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Novel dynamic measures of emetic behavior in musk shrews

Charles C. Horn^{a,b,c,d,*}, Hong Wang^e, Laureline Estival^f, Kelly Meyers^a, and Magnus Magnusson^g

^aBiobehavioral Medicine in Oncology Program, Univ. Pittsburgh Cancer Institute, Pittsburgh, PA, USA ^bDept. Medicine: Div. Gastroenterology, Hepatology, and Nutrition. Univ. Pittsburgh School of Medicine, Pittsburgh, PA, USA ^cDept. Anesthesiology, Univ. Pittsburgh School of Medicine, Pittsburgh, PA, USA ^dCenter for Neuroscience, Univ. Pittsburgh, Pittsburgh, PA, USA ^eDept. Biostatistics, Univ. Pittsburgh, Pittsburgh, PA, USA ^fAgroSup Dijon, Dijon, France ^gHuman Behavior Laboratory, University of Iceland, Reykjavik, Iceland

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. Nearly all clinical studies 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.

Keywords

emesis; vomiting; nausea; Suncus; sex; motion sickness

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 (Billig et al., 2000; Horn et al., 2013; Miller et al., 1994). Despite an understanding of the sequence of motor outputs of the emetic

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*Corresponding Author: Charles C. Horn, PhD, University of Pittsburgh Cancer Institute, Hillman Cancer Center – Research Pavilion, G.17b, 5117 Centre Avenue, Pittsburgh, PA 15213, Phone: (+00) 1-412-623-1417, Fax: 412-623-1119, chorn@pitt.edu.

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episode (Andrews et al., 2004; Grelot et al., 1994), the variability in the dynamics of multiple emetic episodes across time remains a mystery. Nearly all clinical studies 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 (Cluny et al., 2008; Horn et al., 2012; Kwiatkowska et al., 2004; Percie du Sert et al., 2010; Rudd et al., 2011), and other small laboratory mammals, such as mice and rats, do not have a vomiting reflex (Horn et al., 2013). Shrews were tested with each of 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., 1987; Beleslin et al., 1983; Fukui et al., 1993; Jovanovic-Micic et al., 1989; Makale et al., 1992). 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) (Chan et al., 2007; Javid et al., 1999; Rudd et al., 1999). 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 (Horn et al., 2011; Magnusson, 2000). 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).

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 females and 42 male musk shrews (N=84). Animals were housed in clear plastic cages (28 × 17 × 12 cm), 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 (~45 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 copper sulfate 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 CuSO₄ comparison testing

Three emetic tests were conducted with a 3 to 4 weeks time interval between tests to allow for recovery (1st test = motion, 2nd test = nicotine, and final test = CuSO₄). 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 $\pm$ 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 CuSO₄ 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 before the start of motion or injection of chemicals and 30 min after these manipulations. All animal behavior was recorded with 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 netbook installed with coding software (JWatcher; <http://www.jwatcher.ucla.edu/>). For motion exposure, the test chambers ($28 \times 17 \times 12$ cm) 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 et al., 1999). In nicotine or CuSO₄ tests, animals were subcutaneously injected with nicotine (5 mg/kg) or using a gavage needle for CuSO₄ (120 mg/kg) and doses were based on previous studies with these chemicals (Chan et al., 2007; Rudd et al., 1999; Yamamoto et al., 2004). 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 CuSO₄ (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 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 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). Two conditioned 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 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 timestamped 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 (Casarrubea et al., 2012; Casarrubea et al., 2010a; Casarrubea et al., 2010b; Horn et al., 2011; Kerepesi et al., 2005; Magnusson, 2000; Magnusson, 2006; Martaresche et al., 2000). 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 event. If no emetic events occurred during a test with a specific animal (e.g., CuSO₄ 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., use of the latency time from the nicotine sc test to define the 2 min of data collection in the saline sc test).

2.8. Data analysis

Primary emesis variables are shown in Figure 1. Total number of emetic episodes (with and without vomiting), duration, rate, the standard deviation of the inter-episode interval (SD-I), abdominal contractions, swaying, and the number of behavioral patterns was conducted using either two-way ANOVA (Study 1; stimulus by sex factorial design) or t-test (Studies 2 and 3; first nicotine vs. second nicotine test or saline vs. CP-99,994). Tukey's test was used to compare means after ANOVA. We applied survival plots and Cox regression analysis to latency data, which permits the use of all data, including censored values (i.e., animals that did not show emesis during the test period). A Pearson product correlation was used to assess the relationships between emetic stimuli. $P < 0.05$ was used to determine statistical significance for ANOVA, t-test, and Cox regression; however, a more stringent level of $p < 0.01$ was used for correlation analysis to control for the use of multiple tests.

3. Results

3.1. Study 1: Motion, nicotine, and CuSO₄ comparison testing

Of the three stimuli, nicotine produced more total emetic episodes, and had fewer emetic episodes with a vomit, compared to motion exposure or CuSO₄ injection [$F(2,54) = 11.2$, $p < 0.0001$, main effect of stimulus and $p < 0.05$ Tukey's tests; Fig. 2A and 2B]. Females displayed significantly fewer emetic episodes without a vomit after nicotine injection compared to males [$F(2,54) = 5.3$, $p < 0.01$, interaction effect of stimulus by sex and $p < 0.05$ Tukey's tests; Fig. 2C]. Females also showed a significant increase in emesis duration after CuSO₄ injection compared to motion exposure or nicotine injection [$F(2,44) = 4.9$, $p < 0.01$, interaction effect of stimulus by sex and $p < 0.05$ Tukey's tests; Fig. 2D]. The emetic rate was highest after nicotine injection but CuSO₄ injection produced the most variable rate (i.e., the SD-I) [$F(2,44 \text{ or } 42) = 15.6$, $p < 0.0001$, main effect of stimulus and $p < 0.05$ Tukey's tests; Fig. 2E and 2F]. More abdominal contractions were produced with nicotine or CuSO₄ injections than motion exposure [$F(2,54) = 8.7$, $p < 0.0005$, interaction effect of stimulus by sex and $p < 0.05$ Tukey's tests; Fig. 2G]. Only nicotine injection produced a

significant increase in swaying behavior [$F(2,54) = 21.8$, $p < 0.0001$, main effect of stimulus and $p < 0.05$ Tukey's tests; Fig. 2H].

Females and males showed no significant differences in the latency for emesis after motion exposure or CuSO_4 injection, but females displayed a shorter latency than males after nicotine injection ($p < 0.05$, Cox regression; Fig. 3A). CuSO_4 injection produced a significantly longer emetic latency than motion exposure or nicotine injection ($p < 0.05$, Cox regression). Both males and females demonstrated emetic latencies after CuSO_4 injection that were similar for episodes with or without vomiting, however, motion exposure and nicotine injection produced much shorter latencies for non-productive emetic episodes compared to episodes with vomiting ($p < 0.05$, Cox regression; Fig. 3B).

For females and males, nicotine injection produced more unique and total behavioral patterns than CuSO_4 injection [$F_s(1,52) = 5.8$, $p < 0.02$, main effect of stimulus; Fig. 6]. Nicotine injection was associated with more behavioral patterns containing event types of emesis, immobility (immob), mobility (mob), clockwise rotation (rot), counter clockwise rotation (rotc), distance moved of the body center (dchi), and movement of the body center (mchi) [$F_s(1,52) = 4.1$, $p < 0.05$, main effect of stimulus; Fig. 4].

Table 2 shows the correlations for these emesis and behavioral pattern variables. Only females showed statistically significant correlations. In females, motion-induced emetic episodes with a vomit were correlated with nicotine- and CuSO_4 -induced emetic episodes with a vomit ($p < 0.05$, Pearson correlation; Table 2). Motion-induced emetic rates were correlated with nicotine-induced emetic rates ($p < 0.05$, Pearson correlation; Table 2). There were no significant correlations for behavioral patterns.

In a control study with one cohort of Study 1 animals we determined whether nicotine and CuSO_4 injections produced behavioral patterns that were different from saline injections (sc or ig). The emetic chemicals produced more unique and total behavioral patterns than saline injection [$F_s(1,12) = 14.5$, $p < 0.005$, main effect of stimulus; Fig. 5A]. Nicotine injection produced more behavioral patterns associated with emesis compared to CuSO_4 injection (Fig. 5B). The emetic chemicals produced more behavioral patterns containing event types of immobility (immob) and movement of the center (mchi) compared to saline [$F_s(1,12) = 7.4$, $p < 0.02$, main effect of emetic stimulus; Fig. 5B]. The subcutaneous route of injection produced more patterns containing event types with distance moved of the body center (dchi) compared to the intragastric route of injection [$F_s(1,12) = 7.5$, $p < 0.02$, main effect of injection route; Fig. 5B].

3.2. Study 2: Retest of nicotine

In a control study we assessed the relationship of emetic events in a cohort of female musk shrews tested twice for nicotine-induced emesis. This study was conducted to determine if emetic measures were consistent across time. Animals displayed 18.5 ± 1.3 and 15.5 ± 1.4 total emetic episodes for the first and second tests, respectively [$t(11) = 1.8$, $p < 0.05$, two-tailed]. There were statistically significant correlations for total number of emetic episodes, emetic rate, and total behavioral patterns ($p < 0.05$, Pearson correlations; Table 2).

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

In a control study we determined whether the proposed new measures of emesis and behavioral patterns could provide additional information in tests of antiemetic effects. CP-99,994 pre-treatment reduced the number of emetic episodes (total, with and with vomiting) [$t(10) = 2.4$, $p < 0.05$; Fig. 6A, 6E, and 6F] and the emetic rate [$t(8) = 3.8$, $p < 0.01$; Fig. 6C] produced by nicotine. CP-99,994 also increased the standard deviation of the

emetic interval [$t(6) = 5.8$, $p < 0.005$; Fig. 6D], but had no effect on the duration of emesis or the number of abdominal contractions (Fig. 6B and 6G). The emetic latency was significantly increased by CP-99,994 treatment ($p < 0.05$, Cox regression; Fig. 6H). CP-99,994 had no significant effects on the number of unique and total behavioral patterns (t-tests; Fig. 7A) and patterns containing specific event types after nicotine injection (t-tests; Fig. 7B).

4. Discussion

The current study used several novel measures to compare the actions of three prototypical emetic stimuli. Calculating the number of emetic episodes provided limited insight into potential differences in emetic behavior between these stimuli (Fig. 2A). For example, although motion and CuSO_4 produced similar levels of emetic activation based on total emetic episodes, they diverged in emetic duration and SD-I (Fig. 2D and 2F). Notably, it is clear that CuSO_4 produced a highly variable interval of responding during the test period (Fig. 2F), and this stimulus was associated with similar latencies for productive (vomiting) and non-productive emetic episodes compared to motion exposure and nicotine injection (Fig. 3B). Potentially these differences could arise because of pharmacokinetics of drug action and adaptation of neurotransmitter receptors. Notably, the current study used only a single dose or set of motion parameters for each stimulus. Future studies will need to vary these parameters to assess the complete stimulus-response relationships.

We also showed that an NK1 receptor antagonist, CP-99,994, can produce a dramatic suppression of the total number of emetic episodes, a result reported previously (Rudd et al., 1999); however, it was less effective for reducing the duration of emesis and abdominal contractions, which suggests that these animals were still experiencing visceral sickness. The number of temporal behavioral patterns was also less affected by CP-99,994 treatment than total number of emetic episodes. This difference could be related to the reported actions of NK1 receptor antagonists to be more effective for the control emesis compared to nausea (Andrews et al., 2004). Evidence also indicates that NK1 antagonists act on the motor outputs of the emetic circuitry (Fukuda et al., 2003). Pre-treatment with CP-99,994 reduced the cumulative incidence of nicotine-induced emesis by 33% (Fig. 6H).

Survival plots of emetic latency could potentially provide a powerful method to compare emetic stimuli and the effects of antiemetic drugs. It is often difficult to determine the appropriate presentation and analysis of emesis latency data since it is unclear whether non-responding animals should be included. Furthermore, behavioral latency data typically do not have a normal distribution; and, therefore, parametric analyses are not appropriate. The current approach, based on analysis of behavioral latencies in rodents (Jahn-Eimermacher et al., 2011), does not require assumptions about the distribution of data, and values from non-responding animals can also be included (i.e., censored values). Plotting the cumulative incidence of emesis shows the number of responding animals, group medians and quartiles, and illustrates the temporal differences between groups that can be analyzed using Cox regression (Jahn-Eimermacher et al., 2011).

A statistical pattern analysis (T-pattern analysis) was applied to the behavioral data from nicotine and CuSO_4 testing to determine behaviors that are potentially associated with visceral sickness or nausea (Horn et al., 2011). We were unable to use the behavioral data from motion exposure because the current methodology did not permit corrections for the movement of the test chamber, which was confounded with the movements of the animal. This problem could be addressed in future experiments by independently tracking the cage position and subtracting these movements from the tracked coordinates of the animal. Our pattern analyses revealed that nicotine had a greater impact on musk shrew behavior

compared to CuSO₄ injection (Fig. 4A). Indeed nicotine injection was associated with more emesis, immobility, and bursts of locomotion than CuSO₄ injection (Fig. 4B). A subgroup analysis showed that total number of behavioral patterns, and those associated with immobility and bursts of locomotion were significantly greater in tests with emetic chemicals compared to control injections of saline (Fig. 5).

Emetic episodes can be classified as productive (with a vomit) or non-productive. We did not directly control the amount of food eaten or the time since the last meal; however, potential differences between experimental conditions were controlled by testing animals at similar times each day. Therefore, it is likely that differences between emetic stimuli reported in Fig. 2 and 3 for emetic episodes with versus without vomiting are reliable. Feeding and gastric fill do not affect the total number of emetic episodes produced by motion exposure or injection of nicotine (or the chemotherapy agent cisplatin) in musk shrews (Horn et al., 2010).

In the current report, female and male musk shrews did not significantly differ in the number of emetic episodes produced by the three emetic stimuli. However, females did display fewer non-productive emetic episodes and a longer duration of emesis after injection of CuSO₄ compared to males (Fig. 2). Females also showed a shorter emetic latency after injection with nicotine in comparison to males (Fig. 3). Other reports also indicate small sex differences in emetic responding in musk shrews. Tropisetron (a 5-HT₃ receptor antagonist) was reported to be less effective for reducing cisplatin-induced emesis in female musk shrews (Matsuki et al., 1997). Female musk shrews also have been reported to show 33% more (Javid et al., 1999) and 17% fewer emetic episodes (Matsuki et al., 1997) compared to males in response to motion exposure (1 Hz). It is possible that these modest differences between male and female shrews are due to the genetic variability of different populations of musk shrews.

Motion-induced emesis (with vomiting) was a significant predictor of vomiting after injection with nicotine or CuSO₄, but only in female musk shrews (Table 2). The emetic rate during motion-induced emesis was also significantly correlated with nicotine-induced emetic rate in females but not in males (Table 2). In addition, the current study showed that emetic correlations are likely stable from test-to-test for individual animals. In other words, individual factors are likely to be highly significant for these test responses, in contrast to environmental factors, age, etc. This was demonstrated in a separate cohort study using two nicotine tests in the same female animals (Table 2; Study 2). The total number of emetic episodes was highly correlated between the first and second tests with nicotine ($r = 0.66$). Furthermore, the total number of behavioral patterns was also correlated ($r = 0.89$). We chose not to do retesting with a motion stimulus because it is known that animals adapt to motion-induced emesis with repeated assessments (Javid et al., 2001; Kaji et al., 1990).

Overall, the current study demonstrates the use of several novel dynamic behavioral measures to more comprehensively assess emesis and the impact of therapies, including survival analysis of emetic latency, duration of emesis, emetic rate, SD-I, and behavioral pattern analysis. Although no differences were detected in the total amount of emesis in males compared to females, there were significant correlations of number of vomits and emetic rate between motion exposure and other emetic stimuli in females. These results suggest that sensitivity to motion sickness and female sex, well known risk factors for nausea and emesis in the clinical domain (PONV and CINV, Apfel et al., 1998; Apfel et al., 1999; Hesketh et al., 2010; Shih et al., 2009), might also apply to this preclinical model (musk shrew). Future studies should focus on determining these potential associations between female sex and susceptibility to motion sickness using emetic testing with anesthesia and chemotherapy. In summary, the new measures of emesis could provide

detailed insight into the dynamics of emesis that is often hidden when reporting only the total number of episodes.

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Fig. 1.

Measures of emesis. The diagram shows emetic episodes that occur during 10 min of motion exposure or after chemical injection. The total number of emetic episodes and latency to the first emetic response are common measures of emetic intensity. Additional measures were used to assess the temporal dynamics of emesis, including the duration (time from first to last episode), rate (episodes/min), and the standard deviation of the interval between episodes (SD-I).

Fig. 2.

Effects of motion exposure (10 min, 1 Hz), nicotine injection (5 mg/kg, sc), and copper sulfate injection (120 mg/kg, ig) on the (A) total number of emetic episodes, (B) emetic episodes with vomiting, (C) emetic episodes without vomiting, (D) duration of emesis, (E) emetic rate, (F) standard deviation of the emetic interval, (G) abdominal contractions, and (H) swaying. See Fig. 1 for diagram showing measures of emesis. Bars with different letters (combining males and females) = $p < 0.05$, Tukey's test. * = $p < 0.05$, Tukey's test, for female (red), male (blue), or female vs. male (black). Data represent means $\pm$ SEMs.

Fig. 3.

Effects of motion exposure (10 min, 1 Hz), nicotine injection (5 mg/kg, sc), and copper sulfate injection (120 mg/kg, ig) on the cumulative latency (incidence) to the first emetic episode: (A) latency to first emetic episode, male versus female, and (B) latency to emetic episode (EE) with vomiting versus without vomiting. Red = female, and blue = male. * = $p < 0.05$, Cox regression.

Fig. 4. Effects of nicotine (5 mg/kg, sc) and copper sulfate injections (120 mg/kg, ig): (A) the number of unique behavioral patterns and the total number of behavioral patterns, and (B) the number of behavioral patterns containing specific event types in different categories (Table 1). $p < 0.05$, ANOVA, main effect of emetic stimulus. Data represent means $\pm$ SEMs.

Fig. 5.

Effects of saline injection (sc or ig) compared to emetic chemicals (nicotine, 5 mg/kg, sc; and, copper sulfate, 120 mg/kg, ig): **(A)** the number of unique behavioral patterns and the total number of behavioral patterns, and **(B)** the number of behavioral patterns containing specific event types in different categories (Table 1). $\alpha = p < 0.05$, ANOVA, main effect of emetic stimulus. $\beta = p < 0.05$, ANOVA, main effect of route of injection. $\gamma = p < 0.05$, t-test, two-tailed, nicotine vs. CuSO₄. Data represent means $\pm$ SEMs.

Fig. 6.

Effect of CP-99,994 compared to saline injection on nicotine-induced emesis (5 mg/kg, sc): (A) the number of emetic episodes, (B) the duration of emesis, (C) the emetic rate, (D) the standard deviation of the emetic interval, (E) the number of emetic episodes with a vomit, and (F) the number of emetic episodes without a vomit. Data represent means ± SEM. * = $p < 0.05$, t-test, two-tailed. (G) the number of abdominal contractions, and (H) the cumulative incidence of emesis are also shown. * = $p < 0.05$, Cox regression.

Fig. 7.

Effect of CP-99,994 compared to saline injection on nicotine-induced (5 mg/kg, sc) behavioral patterns: (A) the number of unique behavioral patterns and the total number of behavioral patterns, and (B) behavioral patterns containing specific event types in different categories (Table 1). Data represent means $\pm$ SEM.

Table 1

Behavioral event types and categories

emesis	a sequence of contractions of the abdomen (retching, with or without a vomit)
<u>No movement</u>	
Immob	Immobile (< 1% change in body contour)
norm	normal body contour
<u>Contour change</u>	
abcon	a single contraction of the abdominal region
sway	swaying the abdominal portion of the body from side to side
con	contracted body contour, 60% of normal
long	elongated body contour, 80% of normal
<u>General movement</u>	
mob	mobile (> 1% and < 8% change in body contour)
dnhi	distance moved, nose, high
mnhi	Movement of the nose (velocity, > 2 cm/s)
mobhi	highly mobile (> 8%; change in body contour)
<u>Turning</u>	
rot	rotation-clockwise (a turn of 360 degrees)
rotc	rotation-counter clockwise
tachi	turn angle of the body center, high
tanhi	turn angle of the nose, high
<u>Locomotion</u>	
dchi	distance moved, body center, high
mchi	Movement of the body center (velocity, > 2 cm/s)

Table 2

Correlations between tests

	Study 1				Study 2	
	Females		Males		Females	
	Nicotine	CuSO ₄	Nicotine	CuSO ₄	Nicotine (retest)	
All emetic episodes	Motion	0.36	0.16	0.11	0.36	
	Nicotine	--	0.36		0.16	0.66 *
EE w/ vomit	Motion	0.48 *	0.49 *	0.00	-0.35	
	Nicotine	--	0.14		-0.07	0.03
EE w/o vomit	Motion	0.19	-0.07	0.01	0.30	
	Nicotine	--	0.22	--	0.14	0.53
Emetic duration	Motion	-0.11	0.06	0.06	0.12	
	Nicotine	--	0.04	--	-0.21	-0.05
Emetic rate	Motion	0.06	0.57 *	0.28	0.49	
	Nicotine	--	-0.03	--	0.03	0.71 *
SD of interval	Motion	0.14	0.48	0.02	-0.25	
	Nicotine	--	0.14	--	0.00	-0.17
Total patterns	Nicotine	--	0.08	--	-0.08	0.89 *
Immobility patterns	Nicotine	--	-0.12	--	0.12	-0.25

* p<0.01, Pearson correlation