Role of tarsal gustatory sensilla in host plant recognition and oviposition preference in *Papilio hospiton*.

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Short title: Tarsal taste input related to egg-laying in *P. hospiton*

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Abstract

In herbivorous insects, host selection involves different sensory modalities (sight, smell, taste), but the contact chemoreceptors capable of detecting stimuli both from host and non-host plants play an important role in the final steps of the oviposition behaviour. Female butterflies scratch and drum the leaf surface and taste the compounds present in the plant saps with the tarsal chemosensilla. We assumed that tarsal taste sensitivity may be related to the width of host selection in ovipositing females of *Papilio hospiton* Géné. The spike activity of tarsal taste basiconic sensilla was recorded in response to stimulation with NaCl, bitter compounds and carbohydrates, with the aim of characterizing the gustatory receptor neurons (GRNs) and of comparing the response patterns in the light of the different acceptability of host plants. Then we studied the sensitivity of GRNs to saps of host plants *Ferula communis L.*, *Peucedanum paniculatum* Loisel, *Pastinaca latifolia* (Duby) DC. and *Ruta lamarmorae* Bacch., Brullo et Giusso and evaluated the relationship between taste sensitivity and oviposition preference. The results show that: a) each sensillum houses one water-, one sugar-, one bitter- and one salt-sensitive cell; b) the spike activity of the gustatory neurons in response to plant saps produces different across neuron patterns; c) the number of eggs laid on each plant is highest on *F. communis* and lowest on *R. lamarmorae*. These results suggest that the different activity of the tarsal GRNs may affect the host plant acceptability and that the ovipositing females of *P. hospiton* seem to be able to discriminate between different host plants.

Introduction

In insects, host selection behaviour both in terms of search of oviposition sites and of food sources, is strongly influenced by sensory input arising from their chemosensilla (Dangles et al., 2009; Feeny et al., 1989; Nishida, 2005; Masala et al., 2008; Masala et al., 2009; Ozaki et al., 2011; Sollai et al., 2007; Sollai et al., 2010).

In Lepidoptera, a sophisticated olfactory system attracts female butterflies towards a potential host by means of volatiles compounds, while the gustatory system has adapted to mediate the acceptance or rejection of a plant as a result of drumming with the legs (Zhang et al., 2013).

Although host recognition by herbivorous insects involves multiple sensory modalities, including visual cues, smell and taste, and the range of hosts accepted is highly variable, depending on many factors such as quantity and type of hosts available, environmental factors and the physiological state of the insect (Singer, 1982; Thompson & Pellmyr, 1991), the contact chemoreceptors capable of detecting stimuli both from host and non-host plants play an important role in the final steps of oviposition behaviour (Nishida, 2005). Several studies on Papilionidae, Pieridae and Danaidae showed that the main signals that allow females to discriminate and choose the plant for oviposition are mostly non-volatile secondary metabolites (Honda & Nishida, 1999; Nakayama & Honda, 2004). In fact, in the insect host-plant interaction, and particularly in host recognition, the acceptability of a plant depends on the total sensory impression obtained from the response to multiple components of plants, rather than on the presence or absence of single stimulating or deterrent compounds (Dethier, 1973). This has brought many lepidopteran species to adapt to a restricted number of plants (oligophagy), with the extreme case of adaptation to a single plant (monophagy) (Ozaki et al., 2011).

Upon alighting on a potential host plant female butterflies start drumming and scratching the leaf surface with the foretarsi and this exposes the compounds present in the plant saps to the tarsal chemosensilla. These chemosensilla are located mainly on the fifth tarsomere of the foreleg tarsi, and their role in the oviposition behaviour has been widely studied in some species of lepidopterans,

such as *Pieris brassicae* and *Papilio xuthus* (Chun & Schoonhoven, 1973; Ozaki et al., 2011). Each sensillum houses 4 chemosensory neurons and one mechanoreceptor: the chemoreceptors appear to be sensitive to water, salt, bitters and oviposition stimulants, suggesting a role in the oviposition behaviour (Chun & Schoonhoven, 1973; Ozaki et al., 2011).

The goal of our study was to evaluate whether the chemical composition of the plant could be responsible for the oviposition preference hierarchies that characterize insect/host plant interaction. In general, ovipositing females prefer to use a particular plant species even when multiple host plants are available in the same habitat (Nakayama & Honda, 2004). We chose, as an experimental model *Papilio hospiton* Géné, an oligophagous lepidopteran endemic of the islands of Sardinia and Corsica, which uses as host only plants belonging at the Apiaceae and Rutaceae families. In Sardinia, *P. hospiton* can be considered almost monophagous since it actually lays eggs only on the giant fennel (*Ferula communis*): when and where *F. communis* is unavailable, two other plants are used, one narrowly endemic (*Ferula arrigonii*) and the other rare and confined to two small stands (*Ruta lamarmorae*) (Bacchetta et al., 2006); on the contrary, in Corsica it feeds on several species: *Peucedanum paniculatum*, *Ferula communis*, *Ruta corsica* and *Pastinaca latifolia* (Aubert et al., 1996). To this end, we first stimulated foreleg tarsal sensilla with sugars, one sugar alcohol, salts and bitter compounds, to provide a functional characterization of each GRN. Secondly, we stimulated tarsal sensilla with leaf saps of different host plants (*Ferula communis, Peucedanum paniculatum, Pastinaca latifolia, Ruta lamarmorae*), and evaluated qualitative and quantitative differences in the response profiles of GRNs between the taste stimuli. We expected these sensilla, to show such differences in their spike response patterns to different plant saps, as to reflect somehow the different degrees of host acceptance by egg-laying females. Finally, we evaluated the relationship between the number of eggs laid on each plant by *P. hospiton* females and the electrophysiological recordings.

Materials and Methods

Insects and rearing

Papilio hospiton Géné adults were obtained from lab stock overwintering pupae reared the previous year as larvae. in 1500-ml plastic cups (4-5 per cup) in an environmental growth chamber (24-25 °C, 70% R.H., 16L/8D photoperiodic regime). Larvae had hatched from eggs laid on giant fennel plants (*Ferula comm*unis L) in the butterfly oviposition annex (a 3 x 3 x 3m cage) of the Physiology Laboratories (University of Cagliari). After emergence in a separate cage, female adults were released in the insectary annex, where they were free to feed on *Lantana camara* L. flowers. For behavioural experiments, two adult females per day were left free to lay eggs for 24 hours starting the next day after mating and were then removed. For the electrophysiological experiments, the day after mating females were removed from the cage and transferred to smaller boxes and fed with a sugar solution until used for electrophysiological recordings.

Morphological observations

Tarsi of adult females were collected and treated according to the technique described by Loy et al. (2016). Samples were sonicated twice in a Triton X-100 1% solution in bidistilled water. After several washes in bidistilled water, samples were dehydrated in acetone, dried in air and coated with 2 nm platinum by means of an Emitech K575 Sputter Coater. Tarsi were then observed by a Field Emission Scanning Electron Microscopy Hitachi s4000 and photos were collected by a Quartz PCI v. 5 software (Quartz Imaging Corporation, Vancouver, BC, Canada).

Electrophysiological experiments

Forelegs of female butterflies were removed from the insect body using fine forceps and the electrophysiological recordings were obtained from the sensilla of the fifth tarsomere by means of the “tip-recording” technique (Hodgson et al., 1955). The reference electrode, a thin Ag/AgCl, was inserted into the amputated leg, while 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 (Sollai et al., 2008).

Each sensillum was tested with aqueous solution of 1÷500 mM NaCl, 0.1÷10 mM nicotine, caffeine and salicin, 1÷100 mM sucrose, glucose, fructose and inositol all added with KCl 50 mM (control). In addition, we tested four complex stimuli represented by freshly-pressed leaf extracts of three plants belonging to the Apiaceae family: *Ferula communis* L. (giant fennel; hereafter Fcom), *Peucedanum paniculatum* Loisel (Peuc), *Pastinaca latifolia* (Duby) DC. (Past) and one plant belonging to Rutaceae family: *Ruta lamarmorae* Bacch., Brullo et Giusso (Ruta).

The chemical stimuli were purchased from Sigma-Aldrich (Italy). Stimuli were applied to the sensilla for 2-3 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 obtained according to Dethier & Crnjar (1982) and Sollai et al. (2017), and were tested within 30 s after being pressed. 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 tarsal surface of the insect was rinsed with distilled water and blotted dry.

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 & Crnjar, 1982; Inoue et al., 2009; Sollai et al., 2012) and spike sorting and

counting were done by means of the Clampfit 10.0 software, based on earlier studies (Dolzer et al., 2003; Dulcis & Levine, 2005; Pézier et al., 2007; Sollai et al., 2014). In detail, by measuring the peak-antipeak amplitude of action potentials we identified 1 to 4 spike types that were labeled as: small (S; range 0.1 ÷ 0.3 mV), large (L; range 1 ÷ 1.5 mV), intermediate 1 (M1; range 0.5 ÷ 1 mV) and intermediate 2 (M2; range 0.3 ÷ 0.5 mV). These spikes were assigned to four different classes by the Clampfit 10.0 software. Figures 1-25 in the supplementary material show that spike amplitude does not increase with the stimulus concentration, thus validating the peak-antipeak amplitude measure of action potentials in the spike sorting process.

Oviposition assays

To test the oviposition preferences we counted the number of eggs laid on each plant, in a multi-choice situation in the butterfly oviposition annex (a 3x3x3m cage) of the Physiology Laboratories (University of Cagliari). Egg counts were performed every day for 8-10 days at the natural emergence peak of *P. hospiton* (tipically with in first two weeks of May) and repeated for 4 years (springs 2013-2016; in total, 37 egg counts were done on each plant species). Each day the eggs were removed from each plant after counting them. Two specimens of each plant species were present inside the cage and were arranged in a random sequence along the sides of the cage: however, being potted, the plants could be easily rotated daily. and this also assured a homogenous sunlight exposure. All host plants were in their vegetative, non-flowering phenological state and had a roughly equivalent foliage volume. The *Lantana* flowering plants were positioned at the center of the cage.

Statistical analysis

Repeated-measures ANOVA was used to analyze the effect of increasing concentration of taste pure stimuli (NaCl, nicotine, caffeine, salicin, sucrose, glucose, fructose and inositol) on the spike

frequency in the first second of discharges of GRNs ("L", "M1", "M2", "S") of the tarsal sensilla, separately for each stimulus.

One-way ANOVA was used to analyze the relationship between: a) the spike activity of each GRN and the stimulus; b) the oviposition choices (number of laid eggs) and the host plant.

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, 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 Ruta), 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).

Data were checked for the assumptions of homogeneity of variance, normality and sphericity (when applicable). When the sphericity assumption was violated, a Green-Geisser correction or Huynh-Feldt correction was applied in order to modify the degrees of freedom. Post-hoc comparisons were conducted with the Tukey test, unless the assumption of homogeneity of variance was violated, in which case Duncan's test was used. Statistical analyses were performed using STATISTICA for WINDOWS (version 7.0; StatSoft Inc, Tulsa, OK, USA). P values < 0.05 were considered significant.

Permits

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

Results

Morphology of tarsal sensilla

All tarsomeres of the forelegs in *Papilio hospiton* present a dense population of sensilla basiconica located on their ventral surface (Fig. 1A and B). These sensilla are arranged in elongated clusters (N=44-47 in the 5th tarsomere), belong to a same morphological type, are uniporous (Fig. 1C and D) and house the GRNs from which the electrophysiological activity was recorded. The tarsi also possess three rows of longitudinally arranged longer spines, two of which run laterally on the tarsomeres and one ventrally traversing the population of sensilla basiconica. These spines present no pores (Fig. 1C).

Functional characterization of gustatory receptor neurons (GRNs) of the adult female tarsal sensilla

Samples of spike discharges from the tarsal GRNs in response to the chemicals tested are shown in figures 2 and 3.

To test for a dose-response relationship, we analyzed the spike activity evoked in the first second of the discharge for each GRN ("L", "M1", "M2" and "S") to increasing concentrations of bitters, several carbohydrates and NaCl, by using a repeated-measures ANOVA (Fig. 5).

Repeated-measures ANOVA showed a significant effect of concentration on the spike frequency of the "M2" GRN in response to nicotine ($F_{[5.9,290.8]}$=49.535; P<0.00001), caffeine ($F_{[4.4,218.1]}$=21.939; P<0.00001) and salicin ($F_{[4,215.1]}$=25.917; P<0.00001). Post-hoc comparisons showed that the spike frequency in response to each concentration was higher than in response to the next lower concentration (P<0.00001; Duncan's test). These results, together with the analysis of the neural traces (Fig. 2), indicate that "M2" neuron is activated by bitter compounds. Repeated-measures ANOVA also showed a significant effect of concentration on the spike frequency of the "M1" GRN in response to sugars (fructose: $F_{[4.7,161.4]}$=31.409; P<0.00001; glucose: $F_{[6,200]}$=63.130; P<0.00001; sucrose: $F_{[4.6,167.1]}$=43.895; P<0.00001) and inositol ($F_{[3.9,136]}$=20.766; P<0.00001), and post-hoc comparisons that the neural activity in response to each concentration was higher than in response to the next lower concentration (P<0.00001; Duncan's test). These findings, together with the analysis of spike traces (Fig. 3), indicate that a same single taste neuron ("M1") is activated by different sugars. Repeated-measures ANOVA revealed a significant effect of concentration on the spike activity of the "S" GRN in response to NaCl ($F_{[6.3,286.5]}$=52.777; P<0.00001) and pairwise comparisons a significant increase of spike frequency for each concentration step (P<0.0001; Duncan's test). These results and the analysis of spike discharges (Fig. 2), suggest that "S" neuron is activated by inorganic salts.

Finally**,** repeated-measures ANOVA showed a significant effect of concentration on the spike frequency of the "L" GRN in response to fructose ($F_{[4.7,161.4]}$=31.409; P<0.00001), glucose ($F_{[6,200]}$=63.130; P<0.00001) and NaCl ($F_{[6.3,286.5]}$=52.777; P<0.00001); in detail, post-hoc comparisons showed decreases of spike activity for each concentration step (P<0.005; Duncan's test). These results, together with the analysis of spike traces (Figs. 2 and 3), indicate that this GRN is activated by low concentrations of inorganic salt and monosaccharides.

241

242 **Effects of plant saps on the spike activity of the tarsal GRNs**

243 Samples of spike discharges of the activity of the tarsal GRNs in response to plant extracts tested
244 are shown in figure 4.

245 To test for a relationship between neural activity of each GRN and the stimulus, we analyzed the
246 spike response evoked in the first second of the discharge for each GRN ("L", "M1", "M2" and
247 "S"), by using an one-way ANOVA.

248 One-way ANOVA showed a significant effect of stimulus on the spike frequency of all GRNs
249 ($F_{[3,216]}$>3.5185; P<0.05; Fig. 6A). In particular, post-hoc comparisons showed that the spike
250 frequency of "M2" neuron in response to Ruta was higher than in response to the other plant saps
251 *(*P<0.01; Tukey test); while, the spike frequency of "L" and "M1" neuron in response to Fcom was
252 higher than in response to all other saps *(*P<0.05; Tukey test).

253 These results indicate that Fcom is the best stimulus for "L" and "M1" neurons, while the extracts
254 of Ruta is the most stimulating for "M2" neuron.

255

256 **Oviposition preferences**

257 To test for a relationship between ovipostions preference and oviposition substrate, we analyzed the
258 number of eggs layed on each plant considered, by using one-way ANOVA. One-way ANOVA
259 showed a significant effect of the substrate on the ovipostion choice ($F_{[3,144]}$=6.6928; P<0.001; Fig.
260 6B). In particular, post-hoc comparisons showed that the number of eggs layed was significant
261 higher on Fcom than on all other plants (P<0.01; Duncan's test) and the number of eggs layed on
262 Peuc was higher than on Ruta (P<0.001; Duncan's test). No differences were found between Peuc
263 and Past and between Past and Ruta. These results indicate that the hierarchy of host-plants choice
264 by ovipositing females is: Fcom > Peuc = Past > Ruta.

265

266 **Sensory code mediating plant discrimination**

We investigated whether insects can discriminate among different plant saps by means of an ensemble, temporal and/or spatio-temporal code. 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 ($F_{[9,864]}$=4.0875; P<0.0001) (Fig. 7A). In detail, the results presented in Table 1A, indicate that plant saps generated a different ensemble code, except that between Peuc and Past. 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 ($F_{[27,8850]}$=0.72291; P=0.85013) (Fig. 7B) (Tab. 1B), indicating 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. Two-way ANOVA revealed a significant interaction of Time × GRN for Fcom, Peuc and Past, but not for Ruta (Tab. 1C). These results show that Fcom, Peuc and Past evoked non-parallel T-I curves in all GRNs, instead, Ruta evoked T-I curves in the 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 Ruta.

Discussion

Our SEM observations show that the foreleg tarsi of *Papilio hospiton* present a population of sensilla basiconica located on their ventral surface. These sensilla are uniporous and house the GRNs from which the electrophysiological activity was recorded. They belong to one single type, unlike *P. xuthus* where two types of sensilla basiconica were described (Ozaki et al., 2011; Ryuda et al., 2013). Three rows of longitudinally arranged longer spines are also present on the ventro-lateral surface of the tarsi. These spines are poreless and, given their size and distribution, they are likely to be involved in the drumming and scratching behaviour exhibited by *P. hospiton* females after alighting on a potential host plant, which causes plant saps to leak out of the plant tissues.

A primary aim of this study was to functionally characterize the GRNs in the basiconic uniporous sensilla of the foreleg tarsi and evaluate the presence of a relationship between peripheral taste sensitivity and oviposition preference by female butterflies. The dose-response relationships we found indicate that one neuron ("M1") specifically detects sugars and sugar alcohols, one is a deterrent cell ("M2") and one is specific to detect inorganic salts ("S"). A fourth GRN, the "L" neuron, seems to be specific to detect water, since its responses are inversely related to increasing concentrations of NaCl, fructose and glucose. There is evidence, in other vertebrates and invertebrates, that one same GRN may respond to different compounds, whether belonging or not to the same chemical class, although this is a still debated issue (Yarmolinsky et al., 2009). Low concentrations of sugars and NaCl can be considered as a water stimulus, which is generally regarded as phagostimulant for insects (Bernays & Chapman, 2001), since responses of "M1" are inversely related to their concentrations, analogously to what reported in *Phormia regina* Meigen and *Protophormia terraenovae* Robineau-Desvoidy (Diptera: Calliphoridae), *D. melanogaster*, female butterflies of *Papilio xuthus* L. and larvae of *P. hsopiton* (Lepidoptera: Papilionidae) (Dethier, 1976; Evans & Mellon, 1962; Hiroi et al., 2002; Ryuda et al., 2013; Solari et al., 2010; Sollai et al., 2014)

The main goal of this work was to evaluate whether differences in the pattern activities of the GRNs housed in the foreleg tarsi sensilla in response to leaf extracts of several plants could explain the difference in the hierarchy of host plants choice for oviposition.

It is known that phytophagous Lepidoptera are highly dependent on the chemical composition of the plant when deciding whether to assign it or not the role of host, and that the acceptance or rejection of a plant by the ovipositing females is determined by the balance between positive and negative stimuli evoked from the plant itself (Honda & Nishida, 1999; Nakayama & Honda, 2004). Although the first steps in the host plant selection process, by an adult female in flight, are primarily visual and olfactory, the final decision as to whether lay eggs or not requires input from the contact tarsal sensilla after alighting on a potential host plant.

We assume that the differential activation of the acceptance neurons, such as sugar and water GRNs, and the deterrence neurons (the bitter sensitive GRN), may somewhat explain the extent of egg-laying on a given host plant. Our results highlight that the extract of Ruta elicits a higher spike frequency from the "M2" cell (whose response increases with increasing concentrations of bitter compounds), as compared to the saps of Fcom, Peuc and Past; instead, the extracts of Fcom evokes a higher activity in "M1" neuron, that increases its spike frequency with increasing concentrations of sugars. Behavioural results about the oviposition preferences showed that Fcom is the preferred plant by the ovipositing females, while Ruta is where the least number of eggs have been counted. Together, these results suggest a direct relationship between the degree of acceptance of a plant as host and the electrophysiological responses elicited by each of them.

Our results revealed that *P. hospiton* females are able to discriminate between host plants by means of an ensemble and spatio-temporal code. In fact, we found that Fcom, Peuc, Past and Ruta generate a different across neuron pattern (ANP) among them, but not Peuc and Past, chosen equally by ovipositing females. Besides, the extracts of the Fcom, Peuc and Past each evoke non-parallel T-I curves in the GRNs, while the extract of Ruta evoked parallel T-I curves, thus indicating a difference in spatio-temporal code. The sensory input goes to the CNS for further processing to produce the final behaviour: thus, the differential activation of tarsal GRNs is a neural code used by the brain to decide whether to accept or reject a host, as also suggested in other Papilionid species (Honda, 1995).

However, the successful choice of a host plant is determined both by the egglaying butterfly and the larva which may or may not feed on the plant: therefore the choice of oviposition site is crucial for larval performance (Nishida, 2005). We recently found that all larvae of *P. hospiton* reared on Fcom, Peuc, Past and Ruta, reached pupation, although with different performance rates (Sollai et al., 2017). In fact, larval performance ranking from best to worst was Fcom=Peuc=Past>Ruta, while oviposition preference was highest on Fcom, lowest on Ruta and intermediate on Peuc=Past. This suggests some degree of correspondence between oviposition preference and larval performance,

Ruta is the least chosen plant for egg-laying and provides the lowest growth performance, while ferula is the best egg-laying choice and is the group of the plants on which larvae perform best. However, whether a positive relationship exists between oviposition preference and larval performance is still a matter of debate. Some authors strongly support the performance-preference hypothesis, according to which females lay their eggs on host plants where the progeny performs best (Jaenike, 1978; Gripenberg et al., 2010), while others argue that the choice by females is not related to larval performance, and the insects sometimes lay eggs on host plants unsuitable for their offspring (Konig et al., 2016; Larsson & Ekbom, 1995). Further experiments are needed to better evaluate the two hypotheses in the case of *P. hospiton*.

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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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466

Legends of Figures

Fig. 1 – Side view (A) and ventral view (B) of the 5th tarsomere of a foreleg in a *Papilio hospiton* female. Three rows of long spines (one medial and two lateral) delimit an elongated cluster of uniporous sensilla basiconica. The apical pore of these sensilla is visible in C (short arrows) and (D). The asterisk in C denotes one of the poreless spines.

Fig. 2 – Sample traces showing spike firing frequency of a tarsal sensillum following stimulation with KCl (control), NaCl, caffeine (Caf), nicotine (Nic) and salicin (Sal).

Fig. 3 – Sample traces showing spike firing frequency of a tarsal sensillum following stimulation with glucose (Glu), fructose (Fru), sucrose (Suc) and inositol (Ino).

Fig. 4 – Sample traces showing spike firing frequency of tarsal sensillum following stimulation with leaf sap of *F. communis* (Fcom), *P. paniculatum* (Peuc), *P. latifolia* (Past) and *R. lamarmorae* (Ruta).

Fig. 5 – Dose-response relationship between spike activity of GRNs and different taste stimuli. All values are mean ± s.e.m. N=26-41. Filled symbols indicate significant differences between a concentration and that next lower ($P<0.005$; Duncan's test subsequent to repeted-measures ANOVA). Circle symbols indicate the GRN responses to 50 mM KCl (K).

Fig. 6 – (A) Mean values ± s.e.m. of number of spikes evoked in each GRN of the tarsal sensillum during the first second of stimulation with leaf sap of *F. communis* (Fcom), *P. paniculatum* (Peuc), *P. latifolia* (Past) and *R. lamarmorae* (Ruta). N=44-57. Different letters indicate significant differences between the spike activity of the same GRN in response to different taste stimuli ($p<0.01$; Tukey test subsequent to one-way ANOVA).

493 (B) Mean values ± s.e.m. of percentage of eggs layed on *F. communis* (Fcom), *P. paniculatum*
494 (Peuc), *P. latifolia* (Past) and *R. lamarmorae* (Ruta). N=37. Different letter indicates significant
495 differences ($p<0.01$; Duncan's test subsequent to one-way ANOVA)
496
497 **Fig. 7 –** (A) Significant interaction of the Stimulus × GRN on the spike frequency and (B) Time-
498 Intensity curves (i.e., number of spikes during 10 consecutive 100 ms intervals) elicited by *F.*
499 *communis* (Fcom), *P. paniculatum* (Peuc), *P. latifolia* (Past) and *R. lamarmorae* (Ruta). N=44-57.
500
501 **Table 1** – (A) Ensemble code anlyses: we inferred a difference in ensemble code, e.g. between
502 Fcom and Ruta, if the was a significant interaction of the Stimulus × GRN on the spikes frequency
503 during the first second of stimulation. (B) Temporal code analyses: we inferred a difference in
504 temporal code (e.g., between Fcom and Ruta), if there was a significant interaction of Time ×
505 Stimulus on the spikes frequency during the first second of stimulation. (C) Spatio-temporal code
506 analyses: we inferred a difference in spatio-temporal code (e.g., between Fcom and Ruta), if there
507 was a significant interaction of Time × GRN on the spikes frequency during the first second of
508 stimulation.

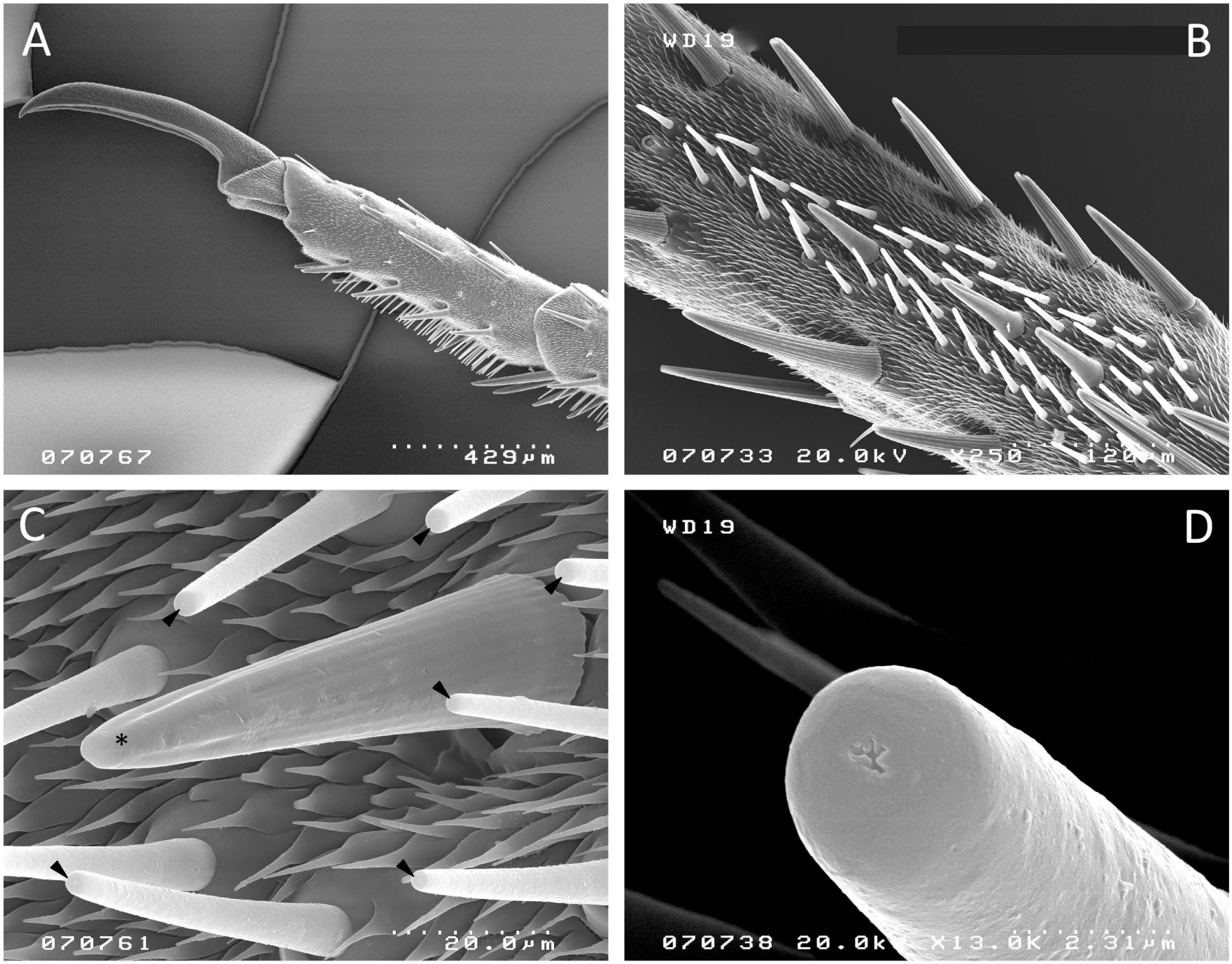
A
070767
429µm
WD19
B
070733 20.0kV X250 120µm
C
*
070761
20.0µm
WD19
D
070738 20.0kV X13.0K 2.31µm

Figure 1

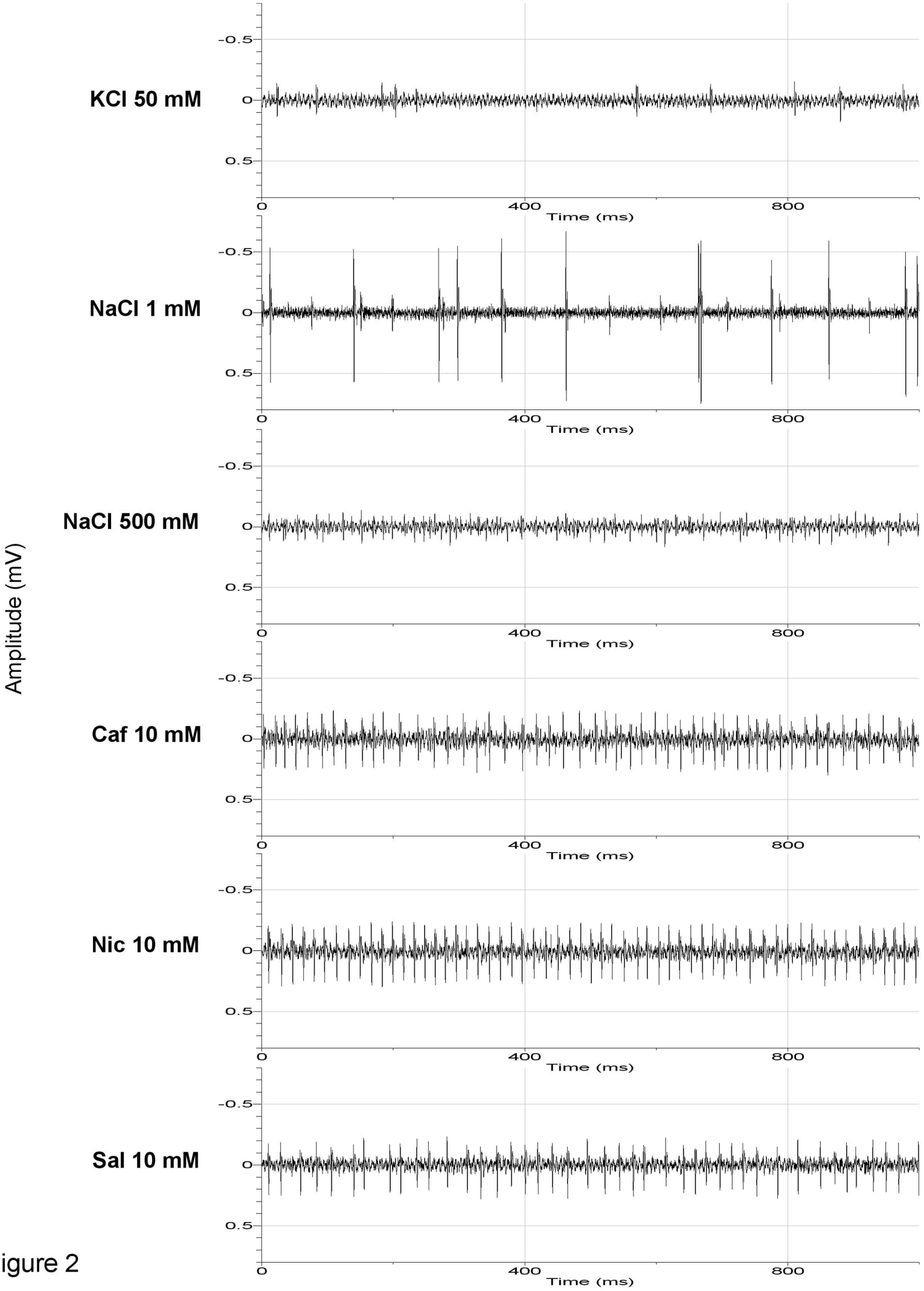
KCl 50 mM
NaCl 1 mM
NaCl 500 mM
Caf 10 mM
Nic 10 mM
Sal 10 mM
Amplitude (mV)
-0.5
0
0.5
0
400
800
Time (ms)

Figure 2

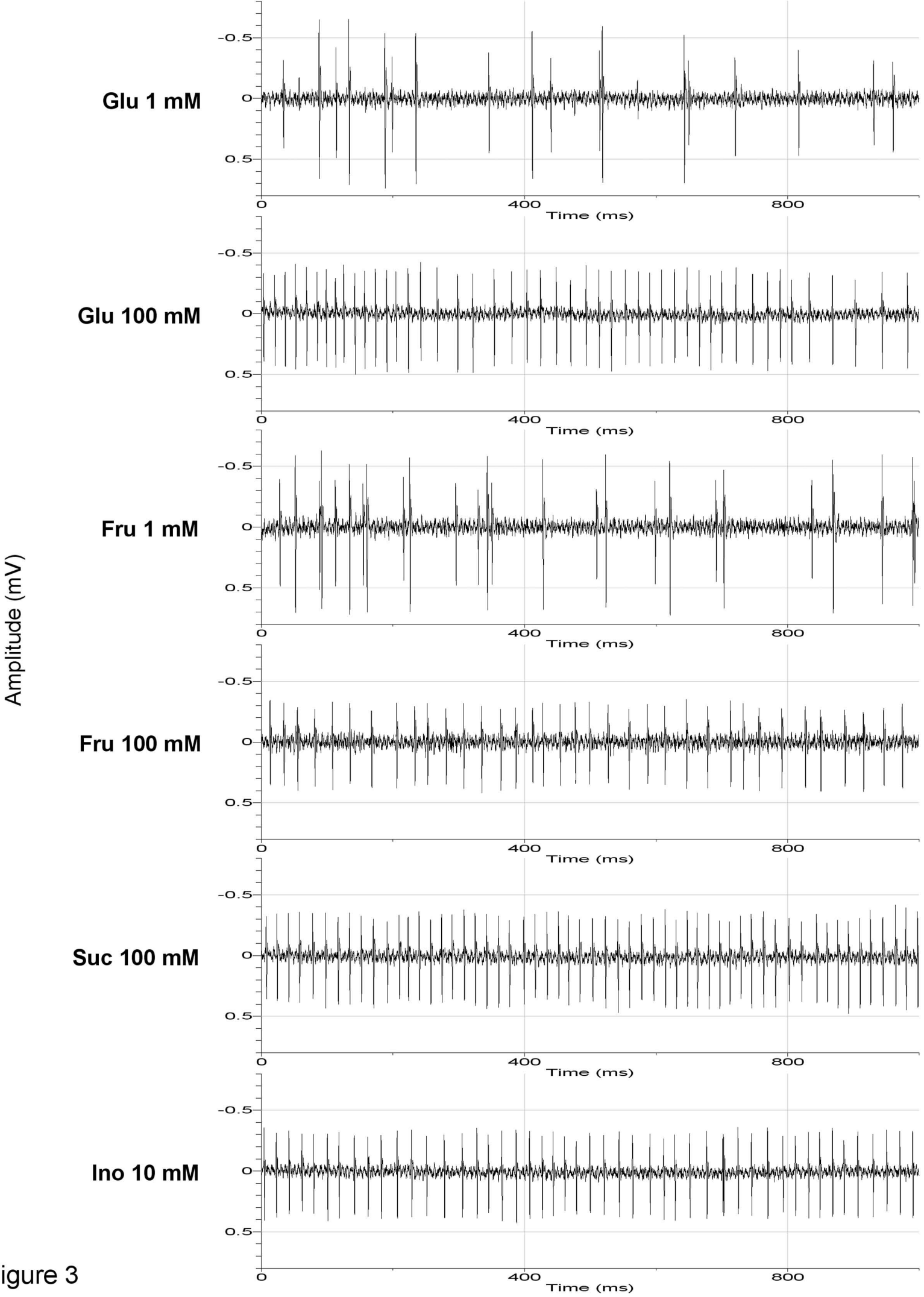
Glu 1 mM
Glu 100 mM
Fru 1 mM
Fru 100 mM
Suc 100 mM
Ino 10 mM
Amplitude (mV)
-0.5
0
0.5
0
400
800
Time (ms)

Figure 3

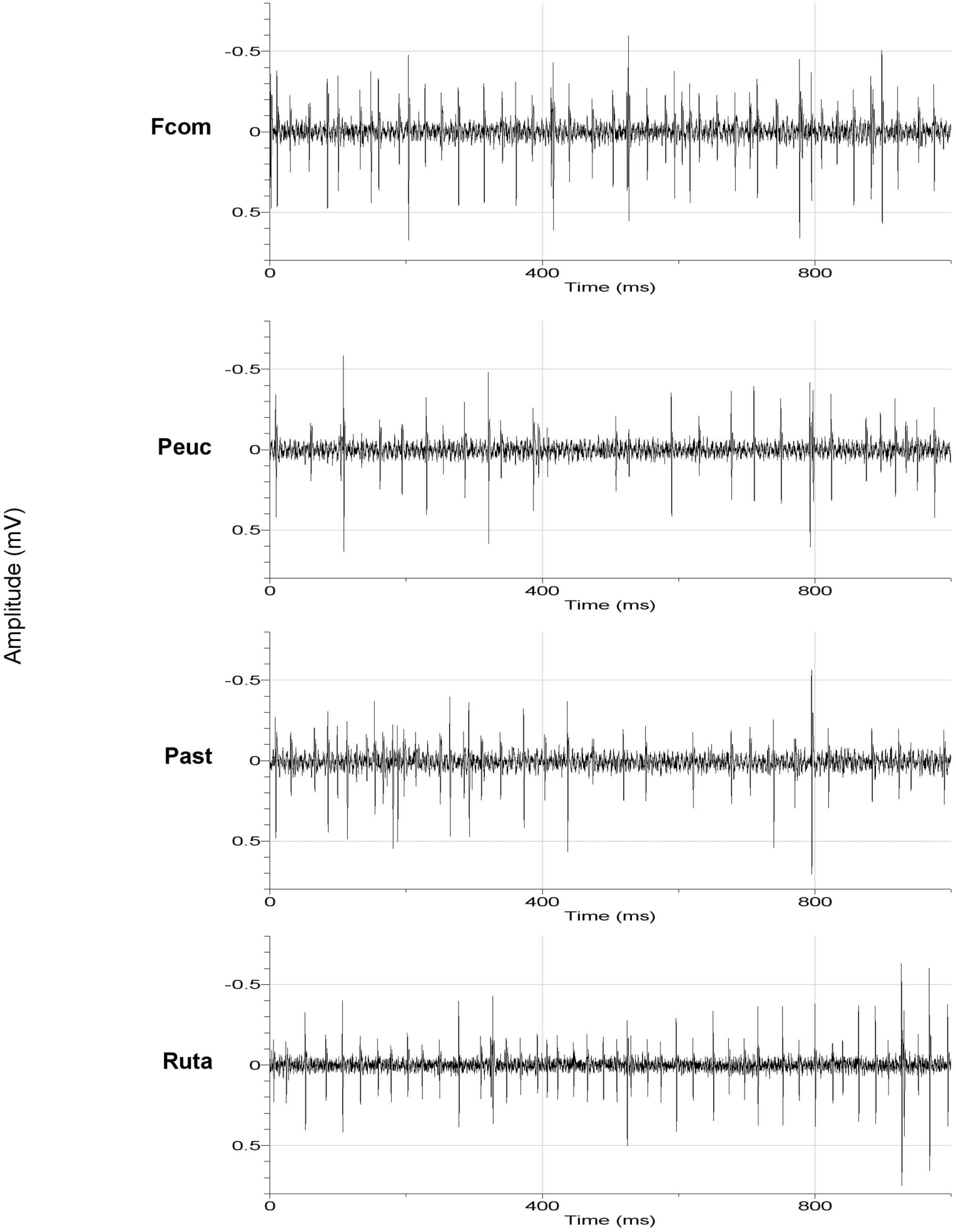

Figure 4

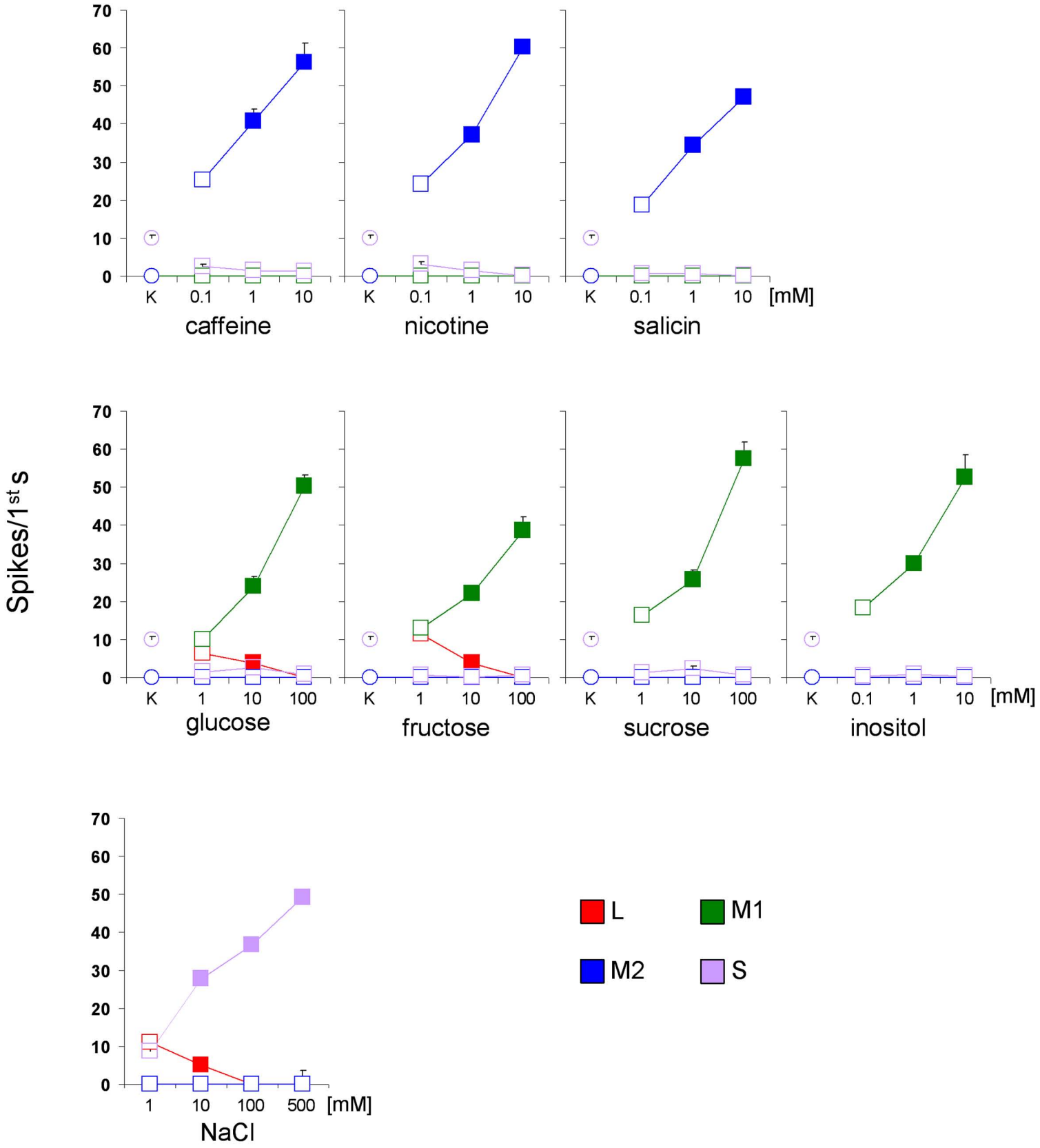
Spikes/1st s
70
60
50
40
30
20
10
0
K
0.1
1
10
[mM]
caffeine
nicotine
salicin
100
glucose
fructose
sucrose
inositol
500
NaCl
L
M1
M2
S

Figure 5

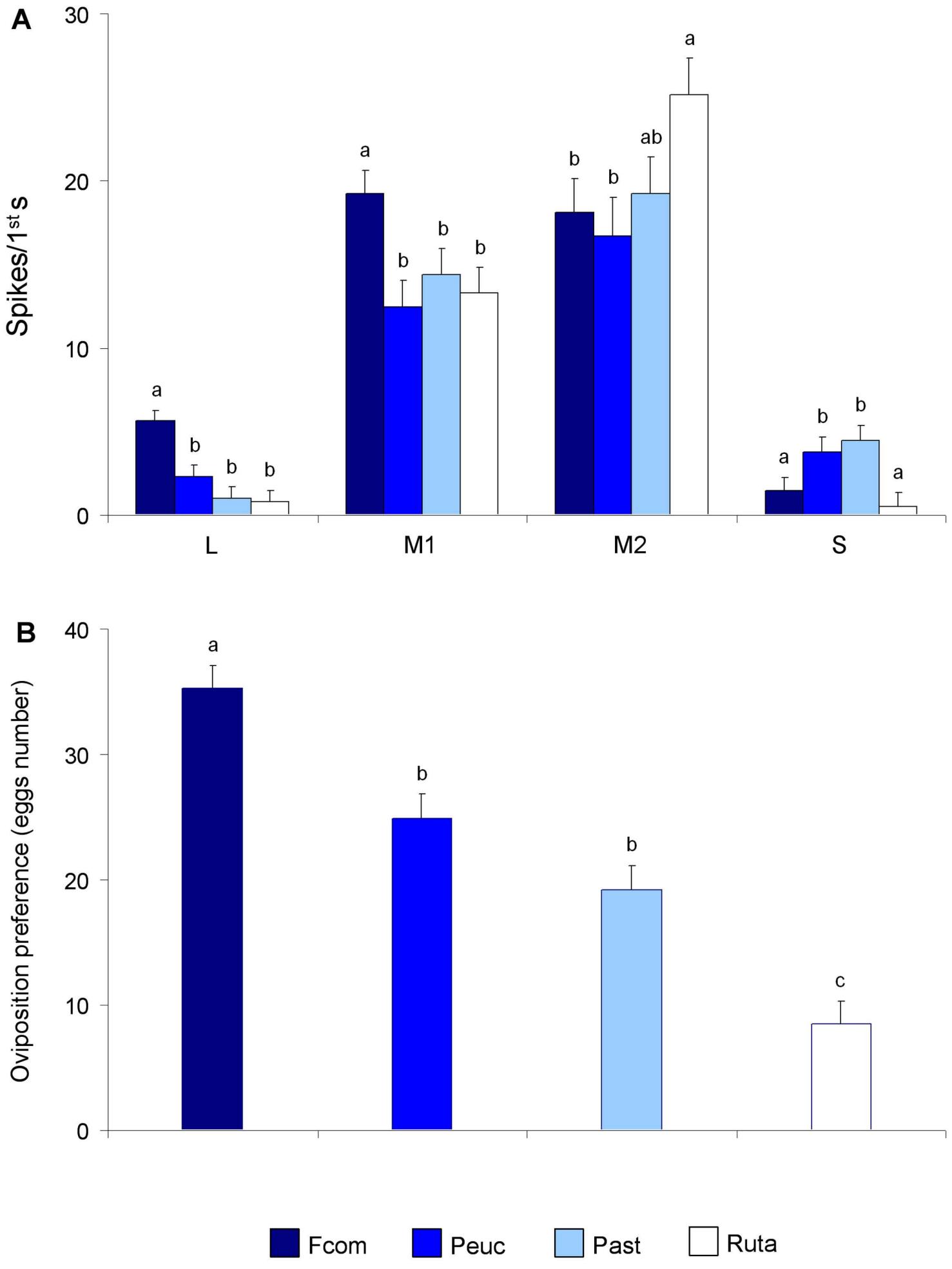
A
Spikes/1st s
30
20
10
0
a
b
b
b
a
b
b
b
b
b
ab
a
a
b
b
a
L
M1
M2
S
B
Oviposition preference (eggs number)
40
30
20
10
0
a
b
b
c
Fcom
Peuc
Past
Ruta

Figure 6

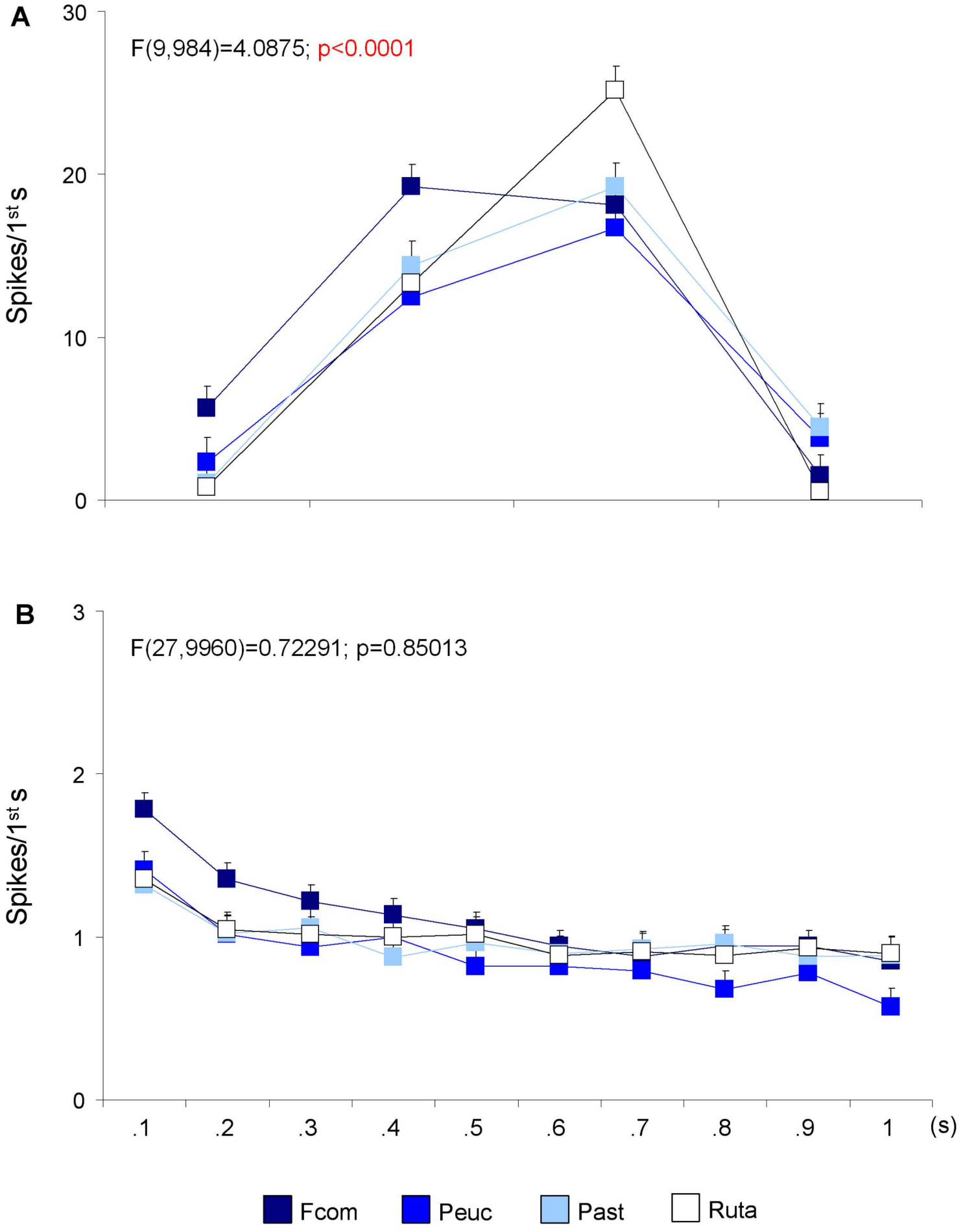
A
F(9,984)=4.0875; p<0.0001
Spikes/1st s
30
20
10
0
B
F(27,9960)=0.72291; p=0.85013
Spikes/1st s
3
2
1
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1
(s)
Fcom
Peuc
Past
Ruta

Figure 7

A

Stimulus pair	Ensemble code
Fcom-Peuc	F(3, 396)=3,0208; p=,02939
Fcom-Past	F(3, 404)=3,1434; p=,02492
Fcom-Ruta	F(3, 408)=7,6345; p=,00005
Peuc-Past	F(3, 456)=,58625; p=,62428
Peuc-Ruta	F(3, 460)=6,4217; p=,00029
Past-Ruta	F(3, 468)=3,8034; p=,01028

B

Stimulus pair	Temporal code
Fcom-Peuc	F(9,4080)=,39945; p=,93593
Fcom-Past	F(9, 4160)=1,2134; p=,28148
Fcom-Ruta	F(9, 4230)=1,0094; p=,42968
Peuc-Past	F(9, 4620)=,87610; p=,54586
Peuc-Ruta	F(9, 4660)=,57006; p=,82269
Past-Ruta	F(9, 4740)=,12791; p=,99900

C

Stimulus	Spatio-temporal code
F. communis	F(27, 1780)=4,0296; p=,00000
P. paniculatum	F(27, 2240)=1,7052; p=,01328
P. latifolia	F(27, 2320)=2,3594; p=,00102
R. lamarmorae	F(27, 2360)=1,3594; p=,10255

Table 1