Bumblebee mouthparts exhibit poor acuity for the detection of pesticides in nectar

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Abstract (max 200 words)

Bees are important pollinators of agricultural crops, but their populations are at risk when pesticides are used. One of the largest risks bees face is poisoning of floral nectar and pollen by insecticides. Studies of bee detection of neonicotinoids have reported contradictory evidence about whether bees can taste these pesticides in sucrose solutions and hence avoid them. Here, we use an assay for the detection of food aversion combined with single-sensillum electrophysiology to test whether the mouthparts of the buff-tailed bumblebee (*Bombus terrestris*) detect the presence of pesticides in a solution that mimicked the nectar of oilseed rape (*Brassica napus*). Bees did not avoid consuming solutions containing concentrations of imidacloprid, thiamethoxam, clothianidin, or sulfoxaflor spanning six orders of magnitude, even when these solutions contained lethal doses. Only extremely high concentrations of the pesticides altered spiking in gustatory neurons through a slight reduction in firing rate or change in the rate of adaptation. These data provide strong evidence that bumblebees cannot detect or avoid field-relevant concentrations of pesticides using information from their mouthparts. As bees rarely contact floral nectar with other body parts, we predict that they are at high risk of unwittingly consuming pesticides in the nectar of pesticide-treated crops.

Keywords: Neonicotinoids, sulfoxaflor, bumblebees, oil seed rape, taste perception

Main text

Insect pollination is a critical ecosystem service. The most economically valuable pollinators are bees (Calderone, 2012; Garibaldi et al., 2014; Huang and An, 2018), especially domesticated bees that secure crop yield and quality (Meehan et al., 2011). In agricultural ecosystems, however, insecticides are widely used to protect crop yields (Klein et al., 2007). Using insecticides impacts the health and survival of bees, contributing to the recent population declines of all bee species (Goulson et al., 2015; Potts et al., 2016; van der Sluijs et al., 2013; Vanbergen et al., 2013). Hundreds of studies have shown that exposure to sublethal concentrations of neonicotinoid pesticides (i.e. imidacloprid, IMD, thiamethoxam, TMX, and clothianidin, CLO) in food impairs foraging behaviour, homing and orientation behaviour, and olfactory learning and memory (e.g., Gill et al., 2012; Henry et al., 2012; Parkinson et al., 2022a; Schneider et al., 2012a; Williamson et al., 2014; Wright et al., 2015). Most studies administer pesticides in sugar solutions; bees consume the solution, and then a change in behaviour or performance is recorded. If bees could detect and avoid pesticides in food, however, these substances could be used to defend crops against pests without risk to bees. Only a few have explicitly tested whether bees can taste and avoid neonicotinoid pesticides in nectar (Arce et al., 2018; Kessler et al., 2015; Muth et al., 2020), but the conclusions of these studies are contradictory. In addition, other compounds that affect cholinergic signalling in the insect nervous system, such as sulfoxaflor, have been proposed as alternatives to neonicotinoids, but whether or not these compounds can be detected by the bee's sense of taste has not previously been tested.

Insects detect non-volatile compounds (i.e. tastants) through activation of GRNs in contact chemosensilla on mouthparts, antennae, and tarsi which express receptor proteins that bind with specific types of ligands (e.g. sugars, non-nutrient 'bitter' compounds, salts, etc.) (Wright, 2016). When a taste receptor binds with its ligand, this sets off a signal transduction cascade that results in depolarization of the neuron (Dethier, 1976). For example, several types of receptors tuned to bitter substances are expressed

60 in a specialized subset of GRNs (Weiss et al., 2011). When these GRNs spike in
61 response to stimulation, feeding reflexes are inhibited (French et al., 2015). Bitter
62 compound detection is also accomplished by the inactivation of sugar-sensing GRNs
63 (French et al., 2015). For compounds which are potentially toxic, simultaneous activation
64 of GRNs that directly inhibit feeding reflexes and silencing of GRNs that activate feeding
65 make it possible to form a rapid response in reaction to contact (Wright, 2016). Thus, we
66 expected that if bees could detect neonicotinoids as bitter compounds, we would see
67 activation of bitter-sensing GRNs and/or inactivation of sugar-sensing GRNs. Previously,
68 we observed that stimulation of the mouthpart's galeal sensilla with neonicotinoids in
69 water did not elicit spikes from any GRNs, nor did we see a reduction in the rate of firing
70 of sugar-sensing GRNs in the sensilla when stimulated with a mixture of sucrose and
71 neonicotinoid compound (Kessler et al., 2015).

72 In a two choice assay, we unexpectedly found that when bees were given a choice
73 between sucrose solution and sucrose containing field-relevant doses of IMD and TMX
74 over a 24 h period, the bees choose the neonicotinoid solution (Kessler et al., 2015). This
75 could indicate that bees find neonicotinoid pesticides phagostimulatory, like sugars.
76 However, we also failed to find evidence that neonicotinoid pesticides in water elicited
77 spikes in any galeal GRNs including the nutrient/sugar sensing neurons. For this reason,
78 we concluded that bees could not taste neonicotinoids in nectar or on their own in water.
79 This conclusion, however, has been challenged by a study in free-flying *B. terrestris*
80 foragers (Arce et al., 2018). They found that free-flying bumblebees preferred specific
81 concentrations of solutions laced with TMX, but only after a period of at least 10 days of
82 feeding on the solutions (Arce et al., 2018). In this study, the location of the solutions was
83 randomized, forcing a choice at the point of feeding. These authors concluded that the
84 only way bees could solve this problem was if they used their sense of taste to identify
85 the preferred solution, arguing that further work needed to be done to identify the
86 mechanism for sensation. Subsequently, however, another study in a different species of
87 bumblebees (*B. impatiens*) using methods similar to ours reported that bees are neither
88 attracted nor deterred from consuming sugar solutions containing neonicotinoids,

consistent with the idea that they cannot detect the pesticides (Muth et al., 2020). Thus, whether or not bumblebees can taste neonicotinoids in food remains unresolved.

Bees have highly specialized mechanisms for encoding sugars which are different to those reported for any other insect species. For example, in bumblebee galeal sensilla, stimulation with sugars produces coherent spiking in 2 GRNs which burst in response to stimulation with high-value saccharide compounds (Miriyala et al., 2018; Parkinson et al., 2022b). We recently discovered that bumblebees also highly value the monosaccharide, fructose, and have a GRN tuned to detect it (Parkinson et al., 2022b). It is possible that changes to the burst pattern of firing could indicate that the presence of a compound such as a neonicotinoid in food. Furthermore, the type of sugar used to stimulate the sensilla could influence whether bees could taste neonicotinoids (Parkinson et al., 2022b). We also know that the labial palps are critical to toxin detection in other insect species (Chapman and Sword, 1993), and could house additional GRNs sensitive to bitter compounds like pesticides. Our previous work on neonicotinoid taste used sucrose to stimulate the galeal sensilla and did not analyse how pesticides might alter the burst structure of the galeal GRN response to sugars or test the labial palps. Clearly, deeper investigations could determine whether the mouthparts GRNs possess novel gustatory mechanisms for pesticide detection and provide critical information about the risk to bees of consuming the nectar of pesticide-defended crops in the field. They would also elucidate novel mechanisms for the detection of bitter compounds.

Here, we use a combination of sensitive behavioural assays and electrophysiology to test in detail whether bumblebee mouthparts have mechanisms to detect pesticides in nectar. Feeding assays using freely moving foragers make it possible to assess if bees detect and avoid potential toxins in food (Ma et al., 2016). To definitively test whether bumblebees detect neonicotinoids on their mouthparts, we quantified the structure of feeding when the mouthparts of freely-moving bumblebees (*B. terrestris)* were stimulated with nectar-like solutions containing pesticides over a 2 min period (Ma et al., 2016). We specifically tested a mixture of primarily fructose and glucose that mimicked the nectar of oilseed rape (*Brassica napus*) with field-relevant concentrations of neonicotinoids. In addition, we comprehensively tested these solutions on the gustatory receptor neurons

119 in the A-type sensilla on the galea and the labial palps of the bees' mouthparts using
120 electrophysiology to determine whether sensilla in multiple locations are able to detect
121 neonicotinoids (IMD, CLO, and TMX) and the previously untested sulfoxamine pesticide,
122 sulfoxaflor (SFX). The burst-spiking of the galeal GRNs was analysed for changes to
123 structure and compared to the GRN response to the bitter compound, quinine (QUI),
124 which has been reported previously to be a feeding deterrent to bees (Ma et al., 2016;
125 Wright et al., 2010).

126

127 **Methods**

128 *Bumblebee colonies*

129 Bumblebee colonies (*Bombus terrestris audax,* Biobest, Westerlo, Belgium*)* were
130 maintained at laboratory conditions (22-27°C and 35-40% RH) at the University of Oxford
131 and fed *ad libitum* with the proprietary sugar syrup provided with the colonies (BioGluc®,
132 Biobest, Westerlo, Belgium) containing fructose (37.5%), glucose (34.5%), sucrose
133 (25%), maltose (2%), oligosaccharides (1%), and the preservatives potassium sorbate
134 (E202) 0.15% and citric acid (E330) 0.06% (Wäckers et al., 2017). Bumblebees were
135 provided with freeze-dried honeybee collected pollen (approx. 10 g) (Agralan Growers,
136 Wiltshire, UK) three times/week.
137
138 In total, 12 colonies were used for the taste assays and 6 colonies were used for the
139 electrophysiological study. Bees were randomly assigned to a treatment and colonies
140 were counterbalanced across treatments to control for intercolony variation.

141 *Solutions*

142

143 An artificial oilseed rape nectar sugar solution was created containing 1.04 M glucose (D-
144 (+)-Glucose, Sigma Aldrich), 0.746 M fructose (D-(-)-Fructose, Sigma Aldrich), and 0.007
145 M sucrose (Sigma Aldrich) (Carruthers et al., 2017), hereafter referred to as 'OSR'.
146

147 Stock solutions (1 mM) were made for each pesticide in OSR solution. No solvents are
148 needed at these concentrations as all pesticides used are readily soluble in water at
149 concentrations far exceeding 1 mM. Concentrations of imidacloprid (IMD), thiamethoxam
150 (TMX), and clothianidin (CLO) in floral nectar range from 1.3 to 64 nM (Wood and
151 Goulson, 2017), and sulfoxaflor (SFX) can reach concentrations as high as 71.4 μM (U.S.
152 Environmental Protection Agency (EPA), 2014). In order to assess field-relevant
153 concentrations of each chemical, a concentration series of each of IMD, TMX, CLO
154 (Sigma Aldrich) and SFX (Chem Service) was made via tenfold serial dilution using OSR
155 to produce nectar containing the pesticides at 1000, 100, 10, and 1, and 0 nM (pure OSR).
156 Additionally, 0.1 mM of each chemical dissolved in OSR was assessed alongside 0.1 mM
157 and 1.0 mM QUI ((-)-Quinine hydrochloride dihydrate, Sigma Aldrich) in OSR, which
158 functioned as positive controls for the effects of a bitter tastant. A concentration of 0.1
159 mM was chosen for the pesticides as this encompasses the higher range of SFX found
160 in nectar, and allowed us to see if extremely high concentrations of IMD, TMX and CLO
161 could be detected by bees.

162 *Aversive taste assay*

163 Bumblebee workers were collected directly from the colony under red light and placed
164 individually into plastic holding vials with ventilation holes drilled into the lid. To minimise
165 inclusion of nest bees (i.e. bees that never or rarely forage), only workers with a thorax
166 width > 4.0 mm were used in these experiments (Goulson et al., 2002). In order to
167 motivate the bees to feed during the assay, bees were deprived of food between 3-6 h.
168 Bees were held in individual plastic holding vials at laboratory conditions in total darkness
169 throughout the starvation period. Following the starvation period, individual bees were
170 transferred into a 15 ml falcon tube, modified such that the tip of the tube was removed
171 and a small (10 mm x 20 mm) steel mesh was affixed contiguous with the resulting hole.
172 Bees could extend their proboscis through this hole to reach the feeding solutions, whilst
173 the mesh served as a surface for the bees to grip onto to maintain their position during
174 feeding. The tube was then affixed to a polystyrene holder with dental wax (Ma et al.,
175 2016).

176

177 A 60 mm long, 100 µL glass capillary tube was filled with a test solution and scanned onto
178 a computer at 600 dpi to produce an image from which the start volume could be
179 measured. The capillary tube was then connected to a silicone tube which was in turn
180 connected to a 1.0 ml syringe via a female connector, as in (Ma et al., 2016). This syringe
181 functioned as a pipette bulb to maintain the feeding solution at the tip of the microcapillary.
182 The capillary was held in place in the apparatus via a 1.0 ml modified syringe affixed to a
183 micromanipulator. A DinoLite digital microscope camera (Model AM4815ZT, DinoLite,
184 The Netherlands) was positioned 20 cm above the feeding site where the bees accessed
185 the test solutions. This was connected to a computer and set to record using DinoLite
186 Digital Microscope software (DinoLite, The Netherlands) at a frame rate of 640 X 480 at
187 25X magnification. All feeding behaviour was recorded for later analysis.
188

189 Once a bee was positioned in the apparatus, they were given 3 min to habituate to their
190 environment before the assay began. Following this, bees were encouraged to extend
191 their proboscis by touching the bee's terminal antennomers with a droplet (~3.5 µL) of 0.5
192 M sucrose (Sigma Aldrich) dissolved in deionised water. Once the bee extended their
193 proboscis, the droplet was presented to the bees' mouthparts for them to consume. Each
194 bee was given up to 5 min to extend their proboscis and consume the droplet. Bees that
195 did not do so were removed from the experiment. When the bee finished consuming the
196 droplet, the microcapillary tube containing the test solution was presented to the bees
197 extended mouthparts. The test phase began once the bee's proboscis contacted the test
198 solution, and the bee was given 2 min to consume the solution. After feeding,
199 microcapillaries were re-scanned to measure the end volume.

200 All experimenters were blind to the experimental treatments. All solutions were tested in
201 a randomised order and treatments were counterbalanced over time to eliminate any
202 effects of starvation time on feeding behaviour.

203 *Measuring solution consumption and feeding behaviour*

204 ImageJ (Schneider et al., 2012b) was used to produce measurements of the length of
205 solution in each microcapillary before and after the assay. The reference scale was set
206 to 60.0 mm. Image files were zoomed in to 400% and the length of the solution inside the
207 microcapillary was measured meniscus to meniscus. The length of test solution
208 consumed by each bee was calculated as the difference between the measured length
209 of the liquid inside the microcapillary tube before and after the test phase. These lengths
210 were then converted to volumes using the formula:
211
212 $$(100\ \mu L \times X\ mm) / 60.0\ mm$$
213
214 Where 100 µL is the maximum volume of the capillary tube, 60.0 mm is the length of the
215 capillary tube, and X mm is the amount of solution consumed as a measured length within
216 the capillary.
217
218 A feeding bout is defined as a period in which contact is made between the proboscis and
219 test solution that is not separated by an absence of contacts for 5 s or more. The number
220 of feeding bouts and their duration can be used to evaluate the phagostimulatory or
221 deterrent activity of the compounds tested (Ma et al., 2016). To identify bouts of feeding
222 behaviour, videos were played back at 50 % speed and periods of contact between the
223 bees' mouthparts and the solution were scored using the Noldus Observer (Noldus, the
224 Netherlands).

225 *Electrophysiology*

226 We performed extracellular tip recordings on individual taste sensilla on the bumblebee
227 mouthparts to assess the peripheral taste perception of sucrose, a 10% dilution of oilseed
228 rape nectar (10% OSR), and the addition of pesticides or QUI to 10% OSR. We recorded
229 from 'A-type' sensilla from the galea (Gal), which are known to produce bursting spike
230 trains in response to sugars (Miriyala et al., 2018) and the third and fourth segments of
231 the labial palps (LP_{III} and LP_{IV}, respectively, Figure 1 A). Bumblebees were prepared for
232 electrophysiology as described previously (Miriyala et al., 2018; Parkinson et al., 2022b).
233 Sensilla were stimulated for 5 s with a borosilicate (Clark capillary glass 30-066,

234 GC150TF-10) recording electrode (20 µm tip diameter, made with a Narishige PC-10
235 electrode puller) containing the test solution. Signals were acquired with a pre-amplifier
236 (TasteProbe; Syntech, Germany), amplified (AC amplifier 1800, A-M Systems, USA),
237 digitized at 30 kHz (DT9803 Data Translation) and stored using DataView (version 11.5).
238 Pesticides (IMD, CLO, TMX, SFX) were tested in a background of 10% OSR. Pesticides
239 were tested using a concentration gradient (1, 10, 100, and 1000 nM) and also at 0.1 mM.
240 Additionally, QUI was tested in 10% OSR at concentrations of 0.1 and 1.0 mM. A
241 minimum inter-stimulus interval of 3 min was used to prevent sensory adaptation. We
242 obtained recordings from n=12 bee per compound and tested 2-4 sensilla per mouthpart
243 location (6-12 sensilla per bee). To ensure the pesticides or QUI were not imposing a
244 toxic effect on the taste sensilla, we included recordings at the beginning and end of each
245 stimulation series with 10% OSR (SI Figure 1).

246 *Spike detection*

247 Spikes from GRNs on the galea were extracted as described previously (Parkinson et al.,
248 2022b). Briefly, after band-pass filtering (300-2500 Hz, second order butterworth filter)
249 and normalization, spikes were detected using the peakfinder function with adequate
250 thresholds manually set for each recording. Interspike intervals were used to detect the
251 end of burst positions (EOB). Using this method, we detected three GRNs within the
252 galeal electrophysiological recordings, as seen previously (Parkinson et al., 2022b).
253 GRNs of the labial palps were non-bursting, and visual inspection of the data suggested
254 that two GRNs were present in these recordings. The spike waveforms for each recording
255 were compared to differentiate the spikes from the putative units. Waveforms were
256 aligned from the peak +/- 2 ms, and the Matlab singular value decomposition (SVD)
257 function was used to obtain the principal components of the spike waveforms. Waveforms
258 were then projected onto the space spanned by the first two principal components, and
259 k-means clustering was used to assign the waveforms to units for each recording. Using
260 this method, two (or in some cases just one) GRNs were obtained from each recording,
261 with the unit labelled GRN1 represented by larger waveform amplitudes and higher spike
262 frequencies in response to sugars.

263 *Statistical analyses and data presentation*

264 We performed all further analyses in R version 4.2.1 (Team, 2016). Average firing rates
265 of the 7 GRN types (Gal GRNs 1-3, LP_{III} GRNs 1-2, and LP_{IV} GRNs 1-2) across mouthpart
266 locations were compared. Firing rates of a given GRN type were averaged across sensilla
267 from a single animal, and log transformed ($\log_{10}$(average_rate + 1)) to fit a normal
268 distribution. We compared electrophysiological responses across stimuli using linear
269 mixed effects models (LME, lmerTest toolbox) (Kuznetsova et al., 2017) with Gaussian
270 distributions and "bee ID" as the random effect (avg_rate~stimulus*GRN+(1|BeeID)).
271 Models with non-significant interaction terms were re-run without the term
272 (avg_rate~stimulus+GRN+(1|BeeID)). *Post hoc* analyses were performed using
273 estimated marginal means (emmeans toolbox, (Lenth et al., 2018)) with Tukey's
274 adjustment for family-wise error rates. Significant effects were denoted with asterisks (*,
275 <0.05; ** <0.001; ***, <0.0001).
276
277 The temporal patterns of GRN activity over time was also assessed by extracting spike
278 rates for each GRN within 0.1 s bins from 0.1 to 1.1 s after stimulation. Firing rates within
279 each bin were averaged by animal for a given GRN type. As the labial palp GRNs did not
280 display a bursting pattern, we averaged the firing rate within each bin across all four labial
281 palp GRNs. The pattern of bursting of galeal GRNs (GRNs 1 and 2) was assessed using
282 two measures: the burst length (number of GRN1 spikes per burst) and burst rate. Firing
283 rate histograms were constructed using these measurements over 1 s of stimulation, and
284 stimuli were compared with LME models (temporal_parameter~stimulus+bin+(1|BeeID))
285 (lmerTest toolbox) (Kuznetsova et al., 2017). Significant differences in the slopes between
286 stimuli were denoted on plots with letters, assessed with the estimated marginal means
287 (emmeans) toolbox (Lenth et al., 2018) with Tukey's adjustment for family-wise error
288 rates. To assess differences in the complete temporal code of all 7 GRNs across
289 mouthparts, we aligned the binned spike times in series for each GRN. For each animal
290 and stimulus, there was a vector of 70 bins (ten 0.1s bin per GRN type, aligned for 7
291 GRNs). To reduce the variability in responses between animals and balance the scale of
292 responses across GRNs, we normalized the responses across GRN replicates within
293 each animal by dividing by the average responses of each GRN to 10% OSR. The

294 normalized responses of single GRNs were then averaged across replicates for each
295 animal. We used t-distributed stochastic neighbour embedding (t-SNE) to reduce the
296 dimensionality of the temporal data to two axes for each dataset (OSR vs sucrose, QUI,
297 and pesticides). Results from t-SNE were then clustered to reveal grouping of responses
298 by stimulus. Optimal cluster count was predicted using Monte Carlo reference-based
299 consensus clustering (M3C, (John et al., 2020)), an unsupervised learning technique that
300 aggregates across k-means clustering runs. Optimal clustering (if k>1) was included on
301 t-SNE plots.
302
303 Measurements of feeding behaviour (volume consumed, first bout, and cumulative bout)
304 were compared using t-tests (for sucrose vs OSR) or LME for concentration gradient
305 comparisons of pesticide mixtures and quinine versus OSR with "colony" as a random
306 effect (feeding_measurement~stimulus+concentration+(1|ColonyID) (lmerTest toolbox)
307 (Kuznetsova et al., 2017 with emmeans *post hoc* tests (emmeans toolbox) (Lenth et al.,
308 2018) with Tukey's adjustment for family-wise error rates. One-way Analysis of Variance
309 (AOV) was used to compare feeding behaviour measurements across high
310 concentrations of pesticides versus OSR. Behavioural data that was not normally
311 distributed was log transformed. For all boxplots, the median is indicated by a line, bounds
312 of the box mark the 1st and 3rd quartile, and whiskers extend to 1.5 times the interquartile
313 range. Where data points are drawn, these represent the responses of individual GRNs.

314 **Results**

315 ***GRNs encode differences in sugar solution composition***

316 Responses of bumblebee galeal GRNs to individual sugar compounds have previously
317 been described (Miriyala et al., 2018; Parkinson et al., 2022b). Here, we tested whether
318 a mixture of fructose, glucose, and negligible amounts of sucrose that mimics OSR nectar
319 altered the GRN spiking responses compared to sucrose alone. We also expanded our
320 investigation to include responses form GRNs in the labial palps (Figure 1 A). We found
321 that GRNs in each location had specific patterns of activation that characterized their
322 responses to sugar stimulation (Figure 1 B). Stimulation of galeal sensilla with sucrose or
323 with OSR nectar produced bursts of spikes resulting from the co-activation of two GRNs

324 (Gal1 and Gal2), as we found for individual sugars previously (Miriyala et al., 2018;
325 Parkinson et al., 2022b, Figure 1 B). Each labial palp sensillum (LP_{III} and LP_{IV}) contained
326 two sugar-sensitive GRNs (LP_{III} 1 and 2, LP_{IV} 1 and 2), but these GRNs exhibited tonic
327 firing patterns in response to stimulation instead of bursts of spikes (Figure 1 B). The
328 spiking rates averaged over 1 s stimulation of Gal1 and the labial palp GRNs were
329 unchanged towards the two stimuli, but the average rate of Gal2 and Gal3 when
330 stimulated with sucrose was lower than the rate for stimulation with 10% OSR (Figure 1
331 C, LME, stimulus*GRN $F_{6,1607}$ = 9.16, p < 0.0001). (Note: GRN3 was active only when
332 stimulated with 10% OSR, but not by an equimolar sucrose solution, Figure 1 C).
333
334 We compared the time-varying responses of GRNs using binned spikes (0.1 s bins over
335 1 s stimulation). We found that the rate of adaptation, measured by the change in the
336 interspike interval of labial palp GRNs were not significantly different for 10% OSR or
337 sucrose (Figure 1 D, stim: $F_{1,461}$ = 3.11, p > 0.05; time, $F_{1,450}$ = 586, p < 0.0001; SI Figure
338 1 B). However, galeal GRN burst structure (Gal1 and Gal2) differed between stimuli
339 (Figure 1 E, stim*GRN: $F_{1,938}$ = 13.8, p = 0.0002, time: $F_{1,938}$ = 1.42, p > 0.05). OSR
340 elicited a 20% higher burst rate (i.e., Gal2 rate, SI Figure 1 C) and shorter bursts (i.e.,
341 fewer Gal1 spikes per burst, SI Figure 1 D), although the rate of adaptation of Gal1 did
342 not differ as a function of stimulus (Figure 1 E). To understand if the population of neurons
343 we recorded from contained information about stimulus identity, we combined the
344 temporal responses binned at 100 ms intervals over 1 s of recording of all 7 GRNs across
345 mouthparts using a clustering algorithm (t-SNE and k-means clustering). The algorithm
346 predicted two statistically separate stimulation groups, one for OSR and one for sucrose
347 (Figure 1 F).
348
349 In spite of the differences observed in GRN responses, the total volume of the 10% OSR
350 and equimolar sucrose solutions (1.79 M, Figure 1 G) consumed by bumblebees was not
351 significantly different in the free-feeding assay (Figure 1 H, t-test, t = -1.44, df = 28, p =
352 0.49). We also did not observe a difference in the duration of the first feeding bout (Figure
353 1 I, t-test, t = -0.50, df = 27, p = 0.081), or the total time the bees spent in contact with the
354 solutions over 2 min (Figure 1 J, t-test, t = -0.837, df = 27, p = 0.38). We also tested

355 whether the concentration of these sugar solutions influenced behaviour using a
356 concentration gradient of OSR and sucrose solutions at 2.0, 1.5, 1.0, 0.5 M and water. A
357 greater volume of sucrose was consumed for the 1 M and 1.5 M concentrations than for
358 the OSR solution, but there was no difference at any other concentration (SI Figure 2).
359 First bout and total contact duration were not significantly different.
360
361 ***Labial palp GRNs may enable bitter detection***
362 QUI is routinely used to test an animal's sensitivity to bitter substances. Here, we tested
363 a concentration series of QUI in 10% OSR solution to test how GRNs on the labial palps
364 and galea respond to QUI, for use as a positive control for our tests with pesticides. A 1
365 mM QUI concentration suppressed all galeal and labial palp GRNs, while 0.1 mM QUI
366 suppressed the activity of $LP_{III}2$ and $LP_{IV}2$ alone (Figure 2 A, LME, stimulus*GRN $F_{12,1581}$
367 = 11.3, $p < 0.0001$, SI Figure 3 A-C). The rate of adaptation, however, of the labial palp
368 GRNs was faster when they were stimulated with the 0.1 mM QUI solution (Figure 2 B,
369 stim*time: $F_{1,450} = 13.8$, $p = 0.0002$). Galeal GRN burst structure was affected by the
370 addition of QUI (Figure 2 C, stim*GRN: $F_{2,1116} = 308$, $p < 0.0001$, time: $F_{1,1116} = 0.062$, p
371 = 0.803; SI Figure 3 B-C). Gal1 adapted faster when stimulated with 0.1 mM QUI
372 compared to QUI, although there was no effect on Gal2 (Figure 2 C). Clustering of all
373 GRN responses predicted two groups, with the responses to 1 mM QUI separating from
374 the responses to 10% OSR and 0.1 mM QUI (Figure 2 D). OSR and 0.1 mM QUI were
375 not significantly different.
376
377 The detection threshold observed in the behaviour experiments was lower than predicted
378 by the t-SNE clustering but consistent with our measurements of adaptation in labial palp
379 neurons. Bumblebees consumed significantly less OSR solution when it contained 0.1
380 mM QUI, while 1 mM QUI was completely deterrent (Figure 2 E, LME, concentration: $F_{2,35}$
381 = 49.1, $p < 0.0001$). However, the structure of feeding towards 1 mM QUI was similar to
382 the results found for the cluster analysis of the GRNs: bees tested with 1 mM QUI
383 exhibited much shorter average first feeding bout duration (Figure 2 F, LME,
384 concentration: $F_{2,40} = 18.2$, $p < 0.0001$) and shorter average cumulative bout duration

385 (Figure 2 G, LME, concentration: $F_{2,36}$ = 61.5, p < 0.0001) than those fed with OSR or 0.1
386 mM QUI solution.
387
388 ***Extremely high concentrations of pesticides in OSR nectar reduce the rate of***
389 ***spiking of GRNs***
390 In our previous work (Kessler et al. (2015), field-relevent concentrations of neonicotinoid
391 pesticides were not detected by galeal GRNs. Here, we used OSR instead of sucrose
392 and included sulfoxaflor. We also extended this experiment to test the labial palps. As
393 before, we found no significant difference in galeal GRN responses between the control
394 (OSR) solution and all of the solutions containing pesticides at field-relevant
395 concentrations (SI Figure 4). No significant differences were detected in the responses of
396 the labial palp neurons to the pesticide solutions (SI Figure 4).
397
398 To test whether all concentrations of pesticides go undetected by bees, we stimulated
399 GRNs with 0.1 mM of each pesticide mixed with 10% OSR. Such concentrations are 10-
400 100k fold greater than has been reported for neonicotinoids from the nectar and pollen of
401 agricultural crops and are at least 1k fold greater than the LD_{50} values for bumblebees
402 (Cresswell et al., 2014; Mundy-Heisz et al., 2022; Siviter et al., 2022). Sulfoxaflor,
403 however, is present at much higher concentrations in the field (e.g., 71.4 μM, U.S.
404 Environmental Protection Agency (EPA), 2014)). Stimulation with extremely high
405 concentrations of TMX reduced the average firing rates of $LP_{III}2$ and $LP_{IV}2$; none of the
406 other pesticides affected the average GRN firing rates (Figure 3 A, LME, stimulus*GRN:
407 $F_{24,2139}$ = 1.65, p = 0.024.)
408
409 We also examined how the temporal response of galeal and labial palp neurons was
410 influenced by stimulation with extreme concentrations of the pesticides. Labial palp GRNs
411 adapted faster when IMD or TMX were added to 10% OSR (Figure 3 B, stim: $F_{1,821}$ =
412 49.9, p < 0.0001; time, $F_{1,808}$ = 571, p < 0.0001), and firing rates of labial palp GRNs over
413 1 s were significantly reduced (SI Figure 5 A). Gal1 and Gal2 spiking and adaptation rates
414 were affected by CLO and IMD (Figure 3 C, stim*GRN: $F_{4,1653}$ = 3.47, p = 0.0078; time,
415 $F_{1,1653}$ = 2.33, p = 0.127). Galeal GRN bursting rate was slightly reduced when stimulated

416 with OSR mixed with CLO or IMD (SI Figure 5 B), while the burst length was not affected
417 by the addition of pesticides (SI Figure 5 C). We used the same clustering algorithm that
418 was able to differentiate the responses to sucrose and OSR. Clustering using all of the
419 data for each of the sensillum types failed to separate the pesticide responses from
420 responses to the control (10% OSR, Figure 3 D).
421
422 ***Bumblebees readily consume pesticides in nectar***
423 We assessed whether the pesticides were aversive in the range of values that included
424 field-realistic concentrations (0 - 1000 nM) and one of the extreme values (0.1mM) added
425 to a 10% OSR solution. Field-relevant concentrations of the pesticides in OSR nectar did
426 not deter the bees from feeding, as the presence of these compounds did not significantly
427 alter the amount of food consumed (LME, concentration: $F_{4,297}$ = 0.209, p = 0.933), first
428 bout duration (LME, concentration: $F_{4,290}$ = 0.648, p = 0.629) or cumulative bout duration
429 (LME, concentration: $F_{4,288}$ = 0.310, p = 0.871) over the 2 min assay period (Figure 4 A-
430 C). Importantly, we also tested feeding behaviour using an extreme concentration (0.1
431 mM) of the pesticides in OSR. Even at this very high concentration, we did not observe a
432 significant difference in the amount of food consumed (AOV: $F_{4,78}$ = 0.975, p = 0.426),
433 first bout duration (AOV, $F_{4,74}$ = 0.403, p = 0.806), or cumulative bout duration (AOV, $F_{4,74}$
434 = 1.22, p = 0.309) over 2 min (Figure 4 D-F).
435

436 **Discussion and conclusions**

437 For the first time, we report how populations of GRNs on the bee's mouthparts respond
438 to stimulation with putatively bitter compounds. We verified that bees do not have
439 mechanisms on their mouthparts that enable them to taste neonicotinoids or sulfoxaflor
440 when these compounds are present in nectar. Neonicotinoids and sulfoxaflor did not
441 change the bursting of sugar-sensing neurons in the galea, nor did they elicit or inhibit
442 spikes in labial palp neurons. Strikingly, we show that bumblebees do not avoid drinking
443 OSR nectar solutions even when very high concentrations of common pesticides are
444 present. Very high concentrations of these pesticides could produce a small but
445 measurable reduction in the spiking of sugar-sensing GRNs, but these compounds never

446 elicited spikes on their own. What's more, these small changes in the rate of spiking did
447 not translate into features that the clustering algorithm could use to differentiate pesticide
448 laced stimuli from the control OSR solution. From these data, we conclude that the buff-
449 tailed bumblebee's mouthparts do not have mechanisms for the detection and avoidance
450 of common cholinergic pesticides in nectar.
451
452 In the present experiments, we expected that if neonicotinoids and sulfoxaflor could be
453 tasted by bees, they would exhibit the same responses in GRNs produced by other non-
454 nutrient compounds (i.e. bitter responses). For this reason, we were mainly testing for the
455 reduction of GRN spiking caused by the suppression of sugar-sensing GRNs, as all of
456 the 'bitter' compounds we tested were presented to the sensilla in OSR nectar (a mixture
457 of primarily fructose and glucose). The known bitter compound, QUI, clearly suppressed
458 the activity of sugar-sensing GRNs in both labial palps and the galea, but it did not
459 measurably alter the burst pattern of firing of the galeal GRNs. Importantly, our new data
460 were able to confirm that field-relevant concentrations of pesticides did not significantly
461 alter the pattern or rate of spiking of galeal or labial palp GRNs towards a nectar solution.
462
463 We used a clustering algorithm that integrated input from all the sensilla we recorded from
464 over a 1 s time interval with every stimulus to identify whether population-level input made
465 it possible to differentiate the stimuli. Although we were able to measure small but
466 significant reduction in the responses of both labial palp and galeal neurons caused by
467 the high concentrations (100 μM) of IMD, SFX, and TMX, this effect was not sufficient for
468 the clustering algorithm to form classes of distinct stimuli for the OSR-pesticide mixtures.
469 This is similar to what we observed for 0.1 mM QUI; though a small change in firing was
470 detected, the clustering algorithm did not differentiate it from OSR. Our
471 electrophysiological recordings are the first to report GRN responses from the
472 bumblebee's labial palps. The labial palps in other insects such as locusts are used to
473 detect both phagostimulants and deterrant compounds (Blaney and Chapman, 1970), so
474 we might have rationally expected to observe labial palp GRNs that spike in response to
475 QUI and cholinergic pesticides, but we did not. This could indicate that on the mouthparts,

476 bitter compounds are only detected through the suppression of sugar-sensing GRNs (de
477 Brito Sanchez et al., 2005).
478
479 It is interesting to note that in the case of all bitter compounds tested, the output of the
480 clustering algorithm matched the behaviour of the bees, as the bees themselves did not
481 reject any of the pesticide solutions or the 0.1 mM QUI solution. This could indicate that
482 that small differences detected at the sensory periphery do not translate into a sufficient
483 signal across the whole population of gustatory neurons to produce an aversion to foods
484 containing potential toxins. However, it is worth noting that the OSR background solutions
485 in both cases were different: in the behavioural experiments the concentration of OSR
486 was an average value of that naturally found in nectar. Our physiology experiments were
487 performed using a 10% dilution of this solution. Different solutions were used because it
488 was not possible to record from neurons with such a concentrated sugar solution, and in
489 behavioural experiments, most bumblebees do not find 10% OSR sufficiently
490 phagostimulatory to feed.
491
492 Our new data also indicate that gustatory coding is more than the presence or absence
493 of spikes arising from GRNs tuned to detect particular tastants. In bumblebees, it is clear
494 that the pattern of spiking itself conveys information which is a feature that enables the
495 brain to distinguish gustatory stimuli (Parkinson et al., 2022b). This supports the idea that
496 encoding of gustatory stimuli is accomplished by the whole population of GRNs on a given
497 body part (e.g. the proboscis) as was observed in multichannel recordings from the adult
498 hawkmoth maxillary nerve (Reiter et al. 2015). Thus, activity across the mouthparts GRN
499 population that includes temporal input over time is likely to be used by the brain to
500 differentiate taste stimuli and to facilitate the valuation of food quality. Our new data
501 testing the difference between a nectar stimulus composed of a mixture of sugars (OSR)
502 and sucrose clearly illustrates this. The changes in the rate of bursting and spiking in the
503 galeal and labial palps were sufficient for the clustering algorithm to classify sucrose and
504 OSR as separate stimuli. Although our behavioural assay did not show a difference, we
505 would not expect it to, as it is an assay developed to study food rejection behaviour by
506 bees, not differences in phagostimulation. We predict that these two solutions are

507 completely distinguishable by bumblebees; this has exciting implications for the bee's
508 ability to distinguish naturally occurring nectar of different flowering plant species. Future
509 studies will be necessary to confirm this.

510 Previously, Arce et al. (2018) reported that *B. terrestris* were capable of selectively
511 foraging on 30% w/v sucrose solutions containing TMX. They challenged our previous
512 conclusion that honeybee and bumblebee preferences for solutions containing TMX were
513 accomplished in the absence of taste cues in our choice assays (Kessler et al. 2015).
514 However, there are important differences in the experiments of Arce et al. (2018) and
515 those we present now. It is important to note that in their experiments, they did not
516 observe a preference for or against the solutions containing the neonicotinoids when they
517 were presented during the first few days of the experiments. If taste was the mechanism
518 for the preference, we should have expected them to prefer the TMX solution within the
519 first foraging bout, such as the time frame of our behavioural assay (2 min). Though the
520 authors of this study didn't measure the behaviour of bees on initial contact, they instead
521 found that the preference for the TMX solution developed over time. In our previous work,
522 we also found that individual bees developed a preference for solutions containing TMX
523 if they were confined to feed on a choice between sucrose and sucrose laced with
524 pesticide over longer time period (i.e. 24 h, Kessler et al., 2015). In contrast, we concluded
525 that the preference was caused by the pharmacological action of TMX on cholinergic
526 circuits involved in encoding reward in the insect brain (Barnstedt et al., 2016), as we had
527 no electrophysiological evidence to support that bees could taste neonicotinoids (Kessler
528 et al., 2015). A preference resulting from post-ingestive reinforcement caused by
529 amplification of the brain's response to sugars would be expected to develop following
530 long-term, repeated exposure to a pharmacological compound (Wright et al., 2013). If
531 TMX caused bees to associate a higher 'reward value' with food found in a particular
532 location, they would learn to return to it in preference over other solutions. Bees
533 experience post-ingestive reinforcement from feeding as quickly as 30 s after being fed
534 (Simcock et al., 2018). Although Arce et al. (2018) switched the feeders between training
535 and testing phases, it was probably not sufficient to rule out the fact that bees could learn
536 the location of the food, as it is likely they could do this within a single feeding bout.

537 Arce et al. (2018) also used gravity feeders in an enclosed arena in which bees could
538 consume solutions *ad libitum*. The feeders did not have visual or olfactory cues, so they
539 deduced that the bees were capable of tasting the presence of TMX in the solution.
540 However, gravity feeders do not simulate how bees feed on real flowers. Such feeders
541 have large openings in which several bees can feed simultaneously; in this setting, bees
542 can contact the solution with all body parts, including the tarsi and antennae. It is possible
543 that the sensilla on the antennae or tarsi could detect TMX, but unlikely. In honeybees,
544 bitter detection in these locations is limited and is only observed when the sensilla are
545 contacted with concentrations of compounds like QUI that are >1 mM (de Brito Sanchez
546 et al., 2005; de Brito Sanchez et al., 2014). Field-relevant concentrations of the pesticides
547 we tested are between 3-6 orders of magnitude lower in concentration (see Kessler et al.
548 2015 for review); such dilute concentrations of a bitter compound present in a highly
549 concentrated sugar solution would be difficult for most organisms to detect.

550 Floral morphology such as corolla length has often co-evolved with particular groups of
551 pollinators with longer mouthparts; a long corolla protects nectar when visitors are
552 efficient and reliable pollinators. In many flowers, nectar is hidden deep within the corolla
553 such that the only contact that bumblebees have with it is via the distal end of the
554 proboscis (also the location where most of the sensilla are). For example, in OSR flowers,
555 nectaries are located at a depth of 5-8 mm (Cresswell et al., 2001) and the nectar cannot
556 be contacted otherwise unless the bee bites through the petals. In addition, bees learn to
557 handle flowers quickly to improve their foraging efficiency (Heinrich, 1976) and even
558 extend the proboscis in anticipation of entering the corolla prior to landing. Thus, they are
559 unlikely to contact nectar using gustatory sensilla on their antennae or tarsi while foraging.
560 For this reason, it is likely that nectar palatability is largely determined by the sensilla on
561 the proboscis. Based on our current results and those of previous studies (Sculfort et al.,
562 2021; Tiedeken et al., 2014), we predict that bumblebees have a limited ability to detect
563 naturally-occurring concentrations of toxic or bitter compounds in nectar. Future studies
564 that survey a greater variety of compounds will be necessary to confirm this.

565

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Figure legends

Figure 1: Electrophysiological and behavioural responses to sucrose and oilseed rape (OSR) nectar.

A: Diagram of the bumblebee's mouthparts from which tip-recordings were made, including the galea, and segments III and IV of the labial palps. Tip-recordings were made from the longer 'A-type' sensilla.

B: Filtered electrophysiological recordings from galeal (Gal), labial palp segment III (LP_{III}), and labial palp segment IV (LP_{IV}) sensilla. Spikes from GRN 1 at each location are labeled with blue circles, GRN 2 spikes in green, and GRN 3 spikes in magenta (only two GRNs present in labial palp recordings).

C: Average firing rates of GRNs from sensilla on the galea (Gal), segment III of the labial palps (LP_{III}), and segment IV (LP_{IV}) over 1 s of stimulation with 10% OSR or an equimolar (0.173 M) sucrose solution (SUC, n=315 sensilla from 37 bees). Mean and standard deviation illustrated with black bars, data shown with coloured points. Asterisks represent significant differences between SUC and OSR (***, $p < 0.0001$).

D: Linear regression of inter-spike intervals per 0.1 s bin of labial palp GRNs versus time. Slope of regression is the adaptation rate. Shading is standard error. No significant difference between the adaptation rate when stimulated with OSR versus SUC.

E: Firing rates of Gal1 versus Gal2 over 1 s stimulation with 10% OSR and SUC. Points represent mean rate in each 100 ms bin across all trials. A black marker highlights the first bin (i.e., time = 100 ms). *Post hoc* comparisons showed that Gal2 firing rates were significantly different between stimuli.

595 F: t-SNE of all GRN responses for each animal following stimulation with OSR (gray) or
596 SUC (orange) and k-means clusters (k=2, predicted by Monte Carlo reference-based
597 consensus clustering) in gray shading.
598 G: Image of an untethered bumblebee in a holding tube feeding from a capillary in the 2-
599 min feeding assay.
600 H: Total volume consumed of 1.79 M SUC or 100% OSR of freely moving bumblebees
601 during 2 minutes (n = 15 bees per group).
602 I: The duration of the first feeding bout duration of bumblebees feeding on SUC or OSR.
603 J: The cumulative duration of feeding bouts within a 2 min period.
604

605 **Figure 2: Quinine shuts down GRN and behavioural responses to sugars.**

606 A: Average firing rates of 7 GRNs from the galea (Gal), and labial palps (LP_{III}, LP_{IV}) over
607 a 1 s stimulations with 10% OSR (OSR), 10% OSR plus 0.1 mM quinine (QUI 0.1), and
608 10% OSR plus 1 mM quinine (QUI 1). Mean and standard deviation illustrated with black
609 bars, data shown with coloured points (n = 325 sensilla from 37 bees). Asterisks represent
610 significant differences between stimulus and OSR (*, <0.05; ** <0.001; ***, <0.0001).
611 B: Linear regressions of inter-spike intervals per 0.1s bin of labial palp GRNs versus time
612 illustrating the adaptation rate, with SEM in grey shading. The addition of 0.1 mM QUI to
613 OSR significantly affected labial palp adaptation. 1 mM QUI not shown as there was
614 insufficient spiking to calculate adaptation.
615 C: Firing rates of Gal1 versus Gal2 over 1 s stimulation. Points represent mean rate in
616 each 100 ms bin across all trials. A black marker highlights the first bin (i.e., time = 100
617 ms). *Post hoc* comparisons showed that only Gal1 (not Gal2) firing rates were significantly
618 different at 0.1 mM QUI, while both were affected by 1.0 mM QUI.
619 D: t-SNE of all GRN responses for each animal following stimulation with 10% OSR, QUI
620 0.1 or QUI 1, and k-means clusters (k=2, predicted by Monte Carlo reference-based
621 consensus clustering) in gray shading.
622 E: Volume consumed by freely-moving bumblebees of 100% OSR, OSR plus 0.1 mM
623 QUI, or OSR plus 1mM QUI over a 2 min period (n = 15 bees/group). Asterisks denote
624 results of Dunnett's test (***, p<0.0001).

625 F: First bout duration when feeding on OSR, QUI 0.1 or QUI 1 (n = 15 bees/group).
626 Asterisks denote results of Dunnett's test (***, p<0.0001).
627 G: Cumulative bout duration over a 2 min period of bumblebees feeding on OSR, QUI 0.1
628 or QUI 1 (n = 15 bees/group). Asterisks denote results of Dunnett's test (***, p<0.0001).

629 **Figure 3: Pesticides affect the temporal code of bumblebee GRNs.**

630 A: Average firing rates of 7 GRNs from the galea (Gal) and labial palps (LP_{III}, LP_{IV}) over
631 a 1 s stimulation with 10% OSR (OSR), or OSR plus 0.1 mM clothianidin (CLO),
632 imidacloprid (IMD), sulfoxaflor (SFX), or thiamethoxam (TMX, n = 331 sensilla from 37
633 bees). Mean and standard deviation illustrated with black bars, and data shown with
634 coloured points. Asterisks represent significant differences between stimulus and OSR
635 (*, <0.05; ** <0.001; ***, <0.0001).
636 B: Linear regressions of inter-spike intervals per 0.1s bin of labial palp GRNs versus time
637 illustrating the adaptation rate, with SEM in grey shading. Asterisks represent significant
638 differences between stimulus and OSR (*** p<0.0001).
639 C: Firing rates of Gal1 versus Gal2 over 1 s stimulation. Points represent mean rate in
640 each 100 ms bin across all trials. A black marker highlights the first bin (i.e., time = 100
641 ms). *Post hoc* comparisons showed that only Gal1 firing rates were significantly different
642 for CLO and IMD versus OSR, and Gal2 firing rates were affected by IMD only.
643 D: t-SNE of all GRN responses for each animal following stimulation with OSR, CLO,
644 IMD, SFX, or TMX. Monte Carlo reference-based consensus clustering predicted an
645 optimal cluster count of k=1 on the t-SNE.
646

647 **Figure 4: Bumblebees do not avoid consuming pesticides in nectar at field relevant**
648 **concentrations**

649 A: The total volume consumed by freely moving bumblebees during 2 min of 100% OSR
650 solutions containing increasing concentrations (nM) of CLO, IMD, TMX and SFX (n = 15
651 bees per group).
652 B: First feeding bout duration of OSR mixtures with increasing concentrations (nM) of
653 pesticides (CLO, IMD, SFX, TMX, n = 15 bees per group).

654 C: Cumulative feeding duration during 2 min of OSR mixtures with increasing
655 concentrations (nM) of pesticides (n = 15 bees per group).
656 D: Total volume consumed of OSR or OSR plus 0.1 mM CLO, IMD, TMX or SFX over 2
657 min by freely moving bumblebees (n = 15 bees per group).
658 E: First bout duration of bumblebees feeding on OSR and OSR mixed with 0.1 mM
659 pesticides (n = 15 bees per group).
660 F: Cumulative bout duration over 2 min by bees feeding on OSR and OSR mixed with 0.1
661 mM pesticides (n = 15 bees per group).
662

663 **References**

664

665 **Arce, A. N., Ramos Rodrigues, A., Yu, J., Colgan, T. J., Wurm, Y. and Gill, R. J.** (2018).
666 Foraging bumblebees acquire a preference for neonicotinoid-treated food with prolonged
667 exposure. *Proceedings of the Royal Society of London B: Biological Sciences* **285**, 8–11.
668 **Barnstedt, O., Owald, D., Felsenberg, J., Brain, R., Moszynski, J. P., Talbot, C. B., Perrat,**
669 **P. N. and Waddell, S.** (2016). Memory-Relevant Mushroom Body Output Synapses Are
670 Cholinergic. *Neuron* **89**, 1237–1247.
671 **Blaney, W. M. and Chapman, R. F.** (1970). The functions of the maxillary palps of acrididae
672 (Orthoptera). *Entomol Exp Appl* **13**, 363–376.
673 **Calderone, N. W.** (2012). Insect Pollinated Crops , Insect Pollinators and US Agriculture :
674 Trend Analysis of Aggregate Data for the Period 1992 – 2009. *PLoS One* **7**, 24–28.
675 **Carruthers, J. M., Cook, S. M., Wright, G. A., Osborne, J. L., Clark, S. J., Swain, J. L. and**
676 **Haughton, A. J.** (2017). Oilseed rape (Brassica napus) as a resource for farmland insect
677 pollinators: quantifying floral traits in conventional varieties and breeding systems. *GCB*
678 *Bioenergy* **9**, 1370–1379.
679 **Chapman, R. F. and Sword, G.** (1993). The importance of palpation in food selection by a
680 polyphagous grasshopper (Orthoptera: Acrididae). *J Insect Behav* **6**, 79–91.
681 **Cresswell, J. E., Hagen, C. and Woolnough, J. M.** (2001). Attributes of individual flowers of
682 Brassica napus L. are affected by defoliation but not by intraspecific competition. *Ann Bot*
683 **88**, 111–117.
684 **Cresswell, J. E., Robert, F. X. L., Florance, H. and Smirnoff, N.** (2014). Clearance of
685 ingested neonicotinoid pesticide (imidacloprid) in honey bees (Apis mellifera) and
686 bumblebees (Bombus terrestris). *Pest Manag Sci* **70**, 332–337.
687 **de Brito Sanchez, M. G., Giurfa, M., de Paula Mota, T. R. and Gauthier, M.** (2005).
688 Electrophysiological and behavioural characterization of gustatory responses to antennal
689 "bitter" taste in honeybees. *European Journal of Neuroscience* **22**, 3161–3170.
690 **de Brito Sanchez, M. G., Lorenzo, E., Su, S., Liu, F., Zhan, Y. and Giurfa, M.** (2014). The
691 tarsal taste of honey bees: behavioral and electrophysiological analyses. *Front Behav*
692 *Neurosci* **8**, 1–16.
693 **Dethier, V. G.** (1976). *The Hungry Fly: A physiological study of the behavior associated with*
694 *feeding*. Harvard University Press.

French, A. S., Sellier, M.-J., Ali Agha, M., Guigue, A., Chabaud, M.-A., Reeb, P. D., Mitra, A., Grau, Y., Soustelle, L. and Marion-Poll, F. (2015). Dual Mechanism for Bitter Avoidance in Drosophila. *Journal of Neuroscience* **35**, 3990–4004.

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., Kremen, C. and Carvalheiro, L. G. (2014). Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. *Science (1979)* **339**, 1608–1611.

Gill, R. J., Ramos-Rodriguez, O. and Raine, N. E. (2012). Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* **491**, 105–8.

Goulson, D., Peat, J., Stout, J. C., Tucker, J., Darvill, B., Derwent, L. C. and Hughes, W. O. H. (2002). Can alloethism in workers of the bumblebee, bombus terrestris, be explained in terms of foraging efficiency? *Anim Behav* **64**, 123–130.

Goulson, D., Nicholls, E., Botías, C. and Rotheray, E. L. (2015). Bee declines driven by combined Stress from parasites, pesticides, and lack of flowers. *Science (1979)* **347**,.

Heinrich, B. (1976). The Foraging Specializations of Individual Bumblebees. *Ecol Monogr* **46**, 105–128.

Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J., Aupinel, P., Aptel, J., Tchamitchian, S. and Decourtye, A. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science (1979)* **336**, 348–350.

Huang, J. X. and An, J. D. (2018). Species diversity, pollination application and strategy for conservation of the bumblebees of China. *Biodiversity Science* **26**, 486–497.

John, C. R., Watson, D., Russ, D., Goldmann, K., Ehrenstein, M., Pitzalis, C., Lewis, M. and Barnes, M. (2020). M3C: Monte Carlo reference-based consensus clustering. *Sci Rep* **10**, 1–14.

Kessler, S. C., Tiedeken, E. J., Simcock, K. L., Derveau, S., Mitchell, J., Softley, S., Stout, J. C. and Wright, G. a. (2015). Bees prefer foods containing neonicotinoid pesticides. *Nature* **521**, 74–76.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. and Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* **274**, 303–313.

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models . *J Stat Softw* **82**,.

Lenth, R., Singmann, H., Love, J., Buerkner, P. and Herve, M. (2018). emmeans: Estimated Marginal Means, aka Least-Squares Means. *R package version*.

Ma, C., Kessler, S., Simpson, A. and Wright, G. (2016). A Novel Behavioral Assay to Investigate Gustatory Responses of Individual, Freely-moving Bumble Bees. *Journal of Visualized Experiments* **113**, e54233.

Meehan, T. D., Werling, B. P., Landis, D. A. and Gratton, C. (2011). Agricultural landscape simplification and insecticide use in the Midwestern United States. *Proceedings of the National Academy of Sciences* **108**, 11500–11505.

Miriyala, A., Kessler, S., Rind, F. C. and Wright, G. A. (2018). Burst Firing in Bee Gustatory Neurons Prevents Adaptation. *Current Biology* **28**, 1585-1594.e3.

Mundy-Heisz, K. A., Prosser, R. S. and Raine, N. E. (2022). Acute oral toxicity and risks of four classes of systemic insecticide to the Common Eastern Bumblebee (Bombus impatiens). *Chemosphere* **295**, 133771.

Muth, F., Gaxiola, R. and Leonard, A. S. (2020). No evidence for neonicotinoid preferences in the bumblebee Bombus impatiens. *R Soc Open Sci* **7**,.

Parkinson, R. H., Fecher, C. and Gray, J. R. (2022a). Chronic exposure to insecticides impairs honeybee optomotor behaviour. *Frontiers in Insect Science* **2**, 1–14.

Parkinson, R. H., Kessler, S. C., Scott, J., Simpson, A., Bu, J., Al-Esawy, M., Mahdi, A., Miriyala, A. and Wright, G. A. (2022b). Temporal responses of bumblebee gustatory neurons to sugars. *iScience* **25**,.

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., Dicks, L. v., Garibaldi, L. A., Hill, R., Settele, J., et al. (2016). Safeguarding pollinators and their values to human well-being. *Nature* **540**, 220–229.

Schneider, C. W., Tautz, J., Grünewald, B. and Fuchs, S. (2012a). RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of Apis mellifera. *PLoS One* **7**, 1–9.

Schneider, C. A., Rasband, W. S. and Eliceiri, Kevin, W. (2012b). NIH Image to ImageJ: 25 years of Image Analysis. *Nat Methods* **9**, 671–675.

Sculfort, O., Gérard, M., Gekière, A., Nonclercq, D., Gerbaux, P., Duez, P. and Vanderplanck, M. (2021). Specialized Metabolites in Floral Resources: Effects and Detection in Buff-Tailed Bumblebees. *Front Ecol Evol* **9**,.

Simcock, N. K., Gray, H., Bouchebti, S. and Wright, G. A. (2018). Appetitive olfactory learning and memory in the honeybee depend on sugar reward identity. *J Insect Physiol* **106**, 71–77.

Siviter, H., Matthews, A. J. and Brown, M. J. F. (2022). A Combined LD50 for Agrochemicals and Pathogens in Bumblebees (Bombus terrestris [Hymenoptera: Apidae]). *Environ Entomol* **51**, 378–384.

Team, R. C. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Tiedeken, E. J., Stout, J. C., Stevenson, P. C. and Wright, G. A. (2014). Bumblebees are not deterred by ecologically relevant concentrations of nectar toxins. *Journal of Experimental Biology* **217**, 1620–1625.

U.S. Environmental Protection Agency (EPA) (2014). Ecological Risk Assessment for the Registration Review of Sulfoxaflor. 79.

van der Sluijs, J. P., Simon-Delso, N., Goulson, D., Maxim, L., Bonmatin, J. M. and Belzunces, L. P. (2013). Neonicotinoids, bee disorders and the sustainability of pollinator services. *Curr Opin Environ Sustain* **5**, 293–305.

Vanbergen, A. J., Garratt, M. P., Vanbergen, A. J., Baude, M., Biesmeijer, J. C., Britton, N. F., Brown, M. J. F., Brown, M., Bryden, J., Budge, G. E., et al. (2013). Threats to an ecosystem service: Pressures on pollinators. *Front Ecol Environ* **11**, 251–259.

Wäckers, F. L., Alberola, J. S., Garcia-Marí, F. and Pekas, A. (2017). Attract and distract: Manipulation of a food-mediated protective mutualism enhances natural pest control. *Agric Ecosyst Environ* **246**, 168–174.

Weiss, L. A., Dahanukar, A., Kwon, J. Y., Banerjee, D. and Carlson, J. R. (2011). The molecular and cellular basis of bitter taste in Drosophila. *Neuron* **69**, 258–272.

Williamson, S. M., Willis, S. J. and Wright, G. A. (2014). Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicology* **23**, 1409–1418.

Wood, T. J. and Goulson, D. (2017). The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environmental Science and Pollution Research* **24**, 17285–17325.

Wright, G. A. (2016). To feed or not to feed: circuits involved in the control of feeding in insects. *Curr Opin Neurobiol* **41**, 87–91.

Wright, G. A., Mustard, J. A., Simcock, N. K., Ross-Taylor, A. A. R., McNicholas, L. D., Popescu, A. and Marion-Poll, F. (2010). Parallel reinforcement pathways for conditioned food aversions in the honeybee. *Current Biology* **20**, 2234–2240.

Wright, G. A., Baker, D. D., Palmer, M. J., Stabler, D., Mustard, J. A., Power, E. F., Borland, A. M. and Stevenson, P. C. (2013). Caffeine in floral nectar enhances a pollinator's memory of reward. *Science (1979)* **339**, 1202–1204.

Wright, G. A., Softley, S. and Earnshaw, H. (2015). Low doses of neonicotinoid pesticides in food rewards impair short-term olfactory memory in foraging-age honeybees. *Sci Rep* **5**, 1–7.

Figure 1

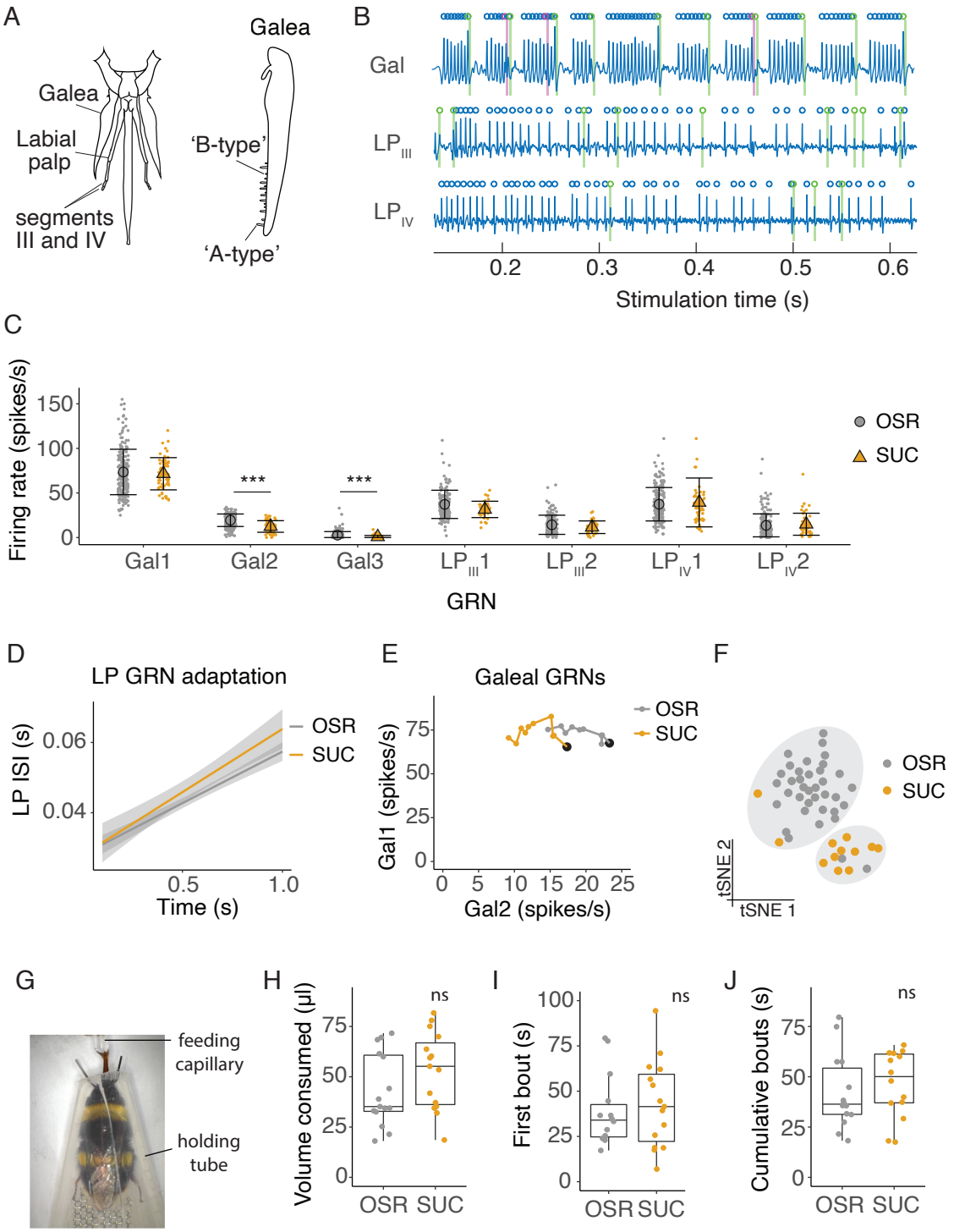

A
Galea
Galea
Labial palp
segments III and IV
'B-type'
'A-type'
B
Gal
LP III
LP IV
0.2
0.3
0.4
0.5
0.6
Stimulation time (s)
C
Firing rate (spikes/s)
150
100
50
0
***
***
OSR
SUC
Gal1
Gal2
Gal3
LP III 1
LP III 2
LP IV 1
LP IV 2
GRN
D
LP GRN adaptation
LP ISI (s)
0.06
0.04
OSR
SUC
0.5
1.0
Time (s)
E
Galeal GRNs
Gal1 (spikes/s)
75
50
25
0
OSR
SUC
0
5
10
15
20
25
Gal2 (spikes/s)
F
OSR
SUC
tSNE 2
tSNE 1
G
feeding capillary
holding tube
H
Volume consumed (µl)
75
50
25
0
ns
OSR
SUC
I
First bout (s)
100
75
50
25
0
ns
OSR
SUC
J
Cumulative bouts (s)
75
50
25
0
ns
OSR
SUC

Figure 2

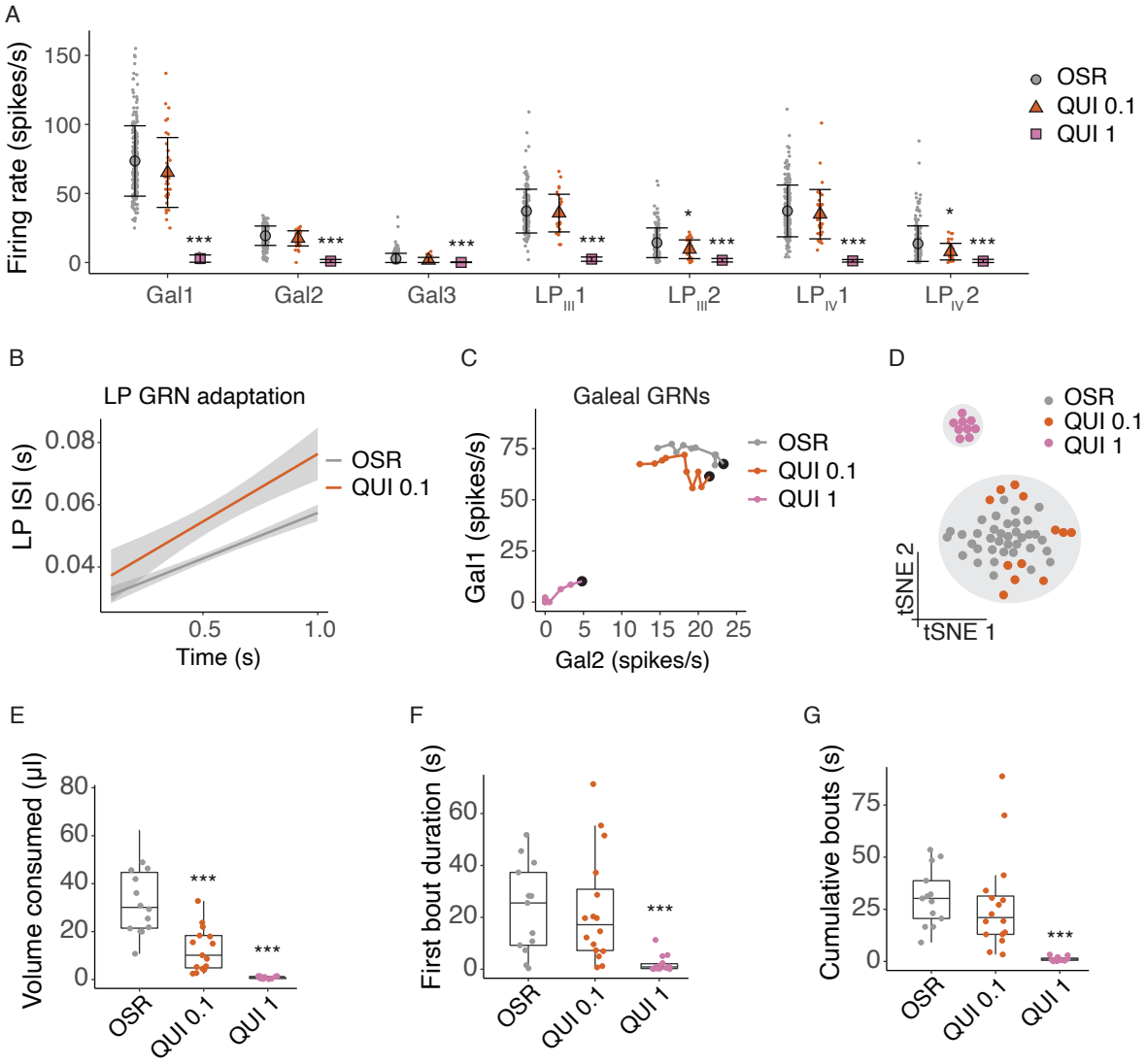

A
Firing rate (spikes/s)
150
100
50
0
OSR
QUI 0.1
QUI 1
***
*
Gal1
Gal2
Gal3
LP$_{III}$1
LP$_{III}$2
LP$_{IV}$1
LP$_{IV}$2
B
LP GRN adaptation
LP ISI (s)
0.08
0.06
0.04
0.5
1.0
Time (s)
C
Galeal GRNs
Gal1 (spikes/s)
75
50
25
0
0 5 10 15 20 25
Gal2 (spikes/s)
D
tSNE 2
tSNE 1
E
Volume consumed (µl)
80
60
40
20
0
F
First bout duration (s)
G
Cumulative bouts (s)

Figure 3

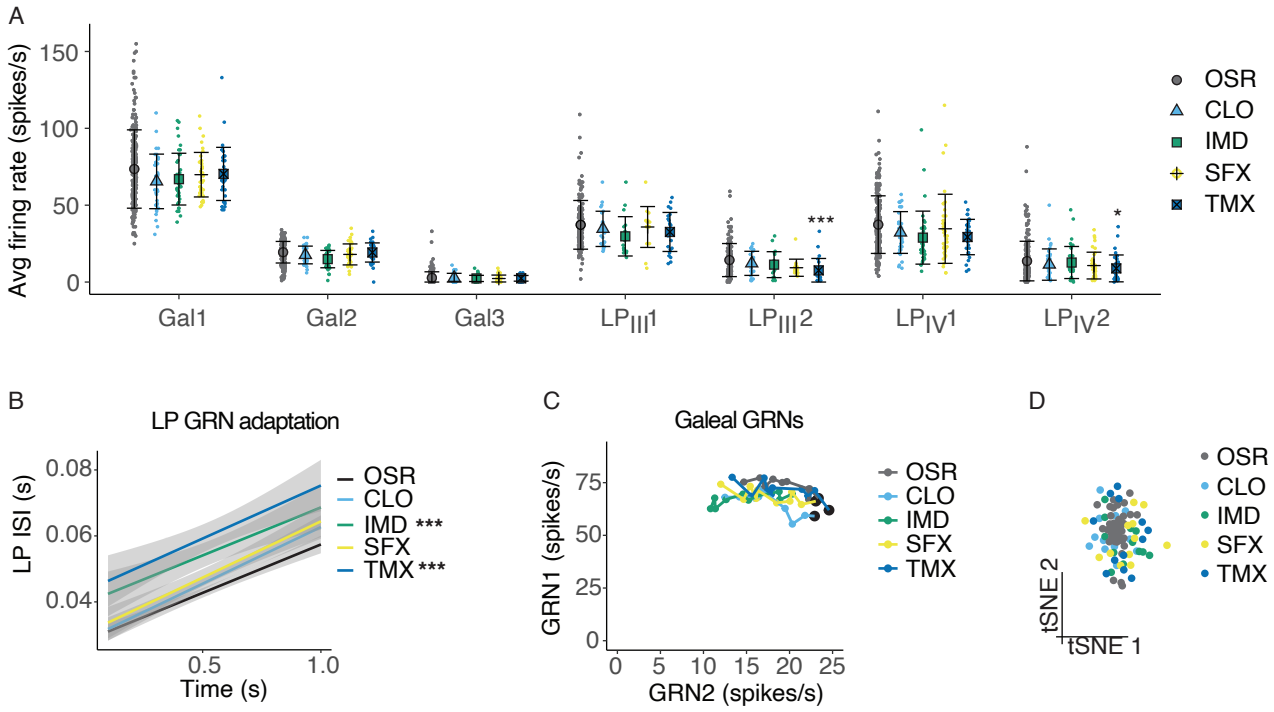
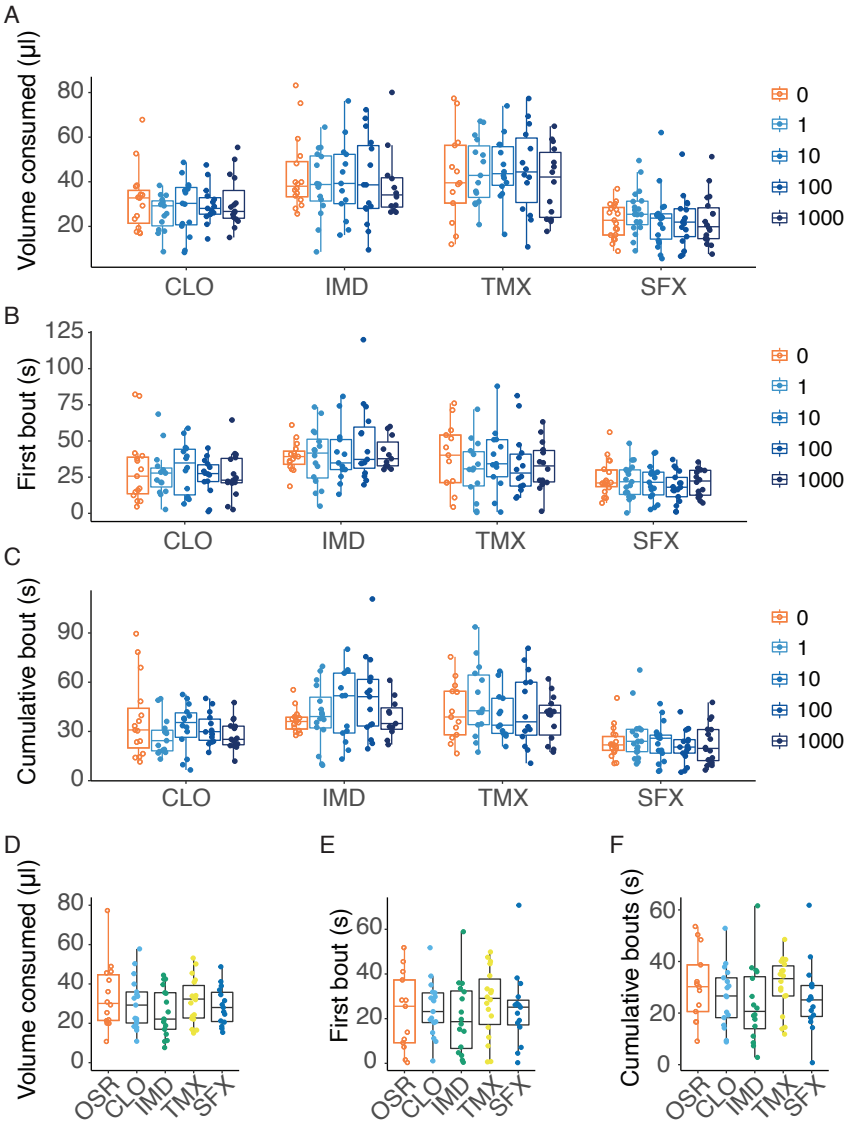

A
Avg firing rate (spikes/s)
150
100
50
0
Gal1
Gal2
Gal3
LPIII1
LPIII2
LPIV1
LPIV2
***
*
OSR
CLO
IMD
SFX
TMX
B
LP GRN adaptation
LP ISI (s)
0.08
0.06
0.04
0.5
1.0
Time (s)
OSR
CLO
IMD ***
SFX
TMX ***
C
Galeal GRNs
GRN1 (spikes/s)
75
50
25
0
0
5
10
15
20
25
GRN2 (spikes/s)
OSR
CLO
IMD
SFX
TMX
D
tSNE 2
tSNE 1
OSR
CLO
IMD
SFX
TMX

Figure 4


A
Volume consumed (µl)
80
60
40
20
CLO
IMD
TMX
SFX
0
1
10
100
1000
B
First bout (s)
125
100
75
50
25
0
CLO
IMD
TMX
SFX
0
1
10
100
1000
C
Cumulative bout (s)
90
60
30
0
CLO
IMD
TMX
SFX
0
1
10
100
1000
D
Volume consumed (µl)
80
60
40
20
0
OSR
CLO
IMD
TMX
SFX
E
First bout (s)
60
40
20
0
OSR
CLO
IMD
TMX
SFX
F
Cumulative bouts (s)
60
40
20
0
OSR
CLO
IMD
TMX
SFX