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Motion sickness: Multiple linear regression identifies behaviours linked to motion-induced emesis in *Suncus murinus* (house musk shrew)

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ABSTRACT

Motion sickness (MS) is a complex syndrome characterized by a spectrum of autonomic and behavioural symptoms, often accompanied by emesis. *Suncus murinus* has become a primary species in which to study the mechanisms of emesis control, yet the behavioural profile associated with motion-induced malaise of this animal has not been thoroughly assessed. In this study, we exposed 129 animals to provocative motion (1 Hz, 4-cm horizontal reciprocating displacement, 30 min) to induce MS and systematically recorded spontaneous behaviours, retching and/or vomiting (R/V), and changes in body surface and tail temperatures. A non-biased MS symptom score was developed based on stepwise multiple linear regression, incorporating nine observable behaviours and physiological variables, namely scratching, stretching, chin on the floor, burrowing, chewing the bedding, micturition, defecation and/or tenesmus, remaining stationary, and body surface and tail temperature changes. Administration of two anti-MS drugs, diphenhydramine (30 mg/kg, s.c.) and scopolamine (10 mg/kg, s.c.), significantly reduced the MS symptom score from 14.25 ± 1.89 to 7.84 ± 0.85 and from 18.11 ± 2.28 to 11.91 ± 0.87 respectively, indicating the validity of the scoring system as a quantitative measure of motion-induced 'sickness', 'malaise', and even 'nausea' in this species. Shapley additive explanation (SHAP) analysis further indicated the contributions of two individual spontaneous behaviours, stationary duration and chin on the floor episode, to MS prediction. Our findings suggest that this behavioural scoring system provides a reliable and translationally relevant tool for studying MS and screening potential anti-emetic treatments for humans.

1. Introduction

Motion sickness (MS) can be caused by a variety of circumstances, including journeys in vehicles and virtual reality (Golding, 2006). It is a common but complex syndrome characterised by a cluster of signs and symptoms, including cold sweating, facial pallor, drowsiness, hypersalivation, hypothermia, and nausea with or without vomiting (Pan et al., 2024; Golding and Gresty, 2015). Various MS rating scales or 'point systems' are used to assess MS without emesis (Golding and Gresty, 2015; Paillard et al., 2013; Murdin et al., 2015). The 'sensory conflict' theory proposes that MS occurs in susceptible individuals because of a mismatch between vestibular, visual, and proprioception inputs, which diverge from learned experience (Reason, 1978). People with a history of MS also have a high incidence of nausea and/or vomiting following chemotherapy, surgery with anaesthesia, and pregnancy (Kottschade et al., 2016; Son and Yoon, 2018), which suggests that the pathways involved in the sensory conflict theory may be easily perturbed by a wide variety of circumstances in these individuals. The treatment of MS includes the use of anti-muscarinic (e.g., scopolamine) or anti-histaminergic drugs (e.g., diphenhydramine) (Golding and Gresty, 2015). However, the efficacy of both classes of drug is variable and limited by unwanted side effects such as sedation, blurred vision, dry mouth/throat, and constipation (Schmäl, 2013; Spinks and Wasiak, 2011).

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The preclinical assessment of drugs to prevent MS commonly involves the use of animal species capable of emesis, including dogs, cats, monkeys, and shrews (Benchaoui et al., 2007; Lucot, 2017; Corcoran et al., 1990; Tu et al., 2020). Many studies have focused on the ability of a test compound to reduce episodes of R/V (retching and/or vomiting), without examining a decrease in other symptoms of MS (Lucot et al., 1997; Chan et al., 2007; Horn et al., 2014). However, few studies have assessed the potential of a compound to reduce both R/V episodes and symptom scores (Lucot, 2017; Suri et al., 1979; Cheung et al., 1992; Yu et al., 2007). As most of the symptoms recorded are a consequence of an activated autonomic nervous system, the methodology may be biased towards detecting drugs with adrenergic/cholinergic receptor-blocking activity (Moullé et al., 2019; Brisinda et al., 2003). Unfortunately, a decrease in any symptom score (e.g. degree of salivation) caused by blocking receptors in the end organs (e.g. blocking of muscarinic receptors in salivary glands) does not necessarily mean that 'nausea' has been reduced, as the sensation of nausea involves circuits in the cortex (Varangot-Reille et al., 2023; Farmer et al., 2015; Sclocco et al., 2016; Toschi et al., 2017).

With regard to the gastrointestinal tract, some studies have reported that nausea associated with MS occurs during a shift of normogastria to tachygastria (Stern et al., 1987; Koch, 2014), but not all studies have found this association (Cheung and Vaitkus, 1998). Studies also have shown that MS can be associated with changes in heart rate variability (Lin et al., 2011) and decreases in core body temperature (Nalivaiko et al., 2014). Nevertheless, even these correlations are subject to regulation by the autonomic nervous system, and therefore, an assessment of the ability of a drug to reduce nausea may be confounded by their own direct or indirect action at the central/peripheral nervous system level. In shrews, we recently identified several behavioural and physiological changes occurring during provocative motion-induced R/V experiments. These include motion-induced R/V; an increase in the number of 'chin on the floor', 'scratching', and 'licking' episodes; and a decrease in exploratory behaviour (Tu et al., 2020). These latter events are mainly 'somatic motor' events and therefore provide a different dimension to the usual components of the MS symptom score.

Considering the complexity of the mechanisms involved in MS, the level of 'sickness' that accompanies R/V is hard to attribute to a single behavioural or physiological change. There might be changes in behavioural/physiological patterns leading up to or following an R/V episode (Horn et al., 2011). In cats, a rating scale that included the symptoms of salivation, panting, urination, and defecation was established (Suri et al., 1979). This scale encompassing behaviours influenced by the autonomic system is rated from 1 to 8 but heavily biased as a score of 16 could be awarded for somatic events, including R/V (Lucot, 2017; Cheung et al., 1992; Lau et al., 2005; Del Vecchio et al., 2014). Comparatively, in mice and rats, which do not vomit, an MS index that encompasses episodes of faecal pellet number, episodes of urination, piloerection, and tremor was scored from 0 to 1.2 in experiments studying the anti-MS potential of modafinil; these events were autonomic or a mix of autonomic and somatic events (Yu et al., 2007). Nevertheless, little research has focused on the quantitative analysis of more categories of behavioural or physiological changes related to MS in species capable of emesis; and a reliable behavioural scale to assess the severity of MS and pharmacological intervention in emetic animal models is still lacking. Therefore, the present study was designed to establish a robust MS symptom score encompassing 15 behavioural and physiological symptoms (sniffing, scratching, grooming, face washing, stretching, chin on the floor, circling, burrowing, chewing the bedding, micturition, defecation or tenesmus, being stationary, lying down, and body surface and tail temperature changes) and stepwise linear regression was used to avoid the bias associated with the previous rating scores. Due to the lack of an objective gold standard for assessing motion-caused sickness, malaise, or even nausea in animal models, R/V episode was employed as an observable proxy outcome.

2. Methods

2.1. Animals

Adult male *Suncus murinus* (house musk shrew; 60–95 g, n = 155) were obtained from The Chinese University of Hong Kong and housed in a room with controlled temperature (24 ± 1 °C) and relative humidity (50 ± 5 %) and a 12-h/12-h light/dark cycle. Water and dry pellet cat chow were provided *ad libitum*. Behavioural experiments were conducted during 08:00–18:00. All experiments were conducted under a license from the Hong Kong SAR government and the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong (Approved NO.: 21-082-GRF).

2.2. Experimental protocols

The study flow chart is shown in Fig. 1. Briefly, 129 *Suncus murinus* animals were individually placed in observation chambers with free access to food and water one night before experimentation. On the experimentation day, chambers were placed on a desktop shaker (Heidolph, Schwabach, Germany) which generated provocative motion (1 Hz, 4-cm horizontal reciprocating displacement) for 30 min. During this period, spontaneous behaviours (see definitions below) and R/V episodes were recorded using JWatcher (version 2.0, University of California Los Angeles and Macquarie University, USA and Australia). Seven days later, animals exhibiting R/V during the pre-screening period were used to validate the MS score. These animals were randomised into three groups using the Latin square design. Diphenhydramine (30 mg/kg, s.c., n = 7), scopolamine (10 mg/kg, s.c., n = 6), or vehicle (saline, 2 ml/kg, s.c.) was administrated 30 min before starting the provocative motion. At selected timepoints, body and tail temperatures were recorded using an infrared camera (E95 FLIR Thermal Imager, Systems AB, Sweden; thermal sensitivity ± 0.03 °C).

2.3. Behaviours

The spontaneous behaviours recorded were sniffing (animal drawing in a scent or air through nose); scratching (animal scratching its body with its hindlimb); grooming (animal cleaning its body, paw, and genital or anal regions with its tongue); face washing (animal grooming its face with both forelimbs); stretching (animal stretching its body, usually accompanied by hindlimb extension); chin on the floor (animal rubbing the floor with its chin); circling (animal rotating more than 180°); burrowing (animal burying its nose under the bedding and/or digging in the bedding with forelimbs); chewing the bedding; micturition, defecation, or tenesmus (animal urinating, attempting to defecate (tenesmus), or defecating, usually accompanied by a raised tail), stationary (animal staying still with its stomach above the floor); and lying down (animal with its stomach fully on the floor). R/V episodes were characterised as episodes of rhythmic abdominal contractions that were followed by the oral expulsion of solid or liquid material from the gastrointestinal tract (i.e., vomiting) or not associated with the passage of material (i.e., retching). Except for stationary and lying down being measured in duration (minutes), other behaviours were counted as episodes. An episode of each behaviour was considered separate when the animal changed its location or when the interval between behaviours exceeded 2 s.

Body surface and tail temperatures were analysed using FLIR Tools (version 6.4.18039.1003, Teledyne FLIR, Wilsonville, USA). Delta temperatures were determined by minimal (body surface) or maximal (tail) temperature occurring during motion compared to the baseline ones (t = 30 min).

2.4. Formulation of drugs

Diphenhydramine hydrochloride and scopolamine hydrochloride

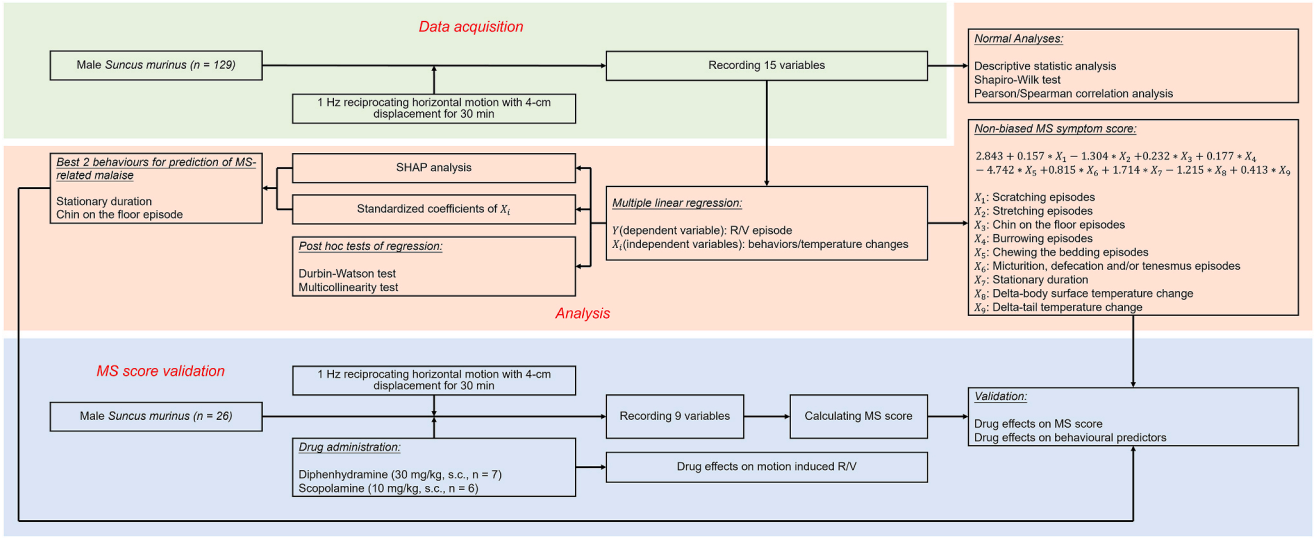

Fig. 1. Flow chart depicting the study.

were purchased from Sigma Aldrich (St. Louis, MO, USA) and dissolved in sterile saline (0.9 %, w/v). All doses are expressed as the free base.

2.5. Statistical analysis

All data were inspected using Shapiro–Wilk tests for all covariates to ascertain whether they were normally distributed. Behaviours that followed a Gaussian distribution were subjected to Pearson's correlation test, whereas those that did not follow a Gaussian distribution were subjected to Spearman's correlation test. A Lollipop plot was generated using R (ggplot2 package, version 3.5.2) to visualize the relative contribution of each behavioural variable to R/V episode. Heatmaps were plotted using graphing software at https://www.bioinformatics.com.cn/ (last accessed on August 30, 2024), an online platform for data analysis and visualisation (Tang et al., 2023). Stepwise multiple linear regression including *post-hoc* assumption tests, was conducted using Jamovi (https://www.jamovi.org/, version 2.3, see below). Standardised coefficients were further calculated and used to compare the importance of every variable. Additionally, a Shapley additive explanation (SHAP) analysis using Python (https://github.com/shap/shap, version 0.47.1) was applied to distinguish which dependent variable had the greatest effect on MS prediction. Score validation was conducted against the vehicle group using unpaired *t*-tests. Other statistical analyses were performed using Prism (version 10.0, GraphPad, California, USA). Data are indicated as the mean ± standard error unless otherwise indicated. Differences were considered significant when $p < 0.05$.

2.6. Construction of MS symptom score

We modelled the number of R/V episodes during motion as the dependent variable. Candidate predictors were prespecified behavioural and physiological variables including scratching, stretching, chin on the floor, burrowing, chewing the bedding, micturition/defecation and/or tenesmus, stationary duration, and changes in body surface and tail temperatures, were used as independent variables. Importantly, R/V itself was not included as a predictor and is not a component of the score. Stepwise multiple linear regression (backward elimination) was conducted with the removal criterion set of 0.20. This relatively lenient removal threshold was chosen to avoid prematurely excluding potentially informative predictors, particularly given the exploratory nature of the analysis and the finite sample size (Mickey and Greenland, 1989). The backward elimination regression model was fitted according to previous statistical studies (Derksen and Keselman, 1992; Fukuchi et al., 2025). Briefly, we started from the full model including all candidate predictors (15 behavioural and physiological variables mentioned above) and iteratively removed the predictor with the largest *p*-value exceeding 0.20 (two-sided *t*-test for the regression coefficient). The elimination procedure stopped when all remaining predictors had $p < 0.20$. The final MS symptom score was defined as the linear combination of retained predictors using unstandardized coefficients (units in parentheses): Score = 2.843 + 0.157 × scratching (episodes) – 1.304 × stretching (episodes) + 0.232 × chin-on-the-floor (episodes) + 0.177 × burrowing (episodes) – 4.742 × chewing-the-bedding (episodes) + 0.815 × micturition/defecation/tenesmus (episodes) + 1.714 × stationary (min) – 1.215 × delta body-surface temperature (°C) + 0.413 × delta tail temperature (°C). We reported regression coefficients with standard errors, 95 % confidence intervals, and *p*-values. *Post-hoc* assumption checks including Durbin–Watson and multicollinearity tests, and Q-Q plot were performed to ensure the validity of the regression model. Additionally, the MS symptom score was subsequently validated in pharmacological experiments using two anti-MS agents, diphenhydramine and scopolamine.

Table 1
Descriptive statistics of behaviours and temperature changes caused by motion. Data represents the mean ± S.E.M of 129 animals. All the behaviours were counted as episodes.

Variables	Mean ± SEM
Sniffing	38.74 ± 1.56
Scratching	5.09 ± 0.51
Grooming	2.42 ± 0.34
Face washing	0.09 ± 0.03
Stretching	0.27 ± 0.06
Chin on the floor	5.94 ± 0.55
Circling	4.12 ± 0.30
Burrowing	2.09 ± 0.30
Chewing the bedding	0.03 ± 0.02
Micturition, defecation and/or tenesmus	0.86 ± 0.07
R/V	11.57 ± 0.58
Stationary (min)	1.88 ± 0.14
Lying down (min)	20.20 ± 0.35
Delta body surface temp (°C)	−1.06 ± 0.05
Delta tail temp (°C)	3.31 ± 0.23

3. Results

3.1. Incidence of behaviours

A descriptive statistical analysis was performed for all behaviours during the 30 min of motion (n = 129, Table 1). Provocative motion induced 11.57 ± 0.58 episodes of R/V, 38.74 ± 1.56 episodes of sniffing, 5.09 ± 0.51 episodes of scratching, 2.42 ± 0.34 episodes of grooming, 0.09 ± 0.03 episodes of face washing, 0.27 ± 0.06 episodes of stretching, 5.94 ± 0.55 episodes of chin on the floor, 4.12 ± 0.30 episodes of circling, 2.09 ± 0.30 episodes of burrowing, 0.03 ± 0.02 episodes of chewing the bedding, and 0.86 ± 0.07 episodes of micturition, defection, and/or tenesmus. The animals also spent 1.88 ± 0.14 min being stationary and 20.20 ± 0.35 min lying flat. The basal body

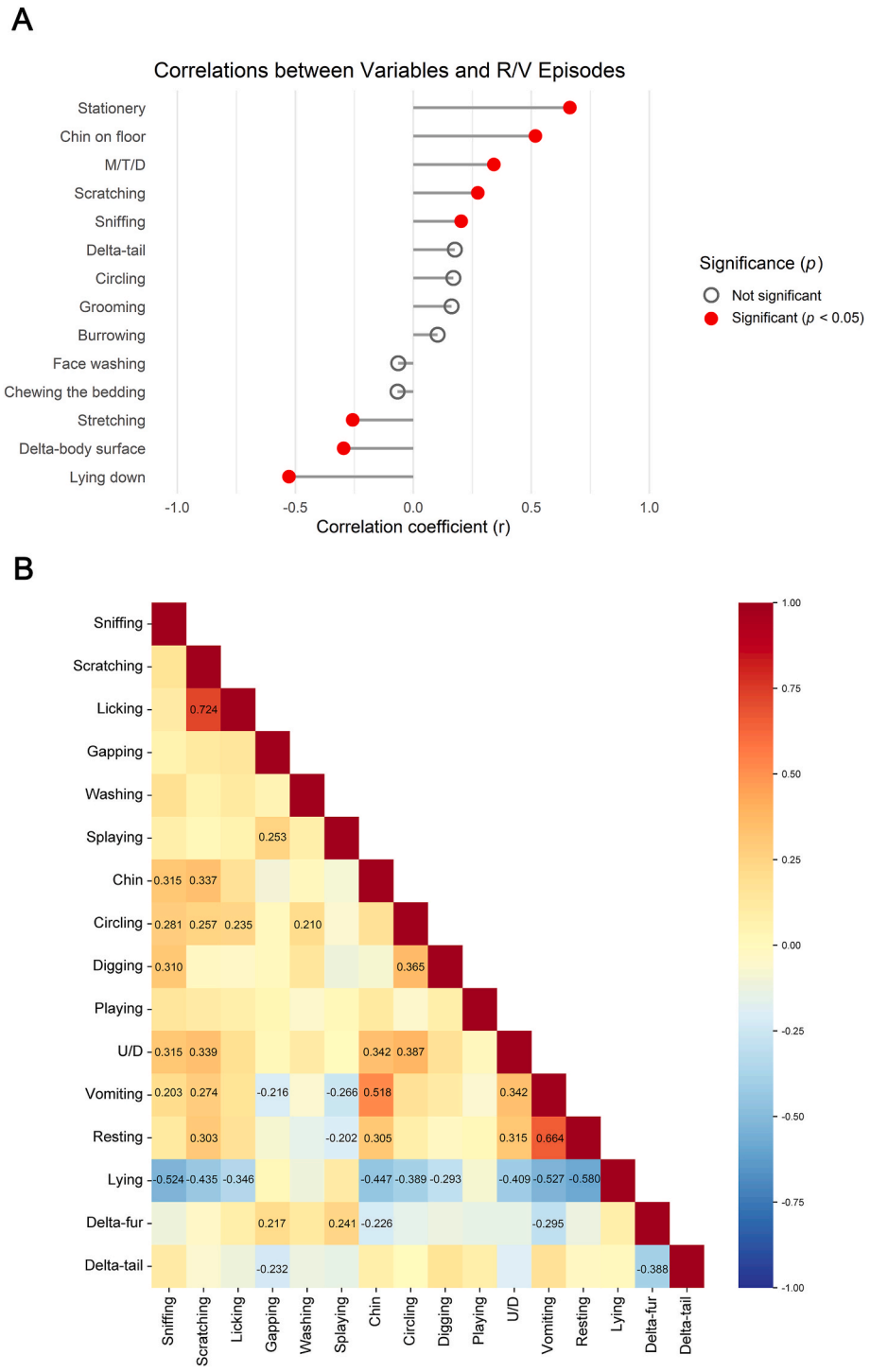

Fig. 2. Correlations between R/V episodes and other variables (n=129). A. Summary Lollipop plot of the correlation coefficients (*r* values), with significance levels of every variable. Each dot represents the correlation coefficient (*r*) for one variable. Red filled circles indicate statistically significant correlations ($p < 0.05$); grey open circles indicate non-significant correlations ($p \geq 0.05$). Variables are ordered by *r* values. B. Heatmap of correlations between behaviours [The colour bar indicates correlation coefficients, ranging from −1 (blue, strong negative correlation) to +1 (red, strong positive correlation). In the heatmap, only $|r|$ values > 0.2 with $p < 0.05$ are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

surface and tail temperatures were 32.33 ± 0.71 °C and 26.16 ± 1.51 °C, respectively, and the maximal body surface and tail temperature changes were −1.06 ± 0.05 °C and 3.31 ± 0.23 °C, respectively.

3.2. Correlations of spontaneous behaviour and temperature changes with R/V episodes

The Shapiro–Wilk tests indicated a normal distribution of data only for the lying down duration and delta-body surface temperature changes ($p = 0.18$ and 0.13 respectively); the distributions of other behaviours were not Gaussian ($p < 0.05$, Supplementary Table S1). Nevertheless, behavioural episodes and temperature changes recorded during provocative motion were then subjected to correlation analyses with the number of R/V episodes for each animal (Fig. 2A). The number of R/V episodes showed weak positive correlations with the numbers of sniffing episodes (r = 0.20), scratching episodes (r = 0.27), and micturition/tenesmus episodes (r = 0.34) and weak negative correlations with the number of stretching episodes (r = −0.26) and changes in body temperature (r = −0.30, $0.2 \leq |r| < 0.4$). The correlations of R/V episodes with chin on the floor episodes (r = 0.52) and the lying down duration (r = −0.53) were moderate ($0.4 \leq |r| < 0.6$). R/V episodes were highly correlated with the time spent stationary (r = 0.67) ($0.6 \leq |r| < 0.8$, Supplementary Figure S1 A–N). Other correlations between R/V episodes and behaviours are shown in Fig. 2B.

3.3. MS symptom score determination using stepwise multiple linear regression analysis and post-hoc assumption checks

Using R/V as the outcome, the stepwise model yielded the following score equation ($p = 0.20$, $r^2 = 0.552$; coefficients unstandardized; Table 2). MS symptom score could be represented as a weighted sum of scratching (coefficient: 0.157), stretching (coefficient: −1.304), chin on the floor (coefficient: 0.232), burrowing (coefficient: 0.177), and chewing the bedding (coefficient: −4.742). Micturition, defection, and/or tenesmus (coefficient: 0.815) episodes, the stationary duration (coefficient: 1.714), and delta-body surface (coefficient: −1.215) and delta-tail (coefficient: 0.413) temperatures functioned as constant terms (coefficient: 2.843).

For assumption checks, Durbin–Watson and multicollinearity tests were performed. The Durbin–Watson test showed that residuals and covariates were independent of each other (autocorrelation value: −0.0276, Durbin–Watson statistic: 2.04, $p = 0.842$). Multicollinearity tests (Table 2) showed that there was no multicollinearity between covariates (variance inflation factor >10 and tolerance <0.5 suggest multicollinearity). These results with quantile–quantile (Q-Q) plots (Supplementary Figure S2) showed high robustness of the constructed multiple linear regression model and indicated that dimensionality reduction was not essential.

According to the standardised coefficients of every variable (Table 2), the three variables with the most important influence on regression were being stationary, chin on the floor, and delta-tail temperature change, with standardised values of 0.628, 0.328, and 0.235, respectively. According to the SHAP analysis, the three variables that had the greatest effects on the overall model prediction were being stationary, chin on the floor, and sniffing, with mean absolute SHAP values of 2.27, 1.19, and 0.63, respectively (Fig. 3). Combining the above two analysis results, the duration of being stationary and number of episodes of chin on the floor were the behaviours that best reflected the degree of MS, indicating that the score captures non-emetic behavioural dimensions co-varying with emesis.

3.4. MS symptom score validity test

The MS symptom score was 11.38 ± 0.41 (Fig. 4A) and obeyed a Gaussian distribution (Shapiro–Wilk test, $p = 0.67$, Fig. 4B). A highly positive correlation was observed between this score and the number of R/V episodes (Spearman's $r = 0.77$, Fig. 4C).

In two separate drug treatment experiments, provocative motion elicited 11.71 ± 3.07 R/V episodes in the vehicle-treated group of the diphenhydramine experiment (n = 7), and 20.83 ± 3.25 episodes in the vehicle-treated group of the scopolamine experiment (n = 6), respectively. Diphenhydramine (30 mg/kg) and scopolamine (10 mg/kg) reduced the number of R/V episodes to 3.71 ± 1.17 (p = 0.004) and 11.17 ± 2.51 (p = 0.003), respectively (Fig. 5A and B). Similarly, motion-induced MS symptom scores were 14.25 ± 1.89 and 18.11 ± 2.28 (arbitrary units) in the vehicle groups of the two experiments. These scores were significantly reduced by diphenhydramine (7.84 ± 0.85, p = 0.004) and scopolamine (11.91 ± 0.87, p = 0.003), respectively (Fig. 5C and D). Although both drugs reduced R/V and the score, their effects on component behaviours diverged. Diphenhydramine significantly reduced the motion-induced stationary time from 2.98 ± 0.89 to 0.45 ± 0.21 min (n = 7, $p < 0.05$) but failed to affect chin on the floor episodes (vehicle: 6.86 ± 0.51, diphenhydramine: 6.71 ± 1.66, n = 7, Fig. 6A and C). Scopolamine significantly reduced the stationary time from 3.13 ± 0.77 to 1.05 ± 0.21 min and inhibited motion induced chin on the floor episodes from 16.67 ± 5.62 to 9.16 ± 1.92 (n = 6, $p < 0.05$, Fig. 6B and D). This pattern illustrates that the model can differentiate how treatments ameliorate malaise beyond the emetic endpoint. Therefore, we interpret the score as a complementary readout trained on an emesis proxy to approximate malaise/nausea severity, rather than as a replacement for R/V.

Notably, while conducting these experiments, diphenhydramine induced 3.14 ± 1.41 R/V episodes during the pretreatment period (observed in six out of seven animals), while 10 mg/kg scopolamine did

Table 2
Model coefficients, p value, VIF, tolerance and standardized coefficient values of every covariate in stepwise multiple linear regression (removal p value = 0.20). Std. Coe: standardized coefficient; Chin: chin on the floor; Chewing: chewing the bedding; M/D/T: Micturition, defecation, and/or tenesmus; Delta-bs: delta body surface temperature change; Delta-tail: delta tail temperature change.

			95 % Confidence Interval						
Predictor	Coefficient	SE	Lower	Upper	t	*p*	Std. Coe	VIF	Tolerance
Intercept	2.843	1.21	0.44	5.25	2.34	0.021	/	/	/
Scratching	0.157	0.08	0.01	0.31	2.05	0.042	0.207	1.17	0.852
Stretching	−1.304	0.62	−2.53	−0.07	−2.10	0.038	−0.200	1.07	0.937
Chin	0.232	0.08	0.08	0.38	3.05	0.003	0.328	1.34	0.745
Burrowing	0.177	0.13	−0.08	0.44	1.32	0.189	0.136	1.11	0.901
Chewing	−4.742	2.37	−9.45	−0.04	−2.00	0.048	−0.186	1.04	0.965
M/D/T	0.815	0.62	−0.41	2.04	1.32	0.189	0.143	1.31	0.762
Stationary	1.714	0.29	1.15	2.28	6.00	<0.001	0.628	1.27	0.786
Delta-bs	−1.215	0.91	−3.02	0.59	−1.34	0.184	−0.151	1.32	0.755
Delta-tail	0.413	0.19	0.03	0.79	2.16	0.033	0.235	1.33	0.754

The MS symptom score equals the intercept plus the weighted sum of the retained predictors using the unstandardized coefficients shown. R/V was the dependent variable and is not included in the score.

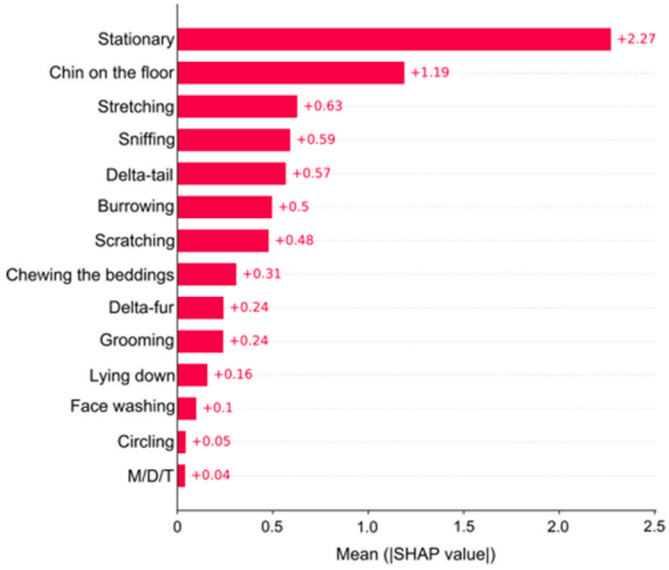

Fig. 3. A Shapley additive explanation (SHAP) feature importance plot with visual ranking of variables by their mean absolute SHAP values.

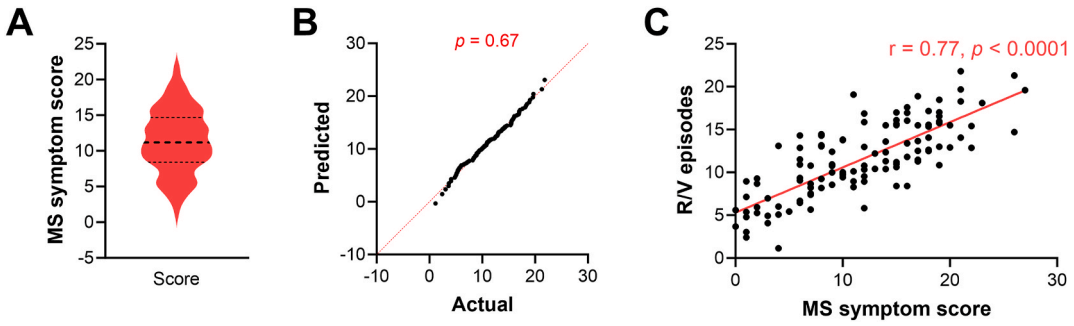

Fig. 4. MS symptom score description and correlation with R/V episodes. A. Descriptive statistics of the MS symptom score. B. Normal Q-Q plot of the normality test of the MS symptom score. C. Spearman correlation between R/V episodes and the MS symptom score.

not (Fig. 5A and B). Both diphenhydramine and scopolamine alone did not change the MS score during pretreatment period (Fig. 5C and D).

4. Discussion

In behavioural studies, it is often assumed that emesis is an indicator of 'malaise' or 'nausea' and that changes in behaviour may occur up to, during, and after an emetic event (Horn et al., 2011). In the absence of an objective gold standard for MS malaise in animals, we trained a regression-based composite on R/V as an observable proxy outcome. This non-biased MS symptom score was then validated using the common anti-MS drugs diphenhydramine and scopolamine. The score included the sum of scores for nine variables, namely the episodes of scratching, stretching, chin on the floor, burrowing, chewing the bedding, and micturition or defecation and/or tenesmus; the duration for which the animals remained stationary; and the changes in their body and tail temperatures. By excluding R/V from predictors, the score aggregates non-emetic features that accompany emesis, thereby complementing—rather than supplanting—R/V.

Several symptoms modulated by the autonomic nervous system have been previously used to document MS in animals (Suri et al., 1979; Yu et al., 2007; Parker et al., 2008). Studies in *Suncus murinus* have documented motion-induced changes in behaviours such as chin on the floor, scratching, licking, and sniffing (Tu et al., 2020). Other studies have examined motion-induced hypothermia (Pan et al., 2024), whereas we assessed motion-induced increases in tail temperature in our previous study (Tu et al., 2017b) and also considered maximal changes in both body surface and tail temperatures as parameters in our model in the present study, offering a more holistic assessment of MS-related malaise. While R/V remains the primary index of emesis, the MS symptom score developed here incorporates additional behavioural parameters, such as stationary duration and chin-on-the-floor episodes, which complement R/V in capturing the broader features of motion-induced malaise or nausea.

As correlation analysis (Supplementary Figure S1) could not indicate the complex behavioural patterns related to motion-induced R/V or 'nausea', we conducted multiple linear regression analyses between behaviours/temperature changes and R/V, which were regarded as more suitable indicators of MS. The regression analysis showed that scratching, stretching, chin on the floor, burrowing, chewing the bedding, micturition and defection and/or tenesmus, time spent stationary, and delta-body surface and delta-tail temperatures were important contributors to the score (Table 2). Further, the standardised coefficients of stretching, chewing the bedding, and delta-body surface

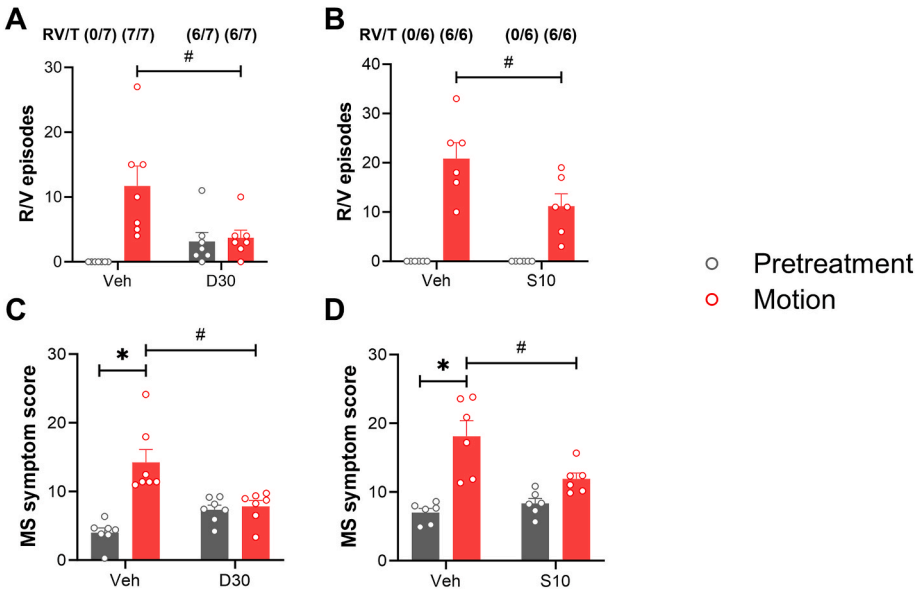

Fig. 5. Effects of 30 mg/kg diphenhydramine (D30) and 10 mg/kg scopolamine (S10) on motion-induced R/V and the MS symptom score. **A**. Diphenhydramine-induced R/V and decreased motion-induced R/V; **B**. Scopolamine did not induce R/V but reduced MS-induced R/V; **C**. Diphenhydramine reduced the MS symptom score from 14.25 ± 1.89 to 7.84 ± 0.85; **D**. Scopolamine reduced the MS symptom score from 18.11 ± 2.28 to 11.91 ± 0.87. For comparisons, significant differences relative to the vehicle group are shown as * $p < 0.05$ (two-way ANOVA followed by Bonferroni multiple tests) when referring to inter-group comparisons and as #$p < 0.05$ (two-way ANOVA followed by Bonferroni multiple tests) when referring to intra-group comparisons.

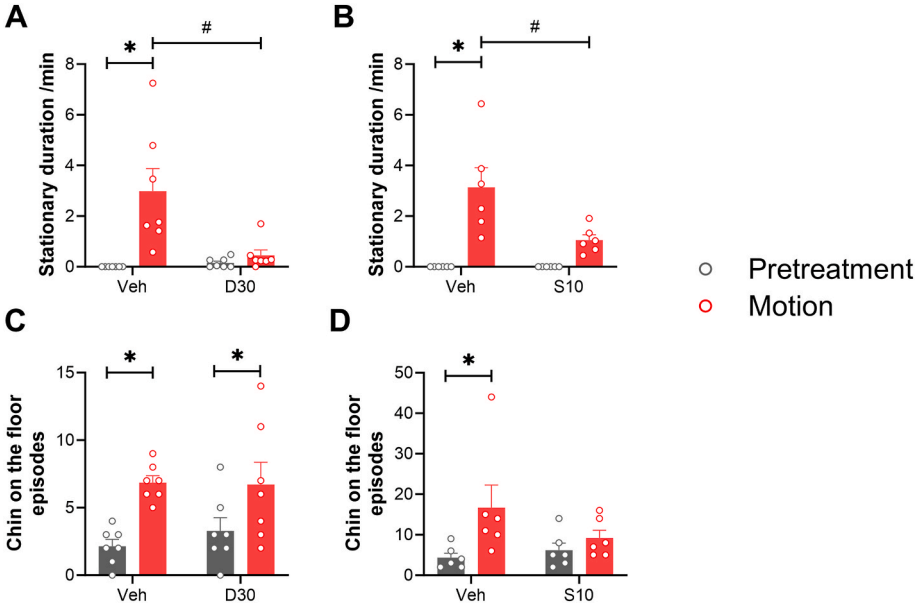

Fig. 6. The effects of 30 mg/kg diphenhydramine (D30) and 10 mg/kg scopolamine (S10) on the motion-induced stationary duration and chin on the floor episodes. **A**. Diphenhydramine significantly reduced the motion-induced stationary duration from 2.98 ± 0.89 to 0.45 ± 0.21 min (n = 7). **B**. Scopolamine significantly reduced the motion-induced stationary duration from 3.13 ± 0.77 to 1.05 ± 0.21 min (n = 6). **C**. Diphenhydramine did not change the motion-induced chin on the floor episodes (Veh: 6.86 ± 0.51, D30: 6.71 ± 1.66, n = 7). **D**. Scopolamine inhibited the motion-induced chin on the floor episodes (Veh: 16.67 ± 5.62, S10: 9.16 ± 1.92, n = 6). *$p < 0.05$ (two-way ANOVA followed by Bonferroni multiple tests) when referring to inter-group comparisons, and #$p < 0.05$ (two-way ANOVA followed by Bonferroni multiple tests) when referring to intra-group comparisons.

temperature changes had negative correlations with the outcome, whereas other behaviours had positive correlations (Table 2). Among all of the behaviours, being stationary and chin on the floor were distinguished as being of the highest importance to MS by regression analysis together with SHAP analysis (Table 2 and Fig. 3). When combined with hypoactivity caused by motion (Qi et al., 2019), we considered the stationary duration parameter to be related to locomotion. It is possible that chin on the floor and/or chewing the bedding allows excessive salivation to transfer to the bedding substrate. Furthermore, a highly positive correlation was observed between the numbers of scratching and grooming episodes (Spearman's $r = 0.72$), encompassing grooming or salivation (Suri et al., 1979) which we were unable to observe in our experiments due to technical limitations. The delta-body surface and delta-tail temperature changes are consistent with our findings that motion decreased the surface body temperature and increased the tail temperature in *Suncus murinus* (Tu et al., 2017a, 2017b).

Multiple linear regression is suitable for estimating relationships between a dependent variable and multiple independent variables. Through our experiments, a prediction equation for the relationships between R/V episodes and multiple behaviours/symptoms was defined. This approach was previously used in a study of galvanic vestibular stimulation-induced MS in cats. In that study, the weighted sum equation consisted of c-Fos-positive cell numbers in the lateral subnucleus of the solitary tract nucleus (coefficient: 0.123), sub-trigeminal nucleus (coefficient: 0.143), and external cuneate nucleus (coefficient: 0.033) and a constant term (−1.567) was established to represent MS severity (Balaban et al., 2014). The obvious advantage of the MS symptom score obtained by multiple linear regression is that the weight of each independent variable is determined by an algorithm in which the bias of artificially assigning weights is fundamentally eliminated. Except for linear regression, a temporal analysis was performed to study behavioural or physiological changes related to emesis. Horn et al. revealed that many patterns of behaviour are associated with cisplatin-induced emesis in *Suncus murinus*, including sniffing and changes in body contraction and locomotion (Horn et al., 2011). However, the readout of temporal analysis was a separate emesis-associated behaviour which cannot be used as an index to test the severity of an emetic stimulation or the anti-emetic potency of drugs.

This study is the first to develop a symptom index of 'sickness', 'malaise', or 'nausea' in animals during provocative motion. Furthermore, the index has been validated using standard anti-emetic drugs, diphenhydramine and scopolamine. As expected, both of these anti-emetic compounds significantly reduced R/V episodes and the MS score. Nevertheless, their effects on stationary duration and chin on the floor episodes, two behaviours that best represent MS, varied. Scopolamine decreased the motion-induced stationary behaviour and chin on the floor activity, but diphenhydramine only affected the motion-induced stationary parameter. This suggests that scopolamine has a higher anti-MS effect than diphenhydramine, which is consistent with a previous finding by Chan and co-workers that 0.3 mg/kg scopolamine reduced motion-induced R/V, whereas 10 mg/kg diphenhydramine did not (Chan et al., 2007). The differential effects of diphenhydramine and scopolamine on individual behavioural symptoms may reflect differences of mechanisms in the action of the two drugs and further validate the inclusion of multiple behavioural parameters in the MS score. The distinct behavioural fingerprints under diphenhydramine versus scopolamine (stationary vs. chin-on-the-floor) suggest the model may help profile mechanisms of anti-MS agents. Such findings underscore the importance of assessing a broader range of symptoms when evaluating anti-MS efficacy, rather than relying solely on emetic endpoints. Surprisingly, diphenhydramine alone caused emesis in *Suncus murinus*, which has never been reported before. Besides H_1 receptors, diphenhydramine has affinity for 5-HT_{2A} and 5-HT_{2C} receptors, with K_i values of 260 and 780 nM, respectively (Krystal et al., 2013). It is not known if this explains the mechanisms of emetic action, but 5-HT_2 agonists can induce emesis in *Suncus murinus* and marmosets. On the other hand, diphenhydramine alone did not increase the MS symptom score. This finding suggests that the score proposed here is limited to MS-caused R/V or 'nausea' and that nausea and vomiting induced by other stimuli might be characterised by other behavioural patterns.

Our MS score has advantages in that it enables an assessment of behavioural changes encompassing both autonomic and somatic events in relation to the degree of R/V (somatic) in *Suncus murinus*. However, we do not claim the score is superior to R/V *per se*; it is intended to complement R/V. It is important to note that R/V is used as a proxy for the severity of MS in animal models only within the constraints of current experimental conditions. The r^2 value of 0.552 from the multiple linear regression model indicates that nine variables included in the model explained 55.2 % of the R/V episodes. Although it is sufficient to derive an MS score (validated using diphenhydramine and scopolamine), the MS score accuracy may be improved by including more variables, e.g., heart rate variability, respiratory changes, and changes in gastrointestinal slow waves (Lu et al., 2017; Gavgani et al., 2017; Percie du Sert et al., 2010; Stern et al., 2011). It would also be advantageous to expand our approach to encompass the end-of-experiment changes in c-Fos activation in the brain (Tu et al., 2017a, 2020; Nalivaiko et al., 2014; Qi et al., 2019; Ngampramuan et al., 2014). Additionally, it may also be useful in future studies to test compounds that act through different mechanisms, such as GABAergic or ghrelin pathways (Chan et al., 2007; Tu et al., 2020). This would help determine whether the model is responsive to a broader range of anti-motion sickness drugs and further enhance its translational value.

5. Conclusion

In summary, a non-biased MS symptom score was determined in the present study. The MS score was based on multiple linear regression of 9 weighted symptoms and effectively represented 'sickness', 'malaise', or even 'nausea' in *Suncus murinus*. The findings provide insights into the mechanism of MS valuable for the development of new anti-MS drugs.

CRediT authorship contribution statement

Zitong Li: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Ji Qi:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis. **Zengbing Lu:** Writing – review & editing, Validation. **Man Piu Ngan:** Resources. **Julia Yuen Hang Liu:** Writing – review & editing. **Lingqing Yang:** Writing – review & editing. **Aleena Khalid:** Writing – review & editing. **Luping Liu:** Writing – review & editing. **Yingyi Deng:** Writing – review & editing. **Xiaofei Huang:** Writing – review & editing. **Sze Wa Chan:** Writing – review & editing, Funding acquisition. **Richard Hau Yue So:** Writing – review & editing, Funding acquisition. **John Anthony Rudd:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Ethical approval

All experiments were conducted under a license from the Hong Kong SAR government and the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong (Approved NO.: 21-082-GRF).

Data and code availability

All the data are available upon request. SHAP analysis and visualisation were performed using the Python package 'shap' (version 0.47.1), available at https://github.com/shap/shap.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2025.178435.

Data availability

Data will be made available on request.

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