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Novel dynamic measures of emetic behavior in musk shrews

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ABSTRACT

The emetic reflex occurs as a pattern of motor responses produced by a network of neurons in the hindbrain. Despite an understanding of the sequence of motor outputs that form an emetic episode (EE), the variability in the dynamics of multiple EEs across time remains a mystery. Many clinical investigations rely on once a day patient recall of total amount of vomiting, and preclinical studies frequently report only the total number of EE per unit time. The aim of the current study was to develop novel temporal measures of emetic activation in a preclinical model. Male and female musk shrews were tested with prototypical emetic stimuli: motion exposure (1 Hz), nicotine (5 mg/kg, sc), and copper sulfate (120 mg/kg, ig). New emetic measures included duration (time from first to last episode), rate, standard deviation of the inter-episode interval (SD-I), and a survival analysis of emetic latency (analyzed with Cox regression). Behavioral patterns associated with emesis were also assessed using statistical temporal pattern (T-pattern) analysis to measure nausea-like behaviors (e.g., immobility). The emetic stimuli produced different levels of total EE number, duration, rate, and SD-I. A typical antiemetic, the neurokinin 1 receptor antagonist CP-99,994, suppressed the number of EEs but was less effective for reducing the duration or prolonging the emetic latency. Overall, the current study shows the use of novel dynamic behavioral measures to more comprehensively assess emesis and the impact of therapies.

1. Introduction

The emetic reflex occurs as a cycle of motor responses produced by a network of neurons that form a central pattern generator in the hindbrain (Miller et al., 1994; Billig et al., 2000; Horn et al., 2013). Despite an understanding of the sequence of motor outputs of the emetic episode (Grelot and Miller, 1994; Andrews and Rudd, 2004), the variability in the dynamics of multiple emetic episodes across time remains a mystery. Most clinical investigations rely on once a day patient recall of the total amount of vomiting, and preclinical studies frequently report only the total number of emetic episodes per unit time (e.g., 1 h).

The aim of the current study was to develop novel temporal measures of emetic activation in a preclinical model. Musk shrews were used because they are a well-established small animal model for emesis research (Kwiatkowska et al., 2004; Cluny et al., 2008; Percie du Sert et al., 2010; Horn et al., 2012; Chan et al., 2013), and other small laboratory mammals, such as mice and rats, do not have a vomiting reflex (Horn et al., 2013). Shrews were tested with three stimuli: (1) motion exposure, (2) subcutaneous nicotine injection, and (3) intragastric copper sulfate ($CuSO_4$), which are believed to activate vestibular, area postrema, and gut vagal afferent pathways, respectively (Beleslin et al., 1983; Beleslin and Krstic, 1987; Jovanovic-Micic et al., 1989; Makale and King, 1992; Fukui et al., 1993). Reported optimal parameters for each emetic stimulus were used, including 1 Hz of reciprocating lateral motion, 5 mg/kg nicotine (sc), and 120 mg/kg $CuSO_4$ (ig), intragastric (Javid and Naylor, 1999; Rudd et al., 1999; Chan et al., 2007). New emetic measures included duration (time from first to last episode), rate, and the variability of the timing of responses (i.e., the standard deviation of the inter-episode interval, SD-I); and, a survival analysis was applied to emetic latency (Jahn-Eimermacher et al., 2011). Behavioral patterns associated with emesis were assessed using statistical temporal pattern (T-pattern) analysis to determine potential sickness or nausea-like behavior (Magnusson, 2000; Horn et al., 2011). We also tested the effects of a neurokinin 1 (NK1) receptor antagonist (CP-99,994) on these novel measures of emesis after injection of nicotine (Lau et al., 2005).

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2. Materials and methods

2.1. Animals

Experimentally naïve adult musk shrews were derived from breeding stock obtained from the Chinese University of Hong Kong; a Taiwanese strain of *Suncus murinus* (Wang, 1994). Three studies were performed using a total of 42 female and 42 male musk shrews (N = 84). Animals were housed in clear plastic cages (28 × 17 ×12 cm; length x width x height), with a filtered air supply, under a 12 h standard light cycle (lights on: 0700 h), in a temperature (~23 °C) and humidity (~40%) controlled environment. Food and drinking water were freely available except during the brief test periods (up to 60 min). Food consisted of a mixture of 75% Purina Cat Chow Complete Formula and 25% Complete Gro-Fur mink food pellets (Temple, 2004). All experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care international-accredited animal care facility.

2.2. Chemicals

Nicotine ((-)-Nicotine, catalog # 36733) and $CuSO_4$ (copper (II) sulfate pentahydrate, catalog # 209198) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Nicotine was made as a 2.5 mg/ml solution in sterile saline (0.15 M NaCl; subcutaneous injection = 5 mg/kg/2 ml) and $CuSO_4$ was dissolved in filtered water (Milli-Q) at a concentration of 24 mg/ml (gavage injection = 120 mg/kg/5 ml). CP-99,994 (dihydrochloride), an NK1 receptor antagonist, was purchased from Tocris Bioscience (Bristol, UK; catalog # 3417) and prepared as a 5 mg/ml solution in sterile saline (0.15 M NaCl; intraperitoneal injection = 10 mg/kg/2 ml). Physiological saline (0.15 M NaCl) was used in subcutaneous and gavage control testing (2 ml/kg, sc; 5 ml/kg, ig).

2.3. Study 1: Motion, nicotine, and $CuSO_4$ comparison testing

Three emetic tests were conducted with a 3 to 4 week time interval between tests to allow for recovery (1st test = motion, 2nd test = nicotine, and final test = $CuSO_4$). Study 1 included 30 male and 30 female musk shrews. At the beginning of experiments, the age of shrews was an average of 207 ± 24 days old (43 ± 1 g; mean ± SEM) for females and 268 ± 34 days old (71 ± 2 g) for males (not significantly different in age; *t*-test, $p > 0.05$). Two females and one male died of apparently natural causes before the last emetic test using $CuSO_4$ gavage. Up to four animals were tested simultaneously between 0800 and 1600 h (light phase). Testing for males and females and emetic stimulus was balanced to control for time of day effects. For all tests, animals had 15 min of adaption in the test chambers (28 x 17 x 12 cm; length x width x height) before the start of motion or injection of chemicals and 30 min after these manipulations. All animal behavior was recorded with a digital video camera (Sony DCR-SR300 or HDR-XR550V, wide field lenses) placed above each test chamber and connected to a computer for storage (Media Recorder; Noldus Information Technology). A trained observer was positioned outside the transparent test chambers to record the occurrence of an emetic episode (with or without a vomit), abdominal contraction, or a swaying movement using a notebook computer installed with coding software (JWatcher; http://www.jwatcher.ucla.edu/). For motion exposure, the test chambers had a clear acrylic lid placed directly on the top. These chambers were placed on a reciprocating shaker (Taitec, Double Shaker R-30, Taiyo Scientific Industrial). Horizontal motion (4 cm displacement; 2 cm left and 2 cm right; 1 Hz) was applied for 10 min. These parameters for motion exposure were determined by past studies to be optimal for inducing emesis in musk shrews (Javid and Naylor, 1999). In nicotine or $CuSO_4$ tests, animals were subcutaneously injected with nicotine (5 mg/kg) or using a gavage needle for $CuSO_4$ (120 mg/kg) and doses were based on previous studies (Rudd et al., 1999; Yamamoto et al., 2004; Chan et al., 2007). Body weight was measured just before the beginning of adaption and at 24 h after the completion of the emetic test. A subset of males (n = 14) were also tested with saline (sc and ig, 3 to 4 weeks between each test) after testing with the final emetic agent. This last test was used to compare saline (sc or ig) with nicotine (sc) or $CuSO_4$ (ig) tests.

2.4. Study 2: Retesting nicotine-induced emesis.

Nicotine tests were conducted similar to Study 1. A new group of 12 females (40.7 ± 1.2 g and 81 ± 7 days of age at the start of testing) were used. Two tests of nicotine-induced emesis were conducted 8.5 months apart. The testing procedures were the same as Study 1.

2.5. Study 3: Testing the effects of a common antiemetic (an NK1 receptor antagonist) on new measures of emesis.

To confirm the appropriateness of the novel parameters of emesis we used the NK1 receptor antagonist CP-99,994 (10 mg/kg) (Rudd et al., 1999). Study 3 included a new group of 12 male musk shrews (65.7 ±1.4 g and 40.2 ± 0.2 days of age). Two groups were tested (0900 to 1230 h): 1) Saline control (n = 6) and 2) CP-99,994 (n = 6). Animals were injected with saline or CP-99,994 (ip) and then 30 min later injected with nicotine (5 mg/kg, sc).

2.6. Coding and tracking of behaviors

Emetic episodes (with and without vomiting), abdominal contractions, and swaying movements were scored by an observer using keystroke entry (JWatcher) and more detailed behavioral movements were recorded as digital video for offline computer tracking and analysis. Animal behavior was automatically tracked with Ethovision (v7.1; Noldus Information Technology). Using gray scaling of the body contour, animals' nose, tail, center of body, and points along the body contour were tracked (NTSC, 29.97 frames/s, 480 × 720, MPEG-2; advanced model based nose–tail detection, 1 pixel erosion and then 1 pixel dilation of the tracked contour). Continuous variables that were automatically tracked (e.g., distance moved and velocity) were converted to discrete events using threshold cutoffs of 2 standard deviations above or below the mean values to generate time-stamped events (Horn et al., 2011) using custom scripts in Matlab (Version 7.1; Mathworks).

2.7. T-pattern analysis

Table 1 shows the set of variables used for T-pattern analysis. Time-stamped data from manual scoring and automatic tracking were exported from Matlab as text files for use in T-pattern software (Theme; Noldus). Theme was used to detect behavioral patterns based on algorithms verified in multiple studies (Magnusson, 2000; Martaresche et al., 2000; Kerepesi et al., 2005; Magnusson, 2006; Casarrubea et al., 2010a; Casarrubea et al., 2010b; Horn et al., 2011; Casarrubea et al., 2012). The Theme settings were: Significance level = 0.01, minimum occurrence = 3, and minimum inter-event time = 3 s. Only the start (beginning) of each behavioral event was used for pattern analysis. Only 2 min of data were analyzed from each test, including 1 min prior and 1 min after the first emetic episode. If no emetic events occurred during a test with a specific animal (e.g., $CuSO_4$ injection), the group median latency was used to define the time period for behavioral analysis (2 min of data). Similarly, for saline tests there were no emetic episodes and the time of the first emetic episode corresponding to a reference emetic test for a given animal was used to define the time period (e.g., the emetic latency time from