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.

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]. Tail
69 vasodilations to provocative motion have been reported to precede emesis events
70 in the musk shrew, serving as an early index for motion-induced nausea in
71 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. It is also 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. This was driven by
91 CGRP's effect in causing diarrhea, which was not observed after SNP
92 administration. However, motion-induced thermoregulation was a robust model of
93 motion sickness. CGRP receptor antagonism by olcegepant was effective in
94 relieving CGRP effects on motion-induced nausea, whereas triptan therapies were
95 not efficacious. Our studies provide a strong premise that antagonizing CGRP
96 signaling will be effective for treating motion-induced nausea of VM, as it has been
97 shown to be highly effective for typical migraine.
98
99

100 **Materials & Methods:**

101 Animals: C57B6/J mice were obtained from Jackson Laboratories (JAX #664) and
102 were housed under a 12-hour day/night cycle under the care of the University
103 Committee on Animal Resources (UCAR) at the University of Rochester. Mice are
104 housed with *ad libitum* access to food, water, bedding, and enrichment materials.
105 A total of 145 mice (73 M/ 72 F) were tested, and all studies were powered
106 sufficiently to detect male/female differences. Prior to behavioral testing, mice
107 were equilibrated in the testing room controlled for an ambient temperature
108 between 22-23°C for at least 30 minutes. Mice were tested around 2.3 to 6 months
109 of age. This age range correlates to 20-30 years in humans and is within the range
110 that migraine symptoms most likely occur in patients [22]. Different cohorts of mice
111 were used to test motion sickness indices and motion-induced nausea. Testing
112 occurred from 9:00 am to 4:30 pm during the day cycle to control for behavioral
113 and thermoregulatory changes that may arise from circadian rhythms. For motion-
114 induced nausea testing, mice were screened for instances of patchy fur (alopecia)
115 and were not included in this study [23].

116 Drug administration: All injections were performed intraperitoneally (IP) with a fine
117 33-gauge insulin syringe. Dulbecco PBS served as the diluent and as the vehicle

118 control. Migraine blockers used were the CGRP-receptor antagonist olcegepant
119 and the selective serotonin receptor agonists sumatriptan and rizatriptan. The
120 concentrations are listed: 0.1x, 0.5x, and 1.0x CGRP were prepared at 0.01, 0.05,
121 and 0.1 mg/kg (rat α-CGRP, Sigma), 1.0x olcegepant was prepared at 1.0 mg/kg
122 (BIBN4096, Tocris), 1.0x sumatriptan was prepared at 0.6 mg/kg (Sigma-Aldrich),
123 1.0x rizatriptan was prepared at 0.6 mg/kg (Sigma-Aldrich), and 0.1x, 0.5x, and
124 1.0x sodium nitroprusside (SNP)- (Sigma-Aldrich) were prepared at 0.25 mg/kg,
125 1.25, and 2.5 mg/kg. After injection, animals were placed in a separate cage from
126 their home cage to recuperate from injection stress. Mice were tested
127 approximately twenty to thirty minutes after delivery of either vehicle, CGRP, SNP,
128 CGRP or SNP co-administered with a blocker, or drug controls. Animals were
129 gently handled and anesthesia was not needed. All animal procedures were
130 approved by the University of Rochester's (IACUC) and performed in accordance
131 with the standards set by the NIH.
132 Off-Vertical Axis Rotation (OVAR): Prior human and rodent studies have used
133 OVAR to assess the otolith-ocular reflex and assess the semicircular canal-
134 otolith interaction [24-26]. Constant velocity OVAR at a tilt can be disorienting and
135 promote motion sickness in human participants [27, 28], and further studies in

136 mice have shown that provocative rotation leads to pica and observations of
137 urination, piloerection, and tremor [29]. In this study, a two-cage rotator (cage
138 dimensions: 12.7 cm x 8.9 cm) was use for twenty minutes (60 rpm, 45° tilt
139 from the vertical, 20 cm from the axis of rotation) as an OVAR vestibular
140 challenge.

141 Motion-Sickness Index (MSI) Testing**:** We adapted Yu et al.'s protocol that
142 previously assessed the drugs scopolamine and modafinil on mitigating motion
143 sickness indicators in rats and mice [12]. We incorporated OVAR (described
144 above) as the vestibular challenge (VC) in this test. We evaluated MSI in mice
145 at the end of the following time points: A) a five-minute baseline (*pre-injection*),
146 B) twenty to thirty minutes after drug injection (*post-injection*), and C) five
147 minutes after the OVAR (*post-VC*). Mice were placed in a testing box separate
148 from their home cage to observe for indicators of motion sickness detailed in
149 **Fig. 1A.** A motion sickness index (MSI) score was determined by the
150 summation of the indicators.

151 Certain actions were taken to normalize the weight distribution of MS
152 indicators during reporting. Fecal granules (Fg) were counted separately at
153 each time point. The unit weight per Fg after vehicle injection was used to

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

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

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

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

binary outcome measure. Tail temperature changes equal to or greater than +1.5^{o}C were designated a Δ tail vasodilation and those less than +1.5^{o}C did not meet the criteria. Mice were excluded from further testing if their Δ tail vasodilation during the vehicle control test did not meet the criteria.

Prior to assessing the efficacy of migraine blockers on thermoregulatory changes due to provocative motion, control experiments were performed to assess i) test-retest reliability of motion-induced thermoregulation and ii) stationary testing (no rotation) after vehicle, 1.0x CGRP, and 1.0x SNP injections. Additionally, we tested motion-induced thermoregulation at 0.1x and 0.5x concentrations of CGRP and SNP to compare with 1.0x effects.

Statistical Analyses: Statistical analyses were conducted in GraphPad Prism 9.5. Multivariate ANOVAs were analyzed for MSI and motion-induced thermoregulation testing and are described in detail in the results. Second-order curve fitting ($B_2*X^2 + B_1*X + B_0$) 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. Head recovery (mins) was approximated by calculating the time to reach baseline using the

208 equation $(-B_1 + \sqrt{(B_1^2)}) / (2B_2)$ and subtracting it from t = 20 mins. For test-
209 retest reliability of head and tail profiles, Pearson's correlation coefficient is
210 listed as r (df), where r is the coefficient and df is the degrees of freedom. R^2
211 values are provided. Values are reported as mean ± SEM unless noted
212 otherwise, and significance was set at $p < 0.05$ for all analyses.
213
214 **Results:**
215 CGRP and SNP's effects on motion sickness index
216 We studied the effects of CGRP release (n = 9M/10F) on motion sickness
217 indicators (MSI) and whether the CGRP receptor antagonist olcegepant or the
218 $5HT_{1D}$ receptor agonist sumatriptan could mitigate MSI according to the timeline
219 shown in **Fig. 1A**. In females, a two-way ANOVA analyzed MSI outcomes
220 between treatments (CGRP versus CGRP co-delivered with blockers) and
221 between pre-injection versus post-vestibular challenge (VC) time points (**Fig. 1B**).
222 Significant effects were observed due to treatment ($F (2.15, 19.35) = 9.94$; $p <$
223 0.001) and post- VC ($F (1.00, 9.00) = 308.20$; $p < 0.001$). Bonferroni post-hoc test
224 showed that this increase was present for every treatment ($p < 0.001$ for all
225 comparisons). Post-VC MSI was higher after 1.0x CGRP ($p < 0.002$) and after

226 1.0x CGRP + sumatriptan ($p = 0.1$, trending) than vehicle, but significant
227 differences were not observed between 1.0x vehicle and 1.0x CGRP + 1.0x
228 olcegepant. This analysis was also conducted in males and the factors of
229 treatment ($F(3.00, 30.00) = 19.09$; $p < 0.001$) and post-VC effects ($F(1.00, 30.00)$
230 $= 634.8$; $p < 0.001$) were also strong. Bonferroni's post-hoc test showed post-VC
231 MSI was higher than pre-injection MSI for every treatment in males ($p < 0.001$).
232 Like females, post-VC MSI after 1.0x CGRP and 1.0x CGRP + 1.0x sumatriptan in
233 males was significantly higher than after vehicle ($p < 0.001$ each), but this
234 difference to vehicle was not seen with 1.0x CGRP + 1.0x olcegepant. In **Fig. 1C**,
235 MSI outcomes after IP injection were compared to post-VC MSI, and 2-way
236 ANOVAs in females and males showed similar trends as was established in the
237 analysis portrayed in **Fig. 1B**. In **Fig. 1D**, pre-injection MSI was compared to post-
238 injection MSI, and with the exception of vehicle, post-injection MSI was higher than
239 pre-injection baseline. **(Fig. 1E-G)** A separate group of mice were tested for MSI
240 after 1.0x SNP (n = 9M/9F), and significant differences were observed when
241 comparing pre-injection to post-VC, post-injection to post-VC, and pre-injection to
242 post-injection. However, the effect of 1.0x SNP was not different from vehicle, and

243 so MSI was not further evaluated with co-delivery of SNP and blockers. A detailed
244 breakdown of F-values and p-statistics is found in **Table 1**.

245 Motion sickness (MSI) outcome is driven by feces criteria

246 A closer observation of MSI outcomes showed that the main driver of MSI is the
247 magnitude of fecal incontinence, and so 2-way ANOVAs were used to assess
248 differences in fecal incontinence after treatment and across time points in males
249 and females (data not shown). Consistent in both sexes, Bonferroni post-hoc
250 analyses comparing post-VC fecal incontinence outcomes indicated high fecal
251 excretion after 1.0x CGRP ($p < 0.001$) and after 1.0x CGRP + 1.0x sumatriptan (p
252 < 0.001) compared to vehicle tests, but not in mice treated with 1.0x CGRP + 1.0x
253 olcegepant ($p = 0.99$). Diarrhea is a frequent observation after 1.0x CGRP and
254 1.0x CGRP + 1.0x sumatriptan but not after 1.0x CGRP + 1.0x olcegepant. Mice
255 treated with SNP did not exhibit diarrhea or increased fecal granules across all
256 time points or when compared to vehicle. In addition, piloerection, urination, and
257 tremor did not significantly differ between CGRP or SNP-treated groups (data not
258 shown). These results suggest that computing an index for motion sickness based
259 on fecal incontinence, urination, piloerection, and tremor is problematic due to how

260 biased the index is towards fecal excretion. Thus, we used a different method to
261 assess motion-sickness based on motion-induced thermoregulation.
262 Hypothermia and tail vasodilations occur during provocative motion and not
263 stationary testing
264 **Fig. 2A** describes the experiment for assessing motion-induced
265 thermoregulation. After a five-minute baseline recording, mice (n = 28M/27F) are
266 challenged with provocative motion (t = 0 mins, rotation = ON) and exhibited
267 gradual hypothermia until the end of the motion (t = 20 mins, rotation = OFF). 2nd
268 order curve fits calculated similar magnitudes of the mouse's head hypothermia to
269 between males and females (**Fig. 2B).** In addition to the hypothermia, mice
270 exhibited transient but significant increases in tail temperature during the first 10
271 minutes of the provocative motion. The Δ tail vasodilations were quantified for
272 females (4.34 ± 0.34 ^{o}C) and males (5.44 ± 0.42 ^{o}C) (**Fig. 2D**). To ensure that the
273 hypothermia and the tail vasodilations were physiological responses to the
274 provocative motion stimulus, a separate group of mice (n = 10M/9F) was tested
275 similarly but for stationary testing after vehicle treatment. No observed
276 hypothermia or transient vasodilations were seen in mice of either sex during the
277 stationary tests (**Fig. 2C** & **2E**).

278 CGRP and SNP's effects during stationary test

279 To exclude the possibility of thermoregulatory changes that may be due to
280 CGRP and SNP effects, mice assessed for vehicle stationary tests (n = 9M/9F)
281 were further tested with 1.0x CGRP **(Fig. 3A & D)** and 1.0x SNP **(Fig. 3B & E)**. No
282 differences were seen in the head or tail temperatures after 1.0x CGRP or 1.0x
283 SNP testing compared to vehicle, and hypothermia or Δ tail vasodilations were not
284 observed.

285 Test-retest reliability during provocative motion

286 After vehicle administration, male and female mice exhibited hypothermia
287 during the provocative rotation and this response was repeatably observed in the
288 retest (n = 9M/8F). In **Fig. 3C**, 2nd order curve fits were generated for test-retest
289 head curves and strong fits were seen for female (Pearson's $r\ (23) = 0.87$, $p <$
290 0.0001) and for male (Pearson's $r\ (23) = 0.89$, $p < 0.0001$). The magnitude of
291 hypothermia measured in the head temperatures was similar between sexes and
292 reproducible during the test-retest. In **Fig. 3F**, similar tail temperature profiles were
293 observed during test-retest for females (Pearson's $r\ (23) = 0.92$, $p < 0.0001$) and
294 for male (Pearson's $r\ (23) = 0.95$, $p < 0.001$). The Δ tail vasodilations were
295 observed to have similar magnitudes for females (mean vasodilation $_{test}$: 4.0 ± 0.8

296 ^{o}C, mean vasodilation $_{retest}$: 4.2 ± 0.8 ^{o}C) and for males (mean vasodilation $_{test}$: 4.9

297 ± 0.8 ^{o}C, mean vasodilation $_{retest}$: 4.9 ± 0.9 ^{o}C).

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

299 As seen in **Fig. 4A and B**, 2nd order curve fits were used to compute the recovery

300 of head temperatures back to baseline values after the hypothermia after 1.0x

301 CGRP delivery (28F/27M). A general trend of extended recovery times was

302 observed due to 1.0x CGRP delivery (female: 24.0 mins, R^2 = 0.73; male: 23.7

303 mins, R^2 = 0.83) compared to their vehicle control responses (female: 20.7 mins,

304 R^2 = 0.76; male: 19.0 mins, R^2 = 0.87). We observed no differences in the

305 magnitude of head hypothermia between CGRP and vehicle control tests in either

306 sex. **Fig. 4E and F** show tail temperature profiles after 1.0x CGRP, and a 2-way

307 ANOVA showed effects of biological sex ($F(1, 53) = 6.79$, $p = 0.01$) and CGRP (F

308 $(1, 53) = 251.7$, $p < 0.001$). 100% of mice exhibited normal Δ tail vasodilations

309 after vehicle control administration but a majority of mice (89% female, 59% male)

310 exhibited diminished Δ tail vasodilations (Bonferroni, $p < 0.001$) after 1.0x CGRP

311 administration when compared to vehicle (**Fig. 5E**). Due to the diminished Δ

312 vasodilations and the extended recovery times detected after CGRP testing, it is

313 inferred that 1.0x CGRP impacted a mouse's natural response to the provocative

314 motion and their nausea response. In parallel, a different group of mice was used
315 to assess SNP's effects on motion-induced thermoregulation (8F/8M), and
316 temperature profiles were analyzed. When measuring head temperatures, we did
317 not observe any differences in recovery time or in the magnitude of head
318 hypothermia (**Fig. 4C and D**). However, similar to CGRP, a 2-way ANOVA
319 indicated SNP impacted ∆tail vasodilations ($F(1, 14) = 38.8$, $p < 0.001$) but saw no
320 effects of biological sex ($F(1, 14) = 0.002$, $p = 0.97$). Bonferroni post hoc analysis
321 indicated 100% of females ($p < 0.001$) and 75% of males ($p < 0.01$) exhibited
322 diminished vasodilations after 1.0x SNP (**Fig. 6G and 6H**).
323 <u>Dose-dependent response of CGRP or SNP on tail vasodilations</u>
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 = 10M/9F) and ∆ tail vasodilations were analyzed for vehicle, 0.1x and 0.5x of IP
329 CGRP or IP SNP, and these mice were compared with the original groups of mice
330 tested for vehicle and 1.0x CGRP (**Fig. 4I-L**). A 2-way mixed effects model and
331 Bonferroni post hoc assessed the factors: biological sex and CGRP/SNP dilutions.

332 0.1x CGRP had no significant effect on Δ tail vasodilations in either sex. However,
333 differences were observed when comparing Δ tail vasodilations between vehicle
334 and 0.5x CGRP **(Supplementary Fig.S1 A.)** in females ($p < 0.001$) and in males
335 ($p = 0.03$). Significant differences in Δ tail vasodilations were seen when
336 comparing vehicle and 1.0x CGRP in both sexes ($p < 0.001$ each). When
337 assessing SNP's effects (n = 8F/8M), no significant differences were seen at 0.1x
338 and 0.5x SNP compared to vehicle control **(Supplementary Fig. S1 B)**, but a
339 significant difference was seen when comparing vehicle to 1.0x SNP in females (p
340 = 0.01) and in males ($p = 0.0002$). The greatest effect of CGRP and SNP on Δ tail
341 vasodilations was observed at their 1.0x concentration and these concentrations
342 were used to assess the migraine blockers.

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

344 Administration of 1.0x CGRP reduced Δ tail vasodilations in nearly all mice
345 (**Fig. 5E**), and so it was hypothesized that these mice would regain their healthy
346 response to provocative motion with IP treatment of either CGRP + olcegepant (n
347 = 10M/10F), CGRP + sumatriptan (m = 10M/8F), or CGRP + rizatriptan (n =
348 7M/7F), and different groups of mice were used. Prior to assessing CGRP +
349 blockers, groups of mice were assessed for blocker-only effects by testing vehicle

350 + 1.0x olcegepant (n = 8F/10M), vehicle + 1.0x sumatriptan (n = 7F/10M), or
351 vehicle + 1.0x rizatriptan (n = 7F/7M), but no differences were seen when
352 compared to their vehicle control so the blockers, by themselves, do not have a
353 secondary effect on tail vasodilations **(Supplementary Fig. S2)**.
354 Tail temperature profiles were recorded and displayed for vehicle and drug
355 treatments (**Fig. 5A-D, G, H, J, K, M & N**). After quantifying Δ tail vasodilations,
356 one-way ANOVAs were used to assess CGRP + blocker effects in the designated
357 groups, and Dunnett post-hoc analysis was used to compare treatments to
358 vehicle. F-values and ANOVA p-values are listed in **Table 1**. We observed
359 olcegepant blocked CGRP-induced changes in males because Δ tail vasodilations
360 resembled vehicle **(Fig. 5F)**, and this protective effect was trending in females ($p =$
361 0.06). In contrast, triptans did not have a protective effect **(Fig. 5L & O)**. Mice after
362 1.0x CGRP + 1.0x sumatriptan still exhibited diminished Δ tail vasodilations
363 compared to their IP vehicle test ($p < 0.001$ in females and $p = 0.004$ in males). A
364 similar observation was seen in mice after treatment of IP 1.0x CGRP + 1.0x
365 rizatriptan, as tail vasodilations were still significantly diminished in both sexes
366 compared their IP vehicle test ($p = 0.003$ in females and $p = 0.004$ in males). In
367 the dose-dependent response study, mice that experienced reduced vasodilations

368 due to the 0.5x CGRP dose were later tested with 0.5x CGRP + 1.0x olcegepant,
369 and 86% of females and 80% of males were rescued after delivery of 1.0x
370 olcegepant (**Fig. 5I).** These findings show that olcegepant blocked the effects of
371 CGRP and allowed for a majority of mice to exhibit their normal response to the
372 provocative motion by blocking CGRP's effects, while both triptans were not
373 effective.
374
375 <u>SNP's effects on tail vasodilations are blocked by olcegepant</u>
376 The effects of olcegepant and sumatriptan were also assessed against 1.0x
377 SNP (n = 8M/8F) that had reduced Δ tail vasodilations in nearly all mice **(Fig. 6E)**,
378 and tail temperatures are depicted (**Fig. 6A-D, F, G, I, & J)**. In order to conserve
379 mice, the same mice were treated with 1.0x CGRP + 1.0x olcegepant and four
380 days later with 1.0x CGRP + 1.0x sumatriptan. 1-way ANOVAs with Dunnett post
381 hoc were computed and F-values and statistics are in **Table 1**. In females and
382 males, robust Δ tail vasodilation responses were observed in 50% of females and
383 50% males after 1.0x CGRP + 1.0x olcegepant **(Fig. 6H)**, and these percentages
384 were also seen when mice were later tested with 1.0x CGRP + 1.0x sumatriptan
385 **(Fig. 6K)**. Post-hoc analyses in both sexes indicated no significant difference in tail

386 vasodilations for either SNP + blocker when compared to vehicle. These mice
387 were not further tested with blockers at lower doses of SNP. **Table 2** is provided to
388 depict the percentage differences in the prevalence of diminished Δ tail
389 vasodilations observed across all treatments (CGRP/SNP + blockers) for both
390 male and female mice.
391
392 **Discussion:**
393 In this study, we show that systemic CGRP injections can induce motion
394 sickness in wildtype C57B6/J mice, and this motion sickness can be strongly
395 antagonized by the CGRP receptor blocker, olcegepant. Published studies
396 assessing CGRP's effects on light-aversive behavior (photophobia) and
397 cutaneous hypersensitivity (allodynia) in rodents indicate triptans are effective
398 in reducing these surrogate behaviors [30, 31], but interestingly in our study,
399 sumatriptan and rizatriptan did not show significant efficacy in blocking
400 CGRP's effects in motion sickness and motion-induced nausea. We have
401 used two different murine motion sickness assays: 1) MSI and 2) motion-
402 induced thermoregulation. In both assays, provocative motion caused distinct
403 changes, but MSI was too weighted on gastric distress. This bias reduced its

404 validity as an assay because gastrointestinal complications do not directly
405 imply motion sickness. However, the motion-induced thermoregulation model
406 is more suitable for studying motion sensitivity as it involves studying motion-
407 induced thermoregulation caused specifically by a vestibular stimulus – the
408 constant orbital rotation. In addition, the motion-induced thermoregulation
409 model was robust for assessing CGRP and SNP's effects. Similar rodent
410 studies suggest tail vasodilations are a precursor to emesis and removal of toxins
411 [11]. Our data showing CGRP's blunting of tail vasodilations to provocative motion
412 suggests that motion-induced nausea may be aggravated with CGRP and that
413 olcegepant is protective but not triptans. This result is in agreement with clinical
414 findings that showed a 2-hour infusion of CGRP caused gastrointestinal
415 hyperactivity and nausea, and pretreatment with a CGRP receptor antagonist
416 ameliorated these clinical symptoms but pretreatment with sumatriptan did not [32].
417 Moreover, a clinical meta-analysis of anti-CGRP signaling treatments for nausea in
418 episodic migraine provided strong support for their effectiveness [33]. Interestingly,
419 we also observed that female mice were more severely affected by systemic
420 CGRP than their male counterparts, which correlates with preclinical studies
421 showing stronger tactile sensitivity in female mice when challenged with

422 durally applied CGRP [34].

423 CGRP signaling and murine motion-sickness indices:

424 Others have shown in human subjects and in mouse models that CGRP
425 causes diarrhea by disrupting peristaltic intestinal activity and promoting ion
426 and water secretion in the intestinal lumen, and that CGRP signaling
427 antagonism can reverse these effects [35-37]. Thus, it is expected that CGRP-
428 induced diarrhea would cause noticeable increases in the motion sickness
429 index (MSI) calculation. The OVAR rotation further increased the motion
430 sickness index (MSI) regardless of CGRP or SNP, highlighting the effect of a
431 strong vestibular perturbation in increasing MSI outcomes.

432 Interestingly, the motion-induced thermoregulation model revealed
433 differences between vehicle and CGRP/SNP injected mice. Differences in
434 motion-induced nausea after CGRP administration were blocked by a CGRP
435 receptor antagonist (olcegepant) but not by triptans (sumatriptan or
436 rizatriptan). We also showed that in the stationary tests, there are no
437 thermoregulatory changes in the absence of provocative motion. While male
438 and female mice both showed disrupted thermoregulatory responses to CGRP
439 injections, dose response experiments at 0.05 and 1 mg/kg CGRP show that

440 olcegepant can rescue CGRP-induced nausea in male yet olcegepant did not
441 fully rescue all female mice. (**Table 2**). This sexual dimorphism is similar to
442 higher incidence of migraine and VM in female patients [4, 38, 39] and reports of
443 sexual-dimorphic effects of CGRP both in dura-induced pain, spinal cord
444 neuropathic pain, and preclinical surrogate of allodynia or touch sensitivities [34].
445 To this end, other studies have demonstrated a sex difference in the
446 expression of CGRP receptor components in the trigeminal nucleus [40].
447 This study is the first to provide preclinical evidence for the use of CGRP
448 antagonists in treating motion hypersensitivities that occur in VM, and should
449 be paired with findings from pertinent clinical trials for validation. We provide
450 data that the motion-induced thermoregulation assay is sensitive to the known
451 migraine triggers, CGRP and SNP, and show the effectiveness of different
452 migraine drugs ('gepants' and triptans) in reducing nausea responses
453 observed in this mouse model. Future experiments will assess if mice over- or
454 under-expressing the CGRP receptor will be more or less susceptible to
455 changes in their thermoregulatory responses to provocative motion, and if
456 migraine blockers can provide protection. These experiments will achieve the

457 greater goal of elucidating the role of CGRP signaling in the symptoms of
458 nausea and motion sickness that occur in migraine and VM.
459
460 **Study Highlight Bullet points:**

461 - Enhanced motion-induced nausea is an observed symptom in migraine and
462 especially vestibular migraine (VM).
463 - We used two different murine motion sickness assays (thermoregulation
464 and MSI), but MSI was too weighted on gastric distress to be effective.
465 - Differences in motion-induced thermoregulation after systemic CGRP
466 were blocked by olcegepant but not by triptans.
467 - Olcegepant was more effective in rescuing CGRP-induced
468 thermoregulatory changes in male compared to female mice.

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591

592

593 **Fig. Legends:**

594 **Fig. 1: CGRP and SNP's effects on MSI:** Female (pink) and male (blue)
595 C57B6/J mice were tested for motion sickness indicators. Treatments were
596 delivered intraperitoneally (IP): vehicle control (open circle/open square), CGRP
597 (closed circle), SNP (closed square), olcegepant + CGRP (top half-filled circle),
598 and sumatriptan + CGRP (bottom half-filled circle). **(A)** Assay timeline is illustrated
599 and a table of criteria and MSI scoring. Motion sickness index (MSI) was
600 computed by a summation of criterion at the appropriate scoring and involved
601 measuring feces, piloerection, urination, and tremor. MSI was recorded at three
602 different time points. The vestibular challenge involved off-vertical axis rotation (60
603 rpm, 45° tilt from the vertical) for 30 minutes. In each instance, the first bar graph
604 in each condition is MSI calculated either pre-injection **(B & E),** post-injection **(C &**
605 **F)**, or pre-injection **(D & G)**, and the second part of bar graphs are either post-
606 vestibular challenge **(B, C, E & F)**, or post-injection **(D & G)**. Two-way ANOVAs
607 were analyzed in males and in females. F-statistics and p-values can be found in
608 **Table 1**.
609

610 **Fig. 2: Stationary and provocative motion testing after IP vehicle control: (A)**
611 Using a FLIR E60 infrared camera, head and tail temperatures for all conditions
612 are recorded before, during, and after a provocative twenty-minute orbital rotation
613 acting as the vestibular challenge. Infrared recordings are 45 minutes in duration.
614 Sample sizes are depicted in the bottom left for female (pink) and male (blue)
615 C57B6/J mice. After administering vehicle (saline), **(B and C)** head and **(D and E)**
616 tail temperatures were compared during **(C and E)** stationary testing or **(B and D)**
617 during the provocative rotation**.** Head temperatures for stationery and VC testing
618 were fit to the second-order curve ($B_2X^2 + B_1X + B_0$) and Δ head temperatures
619 were computed by subtracting temperature at time t = 20 mins from t = 0 mins.
620 Head curves compute similar hypothermia between sexes but different from
621 stationary tests. During the first 10 minutes of the provocative rotation, Δ tail
622 vasodilations were computed to be 4.34 ±0.34^{o}C for females and 5.44 ± 0.42^{o}C for
623 males. Mice during stationary testing did not experience Δ tail vasodilations, and
624 had Δ tail changes of -1.1 ± 0.8^{o}C for females and 0.2 ± 0.6^{o}C for males.
625
626 **Fig. 3: Stationary testing after IP CGRP/SNP and test-retest of IP vehicle**
627 **control.** Sample sizes are listed in the upper left corner. **(A and B)** Head

628 temperature curves were recorded during stationary testing after IP delivery of
629 vehicle, 1.0x CGRP, and 1.0x SNP. **(C and F)** A different cohort of mice was used
630 to assess the test-retest reliability of head and tail temperatures during provocative
631 rotation. **(C)** When assessing test-retest reliability, no significant differences were
632 seen in the approximate magnitude of hypothermia in females (test vs retest: -1.2
633 ^{o}C vs -1.2 ^{o}C) or males (test vs retest: -1.3 vs 1.2 ^{o}C). (**D and E**) Tail
634 temperatures were compared between stationary tests for vehicle, 1.0x CGRP,
635 and 1.0x SNP as similarly done with head temperatures, and no significant
636 differences were seen. **(F)** Similar to head temperatures, test-retest of tail
637 measurements during provocative rotation showed no significant differences in Δ
638 tail vasodilations in either female (test vs retest: 4.00 ± 0.80 ^{o}C vs 4.20 ± 0.80 ^{o}C)
639 or male (test vs retest: 4.90 ± 0.80 ^{o}C vs 4.90 ± 0.90 ^{o}C).
640
641 **Fig. 4: CGRP/SNP effects on hypothermia to provocative motion and dose-**
642 **dependent changes in tail vasodilations.** Sample sizes are shown in the upper
643 right corner. Mice allocated for assessing CGRP's effects on head and tail
644 temperatures were tested for **(A & E)** vehicle and then **(B & F)** 1.0x CGRP. When
645 assessing head temperature recovery, the 2nd-order curve fits extrapolated

646 recovery times for vehicle control (female: 20.7 mins, male: 19.0 mins) and IP
647 CGRP (females: 24.0 mins, male: 23.7 mins). The Δ tail vasodilations observed
648 after vehicle treatment significantly diminished after 1.0x CGRP. A separate mice
649 cohort was allocated for assessing SNP's effects on head and tail and was tested
650 for **(C & G)** vehicle control and later **(D & H)** 1.0x SNP. While head temperature
651 recovery did not differ between vehicle and 1.0x SNP groups, Δ tail vasodilations
652 were diminished after 1.0x SNP. **(I – L)** Dose-dependent changes in tail
653 temperatures were observed for both IP CGRP and IP SNP. For CGRP testing,
654 0.1x, 0.5x, and 1.0x concentrations were prepared at 0.01, 0.05, and 0.1 mg/kg.
655 For SNP testing, 0.1x, 0.5x, and 1x concentrations were prepared at 0.25 mg/kg,
656 1.25, and 2.5 mg/kg.
657
658 **Fig. 5: Olcegepant, but not sumatriptan or rizatriptan, protects against**
659 **CGRP's effects on tail vasodilations.** Sample sizes are labeled in the bottom
660 right corner. Tail temperatures were recorded after IP delivery of **(A & B)** vehicle,
661 **(C & D)** 1.0x CGRP, **(F & G)** 1.0x CGRP + 1.0x olcegepant, **(J & K)** 1.0x CGRP +
662 1.0x sumatriptan and **(M & N)** 1.0x CGRP + 1.0x rizatriptan. **(E)** Bonferroni post-
663 hoc indicated diminished tail vasodilations after 1.0x CGRP in all mice ($p < 0.001$

664 in either sex). **(F)** Dunnett post-hoc comparisons suggested olcegepant protected
665 against CGRP-induced effects more strongly in males than females. When
666 comparing 1.0x CGRP + 1.0x olcegepant to vehicle, no differences were observed
667 in males. In females, differences were deemed not significant but were less certain
668 as $p = 0.057$. **(I)** Mice treated with 0.5x CGRP and exhibited diminished Δ tail
669 vasodilations were treated with 0.5x CGRP + 1.0x olcegepant and recovered their
670 response. **(L & O)** Mice were not protected by 1.0x CGRP + 1.0x sumatriptan or
671 by 1.0x CGRP + 1.0x rizatriptan as Δ tail vasodilations were still diminished
672 compared to their vehicle response (vehicle vs CGRP + sumatriptan: $p < 0.001$ in
673 females and $p = 0.004$ in males; vehicle vs CGRP + rizatriptan: $p = 0.003$ in
674 females and $p = 0.004$ in males).
675 **Fig. 6: Olcegepant and sumatriptan block SNP's effects on tail vasodilations:**
676 Similar to CGRP testing, tail temperatures were recorded after IP delivery of **(A &**
677 **B)** vehicle, **(C & D)** 1.0x SNP, **(F & G)** 1.0x SNP + 1.0x olcegepant, and **(I & J)**
678 1.0x SNP + 1.0x sumatriptan, and sample sizes are labeled in the bottom right
679 corner. **(E)** Dunnett's post-hoc comparisons showed that SNP diminished Δ tail
680 vasodilations compared to vehicle control in both sexes. No differences were

observed between **(H)** vehicle vs 1.0x SNP + 1.0x olcegepant, or **(K)** vehicle vs 1.0x SNP + 1.0x sumatriptan in either sex.

Supplementary Fig. S1: A. 2-way mixed effects model assessed the factors i) sex (male vs female) and ii) CGRP dilutions (0.1x, 0.5x, and 1.0x CGRP). Compared to vehicle responses, significant differences were observed at 0.5x CGRP for females ($p < 0.001$) and for males ($p = 0.03$). These differences were more strongly observed at 1.0x CGRP ($p < 0.001$ for both sexes). 0.1x CGRP did not have a strong effect. Sample sizes are listed: vehicle (34 F/37M), 0.1x, and 0.5x CGRP (9F/10M), 1.0x CGRP (28F/27M). **B.** 2-way mixed effects model assessed the factors i) sex (male vs female) and ii) SNP dilutions (0.1x, 0.5x, and 1.0x SNP). Compared to vehicle responses, significant differences were observed at 1.0x SNP for females ($p = 0.015$) and for males ($p = 0.0002$). Significant differences were not seen at 0.1x and 0.5x SNP. All 8F/8M were tested across dilutions.

Supplementary Fig. S2: In females and males, 1-way mixed effect models with Dunnett's post hoc comparison tests were computed to assess drug controls to the vehicle (Veh), and no significant differences were observed. Sample sizes are

699 listed: vehicle (28 F/27M), Veh + 1x Olceg (8F/10M), Veh + 1.0x Suma (7F/10M),
700 Veh + 1.0x Riza (7F/7M).

701 **Table Legends:**

702 **Table 1:** ANOVA analyses are depicted for motion sickness index (MSI) testing
703 (top half) and for Δ tail vasodilations from motion-induced thermoregulation assay
704 (bottom half). Analyses were conducted separately in males and females. The
705 following abbreviations are listed: pre-injection is pre-inj, post-injection is post-inj,
706 post-vestibular challenge is post-VC, olcegepant is olceg, and sumatriptan is
707 suma. For MSI, two-way repeated measure ANOVAs with Bonferroni post hoc
708 comparisons were computed to assess two factors: treatment (vehicle, CGRP,
709 CGRP+olceg, CGRP+suma) and timepoint differences (pre-inj vs post-VC, post-inj
710 vs post-VC, and pre-inj vs post-inj). For Δ tail vasodilations, 1-way ANOVAs were
711 conducted to study effects of olcegepant, sumatriptan, and rizatriptan. Dunnett
712 post-hoc comparisons were between treatments to vehicle. F and p-values are
713 listed accordingly.

714 **Table 2:** Diminished tail vasodilations indicate a disrupted response to provocative
715 rotation. Percentage of mice that exhibited diminished Δtail vasodilations (<1.5^{o} C)
716 during motion-induced thermoregulation testing across treatment. Treatments are

717 administered at the following concentrations: 0.1x CGRP - 0.01 mg/kg, 0.5x CGRP

718 - 0.05 mg/kg, 1.0x CGRP - 0.1 mg/kg, 1.0x olcegepant - 1 mg/kg, 1.0x sumatriptan

719 - 0.6 mg/kg, 1.0x rizatriptan - 0.6 mg/kg, 1.0x SNP - 2.5 mg/kg.

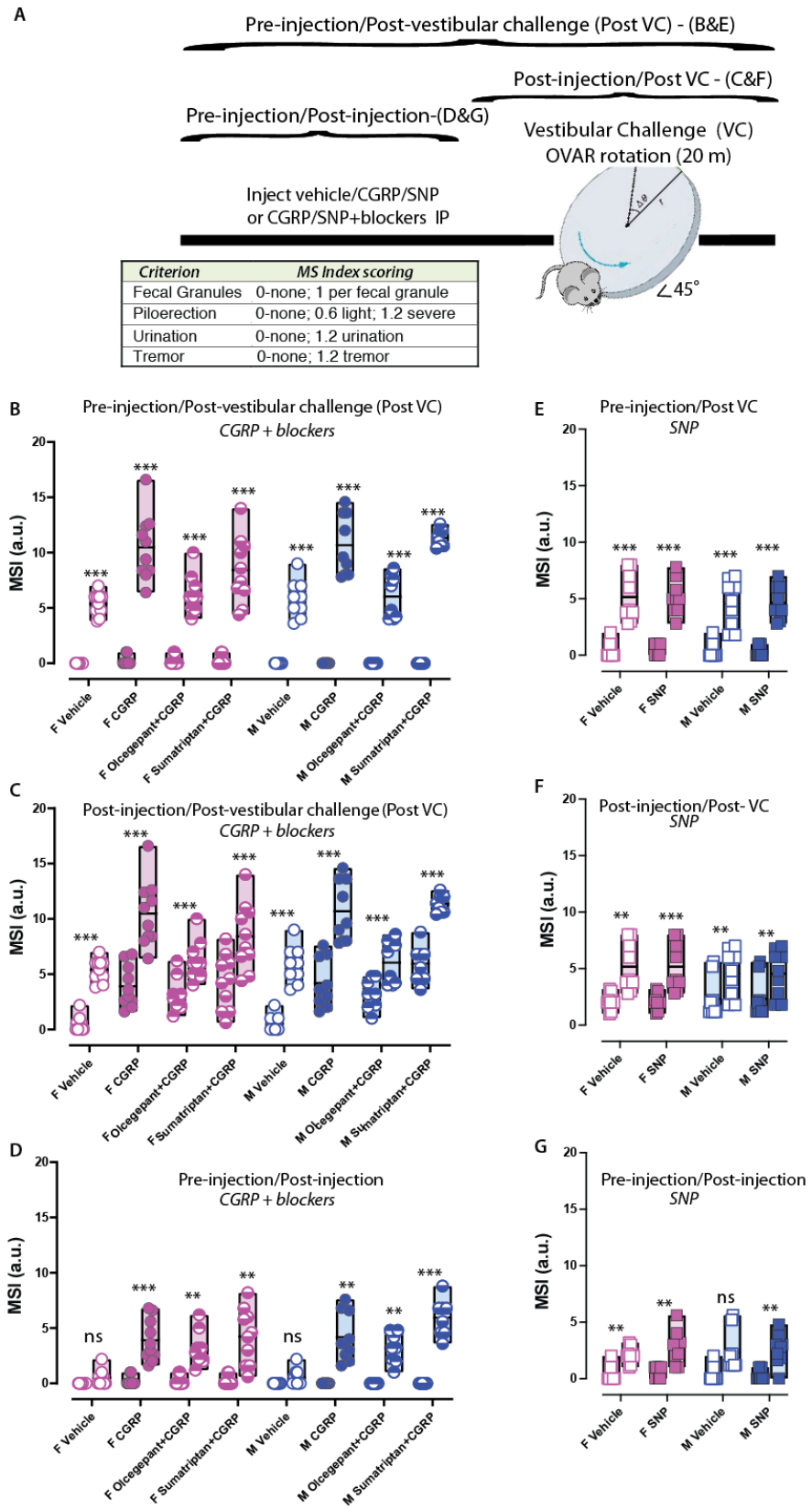

A
Pre-injection/Post-vestibular challenge (Post VC) - (B&E)
Post-injection/Post VC - (C&F)
Pre-injection/Post-injection-(D&G)
Vestibular Challenge (VC)
OVAR rotation (20 m)
Inject vehicle/CGRP/SNP
or CGRP/SNP+blockers IP
∠45°
Criterion | MS Index scoring
Fecal Granules | 0-none; 1 per fecal granule
Piloerection | 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

Fig. 1

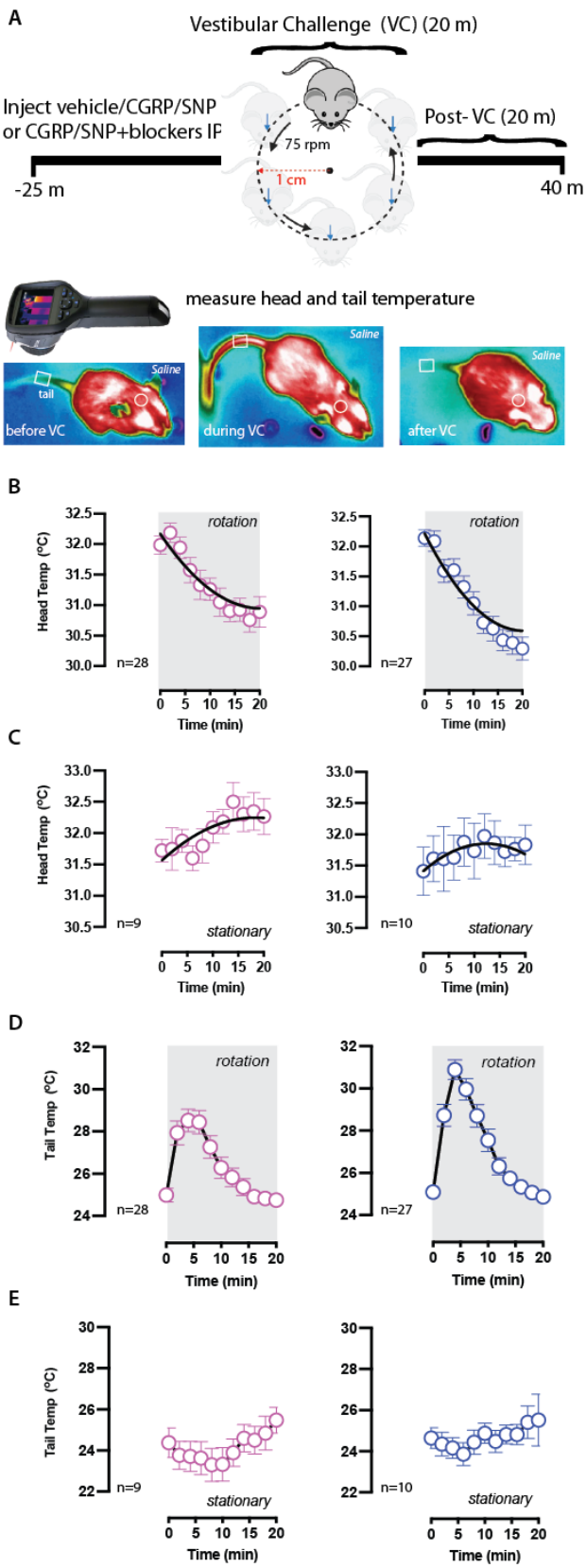

A
Vestibular Challenge (VC) (20 m)
Inject vehicle/CGRP/SNP
or CGRP/SNP+blockers IP
Post- VC (20 m)
75 rpm
1 cm
-25 m
40 m
measure head and tail temperature
Saline
tail
before VC
during VC
after VC
B
Head Temp (°C)
rotation
n=28
n=27
Time (min)
C
stationary
n=9
n=10
D
Tail Temp (°C)
E

Fig. 2

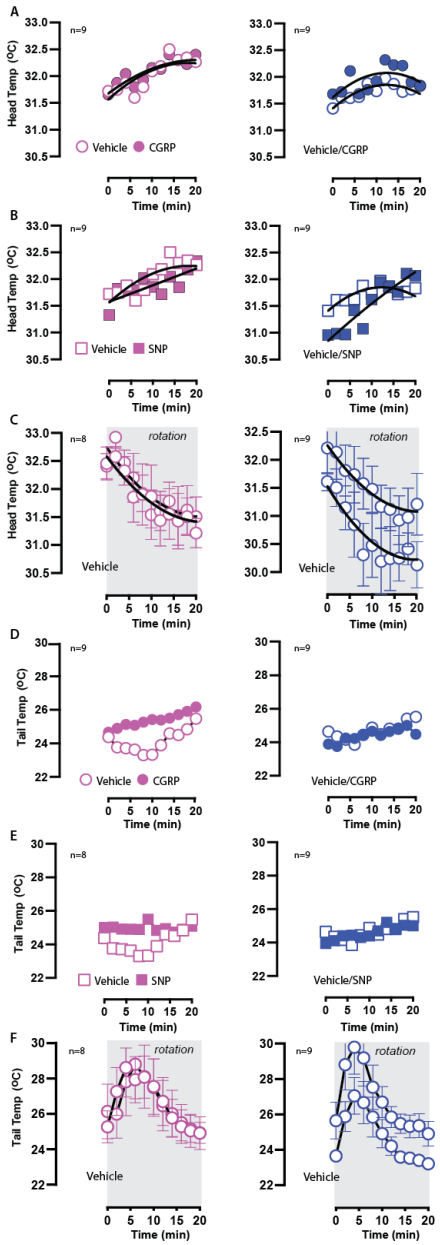

A
Head Temp (ºC)
n=9
Vehicle
CGRP
Vehicle/CGRP
Time (min)
B
Vehicle
SNP
Vehicle/SNP
C
n=8
rotation
Vehicle
D
Tail Temp (ºC)
E
F

Fig. 3

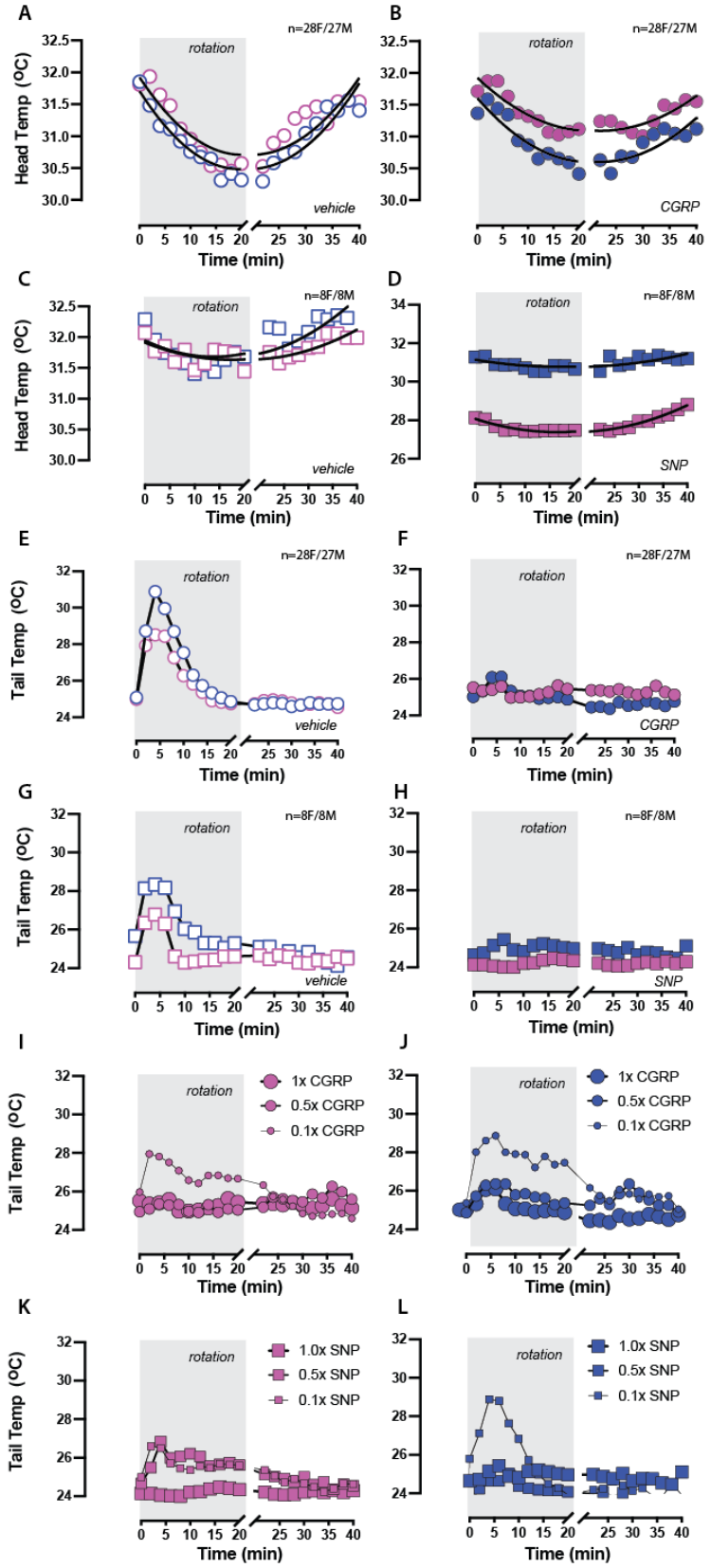

A
B
C
D
E
F
G
H
I
J
K
L
Head Temp (ºC)
Tail Temp (ºC)
Time (min)
rotation
vehicle
CGRP
SNP
n=28F/27M
n=8F/8M
1x CGRP
0.5x CGRP
0.1x CGRP
1.0x SNP
0.5x SNP
0.1x SNP

Fig. 4

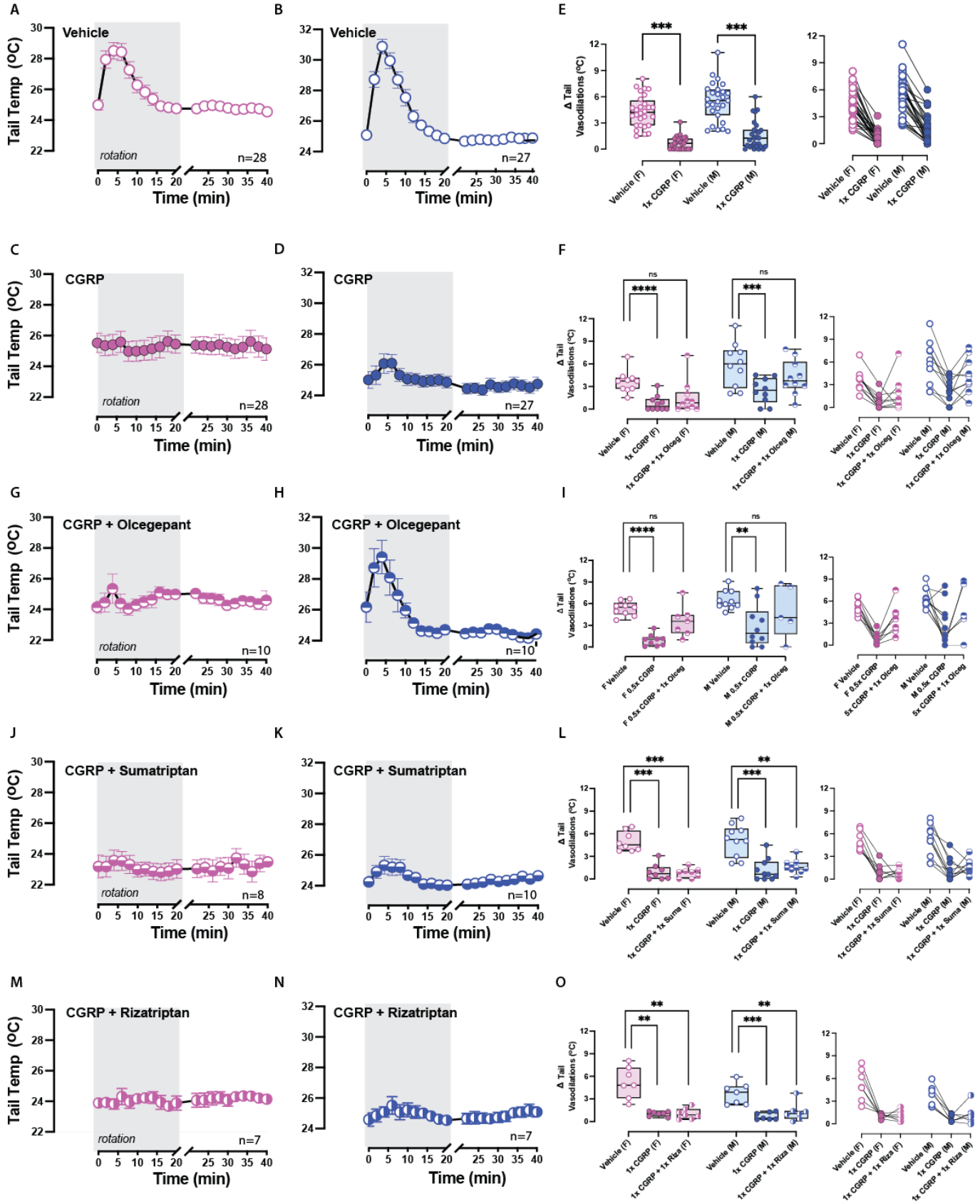

A
Vehicle
Tail Temp (ºC)
rotation
n=28
Time (min)
B
Vehicle
n=27
Time (min)
E
Δ Tail Vasodilations (ºC)
***
***
Vehicle (F)
1x CGRP (F)
Vehicle (M)
1x CGRP (M)
C
CGRP
rotation
n=28
Time (min)
D
CGRP
n=27
Time (min)
F
ns
****
ns
***
1x CGRP + 1x Olceg (F)
1x CGRP + 1x Olceg (M)
G
CGRP + Olcegepant
rotation
n=10
Time (min)
H
CGRP + Olcegepant
n=10
Time (min)
I
ns
****
ns
**
F Vehicle
F 0.5x CGRP
F 0.5x CGRP + 1x Olceg
M Vehicle
M 0.5x CGRP
M 0.5x CGRP + 1x Olceg
5x CGRP + 1x Olceg
J
CGRP + Sumatriptan
rotation
n=8
Time (min)
K
CGRP + Sumatriptan
n=10
Time (min)
L
***
***
**
***
1x CGRP + 1x Suma (F)
1x CGRP + 1x Suma (M)
M
CGRP + Rizatriptan
rotation
n=7
Time (min)
N
CGRP + Rizatriptan
n=7
Time (min)
O
**
**
**
***
1x CGRP + 1x Riza (F)
1x CGRP + 1x Riza (M)

Fig. 5

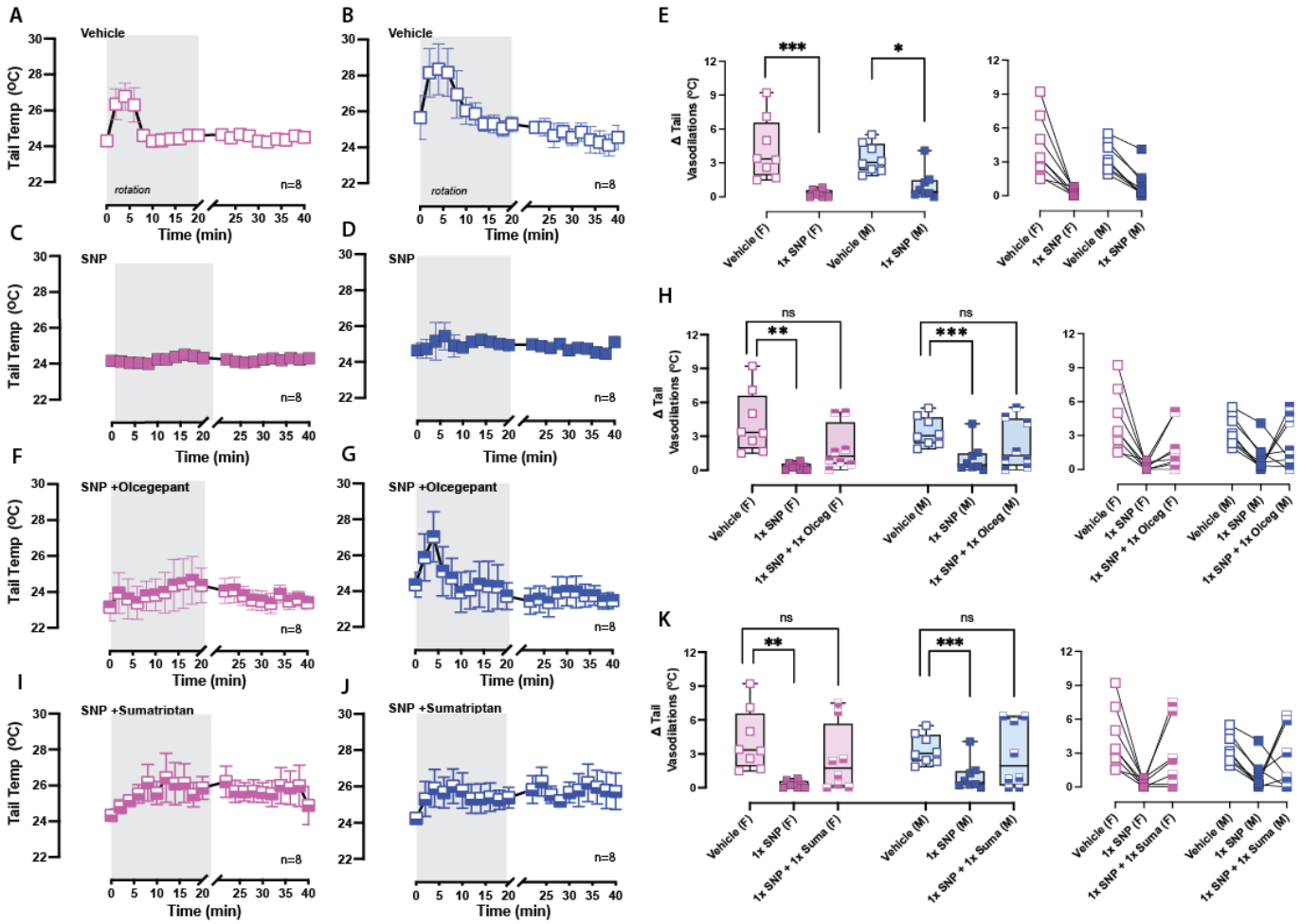
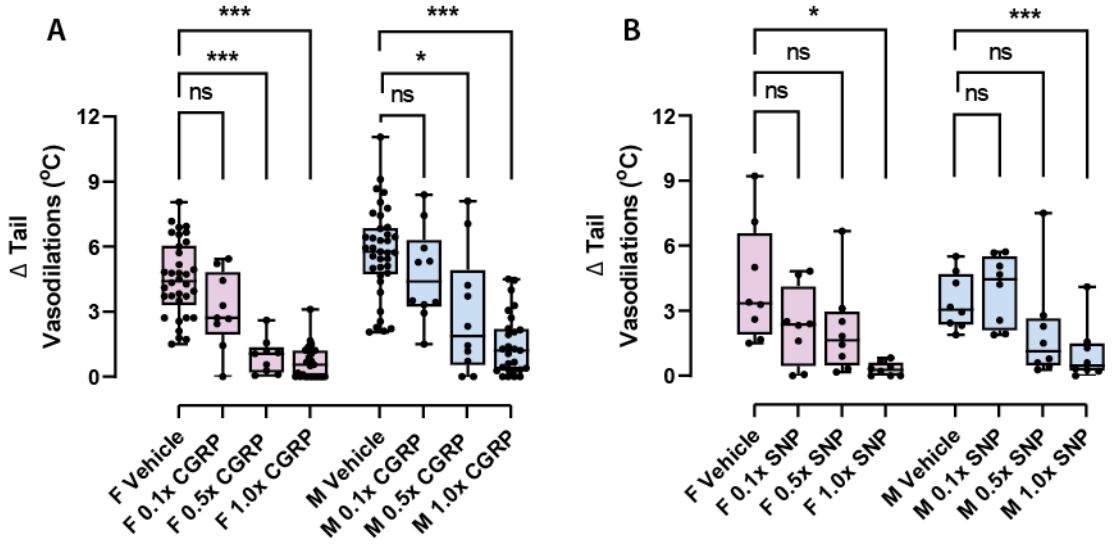
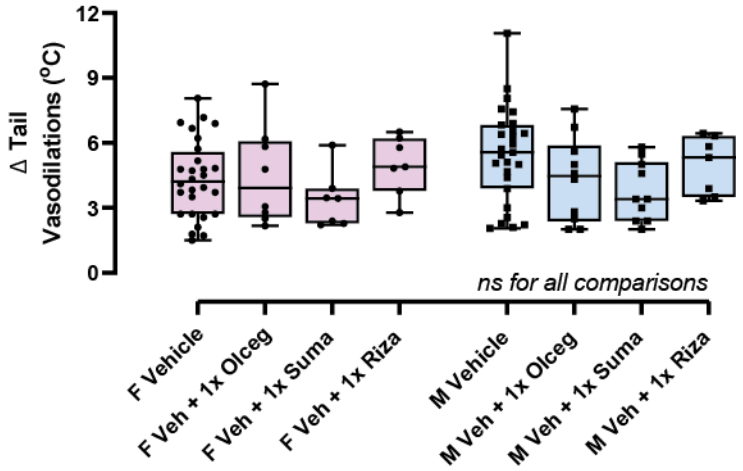

A
Vehicle
Tail Temp (ºC)
rotation
n=8
Time (min)
B
Vehicle
rotation
n=8
Time (min)
C
SNP
n=8
Time (min)
D
SNP
n=8
Time (min)
F
SNP +Olcegepant
n=8
Time (min)
G
SNP +Olcegepant
n=8
Time (min)
I
SNP +Sumatriptan
n=8
Time (min)
J
SNP +Sumatriptan
n=8
Time (min)
E
Δ Tail Vasodilations (ºC)
***
*
Vehicle (F)
1x SNP (F)
Vehicle (M)
1x SNP (M)
H
ns
**
ns
***
1x SNP + 1x Olceg (F)
1x SNP + 1x Olceg (M)
K
ns
**
ns
***
1x SNP + 1x Suma (F)
1x SNP + 1x Suma (M)

Fig. 6



Supplementary Fig. S1



Supplementary Fig. S2

Table 1

Assay	Biological Sex	ANOVAs	Post-Hoc	
Motion Sickness Index (MSI)	Females	pre-inj vs post-VC: F (1.00, 9.00) = 308.2, p < 0.001	vehicle	<0.001
			CGRP	<0.001
		treatment: F (2.15, 19.35) = 9.94, p < 0.001	CGRP + Olceg	<0.001
			CGRP + Suma	<0.001
		post-inj vs post-VC: F (1.00, 9.00) = 296.0, p < 0.001	vehicle	<0.001
			CGRP	<0.001
		treatment: F (2.10, 19.00) = 10.0, p < 0.001	CGRP + Olceg	<0.001
			CGRP + Suma	<0.001
		pre-inj vs post-inj: F (1.00, 9.00) = 91.0, p < 0.001	vehicle	0.200
			CGRP	<0.001
		treatment: F (2.00, 18.00) = 9.0, p = 0.002	CGRP + Olceg	0.001
			CGRP + Suma	0.001
	Males	pre-inj vs post-VC: F (1.00, 30.00) = 634.8, p < 0.001	vehicle	<0.001
			CGRP	<0.001
		treatment: F (3.00, 30.00) = 19.1, p < 0.001	CGRP + Olceg	<0.001
			CGRP + Suma	<0.001
		post-inj vs post-VC: F (1.00, 8.00) = 458.0, p < 0.001	vehicle	<0.001
			CGRP	<0.001
		treatment: F (2.00, 16.00) = 20.0, p < 0.001	CGRP + Olceg	<0.001
			CGRP + Suma	<0.001
		pre-inj vs post-inj: F (1.00, 60.00) = 157.0, p < 0.001	vehicle	0.280
			CGRP	0.002
		treatment: F (2.20, 45.00) = 17.0, p < 0.001	CGRP + Olceg	0.001
			CGRP + Suma	<0.001
Δ Tail Vasodilations	Females (CGRP)	Olcegepant: F (1.21, 10.90) = 10.0, p = 0.007	veh vs CGRP	<0.0001
			veh vs CGRP + Olceg	0.057
		Sumatriptan: F (1.84, 12.90) = 40.4, p < 0.0001	veh vs CGRP	<0.001
			veh vs CGRP + Suma	<0.001
		Rizatriptan: F (1.23, 7.36) = 24.4, p = 0.001	veh vs CGRP	0.005
			veh vs CGRP + Riza	0.003
	Females (SNP)	Olcegepant: F (1.31, 9.19) = 7.7, p = 0.016	veh vs SNP	0.009
			veh vs SNP + Olceg	0.208
		Sumatriptan: F (1.87, 13.10) = 7.0, p = 0.009	veh vs SNP	0.009
			veh vs SNP + Suma	0.332
	Male (CGRP)	Olcegepant: F (1.18, 10.60) = 7.6, p = 0.016	veh vs CGRP	0.001
			veh vs CGRP + Olceg	0.315
		Sumatriptan: F (1.67, 15.00) = 20.8, p < 0.0001	veh vs CGRP	0.000
			veh vs CGRP + Suma	0.004
		Rizatriptan: F (2.00, 11.90) = 25.4, p < 0.0001	veh vs CGRP	0.001
			veh vs CGRP + Riza	0.003
	Male (SNP)	Olcegepant: F (1.10, 7.67) = 3.6, p = 0.094	veh vs SNP	<0.001
			veh vs SNP + Olceg	0.472
		Sumatriptan: F (1.06, 7.41) = 2.7, p = 0.143	veh vs SNP	<0.001
			veh vs SNP + Suma	0.903

Table 2

Reduced Δ tail vasodilations (< 1.5 °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	89%	59.0%
0.5x CGRP + 1.0x olcegepant	14.2%	20.0%
1.0x CGRP + 1.0x olcegepant	70.0%	10.0%
1.0x CGRP + 1.0x sumatriptan	77.8%	50.0%
1.0x CGRP + 1.0x rizatriptan	71.4%	85.7%
0.1x SNP	20.0%	0.0%
0.5x SNP	50.0%	50.0%
1.0x SNP	100%	75.0%
1.0x SNP + 1.0x olcegepant	50.0%	50.0%
1.0x SNP + 1.0x sumatriptan	50.0%	50.0%