

ORIGINAL ARTICLE

Metabotropic glutamate receptor subtype 7 controls maternal care, maternal motivation and maternal aggression in mice

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Abstract

The group III metabotropic glutamate receptor subtype 7 (mGlu7) is an important regulator of glutamatergic and GABAergic neurotransmission and known to mediate emotionality and male social behavior. However, a possible regulatory role in maternal behavior remains unknown to date. Adequate expression of maternal behavior is essential for successful rearing and healthy development of the young. By understanding genetic and neural mechanisms underlying this important prosocial behavior, we gain valuable insights into possible dysregulations. Using genetic ablation as well as pharmacological modulation, we studied various parameters of maternal behavior in two different mouse strains under the influence of mGlu7. We can clearly show a regulatory role of mGlu7 in maternal behavior. Naïve virgin female C57BL/6 mGlu7 knockout mice showed more often nursing postures and less spontaneous maternal aggression compared to their heterozygous and wildtype littermates. In lactating C57BL/6 wildtype mice, acute central activation of mGlu7 by the selective agonist AMN082 reduced arched back nursing and accelerated pup retrieval without affecting maternal aggression. In addition, in lactating CD1 wildtype mice the selective mGlu7 antagonist XAP044 increased both pup retrieval and maternal aggression. With respect to receptor expression levels, mGlu7 mRNA expression was higher in lactating vs virgin C57BL/6 mice in the prefrontal cortex, but not hypothalamus or hippocampus. In conclusion, these findings highlight a significant role of the mGlu7 receptor subtype in mediating maternal behavior in mice. Region-dependent studies are warranted to further extend our knowledge on the specific function of the brain glutamate system in maternal behavior.

KEYWORDS

AMN082, knockout, metabotropic glutamate receptor, social behavior, spontaneous maternal behavior, strain difference, XAP044

1 | INTRODUCTION

L-Glutamate is the major excitatory neurotransmitter essential in many integrative brain functions.¹ While ionotropic glutamate receptors (iGlu)

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mediate rapid neuronal excitation, the metabotropic glutamate receptors (mGlu) produce a more modulatory effect allowing fine tuning of glutamate responses.¹⁻³ The eight known mGlu subtypes can be classified into three groups⁴ according to sequence homology, second messenger coupling and pharmacological profiles.⁵ Group III thereby represents the largest family of mGlu receptors and comprises the subtypes mGlu4, -6, -7 and -8, which are predominantly expressed presynaptically^{6,7} to regulate neurotransmitter release and synaptic plasticity among other functions.² The *Gm7* gene encodes the mGlu7 subtype, which shows the highest degree of evolutionary conservation and is the most widely distributed mGlu family member. Mainly localized in the presynaptic zone of glutamate and GABA neurons,^{8,9} mGlu7 is thought to be an important regulator of glutamatergic and GABAergic neurotransmission with low affinity for glutamate and, thus, becomes activated by excessive glutamate release during very high synaptic activity.¹⁰⁻¹² Importantly, due to its regulatory role of both glutamatergic and GABAergic neurotransmission in emotion and cognition circuitries in the brain, mGlu7 is thought to be a critical factor in the development of psychiatric and neurological disorders.^{10,13} So far, most studies using genetic and pharmacological approaches focused on the role of mGlu7 in fear, anxiety and other stress-related mental and comorbid somatic disorders,¹⁴⁻¹⁶ whereas only few studies investigated the role of mGlu7 in complex social behaviors like intermale aggression. Here, both mGlu7 activation via a selective agonist as well as genetic ablation reduced aggressive behavior toward a conspecific,^{12,17} while intermale sexual behavior even increased in mGlu7 knockout (KO) males.¹⁷

To date, the modulatory role of mGlu7 in the most important prosocial behavior, namely maternal behavior, remains unknown. Its adequate expression ensures the well-being and proper development of the offspring.^{18,19} During the peripartum period, a wide variety of adaptations occur, like increased activity of the brain oxytocin and vasopressin systems (reviewed in^{20,21}). Although maternal behavior occurs mainly in the lactation phase of rodents, virgin female mice display spontaneous maternal behavior within the first minutes after initial pup exposure.²² It is suggested that pup stimuli modify neural circuits regulating maternal responsiveness.²³ Thus, circuits involved in pup-avoidance behavior are downregulated, while circuits for approach behavior are upregulated.²⁴ Such circuits include brain regions like the medial preoptic area (MPOA) and the basolateral/basomedial part of the amygdala, main regulatory sites for maternal behavior^{18,21} and conditioned reward processes of pup stimuli,²⁵ respectively. Moreover, mother-pup interactions are associated with the mesolimbic dopamine system and an interaction with the MPOA, critical for maternal responsiveness and the consolidation of maternal experience²⁶ and reward.²⁷ Interestingly, reward-related brain regions show a high density of mGlu7 expression.¹⁴ In addition, high expression of mGlu7 is also present in the prefrontal cortex (PFC), a brain region not only linked to stress, emotional control and mood regulation,^{28,29} but also to sexual and maternal behavior^{30,31} as well as postpartum mental illnesses.³² Notably, in women suffering from postpartum depression glutamate levels were highly increased within the medial PFC.³³ Together, these findings suggest a potential implication of mGlu7 in the regulation of maternal behavior.

In the present study, we aimed to show the specific role of the mGlu7 subtype in maternal care, maternal motivation to retrieve pups and

maternal aggression toward a conspecific intruder in mice. Firstly, we investigated spontaneous maternal behavior in naïve virgin female C57BL/6 mGlu7 KO mice and their heterozygous (HET) and wildtype (WT) female littermates. Additionally, we centrally manipulated mGlu7 via pharmacological modulation in lactating mice with subsequent behavioral observations. Using lactating C57BL/6 mice, we acutely activated mGlu7 by intracerebroventricular (*icv*) infusion of AMN082 (N,N'-dibenzhydriethane-1,2-diamine dihydrochloride), the first mGlu7-selective allosteric agonist.^{34,35} To test the ubiquitous role of mGlu7 in maternal behavior, we aimed to verify our findings in a different mouse strain; we used lactating CD1 mice, which were acutely infused *icv* with either AMN082 or the mGlu7-selective full antagonist XAP044 (7-hydroxy-3-[4-iodophenoxy]-4H-chromen-4-one³⁶). Finally, we compared virgin vs lactating C57BL/6 mice with respect to their mGlu7 mRNA expression levels in the hypothalamus (HT), hippocampus (HC) and PFC.

2 | MATERIALS AND METHODS

2.1 | Animals

All animals were kept in groups of up to 6 mice under standard laboratory conditions (12:12 hours light-dark cycle, lights on at 0600 hours, 50 ± 5% rel. humidity, 22 ± 2°C) with access to water and food *ad libitum*. Experiments were performed between 0800 hours and 1400 hours. All experimental protocols were approved by the Committee on Animal Health and Care of the local government and conformed to international guidelines on the ethical use of animals and arrive guidelines.³⁷ All efforts were made to minimize the number of animals used and their suffering.

In experiment 1, virgin female mGlu7 KO (mGlu7^{-/-}) mice and their HET (mGlu7^{+/-}) and WT (mGlu7^{+/+}) female littermates (C57BL/6J background, age 7-9 weeks, own breeding, University of Regensburg, DE) were tested. In experiments 2 and 4, C57BL/6N females (age 9-10 weeks, Charles River, Sulzfeld, DE), and in experiment 3, CD1 females (age 9-10 weeks, Charles River) were used. For the mating procedure, virgin female mice were co-housed with sexually experienced C57BL/6N or CD1 males (own breeding). Pregnancy was confirmed by the presence of sperm in the vaginal smears (pregnancy day [PD] 1). From PD 10 on, females were handled daily, underwent stereotaxic surgery on PD 18 and were single-housed in observation cages (25 × 32 × 37 cm). On PD 21, females gave birth and litters were culled to four pups of mixed sexes.

2.2 | Experimental design

2.2.1 | Experiment 1: Effects of mGlu7 KO on spontaneous maternal behavior and maternal aggression in virgin female mice

One day prior to behavioral testing, virgin female mGlu7 KO mice and their virgin female HET and WT littermates were single-housed in

mouse observation cages (25 × 32 × 37 cm; made in-house). Subsequently, spontaneous maternal care was measured in the presence of three unfamiliar pups. Furthermore, spontaneous maternal aggression toward an unfamiliar male intruder was scored in the maternal defense (MD) test in a separate set of virgin mGlu7 WT, HET and KO mice.

2.2.2 | Experiment 2: Effects of acute pharmacological activation of central mGlu7 in lactating C57BL/6 mice

Female C57BL/6 mice implanted with an *icv* guide cannula on PD 18 were randomly assigned to vehicle (VEH; 2 μ L 0.01% DMSO) or AMN082 treatment (30 μ M/2 μ L VEH; Tocris Bioscience, Bristol, UK; dose based on literature¹¹). On lactation day (LD) 1, maternal care was scored. On LD 2, the pup retrieval test (PRT) was performed to measure maternal motivation. On LD 4, females were tested in the MD test to measure maternal aggression toward an unknown male intruder.

2.2.3 | Experiment 3: Effects of acute pharmacological activation or inhibition of central mGlu7 in lactating CD1 mice

To extend our studies to another mouse strain, female CD1 mice were implanted with an *icv* guide cannula on PD 18 and randomly assigned to VEH (2 μ L 0.01% DMSO or 2 μ L 5% DMSO), AMN082 (30 μ M/2 μ L 0.01% DMSO) or XAP044 treatment (100 μ M/2 μ L 5% DMSO; Tocris Bioscience; doses based on literature^{11,36}). All behavioral tests were performed as described in experiment 2.

2.2.4 | Experiment 4: Expression pattern of mGlu7 mRNA in virgin vs lactating C57BL/6 mice

In order to determine lactation-associated changes, we compared the relative mRNA levels of mGlu7 within the HT, HC and PFC of virgin vs lactating C57BL/6 mice.

2.3 | Behavioral tests

2.3.1 | Maternal care

In experiment 1, spontaneous maternal care was measured 1 day after single housing by placing three unfamiliar pups (age between postnatal day 1 to 3; derived from female C57BL/6 WT mice) in the corner of the homecage opposite of the nest of the experimental mouse. The occurrence of spontaneous maternal behavior was scored every minute for 120 minutes. In experiments 2 and 3, maternal care was measured on LD 1 in the homecage every minute for 60 minutes, followed by acute treatment infusion and scoring continued for further 120 minutes. The main parameter for the quality of maternal care

was the occurrence of arched back nursing (ABN), the only active nursing posture, where the mother is engaged in a quiescent kyphosis.^{19,38} Total nursing (the sum of all nursing postures including ABN, hovering and blanket posture) as well as licking and grooming (LG) the pups was used as an indicator for the quantity of maternal care.

2.3.2 | PRT

In experiments 2 and 3, maternal motivation to retrieve the pups was tested in the PRT on LD 2 as described earlier.³⁹ Briefly, pups were separated from their mother 60 minutes prior to testing. Dams received treatment infusion 10 minutes prior to the PRT. Before the test started, the mother was removed from the homecage and four pups were placed in each corner of the homecage. After reintroducing the mother, the latency to retrieve each pup was scored for 5 minutes.

2.3.3 | MD test

In experiment 1, aggressive behavior toward an intruder male mouse was assessed in virgin mGlu7 KO mice and their HET and WT littermates following 120 minutes of cohabitation with three stimulus pups. In experiments 2 and 3, on LD 4 lactating mice were centrally injected with the assigned treatment 10 minutes prior to the MD test, where an unknown male intruder was placed in the homecage of the females in presence of the pups. The behavior was videotaped and following parameters scored by an experienced observer blind to treatment: attack latency, number of attacks, attack duration, total time spent on offensive (including threat behaviors) and maternal behavior.⁴⁰

2.4 | Implantation of *icv* guide cannula

On PD 18, female C57BL/6 and CD1 mice were stereotaxically implanted with a guide cannula (21G, 8 mm length, stainless steel) targeting the right lateral ventricle (relative to bregma: 0.2 mm posterior, 1.0 mm lateral, 1.4 mm depth) under isoflurane anesthesia (Baxter GmbH, Unterschleißheim, DE) and body temperature controlled conditions as described earlier.³⁹ The guide cannula was fixed to the skull using two stainless steel screws and dental cement (Kallocryl, Speiko, Dr. Speier GmbH, München, DE) and closed with a stainless steel stylet. Immediately after surgery, mice underwent antibiotic treatment (s.c., 1.25 mg in 50 μ L, enrofloxacin, Baytril, Bayer Vital GmbH, Leverkusen, DE).

2.5 | Tissue isolation and RNA purification

Total RNA was isolated from brain tissue of virgin and lactating (LD 4) female C57BL/6 mice using Trizol reagent according to manufacturer's instructions (Peqlab, Erlangen, DE). cDNA was prepared from 500 ng of total RNA in a reverse transcription reaction mixture

(Superscript III, Invitrogen, Karlsruhe, DE). Expression levels of mGlu7 and GAPDH mRNA were measured using quantitative PCR (SYBR[®] Green Master Mix; ABI 7500 Fast Sequence Detection System, Applied Biosystems, Darmstadt, DE). Therefore, the following primer sequences were used: mGlu7-forward: 5'-GCAGAAGGAGCCATCA CCAT-3'; mGlu7-reverse: 5'-GTCCGGGATGTGAAGTAAGCA-3'; GAPDH-forward: 5'-TGTGCTCGTCGGATCTGA-3'; GAPDH-reverse: 5'-CCTGCTCACCACCTTCTTGA-3'. Cycling profile 20 seconds at 95°C, 40 cycles 3 seconds at 95°C and 30 seconds at 60°C. Changes in gene expression were determined according to the $2^{-\Delta\Delta CT}$ method^{41,42} by using GAPDH as housekeeping gene for normalization. Virgin animals served as control group.

2.6 | Statistics

Statistical analyzes were performed using SPSS 25.0 (IBM, Armonk, NY). One-way (factor treatment, genotype) or two-way (factors

time × genotype, time × treatment) analysis of variance (ANOVA) for repeated measures followed by Bonferroni *post hoc* testing or independent Student *t* test (mRNA expression levels and separate statistics) were used. All data are presented as mean + SEM. Significance was accepted at $P \leq .05$.

3 | RESULTS

3.1 | Virgin female mGlu7 KO mice displayed increased spontaneous maternal care and reduced spontaneous maternal aggression

During the 120 minutes observation period, the genotypes differed in the display of ABN (one-way ANOVA: $F_{2,48} = 4.444$; $P = .017$) and total nursing positions ($F_{2,48} = 5.499$; $P = .007$; Figure 1A), whereas LG was not changed ($F_{2,48} = 0.881$; $P = .421$; data not shown). In detail, *post hoc* analysis showed higher occurrence of ABN and total

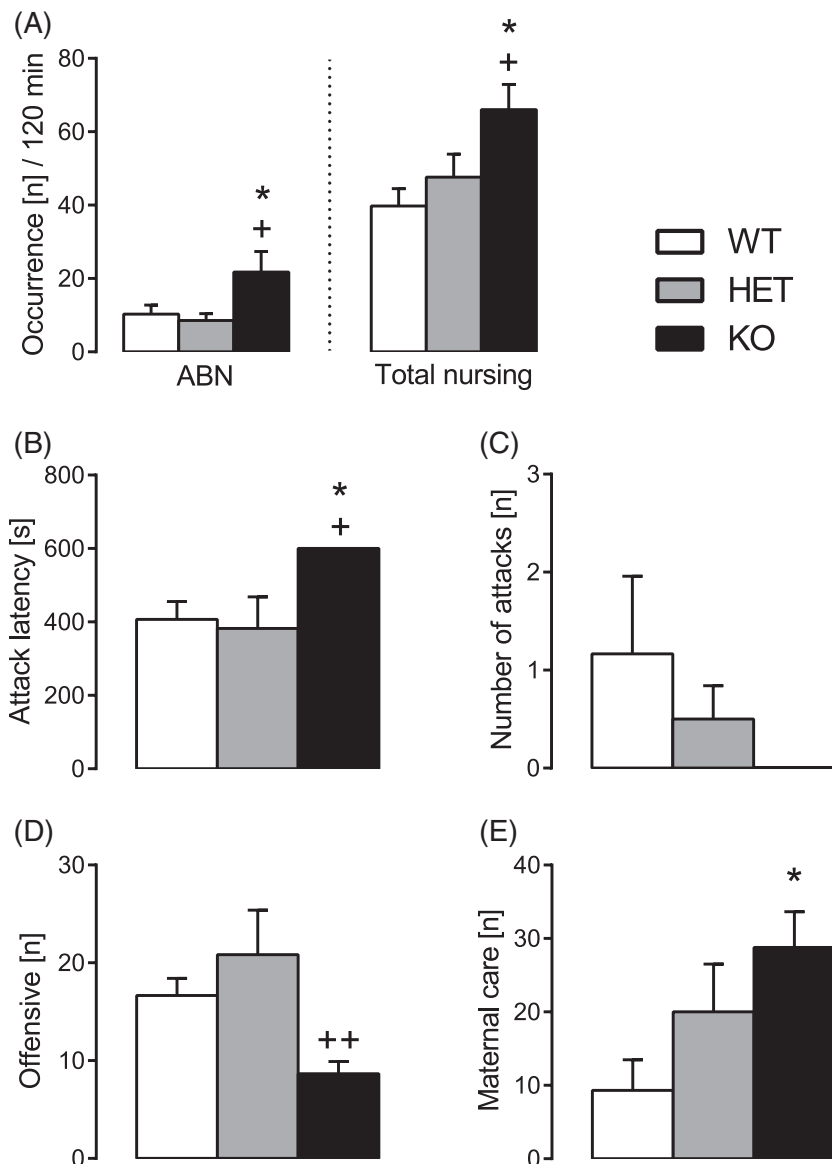


FIGURE 1 Virgin mGlu7 KO mice displayed increased spontaneous maternal care and reduced spontaneous maternal aggression. In virgin mGlu7 KO mice, A, spontaneous maternal care was increased, indicated by an enhanced occurrence of ABN and total nursing within 120 minutes of observation compared to HET and WT females. In contrast, B-E, spontaneous maternal aggression was reduced in mGlu7 KO mice indicated by, B, a higher latency to attack and, D, less offensive behavior compared to HET and WT, whereas, C, the number of attacks remained unchanged. In line with the findings presented in A, E, mGlu7 KO mice spent more time with maternal care compared to WT females even during the MD test. ABN, arched back nursing; HET, heterozygous; MD, maternal defense; WT, wildtype. Data represent mean + SEM. * $P \leq .05$, ** $P \leq .01$ vs HET; * $P \leq .05$, ** $P \leq .01$ vs WT; $n = 11-15$ per group

nursing positions in mGlu7 KO mice compared to HET (ABN: $P = .032$; total nursing: $P = .040$) and WT (ABN: $P = .048$; total nursing: $P = .010$).

Assessing spontaneous maternal aggression during the MD test, there was a significant effect of the genotype with respect to attack latency (one-way ANOVA: $F_{2,18} = 6.760$; $P = .006$; Figure 1B), offensive behavior ($F_{2,18} = 6.233$; $P = .009$; Figure 1D) and maternal behavior ($F_{2,20} = 3.502$; $P = .05$; Figure 1E), but not number of attacks ($F_{2,18} = 1.977$; $P = .167$; Figure 1C). In detail, mGlu7 KO had a longer attack latency compared to HET ($P = .013$) and WT ($P = .03$), showed less offensive behavior compared to HET ($P = .010$) and more maternal care compared to WT ($P = .05$).

3.2 | Acute AMN082-induced activation of central mGlu7 decreased maternal care and increased maternal motivation in lactating C57BL/6 mice

On LD 1, the occurrence of ABN was affected by time (two-way ANOVA for repeated measures: $F_{5,60} = 2.622$; $P = .033$) but not by treatment ($F_{1,12} = 2.895$; $P = .115$) nor was an interaction detected ($F_{5,60} = 1.884$; $P = .110$). However, separate statistics showed reduced ABN 60 minutes after AMN082 injection compared to VEH-treated females (Student t test: $t_{(12)} = 2367$; $P = .036$; Figure 2A). Total nursing was found to depend on time ($F_{5,60} = 3.884$; $P = .004$) but not on treatment ($F_{1,12} = 0.486$; $P = .499$) nor on interaction of time \times treatment ($F_{5,60} = 0.542$; $P = .743$; data not shown). The display of LG was not affected at all (time: $F_{5,60} = 2.302$; $P = .056$; treatment: $F_{1,12} = 0.257$; $P = .621$; interaction: $F_{5,60} = 0.220$; $P = .952$; data not shown).

In the PRT on LD 2, the number of retrieved pups was dependent on time ($F_{10,140} = 51.709$; $P \leq .001$) and treatment ($F_{1,14} = 97.072$; $P \leq .001$) without an interaction of treatment \times time ($F_{10,140} = 0.804$; $P = .625$; Figure 2B). *Post hoc* analysis showed that AMN082-treated dams retrieved their pups faster within the first 60 seconds of the PRT compared to VEH-treated dams (30 seconds: $P = .026$; 60 seconds: $P = .05$).

In the MD test on LD 4, maternal aggression was not affected by AMN082 treatment (Student t test: $t_{(11)} = 1.145$; $P = .277$; data not shown); neither VEH- nor AMN082-treated females showed aggressive behavior toward the intruder, which might be due to the generally low level of maternal aggression in this strain.^{43,44}

3.3 | Acute XAP044-induced blockade of central mGlu7 increased maternal motivation and maternal aggression in lactating CD1 mice

Maternal behaviors were assessed after pharmacological activation or inhibition of mGlu7 by AMN082 or XAP044, respectively. On LD 1, neither ABN (one-way ANOVA: $F_{2,24} = 1.896$; $P = .172$) nor total nursing ($F_{2,24} = 0.567$; $P = .574$; Figure 3A) were affected by any treatment.

In the PRT on LD 2, the number of retrieved pups depended on time (two-way ANOVA for repeated measures: $F_{10,240} = 85.250$;

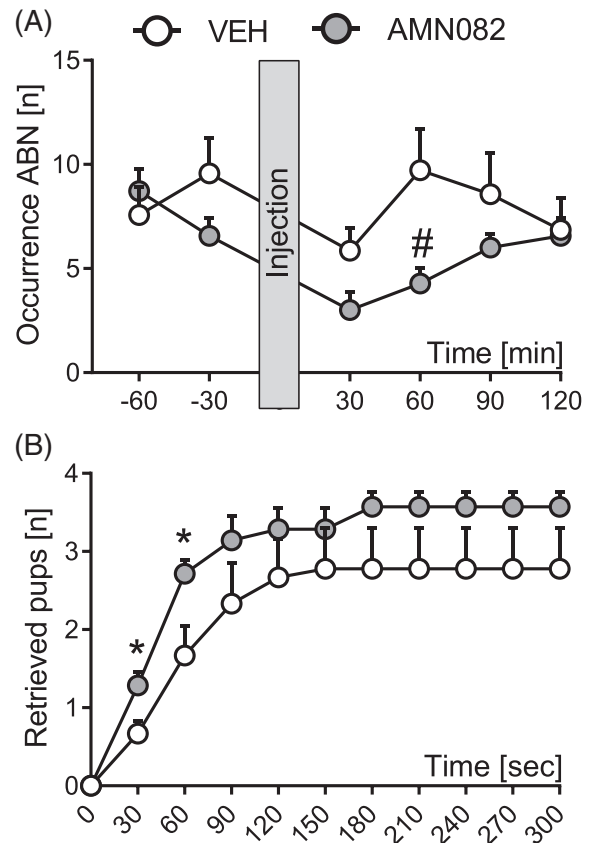


FIGURE 2 Acute pharmacological activation of central mGlu7 using AMN082 injection decreased maternal care and increased maternal motivation in lactating C57BL/6 mice. Maternal care was observed 60 minutes prior to injection as well as 120 minutes afterwards. While basal levels remained unchanged, AMN082 treatment, A, reduced ABN behavior 60 minutes after injection in lactating females compared to VEH-treated mice. In contrast, B, in the PRT AMN082-treated mice retrieved the pups faster compared to VEH-treated females. ABN, arched back nursing; PRT, pup retrieval test; VEH, vehicle. Data represent mean + SEM. # $P \leq .05$ vs VEH (separate statistics: Student t test); * $P \leq .05$ vs VEH; $n = 7-9$ per group

$P \leq .001$) and treatment ($F_{2,24} = 4.725$; $P = .019$), with an interaction of time \times treatment ($F_{20,240} = 2.329$; $P = .001$; Figure 3B). *Post hoc* analysis showed a higher number of retrieved pups in XAP044-treated compared to AMN082-treated mice in the period of 150 seconds to 300 seconds (for each $P \leq .05$) and compared to VEH-treated females in the period of 240 seconds to 300 seconds (for each $P \leq .05$; at 210 seconds: $P = .06$).

In the MD test on LD 4, there was a main treatment effect on attack duration (one-way ANOVA: $F_{2,20} = 6.256$; $P = .008$; Figure 3C) and offensive behavior ($F_{2,20} = 15.739$; $P \leq .001$; Figure 3D), while the attack latency ($F_{2,20} = 1.950$; $P = .168$; data not shown) and maternal care ($F_{2,20} = 2.425$; $P = .114$; data not shown) were not affected. *Post hoc* tests showed an increase in attack duration and offensive behavior in XAP044-treated dams compared to the AMN082-treated (attack duration: $P = .010$; offensive: $P \leq .001$) and VEH-treated dams (attack duration: $P = .009$; offensive: $P \leq .001$).

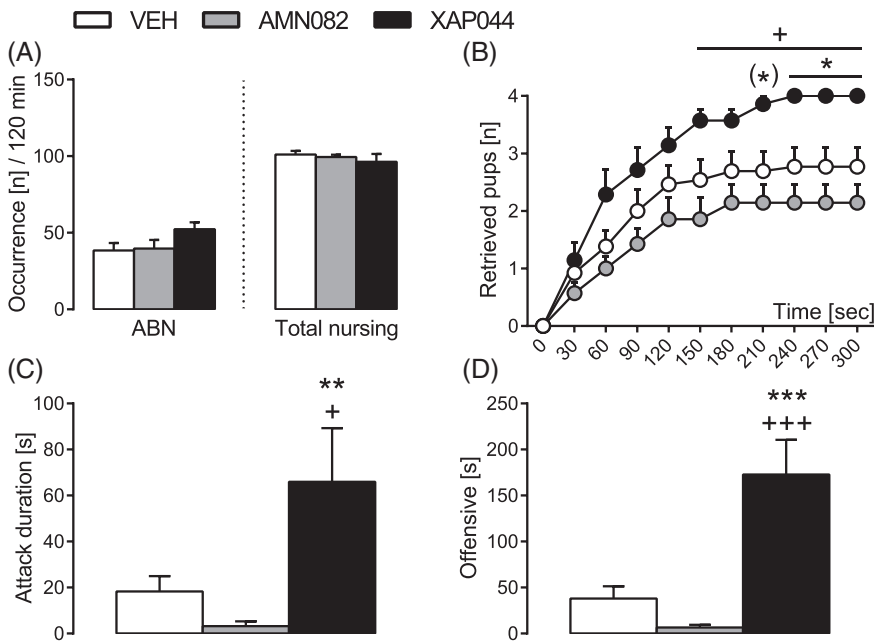


FIGURE 3 Acute pharmacological inhibition of central mGlu7 using XAP044 injection increased maternal motivation and maternal aggression in lactating CD1 mice. A, Central mGlu7 inhibition by XAP044 or activation by AMN082 in lactating females did not change maternal care, whereas, B, XAP044 treatment increased the number of pups retrieved in the PRT. In the MD test, acute XAP044 treatment increased, C, attack duration and, D, offensive behavior compared to VEH- and AMN082-treated females. MD, maternal defense; PRT, pup retrieval test; VEH, vehicle. Data represent mean + SEM. (*) $P = .06$, * $P \leq .05$, ** $P \leq .01$, *** $P \leq .001$ vs VEH; + $P \leq .05$, +++ $P \leq .001$ vs AMN082; $n = 7-13$ per group

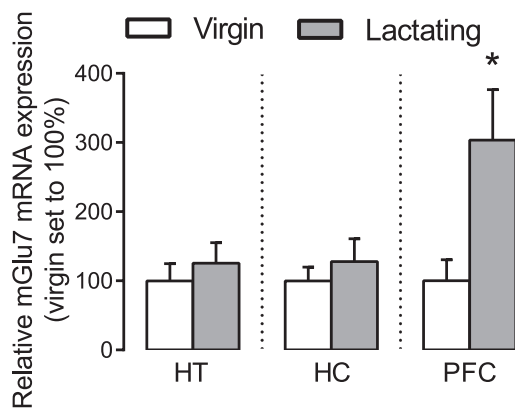


FIGURE 4 mGlu7 mRNA expression was increased in the PFC of lactating C57BL/6 mice. Measuring molecular changes in the expression levels across brain areas of mGlu7 in virgin vs lactating mice on LD4, mGlu7 was found to be increased in the PFC but not HT or HC. HC, hippocampus; HT, hypothalamus; PFC, prefrontal cortex. Data represent mean + SEM. * $P \leq .05$ vs virgin; $n = 6-9$ per group

3.4 | Region-specific increase of mGlu7 mRNA expression in lactating C57BL/6 mice

The mRNA expression of mGlu7 was significantly increased in lactating vs virgin C57BL/6 mice in the PFC (Student t test: $t_{(17)} = -2.680$; $P = .016$) but not in the HT ($t_{(15)} = -0.668$; $P = .514$) or HC ($t_{(21)} = -0.754$; $P = .459$; Figure 4).

4 | DISCUSSION

While mGlu7 has been studied in the context of emotion and cognition, only few studies investigated the role of mGlu7 in complex social behavior

thereby mainly focusing on intermale aggression.^{12,17} However, a potential involvement of the mGlu7 receptor subtype in female social behavior in general, and in maternal behavior as the most important prosocial female behavior in particular, has not been investigated to date.

In the present study, we show a critical involvement of mGlu7 in the control of maternal care, maternal motivation and maternal aggression in mice, using genetic and pharmacological approaches. In detail, mGlu7 KO mice displayed increased spontaneous maternal care and concurrent decreased spontaneous maternal aggression against a male intruder compared to their WT and HET virgin littermates. Moreover, pharmacological modulation of mGlu7 in lactating C57BL/6 females using the allosteric agonist AMN082 decreased maternal care while it increased maternal motivation. In lactating CD1 females, treatment with the mGlu7 antagonist XAP044 increased maternal motivation and maternal aggression while maternal care remained unaffected. Beyond that, we show a brain-region dependent increase of mGlu7 mRNA expression levels in the PFC of lactating compared to virgin C57BL/6 mice, indicating region-specific modulation of the mGlu7 gene during lactation.

To address potential effects of mGlu7 genetic ablation on spontaneous maternal behavior we used virgin mGlu7 KO mice and their HET and WT female littermates. Importantly, while spontaneous maternal behavior requires previous pup-experience in rats,⁴⁵ virgin female mice immediately care for foreign pups.²² Here, genetic lack of mGlu7 resulted in increased spontaneous maternal care, that is, display of ABN and total nursing positions. This suggests that the neural circuits regulating maternal responsiveness are highly sensitive to pup stimuli in mice. In accordance with these findings in virgin mGlu7 KO mice, in lactating C57BL/6 dams central activation of the receptor using the mGlu7-selective agonist AMN082 decreased ABN during maternal care observation. This is in line with previous studies showing the ability of AMN082 to modulate high frequency synaptic

transmission in the basolateral part of the amygdala,⁴⁶ which receives olfactory input, for example, from pups. In turn, these inputs can further activate projections from the medial amygdala causing avoidance behavior and other defensive responses in naïve virgin female rats.⁴⁷ An activation of mGlu7 in the amygdala by AMN082 treatment might therefore also inhibit pup-directed behaviors in lactating mice.

Regarding maternal motivation, lactating C57BL/6 females treated with AMN082 significantly retrieved their pups faster. Surprisingly, the same effect could be seen in lactating CD1 mice after mGlu7 inhibition by XAP044, whereas AMN082 treatment had no effect. These contrary results in C57BL/6 and CD1 females might underlie differently regulated glutamate levels between the strains, similar to the differentially regulated release of the neurotransmitter acetylcholine.⁴⁸ Furthermore, in the nucleus accumbens, a brain region highly involved in maternal motivation,⁴⁹ AMN082 dose-dependently modulated both glutamate and GABA neurotransmission and can therefore act excitatory or inhibitory.⁵⁰ In line with this, presynaptic mGlu7 also acts as an auto- and heteroreceptor regulating glutamate and GABA release, respectively.⁵¹ Thus, activation or inhibition of mGlu7 can change the balance of excitatory and inhibitory neurotransmitter release in the same way or differently, leading to similar or different social behavioral traits, respectively. However, the observed effects are not mediated via alterations in general activity of the females, because neither AMN082 nor XAP044 affects locomotor activity.^{36,52}

With respect to maternal aggression, genetic mGlu7 ablation caused decreased spontaneous maternal aggression, whereas blocking mGlu7 in lactating CD1 mice increased this behavior. These differences might be caused by the strain difference, as mGlu7 KO females have a C57BL/6 background. Secondly, as mGlu7 is likely to play an important role in maternal behavior, compensatory mechanisms might be in place in mGlu7 KO mice to buffer the lack of mGlu7 during development, which is in contrast to acute pharmacological inhibition. Furthermore, with respect to the innate system, in mGlu7 KO mice we tested spontaneous maternal behavior in virgin mice whereas CD1 females were lactating, that is, experiencing pregnancy leading to all necessary peripartum brain adaptations (reviewed in⁵³). Interestingly, in contrast to pharmacological inhibition of mGlu7 in lactating CD1 mice, pharmacological activation by AMN082 in the same strain tended to reduce maternal aggression. Our results are in line with previous studies showing a connection between the glutamate system and offensive aggressive behavior in rodents. For example, male mice lacking the iGlu AMPA receptor show less aggression toward an intruder⁵⁴ while pharmacological blockade of mGlu1 results in decreased offensive behavior.⁵⁵ Moreover, male mice lacking mGlu7 display a strong reduction in intermale aggression toward an intruder, probably caused by olfactory deficits.¹⁷ Regarding the lactating females of the present study, one might assume that reduced olfactory inputs from, for example, pups might inhibit pup-directed behavior in mGlu7 KO mice leading to reduced maternal aggression. While this would be in line with the decrease in maternal aggression in lactating rats following olfactory bulbectomy,⁵⁶ in female mice an impaired olfaction was shown to strongly reduce maternal care.^{57,58} Importantly, this was not the case in our mGlu7 KO females, suggesting either no impairment or a not effective impairment of the mothers' olfactory system.

Taken the behavioral data together, genetic and pharmacological modulation of the mGlu7 subtype differently control the various components of maternal behavior depending on the mouse strain and their reproductive status. Comparable complex results were found in a study on iGlu in mice.⁵⁹ Here, blocking glutamatergic signaling via iGlu-directed inhibition within the dorsal raphe nucleus (DRN), a brain region regulating maternal behavior in rodents,^{60,61} leads to increased maternal aggression but not maternal care. In contrast, iGlu-directed activation of neuronal activity in the DRN enhances maternal care.⁵⁹ These data are in line with our findings and indicate an alternatively regulated glutamate system in different aspects of maternal behavior.

Assessing the influence of the reproductive status of female mice on mGlu7 mRNA transcript levels suggests region-specific modulation of the mGlu7 gene during lactation; mGlu7 mRNA was increased only in the PFC but not in the HT or HC of lactating compared to virgin C57BL/6 mice. The PFC, among other brain regions, regulates social behavior like maternal motivation, nursing and aggressive behavior via sending glutamatergic input to the DRN.^{31,59} Therefore, an increased expression of mGlu7 in the PFC might be an important adaptation during lactation to ensure maternal behavior in mice. However, excessive levels of glutamate may in turn lead to a dysregulation of maternal behavior, as suggested by our data using AMN082 treatment, and even to maternal mental illness, as seen in humans.³³ Nevertheless, an increased expression of mGlu7 in the PFC of lactating vs virgin mice might also contribute to the differential effects of genetic ablation vs pharmacological blockade of mGlu7 on maternal behavior.

In conclusion, our data indicate that the glutamate system, especially the mGlu7 subtype, has a strong impact on the control of maternal behavior. While pharmacological activation of mGlu7 decreased maternal care but accelerated pup retrieval, inhibition of mGlu7 did not affect maternal care but increased maternal motivation and maternal aggression. In contrast, genetic ablation of mGlu7 increased maternal care and reduced maternal aggression, suggesting other mechanisms to compensate for the lack of mGlu7-mediated input to the regulation of maternal behavior. Future studies are warranted to investigate the specific function of the brain glutamate system in maternal behavior in distinct maternal brain regions including the MPOA, DRN and nucleus accumbens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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