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1 **Evidence that the contraction-induced rapid hyperemia in rabbit masseter muscle is based on**
2 **a mechano-sensitive mechanism, not shared by cutaneous vascular beds**

3

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6

7 **Author contributions**

8 Conception and design of the experiments: SR.

9 Collection, analysis and interpretation of data: MT and MM.

10 Drafting the article or revising it critically for important intellectual content: MT and SR.

11

12

13 **running title:** rapid hyperemia in masseter muscle

14

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21 **Abstract**

22

23 Several mechanisms have been hypothesized to contribute to the rapid hyperemia at the onset of
24 exercise. Aim of the present study was to investigate the role played by the mechano-sensitivity of
25 the vascular network.

26 In 12 anesthetized rabbits blood flow was recorded from the exclusively muscular masseteric artery
27 in response to brief spontaneous contractions (BSC) of the masseter muscle, artery occlusion (AO),
28 muscle compression (MC) and muscle stretch (MS). Activation of masseter muscle was monitored
29 by electromyography (EMG). Responses to AO were also recorded from the mostly-cutaneous
30 facial and the central ear arteries. Five animals were also tested in the awake condition.

31 The hyperemic response to BSC (peak amplitude of $394 \pm 82\%$; time-to-peak of 1.8 ± 0.8 s)
32 developed with a latency of 300-400 ms from the beginning of the EMG burst and 200-300 ms from
33 the contraction-induced transient flow reduction. This response was neither different from the
34 response to AO (peak amplitude = $426 \pm 158 \%$), MC and MS ($p=0.23$), nor from the BSC response
35 in the awake condition. As compared to the masseteric artery, the response to AO was markedly
36 smaller both in the facial ($83 \pm 18 \%$) and in the central ear artery ($68 \pm 20 \%$) ($p<0.01$).

37 In conclusion, the rapid contraction-induced hyperemia can be replicated by a variety of stimuli
38 affecting transmural pressure in muscle blood vessels and is thus compatible with the Bayliss effect.

39 This prominent mechano-sensitivity appears to be a characteristic of muscle and not cutaneous
40 vascular beds.

41

42 **Keywords:** muscle stretch, myogenic response, rapid dilatation, reactive hyperemia, muscle
43 compression

44

45

46 **Introduction**

47 Skeletal muscle blood flow has long been known to exhibit a rapid increase at the onset of exercise
48 (1, 9, 12, 22, 27). The mechanisms behind this phenomenon are still matter of debate in spite of the
49 many new insights provided by different research groups in the recent years. In particular it has
50 been shown that the contraction-induced compression of venous compartments (muscle pump)
51 cannot fully account for the blood flow increase and that the additional occurrence of a rapid active
52 dilatation is required (18, 33, 45, 46). Other studies have shown that this rapid dilatation is mediated
53 neither by changes in sympathetic neural drive (7, 41), nor by ACh spillover from the motor
54 endplate (6, 33), nor by endothelium-released NO (6), although this last assertion is debated (see
55 Discussion). As early as 1974, Mohrman and Sparks (31) hypothesized that the rapid dilatation
56 could result from the myogenic response of muscle blood vessels to decreased transmural pressure,
57 which in turn results from the increase in intramuscular (extravascular) pressure produced by the
58 contraction. They showed, in fact, that the rapid hyperemia provoked by a 1-s tetanic stimulation of
59 the isolated calf muscle of the dog could be reproduced, although with lower magnitude, by a brief
60 external compression of the muscle performed through an inflatable cuff (31). The concept of a
61 mechanically-induced dilatation has recently found renewed support, based on *in-vitro* (10) as well
62 as *in-vivo* studies performed on several animal models (19, 33) including humans (6, 11, 24). In
63 particular it has been shown that the increase in extravascular pressure could induce the rapid
64 dilatation of an isolated muscle artery (10) and that the rapid blood flow increase evoked by a brief
65 hand grip was closely reproduced by a brief external compression of the forearm (6, 24). These data
66 led some authors to re-consider the myogenic response as one of the possible mechanisms behind
67 the exercise-induced rapid dilatation (10, 11, 14). For the sake of clarity, it should be emphasized
68 that we here refer to the *rapid* myogenic response that, as first reported by Bayliss, can be evoked *in*
69 *vivo* by a short lasting arterial occlusion in the form of a rapid (reactive) hyperemia (3, 5, 38). At
70 difference with the “classical” (slow) myogenic response, which develops over a period in the order
71 of 1 minute upon sustained changes in transmural pressure and which has been extensively studied

72 and characterized in a variety of experimental conditions, the *rapid* myogenic response has received
73 much less consideration (15, 21) and is still poorly understood.

74 However, a number of intrinsic limitations in the experimental models make the involvement of the
75 mechano-sensitive mechanisms in the contraction-induced hyperemia difficult to quantify. First of
76 all, external compression of the forearm is not an unequivocal mean to induce a myogenic response
77 since the compression-induced depletion of venous compartments may contribute to the hyperemia
78 according to the muscle pump mechanism (26, 45). Surprisingly, the contraction induced
79 hyperemia has never been directly compared to the response to artery occlusion, which would
80 overcome this limitation. Secondly, since blood flow (or blood velocity) is commonly recorded
81 from large arteries supplying musculo-cutaneous vascular compartments, it remains to be assessed
82 to what extent response to compression of cutaneous tissues may contribute to the observed effects.
83 A third issue concerns the accuracy in detecting the latency of the rapid dilatation with respect to
84 muscle contraction, which is crucial to discriminate between the faster mechanical *versus* the slower
85 metabolic mechanism. Based on different experimental models, such latency is currently reported to
86 be “< 1 s” (24, 33, 41, 47), which is considered too short for the metabolic action to develop (11,
87 50). However a recent study indicates that K^+ , released by contracting muscle fibers, could also
88 mediate an early dilatatory effect (2), which leaves the issue unsettled.

89 Based on the similarity in the rapid hyperemic responses that have been reported in different
90 preparations in response to short lasting muscle contractions and mechanical stimuli (6, 8, 10, 18,
91 24, 33, 38, 47) we hypothesized that a single mechano-sensitive mechanism, activated by changes
92 in transmural pressure, could underlie the different responses. Moreover, since it has been proposed
93 that this rapid hyperemia is functionally meant to provide a prompt increase in muscle perfusion at
94 the onset of exercise (9), we aimed to investigate whether this reactivity to the mechanical stimulus
95 were differently exhibited by muscular and cutaneous vascular beds.

96 To test these hypotheses, the rapid hyperemia induced by the following manoeuvres, i.e.,
97 spontaneous muscle contraction, external compression of the muscle, stretch of the passive muscle

98 and occlusion of the relevant artery, is elicited and compared in the same experimental model. In
99 addition the tissue-specificity of this vascular responsiveness is investigated in arteries exclusively
100 supplying skin or muscle.

101 The study is based on a recently developed rabbit experimental model (37) that allows continuous
102 monitoring of muscle blood flow from the masseteric branch of the facial artery, which exclusively
103 supplies the anterior portion of the masseter muscle. This muscle exhibits spontaneous isolated
104 short bursts of activity producing a transient hyperemia (35) thus offering an ideal model to
105 investigate the contraction-induced rapid hyperemia. In addition, the superficial localization of both
106 the masseter muscle and the masseteric artery allows for convenient application of external stimuli
107 such as muscle compression and artery occlusion.

108 The obtained results strongly support the notion that the contraction-induced rapid hyperemia can
109 be largely mediated by mechano-sensitive dilatory mechanisms.

110

111 **Methods**

112

113 *Ethical approval*

114 The study was performed at the University of Torino in accordance with the principles of laboratory
115 animal care. Purposes and protocols were approved by the Ethical Committee for Animal
116 Experiments at the University of Torino.

117 Experiments were carried out on 12 anesthetized rabbits. The rabbits were previously implanted
118 with chronic probes and in five of them recordings were also collected in the awake condition, the
119 results being separately presented.

120

121 *Surgical procedures*

122 Twelve male European rabbits (*Oryctolagus cuniculus*) weighing between 2.8 and 3.3 kg were
123 anesthetized with ketamine (Ketalar, Parke-Davis) and xylazine (Rompun, Bayer) at a dose of 20

124 and 5.5 mg/kg, respectively, injected into the marginal ear vein. Surgical anesthesia was
125 subsequently maintained by continuous i.v. infusion of the same drugs (infusion pump Terumo
126 Europe STC-521, Leuven, Belgium).

127 The surgery was performed in sterile conditions. The masseteric artery was isolated just after
128 branching from the facial artery and accommodated into a perivascular flow probe (model 0.7PSB,
129 Transonic Systems Inc, Itaha, NY, USA), adequate for vessels of diameter < 0.7 mm. The
130 masseteric artery, which exclusively supplies the rostral portion of the masseter muscle, was
131 isolated medially to the mandibular margin, immediately after its branching from the facial artery
132 (37). The probe wire was tunneled below the platysma to the angle of the mandible and then
133 subcutaneously around the neck. Particular attention was paid to avoid torsion of the cable that
134 would have been transmitted by the probe to the vessel and cause interruption of blood flow. The
135 small probe connector was left protruding from the dorsal cervical region, for subsequent
136 connection to the flow meter, and sutured to the skin by means of a Delrin skin holder (Transonic
137 Systems Inc.).

138 In five rabbits additional blood flow probes were implanted around the facial artery (model 1PSB
139 Transonic Systems Inc, Itaha, NY, USA), proximal to the origin of the masseteric artery (35) and
140 around the central ear artery (model 1PSL Transonic System Inc. Itaha, NY, USA), which supplies
141 an almost exclusively cutaneous vascular bed (51).

142 A telemetric blood pressure transducer (TA11PA-D70, DSI USA) was implanted for continuous
143 monitoring of arterial blood pressure (ABP). The catheter was inserted through the right femoral
144 artery into the abdominal aorta while the transmitter was located in a subcutaneous abdominal
145 pouch (35).

146 After surgery all animals underwent analgesic (Finadyne, Schering-Plough, 2 mg/kg i.m.) and
147 antibiotic (Rubrocillin retard, Intervet, 0.1 ml/kg i.m.) treatment for the following 2 and 7 days,
148 respectively.

149

150 After 7-10 days from surgery the animal was re-anesthetized as described above, the trachea was
151 cannulated and the head was fixed in a stereotaxic frame by screws implanted in the frontal bone. In
152 5 rabbits a catheter was inserted in the left femoral vein for drug administration. In 4 animals
153 electromyographic (EMG) activity of the masseter muscle was recorded ipsilaterally to the
154 masseteric artery under investigation. EMG was detected by means of hooked copper wires, teflon-
155 insulated except for their tips, inserted through a thin needle into the anterior portion of the muscle.
156 The experimental procedures started after stabilization of the hemodynamic variables, about 1 h
157 after the surgical preparation was completed.

158

159 *Experimental protocol*

160 The responses to the following stimuli were investigated: i) brief spontaneous contractions (BSC) of
161 the masseter muscle, occurring in the absence of any external stimulus, ii) artery occlusion (AO),
162 iii) muscle compression (MC) and iv) muscle stretch (MS) (Fig. 1).

163 Artery occlusions of short duration (about 1 s) were performed by the experimenter by means of a
164 moderate compression of the skin against the inferior margin of the mandible at the point where the
165 masseteric artery passes over the bone (Fig 1). AO lasting 1 s were later selected for analysis.

166 Effectiveness of the maneuver was confirmed by the interruption of the blood flow in the
167 recording. Muscle compression was performed by means of a shaker (model V2, Data Physics
168 corporation, USA) driven by a computer-generated trapezoidal signal (rise time: 0.1 s; plateau: 1s;
169 return: 0.1s, delivered by a1401 micro board, CED, UK). The shaker was armed with a semi-
170 spherical tip (radius of 1 cm), having a linear excursion of 3-5 mm. This device allowed us to
171 perform repeatable compressions of the cheek at the level of the anterior portion of the masseter
172 muscle. Preliminary experiments showed that the amplitude of the hemodynamic response to MC
173 has a limited dependence on the exerted force, in the range 0.9 – 3.6 N and that the responses are
174 highly reproducible (manuscript in preparation). Muscle stretch was performed by manually

175 lowering the mandible by a fixed amount of 5-7 mm and maintaining the masseter muscle stretched
176 for 1-2 s before returning the mandible to the resting position.

177 Artery occlusion was also operated both on the central ear artery, by manually compressing the
178 vessel just distally to the flow probe, and on the facial artery, by manually exerting a pressure
179 against the inferior margin of the mandible (Fig 1). Mechanical stimuli (AO, MC and MS) were
180 provided in randomized order, separated by resting intervals of at least 2 min.

181

182 Vascular responses to MS were tested before and after administration of the neuromuscular
183 blocking agent succinylcholine chloride (Sigma, Steinheim, Germany; 200-400 mg/kg, 3 animals).
184 During neuromuscular blockade the animal was artificially ventilated so as to maintain the end-
185 tidal CO₂ concentration at the pre-curarization level (in spontaneous breathing), measured by ossi-
186 capnometer (Engstrom, Eliza Duo, Sweden). Effectiveness of neuromuscular blockade was assessed
187 by monitoring the extensor movement of the paw elicited by electrical stimulation of the peripheral
188 stump of the tibialis nerve.

189

190 At the end of the recording session the animal was sacrificed with an i.v. injection of a lethal dose
191 of anesthetic.

192

193 *Recording from conscious animals*

194 Most of the recordings in awake animals concerned the investigation of hemodynamic responses to
195 acute stress stimuli (36) that are not considered in the present study. Only recordings related to
196 simultaneous monitoring of MaBF and EMG in resting conditions (5 animals) are included in the
197 present study.

198 Measurements in awake animals started 5-7 days after implant of chronic probes and were repeated
199 in subsequent days.

200 The animal was kept in a large box (50-36-20 cm) and familiarized with the stimuli performed by
201 the experimenters, including touching the head and exerting light pressures with the fingers on the
202 cheeks and mandible.

203 During the recording session the animal was let free to rest, move and eat in the box. Blood flow in
204 masseteric artery was continuously recorded during rest and while handling the animal to provide
205 different vascular stimuli: AO could be performed as described above while MC was performed by
206 bilaterally compressing the cheeks with two fingers (thumb and index finger) at the level of the
207 anterior portion of the masseter muscle, for a duration of about 1s.

208 Trials were considered valid when the maneuvers did not produce stress, as assessed by the absence
209 of relevant changes in ABP, heart rate and blood flow in the artery under study (36).

210

211 *Data acquisition and processing*

212 During recording sessions, the arterial blood pressure signals were radio-transmitted from the
213 implanted transducer to a nearby located antenna and reconverted to an analogue voltage signal
214 (TA11PA-D70, DSI USA) while the flow probes were connected to the flow meter (2-channels
215 TS420, Transonics, USA) via 1.5 m extension cables. Bipolar EMG recordings were amplified
216 (P511 Grass, USA) and band-pass filtered (100-2000 Hz). All signals were then digitally sampled
217 (1401micro, CED, UK) (sampling rate: 200 Hz for blood flow and arterial blood pressure, 5 kHz for
218 EMG) and continuously acquired and stored on a personal computer. Acquisition and off-line
219 processing were performed with Spike2 (CED, UK). Simple algorithms were implemented in the
220 Spike2 script language aimed at identifying single cardiac cycles (based on systolic peak detection
221 on the ABP signal) from which time averages of the different signals (one value per cardiac cycle)
222 were computed, unless otherwise specified. Hyperemic responses were normalized with respect to
223 their pre-stimulus value. Average responses to the different stimuli (BSC, AO, MC, MS) were
224 obtained for each animal (n trials: 5-10) and then across animals. The average blood flow trace was
225 computed after aligning single trials with respect to the start of the steep rising phase of hyperemic

226 responses, unless otherwise specified. In order to characterize and compare these hyperemic
227 responses the following variables were calculated: the maximum blood flow increase (peak
228 amplitude), the time elapsed from the beginning of the response to the flow peak (time-to-peak) and
229 the time required to return from the peak to the mid value between peak and control level (half-
230 return time).

231 All values are reported as mean \pm standard deviation.

232 The comparison of responses to the different stimuli in anesthetized animals was performed by 1-
233 way multi-variate ANOVA for the 3 variables: peak amplitude, time-to-peak and half return time.

234 The comparison of BSC responses in anesthetized and conscious animals was performed by a 1-
235 way multi-variate ANOVA. The comparisons between AO responses in masseteric, ear and facial
236 arteries was tested by a 1-way ANOVA. Accepted significance level: $P < 0.05$.

237

238 **Results**

239

240 *Hyperemic responses in anesthetized animals*

241 In the anesthetized rabbit BSCs of the masseter muscle were often observed. They are characterized
242 by a short EMG activation immediately followed by a marked increase in MaBF, one example
243 being shown in Fig. 2A. This motor pattern, which is spontaneously exhibited at intervals ranging
244 between 10 s to several minutes, appears to be rather stereotyped also across different animals. Fig.
245 2B reports the average curve obtained from 4 animals in which EMG and MaBF were
246 simultaneously recorded. Note that the EMG curve well preserves its brief and sharp activation
247 pattern. The fast transients are magnified in Fig. 2C. It can be observed that the latency between the
248 beginning of the EMG burst and the increase in blood flow is in the order of 300-400 ms and that a
249 brief reduction in blood flow occurs during muscle activation, preceding the immediate blood flow
250 increase.

251 Very similar hyperemic responses were evoked by AO, MC and by MS. The responses developed
252 immediately after the release of the stimulus and terminated within 10-20 s. Responses to BSC, AO,
253 MC and MS were collected from 8 rabbits and the average curves are superimposed for comparison
254 in Fig. 3A after normalization with respect to the control (pre-stimulus) value. Average parameters
255 are as follows, BSC: peak amplitude = 394 ± 82 % (1.15 ± 0.54 ml/min), time-to-peak = 1.8 ± 0.8 s,
256 half-return time = 6.1 ± 1.0 s; AO: peak amplitude = 426 ± 158 % (0.95 ± 0.50 ml/min), time-to-
257 peak = 1.9 ± 0.9 s, half-return time = 7.5 ± 2.7 s; MC: peak amplitude = 469 ± 200 % (0.87 ± 0.40
258 ml/min), time-to-peak = 1.8 ± 0.6 s, half-return time = 8.0 ± 3.7 s; MS: peak amplitude = 384 ± 171
259 % (0.75 ± 0.52 ml/min), time-to-peak = 2.4 ± 0.7 s, half-return time = 7.4 ± 1.6 s. None of the
260 parameters was significantly dependent on the type of stimulus ($p=0.23$).

261 None of the stimuli affected arterial blood pressure. Thus, the relative changes in MaBF depicted in
262 Fig. 3 can be read in terms of changes in vascular conductance as well. For the sake of clarity
263 average curves are also reported in non-normalized form in Fig. 3B.

264

265 *Response to muscle stretch after neuro-muscular blockade*

266 Reflex activation of the masseter muscle was observed to occur in response to MS. In order to
267 ascertain the role of muscle contraction in the MS-induced hyperemia, in 3 animals the maneuver
268 was performed before and during transient muscle paralysis produced by systemic administration of
269 succinylcholine. The effectiveness of neuro-muscular blockade was confirmed by the disappearance
270 of reflexly-activated EMG activity. In all tested animals the rapid increase in blood flow occurring
271 at the time of muscle release (peak amplitude: 394 ± 52 %) was still present after neuromuscular
272 blockade (465 ± 77 %).

273

274 *Hyperemic responses in conscious animals*

275 Basal MaBF in the conscious rabbit ranged between 0.1 and 0.3 ml/min and always increased in
276 connection with the activity of the masseter muscle, as detected by EMG. The recordings reported

277 in Fig. 4 show the hyperemic events associated with one typical BSC and with a sustained
278 masticatory activity, clearly indicated by EMG. Brief spontaneous contractions occurred
279 occasionally during rest; they evoked a hyperemic response characterized by: peak amplitude = 382
280 $\pm 119\%$, time-to-peak = 1.8 ± 0.8 s and half-return time = 4.4 ± 1.5 (n=5) that was not statistically
281 different from the BSC response recorded in anesthetized animals. Details of the rapid hyperemia at
282 the onset of muscle activity are presented in Fig. 4a and b. In Fig. 4a it can be observed that the
283 latency of blood flow increase, with respect to the beginning of EMG activation, can be as low as
284 0.4 s. The recordings also clearly evidence the transient flow decrease or even flow reversal that
285 occurs during the contraction.

286 Hemodynamic response to mechanical stimuli were also evoked in awake rabbits and confirmed
287 that the transient hyperemia produced by BSC can be reproduced by AO and MC. A representative
288 recording is presented in Fig. 5 showing MaBF increases of comparable magnitude in the response
289 to BSC, AO and MC evoked in the same awake rabbit.

290

291 *Hyperemic responses in facial and ear arteries*

292 In order to ascertain the role of cutaneous circulation in mechanically-induced hyperemia the
293 occurrence of the myogenic response was specifically tested in the facial artery and in the central
294 ear artery, which supply a mostly- and exclusively-cutaneous vascular bed, respectively. In this
295 respect, note that occlusion of the facial artery is performed distally to the branching point of the
296 masseteric artery (as indicated by the open triangle in Fig. 1) which means that the mechanical
297 stimulus did not affect the vascular areas of the masseter and pterygoid muscles. Basal blood flow
298 ranged, in different animals and experimental conditions, between 4 and 12 ml/min in the facial
299 artery and between 0.5 and 8 ml/min in the ear artery. As shown in Fig. 6, the blood flow response
300 to 1-s artery occlusion was markedly smaller for the facial (peak amplitude: $83 \pm 18\%$) and for the
301 ear artery ($68 \pm 20\%$) as compared to the masseteric artery ($521 \pm 105\%$), in the same animals
302 ($p < 0.01$, n=5).

303

304 **Discussion:**

305 The rapid contraction-induced hyperemia has been investigated *in vivo*, in an experimental model
306 characterized by two innovative features: i) blood flow is recorded from an artery supplying
307 exclusively skeletal muscle tissue, which allows to quantify the real changes in muscle perfusion,
308 without the confounding action of possible changes in cutaneous circulation; ii) EMG activity is
309 simultaneously recorded from the relevant muscle, which allows to accurately analyze the time
310 relationship between the onset of motor activity and the hyperemic response.

311 For the first time, the hyperemia evoked by brief spontaneous muscle contractions was shown to
312 develop with a latency as low as 300-400 ms from the beginning of the EMG activation and to be
313 closely reproduced by brief occlusions of the artery supplying the muscle, as well as by other
314 mechanical stimuli. In addition, cutaneous vascular beds supplied by facial and ear arteries
315 exhibited a much lower mechano-sensitivity than the muscular vascular bed supplied the masseteric
316 artery.

317

318 *The rapid hyperemic response to brief spontaneous contractions*

319 The rapid increase in blood flow or arteriolar diameter in response to spontaneous or stimulated
320 contractions was investigated by several authors in both animal and human models (2, 22, 24, 31,
321 33, 41, 47). However, with a few exceptions in which skinned hind limbs or isolated muscle
322 preparations were investigated (e.g. Mohrman and Sparks (31)), in most studies blood flow was
323 measured from large arteries, supplying both cutaneous and muscular vascular beds. As a
324 consequence, the increase of muscle blood flow was “diluted” with unaffected cutaneous blood
325 flow, resulting in attenuated blood flow changes in the artery under study. In addition, the
326 previously employed experimental models did not allow to investigate the flow changes occurring
327 within the first second after the contraction and did not relate the hyperemic response to the onset of
328 EMG activity in the relevant muscle. This may be partly due to the fact that in many studies muscle

329 contraction was obtained by electrical stimulation (31-33, 47). In these studies the hyperemia was
330 reported to develop within 1 s from the end of the 1-s lasting stimulation, i.e., within 2 s from the
331 onset of muscle contraction. Human studies based on voluntary muscle contraction (1-s lasting
332 isometric handgrip) provided a similar indication (19, 24). In the present study, the possibility to
333 monitor blood flow in a purely muscular artery during stereotyped BSCs allowed to detect a sharp
334 hyperemic response exhibiting a 5-fold blood flow increase in less than 2 s and developing with a
335 delay as low as 300-400 ms from the onset of EMG activity and with no detectable latency from the
336 end of the contraction (Fig. 2C). Responses with similar magnitude and time course were observed
337 in awake animals (Fig. 5A), supporting the notion that vascular reactivity is not affected by the
338 anesthesia.

339

340 *Possible role of metabolic mechanisms in the response to brief spontaneous contractions.*

341 The possibility that metabolic signals could take part to the rapid dilatation at the onset of exercise
342 has long been discussed (7, 16, 45, 50). By administering different dilatory metabolites to isolated
343 skeletal muscle arterioles it was shown that at least 4 s are required to elicit dilatation (50). More
344 recently Armstrong et al. (2) suggested that potassium released by the excitation of muscle fibers
345 could play a major role in the early dilatory process, based on the observation that the dilatation
346 (evaluated at 4 s after muscle contraction) was markedly reduced by application of various blockers
347 of the K^+ signalling pathway. However, these data have been questioned (9, 23) on the basis of the
348 following two facts: i) some of the pharmacological interventions weakened the contraction of
349 skeletal muscle fibers, thus also attenuating the mechanical action exerted on blood vessels ii)
350 voltage-gated K^+ channels are also implicated in the myogenic response (15), thus their blockade
351 might have impaired the myogenic reactivity of the vascular network. Therefore, it is argued that
352 production and diffusion of vasodilatory metabolites cannot account for the rapid onset of
353 vasodilation with contractions (9), although this remains to be determined

354

355 Based on the present results several lines of evidence argue against a role of metabolic signals in the
356 response to BSC: i) the hyperemic response develops as early as 300-400 ms from the onset of
357 EMG activation, with a time-to-peak of 1.8 s, i.e. a time course that, on the basis of the available
358 knowledge is not compatible with the metabolic dilatation, ii) the hyperemic response to BSC is
359 replicated by mechanical stimuli, such as AO and MC, that do not activate metabolic pathways; iii)
360 the hyperemic response to MS was not prevented by blockade of the reflexly-induced muscle
361 contraction.

362

363 *Muscle pump and veno-arteriolar reflex*

364 Several studies have compared the hyperemia induced by a single contraction with that evoked by a
365 brief increase in external pressure (6, 24, 31). While this approach provided good evidence of a
366 mechanically-dependent hyperemia, it did not help in understanding the possible contribution by the
367 “muscle pump” mechanism (26, 45). In fact, both maneuvers squeeze and empty intramuscular
368 vessels thus reducing venous blood pressure and increasing perfusion pressure (muscle pump). In
369 addition the reduced venous pressure could stimulate a rapid dilatation through the venous-
370 arteriolar reflex (44). When external compression is exerted on an intact limb, these actions are also
371 exerted on cutaneous layers, as well as to muscles not involved in the contraction, which
372 complicates the comparison of the response with the contraction-induced hyperemia.

373 In the present study we showed, in both anesthetized and conscious animals, that the rapid
374 hyperaemia evoked by spontaneous contractions and external compressions is also reproduced by
375 short lasting AO. Occlusion of the masseteric artery exclusively affects the musculo-vascular bed
376 under investigation, with no involvement of the cutaneous layer and no activation of muscle pump
377 mechanisms. In particular, due to the absence of mechanical emptying of the venous compartment
378 and to the dampening effect of upstream vascular districts, the drop in venous pressure is expected
379 to be smaller during AO than during BSC and MC. This suggests that the involvement of the

380 muscle pump mechanism and of the veno-arteriolar reflex is not a necessary condition for the
381 development of a rapid hyperemia such as is produced by BSC and single short-lasting MC.

382

383 *Mechano-sensitivity of the vascular network*

384 What BSC, MC and AO have in common is that they all produce a transient transmural pressure
385 decrease in the vascular network of the muscle. However, while BSC and MC potentially activate
386 also other mechanisms, such as the release of dilatory metabolites and the muscle pump as
387 discussed above, the effects of AO are almost exclusively limited to the decrease in transmural
388 pressure in the vascular bed located down stream to the occlusion site. Thus, the observation that
389 the response to AO mimics the response to BSC and MC strongly suggests that the reduction in
390 transmural pressure is the relevant stimulus for the rapid hyperemia.

391 On the other hand, the same rapid hyperemic response was produced by MS in both paralyzed and
392 non-paralyzed muscles. Whether MS also resulted in increased intramuscular pressure and reduced
393 vessels' transmural pressure is not known, although it produced, like BSC, a slight decrease in
394 blood flow immediately prior to the hyperemia (Fig 3), indicating some compression of the vascular
395 network. This possibility is supported by observation of a slight increase in intramuscular pressure
396 of the quadriceps during passive flexion of the knee (34). In addition blood vessels may be
397 stretched/squeezed together with muscle fibers and these actions may be as effective as the decrease
398 in transmural pressure in inducing a dilatory response. Thus, it seems reasonable to hypothesize that
399 MS activates the same mechano-sensitive pathways as the other stimuli. This observation fits with
400 several reports of hyperemia induced by passive limb movement (34, 43, 48).

401

402 *Rapid dilatation and the myogenic response*

403 As early as in 1974, Mohrman & Sparks (31) put forward the hypothesis that the decrease in
404 transmural pressure occurring during a brief contraction could produce an active dilatation,
405 according to the myogenic response.

406 In fact, the myogenic response, also called Bayliss effect, is usually described as the
407 increase/decrease in vessel tone in response to the increase/decrease of transmural pressure (3, 15,
408 21, 52). It has been well characterized *in vitro*, in cannulated arterial segments subjected to
409 sustained step changes in transmural pressure (13, 15, 21), while the rapid response to short lasting
410 changes has been comparatively less investigated. This is surprising if we consider that the first
411 observation of the phenomenon by Sir William Bayliss was in fact a rapid blood flow increase in
412 the hind limbs in response to a brief occlusion of the abdominal aorta (3). The existence of a rapid
413 myogenic constriction (28, 29, 49) and dilatation (5, 38) has then been documented in different
414 preparations.

415 However, whether the rapid dilatation evoked by transmural pressure changes in muscle blood
416 vessels is exclusively myogenic in nature is still debated. In particular, in isolated muscle arterioles
417 it has been recently shown that endothelium-mediated dilatation can develop as early as 1-2 s from
418 a step increase in flow delivered in the absence of transmural pressure changes (4). This mechanism
419 may have contributed to the hyperemic responses described in the present study. In addition it has
420 been hypothesized that increased NO release and dilatation could also result from direct mechanical
421 deformation of the endothelium (14, 25). The rapid dilatation induced by external compression of
422 isolated muscle feed arteries was shown to be partly dependent on endothelium integrity (10). In
423 humans, blockade of NO-synthesis by l-NMMA markedly reduced the rapid hyperaemia elicited by
424 passive movements (43) while it did not substantially affect the hyperemic response to 1-s lasting
425 compressions of the forearm (6). In the present study the presence of an endothelium-mediated
426 component in the observed hyperemic responses was not investigated and cannot be excluded. For
427 this reason, the term “rapid *myogenic* response” may be inappropriate and the alternative notation
428 “Bayliss effect” is here preferred. Further studies are required to clarify what is the role played by
429 the endothelium.

430

431 In humans, Kirby et al. (24) evidenced a temporal dissociation between the hyperemic responses to
432 forearm compression and to muscle contraction, the former peaking earlier (1-2 cardiac cycles) than
433 the latter (4 cardiac cycles). On this basis they left the possibility open for other mechanisms to
434 contribute to the contraction-induced rapid dilatation (24). Our results did not evidence the same
435 temporal dissociation: the responses to all types of stimuli employed peaked between 1.8 and 2.4 s
436 (Fig. 3) and were not significantly different; however, many factors may justify small differences
437 between the responses. In particular, in their study, blood flow measurement included adipose and
438 cutaneous blood flow, although the influence of the latter was minimized by skin cooling. It is
439 likely that this might have differently affected the response to spontaneous contraction and to cuff
440 compression of the forearm, also considering the potentially different vascular mechano-sensitivity
441 of the different tissues, as observed in the present study for skin and muscle blood flow.

442

443 *Muscle vs. cutaneous vascular reactivity*

444 The myogenic response is known to be differently expressed by vessels belonging to different
445 tissues and organs (21, 30), as well as by the different segments of the same vascular bed (13, 17).
446 In this respect, a prominent myogenic reactivity is described for arterial and arteriolar segments of
447 brain, skeletal muscle (5) and kidney while no explicit reference is usually made to skin blood
448 vessels (30, 39, 40). To our knowledge, only few studies described the occurrence of a *slow*
449 myogenic response in cutaneous vascular beds (13, 20, 42) developing in 1-2 min upon sustained
450 changes in transmural pressure, while the *rapid* component has never been investigated.
451 In the present study both the exclusively cutaneous ear artery and the mostly cutaneous facial artery
452 showed only a slight blood flow response to AO (Fig. 6). It is tempting to speculate that a high
453 mechano-sensitivity is a prominent characteristics of muscular vessels that is not shared by
454 cutaneous vessels. Alternatively, it could partly be attributed to a greater dilatory reserve of resting
455 muscle, as compared to cutaneous tissues. Irrespective of the underlying mechanisms, the cutaneous

456 vascular bed exhibited a much lower response to mechanical stimulation, as compared to the
457 muscular bed, in physiological conditions.

458

459 *Concluding remarks*

460 In summary, several lines of evidence were gathered indicating that a mechano-sensitive dilatation
461 responding to changes in transmural pressure mediates the rapid hyperemia at the onset of exercise:
462 i) the contraction-induced hyperemia occurs as early as 300-400 ms from the onset of EMG, which
463 makes highly unlikely a role of diffusible metabolic signals; ii) contraction-induced hyperemia is
464 qualitatively and quantitatively mimicked by the response to mechanical stimuli that do not affect
465 metabolic activity, i.e., AO, MC and MS in paralysed muscles; and do not involve the muscle pump
466 nor the veno-arteriolar reflex, i.e., AO. Thus, the rapid hyperemic responses to these different
467 stimuli are all consistent with the Bayliss effect, while the relative contribution of myogenic and
468 endothelial mechanisms remains to be ascertained.

469 These rapid hyperemic events, observed in the rabbit masseter muscle, are strikingly similar to
470 those reported in response to artery occlusion, muscle compression, electrically-evoked and
471 spontaneous contractions in different muscle groups and animal species (6, 8, 10, 18, 24, 33, 38,
472 47). This indicates that such rapid dilatation is a characteristic of the musculo-vascular network that
473 is highly preserved across different species and that the masseteric artery of the rabbit may be a
474 good model for its investigation.

475 The preliminary observation that the rapid dilatation is only expressed by muscular and not by
476 cutaneous vascular networks supports the hypothesis that it is meant to subserve a feedforward
477 control of muscle blood flow (9). Based on this mechanism, a prompt increase in blood supply is
478 granted to the muscle at the very onset of exercise, before slower metabolic feedback mechanisms
479 accurately match perfusion to the actual metabolic demand.

480

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487

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492

493 **Author contributions**

494 Conception and design of the experiments: SR.

495 Collection, analysis and interpretation of data: MT and MM.

496 Drafting the article or revising it critically for important intellectual content: MT and SR.

497

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624

625

626

627 **Figure legends**

628

629 Fig 1: Anatomical scheme illustrating the masseteric branch of the facial artery and the location of
630 the perivascular blood flow probe. The different mechanical maneuvers used in this work are
631 indicated by dashed arrows: mandibular stretch (MS), muscle compression (MC) and artery
632 occlusion (AO). The closed and open triangles indicate the point where the masseteric and the facial
633 artery, respectively, are occluded.

634

635 FIG 2: Masseter artery blood flow (MaBF, grey line) and EMG activity of the masseter muscle
636 (EMG, black line) are shown during brief spontaneous contractions (BSC) of the masseter muscle
637 in anesthetized animals. A) original recordings of a single BSC; B) average curves (n = 4 animals);
638 C) time zoom of the fast transients in part B: note the short latency between the rapid dilatation and
639 the EMG burst, indicated by vertical dashed lines.

640 In B and C, the EMG signal was rectified and low pass filtered (moving average width: 20 ms),
641 MaBF was low-pass filtered (cut-off frequency: 13 Hz) before averaging. The trigger signal used for
642 the average was based on the positive peak of the EMG signal. In C the thin grey line represents the
643 average curve obtained from unfiltered blood flow tracings. This overlaps the filtered MaBF (thick
644 grey line), indicating that the filtering process did not introduce time shifts.

645

646 FIG 3: Masseteric artery blood flow (MaBF) responses to different mechanical stimuli are
647 superimposed on the response to brief spontaneous contractions (BSC) reproduced from Fig. 2.
648 In A) Normalized responses expressed as % of control (pre-stimulus) value. B) Non normalized
649 responses. AO= artery occlusion; MC= muscle compression; MS= muscle stretch; (n= 8 animals).

650

651

652 FIG 4: Simultaneous recording of blood flow from the masseteric artery (MaBF) and electro-
653 myographic (EMG) activity of the masseter muscle in a conscious rabbit. Large increase in MaBF
654 can be observed both during one brief spontaneous contraction (BSC) and during mastication of a
655 carrot, as indicated. The phase of rapid increase in blood flow is magnified in insets a) and b)
656 respectively. Note the short latency, below 400 ms, between the EMG activation and the increase in
657 blood flow, preceded by a slight decrease (inset a).

658

659

660 FIG 5: Hyperemic responses evoked in an awake rabbit. A) brief spontaneous contraction; B)
661 occlusion of the masseteric artery and C) compression of the masseter muscle.

662

663 FIG.6. Comparison of the myogenic responses of different vascular beds evoked by brief occlusion
664 of the relevant arteries. Facial artery blood flow (FaBF, light grey) , masseteric artery blood flow
665 (MaBF, black) and ear artery blood flow (EaBF, dark grey). Occlusion of the facial artery was
666 performed distally to the origin of the masseteric branch (see Fig. 1, open triangle). Note the little
667 responsiveness of blood flow to occlusion of the facial and ear arteries. Each curve is the average of
668 responses collected from 5 animals)

669

670

Fig. 1

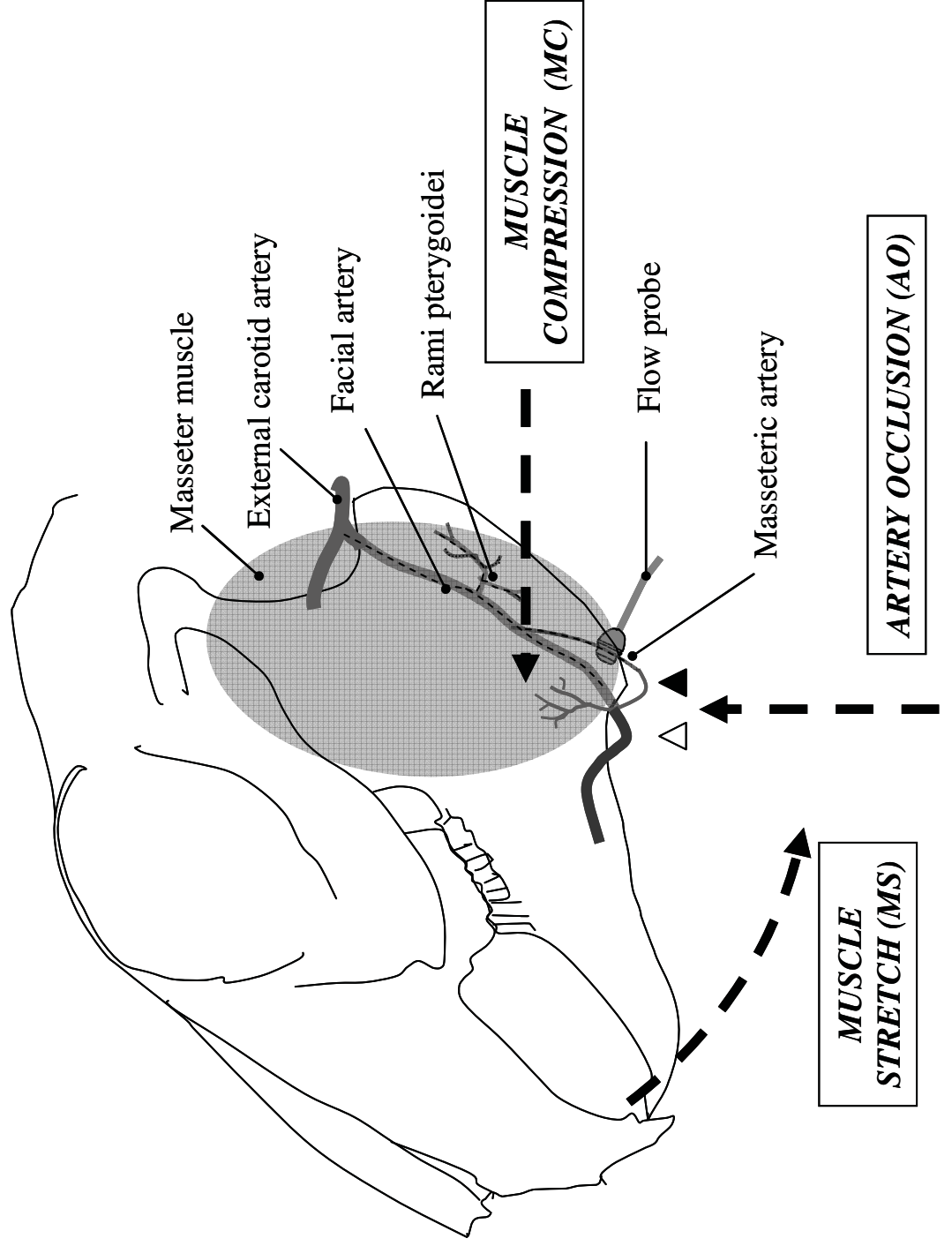


Fig. 2

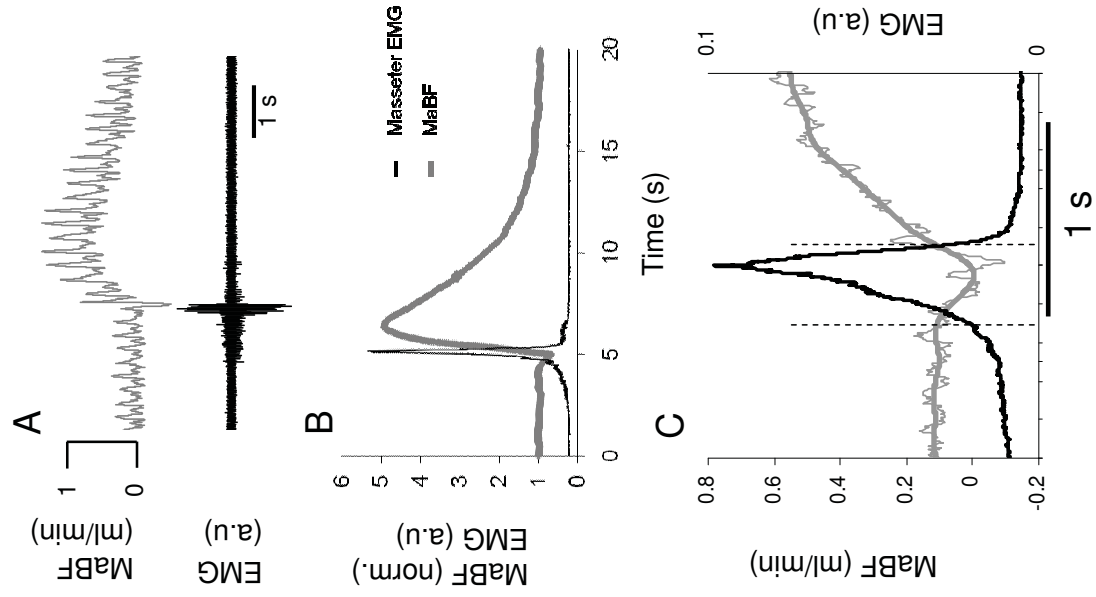


Fig. 3

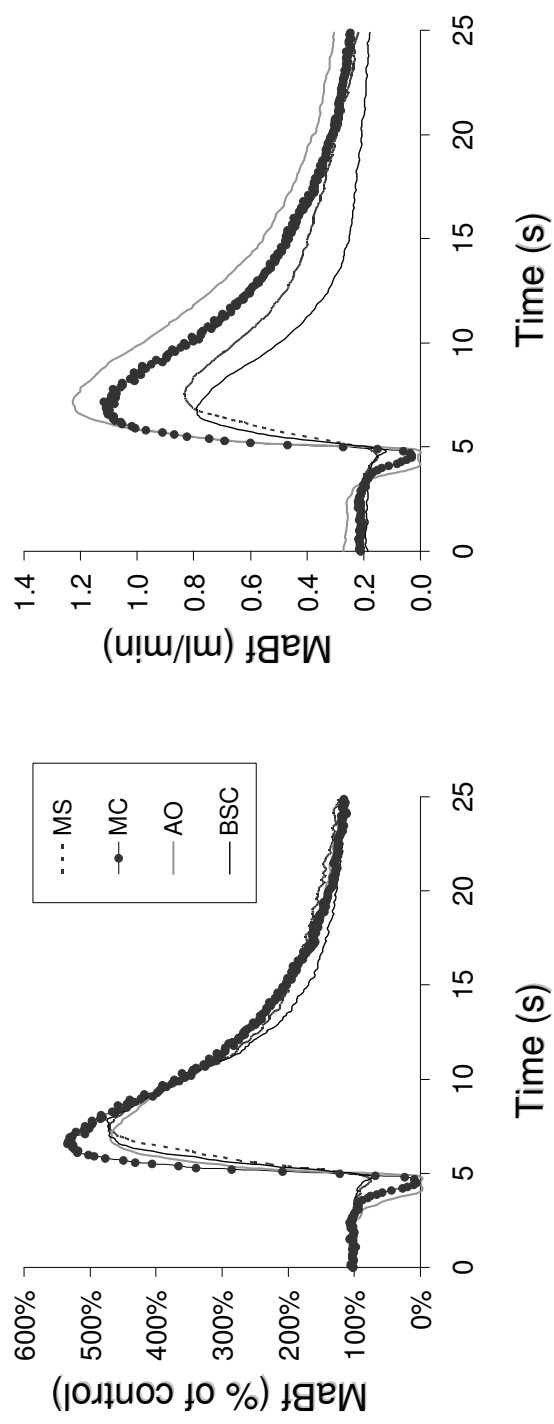


Fig. 4

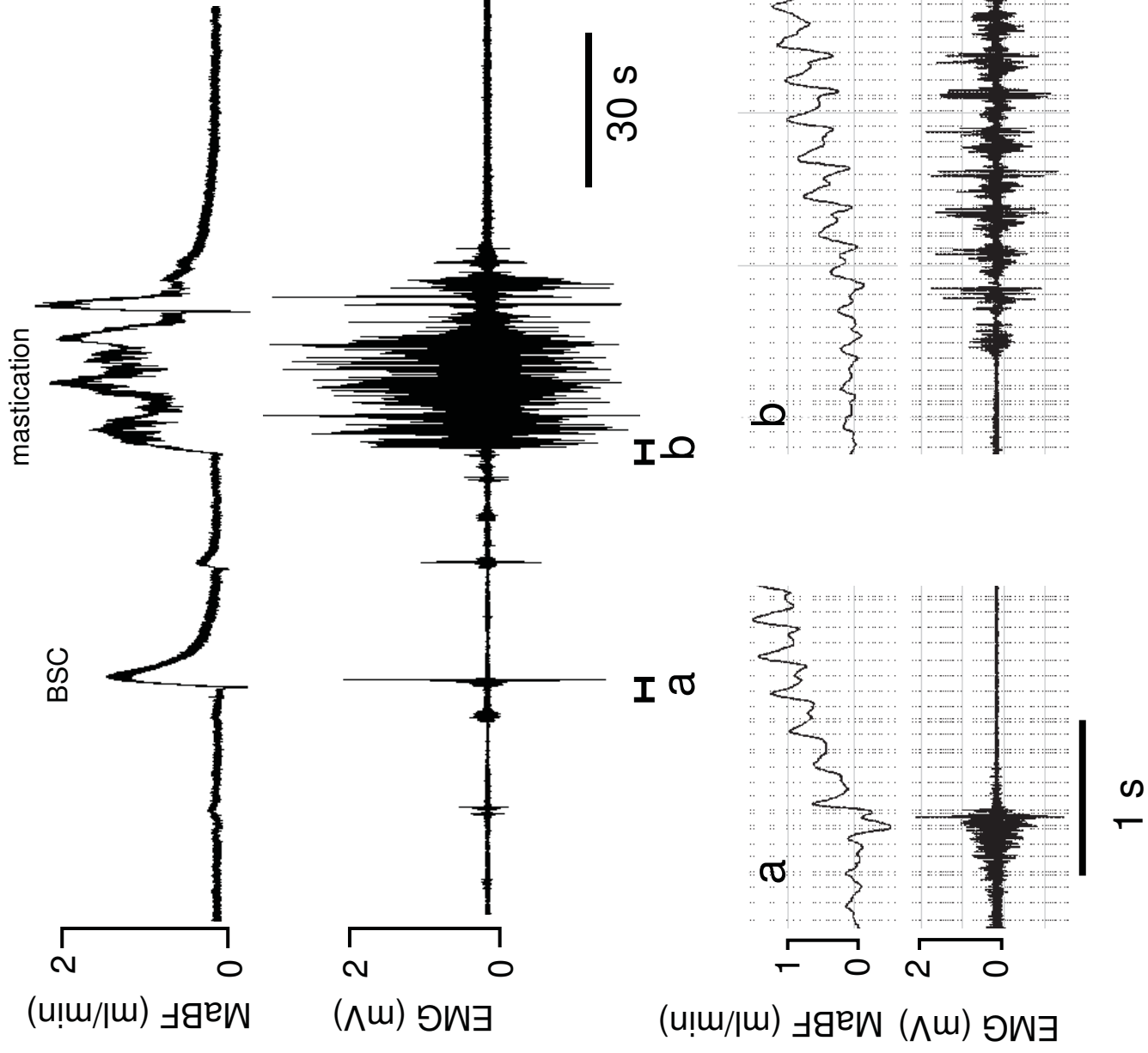


Fig. 5

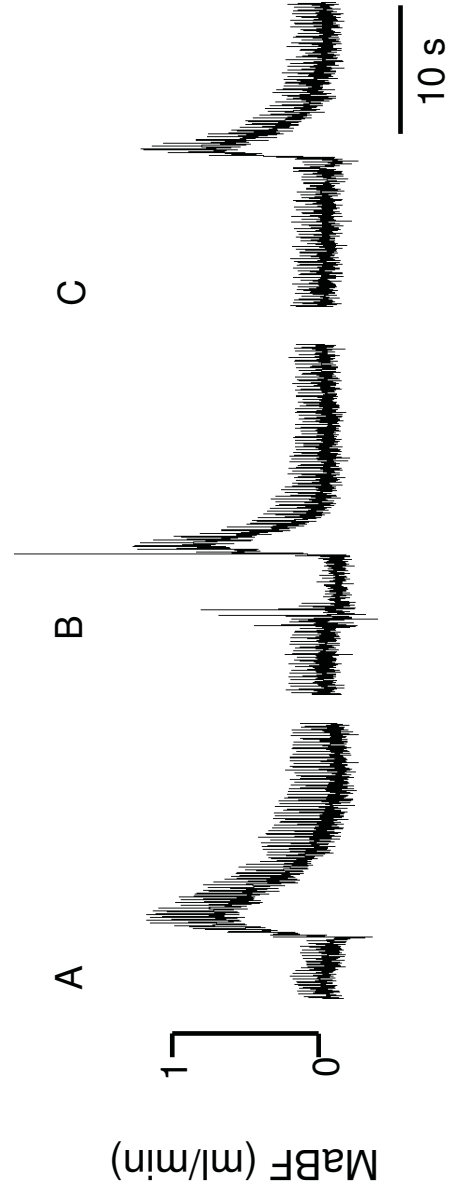


Fig. 6

