1. Title: Calcitonin Gene-Related Peptide (CGRP) Receptor Antagonism Reduces Motion Sickness Indicators in Mouse Migraine Models

Abbreviated Title: CGRP Receptor Antagonism Reduces Motion Sickness

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4. Structured Abstract:

Background: Migraine and especially vestibular migraine are associated with enhanced motion sickness and motion-induced nausea. Murine models of migraine have used injections of Calcitonin Gene-Related Peptide (CGRP) or other migraine triggers [i.e., sodium nitroprusside (SNP)] to induce migraine sensitivities to touch and light. Yet, there is limited research on whether these

triggers increase motion-induced nausea, and if migraine blockers can reduce these migraine symptoms. We hypothesized that systemic delivery of CGRP or SNP will increase motion sickness susceptibility and motion-induced nausea in mouse models, and that migraine blockers can block these changes induced by systemically delivered CGRP or SNP.

Methods: We investigated two measures of motion sickness assessment [motion sickness index (MSI) scoring and motion-induced thermoregulation] after intraperitoneal injections of either CGRP or SNP in C57B6/J mice. The drugs olcegepant, sumatriptan, and rizatriptan were used to assess the efficacy of migraine blockers.

Results: MSI measures were confounded by CGRP's effect on gastric distress. However, analysis of tail vasodilations as a surrogate for motion-induced nausea was robust for both migraine triggers. Only olcegepant treatment rescued tail vasodilations.

Conclusion: These preclinical findings support the use of small molecule CGRP receptor antagonists for the treatment of motion-induced nausea of migraine, and show that triptan therapeutics are ineffective against motion-induced nausea of migraine.

35

36 **5. Trial Registration: NA**

37 **6. Keywords:** CGRP, vestibular, gastric distress, motion-sickness, nausea,

38 migraine, mouse, olcegepant, rizatriptan, sumatriptan

39 **Acknowledgments**: We would like to acknowledge assistance with data collection

40 and analysis from Vedat Duzgezen, Stefanie Faucher, Elana Fine, Catherine

41 Hauser, Benjamin Liang, and Blaze Strangio. This work was fully supported by

42 NIH R01DC017261 (AEL).

43 **Competing interests:** No authors have any financial or non-financial competing

44 interests.

45

46 **Introduction:**

47 Motion sickness and motion-induced nausea are symptoms of migraine and
48 especially vestibular migraine (VM) [1-7]. VM patients show lower motion perception
49 thresholds compared to healthy controls and also show enhanced susceptibility to
50 motion sickness[3]. Interestingly, Wang and Lewis (2016) found that in VM patients,
51 but not in migraine or healthy controls, the residual sensory conflict between
52 gravitational (otolith) and rotational (semicircular canal) cues correlated with
53 motion sickness susceptibility [8].

54 The diagnostic criteria for human motion sickness have recently been
55 updated [9] and are included in the international classification of vestibular
56 disorders. The major symptoms of motion sickness include facial pallor, nausea,
57 vomiting, gastric awareness and discomfort, sweating, and hypothermia. Motion
58 sickness-related hypothermia is broadly expressed phylogenetically in humans,
59 mice, rats, and musk shrews [10, 11]. However, rodent models of motion sickness
60 have been constrained because rodents do not have an emesis reflex. Pica-
61 eating behavior is thought to be an alternative to vomiting, yet, pica has not been
62 shown to be a sensitive measure of motion sickness [12]. Instead, piloerection,
63 tremor, fecal, and urinal incontinence have been used in scoring criteria called the

64 motion sickness index (MSI) to quantify the degree of motion sickness-like
65 behavior experienced by rats and mice to emetic stimuli [12, 13]. In addition,
66 thermoregulatory changes can also be used to assess motion sickness, which in
67 the mouse model involves a decrease in head temperature (hypothermia) and
68 transient tail-skin vasodilation early in the onset of provocative motion [11].
69 Moreover, tail vasodilations to provocative motion have been reported to precede
70 emesis events in the musk shrew, serving as an early index for motion-induced
71 nausea in experimental rodent models.

72 Despite the high prevalence of motion-induced nausea in VM, the
73 underlying mechanisms have yet to be defined. It is accepted that migraine
74 involves the neuropeptide Calcitonin Gene-Related Peptide (CGRP). CGRP is
75 upregulated during migraine attacks[14, 15], infusion of CGRP can induce migraine [15],
76 and antibodies that block CGRP or its receptor can effectively treat most migraines
77 [16, 17]. Yet, it is unclear if CGRP signaling antagonism can also alleviate motion-
78 induced nausea of VM.

79 Animal models of migraine have used injections of CGRP or other migraine
80 triggers such as sodium nitroprusside (SNP) - which generates nitric oxide and
81 stimulates the release of CGRP – to induce allodynic responses to touch [18, 19] and

82 light-aversive behavior [20, 21]. However, it is not known if these triggers can induce
83 motion-induced nausea in preclinical models. Moreover, it is not known if migraine
84 blockers used in preclinical models of touch and light sensitivities can also block
85 motion-induced nausea and motion sensitivity characterized in VM.

86 To better understand CGRP's role in VM motion-sickness susceptibility, we
87 investigated two measures of motion sickness assessment (MSI scoring and
88 motion-induced thermoregulation) after systemic injections of either CGRP or SNP
89 in the wild-type C57B6/J mouse. In this study, we found that MSI measures based
90 on gastric distress were lessened by CGRP antagonism. Yet, this was driven by
91 CGRP's effect in causing diarrhea, which was not observed after SNP
92 administration. However, the motion-induced thermoregulation was a very robust
93 model of motion sickness. Moreover, CGRP receptor antagonism by olcegepant
94 was effective in relieving CGRP effects on motion-induced nausea, whereas
95 triptan therapies were not efficacious. Our studies provide a strong premise that
96 antagonizing CGRP signaling will be effective for treating notion-induced nausea
97 of VM, as it has been shown to be highly effective for typical migraine.

98 **Materials & Methods:**

99 Animals: C57B6/J mice were obtained from Jackson Laboratories (JAX #664) and
100 were housed under a 12 hour day/night cycle under the care of the University
101 Committee on Animal Resources (UCAR) at the University of Rochester. Mice are
102 housed with *ad libitum* access to food, water, bedding, and enrichment materials.
103 A total of 213 mice (106 M/ 107 F) were tested, and all studies were powered
104 sufficiently to detect male/female differences. Prior to behavioral testing, mice
105 were equilibrated in the testing room controlled for an ambient temperature
106 between 22-23°C for at least 30 minutes. Mice were tested around 2.3 to 6 months
107 of age. This age range correlates to 20-30 years in humans and is within the range
108 that migraine symptoms most likely occur in patients [22]. Different cohorts of mice
109 were used to test motion sickness indices and motion-induced nausea. Testing
110 occurred from 9:00 am to 4:30 pm during the day cycle to control for behavioral
111 and thermoregulatory changes that may arise from circadian rhythms. For motion-
112 induced nausea testing, mice were screened for instances of patchy fur (alopecia)
113 that could add uncontrolled variance to thermoregulation analysis. Mice with
114 alopecia were not included in this study [23].
115 Drug administration: Injections were performed intraperitoneally (IP) with a fine 33
116 gauge insulin syringe. Dulbecco PBS served as the diluent and as the vehicle

117 control. Migraine blockers used were the CGRP-receptor antagonist olcegepant
118 and the selective serotonin receptor agonists sumatriptan and rizatriptan. The
119 concentrations are listed: 0.1x, 0.5x, and 1x CGRP were prepared at 0.01, 0.05,
120 and 0.1 mg/kg (rat α-CGRP, Sigma), 1x olcegepant was prepared at 1 mg/kg
121 (BIBN4096, Tocris), 1x sumatriptan was prepared at 0.6 mg/kg (Sigma-Aldrich), 1x
122 rizatriptan was prepared at 0.6 mg/kg (Sigma-Aldrich), and 0.1x, 0.5x, and 1x
123 sodium nitroprusside (SNP)- (Sigma-Aldrich) were prepared at 0.25 mg/kg, 1.25,
124 and 2.5 mg/kg. After injection, animals were placed in a separate cage from their
125 home cage to recuperate from injection stress. Mice were tested approximately
126 twenty to thirty minutes after delivery of either vehicle, CGRP, SNP, CGRP or SNP
127 co-administered with a blocker, or drug controls. Animals were gently handled and
128 anesthesia was not needed. All animal procedures were approved by the
129 University of Rochester's (IACUC) and performed in accordance with the
130 standards set by the NIH.
131 Off-Vertical Axis Rotation (OVAR): Prior human and rodent studies have used
132 OVAR to assess the otolith-ocular reflex and assess the semicircular canal-
133 otolith interaction [24-26]. Constant velocity OVAR at a tilt can be disorienting and
134 promote motion sickness in human participants [27, 28], and further studies in

135 mice have shown that provocative rotation leads to pica and observations of
136 urination, piloerection, and tremor [29]. In this study, a two-cage rotator (cage
137 dimensions: 12.7 cm x 8.9 cm) was use for twenty minutes (60 rpm, 45° tilt
138 from the vertical, 20 cm from the axis of rotation) as an OVAR vestibular
139 challenge.
140 Motion-Sickness Index (MSI) Testing**:** We adapted Yu et al.'s protocol that
141 previously assessed the drugs scopolamine and modafinil on mitigating motion
142 sickness indicators in rats and mice [12]. We incorporated OVAR (described
143 above) as the vestibular challenge (VC) in this test. We evaluated MSI in mice
144 at the end of the following time points: A) a five-minute baseline (*pre-*
145 *injection/pre-VC*), B) twenty to thirty minutes after drug injection (*post-*
146 *injection/pre-VC*), and C) five minutes after the OVAR (*post-injection/post-VC*).
147 Mice were placed in a testing box separate from their home cage to observe
148 for indicators of motion sickness detailed in **Figure 1A.** A motion sickness
149 index (MSI) score was determined by the summation of the indicators.
150 Certain actions were taken to normalize the weight distribution of MS
151 indicators during reporting. Fecal granules (Fg) were counted separately at
152 each time point. The unit weight per Fg after vehicle injection was used to

153 back-calculate the granule number in cases where the weight of fecal
154 incontinence (in grams) was instead measured, for example, in cases of
155 gastric distress. This calculation was necessary during CGRP testing as mice
156 experienced diarrhea, which made it difficult to count fecal.

157 Urination was assigned a 1.2 score and was only counted once throughout
158 the time points. Piloerection was measured as either mild (0.6) or severe (1.2),
159 and tremors were assigned a 1.2.

160 Motion-Induced Thermoregulation Testing**:** We adapted Tu et al.'s protocol
161 who first noticed these thermoregulatory changes when measuring the
162 temperatures of the heads, bodies, and tails of mice to provocative motion [11].
163 In this study, head and tail temperatures of C57B6/J mice were measured for a
164 total 45 minutes using a FLIR E60 IR camera (model: E64501). This camera is
165 connected to a tripod and is positioned approximately 43 cm above an open,
166 plexiglass box (mouse box) used to house an individual mouse during testing.
167 Both the tripod and mouse box are securely attached to the shaker's base. Briefly,
168 baseline measurements were recorded for five minutes prior to the provocative
169 motion (-5 to 0 mins). The provocative motion was an orbital rotation (75 rpm, 2-
170 cm orbital displacement), and mice were recorded for 20 minutes (0 to 20 mins).

171 After 20 minutes, the provocative motion was turned off, and mice were recorded
172 for an additional 20 minutes to measure recovery to baseline (20 to 40 mins) as
173 schematized in Fig. 2. Head and tail temperatures were measured after data
174 retrieval using the software FLIR Tools+. Tail and head temperatures were
175 measured within predefined field of views: square region (3x3 mm) for tail, and
176 circular region (10x10 mm) for head. Tail measurements occurred 2 cm from the
177 base of the tail and head measurements occurred at the center of the head image,
178 in between the mouse's ears. Infrared imaging data was collected every minute
179 during baseline measurements, and every 2 minutes during and after the
180 provocative motion. We quantified thermoregulatory changes to provocative
181 motion by comparing changes in tail vasodilations, and we approximated the
182 magnitude of the head hypothermia based on second order curve fit estimates.
183 Transient increases in the tail temperature of the mouse to provocative motion are
184 referred to as *delta tail vasodilations* (^{o}C), and were computed by subtracting the
185 tail temperature at time t = 0 minutes (rotation ON) from the max tail temperature
186 measured during the first 10 mins of the rotation ($0 \leq t \leq 10$). In order to facilitate
187 quantification and comparisons of the delta tail vasodilations between treatment
188 groups, a threshold of 1.5^{o}C was imposed upon the data to make it a binary

189 outcome measure. Tail temperature changes equal to or greater than +1.5^{o}C were
190 designated a delta tail vasodilation and those less than +1.5^{o}C did not meet the
191 criteria.

192 Prior to assessing the efficacy of migraine blockers on thermoregulatory
193 changes due to provocative motion, control experiments were performed to assess
194 i) test-retest reliability of motion-induced thermoregulation and ii) stationary testing
195 (no rotation) after vehicle, 1x CGRP, and 1x SNP injections. Additionally, we
196 tested motion-induced thermoregulation at 0.1x and 0.5x concentrations of CGRP
197 and SNP to compare with 1x effects.

198 Statistical Analyses: Statistical analyses were conducted in GraphPad Prism
199 9.0. Repeated measures ANOVA is not capable of analyzing data with missing
200 values (i.e., excluded mice), and so a mixed-effects (ME) model approach was
201 the statistical substitute. ME models use a compound symmetry covariance
202 matrix and are fit using Restricted Maximum Likelihood (REML). In the presence of
203 missing values, these results can be interpreted like repeated measures
204 ANOVAs. Sphericity was not assumed during analyses and so a Greenhouse-
205 Geisser correction was applied to results. When comparing effects of migraine
206 blockers on motion sickness indices (feces and total MSI), we ran 3-way ME

models with the first factor assessing timepoint differences, the second factor assessing sex, and the third factor comparing treatments. For motion-induced thermoregulation data, two-way ME models were applied to delta tail vasodilations across the two factors (sex and treatment) for both triggers. Second-order curve fitting were used to generalize trends in head temperature profiles and approximate the magnitude of head hypothermia, a single value with no standard error, computed by subtracting the curve's predicted head temperature at t = 20 mins from t= 0 mins. Across both assays, Tukey multiple-comparisons test was the preferred post-hoc analysis. For test-retest reliability of head and tail profiles, Pearson's correlation coefficient is listed as r (df), where r is the coefficient and df is the degrees of freedom. Head temperature curves are computed by 2nd order curve fitting and an R^2 value is provided. Values are reported as mean ± SEM unless noted otherwise, and significance was set at $p < 0.05$ for all analyses.

Results:

CGRP and SNP's effects on motion sickness index

We studied the effects of CGRP release (n = 9M/10F) on motion sickness indicators (MSI) and whether the CGRP receptor antagonist olcegepant or the

225 $5HT_{1D}$ receptor agonist sumatriptan could mitigate MSO according to the timeline
226 shown in Fig. 1. While no effect of sex was seen on motion sickness indicators,
227 increased MSI were observed both after CGRP/SNP injection and after a
228 vestibular challentge (VC) **(Fig. 1B, C, and D).** Significant differences were seen
229 between measurements as described: pre-injection versus post-VC ($F(1, 18) =$
230 $846.7, p < 0.0001$), post-injection vs post-VC ($F(1,18) = 904.1, p < 0.0001$), and
231 pre-injection versus post-injection ($F(1,18) = 74.7, p < 0.0001$)**.** Tukey post hoc
232 analysis compared MSI outcomes between treatments after the vestibular
233 challenge, and significant increases in MSI were seen after delivery of 1x IP
234 CGRP ($p < 0.0001$) or 1x IP CGRP + sumatriptan ($p = 0.0002$) compared to
235 vehicle control. No difference in MSI was seen between vehicle and 1x IP CGRP +
236 olcegepant ($p = 0.98$). A similar observation of increasing MSI score was seen
237 with IP SNP administration at similar time points **(Figure 1E, F, and G)**. For SNP
238 treated animals (n = 9M/9F), significant differences were seen in MSI outcomes
239 when comparing pre-injection vs post-VC ($F(1,17) = 267.8, p < 0.0001$), post-
240 injection vs post-VC ($F(1,17) = 136.6, p < 0.0001$) and pre-injection versus post-
241 injection ($F(1,17) = 105.6, p < 0.0001$). However, Tukey post hoc showed no

differences between vehicle and IP SNP groups at each time point, and so blockers were not further evaluated for SNP's effects on MSI**).**

Motion sickness (MSI) outcome is driven by feces criteria

A closer observation of MSI outcomes showed that the main driver of MSI is the magnitude of feces collected. Across the three time points, differences in collected feces were significant and mimicked MSI outcomes for both IP CGRP and IP SNP**.** Mixed-effects analyses were used to assess differences in feces collected. Tukey post-hoc analyses after the vestibular challenge showed increased fecal excretion after 1x CGRP (adj. $p < 0.0001$) and after 1x CGRP + 1x sumatriptan (adj. $p = 0.0001$). In contrast, mice treated with 1x CGRP + 1x olcegepant showed no significant difference in excreted feces compared to vehicle control (adj p. = 0.97). During the test, we observed that mice treated with 1x CGRP or 1x CGRP + 1x sumatriptan exhibited diarrhea while mice treated with 1x CGRP + 1x olcegepant did not. Mice treated with 1x SNP did not exhibit diarrhea across all time points, and no differences were observed in collected feces across treatment. In addition, piloerection, urination, and tremor did not significantly differ between CGRP or SNP-treated groups (data not shown). These results suggest that computing an index for motion sickness based on fecal incontinence, urination, piloerection, and

260 tremor is problematic due to how biased the index is towards fecal excretion. We
261 used a different method to assess motion-sickness based on motion-induced
262 thermoregulation.

263 Hypothermia and tail vasodilations occur during provocative motion and not
264 stationary testing

265 **Figure 2A** describes the experiment for assessing motion-induced
266 thermoregulation. After a five-minute baseline recording, mice (n = 32M/25F) are
267 challenged with provocative motion (t = 0 mins, rotation = ON) and exhibited
268 gradual hypothermia until the end of the motion (t = 20 mins, rotation = OFF). 2nd
269 order curve fits calculated the magnitude of the mouse's head hypothermia to be -
270 1.22^{o}C for females and -1.62 ^{o}C for males with R^2 fits equal 0.9 for both curves
271 (**Figure 2B).** In addition to the hypothermia, mice exhibited transient but significant
272 increases in tail temperature during the first 10 minutes of the provocative motion.
273 The delta tail vasodilations were quantified for females (4.6 ± 0.3 ^{o}C) and males
274 (6.1 ± 0.4 ^{o}C) (**Figure 2D**). To ensure that the hypothermia and the tail
275 vasodilations were physiological responses to the provocative motion stimulus, a
276 separate group of mice (n = 9F/10M) was tested similarly but for stationary testing.

277 No observed hypothermia or transient vasodilations were seen in mice of either
278 sex during the stationary tests (**Figure 2C** and **2E**).

279 CGRP and SNP's effects during stationary test

280 To exclude the possibility of thermoregulatory changes that may be due to
281 the treatments - CGRP and SNP - and not the rotation, a secondary stationary test
282 was performed on mice (n = 9M/9F) who were tested with 1x CGRP and 1x SNP.
283 No differences were seen in the head or tail temperatures after 1x CGRP or 1x
284 SNP testing compared to vehicle control, and hypothermia or delta tail
285 vasodilations were not observed (**Figure 3A, B, D, E).**

286 Test-retest reliability during provocative motion

287 When assessing IP vehicle delivery, male and female mice exhibited
288 hypothermia during the provocative rotation and this response was repeatably
289 observed in the retest (n = 9M/8F). In **Figure 3C**, 2nd order curve fits were
290 generated for test-retest head curves and strong fits were seen for female
291 (Pearson's $r\ (23)$ = 0.87, $p < 0.0001$) and for male (Pearson's $r\ (23)$ = 0.89, $p<$
292 0.0001). The magnitude of hypothermia measured in the head temperatures was
293 similar between sexes and reproducible during the test-retest (data not shown). In
294 **Figure 3F**, similar tail temperature profiles were observed during test-retest for

295 females (Pearson's *r* (23) = 0.92, $p < 0.0001$) and for male (Pearson's *r (23)* =
296 0.95, $p < 0.001$). Delta tail vasodilations were observed to be similar in magnitude
297 for females (mean vasodilation $_{test}$: 4.0 ± 0.8 ^{o}C, mean vasodilation $_{retest}$: 4.2 ± 0.8
298 ^{o}C) and for males (mean vasodilation $_{test}$: 4.9 ± 0.8 ^{o}C, mean vasodilation $_{retest}$: 4.9
299 ± 0.9 ^{o}C).

300 <u>CGRP and SNP affect motion-induced thermoregulation in C57/B6J mice</u>

301 As seen in **Figure 4A and B**, 2nd order curve fits were used to compute the
302 recovery of head temperatures back to baseline values after the hypothermia after
303 1.0x CGRP delivery, and a general trend of extended recovery times was
304 observed due to 1.0x CGRP delivery (female: 28.4 mins, R^2 = 0.73; male: 25.4
305 mins, R^2 = 0.83) compared to their vehicle control responses (female: 20.3 mins,
306 R^2 = 0.86; male: 20.4 mins, R^2 = 0.85). We observed no differences in the
307 magnitude of head hypothermia between CGRP and vehicle control tests. **Figure**
308 **4E and F** show tail temperature profiles after 1.0x CGRP. 100% of mice exhibited
309 normal delta tail vasodilations after vehicle control administration but a majority of
310 mice (83.3% female, 45% male) exhibited diminished delta tail vasodilations (<
311 1.5^{o}C) after 1.0x CGRP administration. Due to the diminished delta vasodilations
312 and the extended recovery times detected after CGRP testing, it is inferred that

313 1.0x CGRP impacted a mouse's natural response to the provocative motion and
314 their nausea response. In parallel, a different group of mice was used to assess
315 SNP's effects on motion-induced thermoregulation, and temperature profiles were
316 analyzed similarly. When measuring head temperatures, we did not observe any
317 differences in recovery time or in the magnitude of head hypothermia (**Figure 4C**
318 **and D**). However, similar to CGRP, 1.0x SNP impacted delta tail vasodilations.
319 After vehicle control, 100% of mice showed observable, normal delta tail
320 vasodilations assumed for this behavior but a majority of mice in this testing cohort
321 (100% females, 75% males) exhibited diminished vasodilations after 1.0x SNP
322 similarly to what was observed with 1.0x CGRP (**Figure 4G and H**).
323 Dose-dependent response of CGRP or SNP on tail vasodilations
324 While 1.0x concentrations were used for the motion sickness assay in this study
325 and these concentrations are based on previous studies assessing these migraine
326 triggers, it was unclear whether lower doses would also elicit observed responses.
327 To answer this question, a dose-dependent response curve was tested in mice (n
328 = 9M/9F) and delta tail vasodilations were analyzed at 0.1x, 0.5x, and 1.0x
329 concentrations of IP CGRP or IP SNP (**Figure 4I, J, and L**). In the CGRP group
330 (n = 10M/9F), 0.1x CGRP had no significant effects on delta tail vasodilations in

331 either sex. However, significant differences were observed when comparing delta
332 tail vasodilations between vehicle and 0.5x CGRP in females ($p < 0.0002$) and in
333 males ($p < 0.02$). Significant differences in delta tail vasodilations were also seen
334 when comparing vehicle and 1.0x CGRP in both sexes ($p < 0.0001$). When
335 assessing SNP's effects (n = 8F/8M), no significant differences were seen at 0.1x
336 and 0.5x SNP compared to vehicle control, but a significant difference was seen
337 when comparing vehicle to 1.0x SNP in females ($p < 0.02$) and in males ($p <$
338 0.0002). The greatest effect of CGRP and SNP on delta tail vasodilations was
339 observed at their 1.0x concentration and these concentrations were used to
340 assess the migraine blockers.

341 <u>Olcegepant but not triptans protect against CGRP's effects on tail vasodilations</u>

342 Administration of 1x IP CGRP reduced delta tail vasodilations in nearly all
343 mice, and so it was hypothesized that mice would regain their healthy response to
344 provocative motion with IP treatment of either CGRP + olcegepant (n = 10M/7F),
345 CGRP + sumatriptan (m = 10M/8F), or CGRP + rizatriptan (n = 7M/7F). Prior to
346 assessing CGRP + blockers, groups of mice were assessed for blocker-only
347 effects by testing 1x IP olcegepant (n = 7F/10M) or 1x IP sumatriptan (n = 6F/10M)

348 , but no differences were seen when compared to their vehicle control so the
349 blockers, by themselves, do not have a secondary effect on tail vasodilations.
350 Tail temperature profiles were recorded and displayed for vehicle and drug
351 treatments (**Figure 5A-J**). A floating bar plot is used to compare all animals for
352 assessing CGRP's effects on delta tail vasodilations (**Figure 5K**). Mixed-effects
353 analysis on delta tail vasodilations with Dunnett post-hoc analysis showed that the
354 factors CGRP/CGRP+Blockers ($F(4, 93) = 61.6, p < 0.001$) and sex ($F(1, 69) =$
355 $10.2, p = 0.002$) to be significant (**Table 1).** Tukey post-hoc analysis showed a
356 significant difference between vehicle and 1.0x CGRP (adj. $p < 0.0001$) and
357 vehicle versus 1.0x CGRP + 1.0x sumatriptan (adj. $p<0.001$), and 1.0x
358 CGRP+1.0x rizatriptan (<0.005) for male and female mice, while no significant
359 differences were seen between vehicle vs 1.0x CGRP + 1.0x olcegepant. I n a
360 dose-dependent response study, while no significant effects were seen with 0.1x
361 CGRP, mice that experienced reduced vasodilations due to the 0.5x CGRP dose
362 were later tested with 0.5x CGRP + 1.0x olcegepant, and a majority of these mice
363 were rescued after delivery of 1.0x olcegepant (**Figure 5K**). These findings show
364 that olcegepant blocked the effects of CGRP and allowed for a majority of mice to

365 exhibit their normal response to the provocative motion by blocking CGRP's
366 effects, while both triptans were not effective.
367
368 <u>SNP's effects on tail vasodilations are blocked by olcegepant</u>
369 Similar to CGRP testing, the effects of the blockers olcegepant and
370 sumatriptan were also assessed against 1x IP SNP (n = 8M/8F), and tail
371 temperatures are depicted (**Figure 6A, B, C, D, E, F, G, and H**). A floating bar plot
372 is used to compare all tested animals used for assessing SNP's effects (**Figure 6I,**
373 **J and K**). Mixed-effects analyses with Dunnett post hoc analyses showed that the
374 factor SNP/SNP + Blockers ($F\ (2.4, 44.5) = 6.0,\ p = 0.003$) to be significant but
375 that sex was not significant ($F\ (1, 56) = 0.1,\ p = 0.79$). Dunnett's post-hoc analysis
376 indicated that 1x IP SNP significantly reduced tail vasodilations in males ($p =$
377 0.0002) and females ($p = 0.01$), but saw no differences in tail vasodilations
378 between IP vehicle and IP SNP co-administered with either olcegepant or
379 sumatriptan (**Table 1**). Blockers were partially effective in regaining tail
380 vasodilations in SNP-treated mice, as half of mice were still affected by SNP's
381 effects (50% F/50% M). **Table 2** is provided to depict the differences in diminished

382 tail vasodilations observed across all treatments (CGRP/SNP + blockers) for both
383 male and female mice.

384 **Discussion:**

385 In this study, we show that systemic CGRP injections can induce motion
386 sickness in wildtype C57B6/J mice, and this motion sickness can be strongly
387 antagonized by the CGRP receptor blocker, olcegepant. Published studies
388 assessing CGRP's effects on light-aversive behavior (photophobia) and
389 cutaneous hypersensitivity (allodynia) in rodents indicate triptans are effective
390 in reducing these surrogate behaviors [30, 31], but interestingly in our study,
391 sumatriptan and rizatriptan did not show significant efficacy in blocking
392 CGRP's effects in motion sickness and motion-induced nausea. We have
393 used two different murine motion sickness assays: 1) MSI and 2) motion-
394 induced thermoregulation. In both assays, provocative motion caused distinct
395 changes, but MSI was too weighted on gastric distress. This bias reduced its
396 validity as an assay because gastrointestinal complications do not directly
397 imply motion sickness. However, the motion-induced thermoregulation model
398 is more suitable for studying motion sensitivity as it involves studying motion-
399 induced thermoregulation caused specifically by a vestibular stimulus – the

400 constant orbital rotation. In addition, the motion-induced thermoregulation
401 model was robust for assessing CGRP and SNP's effects. Similar rodent
402 studies suggest tail vasodilations are a precursor to emesis and removal of toxins
403 [11]. Our data showing CGRP's blunting of tail vasodilations to provocative motion
404 suggests that motion-induced nausea may be aggravated with CGRP and that
405 olcegepant is protective but not sumatriptan or rizatriptan. This finding is in
406 agreement with clinical findings that showed a 2-hour infusion of CGRP caused
407 gastrointestinal hyperactivity and nausea, and pretreatment with a CGRP receptor
408 antagonist ameliorated these clinical symptoms but pretreatment with sumatriptan
409 did not [32]. Moreover, a clinical meta-analysis of anti-CGRP signaling treatments
410 for nausea in episodic migraine provided strong support for their effectiveness [33].
411 Interestingly, we also observed that female mice were more severely affected
412 by systemic CGRP than their male counterparts, which correlates with
413 preclinical studies showing stronger tactile sensitivity in female mice when
414 challenged with durally applied CGRP [34].
415 <u>CGRP signaling and murine motion-sickness indices:</u>
416 Others have shown in human subjects and in mouse models that CGRP
417 causes diarrhea by disrupting peristaltic intestinal activity and promoting ion

418 and water secretion in the intestinal lumen, and that CGRP signaling
419 antagonism can reverse these effects [35-37]. Thus, it is expected that CGRP-
420 induced diarrhea would cause noticeable increases in the motion sickness
421 index (MSI) calculation. The OVAR rotation further increased the motion
422 sickness index (MSI) regardless of CGRP or SNP, highlighting the effect of a
423 strong vestibular perturbation in increasing MSI outcomes.
424 Interestingly, the motion-induced thermoregulation model revealed
425 differences between vehicle and CGRP/SNP injected mice. Differences in
426 motion-induced nausea after CGRP administration were significantly blocked
427 by a CGRP receptor antagonist (olcegepant) but not by triptans (sumatriptan
428 or rizatriptan). We also showed that in the stationary tests, there are no
429 thermoregulatory changes in the absence of provocative motion. While male
430 and female mice both showed disrupted thermoregulatory responses to IP
431 CGRP injections, dose response experiments at 0.05 and 1 mg/kg CGRP
432 show that olcegepant can rescue CGRP-induced nausea in male yet
433 olcegepant did not fully rescue all female mice. (**Table 2**). This sexual
434 dimorphism is similar to higher incidence of migraine and VM in female
435 patients [4, 38, 39] and reports of sexual-dimorphic effects of CGRP both in dura-

436 induced pain, spinal cord neuropathic pain, and preclinical surrogate of
437 allodynia or touch sensitivities [34]. To this end, other studies have
438 demonstrated a sex difference in the expression of CGRP receptor
439 components in the trigeminal nucleus [40].
440 This study is the first to provide preclinical evidence for the use of CGRP
441 antagonists in treating motion hypersensitivities that occur in VM, and should
442 be paired with findings from pertinent clinical trials for validation. We provide
443 data that the motion-induced thermoregulation assay is sensitive to the known
444 migraine triggers, CGRP and SNP, and show the effectiveness of different
445 migraine drugs ('gepants' and triptans) in reducing nausea responses
446 observed in this mouse model. Future experiments will assess if mice over- or
447 under-expressing the CGRP receptor will be more or less susceptible to
448 changes in their thermoregulatory responses to provocative motion, and if
449 migraine blockers can provide protection, to achieve the greater goal of
450 elucidating the role of CGRP signaling in the symptoms of nausea and motion
451 sickness that occur in migraine and VM.
452
453 **Study Highlight Bullet points:**

454 • Enhanced motion-induced nausea is an observed symptom in migraine and
455 especially vestibular migraine (VM).
456 • We used two different murine motion sickness assays (thermoregulation
457 and MSI), but MSI was too weighted on gastric distress to be effective.
458 • Differences in motion-induced thermoregulation after systemic CGRP
459 were blocked by olcegepant but not by triptans.
460 • Olcegepant was more effective in rescuing CGRP-induced nausea in
461 male mice then in female mice who were more affected by CGRP
462 injection..

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585 **Figure Legends:**

586 **Figure 1: CGRP and SNP's effects on MSI:** Female (pink) and male (blue)
587 C57B6J mice were tested for motion sickness indicators. Treatments were
588 delivered intraperitoneally (IP): vehicle control (open circle/open square), CGRP
589 (closed circle), SNP (closed square), olcegepant + CGRP (top half-filled circle),
590 and sumatriptan + CGRP (bottom half-filled circle). **(A)** Assay timeline is illustrated
591 and a table of criteria and MSI scoring. Motion sickness index (MSI) was
592 computed by a summation of criterion at the appropriate scoring and involved
593 measuring feces, piloerection, urination, and tremor. MSI was recorded at three
594 different time points. The vestibular challenge involved off-vertical axis rotation (60
595 rpm, 45° tilt from the vertical) for 30 minutes. **(B-D).** In each instance, the first
596 bar graph in each condition is MSI calculated either pre-injection (B), post-injection

597 (C), or pre-injection (D), and the second part of bar graphs are either post-
598 vestibular challenge (B & C), or post-injection (D) to tease apart effects of injection
599 with CGRP and vestibular challenge on MSI. In animals allocated for assessing
600 CGRP's effects, MSI outcomes significantly increased after injection and after the
601 vestibular challenge for all treatments**.** Tukey post-hoc indicated increased MSI
602 after IP CGRP (adj. $p < 0.0001$) or after IP CGRP+sumatriptan (adj. $p = 0.0002$)
603 compared to vehicle control. **(B-D). (E-G).** When testing SNP's effects,
604 significant differences were seen in MSI outcomes after the vestibular challenge,
605 but no differences were seen when comparing vehicle control versus SNP.
606
607 **Figure 2: Stationary and provocative motion testing after IP vehicle control:**
608 **(A)** Using a FLIR E60 infrared camera, head and tail temperatures for all
609 conditions are recorded before, during, and after a provocative twenty-minute
610 orbital rotation acting as the vestibular challenge. Infrared recordings are 45
611 minutes in duration. (**B-E**). Sample sizes are depicted in the bottom left for female
612 (pink) and male (blue) C57B6/J mice. After administering vehicle control (saline),
613 **(B and C)** head and **(D and E)** tail temperatures were compared during **(C and E)**
614 stationary testing or **(B and D)** during the provocative rotation**.** Head

615 temperatures for stationery and VC testing were fit to the second-order curve
616 ($B_2X^2 + B_1X + B_0$) and delta head temperatures were computed by subtracting
617 temperature at time t = 20 mins from t = 0 mins. Head curves compute an
618 approximate hypothermia of -1.22^{o}C for females and -1.62^{o}C for males during VC
619 testing and a difference of 0.70^{o}C for females and 0.27^{o}C for males during
620 stationary testing. Delta tail vasodilations were computed by averaging individual
621 tail responses and are depicted as group averages +/- standard error of the mean
622 (SEM). During the first 10 minutes of the provocative rotation, delta tail
623 vasodilations were computed to be 4.6 ±0.3^{o}C for females and 6.1 ± 0.4^{o}C for
624 males. Mice during stationary testing did not experience delta tail vasodilations,
625 and thus a similar computation led to delta tail changes of -1.1 ± 0.8^{o}C for females
626 and 0.2 ± 0.6^{o}C for males.
627
628 **Figure 3: Stationary testing after IP CGRP/SNP and test-retest of IP vehicle**
629 **control.** Sample sizes are listed in the upper left corner. **(A and B)** Head
630 temperature curves were recorded during stationary testing after IP delivery of
631 vehicle control, 1x CGRP, and 1x SNP. **(C and F)** A different cohort of mice was
632 used to assess the test-retest reliability of head and tail temperatures during

633 provocative rotation. **(C)** When assessing test-retest reliability, no significant
634 differences were seen in the approximate magnitude of hypothermia in females
635 (test vs retest: -1.2 ^{o}C vs -1.2 ^{o}C) or males (test vs retest: -1.3 vs 1.2 ^{o}C). (**D and**
636 **E**) Tail temperatures were compared between stationary tests for IP vehicle, IP
637 CGRP, and IP SNP as similarly done with head temperatures, and no significant
638 differences were seen. **(F)** Similar to head temperatures, test-retest of tail
639 measurements during provocative rotation showed no significant differences in
640 delta tail vasodilations in either female (test vs retest: 4.0 ± 0.8 ^{o}C vs 4.2 ± 0.8 ^{o}C)
641 or male (test vs retest: 4.9 ± 0.8 ^{o}C vs 4.9 ± 0.9 ^{o}C).
642
643 **Figure 4: CGRP/SNP effects on hypothermia to provocative motion and**
644 **dose-dependent changes in tail vasodilations.** Sample sizes are shown in the
645 upper right corner. Mice allocated for assessing CGRP's effects on head and tail
646 temperatures were tested for **(A and E)** vehicle control and then **(B and F)** 1x
647 CGRP at 0.1 mg/kg. When assessing head temperature recovery, the 2nd-order
648 curve fits extrapolated recovery times for vehicle control (female: 20.3 mins, male:
649 20.4 mins) and IP CGRP (females: 28.4 mins, male: 25.4 mins). Delta tail
650 vasodilations were observed under vehicle control (female: 4.56 ± 0.31 ^{o}C, male:

651 6.05 ± 0.35 ^{o}C) but were significantly diminished after 1x IP CGRP delivery
652 (female: 0.61 ± 0.20 ^{o}C, male: 1.92 ± 0.40 ^{o}C). A separate mice cohort was
653 allocated for assessing SNP's effects on head and tail and was tested for **(C and**
654 **G)** vehicle control and later **(D and H)** 1x SNP at 2.5 mg/kg. While head
655 temperature recovery did not differ between vehicle and 1x IP SNP groups, delta
656 tail vasodilations were diminished after 1x IP SNP delivery (female: 0.3 ± 0.1 ^{o}C,
657 male: 1.1 ± 0.5 ^{o}C) when compared to vehicle control (female: 4.2 ± 1.0 ^{o}C, male:
658 3.4 ± 0.5 ^{o}C). **(I – L)** Dose-dependent changes in tail temperatures were observed
659 for both IP CGRP and IP SNP. For CGRP testing, 0.1x, 0.5x, and 1x
660 concentrations were prepared at 0.01, 0.05, and 0.1 mg/kg. For SNP testing, 0.1x,
661 0.5x, and 1x concentrations were prepared at 0.25 mg/kg, 1.25, and 2.5 mg/kg.
662
663 **Figure 5: Olcegepant, but not sumatriptan or rizatriptan, protects against**
664 **CGRP's effects on tail vasodilations: (A-J)** Sample sizes are labeled in the
665 bottom right corner. Tail temperatures were recorded IP delivery of vehicle control,
666 CGRP, CGRP+olcegepant, CGRP + sumatriptan and CGRP + rizatriptan. **K**. A
667 floating bar plot is used to compare all animals used to assess CGRP and
668 CGRP+blockers' effects on delta tail vasodilations. Tukey post-hoc analysis

669 showed delta tail vasodilations were significantly diminished after CGRP, after
670 CGRP+sumatriptan, and after CGRP + rizatriptan delivery in both sexes but no
671 significant differences between vehicle control and CGRP+olcegepant. **L.** Mice
672 treated with 0.5x IP CGRP also showed reduced delta tail vasodilations compared
673 to vehicle control, and fully regained near normal vasodilations when treated with
674 IP 0.5x CGRP + olcegepant.

675 **Figure 6: Olcegepant and sumatriptan block SNP's effects on tail**
676 **vasodilations: (A-H)** Similar to CGRP testing, tail temperatures were recorded
677 after IP delivery of vehicle control, SNP, SNP+olcegepant, and SNP+sumatriptan,
678 and sample sizes are labeled in the bottom right corner. Tukey's post-hoc analysis
679 showed that SNP diminished delta tail vasodilations compared to vehicle control in
680 both sexes , yet no difference between vehicle vs IP SNP+olcegepant, or vehicle
681 vs IP SNP+sumatriptan. **(I)** A floating bar graph depicts delta tail values for all
682 mice assessed with SNP and SNP + blockers.

683

684 **<u>Table Legends:</u>**

685 **Table 1:** Two-way mixed-effects models were computed to assess the effects of
686 treatment (CGRP, SNP, or CGRP/SNP + Blockers) on delta tail vasodilations.

687 Mice were separated by sex and the interaction of treatment x sex was also
688 considered. Separate groups of mice were used to compare the effects of
689 CGRP/CGRP+Blockers or SNP/SNP + Blockers. The Geisser-Greenhouse
690 Correction ($\hat{\varepsilon}$) was applied to factors that are repeated measures. Tukey post-hoc
691 comparisons are described in relation to vehicle control in males and females. For
692 tabular results and multiple comparisons, F and P-values are listed accordingly.
693 **Table 2:** Diminished tail vasodilations indicate a disrupted response to provocative
694 rotation. Percentage of mice that exhibited diminished delta tail vasodilations
695 ($<1.5^{o}$ C) during motion-induced thermoregulation testing across treatment.
696 Treatments are administered at the following concentrations: 0.1x CGRP - 0.01
697 mg/kg, 0.5x CGRP - 0.05 mg/kg, 1x CGRP - 0.1 mg/kg, 1x olcegepant - 1 mg/kg,
698 sumatriptan - 0.6 mg/kg, rizatriptan - 0.6 mg/kg, SNP - 2.5 mg/kg.

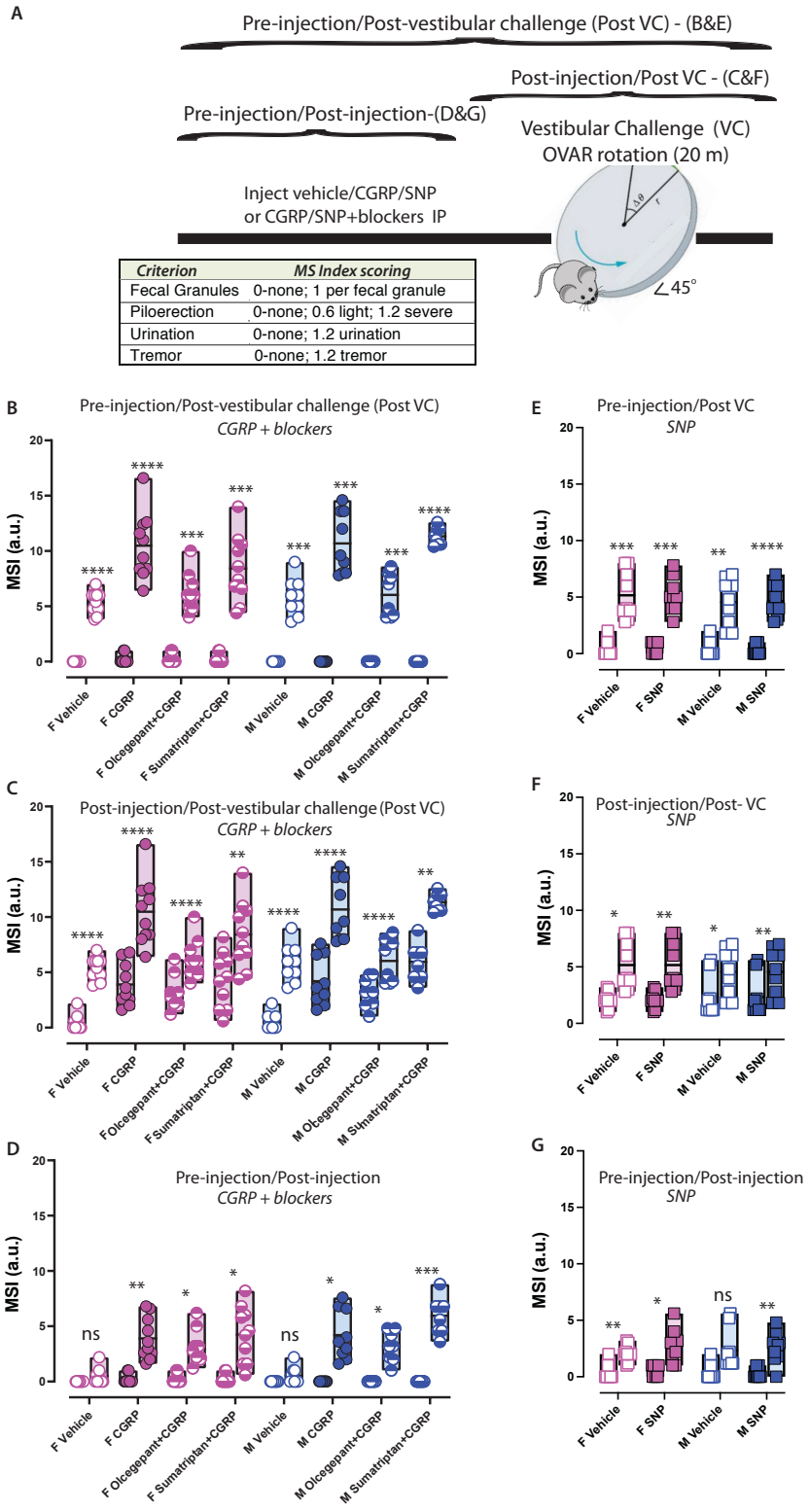

A
Pre-injection/Post-vestibular challenge (Post VC) - (B&E)
Post-injection/Post VC - (C&F)
Pre-injection/Post-injection-(D&G)
Vestibular Challenge (VC)
OVAR rotation (20 m)
Inject vehicle/CGRP/SNP
or CGRP/SNP+blockers IP
∠45°
Criterion | MS Index scoring
Fecal Granules | 0-none; 1 per fecal granule
Piloerection | 0-none; 0.6 light; 1.2 severe
Urination | 0-none; 1.2 urination
Tremor | 0-none; 1.2 tremor
B
Pre-injection/Post-vestibular challenge (Post VC)
CGRP + blockers
C
Post-injection/Post-vestibular challenge (Post VC)
CGRP + blockers
D
Pre-injection/Post-injection
CGRP + blockers
E
Pre-injection/Post VC
SNP
F
Post-injection/Post- VC
SNP
G
Pre-injection/Post-injection
SNP
MSI (a.u.)
F Vehicle
F CGRP
F Olcegepant+CGRP
F Sumatriptan+CGRP
M Vehicle
M CGRP
M Olcegepant+CGRP
M Sumatriptan+CGRP
F SNP
M SNP
ns

Figure 1

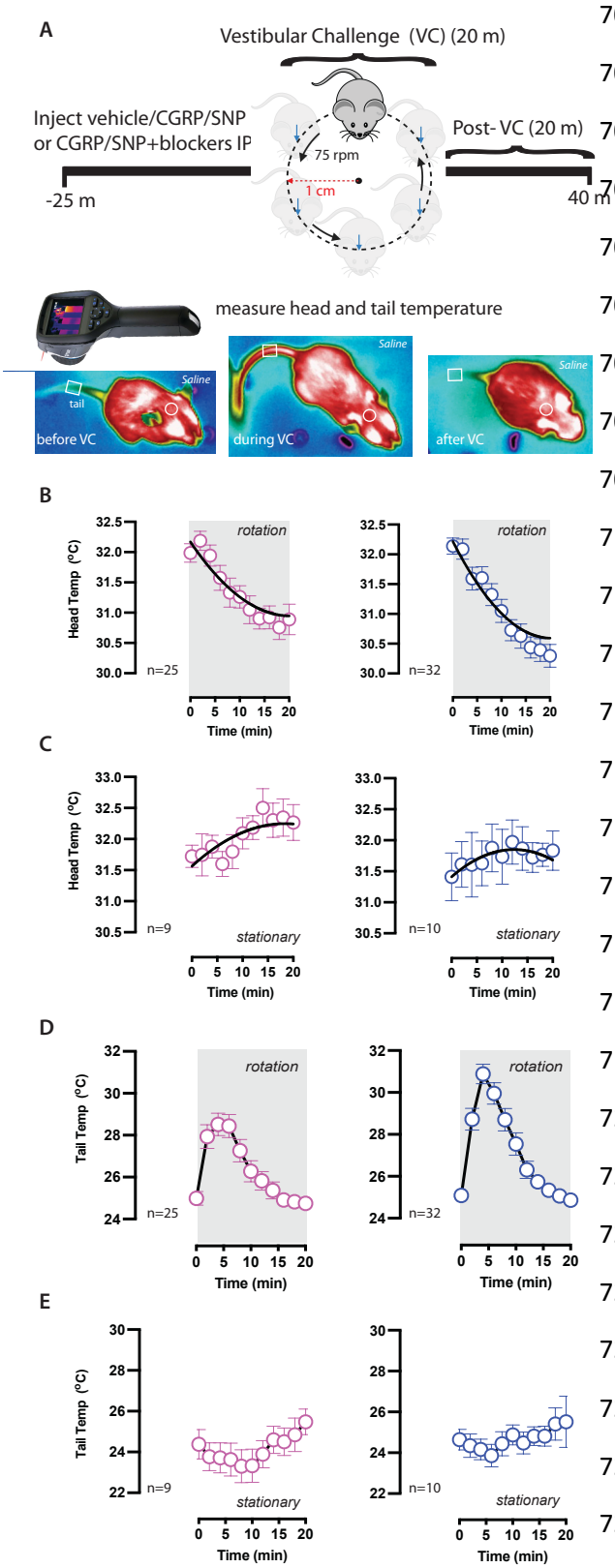
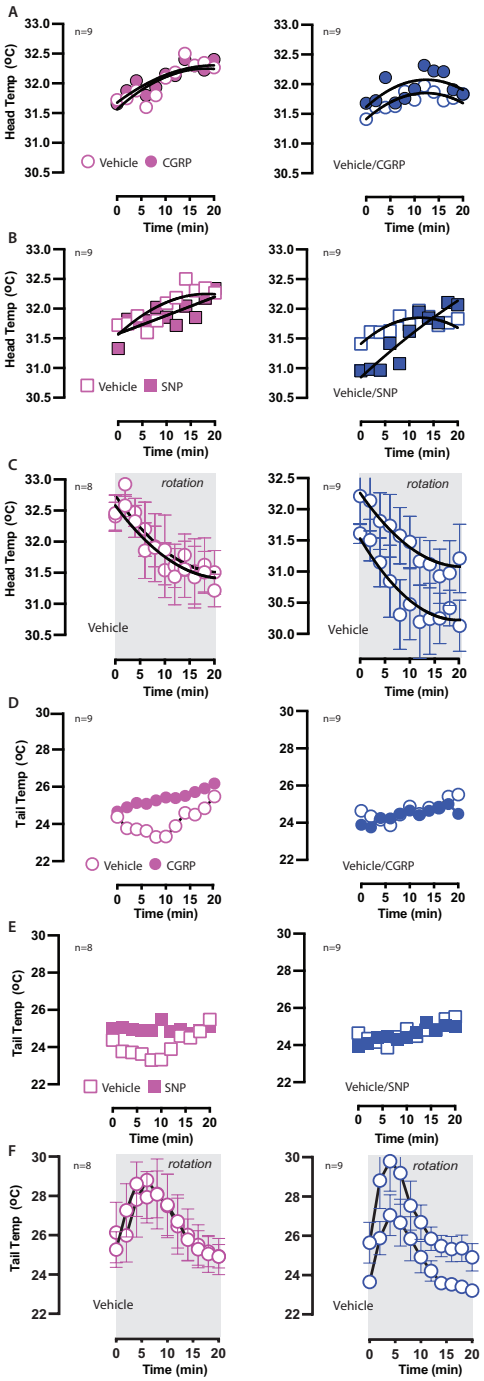

A
Vestibular Challenge (VC) (20 m)
Inject vehicle/CGRP/SNP
or CGRP/SNP+blockers IP
Post- VC (20 m)
75 rpm
1 cm
-25 m
40 m
measure head and tail temperature
Saline
tail
before VC
during VC
after VC
B
Head Temp (°C)
rotation
n=25
n=32
Time (min)
C
stationary
n=9
n=10
D
Tail Temp (°C)
E

Figure 2



730

731 Figure 3

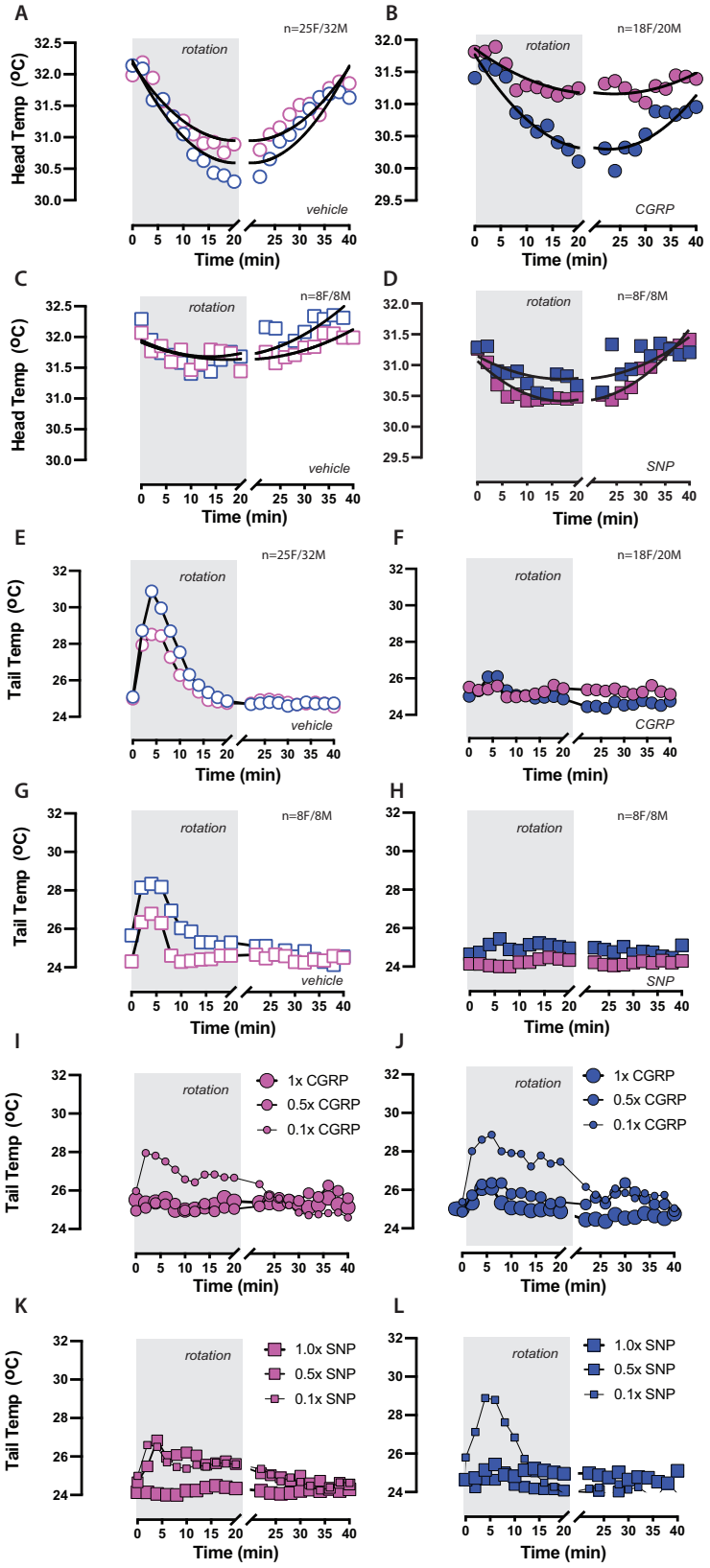

A
n=25F/32M
rotation
vehicle
Head Temp (ºC)
Time (min)
B
n=18F/20M
rotation
CGRP
Time (min)
C
n=8F/8M
rotation
vehicle
Head Temp (ºC)
Time (min)
D
n=8F/8M
rotation
SNP
Time (min)
E
n=25F/32M
rotation
vehicle
Tail Temp (ºC)
Time (min)
F
n=18F/20M
rotation
CGRP
Time (min)
G
n=8F/8M
rotation
vehicle
Tail Temp (ºC)
Time (min)
H
n=8F/8M
rotation
SNP
Time (min)
I
rotation
1x CGRP
0.5x CGRP
0.1x CGRP
Tail Temp (ºC)
Time (min)
J
rotation
1x CGRP
0.5x CGRP
0.1x CGRP
Time (min)
K
rotation
1.0x SNP
0.5x SNP
0.1x SNP
Tail Temp (ºC)
Time (min)
L
rotation
1.0x SNP
0.5x SNP
0.1x SNP
Time (min)

Figure 4

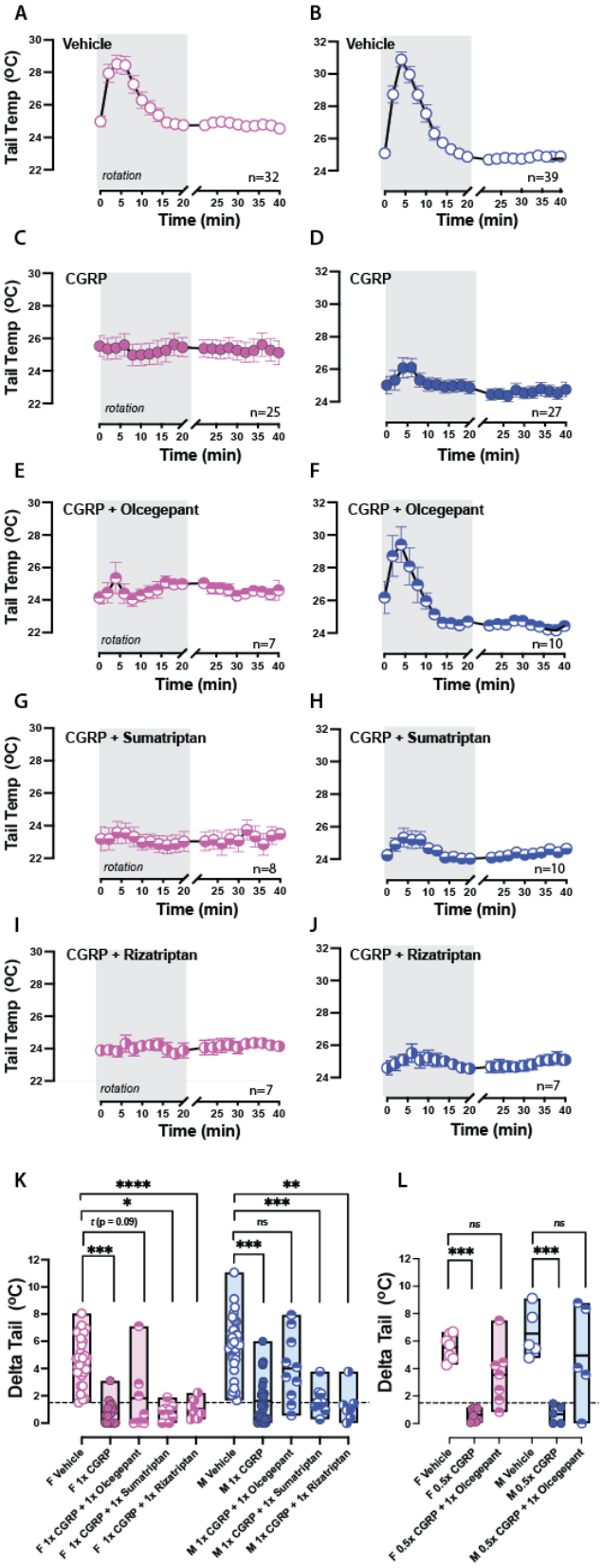

A
Vehicle
Tail Temp (ºC)
rotation
n=32
Time (min)
B
Vehicle
n=39
Time (min)
C
CGRP
rotation
n=25
Time (min)
D
CGRP
n=27
Time (min)
E
CGRP + Olcegepant
rotation
n=7
Time (min)
F
CGRP + Olcegepant
n=10
Time (min)
G
CGRP + Sumatriptan
rotation
n=8
Time (min)
H
CGRP + Sumatriptan
n=10
Time (min)
I
CGRP + Rizatriptan
rotation
n=7
Time (min)
J
CGRP + Rizatriptan
n=7
Time (min)
K
Delta Tail (ºC)
t (p = 0.09)
ns
F Vehicle
F 1x CGRP
F 1x CGRP + 1x Olcegepant
F 1x CGRP + 1x Sumatriptan
F 1x CGRP + 1x Rizatriptan
M Vehicle
M 1x CGRP
M 1x CGRP + 1x Olcegepant
M 1x CGRP + 1x Sumatriptan
M 1x CGRP + 1x Rizatriptan
L
Delta Tail (ºC)
ns
F Vehicle
F 0.5x CGRP
F 0.5x CGRP + 1x Olcegepant
M Vehicle
M 0.5x CGRP
M 0.5x CGRP + 1x Olcegepant

Figure 5

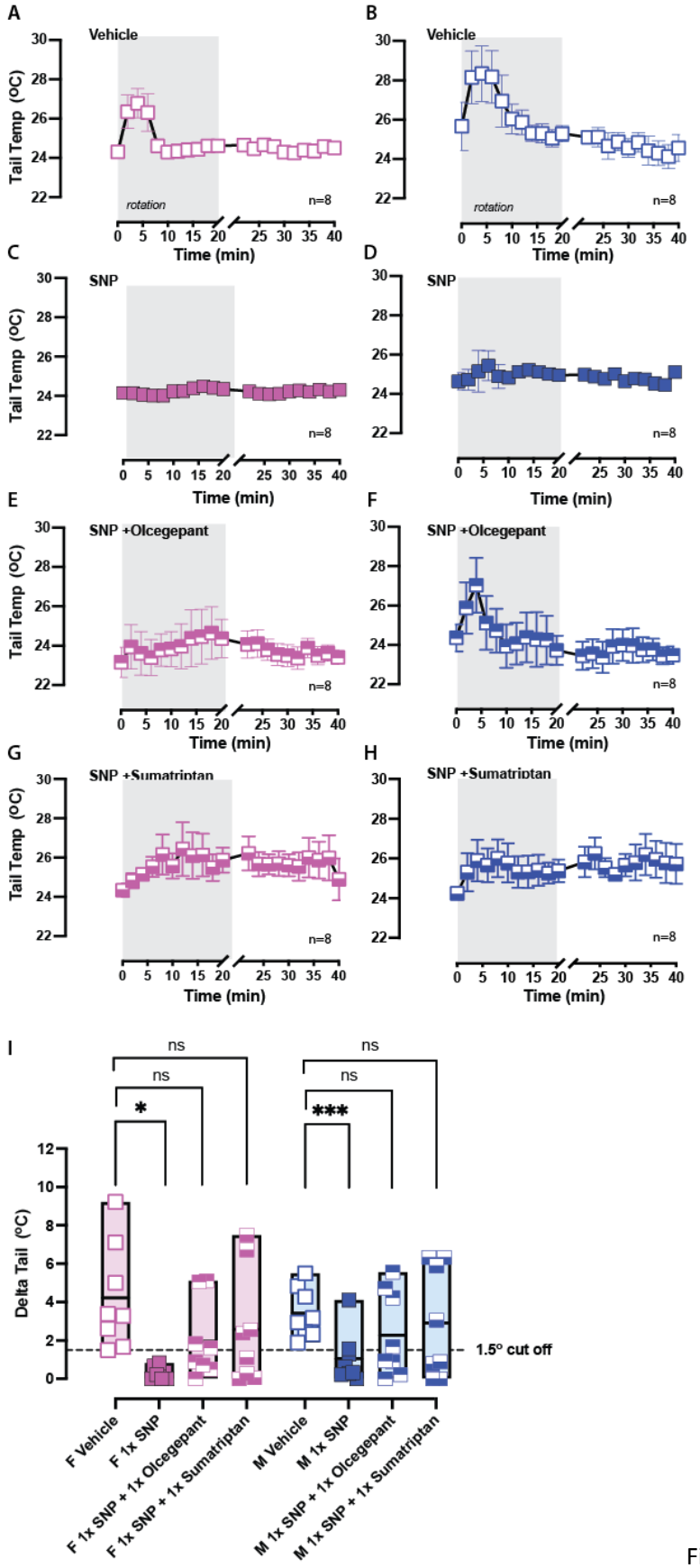

A
Vehicle
Tail Temp (ºC)
rotation
n=8
Time (min)
B
Vehicle
rotation
n=8
Time (min)
C
SNP
n=8
Time (min)
D
SNP
n=8
Time (min)
E
SNP +Olcegepant
n=8
Time (min)
F
SNP +Olcegepant
n=8
Time (min)
G
SNP +Sumatriptan
n=8
Time (min)
H
SNP +Sumatriptan
n=8
Time (min)
I
ns
ns
*
ns
ns
***
Delta Tail (ºC)
1.5º cut off
F Vehicle
F 1x SNP
F 1x SNP + 1x Olcegepant
F 1x SNP + 1x Sumatriptan
M Vehicle
M 1x SNP
M 1x SNP + 1x Olcegepant
M 1x SNP + 1x Sumatriptan

Figure 6

Table 1

Delta Tail - mixed effects models						
Analysis	**Factors**	**P-val**	**F (DFn, DFd)**	**Sex**	**Treatment**	**P-val**
CGRP vs CGRP+Blockers	CGRP/CGRP+Blockers	<0.001	F (4, 93) = 61.62	M	vehicle vs. CGRP	<0.001
					vehicle vs. CGRP + olcegepant	0.5
					vehicle vs. CGRP + sumatriptan	<0.001
	Sex	0.002	F (1, 69) = 10.20		vehicle vs. CGRP + rizatriptan	0.003
				F	vehicle vs. CGRP	<0.001
	CGRP/CGRP Blockers x sex	0.86	F (4, 93) = 0.32		vehicle vs. CGRP + olcegepant	0.1
					vehicle vs. CGRP + sumatriptan	<0.001
					vehicle vs. CGRP + rizatriptan	0.008
SNP vs SNP+Blockers	SNP/SNP+Blockers	0.003	F (2.4, 44.5) = 5.96	M	vehicle vs. SNP	0.0002
					vehicle vs. SNP + olcegepant	0.58
	Sex	0.79	F (1, 56) = 0.07		vehicle vs. SNP + sumatriptan	0.96
				F	vehicle vs. SNP	0.01
	SNP/SNP+Blockers vs Sex	0.77	F (3, 56) = 0.38		vehicle vs. SNP + olcegepant	0.27
					vehicle vs. SNP + sumatriptan	0.42

Table 2

Reduced delta tail vasodilations (< 1.5 ^{o}C) during motion-induced thermoregulation		
Treatment	**Female %**	**Males %**
Vehicle	0.0%	0.0%
0.1x CGRP	22.2%	0.0%
0.5x CGRP	77.7%	50.0%
1.0x CGRP	83.3%	45.0%
0.5x CGRP + 1.0x olcegepant	14.2%	20.0%
1.0x CGRP + 1.0x olcegepant	57.1%	20.0%
1.0x CGRP + 1.0x sumatriptan	87.5%	60.0%
1.0x CGRP + 1.0x rizatriptan	75.0%	87.5%
1.0x SNP	100.0%	75.0%
1.0x SNP + 1.0x olcegepant	50.0%	50.0%
1.0x SNP + 1.0x sumatriptan	50.0%	50.0%