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1 **The connectome from the cerebral cortex to the skeletal muscle using viral**
2 **transneuronal tracers: A review**

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Abstract

Connectomics has developed from an initial observation under an electron microscope to the present unassailable medical imaging research approach. The emergence of the most popular transneuronal tracers has further advanced connectomics research. Researchers use the virus trans-nerve tracing method to trace the whole brain, mark the brain nerve circuit and nerve connection structure, and construct the complete nerve conduction pathway. This review assesses current methods of studying cortical to muscle connections using viral neuronal tracers and demonstrates the application in disease diagnosis and prognosis.

Keywords: Connectomics, skeletal muscle, transneuronal tracers, cerebral cortex

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1. Introduction

The brain has a complex network of neural circuits¹. When examining the unique physiological structure of the topological heterogeneity of the brain, different techniques have been used to analyze the brain's neural circuits and draw the synaptic connections of living brain neurons^{2,3}. The connectome is an integrated histological and imaging tool for studying neural network connections in the brain. It explores the synaptic connections between neurons at the microscopic level, reveals the neural pathways of the cerebral cortex, and compares the connection patterns between neural networks and various regions of the cerebral cortex from different perspectives and multidimensional dimensions^{4,5}. Back in 2013, the Wu-Minn HCP Consortium proposed a research initiative to encourage neuroscience researchers to use advanced imaging techniques in order to explore human connectomics and advance the field of human brain neuroscience⁶.

The development of connectomics is imperative. With the frequent use of various imaging techniques, researchers have increasingly investigated the cerebral cortex. In a recent study, which used functional magnetic resonance imaging (fMRI) to observe 17 patients with vestibular neuritis found that in patients with visual movement after the stimulus, the abnormal activity of vestibular OP2 + area, was accompanied with nystagmus and paroxysmal vertigo, which indicated that several regions in the cerebral cortex partially transmit visual and motor perception through the vestibular OP2+ region⁷. Another study used the fMRI to examine a causal relationship between mood regulation, cognitive dysfunction, and mental illness, after performing a principal component analysis (PCA) strategy of the cingulate cortex in both autism and psychosis. Reduced connectivity between the posterior cingulate cortex and posterior insula and

1 medial temporal lobes was reported to be consistent with the emotional loss and
2 psychiatric abnormalities the patient presents⁸. Nowadays, transneuronal tracers can be
3 used to analyze brain nerve transmission, draw fine neural pathways from the
4 microscopic point of view, and more comprehensive and objective analysis of the
5 morphological study of living brain tissue⁹.

6

7 **2. Current research on viral transneuronal tracers**

8 Information in brain regions travels through synapses between neurons. Transneuronal
9 tracers are currently the most commonly used neuroscience research tools^{10, 11}.

10 Tracers of neuronal receptors rely on protein labeled neuronal or biological nerve
11 tracers, ranging from non-viral fluorescent-labeled proteins first discovered in 1970¹²
12 to the first viral tracer rabies virus (RABV). Neurotropic viruses are among the most
13 promising transneuronal tracer tools with excellent biological characteristics such as
14 self-replication and specific trans-synaptic transmission^{13, 14}. There are two types of
15 viral tracers: retrograde and bidirectional tracers¹⁵. The most frequently used viruses
16 are adeno-associated viruses (AAV)¹⁶, herpes simplex virus 1 (HSV-1)^{17, 18},
17 pseudorabies virus (PRV)^{19, 20}, measles virus (MV)²¹, vesicular stomatitis virus (VSV)²²,
18 and cholera toxin B subunit (CTb)^{23, 24}. For example, the RABV and PRV, which belong
19 to retrograde trans-neuronal tracers that map input neurons, can successfully identify
20 specific central nervous system regions (CNS) in the brain. HSV and AAV can be used
21 as anterograde transneuronal tracers, which project anterograde axonal transport to
22 inferior neurons and labeling output neurons^{16, 25, 26}.

23 Transneuronal tracers are widely used in anatomical studies of central and peripheral
24 nerves²⁷. A neurotropic virus marks primary neurons along the efferent or afferent
25 nerves to secondary and tertiary neurons. It draws a neural circuit conduction map
26 according to the nerves labeled by the virus²⁸. When the H129 strain of HSV-1 was
27 injected into the interscapular brown adipose tissue (IBAT) during central nerve
28 conduction, the virus infected the paraventricular nucleus of the hypothalamus (PVH),
29 periaqueductal gray matter (PAG), and reticular areas. This intuitively confirmed the
30 neural circuit conduction between the IBAT and CNS²⁹. In peripheral nerves, the RABV

1 was injected into the hind legs of mice, and the virus was found to transmit to the spinal
2 cord in the axon of the peripheral femur nerve and marked in the Schwann cells of
3 peripheral nerves³⁰. These techniques have been widely used to trace neural circuits
4 such as visceral nerve circuits³¹; visual nerve conduction³²⁻³⁴, taste conduction^{35, 36},
5 olfactory conduction³⁷, and motor system conduction³⁸.

6 7 **3. Current research progress on brain-skeletal muscle motor circuits**

8 The execution of movement in primates depends on the control of muscle groups. Most
9 of the neural network governing movement comes from the downward projection of
10 the primary motor cortex (M1), which is transmitted to the corticospinal tract (CST) to
11 coordinate fine movement. The reticular spinal lot is mainly responsible for
12 coordinating the overall direction of muscles³⁹. In addition, the frontal lobe-sensory
13 interaction of the cerebral cortex is involved in the neural regulation of fine sensation⁴⁰,
14 ⁴¹.

15 Brain-skeletal muscle connectome research has shifted from the electron microscope to
16 neuron tracer in the past few decades. Studies have reported using bionics to explore
17 brain-skeletal muscle connectomics. By constructing the skeletal muscle system
18 through machine learning, the nerve conduction device of the skeletal muscle
19 innervated by microelectrodes is built to simulate the nerve conduction of the skeletal
20 muscle⁴². Recent research has shown that the dynamic network of cortical-muscle
21 interactions in physiological states can be mapped according to the specific
22 electroencephalogram (EEG) produced by different motor states⁴³. These techniques
23 shed light on mapping brain-skeletal muscle connections from another perspective. At
24 the same time, the exploration of the brain connectome using transneuronal tracers can
25 more intuitively explore the information transmission within nerves. The neural
26 circuitry of the brain-skeletal muscle connectome is revealed, and the duration of viral
27 application to different skeletal muscles is summarized in figure 1 and Table 1.

28 29 **4. Cerebral cortex innervation of masticatory muscles**

30 Masticatory behavior is projected from the orofacial motor cortex (MCtx) to the

1 trigeminal motor nucleus and brainstem reticular structure through the corticospinal
2 tract⁴⁴. Then the masticatory muscle is innervated to produce physiological behaviors
3 such as chewing, speaking, and swallowing⁴⁵. The neural projection from the human
4 masseter single motor unit (SMU) to M1 was examined in a study using focal
5 transcranial magnetic stimulation (TMS) and found that 87% of the SMU was projected
6 to the contralateral M1 and only 25% to the ipsilateral M1, suggesting that the masseter
7 was subjected to monosynaptic corticomotoneuronal (CM) projection⁴⁶.

8 This undoubtedly inspired us to further explore the neural regulation of the trigeminal
9 nerve on the masticatory muscle, where the retrograde tracer pseudorabies virus-Bartha
10 (PRV-Bartha) was injected into the masseter muscle of mice. The virus retrogrades were
11 shown to have infected the cranial motor nucleus V (Mo5). They then projected to the
12 lateral hypothalamus (LH), basolateral and central amygdala (Amy), insular (Ins), and
13 perirhinal cortices (Rhi), indicating that the Mo5 is co-innervated by multi-synaptic
14 neural pathways. The specific projection of individual neurons into masticatory
15 muscles was further examined at the microscopic level. Therefore, the dual-labeled
16 tracers pseudorabies virus-152 (PRV-152) and pseudorabies virus-614 (PRV-614) were
17 injected into the masseter muscle. The neuropeptide melanocortin concentrating
18 hormone (MCH) and orexin neuropeptides were found to be significantly marked,
19 illustrating that the MCH and orexin neurons in the LH could be down projected to the
20 MAS and salivary gland (SAL) involved in the control of chewing behavior. Amy is
21 also known to be involved in neural regulation of feeding behavior, so when the dual-
22 labeling PRV-Bartha and PRV-614 were injected into the SAL and MAS again, it was
23 observed that Nurrl⁺ neurons projected downward and innervated the masseter from the
24 perspective of nerve molecules⁴⁷. Due to the innervation of oropharyngeal muscles by
25 the medial anterior Amy, the retrograde synaptic tracer PRV was injected into the
26 masseter, genioglossus, and thyroarytenoid of rats. The virus was shown to have
27 infected the Mo5, retrogressed into the central nucleus (CE), and then directly projected
28 to the intermediate reticular nucleus (IRt) via GABA neurons, revealing that the CE is
29 innervated by premotor neurons from the pons to the medulla oblongata reticular
30 structure, and is involved in oropharyngeal taste aversion⁴⁸.

1 The retrograde virus tracer was tagged with the trigeminal nucleus (TG) by the
2 masticatory muscle and transferred to peripheral nerve nuclei along the dendrites of
3 motor neurons⁴⁹. The researchers then injected the retrograde synaptic tracer PRV-
4 Bartha into the superficial one-third of the masseter muscle of rats. The virus
5 retrograded to the lateral portion of the ipsilateral Mo5 and then projected to the
6 bilateral vestibular nuclei (VN). It was significantly marked in the ipsilateral caudal
7 prepositus hypoglossi (PH), medial vestibular nucleus (MVe), and ipsilateral spinal
8 vestibular nucleus (SpVe), indicating that the TG was subjected to a neural projection
9 by VN⁵⁰.

10 In another study, optimized rabies glycoprotein deficient retrograde rabies virus trans-
11 synaptic tracer (Δ G-RV) and Cre dependent AAV2 (AAV-retro-Cre) were injected into
12 the masticatory muscles of mice, and viral markers were found in the ipsilateral motor
13 neurons of the Mo5. Significantly labeled anterior motor neurons were seen in the
14 dorsal IRT, supratrigeminal region (SupV), and peripheral trigeminal areas, suggesting
15 that the brainstem reticular structure is involved in orofacial behaviors of masticatory
16 muscles by projecting on the Mo5⁵¹.

17 Barnett et al.⁵² also reported that the trigeminal nerve regulates multi-synaptic
18 projections of the M1 using the HSV-1 type 1 strain H129 (HSV-1 H129) to infect the
19 trigeminal nucleus. The virus infected the laminae IV and Va of the primary
20 somatosensory cortex from the medial geniculate complex thalamus and ventral
21 posterior medial thalamus, marked in the primary somatosensory cortex (S1). In
22 conclusion, these studies indicate that masticatory muscles are innervated by multiple
23 synapses, which are coordinated by various regions in the central nerve of the brain.

24

25 **5. Cerebral cortex innervation of the flexor muscles of fingers**

26 Earlier studies have shown that premotor circuits control grasping, from initial
27 projection to the posterior parietal cortex (PPC) for visual guidance and then to the
28 cerebral cortex for learning control based on memory and imagination⁵³. The flexor
29 digitorum is one of the few muscles directly regulated by the cortical motor neuron
30 (CM) cells in the M1. The tail of the M1 projects into the hand muscle through the

1 single synapse of CM cells is involved in delicate finger movements^{54, 55}. The hand's
2 dominant areas are critical in the M1, with 20% of the site used to regulate delicate
3 hand movements⁵⁶. Studies have shown that the M1 innervated a single synapse in the
4 flexor digitorum muscles, when retrograde virus tracers RABV were injected into the
5 abductor pollicis longus (ABPL), adductor pollicis (ADP), and extensor digitorum
6 communis (EDC) of macaque monkeys. The virus retrograded to the motoneuron (MN)
7 in the lower cervical and upper thoracic segments of the spinal cord, then labeled the
8 CM in layer V of the M1 and afferent nerves of the Ia (second-order neuron), followed
9 by the CM in layer III of the M1 (third-order neuron)⁵⁷.

10 Damage to the M1 or CST can cause problems with delicate finger movement⁵⁸. The
11 retrograde virus tracers PRV was injected into spinal interneurons of the extensor carpi
12 radialis longus (ECRL) muscle in the forelimb of rats, and it was found that the virus
13 significantly marked intermediate neurons in the C6-T1 spinal segments. It was
14 concluded that the spinal cord premotor circuit recovered moderately in rats with
15 cervical spinal cord injury⁵⁹. Because the pathological manifestation of stroke is the
16 interruption of the axon connection between the corticospinal tract and corpus callosum,
17 stroke patients often have a certain degree of physical discoordination and motor
18 dysfunction⁶⁰. Poinssatte et al. injected mice with the pseudorabies virus (PRV-152) into
19 the left forelimb flexor ascending the corticospinal tract to secondary neurons in layers
20 2 and 3 of the M1, followed by layers 5 of the suitable M1 (MOp5) and S1. Compared
21 with the sham group, stroke mice showed a significant decrease in the signal of MOp5
22 virus markers on the right side due to damage to the right corticospinal tract ($P <$
23 0.0001). The destruction of the integrity of the CST, affected the innervation of the M1
24 to the forelimb muscles, which was confirmed by observing the fluorescent signals in
25 the brain after stroke⁶¹.

26 Tosolini et al.⁶² injected retrograde neuronal tracers several times into 11 forelimb
27 muscles of rats. They observed that neurons were significantly labeled in the cervical
28 spinal cord. The motor neurons innervating the flexor digitorum were concentrated in
29 the cervical segments C6-C7. Grasping is usually caused by the transmission of
30 information from the ventral premotor cortex (PMv) to the M1⁶³. The spinothalamic

1 (ST) system is involved in the neuronal regulation of pain and injury sensation. In one
2 study, the transneuronal tracer HSV-1 H129 was injected into the C5-T1 cervical
3 segment of the spinal cord of Cebus monkeys. The virus entered the thalamus along the
4 spinothalamic neurons and was transported anterograde to the cingulate sulcus of the
5 cerebral cortex⁶⁴. This helped to verify the direct regulation effect of proprioceptive
6 spinal cord neurons (PN) on hand extension and grasping behavior. In another study,
7 dual retrograde tracers of the lentiviral vector carrying enhanced tetanus neurotoxin
8 light chain (HiRet-TRE-EGFP. eTeNT) and AAV2 with the Tet-on sequence (AAV2-
9 CMV-RTTAV16) were injected into PN-targeted neuronal regions. Specific blockade
10 of the PN following oral administration of doxycycline (Dox) showed temporary reach
11 and grasping disorders in macaques after the virus entered the motor neuron region of
12 the C6-T1 spinal segment. The complete PN is thus involved in the extension and
13 flexion movement of the hand and arm, and monosynaptic connections of motor cortex
14 neurons with c6-T1 spinal cord interneurons are involved in delicate finger
15 movements⁶⁵.

16

17 **6. Cerebral cortex innervation of the gastrocnemius muscle**

18 Parkinson's disease (PD) is a neurodegenerative disorder in which patients usually
19 present with systemic static tremor myotonia and bradykinesia. Under whole-body
20 vibration (WBV) training, mechanical vibration stimulation at 20Hz was found to help
21 increase the strength of the calf gastrocnemius muscle (GAS) and improve the fluster
22 gait of PD patients⁶⁶. Similarly, a clinical study demonstrated that deep brain
23 stimulation (DBS) of the subthalamic nucleus (STN) improved the forward-leaning
24 posture in PD patients⁶⁷. To more intuitively observe the transmission between the
25 cerebral cortex and basal ganglia, after injection of the RABV in the M1, the virus was
26 transmitted along hypothalamic neurons to neurons in the globus pallidus (GPe),
27 striatum, and STN⁶⁸. Animal studies have also shown that the STN is double dominated
28 by the cerebral cortex; on the one hand, it is directly projected by glutamate and on the
29 other hand, indirectly launched by the GABA from the GPe and striatum and then
30 transmitted along with the CST to motor neurons in the forefoot of the spinal cord to

1 regulate the GAS⁶⁹.

2 The retrovirus tracer can be specifically projected to the spinal cord region in the target
3 organ, which can be used as a projection tool for potential neuroprotective genes. In a
4 study, adenovirus vector carrying the beta-galactosidase (AdV-LacZ) gene was injected
5 into the gastrocnemius muscle. The virus was retrogradely transported one week later
6 along the axon to the motor neurons in the anterior corner of the lumbar spinal cord.
7 The LacZ gene transmission efficiency was 56.6% in the gastrocnemius muscle of the
8 lumbar spinal segment⁷⁰. In another study, the retrograde virus tracers PRV-Bartha were
9 injected into the gastrocnemius muscle. The virus was transmitted along ipsilateral
10 motor neurons to interneurons in the L4-L5 spinal segment, marking Ia inhibitory
11 interneurons in the dorsal, ventral, and medial motoneuron pool. Interneuronal calcium-
12 binding proteins and parvalbumin were projected to motor neurons through single
13 synapses⁷¹. This study confirmed that the virus injected into the gastrocnemius muscle
14 was launched to the lumbar motor neurons via a single synaptic mode.

15 Transnerve tracers determine the single synaptic connection between cortical motor
16 neurons and the gastrocnemius muscle. A study found that injecting PRV-152 into the
17 gastrocnemius muscle, where the sympathetic nerve is severed, marked gastrocnemius
18 motor neurons. The retrograde virus tracers PRV-BaBLU were simultaneously injected
19 into the adrenal glands to label sympathetic preganglionic neurons. At 96h after
20 infection, dual viruses were jointly characterized in the PAG, LH, and PVN. This study
21 confirms that the gastrocnemius muscle is innervated jointly by sympathetic - motor
22 integration^{72, 73}. In another study, PRV-614 and MC4R-GFP were injected into the
23 gastrocnemius muscle of spinal cord transected mice. The virus projected along the
24 intermediolateral column (IML) to the rostral ventromedial medulla (RVMM) and
25 rostral ventrolateral medulla (RVLM), was subsequently marked significantly in the
26 pedunculopontine tegmental nucleus (PPTg) of the midbrain but unmarked in the
27 cuneiform nucleus (CnF). The gastrocnemius muscle was confirmed to be innervated
28 by the melanocortin sympathetic nerve of the midbrain PPTg⁷⁴.

29 In addition, neuropathic pain modulation in the organism is transmitted by cortical-
30 brainstem spinal cord neural network connections. Injury signals in the prefrontal

1 cortex (PFC) project by the CST to the PAG and subsequently to the rostroventral
2 medulla RVM) and to the on and off cells of the locus coeruleus (LC), which in turn
3 moderate pain perception in the body. In a study, PRV-614 was injected into the efferent
4 neurons of the left gastrocnemius muscle of mice. The virus was found to travel
5 retrograde to the PAG and M1 along the sympathetic preganglionic neurons of the IML.
6 This confirmed that the motor cortical-periaqueductal gray matter-spinal motor
7 pathway is involved in sympathetic innervation⁷⁵. PRV-152 and PRV-BaBLU were
8 injected into the gastrocnemius muscle on both sides to investigate whether there was
9 independent innervation in the regulation of blood flow in the gastrocnemius muscle.
10 After transection of the L2 spinal cord in rats, PRV-152 and PRV-BaBlu were
11 injected into the left and right hind limbs of the gastrocnemius muscles. The motor
12 neurons of the left and right gastrocnemius muscle were infected retrograde to the
13 neurons of the sympathetic nerve and subsequently labelled in the bilateral
14 cerebral nerves of the rats. Neuronal cells labelled by viral tracers were observed
15 in the PVN, RVLM, LC, and A5 adrenergic cell group region (A5) of the rat brain.
16 Among them, RVLM served as the major sympathetic efferent site in the CNS, only
17 half of RVLM neurons were labelled, indicating that the CNS had limited effect on
18 regulating the gastrocnemius blood flow⁷⁶.

19 In regenerative medicine, human umbilical cord mesenchymal stem cells (hUCMSCs)
20 can be used to repair nerve injury and regenerate axons. Sciatic nerve injury is usually
21 accompanied by gastrocnemius atrophy. Existing studies have found that hUCMSCs
22 can promote nerve regeneration and improve denervated gastrocnemius atrophy in rats
23 with sciatic nerve transection⁷⁷. A retrograde PRV-BA tracer was used to label the
24 sciatic nerve of rats 35 days after transplantation of human neural stem cells (hNSC).
25 The virus retrogrades entered the frontal cortex, paraventricular nucleus (PVS), giant
26 reticular cells, raphe nucleus, and A5. However, the GAP43 protein was highly
27 expressed in the spinal cord transection region, and the number of axons significantly
28 increased, indicating that the integrity of the motor neural pathway was observed under
29 the tracking of PRV-BA⁷⁸. In another study, the PRV and CTb were injected into the
30 right gastrocnemius muscle of rats after treatment with hNSC for amyotrophic lateral

1 sclerosis (ALS) to mark afferent motor neurons jointly. Compared with the traditional
2 tracer CTb, the PRV showed a significant advantage in trans-nerve tracer labeling. The
3 PRV entered the sciatic nerve from the gastrocnemius muscle and was labeled at the
4 synaptic terminal of hNSC-derived neuron⁷⁹.

5

6 **7. Cerebral cortex innervation of the lumbar muscles**

7 Chronic low back pain (LBP) is a painful joint skeletal muscle disease⁸⁰. One study
8 found that LBP patients had increased local low back pain with type II muscle fibers
9 due to chronic strain of the lumbar muscles over a long period, resulting in persistent
10 low back pain⁸¹. In terms of pain nerve conduction, the PAG is involved in the neural
11 circuit regulation of the CNS for downward pain⁸². In imaging studies, chronic pain is
12 accompanied by changes in the brain functional structure. CLBP patients have
13 increased network connectivity in the anterior insular cortex, dorsolateral prefrontal
14 cortex, and the anterior temporoparietal junction of the S1. It can be seen that chronic
15 pain causes increased pain conduction between the cerebral cortex⁸³. In cLBP patients,
16 remodeling of the S1 has been reported, leading to a decrease in tactile acuity.

17 Assessment of brain structure images of cLBP patients revealed increased gray matter
18 (GM) volume in the S1-back and S1-fingers, which suggest that changes in the GM
19 microstructure of cLBP are related to nerve conduction of back pain⁸⁴. It has also been
20 reported that the norepinephrine locus coeruleus (LC) is involved in neuronal regulation
21 of pain, when HSV-1 H129 was injected into the Amy and posterior-lateral
22 hypothalamic area (PLH). It was found that the virus directly projected into LC neurons
23 anterolateral, indicating that the Amy and PLH through GABAergic could directly
24 project onto LC axons and participate in the neural regulation of sympathetic nerve
25 activity and pain sensation⁸⁵. To further explore the neuronal circuits involved in lumbar
26 muscle pain transmission, PRV-614 was injected into the left lateral lumbar muscle of
27 mice. The virus traveled retrograde to the raphe nucleus, RVLM, A5, LC, pons reticular
28 nucleus (PRN), and PVN along the spinal cord labeled sympathetic neurons in the IML,
29 demonstrating the central innervation of the external lumbar muscle. However, mice
30 undergoing spinal cord transection L2 presented delayed retrograde infection of the

1 IML, indicating that the RVLM, Lateral paragigantocellular reticular nucleus (LPGi),
2 A5, LC and PVN are also involved in the autonomic innervation of the lumbar muscle⁸⁶.
3 The external lumbar muscles are innervated by both motor and autonomic circuits, and
4 the lumbar muscles receive nerve projections from the ventromedial hypothalamic
5 nucleus (VMH) during lordosis⁸⁷. Daniels et al.⁸⁸ injected PRV into the external lumbar
6 muscle of rats. The virus entered the T8-L2 spinal cord neurons and infected the RVLM,
7 then retrograde infected the pons and midbrain regions, and was significantly marked
8 in the PAG, VMH and medial pontomedullary reticular formation (MRF). These results
9 suggest that the PRV enters the CNS network by infecting sympathetic innervated
10 vessels, thereby marking the neural circuitry of the lumbar external axons. Another
11 study used a dopamine β -hydroxylase immunotoxin (DHIT) injection to cut
12 sympathetic innervation by injecting the PRV into the lumbar external muscles in the
13 ventral horn neurons of L3-S1, followed by observation of the PRV immune response
14 of neurons in the MRF, PAG and VMH, which confirmed that the CNS regulates the
15 lordosis by autonomic innervation of the external lumbar muscles⁸⁹. To further visualize
16 VMH neurons, the PRV was injected into the external lumbar muscle, and the virus was
17 marked in the VMH along the axon. The density of dendritic spines in the MVH
18 increased after treatment with double estradiol, indicating that estrogen could induce
19 specific lordosis behavior by increasing VMH dendrites⁹⁰.

20

21 **8. Conclusion**

22 Transneuronal tracers have excellent characteristics of trans-neuronal signal marking,
23 directionality, and non-attenuation. According to our review, the use of transneuronal
24 tracers provides a new approach to brain-skeletal muscle connectomics. From the
25 microscopic point of view, the innervation image of the cerebral cortex to the skeletal
26 muscle is observed more intuitively. There is still a long way to go in the study of this
27 technology. At present, the research on brain-skeletal muscle connectomics should not
28 be limited to the study of complete neural circuits but should also be extended to the
29 study of incomplete or traumatic disease related neural circuits, and further apply it to
30 the study of nerve injury repair⁹¹. It will pave the way for further research on

1 neuroplasticity and traumatic repair. Therefore, neural tracers could widely be used in
2 the study of connectomics related diseases, providing some new perspectives for the
3 subsequent study of neuroanatomy.

4

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10

11 **Disclosure of conflict of interest**

12 None.

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20 **References**

- 21 1. Luo L. Architectures of neuronal circuits. *Science*. 2021;373:eabg7285.
- 22 2. Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, Wedeen VJ and Sporns O. Mapping
23 the structural core of human cerebral cortex. *PLoS Biol*. 2008;6:e159.
- 24 3. Van Essen DC. Cartography and connectomes. *Neuron*. 2013;80:775-90.
- 25 4. Bijsterbosch JD, Valk SL, Wang D and Glasser MF. Recent developments in representations of
26 the connectome. *Neuroimage*. 2021;243:118533.
- 27 5. Behrens TE and Sporns O. Human connectomics. *Curr Opin Neurobiol*. 2012;22:144-53.
- 28 6. Van Essen DC, Smith SM, Barch DM, Behrens TE, Yacoub E and Ugurbil K. The WU-Minn
29 Human Connectome Project: an overview. *Neuroimage*. 2013;80:62-79.
- 30 7. Ibitoye RT, Mallas EJ, Bourke NJ, Kaski D, Bronstein AM and Sharp DJ. The human vestibular
31 cortex: functional anatomy of OP2, its connectivity and the effect of vestibular disease. *Cereb*
32 *Cortex*. 2022.
- 33 8. Lam YS, Li J, Ke Y and Yung WH. Variational dimensions of cingulate cortex functional
34 connectivity and implications in neuropsychiatric disorders. *Cereb Cortex*. 2022.

- 1 9. Jbabdi S and Behrens TE. Long-range connectomics. *Ann N Y Acad Sci.* 2013;1305:83-93.
- 2 10. Lanciego JL and Wouterlood FG. A half century of experimental neuroanatomical tracing. *J*
- 3 *Chem Neuroanat.* 2011;42:157-83.
- 4 11. Ugolini G. Advances in viral transneuronal tracing. *J Neurosci Methods.* 2010;194:2-20.
- 5 12. Kristensson K. Transport of fluorescent protein tracer in peripheral nerves. *Acta Neuropathol.*
- 6 1970;16:293-300.
- 7 13. Lo CC and Chiang AS. Toward Whole-Body Connectomics. *J Neurosci.* 2016;36:11375-11383.
- 8 14. Beier KT. Hitchhiking on the neuronal highway: Mechanisms of transsynaptic specificity. *J*
- 9 *Chem Neuroanat.* 2019;99:9-17.
- 10 15. Nassi JJ, Cepko CL, Born RT and Beier KT. Neuroanatomy goes viral! *Front Neuroanat.*
- 11 2015;9:80.
- 12 16. Zingg B, Chou XL, Zhang ZG, Mesik L, Liang F, Tao HW and Zhang LI. AAV-Mediated
- 13 Anterograde Transsynaptic Tagging: Mapping Corticocollicular Input-Defined Neural Pathways
- 14 for Defense Behaviors. *Neuron.* 2017;93:33-47.
- 15 17. Zemanick MC, Strick PL and Dix RD. Direction of transneuronal transport of herpes simplex
- 16 virus 1 in the primate motor system is strain-dependent. *Proc Natl Acad Sci U S A.* 1991;88:8048-
- 17 51.
- 18 18. Su P, Ying M, Han Z, Xia J, Jin S, Li Y, Wang H and Xu F. High-brightness anterograde
- 19 transneuronal HSV1 H129 tracer modified using a Trojan horse-like strategy. *Mol Brain.* 2020;13:5.
- 20 19. Li X, Su S, Zhao H, Li Y, Xu X, Gao Y, Sun D, Yang Z, Jin W and Ke C. Virus Injection to the
- 21 Pituitary via Transsphenoidal Approach and the Innervation of Anterior and Posterior Pituitary of
- 22 Rat. *Front Endocrinol (Lausanne).* 2020;11:546350.
- 23 20. Aston-Jones G and Card JP. Use of pseudorabies virus to delineate multisynaptic circuits in
- 24 brain: opportunities and limitations. *J Neurosci Methods.* 2000;103:51-61.
- 25 21. McQuaid S, Campbell S, Wallace IJ, Kirk J and Cosby SL. Measles virus infection and replication
- 26 in undifferentiated and differentiated human neuronal cells in culture. *J Virol.* 1998;72:5245-50.
- 27 22. Mundell NA, Beier KT, Pan YA, Lapan SW, Göz Aytürk D, Berezovskii VK, Wark AR,
- 28 Drokhllyansky E, Bielecki J, Born RT, Schier AF and Cepko CL. Vesicular stomatitis virus enables
- 29 gene transfer and transsynaptic tracing in a wide range of organisms. *J Comp Neurol.*
- 30 2015;523:1639-63.
- 31 23. Shehab SA, Spike RC and Todd AJ. Evidence against cholera toxin B subunit as a reliable
- 32 tracer for sprouting of primary afferents following peripheral nerve injury. *Brain Res.* 2003;964:218-
- 33 27.
- 34 24. Nair J, Bezdudnaya T, Zholudeva LV, Detloff MR, Reier PJ, Lane MA and Fuller DD. Histological
- 35 identification of phrenic afferent projections to the spinal cord. *Respir Physiol Neurobiol.*
- 36 2017;236:57-68.
- 37 25. Yang H, Xiong F, Qin HB, Yu QT, Sun JY, Zhao HW, Li D, Zhou Y, Zhang FK, Zhu XW, Wu T,
- 38 Jiang M, Xu X, Lu Y, Shen HJ, Zeng WB, Zhao F and Luo MH. A novel H129-based anterograde
- 39 monosynaptic tracer exhibits features of strong labeling intensity, high tracing efficiency, and
- 40 reduced retrograde labeling. *Mol Neurodegener.* 2022;17:6.
- 41 26. Zeng WB, Jiang HF, Gang YD, Song YG, Shen ZZ, Yang H, Dong X, Tian YL, Ni RJ, Liu Y, Tang
- 42 N, Li X, Jiang X, Gao D, Androulakis M, He XB, Xia HM, Ming YZ, Lu Y, Zhou JN, Zhang C, Xia XS,
- 43 Shu Y, Zeng SQ, Xu F, Zhao F and Luo MH. Anterograde monosynaptic transneuronal tracers
- 44 derived from herpes simplex virus 1 strain H129. *Mol Neurodegener.* 2017;12:38.

- 1 27. Fan L, Xiang B, Xiong J, He Z and Xiang H. Use of viruses for interrogating viscera-specific
2 projections in central nervous system. *J Neurosci Methods*. 2020;341:108757.
- 3 28. Feng M, Xiang B, Fan L, Wang Q, Xu W and Xiang H. Interrogating autonomic peripheral
4 nervous system neurons with viruses - A literature review. *J Neurosci Methods*. 2020;346:108958.
- 5 29. Vaughan CH and Bartness TJ. Anterograde transneuronal viral tract tracing reveals central
6 sensory circuits from brown fat and sensory denervation alters its thermogenic responses. *Am J*
7 *Physiol Regul Integr Comp Physiol*. 2012;302:R1049-58.
- 8 30. Potratz M, Zaack LM, Weigel C, Klein A, Freuling CM, Müller T and Finke S. Neuroglia infection
9 by rabies virus after anterograde virus spread in peripheral neurons. *Acta Neuropathol Commun*.
10 2020;8:199.
- 11 31. Li Z, Li Z, Xu W, Li Y, Wang Q, Xu H, Manyande A, Wu D, Feng M and Xiang H. The connectome
12 from the cerebral cortex to the viscera using viral transneuronal tracers. *Am J Transl Res*.
13 2021;13:12152-12167.
- 14 32. Prevosto V, Graf W and Ugolini G. Proprioceptive pathways to posterior parietal areas MIP
15 and LIPv from the dorsal column nuclei and the postcentral somatosensory cortex. *Eur J Neurosci*.
16 2011;33:444-60.
- 17 33. Andelin AK, Doyle Z, Laing RJ, Turecek J, Lin B and Olavarria JF. Influence of ocular dominance
18 columns and patchy callosal connections on binocularity in lateral striate cortex: Long Evans versus
19 albino rats. *J Comp Neurol*. 2020;528:650-663.
- 20 34. Azzopardi P and Cowey A. Preferential representation of the fovea in the primary visual cortex.
21 *Nature*. 1993;361:719-21.
- 22 35. Sugita M, Yamamoto K, Hirono C and Shiba Y. Information processing in brainstem bitter
23 taste-relaying neurons defined by genetic tracing. *Neuroscience*. 2013;250:166-80.
- 24 36. Matsumoto I. Gustatory neural pathways revealed by genetic tracing from taste receptor cells.
25 *Biosci Biotechnol Biochem*. 2013;77:1359-62.
- 26 37. Boehm U, Zou Z and Buck LB. Feedback loops link odor and pheromone signaling with
27 reproduction. *Cell*. 2005;123:683-95.
- 28 38. Asante CO and Martin JH. Differential joint-specific corticospinal tract projections within the
29 cervical enlargement. *PLoS One*. 2013;8:e74454.
- 30 39. Li N, Daie K, Svoboda K and Druckmann S. Robust neuronal dynamics in premotor cortex
31 during motor planning. *Nature*. 2016;532:459-64.
- 32 40. Teka WW, Hamade KC, Barnett WH, Kim T, Markin SN, Rybak IA and Molkov YI. From the
33 motor cortex to the movement and back again. *PLoS One*. 2017;12:e0179288.
- 34 41. McColgan P, Joubert J, Tabrizi SJ and Rees G. The human motor cortex microcircuit: insights
35 for neurodegenerative disease. *Nat Rev Neurosci*. 2020;21:401-415.
- 36 42. Knothe Tate ML, Detamore M, Capadona JR, Woolley A and Knothe U. Engineering and
37 commercialization of human-device interfaces, from bone to brain. *Biomaterials*. 2016;95:35-46.
- 38 43. Rizzo R, Zhang X, Wang J, Lombardi F and Ivanov PC. Network Physiology of Cortico-
39 Muscular Interactions. *Front Physiol*. 2020;11:558070.
- 40 44. Mercer Lindsay N, Knutsen PM, Lozada AF, Gibbs D, Karten HJ and Kleinfeld D. Orofacial
41 Movements Involve Parallel Corticobulbar Projections from Motor Cortex to Trigeminal Premotor
42 Nuclei. *Neuron*. 2019;104:765-780.e3.
- 43 45. Westberg KG and Kolta A. The trigeminal circuits responsible for chewing. *Int Rev Neurobiol*.
44 2011;97:77-98.

- 1 46. Pearce SL, Miles TS, Thompson PD and Nordstrom MA. Responses of single motor units in
2 human masseter to transcranial magnetic stimulation of either hemisphere. *J Physiol.*
3 2003;549:583-96.
- 4 47. Pérez CA, Stanley SA, Wysocki RW, Havranova J, Ahrens-Nicklas R, Onyimba F and Friedman
5 JM. Molecular annotation of integrative feeding neural circuits. *Cell Metab.* 2011;13:222-32.
- 6 48. Van Daele DJ, Fazan VP, Agassandian K and Cassell MD. Amygdala connections with jaw,
7 tongue and laryngo-pharyngeal premotor neurons. *Neuroscience.* 2011;177:93-113.
- 8 49. Mong FS, Chen YC and Lu CH. Dendritic ramifications of trigeminal motor neurons
9 innervating jaw-closing muscles of rats. *J Neurol Sci.* 1988;86:251-64.
- 10 50. Giaconi E, Deriu F, Tolu E, Cuccurazzu B, Yates BJ and Billig I. Transneuronal tracing of
11 vestibulo-trigeminal pathways innervating the masseter muscle in the rat. *Exp Brain Res.*
12 2006;171:330-9.
- 13 51. Takatoh J, Park JH, Lu J, Li S, Thompson PM, Han BX, Zhao S, Kleinfeld D, Friedman B and
14 Wang F. Constructing an adult orofacial premotor atlas in Allen mouse CCF. *Elife.* 2021;10.
- 15 52. Barnett EM, Evans GD, Sun N, Perlman S and Cassell MD. Anterograde tracing of trigeminal
16 afferent pathways from the murine tooth pulp to cortex using herpes simplex virus type 1. *J*
17 *Neurosci.* 1995;15:2972-84.
- 18 53. Santello M, Flanders M and Soechting JF. Patterns of hand motion during grasping and the
19 influence of sensory guidance. *J Neurosci.* 2002;22:1426-35.
- 20 54. Zaaimi B, Dean LR and Baker SN. Different contributions of primary motor cortex, reticular
21 formation, and spinal cord to fractionated muscle activation. *J Neurophysiol.* 2018;119:235-250.
- 22 55. Perez MA and Rothwell JC. Distinct influence of hand posture on cortical activity during
23 human grasping. *J Neurosci.* 2015;35:4882-9.
- 24 56. Sobinov AR and Bensmaia SJ. The neural mechanisms of manual dexterity. *Nat Rev Neurosci.*
25 2021;22:741-757.
- 26 57. Rathelot JA and Strick PL. Muscle representation in the macaque motor cortex: an anatomical
27 perspective. *Proc Natl Acad Sci U S A.* 2006;103:8257-62.
- 28 58. Bunday KL, Tazoe T, Rothwell JC and Perez MA. Subcortical control of precision grip after
29 human spinal cord injury. *J Neurosci.* 2014;34:7341-50.
- 30 59. Gonzalez-Rothi EJ, Rombola AM, Rousseau CA, Mercier LM, Fitzpatrick GM, Reier PJ, Fuller
31 DD and Lane MA. Spinal interneurons and forelimb plasticity after incomplete cervical spinal cord
32 injury in adult rats. *J Neurotrauma.* 2015;32:893-907.
- 33 60. Grefkes C and Fink GR. Reorganization of cerebral networks after stroke: new insights from
34 neuroimaging with connectivity approaches. *Brain.* 2011;134:1264-76.
- 35 61. Poinsette K, Betz D, Torres VO, Ajay AD, Mirza S, Selvaraj UM, Plautz EJ, Kong X, Gokhale S,
36 Meeks JP, Ramirez DMO, Goldberg MP and Stowe AM. Visualization and Quantification of Post-
37 stroke Neural Connectivity and Neuroinflammation Using Serial Two-Photon Tomography in the
38 Whole Mouse Brain. *Front Neurosci.* 2019;13:1055.
- 39 62. Tosolini AP and Morris R. Spatial characterization of the motor neuron columns supplying the
40 rat forelimb. *Neuroscience.* 2012;200:19-30.
- 41 63. Buch ER, Mars RB, Boorman ED and Rushworth MF. A network centered on ventral premotor
42 cortex exerts both facilitatory and inhibitory control over primary motor cortex during action
43 reprogramming. *J Neurosci.* 2010;30:1395-401.
- 44 64. Dum RP, Levinthal DJ and Strick PL. The spinothalamic system targets motor and sensory

1 areas in the cerebral cortex of monkeys. *J Neurosci*. 2009;29:14223-35.

2 65. Kinoshita M, Matsui R, Kato S, Hasegawa T, Kasahara H, Isa K, Watakabe A, Yamamori T,
3 Nishimura Y, Alstermark B, Watanabe D, Kobayashi K and Isa T. Genetic dissection of the circuit
4 for hand dexterity in primates. *Nature*. 2012;487:235-8.

5 66. Chang CM, Tsai CH, Lu MK, Tseng HC, Lu G, Liu BL and Lin HC. The neuromuscular responses
6 in patients with Parkinson's disease under different conditions during whole-body vibration
7 training. *BMC Complement Med Ther*. 2022;22:2.

8 67. Fransson PA, Nilsson MH, Rehnström S, Tjernström F, Magnusson M, Johansson R and Patel
9 M. Deep brain stimulation in the subthalamic nuclei alters postural alignment and adaptation in
10 Parkinson's disease. *PLoS One*. 2021;16:e0259862.

11 68. Kelly RM and Strick PL. Macro-architecture of basal ganglia loops with the cerebral cortex:
12 use of rabies virus to reveal multisynaptic circuits. *Prog Brain Res*. 2004;143:449-59.

13 69. Polyakova Z, Chiken S, Hatanaka N and Nambu A. Cortical Control of Subthalamic Neuronal
14 Activity through the Hyperdirect and Indirect Pathways in Monkeys. *J Neurosci*. 2020;40:7451-
15 7463.

16 70. Nakajima H, Uchida K, Kobayashi S, Inukai T, Yayama T, Sato R, Mwaka E and Baba H. Target
17 muscles for retrograde gene delivery to specific spinal cord segments. *Neurosci Lett*. 2008;435:1-
18 6.

19 71. Jovanovic K, Pastor AM and O'Donovan MJ. The use of PRV-Bartha to define premotor inputs
20 to lumbar motoneurons in the neonatal spinal cord of the mouse. *PLoS One*. 2010;5:e11743.

21 72. Kerman IA, Akil H and Watson SJ. Rostral elements of sympatho-motor circuitry: a virally
22 mediated transsynaptic tracing study. *J Neurosci*. 2006;26:3423-33.

23 73. Xiang HB, Zhu WZ, Guan XH and Ye DW. The cuneiform nucleus may be involved in the
24 regulation of skeletal muscle tone by motor pathway: a virally mediated trans-synaptic tracing
25 study in surgically sympathectomized mice. *Brain*. 2013;136:e251.

26 74. He ZG, Liu BW, Li ZX, Tian XB, Liu SG, Manyande A, Zhang DY and Xiang HB. The caudal
27 pedunculo-pontine tegmental nucleus may be involved in the regulation of skeletal muscle activity
28 by melanocortin-sympathetic pathway: a virally mediated trans-synaptic tracing study in spinally
29 transected transgenic mice. *Oncotarget*. 2017;8:71859-71866.

30 75. Ye DW, Liu C, Liu TT, Tian XB and Xiang HB. Motor cortex-periaqueductal gray-spinal cord
31 neuronal circuitry may involve in modulation of nociception: a virally mediated transsynaptic
32 tracing study in spinally transected transgenic mouse model. *PLoS One*. 2014;9:e89486.

33 76. Lee TK, Lois JH, Troupe JH, Wilson TD and Yates BJ. Transneuronal tracing of neural pathways
34 that regulate hindlimb muscle blood flow. *Am J Physiol Regul Integr Comp Physiol*.
35 2007;292:R1532-41.

36 77. Ma Y, Dong L, Zhou D, Li L, Zhang W, Zhen Y, Wang T, Su J, Chen D, Mao C and Wang X.
37 Extracellular vesicles from human umbilical cord mesenchymal stem cells improve nerve
38 regeneration after sciatic nerve transection in rats. *J Cell Mol Med*. 2019;23:2822-2835.

39 78. Lee KB, Choi JH, Byun K, Chung KH, Ahn JH, Jeong GB, Hwang IK, Kim S, Won MH and Lee B.
40 Recovery of CNS pathway innervating the sciatic nerve following transplantation of human neural
41 stem cells in rat spinal cord injury. *Cell Mol Neurobiol*. 2012;32:149-57.

42 79. Xu L, Ryugo DK, Pongstaporn T, Johe K and Koliatsos VE. Human neural stem cell grafts in
43 the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the
44 segmental motor circuitry. *J Comp Neurol*. 2009;514:297-309.

- 1 80. Li W, Gong Y, Liu J, Guo Y, Tang H, Qin S, Zhao Y, Wang S, Xu Z and Chen B. Peripheral and
2 Central Pathological Mechanisms of Chronic Low Back Pain: A Narrative Review. *J Pain Res.*
3 2021;14:1483-1494.
- 4 81. Mannion AF, Käser L, Weber E, Rhyner A, Dvorak J and Müntener M. Influence of age and
5 duration of symptoms on fibre type distribution and size of the back muscles in chronic low back
6 pain patients. *Eur Spine J.* 2000;9:273-81.
- 7 82. Mayer DJ, Wolfle TL, Akil H, Carder B and Liebeskind JC. Analgesia from electrical stimulation
8 in the brainstem of the rat. *Science.* 1971;174:1351-4.
- 9 83. Kim J, Mawla I, Kong J, Lee J, Gerber J, Ortiz A, Kim H, Chan ST, Loggia ML, Wasan AD, Edwards
10 RR, Gollub RL, Rosen BR and Napadow V. Somatotopically specific primary somatosensory
11 connectivity to salience and default mode networks encodes clinical pain. *Pain.* 2019;160:1594-
12 1605.
- 13 84. Kim H, Mawla I, Lee J, Gerber J, Walker K, Kim J, Ortiz A, Chan ST, Loggia ML, Wasan AD,
14 Edwards RR, Kong J, Kaptchuk TJ, Gollub RL, Rosen BR and Napadow V. Reduced tactile acuity in
15 chronic low back pain is linked with structural neuroplasticity in primary somatosensory cortex and
16 is modulated by acupuncture therapy. *Neuroimage.* 2020;217:116899.
- 17 85. Dimitrov EL, Yanagawa Y and Usdin TB. Forebrain GABAergic projections to locus coeruleus
18 in mouse. *J Comp Neurol.* 2013;521:2373-97.
- 19 86. Xiang HB, Liu C, Liu TT and Xiong J. Central circuits regulating the sympathetic outflow to
20 lumbar muscles in spinally transected mice by retrograde transsynaptic transport. *Int J Clin Exp*
21 *Pathol.* 2014;7:2987-97.
- 22 87. Tsukahara S, Kanaya M and Yamanouchi K. Neuroanatomy and sex differences of the
23 lordosis-inhibiting system in the lateral septum. *Front Neurosci.* 2014;8:299.
- 24 88. Daniels D, Miselis RR and Flanagan-Cato LM. Central neuronal circuit innervating the
25 lordosis-producing muscles defined by transneuronal transport of pseudorabies virus. *J Neurosci.*
26 1999;19:2823-33.
- 27 89. Daniels D, Miselis RR and Flanagan-Cato LM. Transneuronal tracing from sympathectomized
28 lumbar epaxial muscle in female rats. *J Neurobiol.* 2001;48:278-90.
- 29 90. Flanagan-Cato LM, Calizo LH and Daniels D. The synaptic organization of VMH neurons that
30 mediate the effects of estrogen on sexual behavior. *Horm Behav.* 2001;40:178-82.
- 31 91. Fortino TA, Randelman ML, Hall AA, Singh J, Bloom DC, Engel E, Hoh DJ, Hou S, Zholudeva
32 LV and Lane MA. Transneuronal tracing to map connectivity in injured and transplanted spinal
33 networks. *Exp Neurol.* 2022;351:113990.
- 34 92. Card JP, Enquist LW, Miller AD and Yates BJ. Differential tropism of pseudorabies virus for
35 sensory neurons in the cat. *J Neurovirol.* 1997;3:49-61.
- 36 93. Kim JS, Enquist LW and Card JP. Circuit-specific coinfection of neurons in the rat central
37 nervous system with two pseudorabies virus recombinants. *J Virol.* 1999;73:9521-31.
- 38 94. Rice CD, Weber SA, Waggoner AL, Jessell ME and Yates BJ. Mapping of neural pathways that
39 influence diaphragm activity and project to the lumbar spinal cord in cats. *Exp Brain Res.*
40 2010;203:205-11.
- 41 95. Niedringhaus M, Jackson PG, Pearson R, Shi M, Dretchen K, Gillis RA and Sahibzada N.
42 Brainstem sites controlling the lower esophageal sphincter and crural diaphragm in the ferret: a
43 neuroanatomical study. *Auton Neurosci.* 2008;144:50-60.
- 44 96. Mack SO, Wu M, Kc P and Haxhiu MA. Stimulation of the hypothalamic paraventricular

1 nucleus modulates cardiorespiratory responses via oxytocinergic innervation of neurons in pre-
2 Botzinger complex. *J Appl Physiol (1985)*. 2007;102:189-99.

3 97. Gong S, DeCuyper M, Zhao Y and LeDoux MS. Cerebral cortical control of orbicularis oculi
4 motoneurons. *Brain Res*. 2005;1047:177-93.

5 98. Karnup SV and de Groat WC. Propriospinal Neurons of L3-L4 Segments Involved in Control
6 of the Rat External Urethral Sphincter. *Neuroscience*. 2020;425:12-28.

7 99. Morcuende S, Delgado-Garcia JM and Ugolini G. Neuronal premotor networks involved in
8 eyelid responses: retrograde transneuronal tracing with rabies virus from the orbicularis oculi
9 muscle in the rat. *J Neurosci*. 2002;22:8808-18.

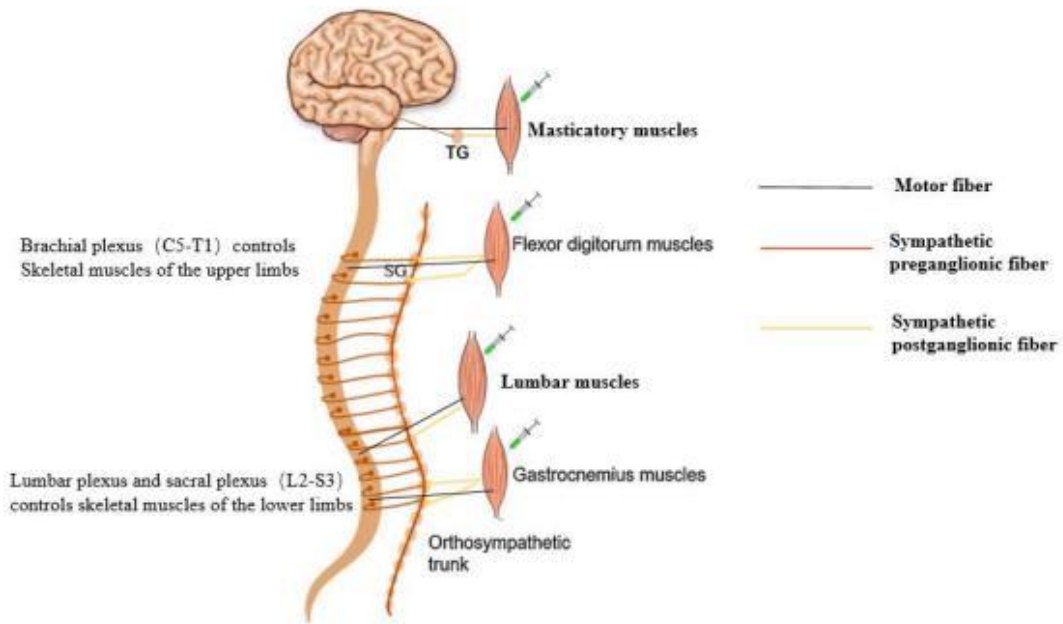
10 100. Grantyn A, Brandi AM, Dubayle D, Graf W, Ugolini G, Hadjidimitrakis K and Moschovakis A.
11 Density gradients of trans-synaptically labeled collicular neurons after injections of rabies virus in
12 the lateral rectus muscle of the rhesus monkey. *J Comp Neurol*. 2002;451:346-61.

13 101. Rubelowski JM, Menge M, Distler C, Rothermel M and Hoffmann KP. Connections of the
14 superior colliculus to shoulder muscles of the rat: a dual tracing study. *Front Neuroanat*. 2013;7:17.

15 102. Sun LW. Transsynaptic tracing of conditioned eyeblink circuits in the mouse cerebellum.
16 *Neuroscience*. 2012;203:122-34.

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Figure 1: Schematic drawing of the peripheral autonomic innervation of the skeletal muscle. PRV injected into the flexor muscles of fingers, lumbar muscles and gastrocnemius muscles was transported to the sympathetic ganglia (SG) (via the sympathetic pathway) and the ventral horn of the spinal cord (via the motor pathway), whereas PRV injected into the masseter muscles was transported to the trigeminal ganglia (TG) via the sympathetic pathway and the motor pathway.

Table 1: Duration of the labeling period upon virus application in different skeletal muscles

Species of the animal model	Species of the virus	Labeling period	Application site	Labeling destination	Reference
Cat	PRV-Becker	4 days	Diaphragm or neck musculature	Dorsal root ganglia	92
Rat	PRV-BaBLU	2 3 days	Stomach musculature	Dorsal motor vagal nucleus	93
Rat	PRV-Bartha	3 days	Masseter muscle	Medial vestibular nucleus (MVe), caudal prepositus hypoglossi (PH), ipsilateral spinal vestibular nucleus (SpVe)	50
Mice	PRV-BaBLU	2 3 days	Masseter muscle	Cranial nerve V (Mo5)	47
Mice	AAV2-reyro-Cre	5 days	Masseter muscle	Intermediate reticular nucleus (IRt)	51
Rat	PRV	4 days	ECRL	Intermediate gray matter (laminae VII and X)	59
Mice	PRV-152	6 days	Forelimb flexor muscle	Primary motor cortex layer 5 neuron (MOp5)	61
Macaques	RABV	4 5 days	ABPL, ADP, EDC	Layer V of primary motor cortex	57
Mice	PRV-Bartha	2 3 days	Tibialis anterior (TA) and gastrocnemius muscles (GC)	Ipsilateral interneurons and ventral grey matter	71
Rat	PRV-152	6 days	Gastrocnemius muscle	The periaqueductal gray and the hypothalamus	72
Mice	PRV-614	4 6 days	Gastrocnemius muscle	Spinal IML, periaqueductal gray and motor cortex	75
Rat	PRV-152, BaBLU	PRV- 4 days	Gastrocnemius muscle	RVLM, RVMN, medullary raphe nuclei, A5 region, LC, SC, and PVN	76
Rat	PRV, CTb	5 days	Gastrocnemius muscle, sciatic nerve	Motor neurons in the dorsolateral column ipsilateral	79
Mice	PRV-614	5 7 days	Lumbar muscle	MRN, PRN, RVLM, A5 region, LC, SubC, PVN, VMH	86
Rat	PRV	4 days	Lumbar epaxial muscle	Medullary reticular formation, periaqueductal gray (PAG), VMN	88
Cat	PRV	4 days	Diaphragm or neck musculature	Dorsal root ganglia and dorsal horn of the spinal cord	92
Mice	PRV, CTb	3 days	Deltoid muscle, biceps muscle, wrist extensor compartment	Corticospinal tract (CST)	38

Cat	RABV	4 days	Diaphragm	Vestibular nuclei (VN) and medial pontomedullary reticular formation (MRF)	94
Ferret	PRV-152, PRV-BaBlu	5 7 days	Crural diaphragm (CD)	Area postrema, DMV, nucleus tractus solitarius (NTS), medial reticular reformation (MRF) and nucleus ambiguous (NA)	95
Rat	PRV	5 days	Genioglossus muscle	Hypothalamic paraventricular nucleus (PVN)	96
Rhesus monkey	RABV	4 5 days	Orbicularis oculi muscles (OO)	Ventrolateral premotor (LPMCv), dorsolateral premotor (LPMCd), and motor cortices (M1)	97
Rat	PRV	2 3 days	External urethral sphincter (EUS)	L3/L4 propriospinal neurons (PSNs) and interneurons	98
Mice	PRV-614	5 days	Gastrocnemius muscle	Pedunculopontine tegmental nucleus (PPTg)	74
Rat	RABV	4 5 days	Orbicularis oculi muscle	Hypothalamus, cerebral cortex and blink-related areas of cerebellar cortex	99
Rhesus monkey	RABV	3 4 days	Lateral rectus muscle	Collicular neurons	100
Rat	PRV-614	3 4 days	Shoulder muscle	Reticular formation, the raphe nucleus and the periaqueductal gray	101
Mice	PRV-152	3 days	Orbicularis oculi muscle	Facial nucleus neurons	102
Rat	PRV	4 5 days	Masseter, genioglossus, thyroarytenoid or inferior constrictor muscles	Central nucleus (CE)	48
