

Astrocytes regulate locomotion by orchestrating neuronal rhythmicity in the spinal network via potassium clearance

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¹**SUMMARY**

2 Neuronal rhythmogenesis in the spinal cord is correlated with variations in extracellular K⁺ levels
3 ([K⁺]_e]. Astrocytes play important role in[K⁺]_e homeostasis and compute neuronal information. Yet it ([K+]e). Astrocytes play important role in[K⁺ 3]e homeostasis and compute neuronal information. Yet it 4 is unclear how neuronal oscillations are regulated by astrocytic K⁺ homeostasis. Here we identify the
5 astrocytic inward-rectifying K⁺ channel Kir4.1 (a.k.a. *Kcni10*) as a key molecular player for neuronal 5 astrocytic inward-rectifying K⁺ channel Kir4.1 (a.k.a. *Kcnj10*) as a key molecular player for neuronal
6 rhythmicity in the spinal central pattern generator (CPG). By combining two-photon calcium imaging 7 with electrophysiology, immunohistochemistry and genetic tools, we report that astrocytes display 7×10^{-11} electrophysiology, immunology, immunology, immunology, we report that astrocytes display 6^{-2} α - Ca⁺ transients before and during oscillations of neighbouring neurons. Inhibition of astrocytic Ca⁺ 8. 8 α transients with BAPTA decreases the barium-sensitive Kir4.1 current responsible of K⁺ clearance.
10 Finally, we show in mice that Kir4.1 knockdown in astrocytes progressively prevents neuronal 11 oscillations and alters the locomotor pattern resulting in lower motor performances in challenging 11 oscillations and alters the locomotor pattern resulting in lower motor performances in challenging 12 tasks. These data identify astroglial Kir4.1 channels as key regulators of neuronal rhythmogenesis in the st 13 the CPG driving locomotion.

14

¹⁵**SIGNIFICANCE STATEMENT**

16 Despite decades of research, the cellular mechanisms responsible of the synchronized rhythmic
17 oscillations driving locomotion remain elusive. To gain insight into the function of the spinal 17 oscillations driving locomotion remain elusive. To gain insight into the function of the spinal 18 locomotor network, numerous studies have characterized diverse classes of locomotor-related 19 neurons to determine their role in generating rhythmic movements during locomotion. In contrast, 20 studies investigating non-neuronal components of the spinal cord are sparse. Our study represents a 21 significant breakthrough by identifying astrocytic K^+ uptake as a key regulator of neuronal
22 rhythmicity synchronization and locomotor pattern at the cellular, microcircuit and system levels. 23 These data provide mechanistic insights into the neuroglial dialogue at play during rhythmogenesis 23 These data provide mechanistic insights into the neuroglial dialogue at play during rhythmogenesis 24 and point to a novel astroglial target for restoring normal neuronal network excitability in brain 25 disorders and neurodegenerative diseases.

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1 **INTRODUCTION**
2 Mammalian locom
3 rhythmic central p:
6 of the lumbar spin
5 network is triggere
6 and dopamine. Se
7 rhythmic oscillation 2 Mamma Contract Pattern generator (CPG) network, which is mainly located in the ventromedial part
2 Mammalian do the lumbar spinal cord (Grillner and El Manira, 2020; Kiehn, 2016). Activation of the spinal CPG
2 Mamma Act 3 of the lumbar spinal cord (Grillner and El Manira, 2020; Kiehn, 2016). Activation of the spinal CPG
3 network is triggered by descending inputs releasing neuromodulators such as glutamate, serotonin
3 and dopamine. Sever metwork is triggered by descending inputs releasing neuromodulators such as glutamate, serotonin

46 and dopamine. Several populations of CPG interneurons - V0, V2a and Hb9 -display conditional

47 rhythmic oscillations in 5 and dopamine. Several populations of CPG interneurons - V0, V2a and Hb9 -display conditional

5 network inconsitions in response to neuromodulators (Grillner and El Manira, 2020; Kiehn, 2016). No

5 single population of 7 rhythmic oscillations in response to neuromodulators (Grillner and El Manira, 2020; Kiehn, 2016). No
8 single population of ventromedial interneurons appears to act alone or to orchestrate the entire CPG
9 pattern. The 7 single population of ventromedial interneurons appears to act alone or to orchestrate the entire CPG

9 pattern. The spinal rhythmogenesis emerges from the synchronization of individual neurons with

10 intrinsic bursti 8 spattern. The spinal rhythmogenesis emerges from the synchronization of individual neurons with

10 intrinsic bursting properties (Brocard et al., 2010). Despite decades of research, the functional

10 organization of th

9 pattern. The spinal represention of the locomotor CPG remains still unclear.

1 organization of the locomotor CPG remains still unclear.

2 In the spinal locomotor CPG, physiological variation in extracellular K⁺ ([K⁺ organization of the locomotor CPG remains still unclear.

12 In the spinal locomotor CPG, physiological variation in extracellular K⁺ ([K⁺]_e) coincides with the

14 emergence of the oscillatory pattern of intrinsic b 12

12 In the spinal locomotor CPG, physiological variation in

14 emergence of the oscillatory pattern of intrinsic burstil

15 powers up the motor output (Bos et al., 2018; Bracci, 1

16 serum levels (Kofuji and Newman, 13
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16
17
18 In the spinal locomotor CPG, physiological variation in extracellular K $([K]_e)$ coincides with the
emergence of the oscillatory pattern of intrinsic bursting neurons (Brocard et al., 2013), and thus
powers up the motor o 15 powers up the motor output (Bos et al., 2018; Bracci, 1998). At rest, $[K^+]_e$ is kept close to 3 mM in
16 serum levels (Kofuji and Newman, 2004; Verkhratsky et al., 2018). The maintenance of K⁺
17 homeostasis is one o powers up the motor output (Bos et al., 2018; Bracci, 1998). At rest, [K₊ 1s kept close to 3 mM in
16 serum levels (Kofuji and Newman, 2004; Verkhratsky et al., 2018). The maintenance of K⁺
17 homeostasis is one of the serum levels (Kofuji and Newman, 2004; Verkhratsky et al., 2018). The maintenance of K
homeostasis is one of the crucial supportive functions mediated by astrocytes (Ben Haim et al.,
2015; Sibille et al., 2015). In additio 2015; Sibille et al., 2015). In addition, to display a high resting K⁺ conductance that facilitates the uptake of neuron released K⁺, astrocytes have a hyperpolarized resting membrane potential (RMP).

20 Both paramete 2015; Sibille et al., 2015). In addition, to display a high resting K-conductance that facilitates the
19 uptake of neuron released K⁺, astrocytes have a hyperpolarized resting membrane potential (RMP).
20 Both parameter uptake of neuron released K⁻, astrocytes have a hyperpolarized resting membrane potential (RMP).

20 Both parameters rely on the activity of the weakly inwardly rectifying Kir4.1 K⁺ channel, which in the

21 nervous sy Both parameters rely on the activity of the weakly inwardly rectifying Kir4.1 K- channel, which in the
nervous system is expressed exclusively in glial cells, with the highest expression in astrocytes (Kelley
et al., 2018) 22 et al., 2018). Astrocytic Kir4.1 channels have been described in many CNS regions, including the spinal cord (Olsen et al., 2006; Ransom and Sontheimer, 1995). Studies using genetic, pharmacological and modeling approac 23 spinal cord (Olsen et al., 2006; Ransom and Sontheimer, 1995). Studies using genetic,
24 pharmacological and modeling approaches in the brain networks identified Kir4.1 as the main
25 astrocytic inwardly rectifying K⁺ 24 pharmacological and modeling approaches in the brain networks identified Kir4.1 as the main
25 astrocytic inwardly rectifying K^{*} channel regulating $[K^+]_e$ and thus influencing neuronal excitability
26 (Nwaobi et al.

25 astrocytic inwardly rectifying K⁺ channel regulating [K⁺]_e and thus influencing neuronal excitability
26 (Nwaobi et al., 2016; Sibille et al., 2015).
27 Astrocytes display ramified processes enwrapping synapses, p astrocytic inwardly rectifying K⁺ channel regulating [K⁺ 25 and thus influencing neuronal excitability

26 (Nwaobi et al., 2016; Sibille et al., 2015).

27 Astrocytes display ramified processes enwrapping synapses, pla 27 Astrocytes display ramified processes er
28 sense synaptic activity (Panatier et a
29 gliotransmitters (Araque et al., 2014;
30 strongly affected in the brain networks
31 modified (Lee et al., 2014; Sheikhbahae
32 oscil 28 sense synaptic activity (Panatier et al., 2011) and (ii) adjust network activity by releasing
29 gliotransmitters (Araque et al., 2014; Savtchouk and Volterra, 2018). Rhythmic oscillations are
30 strongly affected in t 29 gliotransmitters (Araque et al., 2014; Savtchouk and Volterra, 2018). Rhythmic oscillations are

30 strongly affected in the brain networks when gliotransmission is genetically or pharmacologically

31 modified (Lee et 22 strongly affected in the brain networks when gliotransmission is genetically or pharmacologically

2013 modified (Lee et al., 2014; Sheikhbahaei et al., 2018). In the spinal motor network, the locomotor

2020 oscillato 31 modified (Lee et al., 2014; Sheikhbahaei et al., 2018). In the spinal motor network, the locomotor
32 oscillatory rhythm is correlated with enhanced calcium (Ca²⁺) transients in astrocytes (Broadhead
33 and Miles, 20 32 oscillatory rhythm is correlated with enhanced calcium (Ca^{2+}) transients in astrocytes (Broadhead
33 and Miles, 2020), which likely triggers ATP release (Witts et al., 2015), modulating excitatory
34 synaptic transmi oscillatory rhythm is correlated with enhanced calcium (ca⁻) transients in astrocytes (Broadnead
and Miles, 2020), which likely triggers ATP release (Witts et al., 2015), modulating excitatory
synaptic transmission (Carl synaptic transmission (Carlsen and Perrier, 2014). Thus, growing evidence point to the contribution
of gliotransmission in modulating neuronal rhythmogenesis (Montalant et al., 2021). Astrocytes can
also respond to neurotr 35 of gliotransmission in modulating neuronal rhythmogenesis (Montalant et al., 2021). Astrocytes can
36 also respond to neurotransmitters and neuromodulators (Paukert et al., 2014; Rosa et al., 2015) by
37 modifying [K⁺ 36 also respond to neurotransmitters and neuromodulators (Paukert et al., 2014; Rosa et al., 2015) by
37 modifying [K⁺]_e, which greatly impact brain oscillations (Bellot-Saez et al., 2017; Ding et al., 2016;
38 Sibille 37 modifying $[K^+]_e$, which greatly impact brain oscillations (Bellot-Saez et al., 2017; Ding et al., 2016; Sibille et al., 2015). However, the mechanisms underlying the contribution of astrocytic K^+ homeostasis to rhy

modifying [K⁺]_e, which greatly impact brain oscillations (Bellot-Saez et al., 2017; Ding et al., 2016;
Sibille et al., 2015). However, the mechanisms underlying the contribution of astrocytic K⁺
homeostasis to rhyth Sibille et al., 2015). However, the mechanisms underlying the contribution of astrocytic K

homeostasis to rhythmogenesis remain incompletely understood.

40

Here we investigated whether astrocyte K⁺ uptake modulates ne 39 Homeon Christman Income is the remain incompletely protection.
39 Homeon expenditions remain incomplete that most of the ventrom
39 Homeonal oscillations, and that the inwardly-rectifying Kir4.1 change neuronal oscillat - 41
42
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44
45
46 Here we investigated whether astrocyte K+ uptake modulates neuronal oscillations patterns in the
spinal CPG network. We demonstrate that most of the ventromedial astrocytes are active during
neuronal oscillations, and that meuronal oscillations, and that the inwardly-rectifying Kir4.1 channels are crucial for maintaining

neuronal oscillations, which ultimately influence the locomotor activity. This study places astrocytes

as central elemen meuronal oscillations, which ultimately influence the locomotor activity. This study places astrocytes
as central elements of the spinal cord locomotor CPG.
RESULTS
Astrocytes respond to changes in extracellular ions un 44 as central elements of the spinal cord locomotor CPG.
46 **RESULTS**
47 **Astrocytes respond to changes in extracellular ions underlying neuronal oscillations in the spinal
48 CPG.** Astrocytes from the ventromedial part o

RESULTS
45 **RESULTS**
47 Astrocytes respond to changes in extracellular ions of
48 **CPG.** Astrocytes from the ventromedial part of uppe 46 **RESULTS**
47 Astrocytes
48 **CPG**. Astroc

 48 CPG. Astrocytes respond to changes in extracellular ions underlying neuronal oscillations in the spinal CPG. Astrocytes from the ventromedial part of upper lumbar segments (L1–L2), the main locus of 3 48 CPG. Astrocytes from the ventromedial part of upper lumbar segments (L1–L2), the main locus of

 $\mathbf{1}$ 1 the locomotor CPG (El Manira, 2014; Kiehn, 2016), were recorded from transgenic mice expressing 2 GFP under the control of the astrocytic Aldh1L1 promoter (Tsai et al., 2012) (Fig 1A). This spinal
2 region contains interneurons endowed with two types of inherent membrane oscillations at a
24 frequency range similar 3 frequency range similar to stepping rhythms: (i) one is TTX-sensitive dependent on the persistent
3 sodium current (INaP) (Tazerart et al., 2008) and triggered by a rise in $[K^+]_e$ with a concomitant
3 decrease in extra 4 frequency range similar to stepse) (Tazerart et al., 2008) and triggered by a rise in $[K^+]_e$ with a concomitant
6 decrease in extracellular Ca²⁺ levels ([Ca²⁺]_e) (Brocard et al., 2013), (ii) the other is TTX-inse decrease in extracellular Ca⁻ levels ([Ca⁻ \vert _e) (Brocard et al., 2013), (ii) the other is TTX-insensitive
dependent on the Ca²⁺ currents and triggered by a neuromodulator cocktail composed of 5-HT,
dopamine and dependent on the Ca⁻ currents and triggered by a neuromodulator cocktail composed of 5-HT,
dopamine and NMDA, in presence of the voltage-dependent sodium channels blocker, tetrodotoxin
(TTX) (Masino et al., 2012; Wilson (TTX) (Masino et al., 2012; Wilson, 2005; Ziskind-Conhaim et al., 2008). This neuromodulator cocktail

mimics the effect of descending inputs from the hindbrain for inducing a locomotor rhythmicity

(Grillner and El Manir 9 (TTX) (Masino et al., 2012; Wilson, 2005; Ziskind-Conhaim et al., 2008). This neuromodulator cocktail 11 (Grillner and El Manira, 2020). In recording conditions required to generate bursting cells, all

12 astrocytes displayed a reversible depolarisation of their membrane potentials: (i) when $[K^{\dagger}]_e$ was

13 increased astrocytes displayed a reversible depolarisation of their membrane potentials: (i) when $[K^+]_e$ was

11 increased to 6mM and $[Ca^{2+}]_e$ decreased to 0.9mM (Fig.1 B-C) or (ii) when the neuromodulator

11 cocktail was appli increased to 6mM and $[Ca^{-1}]_e$ decreased to 0.9mM (Fig.1 B-C) or (ii) when the neuromodulator
cocktail was applied in presence of TTX (Fig. 1 D-E). We observed that in absence of neuronal
activity, neuromodulators increas 21 activity, neuromodulators increased $[K^+]_0$ to ~6mM (Fig. S1). To explore the temporal dynamics of

216 the astrocytic response compared to neuronal oscillations, we performed dual recordings of a

217 ventromedial Hb9 17 ventromedial Hb9 GFP+ interneuron which is known to display the two types of bursting properties

18 (Tazerart et al., 2008; Ziskind-Conhaim et al., 2008) and an adjacent astrocyte from the upper lumbar

19 segments (L 18 (Tazerart et al., 2008; Ziskind-Conhaim et al., 2008) and an adjacent astrocyte from the upper lumbar
19 segments (L1-L2) (Fig. 1F). In 13 pairs, astrocytic depolarization in response to the TTX-sensitive
20 [K⁺]_e/[19 segments (L1-L2) (Fig. 1F). In 13 pairs, astrocytic depolarization in response to the TTX-sensitive

20 [K⁺]_e/[Ca²⁺]_e variations (Fig. 1 G) or the TTX-insensitive neuromodulator cocktail (Fig. 1H) either

21 pr 19 $\left[\text{K}^{\ast}\right]_e/[\text{Ca}^{2+}]_e$ variations (Fig. 1 G) or the TTX-insensitive neuromodulator cocktail (Fig. 1H) either

21 preceded (69%) or followed (31%) the onset of the neuronal rhythmic bursting of the adjacent GFP+
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bet

22 pacemaker interneurons (Fig. 11), suggesting that astrocytes play an active role in spinal
23 rhythmogenesis.
24 **Enhanced astrocytic Ca²⁺ transients occur during neuronal oscillations in the ventral spinal cord.** To 23 rhythmogenesis.

24 **Enhanced astrocytic Ca²⁺ transients occur during neuronal oscillations in the ventral spinal cord.** To

26 better locate and characterize the dialogue between neurons and astrocytes during spinal 24
25 **Enhanced astrocy**
26 better locate are
27 oscillations, we
28 locomotor CPG a
29 S2A) designed to 25
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31 Enhanced astrocytic Ca⁻
L Eminical district of a characterize the dialogue between neurons and astrocytes during spinal

27 oscillations, we performed two-photon Ca²⁺ imaging of neuronal and astrocyte signals in the

28 locomotor CPG area. Mice 27 oscillations, we performed two-photon Ca²⁺ imaging of neuronal and astrocyte signals in the locomotor CPG area. Mice were injected at birth with adeno-associated virus serotype 9 (AAV9) (Fig. 52A) designed to express 29 S2A) designed to express GCaMp6f in astrocytic processes under the gfaABC1D promoter and

30 jRGECO1a in neurons under the Syn.NES promoter (Fig. S2B). We simultaneously imaged the

31 chromophores in lumbar slices (L1 29 S2A) designed to express GCaMp6f in astrocytic processes under the gfaABC1D promoter and

30 jRGEC01a in neurons under the Syn.NES promoter (Fig. S2B). We simultaneously imaged the

31 chromophores in lumbar slices (L1

31 chromophores in lumbar slices (L1-L2) of post-natal mice (day 13-16) and first confirmed that
32 neuronal signals were prevented by TTX, in contrast to the signals recorded astrocyte processes that
33 remain intact (Fi 32 neuronal signals were prevented by TTX, in contrast to the signals recorded astrocyte processes that
33 remain intact (Fig. S2C-D).
34 We next explored the spatio-temporal dynamics of the astrocytic transients and comp 33 remain intact (Fig. S2C-D).
34 We next explored the spatio-temporal dynamics of the astrocytic transients and compared it to the
35 neuronal transients. In response to changes in extracellular K^{*} and Ca²⁺ levels or 34 We next explored the spate
35 neuronal transients. In res
36 the neuromodulator cock
37 0.03 Hz and 0.15 ± 0.01 Hz
38 transients in processes (Fig. Ca^{2+} signals. Some astrocyt 35 neuronal transients. In response to changes in extracellular K⁺ and Ca²⁺ levels or bath application of
36 the neuromodulator cocktail in presence of TTX, neurons displayed Ca²⁺ oscillatory transients (0.16 ±
37 0 neuronal transients. In response to changes in extracellular K and Ca⁻ levels or bath application of

the neuromodulator cocktail in presence of TTX, neurons displayed Ca²⁺ oscillatory transients (0.16 ±

0.03 Hz and the neuromodulator cocktail in presence of TTX, neurons displayed Ca⁻ oscillatory transients (0.16 ± 0.03 Hz and 0.15 ± 0.01 Hz, respectively) (Fig. 2A-B), which correlated with enhanced astrocytic Ca²⁺ transients in 0.03 Hz and 0.15 ± 0.01 Hz, respectively) (Fig. 2A-B), which correlated with enhanced astrocytic Catransients in processes (Fig. 2A and 2C). Interestingly, we distinguished distinct subsets of astrocytic Ca²⁺ signals. S Ca^{2+} signals. Some astrocytes showed increased Ca^{2+} transients before the onset of the neuronal Ca^{2+} oscillations, in contrast to others, which displayed Ca^{2+} transients during neuronal oscillations (Fig. 2A). oscillations, in contrast to others, which displayed Ca²⁺ transients during neuronal oscillations (Fig. 2A). Quantification showed that the occurrence of Ca²⁺ transients preceded the neuronal oscillations in ~64% of a 2A). Quantification showed that the occurrence of Ca^{2+} transients preceded the neuronal oscillations in ~64% of astrocytes, whereas in ~36% of them the Ca^{2+} transients occurred during neuronal oscillations (Fig. 2D) oscillations (Fig. 2D). These data confirmed our electrophysiological dual recordings (Fig. 11). We also
observed that most of the astrocytes (\sim 70%) showing Ca²⁺ signal increases in response to ionic
variations or pe 44 observed that most of the astrocytes (~70%) showing Ca²⁺ signal increases in response to ionic
45 variations or perfusion of the neuromodulator cocktail were located in the ventromedial part of the
46 spinal cord (Fi

146 spinal cord (Fig. 2E). Altogether, these data suggest that ventromedial astrocytes are functionally

147 coupled with neuronal rhythmicity in the spinal CPG area.

148 The astrocytic Ca²⁺ transients regulate the Kir coupled with neuronal rhythmicity in the spinal CPG area.

48 The astrocytic Ca²⁺ transients regulate the Kir4.1-mediated inwardly rectifying K⁺ current. Because

50 astrocytic Ca²⁺ signals modulate [K⁺]_e in hip 48
49 The astrocytic Ca²⁺ transients regulate the Kir4.1-mediat
50 astrocytic Ca²⁺ signals modulate $[K^{\dagger}]_e$ in hippocampus (Wa
51 al., 2012b), we then investigated whether the increase 49
50
51 The astrocytic Ca $^{-}$ transients regulate the Kir4.1-mediated inwardly rectifying K+ 50 astrocytic Ca²⁺ signals modulate $[K^{\dagger}]_e$ in hippocampus (Wang et al., 2012a) and cerebellum (Wang et al., 2012b), we then investigated whether the increase in astrocytic Ca²⁺ transients related to 4
4 al., 2012b), we then investigated whether the increase in astrocytic Ca²⁺ transients related to al., 2012b), we then investigated whether the increase in astrocytic Ca⁺ transients related to
4
4

neuronal oscillations in the spinal CPG area (Fig. 2) stimulates K uptake. We first recorded the
passive membrane properties of the ventromedial GFP+ astrocytes from upper lumbar slices (Fig.
3 3A-B) and then isolated the 2 BA-B) and then isolated the Ba²⁺-sensitive Kir4.1 current underlying K⁺ uptake in standard artificial

2 cerebro-spinal fluid (aCSF) (Fig. 3C-D). We then switched to oscillatory aCSF by increasing $[K^+]_e$ to

5 6 mM 3A-B) and then isolated the Ba⁻-sensitive Kir4.1 current underlying K- uptake in standard artificial
cerebro-spinal fluid (aCSF) (Fig. 3C-D). We then switched to oscillatory aCSF by increasing $[K^+]_e$ to
6 mM and reduci cerebro-spinal fluid (aCSF) (Fig. 3C-D). We then switched to oscillatory aCSF by increasing [K \vert_e to 6mM and reducing [Ca²⁺]_e to 0.9mM, which leads to neuronal bursting (Fig. 1G and Fig. 2A-B) and intracellular ast 6 mM and reducing $|Ca^2|_e$ to 0.9mM, which leads to neuronal bursting (Fig. 1G and Fig. 2A-B) and intracellular astrocytic Ca^{2+} transients (Fig. 2A and 2C). In this condition, we observed that preventing the astrocyti intracellular astrocytic Ca⁻ transients (Fig. 2A and 2C). In this condition, we observed that

preventing the astrocytic Ca²⁺ transients with BAPTA (30mM) inside the patch pipette significantly

reduced the barium (Ba preventing the astrocytic Ca² transients with BAPTA (30mM) inside the patch pipette significantly

reduced the barium (Ba²⁺)-sensitive current driven by Kir4.1 channels (Fig. 3 E-F). These data strongly

suggest that

reduced the barium (Ba²⁺)-sensitive current driven by Kir4.1 channels (Fig. 3 E-F). These data strongly

suggest that the increase in intracellular Ca²⁺ from ventromedial astrocytes observed during

neuronal oscillati suggest that the increase in intracellular Ca⁻ from ventromedial astrocytes observed during
neuronal oscillations modulates K⁺ uptake via Kir4.1 channels.
Kir4.1 channels are expressed in astrocytes from the spinal lo neuronal oscillations modulates K- uptake via Kir4.1 channels.

11

12 **Kir4.1 channels are expressed in astrocytes from the spi**

investigated whether Kir4.1 channels are expressed in the sp

14 mice which express GFP+ in 12
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19 12 Kir4.1 channels are expressed in astrocytes from the spinal locomotor CPG region. We hext

13 investigated whether Kir4.1 channels are expressed in the spinal locomotor CPG. Using Hb9-eGFP

14 mice which express GFP+ in mostigated whether Kir4.1 channels are expressed in the spinal locomotor Cro. Osing Hb9-eGFT

13 mice which express GFP+ interneurons with intrinsic bursting properties (Tazerart et al., 2008;

15 Ziskind-Conhaim et al., 2 21 μ mice (Cahoy et al., 2008), we found that ventromedial GFP+ interneurons were enwrapped by

16 Kir4.1 labeling, which is diffusely expressed in the grey matter of the upper lumbar segments (Fig.

17 S3A). To confir Kir4.1 labeling, which is diffusely expressed in the grey matter of the upper lumbar segments (Fig. 53A). To confirm that Kir4.1 channels in the locomotor CPG area are exclusively expressed in glial
cells with a highest ex 17 S3A). To confirm that Kir4.1 channels in the locomotor CPG area are exclusively expressed in glial
18 cells with a highest expression in astrocytes, we used the *Aldh1L1-eGFP* astrocyte-specific reporter
19 mice (Cahoy cells with a highest expression in astrocytes, we used the *Aldh1L1-eGFP* astrocyte-specific reporter
mice (Cahoy et al., 2008). We examined co-expression of *Aldh1L1-eGFP* with immunolabeling of
Kir4.1 channels and neuron 18 cells with a highest expression in astrocytes, we used the Aldh1L1-eGFP astrocyte specific reporter

18 mice (Cahoy et al., 2008). We examined co-expression of *Aldh1L1-eGFP* with immunolabeling of

The Ba²⁺-sensitive 19 mice (canoy et al., 2006). We examined co-expression of Aldh1L1-eGFP with immunolabeling of

19 Kir4.1 channels and neuronal marker NeuN in lumbar slices. We observed that 100% of the GFP+

19 astrocytes expressed Kir4.

21 astrocytes expressed Kir4.1 channels contrary to NeuN+ cells, which lacked Kir4.1 staining (Fig. S3B-
22 C).
23 The Ba²⁺-sensitive inwardly rectifying K⁺ (Kir4.1) current is crucial for maintaining neuronal
25 osci 27 interneurons from Hb9-eGFP mice in response to $[K^1]_{\alpha}/[Ca^{2+}]}_{\alpha}$ variations (Fig. 4A) or bath perfusion
28 of the neuromodulator cocktail in presence of TTX (Fig. 4B). In both conditions, pharmacological 23

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26 the

27 internal

28 of the - 24 25 26 27 28 29 20 25 oscillations and locomotor-like activity in the CPG. We next studied the role of Kir4.1 channels in
26 the lumbar rhythmogenesis. We first induced rhythmic oscillations in the GFP+ ventromedial
27 interneurons from Hb9 25 **oscillations and locomotor-like activity** in the CFG. We next studied the role of Kir4.1 channels in
26 the lumbar rhythmogenesis. We first induced rhythmic oscillations in the GFP+ ventromedial
29 of the neuromodulat 27 interneurons from Hb9-eGFP mice in response to $[K^+]_e/[Ca^{2+}]_e$ variations (Fig. 4A) or bath perfusion
28 of the neuromodulator cocktail in presence of TTX (Fig. 4B). In both conditions, pharmacological
29 blockade of K blockade of Kir4.1 channels with Ba²⁺ (100 µM) caused a shift in the spiking pattern from bursting to

30 tonic firing in ~70% of the TTX-sensitive neuronal oscillations (Fig. 4C) or resulted in a cessation of

bursting 31 bursting activity (~85%) of the TTX-insensitive neuronal oscillations (Fig. 4D). Interestingly, we noticed that before totally preventing the neuronal oscillations, Ba²⁺ progressively decreased the rhythmic bursting 32 noticed that before totally preventing the neuronal oscillations, Ba²⁺ progressively decreased the rhythmic bursting frequency accompanied with an increase in burst amplitude and duration in both conditions (Fig. S4) 34 conditions (Fig. S4). We next confirmed the Ba²⁺ effect on preventing neuronal oscillations at a larger
35 scale by two-photon Ca²⁺ imaging of cell populations. We observed a progressive loss of Ca²⁺
36 oscillati conditions (Fig. S4). We next confirmed the Ba²⁺ effect on preventing neuronal oscillations at a larger
scale by two-photon Ca²⁺ imaging of cell populations. We observed a progressive loss of Ca²⁺
oscillations in th

scale by two-photon Ca⁻ imaging of cell populations. We observed a progressive loss of Ca⁻ oscillations in the vast majority of neurons in response to Ba²⁺ in presence of the oscillatory aCSF (n=25/28) or the neurom oscillations in the vast majority of neurons in response to Ba²⁺ in presence of the oscillatory aCSF (n=25/28) or the neuromodulator cocktail (n=7/9) (Fig. 4E-F). Bath application of Ba²⁺ also induced a significant ri (n=25/28) or the neuromodulator cocktail (n=7/9) (Fig. 4E-F). Bath application or Ba⁻ also induced a
significant rise in amplitude of the astrocytic Ca²⁺ signal in both conditions (Fig. 4G).
To confirm the functional significant rise in amplitude of the astrocytic Ca⁻ signal in both conditions (Fig. 4G).

To confirm the functional role of the inwardly rectifying K⁺ (Kir4.1) current in

thythmogenesis, we investigated the contribut To confirm the functional role of the inwardly rectifying K (Kir4.1) current in the integrated

A0 rhythmogenesis, we investigated the contribution of Kir4.1 in the locomotor rhythm-generating

network by using ex vivo wh 41 network by using *ex vivo* whole-mount spinal cords. Since rostral lumbar segments (L1–L2) have a
42 more powerful rhythmogenic capacity than the caudal ones, we recorded locomotor-like activities
43 from the contrala 42 more powerful rhythmogenic capacity than the caudal ones, we recorded locomotor-like activities
43 from the contralateral L2 ventral roots (Fig. 5A) in response to ionic variations (Fig. 5B) or bath
44 application of a From the contralateral L2 ventral roots (Fig. 5A) in response to ionic variations (Fig. 5B) or bath
application of a neuromodulator cocktail composed of N-methyl-DL aspartate (NMA) and 5
hydroxytryptamine (5-HT) (Fig. 5C) application of a neuromodulator cocktail composed of N-methyl-DL aspartate (NMA) and 5
hydroxytryptamine (5-HT) (Fig. 5C). In both conditions, Ba²⁺ (100 μ M) slowed down the locomotor
rhythm by increasing the locomoto 45 hydroxytryptamine (5-HT) (Fig. 5C). In both conditions, Ba²⁺ (100 μM) slowed down the locomotor rhythm by increasing the locomotor burst duration and decreasing the burst frequency without apparent effect on burst a apparent effect on burst amplitude (Fig 5B-D). We also observed a decrease in the cross-correlogram
coefficient in presence of Ba²⁺ related to a decreased rhythmic alternation (Fig 5E-F) confirming the
role of Kir4.1 ch $\frac{1}{48}$ coefficient in presence of Ba²⁺ related to a decreased rhythmic alternation (Fig 5E-F) confirming the

49 role of Kir4.1 channels in modulating rhythmogenesis at the network level.

50 coefficient in presence of Ba² related to a decreased rhythmic alternation (Fig 5E-F) confirming the role of Kir4.1 channels in modulating rhythmogenesis at the network level.
50 **SA2+ 1** channels in modulating rhythmoge $\frac{3}{4}$

1 Loss-of-function of Kir4.1 decreases the probability for ventromedial era interneurons to oscillate
1 To further explore the functional role of Kir4.1 channels in spinal rhythmicity, we performed their
13 transfection sp targeted loss-of function by using a short hairpin RNAs (shRNAs). We injected intrathecally at birth at

213–11 level an AAV9 virus encoding the Kir4.1-shRNA with a eGFP reporter (Fig. 6A-B). The viral

12 transfection led 3 T13–L1 level an AAV9 virus encoding the Kir4.1-shRNA with a eGFP reporter (Fig. 6A-B). The viral
3 transfection led to a decrease of ~40% in Kir4.1 membrane protein expression in the lumbar spinal
3 cord (Fig. 6C). Conc 4 Transfection led to a decrease of ~40% in Kir4.1 membrane protein expression in the lumbar spinal

4 Cord (Fig. 6C). Concomitantly, a strong expression of eGFP in the ventromedial part of the spinal cord

4 Transfected 5 transfer of (Fig. 6C). Concomitantly, a strong expression of eGFP in the ventromedial part of the spinal cord

5 was observed from T1 to L5-S1 (Fig. 6D, Fig. S5). We first examined the effect of Kir4.1-shRNA on

5 glial was observed from T1 to L5-S1 (Fig. 6D, Fig. S5). We first examined the effect of Kir4.1-shRNA on
glial and neuronal electrophysiological properties. In the virally transfected GFP+ astrocytes (Fig.
56A-B), we observed in glial and neuronal electrophysiological properties. In the virally transfected GFP+ astrocytes (Fig. 56A-B), we observed in whole-cell configuration a more depolarized RMP (Fig. S6C), a higher input resistance (Fig. S6C), 8 864-B), we observed in whole-cell configuration a more depolarized RMP (Fig. S6C), a higher input

1 resistance (Fig. S6C), a marked change in the current-voltage (I-V) relationship and a reduced Ba²⁺-

2 we also obser resistance (Fig. S6C), a marked change in the current-voltage (I-V) relationship and a reduced Ba²⁺-
sensitive current in mice transduced with Kir4.1-ShRNA compared to Ctrl-ShRNA mice (Fig. S6D-I).
We also observed that We also observed that the RMPs of ventromedial interneurons surrounded by the virally transfected

11 GFP+ astrocytes (Fig. 6E) from mice transduced with Kir4.1-ShRNA were overall more depolarized

14 than the RMPs of inte 12 GFP+ astrocytes (Fig. 6E) from mice transduced with Kir4.1-ShRNA were overall more depolarized

13 GFP+ astrocytes (Fig. 6E) from mice transduced with Kir4.1-ShRNA were overall more depolarized

14 knockdown of Kir4.1 i 14 than the RMPs of interneurons from mice infected with Ctrl-ShRNA (Fig. 6E-F), suggesting that
15 knockdown of Kir4.1 in astrocytes had a widespread impact on the excitability of neighbouring
16 neurons.
17 We then teste

15 knockdown of Kir4.1 in astrocytes had a widespread impact on the excitability of neighbouring
16 neurons.
17 We then tested the ability of the ventromedial interneurons to oscillate after the viral transfection of
18 t 16 meurons.

17 We then tested the ability of the ventromedial interneurons to oscillate after the viral transfection of

18 the spinal cord. We found a significant lower proportion of rhythmic bursting in ventromedial

19 17 We then 1
18 the spina
19 interneur
20 Ctrl-ShRN
21 data den
22 unterne the spinal cord. We found a significant lower proportion of rhythmic bursting in ventromedial

19 interneurons surrounded by GFP+ astrocytes in mice transduced with Kir4.1-ShRNA compared to

20 Ctrl-ShRNA in response to bo 19 interneurons surrounded by GFP+ astrocytes in mice transduced with Kir4.1-ShRNA compared to

20 Ctrl-ShRNA in response to both ionic variations (~24% vs 80%) (Fig. 6G and 6I) or bath perfusion of

21 the neuromodulator 19 interneurons surrounded by GFP+ astrocytes in mice transduced with Kir4.1-ShRNA compared to 21 the neuromodulator cocktail in presence of TTX (\approx 23% vs 75 %) (Fig. 6H and 6J). Altogether, these
22 data demonstrate the pivotal role of astrocytic Kir4.1 channels in the oscillatory pattern of
23 ventromedial int

21 the neuromodulator cocktail in presence of TTX (\sim 23% vs 75 %) (Fig. 6H and 6J). Altogether, these
22 data demonstrate the pivotal role of astrocytic Kir4.1 channels in the oscillatory pattern of
24 **Loss-of-functio** 28 the Kir4.1-shRNA or the Ctrl-shRNA. From 12 to 18 days after the viral injection, we performed a set
29 of behavioural locomotor tests on freely moving mice (Fig. 7A). We first used footprint analysis to 24
25 **Loss-of-function of Kir4.1 slows down the performances.** We then tested whether timpact on the locomotor behaviors. We the Kir4.1-shRNA or the Ctrl-shRNA. From of behavioural locomotor tests on freely 25
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31 25 Loss-of-function of Kir4.1 slows down the *in vivo* locomotor pattern and decreases the locomotor
26 **performances**. We then tested whether the knockdown of Kir4.1 in the spinal cord has a functional
27 the Kir4.1-shRNA performances. We then tested whether the knockdown of Kir4.1 in the spinal cord has a functional
28 impact on the locomotor behaviors. We thus injected intrathecally at birth an AAV9 encoding either
28 of behavioural locom 22 impact on the Ctrl-shRNA. From 12 to 18 days after the viral injection, we performed a set
29 of behavioural locomotor tests on freely moving mice (Fig. 7A). We first used footprint analysis to
29 or locomotor pattern (29 of behavioural locomotor tests on freely moving mice (Fig. 7A). We first used footprint analysis to evaluate the locomotor pattern (Fig. 7B). Mice transduced with Kir4.1-shRNA displayed an increase in both swing and sta evaluate the locomotor pattern (Fig. 7B). Mice transduced with Kir4.1-shRNA displayed an increase

21 in both swing and stand phases compared to Ctrl-ShRNA mice (Fig. 7C-D) related to a slower speed

22 of locomotion and a in both swing and stand phases compared to Ctrl-ShRNA mice (Fig. 7C-D) related to a slower speed
32 of locomotion and a decrease in regularity index without any modification of the base of support
33 (Fig. 7D). We next tes 32 of locomotion and a decrease in regularity index without any modification of the base of support (Fig. 7D). We next tested whether Kir4.1 channels also modulate locomotor behaviours in more challenging tasks. We thus as (Fig. 7D). We next tested whether Kir4.1 channels also modulate locomotor behaviours in more

challenging tasks. We thus assayed motor coordination using the rotarod and swimming forced test

without practice sessions to a challenging tasks. We thus assayed motor coordination using the rotarod and swimming forced test
35 without practice sessions to avoid any compensatory learning mechanisms. Consistently, mice
36 transduced with Kir4.1-shRN 35 without practice sessions to avoid any compensatory learning mechanisms. Consistently, mice
36 transduced with Kir4.1-shRNA failed to adapt to accelerated speed (Fig. 7E) and displayed a lower
37 swimming performance (F 135 transduced with Kir4.1-shRNA failed to adapt to accelerated speed (Fig. 7E) and displayed a lower
37 swimming performance (Fig. 7F). Importantly, we did not observe any modification of the
38 motoneuronal soma size in Solution and the maintenance of the locomotor pattern in freely moving mice.

38 motoneuronal soma size in mice transduced with Kir4.1-ShRNA compared to Ctrl-ShRNA mice (Fig. 57). In sum, the Kir4.1-mediated inwardly recti motoneuronal soma size in mice transduced with Kir4.1-ShRNA compared to Ctrl-ShRNA mice (Fig. 39 S7). In sum, the Kir4.1-mediated inwardly rectifying K⁺ current appears to play a key role for locomotor coordination and t 39 S7). In sum, the Kir4.1-mediated inwardly rectifying K⁺ current appears to play a key role for
40 locomotor coordination and the maintenance of the locomotor pattern in freely moving mice.
41 **DISCUSSION**
43 This stu

51 performances in challenging tasks.

S7). In sum, the Kir4.1-mediated inwardly rectifying K current appears to play a key role for
docomotor coordination and the maintenance of the locomotor pattern in freely moving mice.
DISCUSSION
This study demonstrates **DISCUSSION**
42 **DISCUSSION**
43 This study demonstrates that astrocytic regulation of extracellular K⁺ plays a critical r
44 maintenance of neuronal rhythmogenesis in the spinal cord motor circuitry. We report that
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47 42 **DISCUSSION**
43 This study der
44 maintenance of
45 astrocytes from
46 Inhibiting astro
48 astrocytes env This study demonstrates that astrocytic regulation of extracellular K- plays a critical role in

maintenance of neuronal rhythmogenesis in the spinal cord motor circuitry. We report that spinal

astrocytes from neonatal m 44 astrocytes from neonatal mice display Ca²⁺ signals before and during rhythmic neuronal oscillations.
46 Inhibiting astrocytic Ca²⁺ transients with BAPTA decreases the Ba²⁺-sensitive inwardly rectifying K⁺
47 cu astrocytes from neonatal mice display Ca^{2+} signals before and during rhythmic neuronal oscillations.
46 Inhibiting astrocytic Ca^{2+} transients with BAPTA decreases the Ba²⁺-sensitive inwardly rectifying K⁺ curren current underlying K⁺ uptake. The Kir4.1 channels mediating the K⁺ current are expressed in performances in challenging tasks. astrocytes enwrapping the ventromedial interneurons from the locomotor CPG. We also
demonstrated that knockdown of Kir4.1 decreases the ability of the ventromedial interneurons to
oscillate. This perturbation results in an demonstrated that knockdown of Kir4.1 decreases the ability of the ventromedial interneurons to
50 oscillate. This perturbation results in an alteration of the locomotor pattern and in lower locomotor
51 performances in ch 49 oscillate. This perturbation results in an alteration of the locomotor pattern and in lower locomotor
51 performances in challenging tasks.
6 51 performances in challenging tasks.

Functional coupling of astrocytic Ca²⁺ signals and neuronal oscillations. Recent studies have highlighted unexpected contribution of astrocytic Ca²⁺ signals in regulating rhythmic behaviours including respiration (S including respiration (Sheikhbahaei et al., 2018), mastication (Morquette et al. 2015) and locomotion

(Broadhead and Miles, 2020). However, it is still incompletely understood whether the increased

astrocytic Ca²⁺ tra 3 (Broadhead and Miles, 2020). However, it is still incompletely understood whether the increased
3 astrocytic Ca²⁺ transient is a prerequisite or a consequence of neuronal oscillations. Here, we reveal
3 that ventromed 4 astrocytic Ca²⁺ transient is a prerequisite or a consequence of neuronal oscillations. Here, we reveal
that ventromedial astrocytes depolarize and display an increase in Ca²⁺ signals both before and in
response to i that ventromedial astrocytes depolarize and display an increase in Ca² signals both before and in

response to ions and neuromodulator changes related to neuronal oscillations. Our two-photon Ca²⁺

imaging data reveal response to ions and neuromodulator changes related to neuronal oscillations. Our two-photon Ca⁻

imaging data reveal a functional coupling between spinal cord astrocytes and CPG interneurons. The

simultaneous imaging 8 imaging a functional and astrocytic activity led to the characterization of distinct
8 imaging of neuronal and astrocytic activity led to the characterization of distinct
8 in socillation. This active role of astrocytes 9 subsets of active astrocytes in the spinal CPG area. In one subset, the response precedes neuronal

9 occillation. This active role of astrocytes in rhythmicity has been suggested in the trigeminal

9 sensorimotor circu 11 oscillation. This active role of astrocytes in rhythmicity has been suggested in the trigeminal

12 sensorimotor circuit (Morquette et al., 2015) or in cortical areas where the increase in astrocytic Ca²⁺

13 events 12 sensorimotor circuit (Morquette et al., 2015) or in cortical areas where the increase in astrocytic Ca²⁺ events precedes the switch to the slow-oscillation state (Poskanzer and Yuste, 2016). In another group, the res 14 group, the responses are concomitant to neuronal bursting. In line with this, in Drosophila astrocyte

15 Ca²⁺ transients are driven by rhythmic firing of the octopaminergic neurons (Ma et al., 2016). A

16 recent st Ca^{2+} transients are driven by rhythmic firing of the octopaminergic neurons (Ma et al., 2016). A

16 recent study also demonstrated such a dynamics in fish radial astroglia (analogs of the mammalian

17 astrocyte), whe 17 astrocyte), where the noradrenergic neuronal activity precedes the Ca²⁺ wave observed in radial
18 astroglia (Mu et al., 2019). However, we cannot totally rule out that one astrocyte may belong to
19 both groups. Alt astrocyte), where the noradrenergic neuronal activity precedes the Ca⁻ wave observed in radial
astroglia (Mu et al., 2019). However, we cannot totally rule out that one astrocyte may belong to
both groups. Altogether, o

19 both groups. Altogether, our data highlight a bi-directional coupling between astrocytic Ca²⁺ signal

20 and neuronal oscillations.

21 **The Ca²⁺-dependent K⁺ uptake in spinal astrocytes.** Astrocytic Ca²⁺ trans 21

22 The Ca²⁺-dependent K⁺ up

23 gliotransmission (Araque

24 classical gliotransmission

25 GPCR-mediated Ca²⁺ signa

26 (Wang et al., 2012a) and - 22 23 24 25 26 27 20 The ca^- -dependent K 22 **The Ca²⁺-dependent K^{*} uptake in spinal astrocytes.** Astrocytic Ca²⁺ transients are often associated to gliotransmission (Araque et al. 2014). Here we propose that an alternative mechanism to the classical gliotr 26 (Wang et al., 2012a) and cerebellum (Wang et al., 2012b). This astrocytic Ca²⁺ transients stimulate
27 the Na⁺, K⁺ ATPase pump resulting in a transient decrease in [K⁺]_e. In our study, we provide for the
28 f 25 GPCR-mediated Ca²⁺ signalling in astrocytes is linked to an increase of K⁺ uptake in hippocampus (Wang et al., 2012a) and cerebellum (Wang et al., 2012b). This astrocytic Ca²⁺ transients stimulate the Na⁺, K⁺ (Wang et al., 2012a) and cerebellum (Wang et al., 2012b). This astrocytic Ca⁻ transients stimulate

27 the Na⁺, K⁺ ATPase pump resulting in a transient decrease in [K⁺]_e. In our study, we provide for the

28 fir the Na , K ATPase pump resulting in a transient decrease in [K]_e. In our study, we provide for the

first time evidence that a blockade of the Ca²⁺ transients from ventromedial lumbar astrocytes

significantly reduce , K
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ed cell; First time evidence that a blockade of the Ca²⁺ transients from ventromedial lumbar astrocytes
significantly reduces the Ba²⁺-sensitive current mediated by Kir4.1. This result is in line with
observations showing that significantly reduces the Ba²⁺-sensitive current mediated by Kir4.1. This result is in line with

30 observations showing that menthol-induced Ca²⁺ transients in astrocytes increases the Ba²⁺-sensitive

31 Kir4.1 cu observations showing that menthol-induced Ca⁻ transients in astrocytes increases the Ba⁻-sensitive

Kir4.1 current from a U251 cell line (Ratto et al., 2020). Because (i) the locomotor-like activity is

associated wit

32 associated with $[K^+]_e$ homeostasis (Brocard et al., 2013), and (ii) the astrocytic K⁺ uptake is activated
33 by intracellular Ca²⁺ transients (Wang et al., 2012a), we assume that the Ca²⁺ dependent astrocytic K by intracellular Ca² transients (Wang et al., 2012a), we assume that the Ca² dependent astrocytic K

homeostasis plays a key role in the spinal rhythmicity.
 Astrocytic-dependent K⁺ regulation of the spinal rhythmo 35
35 **Astrocytic-dependent K⁺ regulation of the spinal**
37 homeostasis by astrocytes is crucial in modulating
38 Neprasova et al., 2007). However, whether spinal ameuronal bursting, as suggested by Ding et al. 20
40 co 36
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41 Astrocytic-dependent K+ **Astrocytic-dependent K** regulation of the spinal rhythmogenesis. The maintenance of K

homeostasis by astrocytes is crucial in modulating neuronal excitability (Dallérac et al., 2013;

Neprasova et al., 2007). However, w 38 Neprasova et al., 2007). However, whether spinal astrocytes may exert a "ionostatic control" of
39 neuronal bursting, as suggested by Ding et al. 2016, is still unclear. Here, we evaluated the
30 consequence of blockin 39 neuronal bursting, as suggested by Ding et al. 2016, is still unclear. Here, we evaluated the

40 consequence of blocking the astrocytic inwardly rectifying K⁺ (Kir4.1) channels on neuronal

39 oscillations in the lo 39 neuronal oscillations in the locomotor CPG. Blocking the Kir4.1-mediated astrocytic K⁺ (Kir4.1) channels on neuronal oscillations in the locomotor CPG. Blocking the Kir4.1-mediated astrocytic K⁺ uptake with Ba²⁺ consequence of blocking the astrocytic inwardly rectifying K⁺ (Kir4.1) channels on neuronal
oscillations in the locomotor CPG. Blocking the Kir4.1-mediated astrocytic K⁺ uptake with Ba²⁺ (100
 μ M) (Cui et al., 201 oscillations in the locomotor CPG. Blocking the Kir4.1-mediated astrocytic K⁺ uptake with Ba²⁺ (100 μ M) (Cui et al., 2018; Djukic et al., 2007; Ransom and Sontheimer, 1995) decreases the frequency of neuronal oscil oscillations in the locomotor CPG. Blocking the Kir4.1-mediated astrocytic K uptake with Ba⁻ (100 μ M) (Cui et al., 2018; Djukic et al., 2007; Ransom and Sontheimer, 1995) decreases the frequency of neuronal oscillati 44
44 neuronal oscillations until progressively preventing them. In line with this effect, injection of Kir4.1-
5hRNA resulting in the decrease in Kir4.1 astrocytic membrane expression also significantly reduces
45 the pro ShRNA resulting in the decrease in Kir4.1 astrocytic membrane expression also significantly reduces
the probability of driving neuronal oscillations. These results highlight the key role of astrocytic K^{*}
uptake in mainta the probability of driving neuronal oscillations. These results highlight the key role of astrocytic K⁺
46 uptake in maintaining the neuronal oscillatory pattern. Although the astrocytic Kir4.1 channels are
47 not homoge 47 not homogeneously expressed among and within brain structures (Higashi et al. 2001), we showed a
48 strong Kir4.1 expression in astrocytes enwrapping the ventromedial bursting interneurons and
49 lamina IX motoneurons. 48 strong Kir4.1 expression in astrocytes enwrapping the ventromedial bursting interneurons and
49 lamina IX motoneurons. This strategic distribution of Kir4.1 channels surrounding the bursting spinal
50 neurons may be vie lamina IX motoneurons. This strategic distribution of Kir4.1 channels surrounding the bursting spinal
1908 - Strong Marchans and Strong Marchans in a metal burst of the ventrom in a speed ontromedial burst of Moreover, sin 49 19 19 neurons may be viewed as a powerful mechanism to rapidly modulate network rhythmicity.

49 Moreover, since glutamate tunes the CPG network by exerting a speed control of locomotor-like

49 T 50 neurons may be viewed as a powerful mechanism to rapidly modulate network rhythmicity.
51 Moreover, since glutamate tunes the CPG network by exerting a speed control of locomotor-like
7 51 Moreover, since glutamate tunes the CPG network by exerting a speed control of locomotor-like activity (Talpalar et al., 2013) and that [K]e provides the driving force for glutamate uptake (Nwaobi

et al., 2016; Verkhratsky and Nedergaard, 2018) the effective K⁺ uptake from the extracellular space

through Kir4 et al., 2016; Verkhratsky and Nedergaard, 2018) the effective K- uptake from the extracellular space
through Kir4.1 is thus crucial for tuning the neuron oscillations. In line with this, previous works
highlighted the imp 3 the importance of $[K^+]_e$ for rhythmogenesis in hippocampal (Jensen et al. 1994) or
3 cortical (Bellot-Saez et al., 2018) networks. Furthermore, the increase in $[K^+]_e$ induced by
3 neuromodulators may promote the shift cortical (Bellot-Saez et al., 2018) networks. Furthermore, the increase in $[K]_e$ induced by
neuromodulators may promote the shift between neuronal oscillatory states in cortical slices
electrically silenced by TTX (Ding

Example 12 dectrically silenced by TTX (Ding et al., 2016). A computational work also demonstrated that

8 extracellular K^{*} dynamics can cause transition between fast tonic spiking and slow bursting in

9 neocortical ne Extracellular K⁺ dynamics can cause transition between fast tonic spiking and slow bursting in

neocortical networks (Frohlich et al., 2006) in the same range than the spinal locomotor network.
 The ventromedial astroc 9 neocortical networks (Frohlich et al., 2006) in the same range than the spinal locomotor network. The ventromedial astrocytes regulate the locomotor pattern in vivo. A dense interature pointed out

11 that during locomotion, mice display enhanced Ca²⁺ signals in astrocytes of the cerebellum

12 (Nimmerjahn et al., 20 that during locomotion, mice display enhanced Ca⁻ signals in astrocytes of the cerebellum (Nimmerjahn et al., 2009; Paukert et al., 2014), visual cortex (Paukert et al., 2014), or somatosensory cortex (Bojarskaite et al 13 cortex (Bojarskaite et al., 2020; Dombeck et al., 2007). On the other hand, silencing astrocyte Ca²⁺ activity in brainstem with optogenetics or chemogenetics can modulate the rhythmic respiratory behaviour (Gourine e 15 behaviour (Gourine et al., 2010; Sheikhbahaei et al., 2018). In this study, we used genetic tools with
16 ShRNA to knockdowns Kir4.1 in the locomotor CPG and demonstrate that the astrocytic regulation of
17 K^{*} homeo 16 ShRNA to knockdowns Kir4.1 in the locomotor CPG and demonstrate that the astrocytic regulation of
17 K⁺ homeostasis plays a key role in controlling the locomotor speed and challenging locomotor tasks
18 *in vivo*. Mi K⁺ homeostasis plays a key role in controlling the locomotor speed and challenging locomotor tasks
 in vivo. Mice transduced with Kir4.1-ShRNA showed striking locomotor deficits compared to Ctrl-

ShRNA mice. Kir4.1 h 18 In vivo. Mice transduced with Kir4.1-ShRNA showed striking locomotor denies compared to Ctrl-
19 ShRNA mice. Kir4.1-had been reported to contribute to the maintenance of the soma size of large α-
20 motoneurons and pea 19 19 Shruma Mice. Huntington disease, results in altered locomotor pattern (Tong et al., 2014). We did not observe any
19 The Mice. Kir4.1-ShRNA expression in brain or brainstem structures involved in the locomotor contro 21 lowered locomotor performances from Kir4.1-ShRNA mice do not result from changes in
22 motoneuronal morphological features. Indeed, we did not observe any modification of the
23 motoneuronal soma size in mice transduced 22 motoneuronal morphological features. Indeed, we did not observe any modification of the
23 motoneuronal soma size in mice transduced with Kir4.1-ShRNA compared to Ctrl-ShRNA mice. Tong
24 et al. 2014 observed that the K motoneuronal soma size in mice transduced with Kir4.1-ShRNA compared to Ctrl-ShRNA mice. Tong

24 et al. 2014 observed that the Kir4.1 expression decrease in the striatal network of a mouse model of

25 Huntington disease, et al. 2014 observed that the Kir4.1 expression decrease in the striatal network of a mouse model of

25 Huntington disease, results in altered locomotor pattern (Tong et al., 2014). We did not observe any

26 Kir4.1-ShRNA 25 Huntington disease, results in altered locomotor pattern (Tong et al., 2014). We did not observe any
26 Kir4.1-ShRNA expression in brain or brainstem structures involved in the locomotor control. This
27 result is in li EXECUTE: 125 Huntington discussion in brain or brainstem structures involved in the locomotor control. This
27 Function discussions in alternative and the thoraco-lumbar segments of the AAV9-ShRNA
28 injected intrathecally 27 result is in line with the restricted diffusion to the thoraco-lumbar segments of the AAV9-ShRNA
28 injected intrathecally at birth in the lumbar segments (Bos et al., 2021). Thus we assume that the
29 behavioural conse 28 injected intrathecally at birth in the lumbar segments (Bos et al., 2021). Thus we assume that the
29 behavioural consequence of Kir4.1 knockdown on locomotor performances is due to Kir4.1 decrease
20 in the spinal loc

29 behavioural consequence of Kir4.1 knockdown on locomotor performances is due to Kir4.1 decrease

20 in the spinal locomotor networks.

21 Overall this study defines the contours of a new concept for the spinal locomoto 20 in the spinal locomotor networks.

21 Overall this study defines the contours of a new concept for the spinal locomotor CPG. We propose

22 that the astrocytic Ca²⁺ transients which occurs during the neuronal oscilla Something the spinal determined the spin of the strategy of that the astrocytic Ca²⁺ transient
33 in the strocytic Ca²⁺ transient
33 in the strategy of the spinal parameter of the syncytium mediating K⁺ buffering is 32 that the astrocytic Ca²⁺ transients which occurs during the neuronal oscillations likely maintains extracellular K⁺ homeostasis, which in turn adjusts the locomotor pattern (Graphical abstract).
34 Further studies that the astrocytic Ca²⁺ transients which occurs during the neuronal oscillations likely maintains

extracellular K⁺ homeostasis, which in turn adjusts the locomotor pattern (Graphical abstract).

Further studies will Further studies will be needed to decipher the importance for spinal rhythmicity of the astrocytic
syncytium mediating K⁺ buffering in the locomotor CPG.
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40 35 syncytium mediating K⁺ buffering in the locomotor CPG.
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40 ss Syncytium mediating K- buffering in the locomotor CPG.
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EXPERIMENTAL PROCEDURES

Further details and an outline of resources used in this work can be found in the Supplemental

Procedures.
 Mice. Mice from the 1st to 3rd postnatal week of either sex were used in this st France dures.

8 Mice. Mice from the 1st to 3rd postnatal week of either sex were used in this study. Animals from

1 different litters were used for each experiment. All animal care and use were conformed to the

1 Fr 8
7 **Mice.** Mice 1
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7 French regul
7 en Neuroscie
7 Supplementa** 9012345 Mice. Mice from the 1st to 3^c postnatal week of either sex were used in this study. Animals from

different litters were used for each experiment. All animal care and use were conformed to the

French regulations (Déc 11 French regulations (Décret 2010-118) and approved by the local ethics committee (Comité d'Ethique

12 en Neurosciences INT-Marseille, CE71 NbA1301404, authorization Nb 2018110819197361). See the

13 **Ex vivo Models.** Sl

12 en Neurosciences INT-Marseille, CE71 NbA1301404, authorization Nb 2018110819197361). See the

13 Supplemental Experimental Procedures for more details.

14 Ex vivo Models. Slice preparations were used for whole-cell rec 13 Supplemental Experimental Procedures for more details.

14 Ex vivo Models. Slice preparations were used for whole-cell recordings and two-photon Ca²⁺ imaging

16 experiments, whereas whole spinal cord preparations wer 14
 Ex vivo Models. Slice preparations were used for whole-c

experiments, whereas whole spinal cord preparations we

Preparation procedures are detailed in the Supplemental

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 Intracellular Recordings. For ex vivo e 15
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Ex VIVO MOdels. Slice preparations were used for Whole-cell recordings and two-photon Ca⁻ imaging

experiments, whereas whole spinal cord preparations were used for fictive locomotion experiments.

Preparation procedures 17 Preparation procedures are detailed in the Supplemental Experimental Procedures.

16 Intracellular Recordings. For ex vivo experiments, whole-cell patch-clamp recordings were made

16 Intracellular Recordings. For ex vi 17 **Intracellular Recordings.** For ex vivo experiments, whole-cell patch-clamp record
19 **Intracellular Recordings.** For ex vivo experiments, whole-cell patch-clamp record
20 from L1-L2 ventromedial GFP+ interneurons (*Hb9* 19
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25 19 Intracellular Recordings. For ex vivo experiments, whole-cell patch-clamp recordings were made

19 Intracellular School (Hb9:eGFP mice) or GFP+ astrocytes (Aldh11:eGFP

19 Intervential Procedures.

23 Intervential Proce

21 mice) from L1-L2 ventromedial GFP+ interneurons (Hb9:eGFP+ integ or GFF+ astrocytes (Aldh1L1:eGFP+
22 Experimental Procedures.
23 Extracellular Recordings. Motor outputs were recorded using glass suction electrodes plac Experimental Procedures.
23 Extracellular Recordings. Motor outputs were recorded using glass suction electrodes placed in
25 Extracellular Recordings. Motor outputs were recorded using glass suction electrodes placed in
2 23
24 Extracellular Recordings.
25 contact with right and
26 monoaminergic cocktail. S
27 Two-photon Ca²⁺ imaging 24
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Extracellular Recordings. Motor outputs were recorded using glass suction electrodes placed in

25 contact with right and left lumbar L2 ventral roots in response to bath application of a

27 **Two-photon Ca**²⁺ imaging. T monoaminergic cocktail. See the Supplemental Experimental Procedures for more details.

27
 28 Two-photon Ca²⁺ imaging. Two-photon excitation of Gcamp6f and jRGECO1a was simultaneously

29 evoked with a laser tuned to 27
28 **Two-photon Ca²⁺ imaging.** Two-photon excitation of Gcamp6f and jRGECO1a was simu
29 evoked with a laser tuned to 960 nm. Imaging was done at 30.5202 Hz. The micros
30 equipped with two detection channels for fluo 28
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32 Two-photon Ca **28 Two-photon Ca²⁺ imaging.** Two-photon excitation of Gcamp6f and jRGECO1a was simultaneously

29 evoked with a laser tuned to 960 nm. Imaging was done at 30.5202 Hz. The microscope was

30 equipped with two detection

equipped with two detection channels for fluorescence imaging. See the Supplemental Experimental

21 **contract of the secure of the secure at 31.5202 ShRNA** constructs. Specific shRNA sequence designed to knockdown Kir4.1 31 Procedures for more details.
32 shRNA constructs. Specific shRNA sequence designed to knockdown *Kir4.1* transcript was
33 incorporated into an adeno-associated viral (AAV) vector (serotype 9), which features a H1 promo **Shand constructs.** Specific
33 incorporated into an adeno-a
34 to drive shRNA expression
35 transduced cells (Cui et al. 20
36 **Intrathecal vector delivery.** 33 incorporated into an adeno-associated viral (AAV) vector (serotype 9), which features a H1 promoter
34 to drive shRNA expression and a CAG promoter to drive eGFP expression for identification of
35 transduced cells (Cu

33 intrathecal vector delivery. A minimally-invasive technique was used to micro-inject adeno-
35 **intrathecal vector delivery.** A minimally-invasive technique was used to micro-inject adeno-
38 **intrathecal vector delive** transduced cells (Cui et al. 2018). See the Supplemental Experimental Procedures for more details.
36 **Intrathecal vector delivery.** A minimally-invasive technique was used to micro-inject adeno-
38 associated viral (AAV) transduced cells (Cui et al. 2018). See the Supplemental Experimental Procedures for more details.
36 **Intrathecal vector delivery.** A minimally-invasive technique was used to micro-inject adeno-
38 associated viral (AAV)

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42 37 Intrathecal vector delivery. A minimally-invasive technique was used to inicio-fifect adeno-
38 associated viral (AAV) vectors into the T13-L1 intervertebral space. A total volume of 2 µL /animal
39 was injected. See th 31 **Kir4.1 protein quantification.** Kir4.1 expression was analyzed using the 12-230 kDa separation
32 module (SM-W004 ProteinSimple) on an automated capillary western blotting system ('Jess'
32 ProteinSimple). See the Supp ProteinSimple). See the Supplemental Experimental Procedures for more details. 41
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44 module (SM-W004 Protein Simple) on an automated capillary western blotting system ('Jess' 43 modeinSimple). See the Supplemental Experimental Procedures for more details.
44 modeinSimple). See the Supplemental Experimental Procedures for more details.

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1 Immunohistochemistry. Transverse spinal cord sections at the lumbar L1-L2 level were processed

2 for immunohistochemistry using antibodies against Kir4.1, NeuN. Tissue processing and staining are

3 detailed in the Supp 2 detailed in the Supplemental Experimental Procedures.

2 **Behavioral tests**. Assessment of motor behaviour were performed on neonatal mice with three

2 **Behavioral tests**: walking, rotarod and swimming. See the Suppleme 3 **Behavioral tests.** Assessment of motor behaviour we
3 different tests: walking, rotarod and swimming. See th
3 **Statistical Analysis.** Group measurements were expres
3 are indicated in Figure legends. The level of signi

567890 Solution tests. Assessment of motor behaviour were performed on neonatal mice with three
different tests: walking, rotarod and swimming. See the Supplemental Experimental Procedures for
more details.
Statistical Analysis. 6 11 12 12 13 different tests: walking more details.
 Statistical Analysis. Group measurements were expressed as means \pm S.E.M. All statistical analyses

are indicated in Figure legends. The level of significance was Frame actual
8 **Statistical An**
9 are indicated
0 performed us
1 more details.
2 Statistical Analysis. Group measurements were expressed as means ± S.E.M. All statistical analyses
are indicated in Figure legends. The level of significance was set at $p < 0.05$. Statistical analyses were
performed using 9 are indicated in Figure legends. The level of significance was set at p < 0.05. Statistical analyses were
1 performed using Graphpad Prism 9 software. See the Supplemental Experimental Procedures for
1 **ACKNOWLEDGMENTS** more details.
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25 **AUTHOR CONT** 22 International of vectors used in this work. We also thank Geneviève Rougon for her critical insights on
23 the manuscript.
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AUTHOR CONTRIBUTIONS 22 production of vectors used in the vectors used in the manuscript.
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25 **AUTHOR CONTRIBUTIONS**
26 Research design T.B. E.P. and R.B. : Methodology T.B. E.P. N.B. and R.B. : Investigation and data

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25 **AUTHOR COM
26** Research design --
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**AUTHOR CONTRIBUTIONS
26 Research design, T.B., E.P. and R
27 analysis, T.B., E.P., M.D., and R.B
28 DECLARATION OF INTERES**

- Research design, T.B., E.P. and R.B..; Methodology, T.B., E.P., N.R. and R.B.; Investigation and data

27 analysis, T.B., E.P., M.D., and R.B.; Writing, R.B.; Funding Acquisition, R.B. and F.B..; Supervision, R.B.

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28 **DECLARATION OF INTERESTS**
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30 The authors declare no conflict of interest. **DECLARATION OF INTERESTS**
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4 5 6 7 8 9 567890 5 7 8 9 0 1 789012 **FIGURE LEGENDS**
8 **Figure 1. Astrocytes i**
9 **spinal CPG. A.** Confoc
0 White inset represen
1 recording glass micros
2 for white matter. **B.**
6 mM and [Ca²⁺]_e decn Figure 1. Astrocytes respond to variations in [K+]e and [Ca2+ **Solution** 1. Astrocyces respond to variations in the set of a particle to incident oscillations in the spinal CPG. A. Confocal image of endogenous GFP from a lumbar slice of a P8 Aldh1L1-eGFP mouse.

White inset represen 9 Spinal CPG. A. Confocal image of endogenous GFP from a lumbar slice of a P8 Aldh1L1-eGFP intouse.

9 White inset represents the patch-clamp recording area of the ventromedial GFP+ astrocytes. The

1 recording glass micr 11 recording glass microelectrode is in white. CC stands for central canal, GM for grey matter and WM

12 for white matter. **B.** Representative voltage trace of a GFP+ astrocyte in response to $[K^+]_e$ rise to

13 6mM and 12 for white matter. **B.** Representative voltage trace of a GFP+ astrocyte in response to $[K^+]_e$ rise to 6mM and $[Ca^{2+}]_e$ decrease to 0.9mM. **C.** Quantification of the effect of the $[K^+]_e$ and $[Ca^{2+}]_e$ variations on 6mM and $[Ca^{-1}]_e$ decrease to 0.9mM. C. Quantification of the effect of the $[K^-]_e$ and $[Ca^{-1}]_e$ variations

on the astrocytic membrane potential (n = 22 astrocytes from 16 mice). **D.** Representative voltage

trace of a 14 on the astrocytic membrane potential (n = 22 astrocytes from 16 mice). D. Representative voltage

15 trace of a GFP+ astrocyte in response to bath application of a neuromodulator cocktail (dopamine,

16 serotonin, and 16 serotonin, and NMDA) in presence of TTX. **E.** Quantification of the effect of the neuromodulator cocktail on the astrocytic membrane potential (n = 8 astrocytes from 4 mice). **F.** Confocal image of the endogenous GFP f 17 cocktail on the astrocytic membrane potential (n = 8 astrocytes from 4 mice). F. Confocal image of
18 the endogenous GFP from the ventromedial part of a lumbar slice of a P12 Hb9-eGFP mouse. The
19 green and orange glas 17 cocktail on the astrocytic includibility of the ventromedial part of a lumbar slice of a P12 Hb9-eGFP mouse. The
19 green and orange glass microelectrodes represent the dual recording of a GFP+ interneuron and an
20 adj 19 green and orange glass microelectrodes represent the dual recording of a GFP+ interneuron and an adjacent astrocyte, respectively. CC for central canal. **G-H**. The $[K^+]_e$ and $[Ca^{2+}]_e$ variations (G, light blue) or t 20 adjacent astrocyte, respectively. CC for central canal. **G-H**. The $[K^+]_e$ and $[Ca^{2+}]_e$ variations (G, light blue) or the bath application of the neuromodulator cocktail in presence of TTX (H, light blue) induces burs 22 induces bursting in Hb9 GFP+ interneurons (green traces) preceded by a depolarization of the neighbouring astrocyte (orange traces). The black inset in H represents the TTX-insensitive neuronal oscillations. I. Proporti 23 neighbouring astrocyte (orange traces). The black inset in H represents the TTX-insensitive neuronal

24 oscillations. I. Proportion of the astrocytic membrane potential depolarization preceding (light

25 orange) or n 24 oscillations. I. Proportion of the astrocytic membrane potential depolarization preceding (light

25 orange) or not preceding (dark orange) the onset of neuronal bursting (n=13 pairs from 8 mice). **P

26 < 0.01; ***P Caster oscillations. I. Proportion of the astrocytic membrane potential depolarization preceding (light

25 orange) or not preceding (dark orange) the onset of neuronal bursting (n=13 pairs from 8 mice). **P

26 < 0.01; *

26 < 0.01; ***P < 0.001 (two-tailed Wilcoxon paired test for C and E). Mean ± SEM. For detailed P
values, see Source data file.
28 **Figure 2. Increase in astrocytic Ca²⁺ transients is correlated to neuronal oscillations** 27 values, see Source data file.

28 **Figure 2.** Increase in astrocytic Ca²⁺ transients is correlated to neuronal oscillations A. *Top.*

29 **Figure 2.** Increase in astrocytic Ca²⁺ transients ($\Delta F/Fo$) oscillating in 28
29 Figure 2. Increase in astro
30 Examples of neuronal jRGE
31 decrease (left) or in respon
32 *bottom*. Examples of the incondinary during (bottom) the neuron 29
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34 Figure 2. Increase in astrocytic Ca⁻
-Examples of neuronal jRGECO1a Ca²⁺ transients is correlated to neuronal oscillations A. Top.
29 Examples of neuronal jRGECO1a Ca²⁺ transients ($\Delta F/Fo$) oscillating in response to K⁺ rise and Ca²⁺
31 decrease (left Examples of neuronal JRGECO1a Ca²⁺ transients (ΔF/Fo) oscillating in response to K+ rise and Ca²⁺
decrease (left) or in response to the neuromodulator cocktail in presence of TTX (right). *Middle,*
bottom. Examples of 32 bottom. Examples of the increase of the astrocytic Gcamp6f Ca^{2+} signals ($\Delta F/F0$) before (middle) or during (bottom) the neuronal oscillations (top) from the same field of view. **B.** Quantification of the mean ampli bottom. Examples of the increase of the astrocytic Gcamp6f Ca⁻ signals (ΔF/F0) before (middle) or
during (bottom) the neuronal oscillations (top) from the same field of view. **B.** Quantification of the
mean amplitude (33 during (bottom) the neuronal oscillations (top) from the same field of view. **B.** Quantification of the
33 mean amplitude (left), duration and frequency (right) of the neuronal jRGECO1a Ca²⁺ transients in
35 response mean amplitude (left), duration and frequency (right) of the neuronal jRGECO1a Ca⁻⁺ transients in
response to K⁺ rise and Ca²⁺ decrease (empty blue circles) or in response to monoaminergic cocktail
in presence of TT response to K+ rise and Ca²⁺ decrease (empty blue circles) or in response to monoaminergic cocktail
in presence of TTX (filled blue circles) (n= 9 and 24 dendritic ROIs from 2 and 3 mice in the ionic
variation condition 37 variation condition and in the TTX+cocktail condition, respectively). **C.** Quantification of the mean amplitude (left), duration (middle) and frequency (right) of the astrocytic Gcamp6f Ca²⁺ signals in response to K **Examplitude** (left), duration (middle) and frequency (right) of the astrocytic Gcamp6f Ca²⁺ signals in response to K⁺ rise and Ca²⁺ decrease (empty light blue circles) or in response to monoaminergic cocktail in pr amplitude (left), duration (middle) and frequency (right) of the astrocytic Gcamp6f Ca²⁺ signals in response to K⁺ rise and Ca²⁺ decrease (empty light blue circles) or in response to monoaminergic cocktail in presen astrocytic ROIs which display Ca²⁺ signals increase in amplitude and/or frequency before the onset of neuronal oscillations. **E.** Proportion of astrocytic ROIs which display Ca²⁺ signals increase above (dorsal) or bel response to K inse and Ca²⁺ decrease (empty light blue circles) or in response to monoaminergic
cocktail in presence of TTX (filled light blue circles) (n= 86 and 165 astrocytic ROIs from 2 and 3 mice
in the ionic varia 41 in the ionic variation condition and in the TTX+cocktail condition, respectively). **D.** Proportion of astrocytic ROIs which display Ca²⁺ signals increase in amplitude and/or frequency before the onset of neuronal osc astrocytic ROIs which display Ca²⁺ signals increase in amplitude and/or frequency before the onset of
neuronal oscillations. **E.** Proportion of astrocytic ROIs which display Ca²⁺ signals increase above
(dorsal) or bel astrocytic ROIs which display Ca⁻ signals increase in amplitude and/or frequency before the onset of
neuronal oscillations. **E.** Proportion of astrocytic ROIs which display Ca²⁺ signals increase above
(dorsal) or belo neuronal oscillations. **E.** Proportion of astrocytic ROIs which display Ca² signals increase above

(dorsal) or below (ventral) the central canal (CC) in response to oscillatory conditions (ionic

variations and neuromo variations and neuromodulator cocktail). $*P < 0.05$, $**P < 0.01$, $**P < 0.001$ (two-tailed Wilcoxon

paired test for C). Mean ± SEM. For detailed P values, see Source data file.
 Figure 3. Spinal astrocytes display a Ba

quared test for C). Mean ± SEM. For detailed P values, see Source data file.

47 Figure 3. Spinal astrocytes display a Ba²⁺-sensitive Kir4.1 current modulated by intracellular Ca²⁺.

48 A. *Left*. DIC image of a lumba 47 Figure 3. Spinal astrocytes display a Ba²⁺-sensitive Kir4.1 current modul
48 A. Left. DIC image of a lumbar slice from a P8 Aldh1L1-eGFP mouse Figure 3. Spinal astrocytes display a Ba2+-sensitive Kir4.1 current modulated by intracellular Ca2+ Figure 3. Spinal astrocytes display a Ba²⁺-sensitive Kir4.1 current modulated by intracellular Ca²⁺.
48 A. Left. DIC image of a lumbar slice from a P8 Aldh1L1-eGFP mouse superimposed with the
15 48 A. Left. Dic image of a lumbar slice from a P8 Aldh1L1-eGFP mouse superimposed with the

1 represented in black. *Right.* Representative membrane potentials (top) of the targeted GFP+

1 astrocyte (left image) in response to incremental current pulses (500ms, $\Delta = 50pA$). **B.** Resting

1 membrane potential (R 2 represented in black. Right. Representative including telectricity (top) of the targeted GFT
astrocyte (left image) in response to incremental current pulses (500ms, $\Delta = 50pA$). **B.** Resting
membrane potential (RMP) (l 3 astrocyte (left image) in response to incremental current pulses (500ms, $\Delta = 30\mu$ m). B. Resting

3 membrane potential (RMP) (left) and input resistance (right) of lumbar GFP+ astrocytes recorded in

3 standard aCSF (3 standard aCSF ($[K^+]_e$ 3mM and $[Ca^{2+}]_e$ 1.2mM) (n = 28 astrocytes from n = 15 mice) **C**. Representative membrane currents (top) of one GFP+ astrocyte clamped at the RMP in response to incremental voltages pulses (bott standard aCSF ([K \parallel _e 3mM and [Ca⁻ \parallel _e 1.2mM) (n = 28 astrocytes from n = 15 mice) **C.** Representative
former membrane currents (top) of one GFP+ astrocyte clamped at the RMP in response to incremental
voltages voltages pulses (bottom, 2.5s, -140 to -40mV, Δ = 10mV) before (left) or during (middle) application
of Ba²⁺ (100 µM) in standard aCSF. **D.** The averaged I/V relationship of the Ba²⁺-sensitive currents of
GFP+ astr **EXECUTE 100 μM)** in standard aCSF. **D.** The averaged I/V relationship of the Ba²⁺-sensitive currents of GFP+ astrocytes (n = 17 astrocytes from 9 mice). **E.** Representative Ba²⁺-sensitive currents of two astrocytes of Ba⁻ (100 µM) in standard aCSF. **D.** The averaged I/V relationship of the Ba⁻-sensitive currents of GFP+ astrocytes (n = 17 astrocytes from 9 mice). **E.** Representative Ba²⁺-sensitive currents of two astrocytes cl GFP+ astrocytes (n = 17 astrocytes from 9 mice). **E.** Representative Ba²-sensitive currents of two
astrocytes clamped at the RMP in response to incremental voltages pulses (2.5s, -140 to +0mV, Δ =
10mV) without (blue 10 astrocytes clamped at the RMP in response to the RMP in oscillatory actor (K¹_{le} 6mM and [Ca²⁺_{le} 0.9mM). F. *Left*. The averaged I/V curves from lumbar astrocytes without (blue circles) or with (black circles) i 12 ($[K^+]_e$ 6mM and $[Ca^{2+}]_e$ 0.9mM). F. *Left*. The averaged I/V curves from lumbar astrocytes without (blue circles) or with (black circles) intracellular BAPTA perfusion in oscillatory aCSF. *Right*. Average effect of ([K
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Figu 12 ([K \int_e 6mM and [Ca² \int_e 0.9mM). F. *Left.* The averaged I/V curves from lumbar astrocytes without (blue circles) or with (black circles) intracellular BAPTA perfusion in oscillatory aCSF. *Right*. Average effect 13 (blue circles) or with (black circles) intracellular BAPTA perfusion in oscillatory aCSF. *Right*. Average effect of intracellular perfusion of BAPTA on the peak amplitude of the astrocytic Kir4.1 current at - 140mV (G

18 A-B. Representative voltage traces of a lumbar GFP+ ventromedial interneuron from a P6 Hb9-eGFP
19 mouse before (left) and during (middle) ionic variations ([K⁺]_e = 6mM, [Ca²⁺]_e = 0.9mM) on which Ba²⁺
20 (1 16 (two-tailed Mann–Whitney test for F). Mean ± SEM. For detailed P values, see Source data file.

17 Figure 4. K^{*} homeostasis maintenance through Kir4.1 channels is crucial for neuronal oscillations.

18 A-B. Represen 17 Figure 4. K^{*} homeostasis maintenance through Kir4.1 channels is crucial for neuronal oscilla

18 A-B. Representative voltage traces of a lumbar GFP+ ventromedial interneuron from a P6 Hb9

19 mouse before (left) and Figure 4. K^* homeostasis maintenance through Kir4.1 channels is crucial for neuronal oscillations. **17 18 A-B.** Representative voltage traces of a lumbar GFP+ ventromedial interneuron from a P6 Hb9-eGFP mouse before (left) and during (middle) ionic variations ([K⁺]_e = 6mM, [Ca²⁺]_e = 0.9mM) on which Ba²⁺ (19 mouse before (left) and during (middle) ionic variations ($[K^+]_e = 6$ mM, $[Ca^{2+}]_e = 0.9$ mM) on which Ba²⁺
100 µM) is added (right) (A) or in presence of TTX (left) and following application of the
121 neuromodulator mouse before (left) and during (middle) ionic variations ([K I_e = 6mM, [Ca⁻]_e = 0.9mM) on which Ba⁻

(100 μ M) is added (right) (A) or in presence of TTX (left) and following application of the

neuromodulator c 21 neuromodulator cocktail (middle) on which Ba²⁺ is added (right) (B). **C-D.** Quantification of the
22 proportion of GFP+ interneurons oscillating in response to ionic variations (C, left) or to the
23 neuromodulator c neuromodulator cocktail (middle) on Which Ba⁻ is added (right) (B). **C-D.** Quantification of the
proportion of GFP+ interneurons oscillating in response to ionic variations (C, left) or to the
neuromodulator cocktail (D 23 neuromodulator cocktail (D, left) and following bath application of Ba²⁺ for more than 5 minutes (C-
24 D, right). E. Top. Examples of neuronal jRGECO1a Ca²⁺ signals ($\Delta F/Fo$) before (blue) and during bath
25 appli neuromodulator cocktail (D, left) and following bath application of Ba⁻ for more than 5 minutes (C-
D, right). **E.** Top. Examples of neuronal jRGECO1a Ca²⁺ signals (ΔF /Fo) before (blue) and during bath
application D, right). **E.** Top. Examples of neuronal jRGECO1a Ca⁻ signals (ΔF/Fo) before (blue) and during bath
application of Ba²⁺ (grey) in presence of high [K⁺]_e and low [Ca²⁺]_e (left) or in presence of the
neuromodu application of Ba² (grey) in presence of high [K₋]_e and low [Ca⁻_{1e} (left) or in presence of the

neuromodulator cocktail + TTX (right). *Bottom*. Examples of astrocytic Gcamp6f Ca²⁺ signals ($\Delta F/F0$)

before (neuromodulator cocktail + TTX (right). *Bottom*. Examples of astrocytic Gcamp6f Ca⁻ signals (ΔF/F0)

before (blue) or during (grey) application of Ba²⁺ in presence of high [K⁺]_e and low [Ca²⁺]_e (left) or in
 before (blue) or during (grey) application of Ba²⁺ in presence of high [K⁻]_e and low [Ca²⁺]_e (left) or in
presence of TTX and the neuromodulator cocktail (right). **F.** Proportion of neuronal ROIs still
oscillati by presence of TTX and the neuromodulator cocktail (right). T. Proportion of neuronal ROIs still
consider the neuron of TTX and the neuromodulator cocktail (right). **G.** Effect of Ba²⁺ application
(empty and filled grey oscillating or not in response to Ba² application (>5min) in presence of high [K]_e and low [Ca²]_e (left) or in presence of TTX and the neuromodulator cocktail (right). **G.** Effect of Ba²⁺ application (empty an (left) or in presence of TTX and the neuromodulator cocktail (right). **G.** Effect of Ba² application (empty and filled grey circles) on the mean amplitude (left), duration (middle) and frequency (right) of the astrocyti 32 of the astrocytic Gcamp6f Ca²⁺ signals in presence of K⁺ rise and Ca²⁺ decrease (empty light blue circles) or in presence of the neuromodulator cocktail + TTX (filled light blue circles). Each circle represents o of the astrocytic Gcamp6f Ca² signals in presence of K rise and Ca² decrease (empty light blue
circles) or in presence of the neuromodulator cocktail + TTX (filled light blue circles). Each circle
represents one astroc encies) or in presence of the neuromodulator cocktain + TTX (filled light blue circles). Each circle

33 represents one astrocytic ROI (n= 24 ROIs from 2 mice in the ionic variation condition and n= 157

35 ROIs from 3 mic

34 represents one astrocytic Roll (n= 24 ROIs from 2 mice in the ionic variation condition and n= 157
35 ROIs from 3 mice in the TTX+cocktail condition) ns, no significance, $*P < 0.05$, $**PP < 0.001$ (two-
36 **Figure 5. Mod** 35 40 ventral root lumbar segment 2 right. **B-C.** Extracellular recordings of alternating rhythmic activities of 41 L2 segment ventral roots (integrated signals) in response to $[K^+]_e$ rise and $[Ca^{2+}]_e$ decrease (B, lef 37 **Figure 5. Modulation of astrocytic Kir4.1 channels alters the locomotor-like act**
38 Schematic representation of the whole-mount spinal cord (ventral side up) with t
39 recording glass electrodes. VRL2L stands for ven Schematic representation of astrocytic Kir4.1 channels alters the locomotor-like activity pattern. A.

Schematic representation of the whole-mount spinal cord (ventral side up) with two contralateral

recording glass elec 39 recording glass electrodes. VRL2L stands for ventral root lumbar segment 2 left. VRL2R stands for ventral root lumbar segment 2 right. **B-C.** Extracellular recordings of alternating rhythmic activities of L2 segment ve 39 ventral root lumbar segment 2 right. **B-C.** Extracellular recordings of alternating rhythmic activities of
341 L2 segment ventral roots (integrated signals) in response to $[K^+]_e$ rise and $[Ca^{2+}]_e$ decrease (B, left) 40 ventral root lumbar segment 2 right. B-C. Extracemular recordings of alternating rhythmic activities of
41 L2 segment ventral roots (integrated signals) in response to $[K^+]_e$ rise and $[Ca^{2+}]_e$ decrease (B, left) or
 L2 segment ventral roots (integrated signals) in response to [K]_e rise and [Ca⁻]_e decrease (B, left) or
in response to the neuromodulator cocktail (C, left) and in the presence (B-C, right) of Ba²⁺. **D.**
Quantif in response to the neuromodulator cocktail (C, left) and in the presence (B-C, right) of Ba⁻. **D.**
Quantification of the effect of Ba²⁺ (grey circles) on the amplitude (left), duration (middle), and
frequency (right) Quantification of the effect of Ba²⁺ (grey circles) on the amplitude (left), duration (middle), and
frequency (right) of the rhythmic bursts recorded at the ventral roots (L2 segments) in ionic variation
condition (ligh condition (light blue empty circles) or in presence of the neuromodulator cocktail (light blue filled circles). Each circle represents one individual animal (n = 6 mice in high $[K^+]_e$ and low $[Ca^{2+}]_e$
16 46 circles). Each circle represents one individual animal (n = 6 mice in high $[K^+]_e$ and low $[Ca^{2+}]_e$
16 circles). Each circle represents one individual animal (n = 6 mice in high [K⁺
individual animal (n = 6 mice in high [K⁺
 $\frac{1}{2}$ circles). Each circle represents one individual animal (n = 6 mice in high [K]_e and low [Ca²⁺]_e

1 condition and n= 7 mice in neuromodulator cocktail condition). **L.** Graphical representation of the tase of the L2 segment before (blue trace) or after (red trace) Ba²⁺ application. **F.** Quantification of the mean cro 2 roots of the L2 segment before (blue trace) or after (red trace) Ba²⁺ application. **F.** Quantification of

4 the mean cross-correlation coefficients between the rhythmic activities of the contralateral ventral

5 root roots of the L2 segment before (blue trace) or after (red trace) Ba²⁺ application. F. Quantification of
the mean cross-correlation coefficients between the rhythmic activities of the contralateral ventral
roots of the L 4 to mean correlation coefficients of the L2 segment before (blue) and after (grey) Ba^{2+} application (n = 6 mice in high $[K^+]_e$ and

4 tow $[Ca^{2+}]_e$ condition and n= 7 mice in neuromodulator cocktail condition). ns,

For the LCa²⁺_{le} condition and n= 7 mice in neuromodulator cocktail condition). ns, no significance, *P <

0.05 (two-tailed Wilcoxon paired test for D and F). Mean ± SEM. For detailed P values, see Source

data file.
 low [Ca²⁺ μ condition and n= 7 mice in neuromodulator cocktail condition). ns, no significance, *P <

0.05 (two-tailed Wilcoxon paired test for D and F). Mean ± SEM. For detailed P values, see Source

data file.
 Fi Figure 1. The Tailor Phase of the Tailor Phase of the ACV test for District Form and Scillations. A.

1 Figure 6. Targeted decrease in Kir4.1 channels reduces the occurrence of neuronal oscillations. A.

2 Schematics of th 9 Figure 6.
0 Schemati
1 human H
2 of the ex
3 Kir4.1 ps
4 injected a 9 Figure 6. Targeted decrease in Kir4.1 channels reduces the occurrence of neuronal oscinations. A.

9 Schematics of the AAV vector engineered to overexpress shRNA or dominant-negative Kir4.1. H1,

1 human H1 promoter; CAG 11 human H1 promoter; CAG, CMV early enhancer/chicken *Actb* promoter. **B.** Schematic representation
12 of the experimental design. WT for wild-type, INs for interneurons and P for postnatal day. **C.** Left:
13 Kir4.1 pseud numan H1 promoter, CAG, CMV early emfanter/chicken Actb promoter. **B.** Schematic representation
12 of the experimental design. WT for wild-type, INs for interneurons and P for postnatal day. **C.** Left:
13 Kir4.1 pseudo-gel 13 Kir4.1 pseudo-gel images from Capillary Western Blot of lumbar segments from P14 intrathecally

14 injected at birth with an adeno-associated virus (AAV9) encoding either a scramble shRNA (n = 5

15 mice) or a Kir4.1-t 15 mice) or a Kir4.1-targeting shRNA (n = 5 mice). One mouse per lane. Right: group mean
16 quantification of the ~168 kDa band normalized to scramble-injected controls. **D.** Representative
17 image of GFP in lumbar slice micropology and the many states of the many states and the many states image of GFP in lumbar slices from P10 mice after intrathecal injection at birth of an adeno-
associated virus (AAV9) encoding a Kir4.1-targeting shRNA quantification of the ~168 kDa band normalized to scramble injected controls. D. Representative

17 image of GFP in lumbar slices from P10 mice after intrathecal injection at birth of an adeno-

18 ventromedial part of a l 23 with AAV9 encoding for Ctrl-ShRNA (left, $n = 18$ neurons from 4 mice) or Kir4.1-ShRNA (right, $n = 30$
24 neurons from 5 mice). **G-H.** Representative voltage traces of one interneuron of a mouse injected 18 associated virus (AAV9) encoding a Kir4.1-targeting shRNA (Kir4.1-5hRNA). L. Die linge of the
18 ventromedial part of a lumbar slice and the corresponding one-photon GFP signal (right) of a P10
20 mouse injected with AA 20 mouse injected with AAV9-Ctrl-ShRNA (left) or AAV9-Kir4.1-ShRNA (right). CC means central canal.

21 Scale bars, 20µm. F. Quantification of the mean RMP (left), input resistance (middle) and cocktail-

22 evoked membra Scale bars, 20µm. F. Quantification of the mean RMP (left), input resistance (middle) and cocktail-

evoked membrane depolarisation (right) of ventromedial interneurons from wild-type mice injected

with AAV9 encoding for 22 Scale bars, 20µm. F. Quantification of the mean RMP (left), input resistance (middle) and cocktain
22 evoked membrane depolarisation (right) of ventromedial interneurons from wild-type mice injected
23 with AAV9 encodin 22 with AAV9 encoding for Ctrl-ShRNA (left, n = 18 neurons from 4 mice) or Kir4.1-ShRNA (right, n = 30
24 neurons from 5 mice). **G-H.** Representative voltage traces of one interneuron of a mouse injected
25 with AAV9-Ctrl 25 with AAV9-Ctrl-ShRNA (left) or AAV9-Kir4.1-ShRNA (right) in response to ionic changes (E) or in
26 response to the neuromodulator cocktail in presence of TTX (F). I-J. Proportion of oscillatory
27 interneurons (blue) o 24 neurons from 5 mice). G-H. Representative voltage traces of one interneuron of a mouse injected
25 with AAV9-Ctrl-ShRNA (left) or AAV9-Kir4.1-ShRNA (right) in response to ionic changes (E) or in
26 response to the neur 26 response to the neuromodulator cocktail in presence of TX (F). 1-J. Proportion of oscillatory

27 interneurons (blue) or not (grey) from mice injected at birth with an AAV9 encoding either a

28 Luciferase shRNA (Ctrl 27 interneurons (blue) or not (grey) from mice injected at birth with an AAV9 encoding either a
28 Luciferase shRNA (Ctrl-ShRNA, n = 4 mice) (left) or a Kir4.1-targeting shRNA (Kir4.1-ShRNA, n = 7
29 mice) (right) in pres 28 Luciferase shRNA (Ctrl-ShRNA, n = 4 mice) (left) or a Kir4.1-targeting shRNA (Kir4.1-ShRNA, n = 7 mice) (right) in presence of high $[K^+]_e$ and low $[Ca^{2+}]_e$ (H) or in presence of the neuromodulator cocktail + TTX (I) mice) (right) in presence of high $[K^+]_e$ and low $[Ca^{2+}]_e$ (H) or in presence of the neuromodulator cocktail + TTX (I). ns, no significance, **P < 0.01, ***P < 0.001 (two-tailed Mann-Whitney test for C and F, two-tailed

and F, two-tailed Fisher test for I and J). Mean ± SEM. For detailed P values, see Source data file.

32
 33 Figure 7. Astrocytic Kir4.1 channels influence the locomotor behaviour. A. Schematic

35 representation of the 32
33
33 **Figure 7. Astrocytic Kir4.1 channels influence the locomotor behaviour. A.** Schen
35 representation of the experimental design. WT for wild-type and P for postnatal day. **B.** Top. Bot
36 view of P18 mice injected 33
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39 Figure 7. Astrocytic Kir4.1 channels inhidence the locomotor behaviour. A. Schematic
35 representation of the experimental design. WT for wild-type and P for postnatal day. **B.** Top. Bottom
36 view of P18 mice injected at 135 representation of the experimental design. WT for wild-type and P for postnatal day. B. Top. Bottom
136 view of P18 mice injected at birth with AAV9 encoding either for the Ctrl-ShRNA (left) or the Kir4.1-
137 ShRNA (r ShRNA (right) freely walking on a glass platform (Catwalk). *Bottom*. Side view of the same P18 mice.
 C. Representative footfall diagrams during CatWalk locomotion of a Ctrl-ShRNA P18 mouse (blue,

top) or Kir4.1-ShRNA 37 ShRNA (right) freely walking on a glass platform (Catwalk). Bottom. Side view of the same P18 mice.
38 **C.** Representative footfall diagrams during CatWalk locomotion of a Ctrl-ShRNA P18 mouse (blue,
39 top) or Kir4.1-S C. Representative Footfall diagrams during CatWalk locomotion of a Ctrl-Sinkiva F18 mouse (blue,

39 top) or Kir4.1-ShRNA P18 mice (green, bottom). The stance phase is indicated by horizontal bars and

40 the swing phase b 39 the swing phase by open spaces. RF for Right Forelimb, RH for Right Hindlimb, LF for Left Forelimb,
39 LH for Left Hindlimb. D. Comparative quantification of the normalized step cycle phases (stance,
39 swing) (left), 141 LH for Left Hindlimb. D. Comparative quantification of the normalized step cycle phases (stance, swing) (left), regularity index (middle left), body speed (middle right) and hind limb base of support (right) between Ct 41 LH for Left Hinding. D. Comparative quantification of the normalized step cycle phases (stance, swing) (left), regularity index (middle left), body speed (middle right) and hind limb base of support (right) between Ctrl (right) between Ctrl-ShRNA mice (blue, $n = 14$ mice) and Kir4.1-ShRNA mice (green, $n = 14$ mice).

Each circle represents an individual mouse. **E.** Latency to fall from a rod rotating at accelerated

speed (4–40 rpm) duri Each circle represents an individual mouse. **E.** Latency to fall from a rod rotating at accelerated
speed (4–40 rpm) during 300s of wild-type mice transduced either with the Ctrl-ShRNA (blue, n = 14
mice) or with Kir4.1-Sh Each circle represents an individual mouse. **L.** Latency to fail from a rod rotating at accelerated speed (4–40 rpm) during 300s of wild-type mice transduced either with the Ctrl-ShRNA (blue, n = 14 mice) or with Kir4.1-S mice) or with Kir4.1-ShRNA (green, n = 14 mice). F. *Left*: Heatmaps illustrate the swimming activity of
two wild-type mice transduced either with the Ctrl-ShRNA (top) or with the Kir4.1-shRNA (bottom).
Scale bar, 10 cm. 46 mice) or with Kir4.1-ShRNA (green, n – 14 mice). T. Left: Heatmaps must act the swimming activity or
47 two wild-type mice transduced either with the Ctrl-ShRNA (top) or with the Kir4.1-shRNA (bottom).
48 Scale bar, 10 48 Scale bar, 10 cm. *Middle:* Comparison of the mean swimming distance (middle left) and the mean
velocity (middle right) between Ctrl-shRNA (blue, n = 9 mice) and Kir4.1-shRNA (green, n = 9 mice)
17 scale bar, 10 cm. Middle: Comparison of the mean swimming distance (middle left) and the mean
velocity (middle right) between Ctrl-shRNA (blue, n = 9 mice) and Kir4.1-shRNA (green, n = 9 mice)
17 $\frac{4}{3}$ velocity (middle right) between $\frac{1}{4}$.17

1 mice. Each circle represents one individual mouse. Right: Percentage of mobility and immobility for

2 Ctrl-shRNA mice (blue, n = 9 mice) and Kir4.1-shRNA mice (green, n = 9 mice) during the swimming

5 forced test. ns,

2 Forced test. ns, no significance, *P < 0.05, **P < 0.01, ***P < 0.001 (Mann-Whitney test for D, E right
and F, Two-way ANOVA with Sidak's multiple comparisons test for E left). Mean ± SEM. For detailed
P values, see Sour 3 forced test. ns, no significance, *P < 0.05, **P < 0.01, ***P < 0.001 (Mann–Whitney test for D, E right
and F, Two-way ANOVA with Sidak's multiple comparisons test for E left). Mean ± SEM. For detailed
P values, see Sour

4 and F, Two-way And Two-way A

5 P values, see Source data file.
6

Figure 2

Figure 7

Graphical Abstract. Overview of the astrocytic K⁺ uptake mechanisms influencing the locomotor pattern. Schematic relationship between astrocytes and neurons in the spinal locomotor central pattern generator (CPG) network. K⁺ for potassium, RF for right front limb, LF for left front limb, RH for right hind limb, LH for left hind limb.