Bio342: Behavioral Genomics and Dog Cognition

"Cognition" is a general term describing the mental capacities of an animal, and often includes the ability to categorize, remember, and communicate about objects in the environment. Studies of animal cognition can provide insight into the evolution of cognitive processes in humans (Miklosi et al., 2004; Gomez, 2005). Object permanence, is one measure of cognition and is a universal characteristic among primates (Gomez, 2005). Many studies on object permanence 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; Table 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.

A related area of research focuses on the ability of animals to follow social cues from humans. The most frequently investigated cue is pointing toward a hidden object, which has been studied in a diverse array of mammals including non-human primates, dogs, wolves, goats, and cetaceans (Miklosi & Soproni, 2006). Interestingly, dogs perform much better at this task than do either wolves (*Canis lupus*) or chimpanzees (Pantroglodytes), which originally led some researchers (Hare et al., 2002) to speculate that
Domestication selects for a particular set of cognitive abilities that allow for human-dog communication.

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

Though independently evolved, social cognition in dogs and humans may rely on similar mechanisms that have been co-opted, or represent "deep-homologies". The oxytocin system has been demonstrated to play a critical role in complex human behaviors (Kumsta and Heinrichs, 2012), mating systems in voles (McGraw et al., 2012), and social interaction of fish (Reddon et al., 2012). In many of these organisms, genetic variation in the oxytocin receptor gene has been linked to variation in behavior. Two recent studies suggest that genetic polymorphisms in the canine oxytocin receptor gene (Figure 1) are linked to variation in affiliative behavior directed either to the owner (Persson et al., 2017) or unfamiliar individuals (Kis et al., 2014) in specific breeds. Here we aim to test whether this correlation persists across dog breeds. For simplicity, we will perform a social cueing task and an object permanence task to determine cognitive ability.

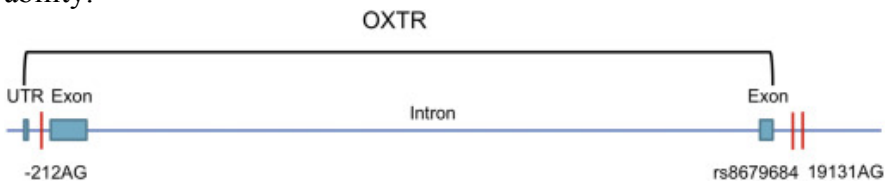

Figure 1. Diagram of the Oxytocin receptor gene showing three common SNP sites associated with different aspects of dog behavior. Two of the SNPs are named according to their location relative to the start site and the two bases that can be found at this site in different alleles. One SNP bears a historical name applied prior to its localization relative to the Oxytocin receptor gene.

In week 1, we will perform behavioral tests with dogs, and collect DNA samples.
In week 2, we will preform genetic tests on these dogs to determine Oxytocin Receptor genotype (protocol not included here).

Supplies per student pair:

- Dog Treats (owner supplied if possible)
- clipboard for recording data,
- stopwatch or timing device (phone),
- three 1 gallon buckets uniform size and color
- one opaque pint glass (Solo)
- String one piece 3 ft. and one 6 ft. long for placing buckets
- Tape to mark center starting position
- Foam tipped buccal swab (individually wrapped)
- Permanent Marker

☐ **Consult the Google Sheet Bio342_Dog_lab_schedule to select dogs to be tested. Each student pair should sign up for 3 dogs.**

☐ **Students should be aware of safety issues involved in working with other peoples' dogs and should involve the owner in the testing procedure as appropriate.**

Before you begin:

1. Greet owner.
2. Greet the dog, in the owner's presence as instructed by the owner.
3. Discuss the testing procedure with the owner and determine:
    a. Should the owner be present during testing?
    b. Should the owner play the role of assistant?
    c. Where is an appropriate and convenient relatively undisturbed testing area?
    d. Will be OK to drop the leash in selected testing area?
    e. What treats should be used as the food reward (12 total rewards will be administered)
4. The goal is to establish a neutral environment in which the dog can attend to the experimenters.
5. For some dogs (depending on anxiety levels and aggressive tendencies) this will be best achieved with the owner present, in other cases it will be best achieved with the owner absent. (Consider how these factors may influence the results)
6. If the owner can be absent during testing, ask them to complete the survey during testing, if not, they may complete the survey as soon as the testing is complete.

DNA Sample (see https://www.youtube.com/watch?v=MiSdFak1T2E**)**

1. Carefully take the foam tipped buccal swab from the individual wrapper, leaving wrapper in tact.
2. Ask the dog owner to collect DNA sample using the foam tipped buccal swab and rubbing across the dog's cheeks and gums (they should have viewed the online instructional video link)
3. Let the swab air dry for a few minutes.
4. Return the buccal swab to it's wrapper without touching it to any contaminated surfaces.
5. Label the swab wrapper with the assigned dog ID # from the google sheet and today's date.

Setup:

1. Use the tape to mark a center starting position (X) and a waiting position (W) (Figure 2).
2. Use the 6 ft string to determine a 6 ft radius.
3. Use the 3ft string to determine bucket placement according to figure 1.
4. (buckets should be equidistant from the dog 3 ft. apart from each other and all 6 ft from the dog)

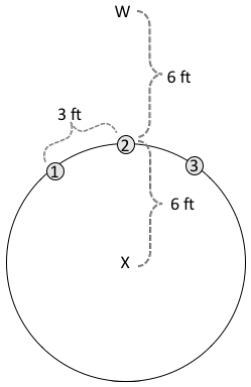

Figure 2. Testing set up. Bucket locations (1,2,3) equidistant to dog starting position (X) and removed from experimenter waiting position (W).

Visible Displacement Test,

1. The assistant (or owner) holds the dog on the center mark (X).
2. The experimenter walks back and forth behind the buckets holding food for the dog to see.
3. Once the experimenter has the dog's attention, they place the food inside one of the buckets.
4. The experimenter steps away from the dog and buckets and retreats to waiting position (W).
5. The assistant starts the timer.
6. At 10 seconds, the assistant releases the dog.
7. Allow time to run an additional 30 seconds.
8. Stop the timer when the dog attempts to move or displace the correct bucket or gets within 1 body length of the incorrect bucket.
9. In your lab notebook, using a table like the one depicted below, record that time.
10. Score the trial as **successful** if the dog attempts to move or displace the correct bucket
11. Score the trial as **unsuccessful** if the dog approaches the incorrect bucket within one body length or does not make an appropriate response for 30 seconds and enter NA in the table.
12. Repeat two more times using the **same** bucket for each trial.
13. If a dog is successful in two or three trials, it has reached Stage 4 (Table 1).

DOG#ID _____ (from google sheet)

Trial#	latency	Successful (Y/N)
1		
2		
3		

Sequential Visible Displacement

1. Repeat the Visible Displacement Test (above) but select a different bucket in which to place the food for each of the three trials.
2. Record the results as before.
3. If a dog is successful in two or three trials, it has reached Stage 5 (Table 1).

Invisible Displacement Test

1. Repeat the Visible Displacement Test (above) but once the experimenter has the dog's attention
2. place the food inside the smaller vessel
    a. lower that smaller vessel inside the bucket.
    b. move the food from the smaller vessel into the bucket (without the dog seeing)
    c. lift up the smaller vessel
    d. show the dog that it is empty.
3. As before, the experimenter then steps away from the dog and buckets to waiting position (W).
4. Score the trial as before and repeat for a total of 3 trials.
5. If a dog is successful in two or three trials, it has reached Stage 6 (Table 1).

Social Cueing

1. The assistant takes the dog out of the room or 20 yards away.
2. Experimenter removes bucket # 2
3. Experimenter flips a coin to decide whether to hide food in bucket #1(heads) or #3(tails).
4. Hide the food.
5. Assistant brings the dog back to the marked position (X)
6. While the assistant holds the dog and starts a timer, the experimenter stands where bucket #2 was and while facing the dog, for 10 seconds points and looks at the bucket in which the food is hidden.
7. The assistant releases the dog.
8. The trial is scored as before and repeated for a total of 3 trials.
9. If a dog is successful in two or three trials, it has succeeded at Social Cueing.

When all 12 trials are complete:

1. Collect the buckets, string and remaining treats.
2. Thank the owner and the dog.
3. Put the completed dog behavior questionnaire into a student lab notebook.
4. Proceed to the next dog appointment.

Back in the lab DNA prep:

1. Put gloves on.
2. Get ES, TPS and NS microcentrifuge tubes from freezer (one for each dog you tested).
3. Label each tube containing 200ul of ES with a dog ID#.
4. Add 25 µl of TPS to the labeled microcentrifuge tube with 200 µl of ES
5. Flick to mix.
6. Carefully take Dog sample foam buccal swab out of its individual wrapper and Rapidly twizzle* it in the correct labeled microfuge tube with TPS+ES. Throw away the buccal swab. (**Highly technical term: slide thumb and forefinger across each other to generate a washing-machine-like action.)*
7. Spin down the sample in small centrifuge. Incubate it at room temperature for ≥10 minutes.
8. Put a lid lock on your microcentrifuge tube to keep it from opening when it gets hot and to protect your fingers from getting burned.
9. Incubate the sample at 95°C (use hot bead bath) for 3 minutes. *Set a timer and be exact about the timing*.
10. Remove the sample from the heat source.
11. Remove (and save) the lid lock.
12. Add 200 µl of NS.
13. Mix by flicking.
14. Check that the label is legible.
15. Store in the freezer -20°C

Back in the lab Data Entry:

1. Student #1 should attach all raw survey responses to individual notebook pages.
2. Open the Google sheet and Record in the Google Sheet, the location of each survey response.
3. Enter the dog survey responses for questions #1-#40 on the appropriate row.
    a. Enter "**0**" for "Almost Never"
    b. Enter "**5**" for "Sometimes"
    c. Enter "**10**" for "Almost Always"
4. Student #2 should double check that data is entered correctly
5. The Google Sheet formulas calculate "Prey Drive", "Pack Drive", "Fight Drive" & "Flight Drive"
6. For stages 4-6, enter 1 if the dog reached that stage according to behavioral trials.
7. Calculate the average latency for the successful trials at each stage and enter that in the class dataset.

The following email was sent to Reed faculty and staff
(OK to send to additional dog owners in the community)

Calling all Dog Owners
The Biology 342 Animal Behavior Class is conducting a dog behavioral genetics lab and looking for subjects. The behavioral tests (conducted in the afternoon Wednesday Oct 2nd and Thursday October 3rd) involve a simple object permanence task, and brief social cueing task. It will take approximately 20 minutes. The dog will receive ~ 12 dog treats for the effort. Treats will be provided by the student unless the owner wishes to provide specific treats. The owner is not required to be present during behavioral testing (provided the dog is friendly and trustworth around students). There will be a very brief questionnaire about the dog's behavior that must be filled out by the owner. We will also ask the dog owners to collect check swabs for DNA samples (supplies provided by students). The class will compile behavioral data and test the DNA for genetic variants of the Oxytocin Receptor. Genetic variation in this neuropeptide receptor gene has been associated with some dog behaviors in some breeds. The class will produce a report of their findings that will be shared with all volunteer dog owners.

To sign up for a specific testing time on Wednesday or Thursday, please go to the google sheet with the link below and enter your name and your location next to a time that is convenient for you.

https://docs.google.com/spreadsheets/d/1J22OJHb6o9grXuY7GigTN1ZjI7Au7TAdZ91339FfmV4/edit?usp=sharing

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