Canine Oxytocin Receptor Genotypes, Cognition, and Social Behavior

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featuring dog 46

ABSTRACT
Domestication efforts may have selected for certain social behaviors and cognitive abilities in dogs that foster mutually beneficial canine-human interactions. The oxytocin system plays a critical role in social bonding in many species, including dogs and humans. Thirty-one dogs of various breeds and ages were tested in a social cueing task and a set of object permanence tasks, and scored according to Piaget's stages of cognitive development. Their owners completed 40-question surveys that were used as measures of their prey, pack, fight, and flight drives. The dogs' cognition indicators were then correlated with their alleles at the oxytocin neuropeptide receptor (OXTR) gene. Polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) analyses were used to determine the genotypes, which were then compared to the behavioral phenotypes. Though data were not statistically significant, the class learned a lot.

Key words: oxytocin – social bonding – behavioral genomics – affiliation

1 INTRODUCTION

Modern domesticated dogs evolved under the influence of strong human-determined selection pressures. Over the course of the domestic dog's evolutionary history, humans placed strong artificial selection on dogs for their tendency to cohabitate peacefully with humans. This artificial selection dramatically transformed the fitness landscape for canines by allowing them an alternate avenue through which to enhance lifetime reproductive fitness. Humans purposely used selective breeding to produce dogs that were useful for tasks, including herding, guarding, or tracking (Miklosi et al., 2013). For most of these selected behavioral phenotypes, low aggression and enhanced communication between the dogs and humans are required. Therefore, dogs with greater ability to form social bonds with humans had increased reproductive fitness. Dogs that formed stronger social bonds with humans were selected. Based on this evolutionary past, dogs are an interesting model organism for the study of the genetic basis of pro-social cognitive skill.

Studies of animal cognition can provide insight into the evolution of cognitive processes in humans (Miklosi et al., 2004; Gomez, 2005). Many comparative studies performed with different non-human primates and other animals rely on a metric conceived by the Swiss developmental psychologists Jean Piaget and Barbel Inhelder as applied to human developmental stages (e.g., Piaget and Inhelder, 1969; Fig. 1). This same scheme has been applied to dogs (Triana and Pasnak, 1981; Gagnon and Dore, 1992, 1993) which perform at a level consistent with a one- to two-year-old human toddler. In the current study, we applied a behavioral task that applies this scale and also an additional social cueing test in which the human experimenter used a pointing cue.

Recently, a growing body of research has indicated that the oxytocin system influences social behavior of dogs (Kis et al. 2014). Oxytocin is a neuropeptide produced in the hypothalamus that acts as a neurotransmitter and has been implicated in a myriad of social behaviors in humans including reproductive behavior, mother-offspring attachment, and social memory (Donaldson and Young 2008; Stallen et al 2012). Intranasal administration of oxytocin in dogs has been

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Stage 1	No response to disappearance
Stage 2	coordinating modalities - e.g. looking for a sound source
	passive expectation - gaze at point of disappearance
	no following of dropped object or anticipation of trajectory
Stage 3	Visual anticipation of trajectory
	responds to "peek-a-boo"
	no retrieval of hidden object
Stage 4	Retrieval of fully hidden object
	makes "A-not-B" errors (persistant looking in previous location)
Stage 5	Succes on "A-not-B" tasks
	Failure on "Invisible displacement"
Stage 6	Success on all above

Figure 1. Jean Piaget's stages of object permanence in human infants and todlers.

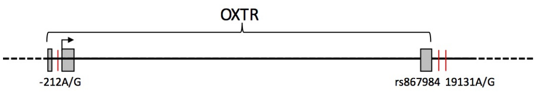

Figure 2. Schematic figure of the dog OXTR gene. The region pictured ranges from 9358932 - 9378248 bp starting with a short un-translated region (UTR) (CanFam 3.1). There are two identified SNPs (rs8679684 and 19131AG) in addition to the -212A/G) addressed in the current study

shown to increase pro-social interactions with humans, but this effect is context specific (Bartz et al. 2011). Researchers have also tested the effect of oxytocin administration on dog performance in an object choice task that utilized a social pointing cue, and found that oxytocin administration not only enhance performance on the task (Oliva et al. 2015).

Among other genetic polymorphisms, the canine oxytocin receptor gene contains a single-nucleotide polymorphism (SNP) 212 bp upstream from the gene start site (Fig. 2). At this SNP, the dog genome contains either an adenine or a guanine base; this gives three possible dog genotypes at this locus: A/A, A/G, and G/G. Differences in oxytocin genotypes may partially account for the variability in social competence within and between breeds of dogs. In dogs with the A/A genotype, the administration of oxytocin resulted in increased seeking of physical contact from experimenters, while the opposite was found in dogs with the GG-genotype (Persson et al. 2017).

Based on the fact that the A allele at the -212A/G SNP of the oxytocin receptor gene is associated with canine affiliation toward humans (Presson et al., 2017), if we assume that a dog's ability at social cueing is positively correlated with its affiliation toward humans than we would predict dogs with the A allele would perform better on social cueing. Similarly, if Piaget's stages of cognition as applied to canine performance on object permanence rely on the same neural mechanisms, then we should expect to find dogs with the AA OXTR genotype performing better (higher success rate, or shorter latency) on these tests than dogs that are GG genotype. If canine affiliation toward humans has evolutionarily co-opted the mechanisms that promote social affiliation among dogs then we would predict dogs with the A allele at -212A/G SNP of the OXTR gene to score higher on measures of pack drive as well. We use owner completed questionnaires, to gather information about dog "personality" drives and student administered behavioral tests of object permanence and social cueing to assess behavioral phenotypes. The dog genotypes at the -212A/G SNP of the OXTR gene are easily survey with a simple Restriction Fragment Length Polymorphism (RFLP) test.

2 METHODS

2.1 Subjects

Dog subjects were identified for the study via an email that was sent to all known staff and faculty dog owners on the Reed College campus, and further transmitted by word of mouth. Dog breeds were determined by owner report. Prior to behavioral testing Owners were asked to take a buccal swab of the dog and then fill out a "Dog Personality Questionnaire," which was adapted from a survey used by Volhard.com (http://www.volhard.com/pages/canine-personality-profile.php). For each question, animals were coded as: Almost always (10), Sometimes (5), or Hardly ever (0) performing that behavior. These values were totaled from 10 questions for a possible score of 100 for each of 4 axes of personality: prey drive, pack drive, fight drive, and flight drive. For unanswered questions the totals were corrected by adding the average of the other scores pertaining to that drive. For fun, dogs were also classified as STEM dogs, non STEM dogs, or CIS dogs according to their owners current primary affiliation with Reed College.

2.2 Behavioral Tests

All behavioral tests were conducted between the hours of 1:00 PM and 3:30 PM on either October 3rd or 4th, 2018. On the day of experimentation, student pairs met owners and dogs at the appointed time and convenient campus location. All behavior tasks relied on a similar spatial design in which the test subject began 6 feet away from equally spaced buckets. To evaluate the cognitive ability of each dog, four behavioral tests were performed.

Each behavioral testing session began with the student experimenter greeting the owner and the dog to first discuss the testing procedure and determine whether or not the owner should participate in the experiment and if so, what role they should play. The owners were also given control over what treats or rewards were used with their dogs (owner supplied or Trader Joe's Brand). The owner also helped identify an appropriate experimentation site that was convenient for the particular dog/owner combination (thus dogs were not tested in a 100% controlled environment). The general setup comprises a the starting location for the dog and assistant which was six feet away three buckets are placed which are in turn three feet apart from each other (Fig. 3). For each test, the experimenter would stand six feet away from the middle bucket in a straight line from the starting position. The four experiments were always conducted in the same order.

Visible displacement test (VDT).
In the VDT, the assistant walks the dog to the starting point. Then, the experimenter walks between the buckets while holding a treat. Once the experiment has the attention of the dog, the treat is placed into one of the buckets, and the experimenter retreats to the waiting position and the assistant starts the timer. After ten seconds, the assistant releases the dog and begins the timer for 30 seconds.

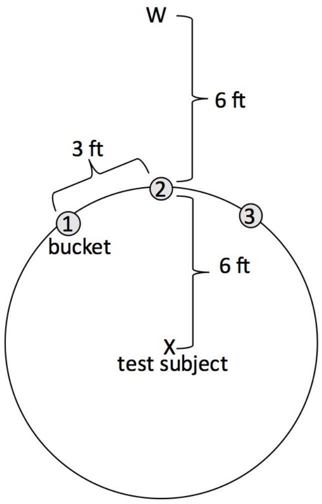

Figure 3. Setup used for the visible displacement test, sequential visible displacement test, and the invisible displacement test. Dogs were placed 6 ft. from the buckets which were placed 3 ft from each other. The experimenter label indicates the waiting position to which the experimenter retreats after placing the food in a bucket. Test Subject denotes the starting position of the dog and assistant.

The trial is marked as a success if the dog touches or attempts to move the correct bucket within 30 seconds. The trial is marked as unsuccessful if the dog comes within one body length of the incorrect bucket or takes longer than 30 seconds. A single session includes three trials using the same bucket for each trial. The dog is said to have achieved Stage 4 of Piaget's developmental stages if at least two of the three trials were marked as success.

Sequential Visible Displacement Test (SVD)
The SVD follows the same protocol as the VDT, except the a different bucket is selected for each of the three trials. The dog is said to have achieved Stage 5 of Piaget's developmental stages if at least two of the three trials were marked as success.

Invisible Displacement Test (IDT)
The IDT follows the same protocol except the dog treat is placed into a cup while the dog is watching and then the cup is placed into the bucket, where the treat is removed out of sight of the dog. The dog is then shown the empty cup. The dog is said to have achieved Stage 6 of Piaget's developmental stages if at least two of the three trials were marked as success.

Social Cueing Test (SCT)
For the SCT the setup is modified by removing bucket number two. The assistant takes the dog out of the room (if the test is being conducted indoors) or 20 feet away where the dog cannot see the bucket or experimenter (if the test is outdoors) while experimenter then places a treat in one of the buckets. The dog and assistant then return to the starting point, and the assistant starts a timer while the experimenter points at the bucket where the dog treat is hidden. After ten seconds, the assistant releases the dog. SCT trials are scored in the same manner as the previous tests.The dog is said to have achieved human-dog socially cueing communication if at least two of the three trials are marked as a success.

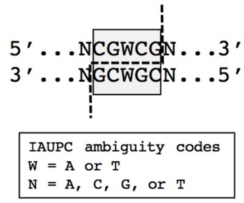

Figure 4. DNA recognition sequence and cut sites for the restriction enzyme Hpy991. N indicates that any nucleotide can be present in that location for effective enzyme function. W indicates that either A or T can be part of the recognition sequence. Dotted line indicate the location of the double-stranded cut and resulting overhang made by the restriction enzyme.

2.3 Genotyping

DNA Sampling
Before the day of behavioral testing, each owner was asked to watch the UC Davis Veterinary Medicine instructional video. To obtain DNA samples for each dog, prior to conducting behavioral experiments, the owners were asked to take buccal swabs from the inside of the cheek of the dog after the animal had not eaten or drank for at least one hour. Swabs were placed in sterile paper containers for transport back to the lab.

DNA Extraction
Approximately 30-90 minutes after the buccal swab was collected, DNA was extracted from the swabs according to the manufacturer's protocol (Sigma Aldrich REDExtract-N-Amp Tissue PCR Kit). Briefly, swabs were swirled in 200 ul of Extraction Solution, and 25 μl of Tissue Preparation Solution was added before allowing the sample to incubate at room temperature for about 10 minutes. The sample was then incubated at 95 °C for three minutes. Then 200 μl of Neutralizing Solution was added, and the sample was stored at -20 °C.

RFLP Analysis
RFLP Analysis was used to determine the dog genotype. A 650 bp product from the region of interest was amplified with polymerase chain reaction (PCR) using oligonucleotide primers (Kis et al 2014) ordered from Integrated DNA Technologies. The reaction mixture contained 1 ul of each 10 μM working stock primer, 4 μl of extracted gDNA, 1 M Betaine, and 10 μl of 2X Red Extract and Amp Polymerase. The Betaine is necessary for amplification of GC rich sequences and is not a standard reagent in PCR. The PCR cycle consisted of 3 minutes of initial denaturation at 94 °C, followed by 35 cycles of 30 seconds at 95 °C of denaturation, 30 seconds at 56 °C of annealing, and 1 minute at 72 °C of extension, and a final 5 minute extension at 72 °C. One half of the PCR product was then restriction digested at 37 °C for 1.5 hours in a 20 μl restriction enzyme mixture containing Hpy991 restriction enzyme and 1x cutsmart buffer (New England Biolabs). Only the A allele of the -212A/G SNP contains the recognition sequence for the restriction enzyme Hpy991 (Fig. 4). When Hpy991 cuts the PCR product at this site the 650bp band is cut, digested, into two fragments of 475bp and 160bp (Fig. 5).

3 RESULTS

3.1 Subjects

Thirty-one dogs underwent behavioral testing for cognitive ability and social cueing. One dog participated twice (ID 18 and 34) but

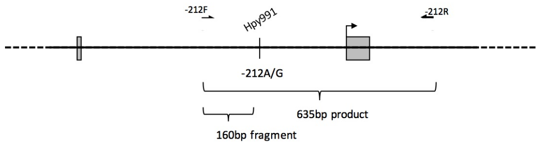

Figure 5. Map of the Canine Oxytocin Receptor gene. An A/G SNP that is 212 bp upstream from the start site of the gene creates an RFLP for the Hpy991 restriction enzyme. Half arrows indicate the forward and reverse primers that create a 635 bp PCR product. There is an additional 3âĂŹ exon not shown several kb away.

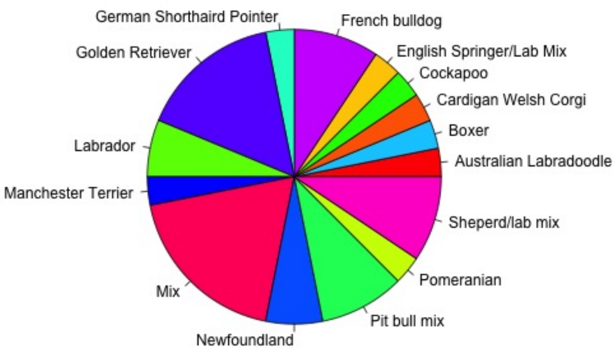

Figure 6. Distribution of dog breeds tested. Dogs are designated as Mix if greater than one breed was named by the owner unless the exact parentage was known or in the case of the Shepherd/Lab mix and Pitbull mix.

only the first trial retained for data analysis. Owner reported breed indicated that the sample included 3 shepherd lab mix, 3 pitbull mix, and other 6 mix breed dogs, in addition to 5 golden retrievers, 3 French bulldogs, and a collection of other purebred dogs (Fig. 6). There were insufficient number of dogs of any given breed to perform meaningful statistical analysis by breed. Sex and age of the dog were not recorded.

3.2 Drives

From the owner completed survey, we calculated 4 axes of personality: prey drive, pack drive, fight drive, and flight drive (see appendix). The questions related to prey drive involved the animals motivation to chase objects or animals while pack drive questions involved the animals tendency to affiliate with animals or humans. These two axes are not mutually exclusive. Fight drive and flight drive however are expected to be negatively correlated because these questions addressed the dogs' behavior toward novel objects, animals, and situations as either approaching/proactive or withdrawing/reactive. Interestingly, raw scores for flight drive and fight drive were strongly positively correlated (PEARSON: r = 0.72138, t30 = 5.7054, p <0.0001) suggesting that owners tended to give either high or low scores across all questions. Similarly raw scores for pack drive and prey drive were also correlated though to a lesser extent (PEARSON: r = 0.3386 , t30 = 1.9712, p = 0.058) (Fig. 7)

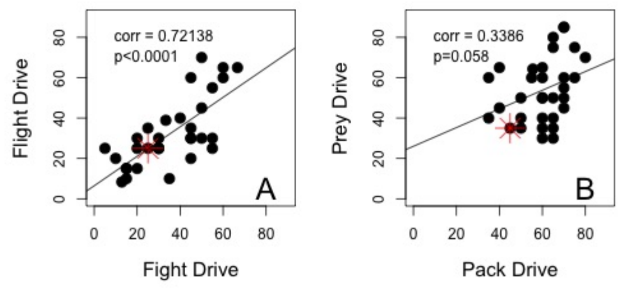

Figure 7. "Personality" Drives as reported by owners. (A) Fight and Flight drives and (B) Prey and Pack drives were correlated. Your individual dog is highlighted in red.

3.3 Object Permanence

The Reed dogs all scored high on the three object permanence tasks. For the visual displacement task (VDT) only five dogs failed. Because dogs were allowed to move on to the next test even if they failed one task, a few dogs were successful at tasks that should be associated with higher cognitive function despite having failed at the lower levels. For the sequential visual displacement (SVD) three of the same dogs failed as did two others, and for the invisible displacement task (IVD) two of the same dogs failed as did three additional ones. In total, nine dogs failed on of the three object permanence tasks.

For the purpose of statistical testing, we grouped all 9 dogs with one or more failures on the object permanence tasks. We consider the other 21 dogs that were successful on all three object permanence tasks to have achieve Piaget's stage 6 for cognition (akin to a human child of 18 - 24 months). We then asked whether these stage 6 dogs differed from the others according to any of the owner reported drives. These two groups of dogs were not significantly different from each other according to any calculated drive score (AOV: fight: $F_{1,30}$=0.002 P=0.961; flight: $F_{1,30}$=0.03 P=0.864, prey: $F_{1,30}$=0.22 P=0.643; pack: $F_{1,30}$=0.06 P=0.808) (Fig. 8).

3.4 Social Cueing

Dogs in this study were also tested on a social cueing task which measures their ability to take social cues from a human (in this case student experimenter). Nine of the dogs in this study were not successful on the social cueing task. Because social cueing is not directly related to cognitive ability, it was interesting to ask whether these two abilities are independent. Four of these nine dogs also failed one of the social cueing tasks which suggests (though not statistically significant) that these trait are not independent and dogs that score better on object permanence tasks also do better with social cueing (CHI-SQUARE: X^2=2.96, df=1, P=0.085).

3.5 Behavioral Genetics

Because the Oxytocin neuropeptide system has been linked to prosocial affiliative behaviors in a wide range of animals and genetic variation in for this receptor has been specifically linked to human directed canine behavior in the past, we used RFLP analysis to genotype the dogs in this study. Specifically we investigated the -212A/G locus in the promoter region of the OXTR gene. For ten

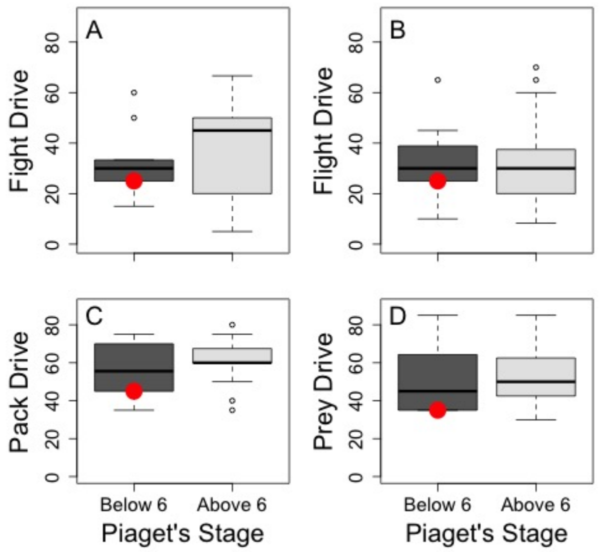

Figure 8. Piaget's stages of cognitive development do not correspond to owner reported Fight (A), Fight (B), Pack (C) or Prey (D) dives. Dogs that failed any one or more of VDT, SVD or IDT are considered below stage 6. Your individual dog is highlighted in red.

of the samples in our study the PCR failed to amplify an fragment. This failure was likely due to poor swabbing technique or improper DNA isolation. We were able to determine the genotype 21 dogs in our study. Among the successful genotyping samples, we identified nine dogs homozygous for the A allele, seven dogs homozygous for the G allele and five dogs that were heterozygous at the -212A/G SNP of the OXTR gene. In general, we did not survey enough of any one breed to determine whether the genotype of the -212A/G locus is associated with breed. While this site has previously been reported to be polymorphic in German Shepherds, and Collies (Kis et al 2014), it has been seen to be fixed for the A allele in a Golden Retriever population (Persson et al 2017). Interestingly, all three golden retriever samples that successfully amplified in our study were also determined to be homozygous for the A allele.

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Table 1. This is my genotpe table. Captions appear above each table. Remember to define the quantities, symbols and units used.

	A/A	A/G	G/G
Social Cueing			
-Success	5	4	6
-Failure	4	1	1
Piaget's Stage			
-Above 6	4	4	6
-Below 6	5	1	1

were also determined to be homozygous for the A allele. We then asked whether the genetic variation at the -212A/G site accounted for the observed variation in behavior. Based on the evidence that the A allele is associated with pro-social human directed behaviors we predicted that dogs with the A allele would perform better on the social cueing task, possibly perform better on the object permanence tasks if social cognition and object permanence cognition rely on similar mechanisms and may have higher pack drive if human directed and dog directed social behavior relies on similar mechanisms. However, in our study dogs homozygous for the A allele or heterozygous, having one A allele were no more likely to be successful on the social cueing task (9 of 14) than dogs that are homozygous for the G allele (6 of 7) (CHI-SQUARE: X^2=0.26, df=1, P=0.608) (Table 1). If anything, the trend is in the opposite directions with a greater percentage of homozygous G allele dogs being successful on the social cueing task.Similarly, whether the dog had A or G alleles at the -212A/G locus of the OXTR gene did not predict their success on object permanence tasks designed to place individuals along Piaget's stages of cognitive development whether the three genotypes were treated as independent (CHI-SQUARE: X^2=0.543, df=2, P=0.171) or whether individuals with the A allele were grouped according to our initial hypothesis (X^2=0.821, df=1, P=0.365)

While our main prediction regarding a genetic correlation between the OXTR gene and behavioral drive related to the dog-dog social affiliation captured by the pack drive, we decided to investigate all four quantified drives. None of the four drives were significantly different for dogs of different genotypes (ANOVA: Fight: $F_{2,18}$= 1.127, P=0.346; Flight: $F_{2,18}$=0.3, P= 0.744; Prey: $F_{2,18}$=2.291, P=0.13; Pack: $F_{2,18}$=2.07, P= 0.155) (Fig. 9)

3.6 Dogs by Division

For fun and because no analysis of the data thus far has been statistically significant, we decided to code the dogs by divisional affiliation and ask whether dogs in different academic departments showed any behavioral differences. We reasoned that this might be the case because dogs and owners are often thought to share some aspects of their phenotypes. We did not hazard any hypotheses and considered this analysis to be purely exploratory. Eighteen of the dogs were classified as STEM, being owned by (or dog sat by) staff, faculty or students in the division of Math and Natural Sciences. Six of the dogs were classified as CIS dogs being owned by staff members in the ETC building. The remaining 8 dogs were classified as nonSTEM though we note one of these hails from Psychology which has recently joined the academic cluster with STEM for the purposes of group requirements. None of theses groups of dogs performed differently from each other on any of the 4 behavior measures (statistics not provided) nor did they differ according to

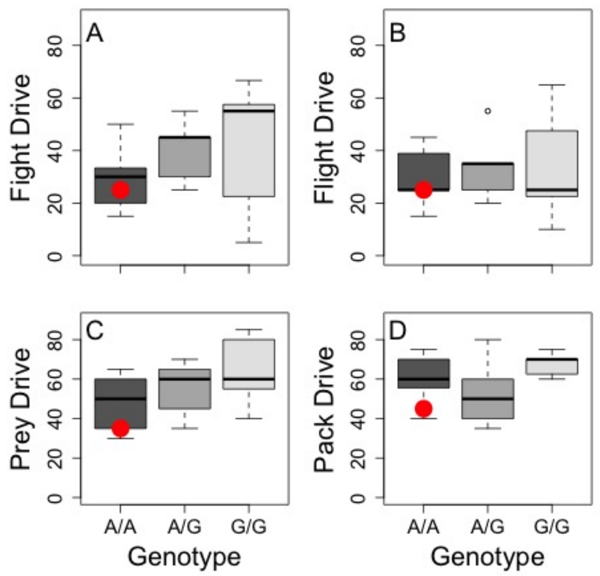

Figure 9. ""Personality" Drive scores for dogs of differing genotypes at the -212A/G OTRX gene. Your individual dog is highlighted in red. If there is no red dot, we were unable to determine the genotype of your dog due to poor DNA sampling/isolation.

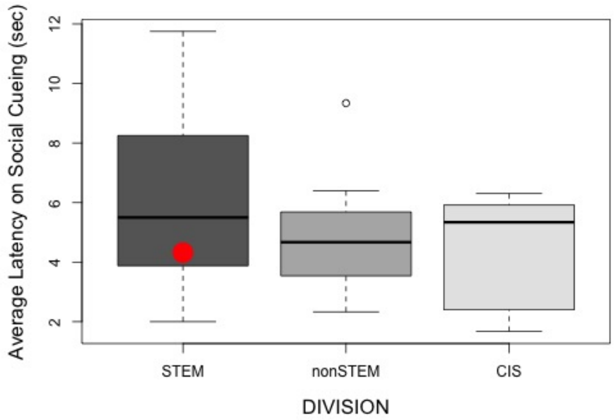

Figure 10. "Latency in Social Cueing for dogs from different academic divisions. Dogs are coded as belonging to a "division" according to the owner's primary affiliation. Latency is calculated as the time from when the dog is released until it successfully approaches the correct bucket. Your individual dog is highlighted in red. If there is no red, your dog was not successful at the social cueing task and therefore there is no measure of latency.

any of the dog personality drive measures calculated from owner report (statistics also not shown). During object permanence and social cueing tasks we also recorded latency, the time the dog took to approach the successful bucket. The latency was very short for nearly all successful trials and the variation probably represents experimenter error as much as anything else. Nonetheless, we tested latency in the social cueing task for dogs from different divisions and (as you'd guess by now) found no statistically significant difference (ANOVA: $F_{2,25}$=0.921 P=0.411) (Fig. 10). If anything, the STEM dogs took longer to respond to social cues.

4 DISCUSSION

This study addressed multiple hypotheses linking genetic variation at the oxtytocin receptor gene to behavior in dogs. We experimentally addressed object permanence and social cueing, and we addressed broader axes of dog personality using a questionnaire administered to the owners. We genotyped dogs for one genetic variant of the oxytocin receptor gene that has previously been shown to be polymorphic in many dog breeds (Kis et al., 2014) and has also been shown to be associated with increased affiliation toward humans (Persson et al 2017). All dogs used in this study reside with members of the Reed College community.

The first surprising result in our study was the finding that several of the axes of drive were positively correlated with each other. The questionnaire that we used has been employed commercially by dog trainers and has been recommended for studies such as the one conducted here (Clotfelter and Hollis, 2008). However, the responses by Reed faculty, staff and students revealed a tendency for owner to score dogs high or low across the board. While this might be interpreted as some dogs having "bigger personalities", it is probably more accurately interpreted as owner bias. We advise future studies to devise a normalization scheme, either requiring owners to work with a limited number of points and distribute them across the questions. This same effect could be achieved by dividing each score by the total for that dog. While we considered this option, we were unsure whether the limit should be applied across all axes or just a subset, for example grouping Flight and Fight, and grouping Prey and Pack. Alternately, one could employ a principle component analysis in order to identify correlated traits within the study group as is often done for studies of "Animal Personality" (Sih et al., 2004). Further study is required to determine the best normalization scheme or to devise a new questionnaire.

The Reed dogs all performed very well on the object permanence tasks suggesting that Reed dogs are smarter than average. According to Piaget's stages of cognitive development, the majority of the Reed dogs perform at the level of an 18-24 month old human toddler. Only two dogs failed at three tests. The fact that several dogs succeeded at a task denoting a higher level than one they had failed is likely due to the similarity among tasks and the fact that they were presented in rapid succession. We attribute success at a higher level to practice and additional opportunity. We suggest that future testing should involve more difficult tasks with greater number of repetitions.

The majority of the dogs were also successful at the social cueing task, though with a lower success rate than the object permanence. This is consistent with similar studies conducted in the past (Lakatos et al., 2011) Again, there was no relationship between performance on this task and any of the other behavioral measures or calculated drives. We did not record the sex of the dog, though it has been shown female dogs see more physical contact from humans than do male dogs (Roth and Jensen 2015) and female dogs respond differently to intranasal administration of oxytocin (Oliva et al 2015). Therefore, future studies should record the sex of the test subject.

Our data do not support the hypothesis that genetic variation of the OXTR genes underlies observed variation in dog behavior with regard to social cueing, object permanence, or the four calculated drives for dog personality. We do not doubt our genotyping results. The replication of a a positive control for the RFLP analysis, was included with each set of samples run by each pair of students. Only genotypes for sets in which the positive control was conclusive were included in the analysis. These data suggest, that given the testing environment on the Reed Campus the genetic locus does not contribute significantly to the observed behavioral variation. This does not mean that the oxytocin system is not involved in dog/human social interactions. Nor does it preclude the possibility that the genetic variation does contribute to behavioral variation, but in our study, we assume that the environmental variation outweighed any contribution from genetic variation. For future studies, we suggest a more controlled setting for the behavioral testing and a genome wide approach. The a canine custom SNP high density microarray can be used to survey >150,000 SNPs across the dog genome (Shannon et al., 2015; Vaysse et al., 2011).

ACKNOWLEDGEMENTS

The students of Biology 431 thank all of the dogs and their owners who participated in this study. We also thank Greta Glover of the Biology stockroom who has prepped labs all semester.

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APPENDIX A: DRIVE QUESTIONNAIRE

<u>Prey drive</u>
1 Does your dog sniff the ground or air a lot?
5 Does your dog get excited by moving objects such as bikes or squirrels?
9 Does your dog stalk cats, other dogs, or things in the grass?
13 Does your dog bark when excited?
17 Does your dog pounce on toys?
21 Does your dog shake and destroy toys?
25 Does your dog steal food or garbage?
29 Does your dog like to carry things?
33 Does your dog "wolf down" food?
37 Does your dog dig and bury objects?

<u>Pack drive</u>
2 Does your dog get along with other dogs?
6 Does your dog get along with people?
10 Does your dog bark when left alone?
14 Does your dog solicit petting or like to snuggle?
18 Does your dog like to be groomed?
22 Does your dog seek eye contact with you?
26 Does your dog follow you around like a shadow?
30 Does your dog play a lot with other dogs?
34 Does your dog jump up to greet people?
38 Does your dog show reproductive behaviors such mounting other dogs?

<u>Fight drive</u>
3 Does your dog stand its ground or investigate strange objects and sounds?
7 Does your dog like to pay tuf-of-war games?
11 Does your dog bark or growl in deep tone?
15 Does your dog guard territory?
19 Does your dog guard food or toys?
23 Does your dog dislike being petted?
27 Does your dog dislike being bathed?
31 Does your dog guard the owners?
35 Does your dog fight with other dogs?
39 Does your dog get picked on by other dogs?

Flight drive

4 Does your dog run away from new situations?
8 Does your dog hide behind you when unable to cope?
12 Does your dog act fearful in unfamiliar situations?
16 Does your Dog tremble or whine when unsure?
20 Does your dog crawl or turn on its back when reprimanded?
24 Is your dog reluctant to come close to you when called?
28 Does your dog have difficulty standing still when groomed?
32 Does your dog cringe when someone strange bends over him/her?
36 Does your dog urinate after greeting other dogs?
40 Does your dog bite when cornered?

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