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Chemogenetic enhancement of functional recovery after a sciatic nerve injury

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Abstract

Designer receptors exclusively activated by designer drugs (DREADDs) are chemogenetic tools used to modulate neuronal excitability. We hypothesized that activation of excitatory (Gq) DREADD by its designer ligand, clozapine-N-oxide (CNO), would increase the excitability of neurons whose axons have been transected following peripheral nerve injury, and that this increase will lead to an enhanced functional recovery. The lateral gastrocnemius (LG) muscle of adult female Lewis rats was injected unilaterally with AAV9-*hsyn-hM3Dq-mCherry* (7.6×10^9 viral genomes/ μ l) to transduce Gq-DREADD expression in LG neurons. The contralateral LG muscle served as an uninjected control. No significant changes in either spontaneous EMG activity or electrically evoked direct muscle (M) responses were found in either muscle after injection of CNO (1mg/kg, i.p.). The amplitude of monosynaptic H-reflexes in LG was increased after CNO treatment exclusively in muscles previously injected with virus, suggesting that Gq-DREADD activation increased neuronal excitability. After bilateral sciatic nerve transection and repair, additional rats were treated similarly with CNO for up to three days after injury. Electrophysiological data were collected at 2, 4 and 6 weeks after injury. Evoked EMG responses were observed as early as 2 weeks after injury only in Gq-DREADD expressing virus injected LG muscle. By 4 weeks after injury, both M-response and H-reflex amplitudes were significantly greater in muscles previously injected with viral vector than contralateral, uninjected muscles. Increases in the excitability of injured neurons produced by this novel use of Gq-DREADD were sufficient to promote an enhancement in functional recovery after a sciatic nerve injury.

Keywords

Designer receptors exclusively activated by designer drugs (DREADDs); Peripheral nerve injury; Electromyography; Rat

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The authors do not have any conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

PBJ and AWE designed the experiments. PBJ conducted all animal surgeries, collected data, analyzed data and wrote the manuscript. AWE and PBJ edited the manuscript.

DATA ACCESSIBILITY STATEMENT

All data and supporting materials can be accessed online at European Journal of Neuroscience archives.

INTRODUCTION

Designer receptors exclusively activated by designer drugs (DREADDs) are a novel chemogenetic tool used to manipulate neuronal excitability (Roth, 2016). These modified muscarinic acetylcholine receptors are activated by a sole ligand, the small molecule designer drug, clozapine-N-oxide (CNO) (Jensen & Roth, 2008), which has no known effect on endogenous receptors (Armbruster *et al.*, 2007). Binding of CNO with excitatory (Gq) DREADDs results in the downstream activation of the Gq pathway (Armbruster *et al.*, 2007) that ultimately results in neuronal excitation (Alexander *et al.*, 2009). Several investigators have successfully employed the use of Gq-DREADDs to produce robust changes in central nervous system excitability (Pei *et al.*, 2008; Goforth *et al.*, 2014; Yau & McNally, 2015; Lopez *et al.*, 2016). The impact of Gq-DREADD activation on functional behaviors such as locomotion (Alexander *et al.*, 2009), food intake (Krashes *et al.*, 2011), learning & memory (Yau & McNally, 2015), pain processing (Peirs *et al.*, 2015) has also been established. We present here results from experiments with the use of Gq-DREADDs in rats following sciatic nerve injury.

Peripheral nerve injuries affect approximately 200,000 individuals every year in the United States alone (Noble *et al.*, 1998; Taylor *et al.*, 2008). Spontaneous recovery of function after a peripheral nerve injury is poor due to the slow and incomplete nature of peripheral axon regeneration and the long distances that injured axons often have to grow to re-innervate their specific targets (Al-Majed *et al.*, 2000; English, 2005; English *et al.*, 2011). The success of experimental therapies aimed at promoting an enhancement of axon regeneration is dependent on their ability to increase the activity of injured neurons (Al-Majed *et al.*, 2000; Udina *et al.*, 2011a; Ward *et al.*, 2016). Maintaining an increased level of activity in neurons whose axons are injured enhances axon regeneration and the degree of target reinnervation by these axons (Udina *et al.*, 2011b). However, despite achieving significant improvements in axon regeneration, only modest improvements in functional recovery have been found (Al-Majed *et al.*, 2000; English, 2005; Gordon *et al.*, 2008; Gordon & English, 2015; Gordon, 2016). Chemogenetic tools such as Gq-DREADDs offer a relatively non-invasive and repeatable alternative to sustaining increases in neuronal excitability.

Measurement of the direct muscle (M) and monosynaptic H-reflex responses evoked by electrical stimulation of the sciatic nerve is an established method for evaluating recovery of motor function after peripheral nerve injury (Moldovan & Krarup, 2004; Wood *et al.*, 2011; Boeltz *et al.*, 2013). The impact of Gq-DREADD activation on functional recovery was evaluated at two, four, and six weeks after peripheral nerve injury. We hypothesized that activation of Gq-DREADDs in sciatic neurons whose axons have been injured will increase their excitability, which will in turn promote the enhancement of functional recovery after peripheral nerve injury. Using intramuscular injection of an AAV9 viral vector encoding Gq-DREADDs under a neuron specific promoter, *hSyn*, we expressed Gq-DREADDs in rat sciatic neurons whose axons innervate the LG muscle. We found that treatments of these animals with CNO increased the excitability of transduced sciatic neurons, promoted enhanced axon regeneration, and led to improvements in functional recovery. A preliminary report of some of these findings has been made (Jaiswal & English, 2015).

MATERIALS & METHODS

Animals

Animals were housed in a 12-hour light/12-hour dark facility with access to water and standard rat chow *ad libitum*. The Institutional Animal Care and Use Committee of Emory University approved all procedures. Female Lewis rats (150–250g; n=16) were used in experiments to determine the effectiveness of Gq-DREADD activation on evoked responses *in vivo*, and to evaluate any Gq-DREADD-mediated improvements in sciatic nerve regeneration after injury. All rats were purchased from Harlan Laboratories, Houston, TX, USA (now ENVIGO, Cambridgeshire, UK) and were allowed to acclimate for one week before experiments.

Intramuscular viral injections

A complete Gq-DREADD (hsyn- hM3Dq-mCherry) construct was obtained as a generous gift from Dr. Bryan L Roth, University of North Carolina Chapel Hill, and inserted into a viral vector (AAV9) by the Viral Vector Core of Emory University. Rats were anesthetized with isoflurane anesthesia (2–3% in O₂). The right lateral gastrocnemius (LG) muscle was exposed and injected with 2µl of the AAV9 vector in sterile 1X PBS (7.6×10⁹ viral genomes/µl). Using a 2.5 µl Hamilton syringe (Hamilton, Reno, NV), four injection sites through the whole LG muscle were injected with 0.5 µl of the viral vector, each. Each injection was performed over 30 seconds and the needle was held in place for approximately 30 seconds after the injection to avoid retrograde leakage of viral vector. The contralateral muscle was not exposed or injected and served as a control. Animals (200–250g, adult n=6; 150–200g, young adult n=9) recovered for up-to six weeks (200–250g, adult n=1 animal recovered for twelve weeks before being used in experiments) after intramuscular injections to allow for sufficient time for retrograde transduction of targeted LG neurons.

Peripheral nerve injury

We used the sciatic nerve transection-repair (Tx-repair) as a model of peripheral nerve injury in our study. Because the recovery from this injury was studied over the course of six weeks, we wanted to make sure that the age of the rats when studied would not be a significant variable. Thus we used young adult rats (n=9) in these experiments. Under isoflurane anesthesia (2–2.5% in oxygen), the sciatic nerves were exposed bilaterally and secured on a small rectangle of SILASTIC film (Dow Corning 501–1) using 10–15µl of fibrin glue: a mixture of fibrinogen, fibronectin, and thrombin (Sigma-Aldrich, St. Louis, MO). This mixture was formulated immediately prior to use. The components of this mixture formed a fibrin-glue ‘clot’ that provided mechanical stabilization of the repaired nerve for approximately 72 hours, until normal tissue fibrosis provided support (MacGillivray, 2003; English, 2005; Sabatier *et al.*, 2011). Once secured to the film, the nerve was cut with sharp scissors near the center of the film. Securing the nerve to the film prevents the withdrawal of the two stumps of the nerve after transection. A second application of 10–15µl of fibrin glue was then applied to secure the ends of the cut nerve together. Injectable meloxicam (2mg/kg, sub cutaneous) was used as an analgesic to minimize pain and distress up to three days during post-operative recovery period. Rats were allowed to recover for at least one week before experimental procedures were performed.

CNO administration

Clozapine-N-oxide (CNO, Sigma Aldrich, St. Louis, MO) was prepared immediately prior to administration. 5mg of CNO was dissolved in 25µl of dimethyl sulfoxide (DMSO, Sigma Aldrich, St. Louis, MO). After ensuring complete dissolution of CNO this mixture was added to 0.9% sterile saline to achieve a final concentration of 1mg/ml CNO. Rats received 1mg/kg dose of CNO via intra-peritoneal injection (i.p.). In experiments to establish increase in neuronal excitability in intact rats (adult n=7), CNO was administered immediately after baseline recordings of electrically evoked responses were made. In experiments involving enhancement of functional recovery after peripheral nerve injury, CNO was administered for one (young adult n=4) or three (young adult n=5) days immediately after peripheral nerve injury.

Evoked responses

Bipolar stimulating electrical cuffs were assembled using a short length of Silastic tubing and stranded stainless steel microwire, AWG size 40 (Cooner Wire, Chatsworth, CA; part# AS631) (Sabatier *et al.*, 2011). These cuffs were placed around both sciatic nerves just proximal to the branching into the common fibular and tibial nerves (n=5 adult; n=9 young adult). In some rats (n=2 adult), stimulating needle electrodes (Neuroline monopolar, 28G, Ambu/AS, Copenhagen, Denmark), positioned 1–2mm apart under the exposed sciatic nerve, were used to evoke responses (Redondo-Castro & Navarro, 2013). Bipolar EMG electrodes (Basmajian & Stecko, 1963), for acute use, were made using fine wire (California Fine Wire Company, Grover Beach, CA; Stablohm 800 A, material number cfw-100189) and were placed into the gastrocnemius muscle using a 25G hypodermic needle. Care was taken to maintain consistency in the recording of these data by placing the electrodes in a common location in the muscle in each animal at each time studied. Electrically evoked EMG activity was recorded from these electrodes in response to sciatic nerve stimulation (0.3 ms pulses). Short latency direct muscle (M) and longer latency monosynaptic H-reflex responses were elicited from sciatic nerve electrical stimulation, under isoflurane anesthesia (1–2% in oxygen), before (Baseline) and at 30mins, 1, 2, 3, 4, and 5 hours after intra-peritoneal injection of 1mg/kg CNO (Post CNO). Long lasting (approximately 9 hours) activation of Gq-DREADD receptor by CNO has been previously reported (Alexander *et al.*, 2009). Stimulus intensity was gradually incremented until maximal M response amplitude was recorded. The inter-stimulation duration was set at three seconds to minimize any muscle fatigue. No further experiments were performed in animals (adult n=7) used in the acute study. The sciatic nerve was then Tx-repaired, bilaterally in animals used for functional recovery experiments (n=9 young adult), as described above. Therefore, these animals (young adult n=9) received CNO treatment approximately 5 hours prior to sciatic nerve Tx-repair. A subset of these animals (young adults n=5) continued to receive CNO treatment for a total of three days. In all these animals bilateral recordings were repeated at 2, 4 and 6 weeks after injury without any additional CNO administration.

Data analysis

The amplitude, latency, and duration of maximal M-responses and H-reflexes were measured from evoked responses, as described in detail in previous publications from our

laboratory (Boeltz *et al.*, 2013; Cannoy *et al.*, 2016). In intact animals, data before (Baseline) and maximal response after (Post CNO) CNO administration were analyzed. Comparisons were made between potentials evoked in the right, virus injected muscles and the left, uninjected controls, in each animal. Levene's test was used to confirm the homogeneity of variances in the groups compared, an assumption for the use of parametric statistics. The difference in response amplitudes from intact animals was compared statistically using ANOVA. Statistical significance of data from animals in the functional recovery study was evaluated using paired t-test or Student's t-tests. Results are expressed as Mean $\pm$ SEM and p-value less than 0.05 were considered statistically significant.

RESULTS

Gq-DREADD activation increased excitability of sciatic neurons in intact rats

As stimulus voltage applied to the sciatic nerves was increased gradually, the amplitude of the M-responses recorded from the right and left LG muscles also increased and eventually plateaued at the maximal M-response (M-max). A second potential was evoked at a slightly longer latency, the monosynaptic H-reflex (Figure 1a). The peak amplitude of the H-reflex (H-max) routinely occurred at lower stimulus intensities than those needed to evoke M-max. The peak amplitudes of the evoked M-responses did not change significantly after CNO treatment in either virus injected muscles or the contralateral control muscles (Figure 1a). The difference in the Post CNO H-reflex response from Baseline was calculated. This difference in H reflex amplitude was significantly greater ($p=0.02$, ANOVA) in virus-injected muscles compared to contralateral control muscles of intact rats after a single injection of CNO (Figure 1b). Such changes were found for up to four hours following a single administration of CNO. Thus, CNO treatments resulted in a significant and long-lasting increase in the amplitude of the monosynaptic H reflex in intact rats whose motoneurons were transduced by Gq-DREADDs.

When compared to values measured at Baseline, no significant differences were found in either the latency or duration of these electrically evoked EMG responses following CNO treatment (paired t-tests), either from virus-injected muscles or from the contralateral control muscles of intact rats (Table 1).

Activation of Gq-DREADD promotes the enhancement of functional recovery after a sciatic nerve injury

Electrically evoked responses were analyzed at two, four and six weeks after sciatic nerve Tx-repair (Figure 2 and Table 2). No significant differences in the amplitude of electrically evoked responses were found between animals treated with CNO for one (young adult $n=4$) or three (young adult $n=5$) days after sciatic nerve Tx-repair. Data from CNO treated animals were therefore pooled, as presented in Figure 2 and Table 2. By two weeks after sciatic nerve Tx-repair, an evoked direct muscle (M) response was observed in ipsilateral LG muscles that had been injected with viral vector encoding Gq-DREADDs in three of the nine animals studied. No similar response was observed in the contralateral uninjected control muscles of any of these rats. A temporally distinct H reflex was visible in the virus-injected muscle of one of these rats. By four weeks after sciatic nerve Tx-repair, M responses and H

reflexes were found following sciatic nerve stimulation in muscles on both sides (Figure 2a). The maximum amplitudes of both responses to sciatic nerve stimulation were significantly greater in virus-injected muscles than in uninjected controls (Figure 2b, M-max, $p=0.01$; Figure 2c, H-max, $p=0.04$, Students t-tests). By six weeks after sciatic nerve Tx-repair, distinct M responses and H reflexes were found in all muscles. No significant differences in the amplitudes of these responses was found between virus-injected muscles and uninjected controls (Fig.2).

Significantly (paired t-tests) longer M response latencies were observed on both sides of animals at two (ipsilateral $p=0.02$), four (ipsilateral $p=0.00004$; contralateral $p=0.001$) and six (ipsilateral $p=0.002$; contralateral $p=0.001$) weeks after sciatic nerve Tx-repair, compared to their respective intact values (Table 2). Likewise, H-reflex latency was significantly increased (paired t-tests) at four (ipsilateral $p=0.0002$; contralateral $p=0.0002$) and six (ipsilateral $p=0.02$; contralateral $p=0.04$) weeks post Tx-repair, relative to intact animals (Table 2). Significantly (paired t-tests) longer M response durations were observed on both sides of animals at two (ipsilateral $p=0.03$) and four (ipsilateral $p=0.005$; contralateral $p=0.004$) weeks after sciatic nerve Tx-repair, compared to their respective intact values (Table 2). H-reflex duration was significantly (paired t-tests) increased at four (ipsilateral $p=0.04$) and six (contralateral $p=0.04$) weeks post Tx-repair, relative to intact animals (Table 2). Treatment with CNO transiently decreased (paired t-tests) M-max duration (Table 2, $p=0.04$) and H-reflex latency (Table 2, $p=0.05$) in the ipsilateral (Gq-DREADD expressing virus-injected) LG muscle, compared to contralateral (uninjected) LG muscle, at four weeks post Tx-repair. Three out of nine animals had an M response and one out of nine animals had a H-reflex (italicized values) response on the ipsilateral Gq-DREADD expressing virus-injected side 2 weeks post Tx-repair.

DISCUSSION

Increasing the activity of neurons whose axons have been injured is *required* (Al-Majed *et al.*, 2000; Gordon & English, 2015; Jaiswal *et al.*, 2016) for the success of experimental therapies that promote an enhancement of axon regeneration (English *et al.*, 2007; Gordon *et al.*, 2008; Ward *et al.*, 2016) and functional recovery (Sabatier *et al.*, 2008; English *et al.*, 2011; Ward *et al.*, 2016) after peripheral nerve injury. However, enhancement of motor axon regeneration using moderate treadmill exercise is extensive (Gordon & English, 2015), and includes axons of motoneurons that are unlikely to have been recruited into activity at all during the exercise period. This apparent paradox stimulated us to hypothesize that even sub-threshold excitation of the injured motoneurons, as might be inferred from the results of studies of fictive locomotion (Perret & Cabelguen, 1980; McCrea & Rybak, 2008; Meehan *et al.*, 2012), might be sufficient to promote enhanced regeneration. Activation of Gq-DREADDs has been shown to increase excitability of various populations of neurons *in-vivo* (Alexander *et al.*, 2009; Whissell *et al.*, 2016). We tested our hypothesis using Gq-DREADDs to increase the excitability of sciatic neurons whose axons had been injured.

The results presented here are consistent with our hypothesis. We showed that CNO-mediated activation of Gq-DREADDs in intact rats significantly increased the efficacy of virus transduced sciatic motoneurons to be recruited into the H-reflex (Figure 1b, c), without

increasing the direct muscle (M) response to peripheral stimulation. We interpret this finding to mean that Gq-DREADD activation increased the excitability of LG motoneurons. Similar to the effects of exercise (Boeltz *et al.*, 2013), recovery of electrically evoked M-responses was first observed at an earlier survival time in CNO treated animals. Activation of Gq-DREADDs after sciatic nerve Tx-repair resulted in an early appearance of an evoked M response, at two weeks after Tx-repair and transiently shortened the duration of M-max and latency of the H-reflex responses, at four weeks after Tx-repair, only in Gq-DREADD expressing virus-injected LG muscles, not in uninjected control muscles. These results are consistent with an interpretation that Gq-DREADD activation after sciatic nerve Tx-repair promotes the enhancement of both motor axon regeneration and functional recovery via a sub-threshold increase in the excitability of motoneurons whose axons are regenerating.

The dose of CNO determines the intensity of responses observed with Gq-DREADD activation and the resulting effects last several hours (Alexander *et al.*, 2009). The recommended doses for *in-vivo* applications range from 0.1–3mg/kg (Roth, 2016). In the present study, we utilized a 1mg/kg dose of CNO and found significant improvements in functional recovery after sciatic nerve Tx-repair. However, unlike exercise (Boeltz *et al.*, 2013), no further enhancements in recovery of functional responses was observed at six weeks after Tx-repair. Also, no significant differences in recovery were found between a single treatment with CNO and three daily treatments. Further studies using varying doses and dosing of CNO are needed to establish the optimal treatment and therapeutic window required to promote axon regeneration and functional recovery. Alterations, if any, in intrinsic function and signaling of neurons expressing Gq-DREADDs also needs to be investigated in detail (Saloman *et al.*, 2016).

The use of DREADD technology provides a novel, non-invasive (Roth, 2016) and repeatable (Alexander *et al.*, 2009) way to activate specific populations of neurons. The availability of Cre-dependent DREADD mouse models (Zhu *et al.*, 2016) further expands the utility of this technology for *in-vivo* investigations involving cell-type specific activation and relative contributions of sensory and motor neurons towards axon regeneration and recovery of function after a peripheral nerve injury. Improvements in viral vector delivery and subsequent application of DREADDs may hold a significant therapeutic potential (Saloman *et al.*, 2016; Whissell *et al.*, 2016) for individuals with peripheral nerve injury. Although many more studies need to be conducted before the clinical use of viral vectors (Castle *et al.*, 2014) and designer receptors, the use of gene therapy to treat individuals is not beyond the realm of possibility (Smith *et al.*, 2017).

CONCLUSION

We employed a retrograde viral-vector mediated delivery and expression of Gq-DREADDs in adult rats. Activation of Gq-DREADDs using the designer drug, clozapine-N-oxide (CNO), in intact rats increased the excitability of transduced motoneurons. Increased amplitudes of monosynaptic H-reflexes were recorded in ipsilateral virus-injected muscles after CNO treatment. Treatment with CNO for up to three days after a peripheral nerve injury resulted in an early recovery of M-response and H-reflexes in LG muscles innervated by Gq-DREADD-expressing motoneurons. We conclude that Gq-DREADD activation in

motoneurons promotes regeneration of their axons and the enhancement of functional recovery after a peripheral nerve injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AAV9	Adeno associated virus serotype 2/9
CNO	Clozapine-N-oxide
DREADDs	Designer receptors exclusively activated by designer drugs
LG	Lateral gastrocnemius muscle

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Figure 1.

(a) Representative traces of M-responses and H-reflexes at Baseline and after CNO administration. No significant differences were found Post CNO in ipsilateral (Gq-DREADD expressing virus-injected) and contralateral (uninjected) LG muscle M-max responses (data not shown). (b) Differences in the amplitude of maximal H reflex responses, relative to Baseline, following CNO injection. Data from seven individual animals (small circles) and group averages (large circle) are shown. * $p=0.02$.

Figure 2.

Recovery of evoked muscle responses. (a) EMG activity evoked from LG muscles in response to supramaximal sciatic nerve stimulation are shown at different times after sciatic nerve Tx-repair in a single rat. Recordings made from the LG muscle previously injected with Gq-DREADD-expressing viral vector are shown on the left, those from the contralateral, uninjected (Control) muscle are shown on the right. Note the much smaller amplitude response found at 4 weeks and the temporally more fragmented responses at 6 weeks in the control muscle. SA=stimulus artifact. M response (b) and H reflex (c) amplitudes following bilateral sciatic nerve Tx-repair. Each symbol represents data from a different rat. Filled symbols are from recordings made in ipsilateral (Gq-DREADD expressing virus-injected). Open symbols represent data from the contralateral, uninjected (Control) muscles. Lines are drawn through group means at the three time-points studied: solid lines = Gq-DREADD, dashed lines = Control, muscles. ** $p=0.01$, * $p=0.04$.

Characteristics of electrically evoked responses in ipsilateral (Gq-DREADD expressing virus-injected) and contralateral (uninjected) LG muscles (n=7 rats). No significant differences in either latency or duration of M-max and H-max were found after CNO administration when compared to Baseline.

Table 1

		Latency (ms)		Duration (ms)	
M-max	Response type	Baseline	Post CNO	Baseline	Post CNO
	Ipsilateral	1.4±0.04	1.4±0.06	3.1±0.14	3.0±0.27
H-max	Contralateral	1.6±0.24	1.5±0.2	3.1±0.3	3.0±0.24
	Ipsilateral	5.7±0.9	4.4±0.25	3.0±0.4	3.3±0.28
	Contralateral	5.2±0.45	5.0±0.5	2.8±0.28	3.0±0.12

Characteristics of electrically evoked responses in ipsilateral (Gq-DREADD expressing virus-injected) and contralateral (uninjected) LG muscles. 3 out of 9 animals had a M-max response and 1 out of 9 animals had a H-max (italicized values) response on the ipsilateral side 2 weeks post Tx-repair.

Table 2

LATENCY (ms)					
Response type	Intact	2wk	4wk	6wk	
	Ipsilateral	1.40±0.04	19.49±2.69***	4.55±0.54***	3.20±0.30***
M-max	Contralateral	1.42±0.06	–	6.19±0.64***	3.53±0.18***
H-max	Ipsilateral	5.17±0.64	24.73	12.59±1.38***	9.32±1.52**
	Contralateral	5.34±0.43	–	17.97±1.69***†	9.49±1.05***
DURATION (ms)					
Response type	Intact	2wk	4wk	6wk	
	Ipsilateral	3.06±0.27	5.13±1.16*	5.61±0.56**	4.43±0.71
M-max	Contralateral	3.50±0.29	–	7.24±1.09***†	4.74±0.74
H-max	Ipsilateral	2.98±0.21	1.77	3.70±0.15*	3.74±0.46
	Contralateral	2.85±0.33	–	4.59±0.84	4.65±0.80*

Significant increases in latency of M-max was observed 2 (ipsilateral *p=0.02), 4 (ipsilateral ***p=0.00004; contralateral ***p=0.001) and 6 (ipsilateral **p=0.002; contralateral ***p=0.001) weeks, and duration of M-max was observed 2 (ipsilateral *p=0.03) and 4 (ipsilateral **p=0.005; contralateral ***p=0.004) weeks after sciatic nerve Tx-repair compared to their respective intact values. H-reflex latency was significantly increased at 4 (ipsilateral ***p=0.0002; contralateral ***p=0.0002) and 6 (ipsilateral *p=0.02; contralateral *p=0.04) weeks and duration was significantly increased at 4 (ipsilateral *p=0.04) and 6 (contralateral *p=0.04) weeks post Tx-repair, relative to intact animals. CNO treatment transiently decreased M-max duration (Tp=0.04) and H-reflex latency (Tp=0.05) in the ipsilateral (Gq-DREADD expressing virus-injected) LG muscle, compared to contralateral (uninjected) muscle, at 4 weeks post Tx-repair.