WHICH WAY DID I GO?

REMOTE TRAINING OF A SPATIAL MEMORY TASK TO ASSESS THE EFFECTS OF DOMOIC ACID EXPOSURE IN STRANDED CALIFORNIA SEA LIONS (*Zalophus californianus*)

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Most animal trainers do the majority of their work with long-term captive animals. Using positive-reinforcement based training with an animal over months and years promotes stimulus control and trust, and this foundation, in turn, clearly facilitates learning new and complicated behaviors. A great many successful collaborations between humans and animals follow this cooperative long-term model. Herein, we describe a successful, ongoing collaboration that does not fit the norm. In our research program at Long Marine Lab in Santa Cruz, California, we have trained more than 20 wild California sea lions (*Zalophus californianus*), to participate in a relatively complicated spatial memory protocol. At least half of these animals had incurred significant brain damage prior to stranding. Therefore, as not to interfere with potential release to the wild, all training has been conducted remotely, that is with no direct or visual contact between trainer and animal.

Our technique will be familiar to any trainer, comprising the most basic tenets of operant conditioning. In essence, we allow the sea lions to take part in self-guided exploratory behavior, and selectively reward them in such a way to develop the sorts of behaviors required for participating in the research task. In this paper, we aim to encourage trainers and researchers to remain open to opportunities involving wild animals in rehabilitation settings, and illustrate some of the challenges and potential benefits of working with these populations remotely, and under severe time constraints.

The main purpose of our research is to better understand the effects of domoic acid toxicity on wild sea lions. Domoic acid is a metabolite of increasingly frequent and widespread algal blooms, and is a looming concern in marine conservation. Fish eat the phytoplankton that produce this neurotoxin, then sea lions (among other higher-level consumers) eat the fish. Once in the blood, the toxin goes to the brain, where it can cause seizures and permanent brain damage. The damage occurs predominately in the hippocampus, a brain area important for spatial, working, and long-term memory in both humans and other animals (Goldstein et al., 2008; Eichenbaum & Lipton, 2008). Thousands of sea lions on the west coast of the United States are exposed to toxic levels of domoic acid each year. While many die, some do survive the initial exposure—we want to examine how brain damage impacts the survivors' long-term prospects in the wild. Further, we believe these sea lions can be used as an alternative to traditional laboratory animals in models of the behavioral effects of brain damage.

Our research focuses on two groups of subjects: animals showing signs of toxic exposure to domoic acid who, following treatment, are deemed stable, and control animals who have no apparent signs of toxic exposure. Each subject comes to us by way of The Marine Mammal Center in Sausalito, California. After medical treatment and acclimation to captivity, they are transported to our lab, where they spend about two weeks involved in training and behavioral testing. On completion of testing, they undergo magnetic resonance imaging (MRI) of their brains at AnimalScan in Redwood City before returning to The Marine Mammal Center for further rehabilitation or release.

Most of their time with us is spent learning to 'alternate' in a T-Maze. Our T-Maze is a chute descending from the animal's pool with two pairs of saloon doors at the end, one pair on the left and one on the right. ***(Photo 1.)*** Our goal is to get the animals to voluntarily and repeatedly traverse the maze, switching doors each time in a left, right, left, right, etc. pattern. Theoretically, animals with healthy brains and animals with hippocampal damage should be able to learn this alternation pattern equally well. This type of repetitive motor learning in humans, such as learning to juggle, does not require the hippocampus. Once our animals learn to successfully alternate in the maze, we test their memory by instituting a delay—forcing them to wait for a short period at the entrance of the maze before each trip through. This is the condition where we expect to see a difference between control and brain-damaged animals. Humans with severe hippocampal damage cannot remember what they have just experienced (if you were to meet such a person they might seem normal, but leave the room and come back a minute later and they will act as if they have never met you before). Delayed alternation in the T-Maze is a way to ask the sea lions, without using language, if they can remember what they have just done. All of our subjects are potential release candidates, and should not learn to associate people with food, so all our training and testing is done remotely, with food dispensed from behind a large visual barrier.

The T-Maze is a chute descending from the animal's pool with two pairs of saloon doors at the end.

A sea lion moving from the pool, down the ramp.

Training begins as soon as the animal arrives and proceeds through three stages before testing. Sessions take place two to four times a day and the majority of the animals' daily allotment of fish is received during training and testing in the T-Maze. All training takes place in the animal's home pen, a large and somewhat isolated empty enclosure with a small aboveground pool.

A sea lion learning to push through saloon doors.

A sea lion exiting ramp after pushing through saloon doors.

❶ Ramp Training

Our first goal is to get the animal to climb out of the pool and walk down a ramp to receive fish. ***(Photo 2.)*** Later in training, the ramp will lead into the T-Maze, but in the early sessions, it leads down to the deck. We use a mixture of baiting and training by approximation during the initial stage of ramp training. First, fish is thrown into the pool to begin the session. Then, fish is thrown at the bottom of the ramp to get the animal out of the pool, but only when the animal's head is up and pointed toward the ramp. More fish is awarded when the animal reaches the bottom of the ramp. Then, fish is thrown to the side of the ramp and then into the pool to get the animal to return to the starting point, completing a circuit. Boxes on either side of the aboveground pool allow the animal to climb back into the pool without returning up the ramp. On subsequent repetitions of this circuit (henceforth referred to as a "trial"), we selectively approximate the animal closer and closer to the ramp from the pool before throwing the fish onto the deck. As soon as possible, we drop out baiting. Then the animal is rewarded only twice on each trial: for reaching the bottom of the ramp and for returning to the pool. Depending somewhat on the animal's motivation and health, about 20 trials constitutes one session. It generally takes the animal one to two training sessions (up to 40 trials) to reliably head down the ramp and back up to the pool without baiting. None of our wild sea lions has required more than 60 trials (1–2 days) to complete this phase of training.

❷ Door Training

Once the animal is reliably completing trials with just the ramp, we train the animal to push through hinged saloon doors in the door-training phase.

The purpose of the door-training phase is to give the animal experience with opening saloon-style doors, which are critical components of the T-Maze. The T-Maze has two sets of doors, one leading to the left and the other leading to the right. Each set comprises two hinged pieces of plywood that open outward. Many animals are at first hesitant to push through these apparent barriers.

During the door-training phase, we use just one set of saloon doors. This is placed centrally at the bottom of the ramp. Walls are also added to the ramp side so the animals cannot climb up or down from the side of the ramp. ***(Photos 3 & 4.)*** To begin the door training, one of the hinged plywood pieces of the pair is in the closed position, while the other is in the open position. As soon as the animal is reliably completing ramp trials with one door closed, we approximate the second door closed on successive trials. Should the animal balk, we encourage the animal to push through by remotely opening the doors using a rope and pulley system. Fish is still provided for returning to the pool, unless the animal is slow to return to the pool, spending more than ~20 seconds on deck before returning. The door-training phase ends when the animal reliably approaches and pushes through the set of saloon doors at the bottom of the ramp. It

A sea lion moving though T-maze with saloon doors at the end. One set on the left as shown, and one set on the right.

A sea lion returning to the pool after exiting the T-maze.

has not yet required more than 2 sessions (40 trials, 1 day) for the sea lions to reliably push through the central set of doors at the bottom of the ramp on each trial without prompting.

❸ T-Maze

The next phase is T-Maze training. The centrally located saloon doors used during Door Training are removed and the ramp now feeds directly into the T-Maze. The T-Maze consists of a chute flanked by two sets of saloon doors, one on the right and one on the left. ***(Photo 5.)***

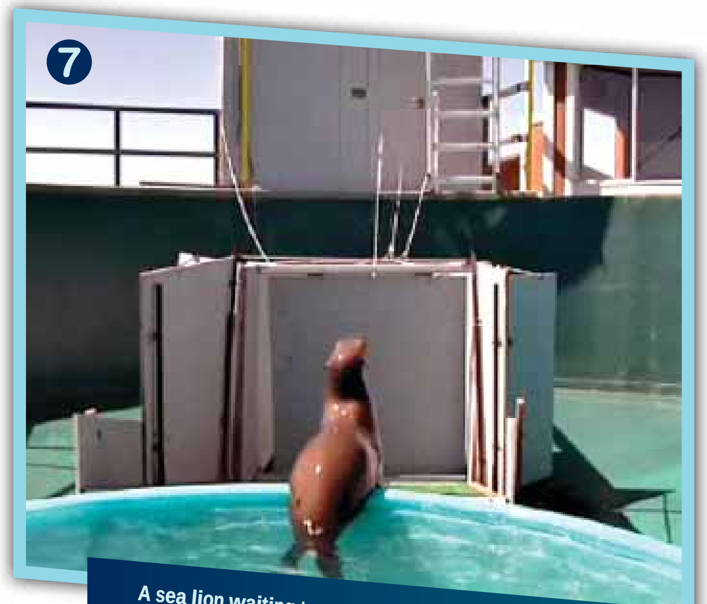
To begin, the sets of doors in the T-Maze are kept open. The animal is rewarded for heading down the ramp and into the chute. Some animals are nervous about entering the semi-enclosed chute—after receiving their first fish reward, some choose to walk back up the ramp to the pool without exiting via either set of doors. We do not provide a food reward for returning to the pool via the ramp.

When the animal is reliably coming down the ramp into the chute, we stop rewarding the animal at the bottom of the ramp, and begin baiting them through the doors. We do so in an alternating pattern, first baiting, for example, to the right, then to the left, then to the right, etc. It does not matter whether the animal begins to the right or the left, as the alternation pattern remains the same. This gives the animal roughly equal reinforcement history with both exits from the T-Maze. It also begins to establish the desired alternation pattern. At this point, we are leading the sea lion in a figure-eight pattern through the maze: down the ramp, through the chute of the maze, out one set of doors, back up to the pool, down the ramp, out the other set of doors, etc. ***(Photo 6.)***

As soon as the animal is comfortably moving through the maze, which usually takes only one session (~20 trials), we close the two sets of doors at the end of the maze and stop baiting. To encourage the animal to continue alternating, we hold closed one set of doors on each trial, rewarding the animal for going through the open set. On the next trial, we switch which set of doors is held closed. At this point, the animal is rewarded when having moved all the way through the correct set of saloon doors. This allows us to close the doors behind the animal. This will be important at a later stage in training.

Getting the sea lions to reliably move through the opened doors usually takes only one session, and has never taken more than two (~20–40 trials). When this is accomplished, we close both sets of doors. The animal must now push through a set of doors in the T-Maze to be rewarded, but we continue holding closed the incorrect set of doors on each trial. As we are able to remotely open the doors, we will open the correct set of doors to prompt the animal if he or she becomes frustrated. When the animal is reliably pushing through the correct set of doors without prompting, which generally takes one to two sessions, and has never taken more than three (~20–60 trials), we leave both sets of doors closed, but stop holding closed the incorrect set of doors on each trial.

Now, for the first time in the training protocol, the animal is fully free to choose whichever door they like, either correctly or incorrectly. If the animal goes through the wrong set of doors, no reward is given. Most newly trained animals make a predictable pattern of mistakes; they perseverate by exiting via the set of doors (left or right) that they selected and were rewarded for on the first trial. To ease frustration following errors, we continue to provide fish rewards for returning to the pool to help keep up momentum in the session. It is important to note here that the animal receives fish in the pool whether completing a correct or incorrect trial. This could constitute a reward, albeit delayed, for selecting the incorrect set of doors. However, on a correct trial, they also receive fish reward immediately after exiting the saloon doors. Thus, there is a higher rate of reinforcement associated with going through the correct set of doors.



A sea lion waiting to access the T-maze during a delay Trial.

With early pilot subjects we attempted to train this phase without rewarding animals for returning to the pool, thus making a correct choice the only avenue to reinforcement. However, this approach, in conjunction with the perseveration in door-choice almost all subjects show, led to the animals' ceasing to participate. In other words, after five or six incorrect trials with no reinforcement what so ever, they would simply stop moving through the maze. By reinforcing for return to pool as well as a correct choice of doors, we are able to keep the animals participating long enough to learn the alternating pattern. The payment on return to the pool is gradually eliminated as the animal's success rate increases. The reinforcement history with both right and left maze exits likely helps during this transition from guided to free door selection as well. Most animals only choose the same set of doors six or seven times in a row before spontaneously switching to the other set of doors. If the animal simply will not switch and appears to be losing momentum/motivation, we will regress to the previous training phase, once again holding closed the incorrect set of doors and/or pulling open the correct set to prompt a switch.

Once the animal begins switching sides on their own, no more training manipulations are required. At this point, animals will often try to get back through a set of doors if they receive no reward, perhaps to try to exit through the other, "correct" set of doors. This is why we have to use doors that we can hold closed, once the animal has made a choice. Their only option on an incorrect trial is to return to the pool and try again. The mean number of T-Maze trials (not counting trials during ramp and door training) required to reach our testing criterion of two consecutive sessions (20 trials) at 85% correct or higher, is ~350 (~17 sessions, ~6 days). At this point most animals are reliably switching between sets of doors on each trial, following a consistent left, right, left, right, etc. pattern. In essence, we train them to go through the maze, but they learn to alternate properly merely by interacting with the maze with the reinforcement contingency we've established.

❹ Testing

When the animal selects the correct set of doors on 85% or more trials in two consecutive sessions, we begin testing. The basic procedure is the same, except now we lower a gate at the entrance to the maze at the beginning of each trial. ***(Photo 7.)*** During this delay, the animal must actively remember which set of doors they went though on the previous trial in order to choose the other set during the up-coming trial, thus maintaining the alternating pattern.

We test the animals at two delay durations, 7 and 20 seconds. In the testing phase, each block of delay trials is run following a matched block of non-delay trials (exactly the same as those detailed in the T-Maze section). Our final measure is performance on delay trials in comparison to performance on matched blocks of non-delay trials. This gives us a measure of how impaired the animals are by the delay.

Conclusion

By the time the animal finishes testing, they have been with us for 12 to 14 days and completed anywhere from 400 to 800 trials overall. By comparing their performance from the delay and non-delay trials, and then comparing these results to the data on brain damage we get from the MRIs, we are learning about the unique challenges faced by sea lions when exposed to domoic acid in the wild. As of February 2011, we had completed testing on 20 animals. We plan to test at least another ten; as such, we do not have enough data to comment on results yet. We can report that the training has been successful, allowing us to obtain data from every subject we have acquired, even those with severe brain damage. Further, our training has not interfered with the animals' releasability—those animals we worked with that were healthy enough to be released have been, and with no apparent difficulty. Anecdotally, this training approach appears to be quite enriching, encouraging active engagement from a many otherwise sedentary individuals.

This project has already shown that training-intensive research can be conducted with non-habituated, releasable animals, remotely, in a very short period, using basic operant conditioning techniques. By carefully constructing the animals' environment and instituting clear rules of reinforcement instead of focusing on tight stimulus control, we have been able to successfully train all of our subjects to take part in a complex spatial memory task. We hope the success of this training can inspire confidence in trainers and researchers working under similar circumstances, and pave the way for other fruitful collaborations outside of the long-term captive setting.

Note: This research was conducted under authorization granted to the Marine Mammal Health and Stranding Response Program by NMFS scientific research permit 932-1489-10. This research has been approved by the Institutional Animal Care and Use Committees at both UCSC and TMMC.

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