Chemosensory basis of larval performance of *Papilio hospiton* on different host plants.

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Abstract

Papilio hospiton Géné is an oligophagous species, endemic of the islands of Corsica and Sardinia, using various Apiaceae and Rutaceae as host plants, such as *Ferula communis, Ferula arrigonii, Peucedanum paniculatum, Ruta lamarmorae* and *Pastinaca latifolia*. We previously found that the lateral maxillary styloconic sensillum in the larva has two deterrent neurons, one phagostimulant and one salt specific, while the medial sensillum has two phagostimulant neurons, one deterrent and one salt specific. In this work we studied the sensitivity of gustatory receptor neurons (GRNs) to saps of *F. communis, F. arrigonii, P. paniculatum, P. latifolia* and *R. lamarmorae* and evaluated the relationship between taste sensitivity to different host-plants and larval growth rate on each of them. The spike activity was recorded from medial and lateral taste sensilla stimulated with plant saps, and GRN response patterns were cross compared in the light of a different feeding acceptance. The phagodeterrent GRNs show a higher activity in response to *F. arrigonii* and *R. lamarmorae* than to *F. communis, P. paniculatum* and *P. latifolia*. Behavioral trials showed that the time to pupation is significantly longer when larvae are reared on *F. arrigonii* and *R. lamarmorae* than on the other host-plants. These results suggest that the different activity of the phagodeterrent GRNs may inhibit food acceptance and extend the duration of the larval stage.

Key Words: chemoreception; host plants discrimination; lepidopterous larvae; feeding acceptance; Papilionidae; neural coding.

1. Introduction

Peripheral taste sensitivity plays a crucial role in the choice of food both in invertebrates and vertebrates, including humans (Caicedo et al., 2002; Chapman, 2003; Dethier, 1976; Melis et al., 2015; Tepper, 2008; Zhang et al., 2013; Zhou et al., 2010). In insects, taste chemoreceptors respond to various chemicals present in potential food sources and their integrated activity plays a role in the balance between appetitive or aversive behaviour toward foods.

In fact, herbivorous insects, and in particular the larvae of Lepidoptera, represent a suitable model to study the relationship between sensory input and behavioural output in the choice of food, as they exhibit clear food preferences and possess a limited number of gustatory neurons, housed within sensilla in the maxillae and epipharynx. The axons of these chemoreceptors project directly to the brain, in a specific area called subesophageal ganglion (SOG) (Asaoka, 2002; del Campo and Miles, 2003; Schoonhoven and van Loon, 2002; Tang et al., 2014).

In the insect host-plant interaction, and particularly in host recognition, the acceptability of a feeding source depends on the total sensory impression obtained from the response to multiple components of plants, rather than to the presence or absence of individual phagostimulating or deterrent compounds (Dethier, 1973; Martin and Shields, 2012).

In the larvae (of lepidopterans) food assessment is performed by gustatory organs localized on the mouthparts: styloconic sensilla on the maxillary galea, basiconic sensilla at the tip of the maxillary palp and sensilla on the epipharynx (Dethier, 1937; Schoonhoven, 1969).

Most of the electrophysiological studies have been focused on the two styloconic sensilla of each maxillary galea, since they are considered the sensory organs primarily involved in feeding: in fact, they mediate the plant recognition as a food source and its selection and seem to play a particularly important role in the acceptance of the host plant (Dethier and Crnjar, 1982; Martin and Shields, 2012; Schoonhoven 1987). Each styloconic sensillum has 4 gustatory receptor neurons (GRNs) with a specific spectrum of response to plant compounds (for a review, see Schoonhoven and van Loon, 2002). Typically, some neurons respond to phagostimulants, that is primary plant metabolites

such as sugars and amino acids that promote feeding. Other GRNs are activated by deterrent compounds, secondary plant metabolites generally bitter to humans, which mediate food aversive behaviour. Feeding does not depend on the presence or absence of specific compounds, but rather on the balance between phagostimulants and deterrents (Dethier, 1973).

We chose, as a experimental model *Papilio hospiton* Géné, an oligophagous lepidopteran endemic of the Sardinian and Corsican islands, which uses as host plants only a few Apiaceae and Rutaceae (*Ferula communis, Ferula arrigonii*, *Peucedanum paniculatum*, *Pastinaca latifolia* and *Ruta lamarmorae*). In the peripheral taste system of *P. hospiton*, the functional characterization of larval styloconic sensilla showed that the lateral sensillum has two deterrent GRNs (L-lat and M2-lat neurons), one phagostimulant (M1-lat neuron) and one salt neuron (S-lat neuron), while the medial sensillum has two phagostimulant GRNs (L-med and M1-med neurons), one deterrent (M2-med neuron) and one salt neuron (S-med neuron) (Sollai et al., 2014). In addition, the L-lat GRN may act as a "labeled-line" which indicates the presence of toxic compounds (Sollai et al., 2015). In this respect, larval peripheral taste sensitivity plays an important role in feeding acceptance; in fact, host specificity of lepidopterans is determined not only by female oviposition preferences, but also by larval food acceptance (Sollai et al., 2014)

On the basis of these considerations, we assumed that an appetitive or aversive behaviour for food plants could reflect differences in the sensitivity profiles of its gustatory receptor neurons. To this end, we stimulated both styloconic sensilla with leaf saps of different host-plants (*Ferula communis, Ferula arrigonii*, *Peucedanum paniculatum*, *Pastinaca latifolia* and *Ruta lamarmorae*), and we evaluated qualitative and quantitative differences in the response profiles of GRNs between the taste stimuli. We expected that these sensilla, that are indeed involved in host recognition, would show differences in their spike response patterns to different plant saps, thus reflecting somehow the different degrees of host acceptance by the larva. In some cases, larvae may have no choice and need to adapt to the plant on which they hatched. In this respect, the discriminating capability of the larval peripheral taste system would play an important role in feeding acceptance

89 governed by the balance between phagostimulant and phagodeterrent inputs and by the ability to
90 discern among chemicals of the different host-plants. In this study, we stimulated the lateral and
91 medial sensilla with complex natural stimuli, such as plant saps and correlated the spike activity of
92 their GRNs with the behavioral responses to these stimuli. To this end, we have put in relation the
93 electrophysiological responses to host-plant saps with the larval growth performance. This could
94 provide a better understanding of the neural code for acceptance or aversion to plants by insect
95 herbivores and this is considered a major objective of studies on coding of taste information (Tang
96 et al., 2014).
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2. Materials and Methods

2.1. Insects and rearing

Papilio hospiton Géné larvae were obtained from eggs laid in the butterfly oviposition annex (a 3 x 3 x 3m cage) of the Physiology Laboratories (University of Cagliari) by lab stock adult females on potted giant fennel (*Ferula communis* L.). Caterpillars were reared at the insectary annex of the Physiology Laboratories (University of Cagliari) in 1500-ml plastic cups (4-5 per cup) kept in an environmental growth chamber (24-25 °C, 70% R.H., 16L/8D photoperiodic regime) and checked daily until fit for the experiments.

Fresh foliage of *F. communis* came from plants grown in a yard adjacent to the butterfly cage and was available ad libitum each day.

2.2. Electrophysiological experiments

Electrophysiological recordings were obtained from 5th instar larvae two days after moulting (Simmonds et al., 1991) from the medial and lateral maxillary styloconic sensilla by means of the "tip-recording" technique (Hodgson et al., 1955). The reference electrode, a thin Ag/AgCl, was inserted into the head and gently pushed into the maxillary-labial complex to fix the maxillae in a prognathous position. The recording electrode, a glass micropipette (tip diameter 20 μm), filled with the stimulating solution, was placed over the sensillum tip. All signals were recorded with a high input impedance (10^{15} Ω) electrometer (WPI, Duo 773), band-pass filtered (0.1-3 KHz), digitized by means of an Axon Digidata 1440A A/D acquisition system (sampling rate 10 KHz) and stored on PC for later analysis.

Medial and lateral sensilla were tested with aqueous solution of KCl 50 mM (control) and five complex stimuli represented by leaf freshly-pressed extracts of four plants belonging to Apiaceae family: *Ferula communis* L. (giant fennel; hereafter Fcom), *Ferula arrigonii* Bocch. (Farr), *Peucedanum paniculatum* Loisel (Peuc), *Pastinaca latifolia* (Duby) DC. (Past) and one plant

belonging to Rutaceae family: *Ruta lamarmorae* Bacch., Brullo et Giusso (Ruta). Dare info sulla tecnica di estrazione dei succhi……

Stimuli were applied to the sensilla for 6-7 s, in a randomized sequence except for KCl that was tested first and a 3 min interval was allowed between consecutive stimulations to minimize adaptation phenomena. All leaf extracts were tested within 30 s after being pressed, according to Dethier and Crnjar (1982). At the end of each sequence, KCl was tested again to assess any shift in chemosensillar responsiveness; whenever significant variations were found, the experiment was discarded. In order to avoid any drift in solution concentration due to evaporation, a clean, dry piece of filter paper was used to draw a small amount of solution from the electrode tip just before each stimulation. After each test, the mouthparts of the insect were rinsed with distilled water and blotted dry. Finally, we recorded only from sensilla of one maxilla for each larva (N=36-58) and no preparation was used in more than one experiment.

2.3. Data analysis

Recordings typically lasted 2-3 s, but spike analysis was performed in the interval 10-1010 ms after contact with the sensillum, the first 10 ms being skipped as containing the contact artifact. The first second of the discharges was chosen as representative of the phasic/phasic-tonic parts of the response (Dethier and Crnjar, 1982; Inoue et al., 2009) and spike sorting and counting were performed by means of the Clampfit 10.0 software, based on earlier studies (Dolzer et al., 2003; Dulcis and Levine, 2005; Pézier et al., 2007; Sollai et al., 2014).

2.4. Larval growth performance

To test the larval growth performance we measured the duration of the larval stage on each host-plant, defined as the period from egg hatch to pupation. The larvae were reared on the host-plant where they hatched from egg, at environmental condition, in the butterfly oviposition annex (a 3x3x3m cage) of the Physiology Laboratories (University of Cagliari). We looked for growth

performance of larvae laid as eggs on the same plants tested for the electrophysiological recordings (N=32 for each plant).

2.5. Statistical analysis

One-way ANOVA was used to analyze the relationship between: a) the spike activity of each GRN and the stimulus; b) the larval growth (days from hatching to pupa) and the host plant.

Main effects ANOVA was used to verify whether any two taste stimuli generated a different rate code, i.e. a different number of action potentials per time unit (frequency code). Thus, we analyzed the total number of spikes generated by each bitter-sensitive GRN in the first second of response and we inferred a difference in rate code, e.g. between Fcom and Farr, whenever there was a significant main effect of the taste stimulus on the spike frequency.

Two-way ANOVA was used to verify whether any two taste stimuli produced: a) a different ensemble code, i.e. a different response pattern across all active GRNs. In this case, we analyzed the total number of spikes generated by each GRN in the first second of response and we inferred a difference in ensemble code if there was a significant interaction of Stimulus × GRN on the spikes frequency; b) a different temporal code, i.e. a different distribution of neural activity over time. Time-intensity (T-I) curves (i.e. the number of action potentials in each successive 100 ms during the first second of activity) were obtained separately for each taste stimulus and GRN. We inferred a difference in temporal code (e.g., between Fcom and Farr), if there was a significant interaction of Time × Stimulus; c) a different spatio-temporal code, according to which stimulus identity is encoded by the time course of the action potential frequency of each neuron activated by the same stimulus. Time-intensity curves (T-I) of each GRN were considered separately for each stimulus, and we wondered whether the T-I curve produced by a GRN was different from that produced by the other GRNs. We inferred a difference in spatio-temporal code (e.g., between Fcom and Farr), if the curves T-I of a taste stimulus produced a significant interaction of Time × GRN, while those of another stimulus produced a non-significant interaction (Sollai et al., 2015).

178 Data were checked for the assumptions of homogeneity of variance and normality. Post-hoc
179 comparisons were conducted with the Tukey test, unless the assumption of homogeneity of variance
180 was violated, in which case Duncan's test was used. Statistical analyses were performed using
181 STATISTICA for WINDOWS (version 7.0; StatSoft Inc, Tulsa, OK, USA). *P* values < 0.05 were
182 considered significant.
183
184 **2.6. Permits**
185 Required permits were obtained for *Papilio hospiton.* Specimens were collected in Sardinia in the
186 spring of 2012, in compliance with the permit issued on 28 May 2012 (Ref. # 0010888) to Roberto
187 Crnjar and his co-workers, by the "Ministero dell'Ambiente e della Protezione del Territorio e del
188 Mare" (Italian Board of Environment and Protection of Land and Sea), in derogation from the
189 provisions set out in the regulation DPR 357/97 concerning the application of the "Council
190 Directive 92/43/EEC of 21 May 1992 on conservation of natural habitats and of wild fauna and
191 flora". No specific permits were required for all host plants tested, as they are not endangered or
192 protected species.
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194 **3. Results**
195 **3.1 Effect of the plant saps on the spike activity of the lateral and medial GRNs**
196 Samples of spike discharges of the activity of the GRNs, recorded from the lateral and medial
197 styloconic sensilla, in response to complex stimuli like leaf extracts of host plants, are shown in
198 Figures 1 and 2. All tested plant saps elicited responses from all GRNs housed in both lateral and
199 medial sensilla (for details, see Supplemental Material).
200 To test for a relationship between neural activity of each GRN and the stimulus, we analyzed the
201 spike response evoked in the first second of the discharge for each GRN ("L", "M1", "M2" and
202 "S") in both lateral and medial sensilla, by using an one-way ANOVA.

For the lateral styloconic sensillum (Fig. 3), one-way ANOVA showed a significant effect of stimulus on the spike frequency of all GRNs ($F_{[4,219]}$>5.4775; P<0.001). In particular, post-hoc comparisons showed that the spike frequency of both deterrent neurons (L and M2) in response to Farr and Ruta was higher than that in response to the other saps plant (P<0.001; Tukey test), that the spike frequency of phagostimulant neuron (M1) in response to Farr and Ruta was higher than that in response to Fcom and Past (P<0.05; Tukey test). Finally, pairwise comparison showed that the activity of salt neuron (S) in response to Fcom was lower than that in response to Farr and Past (P<0.01; Duncan's test). These results indicate that Farr and Ruta are the most stimulating plant saps for all GRNs housed on the lateral sensillum.

For the medial sensillum (Fig.3), one-way ANOVA showed a significant effect of stimulus on the spike frequency of M1 and M2 neurons ($F_{[4,212]}$>3.5444; P<0.01). In particular, post-hoc comparisons showed that the spike frequency of phagostimulant neuron (M1) in response to Fcom was lower than Farr (P<0.005; Tukey test), and that activity of the deterrent neuron (M2) in repsonse to Farr and Ruta was higher than that in response to the other saps plant (P<0.01; Tukey test). Finally, no other stimulus effects were found. These results indicate that, for the medial sensillum, Farr and Ruta are the most stimulating plant saps for the deterrent M2 nurons and only Farr for the phagostimulant M1.

3.2 Sensory code mediating plant discrimination

We investigated whether GRNs can discriminate among different plant saps by means of a rate, ensemble, temporal and/or spatio-temporal code. To verify a difference in rate code, we analyzed the total number of spikes evoked in the first second of response with each plant sap tested. The results show that taste stimulus was not the main effect on the spike frequency, for both lateral and medial styloconic sensilla, except in the comparison Fcom-Farr (Tab. 1), thus indicating that the tested plant saps do not generate different rate codes. To verify a difference in ensemble code, we analyzed the total number of spikes evoked in the first second of response for each GRN and

stimulus separately. A significant interaction of Stimulus × GRN on spike frequency was found in the plant saps comparison for both lateral ($F_{[12,872]}$=5.0769; P<0.00001) and medial sensillum ($F_{[12,848]}$=5.1224; P<0.00001) (Fig. 4). In detail, the results presented in Table 2, indicate that Fcom, Peuc and Past generated a different ensemble code from those by Farr and Ruta.

In order to verify a difference in temporal code, we analyzed the T-I curves for each plant sap and evaluated the presence of a significant interaction of Stimulus × Time by using two-way ANOVA. A non-significant interaction of Stimulus × Time was found in both lateral ($F_{[36,8910]}$=0.37555; P=0.99976) and medial sensillum ($F_{[36,8623]}$=0.64978; P=0.94771) (Fig. 5) (Tab. 3). These results indicate that the plant saps don't generated a different temporal codes. Finally, to verify a difference in spatio-temporal code, we analyzed the T-I curves produced by each GRN separately for each taste stimulus. For the lateral sensillum, there was a significant interaction of Time × GRN for all stimuli tested: this result shows that each stimulus evoked non-parallel T-I curves in all GRNs (Tab. 4). Instead, for the medial sensillum, the interaction of Time × GRN was significant for Fcom, Peuc and Past, but not for the Farr and Ruta (Tab. 4). These results show that Farr and Ruta each evoked T-I curves in the medial GRNs that were essentially parallel to one another. These findings indicate that Fcom, Peuc and Past generated a different spatio-temporal code with respect to Farr and Ruta, in the medial sensillum, but not in the lateral one.

3.3 Larval growth performance

To test for a relationship between larval growth performance and feeding substrate, we analyzed the number of days needed to reach the pupal stage on each host-plant considered, by using one-way ANOVA. One-way ANOVA showed a significant effect of the feeding substrate on the larval performance ($F_{[4,155]}$>84.586; P<0.00001; Fig.6). In particular, post-hoc comparisons showed that the number of days needed to pupation was higher for those larvae raired on Farr and Ruta than those on Fcom, Peuc and Past (P<0.0001; Tukey test). No other feeding substrate effects were

found. These results indicate that the larvae grow more slowly on Farr and Ruta than those on Fcom, Peuc and Past.

4. Discussion

Insects have a gustatory system that allows them to discriminate among different food sources and between host and non host plants (Chapman, 2003; Forister et al., 2012; Schoonhoven et al., 2005). Among all gustatory neurons housed on the mouthparts, the lateral and medial styloconic sensilla are considered the sensory organs primarily involved in feeding: they seem to play an important role in host plant acceptance (Dethier and Crnjar, 1982; Schoonhoven, 1987). In fact, larvae tend to accept a plant more than another and this preference is maintained also after they are surgically deprived of all taste input except that from GRNs of lateral and medial sensilla, and of epipharynx (Dethier and Crnjar, 1982).

The main goal of this work was to evaluate whether differences in the pattern activities of the 4+4 GRNs housed in the lateral and medial styloconic sensilla in response to leaf extracts of several host plants could justify the difference in the degree of their acceptance as food sources.

The electrophysiological results show that each stimulus evoked spike activity in all neurons, but only 6 of them responded with a high frequency. Statistically significant differences were observed in the activity of individual neurons in response to different extracts: in particular, the extract of Farr and Ruta elicit a higher spike frequency from the both bitter and sugar cells (Sollai et al., 2014), as compared to the saps of Fcom, Peuc and Past. Differences in the neuron responses to the plant saps tested are considered consistent with the differences in food preference (Tang et al., 2014). Behavioral results about larval growth performance show that the duration of the larval stage, from egg to pupa, on Fcom, Peuc and Past is statistically lower than Farr and Ruta. Together, these results suggest a direct relationship between the degree of acceptance of a food source (e. g.. a host plant) and the electrophysiological responses elicited by each of them. Plants on which the larvae have the same performance, such as Fcom/Peuc/Past on the one hand and Farr/Ruta on the

other, also give similar electrophysiological responses. Some authors support the hypothesis that the increase in the spikes frequency of a particular GRN (e. g. one neuron that responds to bitter and potentially toxic compound) is correlated with a more rapid and intense behavioral response (e. g. taste rejection) (de Boer et al., 1977), and that the activation of the deterrent GRN by a plant extract, slows down the feeding activity (Glendinning et al., 1998); others argue instead that the chemosensory cells of the maxillary palps produce spontaneous electrical activity that inhibits feeding in the absence of a sufficient excitatory input, suggesting that food rejection is linked more to the absence of phagostimulant inputs that to the presence of deterrent inputs (Ma, 1972). We suppose that the lower larval performance on Farr and Ruta is linked to the fact that the extracts of these plants elicit a higher activity from the L-lat, M2-lat and M2-med neurons, previously identified as bitter cells (Sollai et al., 2014), with respect to saps of Fcom, Peuc and Past; this holds true for to the L-lat neuron which was found to signal the presence of bitter and toxic compounds (Sollai et al., 2015). However, the same saps also evoke a higher spike activity from the phagostimulant neurons (M1-lat and M1-med), in agreement with the fact that all larvae reach the pupal stage. These results support the hypothesis that the peripheral gustatory system plays an important role in the acceptance of a host plant and that the acceptance degree of a specific plant is due to the balance between phagostimulant and deterrent stimuli, rather than to a simple discrimination between them.

Manduca sexta larvae can discriminate among different host plants with only 8 functioning taste receptors (Dethier and Crnjar, 1982). These taste receptors are considered capable of "coding" the chemical complexity of plants transducing the quality of the mixture of plant compounds into spike trains to bring the information up to the CNS (Dethier and Crnjar, 1982). Each of these cells is a labeled line for a gustatory modality (represented by a class of chemical compounds, such as sugars, secondary metabolites or bitters, salts, water, etc.), but other neural codes appear to be important for mixtures of compounds (Dethier and Crnjar, 1982; Glendinning et al., 2006). We have previously showed that the *P. hospiton* larvae can discriminate between toxic and non-toxic bitter compounds

306 by means of a set of neural codes (Sollai et al., 2015). In the present study, the results suggest that
307 mixtures of chemical compounds, such as plant saps, can be discriminated by means of an ensemble
308 and spatio-temporal code. In fact, we found that Fcom/Peuc/Past generate the same across neuron
309 pattern (ANP), but different from that obtained with Farr and Ruta, which were equal to each other.
310 In addition, in the medial sensillum, the extracts of Fcom/Peuc/Past each evoked non-parallel T-I
311 curves in the GRNs, while the extract of Farr/Ruta each evoked parallel T-I curves, indicating a
312 difference in spatio-temporal code. The plants on which the larvae grow faster, as Fcom/Peuc/Past,
313 do not differ in ensembles and spatio-temporal code, thus giving similar electrophysiological
314 responses. Those are the plants that evoke a lower activity in deterrent neurons, in particular in the
315 GRN previously indicated as labeled line for the toxic bitter compounds. On the contrary, the leaf
316 extract of Farr and Ruta evoked a higher activity in the deterrent neurons and produced both
317 ensemble and spatio-temporal codes different from the other plants, thus signaling these plants as
318 non-host, novel or foreign. In *Helicoverpa* the duration of the larval stage for each species was
319 significantly shorter on the host plant preferred by the larvae (Liu et al., 2012). Besides, the evoked
320 ANP may control the degree of acceptance of a food source, as shown in Leptinotarsa sp. (Sperling
321 and Mitchell, 1991). The fact that,all larvae reach the pupal stage, though with different time
322 lengths, suggests that the *P. hospiton* probably recognizes Farr and Ruta as novel or foreign plant,
323 but not as non-host. This is probably due to the fact these same plants also evoked a higher activity
324 in phagostimulant neurons, and so the final decision whether to accept or not a food source is
325 determined by the balance arising from both phagostimulant and phagodeterrent inputs (Dethier,
326 1973).
327 In conclusion, these results suggest that, in *P. hospiton* larvae, the peripheral gustatory system plays
328 an important role in the acceptance of a host plant and that the characteristics of the
329 electrophysiological responses to each plant sap is strongly consistent with that of the feeding
330 preference behaviour. However, we cannot exclude that growth performance be also related to other
331 factors such as nutritional values of host plants: future experiments are needed to elucidate this

aspect. Besides, larvae seem to be able to discriminate among host plants by means of an ensemble and spatio-temporal code. We therefore propose that discrimination may be the outcome of several combined coding mechanisms principally involving the chemosensory neurons of the lateral and medial sensilla. From a functional viewpoint, the discriminating capability among different host-plants may allow larvae to recognize the most favourable one for larval growth. In fact, even if the first choice is done by the egg laying adult female, it may not be uncommon that larvae be confronted with choice situations of feeding substrate: if they come in contact with neighbouring non-host plants, fall or stray from host plant, or in general when sampling host plant tissues for healthy parts vs. withering ones, this all requires some chemosensory discrimination on their part: they will have then to decide whether to eat or not.

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Conflict of interest

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

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Legends of Figures

Fig. 1 – Sample traces showing spike firing frequency of a lateral styloconic sensillum following stimulation with leaf sap of *F. communis* (Fcom), *F. arrigonii* (Farr), *P. paniculatum* (Peuc), *P. latifolia* (Past) and *R. lamermorae* (Ruta).

Fig. 2 – Sample traces showing spike firing frequency of a medial styloconic sensillum following stimulation with leaf sap of *F. communis* (Fcom), *F. arrigonii* (Farr), *P. paniculatum* (Peuc), *P. latifolia* (Past) and *R. lamermorae* (Ruta).

Fig. 3 – Mean values ± s.e.m. of number of spikes evoked in each GRN of the lateral and medial sensillum during the first second of stimulation with leaf sap of *F. communis* (Fcom), *F. arrigonii* (Farr), *P. paniculatum* (Peuc), *P. latifolia* (Past) and *R. lamermorae* (Ruta). N=36-58.
Different letters indicate significant differences between the spike activity of the same GRN in response to different taste stimuli (for L GRN of lateral sensillum: $p<0.00001$; Duncan's test subsequent to one-way ANOVA; for all others GRN: $p<0.05$; Tukey test subsequent to one-way ANOVA).

Fig. 4 – Significant interaction of the Stimulus × GRN on the spike frequency in both lateral and medial sensillum.

Fig. 5 – Time-Intensity curves (i.e., number of spikes during 10 consecutive 100 ms intervals) elicited by *F. communis* (Fcom), *F. arrigonii* (Farr), *P. paniculatum* (Peuc), *P. latifolia* (Past) and *R. lamermorae* (Ruta). N=36-58**.**

Fig. 6 – Mean values ± s.e.m. of the number of days nedeed to pupation on *F. communis* (Fcom), *F. arrigonii* (Farr), *P. paniculatum* (Peuc), *P. latifolia* (Past) and *R. lamermorae* (Ruta). N=32/plant.

460 Different letter indicates significant differences ($p<0.0001$; Tukey test subsequent to one-way
461 ANOVA)
462
463 **Table 1** - Rate code analyses: we inferred a difference in rate code, e.g. between Fcom and Farr, if
464 the main effect on the total number of spikes generated by each GRN in the first second of response
465 was the taste stimulus than the neuron (red typing). L=lateral sensillum; M=medial sensillum.
466
467 **Table 2** - Ensemble code anlyses: we inferred a difference in ensemble code, e.g. between Fcom
468 and Farr, if the was a significant interaction of the Stimulus × GRN on the spikes frequency during
469 the first second of stimulation (red typing).
470
471 **Table 3** - Temporal code analyses: we inferred a difference in temporal code (e.g., between Fcom
472 anf Farr), if there was a significant interaction of Time × Stimulus on the spikes frequency during
473 the first second of stimulation.
474
475 **Table 4** - Spatio-temporal code analyses: we inferred a difference in spatio-temporal code (e.g.,
476 between Fcom anf Farr), if there was a significant interaction of Time × GRN on the spikes
477 frequency during the first second of stimulation (red typing).

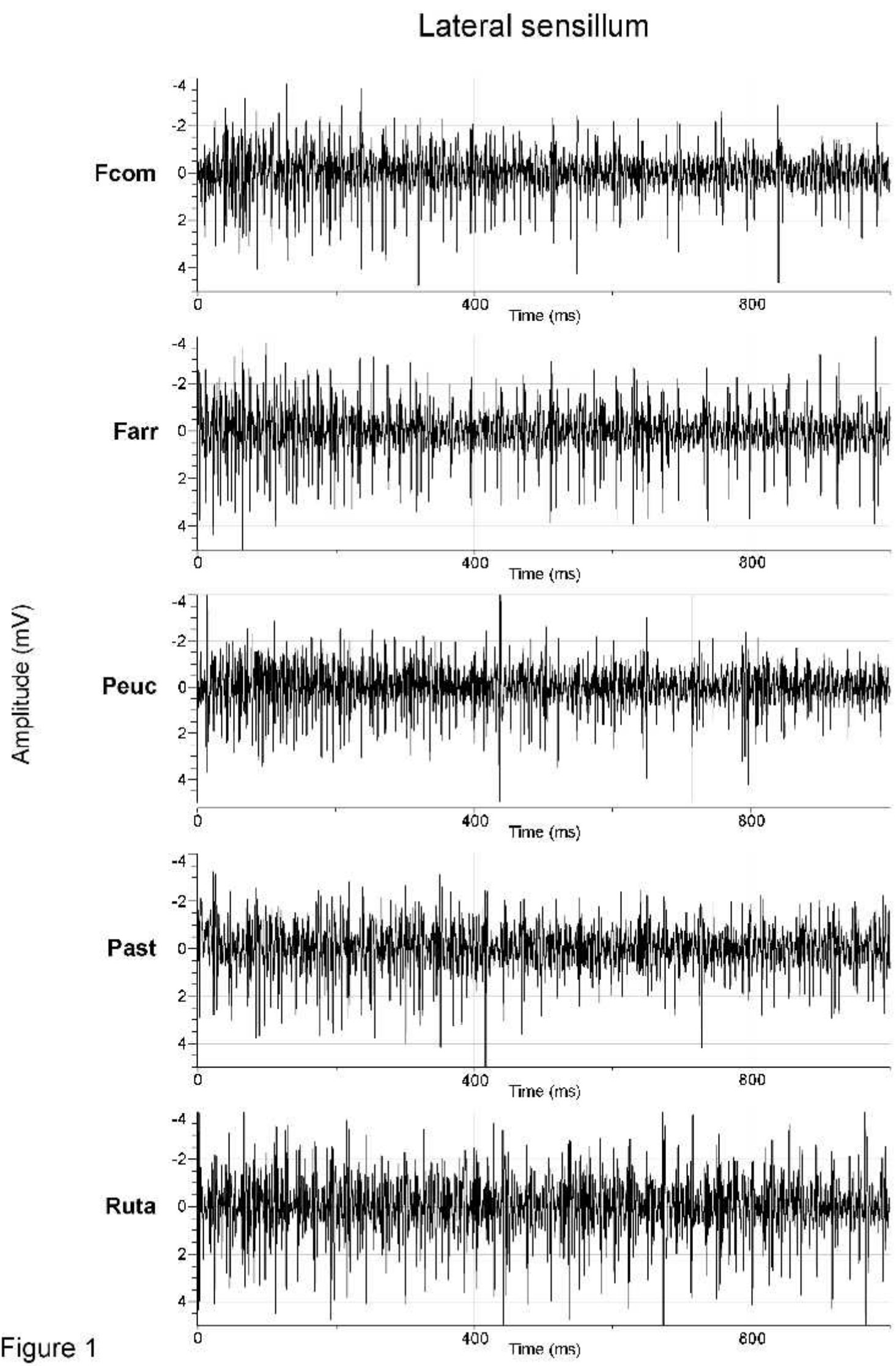
Lateral sensillum
Fcom
Farr
Peuc
Past
Ruta
Amplitude (mV)
-4
-2
0
2
4
0
400
800
Time (ms)

Figure 1

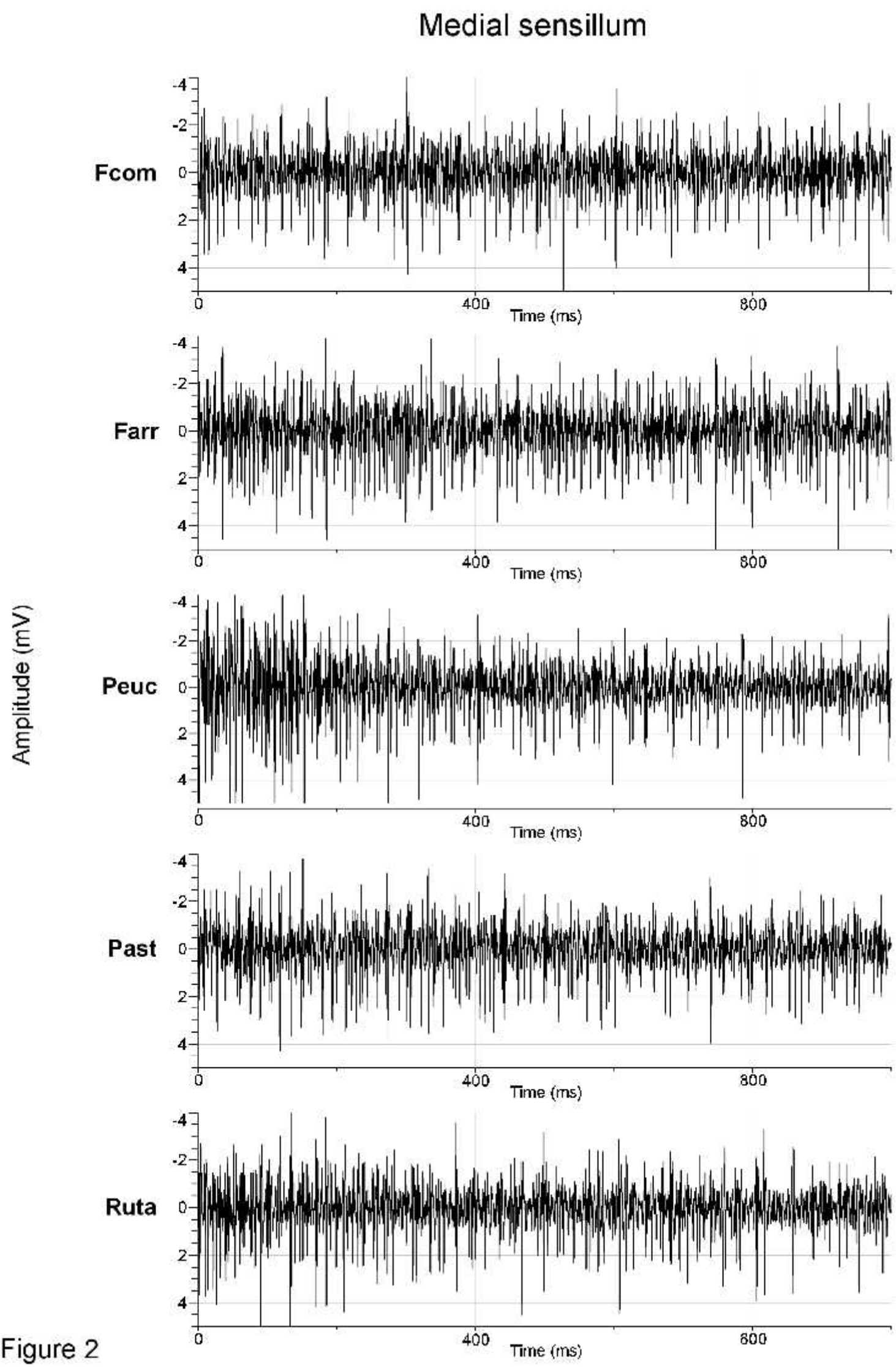
Medial sensillum
Fcom
Farr
Peuc
Past
Ruta
Amplitude (mV)
-4
-2
0
2
4
0
400
800
Time (ms)

Figure 2

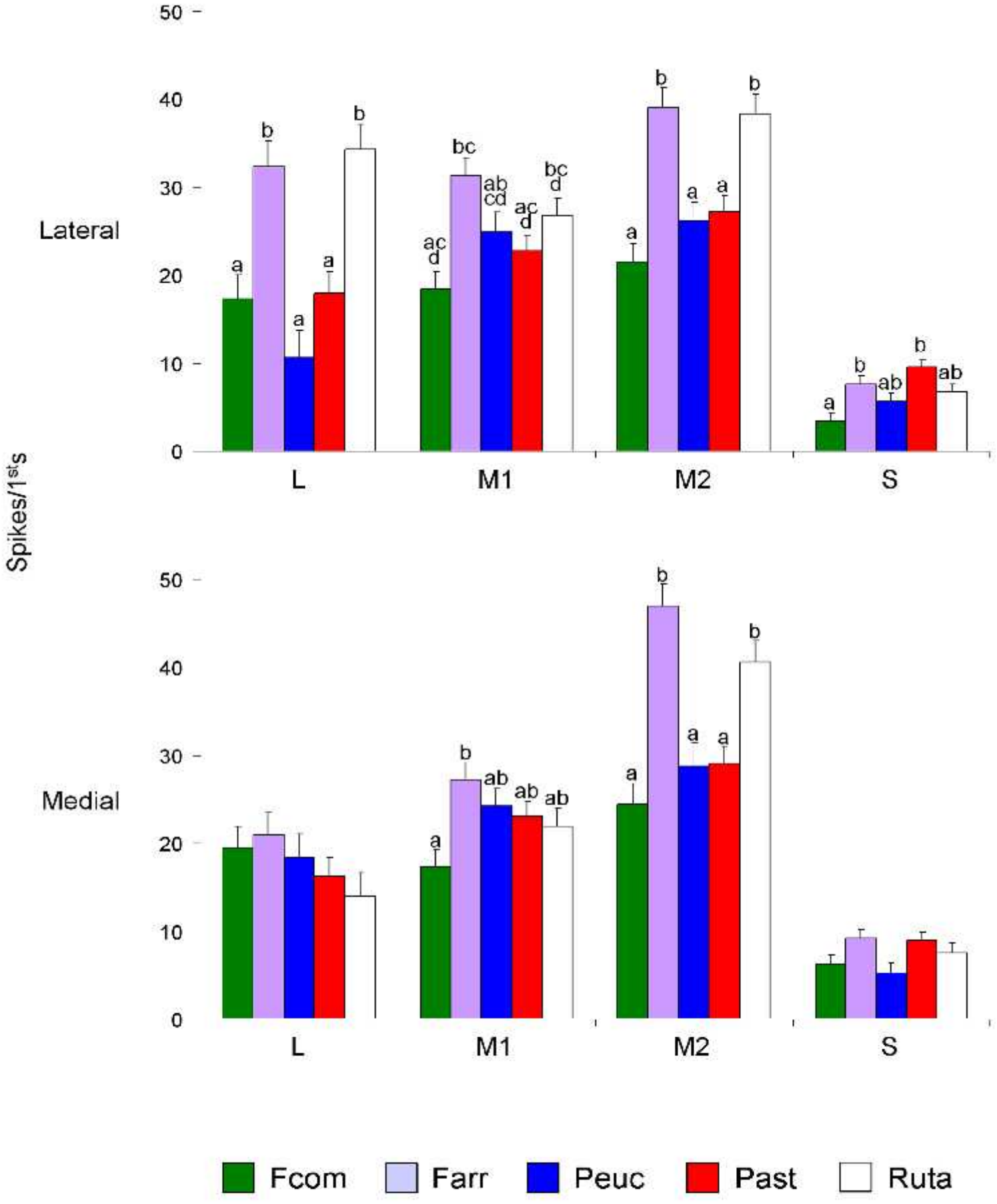
Lateral
Medial
Spikes/1st s
50
40
30
20
10
0
L
M1
M2
S
Fcom
Farr
Peuc
Past
Ruta

Figure 3

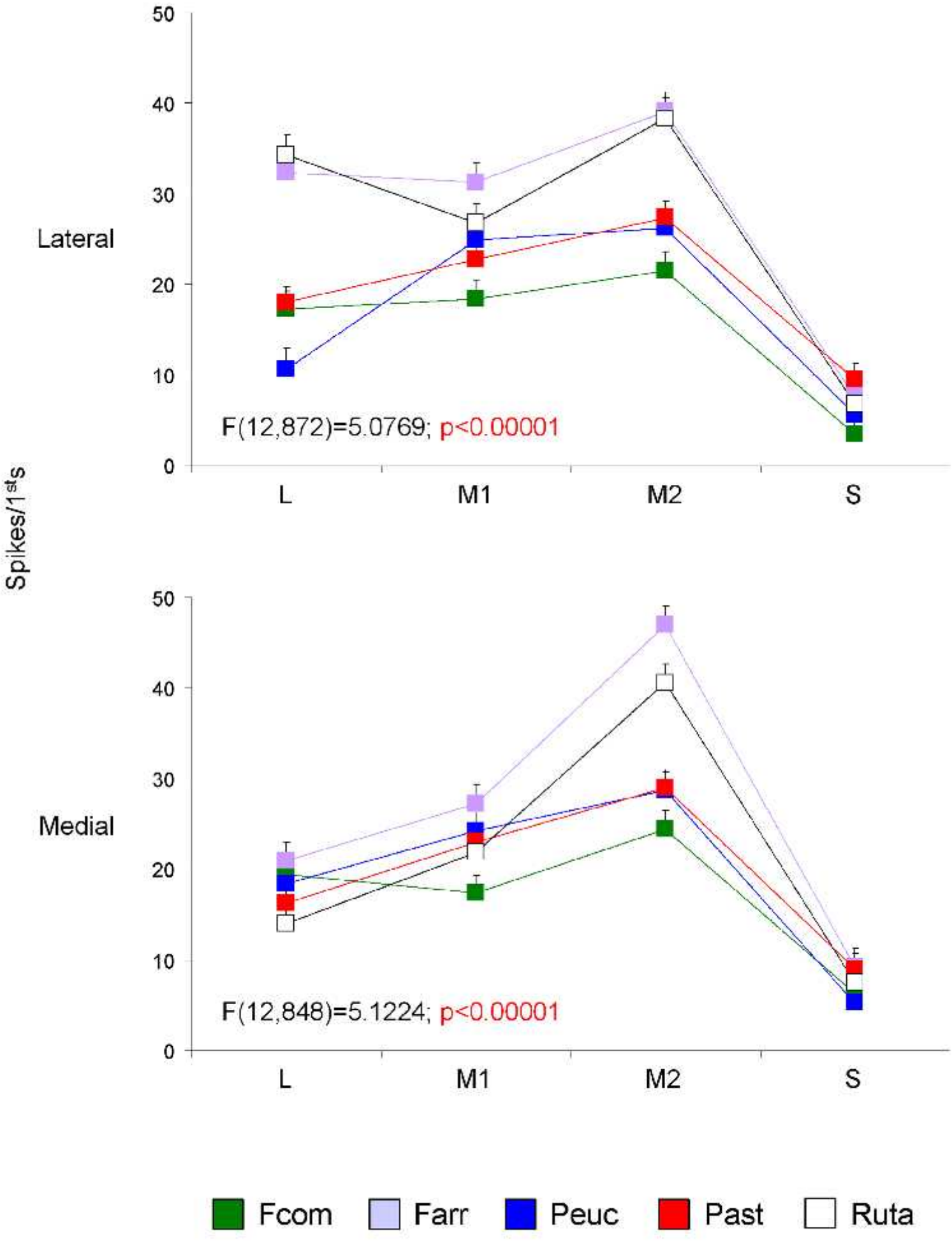
Lateral
Medial
Spikes/1st s
50
40
30
20
10
0
L
M1
M2
S
F(12,872)=5.0769; p<0.00001
F(12,848)=5.1224; p<0.00001
Fcom
Farr
Peuc
Past
Ruta

Figure 4

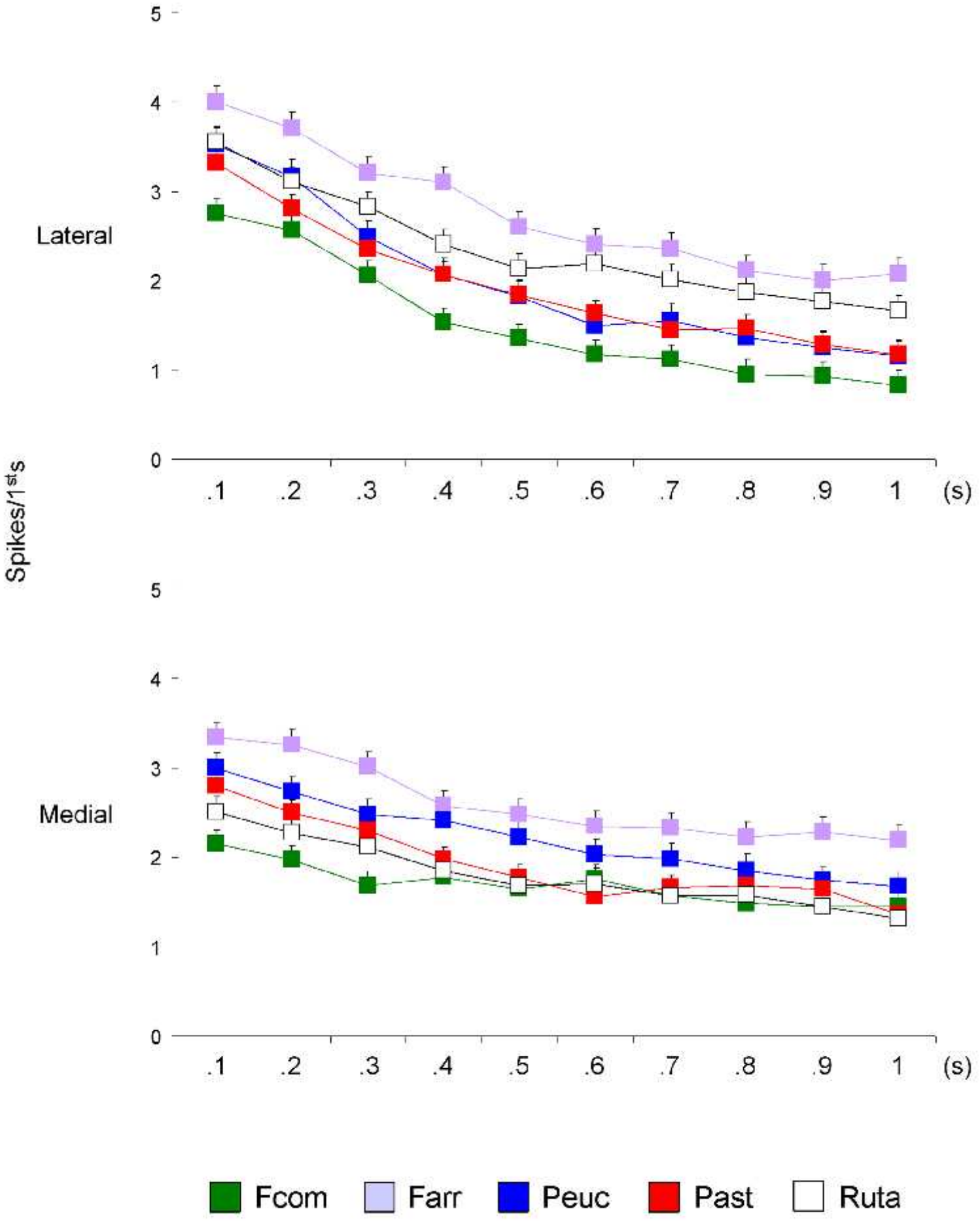
Lateral
Medial
Spikes/1st s
5
4
3
2
1
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1
(s)
Fcom
Farr
Peuc
Past
Ruta

Figure 5

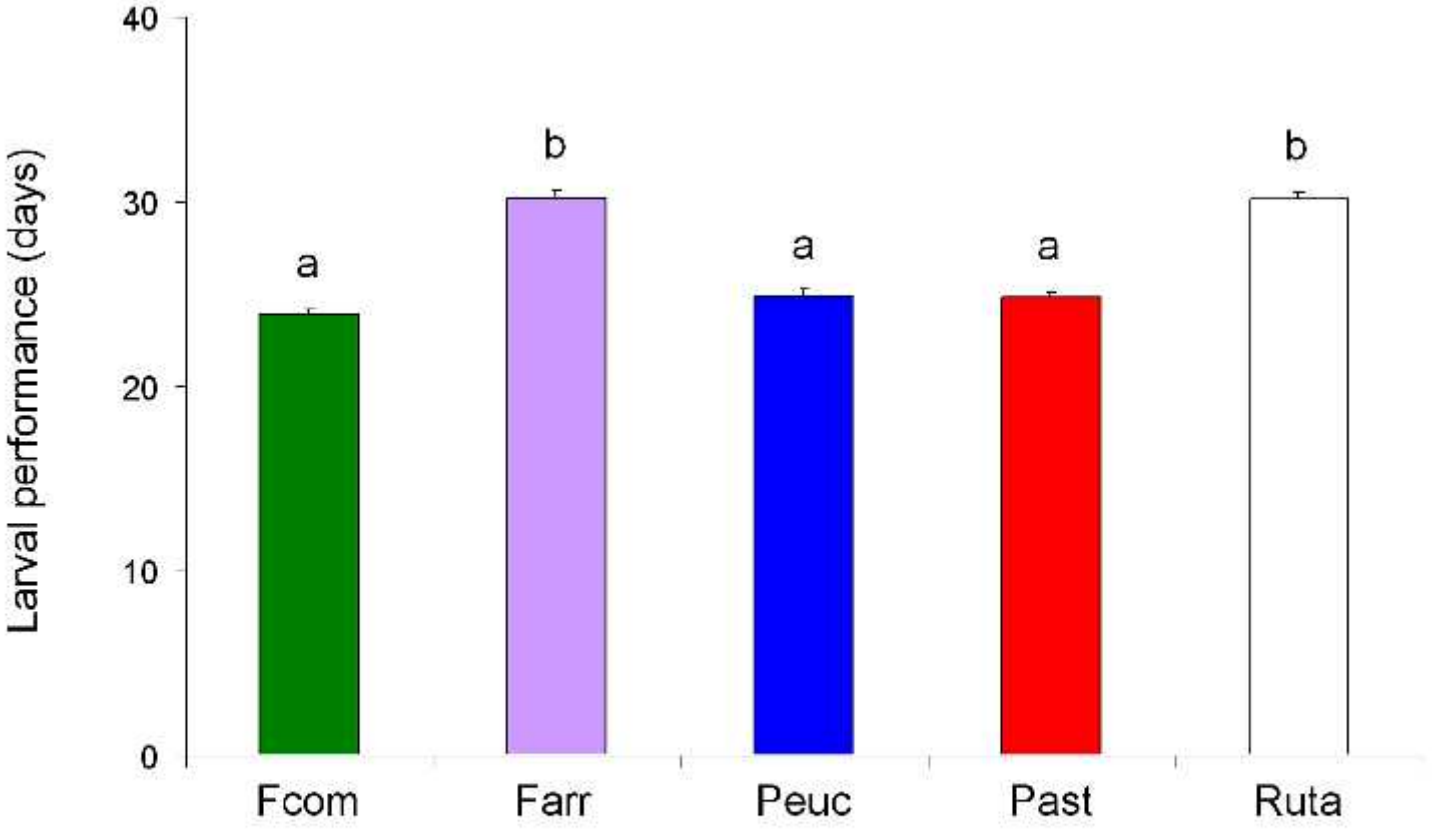
Larval performance (days)
40
30
20
10
0
a
b
a
a
b
Fcom
Farr
Peuc
Past
Ruta

Figure 6

Table 1 - Rate code

Stimulus pairs		Stimulus	Neuron
Fcom-Farr	L	F(1, 335)=74,456; p=,00000	F(3, 335)=55,616; p=0,0000
	M	F(1, 331)=33,288; p=,00000	F(3, 331)=50,379; p=0,0000
Fcom-Peuc	L	F(1, 319)=12,083; p=,00058	F(3, 319)=60,656; p=0,0000
	M	F(1, 327)=13,658; p=,00026	F(3, 327)=57,994; p=0,0000
Fcom-Past	L	F(1, 411)=12,790; p=,00039	F(3, 411)=43,862; p=0,0000
	M	F(1, 403)=3,2615; p=,07167	F(3, 403)=36,475; p=0,0000
Fcom-Ruta	L	F(1, 347)=29,174; p=,00000	F(3, 347)=38,192; p=0,0000
	M	F(1, 315)=,6226; p=,43068	F(3, 315)=32,622; p=0,0000
Farr-Peuc	L	F(1, 299)=22,329; p=,00000	F(3, 299)=67,688; p=0,0000
	M	F(1, 311)=6,6972; p=,01011	F(3, 311)=99,822; p=0,0000
Farr-Past	L	F(1, 391)=33,417; p=,00000	F(3, 391)=50,956; p=0,0000
	M	F(1, 387)=20,307; p=,00001	F(3, 387)=60,807; p=0,0000
Farr-Ruta	L	F(1, 327)=5,1549; p=,02383	F(3, 327)=47,665; p=0,0000
	M	F(1, 299)=24,666; p=,00000	F(3, 299)=61,213; p=0,0000
Peuc-Past	L	F(1, 375)=,13741; p=,71108	F(3, 375)=61,234; p=0,0000
	M	F(1, 383)=4,2320; p=,04035	F(3, 383)=67,630; p=0,0000
Peuc-Ruta	L	F(1, 311)=3,9630; p=,04738	F(3, 311)=36,188; p=0,0000
	M	F(1, 295)=8,4037; p=,00403	F(3, 295)=72,460; p=0,0000
Past-Ruta	L	F(1, 403)=7,5717; p=,00620	F(3, 403)=33,463; p=0,0000
	M	F(1, 371)=,86494; p=,35297	F(3, 371)=43,466; p=0,0000

Table 2 - Ensemble code

Pair stimuli	lateral	medial
Fcom-Farr	F(3, 332)=4,2622; p=,00568	F(3, 328)=9,8796; p=,00000
Fcom-Peuc	F(3, 316)=1,4611; p=,17584	F(3, 312)=2,3952; p=,05611
Fcom-Past	F(3, 408)=1,0745; p=,35968	F(3, 400)=2,1901; p=,08868
Fcom-Ruta	F(3, 344)=4,1655; p=,00645	F(3, 324)=8,9092; p=,00001
Farr-Peuc	F(3, 296)=9,4386; p=,00001	F(3, 380)=5,6874; p=,00081
Farr-Past	F(3, 388)=6,5592; p=,00025	F(3, 384)=6,9826; p=,00014
Farr-Ruta	F(3, 324)=1,3134; p=,12029	F(3, 296)=0,0947; p=,96295
Peuc-Past	F(3, 372)=1,9574; p=,32015	F(3, 308)=,32342; p=,80844
Peuc-Ruta	F(3, 308)=19,285; p=,00000	F(3, 292)=4,3957; p=,00481
Past-Ruta	F(3, 400)=8,7186; p=,00001	F(3, 368)=4,9901; p=,004

Table 3 - Temporal code

Pair stimuli	lateral	medial
Fcom-Farr	F(9, 3380)=,30622; p=,97313	F(9, 3340)=1,1635; p=,31409
Fcom-Peuc	F(9, 3220)=,40148; p=,93487	F(9, 3300)=1,1272; p=,33927
Fcom-Past	F(9, 4180)=,35867; p=,95450	F(9, 4053)=1,0957; p=,07664
Fcom-Ruta	F(9, 3500)=,27182; p=,98225	F(9, 3180)=,80119; p=,61522
Farr-Peuc	F(9, 3020)=,35727; p=,95505	F(9, 3140)=,24954; p=,98692
Farr-Past	F(9, 3980)=,29019; p=,97768	F(9, 3900)=,16148; p=,99747
Farr-Ruta	F(9, 3300)=,31201; p=,97136	F(9, 3020)=,18299; p=,99589
Peuc-Past	F(9, 3820)=,48982; p=,88243	F(9, 3860)=,35051; p=,95778
Peuc-Ruta	F(9, 3140)=,72054; p=,69052	F(9, 2980)=,18703; p=,99553
Past-Ruta	F(9, 4100)=,27899; p=,98055	F(9, 3740)=,29287; p=,97696

Table 4 - Spatio-temporal code

Pair stimuli	lateral	medial
F. communis	F(27, 2360)=5,1858; p=,00000	F(27, 1560)=2,6955; p=,00001
F. arrigonii	F(27, 1560)=5,7327; p=0,0000	F(27, 1720)=1,4253; p=,07274
P. paniculatum	F(27, 1400)=4,6972; p=,00000	F(27, 1520)=2,5703; p=,00002
P. latifolia	F(27, 2360)=5,1858; p=,00000	F(27, 2280)=1,5625; p=,03268
R. lamarmorae	F(27, 1680)=3,3986; p=,00000	F(27, 1400)=1,3998; p=,08396