Walking on the Moon: Hypogravity drives the emergence of a proprioception-dependent locomotor state

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Abstract:

Animals must adapt locomotion to changing environments, but how the nervous system flexibly select gait remains unclear. Gravity is a powerful natural perturbation altering body loading and limb dynamics. Apollo astronauts often skipped on the Moon, adopting an asymmetric gait rarely used on Earth, yet the motor control basis of this behavior is unknown. Here, by studying the effect of hypogravity on locomotion in humans and mice, we identify a conserved strategy for gait adaptation. Muscle synergy analysis in humans shows that skipping in reduced gravity is generated through flexible reuse of existing motor modules rather than construction of new ones. In mice, lunar gravity elicited a skipping-like asymmetric gait and genetic elimination of muscle proprioceptors abolished it. Thus, hypogravity reveals a proprioception-dependent mechanism for flexible gait selection.

A central challenge in neuroscience is understanding how the nervous system controls movement in a robust and flexible way. This is especially evident during locomotion, where coordination must be maintained while the sensorimotor system adapts to changing body state and environmental conditions (*1*–*3*). Somatosensory feedback is central to this flexibility because it continuously informs the nervous system about limb position, loading, contact, and body movement. Gravity represents a powerful perturbation of this control problem because it simultaneously alters body loading, limb dynamics, and the resulting sensory feedback (*4*–*6*). Thus, changes in gravitational conditions offer a unique opportunity to ask how the nervous system selects among available locomotor states when the mechanical and sensory context of movement is altered.

During extravehicular activities on the lunar surface, Apollo astronauts often preferred a unilateral skipping gait over walking or running (Fig. 1A and movie S1) (*7*), citing stability and ease of adaptation as reasons for their choice (*8*–*10*). This behavior is striking because unilateral skipping is rarely used by adults on Earth, yet it emerged spontaneously in the reduced-gravity environment of the Moon. Skipping is a hybrid gait that combines the double support of walking with the flight phase of running and involves an asymmetric use of the limbs, as the trailing and leading legs never switch roles (Fig. 1, B and C) (*7*, *11*). On Earth, skipping is metabolically more costly than running (*7*). This energetic disadvantage progressively diminishes as gravity decreases, and under lunar gravity it becomes negligible (*12*). Therefore, energetic viability alone does not explain why skipping becomes a preferred locomotor solution in hypogravity, an issue that is increasingly relevant as the Artemis program aims to establish a sustained human presence on the Moon (*13*).

How reduced gravity changes the neural control of gait remains unknown. One possibility is that locomotion in hypogravity requires a new motor control architecture. An alternative is that reduced gravity reveals a latent locomotor state by retuning pre-existing control elements. Muscle synergies describe the modular organization of movement and provide a way to distinguish between these two possibilities (*14*). A new control architecture should alter the relative contribution of muscles to each synergy. Reuse of existing synergies should instead preserve the modular structure while changing the timing of recruitment (*15*–*20*). Another unresolved question is whether hypogravity-induced gait selection depends on sensory feedback. Proprioception, the sense of body position and movement, monitors muscle length and tension and is therefore ideally positioned to signal the changes in limb loading produced by reduced gravity (*21*). If the emergence of skipping on the Moon reflects a sensory-dependent adaptation, then disrupting proprioceptive feedback should impair the expression of the asymmetric locomotor state induced by hypogravity. Answering these questions requires an experimental model in which locomotion can be tested under reduced gravity and the sensorimotor system can be causally manipulated.

We therefore combined simulated hypogravity experiments in humans with a newly developed mouse model of reduced-gravity locomotion. In humans, muscle-synergy analysis allowed us to test whether hypogravity changes the structure or timing of locomotor control. In mice, locomotion under simulated lunar gravity allowed us to determine whether reduced gravity induces an asymmetric gait, while mouse genetics allowed us to test whether proprioceptive feedback is required for its expression. Together, this cross-species approach reveals how the nervous system adapts locomotion to reduced gravity by flexibly reusing existing motor control elements.

Simulated lunar gravity reproduces the asymmetric structure of skipping

We modeled human reduced-gravity locomotion in the laboratory using an elastic body-weight support system mounted over a treadmill. Participants were asked to walk, skip, and run under Earth gravity and simulated Martian and lunar gravity while bilateral electromyographic (EMG) activity was recorded from lower-limb muscles (Fig. 1D and movie S2). Stick-figure reconstruction of skipping obtained during simulated lunar gravity recapitulated the kinematic pattern observed in the Apollo footage (Fig. 1B), indicating that the experimental paradigm captured the essential spatiotemporal organization of lunar skipping.

Quantitative analysis confirmed that skipping was the gait characterized by the strongest asymmetry across all gravity conditions (Fig. 1E). Interlimb step-time asymmetry, computed as the normalized absolute difference between lead-to-trail and trail-to-lead touchdown intervals, was higher for skipping than for either walking or running, and this effect was conserved under simulated Martian and lunar gravity.

These results establish that the simulated hypogravity paradigm reproduces the interlimb asymmetry characteristic of lunar skipping.

Humans adapt to hypogravity by adjusting the timing of conserved locomotor modules

To determine whether locomotion in hypogravity requires a new control architecture or the retuning of an existing one, we extracted muscle synergies (*14*) from lower-limb EMG activity (Fig. S1) recorded during walking, skipping, and running under Earth gravity and simulated Martian and lunar gravity. Muscle synergies are composed of time-independent muscle weights (**W**) and time-dependent activation patterns (**P**) describing, respectively, the relative contribution of different muscles to each synergy and the time course of synergy activation (Fig. 2A). The number of synergies (Fig. 2B) and quality of EMG reconstruction (Fig. 2C) were unaffected by gait or gravity, indicating that neither parameter alters the dimensionality of motor output. Four muscle synergies functionally described different phases of the gait cycle in walking, skipping, and running (Fig. 2D, Table S1). Activation patterns (**P**) showed pronounced temporal modulation in response to hypogravity (Fig. 2D), such as shifts (Fig. 2E) and/or changes in the duration of muscle synergy activity (Fig. S2). This is consistent with the idea that adaptation to perturbation is primarily achieved by adjusting the timing of a conserved modular architecture (*15*–*20*).

Because hypogravity affected the activation patterns of a conserved number of muscle synergies, we next asked whether it also altered their dynamical properties. Previous work showed that perturbations make muscle activation patterns less unstable during locomotion (*22*), suggesting that challenging conditions constrain the range of activation dynamics. By contrast, less constrained conditions may permit a wider range of activation solutions. We therefore calculated the short-term maximum Lyapunov exponent of activation patterns to quantify their dynamic stability (*23*). The results show that, on Earth, the gait with the largest instability of activation patterns was walking (Fig. 2F). Yet, under simulated lunar gravity, skipping presented the highest instability (Fig. 2F). Thus, hypogravity reorganized activation pattern dynamics, shifting the greatest instability from walking on Earth to skipping under lunar gravity, providing an explanation to why skipping was preferred by astronauts on the Moon.

Altogether, these findings indicate that hypogravity primarily alters locomotor control by changing the timing of a conserved set of muscle synergies, rather than by changing the modular architecture of the synergies.

Skipping shares a running-like modular architecture

To determine whether skipping arises through reuse of pre-existing locomotor modules, we compared muscle synergies extracted from skipping EMG with those extracted from walking and running. Skipping and running, unlike walking, are bouncing gaits (*7*, *12*) and, although kinematically distinct, their temporal activation patterns under Earth gravity were qualitatively similar (Fig. 3A). By contrast, the corresponding muscle weights, ordered according to the timing of their activation patterns, differed substantially (Fig. 3B). However, reordering the skipping muscle weights from their original sequence 1-2-3-4 to a new sequence of recruitment (3-4-1-2) revealed a marked overlap with the muscle weights of running (Fig. 3C). These observations suggest that the main difference between skipping and running does not lie in the structure of activation patterns, but in the recruitment order of muscle weights.

To test this idea systematically, we performed a cross-reconstruction analysis in which running EMG activity was reconstructed using all possible permutations of running activation patterns and muscle weights from either running or skipping (**W** x **P**; Fig. 3D). As expected, running EMG was best reconstructed by using running muscle weights ordered according to original timing of running activation patterns (1-2-3-4), but reconstruction quality decreased when the temporal sequence of running muscle weights recruitment was changed (Fig. 3D, left). Conversely, reconstruction of running EMG using skipping muscle weights ordered using the original sequence of skipping activation patterns was poor, consistent with a mismatch in the spatial structure of running and skipping (Fig. 3D, right). However, reordering the skipping muscle weights specifically into the 3-4-1-2 sequence yielded high reconstruction quality across gravity levels (Fig. 3E). This effect was not observed when the same analysis was applied to walking and skipping (Fig. S3).

These results indicate that skipping is generated by reconfiguring a running-like architecture rather than by using a new set of locomotor modules.

Lunar gravity induces a skipping-like asymmetric gait in mice

To determine whether the emergence of skipping is a conserved adaptation to hypogravity and identify the underlying mechanisms, we established a mouse model of reduced-gravity locomotion. We adapted the human experimental paradigm to mice by studying animals wearing a harness connected to an elastic body-weight support system over a treadmill (Fig. 4A and fig. S4) and quantified 103 kinematic parameters describing locomotion (Table S2) under Earth gravity and simulated lunar gravity using high-speed video and markerless motion tracking (*24*). Unlike in the human experiments, gait was self-selected and not instructed or trained.

Under Earth gravity, mice typically locomoted with a symmetrical trot (Fig. 4B and movie S3) (*25*). In contrast, under simulated lunar gravity, the animals frequently adopted an asymmetric gait characterized by prolonged swing duration of one limb relative to the other, such that one paw remained lifted for one or more gait cycles (Fig. 4B and movie S3). Although quadrupedal in expression, this behavior resembled a skipping-like gait because it introduced an asymmetry in left-right limb timing analogous to the unilateral skipping observed in humans. To quantify this effect, we calculated left-right airtime symmetry for both forelimbs and hindlimbs. Simulated lunar gravity increased asymmetry in the hindlimbs, whereas the forelimbs were not significantly affected (Fig. 4C). Thus, hypogravity induced a specific reorganization of hindlimb step timing consistent with the emergence of a quadrupedal skipping-like gait.

We next asked whether this adaptation represented a distinct locomotor state. Principal component analysis of kinematic parameters revealed that locomotion under Earth gravity and simulated lunar gravity occupied separate regions of the kinematic space indicating that reduced gravity induces a distinct locomotor state in mice (Fig. 4D).

These findings show that simulated lunar gravity elicits a skipping-like asymmetric gait in mice, indicating that hypogravity reveals a conserved locomotor adaptation principle across limbed vertebrates.

Proprioceptive feedback is required for locomotor adaptation to lunar gravity

The emergence of skipping-like behavior in mice locomoting under lunar gravity provides a tractable experimental model for testing the sensory mechanisms that enable this adaptation. Proprioception is ideally positioned to detect changes in gravitational load because muscle spindles and Golgi tendon organs continuously monitor muscle length and tension, thereby signaling how limb mechanics are altered when gravity changes.

To test whether proprioceptive feedback is required for the emergence of skipping-like gait under reduced gravity conditions, we took advantage of an intersectional mouse genetics strategy to selectively eliminate proprioceptive sensory neurons (*26*). We generated a mouse line in which the human diphtheria toxin receptor and a red fluorescent protein (tdTomato) were selectively targeted to muscle spindle- and Golgi tendon organ-afferents in a Cre- and Flp-dependent manner (Fig. S5). Following diphtheria toxin administration in adult mice, we quantified ablation efficiency over the course of a week by histological analysis of dorsal root ganglia (Fig. 5A). We observed progressive loss of tdTomato-labelled proprioceptive neurons starting 24 hours after injection and reaching maximum by 72 hours. As expected, we found that the extent of proprioceptor loss correlated with the effect on kinematic parameters describing locomotion (Fig. 5B and fig. S6). Consistently, locomotor impairment reached a plateau by 72 hours, with no further degradation at one week. At these late time points, locomotion was severely compromised (Fig. S6 and movie S4). In contrast, locomotion remained largely preserved at the 24- and 48-hour time points (Fig. 5C and movie S5), defining a window in which partial sensory loss could be studied before severe locomotor deficits occurred.

We therefore examined locomotion under lunar gravity before diphtheria toxin injection and during the first 48 hours after injection, when neuronal ablation had begun but motor impairment remained limited. Principal component analysis of kinematic parameters (Table S2) confirmed that locomotor changes during this period were relatively minor and were expressed primarily along the second principal component, whereas the first principal component, which captured most of the variance, remained preserved (Fig. 5C). These findings indicate that the earliest effects of proprioceptor loss did not result in a global collapse of locomotor function. We next examined whether the skipping-like hindlimb asymmetry induced by simulated lunar gravity was retained during this early period of neuronal ablation. Before diphtheria toxin injection and 24 hours after injection, simulated lunar gravity induced hindlimb asymmetry; however, this effect disappeared at 48 hours (Fig. 5D and movie S6). Consistent with this result, the effect size of lunar gravity on hindlimb gait asymmetry gradually declined from large values before and 24 hours after injection to negligible values at 48 hours (Fig. 5E).

Together, these findings indicate that proprioceptive feedback is necessary for the expression of hypogravity-induced skipping-like locomotor adaptation in mice.

Discussion

Changing gravity perturbs the mechanical and sensory context in which locomotion is controlled: limb loading is reduced, body dynamics are reshaped, and the proprioceptive consequences of each step are altered (*1*). For these reasons, the preference of Apollo astronauts for skipping on the Moon offers a unique perspective on a broad motor control question: how does the nervous system flexibly select a gait when the environment constrains the available control solutions? Our findings suggest that this adaptation does not require a new motor control architecture. In humans, simulated Martian and lunar gravity preserved the number and composition of muscle synergies, while selectively changing their temporal recruitment. Thus, reduced gravity does not reshape the identity of muscles engaged in different synergies, but rather changes the phase of the gait cycle in which they are recruited. From this perspective, skipping is a latent locomotor state that becomes accessible when changes in gravitational load perturb the sensorimotor system.

Previous studies have defined skipping as a hybrid gait that combines the double support of walking with the flight phase of running (*7*, *11*). Our analyses extend this concept from biomechanics to motor control. Running muscle activity can be accurately reconstructed when running activation patterns are combined with an appropriate assignment of skipping muscle weights. Thus, skipping appears to arise through the flexible reuse of an existing locomotor architecture rather than the recruitment of a new set of muscle synergies. Comparative observations are consistent with this view. Jerboas frequently use hopping and skipping (*27*, *28*); crows and magpies can produce running-like mechanics through altered interlimb phasing (*27*, *29*). Within primates, lemurs can use unilateral skipping or hopping despite lacking a human-like running gait (*30*), suggesting that asymmetric bouncing patterns are part of a broader locomotor repertoire across vertebrates. In humans, children spontaneously express skipping during development (*7*), indicating that this gait remains available even though it is rarely used in adulthood.

The mouse experiments in simulated lunar gravity provide a model in which the sensory mechanisms underlying this flexible adaptation can be tested. While the changes in locomotor behavior of mice cannot be considered kinematically homologous to human skipping, simulated lunar gravity induced the key organizational feature observed in Apollo astronauts: persistent left-right asymmetry in limb timing. Under Earth's gravity, mice locomoted with a symmetric trot. However, under simulated lunar gravity, they adopted a hindlimb-asymmetric state, in which one hind paw remained in swing for one or more gait cycles. This behavior emerged during otherwise coordinated treadmill locomotion and affected only the hindlimbs, indicating that hypogravity did not simply disrupt gait control, but revealed a distinct asymmetric locomotor state that parallels the defining kinematic feature of human skipping on the Moon.

We used this framework to test whether proprioception is necessary for flexible locomotor adaptation to reduced gravity. Muscle spindles and Golgi tendon organs detect changes in muscle length and tension. This information is necessary for robust control of locomotor output in the face of internal or external perturbations (*1*, *2*, *31*, *32*). Hypogravity alters these very same mechanical variables by reducing limb loading and altering the sensory consequences of each step. In intact mice, this altered sensory context enabled expression of the hindlimb-asymmetric state under simulated lunar gravity. After partial proprioceptor ablation, locomotor kinematics remained largely preserved, but the asymmetry induced by lunar gravity disappeared. Because this loss occurred before severe locomotor impairment, it cannot be explained by ataxia or global motor deterioration alone. Rather, proprioceptive feedback appears necessary for expressing the skipping-like asymmetric gait in hypogravity. These data indicate that proprioception is not only necessary to maintain robust gait control, but also to enable adaptive changes in response to reduced gravitational load. This observation is consistent with a broader view in which sensory feedback is not merely corrective, but actively shapes motor control by regulating which locomotor states can be expressed.

Why should hypogravity favor the expression of skipping? Reduced gravity changes the physical constraints under which movement is controlled. On land, locomotion requires animals to coordinate propulsion while maintaining body support, making limb loading, stance-phase control, and balance central constraints on locomotor control (*1*, *3*, *33*). Lunar gravity partially relaxes these constraints while preserving the need to coordinate propulsion, balance, and ground contact. Previous metabolic measurements showed that skipping is more costly than running on Earth, but that this energetic disadvantage becomes negligible under lunar gravity. Thus, hypogravity may favor skipping not by making it metabolically cheaper than running, but by reducing the energetic penalty that normally suppresses it and allowing other factors to influence gait selection (*12*). By combining an aerial phase with double support, skipping may extend the window over which body motion can be corrected before initiating the flight phase (*12*). In this view, reduced gravity shifts locomotion into a regime in which a latent asymmetric bouncing solution becomes viable, while proprioceptive feedback provides the limb load- and position-dependent information required for its expression.

More broadly, hypogravity acts as a perturbation that reveals the robustness and flexibility of the locomotor control system. In humans, reduced gravity changed the temporal deployment of conserved muscle synergies without altering their composition. The increased local instability of skipping activation patterns under lunar gravity indicates that hypogravity broadens the dynamical range in which muscle synergies can produce robust locomotor control. In mice, hypogravity induced proprioception-dependent expression of a skipping-like asymmetric gait, suggesting a conserved sensorimotor strategy for adapting to reduced gravity. Together, these findings provide a mechanistic account of a classic observation from lunar exploration and establish hypogravity as a powerful paradigm for revealing how the nervous system flexibly adapts to perturbations.

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Visualization: AS

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Supplementary Materials

Materials and Methods

Figs. S1 to S6

Tables S1 and S2

Movies S1 to S6

Fig. 1. Lunar skipping is an asymmetric gait. (**A**) Two-dimensional, markerless tracking of Eugene Cernan skipping on the Moon during the Apollo 17 mission. (**B**) Sagittal-plane stick diagrams reconstructed from the Apollo archival footage (top) and from simulated hypogravity on a treadmill with body weight support (bottom). (**C**) Schematic footfall pattern of walking (left), unilateral skipping (center), and running (right). Unlike walking and running, skipping maintains the same trailing limb across cycles and combines a walking-like double stance with a running-like flight phase. (**D**) Human simulated hypogravity locomotion setup. Participants locomoted on a treadmill under Earth's gravity, as well as under simulated Martian and lunar gravity, while supported by an elastic body-weight suspension system. Surface electromyography (EMG) was recorded bilaterally from the *vastus medialis* (vm), *semitendinosus* (st), *biceps femoris* long head (bf), *tibialis anterior* (ta), *gastrocnemius medialis* (gm), and *soleus* (so). (**E**) Interlimb step-time asymmetry across gravity conditions and gaits ($N = 12$ participants). Asymmetry was quantified as the absolute difference between lead-to-trail and trail-to-lead touchdown intervals, normalized to mean gait-cycle duration. Different letters indicate significant *post-hoc* differences.

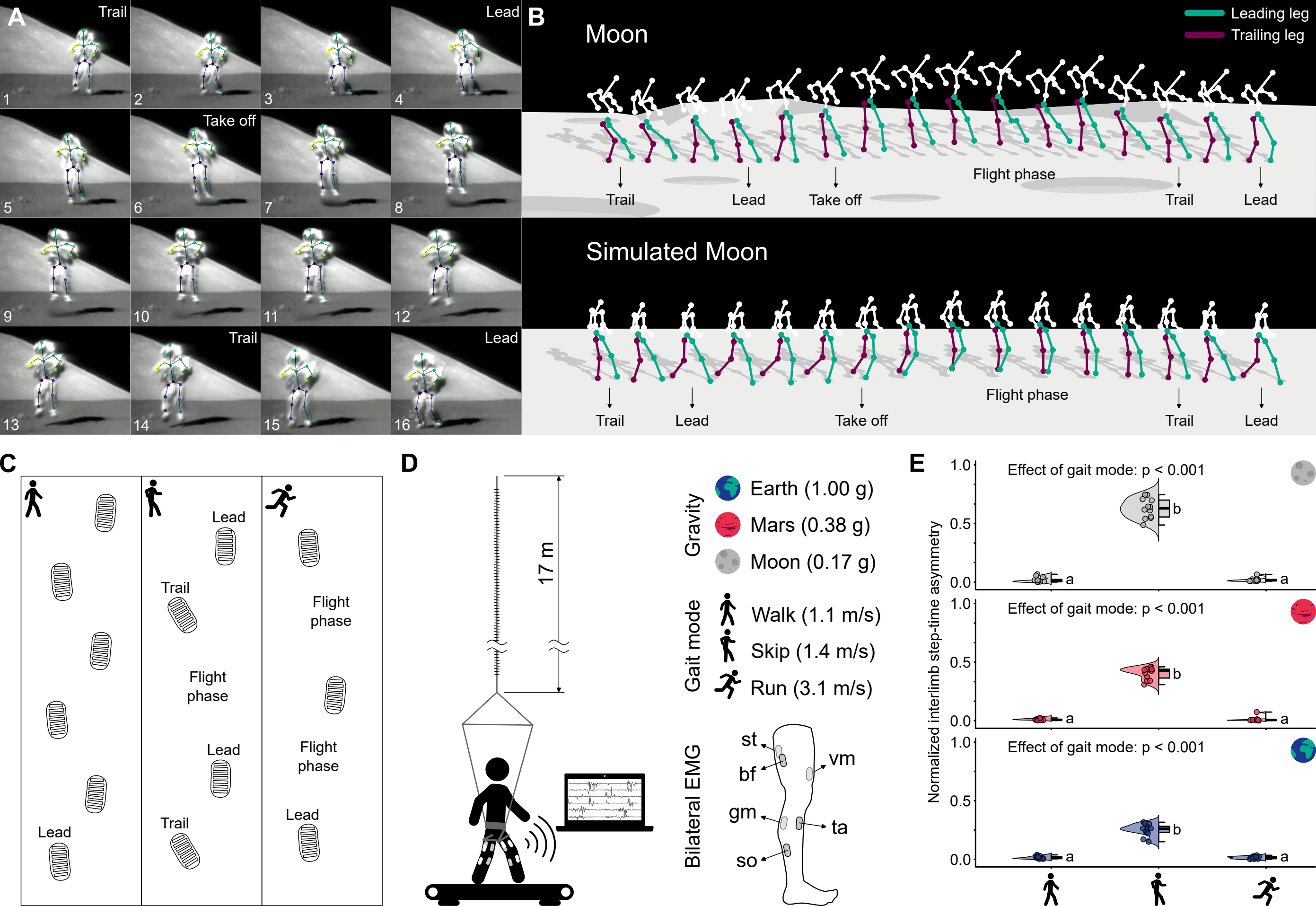
Fig. 2. Hypogravity induces the modulation of muscle synergy activation patterns. (**A**) Mathematical intuition of non-negative matrix factorization of EMG activity to extract muscle synergies. Each synergy is defined by a set of time-independent muscle weights **W**, representing the relative contribution of each muscle within a synergy, and a set of time-dependent activation patterns **P**, representing the time course of synergistic activation. (**B**) Minimum number of synergies (factorization rank) required to reconstruct locomotor EMG patterns across gaits and gravity conditions. (**C**) Reconstruction quality of the synergy model, expressed as variability accounted for (VAF), across recorded muscles, gaits, and gravity conditions. (**D**) Muscle weights **W** and activation patterns **P** of muscle synergies extracted from walking, skipping, and running under Earth gravity, and under simulated Martian and lunar gravity. (**E**) Center of activity (CoA) of synergy activation patterns across gaits and gravities. (**F**) Local dynamic stability of activation patterns, quantified by the short-term maximum Lyapunov exponent (sMLE), across gaits and gravities ($N = 12$ participants). Different letters indicate significant *post-hoc* differences.

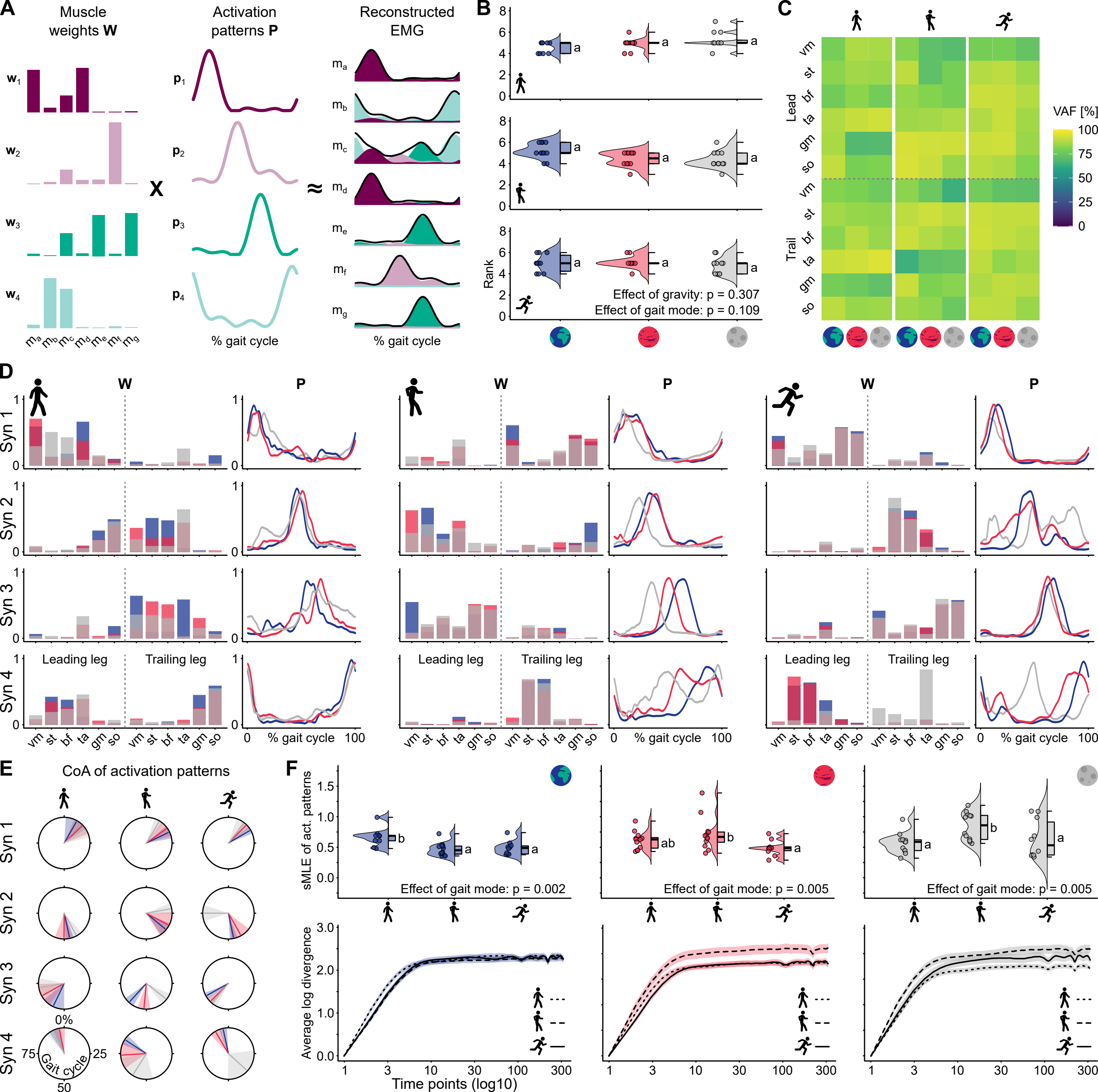
Fig. 3. Skipping emerges from a running-like modular architecture. (**A**) Average synergy activation patterns **P** for running and skipping under Earth gravity. (**B**) Synergy muscle weights **W** for running and skipping under Earth gravity. (**C**) Reordered skipping muscle weights overlaid on running weights. (**D**) Cross-reconstruction analysis of running EMG using running activation patterns combined with running weights or skipping weights. Reconstruction quality

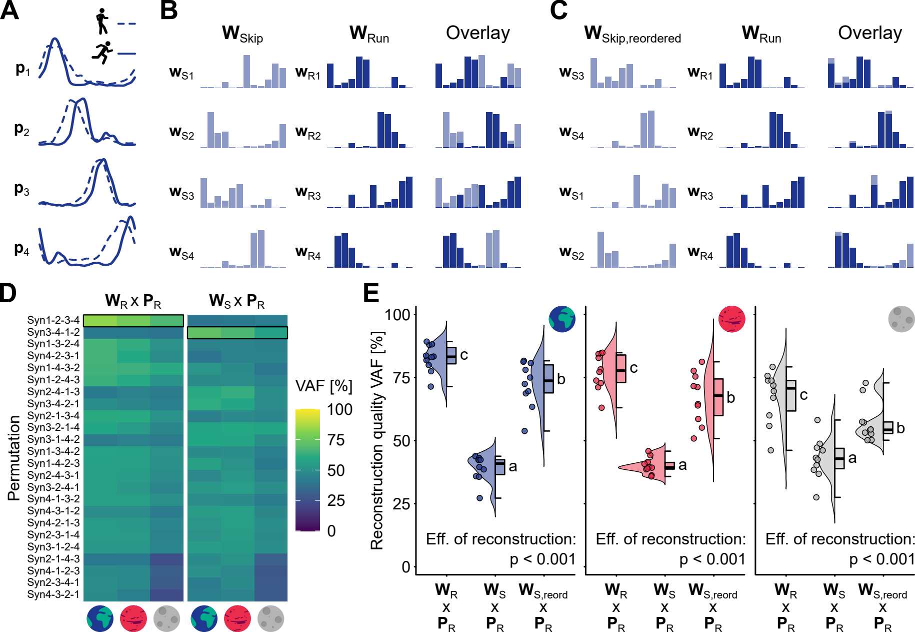
was quantified for all possible weight-order permutations. (**E**) Reconstruction quality of running across gravity conditions using i) running weights and activations, ii) skipping weights and running activations, or iii) reordered skipping weights and running activations. Different letters indicate significant *post-hoc* differences (*N* = 12 participants).

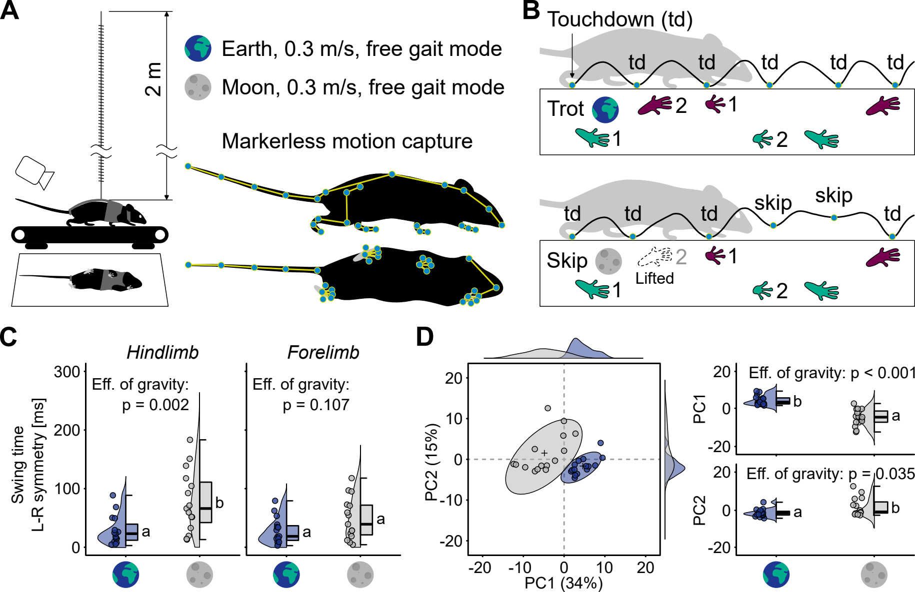
Fig. 4. Hypogravity induces skipping-like asymmetric gait in mice. (**A**) Mouse simulated hypogravity locomotion setup. Mice walked on a treadmill at 0.3 m/s under Earth gravity or simulated lunar gravity while supported by an elastic body-weight suspension system. Limb kinematics were quantified from high-speed video using markerless tracking. (**B**) Representative limb trajectories under Earth gravity and simulated lunar gravity. Under Earth gravity, mice typically used a symmetric trot (top). Under simulated lunar gravity, mice frequently prolonged swing of one hindlimb across one or more gait cycles, producing a persistent hindlimb asymmetry (bottom). (**C**) Left–right airtime asymmetry during Earth and simulated lunar gravity locomotion for hindlimbs and forelimbs (*N* = 15 mice). (**D**) Principal component analysis of kinematic variables during Earth and simulated lunar locomotion (*N* = 15 mice). Different letters indicate significant *post-hoc* differences.

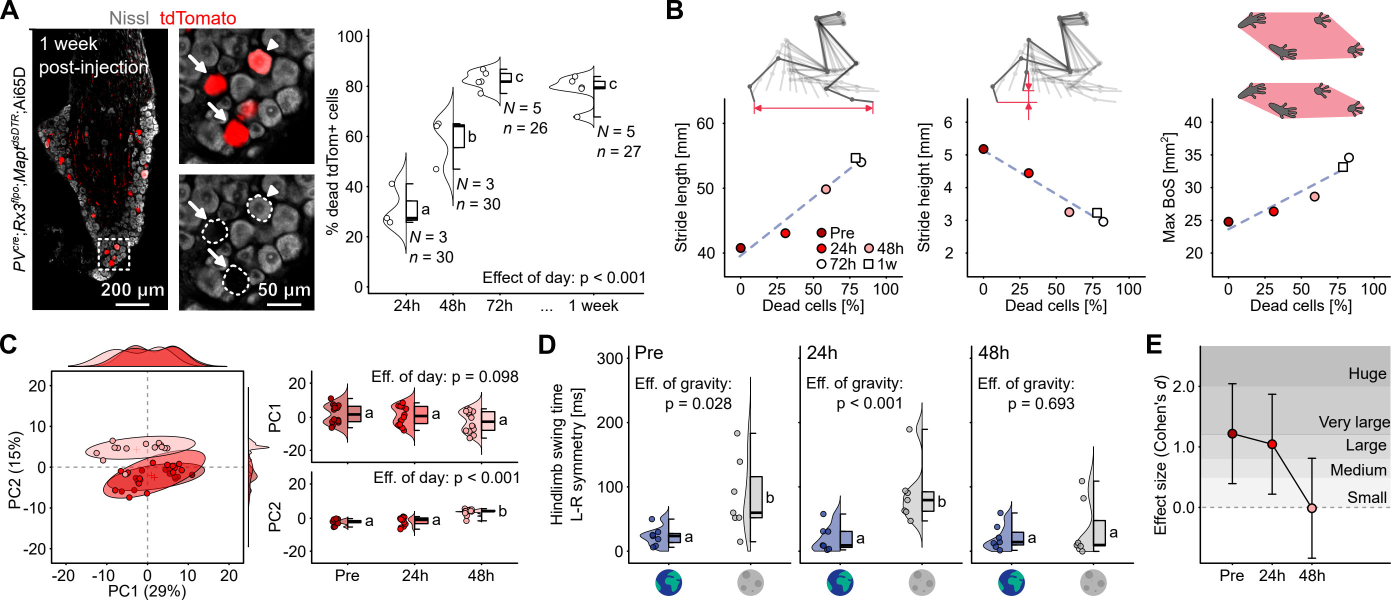
Fig. 5**. Proprioceptive feedback is required for locomotor adaptation to hypogravity.** (**A**) Representative image of a lumbar dorsal root ganglion one week after diphtheria toxin administration showing loss of proprioceptive sensory neurons (left, arrows indicate ablated neurons, tdTomato$^+$; Nissl$^-$; arrowhead shows a spared neuron, tdTomato$^+$; Nissl$^+$) and time course of neuronal ablation (right). (**B**) Relationship between proprioceptor loss and kinematic changes, using as representative examples thre key paramters describing locomotion: stride length (left), stride height (center), and base of support (right). (**C**) Principal component analysis of kinematic variables before and after diphtheria toxin injection. (**D**) Hindlimb left-right swing-time asymmetry under Earth and simulated lunar gravity before (left), 24 hours (center) and 48 hours (right) after proprioceptor ablation (*N* = 7 mice). (**E**) Effect size of simulated lunar gravity on hindlimb asymmetry over time after diphtheria toxin injection (*N* = 14 mice). Different letters indicate significant *post-hoc* differences.


A
Trail
Lead
Take off
Trail
Lead
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
B
Moon
Leading leg
Trailing leg
Trail
Lead
Take off
Flight phase
Trail
Lead
Simulated Moon
Trail
Lead
Take off
Flight phase
Trail
Lead
C
Lead
Lead
Trail
Flight phase
Lead
Trail
Flight phase
Flight phase
Lead
D
17 m
Gravity
Earth (1.00 g)
Mars (0.38 g)
Moon (0.17 g)
Gait mode
Walk (1.1 m/s)
Skip (1.4 m/s)
Run (3.1 m/s)
Bilateral EMG
st
bf
gm
so
vm
ta
E
Normalized interlimb step-time asymmetry
Effect of gait mode: p < 0.001
Effect of gait mode: p < 0.001
Effect of gait mode: p < 0.001
1.0
0.5
0.0
a
b
a


A
Muscle weights W
Activation patterns P
Reconstructed EMG
w1
w2
w3
w4
p1
p2
p3
p4
X
≈
ma
mb
mc
md
me
mf
mg
% gait cycle
% gait cycle
B
Rank
a
Effect of gravity: p = 0.307
Effect of gait mode: p = 0.109
C
VAF [%]
100
75
50
25
0
Lead
Trail
vm
st
bf
ta
gm
so
D
W
P
Syn 1
Syn 2
Syn 3
Syn 4
Leading leg
Trailing leg
vm st bf ta gm so
0 % gait cycle 100
E
CoA of activation patterns
0%
25
50
75
Gait cycle
F
sMLE of act. patterns
a
b
ab
Effect of gait mode: p = 0.002
Effect of gait mode: p = 0.005
Effect of gait mode: p = 0.005
Average log divergence
Time points (log10)
1
3
10
30
100
300


A
p$_1$
p$_2$
p$_3$
p$_4$
B
W$_{Skip}$
W$_{Run}$
Overlay
w$_{S1}$
w$_{S2}$
w$_{S3}$
w$_{S4}$
w$_{R1}$
w$_{R2}$
w$_{R3}$
w$_{R4}$
C
W$_{Skip,reordered}$
W$_{Run}$
Overlay
w$_{S3}$
w$_{S4}$
w$_{S1}$
w$_{S2}$
w$_{R1}$
w$_{R2}$
w$_{R3}$
w$_{R4}$
D
W$_R$ x P$_R$
W$_S$ x P$_R$
Permutation
Syn1-2-3-4
Syn3-4-1-2
Syn1-3-2-4
Syn4-2-3-1
Syn1-4-3-2
Syn1-2-4-3
Syn2-4-1-3
Syn3-4-2-1
Syn2-1-3-4
Syn3-2-1-4
Syn3-1-4-2
Syn1-3-4-2
Syn1-4-2-3
Syn2-4-3-1
Syn3-2-4-1
Syn4-1-3-2
Syn4-3-1-2
Syn4-2-1-3
Syn2-3-1-4
Syn3-1-2-4
Syn2-1-4-3
Syn4-1-2-3
Syn2-3-4-1
Syn4-3-2-1
VAF [%]
100
75
50
25
0
E
Reconstruction quality VAF [%]
100
75
50
25
0
c
a
b
c
a
b
c
a
b
Eff. of reconstruction:
p < 0.001
Eff. of reconstruction:
p < 0.001
Eff. of reconstruction:
p < 0.001
W$_R$ x P$_R$
W$_S$ x P$_R$
W$_{S,reord}$ x P$_R$
W$_R$ x P$_R$
W$_S$ x P$_R$
W$_{S,reord}$ x P$_R$
W$_R$ x P$_R$
W$_S$ x P$_R$
W$_{S,reord}$ x P$_R$


A
2 m
Earth, 0.3 m/s, free gait mode
Moon, 0.3 m/s, free gait mode
Markerless motion capture
B
Touchdown (td)
td
td
td
td
td
Trot
2
1
1
2
td
td
td
skip
skip
td
Skip
2
Lifted
1
1
2
C
Hindlimb
Forelimb
Swing time
L-R symmetry [ms]
300
200
100
0
Eff. of gravity:
p = 0.002
a
b
Eff. of gravity:
p = 0.107
a
a
D
20
10
0
-10
-20
PC2 (15%)
-20
-10
0
10
20
PC1 (34%)
Eff. of gravity: p < 0.001
PC1
b
a
Eff. of gravity: p = 0.035
PC2
a
b


A
Nissl tdTomato
PV^{cre};$Rx3^{flpo}$;$Mapt^{dsDTR}$;Ai65D
1 week post-injection
200 µm
50 µm
% dead tdTom+ cells
100
80
60
40
20
0
a
b
c
c
N = 3
n = 30
N = 3
n = 30
N = 5
n = 26
N = 5
n = 27
Effect of day: p < 0.001
24h
48h
72h
...
1 week
B
Stride length [mm]
60
50
40
Pre
24h
48h
72h
1w
Dead cells [%]
0
25
50
75
100
Stride height [mm]
6
5
4
3
2
Max BoS [mm²]
40
35
30
25
20
C
PC2 (15%)
PC1 (29%)
20
10
0
-10
-20
Eff. of day: p = 0.098
Eff. of day: p < 0.001
PC1
PC2
a
a
a
a
a
b
Pre
24h
48h
D
Hindlimb swing time L-R symmetry [ms]
300
200
100
0
Pre
24h
48h
Eff. of gravity: p = 0.028
Eff. of gravity: p < 0.001
Eff. of gravity: p = 0.693
a
b
a
b
a
a
E
Effect size (Cohen's d)
2.0
1.0
0.0
Huge
Very large
Large
Medium
Small
Pre
24h
48h