Appetite 83 (2014) 178–184

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Research report

Running-based pica in rats. Evidence for the gastrointestinal discomfort hypothesis of running-based taste aversion ☆

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ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 18 August 2014
Accepted 23 August 2014
Available online 27 August 2014

Keywords:
Conditioned taste aversion
Wheel running
Pica
Kaolin
Lithium chloride
Rats

ABSTRACT

Voluntary running in an activity wheel establishes aversion to paired taste in rats. A proposed mechanism underlying this taste aversion learning is gastrointestinal discomfort caused by running. We tested this hypothesis by measuring the pica behavior (kaolin clay intake) of rats, because it is known that rats engage in pica behavior after various nausea-inducing treatments including irradiation, motion sickness, and injection of emetic drugs such as lithium chloride (LiCl). Following a demonstration of the already-known phenomenon of LiCl-based pica in Experiment 1, we successfully showed running-based pica behavior in Experiment 2 where the running treatment was compared with a non-running control treatment (i.e., confinement in a locked wheel). These results suggest that not only LiCl but also running induces nausea in rats, supporting the gastrointestinal discomfort hypothesis of running-based taste aversion learning.

Introduction

Lett and Grant (1996) reported that voluntary running in an activity wheel establishes Pavlovian conditioned taste aversion (CTA) in laboratory rats to a substance consumed shortly before running. Running-based CTA has been replicated in their own and other researchers' laboratories (e.g., Heth, Inglis, Russell, & Pierce, 2001; Lett, Grant, & Gaborko, 1998; Nakajima, Hayashi, & Kato, 2000), and subsequent studies have revealed many features of this relatively new Pavlovian conditioning phenomenon (see Boakes & Nakajima, 2009, for a review).

One of the major hypotheses that addresses the underlying physiological mechanism of running-based CTA was proposed by John Garcia in a personal communication to Lett, Grant, Koh, and Parsons (1999), where he ascribed the physiological cause of this phenomenon to gastrointestinal discomfort (e.g., nausea) induced by running. Now, we have at least three pieces of evidence for this hypothesis.

First, Eccles, Kim, and O'Hare (2005) reported prevention of running-based CTA by an anti-emetic drug (granisetron) injection, suggesting that nausea plays a major role in establishing running-based taste aversion. Second, Nakajima, Urata, and Ogawa (2006) demonstrated that running-based CTA is alleviated not only by preexposure to running but also by prior injection of lithium chloride (LiCl), which is the most popular nausea-inducing drug in rat CTA studies (Riley & Freeman, 2004). This finding implies that a common process (presumably nausea) is physiologically habituated by preexposure. Third, by analyzing the microstructure of rats' licking, Dwyer, Boakes, and Hayward (2008) measured the change in the palatability of the taste solution paired with running, LiCl, or a rewarding drug (amphetamine), and they found that a reduction in taste palatability accompanies running- and LiCl-based CTAs but not amphetamine-based CTA. They concluded that running- and LiCl-based CTAs are commonly caused by nausea, while the dopamine system plays a major role in amphetamine-based CTA.

In the present study, we provide another piece of evidence for the gastrointestinal discomfort hypothesis of running-based CTA by measuring the pica behavior of rats. Almost 40 years ago, Mitchell and his colleagues found that geophagia (consumption of soil or kaolin clay) is generated in a majority of rats by a nausea-inducing drug (injection of LiCl or cyclophosphamide, or intragastric intubation of a rodenticide: Mitchell, Beatty, & Cox, 1977; Mitchell et al., 1976), or by rotation-induced motion sickness (Mitchell, Krusemark, & Hafner, 1977; Mitchell, Laycock, & Stephens, 1977). According to

☆ *Acknowledgements:* We thank Takefumi Kikusui for directing our attention to nausea-based pica behavior in rats. This study was supported by JSPS KAKENHI (24530931) to the first author, who designed the experiments and prepared the manuscript. The second author administered the experiment and collected the data in partial fulfillment of requirements for the BA degree in Psychology under the close supervision of the first author. The data were analyzed by the first author. This research project and the animal facility were approved by the Animal Care and Use Committee of KGU, based on a Japanese law (the Act on Welfare and Management of Animals) and the guideline published by the Science Council of Japan (the Guidelines for Proper Conduct of Animals Experiments) in 2006, which is shown in the following web site: http://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-20-k16-2e.pdf

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http://dx.doi.org/10.1016/j.appet.2014.08.031
0195-6663/

Mitchell et al. (1976), quantification of gastrointestinal discomfort in the rat is easily achieved when we use this aberrant pica behavior.

Nausea-based pica behavior has been replicated by other researchers with a variety of emetogenic drugs including LiCl (e.g., McCutcheon, Ballard, & McCaffrey, 1992; Watson & Leitner, 1988), cyclophosphamide (e.g., Tohei, Kojima, Ikeda, Hokao, & Shinoda, 2011; Yamamoto, Nakai, Nohara, & Yamatodani, 2007), morphine (e.g., Aung, Mehendale, Xie, Moss, & Yuan, 2004), apomorphine (e.g., Takeda, Hasegawa, Morita, & Matsunaga, 1993; Takeda et al., 1995a), nicotine (Yamamoto, Ngan, Takeda, Yamatodani, & Rudd, 2004), copper sulfate (e.g., Hasegawa et al., 1992; Takeda et al., 1993), cisplatin (e.g., Liu, Malik, Sanger, Friedman, & Andrews, 2005; Takeda et al., 1993, 1995b), 2-deoxy-D-glucose (2DG: e.g., Watson & Leitner, 1988; Watson et al., 1987), ritonavir (e.g., Aung et al., 2005), cholecystokinin octapeptide (CCK-8: McCutcheon et al., 1992), actinomycin D (Yamamoto et al., 2007), 5-fluorouracil (Yamamoto et al., 2007), and ethanol (in a case of gavage administration: Constancio, Pereira-Derderian, Menani, & De Luca, 2011), as well as irradiation (e.g., Yamamoto, Takeda, & Yamatodani, 2002) and motion sickness (e.g., McCaffrey, 1985; Morita, Takeda, Kubo, & Matsunaga, 1988).

Furthermore, pica caused by these agents is attenuated by anti-emetic drugs. For example, Takeda et al. (1993) demonstrated that domperidone and ondansetron, respectively, inhibit apomorphine- and cisplatin-based pica in rats. Notably, prevention of cisplatin-based pica has been extensively explored, because cisplatin is one of the most frequently used drugs in cancer chemotherapy. The list of drugs that have been shown to attenuate cisplatin-based pica includes ondansetron (Malik, Liu, Cole, Sanger, & Andrews, 2007; Takeda et al., 1993, 1995b), granisetron (Han et al., 2014; Yamamoto et al., 2014), diphenidol (Takeda et al., 1995b), thalidomide (Han et al., 2014), dexamethasone (Malik et al., 2007; Rudd, Yamamoto, Yamatodani, & Takeda, 2002), tachykinin NK_1-receptor antagonists (HSP-117: Saeki et al., 2001; GR205171: Malik et al., 2007), several antioxidants (Sherma, Gupta, Kochupillai, Seth, & Gupta, 1997), and herbal medicines (Aung et al., 2003, 2005; Mehendale et al., 2004, 2005; Qian et al., 2011; Raghavendran et al., 2011; Wang et al., 2005). Pica caused by cyclophosphamide, morphine, apomorphine, ritonavir, irradiation, and motion sickness has also been attenuated by anti-emetics (e.g., Aung et al., 2004; Morita et al., 1988; Takeda et al., 1995a; Tohei et al., 2011; Yamamoto et al., 2002; Yuan et al., 2009).

Therefore, we now have ample evidence to consider that pica behavior is a good tool for measuring nausea in rats. The present study, thus, uses pica as an index of nausea to test the hypothesis that running-based taste aversion learning is mediated by nausea. Because we had not conducted any research on pica behavior in rats, the first experiment of the present study aimed to replicate rats' pica with a nausea-inducing drug (LiCl), before conducting the second experiment, which would explore whether running produces pica in rats. If pica is caused not only by LiCl injection but also by wheel running in similar settings in the same laboratory, we may then have a strong piece of evidence that LiCl and running share a similar physiological process (seemingly nausea) in taste aversion learning.

Experiment 1

The aim of Experiment 1 was to ensure nausea-based pica in our laboratory. We used LiCl as the nausea-inducing drug in this experiment, not only because it has been used in nausea-based pica studies (McCutcheon et al., 1992; Mitchell et al., 1976; Watson & Leitner, 1988; Yamamoto et al., 2004), but also because it is the most conventional emetogenic drug in rat taste aversion studies (see Riley & Freeman, 2004, for a database). A collateral aim of Experiment 1 was to set up a proper procedure for measuring rats' pica behavior in our laboratory, by emulating the techniques of previous reports on nausea-based kaolin intake in rats. As rats chew pellets into small pieces, we needed a couple of days to refine the procedure of collecting the kaolin splinters for measuring precisely the amount of kaolin intake.

Method

Subjects and apparatus

Eight experimentally naïve male rats (Slc: Wistar/ST) were housed in individual hanging wire home cages (20 cm wide, 25 cm long, and 18.7 cm high) in a vivarium on a 16:8-h light–dark cycle (lights on at 0800 h) at 23 °C and 55% humidity. The animals were 9–10 weeks old on the first day of this experiment, and they were maintained with food pellets, tap water, and kaolin pellets available ad libitum throughout the experiment.

The food pellets (MF diet; Oriental Yeast Co., Tokyo, Japan) were placed in a stainless container positioned inwards with its end apertures 3.5 cm above the cage floor. The tap water was accessible from a stainless needle-pin nozzle protruding through a hole in the center of the back wall of each cage. The kaolin pellets were made of kaolin powder (Shin Nihon Zokei Co., Tokyo, Japan) and gum arabic (Holbein Works, Ltd., Osaka, Japan) at a 99:1 (w/w) ratio; they were mixed with tap water to form cylindrical pellets and were completely dried at room temperature. Each day, three or four kaolin pellets (about 20–25 g in total) were presented to each rat in a stainless steel bowl (8 cm in diameter and 3.5 cm deep) clipped to the cage wall at floor level with an iron hoop holder. A plastic tray (22.5 cm wide, 32 cm long, and 5.5 cm deep) with paper bedding was positioned 10 cm below each cage to collect excreta, food shatters, and, most importantly, kaolin splinters. Split kaolin and crushed food were collected with a spoon and chopsticks, dried for a day, segregated, and weighed to obtain correct amounts of kaolin and food intake.

Procedure

Each day, the food and kaolin containers were removed at 1200 h, weighed with an electric balance (BJ-1500, Sartorius, K.K., Tokyo, Japan) to the nearest 0.1, refilled, and replaced at 1300 h. In short, we recorded the amounts of food and kaolin consumed in the preceding 23-h period every day. The rats were also weighed in the vivarium with an electric balance (KS-251, Dretec Co., Koshigaya, Japan) to the nearest 1 g between 1200 and 1300 h.

After a six-day baseline phase, half of the rats were given an intraperitoneal (i.p.) injection of 0.15 M LiCl at 1% body weight (i.e., 63.6 mg/kg) while the remaining rats received physiological saline of the same amount. Three days later, the roles of the experimental and control treatments were exchanged: the formerly LiCl-injected rats were now given an i.p. injection of saline, and vice versa. After an additional three baseline days, the group roles were changed again, but the dose of LiCl and saline was doubled to 2% body weight. Finally, three days later, the treatments were switched using the same dose, followed by two non-treatment days.

All statistical analyses in this and the following experiments are based on an alpha level set at $p < .050$.

Results and discussion

Figure 1 illustrates the kaolin intake of the two groups of rats across days. The group names stand for the order of treatment: Group LiCl-Sal received LiCl and then saline, while Group Sal-LiCl was given saline and then LiCl. The data are shown from the fourth day of the initial baseline phase, because we needed three days to establish a procedure for precisely measuring the rats' kaolin intake in our laboratory.