1 **IL-1 receptor activation undermines respiratory motor plasticity after systemic inflammation.**

2 Austin D. Hocker, Adrianne G. Huxtable

3 Department of Human Physiology, University of Oregon, Eugene, OR, 97403;

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5 **Running Title:** IL-1 signaling undermines respiratory plasticity

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7 **Correspondence:** **Adrianne G. Huxtable, PhD**

8 **Department of Human Physiology**

9 **1240 University of Oregon**

10 **Eugene, OR, 97401**

11

12 TEL: 541-346-9057

13 Email: huxtable@uoregon.edu

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15 **Support:** Supported by Francis Family Foundation (AGH)

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17 **Key Words:** respiratory motor plasticity, inflammation, Interleukin-1, phrenic motor neurons

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23 **ABSTRACT:**

24 Inflammation undermines respiratory motor plasticity, yet we are just beginning to understand
25 the inflammatory signaling involved. Since interleukin-1 (IL-1) signaling promotes or inhibits
26 plasticity in other CNS regions, we tested the following hypotheses: 1) IL-1 receptor (IL-1R)
27 activation after systemic inflammation is necessary to undermine phrenic long-term facilitation
28 (pLTF), a model of respiratory motor plasticity induced by acute intermittent hypoxia (AIH), and
29 2) spinal IL-1β is sufficient to undermine pLTF. pLTF is significantly reduced 24 hours after
30 lipopolysaccharide (LPS; 100μg/kg; i.p.; 12±18%, n=5) compared to control (57±25%, n=6) and
31 restored by peripheral IL-1R antagonism (63±13%; n=5; AF-12198, 0.5mg/kg i.p., 24 hours).
32 Furthermore, acute, spinal IL-1R antagonism (1mM AF-12198; 15 μL; intrathecal) restored pLTF
33 (53±15%; n=4) compared to LPS treated rats (11±10%; n=5), demonstrating IL-1R activation is
34 necessary to undermine pLTF after systemic inflammation. However, in healthy animals, pLTF
35 persisted after spinal, exogenous rIL-1β (1ng ± AIH; 66±26%, n=3, 10ng ± AIH; 102±49%, n=4,
36 100ng + AIH; 93±51%, n=3, 300ng ± AIH; 37±40%, n=3; $p<0.05$ from baseline). Spinal rIL-1β in
37 the absence of AIH, induced progressive, dose-dependent phrenic amplitude facilitation (1 ng; -
38 3±5%, n=3, 10 ng; 8±22%, n=3, 100 ng; 31±12%, $p<0.05$, n=4, 300 ng; 51±17%, $p<0.01$ from
39 baseline, n=4). In sum, IL-1R activation, both systemically and spinally, undermines pLTF after
40 LPS-induced systemic inflammation, but IL-1R activation is not sufficient to abolish plasticity.
41 Understanding the inflammatory signaling inhibiting respiratory plasticity is crucial to
42 developing treatment strategies utilizing respiratory plasticity to promote breathing during
43 ventilatory control disorders.

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44 **New & Noteworthy**

45 This study gives novel insights concerning mechanisms by which systemic inflammation

46 undermines respiratory motor plasticity. We demonstrate that interleukin-1 signaling, both

47 peripherally and centrally, undermines respiratory motor plasticity. However, acute, exogenous

48 interleukin-1 signaling is not sufficient to undermine respiratory motor plasticity.

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49 **Introduction**

50 Our understanding of how systemic inflammation alters CNS function has accelerated
51 with the identification of peripheral cytokines inducing CNS inflammation by crossing the blood
52 brain barrier, entering the CNS at circumventricular organs, activating vagal afferents (22, 29),
53 or activating blood brain barrier endothelial cells and glia (9, 26, 37). In turn, this peripheral to
54 central inflammatory signaling alters CNS function by modulating glia (30) and significantly
55 affecting neuroplasticity (16).

56 A complicated relationship exists between inflammatory signaling and neuroplasticity.
57 Inflammatory signaling in the CNS promotes and inhibits distinct forms of neuroplasticity in
58 different CNS regions through the actions of the interleukin-1 (IL-1) signaling cascade. CNS IL-1β
59 and its corresponding IL-1 receptor (IL-1R) are upregulated after systemic inflammation induced
60 experimentally by lipopolysaccharide (LPS) (16, 25, 48). IL-1β is sufficient to inhibit hippocampal
61 dependent memory formation and long-term potentiation, a form of plasticity important for
62 learning and memory formation (27). However, a genetic knockout of the IL-1R undermines
63 hippocampal-dependent memory formation and abolishes plasticity in the form of long-term
64 potentiation (2, 15, 41), demonstrating IL-1 signaling both contributes to and undermines
65 hippocampal plasticity. Conversely, spinal pain hypersensitivity, a form of neuroplasticity in the
66 spinal dorsal horn, is induced by inflammatory stimuli involving IL-1R activation (10, 31, 49).
67 Thus, IL-1β and IL-1Rs have complicated effects on neuroplasticity, whereby IL-1R signaling can
68 either promote or inhibit plasticity depending on the CNS region in which this plasticity is
69 occurring.

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70 The respiratory control system, like other CNS regions, undergoes plasticity to confer
71 stability and adaptability to respiratory output (13, 33). Phrenic long-term facilitation (pLTF) is a
72 frequently studied model of neuroplasticity in the respiratory control system (34). pLTF is
73 induced by acute intermittent hypoxia (AIH: three 5-min hypoxic episodes, separated by 5 min
74 normoxic periods) and manifests as a progressive increase in phrenic nerve burst amplitude
75 following AIH. Previously, we have shown pLTF is abolished by systemic inflammation and IL-1β
76 gene expression increases after systemic inflammation in the cervical spinal cord (24, 25).
77 However, the inflammatory signaling cascades involved in undermining plasticity in the
78 respiratory system are not fully understood.
79 Given the central role for IL-1R signaling in other forms of plasticity and increased IL-1β
80 gene expression in the cervical spinal cord (where purportedly pLTF occurs (3)) after systemic
81 inflammation, we investigated the necessity and sufficiency of IL-1 signaling in undermining
82 pLTF after systemic inflammation. We demonstrate that IL-1R activation, both systemically and
83 centrally, undermines pLTF after LPS-induced systemic inflammation. Yet, in healthy rats, acute,
84 exogenous IL-1β spinally was not sufficient to undermine pLTF. Overall, our findings further our
85 mechanistic understanding of how plasticity in the neural control of breathing is abolished by
86 systemic inflammation.
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89 **Methods**

90 All experiments were approved by the Institutional Animal Care and Use Committee at

91 the University of Oregon and conformed to the policies of the National Institute of Health *Guide*

92 *for the Care and Use of Laboratory Animals*. Male Sprague Dawley Rats (300-400g; 3-4 months;

93 Envigo Colony 217 and 206) were housed under standard conditions with a 12:12h light/dark

94 cycle with food and water *ad libitum*.

95 ***Drugs and Materials***

96 Lipopolysaccharide (LPS; 0111:B4, Sigma Chemical) was dissolved and sonicated in

97 sterile saline to a working concentration of 100 μg/mL given via intraperitoneal (i.p.) injections.

98 The IL-1R antagonist, AF-12198 (Toronto Research Chemicals) was dissolved in 10% ethanol and

99 sterile saline for peripheral injections (0.5 mg/mL) or in fresh artificial cerebrospinal fluid (aCSF;

100 120mM NaCl, 3mM KCl, 2mM $CaCl_2$, 2mM $MgCl_2$, 23mM $NaHCO_3$, and 10mM glucose) for

101 intrathecal (i.t.) injections (1 mM). Recombinant rat IL-1β (rIL-1β, BioVision 4130) was dissolved

102 in aCSF for i.t. injections (0.25-10 ng/μL).

103 ***Experimental Groups***

104 To investigate the necessity of peripheral IL-1R activation in undermining pLTF, we used

105 the following experimental groups: 1) LPS (100 μg/kg i.p., 24 hours before AIH) + AF-12198 (0.5

106 mg/kg i.p., 24 hours before AIH) (n = 5); 2) LPS + Vehicle (10% ethanol in sterile saline) (n = 5);

107 3) Saline (i.p.; LPS Vehicle) + AF-12198 (n = 6); 4) Saline + Vehicle (n = 5); 5) Time control (TC),

108 which consists of rats from each of the previous treatment groups (LPS + AF-12198 (i.p.), n=4;

109 Saline + AF-12198 (i.p.), n=5; LPS + Vehicle (i.p.), n=4; Saline + Vehicle (i.p.) n=4).

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110 To investigate the necessity of spinal IL-1R activation in undermining pLTF after systemic
111 inflammation, we used the following experimental groups: 1) LPS (100 μg/kg i.p., 24 hours
112 before AIH) + AF-12198 (1 mM, 15 μL, i.t., 20 min before AIH) (n = 4); 2) LPS + Vehicle (10%
113 ethanol in sterile saline, i.t.) (n = 5); 3) Saline (i.p.) + AF-12198 (i.t.) (n = 4); 4) Saline + Vehicle
114 (i.t.) (n = 4); 5) TCs, which consist of rats from each of the previous treatment groups (LPS + AF-
115 12198 (i.t.), n=4; Saline + AF-12198 (i.t.), n=3; LPS + Vehicle (i.t.), n=4; Saline + Vehicle (i.t.),
116 n=4).
117 To investigate the sufficiency of acute, exogenous, spinal rIL-1β to undermine pLTF, we
118 performed a dose-response with exogenous rIL-1β (i.t.) at 1 ng, 10 ng, 100 ng, and 300 ng. AIH
119 was performed 20 minutes after spinal rIL-1β injections.
120 To investigate the effects of acute, exogenous, spinal rIL-1β on phrenic burst facilitation
121 in the absence of AIH, rIL-1β (i.t., 1 ng, 10 ng, 100 ng, 300 ng) was applied over the cervical
122 spinal cord and phrenic neural output monitored over the next 105 minutes.
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125 ***Electrophysiological studies***

126 Electrophysiological studies have been described in detail previously (24). Rats were
127 anesthetized with isoflurane, tracheotomized, ventilated (Rat Ventilator, VetEquip®) and
128 bilaterally vagotomized. A venous catheter was placed for drug delivery and fluid replacement,
129 and a femoral arterial catheter was used for arterial blood analysis and monitoring blood
130 pressure. Arterial blood samples were analyzed (PaO_2, $PaCO_2$, pH, base excess; Siemens
131 RAPIDLAB® 248) before AIH, during the first hypoxic response, and 15, 30 and 60 minutes post-
132 AIH. Temperature was measured with a rectal temperature probe (Kent Scientific Corporation)
133 and maintained between 37 and 38°C with a heated table. Using a dorsal approach, hypoglossal
134 and phrenic nerves were dissected, cut distally, and desheathed. Rats were converted to
135 urethane anesthesia (1.8 g/kg i.v.; Sigma-Aldrich), allowed to stabilize over the next hour, and
136 paralyzed with pancuronium dibromide (1 mg/rat; Selleck Chemicals).

137 In rats receiving i.t. injections, a laminectomy was performed at cervical vertebrae 2 (C2)
138 and a primed, silicone catheter was inserted two millimeters through a small incision in the
139 dura. The catheter tip extended toward the rostral margin of C4 (4). AF-12198 (1 mM) or
140 vehicle (aCSF) was injected (15 µL) 20 minutes before AIH. rIL-1β was applied i.t. 20 minutes
141 before the start of AIH at doses of 1ng (4 µL of 0.2 ng/µL), 10ng (10 µL of 1 ng/µL), 100ng (10 µL
142 of 10 ng/µL), and 300ng (30 µL of 10 ng/µL in 10 µL boluses over 3 minutes). In rats receiving
143 rIL-1β (i.t.) but not AIH, all time points were matched with rIL-1β + AIH experiments.

144 Nerves were bathed in mineral oil and placed on bipolar silver wire electrodes. Raw
145 nerve recordings were amplified (10k), filtered (0.1-5 kHz), integrated (50 ms time constant),
146 and recorded (10 kHz sampling rate) for offline analysis (PowerLab and LabChart 8.0, AD

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147 Instruments). Apneic and recruitment CO_2 thresholds were determined by changing inspired
148 CO_2 with continuous end-tidal CO_2 monitoring (Kent Scientific Corporation). End tidal CO_2 was
149 set 2 mmHg above the recruitment threshold and arterial blood samples were used to establish
150 baseline $PaCO_2$, which was maintained within 1.5 mmHg of the baseline value throughout.
151 Blood volume and base excess were maintained (±3 MEq/L) by continuous infusion (1-3 mL/h
152 i.v.) of hetastarch (0.3%) and sodium bicarbonate (0.99%) in lactated ringers. Experiments were
153 excluded if mean arterial pressure deviated more than 20 mmHg from baseline versus 60
154 minutes after AIH.
155 All rats (except for TC rats) received three, 5 minute bouts of hypoxia (~10.5% O_2, PaO_2
156 35-45 mmHg) separated by 5 minutes of normoxia. The average amplitude and frequency of 30
157 consecutive integrated phrenic bursts were analyzed at baseline, during the first acute hypoxic
158 response, and 15, 30, and 60 minutes after AIH and made relative to baseline amplitude.
159 Physiological variables and phrenic nerve activity data for each experimental group were
160 compared using two-way, repeated measures ANOVA with Fisher LSD post hoc tests. Time
161 control data were grouped for each experimental design since no significant differences
162 between experimental groups were found (ANOVA RM, Fisher LSD post-hoc). Mean arterial
163 pressure is reported from baseline, the end of the final hypoxic exposure, and 60 minutes after
164 AIH. Acute hypoxic responses were compared using an ANOVA with Fisher LSD post hoc test.
165 Values are means ± SD.
166

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167 **Results**

168 *Peripheral IL-1R activation is necessary for undermining pLTF after peripheral LPS.*

169 Saline + AF-12198 Vehicle (i.p.) and Saline + AF-12198 (i.p.) rats exhibited pLTF 60

170 minutes after AIH (57 ± 25%, $p < 0.0001$, n=6; 44 ± 14%, $p < 0.0001$, n=5, respectively, Fig. 1). As

171 expected (25), pLTF was eliminated in LPS + Vehicle (i.p.) rats (12 ± 18%, $p < 0.05$, n=6, Fig. 1).

172 pLTF was restored by peripheral antagonism of IL-1Rs (LPS ± AF-12198 i.p.; 63 ± 13%, p <

173 0.0001, n=5, Fig. 1) and was not evident in any TC groups (-1 ± 9 % p = 0.24, n=17). No

174 significant differences in the magnitude of pLTF was evident between LPS + AF-12198 (i.p.) and

175 Saline + AF-12198 (i.p.) groups.

176 The acute hypoxic phrenic response was unaffected by LPS or IL-1R antagonism (Saline +

177 Vehicle (i.p.); 133 ± 39%, LPS + Vehicle (i.p.); 129 ± 49%, Saline + AF-12198 (i.p.); 104 ± 46%, LPS

178 + AF-12198 (i.p.); 129 ± 30%, Fig. 1C); no significant differences were present between groups.

179 LPS caused a small, but significant, increase in baseline phrenic burst frequency

180 compared to saline controls (LPS + Vehicle (i.p.), 51 ± 6 bursts/min; Saline + Vehicle (i.p.), 43 ± 8

181 bursts/min; $p = 0.021$). However, AF-12198 had no effect on baseline burst frequency between

182 groups (LPS ± AF-12198 (i.p), 49 ± 5 bursts/min; Saline + AF-12198 (i.p.), 45 ± 5 bursts/min).

183 Frequency LTF was not evident 60 minutes post-AIH in any experimental group.

184 Small variations were evident in physiological parameters (Table 1), but remained within

185 experimental limits. As expected, all experimental groups displayed significantly decreased

186 MAP and PaO_2 during hypoxia. Temperature 60 minutes after AIH was significantly different in

187 Saline + Vehicle and LPS + AF-12198 (i.p.) groups relative to baseline, but remained within

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188 experimental limits noted above. Similarly, 60 minutes after AIH small differences were evident
189 between groups in PaO_2 and pH, but remained within acceptable limits (Table 1).
190
191 *Spinal IL-1R activation is necessary for undermining pLTF after peripheral LPS.*
192 We next tested the hypothesis that acute, spinal IL-1R activation is necessary to
193 undermine pLTF 24 hours after peripheral LPS (Fig 2). Saline + Vehicle (i.t.) rats exhibited pLTF
194 60 minutes after AIH (60 ± 18%, $p < 0.0001$, n=4). AF-12198 (i.t.) did not significantly increase
195 the magnitude of pLTF in saline treated rats (Saline + AF-12198 (i.t.); (72 ± 26%, $p < 0.0001$,
196 n=4). As expected, LPS (100 µg/kg; i.p.) eliminated pLTF (LPS + Vehicle (i.t.), 11 ± 10%, p = 0.165
197 from baseline, n=5). pLTF was restored after spinal IL-1R antagonism (LPS + AF-12198 (i.t.), 53 ±
198 15%, $p < 0.0001$ from baseline, n=4). pLTF was not apparent in in the TC group (6 ± 17%, p =
199 0.241, n=15).
200 The acute hypoxic phrenic response was not significantly affected by LPS or spinal IL-1R
201 antagonism (Saline + Vehicle (i.t.); 117 ± 23%, LPS + Vehicle (i.t.); 169 ± 45%, Saline + AF-12198
202 (i.t.); 150 ± 61%, LPS ± AF-12198 (i.t.); 104 ± 36%).
203 Baseline phrenic burst frequency was not significantly different between any groups
204 (Saline + Vehicle (i.t.); 45 ± 6 bursts/min, Saline + AF-12198 (i.t.); 45 ± 3 bursts/min, LPS +
205 Vehicle (i.t.); 44 ± 4 bursts/min, LPS ± AF-12198 (i.t); 47 ± 10 bursts/min, p = 0.9528). Frequency
206 LTF was evident in Saline + AF-12198 (i.t.) rats 60 minutes after AIH (4 ± 2 bursts/min, p =
207 0.0138), but not in any other group.
208 Small variations were evident in physiological parameters (Table 2), but they remained
209 within experimental limits. As expected, all experimental groups displayed significantly

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210 decreased MAP and PaO_2 during hypoxia. There were no significant differences in temperature,
211 $PaCO_2$, or pH at baseline. The significant decrease in PaO_2 at 60 min in the Saline + Vehicle (i.t.)
212 group was within normal limits. At baseline, LPS treatment increased MAP relative to saline-
213 treated groups with AF-12198 or vehicle (Table 2).
214
215 *Spinal exogenous rIL-1β does not abolish AIH-induced pLTF in healthy rats.*
216 pLTF remains after spinal, exogenous rIL-1β (1ng ± AIH; 66 ± 26%, n=3, p = 0.012, 10ng ±
217 AIH; 102 ± 49%, n=4, p < 0.0001, 100ng + AIH; 93 ± 51%, n=3, p < 0.0001, 300ng ± AIH; 37 ±
218 40%, n=3, p = 0.028). However, after 300ng rIL-1β, AIH elicited pLTF with lower magnitude than
219 10ng (p = 0.008) and 100ng doses (p = .014, Fig. 3A). Thus, rIL-1β is not sufficient to abolish pLTF
220 in healthy rats.
221 Furthermore, rIL-1β had no effect on the acute hypoxic phrenic responses at any dose
222 (1ng; 154 ± 63%, 10ng; 220 ± 131%, 100ng; 143 ± 55%, 300ng; 96 ± 52%).
223 Minor changes in physiological variables occurred in animals receiving intrathecal rIL-1β
224 and AIH (Table 3). Temperature was decreased at 60 minutes in the 1ng + AIH group, but
225 remained within acceptable ranges. There were no significant differences in $PaCO_2$ and small,
226 but significant, changes in pH during hypoxia in 1ng + AIH and 300ng + AIH groups. PaO_2 and
227 MAP were significantly decreased during hypoxia (Table 3).
228
229 *Spinal exogenous rIL-1β facilitates phrenic amplitude in healthy rats.*
230 Since exogenous IL-1β is sufficient to induce plasticity in the spinal dorsal horn in the
231 form of pain hypersensitivity (Cunha et al., 2008), we next tested the hypothesis that spinal rIL-

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232 1β was sufficient to facilitate phrenic nerve amplitude. Low doses of rIL-1β (1 ng and 10 ng) did

233 not significantly alter phrenic amplitude(1 ng; -3 ± 5%, n=3, p = 0.738, 10 ng; 8 ± 22%, n=3, p =

234 0.380); however, higher doses of rIL-1β induced progressive phrenic amplitude facilitation (100

235 ng; 31 ± 12%, p = 0.005 relative to baseline, n=4, 300 ng; 51 ± 17%, p <0 .0001 relative to

236 baseline, n=4). Therefore, high doses of rIL-1β are sufficient to facilitate phrenic amplitude.

237 Physiological variables were stable throughout electrophysiology experiments without

238 any significant changes (Table 3).

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242 **Discussion**

243 Here, we demonstrate a role for IL-1R activation, both peripherally and centrally, in
244 systemic inflammation-induced impairment of respiratory motor plasticity. In healthy rats,
245 however, activation of IL-1Rs with exogenous rIL-1β is insufficient to abolish AIH-induced pLTF,
246 yet induces phrenic amplitude facilitation in the absence of AIH. Thus, IL-1R activation is
247 necessary to abolish pLTF after systemic inflammation, but not sufficient to abolish pLTF in
248 healthy rats. These data demonstrate IL-1R activation is an important signaling step in
249 abolishing respiratory plasticity after systemic inflammation.

250 CNS responses to IL-1β have been widely studied because IL-1β plays a role in
251 modulating many physiological systems, including thermoregulation and behavioral responses
252 (32, 50), and IL-1β is rapidly upregulated in the CNS after LPS-induced systemic inflammation
253 (40). More recently, IL-1 signaling in the CNS has been shown to modulate different forms of
254 neuroplasticity, including hippocampal dependent learning and pain sensitivity in the dorsal
255 spinal cord (10, 31, 49). We now demonstrate a role for IL-1R activation in undermining
256 respiratory plasticity in the cervical spinal cord.

257 The restoration of pLTF by peripheral IL-1R antagonism suggests IL-1R activation is
258 necessary for transmitting relevant inflammatory information to the CNS after LPS-induced
259 inflammation. Additionally, since IL-1R signaling likely contributes significantly to time-
260 dependent changes in serum cytokine levels (44), peripheral IL-1R antagonism may indirectly
261 mitigate CNS inflammation by inducing changes in other cytokine levels and restore plasticity.
262 While we did not assess CNS inflammation directly in these experiments, others have shown
263 peripheral IL-1R activation is required for brain inflammatory responses after i.p LPS (19, 31)

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264 and peripheral and spinal IL-1β gene expression is upregulated after peripheral LPS (25). Thus,
265 we suggest the primary action of peripheral IL-1R antagonism is to inhibit peripheral
266 inflammatory responses induced by LPS. It remains possible, however, that AF-12198 crosses
267 the blood-brain barrier and also directly reduces CNS inflammatory responses. Since AF-12198
268 is a small peptide, we believe it is unlikely to cross the blood-brain barrier at sufficient
269 concentrations to directly reduce CNS inflammation. Thus, the actions of peripheral IL-1R
270 antagonism are most likely by inhibiting peripheral inflammatory responses. Together, we
271 propose peripheral IL-1R antagonism indirectly reduces relevant spinal inflammation, and thus,
272 restores pLTF.
273 Our data demonstrating restoration of pLTF by spinal IL-1R antagonism is consistent
274 with the hypothesis that persistent IL-1 signaling around the phrenic motor pool is necessary
275 for undermining pLTF (11, 12). IL-1R signaling in phrenic motoneurons could directly alter their
276 properties to eliminate pLTF or could promote the release of other inflammatory cytokines
277 from nearby cells (such as microglia) which undermine pLTF. IL-1R signaling can activate diverse
278 intracellular pro-inflammatory pathways including NF-kB and stress activated protein kinases
279 (14), which may inhibit pLTF. Additionally, in a separate model of systemic inflammation, spinal
280 p38-MAPK activation, a kinase downstream of IL-1R (35), is necessary to undermine pLTF after
281 systemic inflammation (24). Future investigations should assess the link between spinal IL-1
282 signaling and activation of p38-MAPK in undermining pLTF.
283 Though we demonstrate the necessity of IL-1R activation in undermining plasticity, the
284 effects of direct application of rIL-1β are more complicated. Here, no rIL-1β dose abolished
285 plasticity after AIH in healthy rats. The 300ng rIL-1β dose did reduce the magnitude of pLTF

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286 compared to other doses. Thus, it is possible higher doses of rIL-1β may be sufficient to
287 undermine pLTF; however, 300ng is the highest dose possible due to solubility and volume
288 limitations for intrathecal injections. Exogenous rIL-1β may be insufficient to undermine pLTF if
289 complementary inflammatory molecules are necessary to completely undermine pLTF or if
290 systemic inflammation primes the CNS to augment the response to IL-1β. For example, IL-1R
291 expression is upregulated after peripheral LPS (47) such that healthy animals not exposed to
292 LPS have a blunted response to exogenous rIL-1β. Alternatively, the cellular mechanisms
293 undermining pLTF may take longer to develop than 20 minutes after spinal, exogenous rIL-1β.
294 However, in hippocampal slices, rIL-1β inhibits long-term potentiation within 10 minutes of
295 exposure (6), demonstrating rIL-1β can have rapid effects. In the absence of AIH, high doses of
296 rIL-1β facilitated phrenic burst amplitude, demonstrating rIL-1β evoked a lasting increase in
297 phrenic amplitude after rIL-1β is likely degraded (38). While the mechanisms mediating this
298 plasticity are unknown, other reports show dose-dependent IL-1β changes in synaptic strength
299 or cell excitability in response to IL-1R activation, such as increased calcium-dependent
300 glutamate release from hippocampal neurons, upregulated NMDA receptor activity (18), and
301 increased dorsal root ganglion neuron excitability after IL-1β (7, 43). Taken together, rIL-1β
302 exerts dose-dependent alterations of phrenic motor output, capable of both inducing
303 facilitation and reducing the magnitude of, but not abolishing, pLTF.

304 Though our study demonstrates an important role of IL-1 signaling in spinal motor
305 respiratory plasticity, IL-1 signaling is likely not the only inflammatory molecule involved in
306 modulating respiratory neural activity. For example, inactivity-induced phrenic motor
307 facilitation, another model of spinal respiratory motor plasticity, requires the activation of TNF

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308 receptor 1, an inflammatory cytokine receptor (8). Additional inflammatory molecules, such as
309 prostaglandins, have also been shown to modulate central respiratory networks. While
310 prostaglandins mediate profound respiratory modulation (17, 28, 36, 42), they are unlikely to
311 play a significant role in undermining respiratory motor plasticity after systemic inflammation
312 since inhibition of cyclooxygenase, the rate-limiting enzyme in the production of
313 prostaglandins, does not restore pLTF (23). However, the relationship between the various
314 types of inflammatory cytokines and their downstream effects in respiratory control remains
315 mostly unknown. Improving our understanding of the actions of individual and combined
316 inflammatory signaling molecules, as well as their cellular sources, in neural respiratory control
317 will be the topic of future investigations.

318 Our study builds on these and other recent studies investigating the effects of IL-1
319 signaling in respiratory control networks (1, 39). Similar to previous reports (1, 20), we found a
320 tendency for systemic inflammation to increase baseline phrenic burst frequency. While the
321 mechanisms mediating the increased frequency after systemic inflammation are unclear, direct
322 intracerebroventricular rIL-1β increases minute ventilation, suggesting a direct effect of IL-1β
323 on respiratory frequency (1). Additionally, acute hypoxic ventilatory responses are attenuated
324 after intratracheal LPS due to spinal IL-1R activation (39); however, no significant changes were
325 seen in the acute hypoxic phrenic amplitude response after systemic inflammation.
326 Furthermore, frequency LTF was only evident in one experimental group, which supports
327 previous work suggesting frequency LTF is small and inconsistent (5). Less is known about the
328 mechanisms underlying frequency LTF, which may be distinctly different from the amplitude
329 change known as pLTF. Thus, we have focused our analyses on amplitude changes since much

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330 more is known about the mechanism of pLTF using this experimental preparation and the AIH
331 paradigm, where the consistent and significant changes are reflected in amplitude. In sum, IL-1
332 signaling seems to have widespread roles in the neural control of breathing.

333 In conclusion, we demonstrate IL-1R activation peripherally and centrally is necessary
334 for undermining respiratory plasticity after systemic inflammation. Our study is the first to
335 investigate the effects of IL-1 signaling on spinal respiratory plasticity, and builds on other
336 recent studies investigating the effects of IL-1 signaling in medullary respiratory networks (1,
337 39). Taken together, inflammatory signaling through IL-1R attenuates chemosensitivity (1) and
338 respiratory plasticity. Furthermore, such results suggest uncovering how inflammation impacts
339 the neural control of breathing is fundamental to understand changes in respiratory function
340 during pathological conditions. Furthermore, for respiratory motor plasticity to be used
341 clinically (21, 45, 46), we must also understand the interaction between plasticity and
342 inflammation, which is common in many pathologies.
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501 **Figure 1. Peripheral IL-1R antagonism restores pLTF after systemic inflammation**.

502 Representative integrated phrenic neurograms **(A)** for acute intermittent hypoxia (AIH)-treated

503 rats receiving Saline + Vehicle (i.p.), LPS (100 μg/kg; i.p.) + Vehicle (i.p), Saline + AF-12198 (i.p.),

504 LPS + AF-12198 (i.p.), and a time control (no AIH). pLTF is evident as the progressive increase in

505 phrenic nerve amplitude from baseline (dashed line) over 60 minutes following AIH. Group data

506 **(B)** demonstrate pLTF is abolished by LPS (Vehicle treated, black bars) and restored by

507 peripheral IL-1R antagonism (AF-12198 treated, grey bars). Acute hypoxic responses **(C)** were

508 not significantly altered by any treatment (ANOVA). *** $p < 0.0001$ significant difference in

509 phrenic amplitude from baseline; # different from Saline + Vehicle ($p < 0.001$), Saline + AF-

510 12198 ($p < 0.05$), and LPS + AF-12198 ($p < 0.001$); ## $p < 0.001$ different from Saline + Vehicle,

511 Saline + AF-12198, and LPS + AF-12198 (ANOVA RM, Fisher LSD).

512

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515 **Figure 2. Spinal IL-1R antagonism restores pLTF after systemic inflammation.** Representative

516 integrated phrenic neurograms **(A)** for AIH-treated rats receiving Saline + Vehicle (i.t.), LPS (100

517 μg/kg; i.p.) + Vehicle (i.t.), Saline + AF-12198 (1 mM, 15 μL; i.t.), LPS (i.p.) + AF-12198 (i.t.), and a

518 time control (no AIH). pLTF is evident as the progressive increase in phrenic nerve amplitude

519 from baseline (dashed line) over 60 minutes following acute intermittent hypoxia (AIH). Group

520 data **(B)** demonstrate pLTF is abolished by LPS + Vehicle (black bars) and restored by intrathecal

521 IL-1R antagonism (AF-12198 treated, grey bars). Acute hypoxic responses **(C)** were not

522 significantly affected by any treatment. *** $p < 0.0001$ significant difference in phrenic

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amplitude from baseline; # $p < 0.001$ different from Saline + Vehicle, Saline + AF-12198, and LPS + AF-12198 (ANOVA RM, Fisher LSD).

Figure 3. Exogenous, spinal rIL-1β does not abolish pLTF in healthy animals. Representative integrated phrenic neurograms (A) after intrathecal rIL-1β (1ng, 10ng, 100ng, 300ng; indicated by ⬇) 20 minutes before acute intermittent hypoxia (Hx). pLTF is evident as the progressive increase in phrenic nerve amplitude from baseline (dashed line) over 60 minutes following acute intermittent hypoxia. Intrathecal rIL-1β does not abolish pLTF at any dose. Group data (B) demonstrate pLTF at all doses of intrathecal rIL-1β, but the magnitude of pLTF at high doses (300 ng) is reduced compared to lower doses (10 and 100 ng). Acute hypoxic phrenic responses (C) were not significantly affected by any treatment. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ significantly different from baseline; a, $p < 0.05$ significantly different from 10ng + AIH and 100ng + AIH (ANOVA RM, Fisher LSD).

Figure 4. Exogenous, spinal rIL-1β induces dose-dependent facilitation of phrenic amplitude. Representative integrated phrenic neurograms (A) for rats receiving intrathecal rIL-1β (1ng, 10ng, 100ng, 300ng; indicated by ⬇). Phrenic amplitude facilitation is evident as the progressive increase in phrenic nerve amplitude from baseline (dashed line) following intrathecal rIL-1β. Group data (B) demonstrate a dose-dependent increase in phrenic nerve amplitude 60 minutes

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547 after intrathecal rIL-1β. ** $p < 0.01$; *** $p < 0.001$ significantly different from baseline; a, $p <$

548 0.05 significantly different from 1ng + TC; b, $p < 0.01$ significantly different from 1ng + TC and

549 10ng + TC; (ANOVA RM, Fisher LSD).

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555
556 Table 1. Physiological parameters during electrophysiology experiments with systemic AF-12198 (0.5 mg/kg, i.p.) treatment.

Experimental Group		Temperature	PaO_2	$PaCO_2$	pH	MAP
Baseline	AF12198 + LPS	37.4 ± 0.3	315.4 ± 17.3	44.6 ± 3.3	7.34 ± 0.04 [c]	145 ± 5
	AF12198 + Saline	37.6 ± 0.3	281.9 ± 29.1 [d]	43.3 ± 3.5	7.34 ± 0.02 [c]	115 ± 13 [d,e]
	Vehicle + LPS	37.5 ± 0.2 [c]	295.8 ± 19.7	43.5 ± 3.3	7.36 ± 0.01	146 ± 7
	Vehicle + Saline	37.7 ± 0.1	295.8 ± 22.9	41.7 ± 2.7	7.38 ± 0.03	126 ± 12
	Grouped TC	37.6 ± 0.3	295.0 ± 24.8	42.4 ± 3.1	7.36 ± 0.03	131 ± 23
Hypoxia	AF12198 + LPS	37.5 ± 0.2	39.9 ± 3.0 [a,b]	44.9 ± 3.2	7.33 ± 0.05 [a,b]	101 ± 24 [a,b]
	AF12198 + Saline	37.4 ± 0.2	40.9 ± 3.7 [a,b]	43.0 ± 3.4	7.34 ± 0.02 [a]	63 ± 12 [a,b,d]
	Vehicle + LPS	37.6 ± 0.2	41.1 ± 1.5 [a,b]	43.4 ± 3.7	7.36 ± 0.03 [a]	106 ± 15 [a,b,c]
	Vehicle + Saline	37.5 ± 0.2	39.1 ± 2.4 [a,b]	42.0 ± 2.8	7.37 ± 0.03	73 ± 26 [a,b,d]
	Grouped TC	37.5 ± 0.2	298.2 ± 25.0	42.1 ± 3.3	7.36 ± 0.03	127 ± 27
60 min	AF12198 + LPS	37.7 ± 0.1 [a]	297.3 ± 13.5 [a]	44.8 ± 2.7	7.37 ± 0.04	132 ± 12 [a]
	AF12198 + Saline	37.4 ± 0.2	277.0 ± 27.8	44.0 ± 2.7	7.37 ± 0.03	115 ± 9 [e]
	Vehicle + LPS	37.6 ± 0.3	299.9 ± 20.8 [c]	44.1 ± 3.5	7.39 ± 0.01	140 ± 9
	Vehicle + Saline	37.5 ± 0.2 [a]	273.7 ± 26.1 [a,b]	42.5 ± 2.4 [a]	7.38 ± 0.02	119 ± 20
	Grouped TC	37.5 ± 0.2	297.1 ± 22.4	42.6 ± 3.4	7.38 ± 0.03 [a]	128 ± 21

557
558 MAP, mean arterial pressure,
559 [a] $p < 0.05$ different from baseline within group,
560 [b] $p < 0.05$ different from grouped TC within time point,
561 [c] $p < 0.05$ different from Vehicle + Saline within time point,
562 [d] $p < 0.05$ different from AF-12198 + LPS within time point
563 AF12198 + LPS; n=5, AF12198 + Saline; n=6, Vehicle + LPS; n=5, Vehicle + Saline; n=6, Grouped TC; n=17
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566 Table 2. Physiological parameters during electrophysiology experiments with spinal AF-12198 (1 mM, 15uL) treatment.
567

Experimental Group		Temperature	PaO_2	$PaCO_2$	pH	MAP
Baseline	AF12198 + LPS	37.5 ± 0.3	294.2 ± 31.0	47.5 ± 2.5	7.35 ± 0.03	145 ± 19
	AF12198 + Saline	37.4 ± 0.3	276.9 ± 22.1	45.1 ± 2.2	7.37 ± 0.03	127 ± 20 [b]
	Vehicle + LPS	37.5 ± 0.3	284.6 ± 29.0	44.0 ± 2.7	7.37 ± 0.01	144 ± 11 [c]
	Vehicle + Saline	37.7 ± 0.3	288.9 ± 33.5	43.5 ± 3.2	7.38 ± 0.02	102 ± 11 [b]
	Grouped TC	37.5 ± 0.2	278.7 ± 16.1	44.8 ± 3.2	7.35 ± 0.04	125 ± 25
Hypoxia	AF12198 + LPS	37.5 ± 0.2	37.6 ± 2.1 [a,b]	47.6 ± 3.4	7.33 ± 0.02	106 ± 37 [a,c]
	AF12198 + Saline	37.6 ± 0.1	39.1 ± 2.0 [a,b]	44.1 ± 2.3	7.37 ± 0.02	66 ± 14 [a]
	Vehicle + LPS	37.4 ± 0.1	37.5 ± 2.1 [a,b]	44.5 ± 3.8	7.37 ± 0.02	95 ± 10 [a]
	Vehicle + Saline	37.7 ± 0.2	38.6 ± 8.0 [a,b]	43.4 ± 3.8	7.36 ± 0.03	56 ± 12 [a]
	Grouped TC	37.6 ± 0.2	283.7 ± 33.8	44.9 ± 3.2	7.36 ± 0.03	122 ± 25
60 min	AF12198 + LPS	37.4 ± 0.3	289.5 ± 18.8	47.2 ± 2.3	7.37 ± 0.02	137 ± 15
	AF12198 + Saline	37.6 ± 0.2	272.5 ± 19.0	45.4 ± 3.3	7.37 ± 0.03	115 ± 18
	Vehicle + LPS	37.5 ± 0.3	283.7 ± 20.3	43.9 ± 2.3	7.39 ± 0.02	132 ± 13
	Vehicle + Saline	37.5 ± 0.1	260.5 ± 20.9 [a]	43.6 ± 2.8	7.40 ± 0.03	99 ± 23
	Grouped TC	37.4 ± 0.2	270.1 ± 24.7	45.1 ± 3.2	7.38 ± 0.02	120 ± 22

568 MAP, mean arterial pressure,
569 [a] $p < 0.05$ different from baseline within group,
570 [b] $p < 0.05$ different from grouped TC within time point,
571 [c] $p < 0.05$ different from Vehicle + Saline within time point,
572 AF12198 + LPS; n=4, AF12198 + Saline; n=4, Vehicle + LPS; n=5, Vehicle + Saline; n=4, Grouped TC; n=15
573
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575 Table 3. Physiological parameters during electrophysiology experiments with spinal rIL-1β treatment.

Experimental Group		Temperature	PaO_2	$PaCO_2$	pH	MAP
Baseline	1 ng + AIH	37.7 ± 0.3	272.9 ± 34.8	42.8 ± 2.0	7.38 ± 0.01	117 ± 12
	10 ng + AIH	37.5 ± 0.2	267.9 ± 11.1	40.0 ± 4.8	7.40 ± 0.05	111 ± 21
	100 ng + AIH	37.6 ± 0.1	258.7 ± 11.1	43.9 ± 2.8	7.37 ± 0.01	115 ± 18
	300 ng + AIH	37.6 ± 0.4	289.0 ± 6.2	41.5 ± 2.9	7.40 ± 0.03	103 ± 10
Hypoxia	1 ng + AIH	37.4 ± 0.3	38.0 ± 3.5 [a]	42.9 ± 3.7	7.36 ± 0.04 [a]	69 ± 17 [a]
	10 ng + AIH	37.4 ± 0.3	38.2 ± 4.0 [a]	38.8 ± 5.8	7.40 ± 0.05	72 ± 21 [a]
	100 ng + AIH	37.4 ± 0.2 [f]	42.6 ± 1.4 [a]	43.4 ± 2.8	7.36 ± 0.02	60 ± 25 [a]
	300 ng + AIH	37.6 ± 0.3	37.6 ± 4.3 [a]	42.2 ± 3.7	7.40 ± 0.01 [a]	62 ± 15 [a]
60 min	1 ng + AIH	37.3 ± 0.1 [a]	247.7 ± 45.2 [a]	42.9 ± 1.3	7.37 ± 0.02	120 ± 5
	10 ng + AIH	37.3 ± 0.2	281.3 ± 18.4	40.5 ± 4.9	7.39 ± 0.05	123 ± 17
	100 ng + AIH	37.4 ± 0.2	249.5 ± 16.3	44.2 ± 2.3	7.37 ± 0.02	107 ± 20 [a]
	300 ng + AIH	37.5 ± 0.4	262.4 ± 15.0 [a]	42.1 ± 3.2	7.37 ± 0.01	115 ± 14

Experimental Group		Temperature	PaO_2	$PaCO_2$	pH	MAP
Baseline	1 ng	37.6 ± 0.4	282.1 ± 26.1	42.5 ± 1.2	7.38 ± 0.02	127 ± 27
	10 ng	37.5 ± 0.2	255.3 ± 19.0	42.4 ± 2.2	7.37 ± 0.01	117 ± 12
	100 ng	37.6 ± 0.3	264.4 ± 23.6	45.4 ± 2.4	7.37 ± 0.01	135 ± 9
	300 ng	37.7 ± 0.3	258.8 ± 24.3	40.5 ± 6.5	7.40 ± 0.04	123 ± 11
60 min	1 ng	37.6 ± 0.3	279.6 ± 15.0	43.0 ± 0.7	7.39 ± 0.03	132 ± 17
	10 ng	37.7 ± 0.3	254.6 ± 7.2	42.8 ± 1.8	7.38 ± 0.02	120 ± 17
	100 ng	37.4 ± 0.2	263.0 ± 25.2	45.7 ± 2.5	7.36 ± 0.01	131 ± 7
	300 ng	37.6 ± 0.2	234.2 ± 22.6	40.2 ± 7.2	7.38 ± 0.01	134 ± 18

576 MAP, mean arterial pressure,
577 [a] $p < 0.05$ different from baseline within group,
578 [b] $p < 0.05$ different from all AIH experimental groups within time point,
579 1 ng + AIH; n=3, 10 ng + AIH; n=4, 100 ng + AIH; n=3, 300 ng + AIH; n=3,
580 1 ng + TC; n=3, 10 ng + TC; n=3, 100 ng + TC; n=4, 300 ng + TC; n=4,
581

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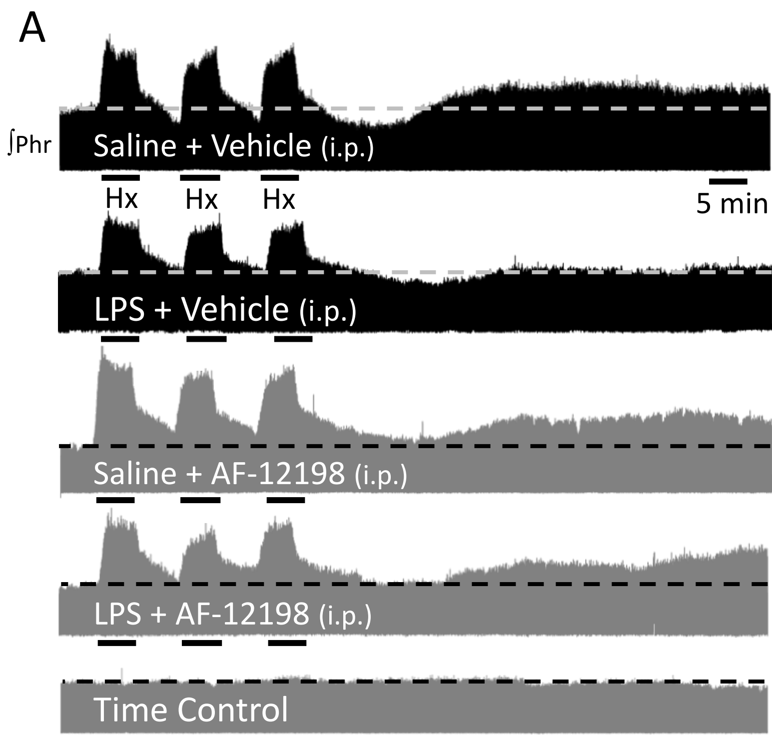

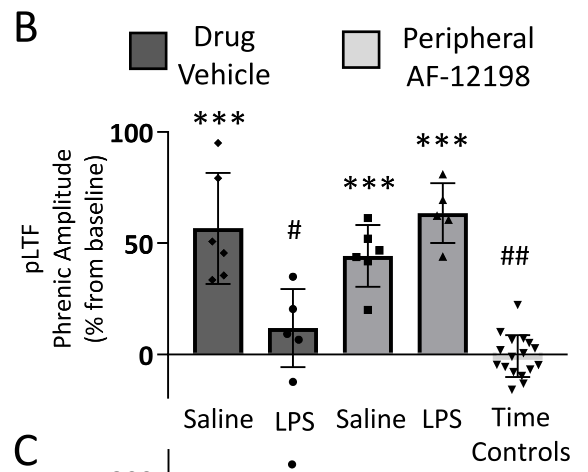

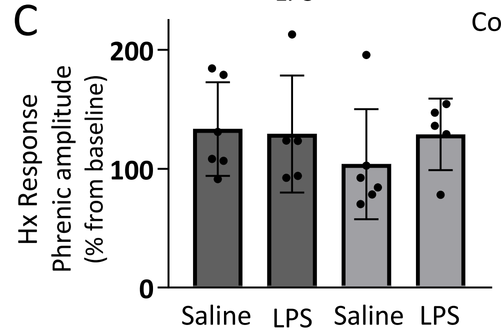

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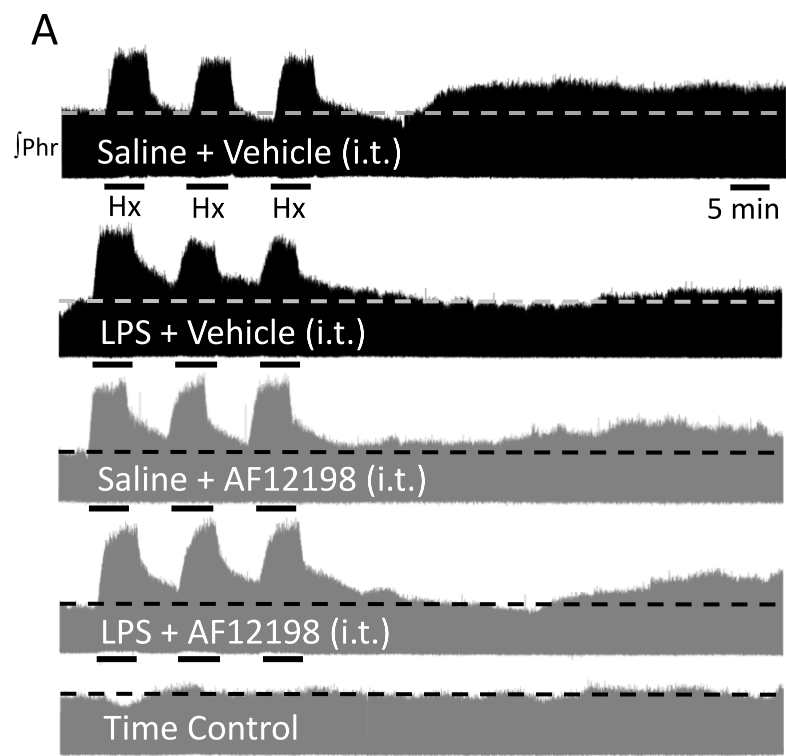

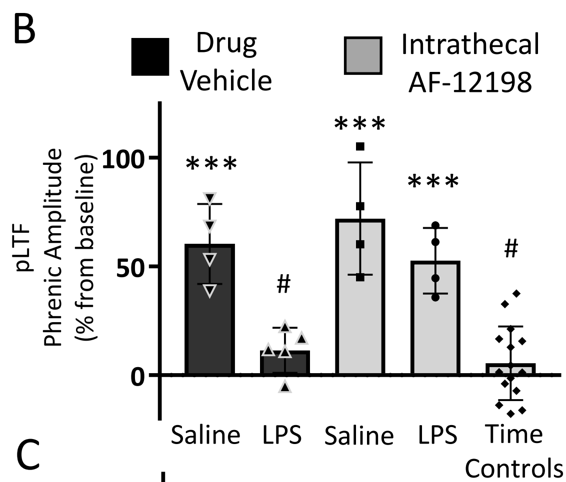

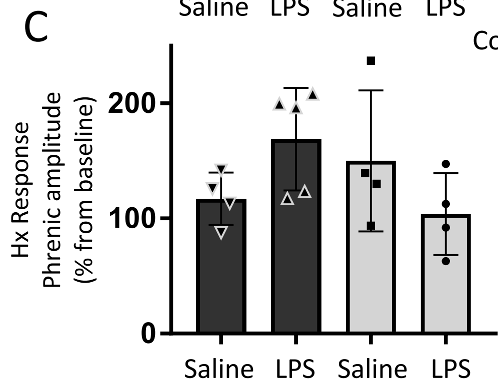

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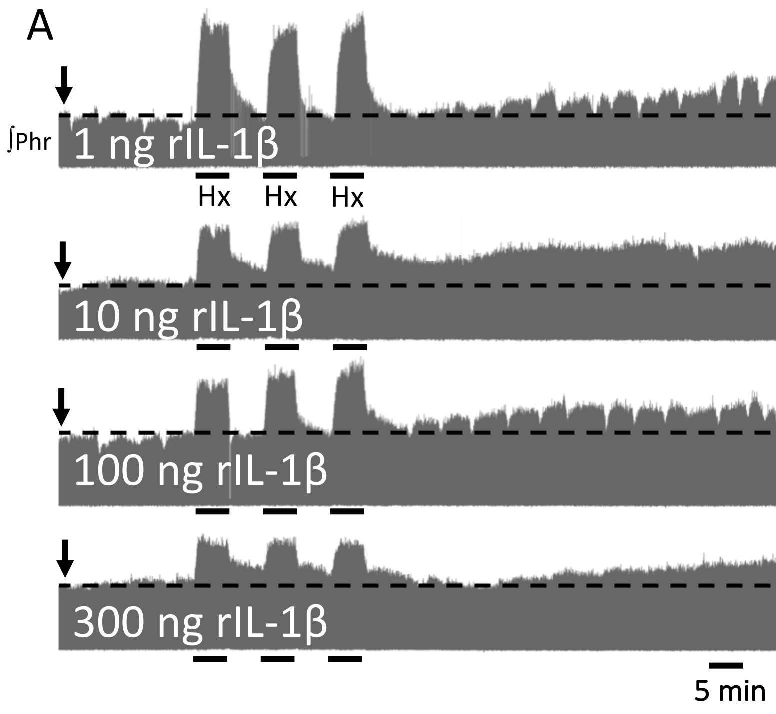

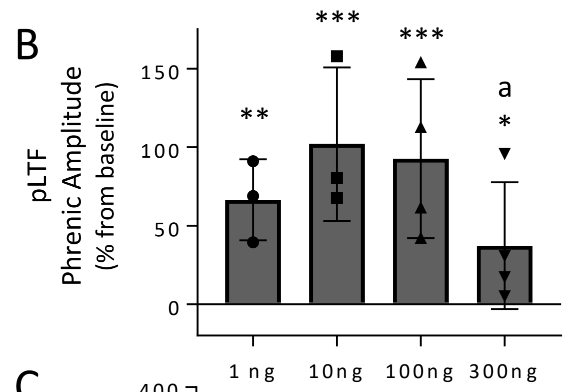

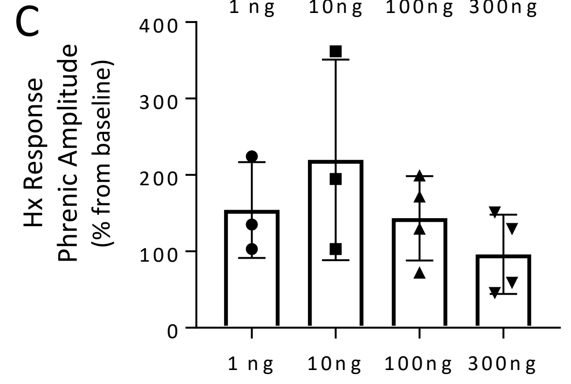

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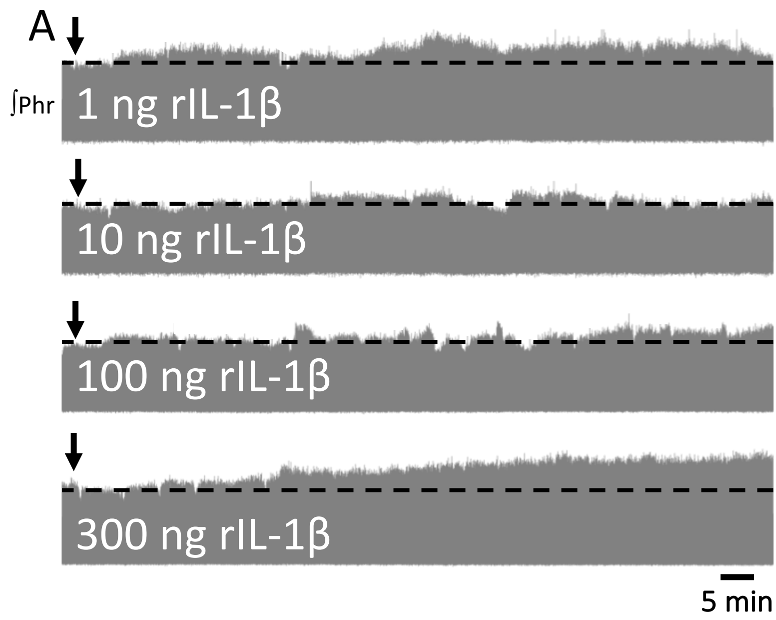

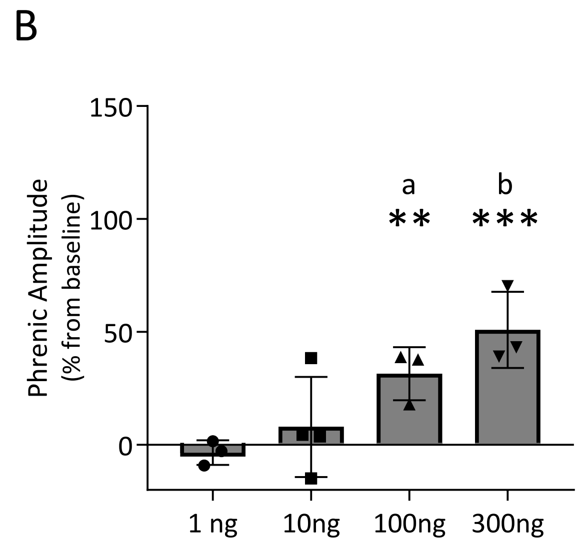

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