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A DIET PROMOTING SUGAR DEPENDENCY CAUSES BEHAVIORAL CROSS-SENSITIZATION TO A LOW DOSE OF AMPHETAMINE

N. M. AVENA AND B. G. HOEBEL*

Princeton University, Department of Psychology, Green Hall, Washington Road, Princeton, NJ 08544, USA

Abstract—Previous research in this laboratory has shown that a diet of intermittent excessive sugar consumption produces a state with neurochemical and behavioral similarities to drug dependency. The present study examined whether female rats on various regimens of sugar access would show behavioral cross-sensitization to a low dose of amphetamine. After a 30-min baseline measure of locomotor activity (day 0), animals were maintained on a cyclic diet of 12-h deprivation followed by 12-h access to 10% sucrose solution and chow pellets (12 h access starting 4 h after onset of the dark period) for 21 days. Locomotor activity was measured again for 30 min at the beginning of days 1 and 21 of sugar access. Beginning on day 22, all rats were maintained on *ad libitum* chow. Nine days later locomotor activity was measured in response to a single low dose of amphetamine (0.5 mg/kg). The animals that had experienced cyclic sucrose and chow were hyperactive in response to amphetamine compared with four control groups (*ad libitum* 10% sucrose and chow followed by amphetamine injection, cyclic chow followed by amphetamine injection, *ad libitum* chow with amphetamine, or cyclic 10% sucrose and chow with a saline injection). These results suggest that a diet comprised of alternating deprivation and access to a sugar solution and chow produces bingeing on sugar that leads to a long lasting state of increased sensitivity to amphetamine, possibly due to a lasting alteration in the dopamine system.

Key words: sucrose, locomotion, eating disorders, addiction, rat.

Animals sensitized to a particular drug will often show an increased locomotor response to a different drug of the same class. This phenomenon, known as cross-sensitization, has been demonstrated with several drugs of abuse (Greenberg and Segal, 1985; Kalivas and Weber, 1988; Schenk et al., 1991; Pierce and Kalivas, 1995; Itzhak and Martin, 1999; Itzhak et al., 1999; Pontieri et al., 2001). Other studies have reported cross-sensitization between drugs of abuse and other non-drug events, such as stress (Antelman and Caggiula, 1977; Prasad et al., 1998; Covington and Miczek, 2001), sexual behaviors (Fiorino and Phillips, 1999a,b; Nocjar and Panksepp, 2002) and consumption of palatable food (Bakshi and Kelley, 1994). It is believed that enhanced mesolimbic dopaminergic or opioid neurotransmission plays a major role in the behavioral effects of drug sensitization as well as cross-sensitization, and may be a factor contributing to drug addiction (Robinson and Berridge, 1993).

Sugar has been shown to have behavioral and neural effects similar, in some ways, to drugs of abuse. Colantuoni et al. (2001) found that rats maintained on a diet of intermittent access to sugar and chow developed a pattern of excessive intake with bingeing during the first hour of daily access. Increased D1 and μ-opioid receptor binding and decreased D2 receptor binding in the nucleus accumbens (NAc) were observed. Also, somatic, behavioral and neurochemical evidence of withdrawal from sugar was seen in these animals (Colantuoni et al., 2002). Rats sensitized to amphetamine demonstrate hyperactivity in response to a taste of sugar, suggesting that sugar may be working on the same neural system as amphetamine (Avena and Hoebel, 2003). Since the behavioral and neurochemical adaptations observed in sugar dependence are similar to those that occur during psychostimulant sensitization, it was hypothesized that sugar-dependent rats would have a heightened sensitivity to amphetamine.

EXPERIMENTAL PROCEDURES

Animals and equipment

Sixty female Sprague–Dawley rats weighing 225–250 g were obtained from Taconic Farms (Germantown, NY, USA) and housed individually on a reversed 12-h light/dark cycle. Water was available *ad libitum* throughout the experiment. Locomotor activity was measured in a computerized 43.2×43.2 cm, open-field activity chamber with 30.5 cm high acrylic sidewalls and 16 infrared photocells on each of the three axes (MED Associates, Georgia, VT, USA). All procedures were carried out in accordance with the National Institutes of Health Guide for the Use and Care of Laboratory Animals and the Princeton University Institutional Animal Care and Use Committee. Efforts were made to use the minimal amount of rats necessary to conduct the experiment and reduce their suffering.

Locomotor activity measures

All locomotor activity tests were conducted between the fourth and fifth hour of the dark period. Baseline locomotor activity measurement was obtained on day 0. Animals were allowed to habituate to the activity chamber for 15 min and then were administered an i.p. injection of saline. Fifteen minutes later activity counts were measured for 30 min. Counts were quantified as the number of infrared beam breaks. Beginning on day 1, subjects were divided into matched groups and maintained on the following diets for the next 21 days: cyclic 10% sucrose (10% w/v in water) and chow (12 h of deprivation followed by 12-h access to 10% sucrose and standard rodent chow beginning 4 h into the dark cycle; $n=15$), cyclic chow (12 h of deprivation followed by 12-h access to chow beginning 4 h

*Corresponding author. Tel: +1-609-258-4463; fax: +1-609-258-1113.
E-mail address: hoebel@princeton.edu (B. Hoebel).
Abbreviations: DA, dopamine; NAc, nucleus accumbens.

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into the dark cycle; $n=15$), *ad libitum* 10% sucrose and chow ($n=15$) and *ad libitum* chow ($n=15$). Locomotor activity measures in response to chow and sucrose intake were taken on day 1 as follows. Before the locomotor test, the experimenter would watch each subject and start and stop a timer while the animal was eating or drinking. For animals on cyclic diets, when sucrose and/or chow was presented for the first time that day, and the animal began to drink sucrose (in the case of the cyclic 10% sucrose and chow group) or eat chow (in the case of the cyclic chow group), another timer would then begin for 3 min. Each animal could drink or eat for a total of 1 min, but could take no longer than 3 min to do so. This procedure was followed to mimic a drug injection. For animals on *ad libitum* diets of 10% sucrose and chow, or just chow, the procedure was followed at the same time of day. All subjects were immediately placed in the locomotor activity chamber, and 15 min later activity counts were measured for 30 min. Animals were then returned to their home cages and treated according to their group designation for the next 21 days. On day 21, another locomotor activity measure was taken in the manner described above. The next day (day 22) feeding regimens ceased and all animals were maintained on *ad libitum* chow for the next 9 days. On day 29, subjects were returned to the activity cages to habituate for 15 min. Each of the four groups was subdivided, with half administered 0.5 mg/kg amphetamine sulfate (Supreme Pharmaceutical Co., New York, NY, USA) i.p. ($n=8$/group), or equal volumes of saline ($n=7$/group). Fifteen min later, locomotor activity was measured for 30 min.

Data analysis

Locomotor activity counts were normalized to baseline (day 0) for each rat by calculating the percent change in locomotion counts (beam breaks). Locomotor activity data were analyzed with a two-way ANOVA (diet group×day) with post hoc Dunnett multiple comparison tests. A one-way ANOVA was used to analyze time spent drinking or eating in the 3 min prior to activity tests. *t*-Tests were used to compare locomotor activity differences between drug treated versus saline treated animals in the same diet group. Body weights were analyzed by a one-way ANOVA and *t*-tests. The null hypothesis was rejected at $P<0.05$.

RESULTS

All rats in the experiment tasted the sugar or chow during the period prior to the locomotor activity tests (for the cyclic groups, this was when the sugar and/or chow were first presented that day). There was no difference between groups that would receive amphetamine or saline in the time spent drinking sucrose or eating chow prior to locomotor activity tests on day 1 ($F(3,29)=0.24$, n.s., $F(3,29)=0.54$, n.s., respectively) nor on day 21 ($F(3,29)=0.16$, n.s., $F(3,29)=0.28$, n.s., respectively). The main effect was a significant interaction between diet group and day ($F(21,99)=4.36$, $P<0.01$). Rats maintained on a diet of cyclic 10% sucrose and chow were hyperactive in response to the low dose of amphetamine compared with rats in any of the other three groups that were also given amphetamine ($P<0.01$; Fig. 1a) or compared with rats on the same diet administered saline ($t(13)=4.71$, $P<0.001$; Fig. 1b). Rats maintained on cyclic chow that later received amphetamine were hyperactive compared with cyclic chow with saline as a control ($t(13)=2.5$, $P<0.03$), but these animals were not hyperactive compared with any other group receiving amphetamine. There was no difference in body weights on the day of the challenge injection between groups or between treatments ($F(3,29)=0.67$, n.s., $t(13)=0.4$, n.s., respectively).

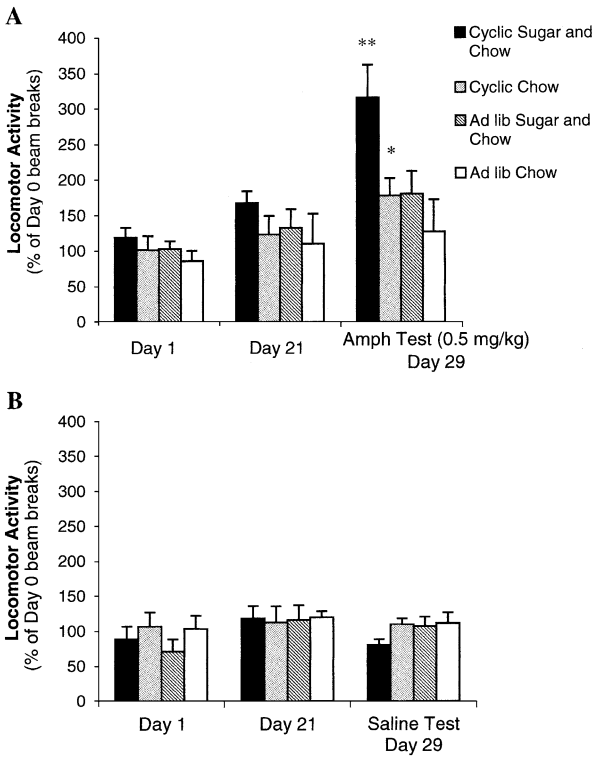

Fig. 1. Locomotor activity in a photocell cage plotted as percent of baseline beam breaks. Rats were on the indicated diet regimen (see Key) for 21 days. A) Rats maintained on a diet of cyclic sugar and chow are hyperactive nine days later in response to a low dose of amphetamine compared with control diet groups (** $P<0.01$). Rats maintained on a diet of cyclic chow are hyperactive in response to amphetamine compared with saline (* $P<0.001$), but not compared with other groups that received amphetamine. B) Control rats injected with saline show no hyperactive responses.

DISCUSSION

The results of this experiment suggest that sugar and amphetamine may be working via the same neural systems, as evidenced by cross-sensitization. These results are similar to those obtained with drugs of abuse (Greenberg and Segal, 1985; Kalivas and Weber, 1988; Schenk et al., 1991; Pierce and Kalivas, 1995; Itzhak and Martin, 1999; Itzhak et al., 1999; Pontieri et al., 2001). This concept has been previously demonstrated in our laboratory with animals first sensitized to amphetamine becoming hyperactive in response to a taste of sugar (Avena and Hoebel, 2003), as well as by others who have shown a relationship between sucrose and amphetamine (Sills and Vaccarino, 1996; DeSousa et al., 2000; Wyvell and Berridge, 2001; Vitale et al., 2003).

Repeated exposure to indirect dopamine (DA) agonists, such as amphetamine, can be timed to produce a state of intermittent DA release following a period of low basal DA transmission (Imperato et al., 1996; Weiss et al., 1997). Intermittent activation of the mesolimbic DA system,

in the context of low DA transmission, may facilitate drug sensitization (Koob and Le Moal, 2001). Supersensitive D1 receptors in the NAc are thought to be involved in the long-term persistence of the sensitized response (Henry and White, 1991, 1995). Palatable foods can release both opioids (Apfelbaum and Mandenoff, 1981; Morley and Levine, 1982; Sclafani et al., 1982; Siviy et al., 1982; Morley et al., 1983; Levine et al., 1985; Tanda and Di Chiara, 1998) and DA (Hernandez and Hoebel, 1988a,b; Radhakishun et al., 1988; Salamone et al., 1994; Tanda and Di Chiara, 1998). Our model of sugar dependency (daily intermittent access to sugar solution and chow) has been shown to produce increased D1 receptor binding in the NAc (Colantuoni et al., 2001). In the present study, a low dose of amphetamine, a known DA agonist, may be releasing DA in a manner that stimulates these up-regulated D1 receptors in sugar-dependent animals, resulting in the increased locomotor response.

It is notable that cross-sensitization was observed a week after the sugar sensitizing procedure was stopped. This suggests that the underlying cause, be it D1 up-regulation or other changes, lasts for at least a week. Another interesting result is that rats maintained on cyclic chow were hyperactive in response to amphetamine compared with saline. The intermittent activation of the DA system due to daily bingeing on chow may have produced a sensitized state, although not as profoundly as in the rats maintained on cyclic sugar and chow.

Previous research from this laboratory has focused on sugar dependency in male rats. The present experiment extends these finding to females. The behavioral paradigm of cyclic 10% sucrose and chow shares some aspects with the pattern of behavior self-imposed by people diagnosed with binge-eating disorder or bulimia nervosa. Bulimics often restrict intake early in the day and then binge later in the evening, usually on palatable high-fat foods (Drewnowski et al., 1992; Gendall et al., 1997). The neurochemical changes that occur under the present feeding regimen, such as increased D1 and μ-opioid receptor binding and decreased D2 receptor binding in the NAc (Colantuoni et al., 2001), could reflect underlying molecular changes that have been proposed to cause addiction (Nestler and Aghajanian, 1997). This same process may play a role in the increased comorbidity with drug abuse in individuals with eating disorders (Mitchell et al., 1991; Brewerton et al., 1995). The present results suggest that a diet comprised of intermittent access to sugar and chow produces a state that makes rats sensitive to amphetamine. Since this suggests a relationship between the neural and behavioral effects of foods and drugs, it may be important to monitor sugar intake in patients recovering from drug addiction, and vice versa, drugs in people who claim to be dependent on sugar.

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