
Research Article: Confirmation | Integrative Systems

Resetting the respiratory rhythm with a spinal central pattern generator

Roberto Meza¹, Nayeli Huidobro¹, Mayra Moreno¹, Abraham Mendez¹, Jorge Flores-Hernández¹, Amira Flores¹ and Elias Manjarrez¹

¹Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla, Puebla, México

<https://doi.org/10.1523/ENEURO.0116-19.2019>

Received: 26 March 2019

Accepted: 26 March 2019

Published: 10 April 2019

Author contributions: R.M., N.H., M.M., A.M., and E.M. performed research; R.M., N.H., M.M., A.M., J.F.-H., A.F., and E.M. analyzed data; R.M., N.H., and E.M. wrote the paper; N.H. and E.M. designed research; J.F.-H., A.F., and E.M. contributed unpublished reagents/analytic tools.

Funding: Consejo Nacional de Ciencia y Tecnología (CONACYT) 253164 ;

Funding: Fundación Marcos Moshinsky (Marcos Moshinsky Foundation) EM

Conflict of Interest: Authors report no conflict of interest.

The following grants supported this research: CONACYT Fronteras de la Ciencia #536 (E.M), Cátedra Moshinsky (E.M), CONACYT Ciencia Básica 253164, F1-62610 (E.M), CONACYT 229866 (E.M), and VIEP-PIFI-FOMES-PROMEP-BUAP-Puebla (E.M), VIEP-BUAP

Correspondence should be addressed to Elias Manjarrez, eliasmanjarrez@gmail.com.

Cite as: eNeuro 2019; 10.1523/ENEURO.0116-19.2019

Alerts: Sign up at www.eneuro.org/alerts to receive customized email alerts when the fully formatted version of this article is published.

Accepted manuscripts are peer-reviewed but have not been through the copyediting, formatting, or proofreading process.

Copyright © 2019 Meza et al.

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

Resetting the respiratory rhythm with a spinal central pattern generator

Abbreviated title: Effects of scratching on the respiratory rhythm

Roberto Meza, Nayeli Huidobro, Mayra Moreno, Abraham Mendez, Jorge Flores-Hernández, Amira Flores, Elias Manjarrez*
Instituto de Fisiología
Benemérita Universidad Autónoma de Puebla.
Puebla, México.

Author contributions: EM and NH designed research, analyzed data and wrote the paper. MM, AM, JF and AF analyzed data, EM, RM, NH, MM and AM performed research.

Correspondence should be addressed to:

Corresponding Author:

Prof. Dr. Elias Manjarrez
Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla.
14 sur 6301, Col. San Manuel A.P. 406, C.P. 72570
Puebla, Pue., México
Tels.: +5222-22-29-5500 Ext 7326, Fax: +5222-22-33-4511
eliasmanjarrez@gmail.com

Number of Figures: 5

Number of Tables: 0

Number of Multimedia: 0

Number of words for Abstract: 165

Number of words for Significance Statement: 56

Number of words for Introduction: 704

Number of words for Discussion: 1061

Acknowledgements:

The following grants supported this research: CONACyT Fronteras de la Ciencia #536 (E.M), Cátedra Moshinsky (E.M), CONACyT Ciencia Básica 253164, F1-62610 (E.M), CONACyT 229866 (E.M), and VIEP-PIFI-FOMES-PROMEPE-BUAP-Puebla (E.M), VIEP-BUAP

Conflict of interest: No (Authors report no conflict of interest)

47 Abstract

48 There is evidence that a variety of central and afferent stimuli, including
49 swallowing, can produce phase-resetting in the respiratory rhythmicity. Also, there are
50 reports about the intrinsic linkage between locomotion and respiration. However, little is
51 known about the interaction between the central pattern generators (CPG) for scratching
52 and respiration. The present study aims to examine whether the activation of scratching-
53 CPG produces phase-resetting of the respiratory rhythm. We employed decerebrate cats to
54 apply brief tactile stimuli to the pinna during the inspiratory-expiratory transition. We
55 observed that those stimuli to the pinna not eliciting fictive scratching did not reset the
56 respiratory rhythm. However, when the pinna stimuli elicited fictive scratching, then the
57 respiratory rhythm exhibited a significant phase-resetting. We also found interneurons in
58 the medulla oblongata exhibiting phase resetting related to scratching-CPG episodes. This
59 second finding suggests that this type of resetting involves brainstem components of the
60 respiratory-CPG. These results shed new light on the resetting action from a spinal central
61 pattern generator on the respiratory rhythm.

62

63 Significant statement

64 Here we report for the first time the resetting influence of the spinal central pattern
65 generator for scratching on the respiratory rhythm. We conclude that fictive scratching, as a
66 “central stimulus” delivered to the respiratory-CPG, can produce phase-resetting in phrenic
67 nerve activity and the firing activity of interneurons from the medulla oblongata in
68 decerebrate paralyzed cats.

69

70

71 **Introduction**

72 Phase-resetting is the transient interruption of activity of an oscillator by a precisely
73 timed perturbation, which prevents the completion of a cycle and restarts it (Winfree,
74 2001). This phenomenon is widespread in the central nervous system, and there is evidence
75 that neurons interact through phase-resetting in order to establish a time frame for
76 information encoding and decoding (Canavier, 2015), among other behaviors. In particular,
77 the analysis of phase resetting is of interest in circuits known as CPG's because the
78 elucidation of their structure and organization is a work in progress and because the
79 cooperation between CPG's is poorly understood (Steuer and Guertin, 2019).

80 Studies performed on the respiratory-CPG have shown that the normal coordination
81 of different physiological functions involving the upper airways (as swallowing) involves
82 phase-resetting, as opposed to a mere interruption of the respiratory rhythm (Paydarfar et al.,
83 1995; McFarland and Lund, 1995; Paydarfar et al., 1986; Paydarfar and Eldridge, 1987,
84 1989, 1998). For instance, electrical stimulation of the midbrain reticular formation and
85 periaqueductal gray matter produces facilitation and a phase resetting of the respiratory
86 rhythm in cats (Paydarfar and Eldridge, 1987). Furthermore, stimulation of the nasal mucosa,
87 the carotid sinus and the radial nerve (Kawahara, 1988) can elicit a response on the
88 respiratory oscillator. Studies also exist correlating specific neuronal types in the
89 preBötzinger Complex and the perturbations in their cycles to specific behaviors like
90 vomiting in decerebrate dogs (Fukuda, 1997). On the other hand, several studies have
91 shown entrainment of the respiratory rhythm by activation of the locomotor-CPG or related
92 afferents both in vivo (Viala, 1983, 1987) and in vitro (LeGal, 2014; Kawahara, 1988) in
93 various animal models and humans (Bramble and Carrier, 1983).

94 In summary, there is evidence of a phase-resetting relationship between the
95 respiratory-CPG with other CPG's that coexist around the same gross anatomical regions,
96 as well as an entrainment relationship with the CPG for locomotion. However, to our
97 knowledge, there are no studies showing the resetting action of the scratching CPG on the
98 respiratory rhythm, even that there is a pioneering report illustrating the relationships
99 between the respiratory rhythm and the scratching-CPG (King, 1931). Our study aims to
100 show that the activation of a spinal CPG can reset the respiratory rhythmicity of the phrenic
101 nerve and single neurons from a respiratory brainstem center. The advantage of our
102 approach using the scratching CPG to reset the respiratory rhythm instead of the
103 locomotion CPG is that the scratching episodes are more easily produced than the
104 locomotion episodes, and just a brief mechanical stimulation to the pinna can be enough to
105 produce scratching.

106 Reasons to pursue the possibility of a physiological resetting interaction from the
107 scratching CPG and the respiration CPG include the fact that there is an activating system
108 for fictive scratching around the bulbar reticular formation (Tapia et al., 2013). The location
109 of this reticular activating system could suggest an overlap or interaction with relevant
110 structures for respiration, as the nucleus tractus solitarius, or the ventral respiratory column.
111 This would guarantee the adequate interaction between non-overlapping and functionally
112 distinct CPG's, necessary for the animal's safeguarding. In particular, here we examine
113 which type of resetting produces the scratching-CPG on the respiratory-CPG. Paydarfar et
114 al., 1986 and Paydarfar and Eldridge, 1987, described three types of reset: type 1, type 0
115 and the "ambiguous". The type 1, is a resetting in which the weak stimuli causes little phase
116 resetting, and the latency from stimulus to subsequent cycles (called the cophase) fall by
117 one cycle as the stimulus time (called the old phase) is advanced through the entire cycle.

118 The type 0, is a resetting in which a strong stimulus causes large perturbations of phrenic
119 nerve rhythm, and there is no net change in the cophase as the old phase increases one full
120 cycle. The ambiguous is a resetting in which a stimulus with moderate strength produces a
121 phase singularity, recorded as highly variable latencies to the next cycles for stimuli given
122 at a single old phase (i.e., there is unpredictable resetting with stimuli given only at a
123 specific old phase). The present study constitutes the first approach to identify whether
124 scratching, as a resetting “stimulus” delivered to the respiratory-CPG, can produce phase-
125 resetting in phrenic nerve activity or brainstem interneurons in decerebrate/paralyzed cats.

126 **Materials and Methods**

127 **Animal preparation**

128 We performed experiments in four adult cats (2.3–3.7 kg, two males and two
129 females). We followed similar experimental procedures as in previous reports from our
130 group (Cuellar et al., 2009, 2018; Perez et al., 2009; Tapia et al., 2013). During surgery, we
131 induced and maintained deep anesthesia with isoflurane at 2%. At the beginning of any
132 surgical procedure, we administered atropine (0.05 mg/kg) and dexamethasone (2 mg/kg).
133 The protocol was approved by the ethics committee (CICUAL-Proyecto-00489) from the
134 Benemérita Universidad Autónoma de Puebla. We followed the guidelines contained in the
135 National Institutes of Health Guide for the Care and Use of Laboratory Animals
136 (publication 85-23, revised in 1985) and the Mexican regulations (NOM-062-ZOO-1999).
137 We verified the level of anesthesia using the monitoring of the arterial blood pressure from
138 the carotid artery and by testing for the lack of withdrawal reflexes and muscle tone. We
139 administered a mix of bicarbonate (100 mm) and glucose (5%) solution at a rate of 5 ml/h
140 throughout the radial vein during all the experiment.

141 We dissected for recording the bilateral tibialis anterior (TA) and the medial
142 gastrocnemius (MG) nerves. The lumbosacral and cervical spinal segments were exposed,
143 and the dura mater was removed. We dissected the C5 root of the phrenic nerve (Eldridge
144 et al., 1989). After these surgical procedures, the animal was mounted on a stereotaxic
145 apparatus. Pools were formed with the skin around the exposed tissues and filled with
146 mineral oil (after placement of the electrodes) and maintained at a constant temperature
147 (37°C). The decerebration consisted of a mechanical precollicular–postmamillary
148 transection with the removal of both cortices and all tissue rostral to the transection. We
149 applied SURGICEL® Absorbable Hemostat on the exposed neural tissues, and the empty
150 cavity was filled with Agar-Agar. We discontinued the anesthesia 3 min after the
151 decerebration. We administered dextran and saline solutions as necessary to maintain blood
152 pressure between 80 and 120 mmHg. Then the animals were paralyzed with pancuronium
153 bromide (Pavulon; Organon) and artificially ventilated. At the end of the experiment, each
154 animal was euthanized with an overdose of pentobarbital. We applied d-tubocurarine
155 (0.1%) on the surface of the C1–C2 segments to induce fictive scratching after the
156 mechanical stimulation of the pinna (Feldberg and Fleischhauer, 1960). Scratching was
157 produced by brief (0.2 s; Tapia et al., 2013) mechanical stimulation of scratch reflex
158 receptive fields located in the left pinna.

159 **Recording**

160 We recorded electroneurograms with conventional hook electrodes from the distal
161 end of the left flexor TA, and extensor MG sectioned nerves, as well as the electrical
162 activity from the left C5 root of the phrenic nerve. An electret microphone was employed to
163 record the sounds during the respiration in the artificially ventilated cats. We employed AC

164 amplifiers Grass-P511 (Astromed) with a 0.05–30 kHz bandpass filter and the Digidata
165 system (Molecular Devices) with a sampling rate of 50 kHz.

166 In two experiments, we used quartz/platinum–tungsten fiber electrodes for the
167 multiunit neuronal activity (impedance 5–7 MOhms) with the Minimatrix system of five
168 channels (Thomas Recording). We obtained recordings of neurons from the medulla
169 oblongata and we performed a histological reconstruction of recording sites.

170 **Spike sorting analysis**

171 We used the unsupervised spike-sorting software “waveclus” developed by Quiroga
172 et al. (2004) to classify unitary spikes from the multiunit recordings. We obtained raster
173 displays of firing activity of medullary neurons following the respiratory rhythm, before,
174 during and after the scratching episodes.

175 **Descriptive statistical Analysis**

176 Correlations were tested with the nonparametric Spearman’s rank coefficient
177 method ($P < 0.001$).

178 **Random experimental paradigm**

179 We applied mechanical stimuli to the pinna randomly during the inspiration-
180 expiration period. Each scratching episode was preceded by four of five control breaths,
181 during which end-tidal P_{CO_2} , blood pressure, and neural respiratory output remained
182 constant. Following each fictive scratching episode, other additional 4-8 breaths were
183 recorded. The mechanical stimulus that produced fictive scratching was given at a time in
184 the respiratory cycle after the inspiratory phrenic burst (Figures 1A-B). We elicited fictive
185 scratching episodes at various random times in the respiratory cycle after the inspiratory
186 phrenic burst.

187 We obtained graphs to examine phase resetting as described by Winfree (2001) and
188 Paydarfar and Eldridge (1987). According to Winfree (1981), the “old phase”, is the phase
189 of the rhythm at which the stimulus begins” and the “new phase (or cophase)” is the phase
190 of the shifted rhythm extrapolated back to the moment the stimulus ends. We adapted these
191 concepts to our experimental paradigm. We defined the old phase as the phase of the
192 inspiratory rhythm at which the fictive scratching episode begins (Figure 1B). The cophase
193 was defined as the phase of the shifted inspiratory rhythm extrapolated back to the moment
194 the scratching episode ends (Figure 1B). Old phase and cophase are in cycle units, i.e. 1 is
195 the period of the control cycle before stimulation. Our definitions of old phase and cophase
196 are also consistent with Paydarfar et al. (1986), Paydarfar and Eldridge (1987) and Paydarfar
197 et al. (1998).

$$198 \quad \text{Old Phase} = (\text{Time at scratching onset} - b) / \text{Period} \quad (1)$$

$$199 \quad \text{Cophase } i = (c_i - \text{Time at scratching offset}) / \text{Period} \quad (2)$$

$$200 \quad \text{Period} = (b - a) / N \quad (3)$$

201 Where $i = 1$ to 4 is the number of phrenic nerve bursts after scratching and N is the
202 number of phrenic nerve bursts before scratching (see Figure 1A-B). We calculated up to
203 four successive cophases ($i = 1$ to 4) to compare our Figures 2C and 3B with the Figures in
204 the articles by Paydarfar et al. (1986), Paydarfar and Eldridge (1987) and Paydarfar et al.
205 (1998), who also used four cophases to illustrate the phase resetting.

206 These definitions of the old phase and cophase are consistent with the concepts
207 stated by Winfree (2001) and Paydarfar and Eldridge (1987). The phase transition graph
208 was obtained by constructing a plot of old phase versus the cophase (for review see
209 Canavier, 2015). The onset of inspiration was represented by the time when rectified, and
210 integrated phrenic nerve activity increased to a level that was twice the baseline noise level

211 (Paydarfar and Eldridge, 1987). The old phase and cophase values were expressed as a
212 normalized fraction of the average period of four or five control breaths before the onset of
213 the scratching episode. For example, in the case of the four control breaths illustrated in
214 Figure 2B, the period was calculated with the formula (3).

215 As a control, we also obtained graphs of cophase versus old phase for the respiratory
216 rhythmicity associated with mechanical stimulation to the pinna that was unable to produce
217 fictive scratching (Figure 1C).

218 We defined the old phase as the phase of the inspiratory rhythm at which the fictive
219 scratching episode begins (Figure 1B). The cophase was defined as the phase of the shifted
220 inspiratory rhythm extrapolated back to the moment the scratching episode ends (Figure
221 1B). Old phase and cophase are in cycle units, i.e. 1 is the period of the control cycle before
222 stimulation.

$$223 \quad \text{Old Phase} = (\text{Time at mechanical stimulus} - b) / \text{Period} \quad (4)$$

$$224 \quad \text{Cophase } i = (c_i - \text{Time mechanical stimulus}) / \text{Period} \quad (5)$$

$$225 \quad \text{Period} = (b-a) / N \quad (6)$$

226 Where $i = 1$ to 4 is the number of phrenic nerve bursts after “the mechanical
227 stimulus applied to the pinna” and N is the number of phrenic nerve bursts before “the
228 mechanical stimulus applied to the pinna” (see Figure 1A-B).

229 **Results**

230 We applied brief mechanical stimulation to the pinna to elicit fictive scratching
231 episodes. As illustrated in Figure 1A, we applied such mechanical stimuli during the
232 inspiration-expiration period. This allowed us to examine changes in the phase of
233 respiratory rhythmicity related to the occurrence of these scratching episodes. The
234 scratching episodes elicited by the brief mechanical stimulation lasted from about 10 to 25

235 seconds. In most of the cases (21 of 25 episodes), the phrenic nerve exhibited an increase of
236 firing activity associated with the occurrence of each scratching episode. Therefore we only
237 included in the analysis those scratching episodes associated with a phrenic nerve
238 excitation. In 21 of 21 episodes, the phrenic nerve excitation during the scratching episode
239 lasted the whole duration of scratching (Figure 1A).

240 For the phase analysis, we measured the timing of phrenic nerve activity relative to
241 the timing of onset and offset of fictive scratching. Figure 2A illustrates some of such
242 timing marks for an animal. The marks in black color indicate the beginning of each
243 rectified and integrated burst of phrenic-nerve activity, whereas the marks in magenta and
244 blue color indicate the onset and offset of fictive scratching, respectively. The triangles in
245 Figure 2 represent the expected periodic activity of the phrenic nerve, which was calculated
246 as the average of four or five periods of phrenic nerve activity before every scratching
247 episode. We can note in Figure 2A the clear phase-shift in phrenic nerve activity after the
248 extinction of every scratching episode (see horizontal red-arrows). This phase-shift
249 qualitatively indicated the occurrence of a resetting behavior in the phrenic nerve rhythm
250 associated with the occurrence of scratching episodes. Therefore, we proceeded with the
251 quantitative analysis of phase resetting using the formulas (1) to (3). The old normalized
252 phase was calculated as “(old phase)/period” and the normalized cophase as
253 “(cophase)/period”. In Figure 2C, we show a phase-transition graph of the “normalized old-
254 phase” versus “normalized cophase” for phrenic nerve activity resulting from the
255 occurrence of scratching episodes from four cats. This graph shows the occurrence of a
256 behavior with a tendency to type 0 resetting. Note how the gray shadows in Figure 2C
257 follow a horizontal tendency, besides the variability in the cophase values.

258 We also analyzed the effects of mechanical stimulation of the pinna (that did not
259 produce fictive scratching) on the phase of the respiratory rhythm for the same animals.
260 This type of analysis served as a control for our observations of phase resetting associated
261 with fictive scratching. Figure 2B shows that such mechanical stimulation to the pinna did
262 not produce a phase shift in the respiratory rhythmicity. Note that the expected period was
263 maintained after the mechanical stimulation. Therefore, we proceeded with the quantitative
264 analysis using the formulas (4) to (5). Figure 2E shows a phase transition graph of the
265 “normalized old phase” versus “normalized cophase” for the phrenic activity associated
266 with this type of mechanical stimulation. We found that there was not a phase resetting of
267 the respiratory rhythm for those mechanical stimuli to the pinna that did not produce fictive
268 scratching. Note that all the points in Figure 2E did not escape from the expected black
269 lines for a normal rhythm without phase shift. Figures 2D and 2F show averaged data from
270 Figures 2C and 2E, respectively.

271 To analyze in more detail the effects of fictive scratching episodes on the respiratory
272 rhythmicity we analyzed the unitary firing activity of neurons from the medulla oblongata
273 of two cats. We found 21 neurons with a rhythmic firing synchronized with the phrenic
274 nerve activity. These neurons changed their firing rhythmicity during the occurrence of the
275 scratching episodes. Figure 3A shows the simultaneous recording of four of these neurons
276 during a scratching episode. Note the synchrony between the rhythmic phrenic nerve
277 activity and the interneuron bursting before scratching. It is clear that during scratching
278 some neurons increase their firing activity becoming tonic, but other neurons decrease their
279 firing activity, or it becomes disorganized. When the fictive scratching episode subsides,
280 the periodic burst of activity of the neurons is recovered but with a phase shift.

281 In Figure 3B, we show a phase-transition graph of the “normalized old phase”
282 versus “normalized cophase” for the interneuronal activity resulting from the occurrence of
283 scratching episodes from two cats. The methods to obtain this graph were similar to those
284 followed for the phase transitions graphs for the phrenic nerve activity. We used similar
285 formulas as those described in (1) to (3) to calculate the “normalized old phase” and
286 “normalized cophase” for the neuronal firing. We found a clear phase shift for all the
287 neurons. Figure 3A shows in pink traces the phase shift for neuron 2. The graph in Figure
288 3B shows the occurrence of a behavior with a tendency from type 1 to type 0 resetting. This
289 finding indicates that in the medulla oblongata there are neurons exhibiting phase resetting
290 associated with the activation of the scratching-CPG. Figure 3C shows averaged data from
291 Figure 3B.

292 We analyzed in more detail the phrenic nerve activity during a more extended
293 period after the scratching episodes. We found that the activity of the phrenic nerve was not
294 synchronized with the respiration during 22.8 ± 2.7 seconds after the end of the scratching
295 episode. It means that after that time the phrenic nerve becomes synchronized again with
296 the artificial respiratory rhythm (see examples for three cats in Figure 4). In Figure 5 we
297 show the rectified and integrated signal from an electret microphone sensing the respiratory
298 sounds in three cats (red traces, inspiration upwards). Note that before the scratching onset,
299 the maximal peak of the rectified and integrated phrenic nerve activity is phase-
300 synchronized with the imposed respiratory rhythm (see green vertical lines)). Furthermore,
301 there is a clear phase shift in the rectified and integrated phrenic nerve activity after the
302 scratching offset (see how the maximal peak of the phrenic nerve activity is displaced on
303 the left, relative to the right green lines. However, approximately 22.8 ± 2.7 seconds after

304 the scratching offset the phrenic nerve activity becomes phase-synchronized again with the
305 artificial respiratory rhythm.

306 **Discussion**

307 We found that those stimuli to the pinna that did not elicit fictive scratching did not
308 reset the respiratory rhythm. However, when the pinna stimuli elicited fictive scratching,
309 then the respiratory rhythm exhibited a significant phase resetting. Our results support the
310 findings by Paydarfar et al., 1986; Paydarfar and Eldridge, 1987 and Eldridge et al., 1989
311 who demonstrated for the first time that a phase resetting exists for the neural oscillator that
312 controls breathing.

313 In 1931, King studied the effect of respiration on the scratch reflex in two dogs and
314 one cat. The scratch movements were registered using a pulley and lever system, whereas
315 the respiration was recorded by a pneumograph and tambour system, or by a compound
316 level and pulley system. He obtained two findings. First, there is a lowering of the
317 scratching threshold during inspiration; and second, the after-discharge may reappear
318 during inspiration for several respiratory cycles. These results were consistent with
319 previous observations from the same author, who reported that there is an inspiratory
320 augmentation of proprioceptive reflexes. He concluded that the scratch reflex is affected by
321 the respiratory activity, assuming that impulses from the respiratory center contribute to the
322 excitatory state in the spinal cord, which in turn is manifested by a lowering of the
323 threshold and by an increase in the number of active motoneurons. However, to our
324 knowledge, there are no reports in the literature examining the resetting effects of the
325 respiratory rhythm on the scratching CPG. In this context, our study is original and
326 contributes to revealing that there is a resetting in the interaction between the scratching
327 and respiratory CPGs.

328 Paydarfar et al. (1986) studied resetting patterns resulting from electrical stimuli of
329 graded intensity applied to the superior laryngeal nerve in anesthetized paralyzed cats. They
330 found results ranging from attenuated stimuli giving type 1 resetting to strong stimuli
331 resulting in type 0 resetting. Based on this finding, we can acknowledge that a limitation of
332 our study is the difficulty to control the intensity and duration of the scratching episodes.
333 Therefore, in our experiments, it is not possible to examine such transition from type 1 to
334 type 0 resetting. However, besides this limitation, our results strongly suggest that there is
335 phase resetting when the scratching-CPG interacts with the respiratory-CPG. The first
336 argument in support of this statement is that our graph showed in Figure 2C tends to type 0
337 resetting (although with high variability). Paydarfar et al., 1986; Paydarfar and Eldridge,
338 1987 and Eldridge et al., 1989 reported the type 0 resetting. A second argument is that
339 Paydarfar and Eldridge (1987) found that the electrical stimulation of the midbrain reticular
340 formation and periaqueductal gray matter can evoke facilitation of phrenic nerve activity in
341 the cat. This is consistent with our observation that the activation of the scratching-CPG,
342 which in turn is related to an activation of the bulbar reticular formation (Tapia et al.,
343 2013), also produces facilitation in the activity of the phrenic nerve (see Figure 1A).

344 On the other hand, the phase resetting has also been useful to advance in the
345 comprehension of the neural architecture of the CPG's for locomotion and scratching.
346 Lafreniere-Roula and McCrea (2005) used phase-resetting as a parameter of interaction
347 among both pattern generators to demonstrate that the same neural substrate is shared by
348 the pattern formation layer of the CPG's for both locomotion and scratching. Therefore, our
349 findings also allow us to suggest the existence of sharing neural substrates in the neural
350 architecture of the respiratory and scratching CPG's.

351 A necessary implication of our study is that the scratching-CPG can reset the
352 respiratory-CPG even that the cats were paralyzed, thus showing that the central interaction
353 between CPG's could be enough to allow phase resetting and entrainment. It means that the
354 muscle machinery during the "motor action" could not be required to guarantee phase
355 resetting in the interaction between both oscillators. Such reasoning is consistent with
356 several observations made in *in vivo* and *in vitro* preparations in the context of locomotion.
357 The first observation is that the respiration rate in cats can increase during fictive
358 locomotion despite the absence of muscular contraction or limb movements (Eldridge et al.,
359 1985, Le Gal et al., 2014). The second observation is that in the isolated neonatal rat
360 brainstem-spinal cord preparation the increase in respiratory rate observed during fictive
361 locomotion is associated with an increase in excitability of pre-inspiratory neurons of the
362 parafacial respiratory group (Le Gal et al., 2014). The third observation is that the
363 respiration rate increases following stimulation of the mesencephalic locomotor region in
364 an *in vitro* lamprey preparation, even in the absence of the spinal cord and caudal brainstem
365 (Garipey et al., 2012; Missaghi et al., 2016). The fourth observation is that the respiratory
366 drive in hindlimb motoneurons in decerebrate cats with neuromuscular blockade is
367 transmitted via elements of the locomotor central pattern generator (Wienecke et al., 2015).
368 This is also in line with the "central command hypothesis" stipulating that central neural
369 connections activate the respiratory centers during exercise to regulate a safe exertion by
370 the body (for review, Garipey et al., 2010).

371 Coupled microcircuits generate respiration, as the preBotzinger Complex (preBotC),
372 which is composed of a master-clock of excitatory rhythmogenic neurons and pattern-
373 formation networks of excitatory and inhibitory interneurons that in concert produce the
374 strong periodic drive for inspiration (for review, Del Negro et al., 2018). Because the

375 preBotC coordinates all phases of the breathing cycle and it is influenced by many inputs
376 including emotion and cognition (Del Negro et al., 2018) it is comprehensible that the
377 central pattern generator circuit for scratching also could produce an effect on it. Our
378 finding of phase resetting of interneurons from the medulla oblongata (near pre-BotC and
379 the ventral respiratory group rVRG) associated with the scratching episodes strongly
380 supports this possibility.

381 It is important to mention that our main finding is not to show the synchrony
382 between brainstem neurons and phrenic nerve activity. Our main finding is that in the
383 brainstem respiratory centers there are neurons exhibiting phase resetting associated with
384 the activation of the scratching CPG. This is a relevant contribution to the understanding of
385 the resetting action of the spinal CPG on the brainstem respiratory CPG.

386 We conclude that fictive scratching, as a “central stimulus” delivered to the
387 respiratory-CPG, can produce phase-resetting in phrenic nerve activity and the firing
388 activity of interneurons from the medulla oblongata in decerebrate/paralyzed cats.

389 **Figure legends**

390 **Figure 1.** Scheme of the experimental paradigm. The phrenic nerve activity was used to
391 examine the respiratory rhythm. **A.** A mechanical stimulus (timing indicated with a short
392 rectangle) applied to the pinna elicits a fictive scratching episode (timing indicated with a
393 large rectangle and the tibial anterior (TA) nerve activity). Such a stimulus was applied
394 time after the fourth phrenic nerve discharge. During the scratching episode, there is an
395 increase in the phrenic nerve activity (Note the increase in the rectified and integrated
396 phrenic nerve activity (PNA)). The periodic respiratory rhythm returns after the scratching
397 episode end. **B.** A zoom of the timing scheme in A illustrating how the old phase and
398 cophase were measured. The horizontal red arrows illustrate how the old phase and cophase

399 were measured. **C-D.** The same as A-B, but for those mechanical stimuli that did not
400 produce fictive scratching.

401 **Figure 2.** Phase analysis performed from recordings of the phrenic nerve activity. **A.** The
402 vertical black marks indicate the phrenic-nerve activity before, during, and after the
403 application of mechanical stimuli applied to the pinna able to produce fictive scratching.
404 The magenta and blue marks represent the onset and offset of the scratching episode. The
405 triangles represent the expected period for the respiratory rhythmicity. The horizontal red
406 arrows show the phase shift, which is the difference in phase in the respiratory rhythm as a
407 consequence of the occurrence of the fictive scratching episode. **B.** The same as A but for
408 those mechanical stimuli not producing fictive scratching. The green marks represent the
409 brief mechanical stimuli. **C.** Phase-transition graph, constructed by the relationship between
410 the normalized old-phase and cophase. Note the tendency of the points towards a horizontal
411 arrangement (i.e., type 0 resetting). Each color is related to each scratching episode that
412 produced a phase shift of the respiratory rhythm. **D.** Averaged data from Figure 2C. **E.** The
413 same as C but for those mechanical stimuli not producing fictive scratching. Each color
414 represents the trials in which the mechanical stimulation to the pinna was unable to change
415 the phase. Note that in this case there was not a phase resetting and all points are arranged
416 on the black lines following the expected period. **F.** Averaged data from Figure 2E. The
417 blue lines in Figures 2D and F represent the linear regression. The colors in Figures 2A and
418 B are not related to the colors in the other graphs. The diagonal black lines (cophase = 1 –
419 old phase) depicts the ideal case in which there is not a phase shift produced by the stimuli.
420 A similar description of these diagonal lines was given by Winfree (1981).

421 **Figure 3.** Phase analysis performed from the unitary neuronal activity recorded from the
422 medulla oblongata. **A.** The same format as Figure 1A but for the firing activity of four

423 medulla oblongata neurons recorded simultaneously with the phrenic nerve (PNA). Pink
424 triangles indicate the expected period for the bursting firing of neuron 2. **B.** Phase-transition
425 graph, constructed by the relationship between the “normalized old-phase” and “normalized
426 cophase” for the rhythmical firing activity of 21 interneurons from the medulla oblongata,
427 as a consequence of the occurrence of fictive scratching episodes. Each color in Figure 3B
428 is related to each interneuron that produced a phase shift of the respiratory rhythm. In
429 Figure 3D, each color represents the location of each recorded interneuron. Note the
430 tendency of the points towards a horizontal arrangement (i.e., type 0 resetting). The
431 diagonal black lines (cophase = 1 – old phase) depicts the ideal case in which there is not a
432 phase shift produced by the stimuli. **C.** Averaged data from Figure 3B. The blue line in
433 Figure 3C represents the linear regression. **D.** Schematic drawing of recording sites of
434 rhythmical neurons from the medulla oblongata.

435 **Figure 4.** The timing of the phrenic nerve activity before and after scratching episodes in
436 three cats. The black vertical lines illustrate the phase shift in the phrenic nerve activity
437 after the scratching offset and its return to the expected rhythm. Such return last 29.6 s, 21.5
438 s and 21.32 s for these animals.

439 **Figure 5.** The timing of phrenic nerve activity and respiratory rhythm imposed by artificial
440 ventilation in three cats as indicated. First trace, phrenic nerve activity (PNA). The second
441 trace, rectified and integrated phrenic nerve activity. Third trace. Rectified and integrated
442 sound signals recorded with an electret microphone (red traces). Fourth trace, sound signals
443 (Microph. activity; i.e., respiratory activity recorded with an electret microphone). The
444 vertical green lines illustrate the timing between the inspiratory activity and the maximal
445 peak of the rectified and integrated phrenic nerve activity. The beginning of the scratching
446 episode is indicated with the pink line (scratching onset). The end of the scratching episode

447 is illustrated with the blue line (scratching offset). There is the same time separation
448 between the vertical green lines before and after the scratching episode.

449 **References**

450 Canavier CC (2015) Phase-resetting as a tool of information transmission. *Current Opinion*
451 *in Neurobiol.* 31: 206-213.

452 Cuellar CA, Tapia JA, Juárez V, Quevedo J, Linares P, Martínez L, Manjarrez E (2009)
453 Propagation of sinusoidal electrical waves along the spinal cord during a fictive motor task.
454 *J Neurosci.* 29: 798-810.

455 Cuellar CA, De La Torre Valdovinos B, Huidobro N, Delgado-Lezama R, Ornelas-
456 Kobayashi R, Manjarrez E (2018) The Spinal Neurons Exhibit an ON-OFF and OFF-ON
457 Firing Activity Around the Onset of Fictive Scratching Episodes in the Cat. *Front Cell*
458 *Neurosci.* 12: 68.

459 Del Negro CA, Funk GD, Feldman JL (2018) Breathing matters. *Nature Rev Neurosci* 19:
460 351-367.

461 Eldridge FL, Millhorn DE, Kiley JP, Waldrop TG (1985) Stimulation by central command
462 of locomotion, respiration and circulation during exercise. *Respir Physiol* 59: 313–337.

463 Eldridge FL1, Paydarfar D, Wagner PG, Dowell RT (1989) Phase resetting of respiratory
464 rhythm: Effect of changing respiratory drive. *Am J Physiol* 257: R271-R277

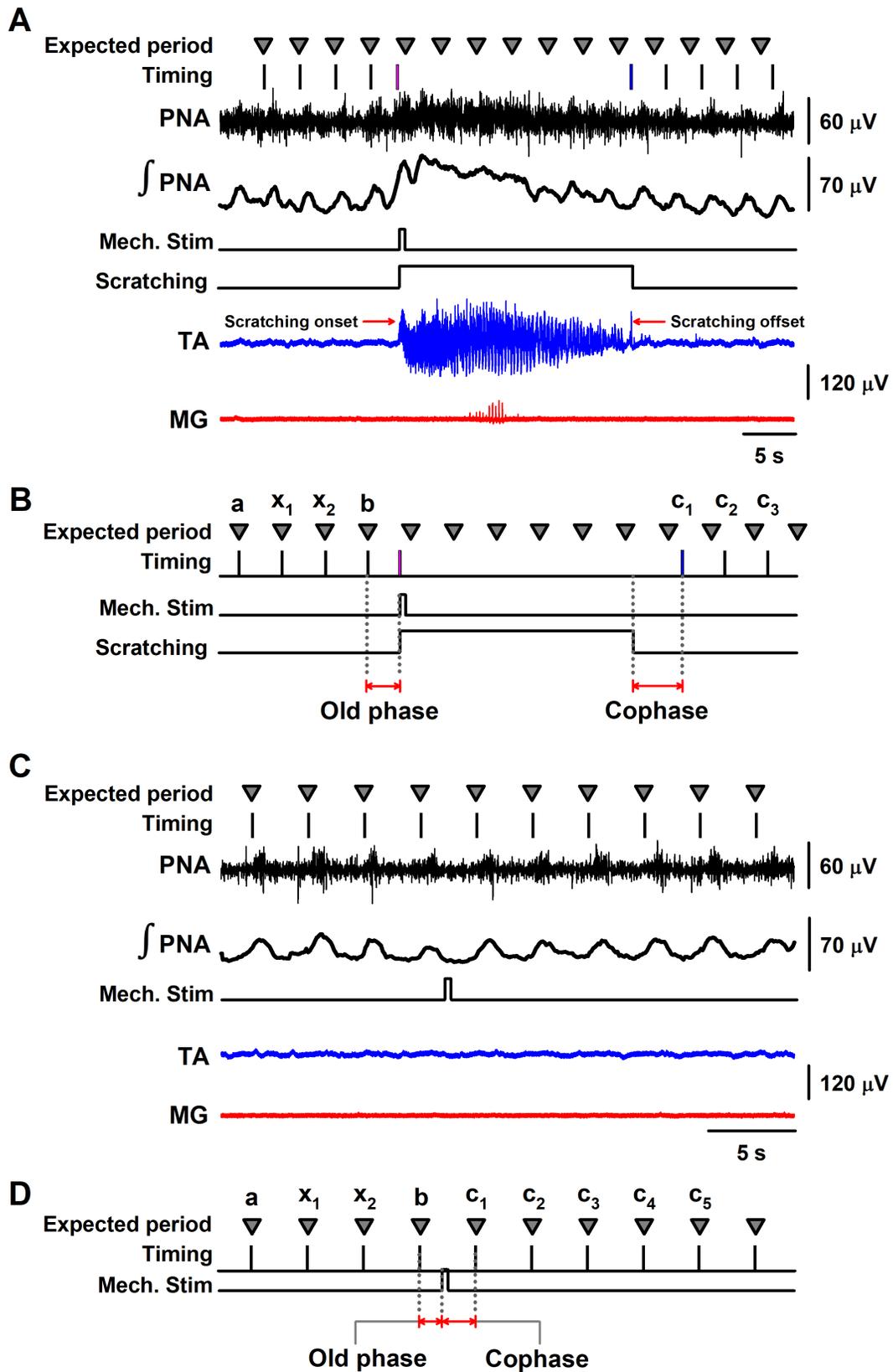
465 Fukuda H and Tomoshige K (1997) Most inspiratory neurons in the pre-Bötzinger complex
466 are suppressed during vomiting in dogs. *Brain Research* 763: 30-38.

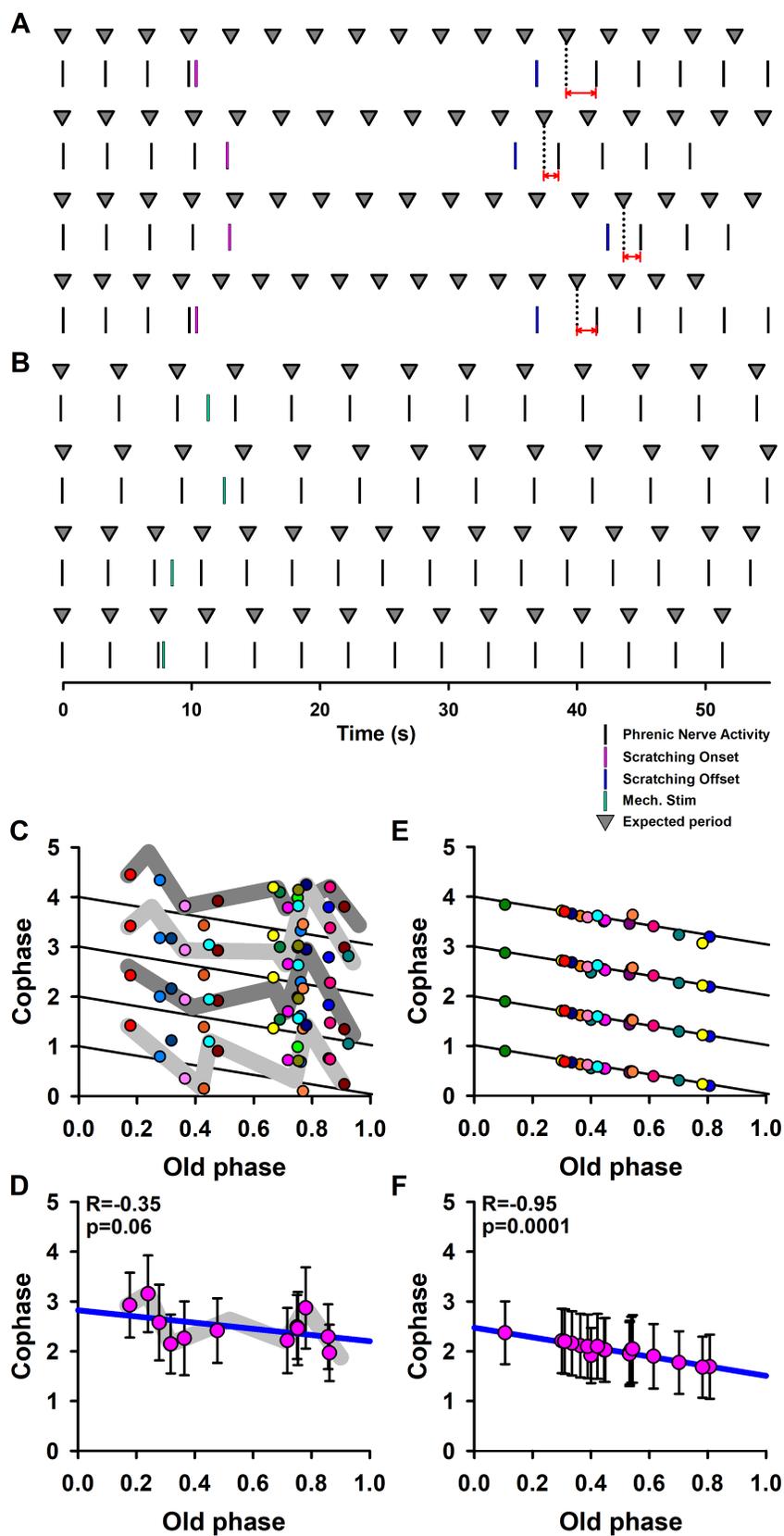
467 Feldberg W, Fleischhauer K (1960) Scratching movements evoked by drugs applied to the
468 upper cervical cord. *J. Physiol.* 151: 502–517.

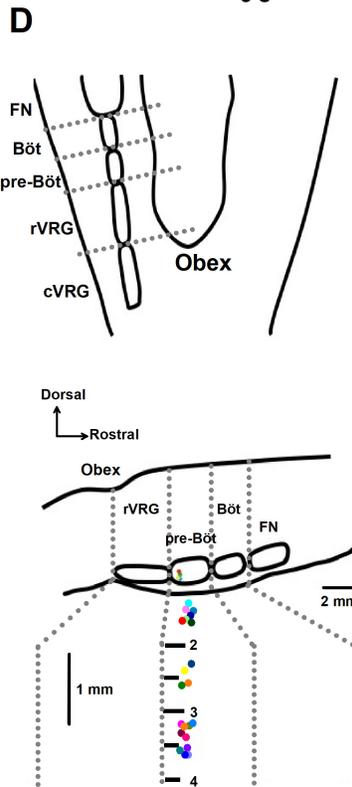
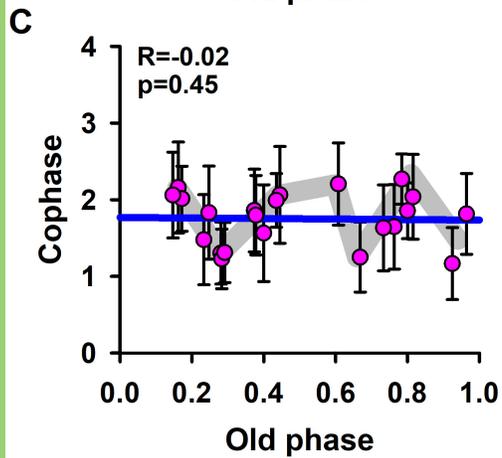
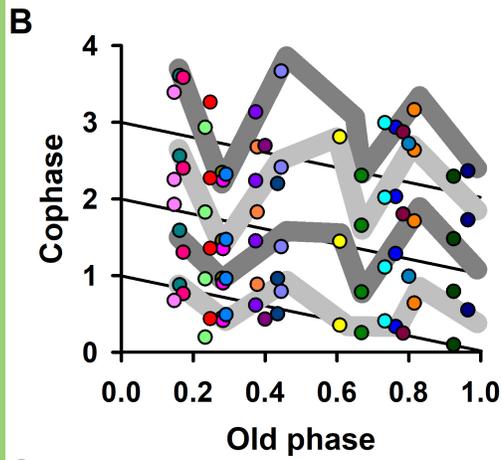
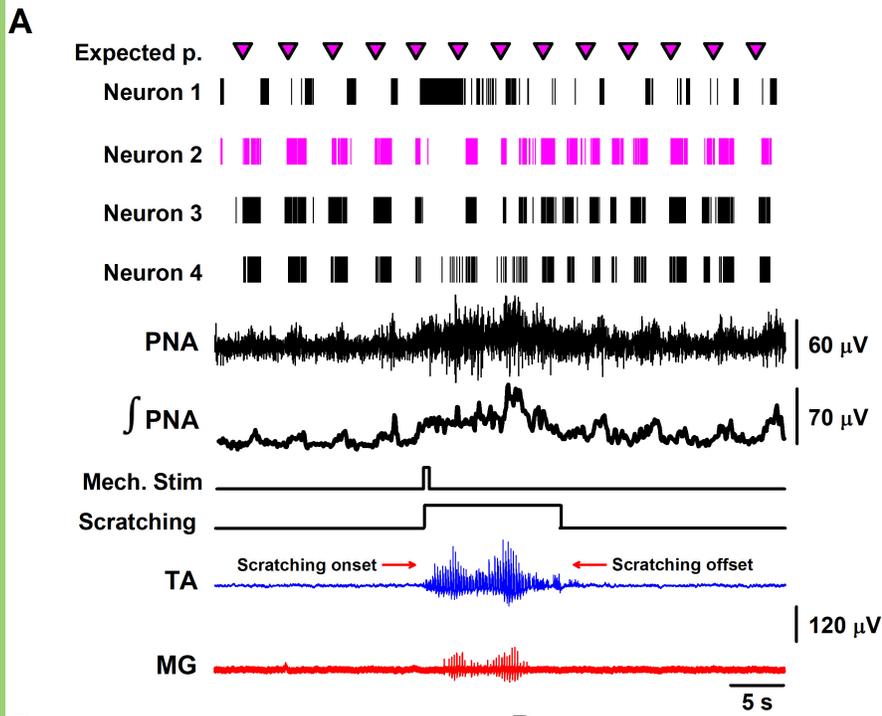
469 Gariépy JF, Missaghi K, Dubuc R (2010) The interactions between locomotion and
470 respiration. *Progress in Brain Research* 187: 173-188.

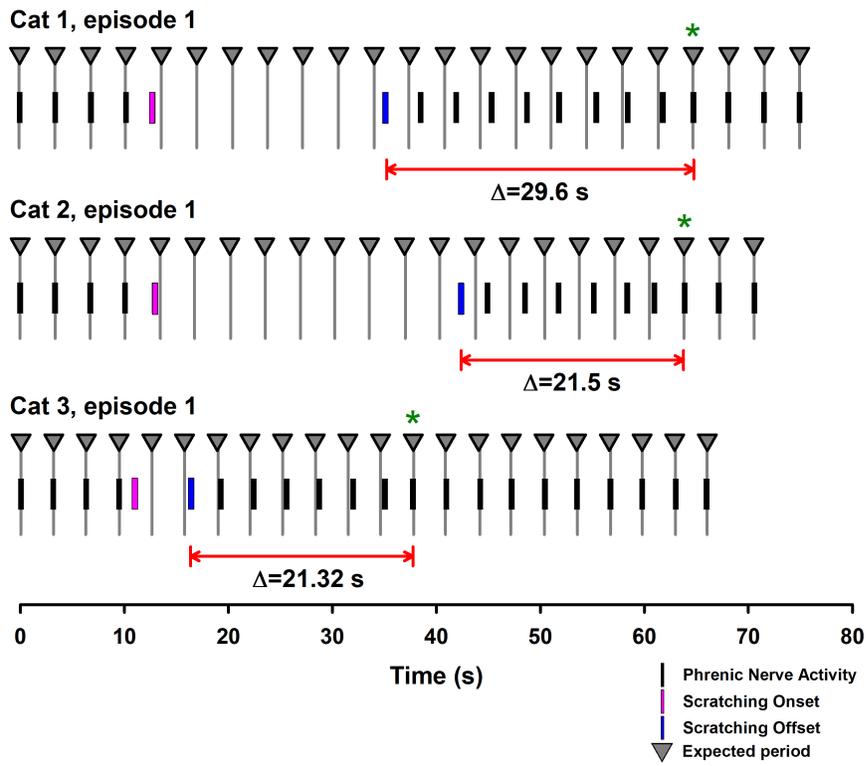
- 471 Gariépy JF, Missaghi K, Chevallier S, Chartre S, Robert M, Auclair F, Lund JP, Dubuc R
472 (2012) Specific neural substrate linking respiration to locomotion. *PNAS* 109: E84-E92.
- 473 Kawahara K1, Kumagai S, Nakazono Y, Miyamoto Y (1988) Analysis of entrainment of
474 respiratory rhythm by somatic afferent stimulation in cats using phase response curves. *Biol*
475 *Cybern* 58: 235-242
- 476 Lafreniere-Roula M and McCrea D (2005) Deletions of rhythmic motoneuron activity
477 during fictive locomotion and scratch provide clues to the organization of the mammalian
478 central pattern generator. *J Neurophysiol* 94:1120-1132
- 479 Le Gal JP, Juvin L, Caroit L, Thoby-Brisson M, Morin D (2014) Remote control of
480 respiratory neural network by spinal locomotor generators. *PLoS ONE* 9(2).
- 481 McFarland DH, Lund JP (1995) Modification of mastication and respiration during
482 swallowing in the adult human. *J Neurophysiol* 74: 1509-1517.
- 483 Mellen NM1, Janczewski WA, Bocchiaro CM, Feldman JL (2003) Opioid induced quantal
484 slowing reveals dual networks for respiratory rhythm generation. *Neuron* 37: 821-826.
- 485 Missaggi K, Le Gal JP, Gray PA, Dubuc R (2016) The neural control of respiration in
486 lampreys. *Respiratory physiology and Neurology* 203: 14-25.
- 487 Paydarfar D, Eldridge FL, Kiley JP (1986) Resetting of mammalian respiratory rhythm:
488 existence of a phase singularity. *Am J Physiol* 250: 721-727
- 489 Paydarfar D and Eldridge F (1987) Phase resetting and dysrhythmic responses of the
490 respiratory oscillator. *Am J Physiol* 252: R55-R62.
- 491 Paydarfar D, Gilbert RJ, Poppel CS, Nassab PF (1995) Respiratory phase resetting and air-
492 flow changes induced by swallowing in humans. *Journal of Physiology (London)* 483: 273-
493 288.

- 494 Paydarfar D1, Eldridge FL, Paydarfar JA (1998) Phase resetting of the respiratory oscillator
495 by carotid sinus nerve stimulation in cats. *Journal of Physiology* 506(2): 515-528.
- 496 Pérez T, Tapia JA, Mirasso CR, García-Ojalvo J, Quevedo J, Cuellar CA, Manjarrez E
497 (2009) An intersegmental neuronal architecture for spinal wave propagation under
498 deletions. *J Neurosci.* 29:10254-10263.
- 499 Tapia JA, Trejo A, Linares P, Alva JM, Kristeva R, Manjarrez E (2013) Reticular
500 activating system of a central pattern generator: premovement electrical potentials. *Physiol*
501 *Rep.* 1(5): e00129.
- 502 Steuer I, Guertin PA (2019) Central pattern generators in the brainstem and spinal cord: an
503 overview of basic principles, similarities and differences. *Reviews in the Neurosciences* 30:
504 107-164.
- 505 Viala D and Fretton E (1983) Evidence for respiratory and locomotor pattern generators in
506 the rabbit cervico-thoracic cord and their interactions. *Exp Brain Res* 49: 247-256
- 507 Viala D, Viala G, Persegol L, Palisses R (1987) Changeover From Alternate to
508 Synchronous Bilateral Pattern of the Phrenic Bursts Entrained by Fictive Locomotion in the
509 Spinal Rabbit Preparation. *Neuroscience Letters* 78: 318-322.
- 510 Wienecke J, Denton ME, Stecina K, Kirkwood PA, Hultborn H (2015) Modulation of
511 spontaneous locomotor and respiratory drives to hindlimb motoneurons temporally related
512 to sympathetic drives as revealed by Mayer waves. *Frontiers in Neural circuits* 9: 1. DOI:
513 10.3389/fncir.2015.00001.
- 514 Winfree, AT *The Geometry of biological time*, 2nd edition Springer, New York, 2001
- 515
- 516
- 517

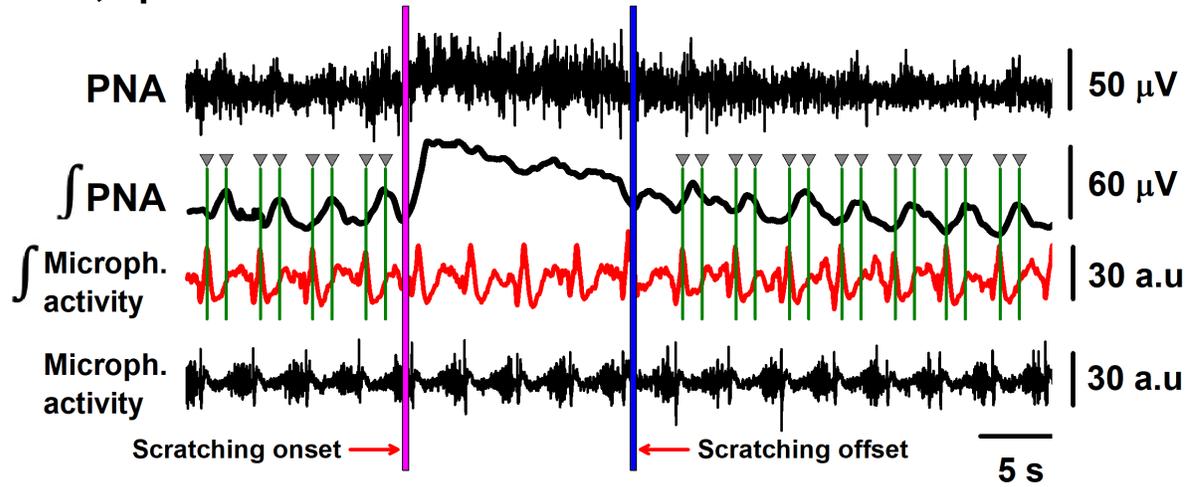




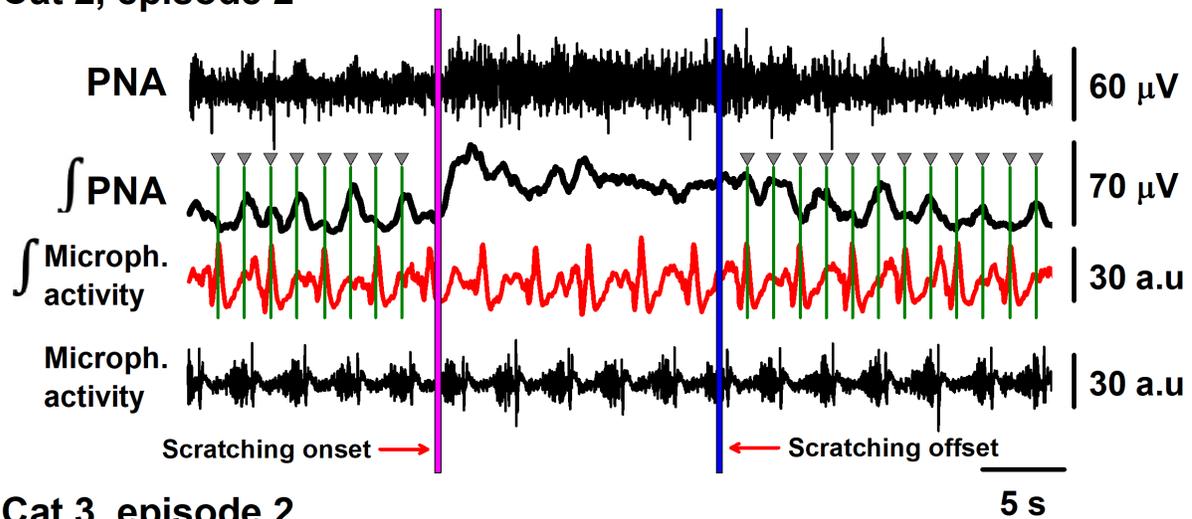




Cat 1, episode 2



Cat 2, episode 2



Cat 3, episode 2

