

1 **Contribution of gustatory input to larval acceptance and female oviposition choice of**  
2 **potential host-plants in *Papilio hospiton* (Géné)**

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4 Giorgia Sollai, Roberto Crnjar

5 Department of Biomedical Sciences, Section of Physiology, University of Cagliari, 09042

6 Monserrato (CA), Italy

7

8 Correspondence to: Giorgia Sollai, Department of Biomedical Sciences, Section of Physiology,

9 University of Cagliari, SP 8 Km 0.700, 09042 Monserrato (CA), Italy. E-mail: [gsollai@unica.it](mailto:gsollai@unica.it);

10 Phone: +39 070 6754160.

11

12 **Abstract**

13 The Lepidopteran *Papilio hospiton* uses only plants belonging to the Apiaceae and the Rutaceae  
14 families as hosts. Both adult females and larvae are equipped with gustatory receptor neurons  
15 (GRNs) capable of detecting sugars, bitters and salts, thus providing information for evaluating the  
16 chemical composition of the plant. Since the activation of these neurons may affect insect behavior,  
17 the aim of this work was: a) to study the gustatory sensitivity of both females and larvae to the sap  
18 of two Apiaceae, *Foeniculum vulgare* (fennel) and *Daucus carota* (carrot), that are not used as host  
19 plants; b) to cross-compare the spike activity evoked from these two plants with that evoked by  
20 *Ferula communis* (ferula), the host plant preferred by ovipositing females of *P. hospiton* and where  
21 the larvae perform best; c) finally, to confirm that the gustatory system can provide the central  
22 nervous system with the necessary information to evaluate differences between plant saps. The  
23 results show that: a) fennel and carrot both evoke a higher neural activity from the bitter-sensitive  
24 neurons and lower from the sugar-sensitive neurons with respect to ferula, in both adult females  
25 and larvae; b) on the basis of the different patterns of neural activity generated in tarsal, lateral and  
26 medial sensilla by fennel and carrot vs. ferula, both adult and larvae possess enough information to  
27 discriminate among these plants; c) adult females of *P. hospiton* lay eggs where the larvae have the  
28 greatest growth success and this confirms the importance of taste sensitivity in host plants selection.

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33 **Key Words:** taste sensitivity; discrimination; oviposition preference; insects; food acceptance.

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## 35 **Introduction**

36 *Papilio hospiton* Gén , an endemic species of the islands of Sardinia and Corsica, is considered an  
37 oligophagous butterfly since it has a narrow range of host plants belonging mainly to the Apiaceae  
38 family and secondarily to the Rutaceae (Prudic, Oliver, & Sperling, 2007). In Corsica, the females  
39 oviposit and the larvae feed on *Ferula communis* L. (fennel) and on other three endemic species of  
40 Apiaceae (*Peucedanum paniculatum* Loisel, *Pastinaca latifolia* (Duby) DC. and *Pastinaca*  
41 *divaricata* R. and C.), and only one of Rutaceae (*Ruta corsica* DC.) (Aubert, Descimon, & Michel,  
42 1996). In Sardinia, instead, *F. communis* represents the primary host plant by *P. hospiton*, albeit  
43 two other plants, rare and restricted to small areas, are used as host: *Ferula arrigonii* Bocch.  
44 (Apiaceae) and *Ruta lamarmorae* Bacch., Brullo et Giusso (Rutaceae) (Sollai, Tomassini  
45 Barbarossa, Solari, Masala, & Crnjar, 2014). Therefore in Sardinia *P. hospiton* can be considered  
46 as an almost monophagous species, in spite of the presence of other plants of the Apiaceae family.  
47 Among the Apiaceae, *Foeniculum vulgare* Mill. (fennel) and *Daucus carota* L. (carrot) deserve  
48 attention because they are widespread in the island like ferula and used, as the main and a rare host  
49 respectively, by *Papilio machaon*, a species phylogenetically related to *P. hospiton*. Neither eggs  
50 nor larvae of *P. hospiton* have ever been found on these two plants during our field observations.  
51 Furthermore, when we tried to raise some larvae on the foliages of these plants, they reached the  
52 third stage at most and then died (personal observation).

53 We have previously found that the sensory input coming from gustatory receptor neurons (GRNs),  
54 plays a key role in controlling the degree of acceptance of a plant as host, both in adult females  
55 (during the choice of the ovipositing sites) and in the larvae (during the choice to eat or not a food  
56 source) (Sollai, Tomassini Barbarossa, Solari, & Crnjar, 2015; Sollai, Biolchini, Solari, & Crnjar,  
57 2017a; Sollai, Biolchini, Loy, Solari, & Crnjar, 2017b; Sollai, Biolchini, & Crnjar, 2018a).  
58 Furthermore, the specific pattern of activation of GRNs, in both adults and larvae, provides  
59 information to the central nervous system (CNS) to discriminate among different chemicals and  
60 plant saps (Dethier & Crnjar, 1982; Glendinning, Davis, & Rai, 2006; Sollai et al., 2015). Finally,

61 chemical senses (taste and olfaction) play in general a primary role in insect-host interaction,  
62 providing information used in different behavioural paradigms, such as the choice of an oviposition  
63 site, a feeding substrate and mating partner (Bernays, Oppenheim, Chapman, Kwon, & Gould,  
64 2000; Biolchini et al., 2017; Chapman, 2003; Dangles, Irschick, Chittka, & Casas, 2009; del Campo  
65 & Miles, 2003; Dethier, 1973; Feeny, Stadler, Ahman, & Carter, 1989; Masala, Solari, Sollai,  
66 Crnjar, & Liscia, 2009; Murata, Mori, & Nishida, 2011; Nishida, 2005; Olianias et al., 2006; Ozaki  
67 et al., 2011; Ryuda et al., 2013; Solari et al., 2007; Solari et al., 2016; Sollai, Solari, Masala, Crnjar,  
68 & Crnjar, 2007; Sollai et al., 2010; Sollai, Solari, & Crnjar, 2018b). On the basis of all these  
69 considerations, our question was whether the peripheral taste sensitivity of adult females and larvae  
70 of *P. hospiton*, could also be involved in the rejection behavior of such plants as fennel and carrot.  
71 To this end we stimulated the tarsal sensilla of adult females and both the lateral and medial  
72 styloconic sensilla of larvae of *P. hospiton* with the foliage extract of the three selected plants  
73 (ferula, fennel and carrot). The electrophysiological responses obtained in response to each plant  
74 sap were compared to one another, since quantitative and qualitative differences in spike activity of  
75 each GRN are used by the CNS in the discrimination process leading to a hierarchy of host  
76 acceptance. Drumming and scratching the leaf surface with the tarsi by the ovipositing female and  
77 the nibbling action by the larvae causes release of plant saps by leaves: taste neurons detect the plant  
78 chemicals and send signals to the CNS. In the brain the stimulating and deterrent inputs are  
79 integrated and processed; their balance provides the final information to the ovipositing females  
80 and to the feeding larvae as to whether to accept or reject the plant (Dethier, 1973; Ichinosè &  
81 Honda, 1978; Nishida, 2005; Renwick & Chew, 1994; Sollai et al., 2015; Sollai et al., 2017a; Sollai,  
82 Sollai et al., 2017b; Zhang et al., 2013). Previous studies revealed that, in *P. hospiton*, adult  
83 females have on the last tarsomere of the forelegs, basiconic sensilla housing at least one sugar-  
84 sensitive and one bitter-sensitive neuron (Sollai et al., 2017b), while the lateral and medial sensilla  
85 of larvae, albeit not the only gustative organs, are considered the most important ones (Dethier &

86 Crnjar, 1982; Martin & Shields, 2012; Schoonhoven, 1987) and possess phagostimulant,  
87 phagodeterrent and salt neurons (Sollai et al., 2014; Sollai et al., 2015).

88

## 89 **Materials and Methods**

### 90 **Insects and rearing**

91 The specimens of *Papilio hospiton* Gén  came from a colony raised for several years (since 2012)  
92 in the butterfly annex (a 3 x 3 x 3m cage) at the Physiology Laboratories (University of Cagliari).  
93 Several pots of giant fennel (*Ferula communis* L.; hereafter ferula), were placed inside the cage  
94 where adult females laid their eggs. After hatching, the larvae were removed from the cage and  
95 reared at the insectary facility on the same plant in 1500-ml plastic cups (4-5 per cup), stored in an  
96 environmental growth chamber (24-25  C, 70% R.H., 16L/8D) and monitored daily until ready for  
97 testing. Fresh foliage of ferula from plants grown in a courtyard near the butterfly annex was  
98 available ad libitum every day. Adult females were free to feed on flowers of *Lantana camara* L.  
99 inside the cage; after mating, each female was transferred into small boxes and fed with a sugar  
100 solution until used for the electrophysiological experiments (Sollai et al., 2017b).

101

### 102 **Electrophysiological experiments**

103 The "tip-recording" technique (Hodgson, Lettvin, & Roeder, 1955) was used to obtain the  
104 electrophysiological recordings from both the basiconic sensilla on the fifth tarsomere of the adult  
105 females and the lateral and medial maxillary styloconic sensilla of the larvae at the fifth instar two  
106 days after moulting (Simmonds, Schoonhoven, & Blaney, 1991). Briefly, the reference electrode (a  
107 thin Ag/AgCl) was inserted into the amputated butterfly leg or the head of the larva, thus  
108 supporting the leg or fixing the maxillae in a prognathous position. The recording electrode  
109 containing the stimulus (glass micropipette, tip diameter 20  m) was brought in contact with the  
110 sensillum tip (Masala, Solari, Sollai, Crnjar, & Liscia, 2008; Solari, Masala, Falchi, Sollai, &  
111 Liscia, 2010). All signals were recorded by means of a high input impedance ( $10^{15}$   ) electrometer

112 (WPI, Duo 773), band-pass filtered (0.1-3 KHz), digitized with an analogical/digital acquisition  
113 system (Axon Digidata 1440A A/D; sampling rate 10 KHz) and stored on PC for subsequent  
114 analysis (Sollai, Solari, Masala, Liscia, & Crnjar, 2008).

115

## 116 **Stimuli**

117 All sensilla were tested with freshly cold-pressed leaf extracts of three plants, *Ferula communis* L.  
118 (ferula), *Foeniculum vulgare* L. (hereafter fennel) and *Daucus carota* L. (hereafter carrot) and with  
119 KCl 50 mM (control). These plants were chosen on the basis of two considerations. First: ferula  
120 represents our reference plant, since it is the main host of *P. hospiton*; in contrast, fennel and carrot  
121 are not used as hosts by ovipositing females, despite their belonging to the same family as ferula  
122 (Apiaceae) and representing, in Sardinia, respectively, the main host plant and a less common one  
123 for *P. machaon*, closely related to *P. hospiton*. Second: rearing trials on fennel revealed that larvae  
124 hardly reached the third stage. Stimuli were delivered for 2-3 s, in a random sequence with a 3 min  
125 interval between consecutive stimulations to minimize adaptation phenomena. Leaf extracts were  
126 tested within 30 s after cold-pressing, according to Dethier and Crnjar (1982) and Sollai et al.  
127 (2017a, 2017b). KCl was tested at the beginning and the end of the recording series to check for  
128 any shift in responsiveness; the experiment was discarded when significant variations between the  
129 initial and final KCl responses were found. After each stimulus, the tarsal surface or the mouthpart  
130 of the insect was rinsed with distilled water and blotted dry. Recordings were obtained from both  
131 sensilla of one maxilla only per larva (N=20 larvae) and from one tarsal sensillum only per adult  
132 female (N=20 butterflies); no preparation was used more than once.

133

## 134 **Data analysis**

135 Spikes analysis was done only on the first second of the discharge, which is representative of the  
136 phasic/phasic-tonic portions of the neuronal response (Dethier & Crnjar, 1982; Inoue, Asaoka, Seta,  
137 Imaeda, & Ozaki, 2009), but discarding the first 10 msec in order to remove the artifact due to

138 electrical contact (Sollai, Solari, Corda, Masala, & Crnjar, 2012). Spike sorting and counting were  
139 performed by means of the Clampfit 10.0 software (Biolchini et al., 2017; Dolzer, Fischer, &  
140 Stengl, 2003; Sollai et al., 2014; Sollai et al., 2017b; Sollai et al., 2017c). For tarsal sensilla, on the  
141 basis of the action potential amplitude, we identified the spikes and assigned them to four different  
142 classes (small S, intermediate M1, intermediate M2 and large L) (Sollai et al., 2017b). For lateral  
143 and medial sensilla, three different classes were identified (small S, intermediate M and large L); in  
144 a next step, intermediate spikes were divided into two additional classes on the basis of action  
145 potential duration: intermediate 1 (M1) and intermediate 2 (M2) (Sollai et al., 2014).

146

### 147 **Statistical analysis**

148 The effect of the taste stimulus on the spike frequency evoked in each GRN of tarsal, lateral and  
149 medial sensillum was evaluated by using repeated measures ANOVA (factor: stimulus, 3 levels).  
150 Two-way ANOVA was used to test for a difference in ensemble, temporal and /or spatio-temporal  
151 code (Sollai et al., 2015). A difference in ensemble code was assumed if a significant interaction of  
152 Stimulus  $\times$  GRN on the spike frequency was found; to this end the total frequency during the first  
153 second of neural activity was evaluated separately for each GRN. A difference in temporal code  
154 was assumed if the interaction of Time  $\times$  Stimulus on the spike frequency was significant: we built  
155 Time-Intensity (T-I) curves counting the spike frequency (separately for each GRN and stimulus)  
156 every 100 msec for the first second of stimulation. A difference in spatio-temporal code (e.g.,  
157 between ferula and carrot), was deduced if a stimulus produced non-parallel Time-Intensity (T-I)  
158 curves (significant interaction of Time  $\times$  GRN), while another stimulus produced parallel Time-  
159 Intensity curves (non-significant interaction of Time  $\times$  GRN). The assumptions of homogeneity of  
160 variance, normality and sphericity (when applicable) were checked for all data. Tukey or Duncan's  
161 test was used for the subsequent post-hoc comparisons. Statistical analyses were performed using  
162 STATISTICA for WINDOWS (version 7.0; StatSoft Inc, Tulsa, OK, USA). *P* values  $< 0.05$  were  
163 considered significant.

164

## 165 **Permits**

166 Required permits were obtained for *Papilio hospiton*. Specimens were collected in Sardinia in the  
167 spring of 2012, in compliance with the permit issued on 28 May 2012 (Ref. # 0010888) to Roberto  
168 Crnjar and his co-workers, by the “Ministero dell’Ambiente e della Protezione del Territorio e del  
169 Mare” (Italian Board of Environment and Protection of Land and Sea), in derogation from the  
170 provisions set out in the regulation DPR 357/97 concerning the application of the “Council  
171 Directive 92/43/EEC of 21 May 1992 on conservation of natural habitats and of wild fauna and  
172 flora”. No specific permits were required for host plants tested, as they are not endangered or  
173 protected species.

174

## 175 **Results and Discussion**

176 The primary aim of this work was to cross-compare the electrophysiological responses of the  
177 gustatory sensilla of both adult females and larvae to the saps of fennel and carrot with those  
178 evoked by ferula. This was aimed at assessing the presence of specific differences in the activation  
179 of stimulant and/or deterrent neurons that could explain the different degree of acceptance of these  
180 three Apiaceae, both as oviposition substrate and food source.

181 For tarsal GRNs, examples of spike discharges of the response to plant extracts tested are shown in  
182 figure 1A, while the mean value  $\pm$  SEM of the neural activity of each GRN evoked by each plant  
183 sap is shown in figure 1B. By means of repeated measures ANOVA, we found a significant effect  
184 of stimulus on the spike frequency of “L”, “M1” and “M2” GRNs ( $F > 5.22$ ;  $df$  2,38;  $p < 0.01$ ), and  
185 post-hoc comparisons showed that the responses were different between ferula and the other two  
186 stimuli ( $P < 0.05$ ; Tukey test), but not between fennel and carrot. In contrast, no stimulus effect  
187 was found for “S” neuron ( $F = 0.56$ ;  $df$  2,38;  $p = 0.5741$ ). These results on the gustatory sensitivity  
188 of adult females indicate that the spike activity evoked in the sugar-sensitive “M1” neuron in  
189 response to ferula is significantly higher than to fennel or carrot, while the opposite was found for



190 the bitter-sensitive “M2” neuron. This may be one of the reasons why, in the field, ovipositing  
191 females of *P. hospiton* do not use fennel and carrot as hosts: in fact, their eggs are nowhere to be  
192 found on either plant. These results are comparable to those previously found about the hierarchy  
193 of host choice by ovipositing females: the most stimulating plant of the bitter-sensitive neuron was  
194 the one where the significant lowest number of eggs was found (Sollai et al., 2017b). The  
195 conclusion that the gustatory information obtained by drumming and scratching the leaf surface  
196 with the foretarsi provides a relevant contribution in the final oviposition choice, does not rule out  
197 the importance of other inputs, such as visual and olfactory ones (Ichinosé & Honda, 1978;  
198 Renwick & Chew, 1994; Zhang et al., 2013). Sight and smell provide information that allows  
199 gravid females to identify a potential host at distance, but once reached the plant, it is the gustative  
200 input that conditions the final decision whether to lay eggs or not (Dethier, 1973; Nishida, 2005;  
201 Sollai et al., 2017b).

202 Similar results were obtained on the spike activity elicited from the GRNs of the styloconic sensilla  
203 of larvae (Figures 2 and 3). For lateral sensilla, repeated measures ANOVA revealed a significant  
204 effect of stimulus on the spike frequency of “L”, “M1” and “M2” GRNs ( $F > 4.54$ ;  $df\ 2,38$ ;  $p <$   
205  $0.05$ ). Pairwise comparisons showed that the spike frequency of “L” and “M1” neurons in response  
206 to both ferula and fennel was different from the response to carrot, while for the neuron “M2”  
207 differences resulted between ferula and carrot vs. fennel ( $P < 0.05$ ; Tukey test), but not between  
208 ferula and carrot ( $P > 0.05$ ; Tukey test). For medial sensilla, repeated measures ANOVA showed a  
209 significant effect of stimulus on the spike frequency of “L” and “S” neurons ( $F > 3.65$ ;  $df\ 2,38$ ;  $p <$   
210  $0.05$ ), and post-hoc comparisons showed that the differences were between ferula and the other two  
211 stimuli for “L” neuron ( $P < 0.01$ ; Tukey test), and between carrot vs. ferula and fennel in the case  
212 of “S” neuron ( $P < 0.05$ ; Tukey test). As a whole, these findings point to the conclusion that ferula  
213 is more stimulating than fennel and carrot for the phagostimulant neurons, while fennel and carrot  
214 are better stimuli for the phagodeterrent ones. Also in the case of larvae, the observed differences  
215 in neuron activity in response to plant saps are considered consistent with the differences in food

216 preference (Tang et al., 2014), and represent an additional convincing reason why larvae are never  
217 found on fennel or carrot in the field.

218 The results obtained both on adult females and larvae, are in agreement with the following two  
219 theories about the relationship between the acceptance of a plant as host and the  
220 electrophysiological responses it elicits. The first one holds that a direct relationship exists between  
221 the neural activity of a specific neuron and the promptness and intensity of the relative behavioral  
222 response: for example, a bitter-sensitive neuron causes a decrement in the feeding (De Boer,  
223 Dethier, & Schoonhoven, 1977; Glendinning, Valcic, & Timmermann, 1998). The second one  
224 suggests instead that host acceptance depends on the presence of stimulating inputs rather than of  
225 deterrent ones (Ma, 1972) [44]. In this respect, it has been reported that sugars need to mask the  
226 presence of bitter compounds and that stimulant inputs balance deterrent ones (Cocco &  
227 Glendinning, 2012; Schoonhoven & Blom, 1988; Sollai et al., 2014). By recalling that the  
228 successful choice of a host plant necessary for survival of the species is determined both by the  
229 ovipositing females and the larval feeding acceptance (Nishida, 2005) [20], these results support  
230 the preference-performance hypothesis, based on which females choose the host plant that ensures  
231 the best performance for the offspring (Gripenberg, Mayhew, Parnell, & Roslin, 2010; Jaenike,  
232 1978). Accordingly, the ovipositing females do not lay eggs on fennel and carrot, two plants on  
233 which the larvae do not survive beyond the third stage.

234 The second aim of this work was to verify whether the different spikes activity (in terms of both  
235 total frequency and time course during the first second of discharge) evoked from each GRN by  
236 plant saps could be used by the central nervous system as a neural code to discriminate between  
237 ferula and the other two plants, as host and non-host respectively. We found a significant  
238 interaction of Stimulus  $\times$  GRN on spike frequency in tarsal sensilla of adult females ( $F = 10.61$ ;  $df$   
239  $6,228$ ;  $p = 0.0000$ ), and in both lateral and medial sensillum of larvae ( $F = 5.43$ ;  $df$   $6,228$ ;  $p =$   
240  $0.0000$ , and  $F = 5.14$ ;  $df$   $6,228$ ;  $p = 0.0000$ , respectively) (Figure 4). As shown in detail in Table 1,  
241 the results indicate that ferula generates a different response pattern across all active GRNs from

242 fennel and carrot, but no difference was found between the latter two stimuli, both in tarsal and  
243 styloconic sensilla.

244 A non-significant interaction of Stimulus  $\times$  Time was found for all sensilla (tarsal:  $F = 0.46$ ;  $df$   
245  $18,2370$ ;  $p = 0.9753$ ; lateral:  $F = 0.29$ ;  $df$   $18,2370$ ;  $p = 0.9984$ ; medial:  $F = 0.30$ ;  $df$   $18,2370$ ;  $p =$   
246  $0.9981$ ) (Fig. 5), thus indicating that time courses of spike frequency in response to plant saps do  
247 not differ from one another (Table 2).

248 As regards the results on the presence of a spatio-temporal code (Table 3), significant interaction of  
249 Time  $\times$  GRN was found only for ferula in the tarsal sensillum and for all saps in the lateral  
250 sensillum, showing that they all evoke non-parallel T-I curves. Instead, for fennel and carrot in the  
251 tarsal sensillum and for all stimuli in the medial sensillum, we found a non-significant interaction  
252 of Time  $\times$  GRN, showing that each stimulus evokes T-I curves that are essentially parallel to one  
253 another.

254 These results confirm what previously reported on the involvement of several combined coding  
255 mechanisms in the discrimination process between host plants by adult females (Sollai et al.,  
256 2017b). In fact, ferula (host) generates a different across neuron pattern (ANP) from fennel and  
257 carrot (non host), and the extracts of fennel and carrot evoke parallel T-I curves in GRNs, while the  
258 extract of ferula evokes non-parallel T-I curves, thus indicating a difference in spatio-temporal  
259 code. Larvae, instead, seem to be able to discriminate between ferula (host) vs. fennel and carrot  
260 (non-host) only by means of an ensemble code. In fact, ferula generates a different response pattern  
261 from fennel and carrot across all active GRNs of both lateral and medial sensilla, while no  
262 difference was found between the latter two, in both sensilla. These results confirm that for larvae a  
263 different ensemble code is sufficient in the discrimination process between host and non-host plants,  
264 as previously found between ferula and *S. tortuosum* (Sollai et al., 2018a). From a functional  
265 viewpoint, the discriminating capability among different plants helps larvae to recognize hosts  
266 from non-hosts, e.g. when they come in contact with neighboring non-host plants, as it often  
267 happens in Sardinia between ferula and fennel.

268

269 **Conclusions**

270 These results, by showing that the different pattern of activity of GRNs evoked by the plant saps  
271 allows both females and larvae to discriminate among them (accepting ferula and rejecting fennel  
272 and carrot as hosts) strengthen the theory that the peripheral taste sensitivity plays a key role in the  
273 host acceptance or rejection, and in the discrimination process between host and non-host plants.

274 In general, we can say that, except for the particular case (in the Asinara island) of divergence  
275 between females and larvae in the acceptance of *S. tortuosum* (Sollai et al., 2018a), in *P. hospiton*  
276 the performance-preference hypothesis is respected, although there is a hierarchy of choice, both  
277 for adults and for larvae, strongly related to peripheral taste sensitivity.

278 These results complete those previously published (Sollai et al., 2017a; Sollai et al., 2017b) and  
279 allow us to speculate that in the case of *P. hospiton* an "ideal" pattern of activity exists for GRNs  
280 housed in the tarsal and styloconic sensilla, represented by the across neuron pattern (ANP)  
281 generated by the extract of *F. communis*. Variations of neuronal activity that lead to across neuron  
282 patterns different from the ideal one, determine a hierarchical behavior in the host choice, which  
283 goes from a lower level of acceptance (i.e. *Ruta lamarmorae*; Sollai et al., 2017a, 2017b) to a total  
284 rejection of a plant as host (i.e. fennel and carrot).

285

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290

291 **Conflict of interest**

292 There are no financial and personal relationships with other people or organizations that may lead  
293 to a conflict of interest.

294

295 **Author Contributions**

296 Conceptualization, G.S. and R.C.; Methodology, G.S.; Software, G.S.; Validation, G.S. and R.C.;  
297 Formal Analysis, G.S.; Investigation, G.S.; Resources, G.S. and R.C.; Data Curation, G.S.; Writing  
298 – Original Draft Preparation, G.S.; Writing – Review & Editing, R.C.; Visualization, G.S. and  
299 R.C.; Supervision, R.C.; Project Administration, R.C.; Funding Acquisition, G.S. and R.C.

300

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448

Pair stimuli	Tarsal	Lateral	Medial
ferula-fennel	$F=18.06$ ; $P=0.0000$ ; $df\ 3,152$	$F=5.79$ ; $P=0.0001$ ; $df\ 3,152$	$F=6.33$ ; $P=0.0005$ ; $df\ 3,152$
ferula-carrot	$F=10.93$ ; $P=0.0000$ ; $df\ 3,152$	$F=7.48$ ; $P=0.0000$ ; $df\ 3,152$	$F=5.77$ ; $P=0.0009$ ; $df\ 3,152$
fennel-carrot	$F=0.45$ ; $P=0.7221$ ; $df\ 3,152$	$F=2.29$ ; $P=0.0806$ ; $df\ 3,152$	$F=2.34$ ; $P=0.0756$ ; $df\ 3,152$

450 Table 1 - Ensemble code analysis: a difference in ensemble code between two saps was inferred,  
 451 whether 2-way ANOVA revealed a significant interaction of the Stimulus  $\times$  GRN on the spike  
 452 frequency during the first second of stimulation (red typing).

453

Pair stimuli	Tarsal	Lateral	Medial
ferula-fennel	$F=0.47$ ; $P=0.8985$ ; $df\ 9,1580$	$F=0.26$ ; $P=0.9845$ ; $df\ 9,1580$	$F=0.25$ ; $P=0.9865$ ; $df\ 9,1580$
ferula-carrot	$F=0.67$ ; $P=0.7372$ ; $df\ 9,1580$	$F=0.18$ ; $P=0.9959$ ; $df\ 9,1580$	$F=0.15$ ; $P=0.9979$ ; $df\ 9,1580$
fennel-carrot	$F=0.22$ ; $P=0.9916$ ; $df\ 9,1580$	$F=0.47$ ; $P=0.8975$ ; $df\ 9,1580$	$F=0.53$ ; $P=0.8504$ ; $df\ 9,1580$

454 Table 2 - Temporal code analysis: a difference in temporal code between two saps was inferred,  
 455 whether there was a significant interaction of the Stimulus  $\times$  Time on the spike frequency during  
 456 the 10 consecutive intervals of 100 ms of stimulation (red typing).

457

Stimulus	Tarsal	Lateral	Medial
ferula	$F=1.56$ ; $P=0.0353$ ; $df\ 27,760$	$F=1.76$ ; $P=0.0105$ ; $df\ 27,760$	$F=0.89$ ; $P=0.6263$ ; $df\ 27,760$
fennel	$F=0.98$ ; $P=0.4920$ ; $df\ 27,760$	$F=1.82$ ; $P=0.0071$ ; $df\ 27,760$	$F=0.89$ ; $P=0.6209$ ; $df\ 27,760$
carrot	$F=0.38$ ; $P=0.9985$ ; $df\ 27,760$	$F=3.31$ ; $P=0.0000$ ; $df\ 27,760$	$F=0.47$ ; $P=0.9903$ ; $df\ 27,760$

458 Table 3 – Spatio-temporal code analysis: a difference in spatio-temporal code between two saps  
 459 was inferred, whether the T-I curves of a stimulus produced a significant interaction of Time  $\times$   
 460 GRN (red typing), while those of another stimulus produced a non-significant interaction.

461

462

463

464 **Legends of Figures**

465 **Figure 1** – (A) Example of traces showing spike activity of a tarsal basiconic sensillum of an adult  
466 *P. hospiton* female following stimulation with leaf sap of *Fe. communis* (ferula), *F. vulgare*  
467 (fennel) and *D. carota* (carrot). (B) Mean values  $\pm$  SEM of number of spikes evoked in each GRN  
468 of the tarsal sensillum during the first second of stimulation with leaf saps of ferula, fennel and  
469 carrot. N = 20 sensilla (one per female). Different letters indicate significant differences between  
470 the spike activity of the same GRN in response to the three taste stimuli ( $p < 0.05$ ; Tukey test).

471

472 **Figure 2** – (A) Example of traces showing spike activity of a lateral styloconic sensillum of a *P.*  
473 *hospiton* fifth instar larva following stimulation with leaf sap of *Fe. communis* (ferula), *Fo. vulgare*  
474 (fennel) and *D. carota* (carrot). (B) Mean values  $\pm$  SEM of number of spikes evoked in each GRN  
475 during the first second of stimulation with leaf saps of ferula, fennel and carrot. N = 20 sensilla  
476 (one per larva). Different letters indicate significant differences between the spike activity of the  
477 same GRN in response to the three taste stimuli ( $p < 0.05$ ; Tukey test).

478

479 **Figure 3** – (A) Example of traces showing spike activity of a medial styloconic sensillum of a *P.*  
480 *hospiton* fifth instar larva following stimulation with leaf sap of *Fe. communis* (ferula), *Fo. vulgare*  
481 (fennel) and *D. carota* (carrot). (B) Mean values  $\pm$  SEM of number of spikes evoked in each GRN  
482 during the first second of stimulation with leaf saps of ferula, fennel and carrot. N = 20 sensilla  
483 (one per larva). Different letters indicate significant differences between the spike activity of the  
484 same GRN in response to the three taste stimuli ( $p < 0.05$ ; Tukey test).

485

486 **Figure 4** – Ensemble code analysis: significant interaction of the Stimulus  $\times$  GRN on the spike  
487 frequency of an adult tarsal sensillum (A), larval lateral (B) and larval medial (C) sensillum of *P.*  
488 *hospiton*, elicited by *Fe. communis* (ferula), *Fo. vulgare* (fennel) and *D. carota* (carrot).

489

490 **Figure 5** – Temporal code analysis: Time-Intensity curves (i.e., number of spikes during 10  
491 consecutive 100 ms intervals) elicited by *Fe. communis* (ferula), *Fo. vulgare* (fennel) and *D. carota*  
492 (carrot) in adult tarsal sensillum (A), larval lateral (B) and larval medial (C) sensillum of *P.*  
493 *hospiton*.

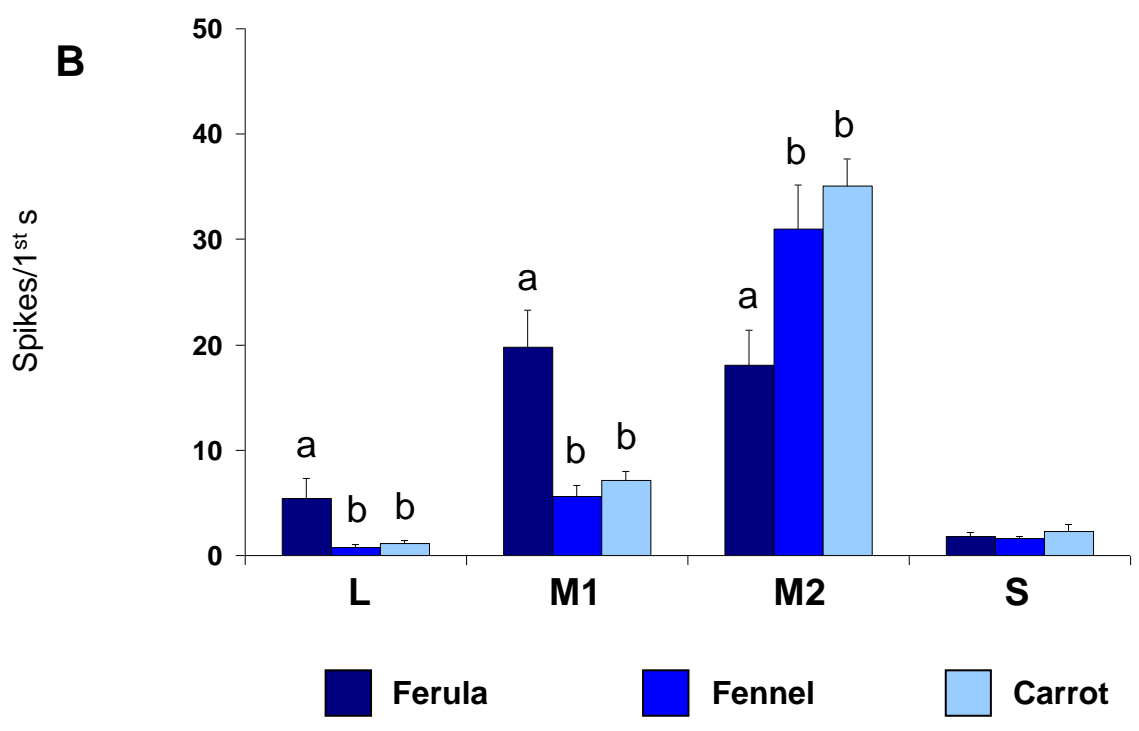
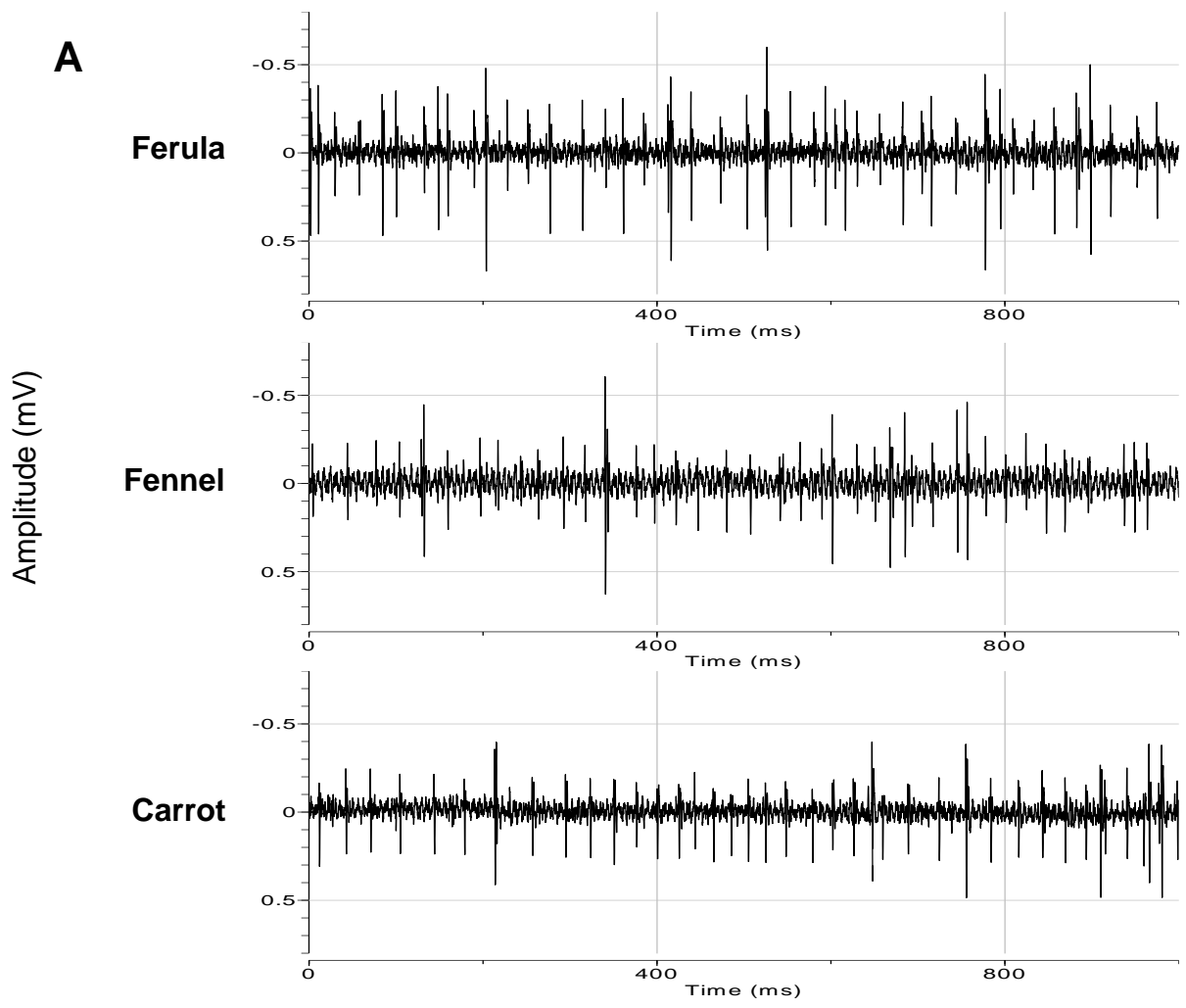


Figure 1

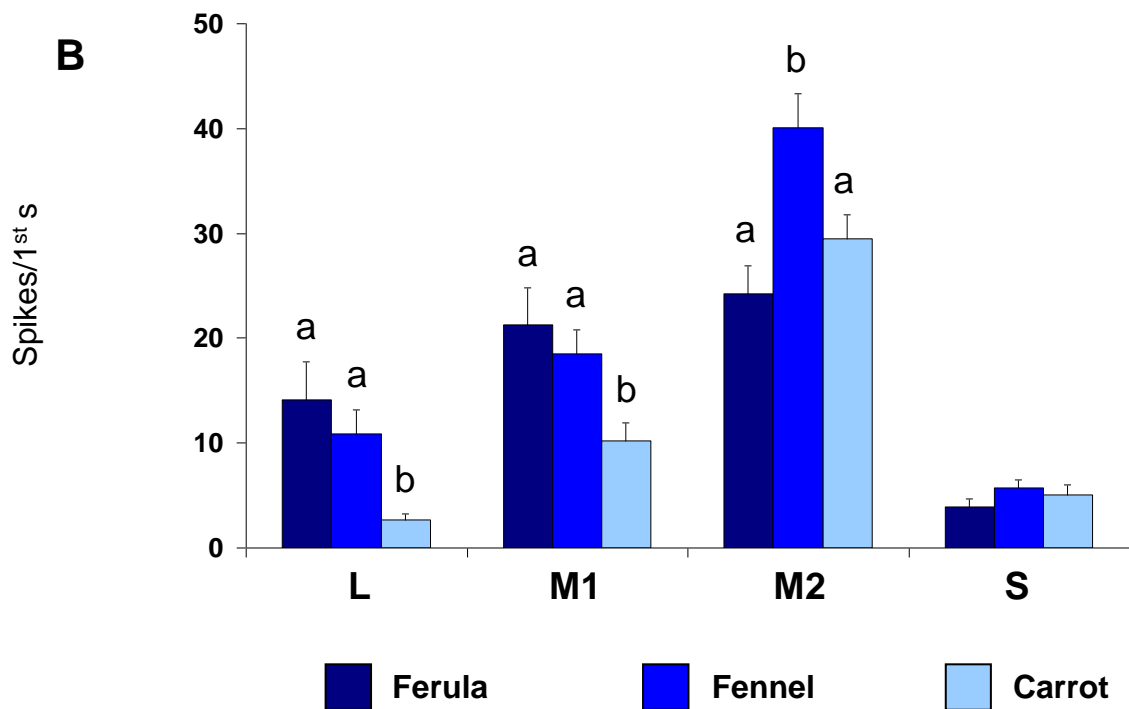
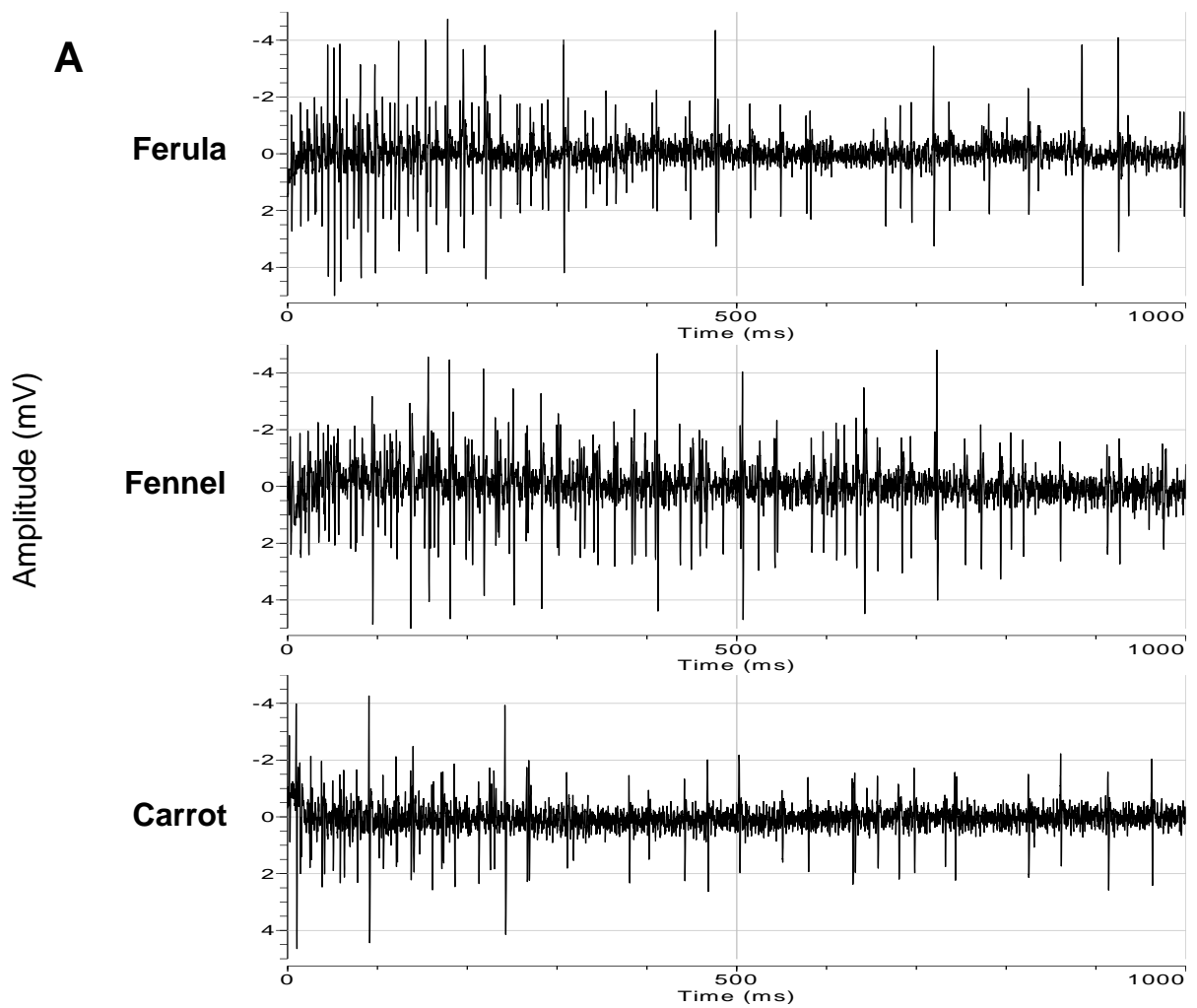


Figure 2

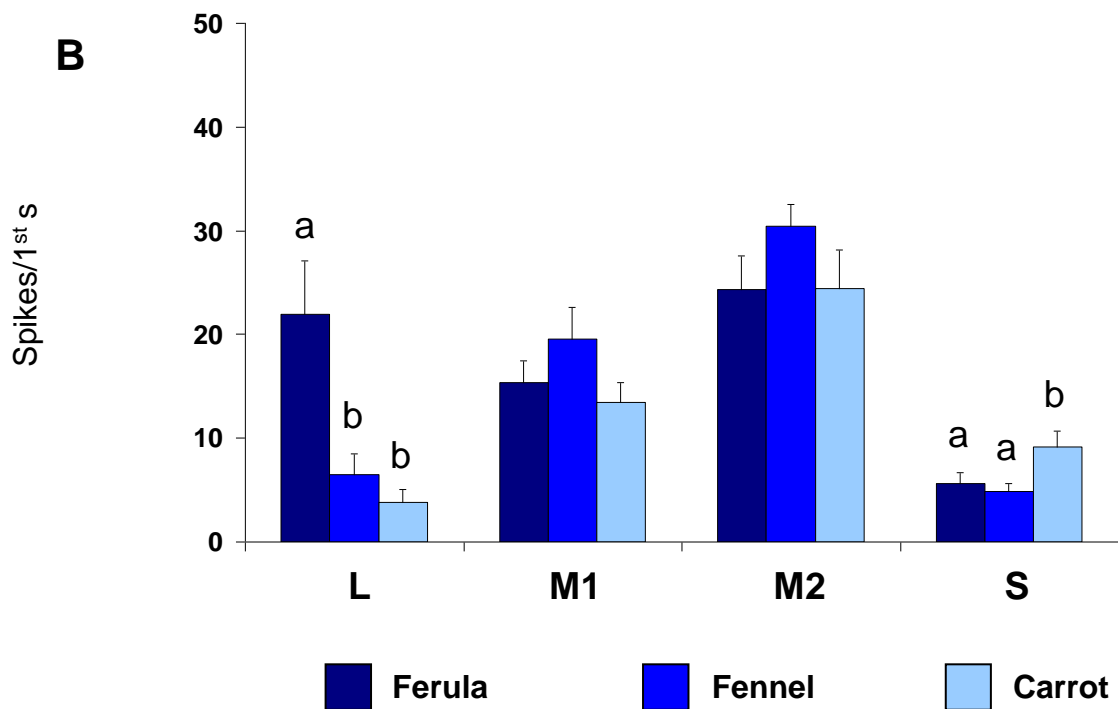
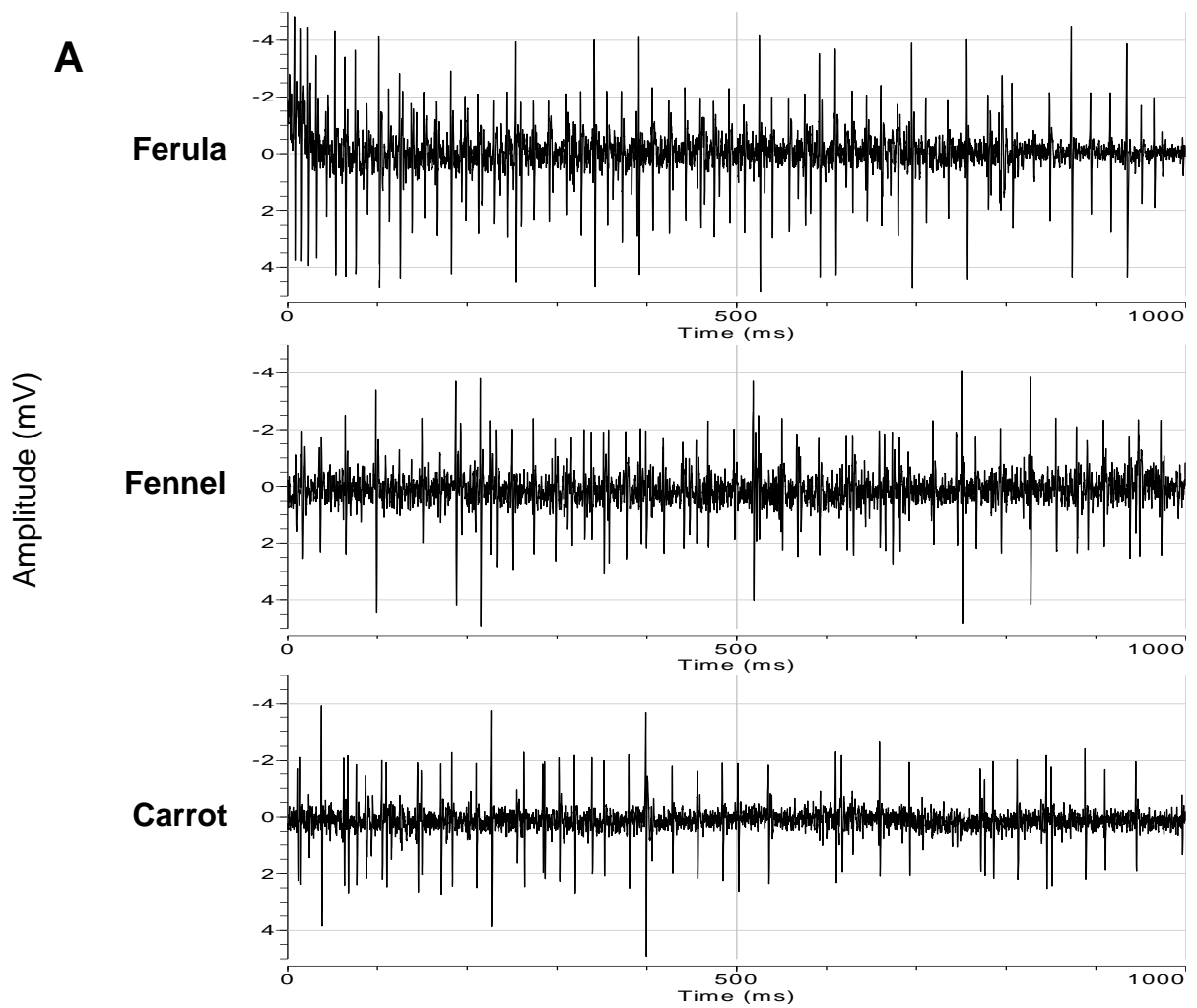


Figure 3

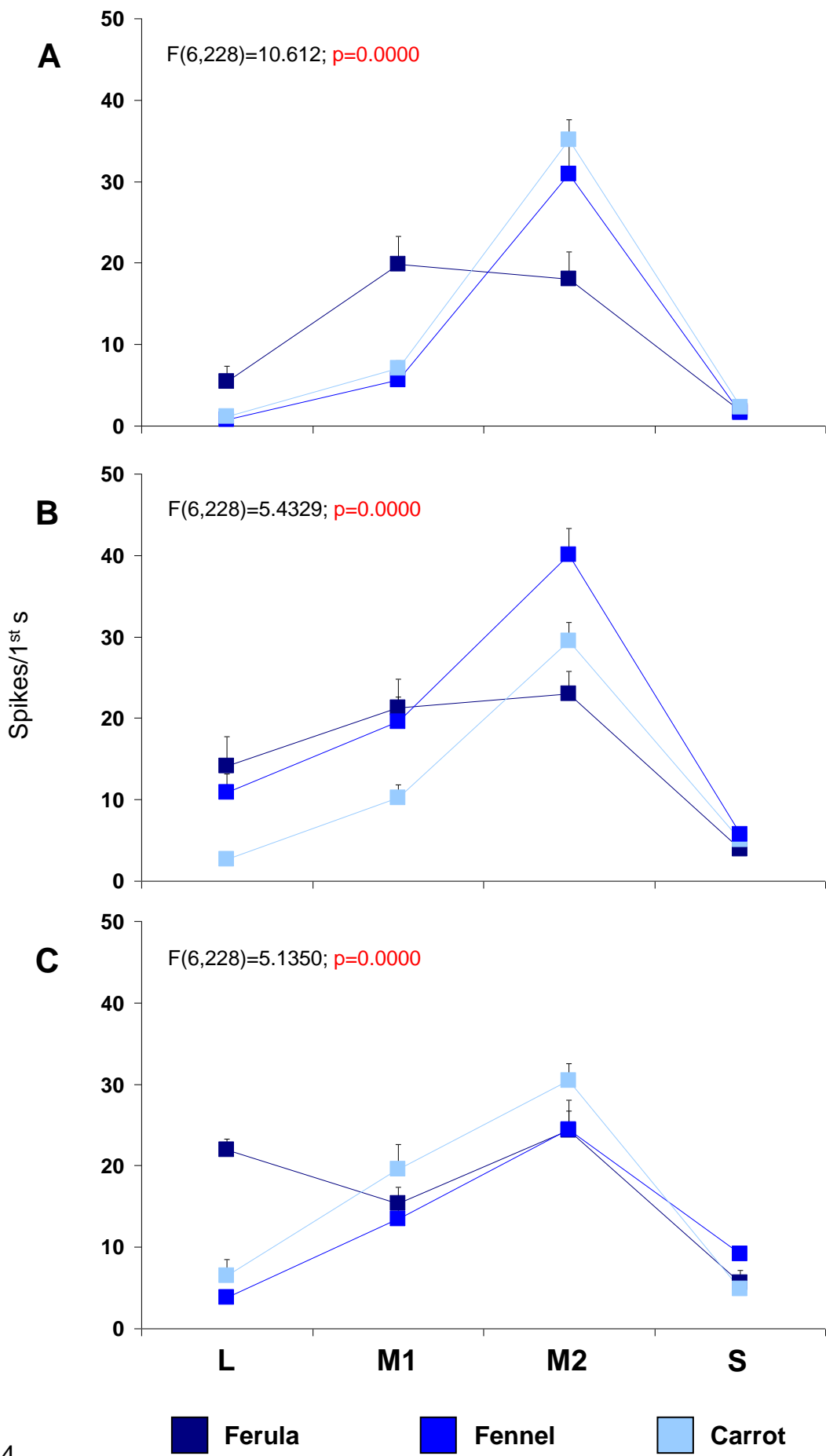


Figure 4



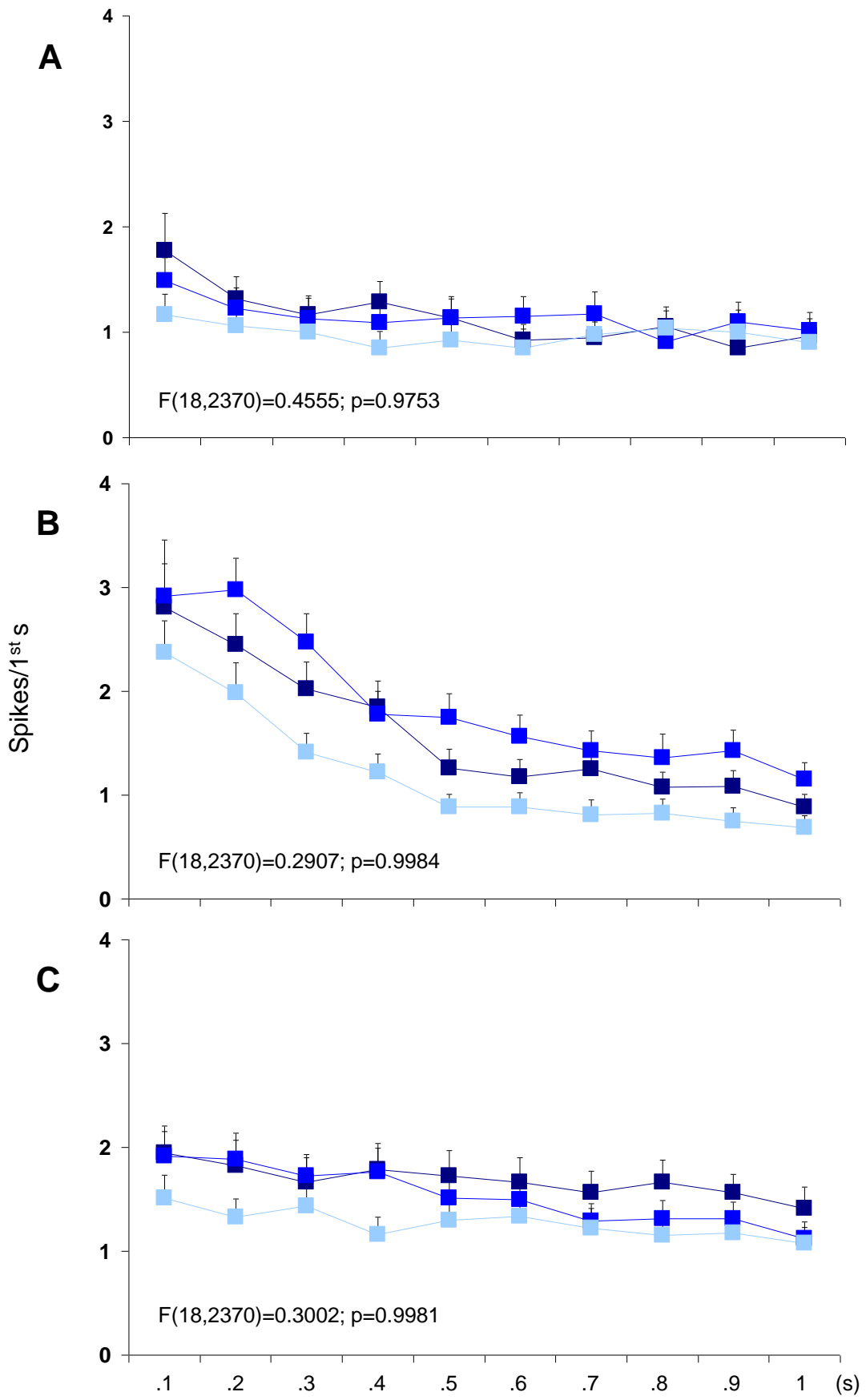


Figure 5