Taste sensitivity and divergence in host plant acceptance between adult females and larvae of *Papilio hospiton* Géné

Giorgia Sollai, Maurizio Biolchini, Roberto Crnjar

Department of Biomedical Sciences, Section of Physiology, University of Cagliari, 09042 Monserrato (CA), Italy

Running head: *P. hospiton* acceptance and rejection of host plant

Corresponding author: Giorgia Sollai, Department of Biomedical Sciences, Section of Physiology, University of Cagliari, SP 8 Km 0.700, 09042 Monserrato (CA), Italy. E-mail: gsollai@unica.it; Phone: +39 070 6754160; Fax: +39 070 6754191

Abstract

In the island of Sardinia the lepidopteran *Papilio hospiton* uses *Ferula communis* as exclusive host-plant. However, on small island of Tavolara, adult females lay eggs on *Seseli tortuosum*, a plant confined to the island. When raised in captivity on *Seseli* only few larvae grew beyond the first-second instar. Host specificity of lepidopterans is determined by female oviposition preferences, but also by larval food acceptance, and adult and larval taste sensitivity may be related to host selection in both cases. Aim of this work was: a) to study the taste sensitivity of larvae and ovipositing females to saps of *Ferula* and *Seseli*; b) to cross-compare the spike activity of gustatory receptor neuron (GRNs) to both taste stimuli; c) to evaluate the discriminating capability between the two saps and determine which neural code/s is/are used. The results show that: a) the spike responses of the tarsal GRNs of adult females to both plant saps are not different and therefore they cannot discriminate the two plants; b) larval L-lat GRN shows a higher activity in response to *Seseli* than *Ferula*, while the opposite occurs for the phagostimulant neurons, and larvae may discriminate between the two saps by means of multiple neural codes; c) the number of eggs laid on the two plants is the same, but the larval growth performance is better on *Ferula* than *Seseli*. Taste sensitivity differences may explain the absence of a positive relationship between oviposition preferences by adult females and plant acceptance and growth performance by larvae.

Key Words: chemoreception; host plant discrimination; oviposition preference; Papilionidae; neural coding; feeding acceptance.

Introduction

In insects, multiple behaviors such as the choice of oviposition site, the acceptance of a food source and the recognition of conspecifics for mating are strongly influenced by the input arising from their chemical senses (Feeny *et al.*, 1989; Nishida, 2005; Solari *et al.*, 2007; Sollai *et al.*, 2007; Masala *et al.*, 2008; Dangles *et al.*, 2009; Masala *et al.*, 2009; Sollai *et al.*, 2010; Ozaki *et al.*, 2011; Biolchini *et al.*, 2017). Gustatory chemoreceptors respond to various chemicals present in potential hosts and their integrated activity plays a role in the balance between acceptance and aversive behavior (Dethier, 1973).

Lepidopterans represent a suitable model to study the relationship between sensory input and behavioral output, both in the identification of suitable oviposition sites by adult females and of potential food sources for the offspring; in fact host specificity is determined not only by female oviposition preferences, but also by larval food acceptance. Female butterflies are initially attracted towards a potential host plant by visual cues and the olfactory perception of volatile compounds, while the gustatory system comes into play only when the butterfly contacts a plant with its legs (Zhang *et al.*, 2013). In fact, 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 sap to the tarsal chemosensilla which, by integrating the total sensory impression obtained from the response to multiple components of plants, play an important role in the final oviposition choice (Dethier, 1973; Nishida, 2005). Each sensillum houses one mechanoreceptor and four chemosensory neurons, that are known to be sensitive to water, salt, bitters, sugars and oviposition stimulants (Chun & Schoonhoven, 1973; Ozaki *et al.*, 2011; Sollai *et al.*, 2017c).

Lepidopteran larvae assess food by means of taste organs situated on the mouthparts: styloconic sensilla on the maxillary galea, basiconic sensilla in the maxillary palp and sensilla on the epipharynx (Dethier, 1973; Schoonhoven, 1969). The two styloconic sensilla are considered the primary sensory organs involved in feeding: indeed, they mediate plant recognition and selection as a food source and appear to have an important role in host plant acceptance (Dethier & Crnjar, 1982; Schoonhoven, 1987; Martin & Shields, 2012; Sollai *et al.*, 2017a). The 4 gustatory receptor neurons (GRNs) located in each styloconic sensillum respond to plant compounds with specific activity patterns (for a review, see Schoonhoven &

van Loon, 2002). Some GRNs are primarily activated by plant metabolites such as sugars and amino acids that promote feeding and are therefore called phagostimulants,. Other GRNs respond to deterrent substances, such as secondary plant metabolites that are normally bitter to humans and mediate food aversive behavior. Feeding is not directly related to the presence or absence of specific substances, but rather on the balance between phagostimulants and deterrents (Dethier, 1973).

To verify whether peripheral taste sensitivity could play a role in the presence or absence of a positive relationship between oviposition preference and larval performance we selected *Papilio hospiton* Géné, an oligophagous butterfly endemic of islands of Sardinia and Corsica. In Sardinia, *P. hospiton* is almost monophagous, as adult females oviposit almost exclusively on the giant fennel (*Ferula communis* L.; Apiaceae). Two other rare plants, *Ferula arrigonii* Bocchieri (Apiaceae) and *Ruta lamarmorae* Bacch., Brullo & Giusso (Rutaceae), are occasionally used as host plants. However, on the small island of Tavolara, just off the northeast coast of Sardinia, we recently found adult females of *P. hospiton* laying eggs on *Seseli tortuosum* L. (Apiaceae), a plant only occurring on this island in the Sardinian region (Brullo *et al.*, 2001). Instead, no larvae of any instar were found on all plants of *S. tortuosum* that we carefully inspected at the same location. On the contrary, larvae of all instars were found on plants of *F. communis* on Tavolara during the same visits. These observations suggested a divergence between acceptance by the egg-laying adult females and rejection by the feeding larvae. We then decided to investigate whether the balance between positive and negative gustatory inputs differed enough, between the two stages of the insect life cycle, to justify acceptance or rejection of *S. tortuosum* as a possible host plant.

To this end, we stimulated the foreleg gustatory basiconic sensilla in adult females and the maxillary styloconic sensilla in the larvae with leaf saps of *F. communis* and *S. tortuosum*, with the aim of evaluating qualitative and quantitative differences in the response profiles of GRNs between the two plant saps. A previous study revealed that, in the peripheral taste system of *P. hospiton*, each of the tarsal chemosensilla of adult females houses sugar-, bitter- and salt-sensitive cells (Sollai *et al.*, 2017c), while in the larval taste system both lateral and medial sensilla contain phagostimulant, phagodeterrent and salt neurons (Sollai *et*

85 *al.*, 2014). Besides, we had found that one phagodeterrent GRN in the larval lateral sensillum may act as a
86 "labeled-line", indicating the presence of toxic compounds (Sollai *et al.*, 2015). The response patterns of
87 these sensilla to the two plant saps were then analysed in order to elucidate, how these plants can produce
88 contradictory effects on the oviposition behavior of adult females and the feeding behavior of larvae, that
89 is host acceptance and food source rejection respectively. In addition, we compared the
90 electrophysiological responses to plant saps with the oviposition preferences and larval growth
91 performance. This could provide a better understanding of the neural code for acceptance or aversion to
92 plants by insect herbivores which is considered a major objective of studies on coding of taste information
93 (Tang *et al.*, 2014). Finally, we evaluated the number of eggs laid on each plant by females, the number of
94 larvae growing to the pupal stage in relation to the number of eggs laid and hatched, and the larval growth
95 performance on the same plants.
96
97 **Materials and Methods**
98 **Insects and rearing**
99 A stock colony of *Papilio hospiton* Géné was raised in the butterfly annex (a 3 x 3 x 3m cage) of the
100 Physiology Laboratories (University of Cagliari). Adult females laid eggs on potted giant fennel (*Ferula*
101 *communis* L.). After hatching larvae were reared on the same plant at the insectary facility in 1500-ml
102 plastic cups (4-5 per cup) kept in an environmental growth chamber (24-25 °C, 70% R.H., 16L/8D
103 photoperiodic regime) and monitored daily until ready for the testing. Fresh foliage of *F. communis* came
104 from plants grown in a yard next to to the butterfly cage and was available ad libitum daily. Female adults
105 were obtained according to Sollai *et al.* (2017c). They were kept in the butterfly annex with free access to
106 *Lantana camara* L. flowers; after mating females were removed from the cage and transferred to smaller
107 boxes and fed with a sugar solution until used for electrophysiological recordings. *S. tortuosum* plants
108 were collected on the island of Tavolara and transferred to the Physiology Laboratories where they were
109 kept in pots.
110

Electrophysiological experiments

Forelegs of female butterflies were removed from the insect body using fine forceps and the electrophysiological recordings were obtained from the basiconic sensilla of the fifth tarsomere by means of the "tip-recording" technique (Hodgson *et al.*, 1955). The same technique was used for the electrophysiological recordings from the medial and lateral maxillary styloconic sensilla of fifth instar larvae two days after moulting (Simmonds *et al.*, 1991). The reference electrode, a thin Ag/AgCl, was inserted into the amputated butterfly leg or the head of the larva and gently pushed into the maxillary-labial complex to fix the maxillae in a prognathous position, while the recording electrode, a glass micropipette (tip diameter 20 μm), filled with the stimulating solution, was placed over the sensillum tip (Masala *et al.*, 2008; Solari *et al.*, 2010). All signals were recorded by means of a high input impedance (10^{15} Ω) electrometer (WPI, Duo 773), band-pass filtered (0.1-3 KHz), digitized with 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 KCl 50 mM (control) and the freshly-pressed leaf extracts of two plants, *Ferula communis* L. (giant fennel; hereafter Fcom) and *Seseli tortuosum* L. (hereafter Stor). 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. Leaf extracts were tested within 30 s after being pressed, according to Dethier and Crnjar (1982) and Sollai *et al.* (2017a, c). At the end of the recording series, KCl was tested again to check for any shift in responsiveness; whenever significant variations were detected, the experiment was discarded. In order to minimize drifts in solution concentration due to evaporation, a small amount of solution was drawn with a dry piece of filter paper from the electrode tip just prior to each stimulation. After each recording series, the tarsal surface or the mouthpart of the insect was rinsed with distilled water and blotted dry.

Data analysis

Spike analysis was performed in the interval 10-1010 ms after contact with the sensillum, with the first 10 ms being discarded as containing the contact artifact (Sollai *et al.*, 2012). This time frame was selected as representative of the phasic/phasic-tonic portions of the GRN response (Dethier & Crnjar, 1982; Inoue *et al.*, 2009). Spike sorting and counting were obtained by means of the Clampfit 10.0 software, on the basis on earlier work (Dolzer *et al.*, 2003; Sollai *et al.*, 2014; Biolchini *et al.*, 2017; Sollai *et al.*, 2017b, c).

Behavioral trials

Oviposition preferences

To test the oviposition preferences we counted the number of eggs laid on each plant, in a double-choice situation, in the butterfly oviposition annex (a 3x3x3m cage) of the Physiology Laboratories (University of Cagliari), according to Sollai *et al.* (2017c). Two plants of each of the two species were randomly placed inside the cage: since the plants were potted, they could be repositioned daily around the cage, thus providing a uniform sunlight exposure. Both plants were in their vegetative, non-flowering phenological state and had approximately the same foliage volume. Eggs were counted daily for 8-10 days at the natural emergence peak season of *P. hospiton* (tipically within first two weeks of May) and the procedure was repeated for 3 years (spring 2014-2016). In total, 28 egg counts were made for each of the two plant species. After counting, eggs were removed daily from the plant with a small patch of the leaf where they had been laid and to which they were still attached.

Larval growth performance

Larval growth performance on Fcom and Stor was evaluated according to Sollai *et al.* (2017a). Briefly, we measured: 1) the duration of the larval stage, as the period from egg hatch to pupation; 2) the maximum larval weight, right after the final evacuation bout preceding pupation. The larvae were reared on foliage of the plant where they hatched from egg, under environmentally controlled conditions, in the insectary facility as previously described. We monitored growth performance of 13 larvae for each host plant, as this was the number of larvae that reached the fifth instar on Stor (all other larvae died in the first and second instar) and the same number of larvae was tested on Fcom.

Statistical analysis

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 plant; c) the larval growth (days from hatching to pupa and weight) and the plant.

Two-way ANOVA was used to verify whether Fcom and Stor 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 successive 100 ms bins 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 (Sollai *et al.*, 2015).

Data were checked for the assumptions of homogeneity of variance and normality. 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 (Sollai *et al.*, 2017b). 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

187 on conservation of natural habitats and of wild fauna and flora". No specific permits were required for host
188 plants tested, as they are not endangered or protected species.
189
190 **Results**
191 **Effect of plant saps on the spike activity of the tarsal GRNs of adult females**
192 Samples of spike discharges of the activity of the tarsal GRNs of adult females in response to plant
193 extracts tested are shown in figure 1A. To test for a relationship between neural activity of each GRN and
194 the stimulus, we analyzed the spike response evoked in the first second of the discharge for each GRN
195 ("L", "M1", "M2" and "S"), by using one-way ANOVA (Fig. 1B).
196 One-way ANOVA revealed a significant effect of stimulus on the spike frequency only for "M1" GRNs
197 ($F_{1,139}$ = 4.1469; P < 0.05), and post-hoc comparisons showed that the spike frequency in response to
198 Fcom was higher than in response to Stor (P < 0.05; Tukey test). No other stimulus effects were found
199 ($F_{1,139}$ < 0.4582; P > 0.05).
200
201 **Effect of the plant saps on the spike activity of the lateral and medial GRNs of larvae**
202 Samples of spike discharges of the activity of the GRNs, recorded from the lateral and medial styloconic
203 sensilla of larvae, in response to leaf extracts of Fcom and Stor, are shown in Figures 2A and 3A. To test
204 for a relationship between neural activity of each GRN and the stimulus, we analyzed the spike response
205 evoked in the first second of the discharge for each GRN ("L", "M1", "M2" and "S") in both lateral and
206 medial sensilla, by using one-way ANOVA. For the lateral styloconic sensillum (Fig. 2B), one-way
207 ANOVA showed a significant effect of stimulus on the spike frequency of "L" and "M1" GRNs ($F_{1,87}$ >
208 5.555; P < 0.05). In particular, post-hoc comparisons showed that the spike frequency of "L" neuron in
209 response to Stor was higher than in response to Fcom, while the opposite was found for the neuron "M1"
210 (P < 0.05; Tukey test). For the medial sensillum (Fig. 3B), one-way ANOVA showed a significant effect
211 of stimulus on the spike frequency of M1 neuron ($F_{1,80}$ = 12.342; P < 0.001), and post-hoc comparisons
212 showed that the spike frequency evoked by Stor was lower than Fcom (P < 0.001; Tukey test). Finally, no

other stimulus effects were found in both lateral ($F_{1,87} < 2.9404$; $P > 0.05$) and medial ($F_{1,80} < 3.9237$; $P > 0.05$) sensilla. These results indicate that Fcom is more stimulating for the phagostimulant "M1" neurons in both lateral and medial sensilla, while Stor for the phagodeterrent "L" lateral neuron.

Oviposition preference and larval growth performance

To test for a relationship between oviposition preferences and oviposition substrates, we analyzed the number of eggs laid on each plant considered, by using one-way ANOVA (Fig. 4A), which revealed a non-significant effect of the substrate on the oviposition choice ($F_{1,54} = 1.4225$; $P > 0.05$); post-hoc comparisons showed that the number of eggs laid on Fcom was not statistically different from Stor ($P > 0.05$; Tukey test). These results indicate that the females equally chose both plants as hosts.

The results in figure 4B show that 226 out of 239 larvae hatched from eggs laid and raised on Stor and 6 out of 238 on Fcom died during the first week after hatching (first/second instar). This means that only 13 larvae survived on Stor and reached the pupal stage. As a consequence, also for Fcom the evaluation of larval growth performance was made on 13 larvae. To test for a relationship between larval growth performance and feeding substrate, we counted the number of days needed to reach pupal stage and we measured the maximum weight of larvae fed on each host-plant just before pupation, by using one-way ANOVA. One-way ANOVA showed a significant effect of the feeding substrate on the number of days needed to reach pupal stage ($F_{1,24} = 548.35$; $P < 0.000001$; Fig. 4C), but not for larval weight ($F_{1,24} = 2.5455$; $P > 0.05$; Fig. 4D). In particular, post-hoc comparisons showed that the number of days needed to pupation was higher for larvae reared on Stor than on Fcom ($P<0.001$; Tukey test). These results indicate that the larvae grow faster on Fcom, but the weight reached at pupation is the same.

Sensory code mediating plant discrimination

We investigated whether insects, both females and larvae, can discriminate between the two plant saps by means of an ensemble and/or 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

239 significant interaction of Stimulus × GRN on spike frequency was found in both lateral and medial
240 sensillum of the larvae ($F_{3,344}$ = 27.026; P < 0.00001 and $F_{3,320}$ = 3.69; P = 0.01228, respectively) (Fig. 5B
241 and C), but not in tarsal sensilla of adult females ($F_{3,556}$ = 1.9444; P = 0.1213) (Fig. 5A). These results
242 indicate that Fcom and Stor generate a different response pattern across all active GRNs only in the larval
243 styloconic sensilla. In order to verify a difference in temporal code, we analyzed the T-I curves for each
244 plant sap and evaluated the presence of a significant interaction of Stimulus × Time by using two-way
245 ANOVA. A significant interaction of Stimulus × Time was found only for the medial sensillum of the
246 larvae ($F_{9,3260}$ = 2.3009; P < 0.05) (Fig. 6), indicating that time course of spike frequency in response to
247 Fcom sap differ from that of Stor only in the medial larval sensillum.
248
249 **Discussion**
250 Insects have a gustatory system that allows them to discriminate among different food sources and host
251 plants (Chapman, 2003; Schoonhoven *et al.*, 2005; Forister *et al.*, 2012; Sollai *et al.*, 2017 a, c). Among all
252 gustatory neurons housed in the foretarsi and in the mouthparts, those located mainly on the fifth
253 tarsomeres of adults and on the lateral and medial styloconic sensilla of larvae, are considered the sensory
254 organs primarily involved in host selection and food recognition, respectively: they seem to play an
255 important role in host plant acceptance (Dethier & Crnjar, 1982; Schoonhoven, 1987; Sollai *et al.*, 2017a,
256 c). The main goal of this work was to evaluate whether the pattern activities of the 4 neurons in the tarsal
257 chemosensilla of adult females and of the 4+4 GRNs in the lateral and medial styloconic sensilla of larvae
258 responding to leaf extracts of Fcom and Stor could explain the degree of their acceptance/aversion both as
259 oviposition substrate and food source. As a first approach, we compared the pattern of activity of each
260 tarsal GRN in response to Fcom in Stor, since the number of eggs laid by female butterflies on the two
261 plants was not statistically different (Fig. 4C). We had previously found that a relationship exists between
262 the degree of acceptance of a plant as host and the electrophysiological responses it evokes from gustatory
263 sensilla (Sollai *et al.*, 2017a, c). The results of the present study show that the spike activity elicited from
264 each tarsal GRN in response to leaf sap of Fcom is not statistically different from that of Stor, and that

265 both plant saps evoke spike responses from all 4 neurons housed in the tarsal sensilla (Fig. 1). In addition,
266 Fcom and Stor do not differ neither in ensemble code (Fig. 5A), as they generated a same across neuron
267 pattern (ANP), nor in temporal code, since T-I curves evoked in the GRNs by both plant saps were
268 essentially parallel (Fig. 6A). By recalling that the sensory input is transmitted to the CNS for further
269 processing and generation of the final behavioral output and that our results revealed that *P. hospiton*
270 females are not able to discriminate between Fcom and Stor, it should not be surprising that no differences
271 were observed in the oviposition preferences between the two plants. In fact, if the plants are similar on the
272 basis of the cues used by the insect for discrimination, then the two plants are perceived as
273 indistinguishable (Larsson & Ekbom, 1995).
274 A second aim of the study was to understand whether the peripheral taste sensitivity of the larvae could
275 explain why only 5% of hatched larvae on Stor reached the larval stage. The electrophysiological results
276 show that statistically significant differences were observed in the activity of individual neurons in
277 response to the two extracts: in particular, the Stor elicited a higher spike frequency from the L-lat
278 bitter/toxic sensitive cell, while Fcom was a better stimulus for the phagostimulant neurons. Differences in
279 the neuron responses to the plant saps tested are considered consistent with the differences in food
280 preference (Tang *et al.*, 2014). Behavioral results about larval growth performance show that the duration
281 of the larval stage, from egg to pupa, is statistically lower on Fcom, and that the maximum weight is the
282 same reached on both plants (Fig. 4C). Together, these results suggest a relationship between the degree of
283 acceptance of a food source (e.g. a host plant) and the electrophysiological responses elicited by each of
284 them. The lower larval growth performance on Stor is linked to the fact that the extracts of this plant elicits
285 a higher activity from the L-lat neuron, previously identified as a deterrent cell signaling the presence of
286 bitter and toxic compounds (Sollai *et al.*, 2014; Sollai *et al.*, 2015). This is in agreement with the
287 hypothesis that a spike frequency increase in a given GRN (e.g. responding to bitter and potentially toxic
288 compounds) is associated with a faster and stronger behavioral response (e.g. taste rejection) (de Boer *et*
289 *al.*, 1977), and that the activation of the deterrent GRN by a plant extract may slow down the feeding
290 activity (Glendinning *et al.*, 1998). Moreover, Stor also evokes a significantly lower spike activity from

291 the phagostimulant neurons (M1-lat and M1-med), consistent with the fact that most larvae died soon after
292 hatching, during the first-second instar, and only less than 5% of the larvae reached the pupal stage: this
293 supports the idea that food rejection could be linked more to the absence of phagostimulant inputs than to
294 the presence of deterrent inputs (Ma, 1972). On the basis of these results we assume that, if the larvae are
295 able to overcome the first two instars, it takes longer to reach the pupal stage because they feed more
296 reluctantly on a plant with an unpleasant taste and this strengthens the hypothesis that the peripheral
297 gustatory system plays an important role in the acceptance of a host plant, as previously suggested (Sollai
298 *et al.*, 2017a). Similar results have been found in *Papilio polytes*, where the high mortality of neonates on
299 *Orixa japonica* seems to be mainly due to inhibition of feeding caused by some anti-feedant(s) present in
300 the plant (Murakami *et al.*, 2003). However, the fact that the larvae in the pre-pupal stage reach a similar
301 weight, suggests that Fcom and Stor do not have different nutritional values for the larvae. Future
302 experiments are needed to elucidate this aspect.
303 We have previously showed that the *P. hospiton* larvae can discriminate between host plants by means of
304 an ensemble and a spatio-temporal code (Sollai *et al.*, 2017a). This is confirmed by the results of the
305 present study which indicate that plant saps can be discriminated also by means of a temporal code, at least
306 in the case of the medial sensillum, substantiating the idea that discrimination may be the outcome of
307 several combined coding mechanisms principally involving the chemosensory neurons of the lateral and
308 medial sensilla. In fact, we found that Fcom generates an across neuron pattern (ANP) different from that
309 of Stor in both styloconic sensilla. In addition, in the medial sensillum, the time course of spike frequency
310 evoked by the extracts of Fcom differed from that of Stor, indicating a difference in temporal code.
311 The main goal of this work was to evaluate whether differences in the pattern activities of the GRNs in
312 foreleg tarsal sensilla of adult females and in the styloconic sensilla of larval maxillae in response to leaf
313 extracts of Fcom and Stor could explain the absence of a positive relationship between oviposition
314 preference and larval performance. In fact, the successful choice of plant as a host is determined both by
315 butterflies that may or may not lay eggs on it and by the larvae that may or may not feed on it: as a
316 consequence, the choice of the oviposition site is crucial for larval performance (Nishida, 2005).

317 Nevertheless, whether a positive relationship exists between oviposition preference and larval
318 performance is still a matter of debate. Some authors support strongly the performance-preference
319 hypothesis, predicting that females will maximize chances of success for their offspring by choosing those
320 host plants for oviposition on which their larvae perform best (Jaenike 1978; Gripenberg *et al.*, 2010).
321 Other authors argue instead that females do not always lay eggs on the plant species on which their
322 offspring grows and survives, and on which their performance is best (Prager *et al.*, 2014; Konig *et al.*,
323 2016). Several explanations for such a lack of positive correlation between oviposition preference and
324 larval growth performance have been suggested: e.g., the rarity of the optimal host plant (Wicklund &
325 Friberg, 2009), or the fact that egg-laying females could be oriented to maximize their performance, rather
326 than that of their progeny (Mayhew, 1997). Indeed, it has been proposed that the oviposition strategies of
327 herbivorous insects vary greatly depending on whether a female is more limited by the time available to
328 oviposit or by the number of eggs it can lay (Jaenike, 1978; Mangel, 1987). Others, finally, talk about
329 oviposition "mistakes": a wider range of host plants used may allow females to save time in the host
330 search, or could be used to select against females who are less specific in the choice of the host plant
331 (Thompson *et al.*, 1991). Alternatively, could it mark the start of a full shift towards a new species of plant
332 (Larsson & Ekbom, 1995).
333 By recalling that phytophagous Lepidoptera are highly dependent on the chemical composition of the
334 plant when deciding whether to assign it or not the role of host, and that the acceptance or rejection of a
335 plant by egg-laying females depends on the balance between positive and negative stimuli of the plant
336 itself (Honda & Nishida, 1999; Nakayama & Honda, 2004; Sollai *et al.*, 2017c), as well as the acceptance
337 of a food source by the larvae (Dethier & Crnjar, 1982; Chapman, 2003; Sollai *et al.*, 2017a), this is the
338 first study that turns its attention to reasons other than environmental ones. In conclusion, our results show
339 that: a) the identical pattern of activity of tarsal GRNs evoked by the two plant saps prevents females to
340 discriminate between them (accepting both of them as hosts), and b) the different pattern of activity of the
341 lateral and medial sensilla, and particularly the different activation of the bitter/toxic-sensitive GRN,
342 determines the rejection of Stor as a food source by the larvae. This suggests that the different peripheral

343 taste sensitivity between parents and progeny toward a host can be added to the reasons for the absence of
344 a positive relationship between oviposition preferences and larval performance.
345
346

347 **Acknowledgements**

348 We are grateful to Dr. Marco Melis, Dept of Biomedical Sciences, University of Cagliari, for technical
349 assistance. This work was supported by the Regione Autonoma della Sardegna [CRP-59859] and the
350 Fondazione Banco di Sardegna [2012/0245].

351

352 **Conflict of interest**

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

355

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Legends of Figures

Fig. 1 – (A) Sample traces showing spike activity of a tarsal basiconic sensillum of an adult *P. hospiton* female following stimulation with leaf sap of *F. communis* (Fcom) and *S. tortuosum* (Stor). (B) 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) and *S. tortuosum* (Stor). N = 67-74. * indicates significant differences between the spike activity of the same GRN in response to the two taste stimuli ($P < 0.05$; Tukey test).

Fig. 2 – (A) Sample traces showing spike activity of a lateral styloconic sensillum of a *P. hospiton* fifth instar larva following stimulation with leaf sap of *F. communis* (Fcom) and *S. tortuosum* (Stor). (B) Mean values ± s.e.m. of number of spikes evoked in each GRN of the lateral sensillum during the first second of stimulation with leaf sap of *F. communis* (Fcom) and *S. tortuosum* (Stor). N = 43-45. ** indicate significant differences between the spike activity of the same GRN in response to the two taste stimuli ($P < 0.01$; Tukey test).

Fig. 3 – Sample traces showing spike activity of a medial styloconic sensillum of a *P. hospiton* fifth instar larva following stimulation with leaf sap of *F. communis* (Fcom) and *S. tortuosum* (Stor). (B) Mean values ± s.e.m. of number of spikes evoked in each GRN of the medial sensillum during the first second of stimulation with leaf sap of *F. communis* (Fcom) and *S. tortuosum* (Stor). N = 38-44. ** indicate significant differences between the spike activity of the same GRN in response to the two taste stimuli ($P < 0.01$; Tukey test).

Fig. 4 – (A) Mean values ± s.e.m. of number of eggs laid daily on *F. communis* (Fcom) and *S. tortuosum* (Stor) by *P. hospiton* adult females (Number of counts = 28). (B) Number of larvae surviving over time on each plant after hatching. They were 99.17% and 99.58% of the total number of eggs laid on *F. communis* (Fcom) and *S. tortuosum* (Stor), respectively. Arrows indicate beginning of pupation: 23.77 ± 0.25 days from egg hatching on *F. communis* (Fcom) and 32.15 ± 0.27 days on *S. tortuosum* (Stor). (C) Mean values ± s.e.m. of the number of days to pupation and (D) maximum weight reached before pupation on *F. communis* (Fcom) and *S. tortuosum* (Stor). N=13 larvae for each plant. *** $P < 0.001$; Tukey test.

Fig. 5 – Significant interaction of the Stimulus × GRN on the spike frequency of an adult tarsal sensillum (A) and larval lateral (B) and larval medial (C) sensillum of *P. hospiton*, elicited by *F. communis* (Fcom) and *S. tortuosum* (Stor).

Fig. 6 – Time-Intensity curves (i.e., number of spikes during 10 consecutive 100 ms intervals) elicited by *F. communis* (Fcom) and *S. tortuosum* (Stor) in the adult tarsal sensillum (A) and larval lateral (B) and larval medial (C) sensillum of *P. hospiton*.

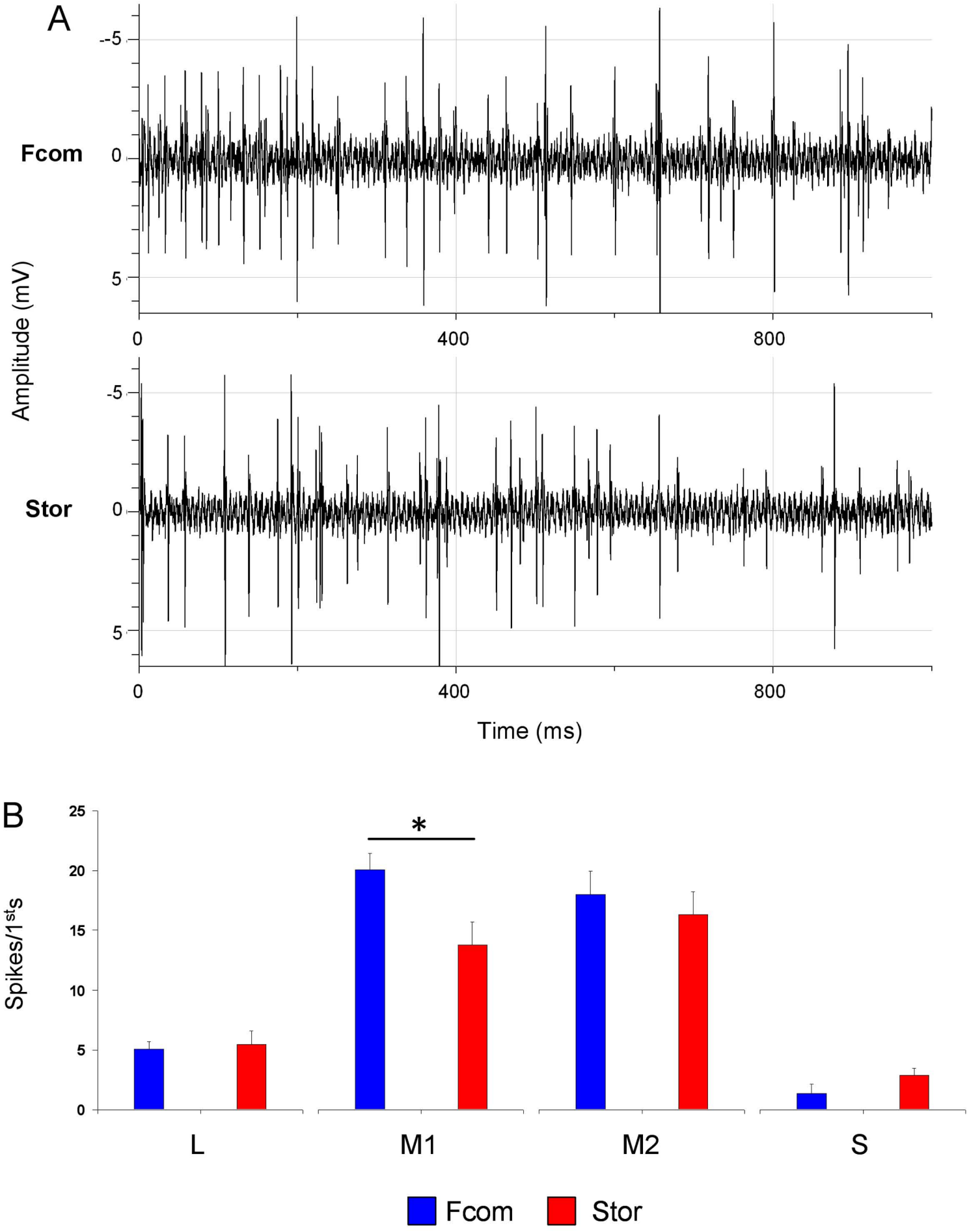

A
Fcom
Stor
Amplitude (mV)
-5
0
5
0
400
800
Time (ms)
B
Spikes/1st s
25
20
15
10
5
0
*
L
M1
M2
S
Fcom
Stor

Figure 1

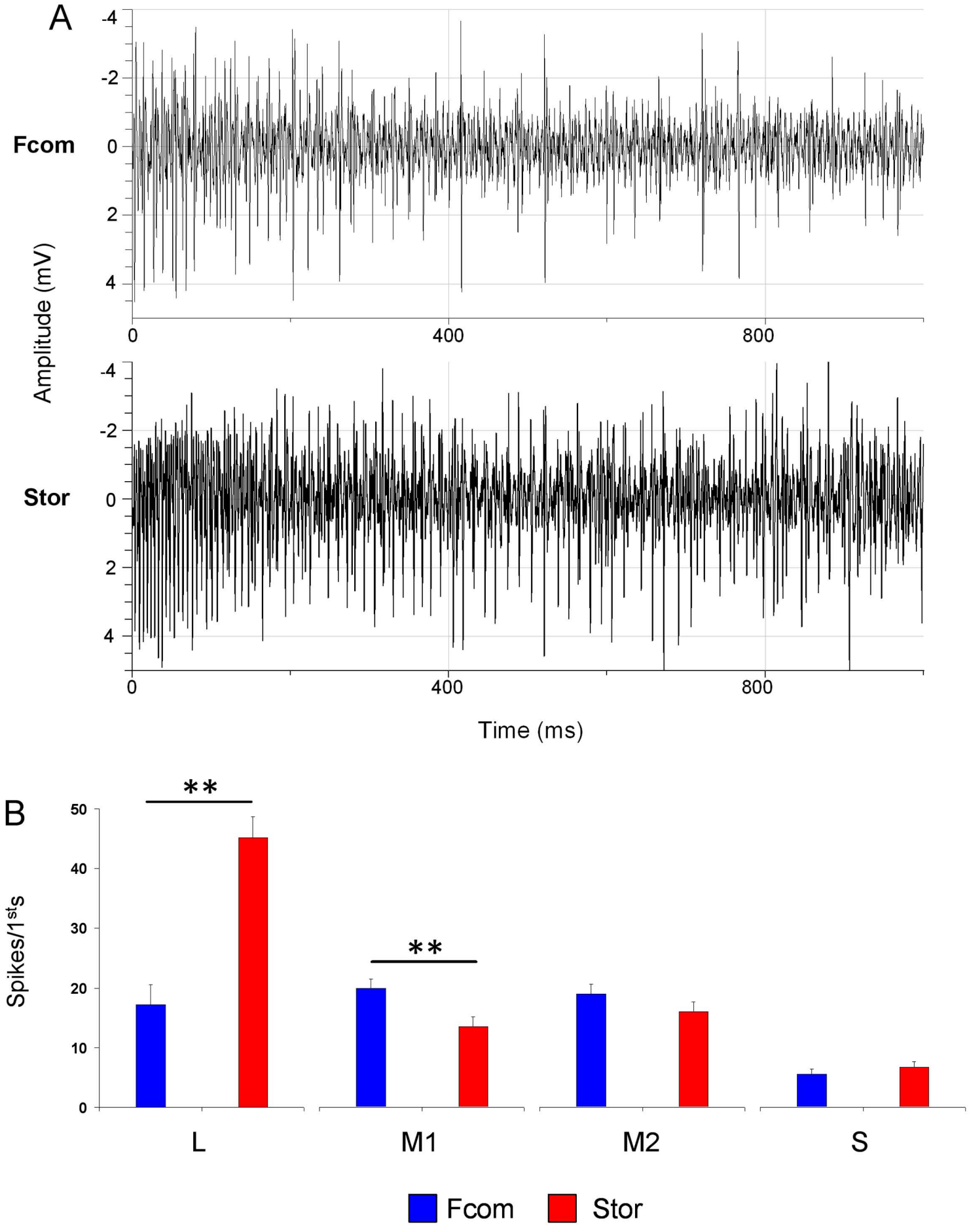
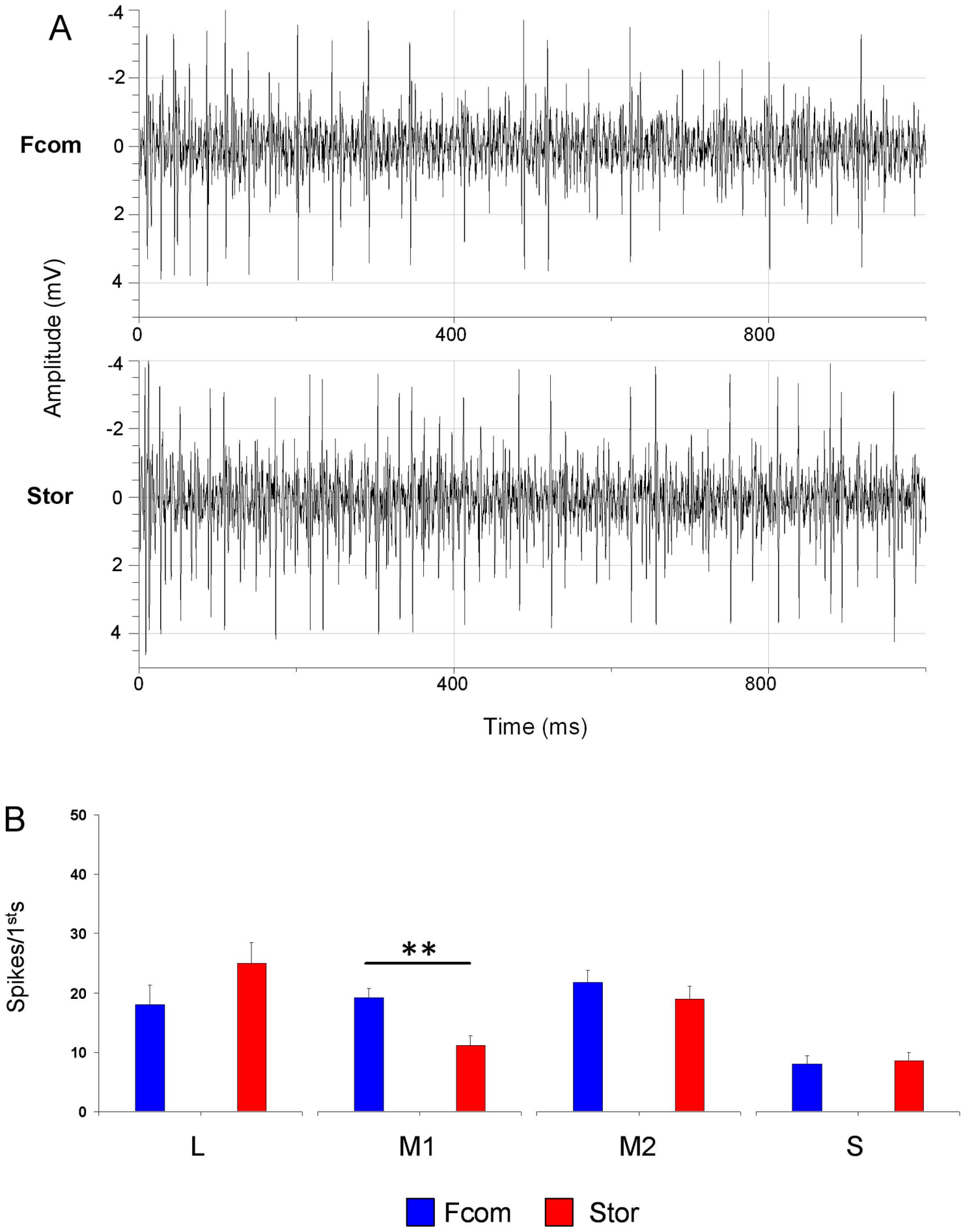

A
Fcom
Stor
Amplitude (mV)
-4
-2
0
2
4
0
400
800
Time (ms)
B
Spikes/1st s
50
40
30
20
10
0
**
**
L
M1
M2
S
Fcom
Stor

Figure 2


A
Fcom
Stor
Amplitude (mV)
-4
-2
0
2
4
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400
800
Time (ms)
B
Spikes/1st s
50
40
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20
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0
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L
M1
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S
Fcom
Stor

Figure 3

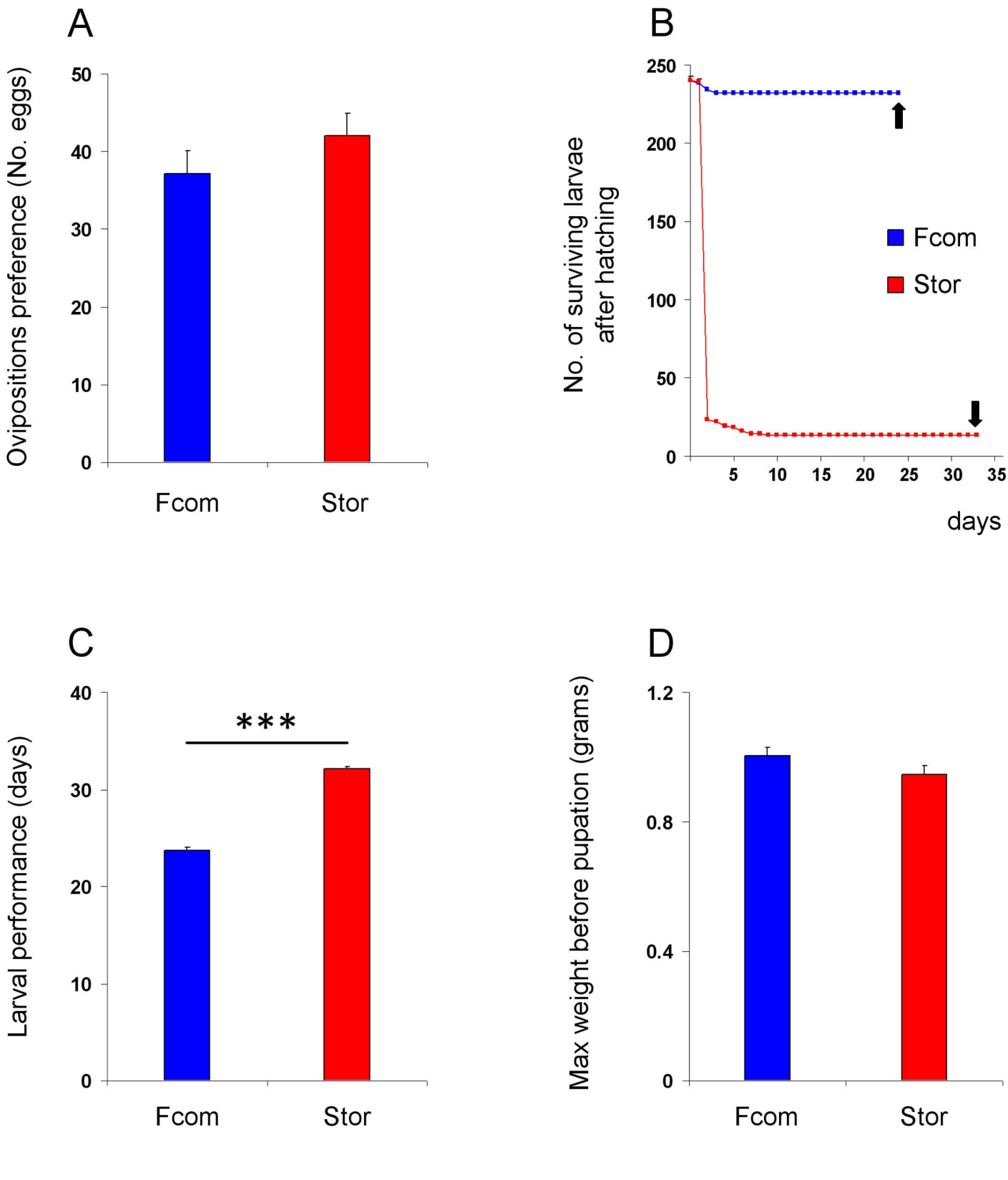
A
Ovipositions preference (No. eggs)
50
40
30
20
10
0
Fcom
Stor
B
No. of surviving larvae after hatching
250
200
150
100
50
0
5
10
15
20
25
30
35
days
Fcom
Stor
C
Larval performance (days)
40
30
20
10
0
***
Fcom
Stor
D
Max weight before pupation (grams)
1.2
0.8
0.4
0
Fcom
Stor

Figure 4

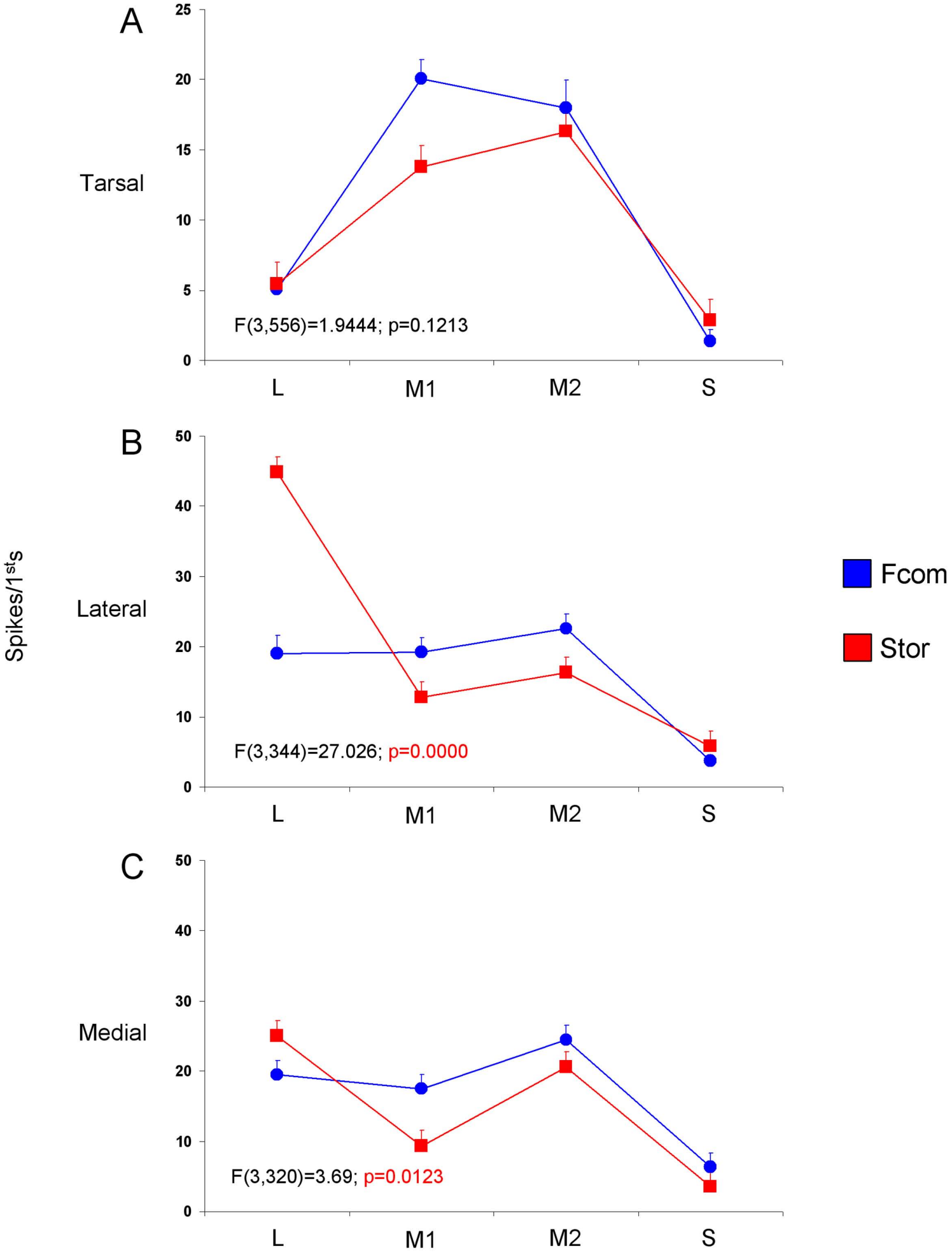
A
Tarsal
25
20
15
10
5
0
F(3,556)=1.9444; p=0.1213
L
M1
M2
S
B
Lateral
50
40
30
20
10
0
F(3,344)=27.026; p=0.0000
L
M1
M2
S
C
Medial
50
40
30
20
10
0
F(3,320)=3.69; p=0.0123
L
M1
M2
S
Spikes/1st s
Fcom
Stor

Figure 5

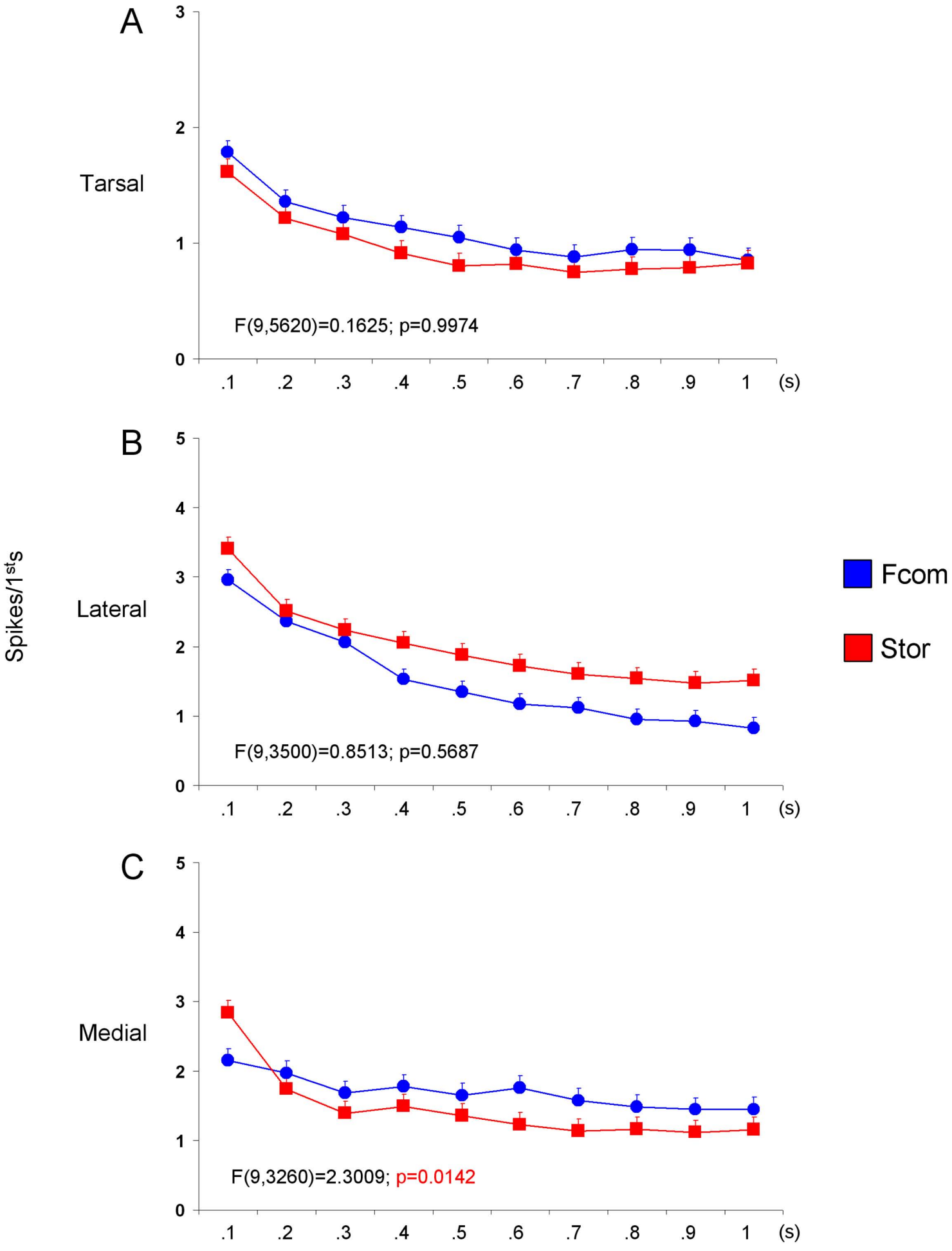
A
Tarsal
F(9,5620)=0.1625; p=0.9974
B
Lateral
F(9,3500)=0.8513; p=0.5687
C
Medial
F(9,3260)=2.3009; p=0.0142
Spikes/1st s
(s)
Fcom
Stor

Figure 6