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How To

A non-invasive method to evaluate gastrointestinal transit behavior in rat

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ABSTRACT

Introduction: Many factors alter gastrointestinal transit. Animal models are useful for preclinical studies of gastrointestinal transit, but terminal methods do not allow later study, and stressful assessment methods will likely alter the transit of the animal. To overcome these factors, we developed a new method to assay rat total gastrointestinal transit.
Methods: Standard plastic cages with their bottoms cut off were placed on wire mesh floors. Custom apparatuses were built to contain fecal pellets as they fell through the floors. Webcams connected to a computer running a security program were placed to image the pellets at regular intervals. Custom food was obtained with and without blue pigment. After habituating to the cages and the non-pigmented food, the pigmented food was administered. The duration to the appearance of the first pigmented pellet was determined by reviewing the photographs. This duration represents the complete gastrointestinal behavior, including feeding. We compared 24-hour fecal pellet counts using images to counts by visual inspection, and also made hourly counts. After establishing baseline transit times and hourly fecal pellet discharge, rats were given buprenorphine, known to alter gastrointestinal transit. Transit times and hourly discharge were obtained again and compared to the baselines.
Results: The methods were successful in determining transit times. Baseline measures were consistent between three groups of 8 rats. Visual and image-based counts were highly correlated. Transit times and hourly pellet discharge were reduced by buprenorphine.
Discussion: The described method offers a relatively simple, inexpensive, and non-invasive means to measure rat gastrointestinal behavior. The method has potential for any study where altered total gastrointestinal transit is an experimental concern.

1. Introduction

Many drugs, disease states, surgery, and aging alter gastrointestinal function, leading to increased or decreased transit in part of or in the entire gastrointestinal tract (Bauer, 2010; Mattei & Rombeau, 2006; Patel et al., 2014; Siemens, Gaertner, & Becker, 2014; Smits & Lefebvre, 1996). In animal models, methods to measure gastrointestinal function include fecal pellet discharge counts, gastrointestinal emptying, gastrointestinal transit, and large intestinal transit, *in vivo* or *ex vivo* (Smits & Lefebvre, 1996; Wehner et al., 2007; Yang et al., 2014). None of these methods give an overall picture of the feeding and digestive behavior of the animal. Moreover, many methods are terminal and otherwise stressful to the animal, which will alter the transit (Nakade et al., 2007; Suda, Setoyama, Nanno, Matsumoto, & Kawai, 2013). We sought to develop a non-invasive method to determine overall gastrointestinal behavior, from the initiation of eating to waste elimination.

2. Methods

The Animal Care and Use Committee of the University of New England approved all experiments. Three groups of 8 adult male Long Evans rats weighing 200–225 g were obtained from Charles River Laboratories. Two groups were used for multiple trials, and the final weight of these rats was 300–350 g. Apparatuses were designed to allow hands-free, 24-hour monitoring of fecal pellet discharge (Fig. 1A). The bottoms of standard plastic cages (47 cm × 26 cm × 21 cm, Allentown USA) were sawn off and the cages placed upon stainless steel wire mesh floors with ½″ openings. The openings allowed passage of fecal pellets, thus largely preventing coprophagy. These assemblies were placed on custom bases constructed of 6.35 mm clear acrylic sheet. Each base accommodated 2 cage-floor assemblies, and the sides were tapered to deflect fecal pellets towards the middle of the open base to help with visualization of the pellets. The entire assembly was placed upon a white absorbent paper sheet (VWR, USA). A 13 cm long × 10 cm diameter stout cardboard tube was placed in each cage for enrichment.

A video surveillance program was installed on a PC (Security Monitor Pro, USA) and linked to 4 HD webcams (Logitech, USA) to monitor fecal pellet discharge. These cameras were installed to view the entire area under one assembly (Fig. 1B). The software allows constant

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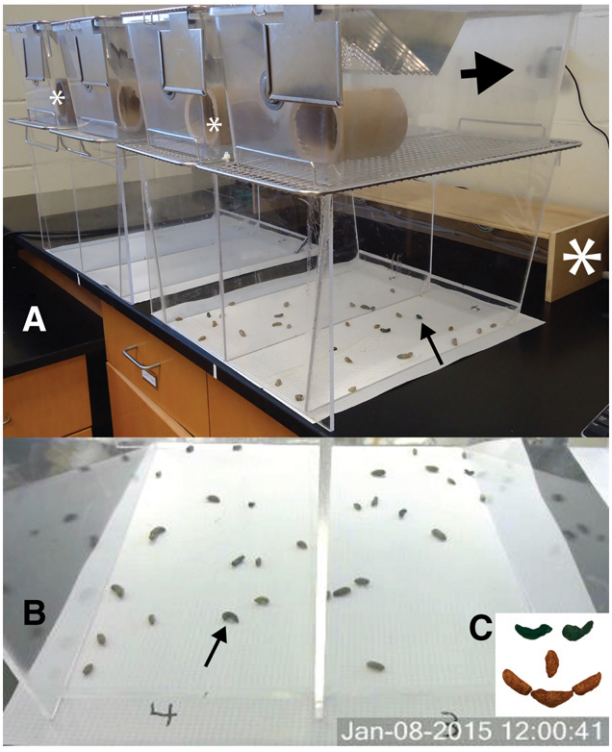

Fig. 1. A. Apparatus used to study transit time. In the closest collection area, there are 15 pellets, which also can be counted using the photograph (B), taken by the webcam (large arrow). The arrows point to the same blue fecal pellet. C. Blue pigmented pellets were readily discerned. Large white asterisk = box containing lights. Smaller white asterisks = light intensity measuring points.

monitoring or still shots to be taken at user-determined intervals. Because most feeding and defecation behavior occur at night, a low level light source was designed. Four 20 W halogen lights were installed in an open 94 cm wide × 20 cm deep × 13 cm tall plywood box (Fig. 1A) that illuminated the pellet deposit area under two assemblies but prevented direct illumination of the rats. These lights were controlled by a timer that turned them on during the dark cycle of the room. The room lights were on from 07:00–19:00, providing 275–325 lx (normal lighting). The low-intensity lights were on from 19:00–07:00, providing 25–30 lx.

Purified custom diets with (TD.130861) or without (TD.94045) blue food coloring were obtained from Harlan (USA). We observed in preliminary studies that this color became clearly evident in the fecal pellets (Fig. 1C). We also observed in preliminary studies that rats readily ate the food, but ate somewhat less of it, which we attributed to the higher caloric content (data not shown). No information existed regarding accommodation to this diet, but our experience suggested that 3 days was sufficient for the rats to accept it, based upon their intake and pellet discharge (Fig. 2A–B).

Testing was performed using the following protocol. Rats in this study were taken from the animal facility and housed in a room with no other rats, 4 days prior to the measurements, to acclimate them to the custom diet and lighting conditions. Rats were individually placed in the custom enclosures, where they had free access to water and the non-colored custom diet. The paper was changed every 24 h. After 3 days, transit time calculations were started. All experiments were started at 08:00. At this time, the white food was replaced with a weighed portion of blue food. The cameras were re-programmed to take images every 12 min. This interval was chosen to balance resolution with convenience (12 min = 0.2 h). After all rats expressed blue pellets, the experiment was stopped (minimum 24 h), and the rats were moved back to their normal cages within the animal facility. Consumption of blue food over 24 h was measured.

Photographs and the white papers under the screens were reviewed and compared for the appearance of the first blue pellet. Transit time was recorded for each rat as the duration between introduction of blue food and observation of the first blue pellet. Pellets were visually counted daily, and compared to counts taken from photographs taken at the same time. Using the photographs, pellets were counted on an hourly basis for each animal.

Group 1 and Group 2 rats were handled for 3–4 min daily for 4 days prior to entering the study. They were then placed in the enclosures, and baseline transit times were obtained. Group 2 rats were tested during increased ambient temperature, and then retested, the second trial serving as their baseline. Group 3 rats were tested prior to and then after handling, the second trial serving as their baseline. After the trial with buprenorphine (see below) we tested if the time of day that the monitoring started affected the transit time by starting monitoring at 14:00 and comparing to the baseline of the same group.

In Group 3 we used buprenorphine, known to affect intestinal motility, as a practical application of this method. After establishing the baseline transit times, 1 dose of buprenorphine (0.05 mg/kg i.p.) was administered at 08:00. One week later, 3 doses of buprenorphine were administered, one at the beginning of the experiment, and then after 12 and 24 h. The dose and interval of buprenorphine are midrange for what is commonly used for postoperative pain control (Kabadi, Kouya, Cohen, & Banik, 2015), and is considered to have an effective duration of action of 12 h.

Data analysis and graphing was performed using Graphpad/Prism 6.0. Data are presented as mean ± SEM. All data sets were subjected to an omnibus normality test. Transit times were analyzed using unpaired two-tailed *t*-tests with Welch's correction and *F*-tests for variance (to compare 2 measures in the same rats) or one-way ANOVAs with Tukey–Kramer post-hoc tests with Bartlett's test for different variances (to compare multiple trials in the same rats). Unpaired *t*-tests were used because analysis reported that pairing was not effective, and repeated measure ANOVAs were not used because the analysis reported that matching was not effective. Pearson's correlation coefficients (2-way) were calculated to compare daily visual counts to photographic counts, and daily fecal pellet discharge to food intake. Hourly pellet counts are graphically depicted, and were analyzed using a two-way repeated measure ANOVA with Tukey–Kramer post-hoc tests. All data were subjected to variance testing. Power calculations were performed using Power & Sample Size Calculator (Statistical Solutions, USA). Statistical significance was set at 0.05 for all results.

3. Results

We report here the results of 8 trials in 3 groups of 8 rats. The appearance of the first blue pellet was readily observed in all cases, by comparing the image taken by the webcams to the direct visual inspection of the pellets. In all cases, the transition from brown to blue pellets was abrupt; about 1 in 8 rats would excrete 1 fecal pellet that was part brown and part blue. This transition indicated that it was unlikely that there was forward leaching of the pigment. The first blue pellets were often not as heavily pigmented as the later ones, which is likely due to the brown and blue chyme mixing in the cecum. We never observed brown pellets appearing after the blue-pigmented pellets appeared.

Fig. 2A–D shows parameters of acclimation to the apparatuses. Fecal pellet discharge did not differ by day for Groups 1 ($F_{3,\ 28} = 2.0$, ns; Bartlett's statistic (BS) $p = 0.97$) or Group 2 ($F_{3,\ 28} = 2.0$, ns, BS $p = 0.72$), which were handled prior to testing (Fig. 2A and B). These data support that the rats acclimated to the enclosures readily. Fecal pellet discharge differed by day for Group 3 rats ($F_{3,\ 28} = 6.7$, $p = 0.0015$; BS $p = 0.002$), which were not handled prior to testing, with the difference driven by the increased first day on the grid (Fig. 2C). Food consumption was also significantly different by day for Group 3 ($F_{3,\ 28} = 8.7$, $p = 0.0003$; BS $p = 0.63$), with the change driven by the low initial day on the grid (Fig. 2D). These effects may reflect increased stress of