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Brief communication

A novel, ecologically relevant, highly preferred, and non-invasive means of oral substance administration for rodents

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ABSTRACT

Prenatal stress and nutrition are well-known to alter a broad range of physiological systems, notably metabolic, endocrine and neurobehavioral function. Commonly used methods for oral administration of xenobiotics can, by acting as a stressor or altering normal nutrition intake, alter these physiological systems as well. Taken together, oral administration methods may unintentionally introduce confounding physiological effects that can mask or enhance toxicity of xenobiotics, particularly if they share biological targets. Consequently, it should be preferable to develop alternative methods without these potential confounds. The aim of this study was to determine the suitability of mealworms as an alternative treat-based method to deliver xenobiotics via the orogastric route. Accurate oral administration is contingent on motivation and preference; mice reliably preferred mealworms over wafer cookie treats. Further, ingestion of wafer cookies significantly increased mouse blood glucose levels, whereas unaltered mealworms produced no such change. Mealworms functioned effectively to orally administer glucose, as glucose-spiked mealworms produced a rise in blood glucose equivalent to the ingestion of the wafer cookie. Mealworms did not interfere with the physiological function of orally administered D-amphetamine, as both mealworm and oral gavage administered D-amphetamine showed similar alterations in locomotor behavior (mice did not fully consume D-amphetamine-dosed cookies and thus could not be compared). Collectively, the findings indicate that mealworms are a preferred and readily consumed treat, which importantly mimics environmental-relevant nutritional intake, and mealworms per se do not alter glucose metabolic pathways. Additionally, mealworms accurately delivered xenobiotics into blood circulation and did not interfere with the physiological function of administered xenobiotics. Thus mealworm-based oral administration may be a preferable and accurate route of xenobiotic administration that eliminates physiological alterations associated with other methods of delivery.

1. Introduction

Implementation of independent variables in experimental studies can sometimes generate unintended confounding factors that at a systems-level may change physiological outcomes. Although physiological complications associated with traditional oral administration methods such as gavage have long been recognized, the power of these methods to mask or enhance the influence of xenobiotic agents for *in vivo* studies has recently become a prominent issue (Vandenberg et al., 2014). Of course, all methods have limitations. For example, oral administration of a chemical in drinking water or food can create variation in dose exposure associated with normal fluctuations and individual differences in daily intake. Also, measuring individual intake for pair-housed animals is difficult. The effects of the drug itself may alter consumption of food or water, therein changing dose paradigms (Atcha et al., 2010). Although food and water dosing is often appropriate, to avoid this variation, acute oral administrations of xenobiotics commonly occurs using oral gavage or dosed-treats.

Orogastric gavage is an extremely common method used to achieve precise dose administration, which is accomplished by the insertion of a gavage needle/syringe directly into an animal's stomach to administer a xenobiotic solution. Oral gavage is known to induce stress (Atcha et al., 2010; Vandenberg et al., 2014; Walker et al., 2012). By increasing allostatic load beyond the physiological capacity of the stress response, either by intense acute or prolonged exposure, immune, digestive, reproductive and nervous system disease risk increases (McEwen, 2004). Exposure to environmental stressors has been shown to alter behavioral, endocrine, neuroanatomical, and neurochemical endpoints,

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particularly when exposure occurs during development (Bale, 2015; Cottrell and Seckl, 2009). In fact, recent research even suggests physiological changes associated with induced stress can be transgenerationally inherited (Bale, 2015; Crews et al., 2012). As a result of the multiple physiological consequences of stress activation, stress can enhance or mask the effects of xenobiotics if they share common substrates (Cao et al., 2013). This is particularly notable in developmental toxicology (Bellinger et al., 2008; Cottrell and Seckl, 2009; Schug et al., 2011). As an example, the interactions between stress and metals toxicity is well-studied; stress can enhance and/or remove effects of metal exposure. Prenatal stress and lead, can work synergistically on common physiological targets (i.e., the hypothalamic-pituitary-adrenal (HPA) axis) enhancing mesocorticolimbic neurotransmitter and behavioral neurotoxicity (Cory-Slechta et al., 2010). Given the potency of stress and potentially shared physiological substrates with experimental manipulations, it has been suggested that oral gavage be avoided in toxicological and regulatory studies focused on endocrine-mediated endpoints (Vandenberg et al., 2014).

A common non-invasive alternative to oral gavage is spiked treat administration, i.e., the incorporation of a xenobiotic into treats, often high in sugar content, such as wafer cookies, honey, milk shakes, or pellets (Ferguson and Boctor, 2009; Küster et al., 2012; Walker et al., 2012). For example, it is common to use a wafer cookie for oral administration to mice and rats (Bansal and Zoeller, 2008; Kelly et al., 2014; McCaffrey et al., 2013; Roegge et al., 2004; Wu et al., 2015). Calculated based on nutrition information provided by commercially available Nilla wafers (Nilla Wafer, Nabisco Brands, Inc., East Hanover, NJ), if given a large cookie size (1/8th of a wafer) is given to mice, simple sugar consumption may be increased 200% per administration in a pregnant dam normally consuming 5 g/day of standard rodent chow (Purina 5001). By adding large boluses of sugar to the diet, nutritional status may be substantially altered. Many toxicants have been shown to disrupt steroid and peptide hormone homeostasis, e.g. glucocorticoids and insulin, altering metabolic pathways (Alonso-Magdalena et al., 2011; Casals-Casas and Desvergne, 2011) and altering maternal nutrition with high sugar and fat diets can purportedly alter early brain development, particularly along reward, mesocorticolimbic dopaminergic, systems inducing life-long behavioral and health deficits (Avena et al., 2008; Geiger et al., 2009; Goran et al., 2013). Such spikes in blood glucose and altered neurodevelopment are potentially problematic for studies of toxicants that share these targets.

Taken together, the development of alternative methods of oral xenobiotic administration that preclude these types of confounders is of clear importance. To that end, we have adapted a non-invasive oral substance administration method utilizing an ecologically-relevant treat that does not alter glucose metabolism, is highly preferred, and readily consumed by rodents. Mice and other rodents are omnivores that derive a large component of their dietary protein from arthropods. In fact, studies of wild rodents have shown protein is the limiting factor for reproduction, with insects making up most of the rodent diet during the spring (Bellocq and Smith, 1994; Pearson et al., 2000; Sharp, 1967). Based on this ecological relevance, we have modified a previously validated method used to administer hormones to other vertebrate species (Breuner et al., 1998). Mealworms (*Tenebrio molitor*) can be administered non-invasively and have significant ecologically-relevant nutrient value. Consequently, we predicted laboratory mice would prefer mealworms over a sugar-containing cookie. Additionally, we hypothesized mealworm consumption would not produce acute spikes in blood sugar after consumption, whereas sugar-based cookies alone and mealworms injected with an equivalent glucose solution would show similar increases in blood glucose concentrations. By spiking mealworms with glucose we can determine if mealworms accurately administered a substance into blood circulation via oral administrations. Furthermore, to determine whether mealworms can accurately administer a drug without altering its effects, we compared effects of a prototypical locomotor-altering agent, D-amphetamine (Moisset, 1977; Yates et al., 2007) administered via mealworms, oral gavage, and on wafer cookies prior to the assessment of locomotor activity.

2. Methods

Animals. Adult C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were used in all testing. All mice were pair-housed in standard cages by sex under a 12 h light-dark cycle maintained at 22 ± 2 °C with food and water *ad libitum*. All experimental activities were approved by the University of Rochester Institutional Animal Care and Use Committee.

Mealworm Injection. Mealworms (*Tenebrio molitor*) were injected using a 1 ml syringe with a 27-gauge needle with 40 μl of a glucose solution prepared in 100 °C deionized water of 2.75 g of glucose/ml, for glucose testing. Mealworms used for D-amphetamine testing were dosed with 8 mg/kg based on mouse body weight of D-amphetamine. The resulting D-amphetamine and saline solution concentration was 2.4 mg/ml and mice were given either 100 μl or 120 μl of D-amphetamine administered using the mealworm depending on each animal's weight 25-30 g or 30–35 g. Prior to injection of substances into mealworms, they were cooled by placement in a 4 °C environment for at least 60 min to reduce movement during injection. The needle was inserted between two segments of the posterior abdomen (Fig. 1a). The mealworm was then placed on a paper-towel to determine if fluids leaked and if so, the mealworm was not used in testing. Immediately following injection, mealworms were placed in a −20 °C environment to be frozen and stored before feeding. Mealworms can be flash frozen in a −80 °C freezer and in that case they freeze solid in seconds and then transfer to a −20 °C freezer. Freezing in a −20 °C freezer also occurs quickly and the cold reduces metabolic activity. Mice readily consume frozen mealworms (Fig. 1b).

Food Preference Test. Six female and 5 male adult mice were each provided concurrently with a mealworm (The Bug Co., Ham Lake, MN) and a cookie treat (Nilla Wafer, Nabisco Brands, Inc., East Hanover, NJ). Naïve mice were first individually familiarized with both the mealworm and cookie in a standard home cage before testing. It is best to give mice live mealworms for the first exposure/familiarization, however from then mice will eat live or frozen mealworm. Mice were typically fed ab libitum, however all food was removed from the cage approximately 15 h before preference testing occurred. The preference test was carried out in the home cage. The first treat approached was defined as the first treat sniffed, touched and/or eaten by the mouse. The first treat consumed was the first treat entirely eaten, even if the mouse moved between treats during the test. Trials were conducted for 10 min and live scored. Mice were given frozen mealworms and ¼ of a vanilla wafer cookie. Only two mice consumed both the cookie and the mealworm during the trial. Mice that did not finish either treat within the time interval were excluded from the analysis (n = 1 male). Each subject was given one trial.

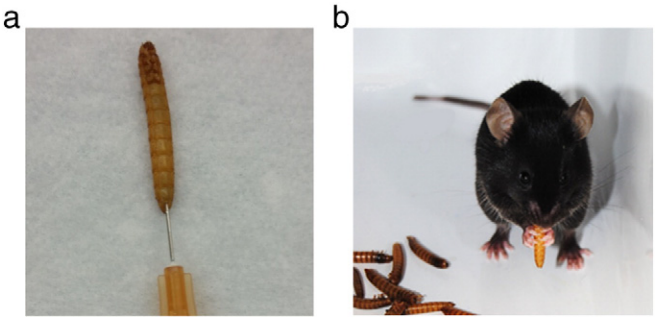

Fig. 1. a and b. Injection and consumption of mealworms. Mealworms were injected between segments in the posterior abdomen (a). Mice readily and preferentially consume mealworms (b).