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Research report

Calorie supply does not alleviate running-based taste aversion learning in rats☆

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ABSTRACT

Voluntary running establishes aversion to the paired taste in rats. A proposed mechanism underlying this taste aversion learning is energy expenditure caused by the running. The energy expenditure hypothesis predicts that running-based taste aversion should be alleviated by a calorie supply since this would compensate for the energy expended by running. Accordingly, running-based taste aversion would be less readily established to a caloric substance (20% sucrose solution) than to a noncaloric substance (0.2% sodium saccharin solution). Because the sucrose and saccharin aversions were equivalent in Experiment 1, the validity of the energy expenditure hypothesis was questioned. Experiments 2 and 3 also pose a problem for this hypothesis, as post-session calorie supply by glucose tablets failed to alleviate running-based aversion to salty water.

Lett and Grant (1996) discovered that voluntary running by a rat in an activity wheel results in aversion to the taste of a substance consumed immediately prior to running. Because the correlation of taste and running is necessary to establish taste aversion, this phenomenon has been considered to be a type of Pavlovian conditioning, with the taste as a conditioned stimulus (CS) and the running as an unconditioned stimulus (US). Although many features of running-based taste aversion learning have been investigated (see Boakes & Nakajima, 2009, for a review), it is still unknown why wheel running works as a US agent in rats. Lett and Grant (1996) have proposed that running activates the mesolimbic dopamine system in the brain, which induces aversion to the paired taste. Meanwhile, in a personal communication to Lett, Grant, Koh, and Parsons (1999), John Garcia expressed his hypothesis that ascribes the cause of this phenomenon to gastrointestinal discomfort through inhibition of stomach emptying by running. On the other hand, Nakajima, Hayashi, and Kato (2000) claimed that energy expenditure caused by running yields aversion to the paired taste. Rats learn to prefer tastes that are associated with caloric restoration (see Capaldi, 1992, 1996; Fedorchak, 1997; Mehiel, 1991; Sclafani, 1990, 1991, for reviews). Conceivably, they can also learn to avoid tastes that are associated with energy (i.e. calorie) expenditure. Indirect support for this presumption is the 'missing calorie effect' in which rats learn to avoid a taste that signals the absence of otherwise expected caloric restoration (Boakes, Colagiuri, & Mahon, 2010).

There are also other accounts of running-based taste aversion in rats. Forristall, Hookey, and Grant (2007) have suggested the possibility that motion sickness induced by back-and-forth swings of a wheel results in nausea, although the generality of this hypothesis is questioned because taste aversion is establishable with motorized wheels, which produce no swings (Eccles, Kim, & O'Hare, 2005; Masaki & Nakajima, 2006). Finally, Nakajima, Urata, and Ogawa (2006) claimed that stress-induced physiological change is a cause of running-based taste aversion, although more specific identification of the critical physical mechanism is required in order to test the validity of this concept.

The present study focuses on the energy expenditure hypothesis for running-based taste aversion. Within the framework of this hypothesis, Nakajima and Masaki (2004) argued that any other physical activities, especially exhausting ones, should also work as a US for establishing taste aversion. As evidence favoring that hypothesis, they reported taste aversion learning based on swimming in a water pool, a phenomenon that was repeatedly replicated thereafter (Masaki & Nakajima, 2004a, 2004b, 2005, 2006,

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2010; Nakajima, 2004). Although the discovery of swimming-based taste aversion learning supports the energy expenditure hypothesis, it may also be explained by the hypotheses that ascribe the cause to the mesolimbic dopamine system, gastrointestinal discomfort, or stress-related physiological changes. Thus, the present study takes another approach to verify the energy expenditure hypothesis. If energy expenditure establishes aversion to the paired taste, then a calorie supply may alleviate running-based taste aversion because it compensates for the energy expended by running. The 3 experiments reported here were designed to test this prediction.

Experiment 1

Experiment 1 attempted to compare the strength of running-based aversion to a caloric sweet taste (sucrose solution) with that to a noncaloric sweet taste (saccharin solution). The concentrations of the sucrose and saccharin solutions were determined by a pilot study to produce equivalent amounts of initial intake by rats. Our expectation was that there would be a weaker conditioned aversion to the former because of the calorie supply.

However, we must be cognizant of the possibility that sucrose and saccharin aversions differ in strength not because of the caloric natures of these solutions, but because of their stimulus salience. In order to assess the salience of sucrose and saccharin solutions employed here, we replaced the wheel running for other groups of rats with injection of a low dose of cyclophosphamide, an emetic drug. With sucrose and saccharin aversions conditioned by cyclophosphamide as reference controls, we could pertinently compare running-based sucrose and saccharin aversions. Notably, we used cyclophosphamide instead of lithium chloride (LiCl), which is the most conventional emetic in rats' taste aversion studies (see Riley & Freeman, 2004, for a database), because our pilot research found a great amount of individual difference in the strength of taste aversion caused by low doses of LiCl.

Cyclophosphamide is a nitrogen mustard derivative used for treatment of tumors (i.e. immunosuppressant), and there have been many studies reporting taste aversion learning in rats with this drug (e.g. Ader, 1973; Barker, Smith, & Suarez, 1977; Dragoin, 1971; Dragoin, McCleary, & McCleary, 1971; Elkins, 1973; Garcia, Ervin, & Koelling, 1967; Grote & Brown, 1971; Wright, Foshee, & McCleary, 1971). This drug is probably the second most popular agent for establishing conditioned taste aversion after LiCl (see Riley & Freeman, 2004, for a database). Rats display orofacial and somatic rejection reactions to tastes that have been conditioned with cyclophosphamide (Limebeer & Parker, 1999; Parker, 1998). The neurophysiological mechanism responsible for cyclophosphamide-based taste aversion has not been well clarified compared with LiCl-based taste aversion, but a recent study reported that these aversions share the same brain mechanisms mediated by the medial and lateral parabranchial nuclei (Mungarndee, Lundy, & Norgren, 2006).

Conditioned taste aversion was assessed here by a two-bottle test after the treatment because it is more sensitive than the one-bottle test for detecting the relatively weak aversions established by running. Even with a very low dose, cyclophosphamide caused stronger taste aversion than did wheel running, and we therefore partially extinguished the cyclophosphamide-based aversion before comparing the cyclophosphamide- and running-based sucrose and saccharin aversions.

Methods

Subjects

The subjects were 32 experimentally naïve, 8-week-old male Wistar rats with a mean weight of 273 g (range: 253–285 g) on the first day of training. The animals were housed in individual hanging wire home cages in the vivarium on a 12:12 h light-dark cycle (lights on at 8:00 am) at 23 °C and a humidity level of 55%. They were deprived of water on the day before the beginning of the adaptation training, and fluid was available only in the experimental sessions unless otherwise noted. Laboratory chow (MF, Oriental Yeast Co., Ltd., Japan) was always available in the home cages.

Apparatus

The experiment was conducted in a conventionally illuminated room where 8 drinking cages and 4 activity wheels were located. The drinking cages on a table were copies of the home cages, and the inner dimensions of each were 20 cm wide, 25 cm long, and 18.7 cm high. Fluid was provided via a glass bottle with a metal spout inserted from the cage ceiling. The end of the spout was positioned 16.5 cm above the cage floor. When two bottles were used, they were positioned 8 cm apart. The fluid in each bottle was tap water, 20% sucrose solution, or 0.2% sodium saccharin solution. The semi-handmade wheels were hung in a line on a wire net on a wall of the experimental room, 140 cm above the room floor, and adjacent wheels were spaced 20 cm apart (side to side). The inner dimensions of each wheel were 15 cm wide and 30 cm in diameter. The wheel walls were perforated metal sheets, and the running surface was made of 2-mm 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. The force necessary to initiate wheel movement was 15 g at the circumference of the wheel.

Procedure

All experimental sessions were conducted with four squads (or sets) of 8 rats, each at a regular time during the lighted phase on successive days. Each rat was initially adapted to consuming tap water in the drinking cage for 3 days. On the first day (Day 1), a water bottle was offered for 30 min. On the remaining 2 days (Days 2–3), two bottles were concurrently presented for 15 min per day: the left bottle contained tap water and the right bottle was empty on Day 2, but the locations of the bottles were interchanged on Day 3. This two-bottle training was conducted with the intention of preparing the rats for the choice test at the end of this experiment. The rats were then assigned to one of four groups of equal number (each $n = 8$) matched for their amount of water intake and bodyweights.

On the next day (Day 4), a group of rats was allowed access to a bottle of 20% sucrose solution for 15 min immediately followed by an intraperitoneal injection of 12.64 mmol/L cyclophosphamide at 1% of bodyweight (i.e. 33.0 mg/kg, Group Suc-Cyc). Another group of rats received identical treatment except that the bottle contained 0.2% sodium saccharin solution (Group Sac-Cyc). These groups were watered for 15 min in the home cages 2 h after the session to facilitate recovery from illness. Over the next 4 days (Days 5–8), these two groups of rats were offered the taste solutions employed on the poisoning day without further poisoning (one-bottle extinction treatment). The 15-min post-session watering was also administered on these days as it was on the poisoning day.

The third and fourth groups of rats were also allowed to drink the 20% sucrose solution and 0.2% sodium saccharin solution, respectively, but the 15-min drinking episode was immediately followed by a 20-min opportunity to run in the activity wheel (Groups Suc-Run and Sac-Run). This pairing treatment lasted for 5 days (Days 4–8). These rats were also watered for 15 min in the home cages 2 h after each daily session, as were Groups Suc-Cyc and Sac-Cyc, to equate the thirst levels across the groups.

Two-bottle choice testing was administered on the next day (Day 9) for all rats. One bottle contained the target solution (i.e. the sucrose solution for Groups Suc-Cyc and Suc-Run; the saccharin