Oromotor and somatic taste reactivity during sucrose meals reveals internal state and stimulus palatability after gastric bypass in rats

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Oromotor and somatic taste reactivity during sucrose meals reveals internal state and stimulus palatability after gastric bypass in rats

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Running title: Taste reactivity reveals internal state after gastric bypass

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ABSTRACT

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32   Following Roux-en-Y gastric bypass, rats consume less high-energy foods and fluids, though

33   whether this reflects a concomitant change in palatability remains unclear. By measuring

34   behavior during intraorally delivered liquid meals across days (1 water, 8 sucrose sessions), we

35   showed that RYGB rats (RYGB, n=8/sex) consumed less 1.0M sucrose than their sham surgery

36   counterparts (SHAM, n=8 males, n=11 females) but displayed similarly high levels of ingestive

37   taste reactivity responses at the start of infusions. Relative to water, both groups increased

38   intake of sucrose, and ingestive responses were dominated by tongue protrusions rather than

39   mouth movements. Thus, RYGB animals still found sucrose palatable despite consuming less

40   than the SHAM group. As the intraoral infusion progressed but prior to meal termination,

41   aversive behavior remained low and both RYGB and SHAM animals showed fewer ingestive

42   responses, predominantly mouth movements as opposed to tongue protrusions. This shift in

43   responsiveness unrelated to surgical manipulation suggests negative alliesthesia, or a

44   decreased palatability, as rats approach satiation. Notably, only in RYGB rats, across sessions

45   there was a striking emergence of aversive behavior immediately *after* the sucrose meal. Thus,

46   while lower intake in RYGB rats seems independent of the hedonic taste properties of sucrose,

47   taste reactivity behavior in these animals immediately *after* termination of a liquid meal appears

48   to be influenced by postoral events and reflects a state of nimiety, or excessive consumption.

49   Measurement of taste reactivity behaviors during an intraorally delivered meal represents a

50   promising way to make inferences about internal state in nonverbal preclinical models.

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INTRODUCTION

Roux-en-Y gastric bypass surgery (RYGB) is a surgical intervention for the treatment of obesity and its complications. Following the procedure, patients lose substantial body mass, especially fat, and maintain a reduced body weight for many years, mitigating typical obesity-associated complications such as cardiovascular disease, Type 2 diabetes mellitus, and cancer (1–3). These outcomes are thought to result from several postoperative behavioral and physiological consequences. RYGB leads to increases in postprandial gut hormone responses, hypertrophy of the jejunal mucosa, changes in bile acid receptors, alterations in the gut microbiota, and modifications of dopamine signaling in the brain (4, 5, 14–17, 6–13). Patients often verbally report eating less food postoperatively, especially items with high fat and high sugar content (3, 18–25), and it is commonly thought that the taste of those foods is also less appealing (26–29). While there are few studies of direct observation and measurement of food intake by humans, the data available call into question whether humans really do consume less high-fat, high-sugar foods (e.g., 3, 30). Rodent models have been a reliable method for studying RYGB and its effects on food intake and selection, with similar profiles for weight loss, glycemic control, intake reductions, and gut hormone changes (26, 31, 40, 41, 32–39). These studies have provided insight into what foods are chosen after surgery, as well as how foods are consumed. RYGB rats decrease preference for foods and fluids containing high amounts of fats and/or sugars but do not avoid them altogether and continue to drink the proffered substances across days (26, 33, 37, 39, 42–45).

One possible explanation for the decreases in preference is that RYGB surgery reduces the palatability of these items. Some work studying the hedonic qualities of sugar- and fat-containing foods and fluids supports this assertion, with patients reporting reductions in the perceived pleasantness of some foods (20, 21, 29, 46–48), although this effect has not been universally observed (33). Similarly in rodent studies, results are somewhat equivocal with

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79 regard to changes in the hedonic qualities of sugars and fats. In brief-access tests, which

80 reduce the influence of postingestive signaling on behavioral responses, animals still lick at

81 similar rates as SHAM rats in a concentration-dependent manner (38, 49) after RYGB, although

82 some studies show lower licking at the higher concentrations (50, 51).

83   Taken together, these results suggest that patients and post-RYGB rats are still

84 motivated to consume sugar and fat, albeit in reduced amounts. However, even traditional

85 short-term intake tests are influenced by both the taste characteristics and the postingestive

86 consequences of a stimulus. The total intake measured by these methods is also the result of

87 both appetitive and consummatory responses to the stimulus. Appetitive responses are

88 approach behaviors that bring the animal towards a stimulus, while consummatory responses

89 are behaviors that follow contact with the stimulus; these latter types of behavior are thought to

90 better reflect palatability of tastants (see 52). To assess whether these changes in intake and

91 licking reflect a decrease in the palatability of the stimulus based on its taste versus its

92 postingestive properties, a different experimental approach that fully excludes appetitive

93 behavior is required. The taste reactivity paradigm (53), in which stereotyped oromotor and

94 somatic responses following contact with tastants is quantified, is well suited for this purpose.

95 Responses systematically change based on stimulus concentration, the physiological state of

96 the animal, and learning processes such as conditioned taste aversion (54–60). Further, taste

97 reactivity allows observation of the palatability of a stimulus across an entire meal. Assessment

98 of taste reactivity has been used to demonstrate altered palatability of the stimulus based on

99 changing physiological state of the animal, as when a taste reactivity test follows an oral or

100 gastric preload to a stimulus (61–63). Consequently, we combined the taste reactivity test with

101 intraoral intake tests to measure palatability of the intraorally infused solution across the entire

102 intake session. Intraoral delivery of the stimulus allows experimenter control over the flow of

103 fluid, thus eliminating the appetitive component of intake tests. The intraoral intake test is

104 similar to more traditional drinking tests in that the volume consumed is dependent on the

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105   concentration of infused stimuli, gastric preloads, conditioning, and pharmacological

106   manipulations (61, 64–67). To date, the use of the taste reactivity paradigm to determine

107   whether palatability has changed after RYGB surgery has been tested once, but the

108   methodology used included an appetitive component by requiring the animals to lick the

109   stimulus from the floor of the chamber (68); an intraoral intake test in RYGB animals has not yet

110   been published. The combination of intraoral intake tests with taste reactivity allows analysis of

111   the animal's responses concomitant with a self-directed meal and provides an opportunity to

112   study whether the hedonic properties of the stimulus are altered by RYGB, whether those

113   changes occur across the course of the meal (within a single session), and whether experience

114   with the stimulus changes subsequent responses (across sessions).

MATERIALS and METHODS

Subjects

Thirty-six male and 36 female Sprague-Dawley rats, aged 10-12 weeks upon arrival to the facility, were used in this study. Rats were single-housed in standard polycarbonate cages in a facility where light (12h light:12h dark), temperature, and humidity were controlled automatically. All handling and testing occurred during the light phase. Standard woodchip bedding was used during the experiment, except during recovery from RYGB surgery (see below). Rats were given ad libitum access to standard rat chow (Purina 5001; Purina, St. Louis, MO, USA) and reverse-osmosis deionized water, except where noted after RYGB surgery (see below). Environmental enrichment (Rattle-A-Round, Otto Environmental) was provided throughout the study. Prior to RYGB surgery, all rats were given prophylactic injections of iron dextran (2.5 mg/kg, SC once weekly) to minimize the potential for iron deficiency after RYGB surgery. Rats that underwent RYGB surgery continued on this protocol throughout the experiment, while SHAM rats were given saline (2.5 ml/kg, SC) injections instead following surgery to preclude the development of iron toxicity. All procedures described were approved by the Florida State University Animal Care and Use Committee.

Surgery and Recovery

For all surgeries, aseptic technique was used to prepare materials and to perform the surgery. For each procedure, the rat was anesthetized with isoflurane (induction at 5%, maintenance on a nosecone at <3% in 1 L oxygen/minute).

Roux-en-Y gastric bypass

Prior to surgery, rats were acclimated for one night to the housing and foods to be used during postsurgical recovery. This postsurgical recovery cage was a standard polycarbonate

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141   cage fitted with an absorbent untreated cageboard (Techboard, Shepherd Specialty Products,

142   Milford, NJ, USA) below a raised stainless-steel wire floor insert. Soft recovery foods such as a

143   chow mash (1 part powdered chow to 4 parts water) and a custom-prepared gelatin diet (corn

144   starch, whey powder, corn oil, gelatin, baby vitamins, and water; see (see 38) were provided.

145   On the night prior to surgery, rats were placed in a clean recovery cage without food but with

146   access to water.

147   These aseptic surgeries were performed in two phases by two surgeons (CMM and

148   GDB), as described elsewhere (31). After a surgical plane of anesthesia was achieved, a

149   midline laparotomy exposed the abdominal cavity. The upper jejunum was transected ~7-10cm

150   from the ligament of Trietz, and each end ligated to form two stumps. The biliopancreatic limb

151   was made by a side-to-side anastomosis of the stump oral to the transection line with a portion

152   of the jejunum ~25-28 cm oral to the cecum. The stomach remained continuous with the

153   biliopancreatic limb, but the majority of the stomach was transected ~5mm aboral to the

154   esophageal junction to form a small gastric pouch and the stomach remnant. The remnant was

155   closed with suture. The gastric pouch was connected to the aboral jejunal stump by a side-to-

156   side anastomosis to create the alimentary limb. Sham surgeries were performed by placing

157   suture at the same locations in the gastrointestinal tract but without transecting tissue.

158   Following each procedure, the abdominal muscles and skin were closed with suture separately.

159   Each RYGB rat received subcutaneous saline (10 ml), and all rats received prophylactic

160   injections of antibiotic (enrofloxacin, 2.3 mg/kg, SC) and analgesic (carprofen, 5 mg/kg, SC) on

161   the day of surgery and for 3 days afterwards. After recovery from anesthesia, rats were

162   returned to a clean recovery cage and left without food but with access to water. Starting the

163   morning after surgery, rats were given small rations of the soft recovery foods to allow time for

164   anastomoses to heal. These rations increased in size and number across days, until the rats

165   were eventually given powdered chow and then standard pellets again. Most rats returned to

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166   pelleted chow by postoperative day 14, but some rats required more time on the soft recovery

167   foods. All rats were recovered from RYGB surgery by postoperative day 18.

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169   <u>Intraoral Cannulation</u>

170   Between 5 and 10 weeks after RYGB surgery, a single intraoral (IO) cannula was

171   implanted. These aseptic surgeries were conducted 10-14 days prior to the start of intraoral

172   infusions, in cohorts of 5-9 rats at a time.

173   After a surgical plane of anesthesia was achieved, a midline incision was made on the

174   skin over the dorsal surface of the skull. Four stainless steel set screws were placed in the

175   skull. A sharpened 19G stainless steel cannula was friction fit into the cannula tubing and used

176   to guide the tubing from its insertion point lateral to the second maxillary molar through the

177   muscle to its exit at the top of the skull. The sharpened guide cannula was removed and

178   replaced with a blunted 19G cannula. A headcap was formed around the intraoral cannula with

179   dental resin. If necessary, the scalp incision was closed around the headcap with silk suture.

180   All rats received prophylactic injections of antibiotic (gentamicin, 8 mg/kg, SC) and

181   analgesic (carprofen, 5 mg/kg, SC) on the day of surgery and for 6 days afterwards. Some rats

182   (from the first phase of RYGB surgeries) were also provided wet mash for the 6 days after IO

183   surgery. All rats were provided powdered and pelleted chow from the day of IO surgery to the

184   end of the experiment. Headcaps were inspected daily and starting at postsurgical day 2, each

185   IO cannula was cleared daily. In some cases, a collection of fluid around the headcap would

186   occur, requiring the headcap to be cleaned and treated topically with a betadine-containing first

187   aid solution. If it was necessary to provide treatment during testing, this occurred after the daily

188   intraoral infusion.

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190   **Intraoral Intake Test**

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191   Starting 10-14 days after IO cannulation, intraoral infusions began. Food was removed

192   from the home cage 45 min prior to the start of the session to minimize the likelihood of a rat

193   consuming a meal immediately prior to the testing session. Rats were given a day of

194   habituation to the test chamber, which consisted of an acrylic cylinder, floor, and lid placed

195   above a mirror. The mirror was set to a 45° angle, allowing a ventral view of the rat. A digital

196   camera (Sony DSC-WX50 HD) on a tripod was pointed at the mirror to video-record the entire

197   session. The lid of the chamber housed a fluid swivel, connected on the interior of the chamber

198   to a length of flexible Silastic tubing (0.6 mm ID x 1.2 mm OD) that could be connected to the IO

199   cannula. The tubing was protected from damage by a stainless-steel spring. The external end

200   of the swivel was connected to a length of Tygon tubing (0.5 mm ID x 1.5 mm OD) and a

201   syringe mounted to a motorized pump set to deliver 1.0 ml/min of solution. The cannula for the

202   rat was cleared and connected to the Silastic tubing, and the rat was placed into the chamber.

203   After habituating to the chamber for ~10 min with no fluid dispensed, the rat was given a short

204   infusion (~30 s) of reverse-osmosis deionized water before being returned to the home cage.

205   Food was returned immediately after the end of the session on this and all test days.

206   On the following day and for all test days thereafter, the following test protocol was used

207   (Figure 1). After a habituation period of ~1 min wherein the rat was connected to the tubing but

208   without infusion, the pump was turned on to deliver 1.0 ml/min of solution. The infusion

209   continued until the stimulus fell from the mouth of the rat or was actively ejected, after which the

210   pump was turned off. After 30 s without infusion, the pump was activated again to ensure that

211   the previous rejection response was not due to accidental fluid discharge by the animal. The

212   infusion continued until fluid dripped or was actively ejected again. If this occurred less than 30

213   s after the start of the next infusion, the test session ended. If this occurred after 30 s, the pump

214   was turned off for 30 s before another infusion was provided. This procedure continued until the

215   rat met the session termination criterion as described (passive or active fluid ejection within 30

216   after the pump was reactivated). The total infusion duration (all infusions combined) and

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217   stimulus volume delivered was recorded. Rats were tested in this way for one day with water,

218   then for 8 days with 1.0 M sucrose as the stimulus.

219   **Taste Reactivity Scoring**

220   Portions of the videorecords from the water session and the first and last sucrose

221   sessions were used to score oromotor behaviors for each rat. Scoring was done by a person

222   blind to the rat and test day. The first 30 s following the start of oromotor behavior (ensuring

223   stimulus delivery through the cannula and into the oral cavity), the 30 s prior to the first fluid

224   rejection, and the 30 s after the final infusion were scored. These timepoints were chosen so

225   that we could compare the oromotor responses to the stimulus at the start of meal before any

226   significant postoral accumulation of fluid occurred and near or at the end of the meal reflecting

227   the relationship between satiation and taste reactivity behavior. The ingestive behaviors, so

228   called because they are accompanied by and facilitate ingestion of the stimulus, scored were:

229   mouth movements, tongue protrusions, lateral tongue protrusions (each scored as individual

230   events), and paw licking (scored by time and converted to licks at a rate of 6/s). The aversive

231   behaviors, which are associated with ejection of the stimulus, scored were: gapes, chin rubs,

232   forelimb flails, and head shakes (each scored as individual events). Passive drip, when fluid

233   falls from the mouth without coincidence to another oromotor behavior, was scored but not

234   analyzed and during sessions was included as a trigger for pump termination. A sum for

235   ingestive behaviors and for aversive behaviors were separately calculated for each rat for each

236   timepoint in each day. For detailed description of each oromotor and somatic behavior, see

237   (53).

238   If oromotor behavior could not be scored (e.g., the rat was out of view of the camera) for

239   more than 1 s of continuous footage, the time was recorded as No Data. The scores for that

240   session were adjusted by dividing the total for each behavior by the ratio of time scored out of

241   30 s. This allowed the scores for each rat on each day to be standardized to the number of

242   behaviors expected if 30 s were counted. In one case, a rat did not have a full 30-s infusion

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243 before rejecting the stimulus (water); the scores for this rat were adjusted in the same way to
244 account for the difference in time.

245

246 **Data Analysis**

247   Only rats that were successfully given all test infusions were included in the analyses.
248 Nineteen rats (11 M, 8 F) were removed from study after complications immediately following
249 RYGB or IO surgery. Thirteen rats (7 M, 6 F) were removed from study for issues with the IO
250 cannula during testing (i.e., clogged or leaking). One rat (RYGB F) was removed from study
251 during the Intraoral Intake phase due to the observance of seizure-like activity. This did not
252 seem related to the infusion as it occurred before the infusion for the day and is unlikely to be
253 related to the IO cannula itself. One RYGB female was removed from study during the Intraoral
254 Intake phase after displaying drooling behavior, indicating an obstruction in the upper
255 anastomosis. This was observed prior to the testing session for the day and was likely
256 unrelated to the testing protocol. Two RYGB male rats lost a significant amount of body mass
257 (15-20% of pre-testing body mass) during the Intraoral Intake phase and were removed from
258 study over health concerns. One RYGB female was removed from study due to repeated
259 removal of the tubing from the IO cannula during infusions. These rats are not included in any
260 analyses. Final group sizes were as follows: SHAM M, n=8; SHAM F, n=11; RYGB M, n=8;
261 RYGB F, n=8.

262   Volumes consumed for each day were compared via mixed 2-way ANOVA (group x
263 day). Ingestive behaviors for each day were summed to calculate a daily total ingestive score
264 for each animal. Aversive behaviors were treated the same way to calculate a daily total
265 aversive score for each animal. Total ingestive and aversive oromotor scores were separately
266 compared at each timepoint in 2-way ANOVAs (sex x surgery). When interactions were
267 significant, appropriate follow-up t-tests were performed and are reported in corresponding
268 figure legends. The unadjusted p-values are reported. Proportion of responses that include

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269   tongue-protruding behaviors were analyzed in 2-way ANOVAs within specific sessions (sex x

270   surgery) or mixed 3-way ANOVAs when comparing across sessions (sex x surgery x day).

271   Paired t-tests comparing Water and Sucrose Day 1 were conducted for intake and taste

272   reactivity behaviors. Statistical significance was considered for any result of $p \leq 0.05$.

11

RESULTS

274   RYGB rats lost body mass after surgery, as expected (Figure 2). These animals were

275 considered recovered from RYGB after returning to standard pelleted rodent chow, which

276 occurred within 18 days from surgery. On average, female RYGB rats returned to their

277 presurgical body mass as has been reported elsewhere for female rats (49, 69, 70), while male

278 RYGB rats stabilized below presurgical values.

279

280 Intraoral Intake

281   All groups consumed a similar amount of water when it was intraorally infused (Figure 3;

282 Table 1). On the first day of the 1.0 M sucrose infusions (Sucrose day 1), all animals, regardless

283 of group or sex, drank more sucrose than they had water (Figure 3, Table 2). Notably, both the

284 male and female RYGB groups consumed about half of the sucrose as their SHAM controls.

285 (Figure 3; Table 1). On the eighth and final sucrose infusion test (Sucrose day 8), RYGB

286 animals still consumed less sucrose than the SHAM group, and females consumed less than

287 their male counterparts, regardless of surgical group. When intakes were compared between

288 the first and last sucrose infusions, only the SHAM males significantly differed across days,

289 consuming more sucrose on Sucrose 8 than on Sucrose 1 (t_7=14.95; p<0.01); intakes for the

290 other groups did not change (SHAM F: t_{10}=0.77, p=0.40; RYGB M: t_7=0.752, p=0.42; RYGB F:

291 t_7=5.01, p=0.06). So, in general, while RYGB decreased overall intake of 1.0 M sucrose in rats,

292 further postsurgical experience with the stimulus did not lead to further decreases in the amount

293 consumed across sessions.

294

295 Ingestive Taste Reactivity

296   Taste reactivity responses were analyzed for water and for the first and last days of

297 sucrose infusion (Sucrose 1 and Sucrose 8, respectively). Overall, there were few effects of

298 surgery or sex on ingestive responses, regardless of testing day or timepoint within the session

299   (Figure 4; Table 3). All groups displayed more ingestive responses to sucrose than to water

300   (Table 2), and there were few differences between groups. There were no group differences in

301   the first 30-s of infusion time, with water or on either of the analyzed sucrose days (Sucrose 1

302   and Sucrose 8). Female rats displayed more ingestive responses for water in the 30 s following

303   the end of infusion and more ingestive responses for sucrose prior to the first rejection on

304   Sucrose 1, but these main effects of sex did not interact with surgery. Independent of sex,

305   RYGB rats displayed more ingestive responses prior to the first rejection on the last day of

306   sucrose testing than did SHAM rats. Although minor, the differences were statistically

307   significant.

308   Not only were there a high number of total ingestive behaviors for sucrose, but the

309   proportion of ingestive behaviors that include a protruding tongue (e.g., tongue protrusions,

310   lateral tongue protrusions, and paw licking) was very high in the first 30 s for all groups (Figure

311   5). While all rats, regardless of group, displayed higher proportions of tongue-protruding

312   behaviors for sucrose compared to water on Sucrose 1 (Table 2), RYGB rats displayed a higher

313   proportion of these behaviors than did SHAM rats (Table 4). As the Sucrose 1 session

314   progressed, despite still showing a high number of ingestive responses (Figure 4), the

315   proportion of ingestive responses that included a protruding tongue decreased substantially

316   prior to the first rejection in all animals, reaching levels similar to those for water (Figure 5; Table

317   2). Animals behaved similarly during the last sucrose session, with high proportions of tongue

318   protrusions in the first 30-s of Sucrose 8 and a decrease in proportion prior to the first rejection

319   (Figure 5; Table 5).

320

321   **Aversive Taste Reactivity**

322   While there were some minor group differences in total ingestive responses to water or

323   to sucrose, some key differences emerged when comparing total aversive responses (Figure 6;

324   Table 3). During the first 30-s of the water infusion, male SHAM rats displayed more aversive

<center>13</center>

325 responses than female SHAM rats, leading to a sex x surgery interaction. All rats, regardless of

326 sex or group, displayed low rates of total aversive behavior during the initial 30 s of the infusion

327 on the first sucrose test session (Sucrose 1). There was, however, no effect of surgery for

328 either males or females for aversive score during the first 30-s of water infusion. Female rats,

329 independent of surgical group, showed more aversive responses prior to the first rejection,

330 though this difference was minor, numerically speaking.   Low levels of aversive responses by

331 all groups during the first sucrose infusion continued until the end of the meal.

332   This pattern had changed by the last sucrose session (Sucrose 8; Figure 6). While all

333 groups showed the same low aversive responding at the start of the infusion, there was a

334 significant sex x surgery interaction prior to the first rejection that was likely caused by higher

335 total aversive responding in RYGB females compared to SHAM females (Figure 6; Table 3).

336 After the infusions had ended, when no stimulus was being actively delivered, both male and

337 female RYGB rats displayed aversive responses. Female rats also showed more aversive

338 responding than male rats, though this was largely driven by the RYGB female rats, leading to a

339 significant sex x surgery interaction at this timepoint. While total aversive scores after the

340 infusion ended on the last sucrose test session (Sucrose 8) were relatively low, it is important to

341 note that these aversive responses take time to execute, particularly the somatic behaviors,

342 which involve moving the head or body rather than just the mouth and made up the majority of

343 the activity after the infusion ended (Figure 6). When the duration of these behaviors is

344 summed (Figure 7), it is evident that RYGB animals spent a larger proportion of their time

345 displaying these responses than did their SHAM counterparts, even in the absence of any fluid

346 delivery.

347

348 **Other Observed Behaviors**

349   The behaviors discussed above are those typically included in taste reactivity studies.

350 However, additional observed responses by RYGB animals are of note. First, one female

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351 RYGB rat was removed from study after repeatedly and intentionally removing the tubing from

352 the IO cannula during infusions. However, this behavior began on sucrose infusion day 7, and

353 the rat had been displaying the same pattern of responses as described above for other RYGB

354 animals. Thus, the removal of the tubing during the infusion may reflect a learned (avoidance)

355 strategy.

356   Second, the oromotor behavior data reported here do include two RYGB rats (one male,

357 one female) that did not display any of the scored aversive responses – gapes, chin rubs,

358 forelimb flails, and head shakes. Instead, both of these rats repeatedly displayed a behavior

359 called paw pushing or paw treading (53, 71). This behavior is usually considered aversive, as it

360 is typically (albeit rarely) observed to non-preferred stimuli such as quinine hydrochloride.

361 However, as this behavior is not usually included in aversive scores in the literature, it was not

362 analyzed in this data set. Importantly, though, this behavior was not observed in any of the

363 SHAM rats throughout the study.

364   Finally, an atypical behavior was observed prior to rejection of the stimulus in many (but

365 not all) RYGB rats, concomitant with the aversive behaviors at the end of sessions. This

366 involved the rat standing in a quadrupedal posture but with forelimbs somewhat extended

367 (weight distributed slightly toward the rear limbs) and ceasing typical oromotor behavior for a

368 short period of time (1-2 seconds). The rat could then be seen to swallow the accumulated fluid

369 while dorsoflexing the neck; this latter action is a component of a chin rub response. This

370 behavior was not noted for SHAM rats or in sessions with RYGB rats when they were not also

371 displaying an increase in aversive responses. Given that this behavior does not seem to be

372 previously described in the taste reactivity literature, it was not counted in the behaviors

373 quantified here.

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DISCUSSION

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378   Congruent with other studies, RYGB rats consumed less of a high concentration of

379   sucrose than did SHAM rats, even when no appetitive behavior was required for its intake.

380   However, the difference in intake was evident even on the first day (Figure 3), which has not

381   always been observed in short-term intake tests (38). In addition, intake did not progressively

382   decrease over sessions which may be due to their already low consumption initially on the first

383   sucrose meal. The apparent discrepancy may lie in the form of stimulus delivery. In short-term

384   intake tests involving a drinking spout, the rat consumes the stimulus freely in bursts of licking,

385   but, in the intraoral intake test, the stimulus is externally delivered at a constant infusion rate.

386   Accordingly, the presence of an appetitive component, providing the opportunity for pauses in

387   ingestion when fluid is obtained by licking a drinking spout, appears to delay the onset of

388   satiation in RYGB rats at least on initial exposure to concentrated sucrose solution.

389   Despite consuming substantially less sucrose overall, RYGB rats displayed similar levels

390   of ingestive taste reactivity as SHAM rats in the first session with the sugar stimulus, a trend that

391   continued throughout the infusions (Sucrose 1). The sum of ingestive responses increases with

392   sucrose concentration and corresponds to acceptance in intake tests (56, 72, 73). Indeed, there

393   was approximately a doubling of the ingestive score from the water session to the first 30 s of

394   the first sucrose session by all groups. In particular, in contrast to the water session, the

395   response profile at the beginning of sucrose infusions was dominated by tongue protrusions by

396   all rats (Figures 4, 5), a behavior shown to increase with higher levels of acceptance and to

397   decrease first when tastants are conditioned to be aversive (54, 58, 60). The nature of and

398   similarity in responses between surgical groups at the beginning of the sucrose sessions

399   suggests that RYGB and SHAM rats both find the taste of 1.0 M sucrose affectively positive

400   despite the large group differences in overall intake here and when preference is assessed in

401   two-bottle tests (e.g., 33, 49, 74). The failure for surgery to affect taste reactivity to sucrose

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402 during the initial stage of the infusion is similar to the results in some other studies that focus on

403 the orosensory hedonic characteristics of sugar and/or fat solutions. In brief-access tests, in

404 which rats are allowed to freely lick varying concentrations of a stimulus in short periods (5-10 s)

405 of access, RYGB rats will sometimes respond in the same concentration-dependent manner as

406 SHAM rats (38, 75; but see 50). Consistent with these findings, RYGB rats tested in a

407 progressive ratio task, which requires the animal to perform progressively higher numbers of

408 responses to obtain a reinforcer, were just as motivated to work for sucrose as SHAM rats (42).

409   In contrast, an earlier study with RYGB rats that measured taste reactivity found a

410 decrease in ingestive responses to 1.0 M sucrose (68). The differences between those results

411 and the ones reported here may have a methodological origin. Shin and colleagues

412 (68)conducted the taste reactivity test by allowing the animals to lap the stimulus from the floor

413 of the chamber; the animal was required to approach and sample the stimulus before any

414 oromotor responses could be measured. As such, their method was unlike most other taste

415 reactivity tests reported in the literature, in which the experimenter rather than the animal is in

416 control of stimulus delivery, because it conflated the appetitive and consummatory

417 responsiveness of the animal. Another methodological difference is in the maintenance diet

418 provided to the rats.   The rats in this study were only given the standard rodent diet, whereas

419 Shin et al. (68) additionally offered a high fat diet. We only gave rats access to chow because

420 there is evidence that maintenance diet can have an impact on ingestive behaviors towards

421 palatable stimuli in rodents (76).   Moreover, it should be noted that Miras et al. (78) reported

422 that male Sprague-Dawley rats fed a high fat diet for 6 weeks did not differ in adiposity from rats

423 having a similar terminal total body mass that were fed chow. Nevertheless, we cannot dismiss

424 the possibility that the use of rats placed on a high fat diet for a longer period of time or that had

425 heavier body weights than those in our study would have led to different outcomes. It would be

426 instructive to test such a possibility so that that the relevant physiological and environmental

427 boundaries of the phenomena described here can be better understood.

17

428   Taste reactivity responses just before rejection during the first sucrose session were also

429   similar in RYGB and SHAM rats. Ingestive scores decreased slightly compared to the

430   beginning of the session, which has been demonstrated in a similar context with extended

431   intraoral infusions (77). What was striking, however, was that while overall ingestive responses

432   by all the rats decreased only slightly, the proportion of tongue-protruding behaviors dropped

433   precipitously, suggesting a reduction in the palatability of the stimulus near the end of the meal

434   (Figures 4, 5). This may also reflect a decrease in acceptance of the stimulus that would have

435   led to cessation of drinking if the animal were freely drinking the stimulus. Importantly, the

436   reduction in overall responses and in the proportion of tongue-protruding behaviors was similar

437   between SHAM and RYGB rats (Tables 1, 2). Accordingly, this reflects a general behavioral

438   process at the end of a meal and is not directly related to the surgery. Aversive responses

439   remained low prior to and following pump termination during the first sucrose session,

440   suggesting a general satiation process rather than an aversive reaction to the stimulus at the

441   end of a meal. Indeed, administering an energy preload decreases ingestive responses in taste

442   reactivity studies (71, 79), as well as decreasing other positive affective-related behaviors such

443   as preference (80). This phenomenon is referred to as alliesthesia (79, 81). Negative

444   alliesthesia, an internal state-induced decrease in positive affective responses, has been

445   demonstrated in humans and rats as a process tied to satiation at the end of a meal. With time

446   (a matter of hours), this effect dissipates, and animals again show positive responding to the

447   stimulus (71, 73, 79, 82).

448   This temporary shift in hedonic valence of a stimulus distinguishes alliesthesia from

449   more long-term learned responses, such as conditioned taste aversion (CTA). In CTA, animals

450   will subsequently reject a taste stimulus that upon previous ingestion led to gastrointestinal

451   malaise. This represents a learned response and is accompanied by a marked increase in

452   aversive taste reactivity responses (60, 63, 83, 84) seen immediately upon start of the infusion.

453   However, in this study, despite RYGB rats developing aversive responding after the conclusion

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454 of the testing session (Sucrose 8; Figure 6), the taste reactivity response profiles at the start of

455 the subsequent sucrose sessions never changed (Figure 4). Therefore, it is unlikely that a CTA

456 was learned by these animals.

457 Conditioned avoidance is another learned response where animals typically consume

458 less of a taste stimulus, but intake of it is unaccompanied by aversive taste reactivity responses

459 (see 85, 86). Because intake did not decrease across the intraoral intake sessions and aversive

460 taste reactivity behavior was not evident at the beginning of sessions, RYGB rats do not seem

461 to be exhibiting either conditioned aversion or conditioned avoidance, as currently understood.

462 Rather, the emergence of aversive oromotor and somatic responses displayed by RYGB rats

463 across sessions after infusions ended appear to be an exaggerated form of alliesthesia

464 compared to that shown by SHAM animals and may reach the threshold of nimiety, a state of

465 being full to excess. One caveat to this interpretation is that conditioned avoidance studies

466 typically do not assess taste reactivity behaviors at the end of an intake session; to our

467 knowledge, only one previously published study quantified taste reactivity responses after the

468 end of the infusion (73). Perhaps a similar study using a typical conditioned avoidance

469 paradigm with drugs of abuse or lactose (adults rodents are lactase insufficient) (83, 86, 87)

470 would find that aversive oromotor responses surface with time across intraoral intake sessions.

471 The mechanism(s) that lead RYGB rats to exhibit aversive oromotor and somatic

472 responses after experience with a taste stimulus is unknown. That the aversive display

473 happens immediately after the end of the session implicates postoral signals. RYGB in humans

474 and rats leads to a profile of gut hormones that favor reduced consumption, such as high

475 postprandial levels of GLP-1 and PYY. At least in humans, high levels of exogenously

476 administered PYY and GLP-1 are reported to induce nausea (88, 89), reminiscent of the

477 aversive responses of RYGB rats at the end of the intraoral intake sessions. It may be, then,

478 that these end-of-meal aversive behaviors by RYGB rats reflect the altered enteroendocrine

479 profile following the procedure. However, the postmeal aversive behavior may also be related

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480 to nonendocrine preabsorptive events. After RYGB, the small gastric pouch volume and lack of

481 pylorus leads to very fast transfer of fluids to the intestines, a process thought to contribute to

482 nausea and dumping syndrome in humans (90); however, no obvious symptoms of dumping

483 syndrome were observed in this study. RYGB rats also have a much smaller reservoir for

484 ingested fluid, given the lack of a stomach and duodenum, and it may be that the rats are

485 consuming as much fluid as the reorganized gastrointestinal system can allow. Becoming

486 overfull would likely lead to mechanosensory signals and potentially pain as the intestines

487 expanded. Of course, one would expect that any of these potential mechanisms would exist

488 with the first exposure to sucrose, but RYGB animals did not display aversive responses after

489 infusions on the first day of sucrose testing. We hypothesize that upon normal satiation, RYGB

490 rats quickly slip into a negative internal state caused by the accumulated ingested load even on

491 the first session. However, it is the expression of this internal state through taste reactivity

492 behavior that requires experience.

493   If these responses are caused by postingestive signaling that grows during a meal, it

494 may be somewhat of a misnomer to refer to these behaviors as being "taste" reactivity. If the

495 rats were responding to the taste alone, then aversive responses should have been observed

496 throughout the infusion session. Indeed, such responses may not require any tastant at all. Of

497 note is the fact that most of the behaviors elicited were somatic (e.g., headshakes), and not

498 oromotor (e.g., gapes) in nature (Figure 6). This may be related to the cessation of intraoral

499 stimulus delivery. Of course, there were likely still taste signals being generated from the oral

500 cavity immediately after pump termination and, at the very least, the taste of sucrose would be

501 expected to still be active in working memory. Thus, it is possible that the animals were

502 responding to a compound conditioned stimulus (the taste + the postingestive signals), and that

503 neither would be sufficient to elicit the responses alone. This has been demonstrated with LiCl

504 previously (59).

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505   It remains unclear what physical features of the stimulus lead to the development of

506   aversive responses immediately after the meal has terminated in the RYGB rats. It is plausible

507   that the colligative or energy/macronutrient content of the solution are critical, especially

508   considering a particularly high concentration of sucrose was chosen precisely because, after

509   RYGB, rats show lowered preference to and intake of this sugar solution in long-term tests

510   despite it being sufficient to reinforce responding (e.g., 33, 38, 42, 49). After RYGB, rats may

511   become particularly susceptible to the postingestive feedback of the high sugar concentration.

512   As with intact rats, in some contexts, RYGB rats will increase intake of low-energy foods and

513   fluids, demonstrating that stimulus concentration can be a factor in post-RYGB outcomes (75,

514   80). Alternatively, or in addition to its colligative and energy properties, the molecular identity of

515   the stimulus may be relevant. It remains to be seen if different sugars or energy sources (e.g.,

516   lipids) might be just as, more, or less effective at generating the aversive behaviors seen

517   immediately after the termination of the meal across sessions.

518   Notably, some significant sex differences were found, namely in relation to the aversive

519   responding after the end of intake sessions, with female RYGB rats displaying the highest

520   aversive responses (Figure 6; Table 2). It is not clear why, after RYGB, female rats would show

521   more aversive responses than male rats. In the only published report of taste reactivity patterns

522   across phases of female estrous cycles of which we are aware, aversive response rates to the

523   bitter tastant quinine hydrochloride changed as estrogen levels cycled, but that maximal

524   aversive responding was still comparable to that of male rats (57). One interesting possibility,

525   though, is that estrogen levels in the female rats used in this study interacted with whatever

526   potential postingestive signals were stimulating the aversive responses seen here. Indeed,

527   estradiol treatments enhance the effects of exogenous treatments of GLP-1 and CCK on food

528   intake and body weight in ovariectomized female rats, including after gastric bypass surgery

529   (70, 91–93). The females in this study were not ovariectomized, and normal cycling has been

530   found in both rats and mice following the procedure (45, 94); this would suggest that the rats in

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531   our study also had normal estrous cycles. As our study was not specifically designed to assess

532   the role of estrus in taste reactivity behaviors, possible interactions between estrous phases and

533   the physiological consequences of RYGB remain to be tested in this context but may be of

534   relevance to female patients.

535   Overall, it appears that postingestive signals generated by a sucrose load, specifically in

536   rats that have a reorganized gastrointestinal tract, trigger behavior associated with aversion

537   immediately after an intraoral meal and, as such, these results provide insight as to why RYGB

538   rats show lower preference for and intake of high concentrations of sucrose. These behaviors

539   only emerge with multiple exposures to the stimulus and are unrelated to total intraoral intake or

540   the taste reactivity observed at the beginning of an infusion, suggesting that some learning

541   process takes place that is distinct from conditioned avoidance of and aversion to taste stimuli.

542   Ultimately, more work needs to be done to determine the physiological mechanisms underlying

543   these aversive responses, which appear to be unique to rats after RYGB, but could represent a

544   general readout of a negative visceral state that is an immediate consequence of profound

545   overeating.

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557 **DECLARATION OF INTEREST**

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860 Figure 1. Timeline of intraoral intake sessions, and a flowchart of the intraoral infusion session
861 with the timepoints scored for taste reactivity behaviors. Top: Infusion schedule. Each session
862 occurred on a separate day. Bottom: a flowchart of intraoral infusions within a session. Dark
863 grey indicates times when the infusion pump was off and no fluid was actively dispensed. Light
864 grey indicates when the infusion pump was on and stimulus was being delivered. *: "Rejection"
865 is here defined as fluid dropping out of the mouth, indicating that the animal was no longer
866 swallowing the stimulus being infused. This could occur passively (e.g., passive drips) or
867 actively (e.g., gapes or head shakes). Taste reactivity scoring epochs are indicated relative to
868 infusions. Group sizes: RYGB males, n=8; RYGB females, n=8; SHAM males, n=8; SHAM
869 females, n=11.

870

871 Figure 2. Average (±SE) body mass as a proportion of ad libitum presurgical body mass during
872 surgical recovery period and testing. Ad libitum body mass was measured on the day before
873 surgery (AD LIB; mean [SE] for each group provided in the legend) for each rat, prior to food
874 deprivation in preparation for surgery (SURG). During testing, ad libitum body mass was
875 measured prior to being placed into the testing chamber for habituation (HABIT), water, and
876 each sucrose test session (S1 – S8). Group sizes: RYGB males, n=8; RYGB females, n=8;
877 SHAM males, n=8; SHAM females, n=11.

878

879 Figure 3. Average total volume (±SE) consumed during the intraoral meal. This value was
880 calculated by the total infusion durations (in minutes) multiplied by the infusion rate (calibrated
881 to 1 ml/min). ANOVAs comparing groups for these data are presented in Table 1. Paired t-
882 tests comparing intake for Water to Sucrose 1 are presented in Table 2. Group sizes: RYGB
883 males, n=8; RYGB females, n=8; SHAM males, n=8; SHAM females, n=11.

884

885 Figure 4. Average total ingestive responses (height of bars; ±SE) during water, first sucrose
886 (S1), and last sucrose (S8) sessions, separated by timepoint within the session. Average
887 scores for individual behaviors (MM: mouth movements; TP: tongue protrusions; LTP: lateral
888 tongue protrusions; PL: paw licking) are indicated by the separate colors within the bars. Two-
889 way ANOVAs (sex x surgery) are presented in Table 3, and significant effects are indicated
890 above the bars by a solid line (main effect of sex) or a dashed line (main effect of surgery).
891 There were no significant interactions for these data. *: significant difference between ingestive
892 responding to Water and Sucrose 1 in paired t-tests (Table 2). Group sizes: RYGB males, n=8;
893 RYGB females, n=8; SHAM males, n=8; SHAM females, n=11.

894

895 Figure 5. The proportion of ingestive responses that included a protruding tongue (tongue
896 protrusions, lateral tongue protrusions, and paw licking) were calculated for the first 30 s and the
897 30 s before the first rejection for all three scored infusion sessions. Dashed line: significant
898 effect of surgery in two-way ANOVAs for the first 30-s of infusions (Table 4). *: significant
899 difference between water and Sucrose 1 in paired t-tests (Table 2). #: significantly lower
900 proportion of tongue protrusions than during first 30-s of infusions in paired t-tests (Sucrose 1—
901 RYGB M: $t(7)=5.97$, $p<0.01$; RYGB F: $t(7)=5.48$, $p<0.01$;SHAM M: $t(7)=5.25$, $p<0.01$;SHAM F:
902 $t(10)=5.89$, $p<0.01$. Sucrose 8—RYGB M: $t(7)=13.97$, $p<0.01$; RYGB F: $t(7)=3.80$, $p<0.01$
903 ;SHAM M: $t(7)=3.24$, $p<0.01$;SHAM F: $t(10)=7.07$, $p<0.01$). Results of 3-way ANOVAs

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904   comparing Sucrose 1 and Sucrose 8 are found in Table 5.   Group sizes: RYGB males, n=8;
905   RYGB females, n=8; SHAM males, n=8; SHAM females, n=11.

906

907   Figure 6. Average total aversive responses (height of bars; ±SE) during water, first sucrose
908   (Sucrose 1), and last sucrose (Sucrose 8) sessions, separated by timepoint within the session.
909   Average scores for individual behaviors (G: gapes; FF: forelimb flails; HS: headshakes; CR:
910   chin rubs) are indicated by the separate colors within the bars. Two-way ANOVAs (sex x
911   surgery) are presented in Table 3, and significant effects are indicated above the bars by a solid
912   line (main effect of sex) or a dashed line (main effect of surgery). Where significant interactions
913   were found, follow-up t-tests were conducted. #: significant difference between sexes for the
914   marked surgical group. *: significant difference between surgery groups for the marked sex.
915   For water in the first 30-s infusion, within sex: RYGB $t_{14}=0.26$, p=0.80, SHAM $t_{17}=2.42$, p=0.03;
916   within surgery: males $t_{14}=1.60$, p=0.13; males $t_{17}=1.6$, p=0.13. For Sucrose 8 before the first
917   rejection, within sex: RYGB $t_{14}=1.89$, p=0.08, SHAM $t_{17}=0.67$, p=0.51; within surgery: males
918     $t_{14}=0.24$, p=0.82; males $t_{17}=2.54$, p=0.02. For Sucrose 8 after the last rejection, within sex:
919   RYGB $t_{14}=3.50$, p<0.01, SHAM $t_{17}=0.65$, p=0.52; within surgery: males $t_{14}=2.00$, p=0.07; males
920     $t_{17}=5.44$, p<0.01. Paired t-tests comparing aversive responses for Water and Sucrose 1 are
921   presented in Table 2. Group sizes: RYGB males, n=8; RYGB females, n=8; SHAM males, n=8;
922   SHAM females, n=11.

923

924   Figure 7. Average duration (±SE) of aversive responses for the 30-s following infusions on
925   Sucrose 8. The dashed line indicates a significant effect of surgery ($F_{1,23}=56.22$; p<0.01) in a
926   two-way ANOVA (sex x surgery). There was no main effect of sex ($F_{1,23}=2.03$, p=0.17) and no
927   interaction ($F_{1,23}=1.55$, p=0.23). Group sizes: RYGB males, n=8; RYGB females, n=8; SHAM
928   males, n=8; SHAM females, n=11.

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930

931   Table 1. ANOVAs comparing group intraoral intakes.

	WATER	S1	S8
SEX	$F_{(1,31)}=0.75$; p=0.85	$F_{(1,31)}=0.13$; p=0.72	**$F_{(1,31)}=7.685$; p<0.01**
SURGERY	$F_{(1,31)}=0.32$; p=0.58	**$F_{(1,31)}=35.64$; p<0.01**	**$F_{(1,31)}=99.525$; p<0.01**
SEX x SURGERY	$F_{(1,31)}<0.01$; p=0.94	$F_{(1,31)}=0.78$; p=0.38	$F_{(1,31)}=1.973$; p=0.17

932   Bolded values represent statistical significance (p≤0.05).

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934   Table 2. Paired t-tests comparing Water and Sucrose 1 within each group.

935

Water vs. S1	SHAM		RYGB	
	M	F	M	F
Intake (Fig. 2)	**t(7)=6.58; p<0.01**	**t(10)=5.22; p<0.01**	**t(7)=3.72; p<0.01**	**t(7)=3.26; p=0.01**
Taste Reactivity (Figures 4 & 6)				
First 30 s				
Ingestive	**t(7)=5.91; p<0.01**	**t(10)=3.35; p<0.01**	**t(7)=3.06; p=0.02**	**t(7)=3.20; p=0.02**
Aversive	t(7)=2.25; p=0.06	t(10)=1.32; p=0.22	t(7)=1.16; p=0.29	t(7)=2.30; p=0.06
Before First Rejection				
Ingestive	t(7)=2.08; p=0.08	**t(10)=2.80; p=0.02**	t(7)=1.19; p=0.27	t(7)=1.97; p=0.09
Aversive	t(7)=2.31; p>0.05	t(10)=0.65; p=0.53	t(7)=0.76; p=0.47	t(7)=0.41; p=0.69
After Infusion				
Ingestive	t(7)=1.36; p=0.22	**t(10)=4.12; p<0.01**	t(7)=0.77; p=0.47	t(7)=0.20; p=0.85
Aversive	t(7)=0.43; p=0.68	t(10)=1.17; p=0.27	t(7)=0.60; p=0.57	t(7)=0.94; p=0.38
Tongue Protruding Behaviors (Figure 5)				
First 30 s	**t(7)=3.07; p=0.02**	**t(10)=6.64; p<0.01**	**t(7)=6.49; p<0.01**	**t(7)=3.69; p<0.01**
Before Rej	t(7)=1.23; p=0.27	t(10)=0.44; p=0.67	t(7)=1.36; p=0.22	t(7)=1.36; p=0.22

936   Bolded values indicate statistical significance (p≤0.05).

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938

939   Table 3. Two-way ANOVA results for total ingestive and aversive responses

INGESTIVE	WATER	S1	S8
First 30s			
SEX	$F(1,31)=0.24$, $p=0.63$	$F(1,31)=0.71$, $p=0.41$	$F(1,31)=0.35$, $p=0.56$
SURGERY	$F(1,31)=0.46$, $p=0.50$	$F(1,31)=2.06$, $p=0.16$	$F(1,31)=1.27$, $p=0.27$
SEX x SURGERY	$F(1,31)=3.16$, $p=0.09$	$F(1,31)=0.02$, $p=0.88$	$F(1,31)=0.31$, $p=0.58$
Before First Rejection			
SEX	$F(1,31)=0.56$, $p=0.46$	**$F(1,31)=4.85$, $p=0.04$**	$F(1,31)=3.42$, $p=0.07$
SURGERY	$F(1,31)=0.72$, $p=0.40$	$F(1,31)=0.72$, $p=0.40$	**$F(1,31)=4.75$, $p=0.04$**
SEX x SURGERY	$F(1,31)=2.83$, $p=0.10$	$F(1,31)=0.35$, $p=0.56$	$F(1,31)=2.16$, $p=0.15$
After Infusion			
SEX	**$F(1,31)=6.68$, $p=0.02$**	$F(1,31)=0.02$, $p=0.90$	$F(1,31)=0.03$, $p=0.87$
SURGERY	$F(1,31)=3.72$, $p=0.07$	$F(1,31)=1.55$, $p=0.22$	$F(1,31)=0.08$, $p=0.78$
SEX x SURGERY	$F(1,31)=2.37$, $p=0.13$	$F(1,31)=0.13$, $p=0.72$	$F(1,31)=0.89$, $p=0.77$
AVERSIVE	WATER	S1	S8
First 30s			
SEX	$F(1,31)=3.65$, $p=0.07$	**$F(1,31)=4.94$, $p=0.03$**	$F(1,31)=0.68$, $p=0.42$
SURGERY	$F(1,31)=1.31$, $p=0.26$	$F(1,31)=0.30$, $p=0.59$	$F(1,31)=0.26$, $p=0.61$
SEX x SURGERY	**$F(1,31)=4.59$, $p=0.04$**	$F(1,31)=0.40$, $p=0.53$	$F(1,31)=3.40$, $p=0.08$
Before First Rejection			
SEX	$F(1,31)=0.02$, $p=0.90$	$F(1,31)=0.30$, $p=0.59$	$F(1,31)=2.39$, $p=0.13$
SURGERY	$F(1,31)=2.46$, $p=0.13$	$F(1,31)=0.25$, $p=0.62$	$F(1,31)=3.37$, $p=0.08$
SEX x SURGERY	$F(1,31)=2.33$, $p=0.14$	$F(1,31)=0.04$, $p=0.84$	**$F(1,31)=4.45$, $p=0.04$**
After Infusion			
SEX	$F(1,31)=0.04$, $p=0.95$	$F(1,31)=1.04$, $p=0.32$	**$F(1,31)=13.67$, $p<0.01$**
SURGERY	$F(1,31)=0.02$, $p=0.96$	$F(1,31)=1.44$, $p=0.24$	**$F(1,31)=29.23$, $p<0.01$**
SEX x SURGERY	$F(1,31)=0.84$, $p=0.37$	$F(1,31)=0.21$, $p=0.65$	**$F(1,31)=10.08$, $p<0.01$**

940   Bolded values indicate statistical significance ($p \leq 0.05$).
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942 Table 4. Two-way ANOVA results comparing proportion of ingestive responses that included a
943 protruding tongue.

	WATER	S1	S8
First 30s			
SEX	$F_{(1,31)}=1.91$, p=0.18	$F_{(1,31)}=3.87$, p=0.06	$F_{(1,31)}=2.58$, p=0.44
SURGERY	$F_{(1,31)}=0.18$, p=0.68	**$F_{(1,31)}=5.52$, p=0.03**	$F_{(1,31)}=0.44$, p=0.51
SEX x SURGERY	$F_{(1,31)}<0.01$, p=0.99	$F_{(1,31)}=0.20$, p=0.73	$F_{(1,31)}=0.68$, p=0.42
Before First Rejection			
SEX	$F_{(1,31)}=0.12$, p=0.73	$F_{(1,31)}=1.10$, p=0.30	$F_{(1,31)}=2.13$, p=0.15
SURGERY	$F_{(1,31)}=0.72$, p=0.40	$F_{(1,31)}=0.03$, p=0.86	$F_{(1,31)}=0.30$, p=0.59
SEX x SURGERY	$F_{(1,31)}=0.06$, p=0.81	$F_{(1,31)}=0.02$, p=0.90	$F_{(1,31)}=0.98$, p=0.33

944 Bolded values indicate statistical significance (p≤0.05).
945

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946 Table 5. Three-way ANOVAs comparing tongue-protruding behaviors during the first and last
947 sucrose sessions.
948

SUCROSE 1 vs. SUCROSE 8	First 30s	Before First Rejection
SEX	F(1,31)=5.65, p=0.02	F(1,31)=2.54, p=0.12
SURGERY	F(1,31)=3.52, p=0.07	F(1,31)=0.21, p=0.65
DAY	F(1,31)=0.09, p=0.76	F(1,31)=2.32, p=0.14
SEX x SURGERY	F(1,31)=0.69, p=0.41	F(1,31)=0.28, p=0.60
SEX x DAY	F(1,31)<0.01, p=0.93	F(1,31)=0.08, p=0.78
SURGERY x DAY	F(1,31)=0.87, p=0.36	F(1,31)=0.08, p=0.78
SEX X SURGERY x DAY	F(1,31)=0.22, p=0.65	F(1,31)=0.78, p=0.38

949

Infusion Schedule

HABITUATION one session	WATER one session	SUCROSE			NO INFUSION two days	SUCROSE				
		1	2	3		4	5	6	7	8

Intraoral Intake Sessions

Intraoral Intake Test

Taste Reactivity Scoring Epochs

HABITUATION
~1 min

INFUSION
1 ml/min until
fluid is rejected* ⟶ *first 30-s of infusion*

PAUSE
30 sec

INFUSION
1 ml/min until
fluid is rejected* ⟶ *30-s before fluid rejection**

Infusion duration
≥ 30 sec

Infusion duration
< 30 sec

End Session
30 sec ⟶ *30-s after infusions*
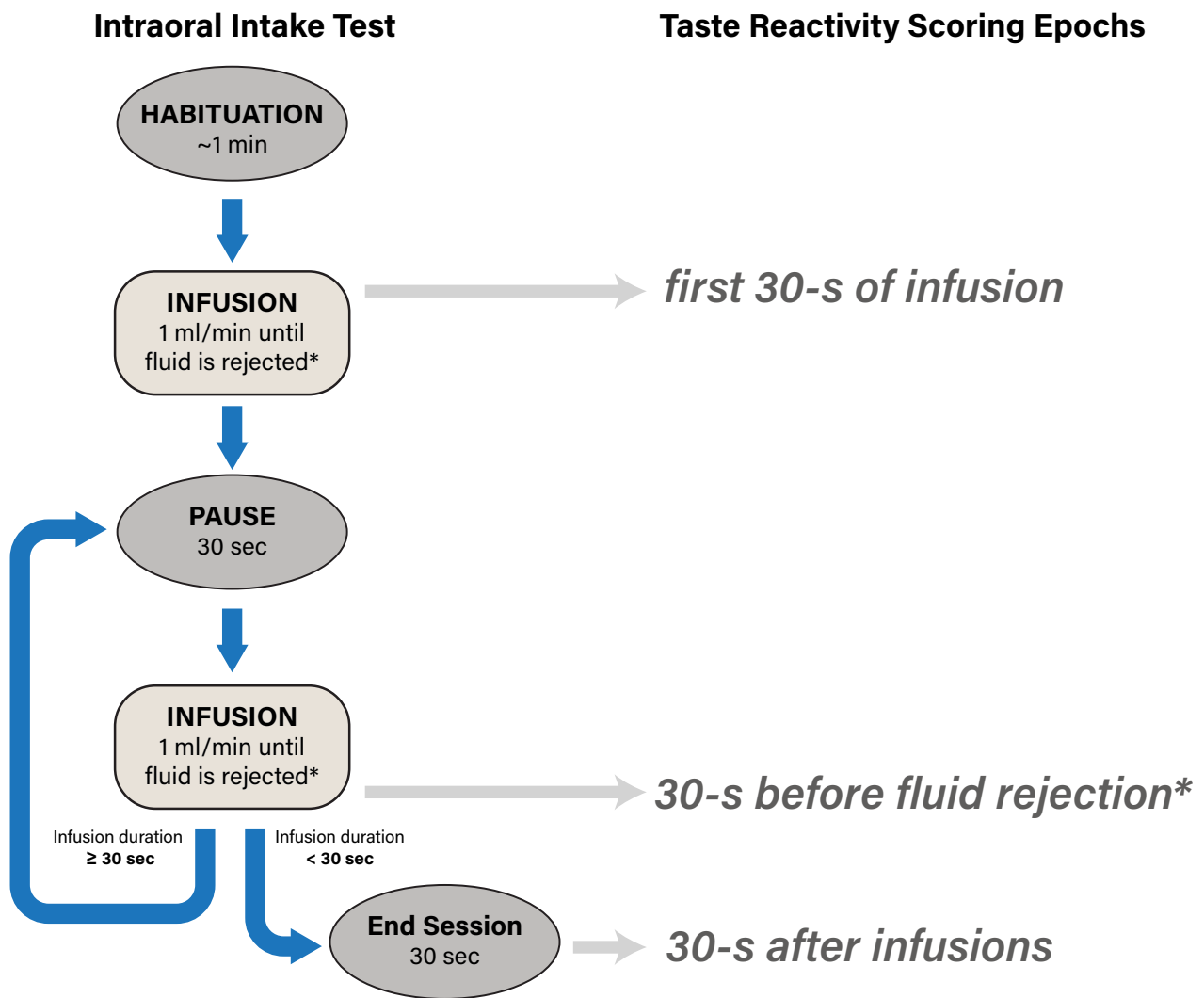
BODY MASS DURING SURGICAL RECOVERY

BODY MASS DURING TESTING

PROPORTION OF PRESURGICAL BODY MASS

DAY FROM SURGERY

DAY OF TESTING

- ● RYGB M (586.5 [12.8])
- ○ RYGB F (329.8 [6.7])
- ▼ SHAM M (564.6 [19.1])
- △ SHAM F (327.9 [4.3])
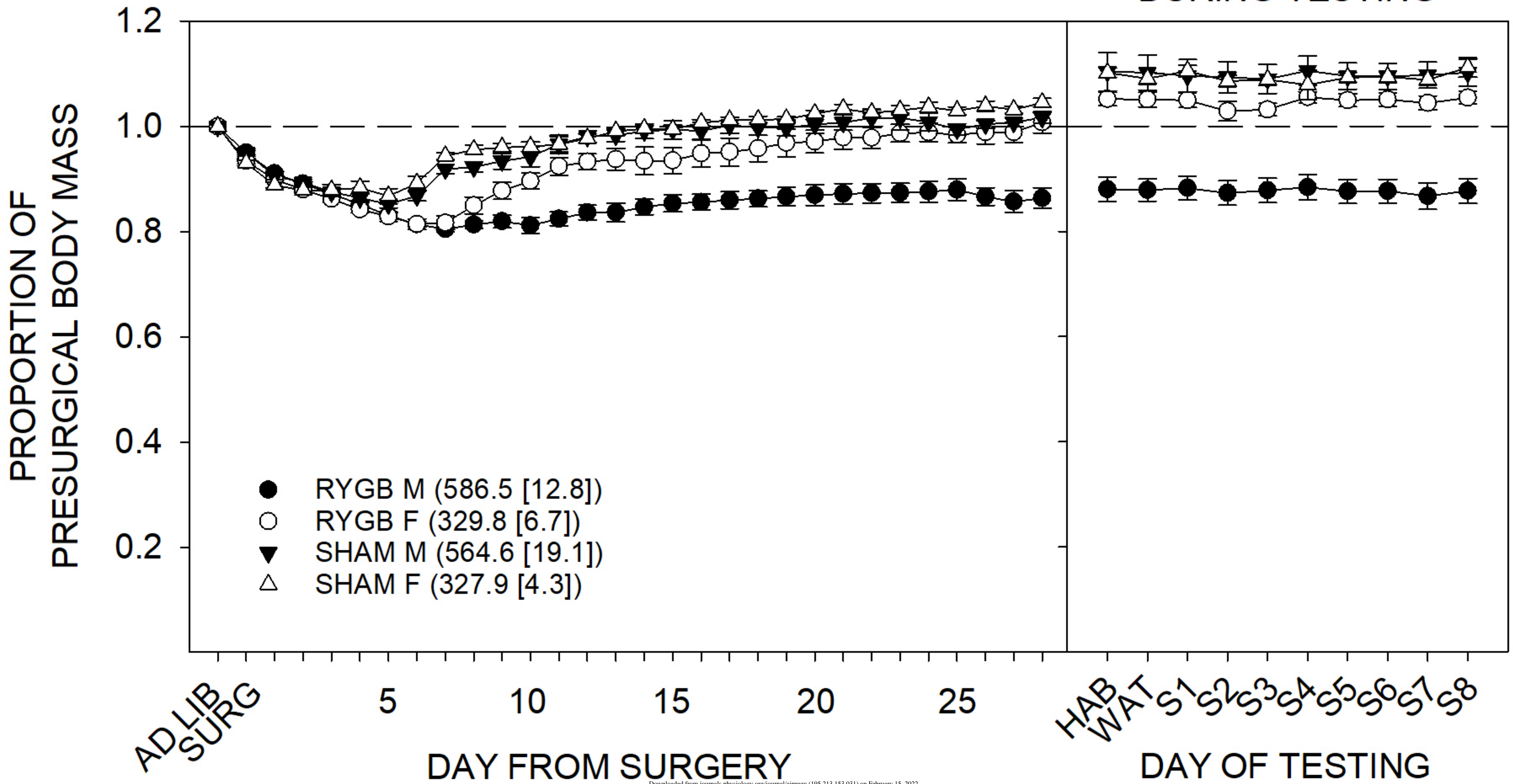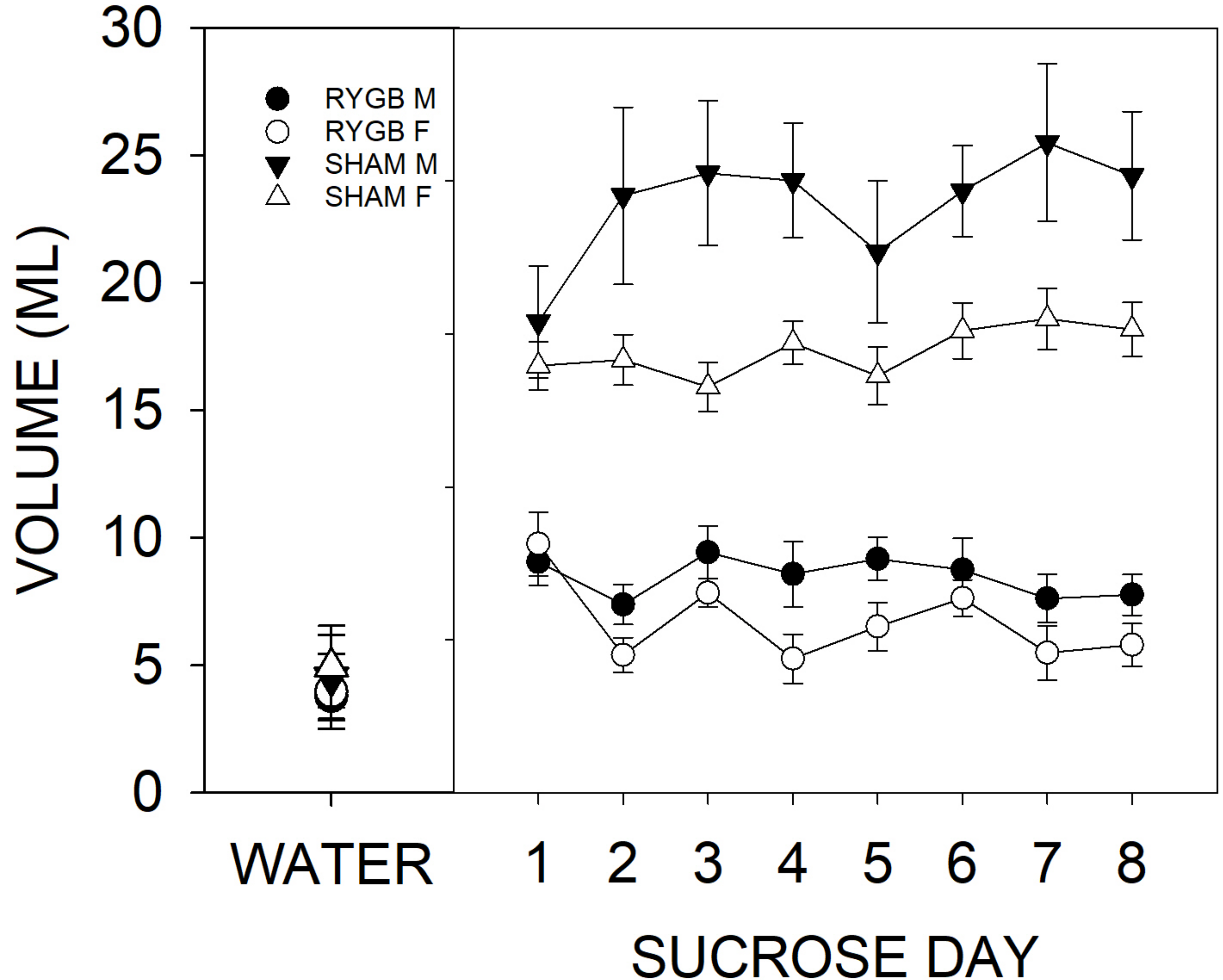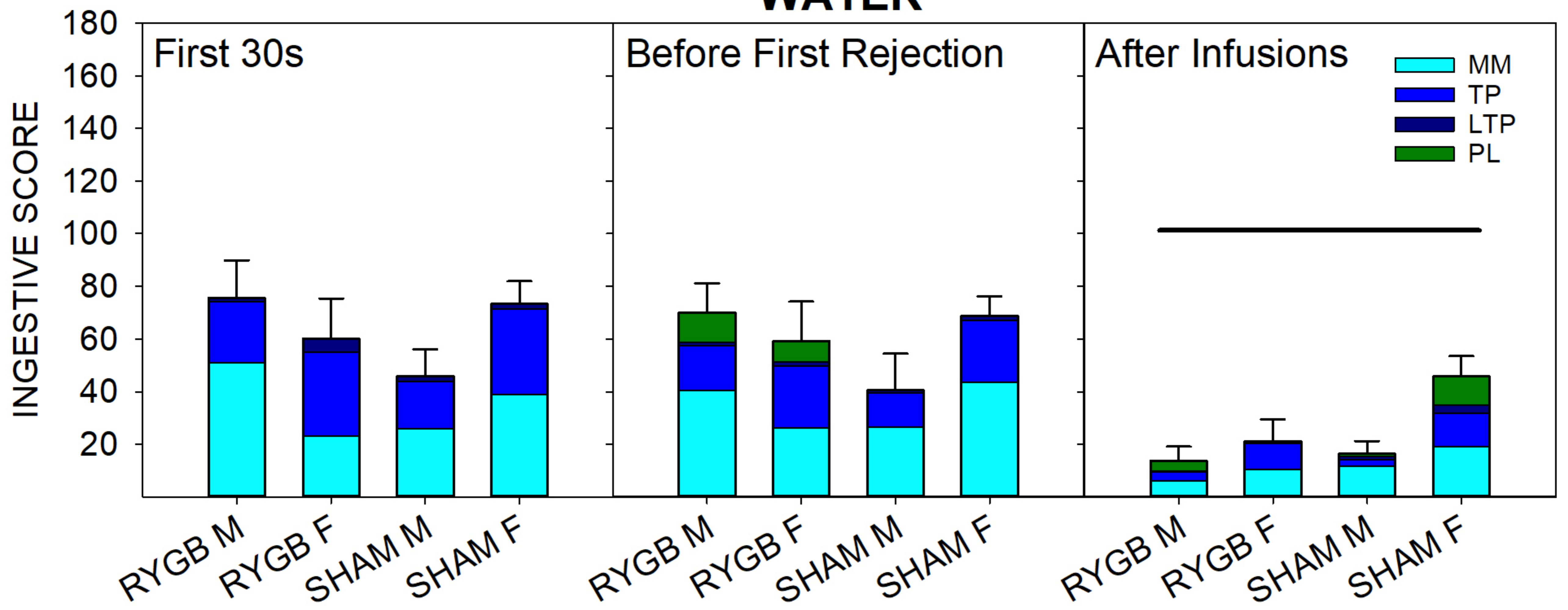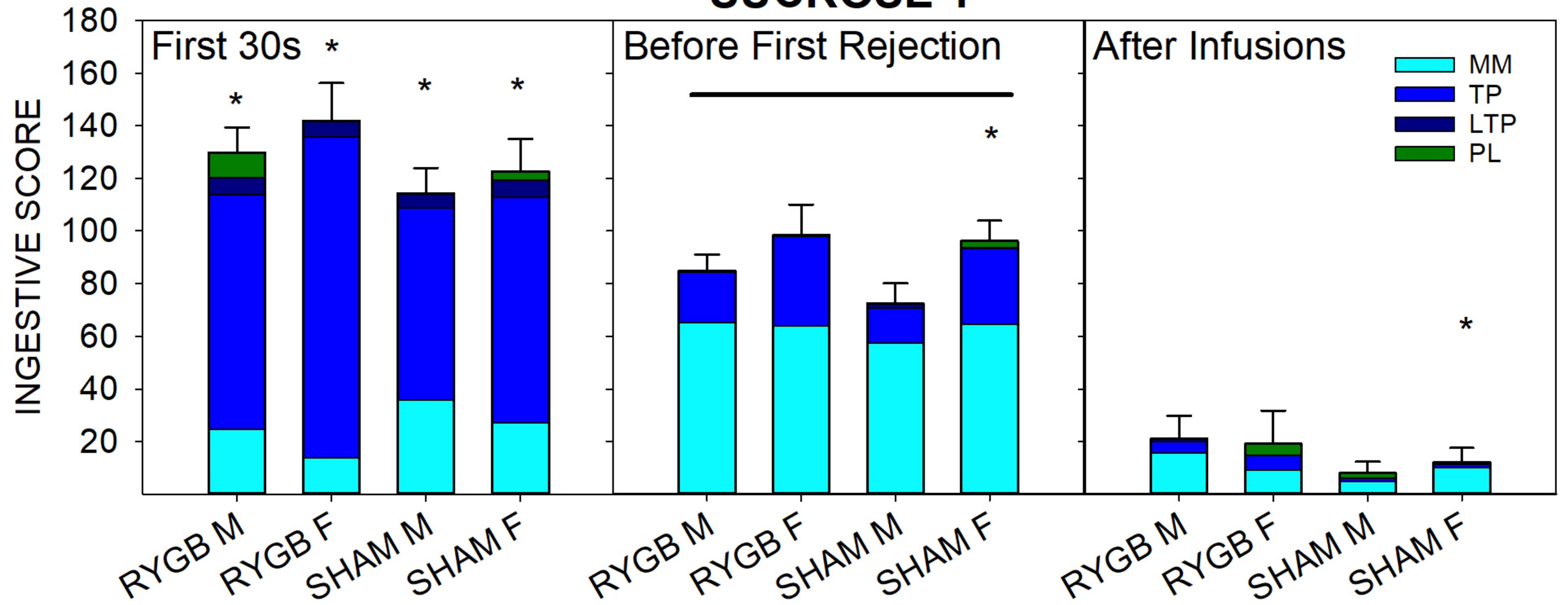
WATER

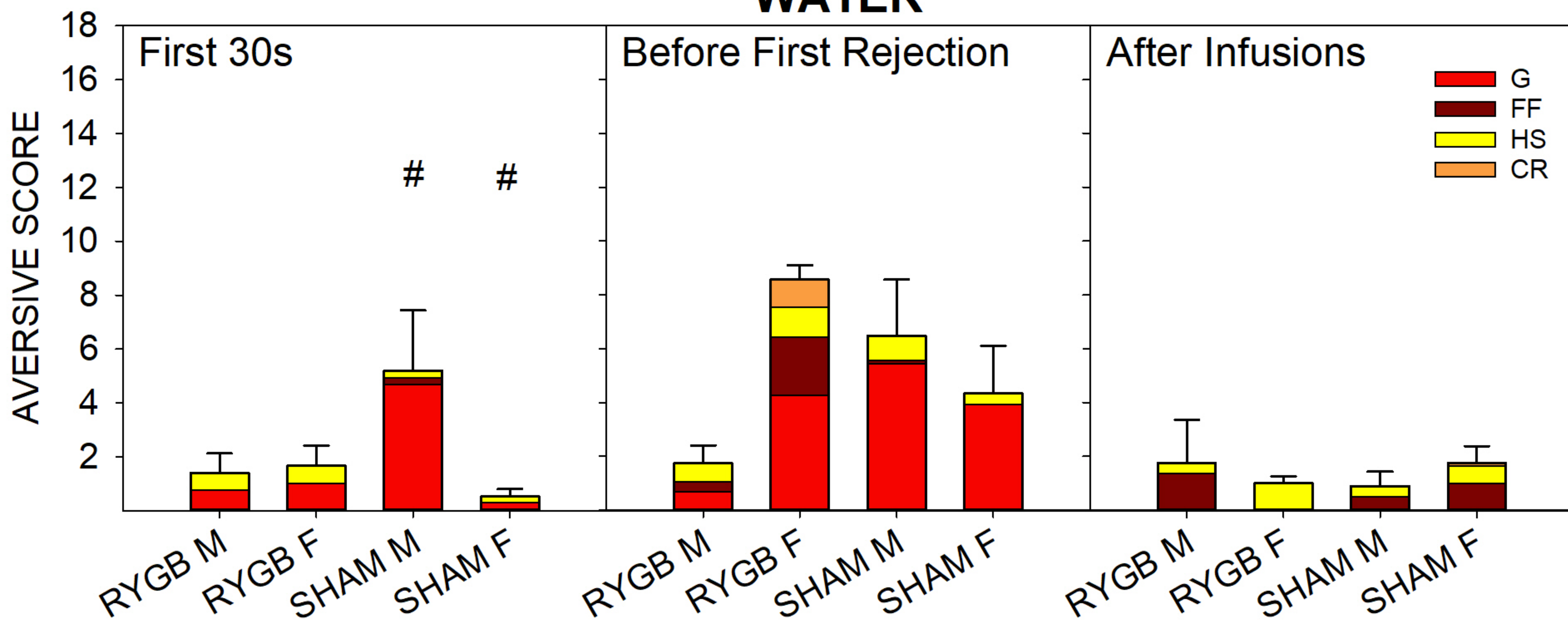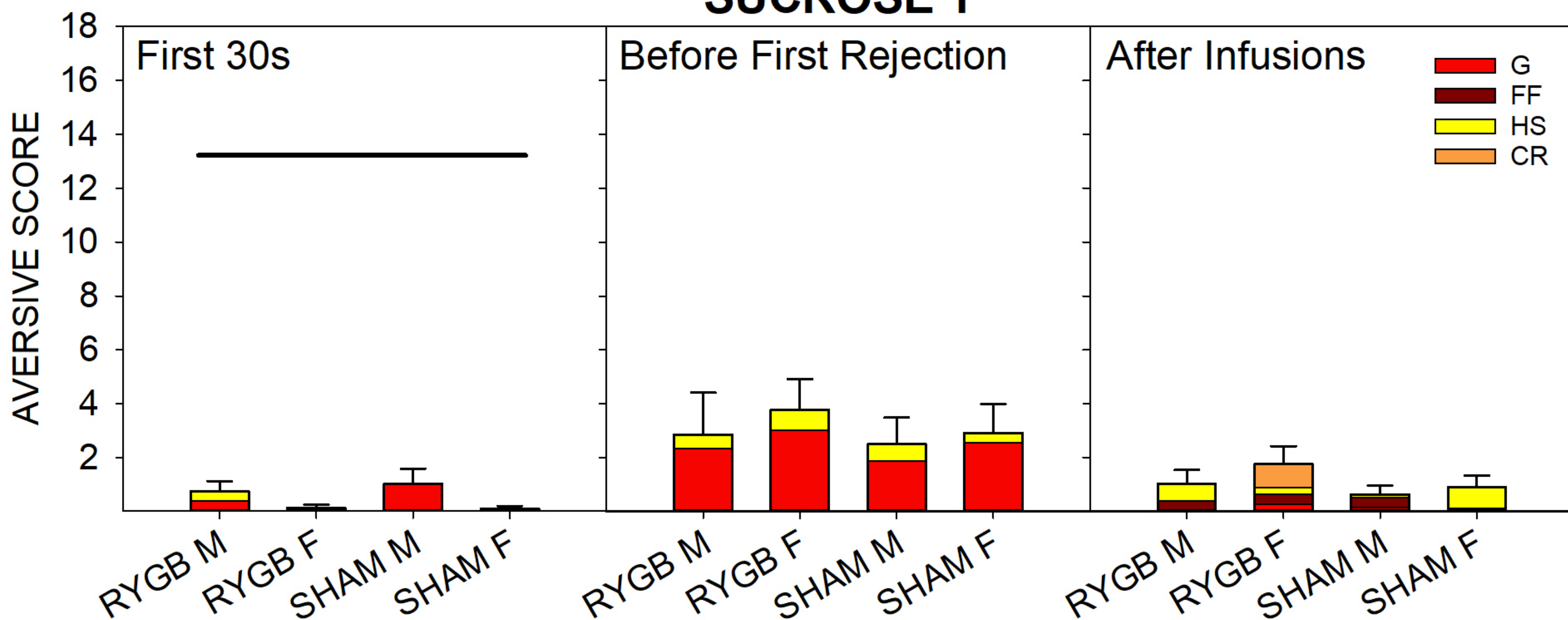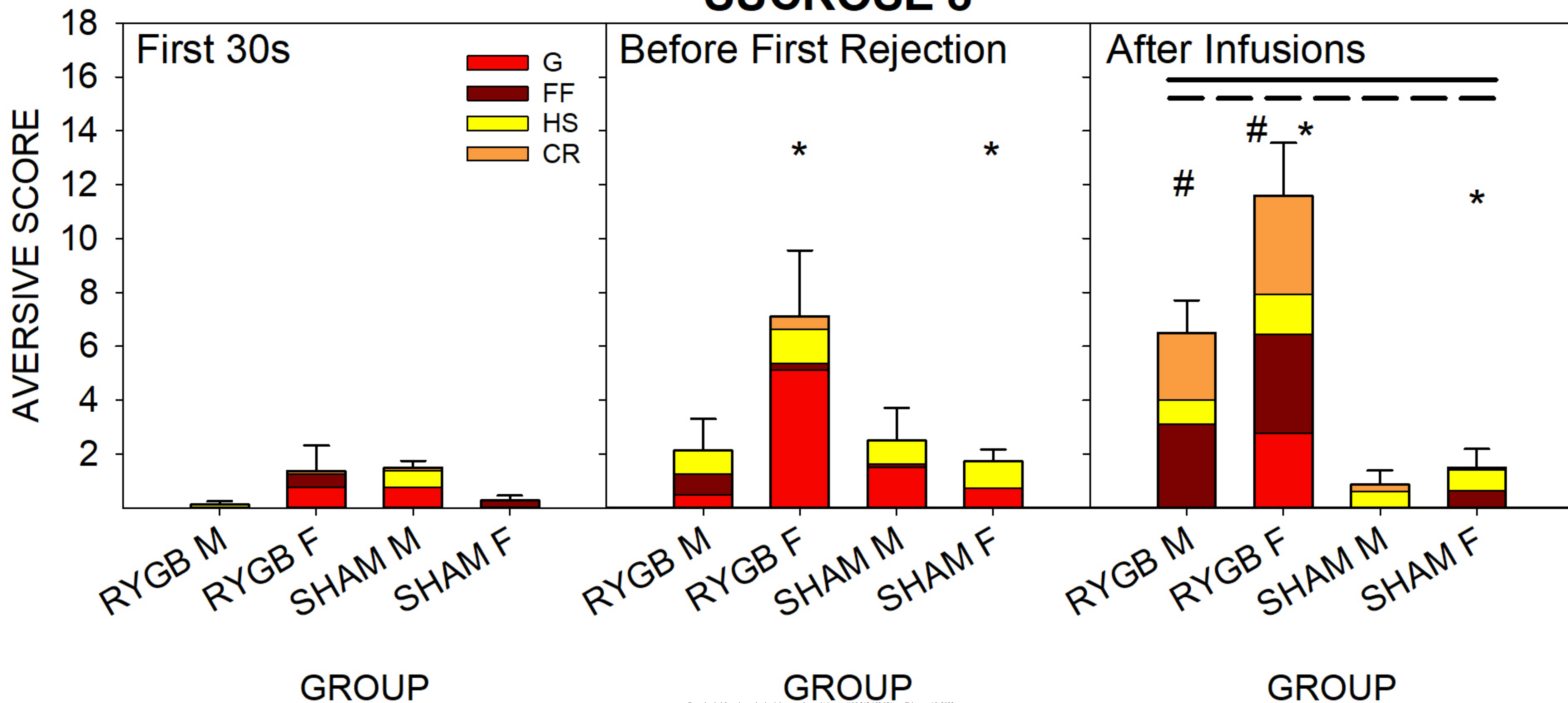# SUCROSE 1

SUCROSE 8

SUCROSE 8
AFTER LAST INFUSION
AVERSIVE RESPONSES