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Baclofen differentially mediates fructose-conditioned flavor preference and quinine-conditioned flavor avoidance in rats

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ABSTRACT

Rats display both fructose-conditioned flavor preference (CFP) and quinine conditioned flavor avoidance (CFA). Dopamine (D_1 and D_2), muscarinic and nicotinic, but not NMDA or opioid receptor antagonists reduced fructose-CFP expression. Dopamine D_1, dopamine D_2, muscarinic or NMDA, but not opioid or nicotinic receptor antagonists reduced fructose-CFP acquisition. Dopamine D_1, NMDA, nicotinic or opioid, but not dopamine D_2 or muscarinic receptor antagonists enhanced quinine-CFA acquisition. Baclofen (BAC), a $GABA_B$ receptor agonist, alternately enhances or reduces feeding under specific conditions. The present study examined whether systemic BAC administration mediated fructose-CFP expression and acquisition or quinine-CFA acquisition. Fructose-CFP expression studies trained rats with one flavor (CS+) in 8% fructose and 0.2% saccharin and a second (CS−) flavor in 0.2% saccharin, followed by vehicle (VEH) and BAC (0.5–5 mg/kg) preceding 2-bottle (CS+, CS−) 0.2% saccharin choice tests. Fructose-CFP acquisition studies administered VEH or BAC (3 or 5 mg/kg) prior to CS+ and CS− training sessions followed by six 2-bottle (CS+, CS−) 0.2% saccharin choice tests. Quinine-CFA acquisition studies administered VEH or BAC (3 or 5 mg/kg) prior to CS− (8% fructose+0.2% saccharin) and CS+ (fructose+saccharin+0.030% quinine) training sessions followed by six 2-bottle (CS−, CS+) fructose+saccharin choice tests. BAC (3 mg/kg) minimally (66%) reduced fructose-CFP expression. BAC failed to alter fructose-CFP acquisition. Quinine-CFA acquisition was enhanced by the 5 mg/kg BAC dose (15–25%) relative to VEH (34–48%). These data implicate $GABA_B$ receptor signaling in acquisition of quinine avoidance with minimal or no effects upon fructose preferences.

1. Introduction

Flavor cues guide rodent learning of food preferences (e.g., sugar-conditioned flavor preferences (CFP): Capaldi, 1996; Sclafani, 1995) and avoidance of aversive tastes (e.g., quinine-conditioned flavor avoidance (CFA): Dwyer, 2011; Fanselow and Birk, 1982). Acquisition and expression of flavor-flavor conditioning was examined for sucrose in sham-feeding rats (Yu et al., 1999, 2000), and for fructose in real-feeding rats (Baker et al., 2003, 2004). Dopamine D_1 or D_2 receptor antagonists eliminated acquisition and expression of fructose- and sucrose-CFP (Baker et al., 2003; Yu et al., 2000). Whereas NMDA receptor antagonism eliminated fructose-CFP acquisition, but not expression (Golden and Houpt, 2007), cannabinoid CB_1 receptor interference reduced fructose-CFP expression, but not acquisition (Miner et al., 2008). Although muscarinic and nicotinic cholinergic receptor antagonists reduced fructose-CFP expression, scopolamine, but not mecamylamine eliminated fructose-CFP acquisition (Rotella et al., 2015). Opioid receptor antagonism failed to alter flavor-flavor-mediated sugar-CFP (Baker et al., 2004; Yu et al., 1999). Quinine-CFA acquisition was enhanced following dopamine D_1, NMDA, opioid and nicotinic cholinergic, but not dopamine D_2 or muscarinic cholinergic receptor antagonists (Rotella et al., 2014, 2015).

γ-Aminobutyric acid (GABA) or its agonizts administered into limbic and hypothalamic sites increase food intake (e.g., Arnt and Scheel-Kruger, 1979; Echo et al., 2002; Grandison and Guidotti, 1977; Soderpalm and Berridge, 2000; Stratford and Kelley, 1997; Ward et al., 2000; Wirtshafter et al., 1993). Feeding elicited by the $GABA_B$ agonist, baclofen (BAC) is mediated through GABA receptor interactions between the ventral tegmental area (VTA) and nucleus accumbens (NAC: Miner et al., 2010). Indeed, brain dopamine and cholinergic systems modulate medium spiny NAC GABA

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output and VTA dopamine output (see reviews: Avena and Rada, 2012; Hoebel et al., 2007). Although peripheral BAC increased rodent chow and fat intake under specific dose regimens and intake conditions (e.g., Bains and Ebenezer, 2013; Ebenezer and Patel, 2011), it decreased food intake in diabetic and diet-induced obese mice (Sato et al., 2007). Peripheral BAC also selectively reduced fat intake under normal, limited-access and "binge-type" conditions (as well as intakes of pure fat or a sugar-fat mixture (Avena et al., 2014; Berner et al., 2009; Buda-Levin et al., 2005; Corwin and Wojnicki, 2009; Rao et al., 2008; Wojnicki et al., 2014; Wong et al., 2009 but see Covelo et al. (2014)). Given the complex effects of $GABA_B$ receptor signaling on feeding, the present study investigated whether systemic BAC mediated expression and acquisition of fructose-CFP as well as quinine-CFA in rats.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats (n=74, 250–275 g), obtained from Charles River Laboratories (Wilmington, MA), were housed individually in wire mesh cages, maintained on a 12:12 h light/dark cycle (lights on: 7 AM, lights off: 7 PM) at a constant ambient temperature of 22 °C with chow (5001, PMI Nutrition International, Brentwood, MO) and water available *ad libitum* for the first week. All animals were then food-restricted to 85–90% of their body weight throughout behavioral testing to insure short-latency responses to presentation of the training and test solutions. Food rations were provided 1 h after the end of daily training and testing sessions. The experimental protocols were approved by the Queens College Institutional Animal Care and Use Committee certifying that all subjects and procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Fructose-CFP initial training and test solutions

During initial training in the fructose-CFP paradigm, rats were trained to drink an unflavored 0.2% sodium saccharin (Sigma Chemical Co., St. Louis, MO) solution during five daily 1-h sessions to guarantee sampling as previously described (Baker et al., 2003, 2004); this initial unflavored training solution was the same concentration as the flavored CS− solutions used in the subsequent conditioning paradigms. The sipper tube was mounted on the front of the cage held by a taut steel spring, and was positioned 3–6 cm above the cage floor. Solution measurement (0.1 ml gradations and accuracy) was insured by using a retrofitted testing sipper tube that has been previously validated (Baker et al., 2003, 2004; Rotella et al., 2014, 2015; Yu et al., 1999, 2000). This training procedure was repeated daily until all rats approached the sipper tubes with short (<1 min) latency, typically within three days. The limited food rations were given 1 h after each training session.

The two training solutions in the *fructose-CFP expression and acquisition* studies were an 8% fructose+0.2% saccharin solution and a saccharin (0.2%) solution, each flavored with 0.05% unsweetened grape or cherry Kool-Aid (General Foods, White Plains, NY). The 8% fructose+0.2% saccharin-paired flavor is referred to as the CS+/Fs, and the 0.2% saccharin-paired flavor as the CS−/s. Half of the rats in each drug paradigm had the cherry flavor added to the CS+/Fs solution and the grape flavor added to the CS−/s solution; flavors were reversed for the remaining rats. In all two-bottle preference choice tests for fructose-CFP, the cherry and grape flavors were presented in 0.2% saccharin solutions (CS+, CS−). All training and testing in both paradigms took place in the rat's home cage during the mid-light phase (~11 AM–4 PM) of the light/dark cycle.

2.3. BAC and fructose-CFP expression

Seventeen rats were given ten daily 1-bottle training sessions (0.5 h/day) with 16 ml of the CS+/Fs solution presented on odd-numbered days, and 16 ml of the CS−/s solution presented on even-numbered days. On days 9 and 10, the rats had access to a second sipper tube containing water. This familiarized the rats to the presence of two sipper tubes used during the choice tests; water intake was negligible in these training trials. The left-right position of the CS and water sipper tubes was counterbalanced over the two days. The order of presentation of the CS+/Fs first followed by subsequent presentation of the CS−/s solution during training was identical to that used in our and other previous studies examining the pharmacological substrates of fructose-CFP (Baker et al., 2004; Golden and Houpt, 2007). Following training, the rats were given eight 2-bottle choice test sessions (0.5 h/day) with unlimited (~45 ml) access to the CS+ and CS− flavors mixed in 0.2% saccharin solutions. Solution intakes during training and testing were measured by weighing (0.1 g) the bottles before and after the sessions. The animals were limited to eight 2-bottle sessions because previous research (Baker et al., 2003, 2004; Yu et al., 2000) demonstrated that the magnitude of the preference did not change during this testing interval. Therefore, each animal received vehicle (VEH), and three BAC doses. All 17 rats initially received a pair of VEH injections that were used to match the animals across subsequent pairs of BAC doses of 0.5 (n=14), 1.5 (n=15), 3 (n=14) and 5 (n=8) mg/kg 30 min prior to the two-bottle choice test. The 30-min interval for systemic BAC administration prior to the experimental condition in this and the other two paradigms was based on this commonly-used interval in many other systemic studies. Thus, all groups of rats were tested in two consecutive daily sessions at VEH and three drug doses with the left–right position of the CS+ and CS− solutions counterbalanced across sessions to control for position effects. To control for drug dose order effects, half of the rats in each group were tested with an ascending dose order, and the remaining rats were tested with a descending dose order.

2.4. BAC and fructose-CFP acquisition

Three groups of rats, matched for their intakes of the unflavored 0.2% saccharin solution prior to training, were given ten 1-bottle training sessions (1 h/day) with 16 ml of the CS+/Fs solution presented on odd-numbered days, and 16 ml of the CS−/s solution presented on even-numbered days. The first group (VEH) of eight rats received daily VEH injections 30 min prior to each training session. The second (BAC 3) and third (BAC 5) groups received daily injections of BAC at doses of 3 (n=7) and 5 (n=7) mg/kg respectively, 30 min prior to each training session. Following training, all groups were given six daily 2-bottle choice sessions (1 h/day) with unlimited (~45 ml) access to the CS+ and CS− flavors mixed in 0.2% saccharin solutions; no drugs were administered prior to these sessions. The positions of the CS+ and CS− solutions were counterbalanced across sessions.

2.5. Quinine-CFA initial training and test solutions

During initial training in the quinine-CFA paradigm, rats were trained to drink an unflavored 8% fructose (Sigma Chemical Co.) and 0.2% sodium saccharin solution as previously described (Rotella et al., 2014, 2015); this initial unflavored training solution was the same concentrations as the flavored CS− solution used in the