Population Variation Altered Aggression-associated Oxytocin and Vasopressin Expressions in Brains of Brandt's Voles in Field Conditions

Shuli Huang
Institute of Zoology Chinese Academy of Sciences
Guoliang Li
Institute of Zoology Chinese Academy of Sciences
Yongliang Pan
Huzhou University
Jing Liu
Institute of Zoology Chinese Academy of Sciences
Jidong Zhao
CAS: Center for Excellence in Biotic Interactions, Chinese Academy of Sciences
Xin Zhang
Institute of Zoology Chinese Academy of Sciences
Wei Lu
Institute of Zoology Chinese Academy of Sciences
Xinrong Wan
Institute of Zoology Chinese Academy of Sciences
Charles J Krebs
Department of zoology, University of British Columbia
Zuoxin Wang
Florida State University Department of Psychology
Wenxuan Han
China Agricultural University College of Resources and Environmental Sciences
Zhibin Zhang (zhangzb@ioz.ac.cn)
Institute of Zoology Chinese Academy of Sciences https://orcid.org/0000-0003-2090-7999

Research

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Abstract

Density-dependent change in aggressive behavior is essential for regulating the population dynamics of many small rodents, but the underlying neurological mechanisms have not been examined in field conditions. We hypothesized that aggression-associated oxytocin (OT) and vasopressin (AVP) in specific regions of the brain may be closely related to aggressive behaviors and population changes of small rodents. In this study, we analyzed the associations of OT and AVP expression as well as aggressive behavior with population density of Brandt's voles in 24 large semi-natural enclosures (0.48 ha for each enclosure) in Inner Mongolia grassland. Then we tested the effects of population density on the OT/AVP system and aggressive behavior in experimentally manipulated populations of Brandt's voles in the semi-natural enclosures. High density was positively and significantly associated with more aggressive behavior, and increased expression of mRNA and protein of AVP and its receptor, but decreased expression of mRNA and protein of OT and its receptor in specific brain regions of the voles. Our study suggests that OT/AVP systems are important in regulating the density-dependent population dynamics via aggressive behavior of small rodents, and they can be used as indicators of population variation or density-dependent stressors.

Background

Understanding the mechanisms of population regulation in small mammals has long been a fundamental topic in population ecology [1, 2]. Hypotheses addressing population fluctuations and regulation in small rodents are generally classified into two categories: extrinsic or intrinsic hypotheses. Extrinsic hypotheses emphasize the role of climate [3], predators [4] and food [5] in causing population fluctuations, while intrinsic hypotheses emphasize the role of density-dependent genetics [6], physiology [7] and behavior [8, 9] . Both extrinsic and intrinsic factors can jointly contribute to population regulation in small mammals.

Density-dependence is well recognized in studies of population regulation driven by various intrinsic factors [10, 11]. The genetic regulation hypothesis [6], the physiological regulation hypothesis [7] and the social behavioral regulation hypothesis [8] are widely used to explain density-dependence in the population regulation of small rodents. The genetic hypothesis suggests that population density exerts selective pressure on different genotypes, favoring highly aggressive and low reproductive animals in high density, and vice versa. The behavioral regulation hypothesis suggests that territory defense, social rank and aggressive behavior play key roles in regulating populations; animals with low aggressive behavior or social rank suffer low reproduction and high mortality due to lack of resources[8]. The physiological regulation hypothesis suggests that high population density induces high aggression and social stress, which result in disorders of the endocrine system including increases in corticosterone (CORT) and glucose levels, decreases in growth or reproductive hormones, followed by a population crash or decline [7]. It is notable that all the three core hypotheses include the role of density-dependent aggressive behavior in regulating population fluctuations. Under laboratory condition, it is found that high density induced aggression or fighting could alter oxytocin (OT) and vasopressin (AVP) expressions in

brains of Brandt's voles [12], however, how population density affects aggressive behavior (and then population growth) of animals via neurological pathways has not been investigated in field conditions.

OT and AVP play a significant role in social recognition, aggression, parental care, mating [13-15]. Many previous studies have documented that OT, AVP and their receptors (i.e. OTR and AVPR) regulated aggressive behavior in mammals. High levels of aggression behavior in hamsters were closely associated with high AVP expression [16-18] . A high level of OT expression significantly decreased aggressive behavior in female rats (*Rattus norvegicus*)[19, 20].

Stress can change expression of AVP a nd OT and their releases [21], and then regulate the aggressive behavior of animals. In general, AVP could increase the stress responses, whereas OT could decrease stress responses [22, 23]. Population density of animals often fluctuates greatly in the field under the influence of climate, food, predators or parasites. High density is often accompanied by an increase in crowding, unfamiliar encounters and shortages of food or shelter, which may act as social or physiological stressors in animals. Our recent study indicates that under laboratory condition, high density as an environmental stressor could decrease OT expression, but increase AVP expression of Brandt's voles; fighting could decrease OT expression but increase AVP expression of voles[12]. In another experiment, injection of OT in brains reduced aggression of Brandt's voles, but injection OT and OTR antagonists increased aggression of Brandt's voles [24]. Injection of AVP in brains could increase aggression of several other rodent species [25-27]. Decrease of OT or increase of AVP could mediate stress and reproduction hormone [28-30].

Based on the current knowledge (Fig. 1), we hypothesize that variation in population density will be reflected in variation of AVP and OT in the brains of small rodents in field populations. High population density as a stressor would increase both crowding stress and aggressive stress due to the increased encounters with strangers, which would then increase AVP expression but decrease OT expression in brains of animals. The high density induced the change of OT and AVP, further increase aggression behaviors of animals, and again increased the social stress, which in return increased AVP expression but decreased OT expression. The high density induced reciprocal enhancement between OT/AVP and aggression behavior would promote the level of aggression and stressful neuro-peptides. High levels of aggression should negatively affect population growth rates due to increased mortality or reproductive failure caused by fighting or interference. High level AVP or low level OT would increase release of stressful hormone (CORT) or decrease of reproductive hormone (e.g. GnRH, FSH, LH), and then reduce the reproduction or survival of individuals.

Brandt's voles (*Lasiopodomys brandtii*) occur in the steppe grasslands of Inner Mongolia, China and Mongolia. They are social small herbivores which show large variations of population density in different years. Both extrinsic and intrinsic factors would affect their population fluctuations[31-33]. Our previous study on Brandt's voles in laboratory condition demonstrated that at high density, AVP expression increased, and OT expression decreased in association with the observed increase of aggression behavior[12]. We now test whether such associations occur in field conditions. We test our prediction that

high density will increase AVP expression but reduce OT expression, and increase aggressive behavior of Brandt's voles in large semi-natural field enclosures.

Materials And Methods

Field enclosure experiment 1

Since 2010, large manipulation experiments studying the population dynamics of Brandt's voles have been conducted at the Maodeng pasture (44°11'N, 116°27'E) of Xilinhot, Inner Mongolia, China[34, 35]. We constructed 24 enclosures (each 60×80 m; 0.48 ha, Fig. S1) with treatments of control, food supplementation (adding 100 g laboratory rodent chow pellets to each family weekly during the growing season), livestock grazing (allowing 40 adult sheep to graze for a half day or a whole day biweekly during the growing season to create light and moderate grazing pressure) and rainfall supplementation (adding extra 50 or 100 mm precipitation during the growing season, creating a light and moderate rainfall supplement; the local annual precipitation is less than 300 mm). The walls of the enclosures were built with steel plates, with 1m deep under the ground to prevent immigration and emigration of rodents and immigration of small predators. The top of the enclosures was cover by nylon wire net to prevent avian predation.

This study was conducted in 2014. Thirteen male-female pairs of voles were released into each of the 24 enclosures as founder populations in late April, 2014. The population density was surveyed monthly by the use of live traps. By early October, population density of voles ranged from 64 to 492 voles in the various enclosures, which is comparable to density variation observed in natural populations. Variation in population density among treatments and replicates was large, which provided an opportunity of studying the density-dependent changes in the OT/AVP systems of Brandt's voles. Seven adult male Brandt's voles in each enclosure were sacrificed in early October for our neurobiological research. Once captured, they were immediately anesthetized with sodium pentobarbital (1 mg /10 g body mass) and terminated by decapitation. The whole brain was immediately removed from the skull to a tinfoil paper within approximately 30 seconds and then was put on dry ice to freeze rapidly (5 minutes). The fresh brain tissues were then kept in a liquid nitrogen and transported to our lab in Beijing. Because vole populations did not successfully establish in seven enclosures in 2014, only seventeen enclosures were available for statistical analysis in this study.

To study the association between aggressive behavior and population density of voles, behaviors of voles were surveyed in one high and one low enclosure in early October 2014. Because these observations lacked replicates, we repeated behavioral observations in 2018. By the use of video cameras, we repeated the study to collect behavior data in twenty-four different enclosures for a succession of 11 days in September 2018 to determine the relationship between aggression behavior and population density in field conditions. Observations were made in late afternoon (16:00–18:00). We chose randomly three families in each enclosure and recorded the behavior of voles on the ground for at

least 0.5 hours during each observation. We classified the observed behaviors into five types: foraging, grooming, aggression, chasing and resting following [36].

Field enclosure experiment 2

The density variation in the first enclosure experiment was created by food addition, grazing, and rainfall addition which may obscure the association between population density and OT/AVP expression.

To study the impact of population density on aggressive behavior, we recorded the behaviors of voles in these enclosures in late September 2018 by means of video cameras. Observations were made in late afternoon (16:00–18:00). We randomly chose three families in each enclosure and recorded the behaviors of voles on the ground for at least 0.5 hours during each observation. We classified the observed behaviors into five types: foraging, grooming, aggression, chasing and resting.

Real-Time PCR

The Real-Time PCR and Western blotting were conducted exactly by following Huang *et al* [12].

Statistical analyses

In the enclosure experiment, we used linear models to test the effects of population density on aggressive behavior, protein expression and mRNA expression levels for a cohort of genes (including OT, OTR, AVP and AVPR) in different brain areas (including AMYG, MPOA and PVN). We used ANOVA to test the significant effects of enclosure density on the expression levels of mRNA and relevant protein for four genes (OT, OTR, AVP and AVPR) in three brain areas. We used the Shapiro-Wilk test and Levene's test to check the normality and the homogeneity of variance assumptions. All statistical analysis was conducted in R (version 3.5.1).

Results

Association of OT/AVP expression with aggression and chasing behavior

For the field enclosure experiment 1 (2014), high-density enclosure showed significant higher frequency of aggression and chasing behavior than low-density enclosure (Fig. S2). For the field enclosure experiment 2 (2018), aggression frequency between voles was positively associated with increased population density in 24 enclosures ($F = 17.5$, $P < 0.001$; Fig. 2A). The chasing frequency between voles was also positively related to population density ($F = 14$, $P < 0.001$; Fig. 2B). The behavior observed the

2018 experiment showed the same pattern: high density enclosures showed significant higher aggression frequency (Fig.2C) and aggression duration (Fig.2D) compared to low density enclosures.

Effects of population density on mRNA expression of OT/AVP

For the 2014 field enclosure experiment, population density showed significant and positive effects on mRNA expression of AVP and its receptor (AVPR) in all brain regions of voles in the enclosure (AVP: $P < 0.001$; AVPR: $P < 0.001$; Fig.3). In addition, mRNA expression of AVP/AVPR were lower in livestock grazing enclosures compared with that in control enclosures. The difference in mRNA expression of AVP/AVPR was not significant among different brain areas (all $P > 0.05$; Fig. 3). Population density showed significant and negative effects on mRNA expression of OT and its receptor (OTR) of voles in the enclosure (OT: $P < 0.001$; OTR: $P < 0.001$), except OTR in the brain areas of AMYG (OTR: $F_{1,67}$=2.38, P= 0.127; Fig. 3). There were no significant effects of enclosure treatments (i.e. food supplementation, livestock grazing and rainfall supplementation) on the mRNA expression of OT/OTR of voles.

For the 2018 field enclosure experiment, voles in the high-density group exhibited higher levels of AVP and AVPR mRNA expression in AMYG and PVN compared to those in low or moderate density groups (Fig. 4a,b). We found OT and OTR RNA expressions in AMYG, MPOA and PVN for voles in moderate and high density groups were significantly lower compared with those from low density groups (Fig. 4c,d).

Effects of population density treatment on protein expressions of OT/AVP systems

For the field enclosure experiment 2 (2018), high population density increased the protein expressions for both AVP and AVPR of voles (AVPR: $F_{2, 45}$ =10.7, $P < 0.001$). In all tested brain areas, we found the protein expression of AVPR was significantly higher in high density group than that of low density group (Fig. 4e,f). We found high population density decreased the protein expression of OT/OTR of voles (OT: $F_{2, 45}$ =5.3, $P = 0.008$; OTR: $F_{2, 45}$ = 6.7, $P = 0.002$). In all brain areas, we found that protein expressions of OT were significantly higher in low density group than those at high density group (Fig. 4g). The protein expressions of OTR is similar with OT that were all higher in low density group than those of high density group (Fig. 4h).

Discussion

Although OT/AVP are well known to regulate aggressive behaviors in mammals, their roles in the density-dependent behavioral population regulation of small mammals have not been investigated in field conditions. In this study, by the use of large-scale field enclosures, we found in support of our hypothesis that in high population density, mRNA and protein expression of AVP/AVPR increased, but mRNA and

protein expression of OT/OTR decreased in the specific brain regions of Brandt's voles, which are well linked to the increased aggression frequency/duration and chasing behaviors. Our results suggest that OT/AVP systems are important in regulating density-dependent aggressive behavior, and possibly thereby affecting the population dynamics of small rodents. Our study thus provides evidence for a neurological mechanism implicated in the behavioral population regulation of small rodents.

Our results are consistent with those in laboratory studies of the function of OT and its receptor (OTR) in regulating aggressive behavior of animals [33, 37-40]. Increasing OT level in brains could promote the social affiliation and social bonding or reduce aggressive behaviors of animals [22, 23, 41] , while decreasing OT levels would increase aggression behavior [42]. Similarly, AVP and its receptor are important in regulating the aggression behaviors [43]. Increase of AVP or its receptor levels could increase aggression [25-27].

In this study, we found that, in two field experiments, high population density was associated with increased AVP/AVPR expression, and decreased the OT/OTR expression, which correspond well to the increased frequency of aggression and chasing behaviors. These results are exactly consistent with our previous study under laboratory condition [12]. In another study, we found aggression of Brandt's voles was reduced by injection of OT but increased by injection of OT antagonists and OTR antagonists [24]. Thus, density-induced OT or AVP and their receptors are able to regulate aggression behavior which then regulate the population density. Therefore, OT/AVP systems in brain are essential in mediating the density-dependent aggressive behavior and population regulation.

Many environmental stressors could increase AVP expression of animals, such as repeated restraint stress on rats, forced swimming on rats[44]. AVP was observed as a major factor with depression, especially in two hypothalamic structures SON and PVN, where product plasma vasopressin [45]. High population density could act as an environmental stressor (e.g. crowding or aggression, food or space shortage). Our previous study indicated that high housing density could decrease OT or its receptor but increase AVP of its receptors [12], but it is not clear whether such associations operate in field conditions. In this study, we showed that high population density increased AVP/AVPR expression but decreased the OT/OTR expression, and aggression behavior of voles was also increased in semi-natural conditions. Based on manipulation experiments of OT or its receptor of voles [24] as well as many previous studies on rodents (see above), the high density induced decrease of OT or increase of AVP could increase aggressive behaviors of voles. Based on our fighting experiments of Brandt's voles in the laboratory [12], aggression behavior as a stressor would further decrease OT expression, but increase AVP expression. Thus, the reciprocal facilitation between OT/AVP expression and aggressive behavior could be an important component of population regulation in small rodents (Fig. 1).

Food in the natural environment is limited and can act as a key factor to control animal populations. Free competition among individuals for food would ultimately exhaust resources and result in habitat destruction and mass starvation. There are many studies indicating that the frequency and intensity of aggression are positively associated with population density [46]. In our study, we found that the

frequency of aggression behavior of voles is positively associated with the population density in field enclosure, supporting our prediction 1. In our study, we also revealed that high density related crowding density and fighting were associated with increased AVP/AVPR expression but decreased OT/OTR expression, and finally increased frequency of aggressive behaviors. Our study revealed the novel mechanism of density-dependent behavioral and population regulation via the OT/AVP systems, and provide a neurological explanation for the density-dependent behavioral regulation.

Some physiological indicators such as blood CORT level, adrenal gland size, and reproductive organ size have been used to index the level of density-dependent stress on population of animals [7, 47-50]. A recent study reveals that GnRH expression in brains is closely associated with population density of wild meadow voles (*Microtus pennsylvanicus*) using large-scale field enclosures[51]. Because OT/AVP expression in the brain is strongly correlated with population density of voles, we recommend that they can be used as a fundamental indicator reflecting population changes or density-dependence stress of small rodents.

Conclusions

Density-dependency is an important phenomenon in regulation of animal populations, however, its neurobiological mechanism remains unclear. In this study, by using large-scale field enclosures, we firstly demonstrated that the mRNA and protein expression of oxytocin (OT) and vasopressin (AVP) in specific brain regions are significantly associated with aggression behaviors and population density of Brandt's voles in field condition. Our results indicate that OT/AVP systems may play a significant role in population regulation of small rodents by altering their density-dependent aggressive behaviors.

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical statement

The study conforms to the legal requirements of China in which it was carried out, including those relating to conservation and welfare and to the journal's policy on these matters. The experimental protocol on animal behavioral experiments were consistent with regulations of the Institute of Zoology, Chinese Academy of Sciences. Ms. Shuli Huang, Jing Liu and Mr. Jidong Zhao, Xin Zhang who conducted the animal behavioral experiments have been trained by the Beijing Agency for Experimental Animals, China, with authorized diploma.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author contributions

Z. Z. designed this study; G. L. performed the density manipulation experiment in the enclosure; S. H. performed the density experiment and all the neurobiological measurements of OT/AVP system; G. L., Z. W., Z. Z. carried out the statistical analyses and plotting; S. H., G. L., Z. Z. drafted the manuscript; Z. W., Y. P., J. L., J. Z., X. Z., X. W., C.K. and W. H. helped modify the manuscript. All authors gave final approval for publication.

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Figures

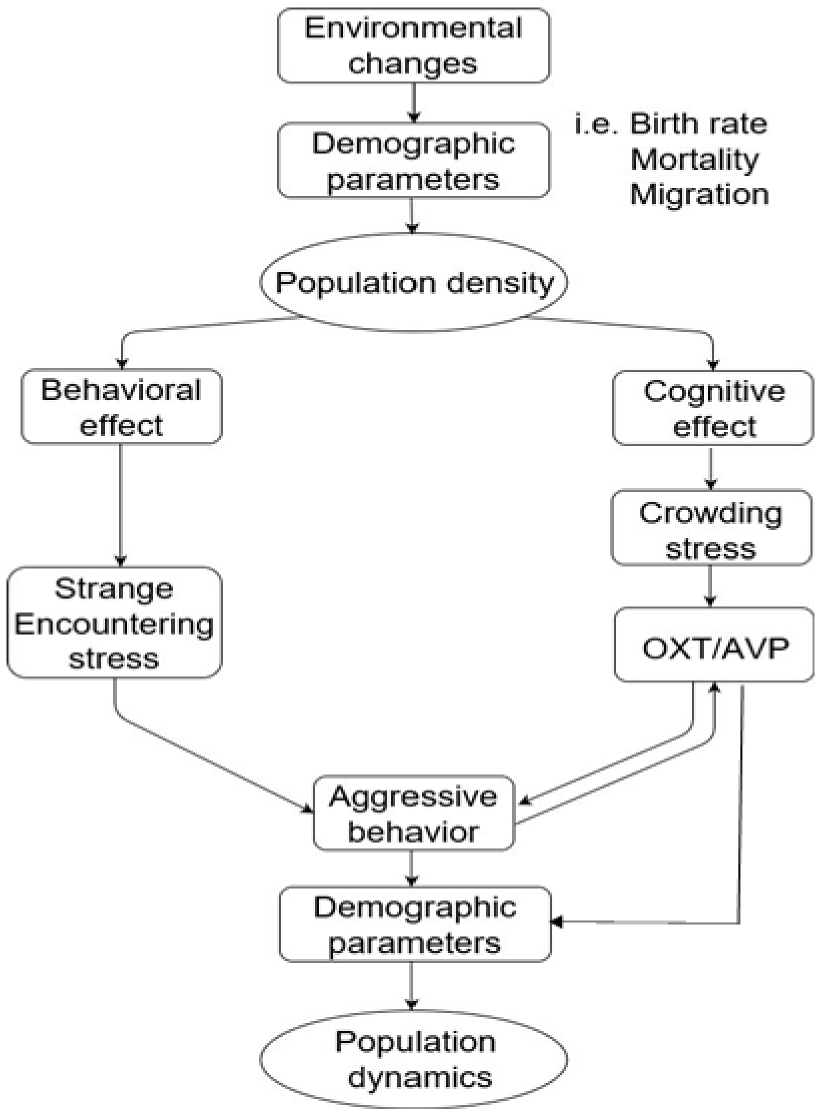

Figure 1

Hypothesis of density-dependent behavioral regulation (and then population regulation) through OT/AVP systems. High density would increase crowding stress due to shortage of space, and aggressive stress due to increased interactions with strangers, which would increase the AVP expression or decrease the OT expression. High-level of AVP and low-level of OT would further elevate aggressive behavior. OT/AVP expression could regulate reproductive or stress hormones.

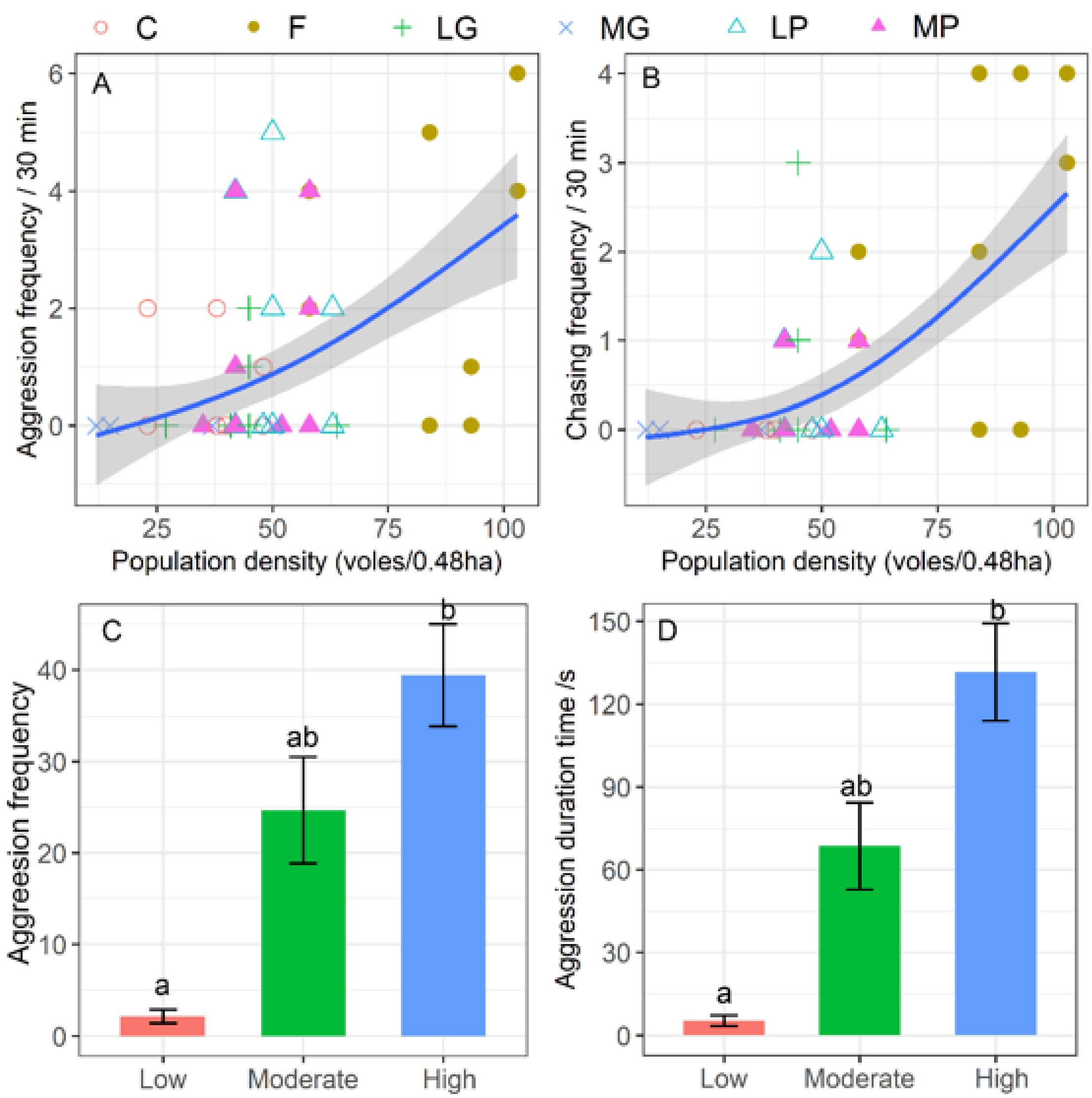

Figure 2

Effects of population density in field enclosures (A, B) on frequency of aggression or chasing behaviors between voles. For Fig. 2A&B, C: control group; F: food supplementation; LG: light grazing; MG: moderate grazing; LP: light precipitation supplementation; MP: moderate precipitation supplementation. Data are shown as mean ± SE. Asterisks indicate significant differences between density groups (* P < 0.05, ** P < 0.01, *** P < 0.001)

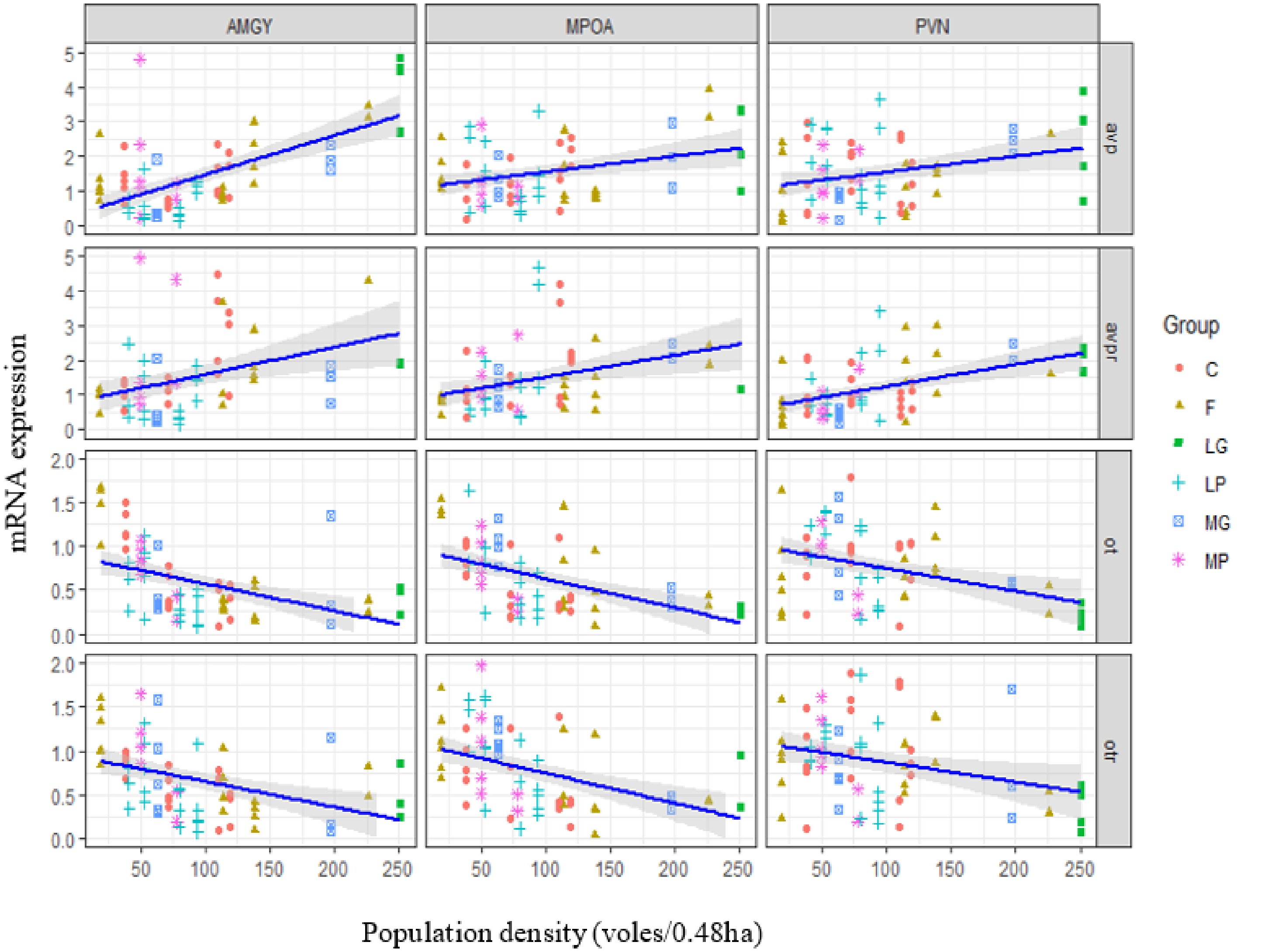

Figure 3

The association between population density and mRNA expression of AVP, AVPR, OT and OTR in brain areas of AMYG, MPOA and PVN. The fitted lines are presented with their 95% confidence interval. C, control enclosures; F, food supplementation enclosures; LG, light grazing enclosure; MG, moderate grazing enclosure; LP, light rain supplementation enclosure; MP, moderate rain supplementation enclosure. Data are shown as mean ± SE. Asterisks indicate significant differences between density groups (* P < 0.05, ** P < 0.01, *** P < 0.001).

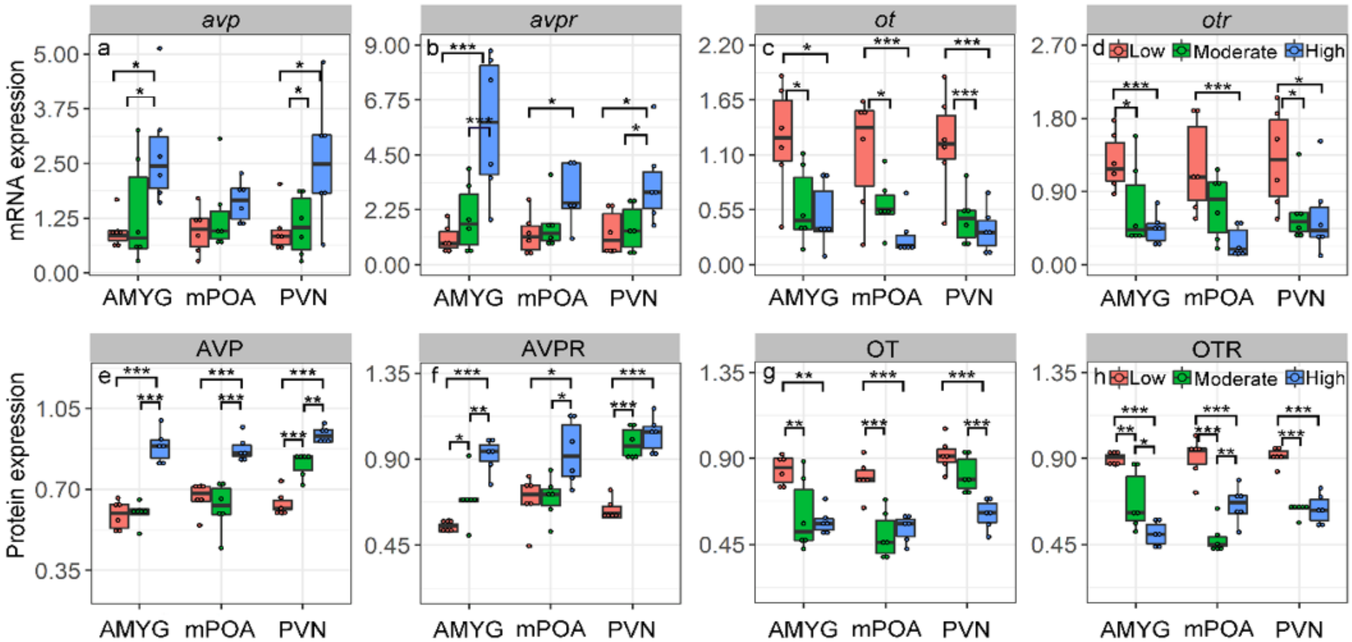

Figure 4

Effects of population density (low, moderate and high density) on the mRNA and protein expression of AVP, AVPR, OT and OTR in brain areas of AMYG, MPOA and PVN. Data are shown as mean ± SE. Asterisks indicate significant differences between density groups (* P < 0.05, ** P < 0.01, *** P < 0.001).

Supplementary Files

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- SupplementaryMaterial.docx