Am J Transl Res 2022;14(7):4864-4879
www.ajtr.org /ISSN:1943-8141/AJTR0143581

Review Article

The connectome from the cerebral cortex to skeletal muscle using viral transneuronal tracers: a review

Yan Huang[1,2*], Yunhua Zhang[3,4,5*], Zhigang He[1*], Anne Manyande[6], Duozhi Wu[7], Maohui Feng[8], Hongbing Xiang[1]

[1]Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, P. R. China; [2]Department of Interventional Therapy, The First Affiliated Hospital of Dalian Medical University, Dalian 116000, Liaoning, P. R. China; [3]Hubei Provincial Hospital of Traditional Chinese Medicine, Wuhan 430061, Hubei, P. R. China; [4]Clinical Medical College of Hubei University of Chinese Medicine, Wuhan 430061, Hubei, P. R. China; [5]Hubei Province Academy of Traditional Chinese Medicine, Wuhan 430061, Hubei, P. R. China; [6]School of Human and Social Sciences, University of West London, London, UK; [7]Department of Anesthesiology, Hainan General Hospital, Haikou 570311, Hainan, P. R. China; [8]Department of Gastrointestinal Surgery, Wuhan Peritoneal Cancer Clinical Medical Research Center, Zhongnan Hospital of Wuhan University, Hubei Key Laboratory of Tumor Biological Behaviors and Hubei Cancer Clinical Study Center, Wuhan 430071, Hubei, P. R. China. [*]Equal contributors.

Received April 17, 2022; Accepted June 22, 2022; Epub July 15, 2022; Published July 30, 2022

Abstract: Connectomics has developed from an initial observation under an electron microscope to the present well-known 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 a complete nerve conduction pathway. This review assesses current methods of studying cortical to muscle connections using viral neuronal tracers and demonstrates their application in disease diagnosis and prognosis.

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

Introduction

The brain has a complex network of neural circuits [1, 2]. 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 [3-11]. The connectome is an integrated histologic 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 [12, 13]. 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 neuroscience [14].

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, used functional magnetic resonance imaging (fMRI) to observe 17 patients with vestibular neuritis. It 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+ region [15]. 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 con-

nectivity between the posterior cingulate cortex and posterior insula and medial temporal lobes was reported to be consistent with the emotional loss and psychiatric abnormalities the patient presents [16]. Nowadays, transneuronal tracers can be used to analyze brain nerve transmission, draw fine neural pathways from the microscopic point of view, and more comprehensive and objective analysis of the morphological study of living brain tissue [17].

Current research on viral transneuronal tracers

Information in brain regions travels through synapses between neurons. Traditional neural tracers are based on this to reach the role of tracking connections between synapses through the neural conduction of labelled neurons and cell bodies along axon terminals, such as horseradish peroxidase (HRP), cholera toxin B (CTb) and fluorescein tracers, but have the following disadvantages: they can only label single neurons; they have decreasing labeling capacity step by step as neurons are transmitted; and they have a transneural transmission inefficiency [18, 19].

Transneuronal tracers are currently the most commonly used neuroscience research tools [20, 21]. Tracers of neuronal receptors rely on protein labeled neuronal or biological nerve tracers, ranging from non-viral fluorescent-labeled proteins first discovered in 1970 [22] to the first viral tracer rabies virus (RABV). In **Figure 1**, we attempt to trace the timeline of viral neural tracers, contributing to an understanding of the progress of neural tracers in reconstructing brain neurology and brain connectomics studies, which have played an important role in the development of neuroanatomy throughout history.

Neurotropic viruses are transmitted by infecting neuronal cells in the organism, which in turn proliferate and spread the virus along nerve loops.

Neurotropic viruses are among the most promising transneuronal tracer tools with excellent biological characteristics such as self-replication and specific trans-synaptic transmission [23, 24]. Compared to conventional tracers, neurotropic viruses have the following significant advantages: they can propagate across synapses; they have flexible tracer directionality and manipulability; they can self-replicate and propagate signals along axons without attenuation; they can carry multiple tracers and functional probes for the tracing of specific neural circuits. There are two types of viral tracers: retrograde and bidirectional tracers [25]. The most frequently used viruses are adeno-associated viruses (AAV) [26], herpes simplex virus 1 (HSV-1) [27, 28], pseudorabies virus (PRV) [29, 30], measles virus (MV) [31], vesicular stomatitis virus (VSV) [32]. For example, the RABV and PRV, which belong to retrograde trans-neuronal tracers that map input neurons, can successfully identify specific central nervous system regions (CNS) in the brain. HSV and AAV can be used as anterograde transneuronal tracers, which project anterograde axonal transport to inferior neurons and labeling output neurons [26, 33, 34].

Transneuronal tracers are widely used in anatomical studies of central and peripheral nerves [35]. A neurotropic virus marks primary neurons along the efferent or afferent nerves to secondary and tertiary neurons. It draws a neural circuit conduction map according to the nerves labeled by the virus [36]. When the H129 strain of HSV-1 was injected into the interscapular brown adipose tissue (IBAT) during central nerve conduction, the virus infected the paraventricular nucleus of the hypothalamus (PVH), periaqueductal gray matter (PAG), and reticular areas. This intuitively confirmed the neural circuit conduction between the IBAT and CNS [37]. In peripheral nerves, the RABV was injected into the hind legs of mice, and the virus was found to transmit to the spinal cord in the axon of the peripheral femoral nerve and marked in the Schwann cells of peripheral nerves [38]. These techniques have been widely used to trace neural circuits such as visceral nerve circuits [39]; visual nerve conduction [40-42], taste conduction [43, 44], olfactory conduction [45], and motor system conduction [46].

Current research progress on brain-skeletal muscle motor circuits

The execution of movement in primates depends on the control of muscle groups. Most of the neural network governing movement comes from the downward projection of the primary motor cortex (M1), which is transmitted to

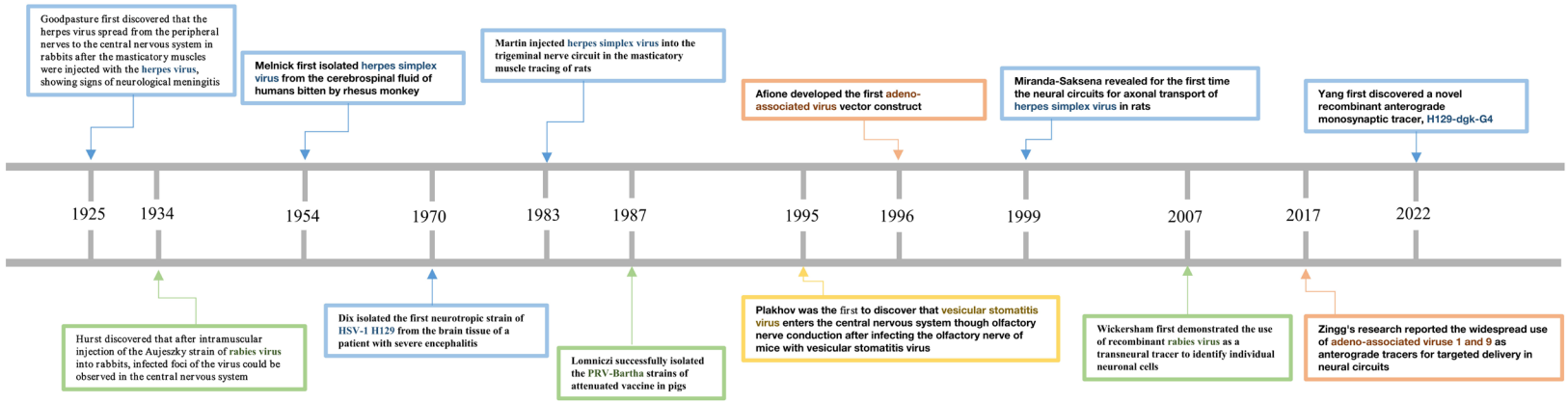

Figure 1. Timeline for the development of neurotropic viruses that are currently frequently used for neural loop tracing. These are herpes simplex virus, pseudorabies virus, rabies virus, adeno-associated virus, and vesicular stomatitis virus.

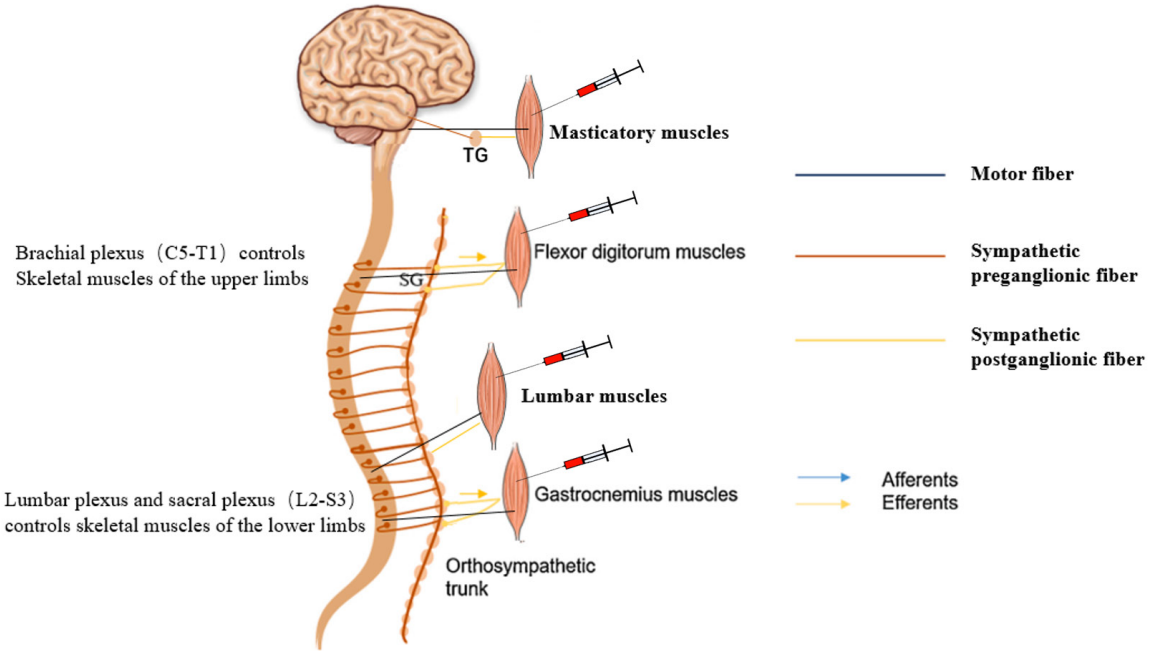

Figure 2. Schematic drawing of the peripheral autonomic innervation of the skeletal muscle. PRV injected into the flexor muscles of fingers, lumbar muscles, and gastrocnemius muscles was transported to the sympathetic ganglia (SG) (by the sympathetic pathway) and the ventral horn of the spinal cord (by the motor pathway), whereas PRV injected into the masseter muscles was transported to the trigeminal ganglia (TG) by the sympathetic pathway and the motor pathway.

the corticospinal tract (CST) to coordinate fine movement. The reticular spinal lot is mainly responsible for coordinating the overall direction of muscles [47]. In addition, the frontal lobe-sensory interaction of the cerebral cortex is involved in the neural regulation of fine sensation [48, 49].

Brain-skeletal muscle connectome research has shifted from the electron microscope to neuron tracer in the past few decades [50, 51]. Studies have reported using bionics to explore brain-skeletal muscle connectomics. By constructing the skeletal muscle system through machine learning, the nerve conduction device of the skeletal muscle innervated by microelectrodes is built to simulate the nerve conduction of the skeletal muscle [52]. Recent research has shown that the dynamic network of cortical-muscle interactions in physiological states can be mapped according to the specific electroencephalogram (EEG) produced by different motor states [53]. These techniques shed light on mapping brain-skeletal muscle connections from another perspective. At the same time, the exploration of the brain connectome using transneuronal tracers can more intuitively explore the information transmission within nerves. The neural circuitry of the brain-skeletal muscle connectome is revealed, and the duration of viral application to different skeletal muscles is summarized in **Figure 2** and **Table 1**.

Cerebral cortex innervation of masticatory muscles

Masticatory behavior is projected from the orofacial motor cortex (MCtx) to the trigeminal motor nucleus and brainstem reticular structure through the CST [54]. Then the masticatory muscle is innervated to produce physiological behaviors such as chewing, speaking, and swallowing [55]. The neural projection from the human masseter single motor unit (SMU) to M1 was examined in a study using focal transcranial magnetic stimulation (TMS) and found that 87% of the SMU was projected to the contralateral M1 and only 25% to the ipsilateral M1, suggesting that the masseter was subjected to monosynaptic corticomotoneuronal (CM) projection [56].

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

Species of 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	[104]
Rat	PRV-BaBLU	2 3 days	Stomach musculature	Dorsal motor vagal nucleus	[105]
Rat	PRV-Bartha	3 days	Masseter muscle	Medial vestibular nucleus (MVe), caudal prepositus hypoglossi (PH), ipsilateral spinal vestibular nucleus (SpVe)	[60]
Mice	PRV-BaBLU	2 3 days	Masseter muscle	Cranial nerve V (Mo5)	[57]
Mice	AAV2-reyro-Cre	5 days	Masseter muscle	Intermediate reticular nucleus (IRt)	[61]
Rat	PRV	4 days	extensor carpi radialis longus (ECRL)	Intermediate gray matter (laminae VII and X)	[69]
Mice	PRV-152	6 days	Forelimb flexor muscle	Primary motor cortex layer 5 neuron (MOp5)	[71]
Macaques	RABV	4-5 days	abductor pollicis longus (ABPL), adductor pollicis (ADP), extensor digitorum communis (EDC)	Layer V of primary motor cortex	[67]
Mice	PRV-Bartha	2-3 days	Tibialis anterior (TA) and gastrocnemius muscles (GC)	Ipsilateral interneurons and ventral grey matter	[81]
Rat	PRV-152	6 days	Gastrocnemius muscle	The periaqueductal gray and the hypothalamus	[82]
Mice	PRV-614	4-6 days	Gastrocnemius muscle	Spinal IML, periaqueductal gray and motor cortex	[87]
Rat	PRV-152, PRV-BaBLU	4 days	Gastrocnemius muscle	rostral ventrolateral medulla (RVLM), medullary raphe nuclei, A5 region, locus coeruleus (LC), and Hypothalamic paraventricular nucleus (PVN)	[88]
Rat	PRV, CTb	5 days	Gastrocnemius muscle, sciatic nerve	Motor neurons in the dorsolateral column ipsilateral	[91]
Mice	PRV-614	5-7 days	Lumbar muscle	Medullary reticular formation nucleus (MRN), pons reticular nucleus (PRN), RVLM, A5 region, LC, SubC, PVN, ventromedial hypothalamic nucleus (VMH)	[98]
Rat	PRV	4 days	Lumbar epaxial muscle	Medullary reticular formation, periaqueductal gray (PAG), VMN	[100]
Cat	PRV	4 days	Diaphragm or neck musculature	Dorsal root ganglia and dorsal horn of the spinal cord	[104]
Mice	PRV, CTb	3 days	Deltoid muscle, biceps muscle, wrist extensor compartment	Corticospinal tract (CST)	[46]
Cat	RABV	4 days	Diaphragm	Vestibular nuclei (VN) and medial pontomedullary reticular formation (MRF)	[106]
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)	[107]
Rat	PRV	5 days	Genioglossus muscle	PVN	[108]
Rhesus monkey	RABV	4-5 days	Orbicularis oculi muscles (OO)	Ventrolateral premotor (LPMCv), dorsolateral premotor (LPMCd), and motor cortices (M1)	[109]
Rat	PRV	2 3 days	External urethral sphincter (EUS)	L3/L4 propriospinal neurons (PSNs) and interneurons	[110]
Mice	PRV-614	5 days	Gastrocnemius muscle	Pedunculopontine tegmental nucleus (PPTg)	[84]
Rat	RABV	4-5 days	Orbicularis oculi muscle	Hypothalamus, cerebral cortex and blink-related areas of cerebellar cortex	[111]
Rhesus monkey	RABV	3-4 days	Lateral rectus muscle	Collicular neurons	[112]
Rat	PRV-614	3-4 days	Shoulder muscle	Reticular formation, the raphe nucleus and the periaqueductal gray	[113]
Mice	PRV-152	3 days	Orbicularis oculi muscle	Facial nucleus neurons	[114]
Rat	PRV	4-5 days	Masseter, genioglossus, thyroarytenoid or inferior constrictor muscles	Central nucleus (CE)	[58]

This inspired us to further explore the neural regulation of the trigeminal nerve on the masticatory muscle, where the retrograde tracer pseudorabies virus-Bartha (PRV-Bartha) was injected into the masseter muscle of mice. The virus retrogrades were shown to have infected the cranial motor nucleus V (Mo5). They then projected to the lateral hypothalamus (LH), basolateral and central amygdala (Amy), insular (Ins), and perirhinal cortices (Rhi), indicating that the Mo5 is co-innervated by multi-synaptic neural pathways. The specific projection of individual neurons into masticatory muscles was further examined at the microscopic level. Therefore, the dual-labeled tracers pseudorabies virus-152 (PRV-152) and pseudorabies virus-614 (PRV-614) were injected into the masseter muscle. The neuropeptide melanocortin concentrating hormone (MCH) and orexin neuropeptides were found to be significantly marked, illustrating that the MCH and orexin neurons in the LH could be down projected to the MAS and salivary gland (SAL) involved in the control of chewing behavior. The Amy is also known to be involved in neural regulation of feeding behavior, so when the dual-labeling PRV-Bartha and PRV-614 were injected into the SAL and MAS again, it was observed that $Nurrl^+$ neurons projected downward and innervated the masseter from the perspective of nerve molecules [57]. Due to the innervation of oropharyngeal muscles by the medial anterior Amy, the retrograde synaptic tracer PRV was injected into the masseter, genioglossus, and thyroarytenoid of rats. The virus was shown to have infected the Mo5, retrogressed into the central nucleus (CE), and then directly projected to the intermediate reticular nucleus (IRt) through GABA neurons, revealing that the CE is innervated by premotor neurons from the pons to the medulla oblongata reticular structure, and is involved in oropharyngeal taste aversion [58].

The retrograde virus tracer was tagged with the trigeminal nucleus (TG) by the masticatory muscle and transferred to peripheral nerve nuclei along the dendrites of motor neurons [59]. The researchers then injected the retrograde synaptic tracer PRV-Bartha into the superficial one-third of the masseter muscle of rats. The virus retrograded to the lateral portion of the ipsilateral Mo5 and then projected to the bilateral vestibular nuclei (VN). It was significantly marked in the ipsilateral caudal prepositus hypoglossi (PH), medial vestibular nucleus (MVe), and ipsilateral spinal vestibular nucleus (SpVe), indicating that the TG was subjected to a neural projection by VN [60].

In another study, optimized rabies glycoprotein deficient retrograde rabies virus trans-synaptic tracer (ΔG-RV) and Cre dependent AAV2 (AAV-retro-Cre) were injected into the masticatory muscles of mice, and viral markers were found in the ipsilateral motor neurons of the Mo5. Significantly labeled anterior motor neurons were seen in the dorsal IRt, supratrigeminal region (SupV), and peripheral trigeminal areas, suggesting that the brainstem reticular structure is involved in orofacial behaviors of masticatory muscles by projecting on the Mo5 [61].

Barnett [62] also reported that the trigeminal nerve regulates multi-synaptic projections of the M1 using the HSV-1 type 1 strain H129 (HSV-1 H129) to infect the trigeminal nucleus. The virus infected the laminae IV and Va of the primary somatosensory cortex from the medial geniculate complex thalamus and ventral posterior medial thalamus, marked in the primary somatosensory cortex (S1). In conclusion, these studies indicate that masticatory muscles are innervated by multiple synapses, which are coordinated by various regions in the central nerve of the brain (**Figure 3**).

Cerebral cortex innervation of the flexor muscles of fingers

Earlier studies have shown that premotor circuits control grasping, from initial projection to the posterior parietal cortex (PPC) for visual guidance and then to the cerebral cortex for learning control based on memory and imagination [63]. The flexor digitorum is one of the few muscles directly regulated by the cortical motor neuron (CM) cells in the M1. The tail of the M1 projects into the hand muscle through the single synapse of CM cells is involved in delicate finger movements [64, 65]. The hand's dominant areas are critical to the M1, with 20% of the site used to regulate delicate hand movements [66]. Studies have shown that the M1 innervated a single synapse in the flexor digitorum muscles, when retrograde virus tracers RABV were injected into the abductor pollicis longus (ABPL), adductor pollicis (ADP), and extensor digitorum communis

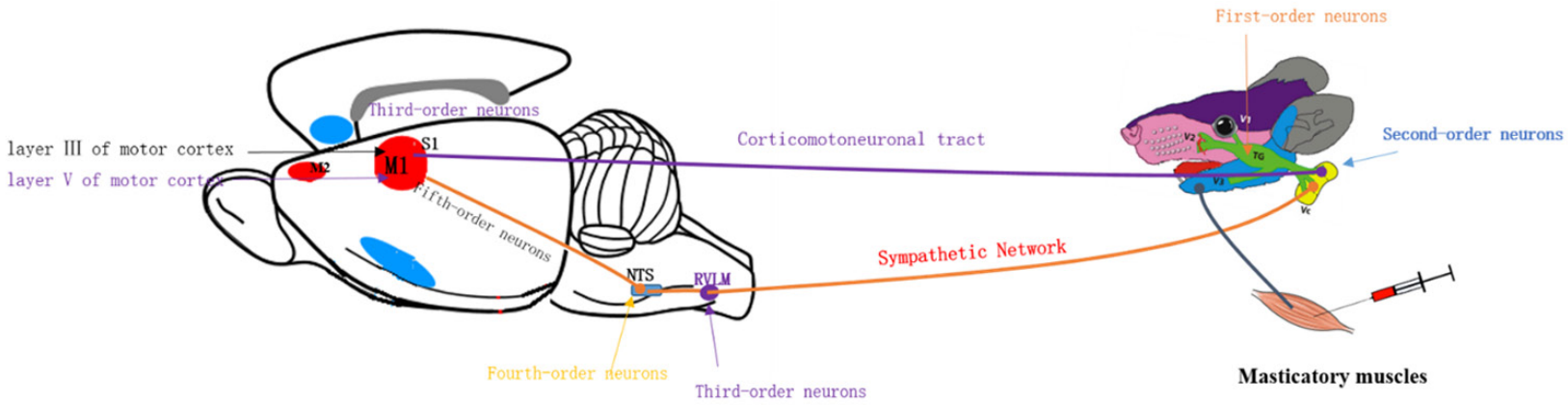

Figure 3. Schematic drawing of the connectivity from the masticatory muscles to the cerebral cortex. When the virus is injected into the masticatory muscles, it is transported in the retrograde direction to infect the trigeminal ganglia (TG) (i.e., first-order neurons) that innervate the masticatory muscles. Then the virus is transported transneuronally in the retrograde direction to label the trigeminal subnucleus caudalis (Vc) (i.e., second-order neurons) that synapse onto the infected TG neurons. NTS, nucleus of the solitary tract; RVLM, rostral ventrolateral medulla. Schematic illustration of the orofacial and trigeminal nervous system came from Kim YS et al. Central terminal sensitization of TRPV1 by descending serotonergic facilitation modulates chronic pain. Neuron. 2014;81:873-887.

(EDC) of macaque monkeys. The virus retrograded to the motoneuron (MN) in the lower cervical and upper thoracic segments of the spinal cord, then labeled the CM in layer V of the M1 and afferent nerves of the Ia (second-order neuron), followed by the CM in layer III of the M1 (third-order neuron) [67].

Damage to the M1 or CST can cause problems with delicate finger movement [68]. The retrograde virus tracers PRV was injected into spinal interneurons of the extensor carpi radialis longus (ECRL) muscle in the forelimb of rats, and it was found that the virus significantly marked intermediate neurons in the C6-T1 spinal segments. It was concluded that the spinal cord premotor circuit recovered moderately in rats with cervical spinal cord injury [69]. Because the pathologic manifestation of stroke is the interruption of the axon connection between the CST and corpus callosum, stroke patients often have a certain degree of physical discoordination and motor dysfunction [70]. Poinsatte injected mice with the pseudorabies virus (PRV-152) into the left forelimb flexor ascending the CST to secondary neurons in layers 2 and 3 of the M1, followed by layers 5 of the suitable M1 (MOp5) and S1. Compared to the sham group, stroke mice showed a significant decrease in the signal of MOp5 virus markers on the right side due to damage to the right CST ($P<0.0001$). The destruction of the integrity of the CST affected the innervation of the M1 to the forelimb muscles, which was confirmed by observing the fluorescent signals in the brain after a stroke [71].

Tosolini [72] injected retrograde neuronal tracers several times into 11 forelimb muscles of rats. They observed that neurons were significantly labeled in the cervical spinal cord. The motor neurons innervating the flexor digitorum were concentrated in the cervical segments C6-C7. Grasping is usually caused by the transmission of information from the ventral premotor cortex (PMv) to the M1 [73]. The spinothalamic (ST) system is involved in the neuronal regulation of pain and injury sensation. In one study, the transneuronal tracer HSV-1 H129 was injected into the C5-T1 cervical segment of the spinal cord of Cebus monkeys. The virus entered the thalamus along the spinothalamic neurons and was transported anterograde to the cingulate sulcus of the cerebral cortex [74]. This helped to verify the direct regulation effect of proprioceptive spinal cord neurons (PN) on hand extension and grasping behavior. In another study, dual retrograde tracers of the lentiviral vector carrying enhanced tetanus neurotoxin light chain (HiRet-TRE-EGFP. eTeNT) and AAV2 with the Tet-on sequence (AAV2-CMV-RTTAV16) were injected into PN-targeted neuronal regions. Specific blockade of the PN following oral administration of doxycycline (Dox) showed temporary reach and grasping disorders in macaques after the virus entered the motor neuron region of the C6-T1 spinal segment. The complete PN is thus involved in the extension and flexion movement of the hand and arm, and monosynaptic connections of motor cortex neurons with C6-T1 spinal cord interneurons are involved in delicate finger movements [75].

Cerebral cortex innervation of the gastrocnemius muscle

Parkinson's disease (PD) is a neurodegenerative disorder in which patients usually present with systemic static tremor myotonia and bradykinesia. Under whole-body vibration (WBV) training, mechanical vibration stimulation at 20 Hz was found to help increase the strength of the calf gastrocnemius muscle (GAS) and improve the fluster gait of PD patients [76]. Similarly, a clinical study demonstrated that deep brain stimulation (DBS) of the subthalamic nucleus (STN) improved the forward-leaning posture in PD patients [77]. To more intuitively observe the transmission between the cerebral cortex and basal ganglia, after injection of the RABV in the M1, the virus was transmitted along hypothalamic neurons to neurons in the globus pallidus (GPe), striatum, and STN [78]. Animal studies have also shown that the STN is double dominated by the cerebral cortex. On the one hand, it is directly projected by glutamate and on the other hand, it is indirectly launched by the GABA from the GPe and striatum and then transmitted along with the CST to motor neurons in the forefoot of the spinal cord to regulate the GAS [79].

The retrovirus tracer can be specifically projected to the spinal cord region in the target organ, which can be used as a projection tool for potential neuroprotective genes. In a study, adenovirus vector carrying the beta-galactosidase (AdV-LacZ) gene was injected into the gastrocnemius muscle. The virus was retro-

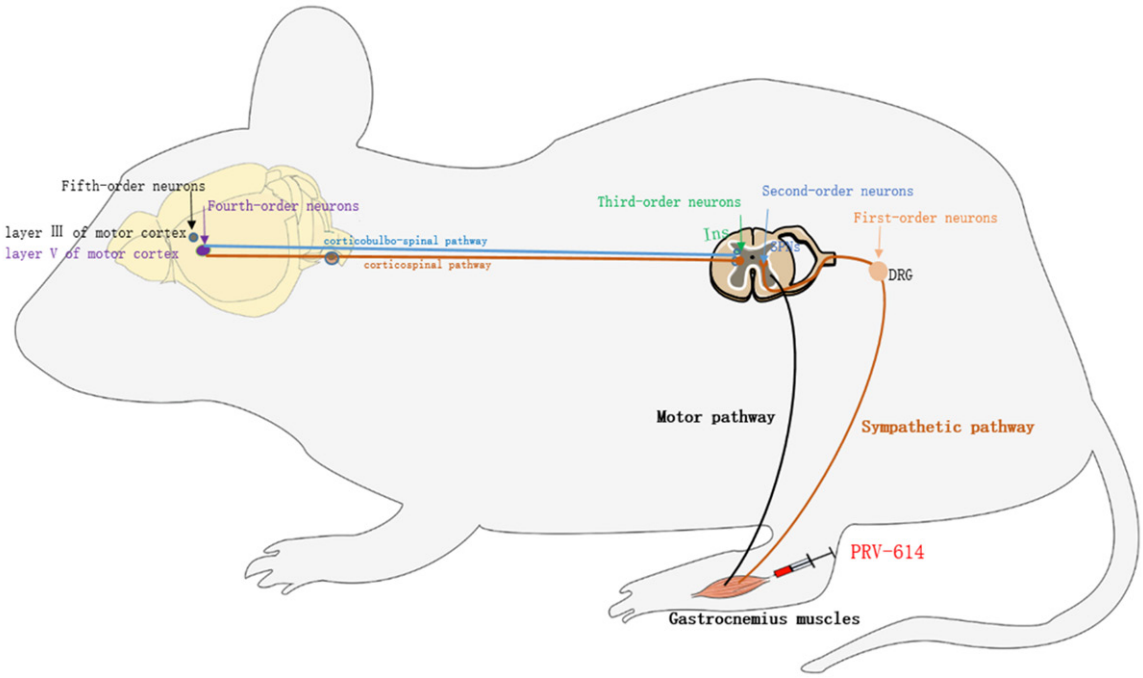

Figure 4. Schematic drawing of the connectivity from the gastrocnemius muscles to the cerebral cortex. When the PRV virus is injected into the gastrocnemius muscles, it is transported in the retrograde direction to infect the dorsal root ganglia (DRG) (i.e., first-order neurons) that innervate the gastrocnemius muscles. Then the virus is transported transneuronally in the retrograde direction to label spinal cord ventral horn and sympathetic preganglionic neurons (SPNs) (i.e., second-order neurons) that synapse onto the infected DRG neurons. DRG, dorsal root ganglia; Ins, interneurons; SPNs, sympathetic preganglionic neurons.

gradely transported one week later along the axon to the motor neurons in the anterior corner of the lumbar spinal cord. The LacZ gene transmission efficiency was 56.6% in the gastrocnemius muscle of the lumbar spinal segment [80]. In another study, the retrograde virus tracers PRV-Bartha were injected into the gastrocnemius muscle. The virus was transmitted along ipsilateral motor neurons to interneurons in the L4-L5 spinal segment, marking Ia inhibitory interneurons in the dorsal, ventral, and medial motoneuron pool. Interneuronal calcium-binding proteins and parvalbumin were projected to motor neurons through single synapses [81]. This study confirmed that the virus injected into the gastrocnemius muscle was launched to the lumbar motor neurons by a single synaptic mode.

Transneuronal tracers determine the single synaptic connection between cortical motor neurons and the gastrocnemius muscle (**Figure 4**). A study found that injecting PRV-152 into the gastrocnemius muscle, where the sympathetic nerve is severed, marked gastrocnemius motor neurons. The retrograde virus tracer PRV-BaBLU was simultaneously injected into the adrenal glands to label sympathetic preganglionic neurons. At 96 h after infection, dual viruses were jointly characterized in the PAG, LH, and Hypothalamic paraventricular nucleus (PVN). This study confirms that the gastrocnemius muscle is innervated jointly by sympathetic - motor integration [82, 83]. In another study, PRV-614 and MC4R-GFP were injected into the gastrocnemius muscle of spinal cord transected mice. The virus projected along the intermediolateral column (IML) to the rostral ventromedial medulla (RVMM) and rostral ventrolateral medulla (RVLM), was subsequently marked significantly in the pedunculopontine tegmental nucleus (PPTg) of the midbrain but unmarked in the cuneiform nucleus (CnF). The gastrocnemius muscle was confirmed to be innervated by the melanocortin sympathetic nerve of the midbrain PPTg [84].

In addition, neuropathic pain modulation in the organism is transmitted by cortical-brainstem-spinal cord neural network connections [85]. Injury signals in the prefrontal cortex (PFC) project by the CST to the PAG and subsequently to

the rostroventral medulla (RVM) and to the on and off cells of the locus coeruleus (LC), which in turn modulate pain perception in the body [86]. In a study, PRV-614 was injected into the efferent neurons of the left gastrocnemius muscle of mice. The virus was found to travel retrograde to the PAG and M1 along the sympathetic preganglionic neurons of the IML. This confirmed that the motor cortical-periaqueductal gray matter-spinal motor pathway is involved in sympathetic innervation [87]. PRV-152 and PRV-BaBlU were injected into the gastrocnemius muscle on both sides to investigate whether there was independent innervation in the regulation of blood flow in the gastrocnemius muscle. After transection of the L2 spinal cord in rats, PRV-152 and PRV-BaBlu were injected into the left and right hind limbs of the gastrocnemius muscles. The motor neurons of the left and right gastrocnemius muscle were infected retrograde to the neurons of the sympathetic nerve and subsequently labeled in the bilateral cerebral nerves of the rats. Neuronal cells labeled by viral tracers were observed in the PVN, RVLM, LC, and A5 adrenergic cell group region (A5) of the rat brain. Among them, RVLM served as the major sympathetic efferent site in the CNS, only half of RVLM neurons were labeled, indicating that the CNS had limited effect on regulating the gastrocnemius blood flow [88].

In regenerative medicine, human umbilical cord mesenchymal stem cells (hUCMSCs) can be used to repair nerve injury and regenerate axons. Sciatic nerve injury is usually accompanied by gastrocnemius atrophy. Existing studies have found that hUCMSCs can promote nerve regeneration and improve denervated gastrocnemius atrophy in rats with sciatic nerve transection [89]. A retrograde PRV-BA tracer was used to label the sciatic nerve of rats 35 days after transplantation of human neural stem cells (hNSC). The virus retrogrades entered the frontal cortex, paraventricular nucleus (PVS), giant reticular cells, raphe nucleus, and A5 region. However, the GAP43 protein was highly expressed in the spinal cord transection region, and the number of axons significantly increased, indicating that the integrity of the motor neural pathway was observed under the tracking of PRV-BA [90]. In another study, the PRV and CTb were injected into the right gastrocnemius muscle of rats after treatment with hNSC for amyotrophic lateral sclerosis (ALS) to mark afferent motor neurons jointly. Compared with the traditional tracer CTb, the PRV showed a significant advantage in trans-nerve tracer labeling. The PRV entered the sciatic nerve from the gastrocnemius muscle and was labeled at the synaptic terminal of hNSC-derived neuron [91].

Cerebral cortex innervation of the lumbar muscles

Chronic low back pain (LBP) is a painful joint skeletal muscle disease [92]. One study found that LBP patients had increased local low back pain with type II muscle fibers due to chronic strain of the lumbar muscles over a long period, resulting in persistent low back pain [93]. In terms of pain nerve conduction, the PAG is involved in neural circuit regulation of the CNS for downward pain [94]. In imaging studies, chronic pain is accompanied by changes in the brain functional structure. CLBP patients have increased network connectivity in the anterior insular cortex, dorsolateral prefrontal cortex, and the anterior temporoparietal junction of the S1. It can be seen that chronic pain causes increased pain conduction between the cerebral cortex [95]. In cLBP patients, remodeling of the S1 has been reported, leading to a decrease in tactile acuity.

Assessment of brain structure images of cLBP patients revealed increased gray matter (GM) volume in the S1-back and S1-fingers, which suggest that changes in the GM microstructure of cLBP are related to nerve conduction of back pain [96]. It has also been reported that the LC is involved in neuronal regulation of pain, when HSV-1 H129 was injected into the Amy and posterior-lateral hypothalamic area (PLH). It was found that the virus directly projected into LC neurons anterolateral, indicating that the Amy and PLH through GABAergic could directly project onto LC axons and participate in the neural regulation of sympathetic nerve activity and pain sensation [97]. To further explore the neuronal circuits involved in lumbar muscle pain transmission, PRV-614 was injected into the left lateral lumbar muscle of mice. The virus traveled retrograde to the raphe nucleus, RVLM, A5 region, LC, pons reticular nucleus (PRN), and PVN along the spinal cord labeled sympathetic neurons in the IML, demonstrating the central innervation of the external lum-

bar muscle. However, mice undergoing spinal cord transection L2 presented delayed retrograde infection of the IML, indicating that the RVLM, Lateral paragigantocellular reticular nucleus (LPGi), A5 region, LC and PVN are also involved in the autonomic innervation of the lumbar muscle [98].

The external lumbar muscles are innervated by both motor and autonomic circuits, and the lumbar muscles receive nerve projections from the ventromedial hypothalamic nucleus (VMH) during lordosis [99]. Daniels [100] injected PRV into the external lumbar muscle of rats. The virus entered the T8-L2 spinal cord neurons and infected the RVLM, then retrograde infected the pons and midbrain regions, and was significantly marked in the PAG, VMH and medial pontomedullary reticular formation (MRF). These results suggest that the PRV enters the CNS network by infecting sympathetic innervated vessels, thereby marking the neural circuitry of the lumbar external axons. Another study used a dopamine-β-hydroxylase immunotoxin (DHIT) injection to cut sympathetic innervation by injecting the PRV into the lumbar external muscles in the ventral horn neurons of L3-S1, followed by observation of the PRV immune response of neurons in the MRF, PAG, and VMH, which confirmed that the CNS regulates the lordosis by autonomic innervation of the external lumbar muscles [101]. To further visualize VMH neurons, the PRV was injected into the external lumbar muscle, and the virus was marked in the VMH along the axon. The density of dendritic spines in the MVH increased after treatment with double estradiol, indicating that estrogen could induce specific lordosis behavior by increasing VMH dendrites [102].

Conclusion

Transneuronal tracers have excellent characteristics of trans-neuronal signal marking, directionality, and non-attenuation. According to our review, the use of transneuronal tracers provides a new approach to brain-skeletal muscle connectomics. From the microscopic point of view, the innervation image of the cerebral cortex to the skeletal muscle is observed more intuitively. There is still a long way to go in the study of this technology. At present, the research on brain-skeletal muscle connectomics should not be limited to the study of complete neural circuits, but should also be extended to the study of incomplete or traumatic disease related neural circuits, and further apply it to the study of nerve injury repair [103]. This will pave the way for further research on neuroplasticity and traumatic repair. Therefore, neural tracers could widely be used in the study of connectomics related diseases, providing new perspective for the subsequent study of neuroanatomy.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81873467, 81770283, 82070302, 81902018), and Hainan Province Clinical Medical Center and the Key Research and Development Program of Hainan Province (ZDYF2021SHFZ087).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hongbing Xiang, Department of Anesthesiology and Pain Medicine, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jie Fang Avenue, Wuhan 430030, Hubei, P. R. China. Tel: +86-15071379880; E-mail: xhbtj2004@163.com; Dr. Maohui Feng, Department of Gastrointestinal Surgery, Wuhan Peritoneal Cancer Clinical Medical Research Center, Zhongnan Hospital of Wuhan University, Hubei Key Laboratory of Tumor Biological Behaviors and Hubei Cancer Clinical Study Center, 169 Donghu Road, Wuhan 430071, Hubei, P. R. China. Tel: +86-18971175688; E-mail: Fengmh@whu.edu.cn

References

[1] Luo L. Architectures of neuronal circuits. Science 2021; 373: eabg7285.
[2] Zheng N, Li M, Wu Y, Kaewborisuth C, Li Z, Gui Z, Wu J, Cai A, Lin K, Su KP, Xiang H, Tian X, Manyande A, Xu F and Wang J. A novel technology for in vivo detection of cell type-specific neural connection with AQP1-encoding rAAV2-retro vector and metal-free MRI. NeuroImage 2022; 258: 119402.
[3] Hagmann P, Cammoun L, Gigandet X, Meuli R, Honey CJ, Wedeen VJ and Sporns O. Mapping the structural core of human cerebral cortex. PLoS Biol 2008; 6: e159.
[4] Van Essen DC. Cartography and connectomes. Neuron 2013; 80: 775-790.

[5] Hao Y, Tian XB, Liu C and Xiang HB. Retrograde tracing of medial vestibular nuclei connections to the kidney in mice. Int J Clin Exp Pathol 2014; 7: 5348-5354.
[6] Feng L, Liu TT, Ye DW, Qiu Q, Xiang HB and Cheung CW. Stimulation of the dorsal portion of subthalamic nucleus may be a viable therapeutic approach in pharmacoresistant epilepsy: a virally mediated transsynaptic tracing study in transgenic mouse model. Epilepsy Behav 2014; 31: 114-116.
[7] Xiang HB, Zhu WZ, Guan XH and Ye DW. Possible mechanism of deep brain stimulation for pedunculopontine nucleus-induced urinary incontinence: a virally mediated transsynaptic tracing study in a transgenic mouse model. Acta Neurochir (Wien) 2013; 155: 1667-1669.
[8] Xiang HB, Zhu WZ, Bu HL, Liu TT and Liu C. Possible mechanism of subthalamic nucleus stimulation-induced acute renal failure: a virally mediated transsynaptic tracing study in transgenic mouse model. Mov Disord 2013; 28: 2037-2038.
[9] Xiang HB, Liu C, Ye DW and Zhu WZ. Possible mechanism of spinal T9 stimulation-induced acute renal failure: a virally mediatedtranssynaptic tracing study in transgenic mouse model. Pain Physician 2013; 16: E47-49.
[10] Liu C, Ye DW, Guan XH, Li RC, Xiang HB and Zhu WZ. Stimulation of the pedunculopontine tegmental nucleus may affect renal function by melanocortinergic signaling. Med Hypotheses 2013; 81: 114-116.
[11] Xiang HB, Liu C, Guo QQ, Li RC and Ye DW. Deep brain stimulation of the pedunculopontine tegmental nucleus may influence renal function. Med Hypotheses 2011; 77: 1135-1138.
[12] Bijsterbosch JD, Valk SL, Wang D and Glasser MF. Recent developments in representations of the connectome. Neuroimage 2021; 243: 118533.
[13] Behrens TE and Sporns O. Human connectomics. Curr Opin Neurobiol 2012; 22: 144-153.
[14] Van Essen DC, Smith SM, Barch DM, Behrens TE, Yacoub E and Ugurbil K. The WU-Minn Human Connectome Project: an overview. Neuroimage 2013; 80: 62-79.
[15] Ibitoye RT, Mallas EJ, Bourke NJ, Kaski D, Bronstein AM and Sharp DJ. The human vestibular cortex: functional anatomy of OP2, its connectivity and the effect of vestibular disease. Cereb Cortex 2022; bhac085.
[16] Lam YS, Li J, Ke Y and Yung WH. Variational dimensions of cingulate cortex functional connectivity and implications in neuropsychiatric disorders. Cereb Cortex 2022; bhac045.
[17] Jbabdi S and Behrens TE. Long-range connectomics. Ann N Y Acad Sci 2013; 1305: 83-93.
[18] Kageyama GH and Robertson RT. Transcellular retrograde labeling of radial glial cells with WGA-HRP and DiI in neonatal rat and hamster. Glia 1993; 9: 70-81.
[19] Itaya SK, Itaya PW and Van Hoesen GW. Intracortical termination of the retino-geniculo-striate pathway studied with transsynaptic tracer (wheat germ agglutinin-horseradish peroxidase) and cytochrome oxidase staining in the macaque monkey. Brain Res 1984; 304: 303-310.
[20] Lanciego JL and Wouterlood FG. A half century of experimental neuroanatomical tracing. J Chem Neuroanat 2011; 42: 157-183.
[21] Ugolini G. Advances in viral transneuronal tracing. J Neurosci Methods 2010; 194: 2-20.
[22] Kristensson K. Transport of fluorescent protein tracer in peripheral nerves. Acta Neuropathol 1970; 16: 293-300.
[23] Lo CC and Chiang AS. Toward whole-body connectomics. J Neurosci 2016; 36: 11375-11383.
[24] Beier KT. Hitchhiking on the neuronal highway: mechanisms of transsynaptic specificity. J Chem Neuroanat 2019; 99: 9-17.
[25] Nassi JJ, Cepko CL, Born RT and Beier KT. Neuroanatomy goes viral! Front Neuroanat 2015; 9: 80.
[26] Zingg B, Chou XL, Zhang ZG, Mesik L, Liang F, Tao HW and Zhang LI. AAV-mediated anterograde transsynaptic tagging: mapping corticocollicular input-defined neural pathways for defense behaviors. Neuron 2017; 93: 33-47.
[27] Zemanick MC, Strick PL and Dix RD. Direction of transneuronal transport of herpes simplex virus 1 in the primate motor system is strain-dependent. Proc Natl Acad Sci U S A 1991; 88: 8048-8051.
[28] Su P, Ying M, Han Z, Xia J, Jin S, Li Y, Wang H and Xu F. High-brightness anterograde transneuronal HSV1 H129 tracer modified using a Trojan horse-like strategy. Mol Brain 2020; 13: 5.
[29] 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 pituitary via transsphenoidal approach and the innervation of anterior and posterior pituitary of rat. Front Endocrinol (Lausanne) 2020; 11: 546350.
[30] Aston-Jones G and Card JP. Use of pseudorabies virus to delineate multisynaptic circuits in brain: opportunities and limitations. J Neurosci Methods 2000; 103: 51-61.
[31] McQuaid S, Campbell S, Wallace IJ, Kirk J and Cosby SL. Measles virus infection and replication in undifferentiated and differentiated human neuronal cells in culture. J Virol 1998; 72: 5245-5250.
[32] Mundell NA, Beier KT, Pan YA, Lapan SW, Göz Aytürk D, Berezovskii VK, Wark AR, Drokhlyan-

sky E, Bielecki J, Born RT, Schier AF and Cepko CL. Vesicular stomatitis virus enables gene transfer and transsynaptic tracing in a wide range of organisms. J Comp Neurol 2015; 523: 1639-1663.
[33] Yang H, Xiong F, Qin HB, Yu QT, Sun JY, Zhao HW, Li D, Zhou Y, Zhang FK, Zhu XW, Wu T, Jiang M, Xu X, Lu Y, Shen HJ, Zeng WB, Zhao F and Luo MH. A novel H129-based anterograde monosynaptic tracer exhibits features of strong labeling intensity, high tracing efficiency, and reduced retrograde labeling. Mol Neurodegener 2022; 17: 6.
[34] Zeng WB, Jiang HF, Gang YD, Song YG, Shen ZZ, Yang H, Dong X, Tian YL, Ni RJ, Liu Y, Tang N, Li X, Jiang X, Gao D, Androulakis M, He XB, Xia HM, Ming YZ, Lu Y, Zhou JN, Zhang C, Xia XS, Shu Y, Zeng SQ, Xu F, Zhao F and Luo MH. Anterograde monosynaptic transneuronal tracers derived from herpes simplex virus 1 strain H129. Mol Neurodegener 2017; 12: 38.
[35] Rao X and Wang J. Neuronal Network Dissection with Neurotropic Virus Tracing. Neurosci Bull 2020; 36: 199-201.
[36] Feng M, Xiang B, Fan L, Wang Q, Xu W and Xiang H. Interrogating autonomic peripheral nervous system neurons with viruses - a literature review. J Neurosci Methods 2020; 346: 108958.
[37] Vaughan CH and Bartness TJ. Anterograde transneuronal viral tract tracing reveals central sensory circuits from brown fat and sensory denervation alters its thermogenic responses. Am J Physiol Regul Integr Comp Physiol 2012; 302: R1049-1058.
[38] Potratz M, Zaeck LM, Weigel C, Klein A, Freuling CM, Müller T and Finke S. Neuroglia infection by rabies virus after anterograde virus spread in peripheral neurons. Acta Neuropathol Commun 2020; 8: 199.
[39] Li Z, Li Z, Xu W, Li Y, Wang Q, Xu H, Manyande A, Wu D, Feng M and Xiang H. The connectome from the cerebral cortex to the viscera using viral transneuronal tracers. Am J Transl Res 2021; 13: 12152-12167.
[40] Prevosto V, Graf W and Ugolini G. Proprioceptive pathways to posterior parietal areas MIP and LIPv from the dorsal column nuclei and the postcentral somatosensory cortex. Eur J Neurosci 2011; 33: 444-460.
[41] Andelin AK, Doyle Z, Laing RJ, Turecek J, Lin B and Olavarria JF. Influence of ocular dominance columns and patchy callosal connections on binocularity in lateral striate cortex: long Evans versus albino rats. J Comp Neurol 2020; 528: 650-663.
[42] Azzopardi P and Cowey A. Preferential representation of the fovea in the primary visual cortex. Nature 1993; 361: 719-721.
[43] Sugita M, Yamamoto K, Hirono C and Shiba Y. Information processing in brainstem bitter taste-relaying neurons defined by genetic tracing. Neuroscience 2013; 250: 166-180.
[44] Matsumoto I. Gustatory neural pathways revealed by genetic tracing from taste receptor cells. Biosci Biotechnol Biochem 2013; 77: 1359-1362.
[45] Boehm U, Zou Z and Buck LB. Feedback loops link odor and pheromone signaling with reproduction. Cell 2005; 123: 683-695.
[46] Asante CO and Martin JH. Differential joint-specific corticospinal tract projections within the cervical enlargement. PLoS One 2013; 8: e74454.
[47] Li N, Daie K, Svoboda K and Druckmann S. Robust neuronal dynamics in premotor cortex during motor planning. Nature 2016; 532: 459-464.
[48] Teka WW, Hamade KC, Barnett WH, Kim T, Markin SN, Rybak IA and Molkov YI. From the motor cortex to the movement and back again. PLoS One 2017; 12: e0179288.
[49] McColgan P, Joubert J, Tabrizi SJ and Rees G. The human motor cortex microcircuit: insights for neurodegenerative disease. Nat Rev Neurosci 2020; 21: 401-415.
[50] Chen D, Cheng X, Yang X, Zhang Y, He Z, Wang Q, Yao G, Liu X, Zeng S, Chen J and Xiang H. Mapping the brain-wide cholinergic neurons projecting to skeletal muscle in mice by high-throughput light sheet tomography. Neurosci Bull 2021; 37: 267-270.
[51] Fan L, Xiang B, Xiong J, He Z and Xiang HB. Use of viruses for interrogating viscera-specific projections in central nervous system. J Neurosci Methods 2020; 341: 108757.
[52] Knothe Tate ML, Detamore M, Capadona JR, Woolley A and Knothe U. Engineering and commercialization of human-device interfaces, from bone to brain. Biomaterials 2016; 95: 35-46.
[53] Rizzo R, Zhang X, Wang J, Lombardi F and Ivanov PC. Network physiology of cortico-muscular interactions. Front Physiol 2020; 11: 558070.
[54] Mercer Lindsay N, Knutsen PM, Lozada AF, Gibbs D, Karten HJ and Kleinfeld D. Orofacial movements involve parallel corticobulbar projections from motor cortex to trigeminal premotor nuclei. Neuron 2019; 104: 765-780, e763.
[55] Westberg KG and Kolta A. The trigeminal circuits responsible for chewing. Int Rev Neurobiol 2011; 97: 77-98.
[56] Pearce SL, Miles TS, Thompson PD and Nordstrom MA. Responses of single motor units in human masseter to transcranial magnetic stimulation of either hemisphere. J Physiol 2003; 549: 583-596.

[57] Perez CA, Stanley SA, Wysocki RW, Havranova J, Ahrens-Nicklas R, Onyimba F and Friedman JM. Molecular annotation of integrative feeding neural circuits. Cell Metab 2011; 13: 222-232.
[58] Van Daele DJ, Fazan VP, Agassandian K and Cassell MD. Amygdala connections with jaw, tongue and laryngo-pharyngeal premotor neurons. Neuroscience 2011; 177: 93-113.
[59] Mong FS, Chen YC and Lu CH. Dendritic ramifications of trigeminal motor neurons innervating jaw-closing muscles of rats. J Neurol Sci 1988; 86: 251-264.
[60] Giaconi E, Deriu F, Tolu E, Cuccurazzu B, Yates BJ and Billig I. Transneuronal tracing of vestibulo-trigeminal pathways innervating the masseter muscle in the rat. Exp Brain Res 2006; 171: 330-339.
[61] Takatoh J, Park JH, Lu J, Li S, Thompson PM, Han BX, Zhao S, Kleinfeld D, Friedman B and Wang F. Constructing an adult orofacial premotor atlas in Allen mouse CCF. Elife 2021; 10: e67291.
[62] Barnett EM, Evans GD, Sun N, Perlman S and Cassell MD. Anterograde tracing of trigeminal afferent pathways from the murine tooth pulp to cortex using herpes simplex virus type 1. J Neurosci 1995; 15: 2972-2984.
[63] Santello M, Flanders M and Soechting JF. Patterns of hand motion during grasping and the influence of sensory guidance. J Neurosci 2002; 22: 1426-1435.
[64] Zaaimi B, Dean LR and Baker SN. Different contributions of primary motor cortex, reticular formation, and spinal cord to fractionated muscle activation. J Neurophysiol 2018; 119: 235-250.
[65] Perez MA and Rothwell JC. Distinct influence of hand posture on cortical activity during human grasping. J Neurosci 2015; 35: 4882-4889.
[66] Sobinov AR and Bensmaia SJ. The neural mechanisms of manual dexterity. Nat Rev Neurosci 2021; 22: 741-757.
[67] Rathelot JA and Strick PL. Muscle representation in the macaque motor cortex: an anatomical perspective. Proc Natl Acad Sci U S A 2006; 103: 8257-8262.
[68] Bunday KL, Tazoe T, Rothwell JC and Perez MA. Subcortical control of precision grip after human spinal cord injury. J Neurosci 2014; 34: 7341-7350.
[69] Gonzalez-Rothi EJ, Rombola AM, Rousseau CA, Mercier LM, Fitzpatrick GM, Reier PJ, Fuller DD and Lane MA. Spinal interneurons and forelimb plasticity after incomplete cervical spinal cord injury in adult rats. J Neurotrauma 2015; 32: 893-907.
[70] Grefkes C and Fink GR. Reorganization of cerebral networks after stroke: new insights from neuroimaging with connectivity approaches. Brain 2011; 134: 1264-1276.
[71] Poinsatte K, Betz D, Torres VO, Ajay AD, Mirza S, Selvaraj UM, Plautz EJ, Kong X, Gokhale S, Meeks JP, Ramirez DMO, Goldberg MP and Stowe AM. Visualization and quantification of post-stroke neural connectivity and neuroinflammation using serial two-photon tomography in the whole mouse brain. Front Neurosci 2019; 13: 1055.
[72] Tosolini AP and Morris R. Spatial characterization of the motor neuron columns supplying the rat forelimb. Neuroscience 2012; 200: 19-30.
[73] Buch ER, Mars RB, Boorman ED and Rushworth MF. A network centered on ventral premotor cortex exerts both facilitatory and inhibitory control over primary motor cortex during action reprogramming. J Neurosci 2010; 30: 1395-1401.
[74] Dum RP, Levinthal DJ and Strick PL. The spinothalamic system targets motor and sensory areas in the cerebral cortex of monkeys. J Neurosci 2009; 29: 14223-14235.
[75] Kinoshita M, Matsui R, Kato S, Hasegawa T, Kasahara H, Isa K, Watakabe A, Yamamori T, Nishimura Y, Alstermark B, Watanabe D, Kobayashi K and Isa T. Genetic dissection of the circuit for hand dexterity in primates. Nature 2012; 487: 235-238.
[76] Chang CM, Tsai CH, Lu MK, Tseng HC, Lu G, Liu BL and Lin HC. The neuromuscular responses in patients with Parkinson's disease under different conditions during whole-body vibration training. BMC Complement Med Ther 2022; 22: 2.
[77] Fransson PA, Nilsson MH, Rehncrona S, Tjernström F, Magnusson M, Johansson R and Patel M. Deep brain stimulation in the subthalamic nuclei alters postural alignment and adaptation in Parkinson's disease. PLoS One 2021; 16: e0259862.
[78] Kelly RM and Strick PL. Macro-architecture of basal ganglia loops with the cerebral cortex: use of rabies virus to reveal multisynaptic circuits. Prog Brain Res 2004; 143: 449-459.
[79] Polyakova Z, Chiken S, Hatanaka N and Nambu A. Cortical control of subthalamic neuronal activity through the hyperdirect and indirect pathways in monkeys. J Neurosci 2020; 40: 7451-7463.
[80] Nakajima H, Uchida K, Kobayashi S, Inukai T, Yayama T, Sato R, Mwaka E and Baba H. Target muscles for retrograde gene delivery to specific spinal cord segments. Neurosci Lett 2008; 435: 1-6.
[81] Jovanovic K, Pastor AM and O'Donovan MJ. The use of PRV-Bartha to define premotor inputs to lumbar motoneurons in the neonatal

spinal cord of the mouse. PLoS One 2010; 5: e11743.
[82] Kerman IA, Akil H and Watson SJ. Rostral elements of sympatho-motor circuitry: a virally mediated transsynaptic tracing study. J Neurosci 2006; 26: 3423-3433.
[83] Xiang HB, Zhu WZ, Guan XH and Ye DW. The cuneiform nucleus may be involved in the regulation of skeletal muscle tone by motor pathway: a virally mediated trans-synaptic tracing study in surgically sympathectomized mice. Brain 2013; 136: e251.
[84] He ZG, Liu BW, Li ZX, Tian XB, Liu SG, Manyande A, Zhang DY and Xiang HB. The caudal pedunculopontine tegmental nucleus may be involved in the regulation of skeletal muscle activity by melanocortin-sympathetic pathway: a virally mediated trans-synaptic tracing study in spinally transected transgenic mice. Oncotarget 2017; 8: 71859-71866.
[85] Kyathanahally SP, Azzarito M, Rosner J, Calhoun VD, Blaiotta C, Ashburner J, Weiskopf N, Wiech K, Friston K, Ziegler G and Freund P. Microstructural plasticity in nociceptive pathways after spinal cord injury. J Neurol Neurosurg Psychiatry 2021; 92: 863-871.
[86] de Andrade EM, Martinez RCR, Pagano RL, Lopes PSS, Auada AVV, Gouveia FV, Antunes GF, Assis DV, Lebrun I and Fonoff ET. Neurochemical effects of motor cortex stimulation in the periaqueductal gray during neuropathic pain. J Neurosurg 2019; 132: 239-251.
[87] Ye DW, Liu C, Liu TT, Tian XB and Xiang HB. Motor cortex-periaqueductal gray-spinal cord neuronal circuitry may involve in modulation of nociception: a virally mediated transsynaptic tracing study in spinally transected transgenic mouse model. PLoS One 2014; 9: e89486.
[88] Lee TK, Lois JH, Troupe JH, Wilson TD and Yates BJ. Transneuronal tracing of neural pathways that regulate hindlimb muscle blood flow. Am J Physiol Regul Integr Comp Physiol 2007; 292: R1532-1541.
[89] Ma Y, Dong L, Zhou D, Li L, Zhang W, Zhen Y, Wang T, Su J, Chen D, Mao C and Wang X. Extracellular vesicles from human umbilical cord mesenchymal stem cells improve nerve regeneration after sciatic nerve transection in rats. J Cell Mol Med 2019; 23: 2822-2835.
[90] Lee KB, Choi JH, Byun K, Chung KH, Ahn JH, Jeong GB, Hwang IK, Kim S, Won MH and Lee B. Recovery of CNS pathway innervating the sciatic nerve following transplantation of human neural stem cells in rat spinal cord injury. Cell Mol Neurobiol 2012; 32: 149-157.
[91] Xu L, Ryugo DK, Pongstaporn T, Johe K and Koliatsos VE. Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the segmental motor circuitry. J Comp Neurol 2009; 514: 297-309.
[92] Li W, Gong Y, Liu J, Guo Y, Tang H, Qin S, Zhao Y, Wang S, Xu Z and Chen B. Peripheral and central pathological mechanisms of chronic low back pain: a narrative review. J Pain Res 2021; 14: 1483-1494.
[93] Mannion AF, Käser L, Weber E, Rhyner A, Dvorak J and Müntener M. Influence of age and duration of symptoms on fibre type distribution and size of the back muscles in chronic low back pain patients. Eur Spine J 2000; 9: 273-281.
[94] Mayer DJ, Wolfle TL, Akil H, Carder B and Liebeskind JC. Analgesia from electrical stimulation in the brainstem of the rat. Science 1971; 174: 1351-1354.
[95] Kim J, Mawla I, Kong J, Lee J, Gerber J, Ortiz A, Kim H, Chan ST, Loggia ML, Wasan AD, Edwards RR, Gollub RL, Rosen BR and Napadow V. Somatotopically specific primary somatosensory connectivity to salience and default mode networks encodes clinical pain. Pain 2019; 160: 1594-1605.
[96] Kim H, Mawla I, Lee J, Gerber J, Walker K, Kim J, Ortiz A, Chan ST, Loggia ML, Wasan AD, Edwards RR, Kong J, Kaptchuk TJ, Gollub RL, Rosen BR and Napadow V. Reduced tactile acuity in chronic low back pain is linked with structural neuroplasticity in primary somatosensory cortex and is modulated by acupuncture therapy. Neuroimage 2020; 217: 116899.
[97] Dimitrov EL, Yanagawa Y and Usdin TB. Forebrain GABAergic projections to locus coeruleus in mouse. J Comp Neurol 2013; 521: 2373-2397.
[98] Xiang HB, Liu C, Liu TT and Xiong J. Central circuits regulating the sympathetic outflow to lumbar muscles in spinally transected mice by retrograde transsynaptic transport. Int J Clin Exp Pathol 2014; 7: 2987-2997.
[99] Tsukahara S, Kanaya M and Yamanouchi K. Neuroanatomy and sex differences of the lordosis-inhibiting system in the lateral septum. Front Neurosci 2014; 8: 299.
[100] Daniels D, Miselis RR and Flanagan-Cato LM. Central neuronal circuit innervating the lordosis-producing muscles defined by transneuronal transport of pseudorabies virus. J Neurosci 1999; 19: 2823-2833.
[101] Daniels D, Miselis RR and Flanagan-Cato LM. Transneuronal tracing from sympathectomized lumbar epaxial muscle in female rats. J Neurobiol 2001; 48: 278-290.
[102] Flanagan-Cato LM, Calizo LH and Daniels D. The synaptic organization of VMH neurons that mediate the effects of estrogen on sexual behavior. Horm Behav 2001; 40: 178-182.

[103] Fortino TA, Randelman ML, Hall AA, Singh J, Bloom DC, Engel E, Hoh DJ, Hou S, Zholudeva LV and Lane MA. Transneuronal tracing to map connectivity in injured and transplanted spinal networks. Exp Neurol 2022; 351: 113990.
[104] Card JP, Enquist LW, Miller AD and Yates BJ. Differential tropism of pseudorabies virus for sensory neurons in the cat. J Neurovirol 1997; 3: 49-61.
[105] Kim JS, Enquist LW and Card JP. Circuit-specific coinfection of neurons in the rat central nervous system with two pseudorabies virus recombinants. J Virol 1999; 73: 9521-9531.
[106] Rice CD, Weber SA, Waggoner AL, Jessell ME and Yates BJ. Mapping of neural pathways that influence diaphragm activity and project to the lumbar spinal cord in cats. Exp Brain Res 2010; 203: 205-211.
[107] Niedringhaus M, Jackson PG, Pearson R, Shi M, Dretchen K, Gillis RA and Sahibzada N. Brainstem sites controlling the lower esophageal sphincter and crural diaphragm in the ferret: a neuroanatomical study. Auton Neurosci 2008; 144: 50-60.
[108] Mack SO, Wu M, Kc P and Haxhiu MA. Stimulation of the hypothalamic paraventricular nucleus modulates cardiorespiratory responses via oxytocinergic innervation of neurons in pre-Botzinger complex. J Appl Physiol (1985) 2007; 102: 189-199.
[109] Gong S, DeCuypere M, Zhao Y and LeDoux MS. Cerebral cortical control of orbicularis oculi motoneurons. Brain Res 2005; 1047: 177-193.
[110] Karnup SV and de Groat WC. Propriospinal neurons of L3-L4 segments involved in control of the rat external urethral sphincter. Neuroscience 2020; 425: 12-28.
[111] Morcuende S, Delgado-Garcia JM and Ugolini G. Neuronal premotor networks involved in eyelid responses: retrograde transneuronal tracing with rabies virus from the orbicularis oculi muscle in the rat. J Neurosci 2002; 22: 8808-8818.
[112] Grantyn A, Brandi AM, Dubayle D, Graf W, Ugolini G, Hadjidimitrakis K and Moschovakis A. Density gradients of trans-synaptically labeled collicular neurons after injections of rabies virus in the lateral rectus muscle of the rhesus monkey. J Comp Neurol 2002; 451: 346-361.
[113] Rubelowski JM, Menge M, Distler C, Rothermel M and Hoffmann KP. Connections of the superior colliculus to shoulder muscles of the rat: a dual tracing study. Front Neuroanat 2013; 7: 17.
[114] Sun LW. Transsynaptic tracing of conditioned eyeblink circuits in the mouse cerebellum. Neuroscience 2012; 203: 122-134.