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# Reduction in 50-kHz call-numbers and suppression of tickling-associated positive affective behaviour after lesioning of the lateral hypothalamic parvafox nucleus in rats

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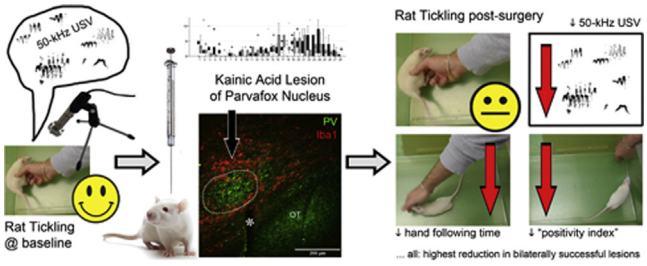
## HIGHLIGHTS

- Wistar rats were tickled before and after excitotoxic lesioning of the lateral hypothalamic parvafox nucleus.
- Ultrasonic vocalisations (USVs) that were emitted during the tickling of rats and their tendency to approach and follow the experimenter's hand were analysed.
- Lesioning was considered successful if the number of parvalbumin-immunoreactive (PV-ir) cells in the area of the parvafox nucleus was reduced beyond a threshold level.
- Rats with bilaterally successful lesions manifested the most profound surgery-associated reduction in the number of 50-kHz USVs and in the tendency to approach and follow the experimenter's hand.
- Positive correlations were found between each of the four investigated parameters.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The parvafox nucleus is located ventrolaterally in the lateral hypothalamic area (LHA). Its core and shell are composed of neurons expressing the calcium-binding protein parvalbumin (PV) and the transcription factor Foxb1, respectively. Given the known functions of the LHA and that the parvafox nucleus receives afferents from the lateral orbitofrontal cortex and projects to the periaqueductal gray matter, a functional role of this entity in the expression of positive emotions has been postulated.

The purpose of the present study was to ascertain whether the deletion of neurons in the parvafox nucleus influenced the tickling-induced 50-kHz calls, which are thought to reflect positive affective states, in rats. To this end, tickling of the animals (heterospecific play) was combined with intracerebral injections of the excitotoxin kainic acid into the parvafox nucleus.

The most pronounced surgery-associated reduction in 50-kHz call-numbers was observed in the group of rats in which, on the basis of PV-immunoreactive-cell counts in the parvafox nucleus, bilateral lesions had been successfully produced. Two other parameters that were implemented to quantify positive affective behaviour, namely, an approach towards and a following of the hand of the tickling

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experimenter, were likewise most markedly suppressed in the group of rats with bilaterally successful lesions. Furthermore, positive correlations were found between each of the investigated parameters. Our data afford evidence that the parvafox nucleus plays a role in the production of 50-kHz calls in rats, and, more generally, in the expression of positive emotions.



## 1. Introduction

Evidence that has accumulated over the past decades implicates the lateral hypothalamus in multifarious physiological responses, which include the modulation of not only autonomic and endocrine, but also skeletomotor and even cognitive functions (reviewed in [1]). Anatomically, it can be sub-divided into a preoptic, tuberal and posterior part along the rostrocaudal axis (reviewed in [2]). It contains two major fibre bundles – the fornix and the medial forebrain bundle (MFB) – as well as well-circumscribed cell aggregates (nuclei). The emergence of specific markers for distinct neuronal sub-populations, such as orexin/hypocretin [3], has facilitated a characterization of the functional units within the lateral hypothalamic area (LHA) and their connectivities. We have previously shown that the calcium-binding protein parvalbumin (PV) serves as a marker for yet another neuronal sub-population, which populates the core region of the parvafox nucleus (formerly "PV1-Foxb1 nucleus"). The shell surrounding the core of the nucleus is composed of small neurons, which express the transcription factor Foxb1 [4]. The nucleus is located in the tuberal part of the ventrolateral hypothalamus, between the optic tract and the fornix [5–7]. Experiments in which anterograde tracers, particularly Cre-recombinase-dependent viral ones, have been injected into PV-Cre or Foxb1-Cre mice, have revealed the parvafox nucleus to project mainly to the periaqueductal grey matter (PAG), and, more specifically, to the ventrolateral [8] and the dorsolateral [9] columns. Furthermore, *in-situ* hybridization has disclosed PV-expressing neurons of the parvafox nucleus to be glutamatergic and thus excitatory [10], in contrast to those in most other brain areas, such as the neocortex, the hippocampus and the cerebellum [11].

Hypotheses about possible functions of the parvafox neurons can be generated based on insights gleaned from manipulations in experimental animals, from patients in which alterations in the corresponding region of the LHA are manifested, and from imaging and stimulation studies that have investigated, in animals or humans, the LHA or the PAG, the main target of the axons emanating from the parvafox nucleus.

In humans, hamartomas arising from the lateral tuberal hypothalamus can evoke the onset of gelastic seizures, a form of epilepsy that is characterized by involuntary laughter, which may or may not be accompanied by a pleasant sensation [12,13]. The lateral tuberal nucleus (LTN) of primates shares topographical as well as some neurochemical features in common with the rodent parvafox nucleus [10,14]. Neurons of the LTN manifest pathological alterations in Pick's disease, in which loss of speech is one of the symptoms [15]. Stimulation of the hypothalamic area can induce vocalization in rats [16], cats [17] and squirrel monkeys [18]. In humans, tickling-induced, involuntary (Duchenne-type) laughter is associated with the activation of several brain regions, including the lateral hypothalamus [19]. The lateral hypothalamus has been ascribed a role in the functioning of the larynx [20] and pharynx [21], as well as in the control of vocalization [22]. Preliminary experiments in which Cre-dependent tracers were injected into the parvafox nucleus have disclosed a projection to the nucleus retroambiguus [9], which contains premotor interneurons capable to produce the motor actions required for vocalization [23]. In addition to its role in the control of vocalization, the lateral hypothalamus also harbours neuronal representations of reward value [24–26].

The ventrolateral and the dorsolateral PAG are involved in the mediation of two opposing types of emotional coping strategy (passive and active, respectively) [27–29]. The PAG is also implicated in the control of vocalization. As a relay station in the limbic vocalization-control pathway, it serves a gating function in the initiation of involuntarily produced vocalisations ([18,30]; research mainly in monkeys). Although the exact mechanisms remain elusive, the PAG appears to play a role in the production of human laughter [12,31–33]. Functional magnetic resonance imaging has revealed PAG-activity to occur in conjunction with involuntarily produced laughter [19]. Electrical [34–36] or pharmacological [37,38] stimulation of the PAG induces the production of naturally sounding, species-specific vocalisations in all mammalian species that have been thus far investigated. More specifically, the caudal portion of the ventrolateral PAG has been implicated in the induction of relaxed emotional states in rats, cats and monkeys [18,27,39] .

In appetitive and neutral situations, rats emit ultrasonic vocalisations (USVs) in a high-frequency range (50-kHz USV, high-frequency calls), whereas in aversive ones, they produce USVs in a low frequency band (22-kHz USV, low-frequency calls) (reviewed in [40]). High-frequency calls are produced by juvenile or adult rats during rough-and-tumble play [41], during mating [41–44], as an expression of reward during voluntary exercise [45], as well as in response to drugs of abuse [46,47], and it is widely accepted today that they reflect a positive affective state [46,48,49]. Consequently, one of their functions appears to be the communication of emotional states to conspecifics [50]. The 50-kHz calls in rats have even been compared to laughter in humans [51,52]. Rough-and-tumble play in rats can be mimicked by tickling [51]. A short period of social isolation has been shown to boost the rate of tickling-induced 50-kHz USVs [51,53] by enhancing the social motivation of the animals. Rats emitting high numbers of tickling-induced 50-kHz USVs have been shown to approach the hand of the tickling experimenter with a short latency period [54]. In addition to conveying information appertaining to affective states, 50-kHz USVs are suggested to fulfil important communicative functions and to be involved in the establishment and maintenance of close social contacts [44,55–58]. Rats use low- frequency (22-kHz) alarm calls as a warning to conspecifics of imminent dangers, with a view to promoting a survival-enhancing behavioural strategy in the colony as a whole [59–61]. 22-kHz USVs can be elicited by pharmacological stimulation of the lateral PAG [62], but also by electrical stimulation of the dorsal (dorsomedial + dorsolateral) and even ventrolateral PAG [63].

The purpose of the present study was to ascertain whether neurons of the parvafox nucleus play a role in the expression of positive emotions in rats, as evidenced by the production of 50-kHz USVs. To this end, the tickling-induced vocalisations that were emitted by adolescent Wistar rats, as well as the tendency to seek (approach and follow) the experimenter's hand, were analysed before and