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Research report

An analysis of licking microstructure in three strains of mice

A.W. Johnson *, A. Sherwood, D.R. Smith, M. Wosiski-Kuhn, M. Gallagher, P.C. Holland

Neurogenetics and Behavior Center, Department of Psychological and Brain Sciences, Johns Hopkins University, 3400 N Charles Street, Baltimore, MD 21218, United States

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ABSTRACT

Mouse models of feeding provide a useful tool for elucidating the molecular pathways of energy regulation. The majority of studies in mice have been limited to intake analyses conducted over extended periods of time, which fail to distinguish between a variety of factors that influence nutrient intake. Using licking microstructure analyses we examined both the size and number of licking bursts for water, polycose, sucrose and lecithin in three strains of mice (C57BL/6J, 129Sv/ImJ and C57129F1 hybrids), using pause criteria (250–500, >500 and >1000 ms) that have previously been described in the rat. Burst size and number varied both as a function of tastant concentration and mouse strain; however, these differences were most evident with the >1000 ms pause criterion. Consistent with previous reports, during water consumption C57 mice showed longer mean interlick intervals, a larger number of bursts but reduced burst size relative to the two other strains. F1 mice showed larger burst sizes for polycose, while C57 mice displayed a greater number of bursts for both polycose and sucrose. Both 129 and F1 mice were insensitive to sucrose concentration, whereas C57 mice showed attenuated lecithin intake influenced by a reduction in the size of bursts for this tastant. These results suggest that these strains of mice display differences in the pattern of licking that are most evident with the use of larger pause criteria. These differences in licking behavior might reflect influences of genetic background on pre- and post-ingestive factors controlling intake, the reinforcing properties of each tastant, or native differences in licking style.

Introduction

Animal models provide an essential contribution for understanding the basic parameters that regulate components of energy balance. In mice, a variety of genetic models of obesity have proven useful for elucidating the molecular basis of energy regulation, with the hope that such studies will aid in the identification of new drug targets and novel therapeutic strategies (Robinson, Dinulescu, & Cone, 2000). However, the majority of studies concerning energy regulation and food intake in mice have been limited to intake analyses over longer time frames, which fail to distinguish between a variety of variables that are known to influence intake (Davis & Smith, 1992; Sclafani, Cardieri, Tucker, Blusk, & Ackroff, 1993). Here we describe a detailed analysis of licking microstructure, which provides an advantageous strategy by which to gauge the possible contributions of tastant palatability and post-ingestive inhibitory feedback on consumption (Smith, 2001).

Licking in rodents is a highly stereotyped behavior that involves the rhythmic cycling of tongue extensions and retractions thought to be under the control of a group of neurons in medulla oblongata nuclei V, VII and XII, which collectively function as a central pattern generator (Nakamura & Katakura, 1995; Norgren, 1995; Travers & Norgren, 1991). In microstructure analyses the rate of licking is defined by the interlick intervals (ILIs), where the majority of ILIs fall <250 ms and reflect continuous licking bursts (Davis & Smith, 1992). Longer pauses between bursts of licking appear to be relevant for dissecting the various components of meal intake. Davis (1996), Davis and Perez (1993) and Davis and Smith (1992) used two criteria to divide pauses. The pauses between 250 and 500 ms were thought to reflect brief interruptions of licking, such as lateral tongue movements (Grill & Norgren, 1978), whereas pauses >500 ms indicated longer interruptions of licking due to the active engagement of other competing behavior, such as grooming or leaving the food area. The number of licks occurring before the pause intervals defined the size of the licking bursts. This measure was unaffected by sham-feeding preparations and increased as a function of sucrose concentration, indicative of pre-ingestive influences (Davis & Smith, 1992). By contrast, increases in both burst and pause number were seen at 250–500 and >500 ms with sham-feeding preparations. Under normal feeding conditions, the number of bursts in a meal displayed an inverted U-shaped function of concentration with sucrose, reflecting post-ingestive negative feedback (Davis & Smith, 1992; Smith, 2001). Spector, Klumpp, and Kaplan (1998) conducted a systematic assessment to examine pause criteria for the study of licking behaviors in rats, deciding upon a >1000 ms pause criterion, whose burst size and

* Corresponding author.
E-mail address: awj@jhu.edu (A.W. Johnson).

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number were associated with pre- and post-ingestive factors, respectively. These results suggest that during the initial stages of sucrose intake, rats rapidly reinstate licking behavior following termination of a licking burst. This pattern leads to increases in both the number of pauses and bursts in the meal. Typically, as the meal progresses the frequency of these events decreases as influences of post-ingestive inhibitory feedback develop.

In the current experiments we evaluated strains of mice that are commonly used in genetic research: C57BL/6J (C57), 129Sv/ImJ (129), and C57129F1 (F1), a hybrid of the previous two. In particular, we were interested in assessing the pattern of licking in these different strains of mice with respect to microstructural variables of burst size and number using pause criteria that had previously been described in the rat (Davis & Smith, 1992; Spector et al., 1998). Previous studies suggest mouse strain differences in lick rates and its microstructure (Boughter, Baird, Bryant, St. John, & Heck, 2007; Glendinning, Feld, Goodman, & Bayor, 2008; Horowitz, Stephan, Smith, & Whitney, 1977). Horowitz et al. (1977) examined licking for water in C57, DBA, and an F1-hybrid strain. C57 mice exhibited the slowest lick rate, F1 an intermediate, and DBA the highest lick rate. More recent examinations with water confirmed slower lick rates and fewer bursts in C57 mice relative to D2, 129 and SWR strains (Boughter et al., 2007; Dotson & Spector, 2005; Glendinning et al., 2005).

Strain differences in licking have also been reported with nutritive tastants. C57 mice showed elevated initial lick rates for sucrose (Glendinning et al., 2008) and increased sucrose and polycose intake (Sclafani, 2006) relative to 129 mice (Glendinning et al., 2005, 2008; Sclafani, 2006). While the taste receptor that mediates glucose polymer taste is unknown, strain differences at low concentrations of sucrose are thought to reflect allelic variations to the T1R3 receptor (Glendinning et al., 2005; Inoue et al., 2007). These results suggest that variations in background genetic strain contribute to factors that influence meal intake. Moreover, targeted gene manipulations of specific neuropeptides (Fintini et al., 2005; Lakaye, Adamantidis, Coumans, & Grisar, 2004) have been shown to influence energy regulation and food intake; however, to date only a few studies have used licking microstructure to assess the role of neuropeptides on feeding behavior (e.g., Baird et al., 2006).

Here we examined lick patterns in C57, F1 and 129 mice during consumption of water and three tastants that differ in their nutritive and caloric properties. In addition to the total intake of each tastant, we examined the size and number of licking bursts. As part of our analysis of licking bursts, we compared three pause criteria (250–500, >500 and >1000 ms) used to define such bursts. In Experiment 1, we examined water consumption to provide an initial assessment of native licking style to a non-nutritive solution in these mice. Next, we assessed the patterns of licking of two commonly studied tastants, the complex polysaccharide polycose and the disaccharide sucrose (Experiments 2 and 3, respectively). Finally, we examined licking microstructure for lecithin (Experiment 4), a principal phospholipid that is used extensively in the food industry as a food additive but has yet to be assessed in animal models.

Methods

Subjects

A total of 66 male mice were used from three strains: C57BL/6J (C57), 129Sv/ImJ (129), and C57129F1 hybrids (F1), obtained from Jackson Laboratory (Bar Harbor, ME). In Experiment 1, eight F1 and 129, and six C57 mice were used, which prior to behavioral testing weighed 33.2 ± 1.5, 31 ± 1.3 and 32.5 ± 1.8 g, respectively. The same mice were also used in Experiment 2. In Experiment 3, seven C57 (23.7 ± 3.5 g) and F1 mice (26.6 ± 0.8 g), and six 129 mice (23.7 ± 1.1 g) from each strain were used. Finally, in Experiment 4, eight mice from each strain were used (weighing 24.3 ± 1.6, 22.6 ± 1.1, and 26.2 ± 1.1 g, respectively). Mice were transferred to the Neurogenetics and Behavior Center at Johns Hopkins University at 6–8 weeks of age and kept on a 12 h light:dark cycle, with lights off at 7 P.M. Following 2 weeks of acclimatization, mice were food-deprived to 85% of their free-feeding weight and were maintained at that weight for the duration of the experiment. Testing took place between the hours of 12 P.M.–5 P.M. Following testing, each mouse was subsequently fed a small food pellet prior to transportation back to the colony room (approximately 5 P.M.). All experiments were conducted under the auspices of the Johns Hopkins University Institutional Animal Care and Use Committee.

Apparatus

Behavioral training took place in eight identical chambers, which consisted of clear polycarbonate sides and ceiling, aluminum front and back walls, and floor comprised of parallel, stainless steel rods, all housed in sound-attenuating shells (Med Associates, St. Albans, VT). Chambers were outfitted with a custom-built food cup into which 0.05 ml of liquid reward could be delivered. Food cups were connected to programmable vacuums, which could suction off reward when desired. Infrared photocells installed in the food cup monitored the time spent and the number of entries into the cup. The food cups also contained custom lickometers (Schoenbaum, Garmon, & Setlow, 2001), which used fiber optics to introduce a light beam through the fluid–air interface of a fluid bolus. Licks were detected as disturbances in the amplified light surface at the interface when the fluid was contacted, permitting time-stamping of individual licks. We previously conducted an extensive set of parametric studies to validate the lickometer counts, using a comparison of lick count to slow-motion video of mouse licking behavior that showed accurate lick counting was uncontaminated by licks missed because of fluid bridges sometimes formed with conventional lickometers. This feature, together with the small size of the fiber optics, allows compatibility with a variety of fluid wells, often useful for adapting the experimental apparatus to use with different mouse phenotypes. Furthermore, as with other optical lick detection systems, our device is useful for future studies aimed at examining neural encoding of licking microstructure, unlike more traditional lick sensors that make the animal a part of the electrical circuit (e.g., Davis and Smith, 1992). The time-stamped data were subsequently analyzed for the microstructure of licking of water, polycose (Ross Nutrition, Columbus, OH), sucrose (J.T. Baker, Phillipsburg, NJ) and lecithin (Lewis Labs, Westport, CT), using custom-made programs.

Procedure

Mice received consumption tests for water (Experiment 1), varying concentrations of polycose (2.5%, 5%, 10% and 20%, w/v; Experiment 2), sucrose (2.5%, 5%, 10% and 20%, w/v; Experiment 3) and lecithin (1.25%, 2.5%, 3.75% and 5%, w/v; Experiment 4).

Prior to commencing each consumption test, mice were transferred from the colony to the experimental room. For each experiment, each mouse was assigned an experimental chamber and thereafter tested in that chamber. Mice initially received food cup training, to habituate them to both the experimental context and tastant. In each of the two daily sessions, 60 deliveries of water (Experiment 1) or the second concentration in the series of the particular tastant was provided on a random-time 60 s schedule (i.e., Experiment 2 = 5% polycose; Experiment 3 = 5% sucrose; Experiment 4 = 2.5% lecithin). Next, mice received consumption tests with water (Experiment 1) or each