The oxytocin receptor gene (OXTR) polymorphism in cats (Felis catus) is associated with “Roughness” assessed by owners

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Introduction

In recent years, researchers have become increasingly interested in oxytocin, the peptide hormone, which is believed to be related to various social behavior in humans, such as pair bonding, nursing for children, and coping with stressors (for review; Carter, 2014). Many studies have also focused on oxytocin receptor gene (OXTR) polymorphisms in humans, which underlie individual variation in behavioral tendencies including empathy (e.g., Wu et al., 2012), cooperation (e.g., Haas et al., 2013), psychiatric disorder, such as autism (e.g., Lerer et al., 2008), and prosociality (e.g., Israel et al., 2009; Kogan et al., 2011). OXTR polymorphisms are also associated with individual differences in nonhuman primates and other mammals (e.g., Kis et al., 2014; Staes et al., 2014). In particular, recent studies suggest that the oxytocin system of dogs (Canis familiaris), which show human-like communicative skills due to their long history of domestication, correlates with their behavior toward humans. For example, dogs’ behavior (e.g., proximity seeking) to both strangers and owners in unfamiliar situations were different based on their genotype of OXTR (Kis et al., 2014). Dogs taking oxytocin spray showed more affiliation behavior to their owners (Romero et al., 2014) and showed positive expectation bias (Kis et al., 2015) than dogs with placebo spray.

By contrast, little research has been done on cats (Felis catus), another companion animal with a long history of domestication (Driscoll et al., 2007; Hu et al., 2014). Like dogs, cats show several human-like cognitive skills, including social referencing (Merola et al., 2015) and possess human-like cognitive skills, including social referencing. OXTR polymorphisms in cats, which have long history of domestication by humans, may be useful from the viewpoint of companion animal welfare; for example, to match potential good owners and cats using genetic information.
et al., 2015), following human pointing (Kraus et al., 2014), and vocal recognition (Saito and Shinozuka, 2013). To reveal genetic influences on cat behavior could further reveal the effects of domestication on social behavior as a result of genetic change. However, as yet it is unknown whether genetic polymorphisms in cats influence their social behavior or personality.

We examined whether OXTR polymorphisms in domestic cats were linked to their owner-assessed personality. We conducted a preliminary study to investigate how genetic variation in the oxytocin system affects cats’ personality, as assessed by cat owners.

Material and methods

Subjects and DNA collection

We collected buccal cell samples from 94 cats in Japan (57 males, 37 females; mean age = 4 years 6.99 months. Standard deviation = 47.90 months) after obtaining permission from the owners. Seventy-two cats were neutered (46 males and 26 females). Thirty-eight cats belonged to 6 “cat cafés,” where customers can freely interact with cats, and 56 cats were pets in their owner’s house. The numbers of cats in each cat café were 13, 6, 6, 5, 5, and 4, respectively. In Japan, more than 80% of cats are reported as mixed breed (mongrel/moggy) by the owners or as of unidentified breed (Japan Pet Food Association, 2014). Sixty-six cats (70.20%) in the present were described as mixed breed. The samples included one mother-offspring pair, one pair of sisters, and a trio of brothers.

Genetic analysis

DNA was extracted from buccal cells using QIAamp Blood and Tissue Kit (QIAGEN, Valencia, CA). We amplified exon1 region in OXTR by polymerase chain reaction (PCR) with 10 μL mixture for each sample, containing LA Taq, dNTPs, GC Buffer I (TaKaRa, Otsu, Shiga, Japan), template DNA, and the forward (OXTR_cat1F: 5'-GCAGCGCTATTCCTTAAA-3') and the reverse (OXTR_cat1R: 5'-CATGTTCGCCTCCACCTACT-3') primers. PCR condition consisted of 95°C preheating for 2 minutes, 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 74°C for 1 minute, and 74°C for 10 minutes as last extension. After PCR, we purified for PCR products using High Pure PCR Product Purification Kit (Roche Diagnostics K.K., Minato, Tokyo, Japan). After checking the purity status by electrophoresing on 1.5% agarose gel, we conducted the sequence analysis by polymerase chain reaction (PCR) with 10 μL mixture for each sample, containing LA Taq, dNTPs, GC Buffer I (TaKaRa, Otsu, Shiga, Japan), template DNA, and the forward (OXTR_cat1F: 5'-GCAGCGCTATTCCTTAAA-3') and the reverse (OXTR_cat1R: 5'-CATGTTCGCCTCCACCTACT-3') primers. PCR condition consisted of 95°C preheating for 2 minutes, 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 74°C for 1 minute, and 74°C for 10 minutes as last extension. After PCR, we purified for PCR products using High Pure PCR Product Purification Kit (Roche Diagnostics K.K., Minato, Tokyo, Japan). After checking the purity status by electrophoresing on 1.5% agarose gel, we conducted the sequence reaction with the condition of preheating of 96°C for 1 minute, 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 1 minute. After that, we conducted ethanol precipitation, after which we sequenced the PCR product using the Applied Biosystems 3130xl/3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA).

Rating of cat personality

We adapted questionnaires that were used to identify personality in Japanese Akita dogs for cats (Konno et al., 2011). The questionnaire contained 30 questions with 6-point scales asking about the personality of the cat, and owners answered it about their cats. We performed parallel analysis and factor analysis to reveal the personality structure of cats and used a generalized linear mixed model (GLMM) to examine the effects of the target genotype, age, breed, sex × neutering (because neutering effect was predicted to be different depending on sex, we used 4 categories; male/female × neutered/nonneutered), and environment (cat café or home) on scale scores on each factor. Because 70.20% of the sample was mixed, we included breed as a random factor. We used R (version 3.0.3) for all statistical analysis (R Core Team, 2014), and also used the libraries such as “psych (Revelle, 2015), “GPA rotation (Bernards and Jennrich, 2005),” “lme4 (Bates et al., 2014),” “car (Fox and Weisberg, 2011),” and “genetics (Warner et al., 2013).”

Results

Genotyping

Among sequenced 1,056 bp, dog and human sequences were different from those of cats in 127 bp (12.03%) and 132 bp (12.50%), respectively. Among 307 amino acids, dogs and humans differed from cats in 19 (6.19%) and 20 (6.51%) amino acids, respectively (Figure S1). We found 3 synonymous single nucleotide polymorphisms (SNPs; C474T, G723A, and G738A) in exon1. Allele frequencies of the SNP G723A were extremely high, and C474T and G738A were strongly linked (Pairwise linkage disequilibrium: $\chi^2 = 59.87, P < 0.001$), so we focused on G738A (Table). Allele frequencies did not overstep in G738A as a result of Hardy-Weinberg equilibrium (Fisher exact test; $P = 1.00$). The OXTR sequence in cats in our study was deposited in the DDBJ database (accession no. LC029887). We divided genotypes into 2 groups for analysis; A allele carriers and non A allele carriers.

Personality structure of cats

The number of factors was determined via parallel analysis and the scree plot. Factor analysis with maximum likelihood estimation and a varimax rotation detected 4 factors (Table S1), which we named “Openness” (highest positive loadings: playful, inquisitive, and curious), “Friendliness” (adaptable, calm, and friendly), “Roughness” (irritable, dominant, forceful, and moody) and “Neuroticism” (vigilant, nervous, and fearful). We defined factor loadings $\geq 0.5$ as salient items. No item had salient loading on more than one factor. We calculated scale score of each factor for each cat. The internal consistencies of the 4 factors assessed by Cronbach alpha were 0.85 for neuroticism, 0.87 for roughness, 0.92 for openness, and 0.90 for friendliness; hence, the reliability of the questionnaire was sufficiently high. To evaluate test-retest reliability and consistency of individual personality scores across time, we collected a second questionnaire randomly from the same owners for almost 25% of the sample (12 males and 12 females). The lag between the first and second questionnaires varied from 1 to 19 months. Pearson correlation coefficients ($r$) were 0.63 for openness, 0.65 for friendliness, 0.70 for neuroticism, and 0.76 for roughness and were all significant at $P < 0.01$, indicating that personality scores of each cat were stable according to the criteria of Konno et al. (2011).

Association between the genotype and scale scores

A GLMM analysis and type II Wald chi-square tests revealed no significant effect of any explanatory variables on friendliness and neuroticism, but there was an effect of age on openness. Younger cats showed higher openness scores ($\beta = -0.01$, standard error = 0.003. 95% Wald confidence interval (CI) = [-0.02, , -0.005], $\chi^2(1) = 15.36, P < 0.001$). GLMM showed
an effect of OXTR genotype ($\hat{\beta} = -0.55$, standard error = 0.24, 95% CI = [-1.03, -0.07], $\chi^2(1) = 5.10, P = 0.02$), and age ($\hat{\beta} = 0.006$, standard error = 0.003. 95% CI = [0.0007, 0.01], $\chi^2(1) = 4.89, P = 0.03$) on roughness, with older cats and those with the A allele showing higher roughness scores. When dividing samples into 4 groups (neutered male, neutered female, intact male, and intact female) and conducting GLMM for each group, neutered cats of both sexes showed the same tendency in roughness scores, but only neutered females showed a statistically significant effect of genotype (male: $\hat{\beta} = -0.36$, standard error = 0.33, 95% CI = [-1.01, 0.29], $\chi^2(1) = 1.16, P = 0.28$; female: $\hat{\beta} = -1.35$, standard error = 0.47, 95% CI = [-2.72, -0.43], $\chi^2(1) = 8.35, P = 0.01$). In intact cats of both sexes, the tendency was reversed, although the sample size was small and the effect was not statistically significant (male: $\hat{\beta} = -0.41$, standard error = 0.57, 95% CI = [-1.53, -0.71], $\chi^2(1) = 0.51, P = 0.47$; female: $\hat{\beta} = -1.10$, standard error = 0.72, 95% CI = [-2.51, 0.31], $\chi^2(1) = 2.35, P = 0.13$). To test whether differences among subgroups statistically significant, we included sex $\times$ genotype, neutering $\times$ genotype, and sex $\times$ neutering $\times$ genotype interaction terms for roughness score, but there was no significant effect.

**Discussion**

We found 3 SNPs in OXTR in cats and showed that one of these SNPs had an effect on owner-assessed roughness scores. To our knowledge, this is the first demonstration of a link between genetic polymorphisms and personality variation in cats.

The present study also gives new information on personality structure in cats. In a previous study that included an analysis of domestic cat personality (Gartner et al., 2014), 3 factors emerged: neuroticism (highest positive loadings: anxious, insecure, and tense), impulsiveness (excitable, active, and playful), and dominance (aggressive to conspecifics, bullying, and not submissive). These factors are similar to those that we found, but we also found a factor that was not identified in the previous study (Gartner et al., 2014). It was possibly because of procedural differences between these 2 studies (e.g., the number of raters, items of the questionnaires, living environment such as home, cat cafes, or shelters, and whether cats are pets).

In recent years, felids other than domestic cats (e.g., Gartner and Powell, 2012; Gartner and Weiss, 2013; Gartner et al., 2014) have been being examined from the viewpoint of personality. Our finding about OXTR in cats may be an indicator for personality evolution of felids and its genetic basis. For example, several studies have compared genetic polymorphisms in dopamine D4 receptor gene between dogs and wolves (Canis lupus) and reveal that wolves often have polymorphisms which show high activity-impulsivity in dogs, suggesting natural selection through domestication (e.g., Heijas et al., 2007; Inoue-Murayama, 2009). Comparing our results to those from wild cats and other felids may also reveal whether the mutation in these SNPs took place in cats during domestication, as an adaptation to living in close contact with people.

There were limitations on this study. First, the G738A SNP is a synonymous SNP coding the same amino acid, but the role of synonymous SNPs is unknown. Several studies reported that synonymous SNPs (e.g., rs2228485, rs237902) have an effect on social behavior in humans (Stankova et al., 2012; Lucht et al., 2013). Silent SNPs may sometimes change the process of protein folding (Komar, 2007), but the mechanism underlying this phenomenon reported in our study is a remaining issue. Second, whether the oxytocin system in cats is generally sex dependent remains unknown although the significant differences in roughness depending on genotypes in neutered female cats in this study are noteworthy. There is evidence of a significant sex $\times$ genotype of OXTR in “Harm Avoidance” scores in humans with the effect of the genotype being stringer in women than in men (Stankova et al., 2012). No significant interaction among sex, neutering, and genotype was found in this study, and sex and neutering effect are also unclear, so we need to add sample especially to females to sufficiently conduct subgroup analysis.

Third, we found age effect, but we cannot discuss it because of the small sample size and several very old cats (more than 10 years old) in subjects. One possibility for the relationship of age and roughness is that a few items (e.g., “moody”) were strongly related to old cats.

In previous studies showing a link between oxytocin system and dogs’ social behavior (Kis et al., 2014; Romero et al., 2014; Kis et al., 2015), the main dependent variable was the dogs’ responses toward humans. In the questionnaire used here, owners were asked about their cats’ everyday activities. Therefore, the object of the cats’ interactions varied across questions (e.g., stranger, owner’s family, other cats). Future researches may wish to conduct a test battery measuring cats’ behavior toward owners will be needed to focus on human-cat relationships, to allow comparison of results with those reported in dogs.

**Conclusion**

This study demonstrated that cats have 4 personality factors: openness, friendliness, roughness, and neuroticism. This study also demonstrated the relationships between genetic polymorphisms and personality traits in cats. G738A, 1 of 3 SNPs in exon1 of OXTR found in this study, was associated with roughness scores assessed by owners. This finding may have implications for animal welfare, for example, in predicting compatibility in cat-cat or cat-owner relationships, developing data that shows what genetic types of cats would match potential owners.

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![Figure](Figure. “Roughness” score for each group of sex $\times$ neutering $\times$ the single nucleotide polymorphism G738A genotype (±standard error).)
questionnaires, conducted genotyping, analyzed data, and drafted the article. Atsuko Saito, Hitomi Chijiwa, Saho Takagi, Yuki Ito, and Arii Watanabe contributed to data collection. Yusuke Hori and Miho Inoue-Murayama conducted data analysis, and Miho Inoue-Murayama and Kazuo Fujita provided critical discussion regarding the analyses and the article. All authors have approved the final article.

Ethical considerations

This study adhered to the ethical guidelines of Kyoto University and was approved by the Animal Experiments Committee of the Graduate School of Letters of Kyoto University.

Conflict of interest

The authors have no competing interests.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jveb.2015.07.039.

References


