General Issues for Animal Testing

Any use of animals in research is governed by laws interpreted by regulatory agencies and local institutions with which researchers must be conversant and compliant. Studies of cognition of necessity require measurements of behavior, and the behavior must be controlled in the context of the experiment. Verbal instructions are not effective, so animals must be coaxed to perform a task. The motivation to perform the task can be appetitive or aversive. Appetitive control occurs through pairing an event or behavior with a food or fluid reinforcement. Aversive control occurs through pairing an event or behavior with an undesirable stimulus such as electric shock or a brief loud noise. Aversive control can be used only if scientifically justified as, for example, in studies of anxiety.

Classical (Pavlovian) conditioning is accomplished by repeatedly pairing a conditioned stimulus (e.g., a tone) with an unconditioned stimulus (e.g., a puff of air) that reliably elicits an unconditioned response (e.g., an eye blink). The sensitivity of the animal to the conditioned stimulus can then be assessed by measuring the occurrence or magnitude of the unconditioned response as some characteristic of the conditioned stimulus (intensity, frequency, etc.) is varied systematically. Operant (instrumental) conditioning is accomplished by reinforcing an emitted behavior (e.g., looking at a spot of light) with a reward (e.g., a food item or fluid) or a punishment (e.g., a mild electric shock). Behaviors followed by positive reinforcements are more likely to be emitted in the future by an organism motivated to receive the reward. Behaviors that are followed by a negative reinforcement are less likely to be emitted in the future. Decades of research has detailed many principles and nuances of operant conditioning that the interested student can learn from a textbook on learning and memory. Also, detailed procedures for various species and situations can be found in the methods sections of peer-reviewed publications. Finally, we should also note the obvious specializations of behavior and associated cognition across the phylogenetic scale.

Taxonomy of Cognition

Stimuli can be presented and overt responses measured. However, to explain orderly and arbitrary relationships between responses and stimuli, it is now regarded as useful, if not necessary, to hypothesize certain covert processes mediating the encoding, selection, and categorization of stimuli and preparation of responses.

Before reviewing methods used to investigate higher order cognition in animals, we should begin by determining how to distinguish cognition from simple sensation and movement. We can also formulate at least a provisional minimal taxonomy of cognition. Reflexes are the most direct link between sensory and motor processes. It could be argued that any behavior that is not a reflex must include some cognitive component. It could also be argued that only behaviors that arise from or are concomitant with conscious mental content qualify as cognition. For the purposes of this article, we can be satisfied with an intermediate position that only holds that processes that intervene between or adaptively adjust sensation and movement constitute cognition. If so, then we can ask, how many cognitive processes can be distinguished reasonably? We will work with the following list:

- Perception, including categorization
- Attention
- Memory, including long-term and working memory
- Response selection and preparation
- Emotion
- Executive control

We are also interested in more complex activities that involve all of the processes listed previously:

- Decision making
- Problem solving
- Communication

Less space is devoted to processes that are described elsewhere in detail, and more space is devoted to less well-studied processes.

Methods to Study Perception

Discrimination and Categorization

Distinguishing the characteristics of stimuli for the purpose of guiding behavior has been investigated in many species. The testing requires designing alternative stimuli appropriate for the sensory apparatus of the particular animal and is based on the principle that two (or more) physically distinct stimuli can be rendered indistinguishable from one another by manipulation of
the magnitude of their physical differences. Thus, a natural component of an animal’s behavior is placed under control of the stimulus and then the characteristics of the stimuli are varied to determine how sensitive the animal is to the variation of the stimulus. For example, monkeys can be trained to shift gaze or press bars to signal whether a visual stimulus has a particular color, shape, or pattern of motion. Similarly, rats can be trained to perform tactile discriminations with their whiskers, such as judging the width of an aperture, the texture of a surface, or the distance of a gap, or also to signal their discrimination of mixtures of odors. Animals are capable of making more complex sensory discriminations. For example, animals as diverse as monkeys and pigeons can learn rapidly to discriminate among color photographs of different categories of objects, such as trees, humans, fish, and water, and these discriminations are generalized to novel examples from the categories of objects.

Awareness

A fruitful line of inquiry in the past decade has investigated neural correlates of awareness in monkeys. This has been accomplished by presenting a single ambiguous stimulus that can be perceived as either of (usually) two alternatives to macaque monkeys trained to report which of the alternatives they perceive. One way to dissociate the presentation of a stimulus from the phenomenal awareness of that stimulus is to employ binocular rivalry. Binocular rivalry occurs when markedly different stimuli are presented to the two eyes (e.g., horizontal stripes to the left eye and vertical stripes to the right). The perception is not of some plaid combination but instead of the stimulus in one eye and then the other, alternating randomly until one or both stimuli are removed.

Another means of dissociating presentation from report is through visual masking. This is accomplished by presenting a stimulus that may be weak in some respect but not below threshold to detect and then rapidly thereafter presenting a stronger stimulus at the same or nearby location. The presence of the subsequent stronger stimulus results in a lack of awareness of the earlier stimulus; this is referred to as backward masking. Critical to these studies is some objective measure of how the sensory system registers the stimuli because animals are very likely to respond in a biased and stereotyped manner to ambiguous stimuli unless reinforcement contingencies prevent this.

Methods to Study Attention

To study the allocation of attention, the most common procedure is to present a cue that informs the animal that a response is required for a subsequent stimulus at the same location. In a fraction of trials, the test stimulus is presented at an uncued location. The difference in response time or discriminability of the test stimulus at the cued versus the uncued location is taken as a measure of the allocation of attention. Alternatively, multiple stimuli can be presented simultaneously with one of the stimuli distinguished by sensory properties, a prior cue, or a memory template to guide the response. The quality of response to the target in the presence versus the absence of distractor stimuli is taken as a measure of the allocation of attention. The more diverse the distractors’ appearance and the more similar the distractors are to the target, the more attention demanding the task becomes. In addition, a stimulus with multiple attributes (e.g., color and shape) can be presented following a cue that instructs the animal to respond according to one of the attributes. The demand for attention can be manipulated by making the cued attribute more or less difficult to discriminate and by making the noncued attribute more or less potent.

Attention has been investigated predominantly with nonhuman primates, but procedures have also been devised for rodents and even birds such as pigeons. Procedures to assess attention in a less specific sense have been formulated to study mouse models of attention deficit disorder. For example, mice can be trained to poke their nose into a hole to receive a reinforcement in response to a visual stimulus. By offering multiple alternative holes signaled by different lights turned on for short durations, the mice must attend to the stimuli to respond quickly enough to receive reinforcement. The attention capacity of the mice can then be assessed by manipulations of target light presentation rate, duration, salience, and temporal predictability.

Methods to Study Memory

Memory can be inferred by persistent and consistent changes in behavior following learning. An overview of classical and operant conditioning learning procedures was provided previously. Memory can be divided into short-term working memory and long-term procedural and declarative memory.

Short-Term Working Memory

Short-term memory has been studied using delay-response tasks in which a stimulus or set of stimuli are presented briefly but a response cannot be produced
until after a retention interval typically lasting approximately 1–10 s. Animals can guide their responses by the location or identity of the stimuli. The task has been elaborated in various ways. Delayed alternation requires monkeys to select the object at the location that was not selected in the previous trial (delayed spatial alternation) or to select the object that was not selected in the previous trial (delayed object alternation). This task requires working memory retention across as well as within trials. Delayed match to sample exposes monkeys briefly to an object and then after an interval presents the target object plus a distractor object. Monkeys are reinforced for selecting through a gaze shift or a reaching movement the target object that they had observed previously. Delayed nonmatch to sample reverses the logic, requiring monkeys to select the object they had not observed previously. It is important to note that delayed-response tasks require not only working memory but also response inhibition to prevent premature movements and may also invoke response preparation if the monkey can anticipate the termination of the retention interval when it can respond.

**Long-Term Memory**

Memory persisting over days or months must be tested in a species-specific manner. For example, rats are commonly tested using spatial navigation through locomotion in a maze to locate a reinforcement. Also, the water maze was developed to assess spatial learning and memory in rats. It is commonly used with mice, but species and strain idiosyncrasies must be recognized in the interpretation of the results. A platform is placed at a particular location beneath the surface of a round pool of murky water. When placed in the pool, mice swim to locate the hidden platform, and learning can be measured by a reduction in the amount of time taken to locate the platform as the mice or rats encode orienting cues in the environment. Contrasting performance when the platform is hidden with that when it is visible permits resolving confounds of sensory or motor impairments.

Implicit in the description of all the complex tasks monkeys can be trained to perform is the fact that they have long-term memory. The nature and capacity of that memory has been probed largely through tests of categorization of stimuli and of relations among stimuli. More evidence for long-term memory is derived from learning sets (learning to learn), an improvement in the speed of acquisition of successive discriminations between arbitrary objects or of reversing responses when reinforcement changes. Learning set has been demonstrated in nonhuman primates as well as in dogs, rats, and some birds.

**Methods to Study Response Selection and Preparation**

All motor responses can be explained in terms of causes (the muscle contracted because the neuron fired), but some responses can be explained also in terms of reasons (the bar was pressed to obtain the food to satisfy the hunger). This section considers movements that can be explained in terms of reasons and not just causes.

**Response Selection**

Requiring an arbitrary response to a particular stimulus invokes a process referred to as stimulus response mapping. A wealth of studies of human subjects can inform the design of experiments with animals; however, it should be clear that different species are incapable of producing more or less complex mappings. The capacity of rodents to learn arbitrary responses through operant conditioning is more limited than that of macaque monkeys, for example. Studies with macaque monkeys have explored progressively more complicated degrees of mapping a response onto a stimulus so that we can say that monkeys are encoding instructions and following rules or even strategies.

At the lowest level, monkeys can produce an arbitrary response not directed toward a stimulus — for example, a shift of gaze to a location directly opposite a target (antisaccade). At the next level, macaque monkeys can be trained to produce arbitrary responses to specific cues on successive trials and to change the mapping if the reinforcement contingencies change (conditional motor learning). Monkeys can also be trained to produce an arbitrary sequence of a few movements guided by particular cues. Perhaps the greatest expression of this ability is seen in apes that learn sign language. In studies with humans, evidence for response selection is an increase in the latency to initiate the first movement with an increase in the number of movements in the sequence. Unfortunately, animal studies have not performed this simple test. Beyond producing sequences of movements in response to sequences of stimuli, monkeys can be trained to produce self-ordered sequences of responses in which successive responses are guided by previous responses.

At perhaps the highest level, macaque monkeys can be trained to respond according to alternative strategies that require flexibility in applying rules with no fixed stimulus–response mapping. For example, monkeys can switch between repeat-stay and change-shift strategies. To perform a task such as this, monkeys must remember the previous response, remember the stimulus or stimulus–response pair preceding that choice, use that memory to evaluate
whether the stimulus on the current trial was different from that on the previous trial, and apply the strategy to choose the same target when the stimulus repeated and to reject that target when the stimulus changed.

Response Preparation

The time of an overt response to a given stimulus is variable and unpredictable. Within that unpredictability, however, certain trends have been observed. For example, when given a warning (‘ready’) before an imperative trigger signal (‘go’), subjects respond earlier and more reliably than when no warning is given. The interval between the warning and trigger signals is referred to as a foreperiod. Response time can also be influenced by repetition of stimuli or responses or by success in previous trials. To explain this variation, one can hypothesize a process that transpires after an instruction or warning signal and is influenced by events in preceding trials to influence the readiness to initiate a movement. Such a covert process may be called response preparation.

The influence of foreperiod on response time varies according to the statistical properties of the foreperiod. If the foreperiod is a fixed value of the appropriate interval, then the animal can learn to anticipate when it will elapse. If the foreperiod is too short (e.g., <100 ms), response time is unaffected because some time is needed for response preparation to engage. If the foreperiod is too long (e.g., >2000 ms), response time is unaffected because it is not possible to sustain response preparation indefinitely. Thus, foreperiods ranging from 100 to 1000 ms afford the largest reduction of response time. The reduction of response time occurs because the passage of time allows a sense of expectation, permitting preparation before the trigger signal so that sometimes the movement might occur before the trigger signal (jump the gun). In fact, such self-generated responses are clear evidence of a covert response preparation process.

Foreperiods can be randomized to reduce predictability. For example, the foreperiod on a given trial could be sampled with equal probability from the range 100 to 1000 ms. This does introduce some variability in response time, but response time tends to decrease as foreperiod increases, and anticipatory responses are often generated especially at the longest foreperiods. This occurs because although the foreperiods are selected randomly across trials, they have a maximum value (i.e., a deadline that the animal can learn through experience). The more time that elapses from the warning stimulus, the higher the probability that the trigger stimulus will occur given that it has not yet. This conditional probability is referred to as the hazard rate of the foreperiod. If the hazard rate increases, the foreperiod is referred to as aging. A nonaging distribution of foreperiods has a flat hazard function; in other words, the passage of time conveys no predictability about when the foreperiod will elapse. Nonaging foreperiod distributions have many short values and exponentially decreasing probability of progressively longer values. Response times obtained with nonaging foreperiods decrease little, if at all, as a function of foreperiod and anticipatory responses are produced rarely.

Further evidence for response preparation is the fact that partially prepared responses can be withheld if an imperative ‘stop’ signal occurs. This ability can be explained by hypothesizing another covert process that prevents movements. The stop signal or countermanding task has been introduced in the past decade into animal studies. The countermanding paradigm probes a subject’s ability to control the initiation of movements by infrequently presenting an imperative stop signal in a response time task. The subjects’ task is to cancel the planned movement if a stop signal is presented. Performance on this task can be accounted for by a race between a process that generates the movement (go process) and a process that cancels the movement (stop process). This race model provides an estimate of the stop signal reaction time, which is the time needed to cancel the planned movement. The stop signal reaction time can be used in tests of neural processes and manipulations.

Intention

The disposition to perform some act is a central feature of an intention, but intention cannot be identified entirely with response preparation. A statement of intention must also answer the question, ‘Why was that done?’ An answer about the causal path through neurons to muscles is not as satisfying as an explanation addressing the reasons for the action based on preferences, goals, and beliefs. In other words, to judge whether a movement was intended, one must refer to the agent’s beliefs about which action must be performed in what circumstances to bring about the desired object of the intention.

These concepts about intention have been formulated in the domain of human interactions, but research describing communication and deception, for example, indicates that the attribution of intention to monkeys at least seems justified. A particular form of intention involves reasoning about the relation between observations of events in the environment and actions in that environment – in other words, about how actions result in consequences. In fact, some have concluded that guiding actions based on reasoning about the causes of events is uniquely
human. However, research has shown that rats can make correct inferences guiding responses for reinforcement on the basis of purely observational learning of causal relations between events. Comparable results have been obtained from nonhuman primates.

**Methods to Study Emotion**

Other articles should be consulted for details on emotion, and this article does not debate whether animals have emotions like humans. Most studies of animal emotion involve fear, anxiety, and depression. Being aversive, the procedures used in these studies deserve special scrutiny and oversight to ensure animal welfare in the pursuit of scientific information.

Fear in animals has been studied most effectively using classically conditioned fear responses. Fear conditioning is a form of learning in which fear is associated with a particular neutral stimulus (e.g., a tone) by pairing the neutral stimulus with an aversive stimulus (e.g., an electric shock, loud noise, or unpleasant odor). Ultimately, the neutral stimulus elicits the state of fear. Fear conditioning has been studied in numerous species, from snails to humans. In animals, conditioned fear can be measured as a period of attentive immobility (freezing), a potentiated startle reflex, as well as changes in heart rate, respiration rate, or muscle tension.

Learned helplessness occurs when an animal is exposed to inescapable aversive stimulation (e.g., electrical shock) and learns that it cannot control its environment. This is regarded as a useful model of human depression.

Anxiety has been investigated by putting mice in an open area and measuring how much time they explore in the exposed interior of the area; more anxious mice spend more time close to the walls.

**Methods to Study Executive Control**

When the environment is ambiguous or presents competing demands, or the mapping of stimulus onto response is complex or contrary to habit making performance prone to errors, then executive control is required to perform the task. Such executive control over the perception, selection, and response systems is seen as a central component of human cognition, and it can be investigated in animals, particularly nonhuman primates, in a variety of ways. The basic approach is to study how performance changes according to the history of trials. For example, if a particular movement is called for in response to a given stimulus on repeated trials, and then in a subsequent trial a different movement is called for in response to the same stimulus, then a cost will be observed in the accuracy and latency of that response. Such experimental paradigms are referred to as task switching. The cost for switching tasks can arise at least in part because of the conflict between producing the rehearsed response and producing the new response.

Response conflict can be investigated by training macaque monkeys extensively to respond to objects with multiple features such as color and shape with alternative responses cued by different features (e.g., red or circle means go and green or square means nogo). Once the mapping is mastered, testing proceeds by presenting congruent (e.g., red circle) stimuli that cue the same response and incongruent stimuli (e.g., red square) that cue the opposite responses. Evidence of conflict evoked by the incongruent features is slowed response times and increased error rates. Response conflict can be elicited in rats by training them to associate a response lever with a particular reinforcement (e.g., food pellet vs. sugar water). Conflict is induced by rewarding the correct responses with the reinforcement opposite the discriminative stimulus. In other words, by correctly pressing the bar associated with the food pellet stimulus, rats receive sugar water reinforcement and vice versa.

When performing in conditions that require executive control, errors are often produced. A cognitive process of error monitoring is believed to be a component of executive control. In human studies of choice responses, a sign of error monitoring is slowing of response time on the trial after errors.

**Decision Making**

This process has many aspects, most of which have been summarized. If we define a choice as an action performed in the context of alternatives to achieve a goal, we can appreciate that decision making occurs when the alternatives are vague or the payoffs are unclear. Accordingly, one approach to studying decision making requires animals to perform particularly challenging sensory discriminations; this can be done in any sensory modality. Another approach is to manipulate reinforcement amount or probability in a more or less predictable manner. A classic finding is that animals will produce alternative responses (e.g., bar presses) in direct proportion to the amount or probability of reinforcement for the alternative responses; this is referred to as matching law.

A popular area of inquiry in the past few years has applied concepts and procedures formulated in game theory and economics to the study of animal behavior. For example, monkeys are trained to perform tasks resembling games such as rock–paper–scissors or matching pennies. These mixed strategy games
allow investigators to explore whether theories developed to explain human decision making in games and economic settings apply to animal behavior. All of these approaches result in behavior that is more or less stochastic but amenable to analysis in terms of quantitative models of the processes hypothesized to produce the responses. This approach has shown that animals express in at least a rudimentary manner many of the biases that characterize human decision making. For example, monkeys show aversion to inequity by refusing a lower valued reinforcement if they observe another individual obtaining a higher valued reinforcement.

**Problem Solving and Tool Use**

Animal cognition is perhaps nowhere more evident than in the solution of problems, especially through the use of tools as highlighted by Kohler’s pioneering studies of the use of tools by apes. Although several species learn to use tools in the wild as well as in the laboratory, it remains unclear to what extent animals understand the physical processes by which these tools are effective. In fact, some of the most interesting evidence to date that animals understand the causal properties of physical objects comes from birds, particularly the corvids (crows and ravens). For example, a New Caledonian crow spontaneously bent a piece of ineffective straight wire into an effective hook tool for retrieving food, and another species of corvid learns rapidly to avoid maneuvering food into a clearly visible and inaccessible trap. Chimpanzees do not fashion tools or avoid such traps without considerable training, but they do keep objects used as tools for future use.

**Communication**

None doubt that animals communicate in rich and diverse manners. For example, vervet monkeys in the wild exhibit a very advanced system of communication, but it is believed to be instinctive, not learned. What is more contentious is whether animals such as great apes can be said to learn language. Investigators have trained chimpanzees, bonobos, gorillas, and orangutans to communicate through symbols on a keyboard or sign language. Individual chimpanzees have acquired vocabularies of at least 200 words or signs and have demonstrated the capacity to respond appropriately and produce meaningfully different sentences.

Learning to produce words is not limited to primates. African gray parrots have gained notoriety through a phenomenal ability to learn words or even phrases, in some cases as complicated as songs. One individual bird has been trained to use English speech to label dozens of objects, features, and categories as well as use commands and requests.

**See also:** Anxiety Disorders; Binocular Rivalry; Cognitive Control and Development; Episodic Memory; Assessment in Animals; Game Theory and the Economics of Animal Communication; Games in Monkeys; Neurophysiology and Motor Decision-Making; Operant Conditioning of Reflexes; Pharmacology of Fear Extinction; Procedural Learning: Classical Conditioning; Reasoning and Problem Solving: Models; Referentiality and Concepts in Animal Cognition; Reward Decision-Making; Spatial Memory: Assessment in Animals.

**Further Reading**


