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Research article

Disorientation effects, circulating small ribonucleic acid, and genetic susceptibility on static postural stability

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ABSTRACT

Background: Motion Sickness increases risk of performance deficits and safety of flight concerns. The etiology of motion sickness is poorly understood. Here, we attempted to quantify the physiological effects of motion sickness on static balance and determine the genetic predictors associated with these effects.

Methods: 16 subjects underwent a disorientation stimulus to induce motion sickness. Motion sickness susceptibility was identified using the Motion Sickness Susceptibility Questionnaire. Postural balance outcomes were measured using two tasks, and small ribonucleic acid profiles were assessed with blood draws before motion sickness stimulus. Differences in postural sway before and after the stimulus as well as effect modification of susceptibility were assessed. A random forest followed by regression tree analysis was constructed for each postural sway variable to determine top genetic and covariate predictors.

Findings: Significant differences existed in mean postural balance responses between before and after stimulus. Individuals with longer stimulus survival experienced a greater (but insignificant) perception of sway, even if not displaying increased sway for all conditions. Circulation small ribonucleic acids were differentially expressed between individuals with long and short stimulus survival, many of these microRNA have purported targets in genes related to vestibular disorders.

Interpretation: We found motion sickness produces transient motor dysfunction in a healthy military population. Small ribonucleic acids were differentially expressed between subjects with long and short stimulus survival times.

1. Introduction

Even as far back as the time of Hippocrates, motion sickness (MS) was a term described of sailing on the sea [1]. Extremely common, it has been documented that roughly one-third of all individuals are very susceptible to this condition [2]. The degree at

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which common symptoms of MS arise in an individual are a result of the stimulus intensity and their predisposition [2]. Several factors that increase one's susceptibility to MS have been observed [3,4]. The theory of etiology of MS most accepted today is one of sensory conflict, first proposed by Reason and Brand [1]. This explanation has been supported by several studies involving a disagreement between visually perceived motion and the vestibular system's sense of movement. Our brain receives information on movement from many sensory organs including vestibular receptors of the inner ears, proprioceptors of the muscles and joints, and eyes. When input from these organs do not correlate, or differ from the brain's positional memory, MS is triggered as a result [5].

The vestibular and proprioceptive systems play a strong role in maintaining postural balance and upright position of which deficits create a greater risk for falling and loss of balance. Previous studies have shown childhood exposure to heavy metal lead (Pb) is associated with postural instability due to damage of the vestibular system [6,7,8]. Mismatches in perceived and actual loss of balance because of modified proprioceptive input from standing on inclined surfaces can also result in an increase in postural sway [9]. Other researchers have observed problems with balance control and coordination in Parkinson's patients [10,11] and in those with otolith disorders [12] due to difficulty in integrating proprioceptive inputs. Dysfunction in balance can result in deleterious effects increasing the chances of workplace accidents and injuries for many types of workers (e.g., roofers, firefighters; [9,13]).

All military personnel subjected to passive motion may have an increased risk of MS based on exposure to these conditions. Of particular interest to us are military aircrew, who experience novel demands to their spatial orientation senses due to the inputs of a distinctive aerial environment, often in suboptimal conditions, which limit the range of visual acuity. Performance deficits in aircrew due to MS reduce mission readiness, combat effectiveness and, in worst cases, can induce human error that leads to safety of flight issues, occasionally to the point of mishap. There is evidence that some military personnel experience MS during flight or shortly following flight and when at sea [14,15]. Decrements in motivation, cognitive performance, and motor tasks have been observed in instances of seasickness [14,16]. Though many countermeasures have been developed and tested for military applications (e.g., scopolamine; [17]), many limitations still exist on their efficacy [18]. There is some evidence in the literature that use of simulations and immersive virtual environments in older subjects as well Parkinson patients have improved balance and gait, but it has not been tried in military personnel [19]. Currently, the effects of MS on postural control in military personnel have not been quantified.

Some researchers have stated individual susceptibility to MS is perhaps the least understood variable and is still relatively unknown [20]. Furthermore, the pathogenesis of MS, onset of symptoms, and adaptive mechanisms to the offensive inputs implies that there could be an underlying connection between the cognitive, physical, and genetic predispositions for this syndrome. Our aims are to further quantify the physiological effects of disorientation stimuli on postural stability function and gene expression in a healthy military population. One previous genome-wide association study of over 80,000 subjects identified 35 single nucleotide polymorphisms (SNPs) associated with MS based on self-reported MS susceptibility [20]. Here we compared the genotypes for these SNPs between the MS subjects separated by how long they endured the motion sickness stimulus (survival time). In addition, we compared the expression of circulating small ribonucleic acid (RNA) molecules with the postural balance results to attempt to identify at-risk individuals. These small RNA molecules include microRNAs, which are endogenous regulators of gene expression, and have been shown to respond to numerous stimuli such as physical exercise [21]. Our final goal was to correlate differences in postural balance to genetic variants and small RNA expression that significantly differ between groups. We hypothesized that postural balance will be markedly changed following induced MS as indicated by an increase in postural sway, and that circulating microRNA could serve as biomarkers of MS susceptibility. While early in development, investigation into the genetic roots of MS may support targeted therapies for afflicted individuals. The ultimate objective of the work described in this pilot study is to refine the methods to investigate motion sickness, identify biomarkers of interest for susceptibility screening and potentially of etiological interest, and finally to determine sample sizes required for future studies.

2. Methods

2.1. Subjects

Healthy subjects were recruited from Wright Patterson Air Force Base through email and word of mouth. Inclusion criteria were working adults 18–50 years old, weighing <250 lbs. Subjects were excluded for the following: previous back injury, neuromusculoskeletal disorders, and pregnancy (pregnancy test completed by all female participants). Prior to testing, informed consent was completed following approval of the protocol by the Naval Medical Research Unit Dayton IRB (NAMRUD.2017.0003), and subjects were instructed to avoid nicotine, alcohol, caffeine, or other drugs for 12 h.

2.2. Disorientation Stimulus

A disorientation stimulus (Neuro-Otologic Test Chair (NOTC), Neuro Kinetics Inc.) was used. The NOTC uses a multi-axis rotating platform to induce MS. The NOTC chair was stationed in the Naval Medical Research Unit- Dayton (NAMRU-D) laboratory and ran by personnel of NAMRU-D. The rotating chair was positioned in a lightproof chamber and subjects were secured with a harness and seatbelt. Coriolis cross-coupling through movement of subject's head side to side was achieved to elicit a MS response. Subjects were required to perform lateral head tilts from neutral position (0°) to 30° each to the left and right for the duration of the stimulus. Subjects were observed through the duration of testing for compliance. This protocol has been widely used and is a reliable method to elicit minor MS symptoms gradually [22]. The stimulus protocol began clockwise rotation at 1 rpm, increasing by 1 rpm every minute for a maximum allotted time of 40 min (40 rpm). The acceleration profile was 200°/s2 [23]. During the protocol, using the Exposure Symptoms Report (ESR), perceived nausea on a scale of 1–10 was reported verbally each minute. Cessation of the stimulus occurred

once moderate nausea (perceived nausea scale >5) was achieved, the maximum allotted time was achieved, or a subject requested to exit ("survival time").

2.3. Protocol

Once consented, subjects were given a briefing of the day. Anthropometric measurements, height, and weight were taken and a Day of Visit Questionnaire (DOV) and Motion Sickness Susceptibility Questionnaire (MSSQ, Pensacola Motion Sickness Questionnaire; [24]) was completed. The DOV recorded general information of the subject's routine in the last 24 h such as meals, sleep schedule, and current health problems and medication. These factors, *despite not appearing serious,* are known to influence motion sickness. *For example, certain type of food, disruptive sleep and certain medication could trigger acute motion sickness. Therefore, these questions were asked on the date of testing.* A repeated measures design was used for this study. Each subject underwent postural balance testing both before and after exposure to the NOTC stimulus. All subjects were assigned the same testing conditions (before and after stimulus) and stimulus protocol, but the order of tests was randomized between subjects. The results outlined here are part of a larger study entailing several other testing protocols [25] however, only the methods and results pertaining to postural balance testing will be discussed.

2.3.1. Postural static balance test

Static balance was measured using the AMTI portable force plate (Accusway +, Advanced Medical Technology Inc. AMTI) and recorded in the Balance Clinic software. This device measures forces and center of pressure (CP) of the testing subject. All subjects performed two 30s trials of each of the two following tests in a repeated counterbalance measure: eyes open on the force platform (EO) and eyes closed on a foam platform (ECF). Averages of the two trials were used in data analysis. The two different tests used in this study are meant to challenge the various afferents (visual, proprioceptive, and vestibular) needed in maintaining upright balance [7]. The variables derived from CP that were used for analysis included sway area (SA), sway length (SL), mediolateral sway (ML), and anteroposterior sway (AP). [26].

2.3.2. Blood collection

Before and after MS stimulus, 10 mL of blood were collected in red-capped tubes (no anticoagulant, Becton Dickinson, Franklin Lakes, NJ) by a phlebotomist and allowed to clot at room temperature for 30 min post draw. Tubes were then centrifuged 20 min at 2,000×*g* at 4 °C to precipitate cellular material. Supernatant was removed and transferred to cryotubes for RNA isolation.

An additional 4 mL of blood were collected into lavender-topped tubes (potassium EDTA, Becton Dickinson, Franklin Lakes, NJ) and transferred to separate cryotubes for DNA isolation.

All blood specimens were stored at −80 °C prior to nucleic acid isolation.

2.3.3. RNA isolation and mircoRNA array analysis

RNA from 200 μL of serum were hybridized on GeneChip™ miR4.0 Arrays (Thermo Fisher Scientific, Waltham, MA) were utilized for small RNA analysis according to manufacturer's instructions. Data summarization was performed by RMA + DABG for the human content of the arrays. Annotation was performed using the most recent version for these arrays (miR-4.0-st-v1.annotations.20160922.csv). Gene expression was filtered by fold-change of at least 1.2, gene-level p-value of less than 0.05, and DABG<0.05.

2.3.4. DNA isolation and genotyping analysis

According to the manufacturer's instructions, DNA was extracted and purified from 200 μL of peripheral blood using a GeneJet Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Waltham, MA). The quantity and quality of the DNA extracted was determined using a Nano Drop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Single nucleotide polymorphisms (SNPs) associated with MS were obtained from the literature [20]. Genotyping was performed with Taqman allele discrimination assays per manufacturer's instructions (Applied Biosystems, Waltham, MA).

2.4. Perceived Sense of Postural instability score (PSPSI)

Following each static trial, subjects were asked a series of four questions pertaining to their perceived sense of postural instability (PSPSI; [27, 28]) Scores ranged from 0 to 2. Totaled scores for each trial, followed by the average for each condition of every subject, were used in data analysis.

2.5. MicroRNA expression variation

MicroRNAs with significant changes in expression (see 2.3.3) between the MS groups ("long survival", LS, or "short survival", SS) were connected to their suspected target messenger RNA (mRNA) molecules according to miRBase release 22.1 [29]. This list of target mRNA was cross-referenced with the literature on genes involved in genetic disorders of the vestibular system [30, 31]. In addition, microRNAs within microRNA families previously shown to be dysregulated in Parkinson's disease [32] were also cross-referenced with differentially expressed microRNAs. The resulting list contains microRNAs that are suspected or demonstrated to regulate the expression of genes known to play a role in the vestibular system and balance, which also are differently expressed between the LS and SS subjects (see 2.6).

2.6. Statistical analysis

Descriptive statistics were expressed as mean ± standard deviation (SD). For all static postural balance variables and subjective PSPSI scores, significant differences between before and after stimulus under both conditions (EO, ECF) were tested using a paired *t*-test. Although MSSQ is a good predictor of motion sickness susceptibility [33], it is not always reliable for predicting specific test outcomes. In Golding and Stott [34], MSSQ score was not related to stimulus time required to induce moderate nausea ($r = -0.17$). Golding 1998 summarizes "Correlations between laboratory measures of motion sickness and general susceptibility to motion sickness, e.g., as measured by the MSSQ, are frequently observed to be low" [35]. Therefore, we converted the continuous variable MSSQ score to the categorical variable "short survival" and "long survival" (SS and LS). Data were initially stratified as susceptible (S) and not susceptible (NS) subjects based upon MSSQ scores, those subjects with a score of 0 being labeled NS ($n = 6$) and any score above 0 were labeled S ($n = 10$). However, comparison of MS stimulus survival time between these groups by unpaired *t*-test with Welch correction did not show significant difference ($p = 0.69$). Therefore, k-means clustering was performed based on NOTC survival time. This resulted in two groups of LS ($n = 5$) and SS ($n = 11$) with cluster centroids of 19.11 and 10.45 min, respectively. Welch's *t*-test for survival time between these groups was highly significant ($p = 0.007$), and these groupings were used for the remaining analyses. Two-sample t-tests were conducted to determine significance between these two susceptibility groups for all static balance variables for each of the conditions before and after the stimulus. A multiple regression model was fitted to the data for each postural sway variable and PSPSI scores under both the test conditions with covariates: Stimulus (binary); NOTC time; Test Order; Susceptibility (binary); Age; Gender (binary); BMI; Foot Area; ESR Score; MSSQ; Sleep. Normality assumption was checked and found to be acceptable. A model selection algorithm (backward elimination procedure) was used adopting the AIC criterion to further confirm the models.

Random forest regression was run on genetic and small RNA expression data for feature selection of each of the static variables. Random forests were performed 20 times to stabilize and select a consensus of the top 10 genetic predictors. Regression trees were built for each static postural balance variable combining both the predictors and cofactors. Separate analyses for minimum splits of 5–8 were conducted hypothetically to find the best model without overfitting the data.

Signal values for each probe on the microarrays were compared between SS and LS groups against the results of the postural static balance test and PSPSI scores.

3. Results

A total of 7 females and 9 males completed the study, ranging in age from 19 to 45 years (mean = 28.3 yrs ± 7.6). Table 1 provides demographics of all subjects who participated. All but 3 subjects received adequate sleep the night before (mean = 7.5 h ±1.09) and most subjects (15/16) had a meal within 6 h of the start of testing based on DOV data. MSSQ ranged from 0 to 97 (mean = 54.9 ± 33). A higher percentage of females (5/7) fell into the shorter survival (SS) group than males (5/9). Subjects survived an average of 13.1 min in the NOTC before surcease. The maximum amount of time a subject survived the stimulus was 25.6 min. Peak ESR scores ranged from 5 to 9 among subjects prior to stimulus cessation and 6/16 of subjects reported some level of nausea on the ESR scale upon completion of all tests. There was a significant difference in NOTC survival time between LS and SS groups ($p = 0.007$).

3.1. Postural Static balance

Results of postural static balance testing revealed an increase in all postural sway variables (SA, SL, ML, AP) and PSPSI for the ECF condition compared to EO condition before and after the stimulus (Table 2). Paired t-tests revealed statistically significant differences ($p < 0.05$) in the following variables: Sway area (SA) for eyes closed foam (ECF) condition ($p = 0.003$), mediolateral sway (ML) for ECF condition ($p = 0.058$), perceived sense of instability score (PSPSI) for the ECF condition ($p = 0.052$), and PSPSI for the eyes open (EO) condition ($p = 0.008$).

Average changes in PSPSI scores (Table 2) reveal subjects perceived significantly greater sway for both EO and ECF conditions (EO: change = 0.438, $p = 0.052$; ECF: change = 0.797, $p = 0.008$).

The observed effect size of the metric Sway Area Eyes Closed on Foam, at the given sample size $n = 16$, has power of 92%. The observed effect size of the metric PSPSI Eyes Closed on Foam, at the given sample size $n = 16$, has power of 80%. All other metrics would require higher sample sizes to detect the observed effect size with 80% power.

Table 1
Characteristics and gender distribution of study participants. Age, years; Time spent in NOTC, mins; Height, cm; Weight, kg; Amount of sleep the night prior to testing, hrs.

Variables	Mean (±SD)	Min	Max	Gender	n
Age (yrs)	28.3 (±7.6)	19	45	Female	7
Time in NOTC (min)	13.1 (±5.0)	7.1	25.6	Male	9
BMI	25.6 (±4.0)	20.5	37.2		
Height (cm)	175.5 (±11.4)	152	190		
Weight (kg)	79.4 (±16)	54.7	107		
Amount of Sleep (hrs.)	7.5 (±1.1)	5.4	9.3		

Table 2
Summary of changes in static balance parameters after stimulus.

	After Stimulus vs. Before Stimulus (n = 16)		
	Change (±SD)	*p*	Effect Size
Sway Area			
Eyes Open	0.164 (±0.6)	0.291	0.273
Eyes Closed on Foam	1.331 (±1.48)	0.003**	0.899
Sway Length			
Eyes Open	1.5 (±4.368)	0.19	0.343
Eyes Closed on Foam	1.876 (±14.808)	0.62	0.127
Mediolateral Sway			
Eyes Open	0.13 (±0.343)	0.149	0.397
Eyes Closed on Foam	0.284 (±0.552)	0.058	0.514
Anteroposterior Sway			
Eyes Open	0.067 (±0.349)	0.457	0.192
Eyes Closed on Foam	0.387 (±0.844)	0.086	0.458
PSPSI			
Eyes Open	0.438 (±0.829)	0.052	0.528
Eyes Closed on Foam	0.797 (±1.05)	0.008*	0.759

PSPSI, perceived sense of instability score. * = $p \leq 0.05$ ** = still significant after Bonferroni correction ($p \leq 0.003$).

Stratified data are presented in Table 3. Subjects within the SS group exhibited, on average, a greater sway response for ML and AP sway, while SA and SL were more equivocal. A comparison of average anteroposterior sway in EO condition after stimulus between the SS and LS groups was significant ($p = 0.02$), but even this was insignificant after multiple testing correction. Short survival subjects had average perceived sense of instability score greater than LS subjects in every condition, indicating greater perception of sway in the SS group.

The observed effect size of the metric Anteroposterior Sway Eyes Open After, at the given sample size n = 16, has power of 89%. All other metrics would require higher sample sizes to detect the observed effect size with 80% power.

For model selection, we used the backward elimination procedure, adopting the AIC (Akaike Information Criterion), which identified the whole set of predictors for each postural balance variable and PSPSI for the case of "eyes open" (Table 4A) and "eyes closed (Table 4B). Stimulus survival time, Age, and BMI were retained in the model selection exercise for all the postural balance variables for the EO test. Survival and Gender were retained in the reduced model for the ECF test. Normality assumption was checked to be valid. All R2 values were significant.

Table 3
Comparison of static balance parameters by stimulus survival group.

	Short Survival (n = 11)	Long Survival (n = 5)		
	Mean (±SD)	Mean (±SD)	*p*	Effect Size
Sway Area				
Eyes Open Before	2.23 (±1.03)	1.89 (±0.60)	0.424	0.366
Eyes Open After	2.43 (±0.92)	1.90 (±0.52)	0.166	0.642
	4.35 (±1.24)	5.04 (±1.62)	0.432	0.508
Eyes Closed After	5.78 (±1.64)	6.17 (±2.69)	0.776	0.195
Sway Length				
Eyes Open Before	33.02 (±6.21)	33.86 (±3.39)	0.732	0.151
Eyes Open After	35.10 (±7.20)	34.08 (±5.65)	0.766	0.150
Eyes Closed Before	67.47 (±21.70)	83.13 (±23.11)	0.242	0.708
Eyes Closed After	69.30 (±18.28)	85.00 (±33.13)	0.365	0.668
Mediolateral Sway				
Eyes Open Before	1.54 (±0.47)	1.35 (±0.29)	0.335	0.446
Eyes Open After	1.62 (±0.46)	1.59 (±0.40)	0.911	0.068
Eyes Closed Before	2.31 (±0.56)	2.33 (±0.53)	0.957	0.036
Eyes Closed After	2.52 (±0.60)	2.77 (±0.66)	0.487	0.405
Anteroposterior Sway				
Eyes Open Before	2.34 (±0.60)	2.19 (±0.39)	0.575	0.274
Eyes Open After	2.53 (±0.52)	1.98 (±0.30)	0.020*	1.170
Eyes Closed Before	3.41 (±0.81)	3.74 (±0.23)	0.229	0.474
Eyes Closed After	3.94 (±0.68)	3.82 (±1.04)	0.814	0.150
PSPSI				
Eyes Open Before	0.48 (±0.56)	0.25 (±0.25)	0.283	0.468
Eyes Open After	0.95 (±1.22)	0.60 (±0.52)	0.43	0.328
Eyes Closed Before	1.64 (±0.98)	1.55 (±0.78)	0.854	0.097
Eyes Closed After	2.50 (±1.59)	2.20 (±0.82)	0.627	0.212

PSPSI, perceived sense of instability score. * = $p \leq 0.05$.

3.2. 3.2 miRNA expression

Of the microRNAs differentially expressed in SS vs LS subjects, 17 were predicted or demonstrated to negatively regulate the expression of genes that have been identified as playing roles in disorders of the vestibular system, while remaining significant after multiple testing correction. (Fig. 1).

Table 4
Cofactors selected by model selection following the backward elimination algorithm for each outcome variable.

Cofactors	Outcome Variables				
	SL	SA	ML	AP	PSPSI
A) The Case of EYES OPEN					
Stimulus	NI	NI	NI	NI	Yes 0.064
NOTC Time	Yes 0.139	Yes 0.019	Yes 0.212	Yes 0.007	NI
Test Order	NI	Yes 0.046	NI	Yes <0.001*	Yes 0.031
Survival group	Yes 0.005*	Yes <0.001*	Yes 0.011	Yes <0.001*	NI
Age	Yes 0.002*	Yes 0.006	Yes 0.076	Yes 0.056	NI
Gender	NI	NI	NI	Yes 0.242	Yes 0.002
BMI	Yes <0.001*	Yes 0.017	Yes 0.094	Yes 0.037	NI
Foot Area	Yes <0.001*	Yes 0.025	Yes 0.022	NI	NI
ESR Score	Yes 0.02	NI	NI	Yes 0.057	Yes 0.002
MSSQ	NI	Yes 0.139	Yes 0.061	Yes 0.064	Yes 0.037
Sleep	Yes 0.006	NI	Yes 0.142	Yes 0.245	NI
Normality R^2	$p = 0.675$ 68.37%	$p = 0.778$ 61.83%	$p = 0.236$ 52.08%	$p = 0.635$ 73.16%	$p = 0.093$ 50.94%
B) The Case of EYES CLOSED					
Stimulus	NI	Yes 0.003*	Yes 0.097	Yes 0.081	Yes 0.027
NOTC Time	NI	Yes 0.148	NI	Yes 0.068	NI
Test Order	Yes 0.15	NI	NI	NI	Yes 0.091
Survival Group	Yes 0.004*	Yes 0.002*	Yes 0.138	Yes 0.03	NI
Age	Yes 0.027	NI	NI	Yes 0.136	NI
Gender	Yes <0.001*	Yes <0.001*	Yes <0.001*	Yes 0.011	NI
BMI	NI	NI	NI	Yes 0.025	Yes 0.158
Foot Area	Yes 0.018	NI	NI	NI	Yes 0.02
ESR Score	NI	Yes 0.076	NI	Yes 0.047	Yes 0.011
MSSQ	NI	Yes 0.072	NI	NI	NI
Sleep	Yes 0.002*	NI	NI	NI	NI
Normality R^2	p=0.902 79.05%	$p = 0.119$ 65.81%	$p = 0.577$ 41.85%	$p = 0.044$ 50.00%	$p = 0.625$ 47.11%

* = $p \leq 0.005$.
* = p≤0.005.
Legend: SL = Sway Length; SA = Sway Area; ML = Mediolateral Sway; AP = Antero-Posterior Sway; PSPSI = Perceived Sense of Instability Score; MSSQ = Motion Sickness Susceptibility Questionnaire Score; ESR = Perceived Nausea Score (1–10); NI = Not Included; Yes = Included, actual p values are reported underneath; Normality assumption is valid if $p > 0.05$.

3.3. Associations of Static balance and miRNA or SNP genotype

Table 5 presents the results of the random forest analysis using feature selection. None of the SNP genotypes examined were significantly associated with static balance parameters.

4. Discussion

The results of this paper provide evidence that postural balance is sensitive to MS stimulus. All postural balance outcomes during the eyes closed foam test condition increase (Table 2) following the MS stimulus, implying the stimulus created a disturbance in possibly in all three afferents (vision, proprioceptive and vestibular systems). Sway plots (not shown, summarized in Table 3) show the dynamics of postural sway patterns among SS and *LS* groups. The significant response to sway area was different than sway length responses after the stimulus but it is not clear the relative contributions of all afferents. In Yasuda et al. [36], researchers tested postural balance among patients with vestibular and proprioceptive disorders. They determined patients with vestibular disorders were more likely to respond with greater sway area, while patients with proprioceptive disorders responded with greater sway length. Other studies have reported similar responses to MS. For instance, Cobb and Nichols [37] found a significant increase in sway path length following a virtual environment stimulus; however, this was seen in an eyes-closed, no foam test using sway magnetometry for sway measures. Nishiike et al. [38] reported an increase in sway area using a force platform during eyes closed tasks following immersion into a virtual reality setting. Clinically, Basta et al. [12] reported their eyes closed foam test condition was most indicative of patients with otolith disorders, showing pathology in 67% of subjects. Some research has even reported postural instabilities in sway displacement before initiation of subjective MS symptoms [39–42], suggesting it is possible to predict MS susceptibility objectively. Thus, postural balance testing may have practical uses within occupations prone to MS, such as aviators in the military.

Several methods were used to separate subjects by motion sickness susceptibility. Initially, MSSQ survey scores were used as a continuous variable. Given the findings of Golding and Stott [34] that MSSQ score may not relate to stimulus time required to induce nausea, we then tried converting MSSQ to a categorical variable; that is, subjects with a MSSQ of 0 were called "not susceptible" and all others were called "susceptible". However, these subjective classifications did not match well with empirical results, as the difference in NOTC survival time was not significant ($p = 0.69$). Therefore, survival time under provocative stimulus (NOTC survival time) was used to stratify data into "short survival" (SS) and "long survival" (LS) groups using k-means clustering. With NOTC time significantly lower now in the SS group ($p = 0.007$ vs LS), differences between these two groups were investigated for the eyes open and eyes closed foam test conditions for all variables. After exposure, there was a significant difference between groups for Anteroposterior Sway in the eyes open condition, but this did not meet significance after multiple testing correction (Table 3). All p-values are above the Bonferroni corrected cutoff, but could be examined in future studies with larger sample size.

Predictors of each sway variable were carried out through backwards elimination for "eyes open" (Table 4A) and "eyes closed" (Table 4B). Gender proved to be a significant predictor for all postural sway variables except PSPSI, indicating males performed worse in postural balance tests in eyes closed foam test, which places relatively more challenge to the vestibular system compared to other afferents. These findings agree with previous studies in an older population [43–45] and in a sample of general population [46]. Some studies show opposite findings [47, 48]; however, only mediolateral and anterior-posterior sway were measured. Consistent with our findings, poor postural sway performance has also been reported in a healthy military male population [49]. It is often presumed men have greater stability than women because of larger feet; however, foot area was not a significant predictor in any of our regression models. We postulate the difference between males and females is a result of significantly higher center of gravity positioning due to

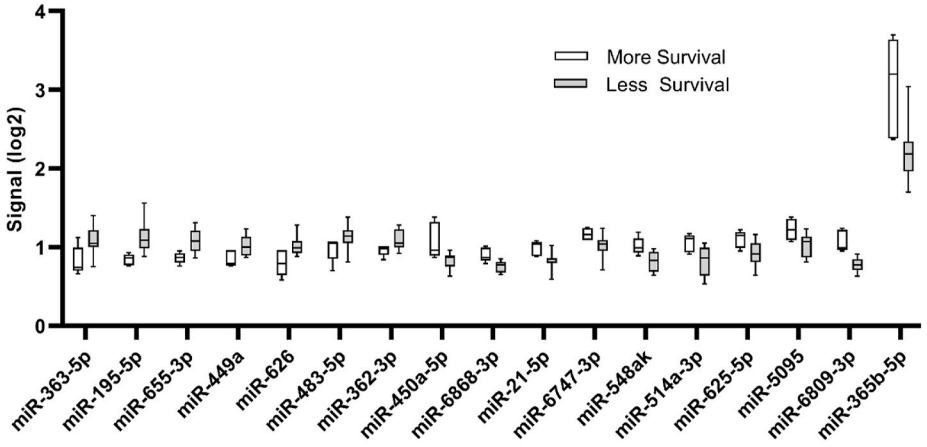

Fig. 1. MicroRNA expression in potential vestibular target genes. Basal levels of circulating microRNA, prior to MS stimulus onset, were compared between the SS and LS subjects. MicroRNA with $p < 0.05$ were cross-referenced with literature identifying genes contributing to disorders of the vestibular system and/or Parkinson's disease. Of these, 17 microRNAs also passed significance by Mann-Whitney test with two-stage step-up false discovery rate ($q < 0.05$), and are differently expressed between the two groups (shown here).

Table 5
Feature selection for predictors of static balance parameter by condition. SA, sway area; SL, sway length; AP, antero-posterior sway; ML, mediolateral sway; NOTC, neuro-otologic test chair (time spent in stimulus in mins); Order, order in testing sway occurred (1–4); EO, eyes open test; ECF, eyes closed on foam test.

Sway Variable	Sway Variable	R^2	Predictors				
SA	EO	0.946	miR-664a-5p	miR-6838-3p	mir-3913-2	miR-6781-3p	
SA	ECF	0.986	ENSG00000238445	ENSG00000200235	miR-4704-3p	Foot Area	
SL	EO	0.86	miR-4704-3p	NOTC	mir-5191		
SL	ECF	0.979	miR-154-5p	Order	miR-6741-3p	Foot Area	NOTC
AP	EO	0.89	miR-612	NOTC	ENSG00000263495	miR-6069	
AP	ECF	0.958	mir-875	miR-6739-5p	mir-944		
ML	EO	0.811	ENSG00000201465	ACA3	Order		
ML	ECF	0.961	miR-323a-5p	mir-3074	miR-3140-3p	ENSG00000238692	

greater body height, providing less stabilization to the ankle joint [45]. Following adjustments for height, Bryant and colleagues [50] reported less variation in postural sway between sexes, further supporting this theory.

Feature selection was used to choose from among our set of features (e.g. subjects' foot width, time in NOTC to induce MS, expression values of small RNAs, etc.) to select for the smallest set that predicted postural sway responses (e.g. sway length and sway area) in each task (Table 5). RNA expression was strongly predictive of some results. For example, in the EO condition, SA was predicted by the values of four microRNA, with an R2 value of 0.946. This indicates that these microRNA alone can account for almost 95% of the variance in the data. One microRNA, miR-4704-3p, was an important predictor of both SA (ECF, condition) and SL (EO). As many of these microRNA have few if any publications, and a given microRNA can potentially target a plethora of mRNA molecules, the biological effects of these microRNA as they relate to MS is a potential area of future study.

In general, perceived sense of instability as captured by PSPSI scores was in the expected direction, following the same direction as objective postural sway variables, suggesting a strong correlation, similar to previous research [51]. On average, subjects perceived more sway following the stimulus and during eyes closed foam test conditions, but were unable to correct for this to reduce their instability, suggesting their afferent pathways were compromised. This disconnect between perceived and actual postural sway, we believe, is a result of the response to the stimulus. In a healthy population, researchers observed a similar disconnect when subjects were placed at a height of 3.2 m above ground [52]. This threat to their posture significantly reduced CP movement; however, conscious perception of movement remained the same. The authors attributed an altered perception to an influx of afferent information from proprioceptors when standing at height. Studies in our lab observed a similar lack of perception in sense of balance at elevated heights when objective measures of postural sway increased without a simultaneous increase in PSPSI [9].

5. Conclusion

Because these data support an important role of the afferent pathways in MS susceptibility, we were interested to see whether microRNA expression differed between the SS and LS groups prior to stimulus exposure. Based on gene ontology results, we searched for microRNAs that regulate vestibular function genes. In the literature, many genes have been shown to be the bases of inherited disorders of the vestibular system. Genetic variants for Usher syndrome, familial episodic ataxia, vestibular migraine, familial migranous vertigo, and Meniere's disease have all been reviewed [30, 31]. Interestingly, 297 of the small RNAs were significantly different in expression between the basal pre-stimulus state of LS and SS subjects. Forty of these were microRNA that have been demonstrated or predicted to target the expression of these few putative vestibular disorder genes. Seventeen of these microRNA passed further statistical filtering (Fig. 1). Significant differences in circulating microRNA suggest that these vestibular function genes could be expressed at different levels in the LS and SS populations. The individual susceptibility to MS is probably rooted at molecular levels, e.g., microRNA, found in the vestibular organ which is considered the significant controlling link in balance maintenance [36, 53].

MicroRNA dysregulation has also been described in Parkinson's disease (PD, reviewed in Goh et al., 2019 [32]). We found suggestion of overlap between dysregulated microRNAs in PD with those included in our search for microRNAs that regulate vestibular function; the microRNA hsa-miR-221-5p that differed significantly between SS and LS individuals (Fig. 1) also appeared in Goh et al. We conclude MS likely produces acute or temporary "Parkinsonism" like symptoms without the underlying features of PD.

Of the microRNA differentially expressed prior to stimulus, many have more than one putative target in gene related to vestibular disfunction. For example, study of a large 4-generation family with dominant inheritance of familial migranous vertigo[54] narrowed down the responsible gene to short list of seven candidates near 5q35. Interestingly, five of these candidates are potentially targeted by microRNA found here to be differentially expressed between LS and SS groups prior to stimulus. In particular, the familial migranous vertigo candidate gene dopamine receptor D1 (DRD1) is a putative target of nine separate differentially expressed microRNAs.

In our random forest analysis, 27 unique small RNAs were found to be predictors of the various sway variables. Among them, miR-4704-3p appears twice, both in sway length and sway area; however, its function is largely unknown. Foot area also appears twice among predictors of SL and SA, but was not a significant predictor in the backwards elimination model. Sway area was largely predicted by miR-664a-5p and miR-6838-3p, which have been shown to promote neuronal differentiation and to be associated with neuropathic pain in spinal cord injury patients [55]. Neuronal differentiation of neuroblastoma cells was significantly regulated by miR-664a-5p. Researchers also identified five predicted target genes (INSM, FLT1, FAT3, EDIL3, and DCX; [56]). Most notably, the

INSM1 gene plays a role in neuroendocrine differentiation in lung tumors [57], the FAT3 gene is associated with meningiomas [58], and the DCX gene is important for neuronal migration during brain development [59]. miR-664a-5p expression levels are decreased in patients with schizophrenia, a neurodevelopmental disorder [60].

Predictors of sway length included miR-5191, which was found to be downregulated in salivary adenoid cystic carcinomas and aided in the progression of the disease [61]. Values of SL during the ECF condition were predicted by miR-154-5p, largely known in the literature as dysregulated in many tumors [62–65] and as a possible marker of endometriosis [66] miR-154-5p has also been shown to play a role in neuropathic pain development [67]. Neuropathic pain is caused by a dysfunction of the somatosensory system, a part of the nervous system that interprets sensory information from receptors and relays them to the brain, including proprioception. As sway length and sway area are likely controlled by the function of the proprioceptive system and vestibular system respectively, this seems like a plausible target to further investigate [36].

Anterior-Posterior sway was predicted by miR-612, a microRNA that is associated with myasthenia gravis [68]. In 2013, Li and colleagues discovered miR-875 directly targets peroxiredoxin III and is associated with the dopaminergic neuron degeneration pathway [69]. However, larger data sets will be needed to develop microRNA-based biomarkers of neurodegenerative diseases [70]. MS -induced neurological deficits, as our results have shown, may also benefit from similar biomarkers in the future.

There were a few limitations to this study. First, because this was a pilot study, the sample size was relatively small with only 128 repeated sway measures in total across 16 subjects. Similarly, the microarrays measured 6631 human small RNAs. Additional studies with a much larger population would reduce the chance of type 1 error. Moreover, while few changes post-stimulus reached statistical significance, the observed change of all variables and conditions was in the expected direction. Our small sample size likely limited the power of our analyses. However, this study does allow calculation of sample size that would be required to achieve 80% power for each variable in any future study. Second, it is possible the stimulus protocol was not aggressive enough to induce complete MS in subjects. By the end of the first test, most subjects had perceived nausea (ESR) scores already $\leq$4 out of 10. Since subjects self-selected to participate, it is also possible that very susceptible persons opted not to enroll or requested to exit the NOTC chair early to avoid the full effects of MS. Attrition of the most MS-susceptible subjects would have decreased the power of the stimulus to induce changes in postural sway. The small MS-induced effect therefore may have subsided before postural sway testing had begun, though this was not explored in our analysis.

Between SS and LS subjects, seventeen microRNAs have known or putative targets in genes previously shown to play roles in disorders of the vestibular system and/or Parkinson's disease (Fig. 1). Motion sickness therefore seems to produce acute or temporary Parkinsonism like symptoms, contributing to the increased postural sway seen here. Additionally, several small RNAs were predictive of sway variables in a random forest analysis (Table 5). However, no SNPs were predictive of these variables nor significantly more or less common in one group than another. The small sample size of this study limits the power to detect such genetic differences. The differences in circulating small RNAs, however, warrant further study and larger cohorts to verify differences that may be biologically meaningful. Furthermore, in future study, with larger sample size, alternate hypothesis around "equilibrium-point" [53, 71, 72], along with molecular markers, may be explored.

Author contribution statement

Michael Markey; Amit Bhattacharya: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ashley Turner: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Peter Le; William Marras: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ali Reiter: Conceived and designed the experiments; Performed the experiments.

Cyndy Cox: Performed the experiments.

Stacy Simmons; Lorenna Altman; Kermit Davis; Dustin Huber: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

MB Rao: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jonathon S Dufour: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data associated with this study has been deposited at GEO. Accession number GSE206921.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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This work is dedicated to the memory of Ali Reiter.

The views expressed in this article reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the United States Government.

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