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Assessing delay discounting in mice

Suzanne H. Mitchell*

Abstract

Delay discounting (also intertemporal choice or impulsive choice) is the process by which delayed outcomes, such as delayed food delivery, are valued less than the same outcomes delivered immediately or with a shorter delay. This process is of interest because many psychopathologies, including substance dependence, pathological gambling, attention deficit hyperactivity disorder and conduct disorder, are characterized by heightened levels of delay discounting. Some of these disorders are heritable, and data indicate that delay discounting also has a genetic component. To identify the genes underlying the delay discounting decision-making process and genetic correlates of heightened discounting, researchers have used mouse models. This unit describes a protocol for generating delay discounting behavior in mice and discusses analysis techniques for such behavior.

Keywords

intertemporal choice; impulsive choice; decision-making; subjective value; indifferent point; hyperbolic; mouse behavior

INTRODUCTION

Delay discounting is a process in which individuals derive the subjective value of a commodity that is only available following a delay by amalgamating the commodity's magnitude and the delay to its receipt (Madden & Johnson, 2010). Researchers are interested in this process for two main reasons. First, calculations of this type are common in everyday life and subjective value calculations are critical drivers of decision-making. Second, the value of delayed commodities is lower in individuals exhibiting specific psychopathologies, including substance dependence, pathological gambling, attention deficit hyperactivity disorder and conduct disorder (Bickel et al., 2012; Perry & Carroll, 2008; Robbins et al., 2012); implying that the discounting process is quantitatively, or possibly qualitatively, different in individuals with diagnosed with these psychopathologies. Many of these psychopathologies have a genetic component, such as alcohol use disorder; making research using mouse models of delay discounting potentially valuable because of the plethora of genetic models and the ease by which researchers can create new, hypothesis-driven models.

Researchers originally created procedures to assess delay discounting in rats or pigeons (Evenden & Ryan, 1996; Mazur, 1988; Rachlin & Green 1972). However, more recently versions of these procedures have been developed for mice to take advantage of the genetic information such models can provide. This article describes the most commonly used procedure to assess delay discounting in mice, which is based on the adjusting amount procedure described by Richards et al. (1997).

*Contact information: Dept of Behavioral Neuroscience, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland OR 97239, Tel (503) 494-1650, Fax (503) 494-6877, mitchesu@ohsu.edu.

BASIC PROTOCOL 1: Adjusting amount procedure

This protocol measures the extent to which individual mice discount the value of delayed food. The description includes training steps, which may require an average of 12-29 sessions depending on the mouse strain, and an experimental phase in which delay discounting is assessed over 5-10 weeks. For consistent results, same time-of-day testing is recommended. Animals may be tested 5 days/week or daily. This protocol also includes information about data processing and analysis.

Materials

Mice, e.g., C57BL/6J from Jackson Laboratories or any other mouse of interest, aged approximately 6 weeks on arrival.

Scale for weighing mice and post-session, supplemental food.

Sucrose solution, e.g., 10% w/v made using store-bought granulated sugar and tap water.

Operant test chambers (commercially available, e.g., Med Associates, St Albans VT): $15.9 \times 14.0 \times 12.7$ cm. Each chamber contains three recesses with head entry detectors and liquid food cups, 3 yellow LED stimulus lights, clicker module, house light. Each chamber should be housed in a ventilated, light- and sound-attenuating cabinet.

Med Associates syringe pumps (motor speed = 3.33 rpm), each containing a syringe (e.g., 10 ml) connected by polyethylene tubes to the liquid food cups of each operant test chamber.

Med Associates behavioral software (Trans-IV and Med-PC version IV) to control the operant test chambers.

Desktop computer with MS Windows XP.

Microsoft Excel with Solver Add-in for data analysis.

Note: Programs for running the operant procedures using Med Associates equipment and conducting curve fitting procedures using MS Excel are available from the author. Alternatively, researchers can easily translate the procedures and analyses protocols to other commercially available systems, if desired.

Note: Please refer to Support Protocols 1 and 2 for detailed instructions on setting up the operant test chamber and configuring behavioral control software, respectively.

Preparing the mice and food restriction—

1. On arrival, mice are housed 2-5/cage and permitted approximately 5 days to acclimate.
2. Beginning on the first Monday after acclimation, mice are marked for identification, then weighed daily for the following 5 days (or 7 days for daily protocols). These free-feeding weights are recorded.

Identification can be accomplished by marking the tail, ear tagging or ear punching.

Weights can be recorded by hand in a logbook, directly into an MS Excel spreadsheet or, if a computer-connected weighing scale is used, directly into a database.

3. Food is removed on Friday, and the food restriction protocol is initiated, which requires each cage to receive 3 g mouse chow/animal/day. Food restriction continues until the end of the study.

The 3 g restriction amount is fixed during the initial restriction phase regardless of strain used, but following that phase, adjustments may be made. Animal weights should be monitored daily and maintained at approximately 85-90% of the free-feeding weights assessed in step 2. Food competition by animals within a box might require that some animals are provided with supplemental food separate from cagemates. Also, if animals are not completing all trials within a session, but are completing elements of the procedure correctly, food restriction may need to be increased, up to 80% free-feeding body weight.

Behavioral training beginning 72 h after food restriction—*Criterion for successful completion of each training step is 1 session on which 60 reinforcers (“free-choice” reinforcers, beginning step 8) are earned by nose poking within 60 minutes. Number of sessions required is estimated for C57BL/6J mice (Helms et al., 2006; Mitchell et al., in prep). Animals that do not successfully complete a training step within 15 sessions are removed from the study.*

4. *Introduction to the center nose poke response contingency (1-3 sessions):* A 20- μ l bolus of sucrose solution is delivered to the center recess according to a variable time (VT) 60-s schedule. In addition, all nose pokes in the center recess should be concurrently reinforced with the same amount of sucrose solution (fixed ratio [FR] 1 schedule).
5. *Initiating nose poking in all recesses (1-3 sessions):* 20- μ l sucrose solution rewards are delivered randomly from the three recesses (VT 60-s schedule, with random assignment to a delivery location). All center nose pokes continue to be reinforced (FR 1).
6. *Chaining center poke and left/right poke (2-10 sessions):* VT-scheduled rewards discontinued. Left or right nose pokes reinforced (FR 1) by delivery of 20 μ l sucrose solution into the poked recess only after a reinforced center poke has occurred.
7. *Reinforcement for center nose poke discontinued (2-3 sessions):* Center pokes are no longer reinforced, but are required immediately prior to a left or right nose poke for that left or right nose poke to be reinforced.
8. *Introduction of forced choice trials, assignment of adjusting contingencies to left/right recess (2-4 sessions):* Adjusting contingencies are assigned to the left or right recess and initiated: amount of sucrose solution delivered for nose-poking in the adjusting recess on first trial of each session is 10 μ l, with changes on successive trials according to the rules described in the Software Program section. Forced-choice of the non-chosen recess following 2 successive choices of the same alternative is initiated.
9. *Introduction of the timeout (6-10 sessions):* After reinforcers are delivered, a variable length timeout is added so that trials begin at 20 s intervals.

Using longer timeouts are associated increasing number of sessions to meet the 60-trials within 60-minute criterion and begin the experimental phase. Shorter timeouts increase the likelihood that no timeout will be experienced for delayed alternative trials once the experimental phase begins because of

slow reaction times to initiate trials by making a center recess nose poke; introducing a lack of symmetry in the structures of trials on which the adjusting or delayed alternatives are chosen.

10. **Experimental phase.** The delays between nose poke and sucrose solution delivery are introduced according to the pseudo-Latin square (Table 1).

The number of occasions that each delay should be experienced is determined by the experimenter but several considerations should influence this decision. First, the initial occasion that each delay is applied often is associated with atypical choice patterns during the session, making removal of these 5 sessions from subsequent data analyses potentially reasonable following data inspection. One possibility to control for these atypical patterns is to program the delays in increasing size across initial sessions, but our data do not indicate that this improved trial completion rates in the long term. Second, there is no data indicating that the delay on the prior session has an impact on behavior during the current session based on data from rats (Richards et al., 1997), but to counterbalance delay orders, it is recommended that the pseudo-Latin square should be completed in its entirety, making the number of sessions in the experimental phase a multiple of 25 (5 delays x 5 repetitions).

Data collection—*The main dependent variable is the size of adjusting reinforce on each free-choice trial, even if the delayed alternative was selected.*

11. During experimental sessions, the following events should be recorded for subsequent analysis, with codes to indicate whether the trial is a free- or forced-choice trial, and whether the adjusting or delayed alternative was ultimately selected:

Time at which the trial was initiated, i.e., when center light turned on

Time at which there was a response in the center recess

Time at which there was a response in the adjusting or delayed recess following a center recess response

12. These events can be used to derive for each trial within a session:

Reaction time from the beginning of the trial (center poke response time minus trial initiation time) – an index of attention

Choice reaction time (left/right nose poke response minus center poke response time) – an index of motivation

Data processing and analysis—*Only data from free-choice trials are included in the analysis. Steps are described for processing data for each individual subject.*

13. **Calculation of “indifference points” for an individual session.** The median size of the adjusting reinforcer for trials following the 30th trial (trials 31-60) should be calculated for each session at each delay. Only sessions where there are at least 45 trials should be included to ensure sufficient trials to calculate a representative median.

A median is calculated, rather than a mean, because the adjustments in amount are proportional to previous values, creating a skewed distribution of values.

Values from the first 30 trials of the session are discarded to permit mice to make the necessary choices to adjust the amount to a point of subjective equality with the delayed alternative reinforcer.

14. Means of the indifference points derived in step 11 should be calculated for each delay.
15. The slope of the discounting curve (k) should be calculated for each subject using the hyperbolic discounting equation (Mazur, 1987):

$$V = \frac{bA}{1+kD} \quad \text{Equation 1}$$

[*Equation 1]

Where V represents the subjective value of the delayed reinforcer (mean indifference point from step 12), b represents response bias (Wilhelm & Mitchell, 2010) which can be calculated by dividing the 0 s-delay indifference point by the size of delayed reinforcer (20 μ l) and provides an index of aversion/preference for the side associated with delayed reinforcers, A represents the amount of sucrose solution available following the delay (20 μ l), k is a fitted parameter that measures steepness of the discounting curve with larger values indicating greater discounting, and D represents the delay to the delivery of the delayed reinforcer (e.g., 0, 2, 4, 8 and 12 s).

Inclusion of a 0-s delay is required to calculate response bias. Response bias can be derived if a 0-s delay is not assessed but this is not recommended because the equation will then include 2 free parameters, possibly resulting in overdetermination of the discounting function.

16. Alternatively to step 15, an area under the delay curve can be calculated by summing that areas of the trapezoids for consecutive delay value, i.e., 0 to 1 s, 1 to 2 s, etc. (also see Myerson et al., 2001).

Support Protocol 1: Operant test chamber configuration

This protocol details operant test chamber configuration. While the instructions follow a stepwise format for clarity, the order of operations is not critical. Importantly, note that the houselight is mounted outside the chamber, rather than the usual in-chamber placement, to prevent mice climbing on it. Reducing the availability of activities that can interfere with the experience of waiting for sucrose solution delivery appears to be critical to subjects exhibiting systematic effects of delay duration.

Materials

Operant test chambers (commercially available, e.g., Med Associates, St Albans VT): 15.9 × 14.0 × 12.7 cm.

Each chamber contains three recesses with head entry detectors and liquid food cups, 3 yellow LED stimulus lights, clicker module, house light.

Each chamber should be housed in a ventilated, light- and sound-attenuating cabinet.

Test chamber set-up—

1. Ensure that operant test chambers are housed in a light- and sound-attenuating, ventilated box (e.g., Med Associates, St. Albans, VT).

2. Mount a clicker to signal sucrose solution delivery on the outside of the left-hand panel, relative to the chamber door.
3. Mount a 100 mA houselight between the top of the left-hand panel and the ceiling panel such that it projects above the test chamber without being accessible to animals from inside the chamber.
4. Make sure that the right-hand panel contains three identical recesses with head entry/nose-poke detectors, and three yellow LED cue lights located above each recess (Figure 1).
5. Also make sure that recesses contain a liquid food cup (feeder cup), connected to a Med-Associates pump (3.33 rpm) housing a 10 ml syringe, via an 18 gauge stainless steel pipe attached to polyethylene tubing.

Software records event times such as lights being turned to signal different trial periods and nose-pokes into the recesses, as well as amounts of sucrose solution delivered. See Support Protocol 2 for software configuration.

Support Protocol 2: Software program configuration

Programs for the adjusting amount procedure can be created using the Trans-IV and Med-PC IV software. Program scripts incorporate the sequences below.

Materials

Trans-IV (Program); Med Associates

Med-PC IV (Program); Med Associates

Desktop computer with MS Windows XP.

1. At the beginning of a trial, the light above the center recess is lit until the subject pokes its nose into that recess.
2. Immediately, on free-choice trials, lights over the left and right recesses are lit until a nose poke occurs in one or the other recess. For each subject, one recess is designated the “delayed” alternative recess and the other is the “adjusting” alternative recess.
3. Following a nose poke into the delayed recess, the light above the adjusting recess is extinguished. After a specific delay (e.g., 0, 2, 4, 8 or 12 s), 20 μ l sucrose solution is delivered and the light above that recess is extinguished. Delay length is constant within a session but varies between sessions using to pseudo-Latin square design (Table 1).

A true Latin square design requires an even number of delay values, and including 6 delays would lengthen the duration of the study with little additional information being added about the subject's degree of discounting.

4. Following a nose poke into the adjusting recess, a specific amount of sucrose solution is delivered immediately and lights above both recesses are extinguished.
5. The size of the immediate, adjusting reinforcer is 10 μ l on the first trial of the session but altered on subsequent trials according to the following rules: (1) if the mouse chooses the adjusting alternative, the size of that reinforcer decreases by 10% for the next trial; (2) if the mouse chooses the delayed alternative, the size of the reinforcer from the adjusting alternative increases by 10% for the next trial.

Limits are set so that the size of the adjusting reinforcer cannot be less than 0.2 μ l (approximately 10 ms of syringe pump activation, the minimal permissible based on MedPC programming parameters) or larger than twice the size of the delayed reward. A proportional decrease or increase is used, rather than an absolute amount, because the psychophysical ability of mice and other organisms to discriminate stimulus differences is relative to stimuli sizes (Weber's fraction: Stevens 1957).

6. After sucrose solution delivery from either alternative, the houselight is turned off, signaling a timeout. The duration of the timeout varies such that each trial is separated by 20 seconds.

Forced-choice trials occur after two consecutive choices of the same alternative. On forced-choice trials only nose pokes into the non-chosen recess are reinforced. The adjusting alternative reinforcer size is not altered following a forced-choice trial.

7. Sessions end after 60 free-choice trials or 60 min, whichever occurs first.

The recess (left or right) to which the delayed and adjusting alternatives are assigned is counterbalanced between subjects but fixed throughout the experiment for each subject. The location can be switched between sessions for individual subjects but training requires significantly more sessions and additional sessions are to accommodate behavioral variability once delays are introduced. Ability to examine the effects of repeatedly pairing the delayed reinforce with a specific chamber location is also compromised if location is varied.

COMMENTARY

Background Information

This article describes the most commonly used procedure to assess delay discounting in mice, which is based on the adjusting amount procedure developed by Richards et al. (1997). Like all procedures to assess delay discounting, the adjusting amount procedure is a psychophysical estimation technique using cross-modality matching (Stevens, 1957). Thus, on each trial, the subject indicates a preference for one of two alternatives. One alternative is the “standard” alternative, which is the alternative of interest; for example, in a delay discounting protocol described earlier, it would be the delayed alternative: 20 μ l sucrose delivered after, say, 4 s. The other is the “adjusting” alternative, which changes according to the individual's expressed preference; for example, the immediate alternative: 10 μ l delivered at once. If the subject prefers the standard alternative, the adjusting alternative is altered to be more attractive. In the adjusting amount procedure, this alteration involves increasing the amount of sucrose provided. If the subject prefers the adjusting alternative, the adjusting alternative is altered to be less attractive. In the adjusting amount procedure, this involves decreasing the amount of sucrose provided. After a series of trials, the subject becomes indifferent between the standard alternative and the amount of sucrose is offered on the adjusting alternative. The indifference point (point of subjective equality) is an index of the subjective value of the standard alternative. By examining a set of standard alternatives that differ only in the delay to the delivery of the reward, a delay discounting function can be created. This function describes how changes in delay affect the subjective value of the standard reward, that is, the extent to which an individual discounts the objective value of the reinforcer alternative as a function of delay.

Various other techniques are available for use with mice than that described earlier. Grahame and colleagues (e.g., Oberlin & Grahame, 2009; O'Tousa et al., 2013) use a

procedure derived from the adjusting amount procedure described here, but, to reduce the duration of the study, fewer delays are assessed. The smaller number of delays facilitates the speed to data collection but also reduces the precision by which discount curves can be assessed, which may be an important consideration depending on the objectives of the study. The 0-s delay is one of the delays omitted preventing calculation of response bias and preventing determinations of whether alterations in the subjective value of delayed alternatives are due to alterations an individual's in sensitivity to delay or whether sensitivity to differences in the size of the alternatives are affected (Locey & Dallery, 2011).

An adjusting delay procedure has been described by Adriani & Laviola (2003), in which the sizes of the immediate and delayed alternatives are constant (e.g., 20 versus 100 μ l respectively) but the delay length to the larger alternative is adjusted according to animals' choices. A choice of the smaller alternative shortens the delay to the large alternative while a choice of the larger alternative increases its delay. A similar procedure has been used for rats (e.g., Perry et al., 2005) and pigeons (e.g., Mazur, 2000), but its utility to address some study objectives may be limited by the inability to easily create a discount curve from data collected in this procedure. An additional limitation is that questions about the sensitivity of the procedure, which have not been fully resolved in studies using rats (Cardinal et al., 2002), have not yet been addressed in studies using mice.

A final type of procedure is the within-session procedure (Evenden & Ryan, 1996; Isles et al., 2003, 2004), in which indifference points are not calculated; rather the main independent variable is the number of occasions on which the larger, delayed alternative is chosen over a smaller but immediate alternative within a block of trials. Length of delay increases systematically across trial blocks, permitting delay discounting to be estimated via shifts in preference indicated by changes in the frequency of delayed alternative choices across blocks. A variant of this procedure is widely used with rats but has not been adopted for use with mice. Reasons for this late-adoption are not clear at the present time.

Critical Parameters

Reward sizes: procedures using mice usually use liquid food rewards (e.g., sucrose solution, milk), which makes it easier to use reinforcers have large differences in size. Differences in size (large reinforcer alternative at least twice as large as the small reinforcer but preferably 3 or 4 times larger) appear to facilitate training and discrimination between the two alternatives at the 0-s delay.

Delays selected: delay values should include 0-s and range up to 12 s. Selection of delays may be influenced by the type of operant response required. A nose poke to a recess where food will be delivered makes it possible to use short delays in the order of 1-s because these are discriminable from a 0-s delay due to the absence of travel time to collect the reinforcer. A lever press or using a central food delivery location may cause differences between some delays to be less discriminable because of travel time. Long delays (12 s and longer) increase the likelihood that mice will engage in other activities during the delay, potentially making interpretation of lower indifference points problematic.

Troubleshooting

Not all trials completed in a session:

Most likely due to a lack of motivation for the sucrose solution.

Possible solutions:

Check weight of subject and if reasonable, food restriction should be increased temporarily and subsequent behavior monitored.

The apparatus (liquid food cups, lines connecting the syringe to food cup, syringe) may be contaminated by mold and the sucrose solution has become less palatable. A complete, thorough, weekly cleaning of the apparatus and replacing syringes and lines at the end of each study should reduce the risk of this problem.

Strain-associated factors may play a role in absolute amounts of sucrose solution consumed during a session prior to satiation or loss of motivation. Assessing consumption by subjects over a time period similar to the duration of a study session should also be included in studies comparing different strains or lines. This would permit researchers to identify differences critical for data interpretation or differences that indicate the amounts suggested for use in this protocol here should be adjusted.

The time taken to initiate a trial by making a center nose poke response should also be examined as a possible reason that not all trials are completed. It is possible that visual deficits, known to develop in some strains of mice, might prevent mice from discriminating the start of the trial. This would be indicated by highly variable times across all trials, as opposed to a motivational explanation which should be characterized by lengthening times across the session. Animals with sensory deficits should be excluded.

No systematic effect of delay on choice between alternatives for an individual subject.

Possible solutions:

Observe the mouse to ensure that bedding in operant chamber is not accessible or other items that may act as distractors and prevent “waiting”/experiencing the delay.

A click is used to signal that sucrose solution availability. It is possible that hearing deficits, known to develop in some strains of mice, might prevent consumption of the sucrose as soon as the delay has elapsed, thereby causing mice to not experience the different lengths of delay to sucrose delivery. Examining head entry times relative the end of the delay will provide information about whether sucrose is consumed promptly after delivery. While low motivation for the sucrose should be ruled out at this point using techniques mentioned previously, animals with potential sensory deficits should be excluded.

If adjusting amounts are relatively large across all delays, indicating a preference for the delayed alternative, increase delay length to determine if the lack of effect is due to low sensitivity to delay. If this proves to be correct, it may still be possible to examine a within subject manipulations by altering the series of delays examined.

The opposite strategy, shortening the delays examined, should be engaged if adjusting amounts are relatively low, indicating extreme aversion to the delayed alternative.

Highly variable preferences at one or more delays between sessions.

Most likely due to inadequate control by the contingencies.

Possible solutions:

If highly variable preferences are seen at the 0-s delay as well as other delays, the mouse may be insensitive to the 10% alteration in adjusting amount and this percentage should be increased to 20%.

If the highly variable preferences are only seen at one specific delay, these data may indicate a lack of discrimination and that delay should be dropped from the analysis or replaced during the experimental phase.

If all delays are affected, the animal should be removed from the study.

Anticipated Results

For each subject on each trial, the size of the reinforcer from the adjusting alternative (adjusting amount) is recorded, even when the delayed alternative is selected, because this provides an index of preference. Increases in the adjusting amount indicate choice of the delayed alternative while decreases indicate choice of the adjusting alternative. Examining adjusting amounts during the sessions at a particular delay will reveal a substantial amount of variability within sessions and between sessions. Figure 2 shows the adjusting amounts on a series of sessions at either the 0-s delay (top panel) or 8-s delay (bottom panel) for a single male C57BL/6J, as well as the average adjusting amount over trials for that subject (#302; Mitchell et al, in prep). Variability between 0-s delay sessions is usually larger than variability between sessions with a particular delay, floor effects might contribute to this effect. Usually no systematic alterations in choice patterns between sessions with a particular delay can be identified.

Once indifference points for each session are calculated based on median adjusting amounts over trials 31–60 (step 11), these are averaged to create a composite value for each delay (step 12). Figure 3 depicts these means (top panel: thick line; bottom panel: open circles). Equation 1 can be fitted to the data using a nonlinear fit to generate a hyperbolic discounting curve and k -values for that individual mouse (step 13; Figure 3 bottom panel: dashed line). The k -value provides a summary statistics describing the extent to which the mouse discounts the objective value of the reinforcer as a function of its delay, and provides an index of the activity of the discounting decision-making process.

Time Considerations

It requires approximately 62–79 sessions to generate data that can be examined with confidence. It is important, however, for data quality to be monitored throughout. Some proportion of mice will not respond sufficiently often during a session to complete 45 free-choice trials. This proportion appears to vary by strain but an estimated 10–15% of animals will fall into this category. In addition, some mice will not progress through the training phases in a timely manner. Steps 7 and 9 are especially problematic and it is recommended that mice not completing the phase within 15 sessions be removed from the study.

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Figure 1. Front panel of the operant test chamber showing the nose poke recesses containing the liquid food cups (feeder cups) and placement of stimulus lights.

Figure 2.

The size of reinforcer from the adjusting amount alternative (adjusting amount) for each session when the delay to the larger reinforcer was 0-s (top panel) or 8-s (bottom panel) for a single representative subject (#302; Mitchell et al., in prep). The average for a particular delay, calculated from sessions on which the mouse completed more than 45 trials is shown as the thicker line in each panel for reference. Horizontal dashed lines in each panel represent the size of the large delayed reinforcer (20 μ l). Vertical dashed lines indicate the 45-trial cutoff point that was used to determine whether data from a session should be included. If data were included, the median adjusting amount was calculated over trials 31-60 (step 11).

Figure 3.

Top panel. The median adjusting amount values for each delay on each repetition within the pseudo-Latin square are plotted (thin lines) along with the mean of these values for each delay (thick line) for a single representative subject (#302; Mitchell et al., in prep). Bottom panel. Means for each delay from the top panel are reproduced as open circles. The dashed line is the hyperbolic function derived from fitting Equation 1 to these data. Parameters from the fit are also shown: the response bias parameter, b (calculated), the gradient of the discount function, k (fitted) and the measure of fit R^2 , adjusted for nonlinearity.

Table 1

Pseudo-Latin square for the delay values used.

Repetition	Delay values for each session (in seconds)				
1	8	0	4	2	12
2	2	4	12	0	8
3	0	12	8	4	2
4	4	8	2	12	0
5	12	2	0	8	4