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MEDICAL TRAINING OF CETACEANS AND PINNIPEDS FOR VETERINARY CARE

GÉRALDINE LACAVE

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Introduction: Why Medical Training?

Medical training is common in marine mammal husbandry and is an essential tool for effective marine mammal medicine. It was originally developed to enhance the welfare of animals and diminish their stress, as well as to reduce the risks of humans and animals being hurt during handling and anesthesia. Medical training refers to the types of husbandry behaviors, based on operant conditioning, that have become integrated into marine mammal training programs. This training can result in the collection of a sample or the needed information in only a few minutes, rather than the hours to days needed in planning restraint or anesthetic procedures. Initially, medical examinations through voluntary approaches were limited to assessing physical parameters and the collection of several body fluids and exudates (Sweeney 1990). They have evolved over the years with the advent of transportable diagnostic imaging tools

(Lacave 1997) and access to newer specific investigation technologies, to allow advanced diagnostics (Lacave 2012a). In many instances, however, especially in zoological environments, the veterinarians are not familiar with operant conditioning, nor realize the importance of the desensitization steps to obtain a specific behavior. This can jeopardize the outcome, due to either an inadequate approach or lack of patience. On the other hand, if the trainers and keepers have good communications with their veterinarian, a correct set-up and an adequate approach for a specific procedure can be defined from the beginning and medical training can enhance marine mammal care, diagnostics, treatment, and well-being.

Preventive medicine has become much more prevalent in zoos and aquaria, and the need to recognize and identify changes in behavior and symptoms early on is a must. Handlers and trainers have adapted standard training procedures, used formerly exclusively for public presentations, to obtain the voluntary participation of marine mammals in many routine clinical examinations. Indeed, if a marine mammal is not trained and it is difficult to obtain a blood sample or approach it closely enough to examine it, problems can develop that could be prevented. The veterinarian will miss the opportunity to pinpoint a problem when there is still time to do something about it. Medical training is for the benefit of the animal but is a vital tool for veterinarians to exercise their expertise. Furthermore, if blood sampling or ultrasound checkups are only performed when an animal is sick, limited reference values will be available for that animal. Establishing normal values for individual animals using different diagnostic procedures has been facilitated through the use of voluntary husbandry behaviors.

Teamwork

For medical training to be successful, teamwork is needed among trainers, animals, and the veterinarians (Clark Price et al. 2016). The veterinarian needs to communicate exactly what he/she needs and what access to the animal is necessary. Without communication, one will not obtain the requested behaviors. If there is a dominancy or bait situation between the handler and the animal, medical behaviors will not occur reliably, particularly when these become more complex or when a there is slight discomfort for the animal, because the animal will not have confidence in its trainer. The most important point to remember is that the trainers have the key role in this teamwork. If they do not have a good relationship with their animals and do not train them for the desired intervention (e.g., blood sampling), the veterinarian will not have that sample. But efforts are also necessary on the part of the veterinarian. It is important for veterinarians to take time and regularly visit the trainers for positive desensitization sessions with the animals, and to get to know the animals better. Otherwise, the veterinarian is assessed by the animal as an unpleasant factor.

The Animals

Although some amazing behaviors can be trained, there will always be animals for which medical training, or at least some specific behavioral training, will not work, although one can generally always teach a little something. As much as marine mammals can be trained, they are—and will remain—"wild" or "nondomesticated" animals, and caution should always be exercised, particularly when working with large animals. Even still, much can be gained through protected or semiprotected contact (see below).

For medical training, there is one essential requirement: the ability to separate the animal physically—or somewhat spatially pending on the behaviour or the animal—and work with it individually—referred to as "gating." Without proper gating or separation, one will very quickly be limited in what can be obtained. If one is busy desensitizing an animal for a needle stick, for example, one cannot have another animal interfering at that moment. Separation and gating are not the easiest behaviors to train, are too often taken for granted by less experienced trainers, and yet are the behaviors that need to be trained and reinforced throughout the life of the animal.

Training: Some Basic Theory

Operant Conditioning

Medical training is based on operant conditioning with positive reinforcement. Operant conditioning is defined as "any training or conditioning where the frequency of a behavior is influenced by the consequence of that behavior—and the subject (in our case the animal) is the one deciding on the behavior" (Skinner 1938). This is the principle used in medical training, with the outcome influenced by positive reinforcement.

Reinforcement

In operant conditioning "positive" means something that is added to the environment, as opposed to "negative", when something is taken away. A positive reinforcement is something pleasant that is likely to augment the frequency of a behavior when it follows that behavior. Primary reinforcements are mandatory for the well-being of the animal (e.g., food), while secondary reinforcements are learned (e.g., petting or praising) and initially paired with a primary reinforcement, and so are animal- or trainer-dependent.

A negative reinforcement is the removal of something to augment the frequency of a behavior (e.g., ceasing to use netting to make an animal gate). This is different from punishment—generally something unpleasant—which will likely diminish the frequency of a behavior. A punishment can be positive by adding something aversive to the environment (e.g., hitting an animal) or negative by retrieving something from the environment (e.g., cutting out food).

These two examples freely adapted from *Animal Training* (Ramirez 1999) illustrate the concept:

1. If a dog approaches a fountain and gets sprayed by water and he likes it, he will likely want to approach it again. His behavior has been conditioned by the result. In his case, he likes being sprayed by water—it is a positive reinforcement. The frequency of his behavior (approaching the fountain) will be augmented because he likes it. He is the one deciding to approach the fountain (he is the "operant" factor).
2. If a cat approaches a fountain and gets sprayed by water and he does not like it, he will likely try to avoid or run away from the fountain. His behavior has been conditioned by the result. In his case, he does not like being sprayed by the water, being wet. It is a positive punishment—something unpleasant has been added to the environment of the animal, water, to diminish a behavior, approaching the fountain. The frequency of his behavior (approaching the fountain) will be diminished because he does not like it. He is also the one deciding on the behavior.

In these examples, the same behavior (approaching the fountain) has different outcomes, because the consequences are experienced differently by the subjects. It was a positive reinforcement for the dog (so the frequency of the behavior will be augmented) and a positive punishment for the cat (the frequency of the behavior will be diminished).

When strong trust is needed for the training of sometimes uncomfortable behaviors in medical training, such as blood sampling or gastroscopy, only positive reinforcement (a pleasant outcome) will work. The key to long-term success in medical training is to reward the animal for allowing us to do something—and never try to force it or trick it.

Bridge

A "bridge" is a signal that tells the animal it has done well and will receive a reinforcement. It bridges the moment the animal is doing something correctly (which could sometimes be far away from the trainers) and the moment it receives its reward, which usually occurs when it returns to the trainer. The timing of the bridge and selective reinforcement of the different steps are very important, as they need to pinpoint accurately the behavior we want the animal to perform and no other behavior. Knowledge and practical experience of operant conditioning are mandatory to being able to shape and bridge correctly the successive approximation steps in medical training, especially once these become more sophisticated. Auditory (e.g., a whistle blow; the word "good!"), visual (e.g., a flashlight or hand movement), or tactile (a small tap of the hand) bridges can all be used.

Discriminative Stimulus (SD)

The term discriminative stimulus (SD) refers to the cue or signal given to the animal to perform a behavior. The SDs need to be discriminative enough for the animals to differentiate the different behaviors asked for. These also can be auditory, visual, or tactile.

Time

Time is of the utmost importance in medical training. One will likely reach some results more quickly using punishment or withholding food from the animal, but reliable and consistent training will never be obtained this way. Having patience, taking the time, and working step by step are the guidelines to follow.

The image of building blocks illustrates this. A child who is putting a block on top of another will very quickly build a high tower. However, one push or movement of the table on which the tower has been built will make it collapse, and then it is much more difficult to rebuild a tower among the collapsed rumble. The same applies to medical training. If one goes too fast ("I bet I can train my sea lion for a blood stick starting from scratch in only a few weeks!"), the behavior is likely to break down very quickly and will probably be hard to regain. On the other hand, if one builds pyramids, as by the Egyptians, they last (we do not have towers from Antiquity any more). Because the base is broad and strong, all the upper levels are well sustained. The blocks will not fall down easily with a push or a movement of the table. However, it takes a long time to build the base, and sometimes trainers do not have the impression they are moving forward with training, or veterinarians get impatient, and by jumping steps, one can jeopardize the final outcome. That "push" on the table can be a new animal, a new area, a new trainer, a new piece of equipment, etc. The stronger and broader the basic behaviors, the faster the more sophisticated medical behaviors will be learned at a later stage.

Basic Behaviors

The six behaviors described below are the most important tools for training of all medical behaviors.

Target

This is the most useful tool in training. "Targeting" is teaching an animal to touch something it is presented with, with any part of its body. Training for targeting is started by using a pole and touching the muzzle or rostrum of the animal with it, bridging and reinforcing it (see **Figure 39.1**). The animal will learn very quickly that it is reinforced when it targets, and control of the animal is achieved. Depending upon the animal, the fist, hand, or fingers can be used instead of a target pole.

Figure 39.1 California sea lion trained to target. (Courtesy of Nausicaa.)

With time, an animal can be taught to touch the pole or the hand with other parts of the body and remain calm while doing so. The animal will learn to touch that target when it is placed in different positions, sometimes at some distance from the trainer, and that is how medical behaviors are shaped. It will, for example, help a sea lion learning to lift a flipper for a turn position while lying down, to fine-tune the position of its body for a radiograph, or to begin to learn to enter a transport cage.

Place

It is very helpful to teach an animal, be it a dolphin, a seal, or a sea lion, to station in front of the trainer, or at an assigned place, while being held under control by a target. With time, the animal should be desensitized to have other people approach and, in the case of pinnipeds, allow them to walk around it. This will allow the veterinarian to look at overall body condition of the animal. If the animal does not trust its trainer, and positive reinforcement is not used for the working relationship (i.e., the classical mistake is to clap behind animals or hose them to make them move), the need to move around the animal to examine it will be a delicate step, particularly when the animal loses sight of the person, because the animal may fear what the person might do then. This lack of trust will complicate, or render impossible, many medical behaviors. Being able to approach and check the animal closely is the starting point for all medical behaviors, yet this very basic behavior unfortunately is still lacking in many zoological settings.

Stay

In husbandry behaviors, teaching the animal to stay at a specific place or in a specific position for a prolonged period of time while staying calm and relaxed, also when people are moving around or picking up material, is a necessary step. Time is a crucial factor in medical training: an ultrasound session can take a while, and if the animal only stays for a short 20 seconds in the requested position, the veterinarian will never be able to obtain useful information. Inspection of the dentition is not possible if the animal does not keep its mouth open in a relaxed way for a certain amount of time. This "staying" behavior is a basic behavior that needs to be trained from the beginning, and is one of the stronger blocks of the pyramid base.

Touch

Desensitizing young animals to touch, working with them from early on, is easier than starting with untrained adult animals. Some animals like to be touched, some will just accept it, and others may hardly ever allow themselves to be touched. With some animals, particularly unpredictable pinnipeds, it may only be possible to touch them with full protected or semiprotected contact (see **Figure 39.2**).

Water

Pinnipeds, being "amphibian" animals, often thrive on being sent into water at any time during a training session. Being in the water often gives them a sense of security, but it is also a safety tool for people.

"A to B"—Separation of Animals—Gating

One needs to be able to gate, or at least spatially separate, a marine mammal, without problem and at any time, from

Figure 39.2 California sea lion trained to stand for radiograph with protected contact. (Courtesy of Nausicaa.)

one area to another, and separate it from the group and work with it without interference from conspecifics. Without this, it is very difficult to desensitize the animal to many behaviors. The basic for this is to have a very strong ability to send the animal from point A to point B, "A to B."

Routine Medical Behaviors

By using the training tools and the basic behaviors described above, routine medical behaviors can be trained and implemented.

Weighing

Weighing is necessary to assess the efficacy of nutrition, the well-being of the animals, weight gain during a pregnancy, possible seasonal fluctuation, and detection of any unexpected weight changes that may reflect onset of disease (Boyd 2016). This should be mandatory and done on a regular basis, preferably every week. Pinnipeds can be targeted or sent by an "A to B" to a scale. Dolphins are generally trained to slide out of the water onto a poolside scale. In some facilities, dolphins have been desensitized to stretcher training and are lifted to be weighed.

Body Examination

A pinniped should learn to station in front of its trainer, lie down on its ventrum, and turn (left and right), and whenever possible, in dorsal recumbency. Too often, animals are trained to turn only one way, and this will hinder examination of the body or complete ultrasound at a later stage in the medical training. Cetaceans should learn to station in front of their trainers and lie in the same positions (ventral, dorsal, and both lateral positions) next to the pool edge (**Figure 39.3**). These will be the basic positions for most medical behaviors. The animals should be desensitized to tactile manipulations of all areas, including anogenital access. Handling of flippers for nail checks is also important in pinnipeds. If the veterinarian cannot have a close look at a wound, a skin problem, a torn nail, or an inflamed joint, for example, it might take a while before such a problem is detected and treated. These positions also allow for auscultation of lungs and heart, local and topical treatments, and, with desensitization, collection of swabs and/or scrapings for cytology and cultures.

Eye Examination

Cetaceans and pinnipeds can be trained to keep their eyes open for an extended period allowing a thorough examination of the eye and surrounding tissues. By target training, it is also possible to teach the animal to move the eye in different directions, permitting visualization of different areas. Close-up pictures are useful to assess and monitor the evolution of a problem over time, so desensitization to camera and flashes should also be trained for (Lacave et al. 2006). Cetaceans can lie laterally, with the head slightly out of the water, and pinnipeds can bend or twist their necks, so that the eye is in a horizontal position to receive drop administration. The important part of the desensitization is to have the animal keep the eye open when drops are given (**Figure 39.4**). Once an animal is trained to receive eye drops, it is of the utmost importance to

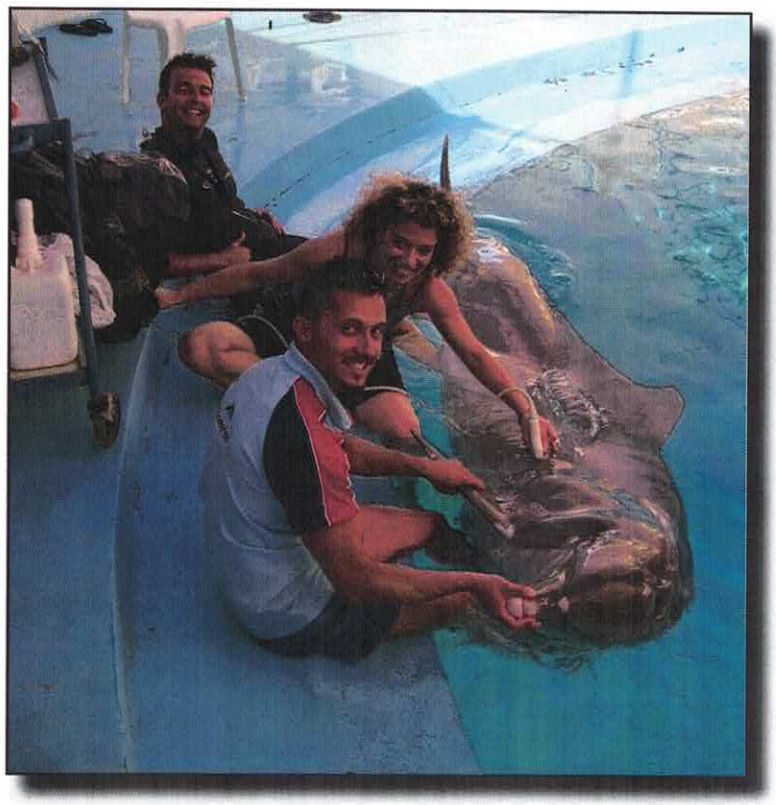

Figure 39.3 Bottlenose dolphin stationed poolside for ultrasound examination. (Courtesy of Zoomarine.)

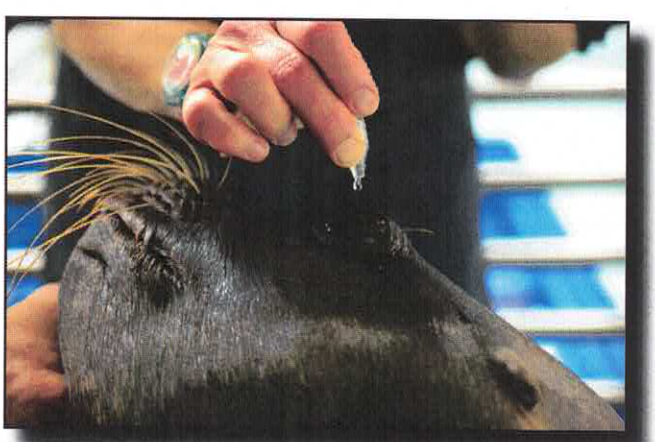

Figure 39.4 California sea lion trained to keep the eye open for application of eye drops. (Courtesy of Amneville.)

desensitize it to several administrations per day for a week or two in succession, to ensure that once such a treatment begins in earnest, the treatment does not fail (Joury et al. 2014). If the animal is not trained for successive treatment regimes, the animal may do it for a day or two and then stop cooperating because frequency had not been trained for. Desensitizing animals to work in a dark environment is a useful tool, too, as their pupil will then be wide open and provide a clear view, when no opacities are present on the cornea, to see to the back of the eye (Huguet et al. 2012). Training for ocular examination and frequent application of eye drops has improved the success of ocular surgery such as cataract removal due to enhanced postoperative care (see **Chapter 23**).

Oral Examination

Both cetaceans and pinnipeds should be desensitized to voluntarily opening the mouth (**Figure 39.5**), as this is the first thing to check if an animal shows sudden anorexia. Gums, tongue, palate, throat, and particularly the teeth should be examined. The animal can also be trained to have fingers or an instrument touch parts of the mouth and the teeth, which can help address simple problems, such as retrieving an object or something squeezed between the teeth. It can also help positioning dental x-ray plate (see **Chapter 22**). A well-trained behavior also allows for local tooth flushing and has enabled capping of teeth in conscious walruses (Oland and Snyder 1997). In general, it is preferable for the trainer to keep contact with the upper and lower jaws of the animal with two hands (or two target poles) when the mouth is open, as it yields better control, and to desensitize the animal to having another person intervene. It is also more reassuring for the veterinarian to have the trainer "controlling" both jaws of an animal when working in the mouth of the patient (**Figure 39.5**). It should be possible to open or close the

Figure 39.5 California sea lion holds his mouth open while at target. (Courtesy of Nausicaa.)

mouth under control and move it vertically and laterally for inspection. When only one hand is used to open the mouth, with several fingers on the upper jaw and the thumb on the lower jaw, the hand it is often in the way of good visualization. Tooth problems are underdiagnosed problems in pinnipeds in zoological settings (Lacave et al. 2004). Being able to brush the teeth of the animals on a daily basis helps alleviate some problems and will also allow a regular thorough checkup of the mouth. With the evolution of diagnostic techniques, particularly in endocrinology, saliva sampling has become a very useful tool. In some facilities, oral medication and daily vitamins are given straight into an open mouth, and the animal is then rewarded and reinforced for swallowing them.

Blowhole or Nostril Sampling

An upper respiratory tract sample (from blowhole or nostrils) can be collected through the training of a forced expiration. Breathing in and out on command has been the basis for more complex behaviors such as receiving aerosol therapy in cetaceans or gas anesthesia induction in pinnipeds. It is also possible to train a dolphin to keep its blowhole open, or a sea lion its nostrils, to allow good visualization of the internal structures and for touching and swabbing.

Gastric Sampling

Obtaining a stomach sample from a fasted animal is extremely helpful for the identification of gastric disorders. With a normal pH of 1–1.5 in a fasted animal, a clear fluid with little to no cells should be collected. It is somewhat easier to train for gastric sampling in cetaceans, since there is no open connection between the respiratory and digestive tracts (the goose beak is held in position through strong muscles in the "nasal" opening dorsally). In recent years, the ability to train for gastric sampling has become a routine behavior in pinnipeds, too. A strong stationing behavior with a consistent two-hands target (hands on upper and lower jaws) with mouth opening is the basic behavior to start with. Transparent equine nasogastric tubes, with soft, rounded ends, 9–25 mm wide and up to 3 m long, depending on the size of the animal, are generally used. At times, cells originating from the upper respiratory tract can be found in a gastric sample, if the animal has swallowed sputum.

The same behavior can be trained for and used for voluntary rehydration, which can be supportive treatment during sickness, long-term antibiotic administration, or molting in pinnipeds. If an animal is very consistently trained for intubation, the more complex procedure of voluntary gastroscopy can be trained for, but it requires excellent control and timing with the animal, since the instruments involved are fragile and expensive. The major advantages of training these behaviors are the ability to observe ulcers, detect delayed emptying of the stomach, and retrieve foreign objects from the upper gastric tract without having to anesthetize the animal. These techniques have been performed on both trained pinnipeds and cetaceans (Bourgain et al. 2008).

Fecal Sampling

A soft and flexible tube (generally a 4- to 5-mm-wide and 60-cm-long, small dog gastric tube) can be delicately inserted into the rectum to collect feces. As fecal material in dolphins is normally semiliquid, it is easily collected when present, but it may be more difficult to sample in pinnipeds, since they have harder stools. Lubricating the external part of the tube with paraffin or lubricant can help insertion of the tube. The behavior can be utilized for rectal temperature checks using a digital thermometer with a sensor probe at the end of a long flexible tube. To have a reliable temperature recording in a cetacean, the probe should be inserted for about 30 cm and stay in place for at least a minute. Rectal temperature can be useful for birth prediction in dolphins, as a drop of 1°C in the

24–48 hours prepartum has been recorded in some animals (Terasawa, Yokoyam, and Kitamura 1999).

Blood Sampling

The first voluntary blood sampling of a dolphin was in 1964 by Dr. Sam Ridgway, at Point Mugu, so that the "capture" of the animals for health checks would not interrupt the hearing studies being performed by Dr. Scott Johnson at the time (Ridgway pers. comm.). Details about blood sampling sites and needle sizes can be found in other chapters of this book (see **Chapters 35, 37, 42 through 44**), and some training specifics are summarized below.

In cetaceans, voluntary samples are generally obtained from the main superficial vessel of the fluke, either dorsally or preferably ventrally. Other sites less commonly used are the dorsal fin or pectoral flipper veins, and the ventral aspect of the peduncle. The animal can be in a head-down vertical position, a horizontal position, or a lateral position. Some animals will stay head-down for a long period, while others will prefer floating on the surface, watching or breathing at regular intervals. Every position is acceptable, and it is best to sometimes accommodate an animal (cetacean or pinniped) for it to be comfortable when doing a somewhat invasive procedure. However, the necessary conditions for blood sampling a dolphin are to have the animal calm and relaxed in the position that is the most suitable for it, for a certain length of time, and with the fluke lying still, without constraint, in the lap of the trainers or poolside. Desensitizing the animal to the presence of the veterinarian, the disinfection, the manipulation, and searching for the correct area to insert the needle are all factors that need to be thoroughly reviewed during the desensitization steps. In training for blood sampling, an important factor to train first is duration. The animal needs to stay calm in the requested position for a certain period, and with exertion of some pressure, before one starts training for the needle. Too often, there is a rush to quickly start inserting a needle, and the behavior is constructed "tower way" instead of "pyramid way." Blood is usually taken on a monthly basis in dolphins, but, once trained and when needed, it is possible to take blood every day or every other day, as a voluntary behavior, in order to follow the course of a disease and the effects of treatment.

In otariids, the best place to sample blood is the caudal gluteal vein, as the vein is a reasonable size, and when hit correctly, a 10 ml sample can be collected quickly. However, because the vein is not visible and is deep, the veterinarian needs to be familiar with the anatomical reference sites. The animal needs to be in straight sternal recumbency with the hind flippers slightly spread but aligned with the length of the body and flat on the floor. A classical mistake is to tuck the front flippers alongside the body. This will often make the animal lean more toward one side or the other, and as such, the anatomical references will be slightly off. It is better to have the animal in a slight spread-eagled position, with the front flippers spread out, for this. The vein is reachable at the first third of the distance between the trochanter and the tip of the tail, and at about one finger's width lateral to the vertebral column. Each animal is a little different, so when bleeding regularly, one can learn how to angle the needle for each animal. Epidural needles are not recommended when working with voluntary sampling as their bevel is not as long and sharp as regular needles, and a greater pressure has to be exerted to pass the needle through the skin; in addition, some animals will hence show discomfort or quit the position because of the strength necessary to insert the epidural needle.

In phocids, the recommended and easiest area to sample blood voluntarily is from the epidural, or extradural intravertebral vein/sinus; when the animal is in ventral recumbency, locate the spines of lumbar vertebrae 3 and 4, and then the needle is inserted perpendicularly between the two vertebral bodies. The animal needs to be straight, and desensitized to being touched near its tail and caudal spine and to allowing strong pressure from the veterinarian to feel the vertebrae (Ferrer et al. 2012). Important desensitization steps for the trainers are to "copy" everything the veterinarian does when taking blood (even, for example, the habit of putting the cap of the needle in the mouth and then pulling the needle out). If not desensitized to this, some animals will take it as the cue for the "real" blood sampling and go away.

In otariids and phocids, blood can also be taken voluntarily from the plexus of veins that run interdigitally. The plexus is reachable on the dorsal part of the hind flipper in otariids on either side of the third phalanx, and on the ventral part of the hind flipper in phocids, running along either side of the second and third phalanx at the base of the web. In phocids, the animals can be trained to stay in ventral recumbency and have their hind flipper twisted gently for this behavior. However, the author's experience is that although seals do not mind much when a needle is inserted intervertebrally, many have a harder time letting their hind flippers be manipulated (Lacave 2002). In otariids, the superficial veins that run dorsally in the webbed skin between the digits of the hind flippers are more accessible for voluntary blood sampling. They are very superficial and tiny, quite visible in Patagonian sea lions (*Otaria byronia*) and in South African fur seals (like *Arctocephalus pusillus*), although less so in females, but are much more difficult to see in California sea lions (*Zalophus californianus*). Putting hot towels on the flippers or using a tourniquet can help dilate the vessels, and the animals need to be desensitized accordingly. Using a vacuum system is generally not recommended here as the aspiration can collapse the vein. Thin butterfly needles (23 gauge) rinsed with anticoagulant should be used. Because the blood will coagulate rapidly, it is a good habit to exert a continuous rolling movement of the syringe when the blood is taken and to aim for no more than 5 ml. The animal should be desensitized to the connecting tube of the butterfly accidently touching its flipper during the manipulation. Some facilities have also had success with voluntary blood as the level of the jugular or in some superficial veins both in the dorsal and ventral part of the fore flipper.

The difficult part for many veterinarians working with the voluntary participation of the animal for blood sampling is learning to be very gentle with the animal. Sticking the animal suddenly is not the way to go, as with time, the animal will not know what or when to expect it and may be quite startled by it (that is also why I do not like the snap of a rubber band to be used in the desensitization process). A lot of patience is necessary, and the needle needs to be inserted gently and slowly. Success will depend upon this gentle and quiet approach in many cases. And the golden rule: one attempt. If the animal was perfect in the position, but the veterinarian did not manage to take blood, abort, reinforce, and retry later (though this could be slightly adapted in very well-trained animals).

Ultrasound

Ultrasound was originally used for monitoring gestation and fetal development (Williamson, Gales, and Lister 1990; Brook 1997; Stone et al. 1999; Lacave et al. 2002), but it is now essential in preventive medicine because there are now portable and reasonably economical machines that can be taken to the animal (see **Chapter 24**). The previously described poolside positions are used in cetaceans for ultrasound behavioral training. A scanning session, particularly if a general checkup is performed and all organs are examined, can take up to 10–20 minutes per side. It is important for the trainers and the veterinarian to be comfortable, so that the exam can be performed swiftly and accurately. Bending over an edge or having to turn the head sideways to see the ultrasound screen will render the session more difficult and lengthen the time to obtain images. The recommendation is to have two trainers sustain the animal—at the level of the head and the tail—and for the scanning veterinarian to be in between. This way, the trainers can easily move the animal forward and backward or slightly roll it laterally. Too often, when only one trainer holds or controls the animal, the animal will tend to drift away or sink down at the unheld side (training-wise, the criteria of the behavior is being lost), or the trainer will be in the way of the scanning veterinarian. For trainers, it is important to realize that the image captured is in 2-D (comparable to a very thin slice of the animal), and exaggerated movements or frequent breathings by the animal will prevent the identification and follow-up of smaller structures (e.g., ovaries). When working outside, it is strongly recommended to work with goggles. The original desensitization to the machine (also potentially the plastic protection, which can be moving and be noisy when there are strong winds), the probe (with different frequencies), the wearing of goggles, and touching all parts of the body also need to be done through all the necessary approximations steps. Cetaceans are excellent patients for ultrasound because their skin is constantly covered by a thin layer of water, which is an excellent wave conductor.

Pinnipeds can also be desensitized to ultrasound examination. Ventral, dorsal, and lateral recumbencies are the basic requirements and most of the time preferred to the stationing ("sitting") position (Lacave 2012b, 2015; **Figure 39.6**). The animal should stay calm and in position when the machine is moved near its body. A basic mistake is to follow the animal with the probe if it moves away. It is important to desensitize the animal to the application of gel. The gel bottle may be shaken forcefully or squeezed to get the gel out, resulting in some noise. This can jeopardize a session if the animal has not been desensitized to these practicalities (harsh movement, noise) of performing an ultrasound. Recently, advances have been made in ophthalmological checkups through immersion ultrasound (Joury, Maillot, and Alerte 2014; Lacave 2014;

Figure 39.6 Ideal position of a California sea lion for abdominal ultrasound. (Courtesy of Amneville.)

Lacave and Huguet 2017). Combining behavior of intubation and ultrasound has also allowed cardiology checkups through transesophageal echocardiography (Rice et al. 2015).

Radiography

The same steps as described above are used for radiography desensitization. The "stay" cue is of great importance to keep the animal in a specific position, but it also allows the trainer to move away from the x-ray beam and the animal. Desensitization to protective equipment (lead aprons, gloves, glasses) is not to be underestimated.

Being able to manipulate the animal to fine-tune a position a couple of times before actually taking the radiograph is a great tool, as care should be taken not to take unnecessary images. The evolution of transportable and handheld machines, together with the advent of digital radiography, has been a great addition to this imaging technique in marine mammals.

Pinnipeds are easy to radiograph on land. Cetaceans can be asked to slide out onto a radiography plate or be desensitized to plates being held against them in the correct position. Excellent dental radiographs can be obtained by training animals to hold plates in their mouth.

Advanced Medical Behaviors

While the formerly described behaviors are part of routine medical checkups, additional behaviors have been obtained in recent years and are added to the medical behavior panel of trained animals. Choices of which behaviors to train for are often triggered by the animal's own medical history. Details about the behaviors will not be reviewed, but they have all been trained for using the basic behaviors referred to above.

Urine Sampling

Urine sampling in dolphins can be trained for by exerting local pressure on the bladder (after ultrasonography to determine that it is full) and/or in other cetaceans by capturing the behavior (bridging when the animal was urinating on its own). In female pinnipeds, manually triggering the genital area and splashing warm water has also proven helpful. In well-trained animals and with careful approximation steps, it is possible, both in males and females, to catheterize the bladder.

Milk Sampling

The ideal position for milk collection in a cetacean is a lateral position, poolside, sustained by the legs of two trainers, with the area of the upper mammary gland lifted above the water (dorsal recumbency makes it too difficult to collect the milk). The animal can be desensitized to an adapted syringe or a breast pump pressed on the mammary slits, and needs to become used to the feel of suction. In larger animals, the same can be achieved with a slide out into a lateral position. Pinnipeds have also been desensitized to the same devices, lying in a lateral recumbency.

Semen Sampling

Conditioning for semen collection in cetaceans is important to reproductive programs in many institutions and is being developed for pinnipeds (Gonzalez et al. 2015). The male is generally in dorsal recumbency and initially is manually stimulated with the slightest reaction being bridged. By further approximation steps, it is eventually possible to trigger a full erection and ejaculation on cue.

Injections

The same basic desensitization of needle insertion can be used for the training of intramuscular (IM) or subcutaneous (SC) injections. An important step in the training is the feeling of having a product injected, which can be desensitized by injecting sterile physiological water.

Killer whales (*Orcinus orca*) are generally desensitized to calmly accept IM injections while dock dried on a lifting platform. Voluntary long-term SC rehydration in pinnipeds and/or IM injections of antibiotics have also been reported and successful in both species (Ruiz, Henderson, and Reid 2015). The same type of desensitization can allow for fine-needle aspirates, as well.

Biopsies

Animals can be desensitized to local anesthesia and biopsies, especially skin biopsies (Molnar et al. 2016). Some facilities have performed buccal and genital biopsies, and even tumor freeze-branding or resections under voluntary behavior (Pereira et al. 2002; Lacave et al. 2003; Massei et al. 2004; **Figure 39.7**).

Endoscopy

Gastroscopy is a complex behavior deriving from intubation. It is also possible to desensitize animals to other types of endoscopies such as cystoscopy (De Souza et al. 1998) or vaginoscopy (Neto et al. 2006), which is an important tool for artificial insemination in cetacean breeding programs.

Anesthesia

Pinnipeds have been trained to breathe voluntarily from a mask, while contained in a squeeze cage, for anesthesia induction.

CT Scan

Dolphins have been desensitized to being repeatedly dry docked and transported to CT scanners, while staying calm and under control of their trainer (Cooper 2016).

Figure 39.7 A bottlenose dolphin trained to allow tumor resection by cryosurgery. (Courtesy of Mike Walsh.)

Prosthetics

In recent years, use of modern technology has made it possible to develop prosthetic equipment for cetaceans and pinnipeds, providing them, through progressive desensitization and adaptation, a better quality of life (Arnold 2016).

Protected Contact

Protected contact is recommended when working with all potentially aggressive animals (though all animals are potentially aggressive) or individual animals that often have enhanced or unpredicted reactions. It is also worth using during behaviors that can elicit unexpected pain, such as a blood sampling, injections, or biopsy, where one might want to protect oneself from an automatic response to pain, which is not necessarily aggressive. Animals can be desensitized to a protecting shield, separation board, or squeeze cage. All voluntary medical behaviors—even the more complex ones—can be performed this way without danger for the people involved (Bourgain et al. 2008; Sleeman and Harris 2015). The safety of a person is much more important than any required sample, and taking risks to obtain one is unacceptable. It is extremely important to desensitize the animals to the protecting devices that will be used, as accidents can happen in case of forceful use (broken tooth or jaw in a squeeze cage for example). Sometimes skittish animals feel safe when separated from people by a board or when in the more confined space of a squeeze cage, and will work quite well this way. Generally, if the protecting device is transparent or allows some vision of what is happening to the animal, the animal will be more willing to perform the requested behavior.

Lifting Floor

Cetaceans should be trained to enter an area with a lifting floor daily, station while the platform is being lifted, allow the presence of multiple people, and be manipulated in this shallow water or dry setup. Desensitization of pregnant cetaceans to the lifting floor is vital (Di Mecola, Lacave, and Biancani 2011). While more and more voluntary behaviors are trained for in marine mammals, there is still a fear to apply these to very young calves (Lacave and Cox 2000). Young animals need these medical checks as well, and the use of and desensitization to a lifting floor have brought changes to their handling in recent years (Lacave et al. 2005). Having their mother comfortable during the handling, because they are used to the platform, is extremely helpful (Salbany, Roque, and Lacave 2004).

Conclusions

One general recommendation I would like to give to veterinarians when working with any animal through voluntary behaviors is that when the trainer or keeper asks to stop, not to ask for "only a few seconds more," but to stop right away (which is sometimes difficult for a veterinarian) and finish on a positive note. There are no limits as to what can be asked as voluntary behavior from our animals. The behaviors mentioned in this chapter are not the limit, and 30 years ago, many would not have imagined all that can be done today. Medical behavior training has been a great help in promoting preventive medicine and research (Madigan and Fahlman 2016). The important factors are a great relationship between the trainer and his/her animal, great communication between the trainers and the veterinarians, excellent teamwork, and the use of positive reinforcement.

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