999), using electrophysiological methods and by Ceccarelli et al. (1999) using the c-Fos immunohistochemistry.

The DNQX 2.5 μ g/mL, a non-NMDA glutamate receptor antagonist, inhibited the antidromic response. Similar results were seen for LEO (0.5 mg/mL) and LIN (0.5 mg/mL). These results suggest that LEO and LIN can be considered a non-NMDA glutamate antagonist (AMPA or Kainate blockers).

In conclusion, our results suggest that LEO and LIN modulate neurogenic and inflammatory pain in the tests of orofacial nociception induced by formalin, capsaicin and glutamate. Antinociceptive activity may be associated with decreased peripheral and central nerve excitability. Our results also support that *Ocimum basilicum* and (-)-linalool has a therapeutic potential for painful facial, dental disorders and a non-NMDA glutamate receptor antagonist.

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***Correspondence**

Lucindo J. Quintans-Júnior
Departamento de Fisiologia, Universidade Federal de Sergipe
Av. Marechal Rondon, São Cristóvão-SE, Brazil
lucindo@ufs.br; lucindo@pq.cnpq.br
Fax: +55 79 3212 6640