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The connectome from the cerebral cortex to the skeletal muscle using viral 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

1. Introduction

The brain has a complex network of neural circuits[1]. 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 neuroscience[6].

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+ region [7]. 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

medial temporal lobes was reported to be consistent with the emotional loss and psychiatric abnormalities the patient presents[8]. 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[9].

2. Current research on viral transneuronal tracers

Information in brain regions travels through synapses between neurons. Transneuronal tracers are currently the most commonly used neuroscience research tools[10, 11].

Tracers of neuronal receptors rely on protein labeled neuronal or biological nerve tracers, ranging from non-viral fluorescent-labeled proteins first discovered in 1970[12] to the first viral tracer rabies virus (RABV). Neurotropic viruses are among the most promising transneuronal tracer tools with excellent biological characteristics such as self-replication and specific trans-synaptic transmission[13, 14]. There are two types of viral tracers: retrograde and bidirectional tracers[15]. The most frequently used viruses are adeno-associated viruses (AAV)[16], herpes simplex virus 1 (HSV-1)[17, 18], pseudorabies virus (PRV)[19, 20], measles virus (MV)[21], vesicular stomatitis virus (VSV)[22], and cholera toxin B subunit (CTb)[23, 24]. 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[16, 25, 26].

Transneuronal tracers are widely used in anatomical studies of central and peripheral nerves[27]. 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[28]. 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[29]. 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 femur nerve and marked in the Schwann cells of peripheral nerves[30]. These techniques have been widely used to trace neural circuits such as visceral nerve circuits[31]; visual nerve conduction[32-34], taste conduction[35, 36], olfactory conduction[37], and motor system conduction[38].

3. 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 the corticospinal tract (CST) to coordinate fine movement. The reticular spinal lot is mainly responsible for coordinating the overall direction of muscles[39]. In addition, the frontal lobe-sensory interaction of the cerebral cortex is involved in the neural regulation of fine sensation[40, 41].

Brain-skeletal muscle connectome research has shifted from the electron microscope to neuron tracer in the past few decades. 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[42]. 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[43]. 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 1 and Table 1.

4. 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 corticospinal tract[44]. Then the masticatory muscle is innervated to produce physiological behaviors such as chewing, speaking, and swallowing[45]. 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[46].

This undoubtedly 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. 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[47]. 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) via 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 [48].

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[49]. 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[50].

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[51].

Barnett et al.[52] 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.

5. 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[53]. 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[54, 55]. The hand's dominant areas are critical in the M1, with 20% of the site used to regulate delicate hand movements[56]. 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 (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)[57].

Damage to the M1 or CST can cause problems with delicate finger movement[58]. 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[59]. Because the pathological manifestation of stroke is the interruption of the axon connection between the corticospinal tract and corpus callosum, stroke patients often have a certain degree of physical discoordination and motor dysfunction[60]. Poinsatte et al. injected mice with the pseudorabies virus (PRV-152) into the left forelimb flexor ascending the corticospinal tract to secondary neurons in layers 2 and 3 of the M1, followed by layers 5 of the suitable M1 (MOp5) and S1. Compared with 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 corticospinal tract ($P < 0.0001$). 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 stroke[61].

Tosolini et al.[62] 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[63]. 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[64]. 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[65].

6. 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 20Hz was found to help increase the strength of the calf gastrocnemius muscle (GAS) and improve the fluster gait of PD patients[66]. Similarly, a clinical study demonstrated that deep brain stimulation (DBS) of the subthalamic nucleus (STN) improved the forward-leaning posture in PD patients[67]. 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[68]. 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, 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[69].

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 retrogradely 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[70]. 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[71]. This study confirmed that the virus injected into the gastrocnemius muscle was launched to the lumbar motor neurons via a single synaptic mode.

Transnerve tracers determine the single synaptic connection between cortical motor neurons and the gastrocnemius muscle. A study found that injecting PRV-152 into the gastrocnemius muscle, where the sympathetic nerve is severed, marked gastrocnemius motor neurons. The retrograde virus tracers PRV-BaBLU were simultaneously injected into the adrenal glands to label sympathetic preganglionic neurons. At 96h after infection, dual viruses were jointly characterized in the PAG, LH, and PVN. This study confirms that the gastrocnemius muscle is innervated jointly by sympathetic - motor integration[72, 73]. 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[74].

In addition, neuropathic pain modulation in the organism is transmitted by cortical-brainstem spinal cord neural network connections. 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 moderate pain perception in the body. 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[75]. 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 labelled in the bilateral cerebral nerves of the rats. Neuronal cells labelled 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 labelled, indicating that the CNS had limited effect on regulating the gastrocnemius blood flow[76].

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[77]. 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. 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[78]. 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[79].

7. Cerebral cortex innervation of the lumbar muscles

Chronic low back pain (LBP) is a painful joint skeletal muscle disease[80]. 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[81]. In terms of pain nerve conduction, the PAG is involved in the neural circuit regulation of the CNS for downward pain[82]. 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[83]. 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[84]. It has also been reported that the norepinephrine locus coeruleus (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[85]. 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, LC, pons reticular nucleus (PRN), and PVN along the spinal cord labeled sympathetic neurons in the IML, demonstrating the central innervation of the external lumbar 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, LC and PVN are also involved in the autonomic innervation of the lumbar muscle[86]. 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[87]. Daniels et al.[88] 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[89]. 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[90].

8. 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[91]. It 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 some new perspectives for the subsequent study of neuroanatomy.

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Disclosure of conflict of interest

None.

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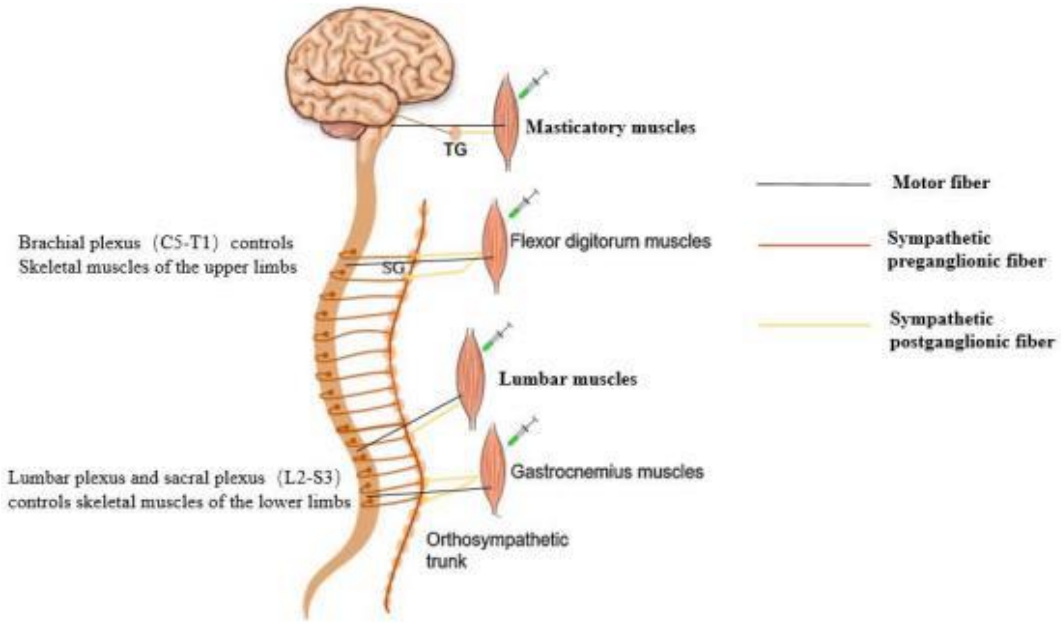

Figure 1: Schematic drawing of the peripheral autonomic innervation of the skeletal muscle. PRV injected into the flexor muscles of fingers, lumbar muscles and gastrocnemius muscles was transported to the sympathetic ganglia (SG) (via the sympathetic pathway) and the ventral horn of the spinal cord (via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