RESEARCH PAPER

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The blockade of μ-opioid receptors in the lateral hypothalamus enhances panic attack-like behaviour and diminishes defensive antinociception

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The lateral hypothalamus (LH) sends neural pathways to structures involved on predator-related defensive behaviours, escape and antinociception. The aim of this study was to investigate the role played by μ-opioid receptors located on LH neurons in defensive behaviour and unconditioned fear-induced antinociception elicited by electric stimulation of LH. To achieve the goals, the μ1-opioid receptor selective antagonist naloxonazine was administered at different concentrations in the LH, and the defensive behaviour and fear-induced antinociception elicited by electrical stimulation of LH were evaluated. The electrical stimulation of LH caused escape behaviour followed by defensive antinociception. Microinjections of naloxonazine in a concentration of 5.0 μg/0.2 μL in the LH decreased the aversive stimulus-induced escape behaviour thresholds, but diminished defensive antinociception. These findings suggest that μ-opioid receptors of LH can be critical to panic attack-related symptoms and facilitate the unconditioned fear-induced antinociception produced by LH neurons activation.

Key words: opioid system, defensive behaviour, fear-induced antinociception, lateral hypothalamus, panic attacks

INTRODUCTION

Defensive behaviours are emotional reactions elaborated by encephalic structures interconnected and triggered against aversive situations (Cheu and Siegel, 1998; Mangieri et al., 2019; Wang et al., 2020). Amongst these structures, we can highlight the periaqueductal grey matter (PAG), the superior (SC) and the inferior (IC) colliculi, the amygdaloid complex (AC) and the hypothalamus (Silveira et al., 1993, Ullah et al., 2015, de Oliveira et al., 2017, Paschoalin-Maurin et al., 2018, Carrero et al., 2019, Calvo et al., 2019a,b,c, dos Anjos Garcia and Coimbra, 2019; 2020). Connections between diencephalic and mesencephalic structures play an important role in the elaboration of panic attack-like behaviours (Ullah et al., 2017), and inhibitory and excitatory projections from LH to PAG evoke predation and escape behaviour, respectively (Li et

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al., 2018). Furthermore, the hypothalamus integrates neural substrates of descending control of pain and its participation on endogenous pain inhibitory systems has been proposed, since there is evidence that several hypothalamic nuclei are involved in pain control. For example, microinjections of nitric oxide donors in the anterior hypothalamus (Falconi-Sobrinho and Coimbra, 2018; Falconi-Sobrinho et al., 2021), microinjections of bicuculline in the posterior hypothalamus (Falconi-Sobrinho et al., 2017a,b) and neurons activated in dorsomedial and ventromedial hypothalamic nuclei cause defensive behaviour followed by unconditioned fear-induced antinociception (Biagioni et al., 2016a, Khan et al., 2020). In laboratory animals, electrical stimulation of LH produces analgesia (Dafny et al., 1996) in trials of short phasic pain and long-term tonic pain (Aimone et al., 1988), but there is a lack of works showing evidence for defensive analgesia organised by LH neurons.

There is a controversy in literature regarding the role played by the opioid system during panic attack-like behavioural responses. The non-selective blockade of opioid receptors decreases anxiety-like responses in the elevated plus maze test, and panic attack- like behaviour displayed by rodents threatened by rattlesnakes (Coimbra et al., 2017; Calvo et al., 2019a,b). Similar panicolytic-like effects were found when opioid receptors were antagonized by opioid receptors non-selective and selective antagonists microinjected in the dorsal midbrain (Coimbra et al., 1996; 2000, Calvo et al., 2019a,b,c). However, the stimulation of μ-opioid receptors inhibits and the stimulation of k-receptors activates the neural substrate of defence in dorsal columns of the PAG (Anseloni et al., 1999). In addition, opioid receptors seem to play a key role in the organisation of defensive behaviour and defensive antinociception (de Luca et al., 2003, Morgan and Clayton, 2005; Twardowschy and Coimbra, 2015, Coimbra et al., 2017). Opioid receptors are reported to be found in structures involved with pain modulation and aversive stimulus-induced defensive behaviour control, including the tectum and more cranial limbic structures, such as anterior and lateral hypothalamic nuclei that show perikarya with moderate to high mRNA expression related to μ and κ receptor types (Mansour et al., 1994). Microinjections of morphine in LH decrease pain response induced in rodents submitted to the formalin test, demonstrating the involvement of the opioid system in antinociceptive processes (Fucks and Melzack, 1995), as also demonstrated elsewhere (Coimbra et al., 2017) considering defensive antinociception.

Regarding the diencephalic distribution of neuronal perikaryon- and axonal-labelled either endogenous opioid or opioid receptors, *in vitro* radioautographic studies performed in the brain of the passerine songbird dark-eyed junco (*Junco hyemalis*) revealed a high density of μ-opioid receptors in ventromedial and lateral hypothalamus (Deviche et al., 1993). There are also μ-opioid receptor binding sites in the hypothalamic paraventricularis nucleus and the infundibular hypothalamic area (Deviche et al., 1993). In the lateral hypothalamus of Sprague-Dawley rats (*Rattus norvegicus*), μ-opioid receptor-like immunoreactive fibres are observed, with a few scattered μ-opioid receptor-labelled perikarya (Mansour et al., 1995). In cynomolgus monkeys (*Macaca fascicularis*), several nuclei of the hypothalamus localised high concentrations of [^{3}H]-diprenorphine, such as the area hypothalamica posterior, the area hypothalamica lateralis, the nucleus perifornicalis, the nucleus tuberomammillaris, the nucleus premammallaris, the nucleus infundibularis and the supraoptic nucleus (Wamsley et al., 1982).

The hypothesis of this work is that laboratory animals submitted to the electrical stimulation of LH will display defensive behaviour followed by fear-induced antinociception and the pretreatment of the LH with naloxonazine will impair these defensive responses. Naloxonazine consists in a dimeric structure, the equivalent of two molecules of naloxone, which binds to high affinity for μ-opioid receptors and has a similar potency of naloxone (Pasternak and Pan, 2013). However, the naloxonazine selectively blocks μ1-opioid receptor in a long-lasting manner through covalent irreversible bonds (Hahn et al., 1982, 1985; Ling et al., 1986).

METHODS

Animals

Male Wistar rats, weighing 200-250 g, from the animal care facility of the Campus of Cuiabá at the Federal University of Mato Grosso (UFMT) were used. Rodents were kept four in a cage with food and water ad libitum and kept in a room with controlled temperature (22 ± 1°C) under a daily light–dark cycle (lights on at 07:00-19:00 h) during the whole length of experiment. All experiments were performed in accordance with the recommendations of the Commission of Ethics in Animal Experimentation, which abides by the ethical principles for animal research adopted by the National Council for Animal Experimentation Control (CONCEA), and were approved by the Commission of Ethics in Animal Research (CEUA-UFMT-process 23108.920455/2018-88).

Surgery procedure

Animals were anaesthetised with intramuscular injections of 0.1 mL of 4% xylazine (9.2 mg/kg; Dopaser, Hertape/Calier, Juatuba, Minas Gerais, Brazil) and 0.2 mL of 10% ketamine (92 mg/kg; Agener, União Química Farmacêutica Nacional, Pouso Alegre, Minas Gerais, Brazil) and fixed in a stereotaxic frame (Bonther, Ribeirão Preto, São Paulo, Brazil), through the temporal bone and upper incisor teeth. The bar of the upper incisor was positioned 2.5 mm below interaural line, so that the skull was in the horizontal position between bregma and lambda. A brain electrode glued to a guide cannula (chemitrode) was vertically introduced into LH using the following coordinates, with the bregma serving as the reference for each plane: anteroposterior: 4.0 mm; mediolateral: 1.8 mm; and dorsoventral: 8.4 mm. The chemitrode was fixed in skull by means of acrylic resin and two stainless steel screws. At the end of the surgery, each guide-cannula was sealed with a stainless steel wire to protect it from obstruction. After surgery, animals received intramuscular injection of benzathine penicillin G, in a dose of 50.000 UI, and the analgesic and non-steroid anti-inflammatory flunixin meglumine at a dose of 2.5 mg/kg.

After the surgical procedure, the laboratory animals were managed individually in a transparent plexiglass-walled with fenestrated roof cages situated close to each other to minimise stress due to social isolation, and both visual and olfactory clues were allowed to rodents in the post-surgery period.

Drugs

Naloxonazine (bis[(5α)-4,5-Epoxy-3,14-dihydroxy-17-(2-propenyl) morphinan-6-ylidene] hydrazone hydrate dihydrochloride; Sigma/Aldrich, St. Louis, MO, USA), a μ1-opioid receptor selective antagonist (Ling et al., 1986), at 0.05, 0.5 and 5.0 μg/0.2 μL, was dissolved in physiological saline (NaCl; 0.9%), which was also used as a control.

Pharmacological treatment

Naloxonazine was administered in LH though a 5 μL Hamilton syringe (Bonaduz, GR, Switzerland), connected to a 0.3 mm (outside diameter) dental needle (Injex, Ourinhos, São Paulo, Brazil), through a PE-10 polyethylene tube. The tip of the needle reached the brain tissue 1 mm below the lower limit of the guide cannula.

Procedure

After four days of the surgery, the animals were placed in a circular transparent acrylic-walled arena (60 cm in diameter, 50 cm in height, with the floor divided in 12 sections), whose experimental compartment was brightened by 40 Watts lux-fluorescent lamp. The rats allowed a 10-min habituation period before each session. Then, LH was electrically stimulated through a sinusoidal current stimulator (Marseillan, Ribeirão Preto, São Paulo, Brazil). The electrical stimulation current was monitored by an oscilloscope (Minipa do Brasil, MO1250S, Joinville, Santa Catarina, Brazil). The encephalic stimulation (60 Hz, 15 s) was performed at 1-min intervals, with current increasing at 10 μA steps (peak to peak), until the aversion threshold was reached.

The following behaviours were recorded in the Wistar rats electrically stimulated in the LH: (I) Non-defensive motor behaviours, such as (i) catching behaviours (movements of the front paws from side to side towards the ground), (ii) digging behaviour (movement of the fore legs as if it were digging), and (iii) exploratory behaviour (head movements from side to side and rearing) and (II) defensive behaviours, such as (iv) defensive attention (interruption of ongoing behaviours for up to five seconds, followed by an attentive posture, as well as behaviours characterised by small head movements, rearing and smelling), (v) defensive immobility (absence of movement for at least six seconds, except for respiration, followed by autonomic reactions, such as defecation, exophthalmia and/or micturition), (vi) escape behaviour (running and/or vertical jumping). The current intensity-producing running from moderate to strong intensity (or jumping) in two successive trials was considered to be the escape behaviour threshold, as previously reported either in a naturalistic experimental model (Coimbra et al., 2017) or in an invasive brain procedure (de Oliveira et al., 2017). Animals with an escape behaviour threshold above 140 μA were discarded from the experiment.

After defensive behaviour threshold determination, the μ-opioid receptor blockade was performed with microinjections of either naloxonazine at different concentrations (0.05, 0.5 and 5.0 μg/0.2 μL) or physiological saline (0.2 μL) into LH, in a randomized manner. Naloxonazine and the vehicle were administered twenty-four hours before the redetermination of defensive behaviour thresholds and tail-flick latencies (Ling et al., 1986; Osaki et al., 2003; Calvo et al., 2019).

Defensive antinociception recording

The rats were submitted to the tail-flick test to record the nociception threshold. Each animal was

placed in an acrylic-walled restraining apparatus (Tail-flick Analgesia Instrument; Insight, Ribeirão Preto, São Paulo, Brazil) so that its tail was placed on a heating sensor filament, in which the calorimetric progressive elevation was automatically interrupted when the animal detached its tail from the apparatus. The electric current was the source to raise the temperature of the coil (Ni/Cr alloy; 26.04 cm in length × 0.02 cm in diameter) at the rate of approximately 9°C/s (Prado and Roberts, 1985), starting from room temperature (approximately 20°C). A small current intensity adjustment could be done, if necessary, in the beginning of the experiment, aiming to obtain three consecutive tail-flick latencies between 2.5 s and 3.5 s. If the animal did not remove its tail from the heating filament within 6 s, the apparatus was turned off in order to prevent damage to receptors of tail skin. Three baselines of control tail-flick latencies were taken at 5 min intervals, before electrical stimulation of the LH. After twenty-four hours of either naloxonazine or physiological saline microinjection, electrical stimulation of the LH was performed, followed by the tail-flick latencies recording at 0, 10, 20, 30, 40 and 60 min after diencephalic electrical stimulation.

Histology

After the experiments, the rats were anaesthetised with xylazine (10 mg/kg) and ketamine (100 mg/kg) and perfused via the left cardiac ventricle. The blood was washed out with cold, oxygen-enriched, Ca^{++}-free Tyrode's buffer (40 mL at 4°C), followed by 200 mL of ice-cold 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.3, for 15 min at a pressure of 50 mmHg. The diencephalon was quickly sectioned, removed and immersed in fresh fixative for 4 h at 4°C. It was then rinsed in 10% and 20% sucrose dissolved in 0.1 M sodium phosphate buffer (pH 7.4) at 4°C, for at least 12 h in each solution. Tissue pieces were immersed in 2-methylbutane (Sigma), frozen on dry ice, embedded in Tissue Tek O.C.T., and cut with a cryostat (Leica CM 1950, Wetzlar, Germany) at -22°C. Brain sections were then mounted on glass slides coated with chrome alum gelatin to prevent detachment and stained with haematoxylin-eosin to be viewed on a photomicroscope (Axio Observer, Zeiss, Oberkochen, Germany). Statistical analysis was performed exclusively with data from the animals that presented histologically confirmed sites of microinjections of drugs and diencephalic electrical stimulation in the LH, according to Paxinos and Watson`s rat brain in stereotaxic coordinates atlas (2005).

Statistical analysis

Data regarding the electrical stimulation of the LH were submitted to a one-way ANOVA followed by the Newman-Keuls *post-hoc* test. Data related to defensive antinociception were submitted to a repeated measure two-way ANOVA followed by Duncan's *post hoc* test. All values were reported as the mean ± standard error of the mean (SEM). $P<0.05$ was considered statistically significant.

RESULTS

The electrical stimulation of the LH elicited head movements from side to side, movements of the front paws from side to side towards the ground (as if catching something), movement of the fore legs as if they were digging, aversive stimulus-induced defensive attention (alertness), in addition to the classic hypothalamic escape behaviour, expressed as moderate intensity running and vertical jumping, interspersed with exploratory behaviour. The escape behaviour was followed by unconditioned fear-induced antinociception. Histological confirmation of the electrode tips situated in the LH and microinjection sites of either naloxonazine or physiological saline are illustrated in Fig. 1.

Effect of LH μ-opioid receptors blockade on defensive behaviour thresholds

According to a one-way ANOVA, followed by Newman-Keuls *post hoc* test, the pretreatment of the LH with naloxonazine in a dose of 5.0 μg/0.2 μL caused a significant decrease on escape thresholds [$F3,25=4.238$, $P<0.05$], compared to the control group. However, naloxonazine at the lower doses of 0.05 and 0.5 μg/0.2 μL did not cause significant effect on escape behaviour thresholds [$F3,25=4.238$, $P>0.05$], compared to the control group. These data are shown in Fig. 2.

Effect of LH μ-opioid receptors blockade on defensive antinociception

The escape behaviour elicited by electrical stimulation of LH was followed by defensive antinociception. LH pretreatment with naloxonazine at the higher concentrations (0.5 and 5.0 μg/0.2 μL) caused a significant decrease of unconditioned fear-induced antinociception recorded immediately after escape behaviour and 10 and 20 min after LH electrical stimulation in comparison to the control group [$F8,192=49.14$;

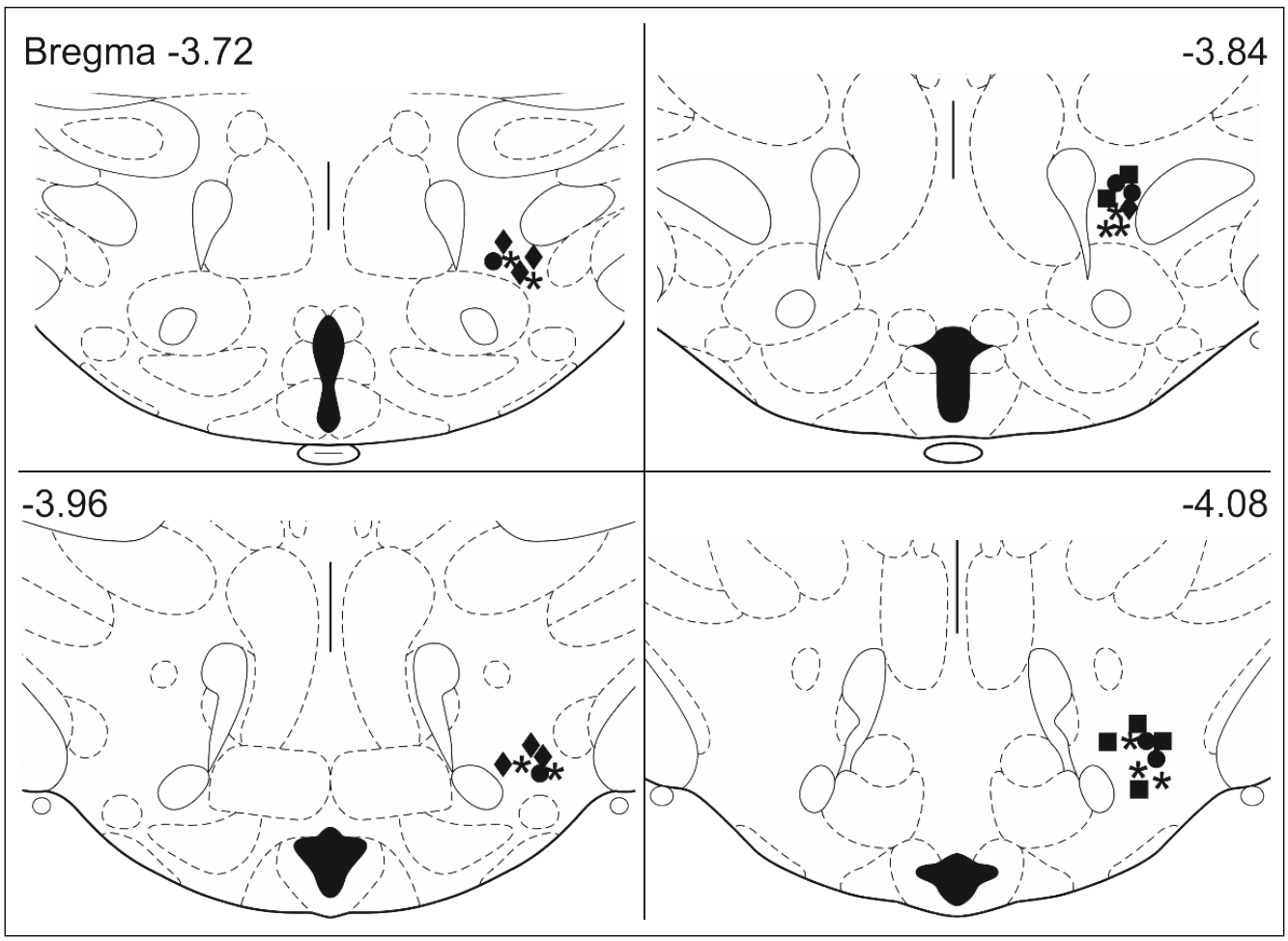

Fig. 1. Schematic coronal sections of the rat brain. This figure shows the sites of electrical diencephalic stimulation and microinjections of either vehicle (*), naloxonazine at 0.05 μg/0.2 μL (♦), naloxonazine at 0.5 μg/ 0.2μL (■) or naloxonazine at 5.0 μg/0.2 μL (●) in the lateral hypothalamus, depicted on modified illustrations from the Paxinos and Watson´s stereotaxic atlas (2005).

P<0.001]. Naloxonazine at the lowest concentration (0.05 μg/0.2 μL) did not cause significant changes in defensive antinociception. These data are shown in Fig. 3.

DISCUSSION

The hypothalamic stimulation in laboratory animals elicits oriented escape behaviour to safe places like those responses elicited by prey confronted with predators in an enriched and dangerous environment (Uribe-Mariño et al., 2012; Twardowschy et al., 2013; dos Anjos-Garcia and Coimbra, 2019; 2020). Amongst the defensive behaviours elicited by hypothalamus neuronal activation, we can highlight the defensive alertness, defensive immobility, oriented escape and vertical jumps, followed by defensive antinociception (Freitas et al., 2009, 2014; Biagioni et al., 2016a,b). The oriented escape elicited by chemical stimulation of the some hypothalamic nuclei, which can also be activated in a prey versus predator encounter (Paschoalin-Maurin et al., 2018; Mendes-Gomes et al., 2020) differs from that elicited by stimulation of mesencephalic structures, such as the PAG and corpora quadrigemina (Almada and Coimbra, 2015; Ullah et al., 2015; Biagioni et al., 2016b).

Several hypothalamic nuclei are responsible for different kinds of defensive and predatory behaviours; for example, the dorsomedial and ventromedial hypothalamic nuclei are responsible for the organisation of oriented escape (Ullah et al., 2015), while the lateral hypothalamic nucleus is related to predation or evasion, depending of efferent neural pathways and neurotransmission connecting the hypothalamus to PAG (Li et al., 2018).

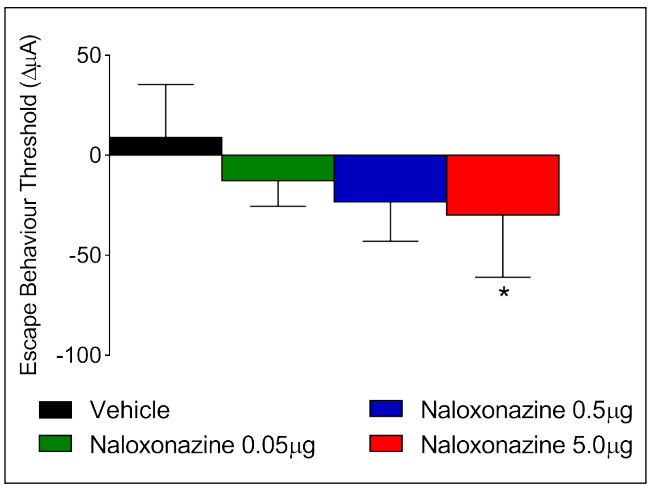

Fig. 2. Effect of the pretreatment of the lateral hypothalamus (LH) with either vehicle, or naloxonazine at different concentrations (0.05, 0.5 and 5.0 μg/0.2 μL) on escape behaviour threshold elicited by electrical stimulation of LH. Data were represented as delta (test – control values) escape behaviour thresholds in microamperes (n= 6-10); * P<0.05, as compared to the vehicle (0.9% NaCl)-LH pretreatment, according to one-way analysis of variance (ANOVA), followed by Newman-Keuls` *post hoc* test.

Interestingly, even panic attack-like non-oriented/explosive escape behaviour displayed by laboratory animals submitted to the stimulation of medial hypothalamic nuclei seems to be dependent on the recruitment of dorsal periaqueductal grey matter neurons (Ullah et al., 2017).

In this work, the electrical stimulation of the lateral part of the hypothalamus also elicited escape behaviour in rats, which seems to be mediated by the endogenous opioid peptides neurotransmission, because the intradiencephalic microinjection of the μ-opioid receptor selective antagonist naloxonazine in a concentration of 5.0 μg /0.2 μL decreased the escape behaviour thresholds, suggesting a proaversive effect. However, other behavioural repertory was also displayed by rats after electrical stimulation of the LH neurons performed by our research team, such as digging and catching behaviours that can be related to feeding behavioural responses, which also recruit the LH opioid system (Ikeda et al., 2015; Ardianto et al., 2016; Romero-Picó et al., 2018). In addition, there is evidence that opioid peptides within the PAG modulate the expression of predatory attack behaviour in *Felis silvestris catus* (Weiner et al., 1991). These authors demonstrated that quiet biting attack is elicited by electrical stimulation of sites

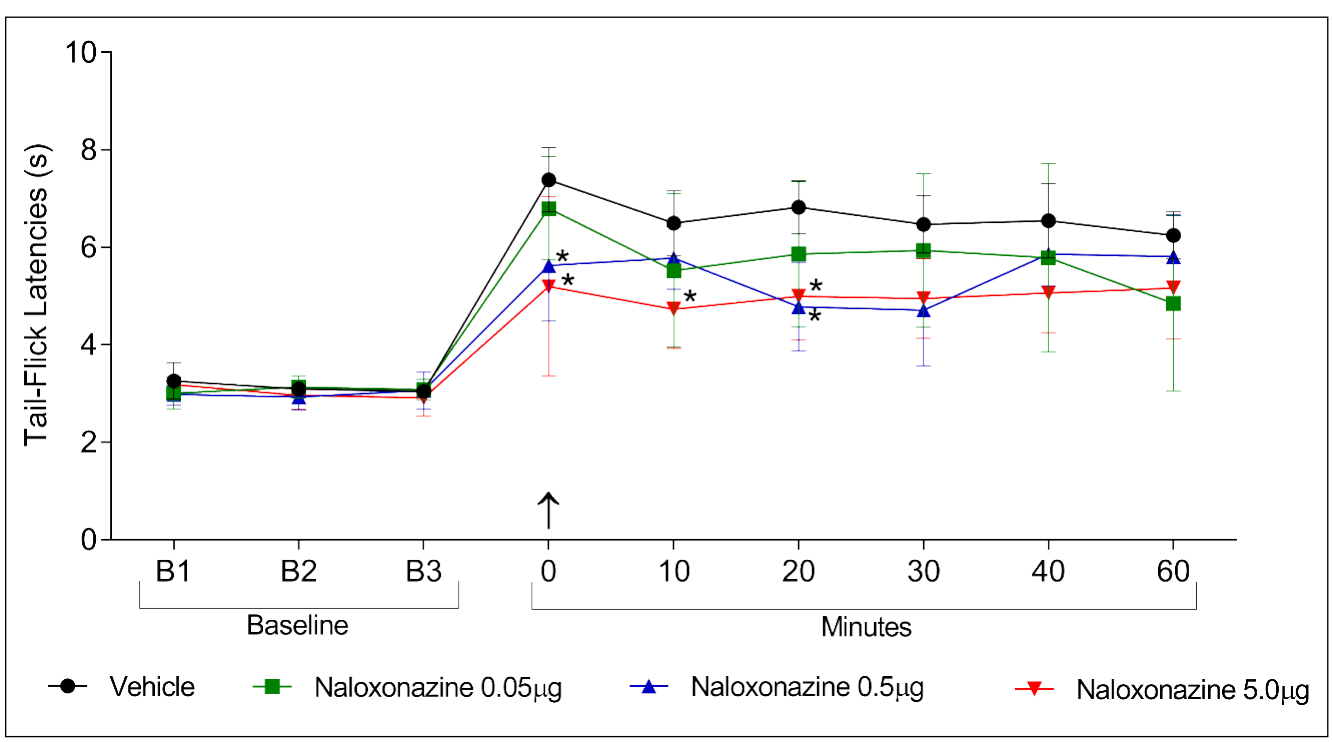

Fig. 3. Effect of either naloxonazine at different concentrations (0.05, 0.5, and 5.0 μg/0.2 μL) or vehicle microinjected in the lateral hypothalamus (LH) on unconditioned fear-induced antinociception (n=6-10). Data were presented as mean ± S.E.M.; * P<0.05, as compared with the vehicle (0.9% NaCl)-LH pretreatment, according to a two-way multivariate repeated measure ANOVA, followed by Duncan's *post hoc* test. B1, B2 and B3 represent baseline measurements of control tail-flick latencies (TFL) and the arrow represents the electrical stimulation of LH.

within the lateral hypothalamus using monopolar electrodes, and they identified modulatory sites within the PAG that when activated either suppress or facilitate LH-induced quiet biting attack. At nine of twelve sites in the PAG where suppression was obtained, administration of naloxone cased a blockade of those effects. Similarly, at six of eight facilitatory sites within the PAG, naloxone also blocked the modulatory effects of PAG stimulation (Weiner et al., 1991). Finally, there is evidence that inhibitory and excitatory projections from the mouse LH to the PAG drive, respectively, predation and evasion (Li et al., 2018).

Hypothalamically elicited predatory attack is facilitated by enkephalinergic mechanisms operating at the midbrain level (Saha et al., 2003).

In Sprague-Dawley rats, several LH targets in the central grey are situated in the ventrolateral columns of the PAG (vlPAG), whose neurons provides a light input to the LH. Opioid mechanisms of vlPAG are also implicated in fear-related responses (de Luca et al., 2003; McNally et al., 2005; McNally and Cole, 2006) and in pain modulation (Chen et al., 2016).

Another limbic structure connected to the LH is the lateral habenula (LHb), an epithalamic diencephalic nucleus that plays a relevant role in aversion-driven learning and behaviour (Sheth et al., 2017). In two models of persistent pain, optogenetic activation of LH neurons or their axonal terminals in the vlPAG decreases nociception, and neural tract tracing reveals that LH neurons send preferentially glutamatergic projections to GABAergic neurons in the vlPAG (Siemian et al., 2021). By contrast, LH outputs to the LHb modulate aversion rather than nociception. Finally, Siemian et al. (2021) showed that LH activation produces a synergistic antinociceptive effect with morphine and restores morphine-induced antinociception after morphine tolerance.

Surprisingly, we found in this work that μ-opioid receptors of LH are also able to diminish panic-like escape behavioural responses in addition to cause an impairment in the defensive antinociception. This is evidence that although defensive behaviour and nociception seems to be elaborated by LH neurons, these responses can be independently organised by the LH network. Indeed, the dorsomedial, the ventromedial and the lateral hypothalamic nuclei consist of diencephalic structures also involved in pain elaboration, since the activation of these diencephalic structures elicits unconditioned fear-induced/defensive antinociception (Aimone et. al. 1988; Biagioni et al., 2013, 2016a; Khan et al., 2020; Safari-Sandiani et al., 2020) similar to that displayed by prey when confronted with a potential predator, such as wild serpents (Coimbra et al., 2017). In this sense, in our work, we verified that the electrical stimulation of LH elicited defensive antinociception and this phenomenon seems to be modulated by the μ-opioid receptor, because microinjections of naloxonazine in the LH significantly diminish the unconditioned fear-induced antinociception. In fact, studies of *in situ* hybridization demonstrated the expression of μ- and μ1-opioid receptors in the diencephalon, including the LH (Mansour et al., 1994).

Although a previous report showed that the electrical stimulation of LH causes antinociception, and this process appears to be mediate by opioid receptors, considering that microinjections of morphine in this structure also cause similar effect (Fucks and Melzack, 1995), this is the first time in literature in which it was demonstrated that LH stimulation-induced unconditioned fear-related antinociception is modulated by μ-opioid receptors.

CONCLUSIONS

In conclusion, the present work suggests that LH μ-opioid receptors modulates the panic attack-like defensive behaviour and unconditioned fear-induced antinociception elicited by electrical stimulation of that diencephalic nucleus.

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REFERENCES

Aimone L, Bauer CA, Gebhart GF (1988) Brain-stem relays mediating stimulation- produced antinociception from the lateral hypothalamus in the rat. J Neurosci 8: 2652–2663.

Almada RC, Coimbra NC (2015) Recruitment of striatonigral disinhibitory and nigrotectal inhibitory GABAergic pathways during the organisation of defensive behaviour by mice in a dangerous environment with the venomous snake *Bothrops alternatus* (Reptilia, Viperidae). Synapse 69: 299–313.

Anseloni VCZ, Coimbra NC, Morato S, Brandão ML (1999) A comparative study of the effects of morphine in the dorsal periaqueductal gray and nucleus accumbens of rats submitted to the elevated plus-maze test. Exp Brain Res 129: 260–268.

Ardianto C, Yonemochi N, Yamamoto S, Yang L, Takenoya F, Shioda S, Nagase H, Ikeda H, Kamei J (2016) Opioid systems in the lateral hypothalamus regulate feeding behaviour through orexin and GABA neurons. Neuroscience 320: 183–193.

Biagioni AF, de Freitas RL, da Silva JA, de Oliveira RC, de Oliveira R, Alves VM, Coimbra NC (2013) Serotonergic neural links from the dorsal raphe nucleus modulate defensive behaviours organised by the dorsomedial hypothalamus and the elaboration of fear-induced antinociception via *locus coeruleus* pathways. Neuropharm 67: 379–394.

Biagioni AF, de Oliveira RC, de Oliveira R, da Silva JA, dos Anjos-Garcia T, Roncon CM, Corrado AP, Zangrossi H Jr, Coimbra NC (2016a) 5-Hydroxytryptamine 1A receptors in the dorsomedial hypothalamus connected to dorsal raphe nucleus inputs modulate defensive behaviours and mediate innate fear-induced antinociception. Eur Neuropsychopharmacol 26: 532–545.

Biagioni AF, dos Anjos-Garcia T, Ullah F, Fisher IR, Falconi-Sobrinho LL, de Freitas RL, Felippotti TT, Coimbra NC (2016b) Neuroethological validation of an experimental apparatus to evaluate oriented and non-oriented escape behaviours: Comparison between the polygonal arena with a burrow and the circular enclosure of an open-field test. Behav Brain Res 298: 65–77.

Calvo F, Almada RC, da Silva JA, Medeiros P, da Silva Soares R Jr, de Paiva YB, Roncon CM, Coimbra NC (2019a) The Blockade of μ1- and κ-opioid receptors in the inferior colliculus decreases the expression of panic attack-like behaviours induced by chemical stimulation of the dorsal midbrain. Neuropsychobiology 78: 218–228.

Calvo F, Almada RC, dos Anjos-Garcia T, Falconi-Sobrinho LL, Paschoalin-Maurin T, Bazaglia-de-Souza G, Medeiros P, da Silva JA, Lobão-Soares B, Coimbra NC (2019b) Panicolytic-like effect of μ1-opioid receptor blockade in the inferior colliculus of prey threatened by *Crotalus durissus terrificus* pit vipers. J Psychopharmacol 33: 577–588.

Calvo F, Lobão-Soares B, de Freitas RL, Paschoalin-Maurin T, dos Anjos-Garcia T, Medeiros P, da Silva JA, Lovick TA, Coimbra NC (2019c) The endogenous opioid system modulates defensive behaviour evoked by *Crotalus durissus terrificus*: Panicolytic-like effect of intracollicular non-selective opioid receptors blockade. J Psychopharmacol 33: 51–61.

Carrero JP, Kaigler KF, Hartshorn GH, Fadel JR, Wilson MA (2019) Mu opioid receptor regulation of glutamate efflux in the central amygdala in response to predator odor. Neurobiol Stress 11: 100–197.

Chen T, Li J, Feng B, Hui R, Dong YL, Huo FQ, Zhang T, Yin JB, Du JQ, Li YQ (2016) Mechanism underlying the analgesic effect exerted by endomorphin-1 in the rat ventrolateral periaqueductal gray. Mol Neurobiol 53: 2036–2053.

Cheu JW, Siegel A (1998) GABA receptor mediated suppression of defensive rage behaviour elicited from the medial hypothalamus of the cat: role of the lateral hypothalamus. Brain Res 783: 293–304.

Coimbra NC, Calvo F, Almada RC, Freitas RL, Paschoalin-Maurin T, dos Anjos-Garcia T, Elias-Filho DH, Ubiali WA, Lobão-Soares B, Tracey I (2017) Opioid neurotransmission modulates defensive behaviour and fear-induced antinociception in dangerous environments. Neuroscience 354: 178–195.

Coimbra NC, Eichenberger GCD, Gorchinski RT, Maisonnette S (1996) Effects of the blockade of opioid receptor on defensive reactions elicited by electrical stimulation within the deep layers of the superior colliculus and DPAG. Brain Res 736): 348–352.

Coimbra NC, Osaki MY, Eichenberger GCD, Ciscato JG Jr, Jucá CEB, Biojone CR (2000) Effects of opioid receptor blockade on defensive behaviour elicited by electrical stimulation of the aversive substrates of the inferior colliculus in *Rattus norvegicus* (Rodentia, Muridae). Psychopharmacology 152: 422–430

Dafny N, Dong WQ, Prieto-Gomez C, Reyes-Vazquez C, Stanford JQ (1996) Lateral hypothalamus: site involved in pain modulation. Neuroscience 70: 449–460.

de Freitas RL, Uribe-Mariño A, Castiblanco-Urbina MA, Elias-Filho DH, Coimbra NC (2009) GABAA receptor blockade in dorsomedial and ventromedial nuclei of the hypothalamus evokes panic-like elaborated defensive behaviour followed by innate fear-induced antinociception. Brain Res 11: 118–131.

de Freitas RL, Salgado-Rohner CJ, Biagioni AF, Medeiros P, Hallak JEC, Crippa JA, Coimbra NC (2014) NMDA and AMPA/kainate glutamatergic receptors in the prelimbic medial prefrontal cortex modulate the elaborated defensive behaviour and innate fear- induced antinociception elicited by GABAA receptor blockade in the medial hypothalamus. Cereb Cortex 24: 1518–1528.

de Luca MC, Brandão ML, Motta VA, Landeira-Fernandez J (2003) Antinociception induced by stimulation of ventrolateral periaqueductal gray at the freezing threshold is regulated by opioid and 5-HT2A receptors as assessed by the tail-flick and formalin tests. Pharmacol Biochem Behav 75: 459–466.

de Oliveira R, de Oliveira RC, Falconi-Sobrinho LL, Soares JR RS, Coimbra NC (2017) 5- Hydroxytryptamine2A/2C receptors of nucleus raphe magnus and gigantocellularis/paragigantocellularis pars α reticular nuclei modulate the unconditioned fear-induced antinociception evoked by electrical stimulation of deep layers of the superior colliculus and dorsal periaqueductal grey matter. Behav Brain Res 316: 294–304.

Deviche P, Cotter P, Gulledge CC (1993) Identification, partial characterization, and hypothalamic distribution of kappa, mu, and delta opioid receptors in a passerine songbird (*Junco hyemalis*). Brain Res 614: 220–226.

dos Anjos-Garcia T, Coimbra NC (2019) Opposing roles of dorsomedial hypothalamic CB1 and TRPV1 receptors in anandamide signaling during the panic-like response elicited in mice by Brazilian rainbow Boidae snakes. Psychopharmacology 236: 1863–1874.

dos Anjos-Garcia T, Coimbra NC (2020) Anandamide in the anterior hypothalamus diminishes defensive responses elicited in mice threatened by *Epicrates cenchria* constrictor serpents. Acta Neurobiol Exp 80: 179–191.

Falconi-Sobrinho LL, Coimbra NC (2018) The nitric oxide donor SIN-1-produced panic- like behaviour and fear-induced antinociception are modulated by NMDA receptors in the anterior hypothalamus. J Psychopharmacol 32: 711–722.

Falconi-Sobrinho LL, dos Anjos-Garcia T, Coimbra NC (2021) Nitric oxide-mediated defensive and antinociceptive responses organised at the anterior hypothalamus of mice are modulated by glutamatergic inputs from area 24b of the cingulate cortex. J Psychopharmacol 35: 78–90.

Falconi-Sobrinho LL, dos Anjos-Garcia T, de Oliveira R, Coimbra NC (2017a) Decrease in NMDA receptor-signalling activity in the anterior cingulate cortex diminishes defensive behaviour and unconditioned fear-induced antinociception elicited by GABAergic tonic inhibition impairment in the posterior hypothalamus. Eur Neuropsychopharmacol 27: 1120–1131.

Falconi-Sobrinho LL, dos Anjos-Garcia T, Elias-Filho DH, Coimbra NC (2017b) Unravelling cortico-hypothalamic pathways regulating unconditioned fear-induced antinociception and defensive behaviours. Neuropharmacology 113: 367–385.

Fucks PN, Melzack R (1995) Analgesia induced by morphine microinjection into the hypothalamus of the rat. Exp Neurol 134: 277–280.

Hahn EF, Carroll-Buatti M and Pasternak GW (1982) Irreversible opiate agonists and antagonists: the 14-hydroxydihydromorphinone azines. J Neurosci 2: 572–576.

Hahn EF, Nishimura S, Goodman RR, et al. (1985) Irreversible opiate agonists and antagonists. II. Evidence against a bivalent mechanism of action for opiate azines and diacylhydrazones. J Pharmacol Exp Therapeut 235: 839–845.

Ikeda H, Ardianto C, Yonemochi N, Yang L, Ohashi T, Ikegami M, Nagase H, Kamei J (2015) Inhibition of opioid systems in the hypothalamus as well as the mesolimbic area suppresses feeding behaviour of mice (2015) Neuroscience 311: 9–21.

Khan AU, Falconi-Sobrinho LL, dos Anjos-Garcia T, dos Santos Sampaio M de F, de Souza Crippa JA, Menescal-de-Oliveira L, Coimbra NC (2020) Cannabidiol-induced panicolytic-like effects and fear-induced antinoci-

ception impairment: the role of the CB1 receptor in the ventromedial hypothalamus. Psychopharmacology 237: 1063–1079.
Li Y, Zeng J, Zhang J, Yue C, Zhong W, Liu Z, Feng Q, Luo M (2018) Hypothalamic circuits for predation and evasion. Neuron 97: 911–924.
Ling GSF, Simantov R, Clark JA, Pasternak, GW (1986) Naloxonazine actions in vivo. Eur J Pharmacol 129: 33–38.
McNally GP, Cole S (2006) Opioid receptors in the midbrain periaqueductal gray regulate prediction errors during pavlovian fear conditioning. Behav Neurosci 120: 313–323.
McNally GP, Lee BW, Chiem JY, Choi EA (2005) The midbrain periaqueductal gray and fear extinction: opioid receptor subtype and roles of cyclic AMP, protein kinase A, and mitogen-activated protein kinase. Behav Neurosci 119: 1023–1033.
Mangieri LR, Jiang Z, Lu Y, Xu Y, Cassidy RM, Justice N, Xu Y, Arenkiel BR, Tong Q (2019) Defensive behaviours driven by a hypothalamic-ventral midbrain circuit. eNeuro 6: 1–19.
Mansour A, Fox CA, Burke S, Akil H, Watson SJ (1995) Immunohistochemical localization of the cloned mu opioid receptor in the rat CNS. J Chem Neuroanat 8: 283–305.
Mansour A, Fox CA, Burke S, Meng F, Thompson RC, Akil H, Watson SJ (1994) Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. J Comp Neurol 350: 412–438.
Mendes-Gomes J, Motta SC, Passoni Bindi R, de Oliveira AR, Ullah F, Baldo MVC, Coimbra NC, Canteras NS, Blanchard DC (2020) Defensive behaviours and brain regional activation changes in rats confronting a snake. Behav Brain Res 381: 112469.
Morgan MM, Clayton CC (2005) Defensive behaviours evoked from the ventrolateral periaqueductal gray of the rat: comparison of opioid and GABA disinhibition. Behav Brain Res 164: 61–66.
Osaki MY, Castellan-Baldan L, Calvo F, Carvalho AD, Felippotti TT, de Oliveira R, Ubiali WA, Paschoalin-Maurin T, Elias-Filho DH, Motta V, da Silva LA, Coimbra NC (2003) Neuroanatomical and neuropharmacological study of opioid pathways in the mesencephalic tectum: effect of μ1- and κ-opioid receptor blockade on escape behaviour induced by electrical stimulation of the inferior colliculus. Brain Res 992: 179–192.
Paschoalin-Maurin T, dos Anjos-Garcia T, Falconi-Sobrinho LL, de Freitas RL, Coimbra JPC, Laure CJ, Coimbra NC (2018) The rodent-versus-wild snake paradigm as a model for studying anxiety- and panic-like behaviours: face, construct and predictive validities. Neuroscience 369: 336–349.
Pasternak GW and Pan YX (2013) Mu opioids and their receptors: evolution of a concept. Pharmacol Rev 65: 1257–1317.
Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates. Elsevier Academic Press, San Diego.
Prado WA, Roberts MH (1985) An assessment of the antinociceptive and aversive effects of stimulating identified sites in the rat brain. Brain Res 340: 219–228.
Romero-Picó A, Sanchez-Rebordelo E, Imbernon M, González-Touceda D, Folgueira C, Senra A, Fernø J, Blouet C, Cabrera R, van Gestel M, Adan RA, López M, Maldonado R, Nogueiras R, Diéguez C (2018) Melanin-concentrating hormone acts through hypothalamic kappa opioid system and p70S6K to stimulate acute food intake. Neuropharmacology 130: 62–70.
Saha SN, Bhatia SC, Nayar U (2003) Enkephalinergic involvement in substantia nigra in the modulation of hypothalamically-induced predatory attack behaviour. Indian J Physiol Pharmacol 47: 311–317.
Safari-Sandiani E, Rahimitabar N, Rezaee L, Behnaz M, Haghparast A (2020) The contribution of orexin receptors within the ventral tegmental area to modulation of antinociception induced by chemical stimulation of the lateral hypothalamus in the animal model of orofacial pain in the rats. Behav Pharmacol 31: 500–509.
Sheth C, Furlong TM, Keefe KA, Taha SA (2017) The lateral hypothalamus to lateral habenula projection, but not the ventral pallidum to lateral habenula projection, regulates voluntary ethanol consumption. Behav Brain Res 328: 195–208.
Siemian JN, Arenivar MA, Sarsfield S, Borja CB, Erbaugh LJ, Eagle AL, Robison AJ, Leinninger G, Aponte Y (2021) An excitatory lateral hypothalamic circuit orchestrating pain behaviours in mice. Elife 10: e66446.
Silveira MCL, Sandner G, Graeff FG (1993) Induction of Fos immunoreactivity in the brain by exposure to the elevated plus-maze. Behav Brain Res 56: 115–118.
Twardowschy A, Castiblanco-Urbina MA, Uribe-Mariño A, Biagioni AF, Salgado- Rohner CJ, de Souza Crippa JA, Coimbra NC (2013) The role of 5-HT1A receptors in the antiaversive effects of cannabidiol on panic attack-like behaviours evoked in the presence of the wild snake *Epicrates cenchria crassus* (Reptilia, Boidae). J Psychopharmacol 27: 1149–1159.
Twardowschy A, Coimbra NC (2015) μ- and κ-opioid receptor activation in the dorsal periaqueductal grey matter differentially modulates panic-like behaviours induced by electrical and chemical stimulation of the inferior colliculus. Brain Res 1597: 168–179.
Ullah F, dos Anjos-Garcia T, dos Santos IR, Biagioni AF, Coimbra NC (2015) Relevance of dorsomedial hypothalamus, dorsomedial division of the ventromedial hypothalamus and the dorsal periaqueductal gray matter in the organisation of freezing or oriented and non- oriented escape emotional behaviours. Behav Brain Res 293: 143–152.
Ullah F, dos Anjos-Garcia T, Mendes-Gomes J, Elias-Filho DH, Falconi-Sobrinho LL, Freitas RL, Khan AU, Oliveira R, Coimbra NC (2017) Connexions between the dorsomedial division of the ventromedial hypothalamus and the dorsal periaqueductal grey matter are critical in the elaboration of hypothalamically mediated panic-like behaviour. Behav Brain Res 319: 135–147.
Uribe-Mariño A, Francisco A, Castiblanco-Urbina MA, Twardowschy A, Salgado- Rohner CJ, Crippa JAS, Hallak JEC, Zuardi AW, Coimbra NC (2012) Anti-aversive effects of cannabidiol on innate fear-induced behaviours evoked by an ethological model of panic attacks based on a prey *vs.* the wild snake *Epicrates cenchria crassus* confrontation paradigm. Neuropsychopharmacology 37: 412–421.
Wamsley JK, Zarbin MA, Young WS III, Kuhar MJ (1982) Distribution of opiate receptors in the monkey brain: an autoradiographic study. Neuroscience 7: 595–613.
Wang Y, Niu L, Meng W, Lin Z, Zou J, Bian T, Huang X, Zhou H, Meng L, Xie P, Zheng H (2020) Ultrasound stimulation of periaqueductal gray induces defensive behaviours. IEEE Trans Ultrason Ferroelectr Freq Control 1–7.
Weiner S, Shaikh MB, Shaikh AB, Siegel A (1991) Enkephalinergic involvement in periaqueductal gray control of hypothalamically elicited predatory attack in the cat. Physiol Behav 49: 1099–1105.