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Extinction of Running-Based Taste Aversion in Rats (*Rattus norvegicus*)

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Wheel running establishes conditioned aversion in rats to a taste solution consumed shortly prior to running. Many studies have shown that this is a case of Pavlovian conditioning, in which the taste and running respectively act as the conditioned stimulus (CS) and the unconditioned stimulus (US), but extinction of this running-based taste aversion has not been demonstrated explicitly. Experiment 1, using a within-subjects design, showed that saccharin aversion formerly established by a single pairing of an exposure to saccharin solution with a running opportunity was extinguished by two daily exposures to the saccharin solution. However, there was no spontaneous recovery from extinction in the tests, which were administered 6 and 27 days after the extinction days. Experiment 2, using a between-groups design, successfully demonstrated extinction and spontaneous recovery of running-based saccharin aversion, when rats were treated with a paradigm of 8 conditioning days, 8 extinction days, and 8 retention days.

Voluntary running in an activity wheel has hedonically bivalent properties in laboratory rats. A large number of studies show that wheel running works as a positive reinforcer for preceding instrumental responses such as lever pressing (e.g., Belke, 1997; Collier & Hirsh, 1971; Iversen, 1993; Kagan & Berkun, 1954). Rats also learn to choose the arm of a T-maze that leads to a running wheel (Livesey, Egger, & Meyer, 1972). Thus, one may regard wheel running as pleasant from the viewpoint of instrumental conditioning. Despite this pleasurable nature of wheel running, it functions as an aversive unconditioned stimulus (US) for rats to establish conditioned avoidance of the taste substance (i.e., flavored solution) consumed before the running (e.g., Lett & Grant, 1996; Heth, Inglis, Russell, & Pierce, 2001; Nakajima, Hayashi, & Kato, 2000; see Boakes & Nakajima, 2009, for a review). In other words, Pavlovian conditioned taste aversion (CTA) is formed by correlational pairing of taste (conditioned stimulus, CS) and wheel running (US). One might argue that instrumental punishment, rather than Pavlovian conditioning, is operating here, because wheel running punishes preceding drinking behavior under some conditions (Mazur, 1975; Terhune & Premack, 1970; see also Premack, 1971)¹. However, rats' avoidance is specific to the taste paired with running in the aforementioned running-based CTA studies: consumption of tap water or unpaired taste is not affected. Furthermore, wheel running generates some kaolin clay intake (Nakajima, 2016, in press; Nakajima & Katayama, 2014), which has been regarded as a marker of nausea in rats (Andrews & Horn, 2006). Thus, it is quite certain that weak nausea induced by running is a genuine US in running-based CTA.

Although the running-based CTA is weak in effect compared with poison-based CTA, many behavioral features of Pavlovian conditioning have been demonstrated in this preparation as well as in poison-

¹ Some researchers have claimed that poison-based CTA is also a kind of punishment learning (e.g., Bitterman, 1976; Li, Hsiao, & Li, 2013). However, poison-based CTA is establishable without active ingestion (Domjan & Wilson, 1972; see also Garcia, Hankins, & Rusiniak, 1976). Furthermore, rats acquire poison-based CTA with intravenously injected taste as a CS (Bellingham & Lloyd, 1987). Aversive orofacial reactions to conditioned taste (e.g., Grill & Norgren, 1978; Pelchat, Grill, Rozin, & Jacobs, 1983; see Parker, 2014, for a review) is another pieces of evidence supporting the view that poison-based CTA is mainly based on the Pavlovian conditioning principle.

based CTA preparations. The features include laws of contiguity and US strength (Hayashi, Nakajima, Urushihara, & Imada, 2002), CS-preexposure effect (or latent inhibition; Heth & Pierce, 2007; Satvat & Eikelboom, 2006; Sparkes, Grant, & Lett, 2003), US-preexposure effect (Baysari & Boakes, 2004; Hughes & Boakes, 2008; Nakajima, 2015; Nakajima, Urata, & Ogawa, 2006; Salvy, Pierce, Heth, & Russell, 2002), degraded contingency effect (Nakajima, 2008), inhibitory learning by backward conditioning (Hughes & Boakes, 2008; Salvy, Pierce, Heth, & Russell, 2004), stimulus overshadowing (Nagaishi & Nakajima, 2010), associative blocking (Pierce & Heth, 2010), and higher-order contextual control (Hashimoto & Nakajima, 2013).

Despite ample literature on running-based CTA in rats, *extinction* of conditioned responding by post-conditioning CS exposure, which is one of the canonical features of Pavlovian conditioning (Pavlov, 1927; Rescorla, 2001, 2002; Vurbic & Bouton, 2014), has not been well documented for running-based CTA. The few reports available are a conference paper (Nakajima & Hashimoto, 2013) and an indirect piece of evidence (i.e., the use of an extinction procedure as a maneuver to increase the sensitivity of detecting CTA in the following choice test: Nakajima, 2015, Experiment 2).

Extinguished responses to a CS *spontaneously recover*, when the animal is tested after a temporal interval (Pavlov, 1927; Rescorla, 2004). Although extinction and spontaneous recovery have been repeatedly demonstrated in poison-based CTA (e.g., Berman, Hazvi, Stehberg, Bahar, & Dudai, 2003; Brooks, Palmatier, Garcia, & Johnson, 1999; Mickley et al., 2007, 2009; Rosas & Bouton, 1996, 1998), there is no explicit report of spontaneous recovery in running-based CTA. Nakajima et al. (2000) administered the second test of running-based CTA seven days after the first test to refresh the performance. The attempt was successful, but it was not assessed critically to determine if the success was truly due to the process of spontaneous recovery.

The shortage of reports on extinction and spontaneous recovery of running-based CTA prompted the author to present this paper, which analyzes data from two experiments. Experiment 1 employed a within-subjects design in attempt to demonstrate extinction and spontaneous recovery of running-based CTA: the former was achieved, but the latter was not. Experiment 2 successfully demonstrated extinction and spontaneous recovery with a between-groups design.

Experiment 1

Method

Subjects. The subjects were 12 experimentally naïve male Jbc:Wistar rats (*Rattus norvegicus*) with a mean weight of 343.2 g (range: 330–358 g), measured on the first adaptation day when they were 10 weeks old. They were purchased from a local supplier (Keari, Osaka, Japan), and housed in individual hanging home cages of the vivarium on a 12:12 h light–dark cycle (lights on at 0800 h) at about 23 °C. Solid food pellets were freely available in the home cages. Water was deprived on the day before the adaptation training. Thereafter, each animal was able to access tap water for 15 min from a metal nozzle protruding through a hole in the center of the back wall of each cage 60 min after the drinking period of experimental treatment (see below).

Apparatus. The rats were transferred, by a carrying cart having individual compartments, from the vivarium to a conventionally illuminated experimental room where four drinking cages and four hand-crafted activity wheels were located. The drinking cages (20 cm wide, 25 cm long, and 18.7 cm high) were copies of the home cages, and they were made of wire with two solid meal walls. Fluid was provided via a plastic bottle with a metal spout inserted from the mesh hole, which was 5 cm from the center of the cage ceiling: the end of the spout was positioned 16.5 cm above the cage floor. When two bottles were used, they were separated 8 cm apart and equally far (5 cm) from the center of the cage ceiling. The activity wheels (15 cm wide and 30 cm in diameter) were hung horizontally in line on a wall of the experimental room, 140 cm above the room floor and the distance between any two adjacent wheels was 20 cm. The wheel walls were perforated metal sheets and the wheel floor was made of 0.2 cm metal rods spaced 1 cm apart. A full

turn of each wheel was counted automatically by a handcrafted system made of a small magnet on the outer rim of the wheel, a reed switch and an electric pedometer fixed on the wall.

Procedure. All experimental treatments were conducted at the same time (1000–1400 h, three squads of four rats each) on successive days. The experimental design is shown in Table 1. On Day 1, each rat was allowed to access a bottle containing tap water for 30 min. On Days 2 and 3, all rats were adapted to a two-bottle choice situation with water and empty bottles for 15 min per day with the left–right locations of the bottles changed across days.

Table 1
Within-Subjects Design of Experiment 1

Days 1–3 (3 days)	Day 4 (1 day)	Day 5 (1 day)	Days 6–7 (2 days)	Day 8 (1 day)	Days 9–12 & 14–33 (4 & 21 days)	Days 13 & 34 (1 day each)
Adaptation water	Conditioning sac → run	Test 0 sac vs water	Extinction sac	Test 1 sac vs water	Retention ----	Tests 2 & 3 sac vs water

Note. water = tap water, sac = sodium saccharin solution, ---- = tap water (in home cage).

On Day 4, all rats were given access to 0.2 % sodium saccharin solution for 15 min, then immediately confined to the individual wheels for 60 min. Notably, a single 60-min running is an effective US for establishing CTA in rats (Masaki & Nakajima, 2006). Testing of saccharin aversion was administered on Day 5 (Test 0) with two bottles available for 15 min in each drinking cage. One of the bottles contained the saccharin solution and the other tap water. The rats were then given extinction training on Days 6–7 with a 15-min access to the saccharin solution in the drinking cages. The choice test was administered on Day 8 (Test 1) in order to assess the effect of extinction treatment on running-based CTA by comparing the performance of that day to the one conducted on Day 5.

On Days 9–12 (4 days) and 14–33 (21 days), the rats were kept in the home cages with a 15-min tap water access. Post-session watering of 15 min was also executed in the home cages as before. Spontaneous recovery of running-based CTA was tested on Days 13 (Test 2) and 34 (Test 3) in exactly the same fashion. The left-right positions of saccharin and water bottles were counterbalanced across rats and consistent within each rat over all test days.

Measurement and analysis. Fluid intake was measured by weighing each bottle before and after the drinking period with an electric balance (BJ-1500, Sartorius Japan, Tokyo) to the nearest 0.1 g. The data are expressed as means ± standard errors (SEs) across subjects, and a paired *t*-test or a one-way within-subjects analysis of variance (ANOVA) was applied to each dataset of interest. All statistical analyses in this study are based on an α level of $p < .05$ and, for simplicity, *t* or *F* values will be reported with the standardized effect sizes only when they are statistically significant. Ryan's procedure was used for post-hoc multiple comparisons with the individually adjusted α levels (Howell, 2007).

Ethical statement. This research project and the animal facility were approved by the Animal Care and Use Committee of Kwansei Gakuin University, based on a Japanese law (the Act on Welfare and Management of Animals) and the guidelines published by the Science Council of Japan (2006). The research also followed the ethical guidelines of the American Psychological Association (APA, 2013).

Results and Discussion

Conditioning and extinction. The rats consumed saccharin solution 11.6 ± 0.7 g on the conditioning day when they turned the wheels 223 ± 24 times in the 60-min period. The saccharin consumption increased to 15.3 ± 1.0 g on the first extinction day. The significant upshift, paired $t(11) = 5.82$, $p < 0.001$, $r = .87$, in saccharin intake despite the conditioning treatment suggests that habituation of neophobic reactions to the novel saccharin masked the expression of conditioned aversion. The saccharin intake significantly increased

to 19.0 ± 1.0 on the second extinction day, paired $t(11) = 4.47, p < 0.001, r = .80$, possibly reflecting continued habituation of saccharin neophobia and/or extinction of saccharin.

Choice test. The total amount of fluid intakes (i.e., saccharin solution + tap water) on the four test days ($18.2 \pm 0.7, 20.1 \pm 0.6, 20.1 \pm 0.9,$ and 17.6 ± 1.8 g, respectively) did not differ significantly, suggesting the thirst levels were comparable across the tests. Test performance was indexed as the choice-preference ratio, which was calculated for each day in the form of $x/(x + y)$, where x is saccharin-solution intake and y is tap-water intake. Lower ratios indicate stronger saccharin aversions. The performance of the four test days are summarized in Figure 1 as a line graph. Although this experiment has no control group for assessing running-based CTA itself, rats would prefer 0.2% saccharin solution to tap water if they had experienced it without any conditioning treatment (Hayashi et al., 2002; Masaki & Nakajima, 2004, 2005, 2006, 2010; Nakajima, 2014). For example, the upper broken line shown in Figure 1 illustrates the average saccharin preference ratio of a group of rats tested on the day following a single session of 15-min saccharin access without running (Masaki & Nakajima, 2006)². Thus, this level is an estimate of no-US control for Pavlovian conditioning.

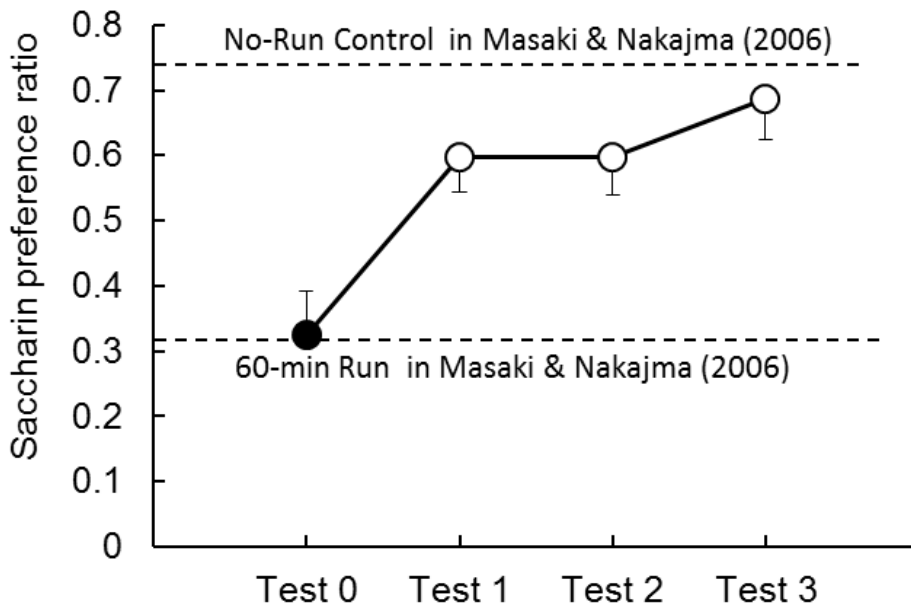


Figure 1. Mean saccharin preference ratio calculated from the test intake data of Experiment 1. Bars of standard errors are shown on either side of the mean values. The upper and lower broken lines, respectively, represent the average test performance of the no-running control rats and the experimental rats with a 60-min running US in Masaki and Nakajima (2006).

On Test 0, the preference ratio was significantly below the chance level of 0.50, $t(11) = 2.73, p = 0.034, r = .64$, reflecting avoidance of the target saccharin solution. Notably, this ratio is almost indistinguishable from the rats that received identical conditioning treatment (a 15-min saccharin CS followed by a 60-min running US) in Masaki and Nakajima (2006). The saccharin avoidance shown in Test 0

² The present experiment was carried out around the same time that Masaki and Nakajima (2006) were conducting the experiment with rats of the same strain, sex, and age.

disappeared after the extinction days, suggesting extinction of running-based CTA. On Tests 1 and 2, the preference ratio did not differ statistically from the chance level of 0.50. On Test 3, the preference ratio was significantly above the chance level of 0.50, $t(11) = 3.10$, $p = 0.021$, $r = .68$, suggesting a weak saccharin preference. This is the opposite direction from what one would predict if the spontaneous recovery took place. A one-way ANOVA, applied for the data of all test days, yielded a significant day effect, $F(3, 33) = 12.18$, $p < 0.001$, $\eta_p^2 = .53$. Post-hoc comparisons revealed that the preference ratio was lower for Test 0 than Test 1, $t(33) = 4.29$, $p < .001$, against adjusted $\alpha = .025$, $r = .60$, Test 2, $t(33) = 4.29$, $p < 0.001$, $\alpha = .013$, $r = .60$, and Test 3, $t(33) = 5.69$, $p < 0.001$, $\alpha = .008$, $r = .70$. The differences between any pairs of the post-extinction tests (Tests 1–3) were all nonsignificant, implying no spontaneous recovery from extinction.

Experiment 2

Experiment 1 successfully demonstrated extinction of running-based CTA in rats, but there was no sign of spontaneous recovery from extinction. Experiment 2 was designed to obtain evidence for spontaneous recovery of extinguished CTA in a between-groups design. The length of wheel confinement per daily trial was shortened to 15 min for the experimenter's convenience, because a relatively large number of animals were employed in Experiment 2 (see below). Notably, this value is effective in establishing running-based CTA (Hayashi et al., 2002; Nakajima, 2004; Nakajima et al., 2000), although the amount of CTA is weak. In order to compensate for a presumably weak US potential in this experiment, the number of conditioning trials was planned to increase from one to eight. The number of extinction trials was also eight. The testing of spontaneous recovery was administered nine days after the extinction treatment.

Method

Subjects and apparatus. The subjects were 40 experimentally naïve male Jbc:Wistar rats were purchased from the same supplier as in Experiment 1. The rats were 9 weeks old with a mean weight of 284.0 g (range: 267–303 g) on the first experimental day. The rats were maintained in the same way as in Experiment 1. The apparatus used in this experiment were the same as those of Experiment 1, but the number of drinking cages in the experimental room was doubled to eight.

Procedure. All experimental treatments were conducted at the same time (1500–1730 h, five squads of eight rats each) on successive days. The experimental design is shown in Table 2. On Days 1–4, each rat was trained to drink tap water from a bottle for 15 min. The two-bottle adaptation technique employed in Experiment 1 was not used in Experiment 2 for procedural simplicity.

The animals were then assigned to one of five groups ($n = 8$ rats per group), matched for their bodyweight and amount of water intake in the drinking cages. The five groups were choice-tested on Day 29 after three phases of treatments (eight days per treatment, see Table 2). The rats of primary interest was Group Rec (the last entry row of Table 2): they received conditioning, extinction, and then retention treatments in Phases 1–3, and thus had an opportunity to show spontaneous recovery in testing. Specifically, in Phase 1 (conditioning), they were given an access to 0.2 % sodium saccharin solution for 15 min, then immediately confined into the individual wheels for 15 min. In Phase 2 (extinction), they were directly returned to the home cages after a 15-min access to the saccharin solution. In Phase 3 (retention), they were kept in the home cages throughout the retention phase with a 15-min tap water access in the home cages. Accordingly, this group was tested to ascertain recovery of saccharin aversion nine days after the extinction treatment. Test performance of this group was compared with that of Group Ext, which received the retention, conditioning, and extinction procedures in this order. Thus, this group was tested on the day next following the extinction of running-based CTA. A strong CTA (i.e., a low saccharin preference ratio) in the Group Rec compared with Group Ext would imply spontaneous recovery from extinction.

Table 2

Between-Groups Design of Experiment 2

	Days 1–4 (4 days)	Days 5–12 (8 days)	Days 13–20 (8 days)	Days 21–28 (8 days)	Day 29 (1 day)
Group (<i>n</i> = 8 each)	Adaptation	Phase 1	Phase 2	Phase 3	Test
Acq-n	water	----	----	sac → run	sac vs water
Acq-m	water	----	sac → run	----	sac vs water
Acq-f	water	sac → run	----	----	sac vs water
Ext	water	----	sac → run	sac	sac vs water
Rec	water	sac → run	sac	----	sac vs water

Note. water = tap water, sac = sodium saccharin solution, ---- = tap water (in home cage).

Extinction of running-based CTA was assessed on the test day (i.e., Day 29) by comparing Group Ext to Groups Acq-n, Acq-m, Acq-f, all of which had no experience of extinction. These three control groups differed in when the CTA acquisition treatment was administered: the suffix of each group name means that the saccharin-running phase was near, middle, or far from the testing. Notably, the traditional control was Group Acq-n, in which the conditioning was executed a day before the testing. On the other hand, the conditioning phases of Groups Acq-m and Acq-f were temporally matched to those of Groups Ext and Rec, respectively, to provide more appropriate controls.

Measurement and analysis. Although the procedure for measurement was identical to that of Experiment 1, a one-way between-groups ANOVAs were applied to each dataset of interest in Experiment 2. An additional planned comparison between Groups Rec and Ext was conducted, because we had an explicit *a priori* prediction that Group Rec would display stronger aversion than Group Ext in the test (cf. Howell, 2007).

Results and Discussion

Conditioning. The left section of Figure 2 illustrates the mean saccharin solution intakes of the five groups of rats in the conditioning phase. Running-based CTA was not evident in the conditioning phase as in some of our previous reports (e.g., Nakajima et al., 2006). This had been expected because a one-bottle assessment is not sensitive to detect weak taste aversion (e.g., Dragoin, McCleary, & McCleary, 1971; Grote & Brown, 1971). A 5 (group) × 8 (day) ANOVA yielded a significant main effect of day, $F(7, 245) = 7.09$, $p < 0.001$, $\eta_p^2 = .17$, but the main effect of group was far from significant. Although the group × day interaction was significant, $F(28, 245) = 2.27$, $p < 0.001$, $\eta_p^2 = .21$, subsequent analyses of the simple main effects found no group differences on any conditioning days. The simple effect of day was significant for all groups, $F_s(7, 245) > 2.11$, $p_s < 0.043$, $\eta_p^2s > .05$.

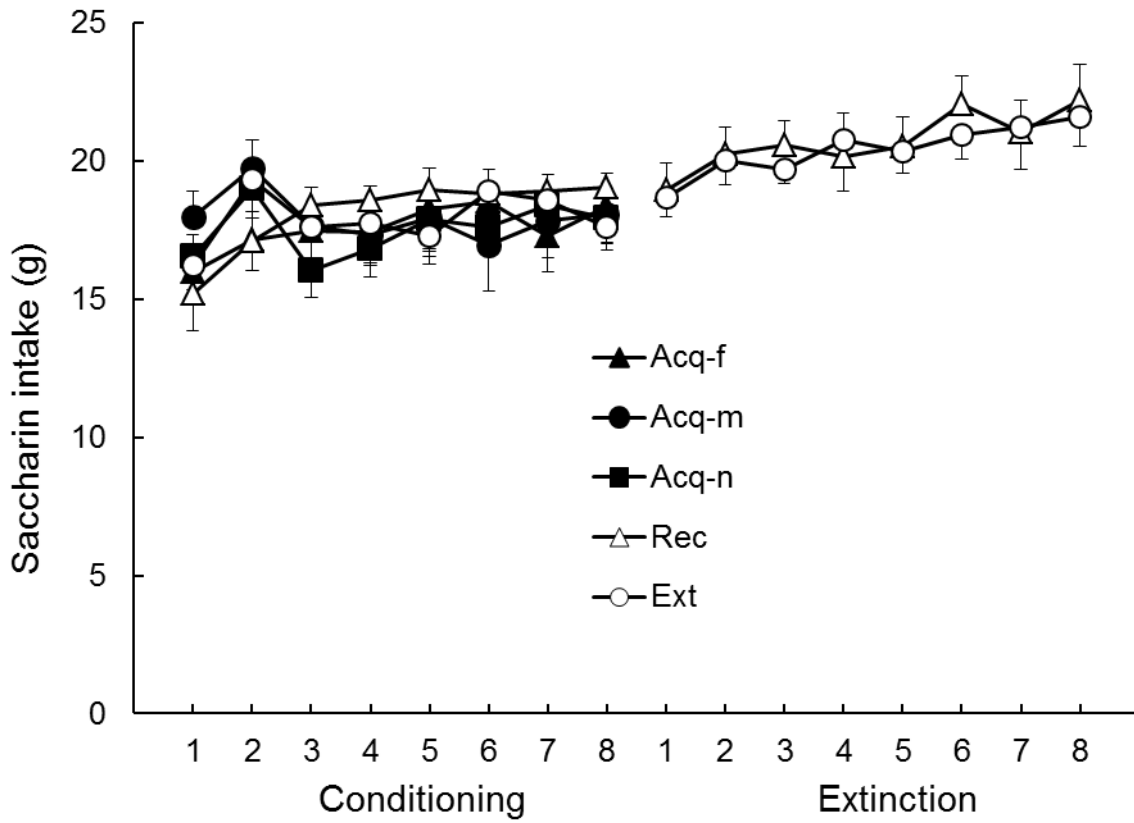


Figure 2. Mean amount of saccharin solution intake in the conditioning and extinction phases of Experiment 2. Bars of standard errors are shown on either side of the mean values.

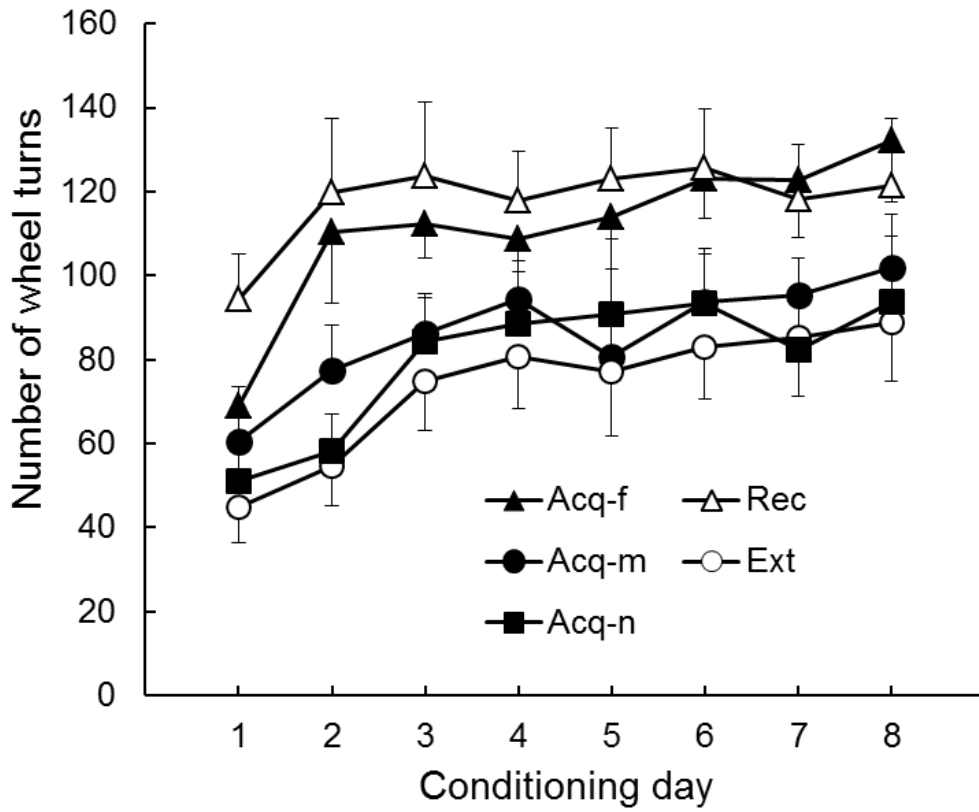


Figure 3. Mean number of wheel turns in the conditioning phase of Experiment 2. Bars of standard errors are shown on either side of the mean values.

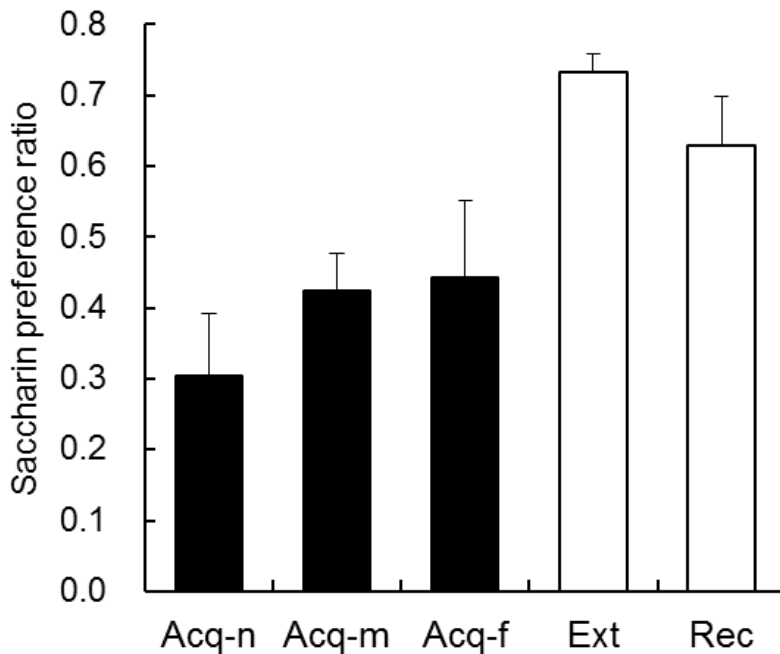


Figure 4. Mean saccharin preference ratio calculated from the test intake data of Experiment 2. Bars of standard errors are shown on the positive side of the mean values.

Wheel running. Figure 3 presents the amount of running throughout the conditioning phase. On average, rats of Groups Rec and Acq-f ran more than those of the other groups. This was unexpected, but may be explained by the fact that these two groups were confined into the wheels when they were younger than the others: wheel activity is conventionally greater in young rats than for older ones (Goodrick, 1980; Goodrick, Ingram, Reynolds, Freeman, & Cider, 1983). A 5 (group) \times 8 (day) ANOVA yielded significant main effects of group, $F(4, 35) = 3.65$, $p = 0.014$, $\eta_p^2 = .29$, and day, $F(7, 245) = 16.2$, $p < 0.001$, $\eta_p^2 = .32$, but their interaction was far from significant. Post-hoc comparisons with Ryan's procedure found a significant difference only between Groups Rec and Ext, $t(35) = 3.04$, $p = 0.004$, against adjusted $\alpha = .005$, $r = .46$. All other combinations failed to reach the adjusted significance levels.

Extinction. The right section of Figure 2 shows that the two groups receiving the extinction treatment (Groups Ext and Rec) did not differ from each other throughout the extinction phase. A 2 (group) \times 8 (day) ANOVA yielded a significant main effect of day, $F(7, 98) = 10.27$, $p < 0.001$, $\eta_p^2 = .42$, but the main effect of group and the interaction were far from significant. The gradual increase might reflect extinction of conditioned negative valence of saccharin, because the saccharin intake had hovered around 18 g in the second half of Phase 1 before it started to increase up to 21.9 ± 0.8 g (collapsed across the two groups) on the last extinction day.

Choice test. The group differences in total amount of fluid intakes (range: 19.0 ± 0.7 to 22.5 ± 1.2 g) failed to reach the statistical significance, suggesting the groups were equally thirsty in the test. The saccharin preference ratios are depicted in Figure 4 in terms of the choice-preference ratio, calculated as in Experiment 1. The three Acq groups showed modest avoidance of saccharin³, the saccharin avoidance was the weakest in Group Ext, and Group Rec was between them. These impressions were supported by a one-way ANOVA, yielding a significant group effect, $F(4, 35) = 5.26, p = 0.002, \eta_p^2 = .38$. Post-hoc comparisons with Ryan's procedure revealed Group Ext significantly differed from Group Acq-n, $t(35) = 4.04, p < 0.001$, against adjusted $\alpha = .005, r = .56$, Group Acq-m, $t(35) = 2.91, p = 0.006, \alpha = .007, r = .44$, and Group Acq-f, $t(35) = 2.74, p = 0.009, \alpha = .010, r = .42$. In addition, the difference between Groups Acq-n and Rec was significant, $t(35) = 3.08, p = 0.004, \alpha = .007, r = .46$. The differences between Groups Acq-n, Acq-m, and Acq-f were all nonsignificant, suggesting that the running-based CTA established in this experiment was resistant to the temporal interval of 8 or 16 days. A planned-comparison between Groups Rec and Ext via a one-tailed *t*-test with the error term of the aforementioned ANOVA revealed that these two groups significantly differed from each other, $t(35) = 2.29, p = 0.014, r = .36$, suggesting spontaneous recovery of extinguished CTA.

As noted, however, Groups Rec and Ext had differed in the amount of wheel activity during the conditioning phase. Thus, it is necessary to control this factor to make a proper comparison between these two groups. Hence, a one-way analysis of covariance (ANCOVA) was performed with the five groups as the independent variable, the test data of all rats as the dependent variable, and the total amount of wheel running in the conditioning phase as the covariant. The results showed that the saccharin avoidance significantly differed among the five groups, $F(4, 34) = 5.14, p = 0.002, \eta_p^2 = .38$. Most importantly, a planned-comparison between Groups Rec and Ext via a one-tailed *t*-test with the error term of the ANCOVA revealed that these two groups significantly differed from each other, $t(34) = 1.90, p = 0.034, r = .31$, supporting the claim that formerly extinguished running-based CTA would spontaneously recover after a temporal interval, though the effect was not so large in this experiment.

General Discussion

The present study consisting of two experiments was undertaken to demonstrate extinction and spontaneous recovery of running-based CTA in rats. Experiments 1 and 2 both showed clear evidence of extinction of saccharin aversion which had been established by running after saccharin intake. Spontaneous recovery, however, was not obtained in Experiment 1 even when the interval between the last extinction day to the test day was extended to 27 days. This failure might be due to the weakness of CTA established by a single saccharin–running pairing. A limited recovery was observed in Experiment 2, where the number of saccharin–running pairings was eight. Because the length of wheel confinement (i.e., the amount of running) was shorter in Experiment 2 than Experiment 1, it is difficult to claim that the saccharin–running association was strong in Experiment 2 compared with Experiment 1. Furthermore, the difference in the experimental designs employed in these experiments prohibits one from drawing any firm conclusions about the reason why spontaneous recovery was obtained in Experiment 2 but not in Experiment 1. Further research is required to find better methods for demonstrating substantial recovery of running-based CTA. Despite the ambiguity in the proper procedural parameters to obtain a strong effect, a successful demonstration of spontaneous recovery after the extinction treatment in Experiment 2 supports our claim that running-based

³ Although there is a weak sign of mitigation in saccharin aversion among the Acq groups as a function of conditioning–test interval, a separate ANOVA applied to the scores of these three groups revealed that this trend was far from significant.

taste aversion is a case of Pavlovian conditioning as is poison-based taste aversion (Boakes & Nakajima, 2009), and it adds another set of features to the list of phenomena reviewed in the introduction of this article.

Extinguished Pavlovian responding may return not only by passage of time, but also by changing background contexts or unpredicted presentations of the US (Bouton, 2000, 2002). Renewal of responding by changing contexts has been repeatedly reported in rats' poison-based CTA (e.g., Bernal-Gamboa et al., 2012; Fujiwara et al., 2012; Revillo, Castello, Paglini, & Arias, 2014; Rosas & Bouton, 1997, 1998; Rosas, García-Gueteirrez, & Callejas-Aguilera, 2007). Reinstatement of responding by unpredicted US presentations is also demonstrated in poison-based CTA (e.g., Revillo et al., 2014; Schachtman, Brown, & Miller, 1985; Schachtman, Gustavson, Chelonis, & Bourne, 1992; but see Bouton, 1982, for some difficulty to obtain this effect). Demonstration of these effects in running-based CTA preparations would lend further credence to the notion that poison- and running-based CTAs share the same Pavlovian mechanism.

In closing, I note an advantage of running-based CTA over poison-based CTA in studying behavioral features of Pavlovian conditioning. As confinement of rats in activity wheels to allow voluntary running for a short period is more humane treatment than poisoning by emetic drugs, the running-based CTA paradigm is more desirable from the humanitarian point of view. For example, the APA guidelines say "Whenever possible behavioral procedures should be used that minimize discomfort to the nonhuman animal" (APA, 2013, Section 5B). Thus, further elaboration and dissemination of this paradigm are called for.

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