Title: Human RAMP1 overexpressing mice are resistant to migraine therapies for motion sensitivity: a mouse model of vestibular migraine

Abbreviated Title: Nausea and sway in RAMP1 overexpression

Author names and Affiliations: Shafaqat M. Rahman[1], Linda Guo[1], Carissa Minarovich[1], Laura Moon[1], Anna Guo[1,] Anne E. Luebke[1,2*]

[1]University of Rochester, Department of Biomedical Engineering, Rochester, NY 14627

[2]University of Rochester Medical Center, Department of Neuroscience, Del Monte Institute of Neuroscience, Rochester, NY 14642

*Corresponding author: Anne E. Luebke, aluebke@ur.rochester.edu

Corresponding author contact details: Anne E. Luebke, aluebke@ur.rochester.edu

Abstract:

Both enhanced motion-induced nausea and increased static imbalance are \observed symptoms in migraine and especially vestibular migraine (VM). Motion-induced nausea and static imbalance were investigated in a mouse model, nestin/hRAMP1, expressing elevated levels of human RAMP1 in the CNS, which enhances CGRP signaling in the nervous system. Behavioral surrogates such as the motion-induced thermoregulation and postural sway center of pressure (CoP) assays were used to assess motion sensitivity.

Tail vasodilation analysis revealed that this model exhibits an increased sensitivity to CGRP's effects at lower doses compared to control mice. In addition, the nestin/hRAMP1 mice exhibit a higher dynamic range in postural sway than their wildtype counterparts, along with increased sway observed in nestin/hRAMP1 male mice that was not present in male littermate controls. Results from migraine blocker experiments were challenging to interpret, but the data suggests that olcegepant is incapable of reversing CGRP-induced alterations in

the nestin/hRAMP1 mice, while rizatriptan was ineffective in both the nestin/hRAMP1 and control mice. The results indicate that overexpression of hRAMP1 leads to heightened endogenous CGRP signaling. Results also suggest that both olcegepant and rizatriptan are ineffective in reducing CGRP-triggered nausea and sway in this hypersensitive CGRP mouse model. This study suggests that hypersensitivity to CGRP may be a mouse model for difficult to treat cases of vestibular migraine.

Introduction

Migraine is a chronic neurological disorder that is marked by pain in the head, nausea, and heightened sensitivity to environmental stimuli such as light and sound. Certain people may also experience an aura before the onset of migraine symptoms [1]. Approximately 40% of patients with a history of migraine may experience vestibular migraine (VM), a unique type of migraine that makes patients vulnerable to recurrent vertigo and imbalances [2-6]. VM affects about 1-4% of adults in the US, with three times as many females affected as males. Furthermore, individuals with VM exhibit greater postural sway when encountering challenging surroundings and are more prone to motion sickness [7-12].

Calcitonin gene-related peptide (CGRP) is commonly considered a crucial factor that causes migraines. It is a vasoactive peptide present in the nervous system that contributes to vasodilation, inflammation, and pain modulation. CGRP signals through the CGRP receptor consisting of three subunits: calcitonin-like receptor (CLR), receptor activity-modifying protein 1 (RAMP1), and receptor component protein (RCP). RAMP1's significance in CGRP signaling has been widely studied as the rate-limiting subunit. It is essential for CLR translocation to the surface and for ligand binding [13-15].

A transgenic mouse model created by Russo et al. manifests sensitivity to CGRP by upregulating the CGRP receptor [14]. The nestin/hRAMP1 mouse model shows increased hRAMP1 expression in the nervous system and heightened sensitivity to the neuropeptide CGRP, as compared to wild-type mice. The mice were subjects of preclinical studies that analyzed surrogate behaviors for photophobia and allodynia. Their aversive reaction to light is amplified by intracerebroventricular (ICV) CGRP injections [14, 16, 17]. Moreover, hind paw withdrawals are detected at markedly lower doses of centrally-administered CGRP compared to their respective controls [18].

The CGRP receptor antagonist olcegepant (BIBN4096) effectively alleviates CGRP-induced behaviors in both human and mouse receptors, as demonstrated by mouse models. Moreover, preclinical models show that light aversion caused by CGRP is reduced through selective serotonin receptor agonists like rizatriptan and sumatriptan. Early studies targeting CGRP with olcegepant have led to the development of improved "gepants" and monoclonal antibodies that can block either the CGRP receptor or the peptide. Numerous treatment options for migraines based on antagonizing CGRP signaling have been investigated in clinical trials, many of which have been approved by the FDA since 2018, and a few studies have indicated that CGRP antagonists have been successful in treating vestibular migraines, particularly in a subset of patients [19, 20]. Alternative treatments, such as vestibular suppressants, have not been shown to alleviate vertigo and may cause drowsiness [21-23]. However, further research is needed to investigate the potential role of CGRP sensitization in causing vestibular symptoms and to elucidate the associated mechanisms. .

CGRP is widely distributed throughout the vestibular central nervous system (CNS) and is present on vestibular efferent fibers that innervate the inner ear [24-26]. In this study, we aim to characterize the effects of CGRP in nestin/hRAMP1 mice by employing motion-induced thermoregulation and postural sway assays. Furthermore, we seek to determine whether olcegepant or rizatriptan can provide protection against any CGRP-induced changes and will compare to C57BL/6J and unaffected littermates.

Materials & Methods

Animals

A total of 175 mice (95F/80M) were tested across all experiments, and all studies were sufficiently powered to detect sex differences. Mice were housed under a 12 to 12 day/night cycle at the University of Rochester's Vivarium under the care of the University of Rochester's Veterinary Services personnel. All animal procedures were approved by the University of Rochester's IACUC committee and performed in accordance with NIH standards.

The nestin/hRAMP1 mice were bred by crossing heterozygous or homozygous hRAMP1 loxP mice with heterozygous nestin-Cre mice to produce offspring that express elevated hRAMP1 in the nervous system. A detailed synopsis of their breeding design can be found in [15]. Control mice used in this study were hRAMP1 loxP mice not expressing nestin-Cre. Mice were housed under a 12-hour day/night cycle under the care of the University Committee on Animal Resources (UCAR) at the University of Rochester. Mice were housed with ad libitum access to necessities (food, water, bedding, and enrichment) with all studies sufficiently powered to detect sex differences. Mice were equilibrated in a testing room with an ambient temperature between 22-23°C for at least 30 minutes prior to testing. Mice were tested between 3-6 months of age. Different cohorts of mice were used to test motion-induced nausea and postural sway. Testing occurred from 9:00 am to 5:30 pm.

Drug administration

All intraperitoneal (IP) injections were performed with a fine 33-gauge insulin syringe. Dulbecco PBS (saline) served as vehicle control diluent. The CGRP-receptor antagonist olcegepant and the selective serotonin receptor agonist rizatriptan were used as

100 migraine blockers in this study. The concentrations are listed: 0.1x, 0.5x, and 1.0x CGRP
101 were prepared at 0.01, 0.05, and 0.1 mg/kg (rat α-CGRP, Sigma), 1.0x olcegepant was
102 prepared at 1.0 mg/kg (BIBN4096, Tocris), and 1.0x rizatriptan was prepared at 0.6 mg/kg
103 (Sigma-Aldrich). Mice were tested approximately twenty to thirty minutes after injections.
104 Animals were gently handled without the need for anesthesia. All animal procedures were
105 approved by the University of Rochester's (IACUC) and performed in accordance with the
106 standards set by the NIH.

107

108 **Motion-induced thermoregulation and center of pressure (CoP) testing for assessing**
109 **motion-induced nausea and postural sway in mouse models**

110 **Fig. 1** provides details regarding experimental methodologies pursued in this study.
111 Postural sway was evaluated using the center of pressure (CoP) assay. Around 10-12 CoP
112 areas were recorded per mouse, and a 10% robust outlier removal (ROUT) was applied to
113 eliminate outlier CoP areas from individual mouse trials. After this exclusion, each mouse
114 had a minimum of six CoP areas available for averaging in each testing condition. No
115 individual mice were excluded from the study.

116 We used a FLIR E60 IR camera (model: E64501) to head and tail temperatures of
117 mice before, during, and after a vestibular perturbation. The measurements were conducted
118 over a duration of 45 minutes. To summarize, we recorded baseline measurements for five
119 minutes prior to initiating the perturbation (-5 mins ≤ t ≤ 0 mins). Subsequently, mice were
120 recorded for 20 minutes (0 mins ≤ t ≤ 20 mins) during an orbital rotation at 75 rpm (2 cm
121 orbital displacement). After the perturbation, mice were additionally recorded for 20 minutes
122 to observe the recovery from hypothermia back to baseline (20 min ≤ t ≤ 40 min). FLIR
123 Tools+ was used for analyzing data.

We defined Δ tail vasodilations (^{o}C) as the transient increases in mouse tail temperature in response to perturbation. These were computed by subtracting the baseline tail temperature at time t = 0 minutes from the maximum tail temperature measured during the first 10 minutes of the rotation ($0 \leq t \leq 10$). Additionally, we calculated the hypothermia magnitude (Δ heads) by subtracting the baseline head temperature at time t = 0 minutes from the minimum head temperature recorded throughout the entire experiment (0 mins ≤ t ≤ 40 mins).

Data analysis and statistics

All statistical analyses were conducted in GraphPad Prism 9.5. Analyses were conducted separately in females and males unless otherwise noted. Multivariate ANOVAs and mixed-effect models were used to analyze Δ tail vasodilations and center of pressure (CoP) ellipse areas, and are further elaborated in the results. Group averages of Δ tail vasodilations and CoP ellipse areas are reported as mean ± SEM, and significance was set at $p < 0.05$ for all analyses.

Analysis of Δ tail vasodilations involved defining a threshold of 1.5^{o}C to examine the data as a binary outcome test. 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, and were labeled as diminished tail vasodilations. Mice were excluded from further testing if their Δ tail vasodilation after vehicle (saline) testing did not meet the criteria.

Second-order curve fitting was used for fitting head temperatures ($B2*X^2 + B1*X + B0$) and R^2 fit were calculated per curve. Head recovery (mins) was approximated by normalizing head temperature at x = 0 mins to y = 0 ^{o}C. An x-intercept quadratic model

147 approximated the recovery by detecting x-intercepts passed 20 minutes (when we stopped
148 the perturbation).

149 **Results**

150 **No difference in Δ Heads/Magnitude of Hypothermia or Hypothermia Recovery**

151 Head temperatures are depicted after intraperitoneal (IP) delivery of vehicle (saline),
152 1.0x CGRP, 1.0x CGRP + 1.0x Olcegepant, and 1.0x CGRP + 1.0x rizatriptan in males and
153 females (**Fig. 2A-J**). The magnitude of hypothermia was computed during an animal's test –
154 designated as the Δ head – and a 2-way ANOVA was computed across the factors i)
155 treatment and ii) strain in males and in females. No differences in hypothermia magnitude
156 were observed in mice regardless of treatment **(Fig. A-J).** In addition, no differences in
157 recovery were observed in the nestin/hRAMP1 mice compared to their controls for either
158 sex (**Table 1**).

159

160 **Dose response on tail vasodilations**

161 Motion-induced thermoregulation was examined at three different IP doses of CGRP
162 – 0.01 mg/kg, 0.05 mg/kg, and 0.1 mg/kg. These doses correspond to 0.1x CGRP, 0.5x
163 CGRP, and 1.0x CGRP respectively. When mice are injected with vehicle (saline), mice
164 exhibit a transient delta tail vasodilation in response to the vestibular perturbation. However,
165 this response is diminished after 1.0x CGRP (**Fig. 3B, E**). Dunnett multiple comparison's
166 test was used to compare Δ tail vasodilations changes after CGRP dose to vehicle, and a
167 significant effect on was observed after intraperitoneal injections of 0.5x CGRP and 1.0x
168 CGRP (**Fig. 3C, F**). Of the male controls, 50% and 53% exhibited diminished tail
169 vasodilations after treatment of 0.5x and 1.0x CGRP (*adj. $p = 0.1$ and adj. $p < 0.001$*
170 *respectively*), whereas 100% and 83% of male nestin/hRAMP1 mice were affected by 0.5x

171 CGRP and 1.0x CGRP (*adj. p = 0.004* and *adj. p = 0.02 respectively)*, highlighting a
172 potential sensitivity to CGRP in the male nestin/hRAMP1. A majority of female controls
173 (78% and 88%) exhibited diminished Δ tail vasodilations with doses of 0.5x and 1.0x CGRP
174 (*adj. p < 0.0001 at each dose*). In comparison, male controls also exhibited diminished Δ tail
175 vasodilations at 0.5x and 1.0x CGRP, whereas 88% and 80% of female nestin/hRAMP1
176 tested at 0.5x CGRP and at 1.0x CGRP exhibited diminished Δ tail vasodilations (*adj. p =*
177 *0.0007 and adj. p = 0.001 respectively*). Based on the dose response experiments, it is
178 evident that titrating the dose of CGRP to 1.0x leads to disruption in motion-induced
179 thermoregulation in a strong majority of mice. It is also observed that nestin/hRAMP1 males
180 are more readily affected by incremental doses of CGRP than their complement controls
181 (**Table 2**).

182 **Olcegepant's efficacy on Δ tail vasodilations is similar between nestin/hRAMP1 and**
183 **controls.**

184 The CGRP-receptor antagonist olcegepant was assessed for its efficacy in motion-
185 induced nausea. Raw temperatures after intraperitoneally delivered (IP) 1.0x CGRP + 1.0x
186 olcegepant treatment are depicted (**Fig. 4A, D**). Post-hoc analysis was conducted using
187 Tukey's multiple comparisons test. In female controls, 1.0x CGRP caused reduced Δ tail
188 vasodilations, but co-administration of olcegepant helped to block this response in a portion
189 of females. This observation was also seen in nestin/hRAMP1 mice, where some females
190 showed restored Δ tail vasodilations with olcegepant (**Fig. 4B**). In males, olcegepant was
191 effective in blocking 1.0x CGRP-induced changes in male controls and male nestin/hRAMP1
192 mice (**Fig. 4E**). A separate group of mice were tested at 0.5x CGRP, and mice with
193 diminished Δ tail vasodilations were further tested with 0.5x CGRP + 1.0x olcegepant. We
194 observed a majority of CGRP-affected female controls and nestin/hRAMP1 to regained their
195 delta tail vasodilations with olcegepant blocking this sub-maximal dose. However, unlike

male controls where a majority regained their tail vasodilation with olcegepant, male nestin/hRAMP1 still presented with diminished Δ tail vasodilations compared to their vehicle response (*0.5x CGRP vs 0.5x CGRP + 1.0x Olceg, p = 0.04*), suggesting an enhanced sensitivity to CGRP at this submaximal dose that is not being blocked with olcegepant (**Fig. 4F**).

Rizatriptan does not block CGRP-induced changes on Δ tail vasodilations

Rizatriptan was also examined across Δ tail vasodilations in a similar testing scheme and analysis as olcegepant. Observed in both sexes of nestin/hRAMP1 and controls, Δ tail vasodilations during CGRP+rizatriptan testing resembled CGRP-induced changes rather than vehicle. Unlike olcegepant, rizatriptan had no protective effect on Δ tail vasodilations (**Fig. 5A-D**).

A subset of nestin/hRAMP1 mice were also assessed after IP delivery of 0.5x CGRP + rizatriptan (**Fig. 5E**). Even at the half dose, rizatriptan did not recover Δ tail vasodilations in these mice.

Nestin/hRAMP1 mice exhibit greater postural sway at baseline than controls

Prior to testing CGRP and the effects of blockers, baseline center of pressure (CoP) responses were compared between nestin/hRAMP1 and controls. Two-way ANOVA with Dunnett's multiple comparisons test computed the following factors: i) male vs female and ii) controls versus nestin/hRAMP1 on sway obtained after vehicle injections (saline). While biological sex had no effect on sway ($F(1, 81) = 2.609$, $p = 0.11$), a significant difference was observed between controls and nestin/hRAMP1 ($F(1, 81) = 35.45$, $p < 0.001$). Female controls exhibited a CoP of 0.91 ± 0.09 cm^2, whereas female nestin/hRAMP1 mice had CoPs of 1.56 ± 0.12 (*adj. p < 0.001*). Similarly, male controls had CoP 0.69 ± 0.06 cm^2, but male nestin/hRAMP1 had larger CoP at 1.41 ± 0.17 (*adj. p < 0.001*). The higher sway in the

220 nestin/hRAMP1 suggest the potential implications of endogenous CGRP having a greater
221 impact on static imbalance due to upregulated hRAMP1 in the nervous system (**Fig. 6A**).

222 **Higher CGRP doses up to 0.1 mg/kg induce significant sway in female controls**

223 Changes in center of pressure (CoP) after intraperitoneally administered 0.1x CGRP,
224 0.5x CGRP, and 1.0x CGRP were examined using the same statistical tools used for the
225 motion-induced thermoregulation experiments. Two-way mixed-effected models were used
226 to assess CGRP dose and controls versus nestin/hRAMP1, and Dunnett's multiple
227 comparisons was used to compare differences in CoP after each CGRP dose to vehicle. In
228 male nestin/hRAMP1 and controls, no differences were detected due to CGRP (**Fig. 6C**).
229 Female controls exhibited significantly higher CoP after 1.0x CGRP compared to their
230 vehicle response (*adj. $p < 0.001$*) (**Fig. 6B**). In contrast, female nestin/hRAMP1 mice did not
231 significantly change their CoP in response to increasing doses of CGRP.

232

233 **Olcegepant and not rizatriptan blocks CGRP-induced changes in female control sway,**
234 **but nestin/hRAMP1 mice are not sensitive to CGRP or either migraine blocker.**

235 Olcegepant and rizatriptan were examined for their ability to protect against 1.0x
236 CGRP-induced changes in CoP. 1.0x CGRP induced significantly higher CoP in female
237 controls than compared to their vehicle response, but co-administration of 1.0x CGRP + 1.0x
238 Olcegepant led to lower CoP compared to 1.0x CGRP alone (*female controls: CGRP vs*
239 *CGRP+olcegepant, adj. $p = 0.0004$*) (**Fig. 7A**). In contrast, rizatriptan did not have an effect
240 on female control CoP, as 1.0x CGRP + 1.0x rizatriptan led to sway that resembled 1.0x
241 CGRP induced changes (**Fig. 7C**). Interestingly, the CoP results on female controls
242 dramatically differ from their nestin/hRAMP1 counterparts. Female nestin/hRAMP1 mice did

243 not further increase their sway after 1.0x CGRP, nor did co-delivery of 1.0x olcegepant or
244 1.0x rizatriptan modulate their CoP any further.

245 When examining male controls and nestinhRAMP1 (**Fig. 7B and 7D**), CGRP and
246 CGRP co-delivered with olcegepant/rizatriptan did not modulate their sway when compared
247 to their response to vehicle. It is clear that male nestin/hRAMP1 mice have higher CoP
248 values after vehicle and 1.0x CGRP than male controls, but olcegepant and rizatriptan do
249 not affect male CoP sway. We did not further test male controls with olcegepant or
250 rizatriptan, since no changes were observed after 1.0x CGRP, and male controls had a
251 much lower dynamic range in their CoP than did the nestin/hRAMP1.

252 **Table 2** is provided and gives specific details regarding the multivariate statistics (F-
253 statistics, p-values, and post-hoc analyses) used in this study for motion-induced nausea
254 and postural sway comparisons.

255

256 **Discussion**

257 Migraine treatment aims not only to address pain and ensure freedom from
258 headache, but also to alleviate the most bothersome symptoms (MBS), such as nausea,
259 vomiting, photophobia, or phonophobia. Photophobia is the most prevalent MBS and affects
260 50% of migraine patients, while nausea -the second most frequent MBS- affects 30% of
261 migraine patients [27]. Furthermore, individuals suffering from migraines also encounter
262 difficulty with postural control and balance, resulting in increased sway area and velocity [3, 4,
263 28, 29]. A study examining various lighting conditions during bipedal and unipedal stances of
264 migraine patients concluded that migraine-associated photophobia is linked to postural sway
265 [30]. Additionally, nausea is linked to calcitonin gene-related peptide (CGRP; and sensory
266 pathways linked to nausea involve the vestibular system and the area postrema, which is a

267 circumventricular organ associated with nausea and vomiting [31]. CGRP receptors have
268 been detected in the area postrema through the localization of RAMP1 and RAMP3 mRNA
269 in this region [32]. Previous research has demonstrated that the neuropeptide amylin, which
270 has a similar structure to CGRP, can stimulates certain subsets of area postrema neurons
271 directly [33]. As the area postrema is not enclosed by the blood-brain barrier, systemic
272 delivery of CGRP can affect area postrema neurons and potentially alter this region during
273 motion-induced nausea and sickness. Moreover, overexpression of RAMP1 may exacerbate
274 this condition.

275 The nestin/hRAMP1 mouse model has been extensively studied to manipulate
276 CGRP's effects, simulate migraine-like surrogate behaviors, and assess the efficacy of
277 migraine drugs. The model first elucidated that the CGRP receptor is a critical aspect of
278 neurogenic inflammation [15]. Later studies examined the mouse model to assess mechanical
279 allodynia and photophobia. . This study examined the impact of CGRP on motion-induced
280 nausea and postural sway using the motion-induced thermoregulation and center of
281 pressure assays. Further, we investigated whether the CGRP-receptor antagonist
282 olcegepant or the selective serotonin receptor agonist rizatriptan could mitigate CGRP-
283 induced changes in the nestin/hRAMP1 and its littermate controls.

284 The study revealed several fascinating findings. Initially, the investigation did not
285 identify significant differences in hypothermia magnitude or time to recover from
286 hypothermia in mice irrespective of treatment. As a result, it is reasonable to conclude that
287 CGRP and its blockers do not partially shape this element of the nausea response in mouse
288 models. Notably, male and female nestin/hRAMP1 mice demonstrated lower baseline head
289 temperatures when compared to wildtype littermate control. Many prior studies investigating
290 cAMP signaling in the regulation of central body temperature have demonstrated that an
291 increase in cAMP levels leads to hypothermia, and conversely, a decrease in cAMP levels

292 leads to hyperthermia. The administration of the adenylyl cyclase activator forskolin or
293 treatment with dibutyryl-cAMP has induced hypothermia in mice and rats [34-36]. The lower
294 baseline temperature in the nestin/hRAMP1 at steady state may be due to increased central
295 cAMP levels resulting from elevated expression of central/neural hRAMP1. The dose of IP
296 CGRP had a significant impact on the presentation of Δ tail vasodilations in both male and
297 female mice. At higher doses (0.5x and 1.0x CGRP), the majority of mice, including both
298 nestin/hRAMP1 and control mice, showed reduced Δ tail vasodilations. At the highest dose
299 of 1.0x CGRP, which is 0.1 mg/kg, most mice do not show a significant increase in Δ tail
300 vasodilation after being stimulated with provocative rotation. The strong effect observed at
301 the maximum dose of CGRP prompted us to investigate the impact of the CGRP-receptor
302 antagonist olcegepant and the serotonin receptor agonist (5HT1B/1D) rizatriptan.

303 Olcegepant effectively restored tail vasodilations in male mice and, to a lesser
304 extent, in female mice, in both nestin/hRAMP1 mice and controls. However, rizatriptan did
305 not offer protection and resulted in changes comparable to those resulting from CGRP-
306 induced alterations. Rizatriptan and other triptans have been extensively researched as
307 drugs that indirectly inhibit the release of CGRP in migraine treatment. Further investigation
308 into the effects of half the maximum dose was conducted through the co-administration of
309 olcegepant or rizatriptan. It was discovered that at half the maximum dose of CGRP,
310 olcegepant improved the response of both nestin/hRAMP1 and control mice; however, it
311 was more effective in control male mice than nestin/hRAMP1 male mice. Rizatriptan did not
312 restore Δ tail vasodilations in nestin/hRAMP1 mice, even at 0.5 CGRP.

313 During the investigation of postural sway, we observed that nestin/hRAMP1 mice
314 showed higher baseline sway levels than control mice. Our previous research has
315 demonstrated that males do not encounter variations in their center of pressure while being
316 exposed to provocation rotation or CGRP. In contrast, female controls treated with 0.1

317 mg/kg CGRP encounter postural sway. In this study, we found that male mice have a higher
318 range of sway than their littermate controls. This change is associated with increased levels
319 of CGRP, which have an effect on sway due to elevated signaling mediated by hRAMP1
320 overexpression. This finding is internally consistent with other behavioral studies in
321 nestin/hRAMP1 mice, who also exhibit light sensitivity to normal room lighting that is not
322 observed in littermate controls.

323 In conclusion, this study demonstrates a heightened sensitivity to motion in the
324 nestin/hRAMP1 model through the induction of nausea and static imbalance changes,
325 compared to unaffected control mice. It is important to understand the mechanisms
326 associated with CGRP signaling, not only for pain but also for other migraine symptoms
327 such as nausea and motion sensitivity, and to develop new therapies aimed at alleviating
328 these debilitating symptoms in migraine patients.

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443

444

445

446

447 **Figure Legends**

448 **Fig.1:** (A) Calcitonin gene-related peptide (CGRP) receptor structure consists of receptor
449 activity modifying protein 1 (RAMP1), calcitonin-receptor like receptor (CLR), and receptor
450 component protein (RCP). The nestin/hRAMP1 mice are designed to have enhanced CGRP
451 signaling due to overexpression of human RAMP1 (hRAMP1). **(B-C)** Methods for motion-
452 induced thermoregulation and center of pressure (CoP) testing as surrogate behaviors for
453 motion-induced nausea and postural sway.

454

455 **Fig. 2:** Head temperatures during motion-induced nausea test after intraperitoneal (IP)
456 injections of vehicle, CGRP, CGRP + olcegepant, or CGRP + rizatriptan in female **(A – D)**
457 and male **(F-I)** nestin/hRAMP1 vs controls**.** **(E-J)** Hypothermia magnitudes (Δ heads) were
458 not different between nestin/hRAMP1 and controls after each treatment in either sex.
459 Sample sizes are listed for male (M) and female (F): vehicle = 17M/18F nestin/hRAMP1 and
460 27M/26F controls; CGRP - 9M/10F nestin/hRAMP1 and 17M/17F controls; CGRP + Olceg -
461 9M/10F nestin/hRAMP1 and 10M/10F controls; CGRP + Riza - 9M/10F nestin/hRAMP1 and
462 7M/7F controls. A legend is provided to indicate color code and symbol shape across
463 treatment, mouse strain, and sex.

464 **Fig. 3:** Tail temperatures during motion-induced nausea test and dose response analyses of
465 Δ tail vasodilations are depicted in females (left) and males (right). **(A, D)** Mice exhibit a
466 transient tail vasodilation approximately 5 to 10 minutes after the rotation starts. However,
467 this response is diminished after 1.0x CGRP as seen in **(B, E**). Dunnett multiple
468 comparison's test is used to compare Δ tail vasodilations at each CGRP dose to vehicle. **(C)**
469 In female nestin/hRAMP1 and controls, a significant effect on Δ tail vasodilations is observed

470 due to 0.5x CGRP and 1.0x CGRP. **(F)** Male controls were affected by 1.0x CGRP but not
471 0.5x CGRP, whereas male nestin/hRAMP1 mice were affected by 0.05x CGRP and 1.0x
472 CGRP, highlighting a potential sensitivity to CGRP in the male nestin/hRAMP1. Sample
473 sizes are listed for male (M) and female (F): vehicle = 17M/18F nestin/hRAMP1 and
474 27M/26F controls; 0.1x and 0.5x CGRP = 8M/8F nestin/hRAMP1 and 10M/9F controls, 1.0x
475 CGRP - 9M/10F nestin/hRAMP1 and 17M/17F controls. A legend is provided, and
476 significance levels are indicated as follows: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

477

478 **Fig. 4:** The CGRP-receptor antagonist olcegepant was assessed for its efficacy in motion-
479 induced nausea. Drugs were injected intraperitoneally at the following concentrations: 0.5x
480 CGRP – 0.05 mg/kg, 1.0x CGRP – 0.1 mg/kg, 1.0x olcegepant – 1 mg/kg. **(A, D)** Tail
481 temperatures after intraperitoneally delivered CGRP + olcegepant treatment are depicted.
482 Post-hoc analysis was conducted using Tukey's multiple comparisons test. **(B)** In female
483 controls, 1.0x CGRP caused reduced Δ tail vasodilations, but co-administration of
484 olcegepant helped some female controls regain a similar response to their vehicle test. This
485 observation was also seen in nestin/hRAMP1 mice, where some females showed restored Δ
486 tail vasodilations with olcegepant. (E) Olcegepant was more effective in blocking CGRP-
487 induced changes in male controls and male nestin/hRAMP1 mice. A separate group of mice
488 were tested at 0.5x CGRP, and mice with Δ tail vasodilations < 1.5 ^{o}C were further tested
489 with 0.5x CGRP + 1.0x olcegepant. **(C)** A majority of CGRP-affected female controls and
490 nestin/hRAMP1 regained their tail vasodilation response with olcegepant blocking this sub-
491 maximal dose. **(F)** While a majority of 0.5x CGRP-affected male controls regained their tail
492 vasodilation with olcegepant, many male nestin/hRAMP1 still presented with diminished Δ
493 tail vasodilations compared to their vehicle response. For detailed F-statistics and p-values,
494 please refer to Table 2. Sample sizes are listed: vehicle, 1.0x CGRP, and 1.0x CGRP + 1.0x

495 olcegepant - 9M/10F nestin/hRAMP1 and 10M/10F controls; 0.5x CGRP = 8M/8F
496 nestin/hRAMP1 and 10M/9F controls; 0.5x CGRP + 1.0x olcegepant = 8M/7F
497 nestin/hRAMP1 and 5M/7F controls. A legend is provided, and significance levels are
498 indicated as follows: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, $p < 0.0001$****

499 **Fig. 5:** The selective serotonin receptor agonist rizatriptan – a clinically prescribed migraine
500 drug – was assessed for its efficacy in motion-induced nausea. Drug concentrations are
501 listed: 0.5x CGRP – 0.05 mg/kg, 1.0x CGRP – 0.1 mg/kg, 1.0x rizatriptan – 0.6 mg/kg. **(A-B)**
502 Raw temperatures after CGRP + rizatriptan treatment are depicted. **(C - D)** In female and
503 male controls, CGRP caused diminished Δ tail vasodilations and CGRP+rizatriptan did not
504 block these effects. The nestin/hRAMP1 mice resembled control mice, as rizatriptan was not
505 effective in blocking CGRP-induced changes. **(E)** A small subset of nestin/hRAMP1 mice
506 assessed rizatriptan's efficacy at half the maximum CGRP dose, but no blocker effects were
507 observed. F-statistics and p-values can be found in Table 2. Sample sizes are as follows:
508 vehicle, 1.0x CGRP, and 1.0x CGRP + 1.0x rizatriptan – 9/10 Nestin/hRAMP1 and 7/7
509 Controls; vehicle, 0.5x CGRP, and 0.5x CGRP + 1.0x rizatriptan – 4F/4M nestin/hRAMP1.
510 Significance levels are listed: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***

511

512 **Fig. 6: (A)** 2-way ANOVA computed the factors i) male vs female and ii) controls versus
513 nestin/hRAMP1 on sway obtained after vehicle injections (saline). While biological sex had
514 no effect on sway ($F(1, 81) = 2.609$, $p = 0.11$), a significant difference was observed
515 between controls and nestin/hRAMP1 ($F(1, 81) = 35.45$, $p < 0.001$). Tukey's multiple
516 comparisons indicated nestin/hRAMP1 males and females have a higher sway baseline
517 than their complement controls. **(B)** CGRP induces higher CoP ellipse areas in female
518 controls at 1.0x CGRP but not in female nestin/hRAMP1. **(C)** While male nestin/hRAMP1
519 sway is significantly higher than male controls at all doses of CGRP, there is no modulation

520 in their sway area with increasing CGRP dose. Before/after plots are placed next to violin
521 plots and show animals that were specifically tested with 1.0x CGRP. Sample sizes are
522 listed: vehicle and 1.0x CGRP– 18M/22F nestin/hRAMP1 and 17M/28F controls; 0.1x and
523 0.5x CGRP -8M/8F nestin/hRAMP1 and 8M/9F controls. Dose response statistics for sway
524 can be found in top half of Table 2. $p < 0.05$*, $p < 0.01$**, $p < 0.001$***

525

526 **Fig. 7**: Changes in sway were observed after vehicle, CGRP, CGRP + olcegepant or CGRP
527 + rizatripta treatment in controls and nestin/hRAMP1, using same concentrations from
528 motion-induced thermoregulation studies. **(A)** In female controls, CGRP causes increases
529 in postural sway but co-administration of olcegepant blocks CGRP-induced changes. In
530 contrast, female nestin/hRAMP1 mice do not appear affected by CGRP, and olcegepant is
531 not an effective blocker. **(B)** Male nestin/hRAMP1 mice do not respond to CGRP or
532 CGRP+olcegepant. For male controls, a paired t-test was used to compare vehicle vs
533 CGRP effects but no differences were observed. The lack of a CGRP-induced change in
534 male control sway provided us the rationale to discontinue testing these mice with blockers.
535 Sample sizes include 9M/14F nestin/hRAMP1 and 10M/18F controls repeatedly tested. Full
536 list of F and p-values for these sway experiments are found in Table 2. Significance levels
537 are indicated as $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, $p < 0.0001$****.

538 **<u>Table Legends</u>**

539 **Table 1.** Time to recover from hypothermia (minutes) was analyzed between
540 nestin/hRAMP1 and controls using an x-intercept quadratic model. Males and females were
541 separated. A constraint to the model was applied so that the x-intercept analyzed occurred
542 after time = 20 minutes. While F-statistics and p-values are listed, comparisons are made
543 more confidently in curve fits with $R^2 \geq 0.60$. No significant differences were observed in
544 hypothermia recovery between nestin/hRAMP1 and controls. While 1.0x CGRP + 1.0x

545 olcegepant testing appeared significant between female nestin/hRAMP1 and controls, curve
546 fits were poor so an interpretation is not made. Sample sizes are listed for male (M) and
547 female (F): vehicle = 17M/18F nestin/hRAMP1 and 27M/26F controls; CGRP - 9M/10F
548 nestin/hRAMP1 and 17M/17F controls; CGRP + Olceg - 9M/10F nestin/hRAMP1 and
549 10M/10F controls; CGRP + Riza - 9M/10F nestin/hRAMP1 and 7M/7F controls/

550 **Table 2.** Percentage of mice with diminished delta tail vasodilations during motion-induced
551 thermoregulation test after each treatment (row). Columns are categorized by
552 nestin/hRAMP1 vs controls and male vs female.

553 **Table 3.** Multivariate statistics are listed per study with corresponding F and p values. Dose
554 responses were analyzed using 2-way repeated measures (RM) mixed effects models to
555 handle missing values since not all animals were repeatedly tested with every CGRP dose,
556 whereas 1.0x CGRP + 1.0x blocker studies used 2-way RM ANOVAs since animals were
557 repeatedly tested.

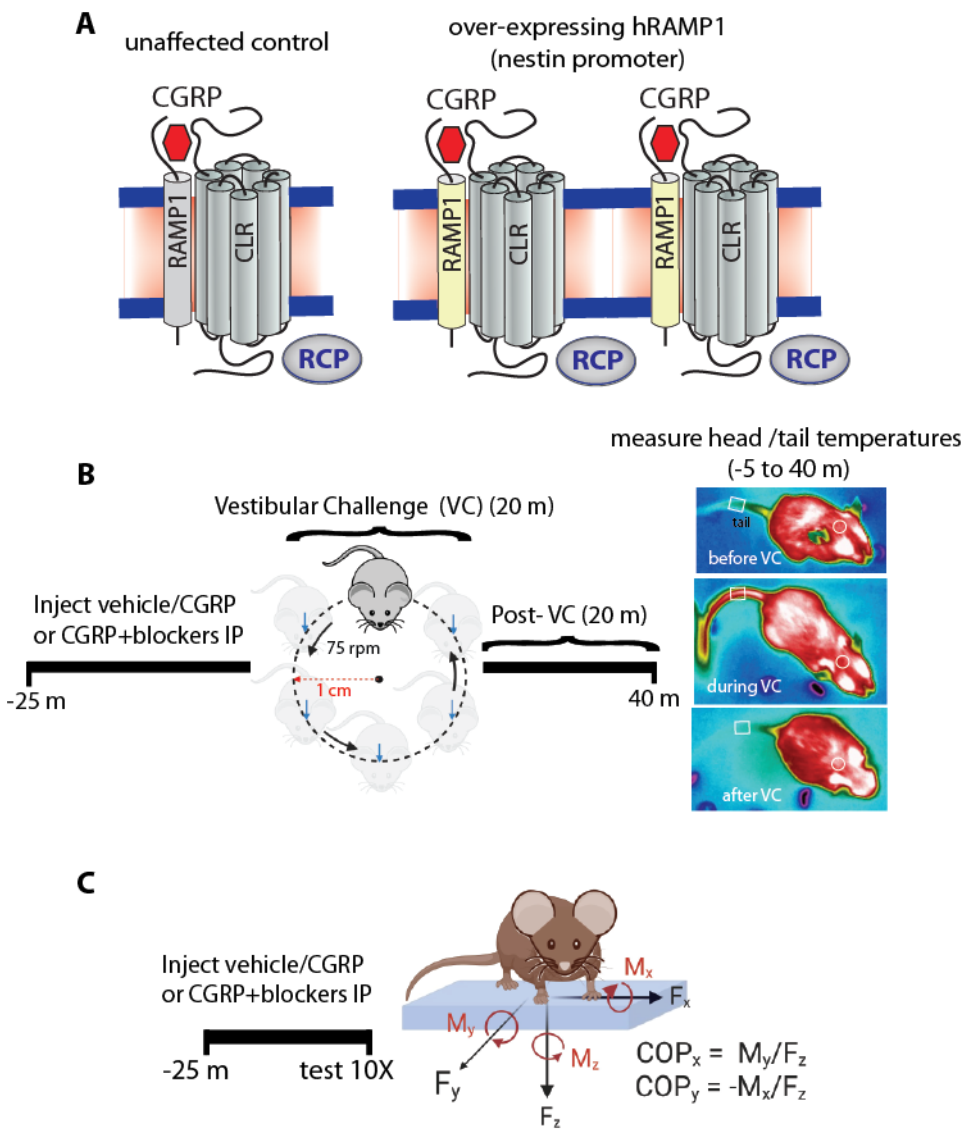

A
unaffected control
over-expressing hRAMP1
(nestin promoter)
CGRP
CGRP
CGRP
RAMP1
CLR
RAMP1
CLR
RAMP1
CLR
RCP
RCP
RCP
B
measure head /tail temperatures
(-5 to 40 m)
Vestibular Challenge (VC) (20 m)
Inject vehicle/CGRP
or CGRP+blockers IP
75 rpm
1 cm
Post- VC (20 m)
-25 m
40 m
tail
before VC
during VC
after VC
C
Inject vehicle/CGRP
or CGRP+blockers IP
-25 m
test 10X
Mx
Fx
My
Mz
Fy
Fz
COPx = My/Fz
COPy = -Mx/Fz

Fig. 1

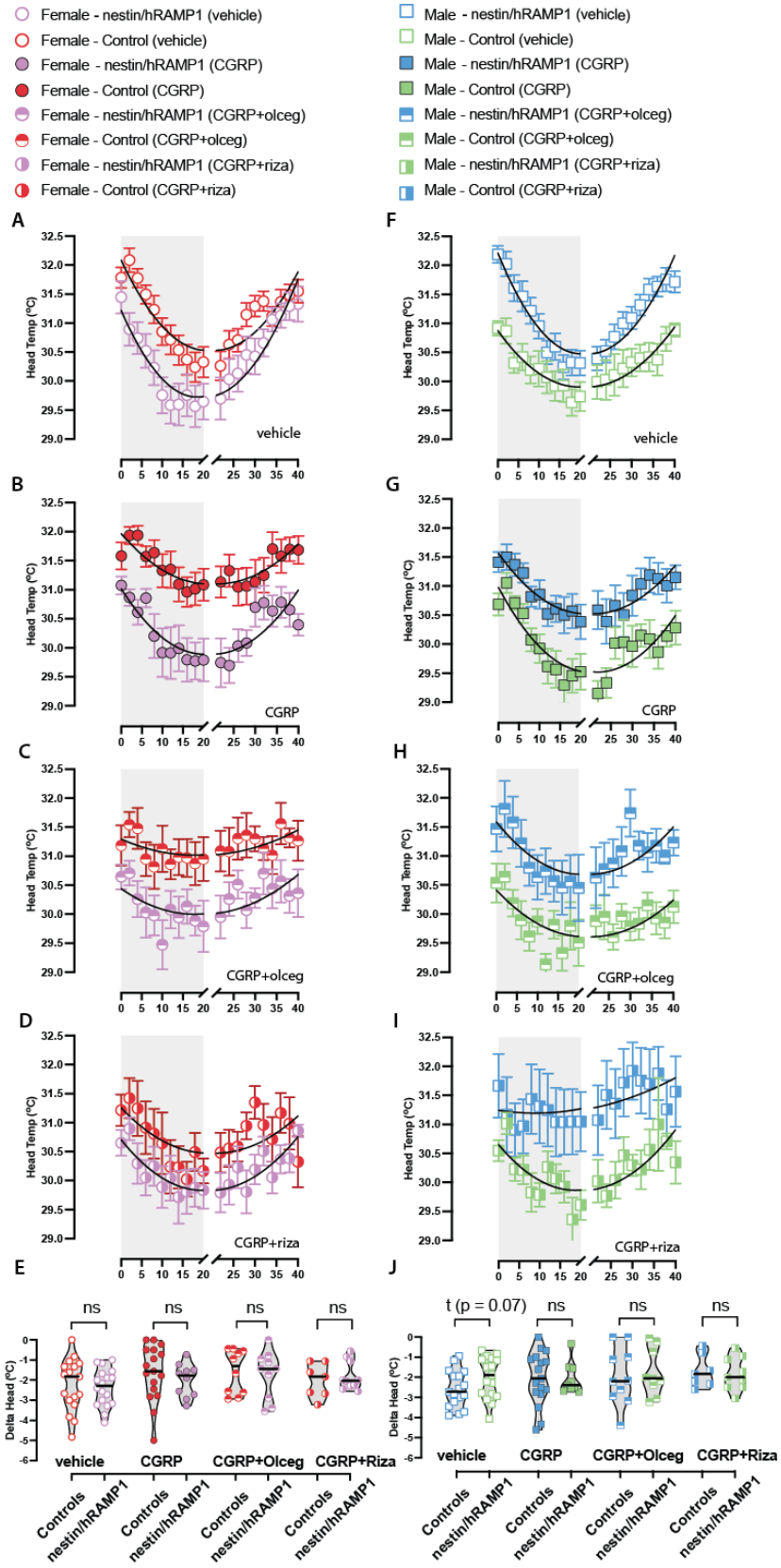

Female - nestin/hRAMP1 (vehicle)
Female - Control (vehicle)
Female - nestin/hRAMP1 (CGRP)
Female - Control (CGRP)
Female - nestin/hRAMP1 (CGRP+olceg)
Female - Control (CGRP+olceg)
Female - nestin/hRAMP1 (CGRP+riza)
Female - Control (CGRP+riza)
Male - nestin/hRAMP1 (vehicle)
Male - Control (vehicle)
Male - nestin/hRAMP1 (CGRP)
Male - Control (CGRP)
Male - nestin/hRAMP1 (CGRP+olceg)
Male - Control (CGRP+olceg)
Male - nestin/hRAMP1 (CGRP+riza)
Male - Control (CGRP+riza)
A
B
C
D
E
F
G
H
I
J
Head Temp (°C)
vehicle
CGRP
CGRP+olceg
CGRP+riza
Delta Head (°C)
ns
t (p = 0.07)
vehicle
CGRP
CGRP+Olceg
CGRP+Riza
Controls
nestin/hRAMP1

Fig. 2.

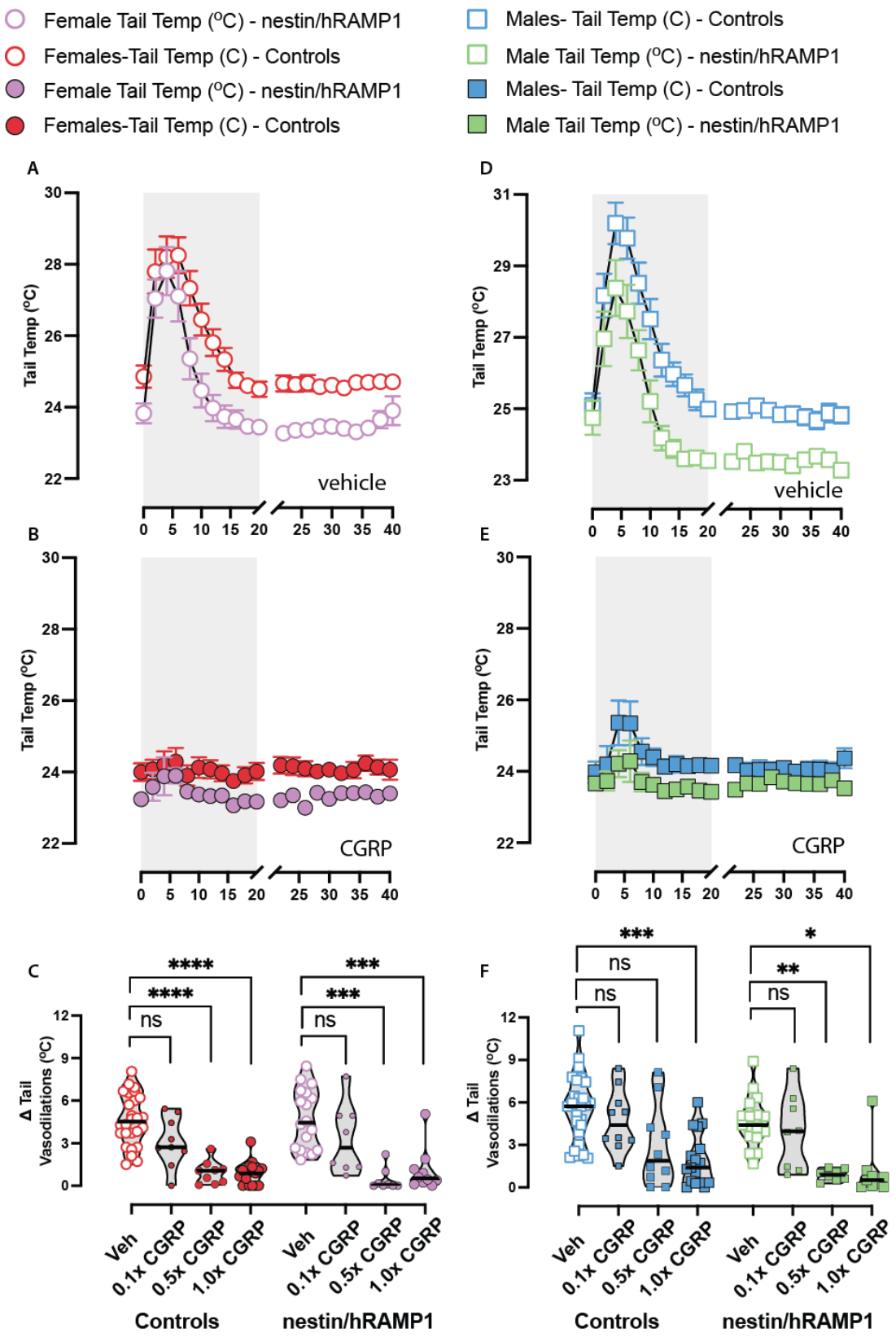

Female Tail Temp (ºC) - nestin/hRAMP1
Females-Tail Temp (C) - Controls
Female Tail Temp (ºC) - nestin/hRAMP1
Females-Tail Temp (C) - Controls
Males- Tail Temp (C) - Controls
Male Tail Temp (ºC) - nestin/hRAMP1
Males- Tail Temp (C) - Controls
Male Tail Temp (ºC) - nestin/hRAMP1
A
B
C
D
E
F
Tail Temp (ºC)
vehicle
CGRP
Δ Tail Vasodilations (ºC)
****
***
ns
*
**
Veh
0.1x CGRP
0.5x CGRP
1.0x CGRP
Controls
nestin/hRAMP1

Fig. 3.

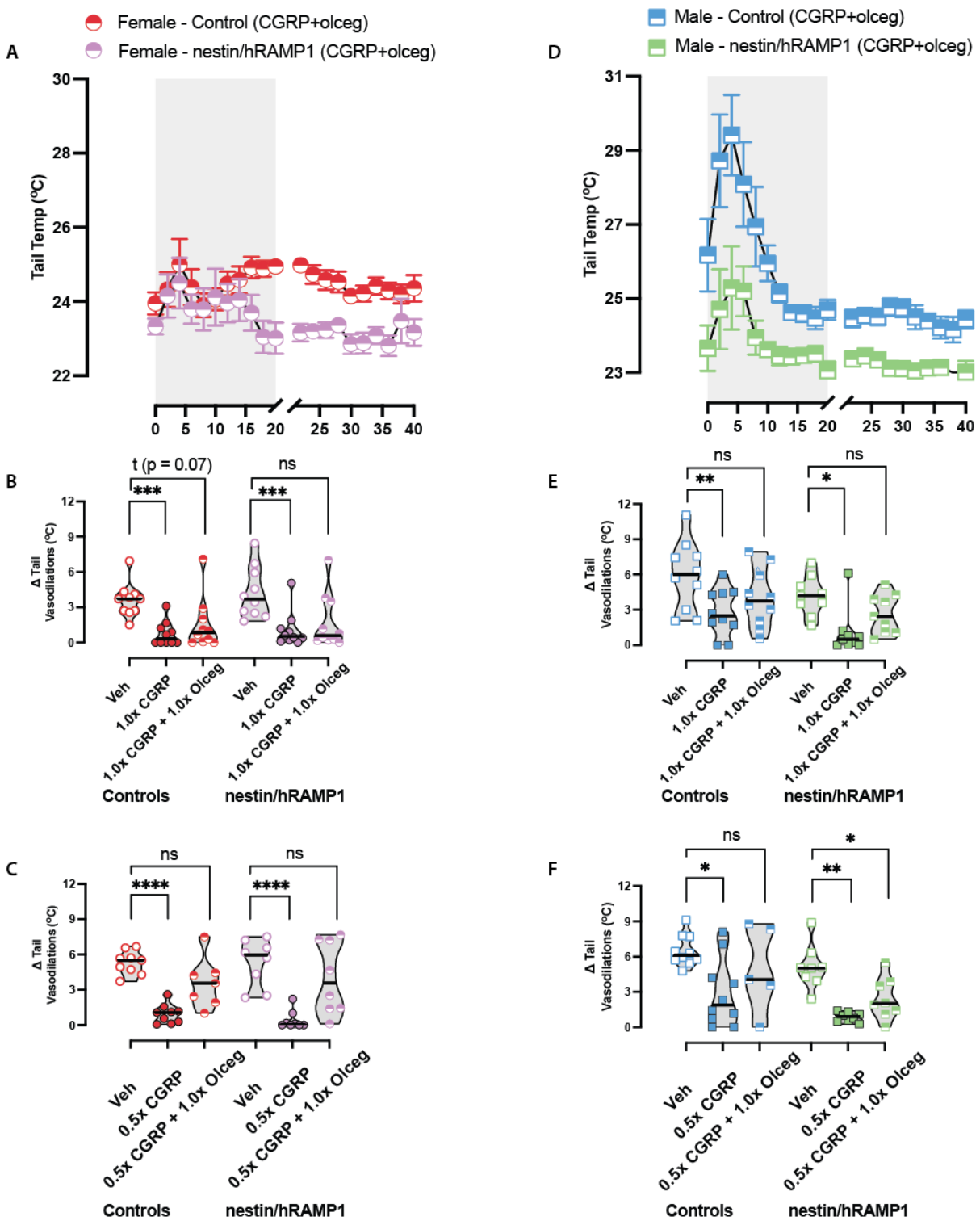

A
Female - Control (CGRP+olceg)
Female - nestin/hRAMP1 (CGRP+olceg)
Tail Temp (ºC)
30
28
26
24
22
0 5 10 15 20 25 30 35 40
D
Male - Control (CGRP+olceg)
Male - nestin/hRAMP1 (CGRP+olceg)
Tail Temp (ºC)
31
29
27
25
23
0 5 10 15 20 25 30 35 40
B
t (p = 0.07)
ns
***
***
Δ Tail Vasodilations (ºC)
Veh
1.0x CGRP
1.0x CGRP + 1.0x Olceg
Controls
nestin/hRAMP1
E
ns
ns
**
*
Δ Tail Vasodilations (ºC)
Veh
1.0x CGRP
1.0x CGRP + 1.0x Olceg
Controls
nestin/hRAMP1
C
ns
ns
****
****
Δ Tail Vasodilations (ºC)
Veh
0.5x CGRP
0.5x CGRP + 1.0x Olceg
Controls
nestin/hRAMP1
F
ns
*
*
**
Δ Tail Vasodilations (ºC)
Veh
0.5x CGRP
0.5x CGRP + 1.0x Olceg
Controls
nestin/hRAMP1

Fig. 4.

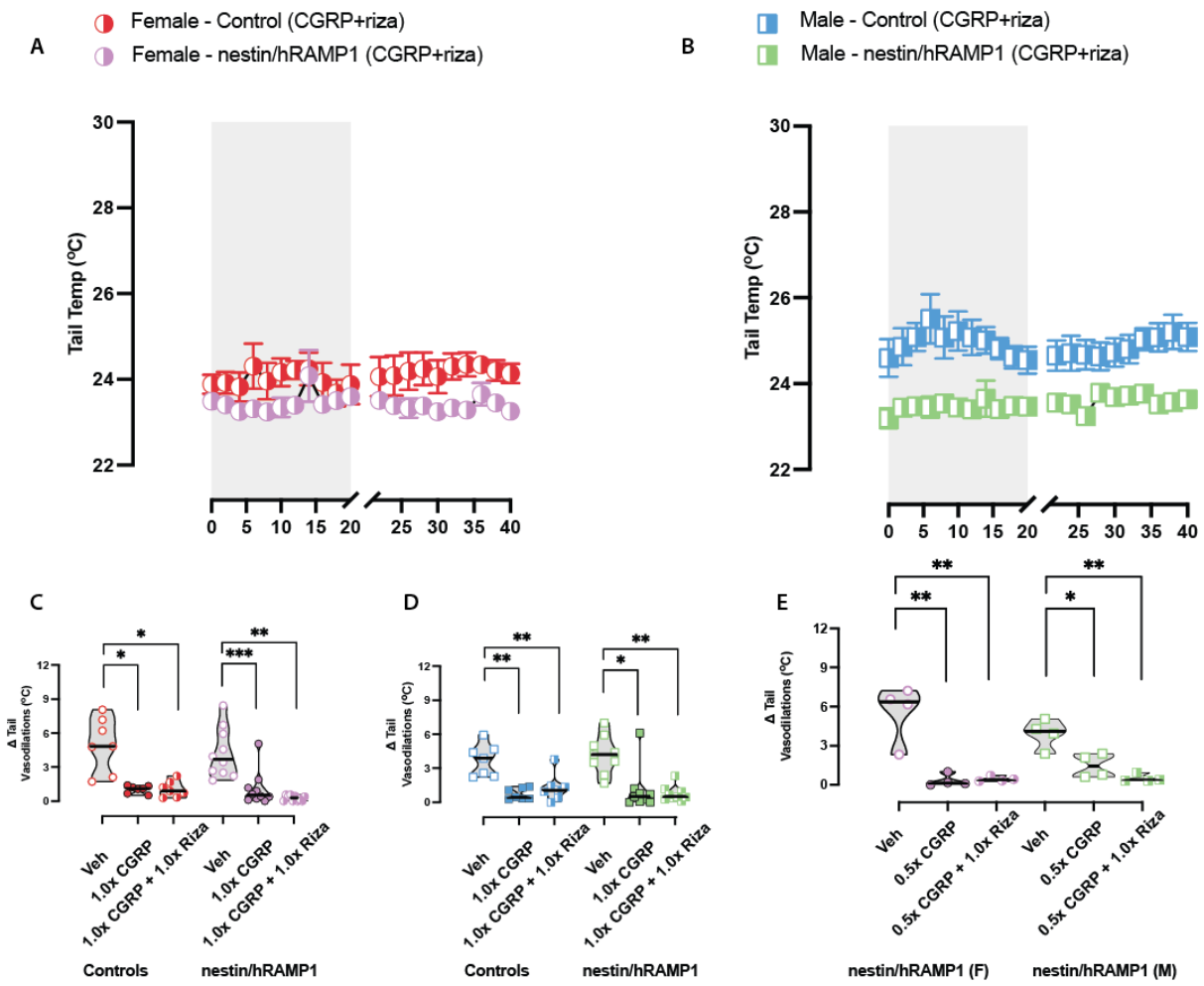

A
Female - Control (CGRP+riza)
Female - nestin/hRAMP1 (CGRP+riza)
Tail Temp (°C)
B
Male - Control (CGRP+riza)
Male - nestin/hRAMP1 (CGRP+riza)
Tail Temp (°C)
C
Δ Tail Vasodilations (°C)
Veh
1.0x CGRP
1.0x CGRP + 1.0x Riza
Controls
nestin/hRAMP1
D
Δ Tail Vasodilations (°C)
Veh
1.0x CGRP
1.0x CGRP + 1.0x Riza
Controls
nestin/hRAMP1
E
Δ Tail Vasodilations (°C)
Veh
0.5x CGRP
0.5x CGRP + 1.0x Riza
nestin/hRAMP1 (F)
nestin/hRAMP1 (M)

Fig. 5.

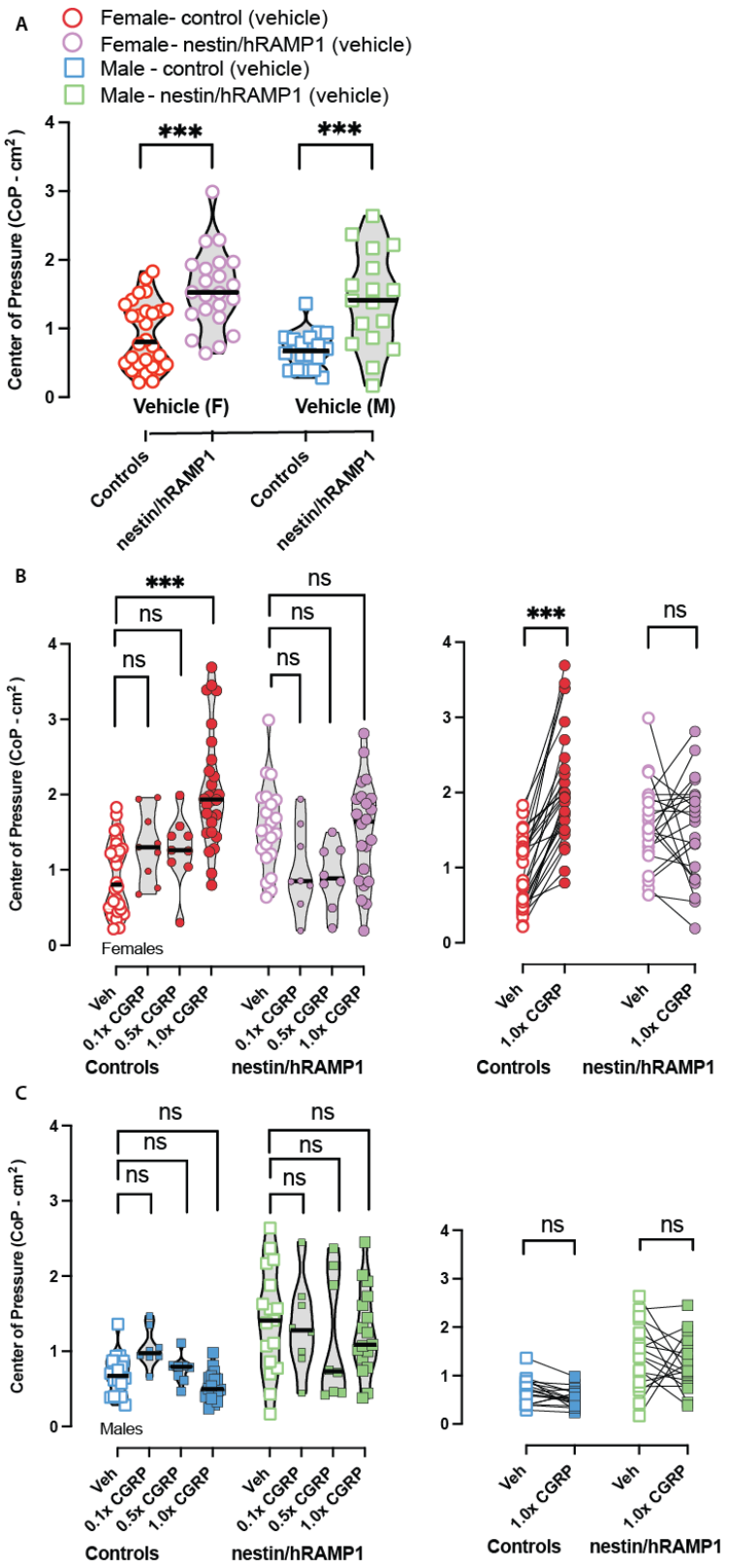
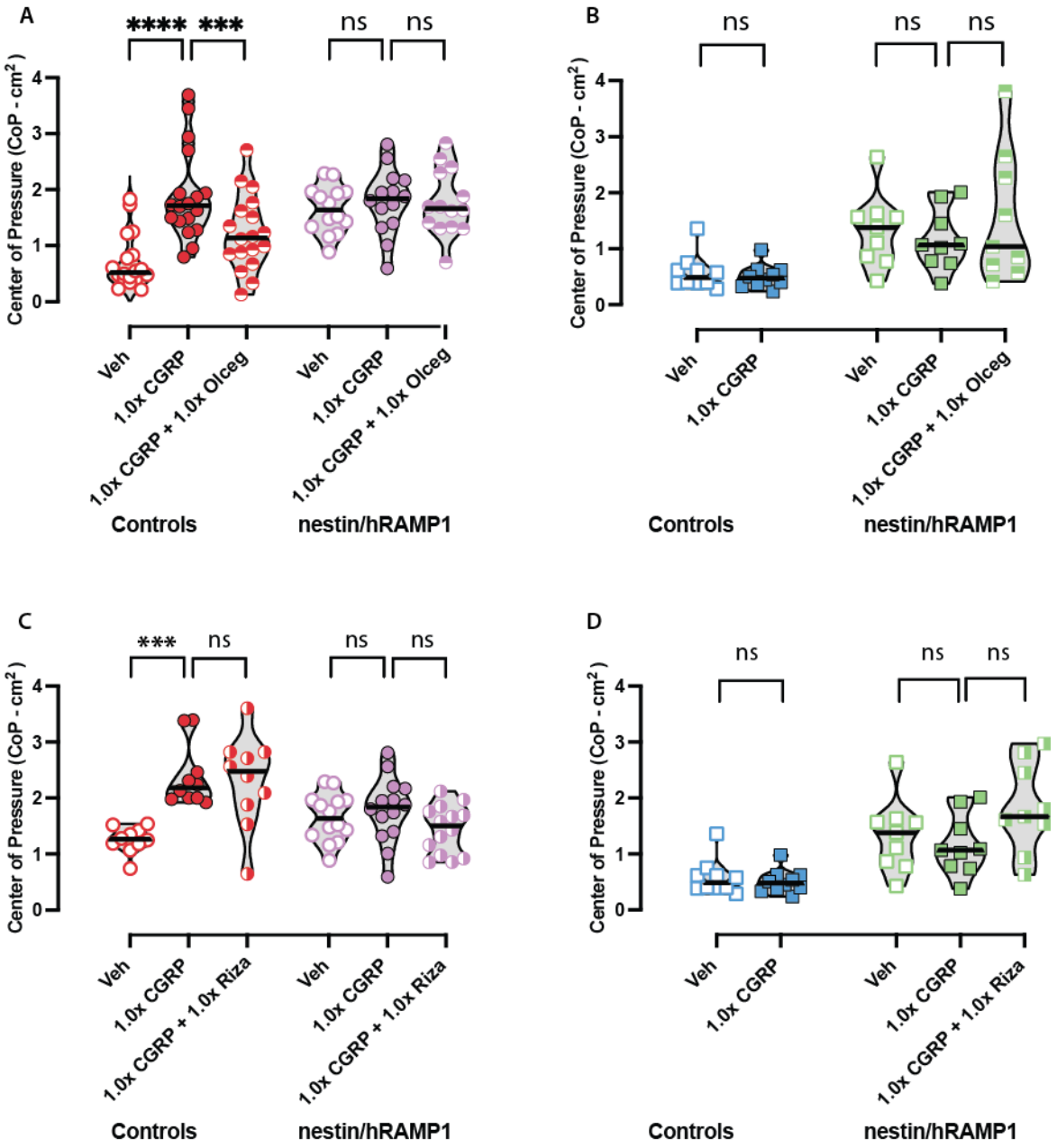

A
Female- control (vehicle)
Female- nestin/hRAMP1 (vehicle)
Male - control (vehicle)
Male - nestin/hRAMP1 (vehicle)
Center of Pressure (CoP - cm²)
***
***
Vehicle (F)
Vehicle (M)
Controls
nestin/hRAMP1
Controls
nestin/hRAMP1
B
***
ns
ns
ns
ns
ns
***
ns
Females
Veh
0.1x CGRP
0.5x CGRP
1.0x CGRP
Controls
nestin/hRAMP1
C
ns
ns
ns
ns
ns
ns
ns
ns
Males
Veh
0.1x CGRP
0.5x CGRP
1.0x CGRP
Controls
nestin/hRAMP1

Fig. 6.



576

577

578 **Fig. 7.**

579

580 **Table 1**

Recovery from Hypothermia - controls vs nestin/hRAMP1								
Sex	**Treatment**	**nestin/hRAMP1**		**controls**		**Recovery Difference**	**F(DFn, DFd)**	**p-val**
		Recovery	**Fit**	**Recovery**	**Fit**			
Male	**vehicle**	40.10	0.90	39.80	0.88	0.30	0.08056 (1, 36)	0.78
	1.0x CGRP	42.00	0.73	40.63	0.82	1.37	0.2664 (1, 36)	0.61
	1.0x CGRP + 1.0x Olceg	44.00	0.50	39.60	0.50	4.40	1.598 (1, 36)	0.21
	1.0x CGRP + 1.0x Riza	35.94	0.55	36.33	0.36	-0.39	0.01133 (1, 36)	0.92
Female	**vehicle**	39.30	0.79	38.28	0.90	1.02	0.7515 (1, 36)	0.39
	1.0x CGRP	40.70	0.67	40.80	0.83	-0.10	0.001123 (1, 36)	0.97
	1.0x CGRP + 1.0x Olceg	39.39	0.39	31.50	0.32	7.89	3.294 (1, 36)	0.08
	1.0x CGRP + 1.0x Riza	38.79	0.74	41.44	0.34	-2.65	0.7528 (1, 36)	0.39

581

582

583

584

585 **Table 2**

586

Diminished Δ tail vasodilations (< 1.5 ^{o} C)				
Treatment	**Controls (F)**	**nestin/hRAMP1(F)**	**Controls (M)**	**nestin/hRAMP1 (M)**
Vehicle	0%	0%	0%	0%
0.1x CGRP	22%	38%	0%	38%
0.5x CGRP	78%	88%	50%	100%
1.0x CGRP	88%	80%	53%	89%
0.5x CGRP + 1.0x Olceg	14%	38%	20%	38%
1.0x CGRP + 1.0x Olceg	60%	70%	20%	33%
1.0x CGRP + 1.0x Riza	71%	100%	86%	89%
0.5x CGRP + 1.0x Riza	NA	100%	NA	100%

587
588

589 **Table 3**

CGRP Dose Response on delta tail vasodilations and center of pressure (CoP) ellipse areas						
Dose Response analysis	**Factors**	**p-value**	**F (DFn, DFd)**	**Comparisons (Dunnett)**	**controls | adj. p val**	**nestinhRAMP1 | adj. p val**
tail vasodilations (females) - 2 way RM ANOVA	CGRP dose	<0.0001	F (1.484, 27.20) = 43.67	Vehicle vs. 0.1x CGRP	0.082	0.557
	controls vs nestin/hRAMP1	0.9785	F (1, 42) = 0.0007370	Vehicle vs. 0.5x CGRP	<0.0001	0.001
				Vehicle vs. 1.0x CGRP	<0.0001	0.000
tail vasodilations (males) - 2 way RM-ANOVA	CGRP dose	<0.001	F (3.000, 56.00) = 20.74	Vehicle vs. 0.1x CGRP	>0.99	>0.99
	controls vs nestin/hRAMP1	0.02	F (1, 42) = 5.495	Vehicle vs. 0.5x CGRP	0.100	0.004
				Vehicle vs. 1.0x CGRP	<0.001	0.020
CoP (females) - 2way RM-ANOVA	CGRP dose	<0.001	F (2.548, 66.24) = 16.03	Vehicle vs. 0.1x CGRP	0.070	0.170
	controls vs nestin/hRAMP1	0.74	F (1, 48) = 0.1078	Vehicle vs. 0.5x CGRP	0.100	0.080
				Vehicle vs. 1.0x CGRP	<0.001	0.980
CoP (males) - 2way RM-ANOVA	CGRP dose	0.13	F (3, 61) = 1.96	Vehicle vs. 0.1x CGRP	0.220	0.870
	controls vs nestin/hRAMP1	<0.001	F (1, 33) = 15.77	Vehicle vs. 0.5x CGRP	>0.99	0.240
				Vehicle vs. 1.0x CGRP	0.560	0.520
***Olcegepant efficacy* on delta tail vasodilations and center of pressure (CoP) ellipse areas**						
Olcegepant analysis	**Factors**	**p-value**	**F (DFn, DFd)**	**Comparisons (Tukey)**	**controls | adj. p val**	**nestinhRAMP1 | adj. p val**
tail vasodilations (females) - 2 way RM ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x olcegepant	<0.001	F (1.330, 23.95) = 15.68	Vehicle vs. 1.0x CGRP	<0.001	<0.001
	controls vs nestin/hRAMP1	0.460	F (1, 18) = 0.5770	Vehicle vs 1.0x CGRP + 1.0x Olceg	0.070	0.110
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	0.570	0.790
tail vasodilations (males) - 2 way RM-ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x olcegepant	< 0.001	F (1.626, 27.64) = 11.39	Vehicle vs. 1.0x CGRP	0.001	0.030
	controls vs nestin/hRAMP1	0.030	F (1, 17) = 5.885	Vehicle vs 1.0x CGRP + 1.0x Olceg	0.320	0.310
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	0.300	0.230
CoP (females) - 2way RM-ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x olcegepant	< 0.0001	F (1.98, 59.4) = 15.1	Vehicle vs. 1.0x CGRP	<0.0001	0.752
	controls vs nestin/hRAMP1	0.023	F (1, 30) = 5.77	Vehicle vs 1.0x CGRP + 1.0x Olceg	0.015	0.830
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	0.000	0.990
CoP (males) - 1 way ANOVA on nestin/hRAMP1	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x olcegepant	0.561	F (1.62, 13.0) = 0.536	Vehicle vs. 1.0x CGRP	NA	0.841
				Vehicle vs 1.0x CGRP + 1.0x Olceg	NA	0.878
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	NA	0.562
tail vasodilations (females) - 2 way RM ANOVA	vehicle vs 0.5x CGRP vs 0.5x CGRP + 1.0x olcegepant	<0.001	F (1.812, 24.46) = 15.61	Vehicle vs. 0.5x CGRP	0.020	0.002
	controls vs nestin/hRAMP1	0.006	F (1, 16) = 10.05	Vehicle vs 0.5x CGRP + 1.0x Olceg	0.580	0.020
				0.5x CGRP vs 0.5x CGRP + 1.0x Olceg	0.520	0.150
tail vasodilations (males) - 2 way RM-ANOVA	vehicle vs 0.5x CGRP vs 0.5x CGRP + 1.0x olcegepant	<0.001	F (1.498, 20.97) = 33.47	Vehicle vs. 0.5x CGRP	<0.001	<0.001
	controls vs nestin/hRAMP1	0.950	F (1, 15) = 0.004	Vehicle vs 0.5x CGRP + 1.0x Olceg	0.120	0.520
				0.5x CGRP vs 0.5x CGRP + 1.0x Olceg	0.020	0.040
***Rizatriptan efficacy* on delta tail vasodilations and center of pressure (CoP) ellipse areas**						
Rizatriptan analysis	**Factors**	**p-value**	**F (DFn, DFd)**	**Comparisons (Tukey)**	**controls | adj. p val**	**nestinhRAMP1 | adj. p val**
tail vasodilations (females) - 2 way RM ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x rizatriptan	< 0.001	F (1.541, 23.11) = 39.84	Vehicle vs. 1.0x CGRP	0.020	<0.001
	controls vs nestin/hRAMP1	0.320	F (1, 15) = 1.076	Vehicle vs 1.0x CGRP + 1.0x Riza	0.010	0.001
				1.0x CGRP vs 1.0x CGRP + 1.0x Riza	>0.99	0.290
tail vasodilations (males) - 2 way RM-ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x rizatriptan	< 0.001	F (1.659, 23.23) = 25.14	Vehicle vs. 1.0x CGRP	0.001	0.030
	controls vs nestin/hRAMP1	0.800	F (1, 14) = 0.06604	Vehicle vs 1.0x CGRP + 1.0x Riza	0.003	0.003
				1.0x CGRP vs 1.0x CGRP + 1.0x Riza	0.500	0.880
CoP (females) - 2way RM-ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x rizatriptan	< 0.001	F (1.99, 43.7) = 9.90	Vehicle vs. 1.0x CGRP	<0.001	0.750
	controls vs nestin/hRAMP1	0.020	F (1, 22) = 6.28	Vehicle vs 1.0x CGRP + 1.0x Riza	0.020	0.260
				1.0x CGRP vs 1.0x CGRP + 1.0x Riza	0.950	0.160
CoP (males) - 1 way ANOVA on nestin/hRAMP1	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x rizatriptan	0.057	F (1.83, 14.6) = 3.59	Vehicle vs. 1.0x CGRP	NA	0.841
				Vehicle vs 1.0x CGRP + 1.0x Olceg	NA	0.130
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	NA	0.069
CoP (females) - 1 way ANOVA on nestin/hRAMP1	vehicle vs 0.5x CGRP vs 0.5x CGRP + 1.0x rizatriptan	0.003	F (2, 6) = 18.3	Vehicle vs. 0.5x CGRP	NA	0.004
				Vehicle vs 0.5x CGRP + 1.0x Olceg	NA	0.005
				0.5x CGRP vs 0.5x CGRP + 1.0x Olceg	NA	0.991
CoP (males) - 1 way ANOVA on nestin/hRAMP1	vehicle vs 0.5x CGRP vs 0.5x CGRP + 1.0x rizatriptan	0.005	F (2, 6) = 14.5	Vehicle vs. 0.5x CGRP	NA	0.023
				Vehicle vs 0.5x CGRP + 1.0x Olceg	NA	0.005
				0.5x CGRP vs 0.5x CGRP + 1.0x Olceg	NA	0.362

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