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HEALTHCARE AND TECHNOLOGY

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by

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Abstract

Animal welfare has been an important issue in laboratory animal science since the mid 1900's. The goal of this project was to discover whether or not training laboratory sheep reduces their stress level during experimental procedures. Based on ethogram data and experimental observation, it was concluded that training did reduce stress. This research could encourage scientists to take a better look at their current husbandry program and improve the life not only of laboratory sheep, but other animals as well.

Introduction

Animal Welfare

Animal welfare has been an important issue since 1876 when the United Kingdom instituted the Cruelty to Animals Act. This act required that the government give licenses to individual scientists who used animals in research. About a century later, in 1966, the United States passed the first legislation governing the use of animals in research called the Laboratory Animal Welfare Act. This law was intended to ensure that research animals were provided with humane care. In 1970 the law was amended and renamed the Animal Welfare Act (Anderson 2007). Every year millions of animals are being used in research to help the lives of others. The health and safety of every research animal should be taken as top priority throughout the scientific community.

There are many countries that have laws meant to improve the living conditions and the health of animals who are used in research. The United Kingdom has an advanced program that puts animal health at its forefront. Their system requires certification and licensing of researchers and their experiments, institutional certification, and the establishment of an Animal Procedures Committee (Monamy 2000). Certification and licensing is probably the most important aspect of their system because this allows the law to look closely at the establishment before experiments begin. First, a university or a research facility must receive a Certificate of Designation by demonstrating that it is a suitable place for research. More importantly, the certificate indicates that the establishment takes care of the laboratory animals before, during, and after an experiment. After institutional certification, the researchers receive personal and project licenses from the British Home Secretary after a period of supervision. Next, the

project license lays out the course of the experiment and limits the severity of animal suffering based on the benefits of the outcome. In addition, the Animal Procedures Committee consists of scientists, physicians, veterinarians, and non-practicing experimenters, which ensures a wide array of insight into the matter (Monamy 2000).

In addition to the United Kingdom, the United States enforces many laws and requirements for laboratory animal research. In 1985, the Food Security Act (also called the Improved Standards for Laboratory Animals Act) required all USDA-registered research facilities to establish an institutional animal care and use committee (IACUC). The IACUC reviews activities involving the animals and determines if the personnel conducting the procedures are trained and qualified. Furthermore, the USDA requires that the veterinary medical officers conduct research facility inspections frequently. In addition to the Improved Standards for Laboratory Animals Act, the Health Research Extension Act of 1985 was established. This law directs the Secretary of Health and Human Services, through the Director of the National Institutes of Health (NIH), to establish rules and regulations for the proper care and treatment of animals in biomedical and behavioral research, to require animal care committees, and to ensure that all personnel are trained (Anderson 2007). Institutions must make sure that "scientists, animal technicians, and other personnel involved with animal care, treatment, and use by the applicant [institution] have available to them instruction or training in the humane practice of animal maintenance and experimentation, and the concept, availability, and use of research or testing methods that limit the use of animals or limit animal distress" (Anderson 2007).

Sheep Behavior

Historically categorized as prey animals, sheep have evolved several defense mechanisms in response to predation. As a result, defense against predation is a strong factor in determining the behavior of sheep. One of the most prevalent reactions to predation in sheep is the flocking instinct (Dwyer 2004). Like most prey animals, the fight or flight response in sheep tends to rely more heavily on the flight aspect. Instead of fighting a potential threat, the flocking response provides them with safety in numbers. Interestingly enough, sheep appear to rely predominantly on visual cues to recognize one another, although they can also make use of olfactory and auditory information (Dwyer 2004). It has even been suggested that a sheep has the ability to remember another sheep's facial features for several years, even if they are not in daily contact with that individual. Sheep on pasture have been observed to have several sentries standing on the outside boundaries of the flock looking for potential threats. If one should arise, they vocalize to alert the other members of the group. This stimulates the other sheep to come together in a tightly packed flock so that they can move as one unit. In comparison to wild species, domesticated species show a reduced alertness and attenuated flight distances (Dwyer 2004). This most likely has to do with their long-standing relationship with man. Domestic sheep are far more used to being handled than wild sheep and are conditioned to expect food to be provided for them by their caretakers. The security of a life in captivity reduces their fight or flight response to situations that could potentially be perceived as a threat. However, when domestic sheep do feel that they are in danger, they still have a strong tendency to flock.

There are several behavioral and physiological responses associated with stress in sheep. As with any animal, stress causes both physiological and behavioral changes. Because of their need to flock, visual and physical isolation from other group members is an intense stressor for sheep. Typical responses to visual isolation include increased vocalizations and general activity, and increased plasma cortisol levels (Baldock et. Al. 1990). In experiments that tested a range of stressors, the main behavioral responses observed were increased immobility or increased locomotion (depending on the situation), decreased sleeping and resting in conjunction with increased alertness, increased vocalization, and increased elimination (Cockram 2004).

The learning experiences of sheep and their responses to subsequent events rely heavily on their previous life experiences, a process known as habituation. Sheep readily learn to associate unpleasant experiences with places, people or auditory stimuli, and they show long-term avoidance of these stimuli, which is not seen in naïve animals (Dwyer 2004). Important to their survival, sheep tend to remember negative events for a much longer period of time than they do positive events. If they have had a traumatic enough experience, memory of that event and a latency to be put in that situation again can stay with them for their entire lifetime. Animals, including sheep, can instantly recognize the voice of a familiar trusted person. They may also become frightened when they hear the voice of a person who abused them (Grandin 1989). Habituation, however, is not always a negative thing. It can also be used as a tool to lessen the fear that farm animals normally experience in novel situations (Kilgour 1987) and to influence the behavioral and physiological responses induced by those situations (Cockram 2004).

Positive Reinforcement and Operant Conditioning

Though there are many schools of thought on how sheep and other livestock should be properly handled, it has been discovered that positive reinforcement and daily gentle handling are most effective in reducing the stress of an animal. There are several experimental and general husbandry applications to positive reinforcement. Reducing stress during handling can improve productivity and prevent physiological changes that could confound research results or lower productivity (Grandin 1989). Any animal that is stressed experiences a range of physiological and behavioral changes that can contribute to inaccurate test results during an experiment or to an inhibition of their ability to thrive in a farm situation. Several interesting studies have been conducted that observed the effects of gentling on sheep. In the first of these studies, sheep raised in a pen in close contact with people were found to have a less intense physiological response to handling than sheep raised on pasture (Grandin 1989). In a similar experiment, researchers discovered that frequent and gentle handling can reduce the stress accompanying isolation and restraint, and might even initiate approach behaviors. It was also observed that gently handled sheep approached more quickly and sniffed the trainer's hand more often than restrained and control group (untrained) sheep (Mateo et. Al. 1991). A third research project involved daily gentling for five weeks and discovered that such treatment of the animals was effective in reducing flight distance and the heart rate response to an approaching human (Hargreaves et. Al. 1990). This data has been vital in demonstrating that sheep, along with other animals, that are allowed to become habituated to human contact through positive reinforcement are both better test subjects and more valuable farm animals.

Operant conditioning is a relatively recent development at the forefront of animal training that utilizes positive reinforcement to reward desired behaviors. Though this technique had its beginning in zoos, it has most recently been used both on farms and in research environments. The basis for operant conditioning lies in the idea that current consequences control future performance. After a desired behavior is performed, a reward (called a reinforcer) immediately follows. Because it is not always possible to deliver a reinforcer at the exact instant the behavior occurs, many trainers have employed the use of a clicker. Animals learn to associate the sound of the clicker with the delivery of a reward soon afterwards. In this way, the clicker acts as a means to mark the correct behavior and as a bridge between the time the behavior is performed and when the reward is delivered. One of the most prevalent uses of operant conditioning on farms is the use of an automatic watering device that requires the animal to push down on a plunger in order to drink. In this case, the water acts as the reinforcer and clicker is not needed as a bridge because the water is received as soon as the plunger is depressed. In order to train more complicated behaviors it is necessary to utilize a process known as shaping. This means that the desired response is obtained after rewarding incremental actions that lead up to the ultimate goal behavior. In cases such as these, a clicker can be an extremely useful tool. In laboratory settings, it has been reported that clicker training has resulted in significant reductions in injuries to the animals and staff, the stress levels of animals and staff, and the number of staff members required for procedures (Blye, Burke, James, Fitzgerald, & Cox, 2006).

There are many benefits to investing time in the training of sheep involved in research studies. Increasing the efficiency of husbandry procedures, reducing the stress of

both the handlers and the animals, and improving the quality of life for both farm and research animals are just a few of many advantages to using positive reinforcement and operant conditioning techniques. It has been suggested that training valuable breeding animals or animals used in long-term research studies to voluntarily enter a restraining device reduces the stress of the people and the animals involved (Grandin 1989). In addition, it has been found that trained sheep can be used to lead naïve sheep through a handling facility with little to no resistance (Grandin 1989). In one research project it was discovered that positive food reinforcement, such as barley, reduced the amount of labor needed to move sheep though a race system into a handling machine (Mateo et. Al.1991). In the interest of improving animal welfare, it is important that such methods be considered by both laboratory personnel and farmers alike.

Measuring Pain and Stress

In animals, pain and stress can be measured by looking at plasma concentrations of cortisol, ACTH (adrenocorticotrophic hormone), lactate, and glutamic oxaloacetic transminase (Apple et al. 1993). However, the best indicator for stress is plasma cortisol levels, which rise when an animal experiences any kind of stress. Cortisol is a corticosteroid hormone produced in the adrenal cortex and is referred to as the "stress hormone" (Voet et al 2006). One might think that the initial blood draw would affect the results when testing on an animal; however, taking the sample quickly will not interfere with results because it takes a few minutes for cortisol levels to rise in sheep (Davidson et al 1968).

The hypothalamic-pituitary-adrenal (HPA) axis is the body's primary stress-response system. HPA axis activation begins when the adrenal glands secrete

glucocorticoid hormones, such as cortisol, into the blood stream. It is the body's primary stress response system and it is especially sensitive to psychological stressors such as separations from attachment of objects and loss of control over environmental occurrence (Henessy et al. 1998).

Certain stressors for sheep can affect cortisol levels for hours or even weeks. According to Dwyer and Bornett (2004), moving sheep indoors from pastures causes a large increase in plasma cortisol levels that can take weeks to normalize. When plasma cortisol levels are raised by other stressors, such as tail docking in lambs, it can take approximately three days for them to normalize (Dwyer and Bornett 2004). Castrating lambs showed a peak in cortisol levels between 15 and 90 minutes after the procedure was finished (Mellor et al. 1991). Moreover, isolation of sheep on consecutive days elevates the cortisol levels and it may take nine hours to establish some semblance of normalization in the baseline values (Dwyer and Bornett 2004).

Upon the completion of this experiment it is expected that the trained sheep will exhibit lower peaks in their plasma cortisol levels after stress than the untrained sheep. In addition, it is expected that the trained sheep will have attenuated flight distances and will even approach their handlers eagerly to be worked with. It is hoped that this research will provide scientific proof that investing time into the operant conditioning of laboratory animals will not only reduce stress but improve experimental results overall.

Materials and Methods

Four mature Finn/Dorset crossbreed female sheep were used in this study. Two of these sheep (# 17582 and # 17583) were handled using standard husbandry procedures including cleaning, feeding, watering, and minimal handling when necessary. The other two sheep (# 17577 and # 17580) were cared for with the same traditional husbandry techniques except that they were trained using operant conditioning methods. The two groups were separated into two different areas. The trained sheep were housed together in an isolated room from which the untrained sheep could not hear the trainers' voices or the sound of the clickers. The untrained sheep were housed in a standard 12'x 12' horse stall with two other sheep for company. The two trained sheep were worked with twice a day for ten days with each session lasting approximately fifteen minutes. Dog training clickers were used to shape desired behaviors and approximately 2 ounces of Nutrena brand grain was given per sheep as reinforcement each session. A Calf-Cart was used for transport purposes when moving sheep from their enclosures to the procedure room and back.

There were several 'goal behaviors' that were predetermined before the beginning of the experiment. These were: willingly approaching a handler, touching their nose to a handler's fist on command, standing calmly for a physical examination or other minor medical procedure, and loading into and out of the Calf-Cart when asked. The four sheep used for this research project all came from a farm background where they had minimal contact with humans. As a result, the first step in the training process was simply getting them to approach and eat grain out of the handler's open palm. In order to accomplish this, it was important for the trainer to kneel down and avert her gaze from the sheep to

avoid intimidating them. Once a sheep had approached and eaten out of hand, the clicker was immediately used to ensure that the animal began to associate its sound with food. This procedure was perpetuated until it was clear that the sheep would approach and take grain from the handler without hesitation. The next step in the process was to hold grain in a closed fist and wait for the sheep to touch her nose to it. Once she did, the clicker was used and grain given as a reward. When this behavior was performed consistently, the command "target" was added just before the sheep touched her nose to the handler's fist. The clicker was sounded and grain given to reinforce the proper response. Eventually, the "target" behavior became a tool with which a trainer could lead sheep around the enclosure. The sheep would follow the handler's fist anywhere she was asked when the command was used.

The second behavior, standing for a physical exam or medical procedure, was trained using the command "touch." Just before physically touching the animal with a finger, the handler would say the word "touch," clicking and rewarding only for calm behavior. If the sheep backed away or fidgeted when touched, no reward was given. For the purposes of this experiment, special attention was given to touching the neck around the jugular vein in hopes that this would desensitize the sheep to the placement of a jugular catheter.

Loading into and out of the Calf-Cart was the last and most complex behavior the sheep had to learn. Using the "target" command, sheep were led into the cart as the trainer clicked and rewarded for one, two, three, and four hooves placed in the cart incrementally. The sheep rarely got all four hooves in the cart in the first session the cart was introduced, so the process was undertaken over a series of several days. In the case

of a more shy animal, simply sniffing the cart and showing interest in it was clicked and rewarded until the first step in was taken. Once a sheep had loaded all the way into the cart several times in succession, one handler continued to "target" them to fist at the head of the cart while another handler gently swung the cart's door back and forth. The sheep was rewarded for not backing out of the cart and remaining calm. If she did back away, she was targeted back into the Calf-Cart and the procedure started from the beginning. As soon as a sheep was comfortable with the door being opened and closed, she was shut into the cart and rewarded for calm behavior. Next, the cart was rolled back and forth in the enclosure while the sheep was, once again, rewarded for staying calm. The final step was to transport the sheep out of her enclosure and into the procedure room where she was asked to perform the "touch" command on the area of her neck where the jugular vein is located. Throughout the transport process the handler would stay at the front of the cart and "target" the sheep to fist, clicking and rewarding for proper response to the command. As soon as she was back in her enclosure, the sheep was asked to back out of the cart and then was targeted back in without closing the door in order to reinforce the cart as a positive experience. An instruction manual detailing the specific steps taken over the ten day period, a training log chronicling the progress of both sheep throughout the learning process, and photographs of the training can be found in Appendix A.

Before the first day of the experiment, a baseline ethogram was recorded of the sheep. This involved setting up a camera and DVD recorder in both the room with the trained sheep and the stall with the untrained sheep. In addition, a cattle marker was used to put a stripe on the back of trained sheep #17580, a stripe on the back of untrained sheep #17582, and two stripes on the back of untrained sheep #17583 as identification.

No marking was put on trained sheep #17577. The recorders were then set to record for two hours in the morning from 7:00 – 9:00 AM. The footage was later reviewed and scored using a specially developed system. Every 60 seconds a check mark was put next to the behavior the observed sheep was performing at that given moment. The behaviors scored consisted of ruminating, eating, drinking, vocalization, defecation, urination, sleeping, aggression, submission, locomotion, standing, lying down, scratching, grooming, yawning, pawing, and offscreen. Refer to Appendix B for an example of the scoring sheet used and a list of behavioral term definitions. Once the footage was scored, the percent of time each behavior was performed was determined. This baseline ethogram data for each sheep would later be compared to the ethogram data taken during the actual experiment.

The experiment itself happened over a period of two days. The first day, trained sheep #17580 and untrained sheep #17582 were studied. First, trained sheep #17580 was loaded into the Calf-Cart and transported to the procedure room using the same operant training techniques that were previously described. Once in the procedure room, an 18-gauge jugular catheter was placed after two tries and the stripe on the sheep's back redrawn with a cattle marker for identification. An initial (T_0) blood sample was taken and placed into a serum separating vial. It would be tested at a later date for its plasma cortisol level. The sheep was then transported back to her enclosure and an experimental ethogram began recording.

Next to be transported to the procedure room was untrained sheep #17582. Because she had minimal experience with human contact it was necessary to catch her and guide her into the calf-cart. Once in the cart, she was moved down to the procedure

room and had an 18-gauge jugular catheter placed after two tries. An initial (T_0) blood sample was also taken for her and put in a serum separating vial. A cattle marker was used to put a stripe across her back for identification. She was then transported back to her enclosure and an experimental ethogram began recording.

For both sheep successive blood samples were taken at T_1 = 30 minutes, T_2 = 1 hour, T_3 = 2 hours, and T_4 = 3 hours. All samples would be sent out for plasma cortisol level analysis. The two experimental ethograms were allowed to record for 2 hours and scored in the same way that the baseline ethograms had previously been.

On the second day, trained sheep #17577 and untrained sheep #17583 were studied. The same procedure was used for these two sheep as the one described above, however there were a few differences. Trained sheep #17577 was marked with two lines on her hindquarters, a line across her shoulders, and a spot on her head for identification and untrained sheep #17583 was marked with a circle on her hindquarters. Instead of jugular catheters, each of the four blood draws on both sheep were taken using a 20 gauge needle on a 6cc syringe. This was a result of the jugular catheters' tendency to bend while in the vein, preventing the passage of blood during draws. Ethograms were taken of these sheep as well and were also allowed to record for 2 hours. All four experimental ethograms were scored and the behavior percentages compared to those determined by the baseline ethograms.

Results

The baseline ethogram results for trained sheep #17580 indicated that she spent most of her time ruminating, lying down, sleeping and eating. A small amount of her time was spent on defecation, locomotion, standing, pawing, showing submission, and being off screen. During the two hours she never drank, vocalized, urinated, showed aggression, scratched, groomed, or yawned. See Table 1 for exact percentages. The experimental ethogram results for trained sheep #17580 indicated that most of her time was spent eating. She only spent a small amount of time drinking, defecating, moving around, standing, and being off screen. During the experiment she never ruminated, vocalized, urinated, slept, showed aggression, showed submission, laid down, scratched, groomed, yawned, or pawed. Table 2 lists the exact percentages. There was a 67.5 % increase in the amount of time this sheep spent eating between baseline and experimental ethograms. There were small increases in the amount of time she spent drinking, lying down, and being off screen. A 34.2% decrease was seen in the amount of time she spent ruminating, a 29.2% decrease in the time she lay down, and a 15.8% decrease was seen in the amount of time she slept on the day of the experiment. Small decreases were observed in the amount of time she showed submission, pawed the ground, and stood off screen. No differences existed between vocalization, defecation, urination, showing aggression, locomotion, scratching, grooming, or yawning. Table 3 lists the differences between the baseline and experimental ethograms. Refer to Figure 1 for a graphical representation of all data for trained sheep #17580.

During her baseline ethogram, trained sheep #17577 spent the most amount of time ruminating, eating, and lying down. She spent some of her time drinking, defecating,

urinating, sleeping, showing aggression, moving around her enclosure, standing, and being off screen. Within the two hours of recording she never vocalized, showed submission, scratched, groomed, yawned, or pawed. Refer to Table 1 for exact percentages. On the day of the experiment this sheep ate, ruminated, and stood the majority of the time, though she also spent a small amount of time moving around, and being off screen. Refer to Table 2. A 28.3% increase was observed in the amount of time she spent standing and a 15.9% increase was seen in the time she spent eating on the day of the experiment. A small increase was seen in the amount of time she spent moving around her enclosure. A 15.0% decrease was observed in the amount of time she ruminated during the experiment while other small decreases were seen in drinking, defecation, urination, sleeping, showing aggression, and standing off screen. Refer to Table 3 for all percentage differences and to Figure 2 for a graphical representation of the results for trained sheep #17577.

The baseline results of untrained sheep #17582 showed a large amount of rumination, eating, sleeping, standing, and laying down behavior. Occasionally, she would defecate, urinate, show aggression, move around, or scratch. During the entire baseline ethogram, however, she never drank, vocalized, showed submission, groomed, yawned, pawed, or was off screen. Refer to Table 1 for the baseline ethogram results. The experimental ethogram results showed a very large amount of eating and standing, and a small amount of ruminating, drinking, and locomotion. Untrained sheep #17582 never vocalized, defecated, urinated, slept, showed aggression or submission, laid down scratched, groomed, yawned, pawed at the ground, or was off screen. Refer to Table 2 for the exact percentage values of the experimental ethogram. The differences in the baseline

and experimental ethograms show a 70% increase in eating, a 25% decrease in rumination, and a 25% decrease in lying down. Other behaviors such as drinking, locomotion, standing, and scratching did not fluctuate much. Refer to Table 3 for the differences in the ethogram results and Figure 3 for a graphical representation of all data for untrained sheep #17582.

Untrained sheep #17583 showed a large percentage of ruminating, locomotion, standing, and lying down during the baseline ethogram. However, she occasionally would eat, drink, defecate, urinate, act submissive, and scratch. During this time, she did not show any vocalization, sleeping, aggression, grooming, yawning, or pawing behaviors. Refer to Table 1 for baseline ethogram results. On the other hand, there was a large percentage of eating and locomotion observed in the experimental ethogram, but a small percentage of rumination, drinking, defecation, urination, and submission. Furthermore, there were no signs of vocalization, sleeping, aggression, standing, laying down, scratching, grooming, yawning, or pawing. Refer to Table 2 for experimental ethogram results. There was a great increase in the amount of eating that this sheep performed and a large decrease in rumination on the day of experimentation. Also, there was a large increase in the percentage of time sheep #17583 spent standing and a subsequent decrease in the time she spent lying down. There were also small decreases in the amount of time spent defecating, urinating, and scratching, while there were increases in submission and locomotion. Refer to Table 3 for the differences in the ethogram results and Figure 4 for a graphical representation of all data for untrained sheep #17583. Though blood samples were taken from each sheep to determine plasma cortisol levels, the results are still pending.

Table 1: *Baseline Ethogram Data*

Date: 12/4/2007
Time: 7:00 AM – 9:00 AM

Sheep:	Trained #17580	Trained #17577	Untrained #17582	Untrained #17583
Behavior				
Ruminating	34.2%	25.0%	25.8%	39.2%
Eating	10.8%	25.8%	10.0%	2.50%
Drinking	0.00%	0.80%	0.00%	1.70%
Vocalization	0.00%	0.00%	0.00%	0.00%
Defecation	0.80%	0.80%	1.70%	1.70%
Urination	0.00%	0.80%	0.80%	2.50%
Sleeping	15.8%	1.70%	15.0%	0.00%
Aggression	0.00%	0.80%	0.80%	0.00%
Submission	0.80%	0.00%	0.00%	0.80%
Locomotion	4.20%	0.80%	0.80%	10.8%
Standing	0.80%	6.70%	18.3%	13.3%
Laying Down	29.2%	35.0%	25.0%	25.8%
Scratching	0.00%	0.00%	1.70%	1.70%
Grooming	0.00%	0.00%	0.00%	0.00%
Yawning	0.00%	0.00%	0.00%	0.00%
Pawing	2.50%	0.00%	0.00%	0.00%
Offscreen	0.80%	1.70%	0.00%	0.00%

Table 1 lists the percentage of time each sheep spent on a particular behavior during the baseline ethogram.

Table 2: *Experimental Ethogram Data*

Date: 12/10/2007 – 12/11/2007
Time (Day 1): 8:30 – 10:30
Time (Day 2): 8:04 AM – 10:04 AM

Sheep:	Trained # 17580	Trained # 17577	Untrained # 17582	Untrained # 17583
Behavior				
Rumination	0.00%	10.0%	0.80%	0.80%
Eating	78.3%	41.7%	80.8%	46.7%
Drinking	3.30%	0.00%	0.80%	1.70%
Vocalization	0.00%	0.00%	0.00%	0.00%
Defecation	0.80%	0.00%	0.00%	0.80%
Urination	0.00%	0.00%	0.00%	0.80%
Sleeping	0.00%	0.00%	0.00%	0.00%
Aggression	0.00%	0.00%	0.00%	0.00%
Submission	0.00%	0.00%	0.00%	2.50%
Locomotion	4.20%	7.50%	2.50%	9.20%
Standing	5.80%	35.0%	15.0%	38.3%
Laying down	0.00%	0.00%	0.00%	0.00%
Scratching	0.00%	0.00%	0.00%	0.00%
Grooming	0.00%	0.00%	0.00%	0.00%
Yawning	0.00%	0.00%	0.00%	0.00%
Pawing	0.00%	0.00%	0.00%	0.00%
Offscreen	7.50%	5.80%	0.00%	0.00%

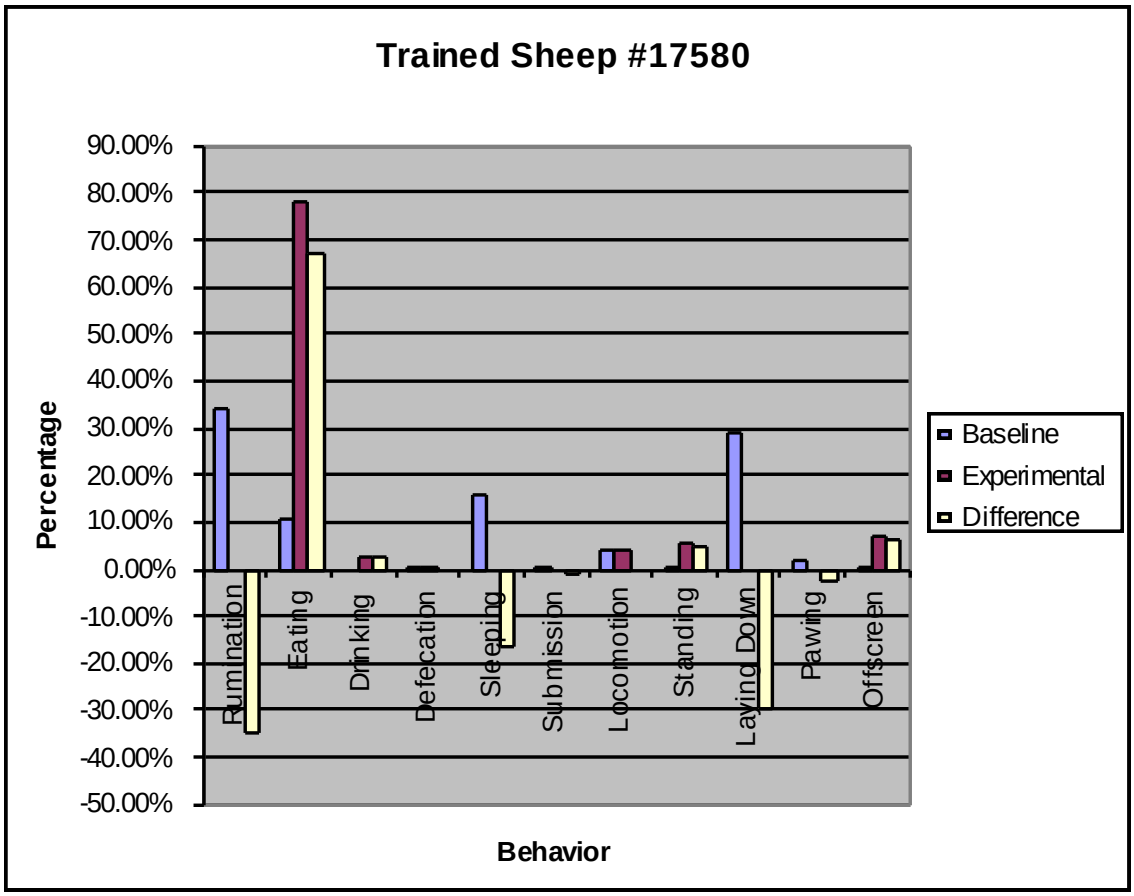
Table 2 lists the percentage of time each sheep spent on a particular behavior during the experimental ethogram.

Table 3: *Percentage Differences Indicating an Increase or Decrease in the Performance of a Behavior from the Baseline Ethogram to the Experimental Ethogram*

Sheep:	Trained #17580	Trained #17577	Untrained #17582	Untrained #17583
Behavior				
Rumination	-34.2%	-15.0%	-25.0%	-32.4%
Eating	+67.5%	+15.9%	+70.8%	+44.2%
Drinking	+3.30%	-0.80%	+0.80%	0.00%
Vocalization	0.00%	0.00%	0.00%	0.00%
Defecation	0.00%	-0.80%	0.00%	-0.90%
Urination	0.00%	-0.80%	0.00%	-1.7%
Sleeping	-15.8%	-1.70%	0.00%	0.00%
Aggression	0.00%	-0.80%	0.00%	0.00%
Submission	-0.80%	0.00%	0.00%	+1.70%
Locomotion	0.00%	+6.70%	+1.70%	-1.60%
Standing	+5.00%	+28.3%	+3.30%	+25.0%
Laying Down	-29.2%	-35.0%	-25.0%	-25.8%
Scratching	0.00%	0.00%	-1.70%	-1.7%
Grooming	0.00%	0.00%	0.00%	0.00%
Yawning	0.00%	0.00%	0.00%	0.00%
Pawing	-2.50%	0.00%	0.00%	0.00%
Offscreen	+6.70%	+4.10%	0.00%	0.00%

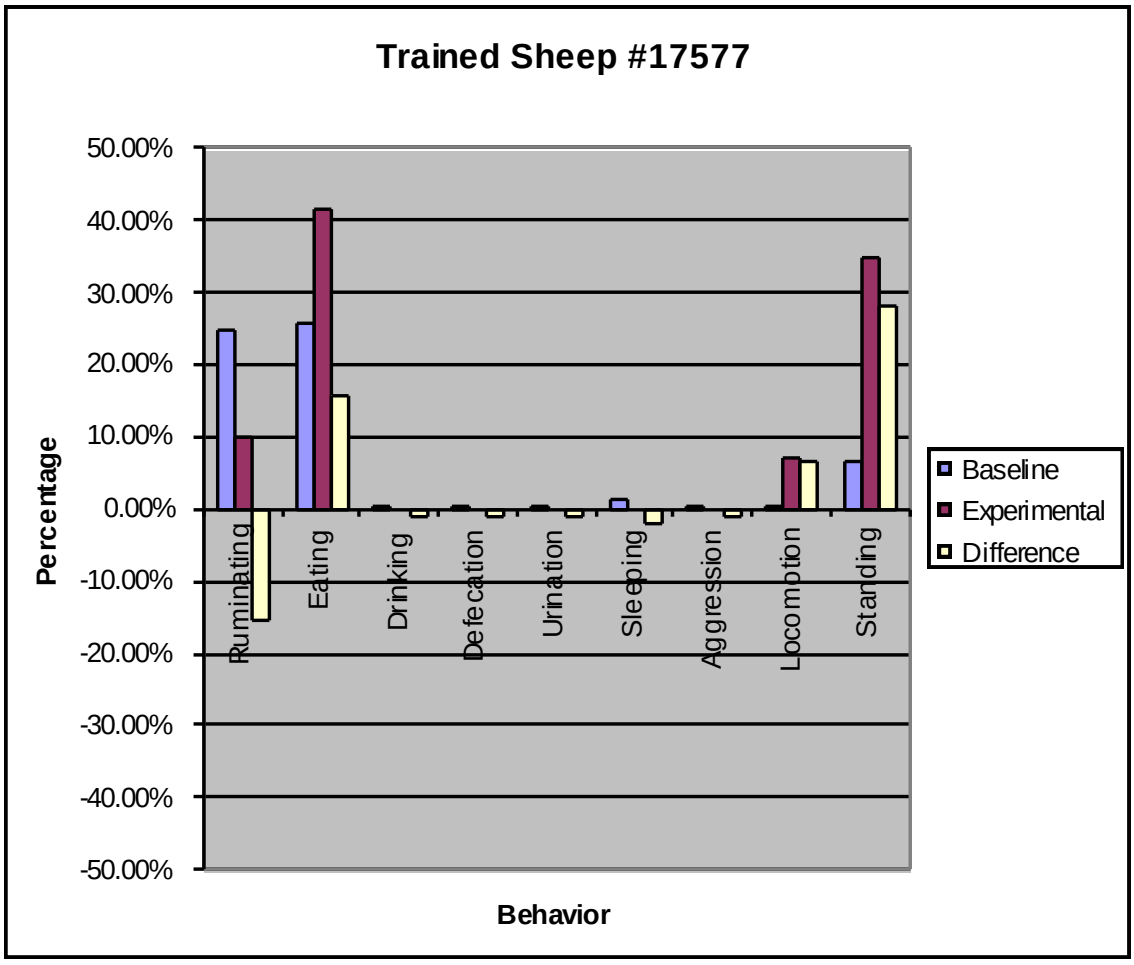
Table 3 lists the increases and decreases in the percentage of time each sheep spent on a particular behavior during the day of the experiment.

Figure 1: Graphical Representation of Tables 1, 2, and 3 for Trained Sheep #17580



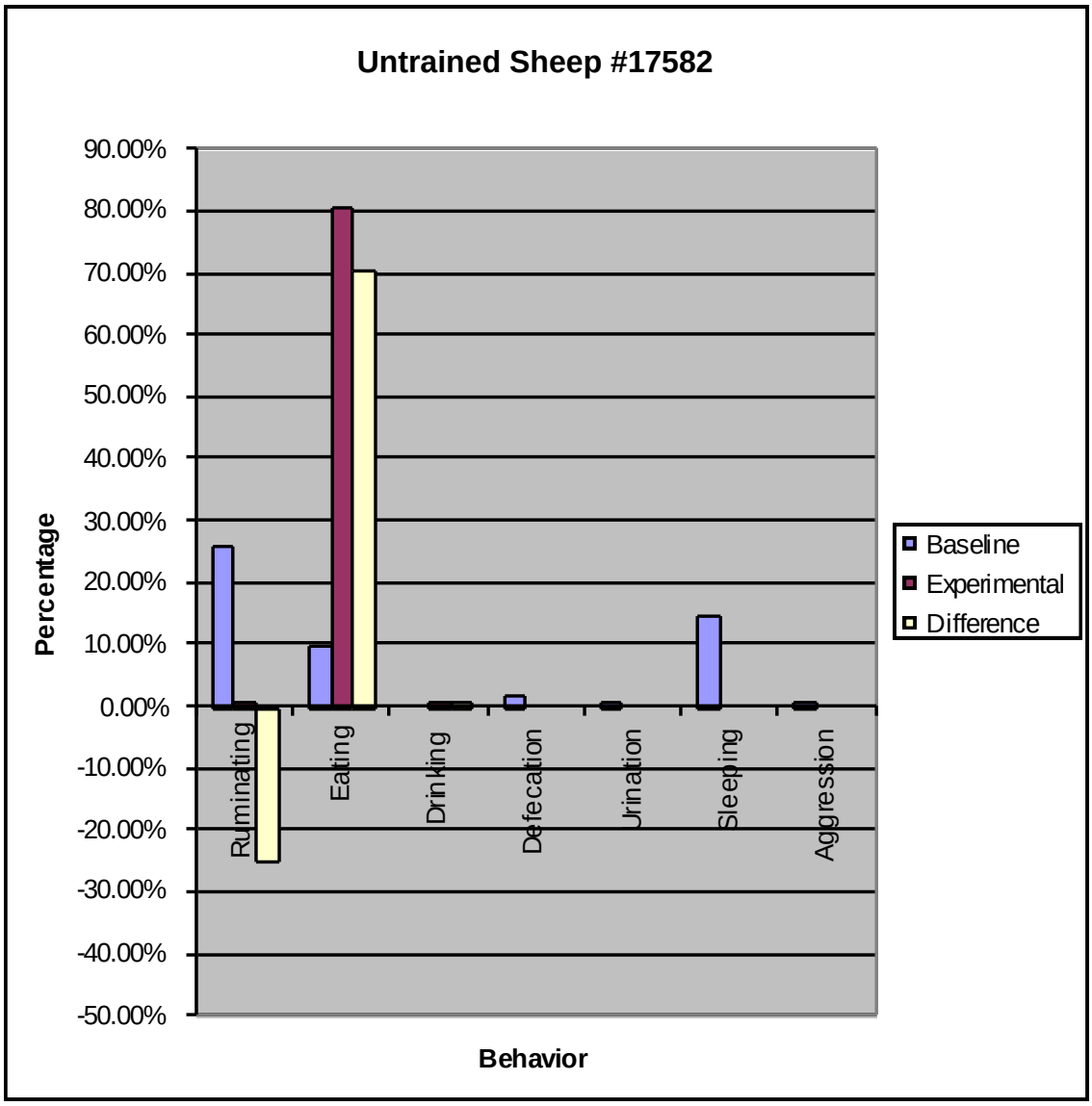
This graph represents the differences between the baseline and experimental ethograms. The blue bar indicates the percent of time spent performing each behavior during the baseline ethogram while the purple line indicates the same data for the experimental ethogram. The yellow bar indicates the positive or negative change in the amount of time spent performing a particular behavior from the baseline to the experimental ethogram. Behaviors that were never observed were not graphed.

Figure 2: Graphical Representation of Tables 1, 2, and 3 for Trained Sheep #17577



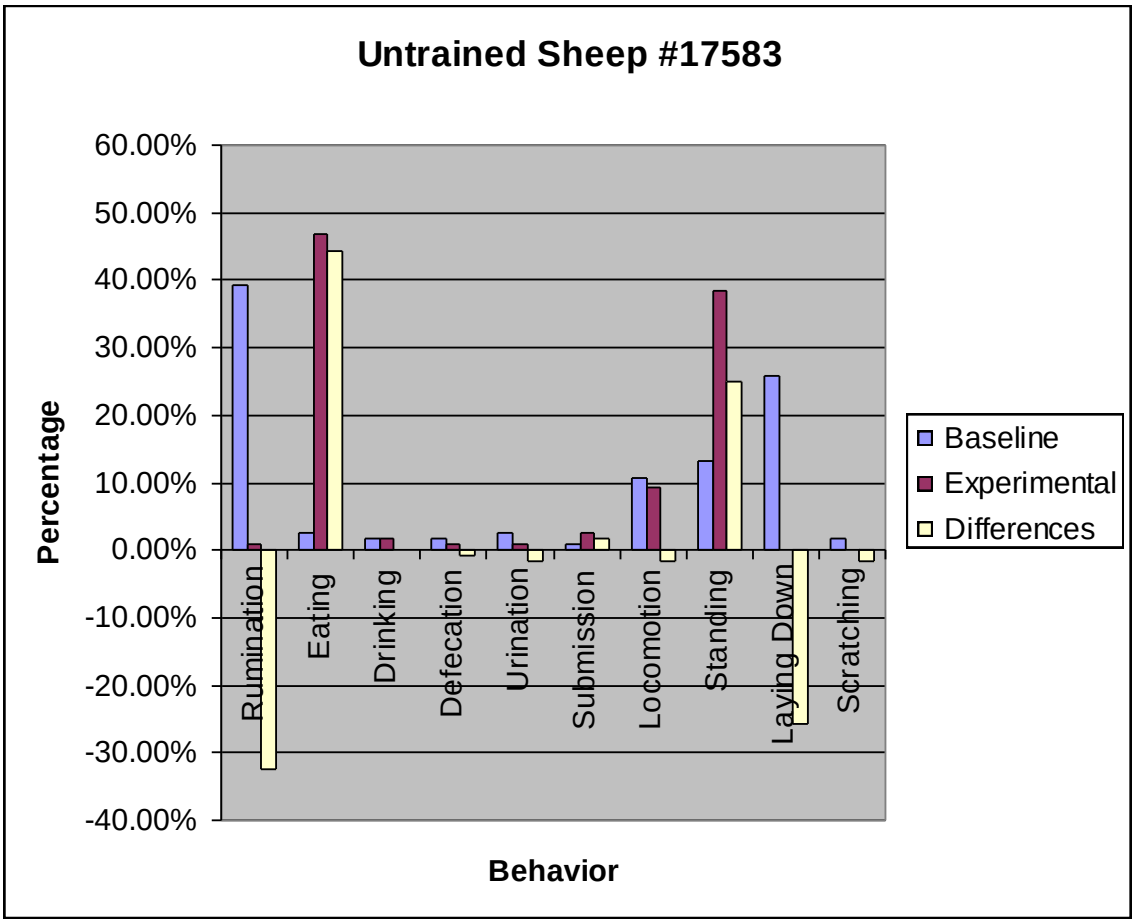
This graph represents the differences between the baseline and experimental ethograms. The blue bar indicates the percent of time spent performing each behavior during the baseline ethogram while the purple line indicates the same data for the experimental ethogram. The yellow bar indicates the positive or negative change in the amount of time spent performing a particular behavior from the baseline to the experimental ethogram. Behaviors that were never observed were not graphed.

Figure 3: Graphical Representation of Tables 1, 2, and 3 for Untrained Sheep #17582



This graph represents the differences between the baseline and experimental ethograms. The blue bar indicates the percent of time spent performing each behavior during the baseline ethogram while the purple line indicates the same data for the experimental ethogram. The yellow bar indicates the positive or negative change in the amount of time spent performing a particular behavior from the baseline to the experimental ethogram. Behaviors that were never observed were not graphed.

Figure 3: Graphical Representation of Tables 1, 2, and 3 for Untrained Sheep #17583



This graph represents the differences between the baseline and experimental ethograms. The blue bar indicates the percent of time spent performing each behavior during the baseline ethogram while the purple line indicates the same data for the experimental ethogram. The yellow bar indicates the positive or negative change in the amount of time spent performing a particular behavior from the baseline to the experimental ethogram. Behaviors that were never observed were not graphed.

Discussion

As previously mentioned, there were a few alterations in the experiment. For instance, from the beginning of the experiment, the trained sheep were isolated together in one room, while the untrained sheep were kept with two other sheep not used in this experiment. This could have caused a more stressful situation when one of the trained sheep left for the procedure because the other trained sheep was left alone in the stall. As a result, the plasma cortisol levels in the trained sheep left alone would rise and could take hours or days to normalize. However, when one of the untrained sheep was taken for catheterization, the sheep left behind was not alone. Therefore her plasma cortisol levels would be less likely to rise from the separation.

In addition to the amount of sheep housed in the stall, there was a catheter difference in the experiment from day one to day two. On the first day, trained sheep #17580 and untrained sheep #17582 both had the catheter in place during the entire experiment. However, on the second day of the experiment both trained sheep #17577 and untrained sheep #17583 managed to bend their catheters after the initial blood draw. Therefore, all other blood draws had to be taken by a 20 gauge needle and 6cc syringe. The extra instances in which needles were used may have caused greater peaks in the plasma cortisol levels for the sheep studied on the second day of the experiment than the sheep studied on the first day. Another difference during this experiment occurred when the ethograms were taken. On the baseline ethograms, both the trained sheep and the untrained sheep were not given hay or grain. However, on both days of the experimental ethograms, all the sheep were fed both hay and grain. This created an extra variable in the baseline and experimental ethograms because it caused the percentage of time sheep

spent eating to be considerably higher on the day of the experiment. This in turn, caused a decrease in the amount of time spent performing different behaviors, most notably sleeping, ruminating, and lying down.

After analyzing the behavior of the sheep, one could see that the trained sheep acted calmer and less stressed than the untrained sheep. On the day of the experiment, trained sheep #17580 did very well with catheterization. Though she was nervous and vocalized during the procedure, she calmed down very quickly once it was finished. Furthermore, she successfully targeted the handler's hand on the way back to the stall. In addition to sheep #17580 doing well with the procedure, trained sheep #17577 also did well on her experimental day. Even though this sheep did not make as much progress with training as previously hoped, she did do well once she was in the Calf-Cart. Sheep #17577 was very calm when being transported down the hall and into the procedure room. As the doctors prepped her for catheterization, she did lie down in the cart, but managed to remain calm during the procedure. Both of the trained sheep, once back in their enclosure, went immediately back to eating.

On the other hand, the behavior of the untrained sheep differed greatly from the trained sheep. Untrained sheep #17582 was extremely nervous throughout the entire experiment. After getting her into the Calf-Cart, she was noticeably uneasy and tried to escape during the entire transport process. When the doctors began to prepare her for catheterization, she started to get very anxious and was trying to not let the doctor touch her. After the first catheter attempt, sheep #17582 turned over on her back and started to hyperventilate. The doctor managed to get the catheter in the jugular vein on the second attempt. When bringing the sheep back to the stall, she lay at the bottom of the Calf-Cart

and refused to move. Once the sheep finally arrived back at her stall, she still looked traumatized and refused to leave the cart for approximately five minutes. Untrained sheep #17583 also had a negative experience during her procedure. As the sheep was brought into the procedure room, she became very anxious and began trying to jump out of the cart. Once the doctor started to prepare her for catheterization, she became quite tense and lay down in the cart while beginning to hyperventilate; much like her stall companion had the day before. After bringing her back into the stall with the other sheep, it took her a little while to calm down.

Based on the ethogram data and the percentage differences listed in Table 3, it was determined that there was a large decrease in the amount of time all four sheep spent ruminating and a large increase in the amount of time they spent eating. This was most likely because food was available on the day of the experiment but not on the day the baseline ethogram was recorded. Because standing still indicates stress in sheep, it would be expected that the amount of time the trained sheep spent standing up would be less than that of the untrained sheep. However, it turned out that trained sheep #17577, who was very timid throughout the training process, and untrained sheep #17583 both stood for approximately the same amount of time more during the experiment. On the other hand, both trained sheep #17580 and untrained sheep #17582 only showed small increases in the amount of time they spent standing. This could simply be a product of a change in the environment from day one to day two since sheep #17580 and #17582 were trained on the first day and #17577 and #17583 were trained on the second day. On the other hand, this large increase could also be a result of the fact that catheters were used for blood draws on the first day, but the second day blood draws were taken by syringe

each time. In addition to the increase in the amount of standing, a large decrease was observed in the amount of time all four sheep spent lying down. This could either be because there was food available and energy was better spent foraging, or it could indicate that the experiment caused the sheep to have an elevated stress level.

Even though the results for the plasma cortisol levels are still pending, three hypotheses can be drawn based on the ethogram results and the behavioral observations made during the procedure. One would expect that the area under the curve for the plasma cortisol levels would be much smaller for the trained sheep than the untrained sheep. In addition to having a smaller area under the curve, the trained sheep should also have a lower cortisol peak at T_0 and at each successive blood draw than the untrained sheep. Furthermore, the time it takes for the plasma cortisol levels to normalize should be quicker for the trained sheep than for the untrained sheep.

In the future, for this research to be valid, it is necessary that more experiments of the same kind be run with a larger test group of sheep. The function of this project was to determine the best way to train sheep over a period of 10 days and to figure out the logistics of both ethogram recordings and experimental procedure. As of now, there are plans to continue this study within the next few years. It is the hope of the researchers that experiments such as these will someday lead to a more humane treatment of laboratory animals. Even if training does not prove to reduce stress levels during experimentation, it is clear that the lives of animals who are given daily training sessions are far more enriched than those who are not. A simple compassion for the animals whose research results save millions of lives yearly will lead to a more humane environment for both them and the people who handle them.

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Appendix A

Training Steps and Daily Training Log

Training Steps for Operant Conditioning in Sheep

To be done over a period of 10 days

Desired Behavior	Training Steps
Desensitization to Human Contact *Willingly approaching a handler and the "target" command*	1. Kneel down and extend arm with an open palm containing grain, be sure to avert gaze from sheep 2. Once a sheep has approached and eaten from hand, click to reinforce the association of the sound with food 3. Continue this several times until the sheep is approaching without hesitation 4. Extend a closed fist containing grain 5. Click when the sheep touches her nose to the fist and immediately reward with grain 6. Once she has done this several times consistently, begin to say "target" just before she touches the fist, then click and reward 7. After doing this several times, say the command "target" then offer the fist. If the sheep touches her nose to the fist, click and reward. If not, go back to step 4 and repeat the process until she seems confident with the command. 8. When a sheep will "target" to fist when asked at a success rate of 90% or higher, she can be asked to follow the fist around her enclosure. This is done by simply asking her to "target" to the fist once again, clicking and rewarding when she does, then moving a little ways away and asking again. She should walk to the fist and touch it. Click and reward and give a lot of praise to reinforce that she has just done something wonderful. Giving extra grain as a reward, called at jackpot,

	for a big step in training like this is very successful for encouraging an animal to perform the same behavior again. 9. If the sheep does not follow the fist when asked, try stepping closer and asking again. If this still does not work, then the sheep is not yet confident enough with the "target" command. Go back to step 7.
Standing for a physical exam or minor medical procedure. *The "touch" command.*	1. Once the sheep has learned to "target" and seems pretty comfortable with human contact the "touch" command can be introduced. First, target the sheep to fist so she is standing close. 2. Next say the word "touch" and then try to place a finger lightly on her forehead. Only click and reward if she stands quietly and does not back away. 3. If the sheep does back away from the contact, "target" her back to the fist and try again. Once the sheep does stand quietly for the forehead "touch," click and reward her with a jackpot. Eventually, she will become desensitized to being touched on the forehead. 4. Because sheep are prey animals and tend to have large flight zones, it is necessary to repeat steps 1-3 of the "touch" command for any part of the body that must be handled during a procedure.
Transporting a sheep away from the flock or to another area. *Loading into and out of the calf cart.*	1. Being the most complex behavior in the sheep's repertoire so far, it is very important that the "target" and "touch" behaviors are already trained and that the sheep is performing them with a success rate of 95% or higher. 2. With the Calf-Cart in the enclosure, open its door. Click and reward for any interest the sheep shows in its

	presence (e.g. sniffing, touching, etc.) 3. Try asking the sheep for the two other commands she already knows. If she performs them and seems not to be concerned with the Calf-Cart being there, she is ready to move on to the next step. If not, keep working on the "target" and "touch" commands with the Calf-Cart still present until she seems unconcerned with it. 4. "Target" the sheep to the fist at the entrance of the Calf-Cart. Click and reward for the right response. 5. Next, put the fist a little ways inside the cart and, again, ask for the sheep to "target". Click and jackpot for one hoof placed inside the cart. 6. Continue to move the fist inside the cart in increments, asking for the sheep to "target" each time. Jackpot for each successive foot placed in the cart (1 hoof, 2 hooves, 3 hooves, 4 hooves). If at any time the sheep gets nervous and backs out of the Calf-Cart, "target" her back to the entrance and ask her to step in once more. Again, click and reward for each successive step taken in. 7. Once a sheep will load with all 4 feet in the cart confidently and at a success rate of 90% or higher, the door to the cart can be gently swung open and closed. Click and reward for calm behavior, if the sheep backs out of the cart, target her back in and try again. 8. When a sheep will stand calmly for the door to the cart opening and closing, the door can be latched shut. Again, click and reward for calm behavior. If the sheep gets nervous, "target" her forward with the fist so that she is focusing on

	the training and not on being confined in the cart. 9. The next step is to move the cart back and forth with the sheep inside. Continue to "target" her forward during the entire experience and click and reward for responding to the command. 10. Once the sheep is calm when the cart is moving, continue to "target" her to fist while moving the cart out of the enclosure. Only go a short distance the first day and jackpot once she is back in the enclosure. Allow her to back out of the cart and then ask her to "target" back in, this time without closing the door. This reinforces that being in the cart is a positive experience. Over the next few days, go farther and farther distances with her in the cart until she no longer seems distressed by the transport.

It is important to remember that every sheep is different. Some sheep take a longer time to learn each step, some take a shorter time. It all depends on past experiences and inherent personality. As a result, there is no set timeline for each step. Though the whole process normally takes ten days for even the most timid of sheep, some animals will never be fully trained by this time. A good trainer will give each animal enough time to become confident with the current behavior it is learning before moving on to something new. It also very important to end a session with a behavior that the sheep is confident with; in this way, the frustration that sometimes comes along with learning a new command can be avoided. This allows the session to end on a good note, reinforcing for the animal that training is a fun experience to look forward to, not something to be nervous about.

Daily Training Journal

Day 1: 11/26/07

8:00 AM

17580: It was obvious upon first meeting her that this sheep was more outgoing than sheep #17577. As long as the trainer was kneeling down with grain extended in an open palm, she would willingly eat out of hand. The trainer began to click when the sheep ate in order to associate the sound of the clicker with food. She was even able to go so far as offering a closed fist, clicking and rewarding when the sheep touched her nose to the fist.

On left #17580; on right #17577.

#17577: This sheep was very nervous around people and her first response was to kneel down on her forelegs to hide under sheep #17580. She would not approach either trainer willingly and, when approached by one of the trainers with grain in her hand, sniffed the food twice but refused to eat. The trainers attempted to hold a metal bowl of grain out to her in hopes that she might eat out of that, but got the same results as with the hand. The bowl was placed on the ground and the trainers left. Observed from outside of the room, she was seen to eat out of the bowl once she was sure the trainers were gone.

A *picture of #17577 kneeling down underneath #17580 to hide from trainers.*

5:00 PM

#17580: This time, as soon as a trainer offered grain, #17580 would eat out of hand without hesitation. A closed fist containing grain was extended and, when she touched it with her nose, the trainer clicked and immediately rewarded her. She learned the command very quickly and would target to fist successfully 90% of the time.

#17577: Because of the shyness she displayed in the morning session, a metal barrier was brought into the enclosure to separate #17577 from #17580. The trainer then kneeled down and averted her gaze while extending an open palm filled with grain. It was obvious that the sheep was still terrified but she did eat a few handfuls of grain from hand.

Day 2: 11/27/2007

8:00 AM

#17580: During this session, #17580 was led by the trainer around the enclosure by targeting to fist and responded correctly to the command 95% of the time. The "touch" command was begun with the trainer saying the word "touch" and then placing a finger on the sheep's forehead and neck. She was rewarded only for calm behavior and was successful 85% of the time.

#17577: Once again, the metal barrier was brought in to separate the two sheep. #17577 ate from an extended palm once again. This time, the trainer clicked each time she ate to reinforce the sound with food. At one point, the sheep got very nervous and ran away. The trainer simply knelt down in front of her once more and offered grain until she took a bite. This way, the session was ended on a good note.

5:00 PM

#17580: This session was much the same as the morning session. #17580 was targeted around the enclosure by fist with a success rate of 95%. The "touch" command was performed on her neck and forehead and she stood calmly 95% of the time.

#17577: The barrier was used once again. After ensuring that she would still eat out of hand without hesitation, sheep #17577 was asked to target to fist. She responded correctly 80% of the time. She was still very timid in her behavior towards the trainers.

Day 3: 11/28/2007

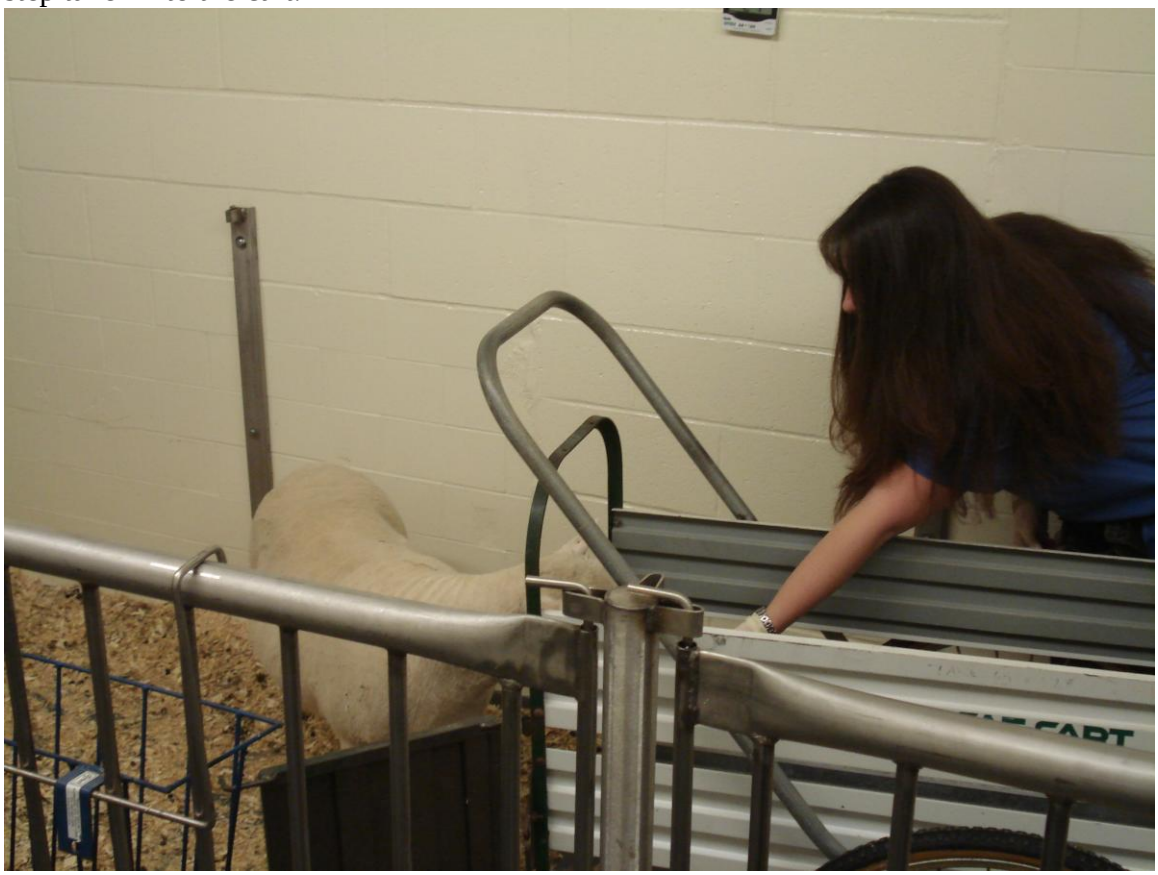
8:00 AM

#17580: This sheep was, once again, targeted around the enclosure to fist. The trainer began asking for variable "targets" which involved asking for multiple responses to the command before rewarding, but clicking for each one performed correctly. The "touch" command was performed on her forehead, neck, back, and forelegs. She stayed calm for the handling 90% of the time.

#17577: The sheep were, again, separated by the barrier for training purposes. This session, sheep #17577 targeted to fist 80% of the time. She was still quite scared of her handler but began showing more interest in the training.

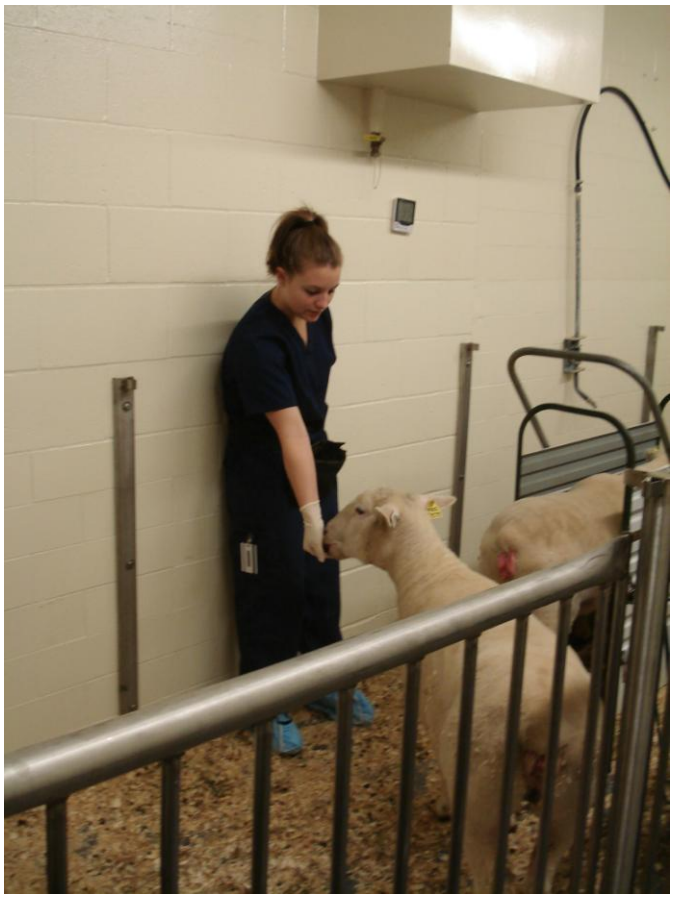
5:00 PM

#17580: Because of her rapid learning rate, it was determined that sheep #17580 was ready to be introduced to the Calf-Cart. After allowing her to explore it, she was asked to perform variable "targets" with a success rate of 100%. The "touch" command was performed on her forehead, neck, back, and legs with her staying calm 95% of the time. At this point, she was asked to "target" to fist inside of the cart and got 3 hooves in with her head through the restraint. Extra grain (a jackpot) was rewarded for each successive step taken into the cart.



#17580 works on loading into the cart for the first time.

#17577: #17577 was nervous about the cart at first, but would sniff it in curiosity. The trainer clicked and rewarded for any interest she showed. She was asked for variable "targets" and performed at a success rate of about 90%. She began to approach the trainers less hesitantly.



#17577 works on targeting with a trainer.

Day 4: 11/29/2007

8:00 AM

#17580: After asking her to target to fist with a success rate of 100%, sheep #17580 was targeted into the cart and got all 4 hooves in. The door was able to be latched while the trainer targeted her forward to fist. This process was able to be performed 80% of the time.

#17577: Sheep #17577 targeted to fist with a success rate of 85% and showed more interest in her trainers. The "touch" command was attempted on her neck, but she ran away. She was rewarded, once again, for any interest shown in the cart.

5:00 PM

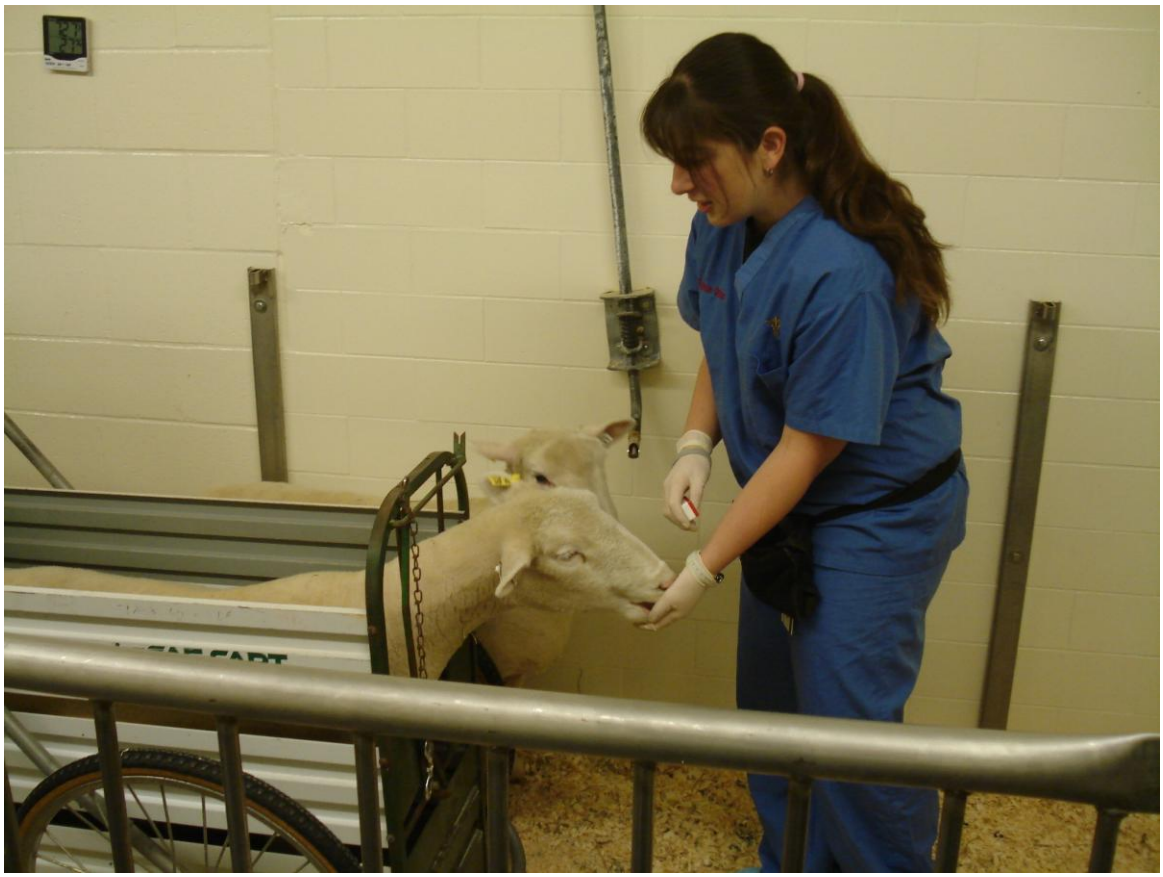
#17580: This sheep targeted 100% of the time and could be touched on the forehead, neck, back, and forelegs 100% of the time. Trainers were able to target her into the Calf-Cart with all 4 hooves in and the door latched 50% of the time. The other 50% of the time she would only load with 3 hooves in the cart.

#17577: Sheep #17577 was extra nervous tonight and would only “target” to fist 75% of the time. She was rewarded for sniffing the Calf-Cart.

Day 5: 11/30/2007

8:00 AM

#17580: Sheep #17580 was successfully targeted to fist 100% of the time and touched on her forehead, neck, back, and forelegs while staying calm 100% of the time as well. She was targeted into the Calf-Cart and got all 4 feet in with the door latched at a 100% success rate.



#17580 with all 4 hooves in the cart and her head through the restraint.

#17577: This sheep was unusually food driven today. Trainers put a pile of grain in the Calf-Cart and clicked when she put a hoof in the cart to get it. When trainers targeted her into the cart, she put 3 hooves in 50% of the time. The cart was positioned in the stall so that, when she walked into it, she would be facing #17580.

5:00 PM

#17580: Trainers targeted her with all 4 hooves into the cart and the door closed 100% of the time. The cart was raised and she was rewarded for calm behavior. With the cart positioned the same way as the morning session, it was discovered that the ultimate reinforcement was not the grain, but allowing her to rejoin #17580 after the session was over.

Day 6: 12/3/2007

8:00 AM

#17580: Sheep #17580 loaded into the cart with all 4 hooves in and the door closed 100% of the time. She was then wheeled back and forth in the enclosure and rewarded for calm behavior. While in the cart, the "touch" command was performed on her neck successfully 95% of the time.

#17577: Sheep #17577 got 3 hooves into the cart 50% of the time when asked to "target" in. The "touch" command was once again attempted outside of the cart, but she still ran away.

On left: One trainer works on targeting with sheep #17580: On right: Another trainer works on loading into the cart with #17577.

5:00 PM

#17580: Once loading into the cart with the door closed, sheep #17580 was transported out of the enclosure and into the hallway for the first time. She was targeted throughout the entire transport and rewarded for calm behavior. Both sheep vocalized quite a bit while separated. Back in the enclosure, #17580 was allowed to back out of the cart and was then targeted back in without closing the door to reinforce the cart as a positive experience.

#17577: Before #17580 was moved out into the hallway, #17577 would "target" into the cart with 2 hooves in 75% of the time. After the other sheep left, she became very nervous and showed distrust of the trainers. She did "target" to fist once or twice more, but stomped a hoof in frustration each time she was asked.

Day 7: 12/4/2007

8:00 AM

#17580: After yesterday's experience, sheep #17580 was a bit wary of the cart. However, she did load all the way in with the door closed 90% of the time. Once she seemed more confident getting in and out of the Calf-Cart, she was moved out into the hallway a bit farther than the night before. Both sheep vocalized as they had before, but seemed less distressed. Again, once she was back in the enclosure, #17580 was allowed to back out of the cart before being targeted back in to reinforce a positive experience.

#17577: In order to avoid making her nervous before training, sheep #17577 was worked with first. She loaded into the cart with 3 hooves 75% of the time. Because she seemed not to respond to the "target" command as well as usual, she was targeted outside of the cart to reinforce it at a 95% success rate. Once again the "touch" command was attempted, but she still ran away.

5:00 PM

#17580: After loading into the cart and closing the door, sheep #17580 was brought out into the hall again and rewarded for targeting calmly to fist. She was successful 95% of the time. While in the hall, the "touch" command was performed on her back and neck with the proper response with a 95% success rate. Both sheep vocalized much less at the separation. Once back in the enclosure, she was allowed to back out of the cart and then targeted back in as always.

#17577: Though trained first, #17577 seemed more skittish than usual and was disinterested in the grain reward. Despite this, perseverance by the trainers finally got her to load into the cart with 3 hooves at a 90% success rate.

Day 8: 12/5/2007

8:00 AM

#17580: Once she had loaded into the cart and the door was latched, #17580 was moved into the hall and stood quietly for the transportation 95% of the time. While in the hall, the "touch" command was performed on her neck and back successfully 100% of the time. The same procedure as the other sessions was followed once back in the enclosure.

#17577: Still hesitant today. She got 3 hooves in the cart 100% of the time, but kept backing out every so often. To remedy this, she was immediately targeted back in each time she backed out and rewarded for each step taken forward into the cart.

5:00 PM

#17580: As with the morning session, #17580 was loaded into the cart and transported into the hall where the "touch" command was performed on her back and neck at a 100% success rate.

#17577: Tonight, #17577 was very wary of the cart and would only put 2 hooves in 50% of the time. Each step taken forward into the cart was rewarded by a click and reward. Trainers did not make eye contact with her to avoid intimidation. She did not seem very interested in the grain reward.

Day 9: 12/6/2007

8:00 AM

#17580: After being targeted into the cart and closing the door, #17580 was wheeled out into the hall and down to the procedure room. Calm behavior and targeting to fist when asked was rewarded during the process. Once in the procedure room, the "touch" command was performed on her back and neck with a success rate of 100%. Back in the enclosure, the sheep was allowed to back out of the cart and then asked to target back in. Though trainers expected her to be nervous in the procedure room, she did not seem to mind at all.

#17577: #17577 was much more eager to work today. She targeted with 3 hooves in the cart and her head through the restraint 90% of the time and did not back out nearly as much. The "touch" command was able to be performed on her forehead twice before she ran away. Each time she stood calmly for handling she was rewarded with a jackpot.

5:00 PM

#17580: Sheep #17580 was targeted into the cart and practiced standing still without backing out. She did this correctly 75% of the time. She was only rewarded when she had all 4 hooves in the cart. Once all 4 hooves were in, the trainer rapidly clicked and rewarded to reinforce standing still in the cart. She was not taken out into the hall this session.

#17577: The same procedure for practicing standing still in the cart was used for sheep #17577. She got 2 hooves in 100% of the time. When she got nervous, she was allowed to back out of the cart but was then immediately targeted back in. The "touch" command was performed 85% successfully on her head and neck. Though she tensed when touched, she did not back away. She got a jackpot for allowing me to handle her.

Day 10: 11/7/2007

8:00 AM

#17580: Once she had loaded into the cart and the door was latched, #17580 was wheeled into the procedure room where the "touch" command was performed on her back and neck. Today, she seemed quite unconcerned about being away from her friend. Once back in the enclosure, she was allowed to back out of the cart and then immediately targeted back in.

#17570: Though she was nervous at first, #17577 seemed very eager to work. When asked, she loaded into the Calf-Cart with 3 hooves 100% of the time. Though she backed out every so often, she immediately targeted back in when requested. The "touch" command was performed on her head 85% of the time successfully, but she back away from being touched on the neck.

5:00 PM

#17580: As in the morning session, #17580 was transported to the procedure room where the "touch" command was performed on her back and neck. Tonight, she seemed more nervous about being separated from the other sheep.

#17577: Sheep #17577 targeted into the cart with 3 hooves 100% of the time. The "touch" command could be performed on her head and neck 85% of the time, though she still backed away from the neck touch. At this point in the training, she is willingly approaching trainers and looking for grain while offering behaviors. She seems much more comfortable with people.

Appendix B

Sample Ethogram Scoring Sheet and Behavioral Term Definitions

Sample Ethogram Scoring Sheet

Date:
Time:
Hour:
Sheep Tag Number:

Behavior	**Time: (min)**	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ruminating																	
Eating																	
Drinking																	
Vocalization																	
Defecation																	
Urination																	
Sleeping																	
Aggression																	
Submission																	
Locomotion																	
Standing																	
Laying Down																	
Scratching																	
Grooming																	
Yawning																	
Pawing																	
Offscreen																	

Behavior	**Time: (min)**	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Ruminating																
Eating																
Drinking																
Vocalization																
Defecation																
Urination																
Sleeping																
Aggression																
Submission																
Locomotion																
Standing																
Laying Down																
Scratching																
Grooming																
Yawning																
Pawing																
Offscreen																

Behavior	**Time: (min)**	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Ruminating																
Eating																
Drinking																
Vocalization																
Defecation																
Urination																
Sleeping																
Aggression																
Submission																
Locomotion																
Standing																
Laying Down																
Scratching																
Grooming																
Yawning																
Pawing																
Offscreen																

Behavior	**Time: (min)**	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Ruminating																
Eating																
Drinking																
Vocalization																
Defecation																
Urination																
Sleeping																
Aggression																
Submission																
Locomotion																
Standing																
Laying Down																
Scratching																
Grooming																
Yawning																
Pawing																
Offscreen																

Definition of Terms

1) **Ruminating:** Chewing cud in any position (laying down, standing, etc.)
2) **Eating:** Eating hay out of manger or off of ground
3) **Drinking:** Drinking water out of either of two buckets
4) **Vocalization:** Any vocal noise made
5) **Defecation:** Excreting fecal matter
6) **Urination:** Excreting urine
7) **Sleeping:** Laying down with chin or whole head resting on the ground
8) **Aggression:** Dominant behavior towards other sheep (head butting, stomping, chasing)
9) **Submission:** Behavior that indicates submission towards an aggressor (running away, backing down, etc.)
10) **Locomotion:** Walking or running
11) **Standing:** Standing still without ruminating, sleeping, eating or any other behavior
12) **Laying down:** Laying on the ground without ruminating, sleeping, eating, or any other behavior
13) **Scratching:** Rubbing up against any object such as the wall, hay manger, etc.
14) **Grooming:** Using mouth to chew or lick any part of the body or eating hay off of another sheep
15) **Yawning:** Opening mouth wide to yawn
16) **Pawing:** Using hoof to paw at hay, door, etc.
17) **Offscreen:** Anytime the animal being observed is not visible in the frame