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Neuromedin U induces self-grooming in socially-stimulated mice

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HIGHLIGHTS

- Neuromedin U (NMU) does not alter sexual behaviors.
- NMU increases self-grooming in mice exposed to other mice or olfactory social cues.
- NMU does not increase self-grooming in mice in a neutral environment.
- NMU induced self-grooming involve the oxytocin system rather than the stress system.
- NMU induced self-grooming are mediated by dopamine within the nucleus accumbens.

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ABSTRACT

Emerging evidence suggest that appetite-regulating peptides modulate social behaviors. We here investigate whether the anorexigenic peptide neuromedin U (NMU) modulates sexual behavior in male mice. However, instead of modulating sexual behaviors, NMU administered into the third ventricle increased self-grooming behavior. In addition, NMU-treatment increased self-grooming behavior when exposed to other mice or olfactory social-cues, but not when exposed to non-social environments. As the neuropeptide oxytocin is released during social investigation and exogenous oxytocin induces self-grooming, its role in NMU-induced self-grooming behavior was investigated. In line with our hypothesis, the oxytocin receptor antagonist inhibited NMU-induced self-grooming behavior in mice exposed to olfactory social-cues. Moreover, dopamine in the mesocorticolimbic system is known to be a key regulator of self-grooming behavior. In line with this, we proved that infusion of NMU into nucleus accumbens increased self-grooming behavior in mice confronted with an olfactory social-cue and that this behavior was inhibited by antagonism of dopamine D2, but not D1/D5, receptors. Moreover repeated NMU treatment enhanced *ex vivo* dopamine levels and decreased the expression of dopamine D2 receptors in nucleus accumbens in socially housed mice. On the other hand, the olfactory stimuli-dependent NMU-induced self-grooming was not affected by a corticotrophin-releasing hormone antagonist, and NMU-treatment did not influence repetitive behaviors in the marble burying test. In conclusion, our results suggest that NMU treatment and, social cues - potentially triggering oxytocin release - together induce excessive grooming behavior in male mice. The mesolimbic dopamine system, including accumbal dopamine D2 receptors, was identified as a crucial downstream mechanism.

1. Introduction

The anorexigenic peptide neuromedin U (NMU) (Egecioglu et al., 2009; Hanada et al., 2004; Howard et al., 2000; Ida et al., 2005; Ivanov et al., 2002; Kojima et al., 2000; Peier et al., 2009; Zeng et al., 2006) has lately been shown to affect various physiological processes. One of these are reinforcement modulation as central NMU signaling reduces reward induced by alcohol (Vallof et al., 2017), amphetamine (Vallof et al., 2016) and cocaine (Kasper et al., 2016). These behavioral responses of addictive drugs involve NMU receptor 2 (NMUR2) in nucleus accumbens (NAc) (Vallof et al., 2016), more specifically NMUR2 dependent activation of GABAergic projections from dorsal raphe nucleus to NAc shell (Kasper et al., 2016). Activation of central NMUR2 has further been shown to influence anxiety as well as depression-like behaviors (Gartlon et al., 2004; Hanada et al., 2001; Tanaka and Telegdy, 2014; Telegdy and Adamik, 2013; Wren et al., 2002; Zeng et al., 2006). The findings that NMU induces stress via corticotrophin releasing hormone (CRH) dependent mechanisms (Gartlon et al., 2004;

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Hanada et al., 2001; Wren et al., 2002) provide additional support for the pleiotropic physiological responses of NMU. The role of NMU signaling for social behaviors, including sexual behaviors, is so far unknown.

Mating behavior is crucial for the survival of the species and is to a large extent innate and is displayed in response to specific social stimuli. Hormones, for example the sex steroids are well-known to be crucial regulators of sexual behaviors such as mate preference, sexual motivation as well as mating behaviors, in males and females (for reviews see (Hull et al., 2004; Pfaff, 1999). In more recent years also other hormones, including some of those released from the gastro-intestinal tract, have been revealed to modulate mating behaviors in rodents. The orexigenic peptide ghrelin activates sexual behaviors (Egecioglu et al., 2016; Hyland et al., 2017; Prieto-Garcia et al., 2015), whereas the anorexigenic peptide amylin decreases sexual behaviors (Clementi et al., 1999). This study intended to investigate whether the anorexigenic peptide neuromedin U (NMU) modulates sexual motivation and mating behavior in male mice.

In our study, we initially investigated how central NMU administered into the third ventricle influence sexual preference, sexual motivation and mating behavior. However, NMU-treatment did not affect these behaviors, but instead induced self-grooming behavior. In further experiments NMU-treatment was revealed to increase self-grooming behavior in mice exposed to other mice or olfactory social-cues but not in mice exposed to non-social environments. To investigate the role of the neuropeptide oxytocin, which is known to be released during social interactions (for review see (Veenema and Neumann, 2008), olfactory social-cue stimulated mice were before NMU- infusion pre-treated with a non-peptidergic oxytocin receptor antagonist. Moreover, previous studies have linked repetitive behaviors to dopamine signaling (for reviews see (Buse et al., 2013; Denys et al., 2004; Kalueff et al., 2016; Nguyen et al., 2014; Wood and Ahmari, 2015)), specifically in brain areas such as NAc and dorsomedial striatum (DMS) (Aliane et al., 2011; Drago et al., 1986). Therefore, the possibility that self-grooming behaviors induced by NMU in social-cue stimulated mice involves dopamine signaling in the NAc shell was investigated. The anatomical specificity of the NMU actions was investigated by local infusion of NMU into NAc shell or DMS. The specificity of the dopamine receptor regulating NMU-induced self-grooming was investigated by treatment with antagonists of dopamine D2 or D1/D5 receptors. To further explore the link between NAc-dopamine, NMU and social cues in self-grooming behavior *ex vivo* levels of dopamine and expression of dopamine receptors in NAc were investigated in socially housed mice after repeated peripheral administration of NMU. Finally, we explored the possibility that the NMU-induced self-grooming behavior in olfactory social-cue stimulated mice involves stress as well as anxiety by using pharmacological inhibition of CRH receptors as well as an anxiety provoking context test on repetitive behavior.

2. Material and methods

2.1. Animals

Adult post-pubertal and sexually naïve male NMRI mice (8–12 weeks old and 25–35 g body weight; Charles River, Susfeldt Germany) were used for the olfactory preference test, partner preference test, sexual interaction test, marble burying test and the biochemistry and expression experiments. In addition, ovariectomized female C57Bl/6N mice (Charles River) were used in the partner preference test and sexual interaction test. The selection of strains was based on earlier studies from our lab (Egecioglu et al., 2016; Prieto-Garcia et al., 2015). In the olfactory preference test bedding from adult post-pubertal and sexually naïve male and female NMRI mice were used. All mice were maintained at 20 °C with 50% humidity and a 12/12 h light/dark cycle (lights on at 7 a.m.). Tap water and food (Normal chow; Harlan Teklad, Norfolk, England) were supplied *ad libitum*, except during the experimental sessions. The Ethics Committee for Animal Experiments in Gothenburg, Sweden approved the studies.

2.2. Drugs

NMU (Tocris Bioscience; Bristol, United Kingdom) was dissolved in vehicle (ringer solution; NaCl 140 mM, Ca Cl_2 1.2 mM, KCl 3.0 mM and $MgCl_2$ 1.0 mM; Merck KGaA, Darmstadt, Germany) for central administration. This intermediate dose of NMU (1 μg in 1 μl, into the third ventricle (icv)) was used since this dose previously have been shown to decrease food intake and block reward induced by alcohol as well as amphetamine, without affecting gross behavior, accumbal dopamine and locomotor activity *per se* (Egecioglu et al., 2009; Howard et al., 2000; Ida et al., 2005; Vallof et al., 2016, 2017). For local and bilateral administration into the NAc shell as well as DMS a dose of 62.5 ng (in 0.5 μl per side) was selected since a previous study reported that this dose into NAc shell had no effect *per se* on locomotor activity or the expression of conditioned place preference in mice, but attenuated amphetamine-induced reward (Vallof et al., 2016). NMU was always administered 20 min prior to the experiment. For peripheral intraperitoneal (ip) administration a dose of 300 μg/kg NMU, dissolved in vehicle (0.9% sodium chloride solution), was used since it passes the blood-brain barrier (Gevaert et al., 2016) and reduces food intake (Kaisho et al., 2017; Peier et al., 2011) as well as alcohol intake in rats (Supplementary material 1). The oxytocin receptor antagonist L368899 (Tocris Bioscience), dissolved in vehicle (0.9% sodium chloride solution), was administered 45 min prior to NMU. The selected dose of L368899 (5 mg/kg, ip) has previously been shown to attenuate place preference for socially conditioned context in mice with no effect *per se* on place preference or locomotor activity (Dolen et al., 2013). Aripiprazole (generously supplied by Bristol Myers Squid; Stockholm Sweden), a dopamine D2 receptor partial agonist with affinity to dopamine D3, D4 serotonin $(5HT)_{1A}$,$5HT_{2A}$, $5HT_{2C}$, $5HT_7$, α_1 and $histamine_1$ receptors (Uzun et al., 2005; Winans, 2003), was dissolved in a few drops of glacial acetic acid and the final volume was made up with lukewarm D-Glucose solution (5.5%). Aripiprazole, is considered as a dopamine stabilizer as it acts as a dopamine D2 receptor antagonist when dopamine levels are high and as a dopamine D2 receptor agonists at a low endogenous dopamine level. The selected dose of Aripiprazole (1.25 mg/kg, ip) has previously been shown to attenuate alcohol as well as amphetamine induced locomotor stimulation in mice with no effect *per se* on locomotor activity (Jerlhag, 2008) and was administered 10 min prior to NMU. The dopamine D1/D5-receptor antagonist SCH23390 (Tocris Bioscience) was dissolved in vehicle (0.9% sodium chloride solution) and was administered 20 min prior to NMU. The selected dose of SCH23390 (0.03 mg/kg, ip) has previously been shown to attenuate alcohol induced locomotor stimulation in mice with no effect *per se* on locomotor activity (Cohen et al., 1997). The CRH receptor antagonist, CP154526 (Tocris Bioscience), was dissolved in vehicle (DMSO and 0.9% sodium chloride solution; 10:90) and was administered 30 min prior to NMU. The selected dose of CP154526 (10 mg/kg, ip), without any effect *per se*, attenuates alcohol induced locomotor sensitization in mice (Fee et al., 2007). The drug challenges in each specific series of experiment were randomized and each experiment was conducted in a new set of mice. Vehicle-treated mice were always included in each experiment to minimize the possibility that batch variation influences the outcome in the present studies.

2.3. Ovariectomy of female mice

Female C57Bl/6N mice, aged 10 weeks at arrival, were anaesthetized with a mixture of ketamine (Ketalar 10 mg/ml; Pfizer, Kronans Apotek, Gothenburg, Sweden) and xylazine (Rompun Vet 20 mg/ml; Bayer Animal Health, Kronans Apotek), ovariectomized via a midline incision and subsequently allowed to recover in temperate cages in groups of eight. Female mice were brought into estrus by giving

sequential injection of 10 and 5 μg of estradiol benzoate 48 and 24 h before the test and 50 μg of progesterone 4 h before the tests. Female mice were used 4–7 h after progesterone injections (Egecioglu et al., 2016; Prieto-Garcia et al., 2015).

2.4. Guide cannula implantation

To enable infusions into the brain, guide cannulas (stainless steel, length 10 mm, with an o.d./i.d. of 0.6/0.45 mm) were implanted three days prior to the initiation of the experiment. The surgery was conducted as previously described (Vestlund et al., 2019). In brief, the mouse was anaesthetized with isofluran (Isofluran Baxter, Kronans Apotek) using a pump (Univentor 400 Anaesthesia Unit, Univentor Ldt., Zejtun, Malta), placed in a stereotaxic apparatus (David Kopf Instruments; Tujunga, CA, USA) and kept on a heating pad to prevent hypothermia. Two drops of Xylocain (10 mg/ml) adrenalin (5 μg/ml) (Astra Zeneca, Kronans Apotek) were used for local anaesthesia. The skull bone was exposed after an incision and two or three holes were drilled, one or two for the guide cannulas and one for the anchoring screw. The coordinates relative to bregma were: icv, AP -0.9, ML ± 0.0 mm and DV -2.1 mm; NAc shell, AP +1.4, ML ± 0.6 mm and DV -4.7 mm; DMS AP +0.5, ML ± 1.5 and DV -2.5 mm (Franklin and Paxinos, 1997), thus enabling unilateral injection into the third ventricle as well as bilateral injection into NAc shell and DMS. The guide cannulas were placed 1 mm below the surface of the brain and they were anchored to the screw and the skull bone with dental cement (DENTALON® plus; Agntho's AB, Lidingö, Sweden). After surgery the mice were injected with carprofen (Rimadyl®, 5 mg/kg subcutaneously; Astra Zeneca, Kronans Apotek) to relieve pain and were kept in individual cages (Macrolon III). At the time of the experiment, the cannula was extended another 1.1 mm, 3.7 mm or 1.5 mm ventrally beyond the tip of the guide cannula aiming for drug administration in the third ventricle, NAc shell and DMS respectively. One hour before initiating each experiment, a dummy cannula was carefully inserted and retracted into the guide cannula to remove clotted blood and hamper spreading depression. The drug was administered over 1 min; the cannula was left in place for another minute and it was then retracted (5 μl Kloehn, microsyringe; Skandinaviska Genetec AB, V. Frölunda, Sweden). The injection sites were following each behavioral test verified and only mice with correct placements were included in the statistical analysis (Supplementary material 2).

2.5. Olfactory preference test

The test was performed in a plastic cage ($37 \times 22 \times 19\ cm^3$, Techniplast, Buguggiate, Italy) as described previously (Egecioglu et al., 2016) and was design to investigate the effects of NMU on sexual behavior or grooming behavior in sexually naïve male mice exposed to an olfactory social cue. In brief, 10 g bedding from group-housed male or female adult free-cycling NMRI mice, not changed for seven days, was placed in opposite side of the cage bottom (area of $11 \times 17\ cm^2$). The placement of the bedding material was altered between left and right side in a balanced design and was prevented from being mixed by a plastic bar. A metal mesh (2 mm wide and 7 mm interval) separated the test mice from the bedding in the bottom of the cage. The mouse was allowed to familiarize to the cage without bedding material for 5 min and was then allowed to freely investigate the arena for 10 min. The behaviors were analyzed by an observer blind to the treatment of individual mice. In between the experiments the bedding was changed, the grid and cage were cleaned with water and alcohol solution (10%).

The first experiment was designed to evaluate the effects of NMU on the time investigating female and male bedding as well as on self-grooming behavior as measured by grooming duration, frequency and latency. Therefore, NMU (1 μg, icv) or equal volume of vehicle was administered centrally into the third ventricle. Directly following this first olfactory preference test the vehicle or NMU treated mice were transferred to a sterile cage and were allowed to investigate this cage for 5 min and self-grooming behavior was scored in this non-social context.

Previous studies have shown that central NMU administration does not affect gross behavior in mice situated in a non-social environment (Egecioglu et al., 2009; Howard et al., 2000; Ida et al., 2005; Vallof et al., 2016, 2017). Mice were following 20 min of habituation in an empty, sterile plastic cage injected with either NMU (1 μg, icv) or an equal volume of vehicle. The mice were then allowed to freely investigate the cage for 10 min allowing confirmation of the hypothesis that NMU does not affect self-grooming behavior in a non-social environment.

Thereafter the mechanisms involved in the NMU-induced self-grooming behavior in mice exposed to a social olfactory stimulus were explored. Therefore, one of the following drugs or an equal volume of the corresponding vehicle was administered prior to NMU (1 μg, icv) or vehicle; i) the oxytocin receptor antagonist L368899 (5 mg/kg, ip). ii) aripiprazole (1.25 mg/kg, ip) a dopamine D2 receptor partial agonist, iii) SCH23390 (0.03 mg/kg, ip) a D1/D5 receptor antagonist, iv) the CRH receptor antagonist CP154526 (10 mg/kg, ip).

The upcoming experiments were designed to identifying brain areas involved in NMU-induced self-grooming behavior. NMU (62.5 ng in 0.5 μl per side) or an equal volume of vehicle was administered locally and bilaterally into the i) NAc shell or ii) DMS. Thereafter, to further establish a role of dopamine signaling in NAc shell in modulation of NMU-induced self-grooming behavior, separate mice were pre-treated with aripiprazole (1.25 mg/kg, ip) or vehicle 10 min prior to NMU (62.5 ng in 0.5 μl per side) or vehicle infusion into NAc shell.

2.6. Partner preference test

The test was performed as previously described (Egecioglu et al., 2016; Prieto-Garcia et al., 2015). In brief, a plastic cage ($50 \times 39 \times 41$ cm, SmartSore Classic; Hammarplast Consumer AB, Gothenburg, Sweden) with holes cut out for cages ($10 \times 10 \times 10$ cm) for incentive animals at opposing sides along the long side of the arena was used. An area of 14×10 cm immediately adjacent to the cages with the incentive animals was designated as the male or female incentive zone. Each mouse was allowed to habituate to the testing area for 10 min in 2 consecutive days before the experiment. Immediately prior to the test, mice were allowed to habituate to the arena for 15 min, whereupon NMU (1 μg, icv) or an equal volume of vehicle was administered. Then, cages with novel stimulus animals, one ovariectomized estrus female and one male were placed at the openings allowing olfactory and visual cues, but not contact, between the animals. Placement of male and female incentive animals was randomized and the arena and mesh barriers were cleaned with water followed by alcohol solution (10%) between the individual tests. Movement patterns were recorded for 15 min and time spent in female and male incentive zone as well as self-grooming behavior, *i.e.* grooming duration, frequency and latency, were manually analyzed by an observer blind to the treatment of individual mice.

2.7. Sexual interaction test

The test was performed as previously described (Egecioglu et al., 2016; Prieto-Garcia et al., 2015) and was designed to evaluate the effect of NMU on behavior of a sexually naïve male mice exposed to social contact with female mouse. Male mice were singled housed for 7 days. 20 min before initiation of the interaction experiment NMU (1 μg, icv) or an equal volume of vehicle was administered. Moreover nesting material and food were removed from the cage 10 min prior to interaction. Thereafter an ovariectomized female mouse in artificial estrus was placed in the home cage of the male mouse and the male mouse was allowed to interact with the female mouse for 20 min. Each female was only used twice per estrus induction. Duration, frequency and

latency to mounting, which is pelvic thrusting as the basic behavior of copulation (for review see (Hull et al., 2004)), sniffing behavior as well as self-grooming behaviors, *i.e.* grooming duration, frequency and latency, was manually scored by an observer blind to the treatment of individual mice.

2.8. Marble burying test

Marble burying test is a valid model to study repetitive symptoms in rodents where the marbles provoke excessive digging behavior performed by the rodent (for review see (de Brouwer et al., 2019). The test was performed to investigate other stereotypic behaviors, i.e. excessive digging, that NMU may induce in an anxiety provoking context. The marble burying test was conducted in a plastic cage filled with wood chip bedding to a depth of 5 cm with flat surface and topped with 20 marbles (1.5 cm in diameter) evenly spaced apart in 4 rows of 5 marbles (4 cm space in between). 10 g of bedding materials from another male cage was added to induce an olfactory environment comparable to the olfactory preference test. NMU (1 μg, icv) or an equal volume of vehicle was administered and the mice were allowed to freely investigate the cage for 30 min. The number of marbles buried, the digging latency, frequency, duration as well as self-grooming behavior (grooming duration, frequency and latency) was manually scored by an observer blind to the treatment of individual mice. Marbles and cage were cleaned with 70% ethanol and the cage was refilled with new wood chip materials and male bedding materials between every individual test.

2.9. Biochemical and expression analysis of specific brain reward areas

The effects of repeated peripheral administration of NMU on noradrenaline, serotonin and dopamine and the metabolites HVA, DOPAC, 5-HIAA, as well as the mRNA expression of *Drd*, *Drd2*, *Drd3*, *Drd4*, *Drd5*, *Comt* and *Th* genes were investigated in mice exposed to a social context. Therefore, in two independent sets of mice NMU (300 μg/kg, ip) or an equal vehicle solution was administered peripherally to group-housed mice for five days. 20 min following the last injection the mice were decapitated and the brains were removed. NAc was rapidly dissected out on a cold glass plate, immediately frozen on dry ice and was kept frozen at −80 °C until the biochemical as well as expression analysis.

2.9.1. Ex vivo biochemical analysis

The biochemical analysis of brain areas from first set of mice was conducted as described previously (Vestlund et al., 2019). The dissected brain areas, from first set of mice (NMU or vehicle), were homogenized by ultrasound homogenization (Sonifier Cell Disruptor B30, Branson Sonic Power Co.; Danbury, CT, USA) in a solution of 0.1 M perchloric acid, 5.37 mM EDTA and 0.65 mM glutathione. Following centrifugation (10000 rpm, 5 °C, 10 min) the supernatant was collected and analyzed for noradrenaline, serotonin, dopamine and the metabolites DOPAC, HVA and 5-HIAA using a split fraction HPLC-ECD system. Noradrenaline, serotonin and dopamine was analyzed on an ion-exchange column (Nucleosil, 5μ, SA, 100 A, 150 × 2 mm, Phenomenex, Torrance, CA, USA) with a mobile phase consisting of 13.3 g citric acid, 5.84 g NaOH, 40 mg EDTA, and 200 ml methanol in distilled water to a total volume of 1000 ml. DOPAC, HVA and 5-HIAA were analyzed on a reverse phase column (Nucleosil, 3μ, C18, 100 A, 50 × 2 mm, Phenomenex) with a mobile phase consisting of 11.22 g citric acid, 3.02 g dipotassium phosphate, 40 mg EDTA, and 60 ml methanol in distilled water to a total volume of 1000 ml. The electrochemical detection was performed by two amperometric detectors (Waters 460) and the currents were recorded with the Dionex Chromeleon software package (Dionex; Sunnyvale, CA, USA).

2.9.2. Gene expression

The brain areas from the second set of mice were used for the gene expression determinations with a method described previously (Kalafateli et al., 2019). For total RNA extraction, the brain tissue samples were homogenized using a TissueLyser II (Qiagen; Sollentuna, Sweden and RNA extraction was performed using RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer's instructions. Concentration and quality of RNA were assessed using a NanoDrop (Thermal Scientific; Willmington, DE, USA) and the RNA concentration was adjusted in all samples prior to cDNA synthesis. RNA concentration was calculated equally for all samples at 1000 ng per sample. After dilution with Milli-Q™ (Millipore Corporation, Billerica, MA, USA) water, the samples were loaded in duplicates in a 96 well plate (Sarstedt AG & Co, Nümbrecht, Germany) and were prepared for a 20 ìl reverse transcription reaction into cDNA using the QuantiTect Reverse Transcription kit (Qiagen) as per manufacturer's instructions.

The quantitative real-time PCR (qRT-PCR) analysis was performed in the facilities of TATAA Biocenter AB Gothenburg, Sweden. Briefly, the samples were corrected for gDNA contamination using the ValidPrime™ (TATAA Biocenter AB) technology and gene expression analysis was performed using the qRT-PCR instrument IntelliQube™ (Douglas Scientific, Alexandria, MN, USA). The selected reference genes were *Tfrc* and *Pgk1* and the genes of interest were *Drd*, *Drd2*, *Drd3*, *Drd4*, *Drd5*, *Comt* and *Th*. The corrected C_T values raw data were analyzed using the comparative C_T method as previously described (Livak and Schmittgen, 2001). Vehicle mice were set as the internal calibrator in these experiments. In brief, the individual ΔC_T values were calculated as: $C_{T\ (gene\ of\ interest)} - C_{T\ (average\ of\ reference\ genes)}$. The $\Delta\Delta C_T$ values were calculated as the average ΔC_T of the internal calibrator (vehicle mice) subtracted from the average ΔC_T of the experimental group (NMU-treated mice). Data analysis was performed on the individual ΔC_T values. The data is visually represented as fold change in the form of $2^{-\Delta\Delta CT}$.

2.10. Statistics

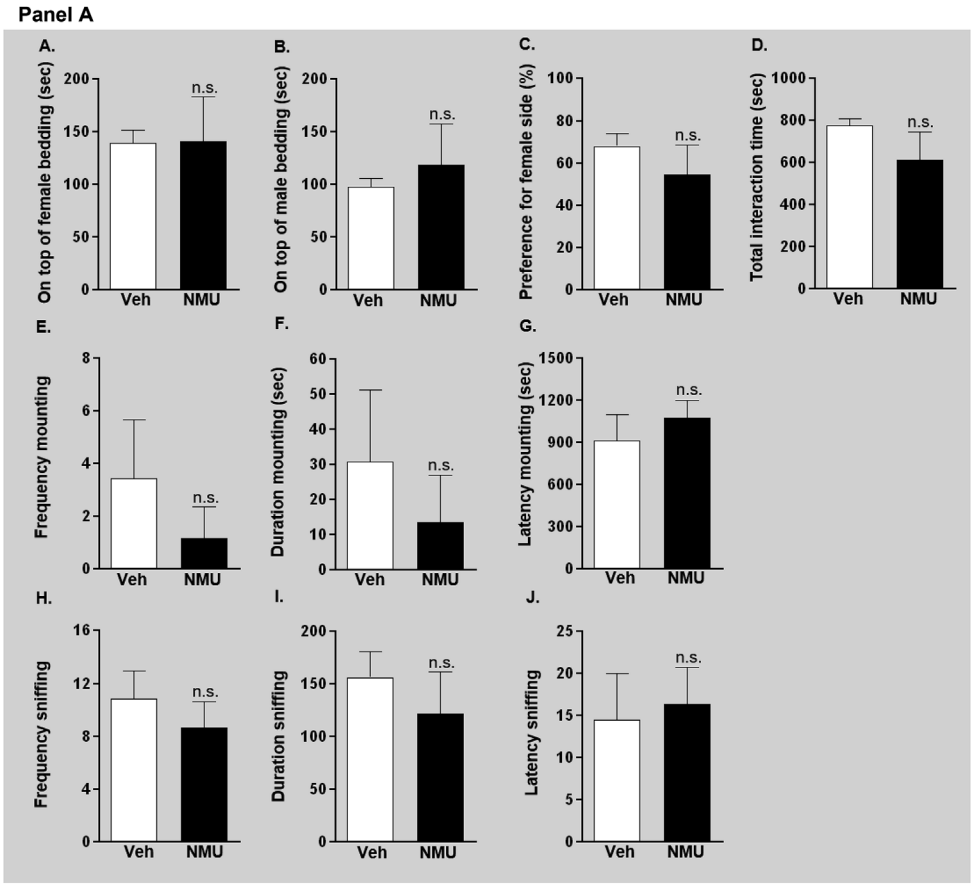
Data from the olfactory preference, partner preference, sexual interaction, marble burying and empty cage test as well as the biochemical and expression experiments (ΔC_T values) were analyzed with an unpaired *t*-test. The interactions between NMU and dopamine, oxytocin and CRH receptors in the olfactory preference test were evaluated by a one-way ANOVA followed by Bonferroni post-hoc test. The behavioral, biochemical and expression data were normally distributed and had homogeneity of variance. Data are presented as mean ± SEM (standard error of the mean). A probability value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. NMU did not affect sexual behavior, but increased self-grooming behavior in male mice exposed to social stimuli

In the bedding test, NMU (n = 7, icv) administration did not affect the time spent over female (P = 0.9610; Fig. 1A) nor male (P = 0.6063; Fig. 1B) bedding compared to vehicle treatment (n = 7). In the preference for female test, NMU treatment (n = 8, icv) did neither alter the preference for the female mouse (P = 0.4168; Fig. 1C) nor total interaction time (P = 0.2768; Fig. 1D) compared to vehicle (n = 7) treatment. The interaction test displayed that NMU (n = 6, icv) infusion did not alter the frequency (P = 0.4106; Fig. 1E), duration (P = 0.5102; Fig. 1F) or the latency (P = 0.4977; Fig. 1G) of mounting compared to vehicle (n = 7) treatment. Neither did NMU affect the frequency (P = 0.4683; Fig. 1H), duration (P = 0.4523; Fig. 1I) nor the latency (P = 0.8023; Fig. 1J) of sniffing compared to vehicle.

The results from the bedding test (same as above) further revealed that NMU increased the duration of (P = 0.0003; Fig. 1K) and



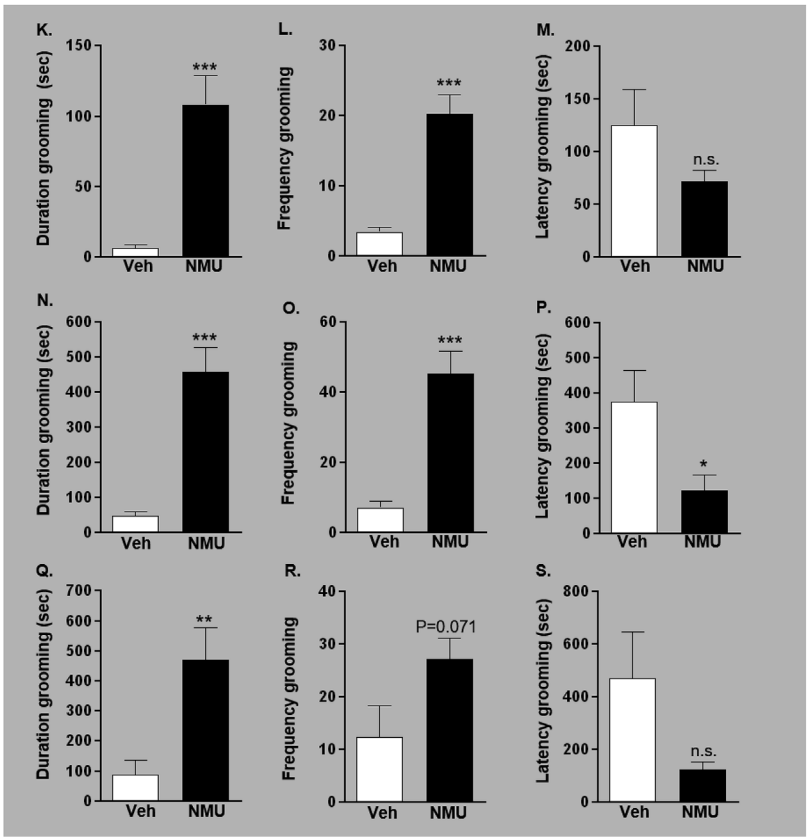


Fig. 1. NMU administration into the third ventricle did not affect sexual behaviors but increased self-grooming behavior in male mice exposed to social stimuli.

Panel A: Sexual behaviors. In the olfactory preference test, NMU into the third ventricle did not alter the time spent over A) the female bedding or B) male bedding compared to vehicle. In the partner preference test NMU neither effected C) the preference for the female mouse or D) the total interaction time compared to vehicle. In the sexual interaction test NMU into the third ventricle had no effect on E) mounting frequency, F) mounting duration, G) mounting latency, H) sniffing frequency, I) sniffing duration or J) sniffing latency compared to vehicle.

Panel B: Self-grooming behaviors. In the olfactory preference test, NMU into the third ventricle K) increased grooming duration, L) enhanced grooming frequency compared to vehicle, M) but did not alter grooming latency compared to vehicle. In the partner preference test central NMU infusion N) elevated grooming duration, O) increased grooming frequency and P) decreased grooming latency compared to vehicle. In the sexual interaction test NMU into the third ventricle Q) increased grooming duration, R) had a tendency to increase grooming frequency and S) did not alter grooming latency compared to vehicle.

Data are presented as mean ± SEM (standard error of the mean); ***P < 0.001, **P < 0.01, *P < 0.05 and n.s. = not significant compared to vehicle; unpaired *t*-test.

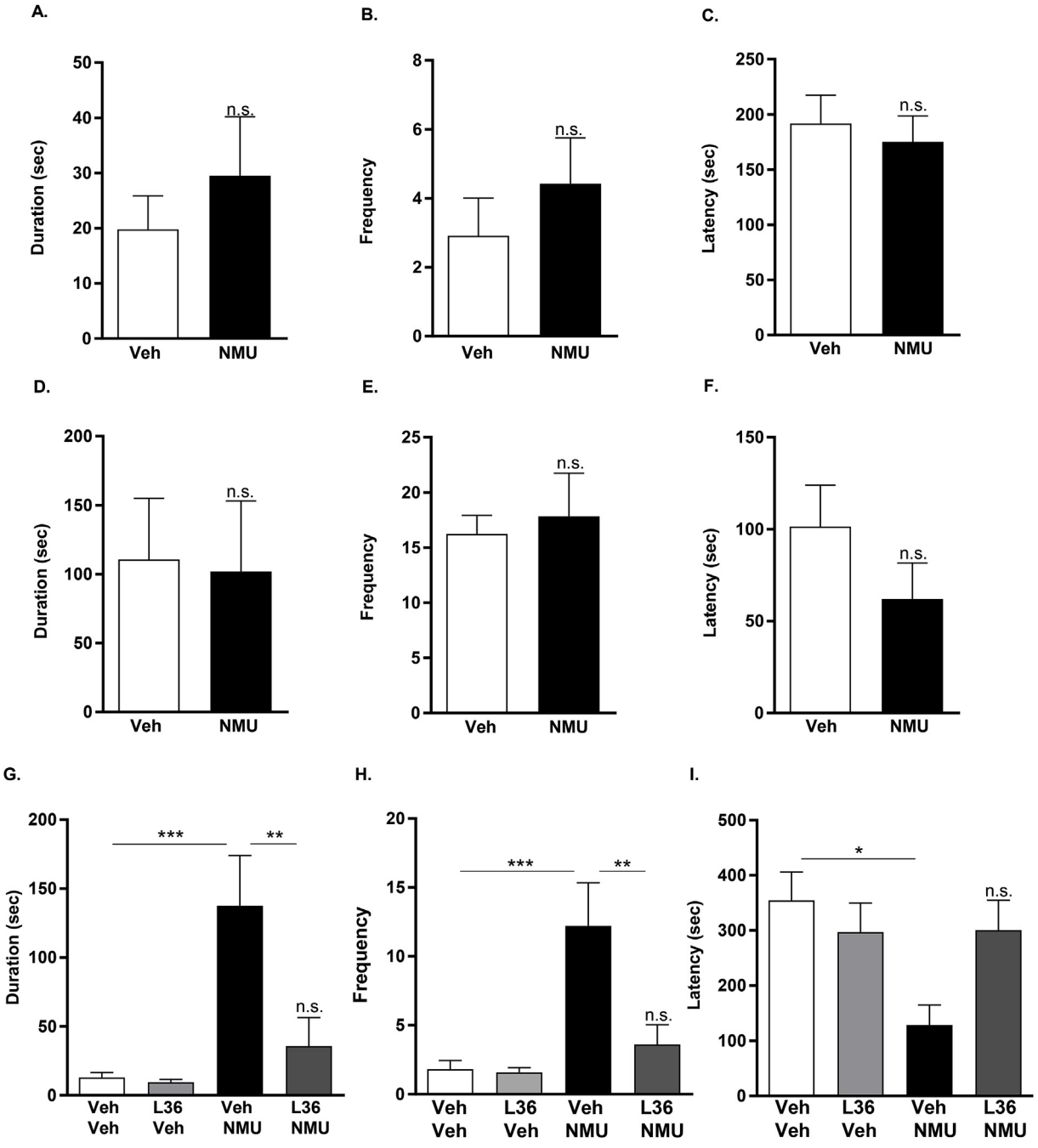


Fig. 2. NMU infusion into the third ventricle did not influence self-grooming behavior in male mice that are not exposed to social stimuli and an oxytocin receptor antagonist prevents NMU induced grooming behavior in male mice exposed to olfactory social-cues.

In mice transferred from the olfactory preference test to a non-social environment, there were no differences between vehicle and NMU treatment when it comes to A) grooming duration B) grooming frequency or C) grooming latency. In mice only exposed to a non-social environment, NMU did not affect D) grooming duration, E) grooming frequency or F) grooming latency compared to vehicle. In the olfactory preference test, pre-treatment with L-368899 (L36) attenuated NMU-enhanced G) grooming duration H) grooming frequency as well as I) blocked the NMU-reduced grooming latency.

Data are presented as mean ± SEM (standard error of the mean); ***P < 0.001, **P < 0.01, *P < 0.05 and n.s. = not significant compared to vehicle; unpaired *t*-test (A–F) and one-way ANOVA followed by Bonferroni post-hoc test (G–I).

frequency of (P < 0.0001; Fig. 1L) self-grooming compared to vehicle treatment, but did not alter the latency to self-grooming (P = 0.1553; Fig. 1M). The preference for female test further demonstrated that NMU treatment increased the duration of (P = 0.0001; Fig. 1N) and frequency of (P = 0.0001; Fig. 1O) self-grooming behavior and reduced the latency to (P = 0.0188; Fig. 1P) this behavior compared with vehicle treatment. In the sexual interaction test, NMU treatment induced self-grooming behavior as shown by increased duration (P = 0.0059; Fig. 1Q) and a tendency in increased frequency (P = 0.0706; Fig. 1R) compared to vehicle treatment. NMU did not affect the latency of grooming (P = 0.1006; Fig. 1S).

3.2. In a non-social context NMU did not influence self-grooming behavior and an oxytocin receptor antagonist prevented the NMU-increased self-grooming behavior in male mice exposed to social stimuli

When mice treated with NMU (n = 14, icv) or vehicle (n = 12) into the third ventricle were transferred to a sterile cage without social stimuli following the end of the bedding test, there were no differences in the duration (P = 0.4566; Fig. 2A), frequency (P = 0.3969; Fig. 2B) nor latency (P = 0.6368; Fig. 2C) to grooming in these mice. Neither did administration of NMU (n = 6, icv) alter grooming duration (P = 0.7273; Fig. 2D), frequency (P = 0.6764; Fig. 2E), nor latency (P = 0.2402; Fig. 2F) compared to vehicle treatment (n = 9) in a sterile environment.

There was an overall main effect on self-grooming duration in mice treated with L368899 and NMU ($F(3,54) = 8.24$, P = 0.0001; Fig. 2G). Post hoc analysis revealed that NMU (n = 14, icv) enhanced the self-grooming duration compared to vehicle treatment (n = 15, $P < 0.001$), and that this increase was attenuated by pretreatment of L368899 ($P = 0.006$, n = 15), at a dose with no effect *per se* compared to vehicle treatment ($P > 0.999$, n = 14). There was no difference in duration between vehicle and L368899-NMU treated mice ($P > 0.999$). There was an overall main effect of treatment on self-grooming frequency ($F(3,54) = 8.29$, P = 0.0001; Fig. 2H). Post hoc analysis revealed that NMU increased the frequency compared to vehicle treatment ($P < 0.001$), and that this enhancement was attenuated by pretreatment of L368899 ($P = 0.005$), at a dose with no effect *per se* compared to vehicle treatment ($P > 0.999$). There was no difference in frequency between vehicle and L368899-NMU treated mice ($P > 0.999$). There was an overall main treatment effect on latency to self-grooming ($F(3,54) = 3.88$, P = 0.0139; Fig. 2I). Post hoc analysis revealed that NMU reduced the latency compared to vehicle treatment ($P = 0.013$). There was no difference in latency between vehicle and L368899-NMU treated mice ($P > 0.999$). However, pretreatment with L368899 reduced, but did not block, the ability of NMU to decrease latency ($P = 0.102$). L368899 had no effect on latency *per se* compared to vehicle ($P > 0.999$).

3.3. Dopamine D2, but not D1/D5, receptors regulate the ability of NMU to increase self-grooming behavior in male mice exposed to social stimuli

There was an overall main effect of aripiprazole and NMU treatment on the self-grooming duration ($F(3,49) = 7.57$, P = 0.0003; Fig. 3A). Post hoc analysis revealed that NMU (n = 14, icv) increased the duration compared to vehicle treatment (n = 12, $P = 0.002$), and that this increase was attenuated by pretreatment of aripiprazole ($P = 0.011$, n = 13), at a dose with no effect *per se* compared to vehicle treatment ($P > 0.999$, n = 14). There was no difference in duration between vehicle and aripiprazole-NMU treated mice ($P > 0.999$). There was an overall main effect of treatment on the self-grooming frequency ($F(3,49) = 10.29$, P < 0.0001; Fig. 3B). Post hoc analysis revealed that NMU increased the frequency compared to vehicle treatment ($P < 0.001$). The NMU enhanced frequency was attenuated by pretreatment of aripiprazole ($P = 0.004$), at a dose with no effect *per se* ($P > 0.999$). There was no difference in duration between vehicle and aripiprazole-NMU treated mice ($P > 0.999$). There was no overall main effect of treatment on the self-grooming latency ($F(3,49) = 1.63$, P = 0.1938; Fig. 3C).

There was an overall main effect of SCH23390-NMU treatment on the self-grooming duration ($F(3,33) = 7.84$, P = 0.0004; Fig. 3D). Post hoc analysis revealed that NMU (n = 9, icv) increased the duration compared to vehicle treatment (n = 9, $P = 0.016$) and that this enhancement was not affected by pretreatment of SCH23390 ($P > 0.999$, n = 9) compared to vehicle-NMU treatment. The duration was higher in SCH23390-NMU treated mice compared to vehicle treated mice ($P = 0.008$). The dose of SCH23390 had no effect *per se* compared to vehicle treatment on duration ($P > 0.999$, n = 10). There was an overall main effect of treatment on frequency of self-grooming ($F(3,33) = 5.15$, P = 0.0049; Fig. 3E). Post hoc analysis revealed that there was no difference in frequency between vehicle-NMU and SCH23390-NMU treated mice ($P > 0.999$). Moreover, the frequency was higher in SCH23390-NMU compared to vehicle treated mice ($P = 0.022$). The dose of SCH23390 had no effect *per se* compared to vehicle treatment on frequency ($P > 0.999$). There was no overall main effect on latency to self-grooming ($F(3,33) = 1.73$, P = 0.1799; Fig. 3F).

3.4. Dopamine signaling within the NAc shell modulated the NMU-increased self-grooming behavior in male mice exposed to social stimuli

NAc shell-NMU increased the grooming duration (P = 0.0029; $n = 18$ per group; Fig. 4A), frequency (P = 0.0012; Fig. 4B) as well as reduced the latency ($P < 0.0001$; Fig. 4C) compared to vehicle in male mice exposed to olfactory social-cues. On the contrary, DMS-NMU did not alter grooming duration (P = 0.9293; $n = 19$ per group; Fig. 4D), frequency (P = 0.2998; Fig. 4E) nor latency (P = 0.1887; Fig. 4F) compared to vehicle in male mice exposed to olfactory social stimuli.

An association between NMU and NAc was further confirmed as repeated systemic administration of NMU (n = 10, ip) in socially housed mice increased the *ex vivo* dopamine levels in NAc compared to vehicle (n = 10) treatment (P = 0.0403, Fig. 4G). Compared to vehicle, NMU did not alter the levels of DOPAC, HVA, noradrenaline, serotonin, nor 5-HIAA (data not shown). The mRNA expression of *DAD2* in NAc was lower (P = 0.0002, Fig. 4H) in group-housed mice exposed to repeated systemic administration of NMU (n = 10, ip) compared to vehicle (n = 10). In comparison to vehicle, NMU did not alter the mRNA expression of *Drd1*, *Drd3*, *Drd5*, *Comt* or *Th genes* (data not shown).

There was an overall effect on the self-grooming duration in mice treated with aripiprazole and NMU-NAc shell ($F(3,46) = 7.33$, P = 0.0004; Fig. 4I). Post hoc analysis revealed that NMU-NAc (n = 12) increased the duration compared to vehicle (n = 13, $P = 0.013$), and that this increase was attenuated by pretreatment of aripiprazole ($P < 0.001$, n = 13), at a dose with no effect *per se* compared to vehicle treatment ($P > 0.999$, n = 12). There was no difference in duration between vehicle and aripiprazole-NMU treated mice ($P > 0.999$). There was an overall treatment effect on the self-grooming frequency ($F(3,46) = 9.50$, P < 0.0001; Fig. 4J). Post hoc analysis revealed that NAc-NMU increased the frequency compared to vehicle treatment ($P = 0.002$). The NMU enhanced frequency was attenuated by pretreatment of aripiprazole ($P < 0.001$), at a dose with no effect *per se* compared to vehicle treatment ($P > 0.999$). There was no difference in frequency between vehicle and aripiprazole-NMU treated mice ($P > 0.999$). There was an overall treatment effect on the latency to grooming ($F(3,46) = 4.32$, P = 0.0091; Fig. 4K). Post hoc analysis revealed that NMU did not alter the latency compared to vehicle ($P = 0.177$). There was no difference in latency between aripiprazole-NMU and vehicle treated mice ($P > 0.999$). There was a higher latency in aripiprazole-NMU treated mice compared to vehicle-NMU treated mice ($P = 0.006$). Aripiprazole had no effect on latency ($P > 0.999$) compared to vehicle.

3.5. NMU-induced self-grooming behavior in socially stimulated mice did not involve CRH receptors nor did it influence on stereotypic digging in an anxiety-provoking context

There was an overall CP154526-NMU treatment effect on self-grooming duration ($F(3,53) = 9.19$, P < 0.0001; Fig. 5A). Post hoc analysis revealed that NMU (n = 14, icv) increased the duration compared to vehicle (n = 13, $P = 0.002$). The NMU enhanced duration was not affected by pretreatment of CP154526 ($P > 0.999$, n = 15) compared to vehicle-NMU treated mice. The duration was higher in CP154526-NMU compared to vehicle treated mice ($P = 0.007$). The dose of CP154526 had no effect *per se* compared to vehicle ($P > 0.999$,

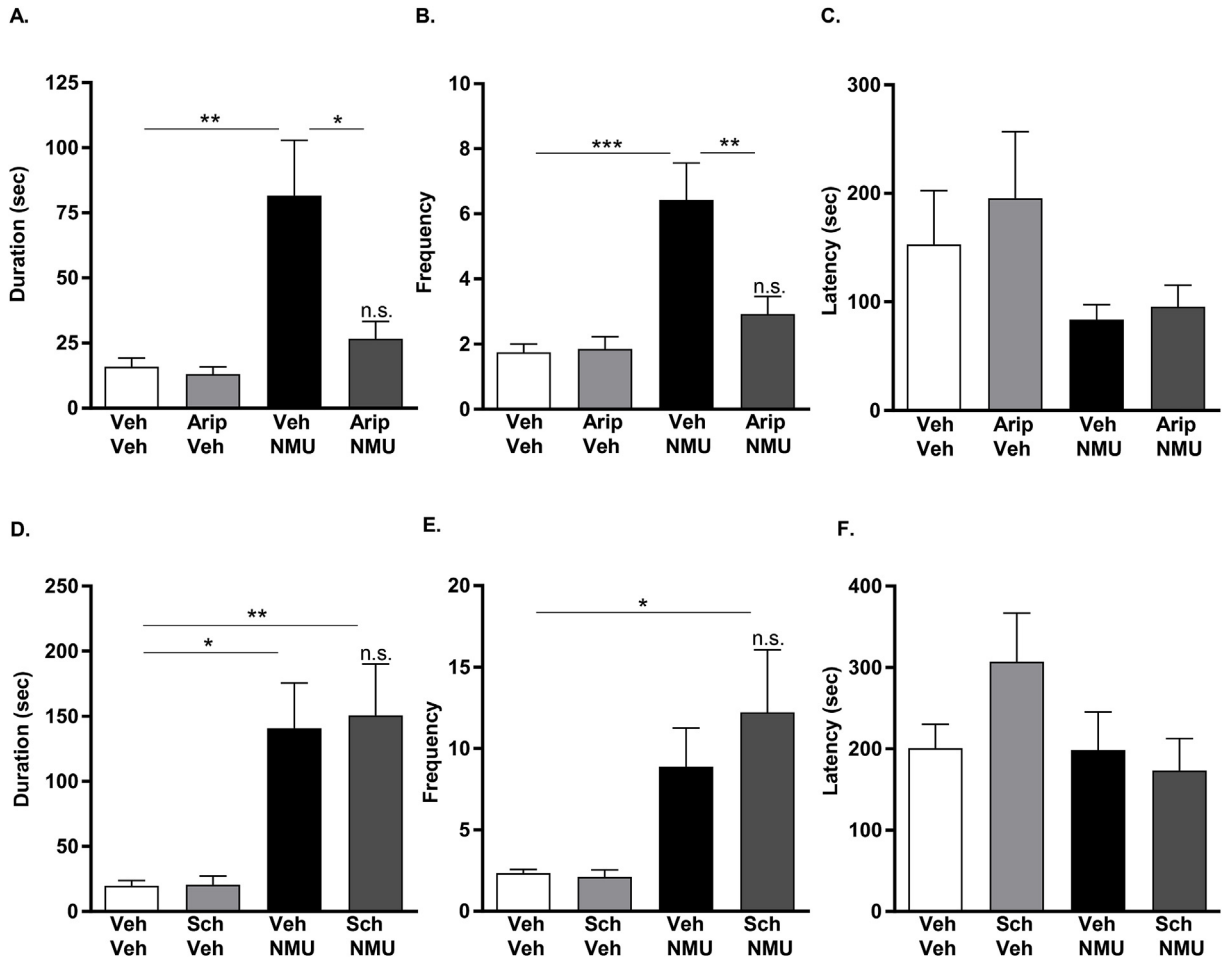


Fig. 3. Dopamine D2, but not D1/D5, receptors regulate the ability of NMU administration into the third ventricle to increase self-grooming behavior in male mice exposed to olfactory social cues.

In the olfactory preference test, pre-treatment with aripiprazole (Arip) blocked the ability of NMU to increase A) the grooming duration as well as B) grooming frequency. C) On the other hand, no robust effect was observed by either treatment on grooming latency. In another olfactory preference test, pre-treatment with SCH23390 (Sch) did not attenuate the NMU-increased D) grooming duration, or E) grooming frequency. F) In these mice, no robust effect on grooming latency was observed by either treatment.

Data are presented as mean ± SEM (standard error of the mean); ***P < 0.001, **P < 0.01, *P < 0.05 and n.s. = not significant compared to vehicle in A-B and compared to vehicle-NMU in D-E; one-way ANOVA followed by Bonferroni post-hoc test.

n = 15). There was an overall treatment effect on self-grooming frequency ($F(3,53) = 7.47$, P = 0.0003; Fig. 5B). Post hoc analysis revealed that NMU did not change the frequency compared to vehicle ($P = 0.100$). There was no difference in frequency between CP154526-NMU and vehicle-NMU treated mice ($P > 0.999$). There was an increased frequency in CP154526-NMU treated mice compared to vehicle treated mice ($P = 0.002$). The dose of CP154526 had no effect *per se* compared to vehicle treatment on frequency ($P > 0.999$). There was a treatment effect on latency to self-grooming ($F(3,53) = 5.24$, P = 0.0030; Fig. 5C). Post hoc analysis revealed that NMU did not alter the latency compared to vehicle ($P = 0.150$). There was no difference in latency between CP154526-NMU and vehicle-NMU treated mice ($P > 0.999$). There was a decreased latency in CP154526-NMU treated mice compared to vehicle treated mice ($P = 0.030$). The dose of CP154526 had no effect *per se* compared to vehicle treatment on frequency ($P > 0.999$).

NMU (n = 6, icv) did not influence the number of marbles buried compared with vehicle (n = 6) treatment (P = 0.0694; Fig. 5D). Compared to vehicle treatment, NMU did neither influence the digging duration (P = 0.0926, Fig. 5E), frequency (P = 0.1255, Fig. 5F) nor digging latency (P = 0.2475, Fig. 5G). On the other hand, NMU increased the self-grooming duration (P = 0.0051; Fig. 5H) and frequency (P = 0.0050; Fig. 5I) but did not alter the latency to grooming (P = 0.4353; Fig. 5J) compared to vehicle. Hence, the tendency in reduced digging behavior, is linked to the increase in self-grooming.

4. Discussion

Herein, we reveal a novel effect of NMU-treatment on self-grooming behavior, which is specific to a social context. Indeed, mice treated centrally with NMU display an increased grooming behavior when exposed to other mice or olfactory social-cues were present, but when moved to an empty cage the excessive grooming behavior is normalized to vehicle level. This is further highlighted by the previous behavioral data showing that central NMU infused mice do not express excessive self-grooming behavior in non-social context paradigms (Vallof et al., 2016, 2017). The self-grooming of vehicle treated mice is higher in those tested in a non-social environment than in the social context. As studies report that social environment increases self-grooming (Ferkin, 2006; Yu et al., 2010), the possibility that the NMU fails to decrease grooming when animals are alone appears less likely. We rather suggest that NMU does not alter grooming-behavior in a non-social context and that batch-differences with regard to behavior exists.

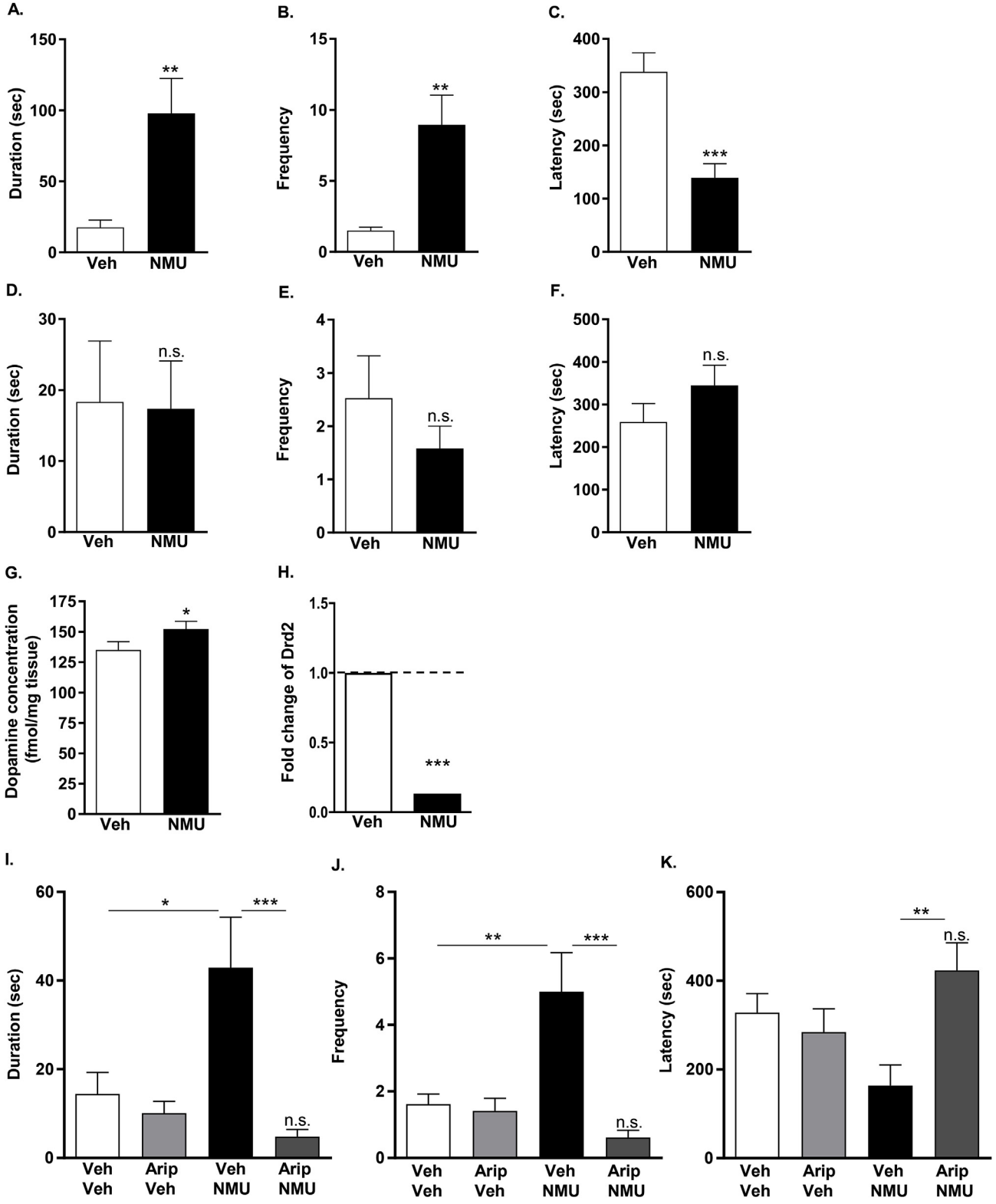


Fig. 4. The NMU enhanced self-grooming behavior involves dopamine signaling in nucleus accumbens in male mice.

In the olfactory preference test, local and bilateral infusion of NMU into nucleus accumbens (NAc) shell increased A) the grooming duration, B) grooming frequency as well as C) reduced grooming latency compared to vehicle. On the other hand, NMU bilaterally into the dorsal medial striatum did not affect the D) grooming duration, E) grooming frequency or F) grooming latency compared to vehicle. Repeated systemic administration of NMU to socially housed mice G) increased the *ex vivo* level of dopamine in NAc compared to vehicle as well as H) decreased the expression of *DRD2* in NAc compared to vehicle. In the olfactory preference test, pre-treatment with Aripiprazole (Arip) attenuated the ability of accumbal NMU to increase I) the grooming duration, J) grooming frequency and K) attenuated the tendency of accumbal NMU-reduced grooming latency.

Data are presented as mean ± SEM (standard error of the mean); ***P < 0.001, **P < 0.01, *P < 0.05 and n.s. = not significant compared to vehicle; unpaired *t*-test (A–H) and one-way ANOVA followed by Bonferroni post-hoc test (I–K). Gene expression is represented as fold change ($2^{-\Delta\Delta CT}$).

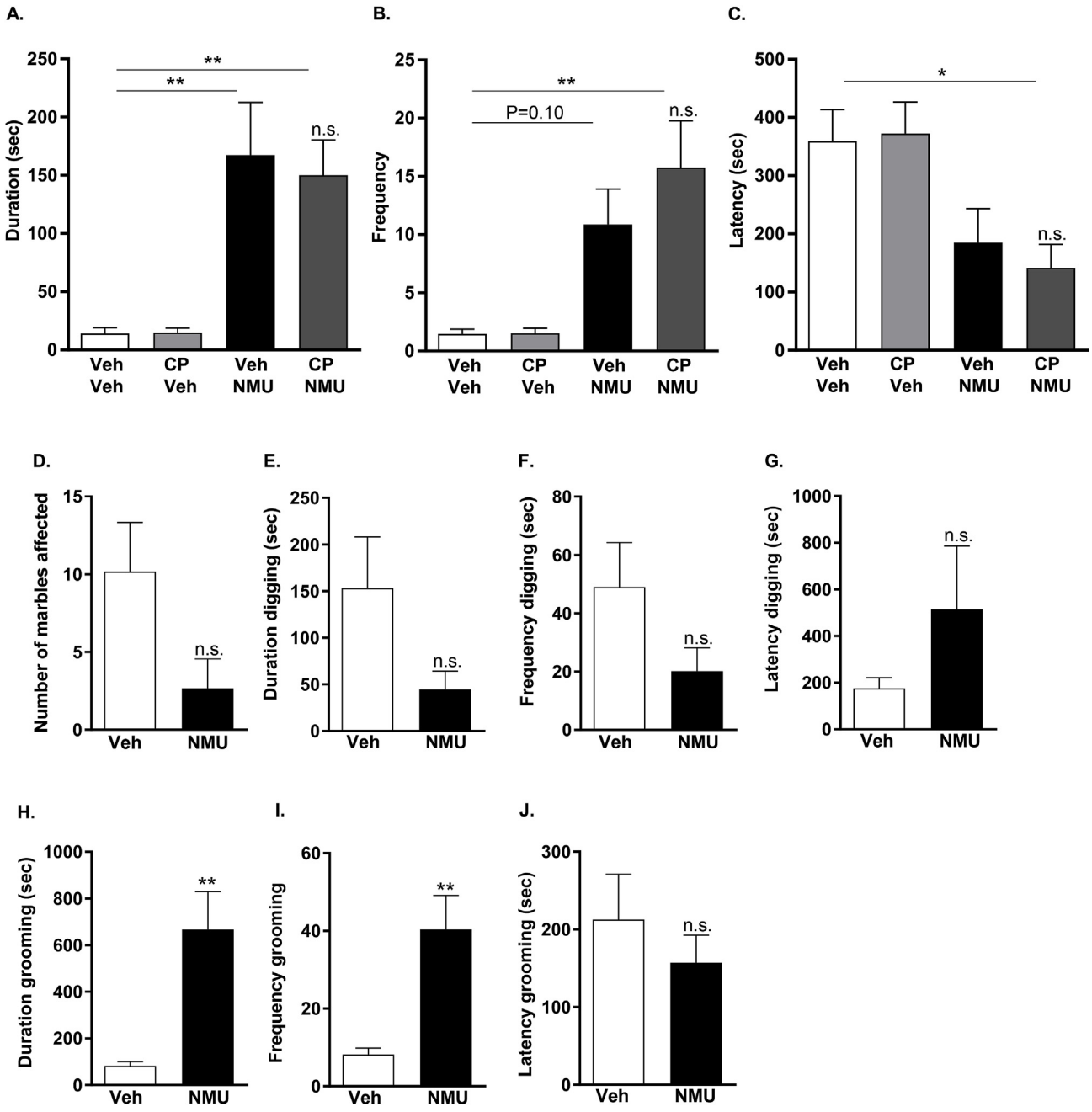


Fig. 5. A CRH receptor antagonist did not prevent the NMU enhanced self-grooming behavior in male mice exposed to olfactory social stimuli and NMU infusion into the third ventricle did not alter stereotypic digging in an anxiety provoking context.

A) Pre-treatment with CP-154526 (CP) did not attenuate the NMU enhanced grooming duration compared to vehicle in the olfactory preference test. Compared to vehicle treatment, CP-NMU B) increased grooming frequency as well as C) reduced grooming latency. In the marble burying test NMU did not influence D) the number of marbles buried, E) the digging duration, F) digging frequency or G) digging latency. NMU did however in this test increase H) the grooming duration, I) grooming frequency, J) but did not alter the grooming latency.

Data are presented as mean ± SEM (standard error of the mean); $^{**}P < 0.01$, $^{*}P < 0.05$ and n.s. = not significant compared to vehicle-NMU in A-C and compared to vehicle for D-J, one-way ANOVA followed by Bonferroni post-hoc test (A–C) and unpaired *t*-test (D–J).

Collectively, these findings indicate that social-cues are necessary for this newly discovered behavior evoked by NMU. The present data showing that an oxytocin receptor antagonist attenuates NMU-increased self-grooming in a social context indicate that oxytocin might be a modulator of this behavior link. As exposure to social stimulus (for review see (Veenema and Neumann, 2008)) or central NMU infusion (Ozaki et al., 2002) independently increases the central levels of oxytocin without altering the self-grooming *per se,* it should be considered that NMU and social stimuli collectively enhances the oxytocin dependent neural circuits that organize self-grooming. This is indirectly supported by the findings that a high dose of centrally infused oxytocin induces excessive self-grooming (Amico et al., 2004). Up-coming studies should therefore evaluate the effect of the combination of social stimulus and NMU infusion on the central oxytocin levels.

A link between NMU-NAc-dopamine-grooming was established in mice exposed to social cues as the partial dopamine D2 receptor agonist, rather than D1/D5 antagonist, attenuates the NMU-induced self-grooming behavior and as infusion of NMU into the NAc shell, but not

DMS, increases grooming behavior. In addition repeated systemic administration of NMU, known to penetrate the brain (Gevaert et al., 2016) and affect centrally mediated behaviors including food and alcohol intake (Kaisho et al., 2017; Peier et al., 2011)(Supplementary material 1), elevates the *ex vivo* dopamine levels and decreases the expression of dopamine D2 receptors in NAc. This collectively may indicate that NMU increases accumbal dopamine in NAc shell only when exposed to social stimuli and that NMU exhibit specific modulatory control in NAc shell during grooming behavior. In support are the findings that elevated dopamine levels induce repetitive behaviors in both humans and rodents (for reviews see (Buse et al., 2013; Denys et al., 2004; Kalueff et al., 2016; Nguyen et al., 2014; Wood and Ahmari, 2015)) and that blockade of accumbal dopamine D2 receptors prevents oxytocin induced self-grooming (Drago et al., 1986). On the other hand, NMU attenuates the dopamine release in NAc shell following exposure to addictive drugs, without altering the rodent's gross-behavior (Vallof et al., 2016, 2017), indicating that the NMU-dopamine-grooming link only is evident in some contexts. This difference should be studied in further detail in upcoming studies. It should be taken into consideration that mice from the *ex vivo* analysis was injected repeatedly and systemically with NMU while the self-grooming behavior was evaluated after acute central infusions of NMU. Thus, upcoming studies should compare the effects of central versus systemic administration of NMU on self-grooming behavior as well as neurotransmission. It should also be mentioned that aripiprazole, a dopamine D2 receptor partial agonist approved to reduce repetitive symptoms in patients with Tourette syndrome, obsessive-compulsive disorders and autism-spectrum disorders (for reviews see (Buse et al., 2013; Denys et al., 2004; Nguyen et al., 2014; Wood and Ahmari, 2015)), also has affinity to dopamine D3, D4, $5HT_{1a}$,$5HT_{2a}$,$5HT_{2c}$, $5HT_7$, α_1 and $histamine_1$ receptors (Uzun et al., 2005; Winans, 2003), which thus may be involved in the pharmacological attenuation of the NMU-induced repetitive behaviors. However, this appears less likely since those other receptors have not yet been studied or associated with excessive self-grooming behavior in mice (for review see (Kalueff et al., 2016)). As elevated dopamine levels are associated with repetitive behaviors in mice and men (for reviews see (Buse et al., 2013; Denys et al., 2004; Nguyen et al., 2014; Wood and Ahmari, 2015)) and that NMU herein was shown to elevate dopamine in NAc shell, aripiprazole should be considered as a dopamine D2 receptor antagonist. However, the use of a partial agonist, rather than antagonist, could be considered as a tentative limitation in the present study.

The mechanisms underlying excessive self-grooming induced by NMU may be mediated via a disinhibition of the dopamine D2 indirect pathway in NAc shell (Ebihara et al., 2017; Zhang et al., 2014) as a result of the ability of NMU to reduce the accumbal GABA levels via activation of NMUR2 expressed on presynaptic GABAergic axons in NAc shell (Kasper et al., 2016). To trigger this grooming response an olfactory social context is required. Both social interaction (Beny-Shefer et al., 2017) and oxytocin (Hung et al., 2017) triggers dopamine release in NAc shell likely through both overlapping and parallel circuits. We speculate that this dopamine release together with decreased GABA release induced by NMU trigger this system over a threshold leading to repetitive self-grooming via the dopamine D2 indirect pathway. As the data revealing an expression and localisation of NMUR2 on presynaptic GABA neurons is conducted in rats (Kasper et al., 2016, 2018), whereas the present findings are from mice the possibility should be considered that different NMU dependent mechanisms may exist between species. However, it seems unlikely since NMU infused into NAc shell attenuates alcohol-mediated behaviors in both mice as well as rats (Vallof et al., 2019). In further support for a physiological role of NMUR2 in mouse NAc shell are the findings that NMU into NAc shell reduces amphetamine-induced locomotor stimulation (Vallof et al., 2016) and decreases peanut butter intake in mice (Vallof et al., 2019).

Past studies have established that CRH receptors modulate stress responses including self-grooming and face washing following central infusion of high NMU doses in mice not exposed to social cues (Gartlon et al., 2004; Hanada et al., 2001; Wren et al., 2002; Zeng et al., 2006). We show here that pre-treatment with a CRH receptor antagonist did not affect the ability of a low NMU dose to increase self-grooming in an olfactory social context, suggesting that the obtained data are independent of stress. This is in line with the established theory that lower as well as intermediate NMU doses do not activate the stress system via CRH signaling (Egecioglu et al., 2009; Howard et al., 2000; Ida et al., 2005; Vallof et al., 2016, 2017). Therefore the novelty of our data compared to these previous studies lie in the fact that we used a lower dose of NMU together with a social stimulus, that we identified that this behavioral response comprise dopamine as well as oxytocin without involvement of the CRH-system. Previous studies have established that lower doses of NMU reduce anxiety when measured with elevated plus maze in mice (Telegdy and Adamik, 2013). However, anxiety do not appear to influence our present data since we show that NMU do not affect excessive digging in the marble-burying test which is considered to be an anxiety-provoking context (for review see (de Brouwer et al., 2019)). In further support for this notion is the data showing that our intermediate dose does not affect anxiety in the elevated plus maze test (Telegdy and Adamik, 2013).

The current experiments display that NMU neither influences sexual motivation or copulation in sexually naïve mice, which are considered natural rewards mandated via the mesolimbic dopamine system (for review see (Hull et al., 2004)). This was rather surprising since NMU modulates reward induced by alcohol, amphetamine and cocaine as well as reduces alcohol drinking in rodents via NMUR2 in NAc shell in non-social environments (Kasper et al., 2016; Vallof et al., 2016, 2017). A tentative explanation to this discrepancy could be that NMU modulates accumbal driven behaviors differently, dependent on social status. In support are the data showing that social environment status profoundly alters the composition as well as function of accumbal neurons thus contributing to resilience and vulnerability to neuropsychiatric disorders (Scala et al., 2018; Zhang et al., 2016). The possibility that other doses of NMU might affect sexual behavior and that NMU alter sexual behavior in sexually experienced male mice should be considered.

In summary, the present experiments show that NMU induces self-grooming behavior in mice exposed to other mice or olfactory social-cues, but not in non-social environments, which may be linked to an activation of oxytocin receptors. Moreover, in these social contexts an association between accumbal dopamine signaling and NMU-induced self-grooming behavior is observed. The findings that excessive self-grooming behaviors in rodents are translated to human compulsions in psychiatric disorders like Tourette syndrome, obsessive-compulsive disorders and autism-spectrum disorders (for review see (Kalueff et al., 2016)) raises the possibility that accumbal NMU signaling might be a contributing mechanism in development of such disorders. Finally, the various experiments conducted have attributed to a deeper understanding of the neurobiological mechanism behind NMU-induced self-grooming behavior in a social context, a novel pharmacological effect of this anorexigenic peptide.

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Author contribution

EJ Designed research; JV, ALK, ES Performed experiments; JV, ALK,

EJ and LW Analyzed data; JV, EJ, LW Wrote the paper. All authors contributed to and have approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2019.107818.

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