

*Chapter 19*

## **BASIC TEMPORAL DISCRIMINATION PROCEDURES**

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### **ABSTRACT**

Many psychophysical temporal discrimination procedures have been designed and used to measure the ability of animals and humans to time interval durations. This chapter introduces three basic psychophysical temporal discrimination procedures that have been used across a wide range of species: the fixed-interval procedure, the peak procedure, and the bisection procedure. This chapter provides an in-depth description of the three procedures as they are executed in standard operant chambers for rats, and methods for data analysis. Standard results, as well as some of the traditional interpretations of the results, are presented. A brief examination of the application of the procedures to different questions and how they have been used to answer these questions is included.

### **INTRODUCTION**

Psychophysical procedures have been developed that use behavior as a measure of time discrimination. This chapter describes three basic psychophysical procedures that have produced similar and robust results in many laboratories and across a variety of species, such as humans (e.g. Allan and Gibbon, 1991; Green Ivry, and Woodruff-Pak, 1999), rats (e.g. Crystal, 2002; Kirkpatrick and Church, 2003), and birds (Machado and Keen, 2003; Santi, Hornyak, and Miki, 2003). These procedures are the fixed-interval procedure (Schneider, 1969; Ferster and Skinner, 1957), the peak procedure (Catania, 1970; Catania and Reynolds, 1968; Roberts, 1981), and the bisection procedure (Stubbs, 1976; Church and Deluty, 1977).

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## Fixed-Interval Procedure

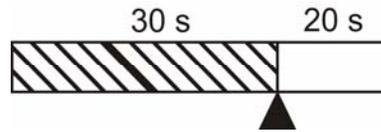


Figure 1. Fixed-interval 30 s procedure.

The fixed-interval procedure is an appetitive procedure that consists of a stimulus that is turned on (e.g. a light), and after a target duration (e.g. 30 s) the next response (e.g. head entry to the food cup) delivers food and terminates the stimulus. After a period with no stimulus the stimulus is turned on again and the cycle is repeated. An example of a cycle from a FI 30-s procedure is shown in Figure 1. The striped bar indicates the stimulus. The black triangle indicates food. The results from three FI procedures, 30, 60, and 120 s, are shown in Figure 2 (data from Guilhardi and Church, 2004). The top left panel shows response rate as a function of time averaged over five sessions (the second half of a total of 10 training sessions consisting of 60 cycles), for the three groups (four rats in a group). The slope of the response gradients decreased as a function of interval duration. Response rate was inversely related to interval duration. When the relative response rate was plotted as a function of relative time since stimulus onset, the three curves superpose. This superposition is shown in the bottom left panel of Figure 2.

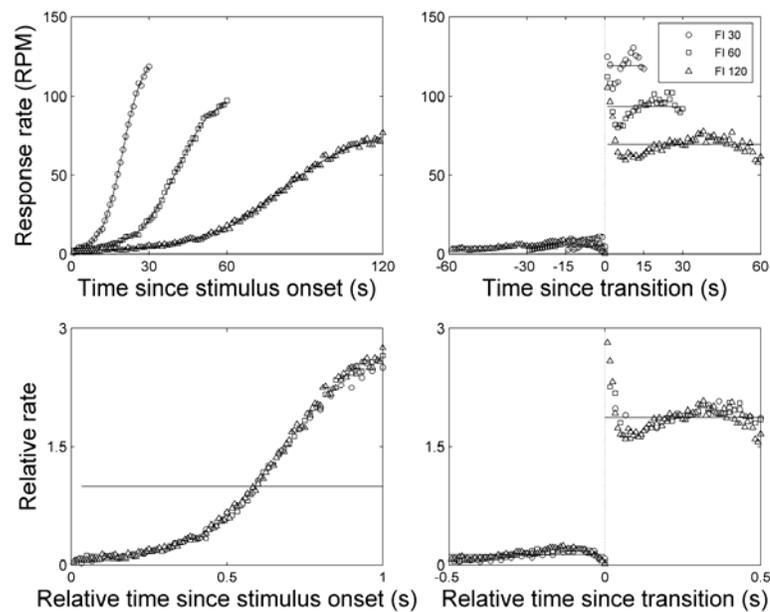


Figure 2. Results from the FI 30-, 60-, and 120-s procedure. (Adapted from Guilhardi and Church, 2004, by Mika L.M. MacInnis).

On a single cycle, responding can be described as a step function, with responses at a low rate in the beginning of the cycle, and at a fast relatively stable rate at the end of the cycle (Schneider, 1969). The ogival response rate gradients shown in the left panels of Figure 2 are the average of step functions with variability in the time of transition from a low to a high state of responding. The top right panel of Figure 2 shows response rate plotted as a function of the transition point (see methods for a description of the mathematical definition of the transition). The difference in response rate across the three fixed intervals is evident following the transition point. When the relative response rate is plotted as a function of relative time, the functions also superposed (bottom right panel of Figure 2).

The results from fixed-interval procedures provide evidence for the four principles of timing (Gibbon, 1977): timescale invariance (when plotted on a relative scale, estimations of different interval durations will superpose), proportionality (time is perceived proportionately rather than absolutely), the scalar property (variability in timing increases linearly with interval duration), and Weber's law in timing (a constant ratio of the standard deviation to the mean of the interval timed). These principles have also been shown in results from FI procedures designed to measure the dynamics of temporal discrimination (e.g. Guilhardi and Church, in press), to examine the ability to time multiple intervals simultaneously (Meck and Church, 1984; Church, Guilhardi, Keen, MacInnis, and Kirkpatrick, 2003; Guilhardi, Keen, MacInnis, and Church, 2005), to investigate the effects of neurological disorders such as Parkinson's disease on timing (e.g. Malapani, Rakitin, Levy, Meck, Dweer, Dubois, and Gibbon, 1998), as well as to compare time discrimination across species (e.g. Lejeune and Wearden, 1991).

### The Peak Procedure



Figure 3. Peak procedure.

The peak procedure is a timing procedure in which animals are exposed to two types of cycles. Standard cycles are identical to cycles in a fixed-interval procedure. On peak cycles the stimulus is turned on but responses do not produce food nor terminate the stimulus that remains on for several times the length of the target duration. Figure 3 shows the standard (top) and peak (bottom) cycles in the peak procedure.

The results from a peak procedure 120 s are shown in Figure 4 (data from Church, Meck, and Gibbon, 1994). The response rate gradients averaged across peak cycles increased until around the time of reinforcement and then decreased relatively symmetrically (top panel). On individual cycles (bottom panels), the pattern of responding was characterized by a low-high-low states of responding with two transition points,  $t_1$  (low-high, left panel) and  $t_2$  (high-low, right panel).

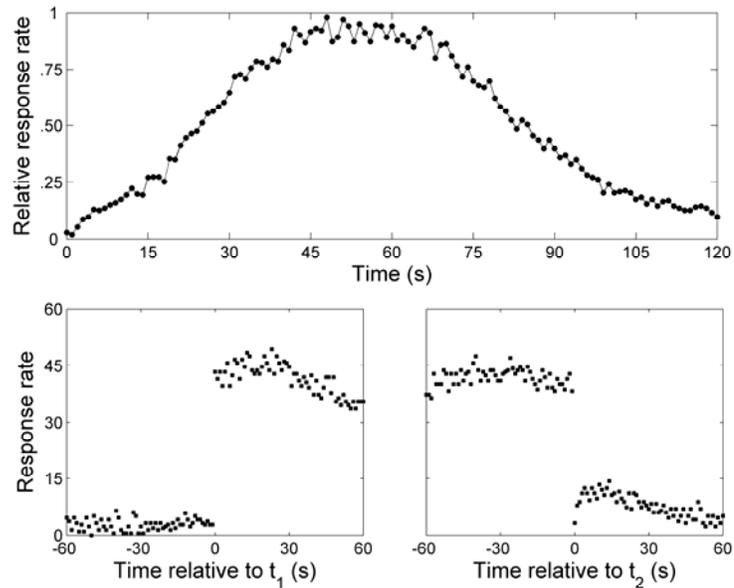


Figure 4. Results from the peak procedure. (Adapted from Church, Meck, and Gibbon, 1994, by Mika L.M. MacInnis).

The peak procedure has been used extensively to study the effects of drugs, as well as neurological disorders (e.g. Parkinson's disease) on time discrimination. The location and spread of the peak in the response gradient is used as a measure of timing accuracy and precision. A shift in the gradient to the left has been produced with the administration of dopamine agonists such as methamphetamine (e.g. Maricq, Roberts, and Church, 1981). A shift to the right has been produced with the administration of haloperidol and other dopamine antagonists (e.g. Drew, Fairhurst, Malapani, Horvitz, and Balsam, 2003). The shifts have been interpreted as an increase and decrease (respectively) in the speed of the internal clock.

### The Bisection Procedure

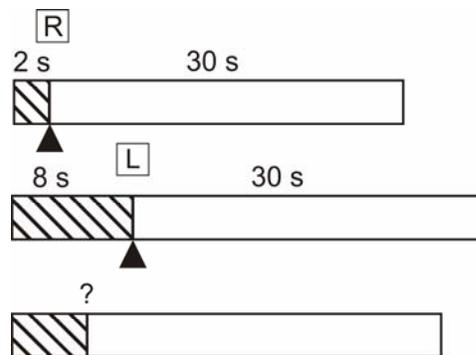


Figure 5. Bisection procedure.

On a bisection procedure, animals are trained to respond differentially (e.g. right and left lever presses) after the presentation of a short (e.g. 2 s) or a long (e.g. 8 s) stimulus durations. Once differential responding to the short and long intervals has been trained, the stimuli are presented for intermediate durations intermixed with the short and long durations. The animal's responses on the intermediate duration and some of the short and longer duration cycles are not reinforced. Church and Deluty (1977) trained eight rats on four pairs of intervals: 1-4 s, 2-8 s, 3-12 s, and 4-16 s (a different pair for each phase of the experiment, the order of which was counterbalanced between rats). After the stimulus was presented, two levers were inserted into the box, and the rat received food if it made a right response for the short interval and a left response for the long interval (counterbalanced across subjects). After a response had been made a 30-s intercycle interval began.

The results from the Church and Deluty (1997) bisection procedure are shown in Figure 6. The proportion of "long" responses increased as a function of the target interval. The slopes of the curves were inversely related to the target interval. The time at which the rat was equally likely to respond "long" as "short" is the point of subjective equality (the bisection point). This point was approximately the geometric mean of the long and short intervals. The proportion "long" functions superposed when plotted as a function of time relative to the geometric mean between the short and long durations (bottom panel). These and similar results (e.g. Meck, Church, and Gibbon, 1985) provided evidence for the principle of timescale invariance.

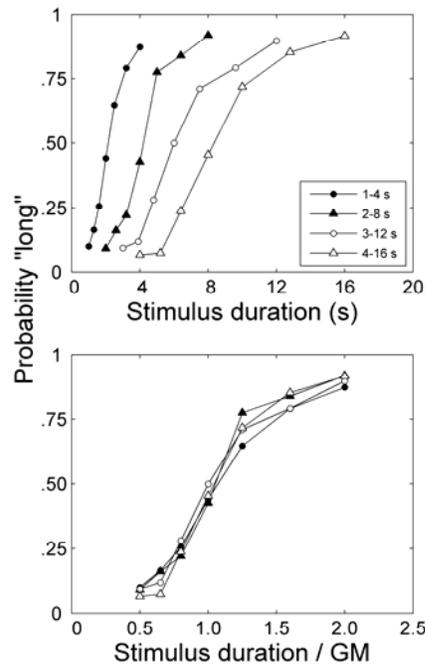


Figure 6. Results from the bisection procedure. (Adapted from Church and Deluty, 1977, by Mika L.M. MacInnis).

The bisection procedure has been used extensively in humans (Allan and Gibbon, 1991), rats (Crystal, Maxwell, and Hohmann, 2003), and pigeons (Machado and Keen, 1999), as well as to study drug effects in rats (Crystal et al., 2003) on temporal discrimination. A leftward shift in the bisection point has been interpreted as an increase, and a rightward shift as a decrease, in the speed of the internal clock. A change in the slope of the function is an indication in the variability of time perception.

## METHODS

This section of this chapter provides a general description of the equipment and basic protocol followed by the Timing Lab at Brown University when conducting temporal discrimination experiments with rats. Moreover, this section provides specific instructions for the use of the three procedures described above and a description of the methods of data analysis.

### Animals

Naive male Sprague Dawley rats (Taconic Laboratories, Germantown, NY) are used. Although female rats can be used, there is evidence that changes in hormones levels may affect timing performance (Ross and Santi, 2000). Traditionally, only male rats have been used to control for potential effects of the estrous cycle on timing behavior. The rats are 30 to 37 days old upon arrival and housed individually in a colony room on a 12:12 hour light/dark cycle (lights off at 9:30 am). Dim red light illuminates the colony room and the testing room during the lights off portion of the day. Because rats are nocturnal animals, this reverse light/dark cycle ensures that the animals are tested during the “waking” part of their day.

During the first week following arrival, the rats are maintained on *ad libitum* food (FormuLab 5008). After the first week, the rats are restricted to 15 grams of food daily. Testing begins following one week of food restriction. After testing begins, the daily ration of food is given in the home cage after the testing session. The rats receive 15 grams of food in addition to the food they receive during the testing sessions provided the total amount of daily food does not exceed 20 g. Weekly weights are taken to ensure a minimum of weight gain of approximately five grams per week. If the minimum weight gain is not reached, an additional three grams of food is given daily until minimum weight gain occurs. Water is available *ad libitum* in both the home cages and the experimental cages throughout the experiments.

The rats are handled daily from arrival to the onset of the experiment. Handling consists of the handler removing the rat from the home cage by lifting it under the forelegs with the ungloved hand, holding it in the gloved hand, and stroking it with the other (ungloved) hand for approximately two minutes. The glove used is a leather gardening glove that protects the handler against getting scratched by the rat’s nails. The more relaxed the handler is when handling, the rat, the calmer the rats, and the more regular the data are.

Testing begins approximately two weeks following arrival. After testing begins, daily handling ceases, although the animals are hand-carried to the experimental chambers in a

manner similar to handling, rat sitting in the gloved hand, and held loosely in place with the ungloved hand.

## Apparatus

Twelve extra tall modular operant test chambers (30.5 x 24.1 x 21.0 cm, Model ENV-007, Med Associates, St. Albans, VT) with stainless steel grid floors (Model ENV-005, Med Associates, St. Albans, VT), each located inside a ventilated, noise-attenuating box (66.0 cm x 55.9 cm x 35.6 cm, Model ENV-016M, Med Associates, St. Albans, VT), are used in testing. Each chamber is equipped with a food cup (5 x 5 x 2 cm, Model ENV-200R2M, Med Associates, St. Albans, VT) located in the middle of the right wall. Each head entry into the food cup is transduced by a LED-photocell (Model, ENV-254, Med Associates, St. Albans, VT). Both the times of head entry and withdrawal to the food cup are recorded. A magazine pellet dispenser (Model ENV-203M, Med Associates, St. Albans, VT) delivers 45-mg Dustless Precision Pellets (Bio-Serv, Rodent Grain-Base Formula, Frenchtown, NJ) into the food cup. To the left and right of the food cup are retractable levers (7 cm above the grid, 4.5 cm wide, extending 2 cm into the chamber and requiring approximately 18 g to operate, Model ENV-112, Med Associates, St. Albans, VT) that can be automatically inserted or removed from the chamber depending upon the procedure. A water bottle is mounted on the outside of the left wall of the experimental chamber. Water is available through a tube that passes through a hole in the middle of the wall (opposite the food cup). Licks on the water tube are recorded via a contact lickometer (Model ENV-250RM). A houselight with a diffuser (Model ENV-227M) is mounted above the water bottle. Two Gateway Pentium computers, running the Med-PC Medstate Notation Version 2.0 (Tatham and Zurn, 1989), control experimental events and record the time at which events occur with 2 ms resolution.

The overall number of responses emitted by each animal during each session is recorded in a notebook at the end of the session in order to have a prompt daily indication of their performance as well as identify unusual, but possible, equipment problems (e.g., light bulbs burned, feeder malfunction). Prior to each test session, a boxtest program is run to ensure that all stimulus generators and response recorders are functioning properly. If some component of the box is malfunctioning, it is either repaired or replaced before the start of the session. If a part malfunctions in the middle of a session, it is recorded in the notebook, with a detailed description of the problem and when it occurred (e.g. at cycle 15), and repaired once all other animals have finished the test session and the animals have been taken back to their home cages. Additionally, any changes in environment that could affect behavior (e.g. animal was dropped on the way to the experimental chamber; there was construction in the building causing excessive noise) are recorded.

## Procedures

### *Fixed-Interval*

A cycle of a FI procedure is shown in Figure 1. A stimulus (e.g. white noise, houselight, clicker) is turned on for the target duration (e.g. 30 s). The reinforcer is made available

(primed) at the target duration, but delivered only after the first response (e.g. head entry into food cup, lever press) following reinforcer prime. This response also terminates the stimulus and begins a 20-s interval with no stimulus. At the end of 20 s, the stimulus is turned on and the cycle is repeated. The cycle is repeated 60 times a session. Rats learn the target duration very quickly within the first five sessions, but performance will continue to change with training (e.g. Guilhardi and Church, 2004). In the timing laboratory at Brown University, rats are generally tested for at least 20 sessions on a fixed interval procedure, and the last 10 session used for steady state performance. In some cases, three intervals signaled by different stimuli are presented intermixed in a session for within subjects comparison (Guilhardi and Church, in press).

### **Peak Procedure**

The peak procedure consists of two types of cycles shown in Figure 3. The reinforced cycle is identical to a cycle from a FI procedure. The stimulus is turned on for a fixed duration (e.g. 30 s) and the first response following reinforcer prime terminates the stimulus and delivers the food. On the peak cycles the stimulus is turned on for an interval duration that is at least four times longer than the target duration (e.g. 120 s), responses have no effect, and no food is delivered. After stimulus termination, the stimulus stays off for 20 s for both cycle types. The cycles are presented randomly, with a determined probability (e.g. .5). At the beginning of each cycle, the computer, using a random number generator, determines what type of cycle is presented (e.g. if the number is under .5, a standard cycle is presented, if the number is above .5, a peak cycle is presented). Generally, 60 cycles are presented in a session.

### ***Bisection Procedure***

In a bisection procedure, there is a long and a short cycle type, shown in the top two panels of Figure 5. The durations for the long and short cycles are generally of a 1:4 ratio (e.g. 1 and 4 s, 2 and 8 s, 3 and 12 s, etc.). On each cycle, the stimulus comes on for its target duration and then terminates (independent of any response from the animal). At stimulus termination the levers are extended into the box. The first lever press response of the animal is recorded, the levers are retracted, and the intercycle interval (e.g. 30 s) begins. The animals are first trained to make the lever press response. The animals are then trained to respond differentially to the short and long stimulus (e.g. right lever press on short cycles, and left lever press on long cycles). Only correct responses are reinforced. During training, a correction procedure can be implemented, such that if the animal makes the incorrect response, that cycle type is repeated on the next cycle, until the correct response is made. In training, short and long cycles are presented with a probability of .5. Following training, cycles of intermediate duration are added to the procedure. There are usually approximately five logarithmically spaced intermediate intervals between the two extremes. The extreme cycles are presented with a probability of .25, and the cycles of intermediate duration are presented with equal probabilities of the other cycles.

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## Data Analysis

The data are recorded with 2-ms resolution. For each session, one file is saved for each animal. File names are recorded using the identification number of the animal. The file extension indicates session number (in three digit format, e.g. '001'). Each input event to the procedure (e.g. stimulus onset, food delivery) and each output event from the animal (e.g. head entry into the food cup, licks to the water tube) are recorded. The resulting data file consists of a single column of numbers, shown to three decimal places. The numbers before the decimal point indicate the time since session start in 2-ms units. The numbers following the decimal point indicate the event that took place at the recorded time. Detailed documentation of the numbers that correspond to the events is maintained to facilitate future analysis of the data. Additionally, this makes secondary data analysis possible, should other aspects of behavior that may have not been the focus of the study at the time it was first conducted come under investigation (e.g. drinking behavior on a fixed-interval schedule of reinforcement).

The data are analyzed in Matlab using existing Matlab functions, as well as specialized functions written to compute dependent measures of interest. All figures are created in Matlab. Inferential statistics are computed in SPSS.

The data are first translated from a single column of numbers into two columns of numbers: time since session start (the numbers before the decimal point) and event (the numbers after the decimal point). Each session's data are then parsed into cycles, and the relative time since cycle start for each event determined. Working with the data in this format makes determining multiple dependent measures straightforward. (For a review of the dependent measures that can be derived from data collected on fixed-interval procedures, see Guilhardi and Church, 2004.)

For any dependent measure, the value is computed for each cycle, of each session, and each subject independently. The calculated values can then be stored in a three dimensional table, with time or cycle as the first dimension, session as the second dimension, and animal as the third dimension. Storing the data in this way enables computation of statistics at several levels of complexity. It allows the experimenter not only to examine overall mean performance of the group, but also the mean performance of any individual either across sessions or for any specific session.

The dependent measures shown in this chapter include responses per minute, the time of transition from a low response state to a high response state (or vice versa), and proportion "long" responses. A computational definition of the dependent measure used in an experiment is a critical component of the description of the results.

### *Responses Per Minute*

Responses per minute in a cycle are computed by dividing the frequency of the response by the opportunity to make that response in each time bin of the cycle, and the units are changed from responses per bin length to responses per minute. The data shown in this paper are in shown in 1-s bins.

### ***Time of Transition***

The times of transition from a low response rate state to a high response rate state ( $t_1$ ) and from a high response rate state to a low response rate state ( $t_2$ ) are defined on individual peak cycles based on the maximization of the index  $A$  (Church, Meck, and Gibbon, 1994):  $A = t_1(r - r_1) + (t_2 - t_1)(r_2 - r) + (t_3 - t_2)(r - r_3)$ , where  $t_3$  is the time of the end of the peak cycle,  $r$  is the overall mean response rate, and  $r_1$ ,  $r_2$ , and  $r_3$  are the rates from cycle start to  $t_1$ , from  $t_1$  to  $t_2$ , and from  $t_2$  to  $t_3$ , respectively. All possible values of  $A$  are calculated with  $t_1$  set as the time of each response in a cycle until the second to the last response, and  $t_2$  set as the time of each response in a cycle from the second response until the last response, with the restriction that  $t_2$  is always greater than  $t_1$ . In fixed-interval procedures, only  $t_1$  is determined with  $A = t_1(r - r_1) + (t_4 - t_1)(r_4 - r)$ , where  $t_4$  is the time of the end of the cycle, and  $r_4$  is the rate from  $t_1$  to  $t_4$  (Guilhardi and Church, 2004).

### ***Proportion “Long” Responses***

The proportion of “long” responses is perhaps the simplest dependent measure shown here. It is used in procedures where the subject makes differential responses depending on what stimulus is presented. It is calculated by dividing the number of “long” responses for a certain cycle type (e.g. 2 s), by the total number of cycles of that type that were presented. In a bisection procedure, this is done for each of the intervals that are presented.

## **Secondary Data Analysis**

Secondary data analysis is becoming more feasible with the development of the world wide web. Data can be uploaded to the web after an experiment is conducted, and made available to other researchers who may have research questions of their own that could be answered by analyzing data that has already been collected, rather than taking the time and the resources to set up a similar, if not identical, study. The Timing Lab at Brown University archives its data, within the lab and online, to facilitate secondary data analysis both by lab members as well as anyone else who might have interest in the data. After testing is complete the data are archived within the lab on CDs. The CDs include a description of the experiment, the data collected, the programs written to run the experiment, and the Matlab programs written for data analysis. When a paper is in press, the data for that paper are uploaded to the Timing Lab archive, with the abstract, as well as any documentation that is needed to analyze the data (e.g. the event codes specific to that experiment). The archive can be accessed at the following address: <http://www.brown.edu/Research/Timelab>.

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