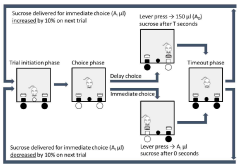
May 07, 2025

Delay Discounting Measured Using an Adjusting Amount Procedure



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Montana Kay Lara[1], Suzanne H. Mitchell[2]

[1]University of California San Diego; [2]Oregon Health & Science University

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Suzanne H. Mitchell
Oregon Health & Science University

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We use this protocol and it's working

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Abstract

Delay discounting is the process by which outcomes (rewards or punishment) are devalued as a function of the delay to their occurrence. The adjusting amount procedure enables researchers to measure how much an individual discounts the value of a reward as a function of delay. Higher levels of delay discounting have been associated with psychopathology, including substance use disorder, and mental illness.

Rats (or mice, if materials are adjusted according to organism size) choose between a small, immediate food reward and a larger reward delivered following a delay. Choices of the immediate reward cause its size to decrease on the next trial. Choices of the delayed reward cause the size of the immediate reward to increase on the next trial. After numerous trials, individuals reach an immediate reward amount at which they are indifferent between the small, immediate and larger, delayed alternatives. This amount at indifference indexes the subjective value of the larger, delayed reward. When the delay to the reward is short, the indifference amount is larger than when the delay is longer.

This protocol is described and used in this paper: https://doi.org/10.1111/gbb.12909
Other related resources can be found here: https://github.com/Palmer-Lab-UCSD/HSrat_delaydiscounting

Attachments

programs.zip
114KB

Guidelines

Vivarium:

- Lights on from 6:00 to 18:00 hours.
- Temperature is maintained at about 70°F.
- Humidity is maintained at about 20%.

Rats:

- Subjects are male and female rats at least 60 days of age, housed in same-sex pairs.
- Rats are transported to the laboratory for behavioral testing on 5-7 days/week in squads that remain in the laboratory for approximately 2 hours/squad.
- Rats are water restricted while in the laboratory but have ad libitum access to water in the vivarium.
- Rats are food restricted prior to the beginning of training.

Acclimating:

- Rats are housed in the vivarium for at least 7 days to acclimate.
- Beginning on the first Monday after acclimation, rats were weighed daily for the following 5 days. These free-feeding weights are recorded directly into an MS Excel spreadsheet through a computer-connected weighing scale.
- Food is removed on Friday, and food restriction is initiated, which requires each cage to receive ~ 10-15 g chow/rat/day.
- Food restriction continues until the end of the study.

Weight:

- Rats are maintained at between 85-95% ad libitum weights.
- Weights are monitored by weighing rats within 60 minutes of experimental sessions.
- Rats receive supplemental feeding immediately after the session (~ 15 g males, ~10 g females).
- Food competition by rats within a box may require that some rats are provided with supplemental food separate from cage mates.
- If rats 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.

- **Testing:**
  Testing occurs between 9:00 and 17:00.

Materials

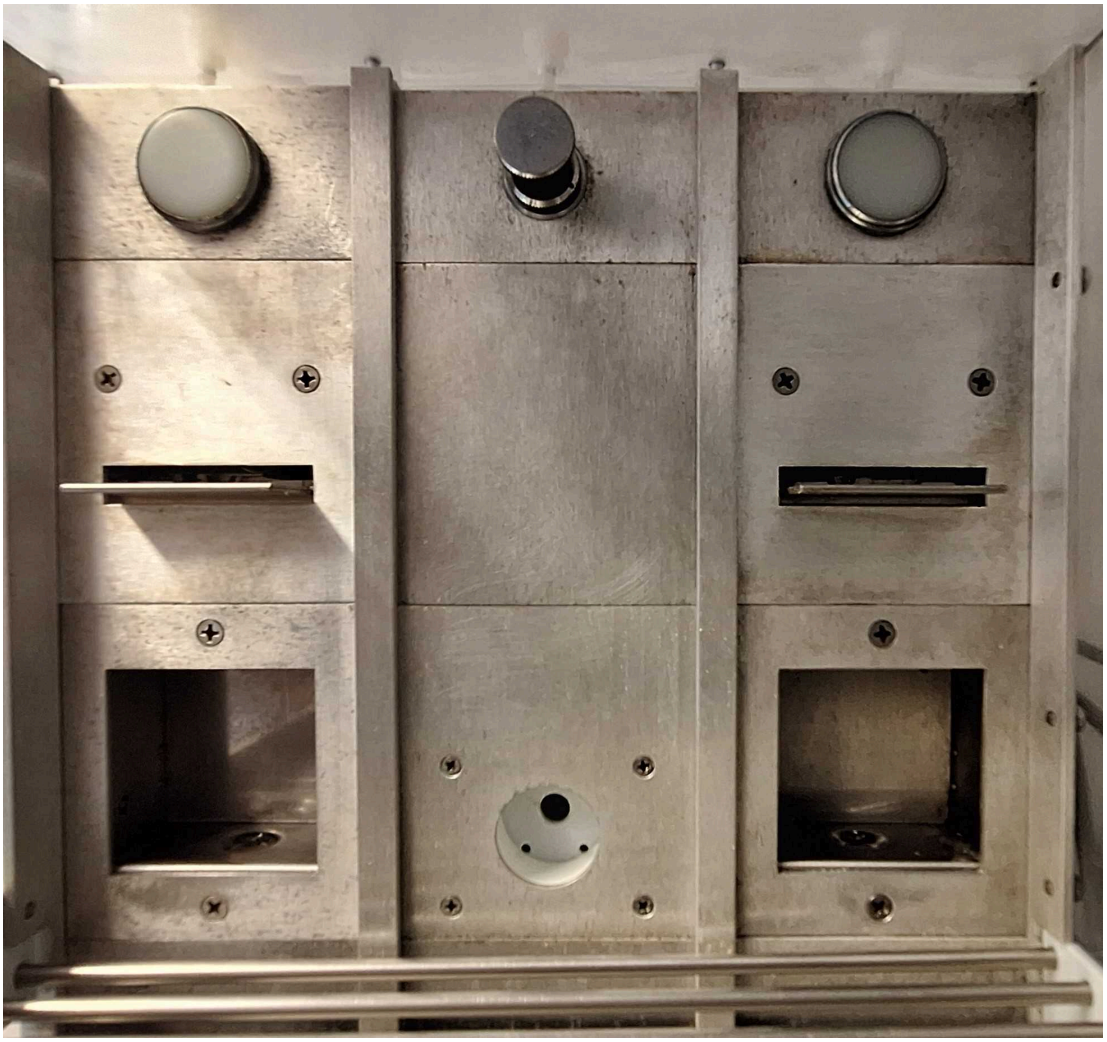
- Scale for weighing rats before experimental sessions (Ohaus Scout II scale, Cole-Palmer, Vernon Hills, IL USA).
- Supplemental food provided after experimental sessions (PicoLab® Laboratory Rodent Diet 5L0D pellets (Rat Specialties, Hubbard OR, USA).
- Sucrose solution, e.g., 10% w/v made using store-bought granulated sugar and deionized water.
- Modular rat test chambers (ENV-008/ENV-088-VP from Med Associates, St Albans VT), each housed within a sound attenuating cubicle (ENV-022MD).

Chambers:

- Chambers have polycarbonate ceiling and door, with front and back 3-module stainless-steel side panels, and a grid floor.
- Below the grid floor is a removable litter pan filled with approximately ¼″ BioFresh™ Performance Bedding (BioFresh, Patterson, NY, USA).
- The back side panel contains a clicker (ENV-135M), and above it, a speaker-tone generator combination (ENV-224AM and ENV-223) in the center module of the panel (**Picture 1**).

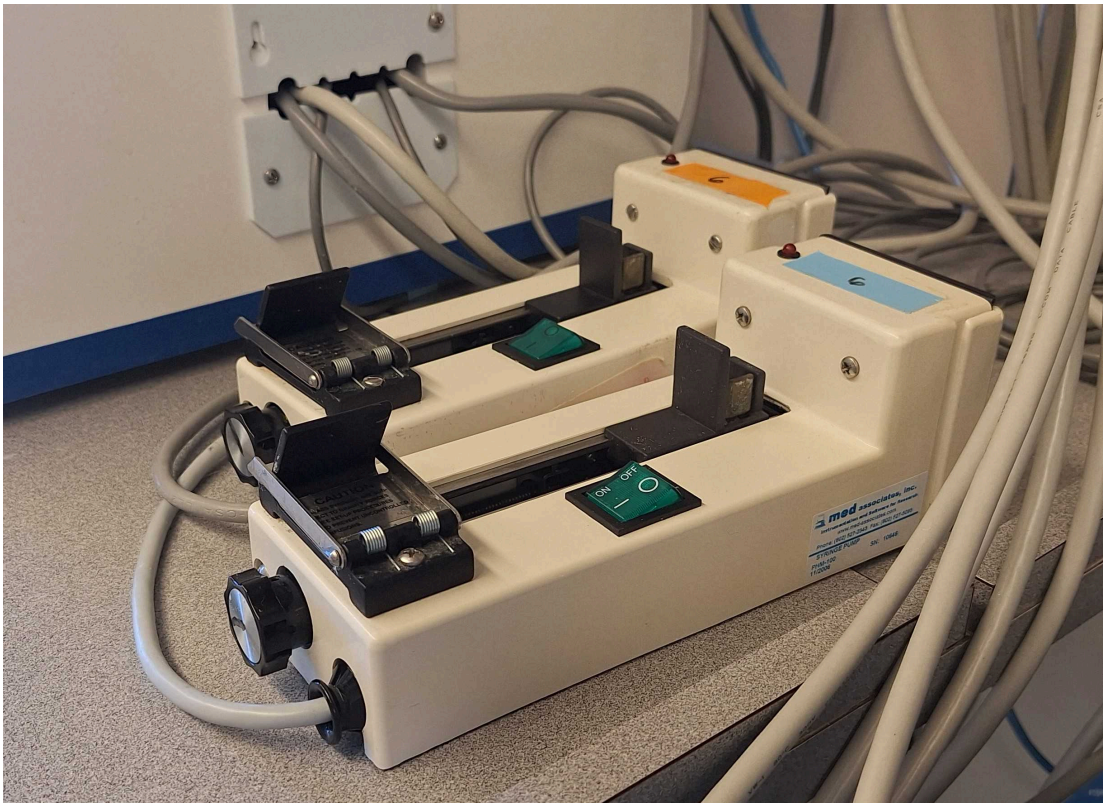
Picture 1. Chamber back panel from the outside showing the central placement of the clicker and speaker, with the tone generator box to the right.

- The front side panel contains a nose poke with a light (ENV-114BM) in the center module, flanked on each side with a single-cup liquid receptacle equipped with a head entry detector (ENV-200R3BM and ENV-254-CB) (**Picture 2**).
- Above each receptacle is a standard, non-retractable lever (ENV-110M), adjusted from manufacturer's specifications to be triggered by pressing with a force > 10g (0.01 N).
- Above each lever is a white stimulus light (ENV-221M).
- A house light (ENV-215M) is positioned in the center module of the panel, aligned with the stimulus lights and oriented to point downwards.



Picture 2. Chamber front panel, as described in the text. Head entry detectors in the left and right liquid receptacles are not shown at this angle.

- Outside each sound attenuating cubicle are two single speed syringe pumps (PHM-100), running at 3.33 rpm, to dispense sucrose solution from a 60 ml syringe to each of the liquid receptacles in the chamber (**Picture 3**).



Picture 3. Syringe pumps (60 ml syringe not shown) for chamber 6. Note the black lines on the dials at the front of the syringes. These were added to facilitate verification that pumps were activating as programmed. Also note the colored tape designating the chamber number. Orange is used for all chambers to indicate left and blue to indicate right liquid receptacles (feeder lines were also tagged) to reduce the possibility that pumps and liquid receptacles would be misaligned.

- The apparatus is controlled by MED-PC V software running on Microsoft Windows 7 computers, with a temporal resolution of 1 millisecond.
- An equipment test is completed daily prior to any sessions and followed any post-training session in which a rat earned fewer than 30 reinforcers. Program is available in the attached zip file (Equipment-Test.MPC).

Before start

- Prior to every session and before a rat is placed in a chamber, syringe pumps deliver a fixed amount of sucrose solution (300 µl) to both left and right liquid receptacles). Program is available in the attached zip file (Feeder-Filling.MPC).
- All food deliveries (response contingent and noncontingent) are signaled by a brief click (approximately 65 dB).

See attached zip file for programs noted in the protocol.

Training Phase 1 (programs: DDPhase1-withVT and DDPhase1-noVT)

1 Rats learn to press either lever to obtain sucrose rewards and learn that the feeder wells below the levers are sources of sucrose.

1.1 Contingency 1: Stimulus lights above each lever are lit. Press on either lever (FR 1) yields 150 µl sucrose solution to feeder well under pressed lever (response contingent reinforcers).

1.2 Contingency 2: Variable time (VT) 60 s schedule delivers 75 µl randomly to the left and right feeder wells (noncontingent food deliveries).

2 Completion of Phase 1: delivery of 60 response contingent reinforcers within 60 minutes on 1 session.

2.1 After 2 sessions without completion, noncontingent food deliveries end (beginning session 3).

2.2 After 10 additional sessions without completion, noncontingent food deliveries resume (beginning session 13).

2.3 After 20 additional sessions without completion, noncontingent food deliveries end (beginning session 33).

Training Phase 2 (programs: DDPhase2-withVT and DDPhase2-noVT)

3 Rats learn to poke their nose in the center nose poke prior to pressing the lever to earn a reinforcer.

3.1 Contingency 1: Light in center nose poke is lit. Single nose poke in the center nose poke (FR 1) turns off the center nose poke light and turns on the stimulus lights above each lever. Then a press on either lever (FR 1) yields a 150 µl sucrose solution delivered to the feeder well under pressed lever (response contingent reinforcers).

3.2 Contingency 2: VT 120 s schedule delivers 75 µl randomly to the left and right feeder wells (noncontingent food deliveries).

4 Completion of Phase 2: delivery of 60 response contingent reinforcers within 60 minutes on 1 session.

4.1 After 10 sessions without completion, noncontingent food deliveries end (beginning session 11)

Training Phase 3 (programs: DDPhase3L or DDPhase3R)

5 Introduction of different amounts of reward associated with pressing the left or right lever (150 µl or 75 µl; L or R in program name designates the side on which the larger reward is delivered).

5.1 Contingencies: As in Phase 2, **except** lever press on one lever yields either a 150 µl sucrose solution and the other yields 75 µl sucrose solution delivered to the feeder well under pressed lever (response contingent reinforcers). Lever designations as larger or small reward are counterbalanced between subjects and are fixed for the remainder of the procedure.

6 Completion of Phase 3: delivery of 60 reinforcers within 60 minutes on 1 session.

6.1 After 3 sessions with less than 30 reinforcers, rat is returned to Phase 2.

Training Phase 4 (programs: DDPhase4L or DDPhase4R)

7 Introduction of a 10-s timeout after reward delivery, which defines separate trials.

7.1 Contingencies: As in Phase 3, **except** after a reward is delivered, the lights over both levers are extinguished for a 10 s period, at the end of which the light in center nose poke is again lit awaiting a nose poke response.

8 Completion of Phase 4: completion of 60 trials (delivery of 60 reinforcers) within 60 minutes on 1 session.

8.1 After 3 sessions with less than 30 reinforcers, rat is returned to Phase 3.

Training Phase 5 (programs: DDPhase5L or DDPhase5R)

9 Introduction of the forced choice trials.

9.1 Contingencies: As in Phase 4, **except** following choice of the same lever on two successive trials prompts the next trial to force the rat to select the nonpreferred lever. On forced choice trials, only the stimulus light above the nonpreferred lever is lit.

10 Completion of Phase 5: completion of 60 trials (delivery of 60 reinforcers) within 60 minutes on 1 session, independent of the number of forced choice trials.

10.1 After 3 sessions with less than 30 reinforcers, rat is returned to Phase 4.

Training Phase 6 (programs: DDPhase6L or DDPhase6R)

11 Introduction of the adjusting amount contingency to the 75 µl reward (adjusting amount) alternative.

11.1 Contingencies: As in Phase 5, **except** a choice of the lever associated with the adjusting amount causes the adjusting amount on the following trial to decrease by 10% on the next trial and a choice of the lever yielding the 150 µl reward causes the adjusting amount to increase by 10%. The adjusting amount is not changed by events on forced choice trials.

12 Completion of Phase 6: 5 sessions, independent of the number of trials completed.

Ascending Phase (programs: DDAALx or DDAARx, where x = delay in seconds)

13 Introduction of the tone marking the delay between a lever press and the delivery of the reward, and introduction of the delays used in the study.

13.1 Contingencies: As in Phase 6, **except** pressing either lever generates a tone (5 kHz, approximately 70 dB) that terminates when the reward is delivered; the duration of the tone associated with pressing the adjusting amount lever is essentially 0 s (1 ms). On successive sessions, the delay between the lever press and delivery of the 150 µl reward lengthens: 0, 2, 4, 8, 16, 24 s. **Notice**: no lever press is required to collect the reward at the end of the delay interval.

14 Completion of the Ascending Phase: 6 sessions, independent of the number of trials completed.

Experimental Phase (Latin Square Phase, programs: DDAALx or DDAARx, where x = delay in seconds)

15 Consecutive sessions apply different delays in an order determined by a Latin Square (**Table 1**; delays in milliseconds).

15.1 **Table 1**. Latin Square used to counterbalance the order of experienced delays.

A	B	C	D	E	F
4000	8000	2000	16000	1	24000
2000	4000	1	8000	24000	16000
8000	16000	4000	24000	2000	1
24000	1	16000	2000	8000	4000
16000	24000	8000	1	4000	2000
1	2000	24000	4000	16000	8000

16 The Latin Square is the same for all rats and required 36 sessions for completion

17 All sessions include free- and forced-choice trials, and end after 60 free-choice trials occur, or 60 minutes elapse.

18 Each free-choice trial includes three phases (**Figure 1**).

19

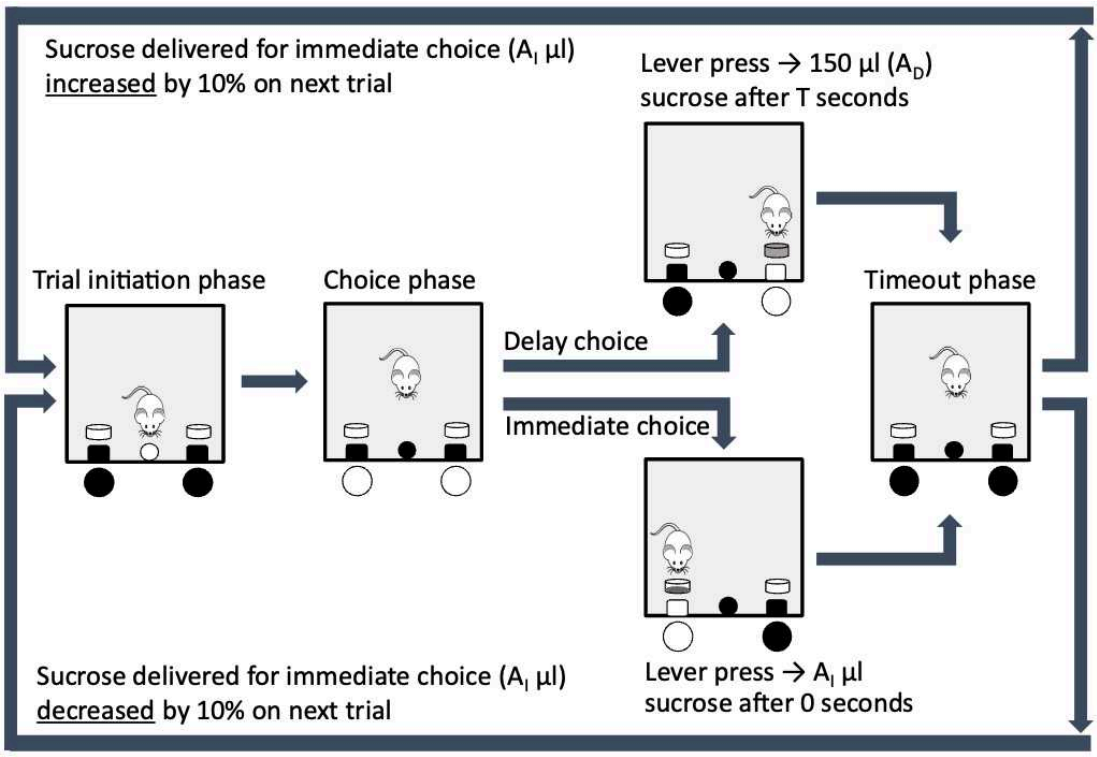


Figure 1. Free-choice trial schematic (see primary manuscript citation Figure 1A for complementary depiction)

Structure of Free-choice Trials

20 Trial Initiation Phase

20.1 The center nose poke is illuminated.

20.2 When the rat makes a nose poke, the light is turned off and the choice phase begins.

21 Choice Phase

21.1 The lights above the left and right levers are illuminated until the rat presses one of them.

21.2 In Figure 1, pressing the left lever is designated as an immediate choice (remember: side counterbalanced between rats). Pressing it causes a click sound, the lights above the left and right levers to turn off, and the delivery of an amount (A_I µl) of 10% w/v sucrose solution (size on trial 1: 75 µl). On the next trial, the A_I µl value was 10% smaller, down to a limit of 5 µl.

21.3 In Figure 1, pressing the right lever is designated as a delay choice. Pressing it causes a click sound, started a tone (5 kHz at approximately 70 dB) and turns off the light above the left lever. After T seconds (0, 2, 4, 8, 16, 24 s varied between sessions), the tone and light above the lever turn off and 150 µl of sucrose solution is delivered (A_D).

21.4 On the next trial, the A_I µl value is 10% larger, up to a limit of 300 µl.

22 Timeout Phase

22.1 The duration of the timeout phase varies so trials begin every 45 s regardless of lever selection in the choice phase (inter-trial interval = 45 s)

23 Forced-choice trials occur after two consecutive choices of either the delay or immediate lever. A_I is not altered following a forced-choice trial.

Data Processing

24 The main dependent measure is the **indifference point**, which is an index of the subjective value of the 150 µl 10% sucrose solution delivered after 0, 2, 4, 8, 16, or 24 s. Indifference points were collected for each session.

25 To assess the indifference point, the A_I is recorded for each free choice trial. The indifference point is the median A_I calculated from the final 30 trials.

26 Three procedures are used to enhance the robustness of indifference point measures:
1. Exclude any session on which fewer than 45 of the 60 free-choice trials were completed by a rat ("incomplete sessions").
2. Exclude any complete session on which choices during the second half of the session are primarily on one lever; operationalized as 80% or more of trials 31-60 during the sessions ("biased-preference" sessions).
3. Exclude individual rats with fewer than 3 complete, unbiased-preference sessions for each delay.

27 Indifference points as a function of delay length can be used to describe the extent to which individual rats discounting the value of the delayed reward.

Protocol references

This protocol is modified from one developed for rats by Jerry B. Richards (Richards et al. 1997) and for mice by Suzanne H. Mitchell (Mitchell, 2014).

Mitchell, S. H. (2014). Assessing delay discounting in mice. *Current Protocols in Neuroscience*, *66*, Unit 8 30. https://doi.org/10.1002/0471142301.ns0830s66
Richards, J. B., Mitchell, S. H., de Wit, H., & Seiden, L. S. (1997). Determination of discount functions in rats with an adjusting-amount procedure. *Journal of the Experimental Analysis of Behavior*, *67*(3), 353-366. https://doi.org/10.1901/jeab.1997.67-353

This protocol is used in the following publications:
Lara MK, Chitre AS, Chen D, Johnson BB, Nguyen KM, Cohen KA, Muckadam SA, Lin B, Ziegler S, Beeson A, Sanches TM, Solberg Woods LC, Polesskaya O, Palmer AA, Mitchell SH. Genome-wide association study of delay discounting in Heterogeneous Stock rats. Genes Brain Behav. 2024 Aug;23(4):e12909. doi: 10.1111/gbb.12909. PMID: 39119916; PMCID: PMC11310854.

Mitchell SH, Sevigny-Resetco D, Garland K. Quantifying delay discounting across species: successes and caveats for human to rat translation. Preprint. Psych Arch. 2023. doi: 10.23668/psycharchives.13032

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