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NOCICEPTIN/ORPHANIN FQ RECEPTOR AGONISTS INCREASE AGGRESSIVENESS IN THE MOUSE RESIDENT-INTRUDER TEST

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Highlights

- NOP agonists increased male mouse aggressiveness.
- The NOP antagonist SB-612111 did not change mouse aggressiveness.
- NOP(-/-) mice did not display any behavior phenotype in the resident-intruder test.
- Lithium, valproate and carbamazepine attenuated mouse aggressiveness.
- An inhibitor of 5-HT synthesis, PCPA, increased mouse aggressive behavior.

Abstract

Aggressive behaviors can be considered symptoms of bipolar disorder, schizophrenia, post-traumatic stress, intermittent explosive, and personality disorders. Nociceptin/orphanin FQ (N/OFQ) is a peptide acting as endogenous ligand of the NOP receptor. Preclinical and clinical findings suggest the NOP receptor as an innovative target for the treatment of psychopathologies, such as anxiety, depression, and drug abuse. This study investigated the effects of NOP ligands and the behavioral phenotype of mice lacking the NOP receptor in an animal model of aggressiveness, the resident-intruder test. Mood stabilizers, such as valproate, lithium, and carbamazepine reduced aggressive behaviors of resident mice, while diazepam was inactive. In contrast, para-chlorophenylalanine (PCPA), an inhibitor of 5-HT synthesis, increased aggressiveness in mice. Similar to PCPA, the treatment with the NOP agonists Ro 65-6570 and AT-090 also increased aggressive behaviors. The systemic administration of the NOP antagonist SB-612111 did not modify the behavior of resident mice, but it prevented the aggressive behavior of Ro 65-6570. NOP receptor knockout mice did not display any behavioral difference compared to wild-type animals in the resident-intruder test. None of the treatments affected non-agonistic behaviors and spontaneous locomotion. In conclusion, NOP receptor agonists increased aggressiveness, while the pharmacological and genetic blockade of NOP receptor signaling did not modify agonistic behaviors. Ultimately, the aggressive profile of action of NOP agonists should be taken into account in the development of innovative psychiatric drugs targeting the NOP receptor.

Keywords: aggressiveness; mouse; resident-intruder test; NOP receptor; NOP ligands; NOP knockout mice.

1. Introduction

Aggressiveness can be defined as a behavior against another individual with the intention to cause harm. In animals, aggressiveness is an adaptive behavior that is used to establish dominance hierarchies, compete for resources, protect offspring and as a form of defense [1]. In humans, aggressiveness is a complex phenomenon associated with genetic, neurobiological, and psychosocial factors. Patients with mental disorders present an elevated rate of aggressive behaviors [2]. In fact, increased aggressiveness is a common symptom observed in patients diagnosed with psychopathologies, such as bipolar disorder, post-traumatic stress disorder, intermittent explosive disorders, personality disorders and schizophrenia [3]. Additionally, psychotic patients are 5 times more likely than the general population to commit physical attacks on other persons, or on one's self (self-mutilation), with deliberate destructive intent sufficiently severe to justify legal measures [4].

Aggressive behaviors are modulated by classical neurotransmitters, i.e., 5-HT and dopamine, but also by neuropeptides such as oxytocin, vasopressin [2] and neuropeptide S [5,6]. The treatment with mood stabilizers such as anticonvulsants (e.g., valproate, lamotrigine, or gabapentin) and lithium may reduce aggressiveness [7], and display advantages compared to classical antipsychotic drugs due to induce smaller side effects. However, the best way to control the aggressiveness may be the result of the treatment of the major psychopathology affecting the patient. Thus, the development of new strategies to pharmacologically treat psychopathologies should include the evaluation of drug effects on aggressive behaviors.

Nociceptin/orphanin FQ (N/OFQ) is a heptadecapeptide acting as the endogenous ligand for the NOP receptor, a member of the opioid G-protein coupled receptor family [8,9]. The NOP receptor is structurally and functionally related to opioid receptors, and the activation of NOP signaling consequently leads to the reduction of Ca^{+2} influx and cAMP production and to the increase of K^{+} efflux [10]. Indeed, N/OFQ has structural similarities with the opioid peptide dynorphin A [11]; however neither N/OFQ has affinity for classic opioid receptors [8] nor the endogenous opioids have affinity for the NOP receptor [12]. In the central nervous system, both N/OFQ and NOP receptor are expressed in limbic structures and brainstem monoaminergic nuclei [13,14,15]. This evidence supports a regulatory role for the N/OFQ – NOP receptor system in

modulating psychiatric disorders, such as depression, anxiety, food consumption-related disorders and drug abuse [10,16,17].

A growing body of evidence suggests a role for NOP agonists as anxiolytic, analgesic, antihypertensive, antitussive drugs, and as innovative treatment for urinary incontinence due to overactive bladder [10]. By contrast, NOP antagonists have been positively implicated in the treatment of major depression and alcohol abuse [17]. The involvement of the N/OFQ-NOP receptor system in the modulation of emotional states, and the therapeutic potential of NOP ligands as innovative treatments for psychopathologies establish the rationale for the investigation of the relationship between the N/OFQ-NOP receptor system and aggressiveness. In this pivotal study, we report the effects of NOP ligands in the resident-intruder test in mice. In addition, the behavioral phenotype of mice lacking the NOP receptor (NOP-/-) was also assessed using the same test. Agonistic and non-agonistic behaviors were registered in treated mice in order to exclude potential biases to the measurement of mouse aggressiveness.

2. Material and Methods

2.1 Animals

Experiments were performed using male Swiss mice (12-14 weeks, weighting 45 g) as residents, and male Swiss mice (7-8 weeks old, weighting 28 g) employed as intruders and as subjects to test the effects of drugs on spontaneous locomotion. Swiss mice were bred at the Federal University of Rio Grande do Norte (Natal, Brazil) while NOP(+/+) and NOP(-/-) mice at the Section of Pharmacology of the Department of Medical Sciences of the University of Ferrara (Ferrara, Italy). Details about the generation of mutant mice have been published previously [18,19]; moreover NOP(+/+) and NOP(-/-) mice have been backcrossed on the CD-1 strain. Mice were housed in groups of maximum 10 in plastic cages (33×40×17 cm) under standard conditions (22 °C; 12-h light:12-h dark cycle, lights on at 6:00 am) with food and water *ad libitum* for at least 7 days before experiments began. A total number of 415 mice were used for this study (227 mice for the resident-intruder test and 188 mice for the open field test). The experiments were performed during the morning between 7:00 and 12:00. All the experimental procedures were conducted in accordance with the Brazilian law no. 11.794/2008 for care and use of experimental animals and were approved by the Local

Ethics Committee for Animal Use of the Federal University of Rio Grande do Norte (License #041/2014). The studies performed at the University of Ferrara (Italy) comply with the European Communities Council directives (2010/63/E) and Italian regulations (D.Lgs, 26/2014). The experiments were approved by the Animal Welfare Body of the University of Ferrara and by the Italian Ministry of Health (License #120/2014). This study is reported following the ARRIVE guidelines [20].

2.2 Drugs and treatments

NOP peptide ligands and Ro 65-6570 were synthesized and purified in the Department of Chemistry and Pharmaceutical Sciences, University of Ferrara (Italy) while AT-090 (1-(1-(cis-4-isopropylcyclohexyl) piperidin-4-yl)indoline-2,3-dione) was from Astraea Therapeutics (Mountain View, USA). SB-612111 was purchased from Tocris Bioscience (Bristol, UK). Valproate, carbamazepine (both from Sanofi-Aventis Farmacêutica, Sao Paulo, Brazil), lithium, *p*-chlorophenylalanine (PCPA; both from Sigma-Aldrich, San Louis, USA), and diazepam (Cristália Produtos Químicos Farmacêuticos, São Paulo, Brazil) were employed in this study. Valproate, carbamazepine, lithium were dissolved in saline while diazepam in 0.5% Tween 80. PCPA was solubilized in 0.8% carboxymethylcellulose and 1% Tween 80. Ro 65-6570 and SB-612111 were solubilized in 1% DMSO, and AT-090 was dissolved in 1% DMSO and 0.5% (2-hydroxypropyl)-b-cyclodextrin (Sigma-Aldrich, San Louis, USA).
The doses, route of administration, and pretreatment time for Ro 65-6570, AT-090 and SB-612111 were selected based on previous studies which demonstrated anxiolytic effects for the NOP agonists [21] and antidepressant effects for the NOP antagonist [22, 23, 24]. Regarding the mood stabilizers valproate, lithium and carbamazepine, and the anxiolytic diazepam, the doses used were selected based on literature information [25,26,27]. All drugs, except PCPA, were injected intraperitoneally (i.p.) 30 min prior the test session. PCPA was injected ip once a day for 3 consecutive days, with the last injection made 24 h before the test [28]. In the co-administration experiments, SB-612111 (10 mg/kg, ip) was injected 5 min prior the administration of Ro 65-6570 (1 mg/kg, ip). The vehicle group was treated with 1% DMSO and 0.5% 2-hydroxypropyl-b-cyclodextrin which were used to solve NOP ligands. All drugs injected ip were given in a volume of 10 ml/kg.

2.3 Resident-intruder test

This assay was based on our previous experience as reported before [5]. The resident mouse was housed individually for 7 days before the experiment in a 30 x 20 x 13 cm plastic cage. Since territoriality is strongly based on the presence of olfactory cues, during the isolation period of resident mice the bedding had never been cleaned. The behavioral evaluation begins when the intruder is placed in the resident cage (basal session) and it lasts for 10 min. Twenty-four hours later the intruder is placed again in the resident cage for 10 min (test session). During each encounter, the resident was exposed to a different intruder. Agonistic (number of attacks and time the resident mouse spends attacking the intruder) and non-agonistic (frequency of rearing – non social exploration – and self grooming) behaviors are manually scored by a researcher blind to drug treatments. The following behaviors are scored as attacks: bites, lateral threat, upright posture by the resident, clinch, keep down, tail rattles and chase. Mice that did not fight during the first session (basal) were excluded from the study (~15%). Before the test session, mice were randomly assigned to the different experimental groups.

2.4 Open field test

In separate experiments, the spontaneous locomotor activity of male Swiss mice was measured using the open field test. The apparatus, made of wood covered with impermeable formica, had a black floor of 40 cm × 40 cm and black walls of 40 cm high. The test room had a controlled illumination (dimly-light condition). Each mouse was placed in the center of the open field and the distance moved (in meters) for 30 min was automatically registered (Anymaze, Stoelting Co.,Wood Dale, IL, USA). After each test, the arena was cleaned with 5% ethanol solution.

2.5 Data analysis

Data are expressed as mean ± SEM of n animals. Data were analyzed using paired or unpaired Student's t test or ANOVA followed by the Dunnett´s test, as specified in figure legends. Statistical data were described as t and p values and degree of freedom. Differences were considered statistically significant when $p<0.05$. For statistical analysis, the GraphPad Prism software version 5.0 (Graph Pad Software Inc., San Diego, USA) was used.

3. Results

3.1 Effects of anti- and pro-aggressive standard drugs on mouse aggressiveness

A first series of experiments was performed to set up and validate the experimental conditions for the resident-intruder test. During the first day of behavioral measurements (basal), untreated resident Swiss mice spent 71 ± 11 s attacking the intruder mouse and the number of attacks was 11 ± 1. When, after 24 h, the same resident mice received an ip injection of saline, no significant changes in agonistic behaviors were recorded (time spent attacking: 67 ± 10 s; number of attacks 11 ± 1).

The effects of the mood stabilizer drugs lithium, valproate and carbamazepine, clinically employed to control aggressive behavior, were tested in the resident-intruder paradigm. Lithium (50 mg/kg) and valproate (300 mg/kg), both injected ip 30 min before the test session, were able to significantly reduce the aggressiveness of resident mice, both in terms of total time spent attacking ($t(9)=2.40$ and $t(15)=5.08$, respectively, $p<0.05$ for both; Fig. 1A) and number of attacks ($t(9)=2.88$ and $t(15)=6.06$, respectively, $p<0.05$ for both; Fig. 1B). Carbamazepine (20 mg/kg, ip) significantly reduced the number of attacks prompted by the resident mouse against the intruder ($t(15)=2.66$, $p<0.05$), while the time spent attacking was not significantly changed (Fig. 1A).

In order to measure the effects of 5-HT depletion on aggressive behavior, mice were treated with PCPA. The administration of PCPA significantly increased the time the resident mouse spent attacking the intruder ($t(8)=2.82$, $p<0.05$, Fig. 1A), without affecting the number of attacks ($p>0.05$, Fig. 1B). The behavioral actions of the anxiolytic drug diazepam were also assessed in this experimental series. At the dose tested, diazepam did not significantly affect the agonistic behaviors of resident mice (Fig. 1).

None of the standard drugs consistently affected non-agonistic behaviors (Table 1) as well as spontaneous locomotion in the open field test (Table 2).

3.2 Effects of NOP ligands on mouse aggressiveness

The systemic injection of vehicle 30 min before testing did not significantly affect the time the resident mouse spent attacking and the number of attacks performed against the intruder during the test session (time spent attacking: basal 94±27 s, test 94±19 s; n of attacks: basal 15±3, test 15±2; n=6, respectively). In this series of experiments, the NOP agonist Ro 65-6570 increased aggressiveness in resident mice. As showed in Fig. 2, the higher dose of Ro 65-6570 (1 mg/kg) significantly increases the time spent attacking the

intruder (t(12)=2.62, p=0.020) and the number of attacks (t(12)= 2.62; p=0.020) of resident mice. A trend to increase aggressiveness was also seen with Ro 65-6570 at lower doses (time spent attacking: Ro 0.1 mg/kg – t(12)=1.99, p=0.070; n of attacks: Ro 0.03 mg/kg – t(10)=2.38, p=0.030). The NOP partial agonist AT-090 at the higher dose tested slightly increased the time residents spent attacking the intruder mice (t(10)=2.85, p=0.020; Fig. 2C,D). On the contrary, the administration of the NOP antagonist SB-612111 in the dose range 1 - 10 mg/kg did not change agonistic behavior of resident mice (Fig. 2E,F).

Importantly, the administration of the NOP ligands Ro 65-6570, AT-090 and SB-612111 did not consistently change non-agonistic behaviors measured in the resident-intruder paradigm and the spontaneous locomotion in the open field test (Table 1 and 2).

3.3 Behavioral phenotype of NOP receptor knockout mice in the resident-intruder test

As showed in Fig. 3, in the resident-intruder test no differences were found between the behavior of resident NOP(+/+) and NOP(-/-) mice neither in terms of number of attacks nor in the time spent attacking the intruder mice.

3.4 *Effects of the co-administration of a NOP agonist and an antagonist on mouse aggressiveness*

In a distinct set of experiments, the administration of the NOP agonist Ro 65-6570, at the higher dose tested – 1 mg/kg, significantly increased the time residents spent attacking (Fig. 4A; t(7)=3.30, p=0.013) the intruder mice and the number of attacks (Fig. 4B; t(7)=3.57, p=0.009). The NOP antagonist SB-612111 (10 mg/kg) did not affect the agonistic behavior of resident mice (Fig. 4, p>0.05). However, the co-administration of SB-612111 blocked the increase of aggressive behavior induced by Ro 65-6570.

Finally, the administration of Ro 65-6570, SB-612111 and the association of Ro 65-6570 and SB-612111 did not significantly change the frequency of rearing and self-grooming in resident mice (Table 1).

4. Discussion

The main finding of this study is that the systemic administration of the NOP agonists Ro 65-6570 and AT-090 affected social behavior of male mice by increasing aggressiveness in the resident-intruder test. Ro 65-6570 was more effective in inducing aggressiveness compared to AT-090. In contrast, the pharmacological and genetic blockade of the NOP receptor did not change mouse agonistic behaviors. This is the first evidence of the role played by the N/OFQ-NOP receptor system in the modulation of aggressiveness. Considering that NOP agonists are promising drugs for the treatment of different diseases including psychiatric disorders [10,17], the pro-aggressive profile of these compounds should be observed in clinical trials performed with such innovative drugs.

Under the present experimental conditions, increase of agonistic behavior was observed in mice pretreated with PCPA, a tryptophan hydroxylase inhibitor. As previously reported, PCPA, after 3 consecutive days of administration at 300 mg/kg, caused a substantial reduction (approx. 70 %) of 5-HT levels in the mouse hippocampus, cerebral cortex and midbrain areas [29]. Similar to our findings, increased aggressive behavior was reported in male mice treated with PCPA [28,30,31]. Thus present data reinforce the view that 5-HT is a core neurotransmitter controlling aggressiveness [2].

In contrast to PCPA, all mood stabilizers tested in the present study, i.e., valproate, lithium and carbamazepine, significantly reduced aggressiveness in mice. Previous studies have reported the effects of mood stabilizers in the resident-intruder test. For instance, the anti-aggressive effects of valproate [5,32] and lithium [32] have been reported for mice subjected to the resident-intruder test. The inhibitory effects of carbamazepine on aggressive behavior induced by clonidine, sleep-deprivation and electric footshocks have been previously demonstrated in mice and rats after acute and chronic administration [33,34,35]. In the present experiments the classical anxiolytic drug diazepam did not statistically change aggressive behavior. Some studies reported reduced aggressiveness [36,37,38,39,40], while others observed no effects for benzodiazepines in the mouse resident-intruder test [41,40]. These discrepant results could be due to differences in route of administration, duration of treatment, particularly doses. In fact, White et al. [41] reported antiaggressive effects in response to diazepam only at doses which also produced motor impairment. Another explanation for the inefficacy of diazepam in counteracting the aggressive behavior of resident mice could be due to the lower aggressiveness basal values presented by diazepam-treated mice. Comparing basal values of our resident mice presented in Fig. 1A, considerable

variation was observed, what could mask the anti-aggressive effects of diazepam. Taken together, the results obtained with control drugs (pro- and anti-aggressive compounds) were instrumental for the pharmacological validation of the resident-intruder test, demonstrating that under the present experimental conditions both pro- and anti-aggressive effects of drugs can be detected. Additionally, our findings support a genuine reduction of aggressiveness for lithium, valproate and carbamazepine, since these treatments exclusively reduced agonistic behavior without changing consistently spontaneous locomotion and non-agonistic parameters.

In the present study, Ro 65-6570 consistently increased agonistic behavior at different doses, while AT-090 slightly increased aggression at the higher dose tested. In contrast, the blockade of the endogenous NOP receptor signaling did not affect aggressive and non aggressive behaviors in mice, when assessed in animals lacking the NOP receptor and after administration of a selective NOP antagonist. Importantly, the co-administration of the NOP antagonist SB-612111 prevented the increase of agonistic effects of the NOP agonist Ro 65-6570. As suggested by *in vitro* studies performed in native and recombinant preparations, the NOP agonist Ro 65-6570 displayed significantly higher efficacy compared to AT-090, which behaved as a partial agonist in the [^{35}S]GTPγS and BRET assays [42]. As far as ligand selectivity is concerned, it is worthy of mention that in elevated plus maze experiments the actions of both Ro 65-6570 and AT-090 were no longer evident in NOP(-/-) mice. Moreover, the effects of Ro 65-6570 were fully prevented by the NOP selective antagonist SB-612111 [21]. Therefore, present findings suggest that the increase of aggressive behavior observed in response to Ro 65-6570 and AT-090 is most likely due to their ability to selectively activate the NOP receptor.

As showed in the present study, the size of the pro-aggressive effects of NOP agonists is quite discrete in the resident-intruder test; actually, the same holds true for the 5-HT inhibitor PCPA. It should be mentioned that paradigm is based on the fact that an adult male mouse will establish a territory when given sufficient living space. As a consequence, the resident will attack unfamiliar co-specific males intruding in its home cage [43]. Considering the causal mechanisms of aggressiveness observed in mice subjected to the resident-intruder test, we conclude that it is difficult to register a further increase of agonistic behaviors in residents, in the present case induced by drugs, in which mouse aggression is already elevated. Thus, further studies aimed at investigating

the pro-aggressive behavior of NOP agonists under distinct animal models of aggression can give additional clues about the role played by this peptidergic system.

Little literature information supports a relationship between N/OFQ-NOP receptor system and aggressiveness. The peptide N/OFQ and its receptor NOP are expressed in brain areas important for the control of aggressive behavior, such as the amygdala, the prefrontal cortex, the lateral and anterior hypothalamus [13,14,15]. Additionally, male mice knockout for the N/OFQ prepropeptide gene developed greater home-cage aggression when housed together compared to wild type animals. However, when tested in the resident-intruder test (in an isolation condition) latency to the first attack was similar in mutants and wild type animals [44]. It should be mentioned that the N/OFQ prepropeptide encodes two other bioactive neuropeptides, nocistatin, and nociceptin II [11]. Thus, the phenotypic changes observed in the N/OFQ precursor knockout mice following social housing stress could not be exclusively attributed to the lack of N/OFQ [44]. Additional studies focused on the effects of NOP ligands on stress-induced aggressiveness are needed in order to better understand the potential therapeutic and/or the side effects of these compounds.

As far as the mechanisms by which NOP agonists facilitate agonistic interactions are concerned, extensive findings support a role for the serotonergic, catecholaminergic and peptidergic systems, mainly oxytocin and vasopressin, in the modulation of aggressiveness [2]. Importantly, the reduction of 5-HT and oxytocin receptor signaling is able to increase agonistic behaviors [45,46]. When activated, NOP receptor promotes inhibitory effects mediated by a Gi-protein thus leading to reduction of neurotransmitters release [47] and neuronal firing rates [48,49,50]. NOP receptors are expressed in 5-HT neurons of the dorsal raphe nucleus [51]. Indeed, *in vitro* and *in vivo* studies demonstrated inhibitory effects of N/OFQ on 5-HT release [52,53,54,55,56] and on neuronal activity of the dorsal raphe nucleus [57,58,59]. Concerning the relationship between oxytocin and N/OFQ, previous electrophysiological studies showed that N/OFQ inhibits oxytocin neurons from the rat supraoptic nucleus [60,61]. Taken together, literature findings suggest an inhibitory role for the N/OFQ-NOP receptor system in the 5-HT and oxytocin release and neuronal firing rates. Thus, the activation of NOP receptor signaling could increase aggressiveness by reducing 5-HT and oxytocin neurotransmission at cortical and limbic areas.

Conclusions

In conclusion this study suggests that NOP agonists increase aggressiveness in male mice assessed in the resident-intruder test. By contrast, the pharmacological and genetic blockade of the NOP receptor signaling did not affect the social behavior in terms of aggressive and non-aggressive interactions between resident and intruder male mice. This research proposes the NOP receptor signaling as mediator of social behaviors and in particular of intra-male aggressive states. Considering the relevance of increased aggressiveness, the effects of NOP agonists on these behaviors should be observed during the clinical development of these compounds, particularly for psychiatric indications.

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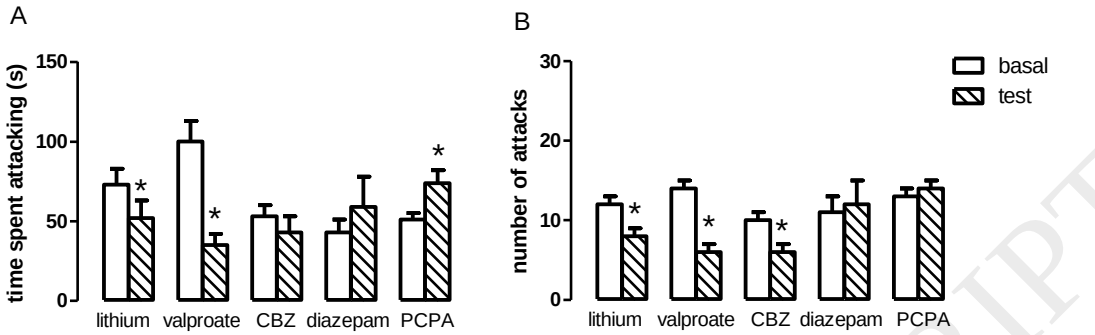

Figure 1 - Effects of the drug stabilizers lithium (50 mg/kg, ip), valproate (300 mg/kg, ip), carbamazepine (CBZ; 20 mg/kg, ip), diazepam (1 mg/kg, ip) and *P*-chlorophenylalanine (PCPA; 300 mg/kg, ip), injected before the test session, on the mouse performance in the resident-intruder test. The total time spent by the resident attacking the intruder (A) and the number of attacks (B) were registered during 10-min basal and test sessions. *$p < 0.05$ vs basal, according to the Student's t test for paired values. Data are the mean ± SEM of 9 (PCPA), 10 (lithium), 12 (diazepam) and 16 (saline, valproate and CBZ) mice.

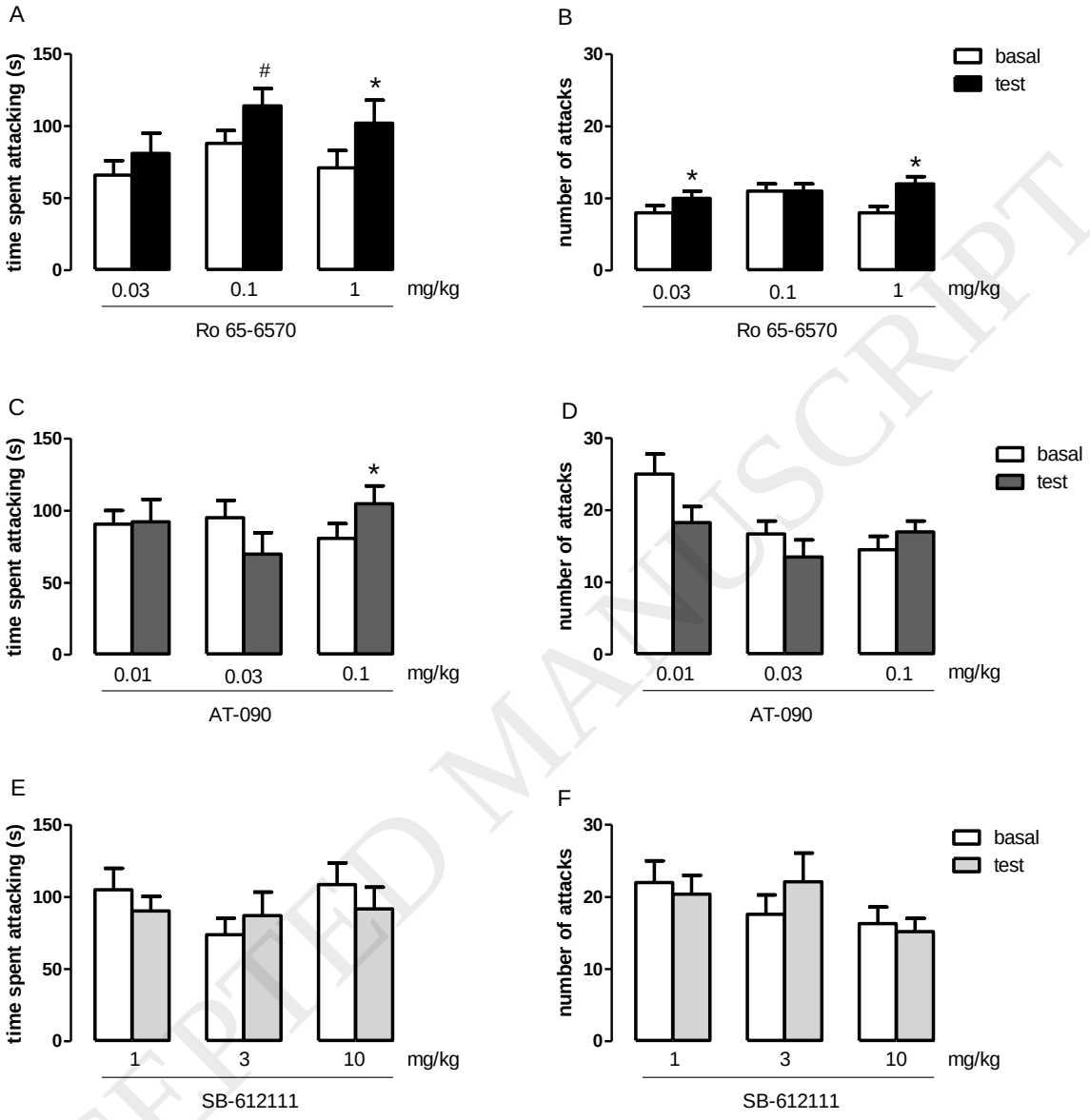

Figure 2 - Effects of NOP agonists Ro 65-6570 (0.03, 0.1 and 1 mg/kg, ip), AT-090 (0.01, 0.03 and 0.1 mg/kg, ip), and SB-612111 (1, 3 and 10 mg/kg, ip), injected 30 min before the test session, on the mouse performance in the resident-intruder test. The total time spent by the resident attacking the intruder (A,C,E) and the number of attacks (B,D,F) were registered during 10-min basal and test sessions. *$p<0.05$ vs basal, #$p=0.07$ vs. basal, according to the Student's t test for paired values. Data are the mean ± SEM of 9 (AT 0.03 and SB 3), 10 (SB 1 and SB 10), 11 (Ro 0.03, AT 0.1 and AT 0.01), and 13 (Ro 0.1 and Ro 1) mice.

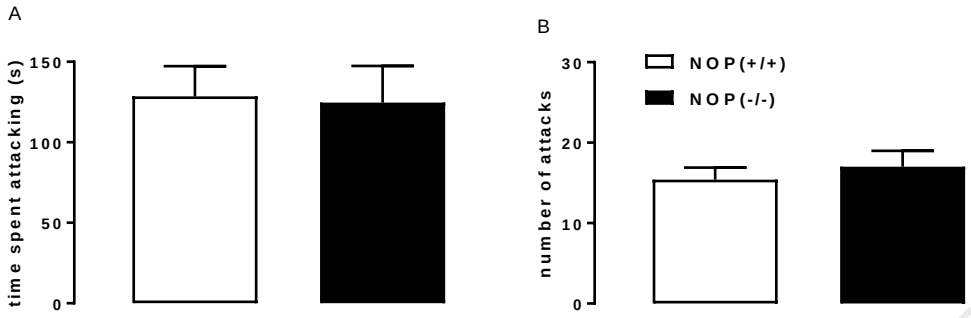

Figure 3 – Phenotype of NOP receptor knockout mice in the resident-intruder test. The total time spent by the resident attacking the intruder (A) and the number of attacks (B) were registered during 10-min sessions. Data are the mean ± SEM of 10 (NOP(+/+)) and 12 (NOP(-/-)) mice.

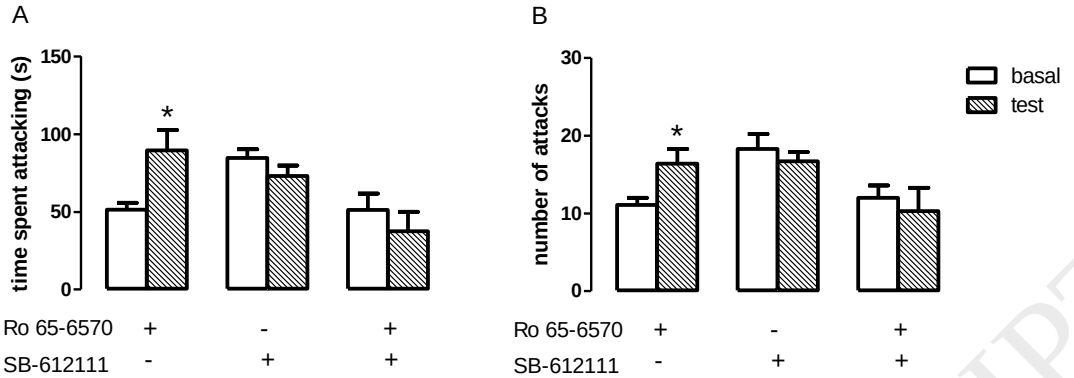

Figure 4 - Effects of the co-administration of the NOP agonist Ro 65-6570 (1 mg/kg, ip, 35 min prior the test) and the NOP antagonist SB-612111 (10 mg/kg, ip, 30 min prior the test) on the mouse performance in the resident-intruder test. The total time spent by the resident attacking the intruder (A) and the number of attacks (B) were registered during 10-min basal and test sessions. *$p<0.05$ vs basal according to the Student's t test for paired values. Data are the mean ± SEM of 8 (Ro and Ro+SB) and 7 (SB) mice.

Table 1 – Effects of drug treatments on non-agonistic behaviors of resident mice registered during a 10-min session in the resident-intruder test.

	Rearing		**Self-grooming**	
Treatments	*Basal*	*Test*	*Basal*	*Test*
Naive	7.1±1.1	8.4±1.6	3.1±0.5	3.0±0.4
Saline	6.3±0.6	4.4±0.4*	5.9±0.5	4.5±0.4
Valproate	7.3±1.0	5.9±1.3	7.1±0.8	1.7±0.5*
Lithium	6.3±0.9	6.6±0.9	4.8±0.8	9.0±3.9
Carbamazepine	9.2±0.9	9.6±2.1	6.5±0.7	7.0±0.7
Diazepam	6.4±1.1	8.2±2.0	4.8±0.7	4,0±0.7
PCPA	3.9±0.4	4.2±0.4	3.4±0.5	3.1±0.4
Vehicle	10.0±2.8	6.3±1.7	4.7±0.8	3.7±1.0
Ro 65-6570 0.03 mg/kg	5.8±0.9	7.3±1.0	4.3±0.5	5.5±1.3
Ro 65-6570 0.1 mg/kg	8.4±1.2	9.2±1.3	5.4±0.7	4.9±0.5
Ro 65-6570 1 mg/kg	8.5±1.3	8.9±1.5	4.5±0.5	4.2±0.7
AT-090 0.01 mg/kg	4.9±0.5	7.8±1.2*	3.8±0.4	4.6±0.5
AT -090 0.03 mg/kg	7.0±1.0	8.9±1.6	4.6±0.9	5.2±0.7
AT-090 0.1 mg/kg	7.0±0.9	8.6±0.9	4.5±0.7	3.6±0.5
SB-612111 1 mg/kg	7.3±0.9	9.5±0.8	4.7±0.5	6.0±1.2
SB-612111 3 mg/kg	9.2±1.0	10.7±1.1	5.4±0.3	6.1±0.6
SB-612111 10 mg/kg	5.8±0.7	7.3±0.7	4.0±0.5	5.0±0.6
Ro 65-6570 1 mg/kg	9.0±0.9	11.1±1.5	6.1±0.6	6.8±0.8
SB-612111 10 mg/kg	8.4±1.0	8.9±0.8	5.1±0.6	7.0±0.4*
Ro 65-6570 + SB-612111	6.1±0.7	6.5±0.7	6.4±0.7	6.1±0.8

Data are the mean ± SEM. *p<0.05 vs. basal levels, according to the Student's t test for paired values.

Table 2 – Effects of drug treatments on the distance moved during 30 min of observation in the open field test.

	Distance moved (m)	
	Vehicle	*Treatment*
Valproate	79.1±5.5 (10)	85.1±12.1 (9)
Lithium	52.6±5.3 (12)	52.8±4.6 (13)
Carbamazepine	52.7±4.7 (8)	56.5±6.4 (8)
Diazepam	60.6±3.3 (8)	60.9±16.7 (7)
Ro 65-6570 0.03 mg/kg	51.5±2.2 (12)	57.6±4.8 (9)
Ro 65-6570 0.1 mg/kg	-	56.8±7.9 (9)
Ro 65-6570 1 mg/kg	-	58.1±8.7 (11)
AT-090 0.01 mg/kg	48.0±4.3 (12)	36.5±4.7 (8)
AT -090 0.03 mg/kg	-	51.1±7.6 (9)
AT-090 0.1 mg/kg	-	56.2±5.4 (10)
SB-612111 1 mg/kg	47.5±4.5 (10)	45.0±6.4 (8)
SB-612111 3 mg/kg	-	51.0±4.1 (9)
SB-612111 10 mg/kg	-	40.9±3.2 (10)

Data are the mean ± SEM of (n) mice. *$p<0.05$ vs. vehicle, according to the Student's t test for unpaired values or ANOVA followed by Dunnett's test.