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# Anxiolytic-like effects of nociceptin/orphanin FQ in the elevated plus maze and in the conditioned defensive burying test in rats

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ABSTRACT

Different reports suggest that nociceptin/orphanin FQ (N/OFQ) may have either anxiolytic- or anxiogenic-like effect in rodents. Since N/OFQ elicits hypolocomotion, which undergoes rapid tolerance, and hypolocomotion may be associated to emotional consequences, the present study was designed to investigate the effect of N/OFQ on anxiety after development of tolerance to its hypolocomotor effect. The effect of single or double intracerebroventricular (i.c.v.) injection of N/OFQ was evaluated on anxiety-related behaviors in rats, in the elevated plus maze (EPM) and conditioned defensive burying (CDB) tests. After single administration, N/OFQ displayed an anxiogenic-like pattern of response on the elevated plus maze but hypolocomotion was also observed. Conversely, in the CDB test, N/OFQ induced a clear-cut anxiolytic pattern. To produce tolerance to N/OFQ-induced hypolocomotion the peptide was administered by two i.c.v. injections separated by 120 min; in these conditions it decreased the expression of anxiety-related behaviors in both tests without affecting locomotor activity. The nociceptin/orphanin FQ peptide (NOP) receptor antagonist UFP-101 significantly reduced the effects of N/OFQ to control values in either tests. Corticosterone levels were significantly increased after a single N/OFQ administration (not in a dose-dependent manner) but this increase did not reach significance after double administration (1 nmol/rat). Our results support the idea that N/OFQ may act as an anxiolytic-like agent in the rat; the apparent anxiogenic-like effect observed following its single administration in the EPM may be consequent to its effect on locomotion.



## 1. Introduction

First identified in 1995, the 17-amino acid peptide nociceptin/orphanin FQ (N/OFQ) is the endogeous ligand for the ''orphan'' opioid receptor ORL-1 (now NOP) [32,45]. N/OFQ is quite selective for NOP receptor, while showing no appreciable affinity for μ opioid (MOP), δ opioid (DOP) or κ opioid (KOP) receptors [34,51,53,24,31]. Nevertheless, studies on the cellular effects and the underlying transduction mechanisms pointed out important commonalities between N/OFQ and classic opioid agonists. Like opioid agonists, N/OFQ inhibits adenylate cyclase and calcium currents and hyperpolarizes neurons by opening potassium channels [20,35].

N/OFQ administration inhibits locomotor activity after intracerebroventricular (i.c.v.) injection of doses of 1–10 nmol [45,9], while it has been reported to increase locomotion at

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very low doses (0.005–0.05 nmol) [14]. N/OFQ modulates responses to nociceptive stimuli, stimulates feeding behavior, blocks morphine place conditioning and the reinforcing effects of ethanol, interfers with learning and memory functions and alters sensory processing as well [3,5,33].

N/OFQ and NOP receptors are abundantly expressed in limbic and limbic-associated brain structures [16,15,39,40] that are involved in processing of emotionally relevant stimuli, thus raising the hypothesis that N/OFQ might contribute to regulation of the hypothalamic–pituitary–adrenal (HPA) axis, and to regulation of emotional states. Accordingly, i.c.v. injection of N/OFQ or of the NOP receptor agonist Ro 64-6198 were shown to decrease behavioral responses to stress [18,21–23,50]. PreproN/OFQ knockout mice express high levels of anxiety-related behavior in neophobic tests, suggesting that an intact N/OFQ system is necessary for normal anxiety responses [23]. Furthermore, acute stress exposure decreases N/OFQ content in forebrain neurons, implicating endogenous N/OFQ neurotransmission in physiological stress responses [12]. However, N/OFQ was also reported to elicit anxiogenic-like responses, to increase plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) in unstressed rats and to augment hormonal responses in mildly stressed rats [11].

Thus, the literature on this subject is not unanimous: there are reports suggesting an anxiolytic-like effect of N/OFQ [21,18,22,52,7,17], but there are also reports suggesting an anxiogenic-like effect for the peptide [13]. N/OFQ is well known to elicit a clear-cut hypolocomotor effect in rats, which undergoes rapid tolerance [9]. Since hypolocomotion may be associated to emotional consequences, particularly in stressful conditions, the present study was designed to investigate the effect of N/OFQ both following acute and repeated administration of the peptide with the aim of minimizing its effect on locomotion.

Two different behavioral tests were used: the elevated plus maze and the conditioned defensive burying in rats, which measure fear-like responses of a composite nature, generated by exposure to stressful environmental conditions. These classic paradigms were established for their sensitivity to conventional anxiolytic tranquilizers and anxiogenic compounds of various structural classes and mechanisms of action. In particular, the elevated plus maze model is based on rats' innate aversion to open and high places and belongs to the group of unconditioned anxiety paradigms used for the development of putative anxiolytic compounds [25,28]. On the other hand, defensive burying is an innate response exhibited by rats towards aversive stimuli. The defensive burying behavior test has been extensively used to study the neuronal mechanisms underlying anxiety regulation, and was chosen for the present investigation on the basis of its sensitivity to manifest both physiological and pharmacological changes in anxiety [49]. The tests had been previously validated, in our experimental conditions, with prototypical anxiolytic compounds (i.e. diazepam) and had been controlled for effects on sensorimotor function (data not shown). Serum corticosterone (CORT) levels were also evaluated in rats during exposure to the anxiety test after either a single or a double N/OFQ treatment. Moreover, the NOP receptor antagonist UFP-101 was tested in the double N/OFQ administration experiments where a rapid development of tolerance to hypolocomotion can occur. Indeed, data obtained in "in vitro" and in "in vivo" tests have demonstrated that UFP-101 behaves as a potent, competitive and selective antagonist at NOP receptors (for a review, see Calò et al. [4]).

The present study sought to shed new light on the contrasting evidences regarding the N/OFQ effects on anxiety-related behaviors; for this purpose we operated in conditions devoid of locomotor impairment, we utilized differential anxiety tests and also examined how the blockade of N/OFQ-NOP signaling can affect this complex emotional behavior.

## 2. Materials and methods

### 2.1. Animals

Two hundred male Wistar rats weighing 180–200 g, at the beginning of the experiments, were housed in Plexiglas® cages in groups of three to four in controlled conditions (free access to food and water; 12-h light:12-h dark cycle; temperature, 22 ± 1 °C; humidity, 60%). All testing sessions were performed between 09:00 and 14:00 h. Ethical guidelines for investigation of experimental pain in conscious animals were followed, and procedures were carried out according to EEC ethical regulations for animal research (EEC Council 86/609; D.Lgs. 27/01/1992, no. 116).

### 2.2. Surgery

The rats were randomly divided into groups of 10 animals each. For i.c.v. injections, stainless-steel guide cannulae (23 ga) (Plastic One, Roanoke, VA, USA) were stereotaxically implanted in the right lateral ventricle, to a depth of 0.5 mm above the ventricle (in mm from the bregma: AP = −0.8; *L* = 1.4; *V* = 3.25) [41], under ketamine plus xylazine anesthesia (115 + 2 mg/kg i.p.; Farmaceutici Gellini, Aprilia, Italy and Bayer, Milan, Italy, respectively) and fixed in place with acrylic dental cement and one skull screw. A removable plug was kept in place except during the drug injections. All i.c.v. injections were in a volume of 5 μl. After the end of the experiment, rats were i.c.v. injected with 5 μl of dye (Evans Blue) and sacrificed under anesthesia. The correct placement of the cannula was ascertained by inspection of dye diffusion in the right lateral ventricle.

### 2.3. Experimental procedure

In the first set of experiments N/OFQ, 0.3, 0.5, 0.75, 1 and 1.5 nmol/rat, or saline was i.c.v. injected in a single administration by means of an injector (1 mm longer than the guide cannula) and the animals ($n = 10$ per group) were subjected either to the elevated plus maze test or to the conditioned defensive burying test 5 min later (amount of rats in experiment 1 = 120). The rats belonging to the groups submitted to the plus maze test were decapitated 20 min after the end of the test, their blood collected from the trunk, centrifuged at 1000 × *g* for 10 min and serum was removed and stored at −80 °C until analysis for corticosterone content