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Acute single non-sedative doses of NOP receptor agonists affect acquisition of object location memory but repeated high doses do not induce long-lasting deficits

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Abstract

The Nociceptin/Orphanin FQ (N/OFQ) system has been shown to modulate various aspects of long-term memory. It is therefore important to study the effects on memory impairment by nociceptin receptor (NOP) agonists under preclinical development. In the present study, we investigated the effect of systemic injection of two small molecule selective NOP agonists, AT-202 and AT-524, in the object location memory task in male and female mice. Since high doses of NOP agonists have been shown to induce sedation, we first determined the sedative doses for the two compounds and found them to be higher in female than in male mice. We then observed that sub-sedative doses of NOP agonists administered before learning, induced memory impairment during a test session performed 24 hours later. Again, female mice were less sensitive to the amnesic effects than males. On the contrary, in male mice, NOP agonists did not produce amnesia when they were injected after learning, suggesting that they do not affect the consolidation of object location memory. Finally, repeated administration of high doses of NOP agonists over 7 days did not impair long-term spatial memory. Together, our data show for the first time that NOP receptor agonists impair the acquisition of object location memory with sex-dependent potency but do not affect memory consolidation, and that repeated stimulation of the receptor does not compromise long-term episodic-like spatial memory.

Keywords: Nociceptin/Orphanin FQ (N/OFQ), NOP receptor, object location memory, acquisition

1. Introduction:

The fourth member of the endogenous neuropeptide opioid family is the 17-amino acid peptide called nociceptin or orphanin FQ, discovered in 1995 (Meunier et al., 1995; Reinscheid et al., 1995). Henceforth referred to as N/OFQ, this neuropeptide acts through a Gi/o coupled receptor called nociceptin opioid peptide receptor (NOP), the fourth opioid receptor subtype, but has no measurable affinity for classical opioid receptors mu, delta and kappa (MOP, DOP, KOP) (Meunier et al., 2000). Since its discovery, the NOP-N/OFQ system has been shown to regulate many physiological functions including pain, inflammation, reward, food intake, stress, and memory (Lambert, 2008), which made the NOP receptor a promising pharmacological target. Preclinical studies have suggested in particular that NOP agonists may be beneficial for the treatment of pain, substance abuse, and anxiety (Toll et al., 2021; Zaveri, 2016). The neuropharmacology of the NOP-N/OFQ system provides compelling rationale that NOP receptor agonism is a viable approach to attenuate addiction-related behaviors and for medication development. Several small-molecule NOP agonists have been investigated in preclinical rodent models of drug reinforcement and drug reward such as self-administration, conditioned place preference (CPP), and reinstatement to drug seeking (Witkin et al., 2014; Zaveri, 2011). We recently showed that the NOP-selective agonist AT-312, from our laboratory, decreased cocaine CPP in mice (Zaveri et al., 2018), decreased cocaine self-administration in rats and reduced conditioned reinstatement of cocaine seeking without modifying food self-administration (Cippitelli et al., 2020). However, while NOP agonists are devoid of the usual opioid-induced side effects such as respiratory depression and abuse liability, the effects of chronic repeated administration of NOP agonists, as would be required for clinical use, has not been studied extensively. One concern is the putative amnesic effect of NOP agonists since N/OFQ has been shown to negatively affect learning and memory in rodents (Mouledous, 2019).

Intracerebroventricular (icv) administration of high doses of N/OFQ in mice or rats compromises learning and memory in the Morris water maze (MWM) (Kuzmin et al., 2009), the fear conditioning (FC) and inhibitory avoidance (IA) paradigms (Fornari et al., 2008; Hiramatsu and Inoue, 1999; Mamiya et al., 2003), and the object recognition (OR) task (Goeldner et al., 2008; Han et al., 2019). Similarly, systemic administration of the NOP agonist Ro 64-6198 (Wichmann et al., 2000) impairs learning and/or memory in the MWM (Higgins et al., 2002; Kuzmin et al., 2009), contextual FC (Goeldner et al., 2009), IA (Adem et al., 2017), and OR (Goeldner et al., 2008). In the FC paradigm, amnesic effects have been described for two other small-molecule NOP agonists, SR-8993 (Andero et al., 2013) and Ro 65-6570 (Rekik et al., 2017). However, the interpretation of the effects of these compounds are complicated by the existence of off-target effects due to their moderate selectivity for the NOP receptor. Indeed, the inhibition of IA learning by Ro 64-6198 was not blocked by the NOP antagonist [Nphe1]N/OFQ(1–13)-NH₂ (Adem et al., 2017). Therefore, from a translational perspective, further evaluation of the amnesic effect of NOP agonists is essential.

Based on the above, this study was designed to assess the potential risks to memory from the use of NOP agonists. For these studies we used two different small-molecule NOP agonists having varying levels of selectivity, namely AT-202 (previously known as SR16835) which displays moderate selectivity for NOP over MOP receptor (Toll et al., 2009) and AT-524, a recently discovered highly selective NOP agonist. We first compared the effects of a single injection of these two NOP agonists on long-term memory using the object location (OL) memory task, an episodic-like memory task highly relevant for daily-life functioning in humans (Postma et al., 2008). We next assessed the consequences of repeated administration of high doses of these two NOP agonists on OL memory. Given the evidence of sex differences in the N/OFQ system regulation and functions (Claiborne et al., 2006; Sinchak et al., 2006; Zhang et al., 2018), the experiments were conducted in both male and female mice.

2. Materials and methods

2.1. Animals

2-4 months old male and female C57BL/6JRj were obtained from Janvier Labs, France. Mice were housed 4-5 mice/cage, under standard conditions (22 °C, 55 % humidity, 12 h light-dark cycle, lights on at 8.00 am) with food and water ad libitum. Nesting material were present in each cage. The experiments were performed in the morning between 8:30 am and 1:00 pm. All experimental procedures were conducted in accordance with the European directive 2010/63/UE and were approved by the French Ministry of Research and local ethic committees (APAFIS#12343-2017111516096417).

2.2. Drugs

NOP agonists AT-202 and AT-524 were synthesized as hydrochloride salts at Astraera Therapeutics by methods previously published (Zaveri et al., 2013). Their in vitro pharmacological profiles at the NOP and other opioid receptors are shown in Table 1S (Supplemental Information). Stock solutions of the NOP agonists were prepared in 100% DMSO, and then diluted in saline (0.9% NaCl) such that maximal final DMSO concentration was 2%. All treatments were administered subcutaneously at predetermined timepoints—30 minutes before the open field and T-maze tests, 30 minutes before exposure to the objects in the OL acquisition session, or right after object exploration for the experiment assessing memory consolidation. The dose range of the NOP agonists and the pretreatment time-points were selected based on previous in vivo studies with AT-202 (Khroyan et al., 2011; Mann et al., 2019) and the pharmacokinetics of brain exposure after systemic administration of these NOP agonists (Data in Supplemental Information, Tables S2 and S3).

2.3. Open field test

Open field test was performed during 10 min in a Plexiglas circular arena (40 cm diameter, 30 cm high). The apparatus was placed in a room that allowed sound attenuation and was equipped with a Light-Emitting Diode (LED) that provided 25 lux in the arena. A camera was fixed to the ceiling above the arena and connected to a computer equipped with Ethovision software (Noldus Tech, Netherlands). Automatic tracking enabled the recording of the total ambulatory distances.

2.4. T-maze test

T-maze test was performed in a Plexiglas maze (each arm 35 cm long, 6 cm wide, 15 cm high). This apparatus was placed in a room that allowed sound attenuation and was equipped with a Light-Emitting Diode (LED) that provided 25 lux in the arena. The session started with a forced-choice trial during which the mouse was placed in the starting arm and one of the two other arms was closed. This trial was followed by 14 free-choice trials during which the mouse was placed in the starting arm and allowed to enter the two other arms. A successful alternation was recorded when mice entered different arms in two successive trials. The session was ended after 14 free-choice trials or after 10 minutes had elapsed. Working memory performance was reflected by the percentage of successful alternation over the session.

2.5. Object location task

The OL test was performed in a Plexiglas square arena (40 cm x 40 cm long x 20cm high) equipped with a pattern of alternating of black and white stripes. A camera was fixed to the ceiling above the arena and connected to a computer equipped with the Ethovision software (Noldus Tech, Netherlands). This apparatus was placed in a room that allowed sound attenuation and was equipped with a Light-Emitting Diode (LED) that provided 25 lux in the arena. The arena and objects were cleaned with 30% ethanol before each trial.

On day 1, the habituation session consisted of letting the animal explore the empty arena for ten minutes.

On day 2, the acquisition session consisted of placing the mouse in the arena with two identical objects and allowing it to explore these objects for ten minutes. Exploration was defined as orienting the head towards the object at a distance of one centimeter or less.

On day 3, the test session consisted of placing the mouse in the arena where one of the two objects had been moved to a new location and allowing it to explore the objects for ten minutes. The exploration time of the two objects was recorded as before and the results were expressed as discrimination ratio: difference between time exploring the displaced object and that spent exploring the non-displaced object divided by the total time spent exploring both objects. All sessions were recorded and the scoring accuracy was confirmed by an experimenter blinded to the treatment conditions.

2.6. Data analysis

Data are expressed as mean \pm SEM. Statistical analyses were performed with GraphPad Prism 6 (GraphPad Software, USA). Data normal distribution was tested using the D'Agostino & Pearson omnibus normality test. The following statistical tests were used: one sample t test for comparison to chance level, Student's t test for comparison between two groups, 2-way ANOVA followed by Tukey post hoc test for analyzing the effect of 2 independent variables (sex and treatment), RM 2-way ANOVA followed by Sidak's multiple comparison test for analyzing the effect of 2 independent variables on matched subjects (time and treatment). Differences were considered statistically significant when $p < 0.05$.

3. Results:

3.1. Sedative effect of AT-202

Putative sedative effects of NOP agonists could interfere with memory acquisition. Therefore, we first evaluated the ability of increasing doses of AT-202 to affect locomotion in the open field in male and female mice (Figure 1A). 2-way ANOVA revealed a significant effect of dose ($F(3, 32) = 24.17, p < 0.0001$) and sex ($F(1, 32) = 11.85, p = 0.0016$) and a significant interaction ($F(3, 32) = 5.716, p = 0.0030$). Males were more sensitive than females, showing strong sedation at the dose of 3 mg/kg ($p < 0.001$, Tukey post-hoc test). Consequently, the doses of 1 mg/kg for males and 3 mg/kg for females were chosen in order to study memory acquisition without interference. We confirmed in the T-maze that these doses do not affect spatial working memory (Figure 1B). Both male and female mice displayed a similar percentage of alternation after s.c. injection of AT-202 or vehicle (males, $p = 0.4916$, females, $p = 0.7815$, unpaired t test).

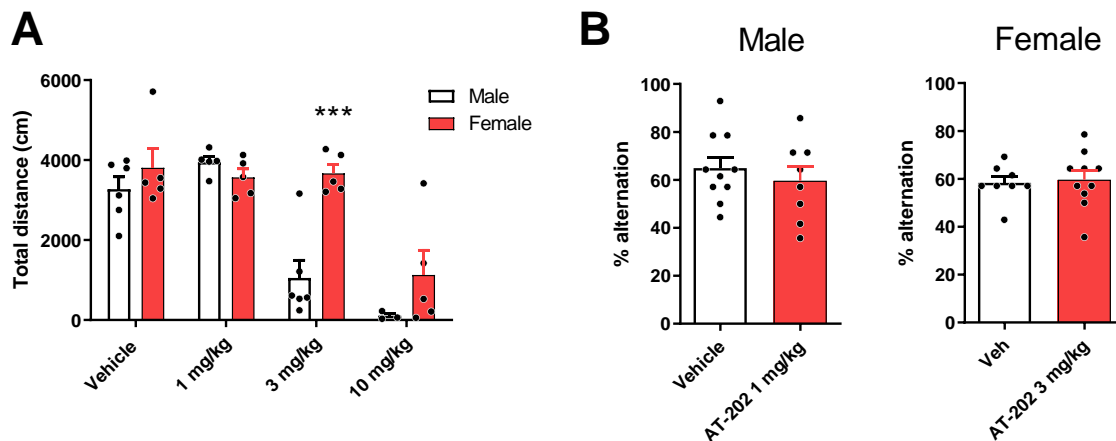


Figure 1: A, locomotion in the open field following AT-202 injection. The total distance traveled by the mice was measured over a 10-minute period. ***, $p < 0.001$; male vs female at the 3 mg/kg dose. $n = 3-6$ mice/group. B, percentage of alternation in the T-maze. The

percentage of correct alternation between the 2 arms was recorded. $n = 8-10$ mice/group. Data are expressed as mean \pm SEM.

3.2. Effect of acute AT-202 treatment on object location memory

In males, 1 mg/kg of AT-202 was injected 30 minutes before exposure to the objects. Memory was tested 24 hours later by measuring the preference for the displaced object (Figure 2A). This dose of AT-202 did not affect locomotor behavior during the acquisition session (Figure 2B, $p = 0.3839$, unpaired t test). Importantly, the total time mice spent exploring the 2 objects decreased between the acquisition session and the test session but was not affected by the AT-202 treatment (Figure 2B, Time effect: $F(1, 28) = 54.46$, $p < 0.0001$; Treatment effect: $F(1, 28) = 0.8163$, $p = 0.3740$; 2-way ANOVA). During the test session, vehicle-treated mice showed a discrimination ratio for the displaced object significantly above chance whereas for AT-202-treated mice the difference was not significant (Figure 2B, vehicle group $p < 0.0001$, AT-202 group $p = 0.1147$, one sample t test). Moreover, when comparing the discrimination ratios between the treatment groups for the acquisition and the test session, a 2-way ANOVA revealed a significant effect of time ($F(1, 28) = 42.64$, $p < 0.0001$) and a significant interaction ($F(1, 28) = 16.78$, $p = 0.0003$). Post-hoc analysis showed that the ratio was significantly lower for the AT-202 group than for the vehicle group during the test session ($p = 0.0005$). Overall, these data demonstrate that object location memory acquisition or consolidation is compromised in male mice treated with a non-sedative dose of AT-202.

Similar results were obtained for female mice treated with 3 mg/kg of AT-202. As for male mice, this dose did not affect locomotor behavior during the acquisition session (Figure 2C, $p = 0.4728$, unpaired t test). The total time mice spent exploring the 2 objects decreased between the acquisition session and the test session but was not affected by the AT-202 treatment (Figure

2C, Time effect: $F(1, 18) = 61.61$, $p < 0.0001$; Treatment effect: $F(1, 18) = 0.005682$, $p = 0.9407$; 2-way ANOVA). During the test session, vehicle-treated mice, but not AT-202-treated mice, showed a discrimination ratio for the displaced object significantly above chance (Figure 2C, vehicle group $p = 0.0002$, AT-202 group $p = 0.079$, one sample t test). Moreover, when comparing the discrimination ratios between the treatment groups for the acquisition and the test session, a 2-way ANOVA revealed a significant effect of time ($F(1, 18) = 35.51$, $p < 0.0001$), of treatment ($F(1, 18) = 6.795$, $p = 0.0178$) and a significant interaction ($F(1, 18) = 7.022$, $p = 0.163$). Post-hoc analysis showed that the ratio was significantly lower for the AT-202 group than for the vehicle group during the test session ($p = 0.0014$). Therefore, as in males, object location memory acquisition or consolidation is compromised in female mice treated with a non-sedative dose of AT-202.

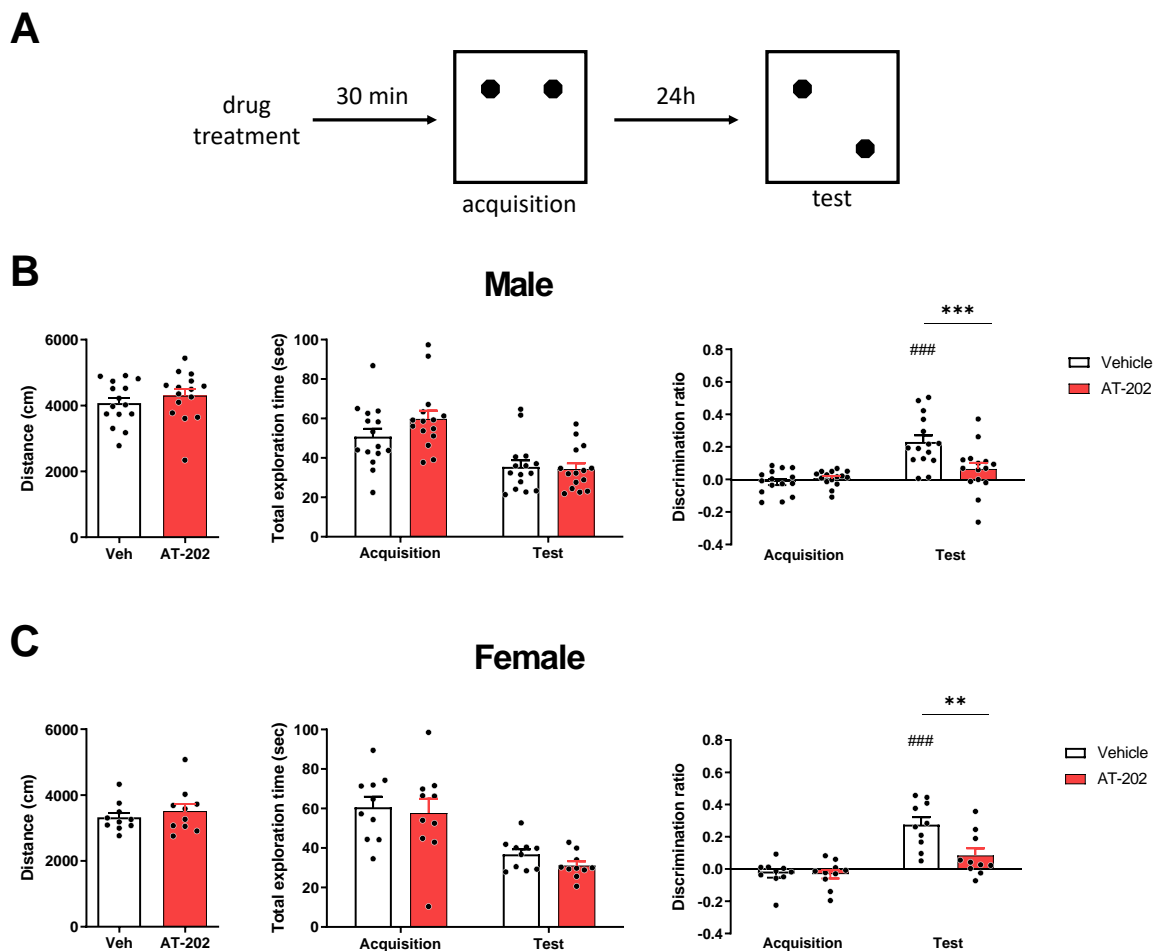


Figure 2: A, Schematic representation of the procedure. B, OL task in male mice treated with 1 mg/kg of AT-202 before memory acquisition. Left, locomotor activity during the acquisition session. Center, total exploration of both objects during the acquisition and the test session. Right, discrimination ratio. ***, $p < 0.001$, post hoc comparison; ###, $p < 0.001$, ratio vs 0. $n = 15$ mice/group. C, OL task in female mice treated with 3 mg/kg of AT-202 before memory acquisition. Left, locomotor activity during the acquisition session. Center, total exploration of both objects during the acquisition and the test session. Right, discrimination ratio. **, $p < 0.01$, post hoc comparison; ###, $p < 0.001$, ratio vs 0. $n = 10$ mice/group. Data are expressed as mean \pm SEM.

The possibility exists that the NOP agonist could produce state dependent learning, resulting in poor performance during the test session when AT-202 is no longer present. To test this hypothesis, we administered AT-202 30 minutes before the test session to mice that had been treated with AT-202 before learning. We also included a group that received AT-202 before test but not before learning to rule out a putative effect of the compound on memory retrieval (Figure 3A). The total time mice spent exploring the 2 objects decreased between the acquisition session and the test session but was not affected by the treatments (Figure 3B, Time effect: $F(1, 27) = 147.8$, $p < 0.0001$; Treatment effect: $F(2, 27) = 0.05916$, $p = 0.9427$; 2-way ANOVA). During the test session, mice treated with AT-202 only before test showed a discrimination ratio for the displaced object significantly above chance whereas the difference was not significant for the two other groups (Figure 3B, vehicle-AT-202 group $p < 0.0001$, AT-202-Vehicle group $p = 0.5915$, AT-202-AT-202 group $p = 0.6957$, one sample t test). Moreover, when comparing the discrimination ratios between the treatment groups for the acquisition and the test session, a 2-way ANOVA revealed a significant effect of time ($F(1, 27) = 7.863$, $p = 0.0092$), of treatment ($F(2, 27) = 7.271$, $p = 0.003$) and a significant interaction ($F(2, 27) =$

14.08, $p < 0.0001$). Post-hoc analysis showed that during the test session the ratio was significantly lower for the AT-202-Vehicle and AT-202-AT-202 groups than for the vehicle-AT-202 group ($p < 0.0001$). Overall, these data demonstrate that the deficit in object discrimination in male mice treated with AT-202 before acquisition is not due to state dependent learning.

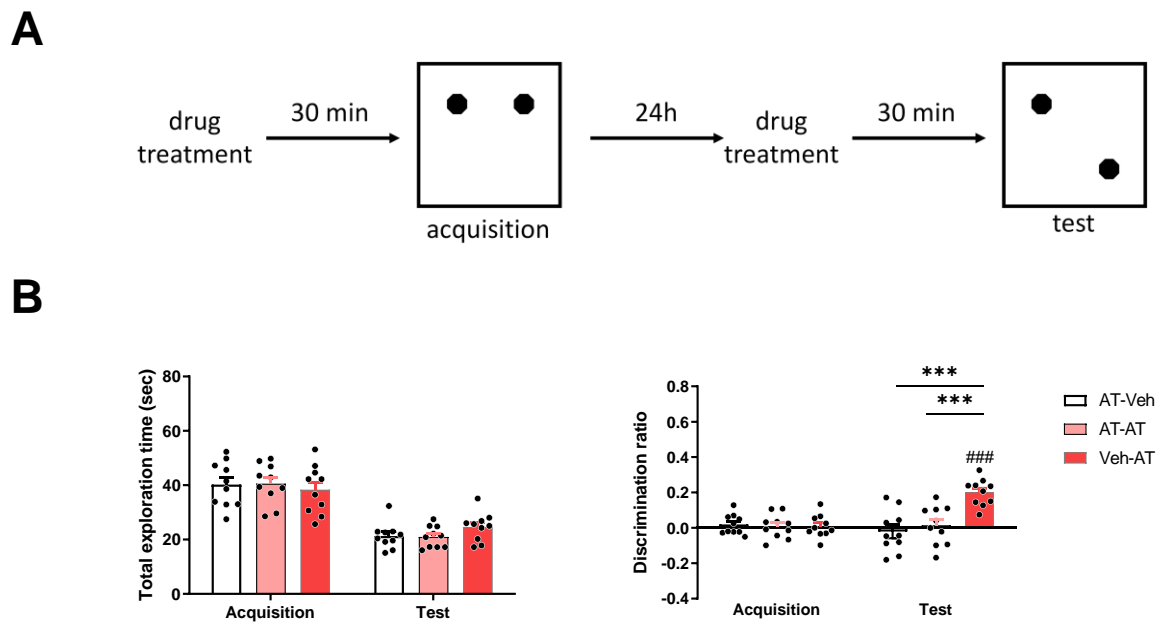


Figure 3: A, Schematic representation of the procedure. B, OL task in male mice treated with 1 mg/kg of AT-202 before memory acquisition and/or before the test session. Left, total exploration of both objects during the acquisition and the test session. Right, discrimination ratio. ***, $p < 0.001$, post hoc comparison; ###, $p < 0.01$, ratio vs 0. $n = 10$ mice/group. AT-Veh, AT-202 before acquisition only; AT-AT, AT-202 before acquisition and test; Veh-AT, AT-202 before test only.

3.3. Effect of acute AT-524 treatment on object location memory

We then wanted to confirm that the deleterious effect of AT-202 on acquisition of object location memory was a general property of NOP agonists by testing a different small-molecule

NOP agonist. AT-524 is a highly selective and potent NOP full agonist (see Supplemental Information for in vitro pharmacological profile at NOP and classical opioid receptors). As done with AT-202, AT-524 was injected s.c. 30 minutes before the acquisition session (Figure 3A). AT-524 being 5 times more potent than AT-202, we chose to test the non-sedative dose of 0.1 mg/kg in both male and female mice. Contrary to the previous experiment with AT-202, we did not use a higher dose in female mice, but compared the effect of the same dose in both sexes. This dose did not affect locomotor activity during the acquisition session, confirming a lack of sedative effect (Figure 4B, $p = 0.5738$, 4 C, $p = 0.9899$, unpaired t test).

In males, 0.1 mg/kg of AT-524 produced the same effect as 1 mg/kg of AT-202. The total time mice spent exploring the 2 objects decreased between the acquisition session and the test session (Figure 4B, Time effect: $F(1, 18) = 95.15$, $p < 0.0001$; 2-way ANOVA). The analysis also revealed a significant effect of treatment ($F(1, 18) = 5.165$, $p = 0.0355$) and a significant interaction ($F(1, 18) = 31.33$, $p < 0.0001$). Indeed, the AT-524 group showed significantly more exploration of the objects than the vehicle group ($p < 0.001$). This difference appears to result from a low level of exploration in the vehicle group. However, in spite of this lower exploration time during memory acquisition, vehicle-treated but not AT-524-treated mice, showed a discrimination ratio for the displaced object significantly above chance (Figure 4B, vehicle group $p = 0.0005$, AT-202 group $p = 0.3434$, one sample t test) during the test session. Moreover, when comparing the discrimination ratios between the treatment groups for the acquisition and the test session, a 2-way ANOVA revealed a significant effect of time ($F(1, 18) = 9.189$, $p = 0.0072$), of treatment ($F(1, 18) = 4.469$, $p = 0.0487$), and a significant interaction ($F(1, 18) = 4.659$, $p = 0.0446$). Post-hoc analysis showed that the ratio was significantly lower for the AT-202 group than for the vehicle group during the test session ($p = 0.0171$). This memory defect cannot be attributed to a deficit of exploration since AT-524 mice explored more the objects than vehicle mice during the acquisition session. Overall, these data

demonstrate that object location memory acquisition or consolidation is compromised in male mice by another different NOP agonist.

This deficit was not found in female mice treated with 0.1 mg/kg of AT-524. In this experiment, the total time mice spent exploring the 2 objects was not different between the acquisition and the test session, and was not affected by the AT-524 treatment (Figure 4C, Time effect: $F(1, 18) = 0.04043$, $p = 0.8429$; Treatment effect: $F(1, 18) = 0.2771$, $p = 0.6050$; 2-way ANOVA). During the test session, both vehicle- and AT-524-treated mice showed a discrimination ratio significantly above chance (Figure 4C, vehicle group $p = 0.0492$, AT-524 group $p = 0.0142$, one sample t test). Moreover, when comparing discrimination ratios between the treatment groups for the acquisition and the test session, a 2-way ANOVA revealed only a significant effect of time ($F(1, 18) = 9.354$, $p = 0.0068$), confirming that OL memory was efficiently acquired in both groups. Therefore, female mice were not as sensitive as males to the amnesic effect of AT-524 in the OL test.

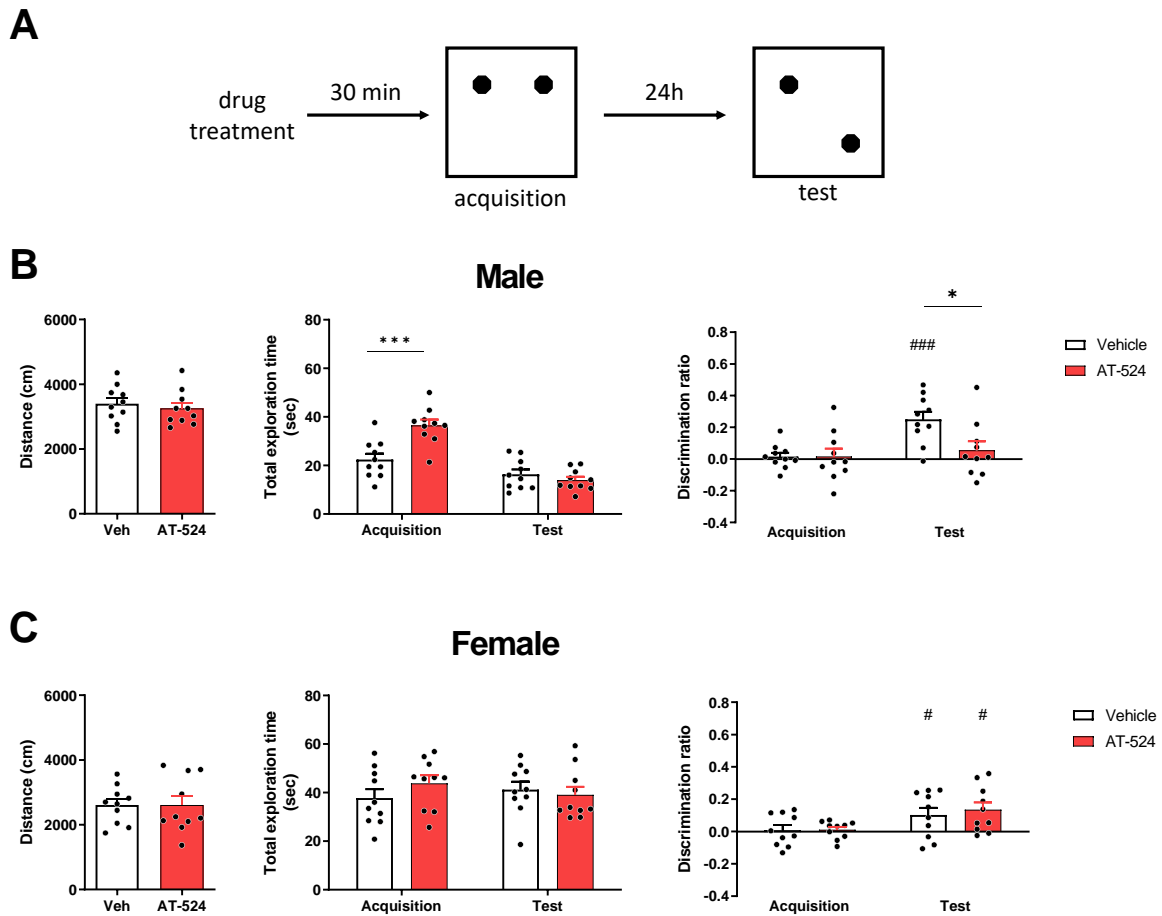


Figure 4: A, Schematic representation of the procedure. B, OL task in male mice treated with 0.1 mg/kg of AT-524 before memory acquisition. Left, locomotor activity during the acquisition session. Center, total exploration of both objects during the acquisition and the test session. Right, discrimination ratio. *, $p < 0.05$, ***, $p < 0.001$, post hoc comparison; ###, $p < 0.001$, ratio vs 0. $n = 10$ mice/group. C, OL task in female mice treated with 0.1 mg/kg of AT-524 before memory acquisition. Left, locomotor activity during the acquisition session. Center, total exploration of both objects during the acquisition and the test session. Right, discrimination ratio. #, $p < 0.05$, ratio vs 0. $n = 10$ mice/group. Data are expressed as mean \pm SEM.

3.4. Effect of NOP receptor agonists on memory consolidation

In the previous experiments, NOP agonists were administered before the learning session so we cannot decipher whether memory encoding or consolidation was affected. Here male mice were treated after the acquisition session in order to study specifically the consolidation process (Figure 5A). Since animals were not under the effect of the drugs during object exploration, sedation was no longer a concern, so higher doses of both agonists were used (10 mg/kg for AT-202 and 1 mg/kg for AT-524).

The total time mice spent exploring the 2 objects decreased between the acquisition session and the test session (Figure 5B, Time effect: $F(1, 27) = 34.13$, $p < 0.0001$; 2-way ANOVA). The analysis also revealed a significant effect of the treatment ($F(2, 27) = 3.84$, $p = 0.0341$) but no significant interaction ($F(2, 27) = 1.626$, $p = 0.2153$). This treatment effect was driven by the low exploration level of the AT-524 group during acquisition. It is a non-specific effect since the drug was injected after object exploration. During the test session, all groups showed a discrimination ratio significantly above chance (Figure 5B, vehicle group $p = 0.0011$, AT-202 group $p = 0.0006$, AT-524 group $p = 0.0097$, one sample t test). Moreover, when comparing discrimination ratios between the treatment groups for the acquisition and the test session, a 2-way ANOVA revealed a significant effect of time ($F(1, 27) = 49.61$, $p < 0.0001$), but not of treatment ($F(2, 27) = 0.1882$, $p = 0.8295$), nor a significant interaction ($F(2, 27) = 0.0411$, $p = 0.9598$). All groups were therefore equally efficient in acquiring the task, demonstrating that high doses of NOP agonists did not affect OL memory consolidation.

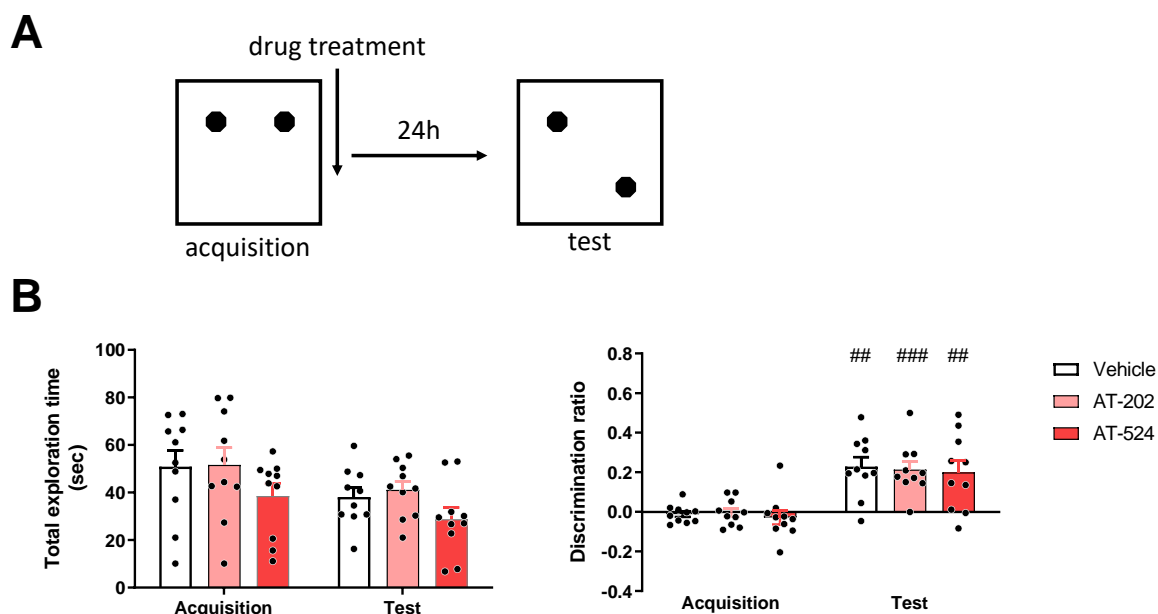


Figure 5: A, Schematic representation of the procedure. B, OL task in male mice with NOP agonist injection after memory acquisition. Left, total exploration of both objects during the acquisition and the test session. Right, discrimination ratio. ##, $p < 0.01$, ###, $p < 0.001$, ratio vs 0. $n = 10$ mice/group. Data are expressed as mean \pm SEM.

3.5. Effect of repeated administration of high doses of NOP agonists on long-term spatial memory

Finally, we investigated whether repeated administration of NOP agonists could durably affect memory performances. Preliminary experiments showed that repeated administration for 5 days produced no tolerance to the sedative effect of NOP agonists (data not shown). Therefore, high sedative doses of AT-202 (10 mg/kg) and AT-524 (1 mg/kg) were administered daily to male mice for 7 days and OL performances were then tested the followings days without any drug administration (Figure 6A).

The total time mice spent exploring the 2 objects decreased between the acquisition session and the test session but was not affected by the AT-202 treatment (Figure 6B, Time effect: $F(1, 18) = 6.534$, $p = 0.0198$; Treatment effect: $F(1, 18) = 0.3857$, $p = 0.5424$; 2-way ANOVA). During

the test session, vehicle- and AT-202-treated mice showed a discrimination ratio significantly above chance (Figure 6B, vehicle group $p = 0.0034$, AT-202 group $p = 0.0017$, one sample t test). Moreover, when comparing discrimination ratios between the treatment groups for the acquisition and the test session, a 2-way ANOVA revealed a significant effect of time ($F(1, 18) = 32.71, p < 0.0001$), but not of treatment ($F(1, 18) = 0.0081, p = 0.9293$), nor a significant interaction ($F(1, 18) = 0.0905, p = 0.7670$).

Similar results were obtained following repeated administration of AT-524. The total time mice spent exploring the 2 objects decreased between the acquisition session and the test session but was not different between treatment groups (Figure 6C, Time effect: $F(1, 15) = 18.32, p = 0.0007$; Treatment effect: $F(1, 15) = 0.2496, p = 0.6246$; 2-way ANOVA). During the test session, both treatment groups showed a discrimination ratio significantly above chance (Figure 6C, vehicle group $p = 0.0331$, AT-524 group $p = 0.0068$, one sample t test). Moreover, when comparing discrimination ratios between the treatment groups for the acquisition and the test session, a 2-way ANOVA revealed a significant effect of time ($F(1, 15) = 14.10, p = 0.0019$), but not of treatment ($F(1, 15) = 0.00539, p = 0.9464$), nor a significant interaction ($F(1, 15) = 0.00467, p = 0.9464$).

Overall, these data demonstrate that repeated administration of high sedative doses of NOP agonists did not irreversibly affect long-term object location memory.

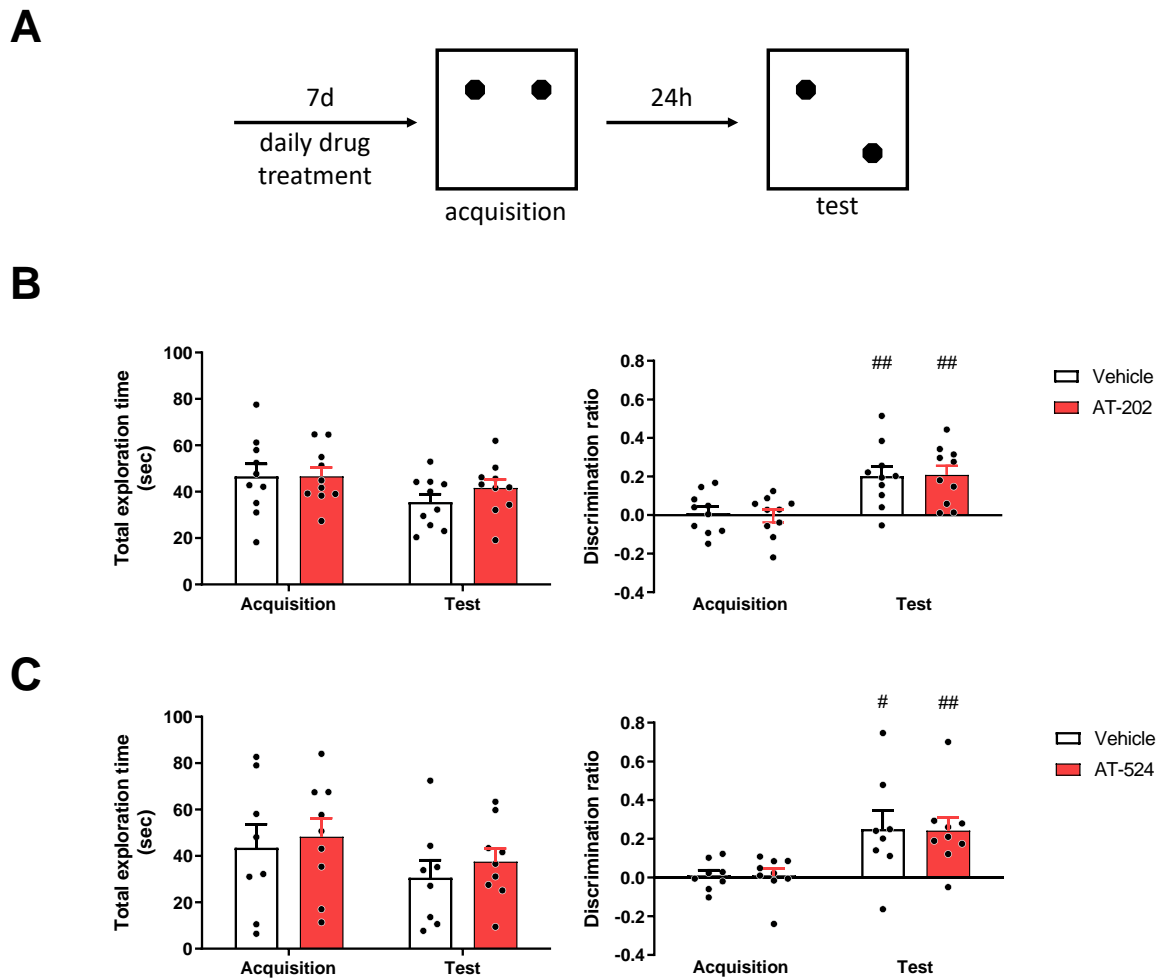


Figure 6: A, Schematic representation of the procedure. B, OL task in male mice following 7 daily injections of AT-202 (10 mg/kg). Left, total exploration of both objects during the acquisition and the test session. Right, discrimination ratio. ##, $p < 0.01$, ratio vs 0. $n = 10$ mice/group. C, OL task in male mice following 7 daily injections of AT-524 (1 mg/kg). Left, total exploration of both objects during the acquisition and the test session. Right, discrimination ratio. #, $p < 0.05$, ##, $p < 0.01$, ratio vs 0. $n = 8-9$ mice/group. Data are expressed as mean \pm SEM.

4. Discussion

The first major conclusion of our study is that the systemic administration of non-sedative doses of two NOP agonists before the acquisition impairs long-term (24h) memory performance in the OL task in male and female mice. These data are in agreement with previous studies in mice and rats investigating other types of long-term memory such as spatial reference memory in the MWM (Higgins et al., 2002; Kuzmin et al., 2009), aversive memory in the FC (Andero et al., 2013; Fornari et al., 2008; Goeldner et al., 2009; Mamiya et al., 2003; Rekik et al., 2017) and the IA tasks (Adem et al., 2017; Hiramatsu and Inoue, 1999), and recognition memory (Goeldner et al., 2008; Han et al., 2019). These amnesic effects were observed with two chemically different NOP agonists, among which AT-524 is highly selective for the NOP receptor, strongly suggesting that they are NOP receptor-specific. From a preclinical perspective, putative amnesic effects should be taken into account as confounding factors in behavioral paradigms that include an associative component such as conditioned place preference or drug self-administration. From a translational perspective, an interference with episodic memory could be a concern for the development of new therapeutics based on NOP receptor activation. However, the magnitude of negative side effects might be different across species. For example, a study with the NOP agonist Ro 64-6198 showed that the therapeutic window between anxiolytic and sedative effects was larger for rats than for mice (Varty et al., 2005). It will therefore be important in future clinical studies to monitor possible memory-related side effects.

Importantly, we also demonstrate in male mice that repeated administration of high doses of the two NOP agonists did not impair durably long-term spatial memory. Following 7 days of treatment, mice recovered normal memory performances in the OL task 24h hours after the last drug injection. Moreover, high sedative doses had no effect when administered after memory

acquisition. We can therefore propose that the main risk, when under treatment, would be an impairment in the acquisition of new information resulting in anterograde amnesia. It is interesting to note that similar effects in memory tasks are observed with benzodiazepines, another class of drugs with anxiolytic and sedative properties (Lister, 1985).

Our study gives important information on the possible phases of long-term memory development specifically affected by NOP agonists. In the post-training treatment, higher doses were used to give the best possible chance of observing potential disruptive effects on memory consolidation. The absence of deleterious effects when the treatment is administered just after the 10 minutes of exploration demonstrates that the compounds at high dose do not affect consolidation of the memory trace in male mice. It is very likely that the lower doses would also spare memory consolidation but this remains to be tested. In any case, a recent study focusing on the amnesic effects of N/OFQ in another episodic-like memory paradigm, the object recognition task, led to a similar conclusion (Han et al., 2019). Intracerebroventricular injection of a moderate non-sedative dose of N/OFQ (1 nmol) before the acquisition session impaired long-term OR memory whereas a higher sedative dose (3 nmol) administered after the learning session had no effect.

The amnesic effect we observed with the small-molecule NOP agonists could be explained by confounding factors that could interfere with memory formation such as effects on sensory motor processes or interference with attention or motivation. This is however unlikely to be the case since the doses we chose did not affect locomotion in the open field and during the OL acquisition session, spatial working memory in the T-maze, or exploratory behavior in the OL set up. Moreover, we controlled that the poor test session performances observed in mice treated with non-sedative doses of one NOP agonist were not due to state dependent learning. We therefore suggest that the observed memory deficits are due to a decrease in the animals' ability to encode spatial information. The dorsal hippocampus is a key region in this process, which

relies in part in NMDA-dependent plasticity (Warburton et al., 2013). Activation of NOP receptors in the hippocampus has been shown to inhibit NMDA-dependent long-term potentiation (Higgins et al., 2002) and NMDA receptor signaling (Goeldner et al., 2008). This mechanism has been proposed to explain N/OFQ-induced memory deficits in several spatial or contextual memory paradigms (Mouledous, 2019). Our data suggests that the same is true for the encoding of object location memory. Interestingly, a study in rats showed that systemic administration of a NMDA receptor antagonist impaired OL memory only when drug treatment occurred before training. Post-training administration had no effect (Larkin et al., 2008). We show here that systemic administration of NOP agonists leads to the same results.

The last major point of this study was the demonstration of increased sensitivity of male mice to the sedative and amnesic effects of NOP agonists. 3 mg/kg of AT-202 induced strong sedation in males whereas the locomotion of female mice was unaffected at this dose. Similarly, OL memory of male mice was strongly impaired after the injection of 1 mg/kg of AT-524 whereas female mice showed control-like performances at this dose. Previous studies have shown that the expression of the N/OFQ-NOP receptor system is modulated by female sex hormones (Flores et al., 2003; Sinchak et al., 2006). Moreover, female rat sensitivity to the analgesic effect of spinal N/OFQ varies depending on hormonal state (Claiborne et al., 2006; Claiborne et al., 2009). A recent study demonstrated a dramatically reduced potency of N/OFQ to activate NOP receptors in the female compared to the male spinal cord (Zhang et al., 2018). Considering our data, it would be interesting to study putative sex differences in the expression of the N/OFQ-NOP receptor system in memory-related brain regions. Behavioral analysis could also be conducted in female mice at different stages of the estrous cycle. In any case, putative sex differences in NOP agonist effects should be taken into account in future investigational studies.

To the best of our knowledge, NOP agonists have never been tested in the OL paradigm. Here we demonstrate that they display the same amnesic effect as in other long-term memory tasks. However, the underlying mechanisms could differ since here consolidation was not affected contrary to what has been observed in some aversive memory tasks (Andero et al., 2013; Mamiya et al., 2003; Rezik et al., 2017). Memory impairment could be a concern for interpreting preclinical results but in the context of drug development, our data suggests that repeated NOP agonist administration does not compromise episodic-like memory irreversibly. Also, the possibility of inducing temporary anterograde amnesia has not been a prohibitive limitation to the development of other drugs such as benzodiazepines. In any case, this risk could be investigated with pharmacovigilance in clinical studies involving NOP receptor agonists.

Supplementary material :

In Vitro Pharmacological Profile of NOP Agonists at the Opioid Receptors

Author contributions statement

FD: data collection and analysis, manuscript revision. NTZ : NOP ligand supply, study design, manuscript writing. LM: study design, data collection and analysis, manuscript writing.

Declaration of Competing Interest

NTZ is an employee of Astraea Therapeutics. All authors declare no other competing interests.

Data Availability

All data is available from the authors upon request.

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References

- Adem A, Madjid N, Kahl U, Holst S, Sadek B, Sandin J, Terenius L and Ogren SO (2017) Nociceptin and the NOP receptor in aversive learning in mice. *Eur Neuropsychopharmacol* **27**:1298-1307.
- Andero R, Brothers SP, Jovanovic T, Chen YT, Salah-Uddin H, Cameron M, Bannister TD, Almlil L, Stevens JS, Bradley B, Binder EB, Wahlestedt C and Ressler KJ (2013) Amygdala-dependent fear is regulated by Oprl1 in mice and humans with PTSD. *Science translational medicine* **5**:188ra173.
- Cippitelli, A., Barnes, M., Zaveri, N. T., & Toll, L. (2020). Potent and selective NOP receptor activation reduces cocaine self-administration in rats by lowering hedonic set point. *Addiction Biology*, **25**: e12844.
- Claiborne J, Nag S and Mokha SS (2006) Activation of opioid receptor like-1 receptor in the spinal cord produces sex-specific antinociception in the rat: estrogen attenuates antinociception in the female, whereas testosterone is required for the expression of antinociception in the male. *J Neurosci* **26**:13048-13053.

- Claiborne JA, Nag S and Mokha SS (2009) Estrogen-dependent, sex-specific modulation of mustard oil-induced secondary thermal hyperalgesia by orphanin FQ in the rat. *Neurosci Lett* **456**:59-63.
- Flores CA, Shughrue P, Petersen SL and Mokha SS (2003) Sex-related differences in the distribution of opioid receptor-like 1 receptor mRNA and colocalization with estrogen receptor mRNA in neurons of the spinal trigeminal nucleus caudalis in the rat. *Neuroscience* **118**:769-778.
- Fornari RV, Soares JC, Ferreira TL, Moreira KM and Oliveira MG (2008) Effects of nociceptin/orphanin FQ in the acquisition of contextual and tone fear conditioning in rats. *Behav Neurosci* **122**:98-106.
- Goeldner C, Reiss D, Wichmann J, Kieffer BL and Ouagazzal AM (2009) Activation of nociceptin opioid peptide (NOP) receptor impairs contextual fear learning in mice through glutamatergic mechanisms. *Neurobiol Learn Mem* **91**:393-401.
- Goeldner C, Reiss D, Wichmann J, Meziane H, Kieffer BL and Ouagazzal AM (2008) Nociceptin receptor impairs recognition memory via interaction with NMDA receptor-dependent mitogen-activated protein kinase/extracellular signal-regulated kinase signaling in the hippocampus. *J Neurosci* **28**:2190-2198.
- Han RW, Tian AW, Lin HR, Chang M, Wei J, Deng KY and Xin HB (2019) Nociceptin impairs acquisition of novel object recognition memory in perirhinal cortex. *Neurobiol Learn Mem* **162**:9-14.
- Higgins GA, Kew JN, Richards JG, Takeshima H, Jenck F, Adam G, Wichmann J, Kemp JA and Grottick AJ (2002) A combined pharmacological and genetic approach to investigate the role of orphanin FQ in learning and memory. *Eur J Neurosci* **15**:911-922.

- Hiramatsu M and Inoue K (1999) Nociceptin/orphanin FQ and nocistatin on learning and memory impairment induced by scopolamine in mice. *Br J Pharmacol* **127**:655-660.
- Khroyan, T. V., Polgar, W. E., Orduna, J., Montenegro, J., Jiang, F., Zaveri, N. T., & Toll, L. (2011). Differential Effects of Nociceptin/Orphanin FQ (NOP) Receptor Agonists in Acute versus Chronic Pain: Studies with Bifunctional NOP/ μ Receptor Agonists in the Sciatic Nerve Ligation Chronic Pain Model in Mice. *Journal of Pharmacology and Experimental Therapeutics*, **339**: 687–693.
- Kuzmin A, Madjid N, Johansson B, Terenius L and Ogren SO (2009) The nociceptin system and hippocampal cognition in mice: a pharmacological and genetic analysis. *Brain Res* **1305 Suppl**:S7-19.
- Lambert DG (2008) The nociceptin/orphanin FQ receptor: a target with broad therapeutic potential. *Nat Rev Drug Discov* **7**:694-710.
- Larkin AE, Fahey B, Gobbo O, Callaghan CK, Cahill E, O'Mara SM and Kelly AM (2008) Blockade of NMDA receptors pre-training, but not post-training, impairs object displacement learning in the rat. *Brain Res* **1199**:126-132.
- Lister RG (1985) The amnesic action of benzodiazepines in man. *Neurosci Biobehav Rev* **9**:87-94.
- Mamiya T, Yamada K, Miyamoto Y, Konig N, Watanabe Y, Noda Y and Nabeshima T (2003) Neuronal mechanism of nociceptin-induced modulation of learning and memory: involvement of N-methyl-D-aspartate receptors. *Molecular psychiatry* **8**:752-765.
- Mann, A., Mouldous, L., Froment, C., O'Neill, P. R., Dasgupta, P., Gunther, T., Brunori, G., Kieffer, B. L., Toll, L., Bruchas, M. R., Zaveri, N. T., & Schulz, S. (2019). Agonist-selective NOP receptor phosphorylation correlates in vitro and in vivo and reveals differential post-activation signaling by chemically diverse agonists. *Sci Signal*, **12**: 574.

- Meunier J, Mouledous L and Topham CM (2000) The nociceptin (ORL1) receptor: molecular cloning and functional architecture. *Peptides* **21**:893-900.
- Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour JL, Guillemot JC, Ferrara P, Monsarrat B and et al. (1995) Isolation and structure of the endogenous agonist of opioid receptor- like ORL1 receptor *Nature* **377**:532-535.
- Mouledous L (2019) The Nociceptin/Orphanin FQ System and the Regulation of Memory. *Handbook of experimental pharmacology* **254**:259-278.
- Postma A, Kessels RP and van Asselen M (2008) How the brain remembers and forgets where things are: the neurocognition of object-location memory. *Neurosci Biobehav Rev* **32**:1339-1345.
- Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ, Jr. and Civelli O (1995) Orphanin FQ: a neuropeptide that activates an opioidlike G protein- coupled receptor. *Science* **270**:792-794.
- Rekik K, Faria Da Silva R, Colom M, Pacifico S, Zaveri NT, Calo G, Rampon C, Frances B and Mouledous L (2017) Activation of nociceptin/orphanin FQ receptors inhibits contextual fear memory reconsolidation. *Neuropharmacology* **125**:39-49.
- Sinchak K, Romeo HE and Micevych PE (2006) Site-specific estrogen and progesterin regulation of orphanin FQ/nociceptin and nociceptin opioid receptor mRNA expression in the female rat limbic hypothalamic system. *J Comp Neurol* **496**:252-268.
- Toll L, Cippitelli A and Ozawa A (2021) The NOP Receptor System in Neurological and Psychiatric Disorders: Discrepancies, Peculiarities and Clinical Progress in Developing Targeted Therapies. *CNS drugs* **35**:591-607.
- Toll L, Khroyan TV, Polgar WE, Jiang F, Olsen C and Zaveri NT (2009) Comparison of the antinociceptive and antirewarding profiles of novel bifunctional nociceptin

- receptor/mu-opioid receptor ligands: implications for therapeutic applications. *J Pharmacol Exp Ther* **331**:954-964.
- Varty GB, Hyde LA, Hodgson RA, Lu SX, McCool MF, Kazdoba TM, Del Vecchio RA, Guthrie DH, Pond AJ, Grzelak ME, Xu X, Korfmacher WA, Tulshian D, Parker EM and Higgins GA (2005) Characterization of the nociceptin receptor (ORL-1) agonist, Ro64-6198, in tests of anxiety across multiple species. *Psychopharmacology (Berl)* **182**:132-143.
- Warburton EC, Barker GR and Brown MW (2013) Investigations into the involvement of NMDA mechanisms in recognition memory. *Neuropharmacology* **74**:41-47.
- Wichmann J, Adam G, Rover S, Hennig M, Scalone M, Cesura AM, Dautzenberg FM and Jenck F (2000) Synthesis of (1S,3aS)-8-(2,3,3a,4,5, 6-hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4. 5]decan-4-one, a potent and selective orphanin FQ (OFQ) receptor agonist with anxiolytic-like properties. *European journal of medicinal chemistry* **35**:839-851.
- Witkin, J. M., Statnick, M. A., Rorick-Kehn, L. M., Pintar, J. E., Ansonoff, M., Chen, Y., Tucker, R. C., & Ciccocioppo, R. (2014). The biology of Nociceptin/Orphanin FQ (N/OFQ) related to obesity, stress, anxiety, mood, and drug dependence. *Pharmacol Ther*, **141**: 283–299.
- Zaveri, N. T. (2011). The nociceptin/orphanin FQ receptor (NOP) as a target for drug abuse medications. *Curr Top Med Chem*, **11**: 1151–1156.
- Zaveri NT (2016) Nociceptin Opioid Receptor (NOP) as a Therapeutic Target: Progress in Translation from Preclinical Research to Clinical Utility. *J Med Chem* **59**:7011-7028.
- Zaveri, N. T., Marquez, P. V., Meyer, M. E., Hamid, A., & Lutfy, K. (2018). The Nociceptin Receptor (NOP) Agonist AT-312 Blocks Acquisition of Morphine- and Cocaine-Induced Conditioned Place Preference in Mice. *Frontiers in Psychiatry*, **9**: 638.

Zaveri NT, Jiang F, Olsen C, Polgar WE and Toll L (2013) Designing bifunctional NOP receptor-mu opioid receptor ligands from NOP receptor-selective scaffolds. Part I. *Bioorg Med Chem Lett* **23**:3308-3313.

Zhang Y, Schalo I, Durand C and Standifer KM (2018) Sex Differences in Nociceptin/Orphanin FQ Peptide Receptor-Mediated Pain and Anxiety Symptoms in a Preclinical Model of Post-traumatic Stress Disorder. *Frontiers in psychiatry* **9**:731.

Highlights :

- Male mice are more sensitive than females to the sedative and amnesic effects of NOP agonists
- Sub-sedative doses of NOP agonists impair memory acquisition in the OL task
- NOP agonists do not impair OL memory consolidation in male mice
- Repeated administration of high doses of NOP agonists do not impair long-term spatial memory

SUPPLEMENTAL INFORMATION

Title: Acute single non-sedative doses of NOP receptor agonists affect acquisition of object location memory but repeated high doses do not induce long-lasting deficits

In Vitro Pharmacological Profile of NOP Agonists at the Opioid Receptors

The receptor binding affinities of AT-202 and AT-524 were determined using radioligand displacement assays conducted in membranes from CHO cells stably expressing the human NOP, MOP, DOP and KOP receptors, using methods we have published previously (Zaveri, et al., 2004; Toll et al., 2009). As shown below in Table 1S, AT-202 showed high binding affinity for the NOP receptor, yielding a K_i value of 4.38 ± 1.2 nM in competition with [3 H]N/OFQ as the radioligand. In similar experiments using [3 H]DAMGO, [3 H]U69593 and [3 H]DPDPE at the MOP, KOP and DOP receptors respectively, AT-202 showed binding selectivity of 10-fold versus MOP, >300-fold versus KOP and DOP receptors. The recently discovered NOP agonist AT-524 was tested in the same assays and showed higher NOP binding affinity with a K_i of 1.41 nM, about 4-fold higher affinity at NOP than AT-202. Also, AT-524 was 20-fold selective versus MOP and 60-fold selective versus KOP. As observed for other piperidinyl NOP ligands from our own compound library as well as those reported in the literature, AT-202 and AT-524 do not show appreciable affinity for the DOP receptor. The high affinity of AT-524 for the NOP receptor is similar to that observed for other reported NOP agonists Ro 64-6198 and MT-7716 (Zaveri, 2016).

The functional efficacy of AT-202 and AT-524 at NOP and opioid receptors was determined using the GTP γ S binding assay conducted in membranes of CHO cells stably transfected with the NOP and classical opioid receptors. Table 1 shows the in vitro functional efficacy profile of AT-202 and AT-524. Both are full agonists at the NOP receptor, showing 100% agonist stimulation compared to the endogenous NOP agonist N/OFQ. AT-524 showed higher potency as a NOP agonist than AT-202. In contrast, it showed only a partial agonist efficacy < 20% at the MOP receptor and significantly lower potency compared to the MOP opioid full agonist DAMGO (Table 1). AT-202 and AT-524 had no agonist efficacy at the KOP receptor.

TABLE 1S: Binding affinities and functional efficacy (inhibition of [³⁵ S]GTPγS binding) at NOP and opioid receptors. [†]									
	Receptor Binding K _i (nM)			[³⁵ S]GTPγS NOP [#]		[³⁵ S]GTPγS μ [#]		[³⁵ S]GTPγS κ [#]	
	NOP	μ	κ	EC ₅₀ nM	% Stim	EC ₅₀ nM	% Stim	EC ₅₀ nM	% Stim
N/OFQ	0.12 ± 0.01		-	3.6 ± 0.7	100 ± 0				
AT-202	4.38 ± 1.2	55.7 ± 15.0	1470 ± 424	37.7 ± 9.6	100.7 ± 4.5	823 ± 113	31.1 ± 2.3	NT*	NT
AT-524	1.41 ± 0.28	32.9 ± 1.16	80.65 ± 19.95	7.8 ± 1.45	97.5 ± 1.07	293 ± 148	18.3 ± 0.2	979.7	4.4

[†] Details of the experimental procedures are as published previously (Zaveri, et al., 2004; Toll et al., 2009)

* NT = Not tested. Compounds with binding affinity K_i >100 nM are not tested in the functional assay.

Values are the Mean ± SD of three independent experiments run in triplicate.

[#] Functional activity was determined by stimulation of [³⁵S]GTPγS binding to cell membranes, % stimulation was obtained as a percentage of stimulation of the standard full agonists, N/OFQ (for NOP), DAMGO (for MOP), and U69,593 (for KOP), which showed at least 2- to 5-fold stimulation over basal, indicative of a robust assay. The stimulation by the standard full agonists was taken as 100% when comparing stimulation by the test compound.

In Vivo Plasma and Brain Exposure after systemic administration of NOP agonists AT-202 and AT-524

(1) AT-202

Species: CD-1 mice (n=3 per time point)

AT-121 Dose: 30 mg/kg

Route: Intraperitoneal

Timepoints: 0.5, 1, 2, 4, and 8 h

Sample collection: Plasma and brain

Bioanalysis: LC-MS-MS

Table S2: In Vivo Plasma and Brain Exposure after systemic administration of NOP agonists AT-202

AT-202	t _{max} (h)	C _{max} (ng/mL)	B/P ratio (C _{max})
Plasma	0.5	1683 ± 40	
Brain	0.5	3227 ± 308	1.92

(2) AT-524

(1) Species: Sprague-Dawley rats (n=3 per time point)

AT-524 Dose: 3 mg/kg

Route: Subcutaneous

Timepoints: 0.5, 1, 2, 4, and 8 h

Sample collection: Plasma and brain

Bioanalysis: LC-MS-MS

Table S3: In Vivo Plasma and Brain Exposure after systemic administration of NOP agonists AT-524

AT-524	t _{max} (h)	C _{max} (ng/mL)	B/P ratio (C _{max})
Plasma	1.0	364 ± 32	
Brain	0.85	185 ± 0	0.52

REFERENCES :

Toll L, Khroyan TV, Polgar WE, Jiang F, Olsen C, Zaveri NT (2009) Comparison of the antinociceptive and antirewarding profiles of novel bifunctional nociceptin receptor/mu-opioid receptor ligands: implications for therapeutic applications. *J Pharmacol Exp Ther* 331:954-964.

Zaveri NT, Jiang F, Olsen Cris M, Deschamps Jeffrey R, Parrish D, Polgar W, Toll L (2004). A novel series of piperidin-4-yl-1,3-dihydroindol-2-ones as agonist and antagonist ligands at the nociceptin receptor. *Journal of Medicinal Chemistry* 47:2973--2976.

Zaveri NT (2016) Nociceptin Opioid Receptor (NOP) as a Therapeutic Target: Progress in Translation from Preclinical Research to Clinical Utility. *J Med Chem* 59:7011-7028.