



# Tonically Active $\alpha_5$ GABA<sub>A</sub> Receptors Reduce Motoneuron Excitability and Decrease the Monosynaptic Reflex

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Received: 12 July 2017

Accepted: 31 August 2017

Published: 19 September 2017

### Citation:

Canto-Bustos M, Loeza-Alcocer E, Cuellar CA, Osuna P, Elías-Viñas D, Granados-Soto V, Manjarrez E, Felix R and Delgado-Lezama R (2017) Tonically Active  $\alpha_5$ GABA<sub>A</sub> Receptors Reduce Motoneuron Excitability and Decrease the Monosynaptic Reflex. *Front. Cell. Neurosci.* 11:283. doi: 10.3389/fncel.2017.00283

Motoneurons, the final common path of the Central Nervous System (CNS), are under a complex control of its excitability in order to precisely translate the interneuronal pattern of activity into skeletal muscle contraction and relaxation. To fulfill this relevant function, motoneurons are provided with a vast repertoire of receptors and channels, including the extrasynaptic GABA<sub>A</sub> receptors which have been poorly investigated. Here, we confirmed that extrasynaptic  $\alpha_5$  subunit-containing GABA<sub>A</sub> receptors localize with choline acetyltransferase (ChAT) positive cells, suggesting that these receptors are expressed in turtle motoneurons as previously reported in rodents. In these cells,  $\alpha_5$ GABA<sub>A</sub> receptors are activated by ambient GABA, producing a tonic shunt that reduces motoneurons' membrane resistance and affects their action potential firing properties. In addition,  $\alpha_5$ GABA<sub>A</sub> receptors shunted the synaptic excitatory inputs depressing the monosynaptic reflex (MSR) induced by activation of primary afferents. Therefore, our results suggest that  $\alpha_5$ GABA<sub>A</sub> receptors may play a relevant physiological role in motor control.

**Keywords:** motoneuron, GABA<sub>A</sub> receptors, tonic inhibition, monosynaptic reflex

## INTRODUCTION

Extrasynaptic GABA<sub>A</sub> receptors play important roles in supra-spinal nuclei of the Central Nervous System (CNS) regulating neuron excitability and network activity by tonically inhibiting and shunting mature neuronal membranes, which set the threshold for action potential generation and temporal window for synaptic integration (Farrant and Nusser, 2005; Włodarczyk et al., 2013). These receptors are conformed mainly by  $\alpha_4$ ,  $\alpha_5$  and  $\alpha_6$  subunits, although sometimes they may contain  $\alpha_2$  and  $\alpha_3$  subunits (Farrant and Nusser, 2005). Though all these subunits are also expressed in the spinal cord their function is poorly understood (Persohn et al., 1991; Wisden et al., 1991; Ma et al., 1993; Ruano et al., 2000; Mody and Pearce, 2004; Petri et al., 2005; Delgado-Lezama et al., 2013; Andres et al., 2014; Loeza-Alcocer et al., 2014; Bravo-Hernández et al., 2016).

In a previous work, we investigated the role of high-affinity GABA<sub>A</sub> receptors in motor behavior. We found that motoneurons presented a tonic current mediated by high affinity

GABA<sub>A</sub> receptors activated by ambient GABA (Castro et al., 2011a). Moreover, we demonstrated that blockade of furosemide-sensitive high affinity GABA<sub>A</sub> receptors facilitated the monosynaptic reflex (MSR; Bautista et al., 2010). We have also provided evidence that motoneurons express a tonic current mediated by  $\alpha_6$  subunit-containing GABA<sub>A</sub> receptors (Andres et al., 2014). Likewise, several immunohistochemical and *in situ* hybridization studies have shown that  $\alpha_5$  subunit-containing GABA<sub>A</sub> ( $\alpha_5$ GABA<sub>A</sub>) receptors are expressed in motoneurons although its role is presently unknown (Persohn et al., 1991; Wisden et al., 1991; Ma et al., 1993; Ruano et al., 2000; Mody and Pearce, 2004; Petri et al., 2005; Loeza-Alcocer et al., 2014). Thus, we hypothesized that  $\alpha_5$ GABA<sub>A</sub> receptors modulating the excitability of motoneurons play a very important role in motor control. In the present report, we investigate whether  $\alpha_5$ GABA<sub>A</sub> receptors are expressed in motoneurons and mediate a GABAergic tonic current that control its excitability and modulate the MSR, using the turtle spinal cord as a model system. We found that  $\alpha_5$ GABA<sub>A</sub> receptors are tonically active by ambient GABA and mediate a tonic current that reduce motoneurons' excitability, decrease the input resistance and increase the rheobase. In addition, here we show that  $\alpha_5$ GABA<sub>A</sub> receptors depress the MSR.

## MATERIALS AND METHODS

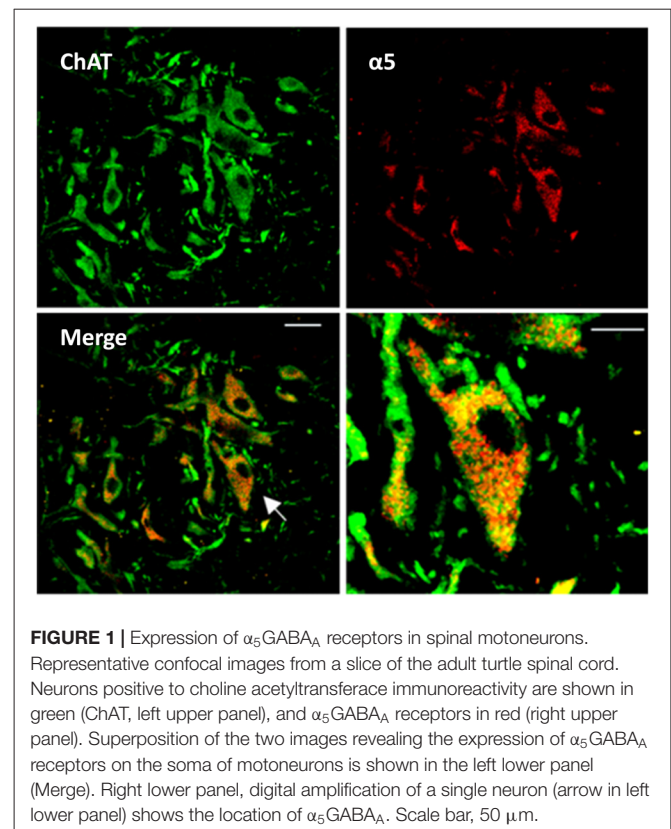
### Preparation

Forty adult turtles (*Trachemys scripta* spp, 15–20 cm carapace length) were anesthetized with pentobarbital (100 mg/kg, i.p.). The plastron was opened and the blood removed by intraventricular perfusion with Ringer solution (~10°C) of the following composition (in mM): 120 NaCl, 5 KCl, 15 NaHCO<sub>3</sub>, 3 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub> and 20 glucose saturated with 2% CO<sub>2</sub> and 98% O<sub>2</sub> to attain a pH value of 7.6. The lumbar spinal enlargement was isolated by a laminectomy and cut transversally to obtain slices of 2–3 mm and 300  $\mu$ m thick. For intracellular and patch-clamp recordings, the slices were placed in a chamber and superfused with Ringer solution (20–22°C). For the extracellular recording two segments of the lumbar spinal cord in continuity with the dorsal and ventral roots were dissected out. At the end of the dissection, the animals were rapidly euthanized by decapitation.

The animals were provided by the National Mexican Turtle Center located in Mazunte, Oaxaca (Mexico) with the authorization DGVS-03821/0907 by the Federal Government Ministry of Environment and Natural Resources (Semarnat). In addition, this study was approved by the Institutional Animal Care and Use Committee (Cinvestav, Mexico City, Mexico; Protocol 0098-16) and followed the guidelines for ethical matters (Drummond, 2009).

### Electrophysiology

Motoneurons were recorded intracellularly in slices of 2–3 mm thick with a sharp electrode (20–40 M $\Omega$ ) filled with potassium acetate (0.8 M) and KCl (0.2 M). Cells were classified as motoneurons if their input resistance was lower than 80 M $\Omega$ , presented an action potential waveform with a fast and slow



posthyperpolarization and show adaptation during repetitive action potential firing (Hounsgaard et al., 1988; Delgado-Lezama et al., 2004). In order to evaluate the excitability of motoneurons, intracellular supra-threshold current pulses were applied in control Ringer and in presence of L-655,708, a selective  $\alpha_5$ GABA<sub>A</sub> receptor inverse agonist (pKi 9.3; Quirk et al., 1996).

In other series of experiments, the presence of the GABAergic tonic current was determined in motoneurons by using the visualized patch clamp technique in its whole-cell configuration. The electrodes were made from thick-walled borosilicate glass capillaries using a Sutter programmable horizontal micropipette puller (Sutter Instrument). The patch pipettes with resistance of 5–10 M $\Omega$  were filled with the following solution (in mM): 122 CsCl; 5 Na<sub>2</sub>-ATP; 2.5 MgCl<sub>2</sub>; 0.0003 CaCl<sub>2</sub>; 5.6 Mg-gluconate; 5 K-HEPES; 5 HEPES. Visualizing the ventral horn with the Olympus microscope (BX51W1) motoneurons were identified by their location and size (Figure 1) and then electrophysiological recordings were performed by using the MultiClamp-700B amplifier (Molecular Devices). The maximal acceptable series resistance compensation was 20%. As described earlier, these neurons presented action potentials with fast and slow posthyperpolarization and adaptation in their firing pattern. Recorded signals were digitized at 20 KHz, filtered using a 8-pole Bessel (3 KHz) and stored in the hard disk of a computer for off-line analysis.

In additional experiments, the MSR was recorded from two spinal cord segments. The dorsal and ventral roots were

suctioned by glass pipettes connected to a source of constant current and to a differential AC amplifier (Grass Instruments), respectively. The dorsal root (DR9) were stimulated with a rectangular current pulse (0.5 ms). The threshold was defined as the minimum stimulus intensity that elicits a measurable ventral root potential. Unless otherwise stated, the recordings shown were the average of 10 stimuli applied every 30 s. The MSR was recorded with a bandwidth of 0.1 Hz to 10 KHz, digitized at 10 KHz and stored for off-line analysis.

## Spinal Cord Immunostaining

Spinal cord sections of 30  $\mu$ m were obtained as described previously (Castro et al., 2011b) and first incubated with an anti-choline acetyltransferase (anti-ChAT) primary antibody (24 h at 4°C, 1:50, Millipore) and then revealed using a FITC donkey anti-goat secondary antibody (2 h at room temperature, 1:200, Jackson ImmunoResearch). Subsequently, sections were incubated with an anti- $\alpha_5$  subunit GABA<sub>A</sub> receptor primary antibody (2 h at 4°C, Sigma; 1:100 dilution), and then exposed 1 h to the secondary antibody (1:200; Dylight 549-conjugated anti-rabbit IgG; Jackson ImmunoResearch). Samples were examined using confocal laser scanning microscopy (Leica TCS SP2, Leyca Microsystems). Images were obtained using the 40 $\times$  oil immersion plan apochromatic objective (NA 0.8) and a subsequent digital amplification using the ImageG software.

## Drugs

GABA<sub>A</sub> receptors were activated with GABA (10–60  $\mu$ M) and blocked with picrotoxin (100  $\mu$ M) and L-655,708 (20  $\mu$ M) applied to the bath solution. Ionotropic glutamatergic and glycinergic receptors were blocked with 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX; 20  $\mu$ M) and (2R)-amino-5-phosphonovaleric acid (APV; 40  $\mu$ M) and strychnine (2  $\mu$ M), respectively. All drugs used in this study were purchased from Sigma-Aldrich.

## Analysis

The effect of GABA<sub>A</sub> receptor activation or blockade in motoneurons recorded intracellularly was quantified by measuring the input resistance and excitability in the absence and presence of L-655,708. The input resistance was determined as the slope of the fitted line to the *I-V* plot. The excitability was evaluated by plotting the current intensity vs. the number of action potentials produced by supra-threshold intracellular current pulses. A change in excitability was indicated by a horizontal shift of the resulting curve. To determine the effect of L-655,708 on the MSR and the dorsolateral funiculus (DLF) induced EPSP we measured the EPSP amplitude and the area under the curve of the MSR in control Ringer and in presence of the drug. The average amplitude of 30 EPSPs and the MSR area were calculated for each condition. Differences between means were determined by the unpaired Student's *t*-test. Means were considered statistically different when  $p < 0.05$ .

The mean holding current recorded in voltage clamp experiments was calculated by generating all-point histograms

of the current values recorded for 5 s in control Ringer and in the presence of L-655,708. A Gaussian distribution was fitted to the histograms. Changes in the holding current were determined as the difference between the means of the Gaussians fitted to the histograms. Differences between Gaussian means were determined by the Student's *t*-test. Means were considered statistically different when  $p < 0.05$ . Values are presented as the mean  $\pm$  SEM.

Intracellular recordings were performed in 2–3 mm thick slices while the patch clamp recordings were made in slices 300  $\mu$ m thick. The results are not comparable because the dendritic tree of motoneurons is severed in thinner slices. In addition, they are recorded at a maximum depth of 40  $\mu$ m, therefore it is not easy to evoke dendritic EPSPs by DR9 stimulation. By the contrary, intracellular recordings of motoneurons were made in normal Ringer at a depth of 200–300  $\mu$ m in which the dendritic tree is almost intact. Likewise, the extracellular GABA concentration is enough to activate the high affinity GABA<sub>A</sub> receptors as was evidenced by the decrease in the rebase and increase in input resistance and excitability, reflected by a leftward shift in the excitability curve when L-655,708 was added. Therefore both set of data were analyzed independently.

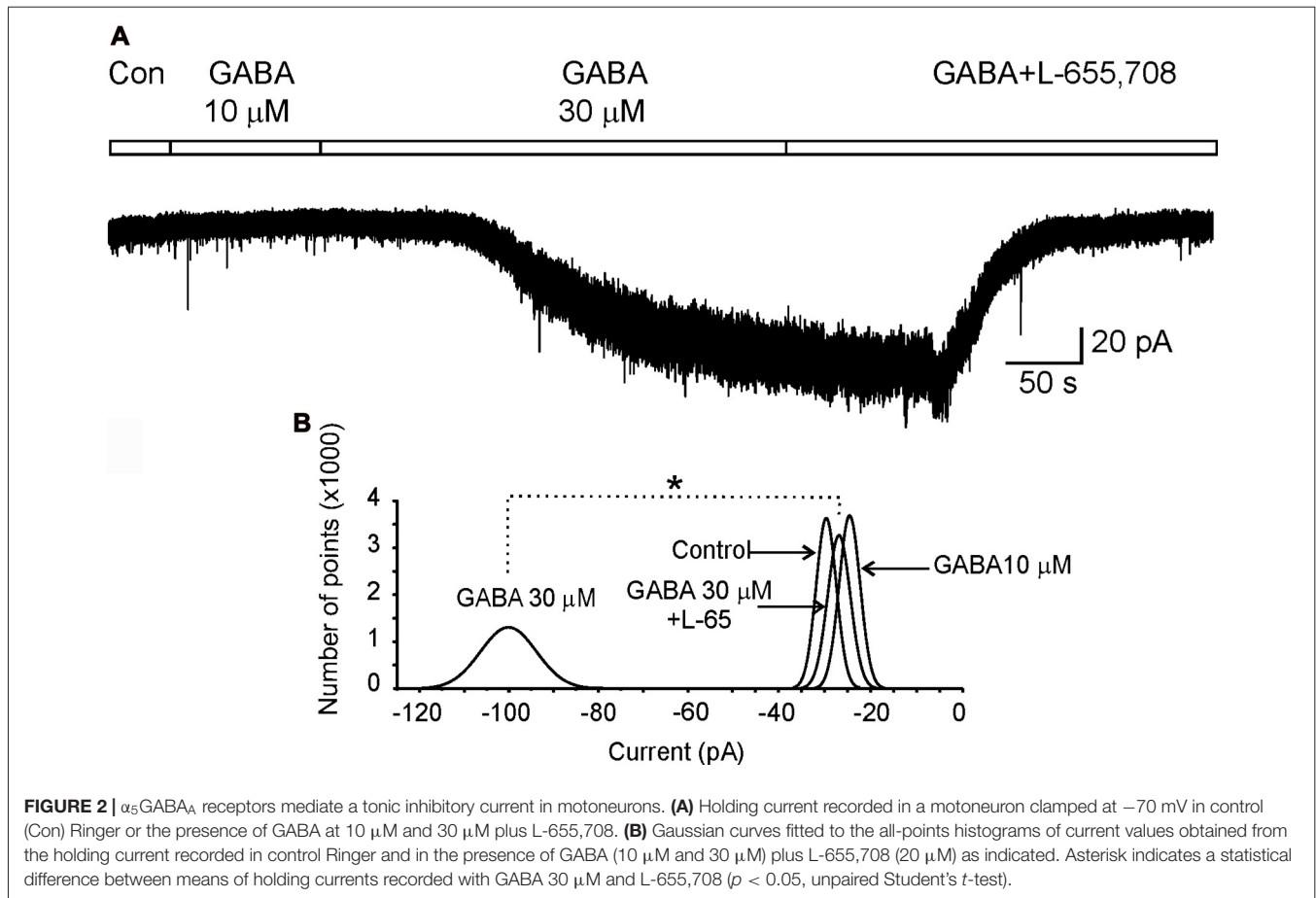
## RESULTS

### $\alpha_5$ GABA<sub>A</sub> Receptor Expression in Turtle Spinal Motoneurons

Our first approach to evaluate the activity of  $\alpha_5$ GABA<sub>A</sub> receptors in motoneurons was to determine their cellular expression. It is worth mentioning that the presence of  $\alpha_5$ GABA<sub>A</sub> receptors in mammalian motoneurons has been suggested previously (Persohn et al., 1991; Wisden et al., 1991; Ruano et al., 2000). In order to determine whether the  $\alpha_5$  subunit is also expressed specifically in turtle motoneurons as previously reported in rodents, immunohistochemical staining was performed on transverse slices of the turtle lumbar spinal cord. The results of this analysis showed that the  $\alpha_5$ GABA<sub>A</sub> immunostaining is prominent in cells expressing ChAT (a marker for motoneurons), where signal was dispersedly distributed in the soma, sparing the nucleus (**Figure 1**). Therefore, our results suggest that extrasynaptic  $\alpha_5$  subunit-containing GABA<sub>A</sub> receptors are also expressed in choline acetyltransferase (ChAT) turtle positive cells as in rodents. No labeling was seen in absence of the primary antibody or in presence of its corresponding antigenic peptide (data not shown).

### $\alpha_5$ GABA<sub>A</sub> Receptors Mediate a Tonic Inhibitory Current in Motoneurons

Next, we decided to perform electrophysiological recordings to characterize the possible role of the  $\alpha_5$ GABA<sub>A</sub> receptors in spinal cord motoneurons. To facilitate the characterization of the  $\alpha_5$ GABA<sub>A</sub> receptors, the tonic current was enhanced by application of GABA (30  $\mu$ M) to the perfusion solution. This was based on the demonstration that the receptors display similar



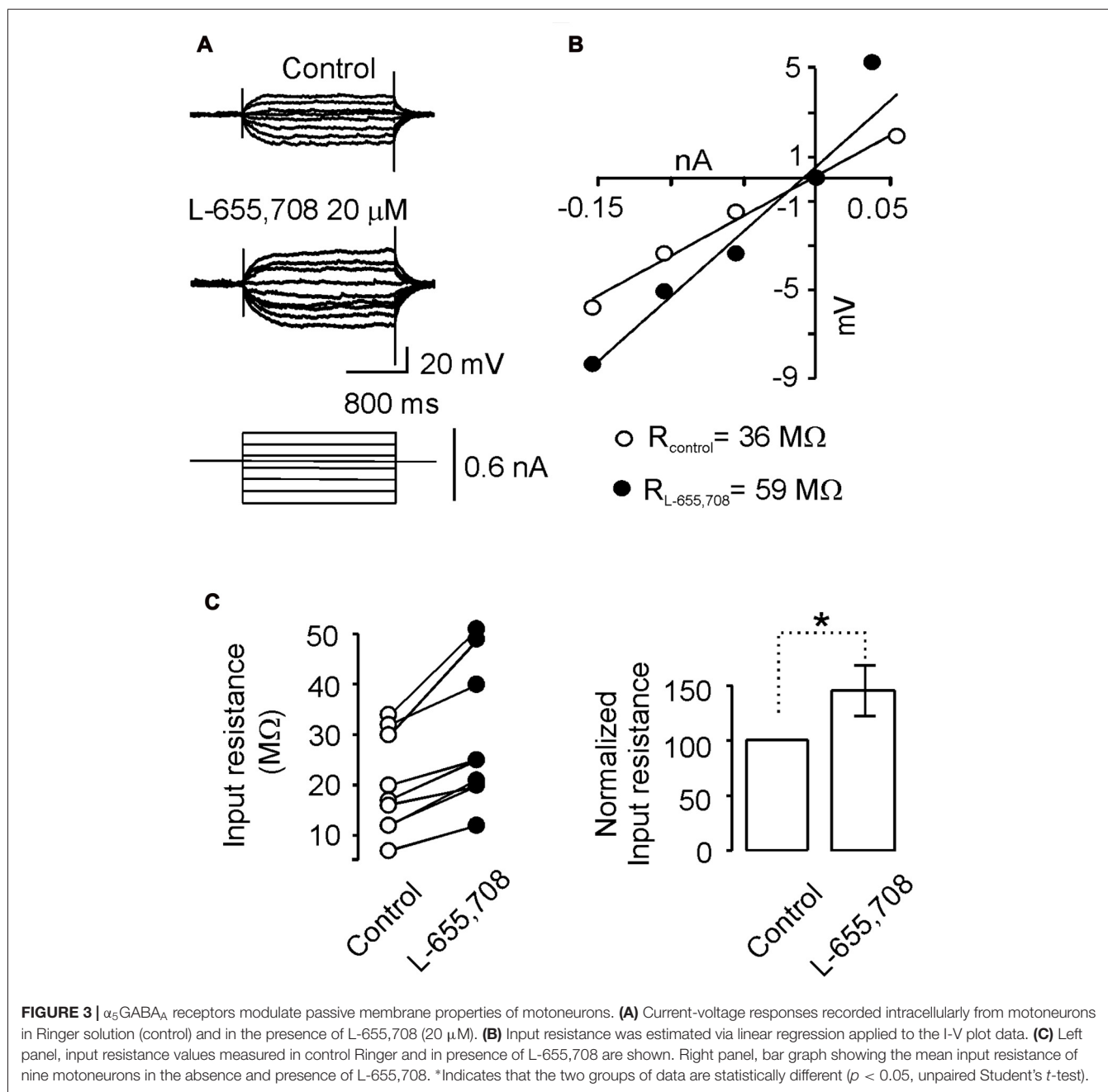
sensitivity and kinetics when exposed to either endogenous or exogenous GABA application (Bai et al., 2001). As can be seen in **Figure 2**, whole-cell patch clamp recordings with a high  $\text{Cl}^-$  internal solution showed a GABA-activated inward current that did not display inactivation.

Previous studies have shown that  $\alpha_5$ GABA<sub>A</sub> receptors mediate a tonic current in different nuclei of the CNS (Farrant and Nusser, 2005; Bonin et al., 2007). To confirm that these receptors also mediate a tonic current in motoneurons, cells were recorded in presence of GABA ( $30$   $\mu$ M) in combination with a cocktail containing (in  $\mu$ M): 2 strychnine, 20 CNQX and 20 APV, to block the activity of glycine, AMPA-kainate and NMDA receptors, respectively. In these conditions, we showed that the holding current of a motoneuron, maintained at a holding potential ( $V_h$ ) of  $-70$  mV and recorded for 50 s in control conditions, was not changed in the presence of GABA ( $10$   $\mu$ M; **Figure 2**). Nevertheless, when GABA concentration was raised to  $30$   $\mu$ M, after about 100 s an inward current of  $75 \pm 0.06$  pA was activated, which was associated to an increase in noise possibly due to stochastic activation of GABA<sub>A</sub> receptors (Brickley et al., 2001). Interestingly, after adding the  $\alpha_5$ GABA<sub>A</sub> inverse agonist L-655,708 the holding current returned to the control level in amplitude and noise as can be seen in the normal fitted current histograms (**Figure 2**). A similar result was observed in a total of four motoneurons. We found that

L-655,708 did not block all the current activated by GABA in two neurons; and the remaining current was blocked by picrotoxin ( $100$   $\mu$ M). These results suggest that the tonic current evoked in motoneurons might be mediated by more than one type of  $\alpha$  subunit. As we previously showed, this subunit could be  $\alpha_6$  (Andres et al., 2014).

### $\alpha_5$ GABA<sub>A</sub> Receptors Modulate Motoneuron Excitability

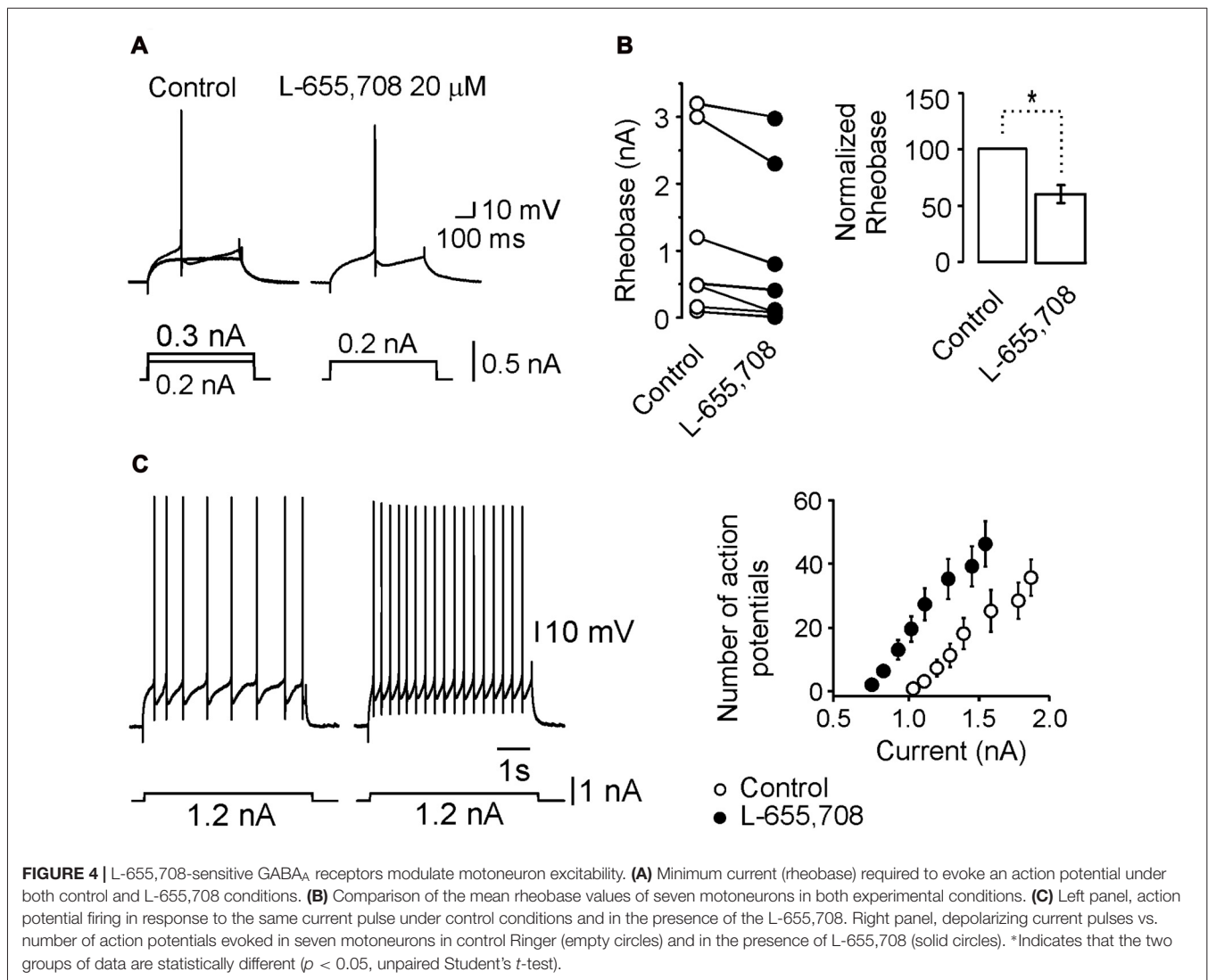
It has been shown that tonic GABAergic current modulates neuronal excitability by shunting the membrane and decreasing the membrane time constant (Mitchell and Silver, 2003; Włodarczyk et al., 2013). Therefore, we next sought to determine whether the  $\alpha_5$ GABA<sub>A</sub> receptors were performing a similar role, by evaluating the action of L-655,708 ( $20$   $\mu$ M) on the passive and active properties of these neurons recorded intracellularly. The cells selected for this series of experiments presented an input resistance of  $21 \pm 3.6$  M $\Omega$ , an action potential waveform with the typical fast and slow post-hyperpolarization, and also showed adaptation of the firing pattern produced by a long intracellular depolarizing current pulse, as occurs in motoneurons according to previous reports (Hounsgaard et al., 1988; Delgado-Lezama et al., 2004).



A typical response of one motoneuron is presented in **Figure 3A**, which shows that the voltage response to the same current pulses was more intense in the presence of L-655,708 to that recorded in control Ringer. This action of L-655,708 was evaluated in the I-V plot. Regression analysis of the individual slopes of the data showed that the input resistance increased from 36 M $\Omega$  to 59 M $\Omega$  after blockade of  $\alpha_5$ GABA<sub>A</sub> receptors (**Figure 3B**). Similar responses were recorded in a total of nine motoneurons. On average, the input resistance increased in  $32 \pm 7\%$  in the presence of L-655,708 compared to the control (**Figure 3C**;  $n = 9$ ;  $p < 0.05$ , unpaired Student's  $t$ -test). These results suggest that  $\alpha_5$ GABA<sub>A</sub> receptors tonically

activated by ambient GABA are shunting the membrane of motoneurons.

We next wanted to know whether the change in input resistance generated by the  $\alpha_5$ GABA<sub>A</sub> receptor activity was sufficient to modify the action potentials firing. As can be seen in **Figures 4A,B**, the minimum current injected to generate an action potential (rheobase) decreased in  $22 \pm 5.3\%$  with respect to control ( $n = 7$ ;  $p < 0.05$ , unpaired Student's  $t$ -test) in presence of L-655,708. As a consequence, in presence of the drug, the number of action potentials activated in response to depolarizing current of increasing intensities was augmented (**Figure 4C**). The action of L-655,708 on the



firing properties of motoneurons may be better visualized in the right panel of **Figure 4C** where blockade of  $\alpha_5$ GABA<sub>A</sub> receptors caused a leftward shift in the excitability curve. The number of action potentials was statistically different for every current in both conditions ( $n = 7$ ;  $p < 0.05$ , unpaired Student's  $t$ -test). These results suggest that  $\alpha_5$ GABA<sub>A</sub> receptors are tonically modulating the firing properties of spinal motoneurons.

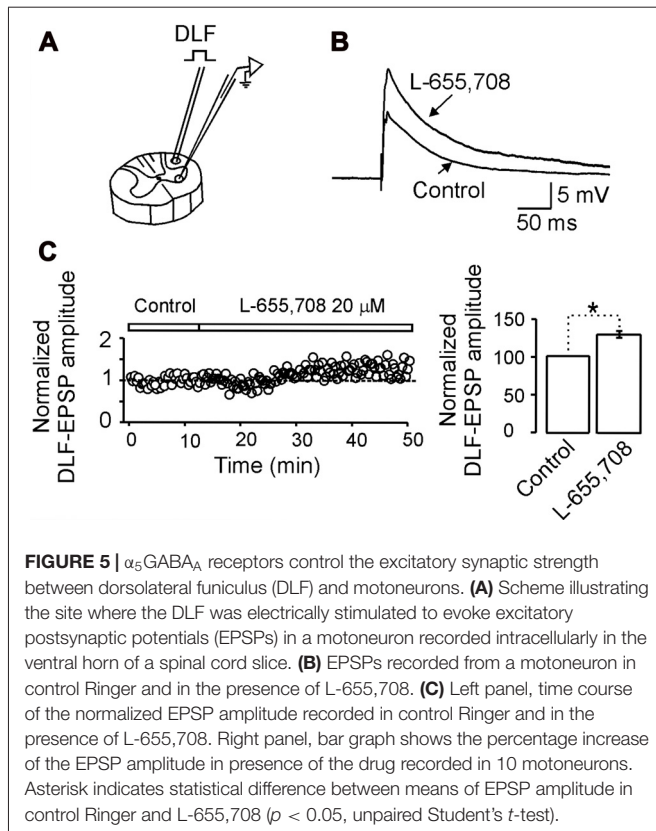
### Postsynaptic $\alpha_5$ GABA<sub>A</sub> Receptors Modulate Synaptic Transmission

In a previous study, we showed that the functional role of the tonic shunting produced by high affinity GABA<sub>A</sub> receptors might be associated to a depression of excitatory synaptic potentials at the dendrites (Delgado-Lezama et al., 2004). To investigate whether the  $\alpha_5$ GABA<sub>A</sub> receptors are participating in this type of synaptic modulation, we next decided to investigate the action of L-655,708 on the excitatory postsynaptic potential (EPSP) evoked by electrical stimulation of the DLF, which do

not express GABA<sub>A</sub> receptors (Delgado-Lezama et al., 2004). **Figure 5A** illustrates the site where the DLF was electrically stimulated to evoke the EPSPs in a motoneuron recorded intracellularly in the ventral horn of a spinal cord slice. **Figure 5B** shows the EPSPs recorded from a motoneuron in control Ringer and in the presence of L-655,708. On average, the EPSP amplitude was facilitated by  $19 \pm 2.5\%$  with respect to control (**Figure 5C**;  $n = 10$ ;  $p < 0.05$ , unpaired Student's  $t$ -test). These results suggest that the tonic activity of  $\alpha_5$ GABA<sub>A</sub> receptors modulate the excitatory synaptic potential of motoneurons.

### $\alpha_5$ GABA<sub>A</sub> Receptors Modulate the Monosynaptic Reflex (MSR)

Previously we also showed that high affinity GABA<sub>A</sub> receptors may modulate the MSR (Bautista et al., 2010). Therefore, if  $\alpha_5$ GABA<sub>A</sub> receptors are regulating the motoneuron excitability we wonder whether they can also modulate the MSR. **Figure 6A** shows the experimental preparation for



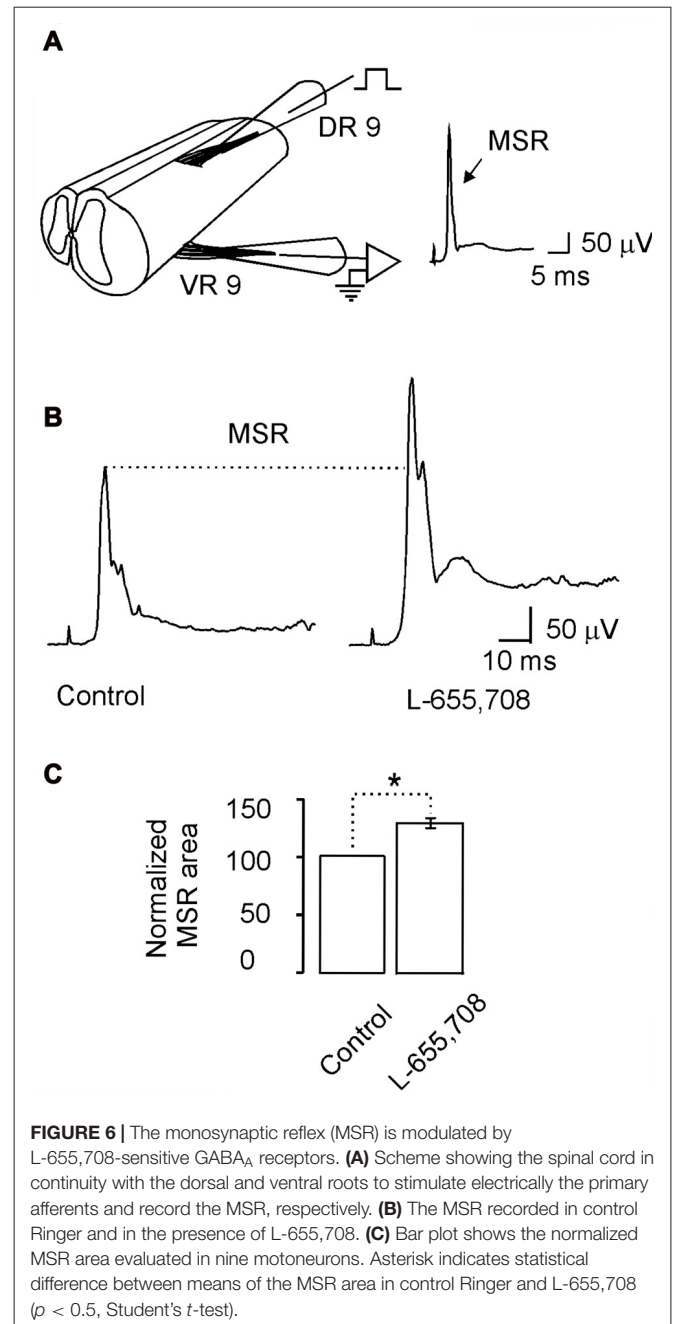
MSR recording. Stimulation of the DR9 evoked a ventral potential (VR9) consisting of mono- and poly-synaptic reflexes (**Figure 6B**). Interestingly, both responses were facilitated after the application of L-655,708 (**Figure 6B**). On average, the MSR was facilitated in about  $20 \pm 4\%$  compared with the control (**Figure 6C**;  $n = 9$ ;  $p < 0.05$ , unpaired Student's *t*-test). These results suggest that  $\alpha_5$ GABA<sub>A</sub> receptors tonically activated by ambient GABA play an important role in motor control.

## DISCUSSION

In the present report we show that the  $\alpha_5$ GABA<sub>A</sub> receptors tonically activated by ambient GABA produce a tonic inhibitory current that modulates the passive and active properties of the turtle spinal motoneurons. These data suggest that GABA<sub>A</sub> receptors may play an important role in motor control.

### $\alpha_5$ GABA<sub>A</sub> Receptors Mediate Tonic Inhibitory Currents in Motoneurons

In order to characterize the tonic current, we perfused exogenously GABA ( $30 \mu\text{M}$ ) into the experimental preparation. Interestingly, the current activated in this condition showed an increase in noise with respect to the control current which returned to the basal level after the blockade of the  $\alpha_5$ GABA<sub>A</sub> receptors with L-655,708. This finding agrees with previous reports in the hippocampus, the dorsal horn and the intermediate area of the spinal cord were tonic inhibitory



currents are mediated mainly by  $\alpha_5$ GABA<sub>A</sub> receptors (Takahashi et al., 2006; Bonin et al., 2007; Glykys and Mody, 2007; Wang et al., 2008; Castro et al., 2011b; Perez-Sanchez et al., 2016). Interestingly, in two neurons the tonic current was resistant to L-655,708, though it was sensitive to blockade by picrotoxin ( $100 \mu\text{M}$ ). Previously, we had observed a similar result by applying furosemide to block  $\alpha_6$ GABA<sub>A</sub> receptors in motoneurons. In this case, the furosemide-resistant tonic current was also completely blocked by  $100 \mu\text{M}$  picrotoxin application (Andres et al., 2014). Taken together, these data suggest that more than one extrasynaptic GABA<sub>A</sub> receptor might be mediating the tonic current in motoneurons as observed

in pyramidal cells of the hippocampus (Mody and Pearce, 2004; Farrant and Nusser, 2005). It is therefore reasonable to propose that extrasynaptic  $\alpha_5$ GABA<sub>A</sub> and  $\alpha_6$ GABA<sub>A</sub> receptors are expressed in motoneurons and are tonically activated by ambient GABA.

### $\alpha_5$ GABA<sub>A</sub> Receptors Modulate Passive and Active Properties of Motoneurons

Blockade of the  $\alpha_5$ GABA<sub>A</sub> receptors by L-655,708 increased the input resistance of motoneurons which implies a  $\alpha_5$ GABA<sub>A</sub>-shunting tonic inhibition. When this inhibition is removed by L-655,708 action, important effects on the motoneuron excitability are unmasked. For instance, the rheobase significantly decreases and the number of action potentials is increased (Figure 4). This observation is in agreement with the result observed in the hippocampus where  $\alpha_5$ GABA<sub>A</sub> receptors regulate the intrinsic excitability of pyramidal cells (Bonin et al., 2007), and in cerebellar granule cells where a tonic inhibitory current, recorded in a free moving mouse, controls its excitability lowering the excitatory synaptic potentials and producing a leftward shift on the frequency–current relationship (Chadderton et al., 2004).

It is possible that shunting inhibition may not be involved in the neuronal gain, but GABAergic tonic shunt might reduce neuronal activity in response to excitatory synaptic input. Indeed, L-655,708 increased the amplitude of EPSPs evoked by electrical stimulation of the DLF (Figure 5). In this context, our interpretation is that tonic activity of  $\alpha_5$ GABA<sub>A</sub> receptors might be a mechanism in the motoneuron to control synaptic input by altering its membrane time constant, thereby narrowing the time window to integrate excitatory synaptic inputs, serving as a filter to preserve the accuracy of the motoneuron response.

### The Monosynaptic Reflex Is Modulated by $\alpha_5$ GABA<sub>A</sub> Receptors

We found also that the  $\alpha_5$ GABA<sub>A</sub> inverse agonist L-655,708 facilitates the MSR. Interestingly, it has been reported that furosemide facilitates the MSR by blocking  $\alpha_6$ GABA<sub>A</sub> receptors, although simultaneous electrophysiological recordings showed that the dorsal root potential (DRP) was not affected (Bautista et al., 2010). In contrast, L-655,708 depressed the DRP, although by using the excitability test we found that primary afferent depolarization associated with presynaptic inhibition was not affected by the drug (Loeza-Alcocer et al., 2013).

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This means that the MSR facilitation was not due to blockade of presynaptic inhibition mediated by synaptic GABA<sub>A</sub> receptors. Therefore, having shown that  $\alpha_5$ GABA<sub>A</sub> receptors presumably control motoneuron excitability in a similar fashion than  $\alpha_6$ GABA<sub>A</sub> receptors, it is therefore reasonable to propose that these receptors may be also modulating the MSR.

### Functional Implications

It has been reported that extrasynaptic high affinity GABA<sub>A</sub> receptors expressed in motoneurons may play an important role in motor control by imposing a tonic shunting that decreases excitability and prevent anomalous firing of action potentials (Bautista et al., 2010). This function was evidenced by studying the regulation of the MSR which is useful to determine motoneuron excitability (Rekling et al., 2000). Interestingly,  $\alpha_5$ GABA<sub>A</sub> as well as  $\alpha_6$ GABA<sub>A</sub> receptors tonically activated by ambient GABA decrease motoneurons excitability by shunting the membrane which prevents its anomalous activation. This is suggested by the facilitation of the MSR followed by a long lasting activation of motoneurons after blockade of these receptors (Bautista et al., 2010).

Taking into consideration that  $\alpha_5$ GABA<sub>A</sub> receptors are also expressed in premotor interneurons (Castro et al., 2011a), our results suggest that these receptors might play an important role in regulating the neuronal network involved in motor control.

## AUTHOR CONTRIBUTIONS

MC-B, EL-A, CAC, RF and RD-L: conceived and designed the experiments. MC-B, EL-A, CAC and PO: performed the experiments. MC-B, EL-A, CAC, PO and RDL: analyzed the data. MC-B, EL-A, CAC, DE-V, VG-S, EM, RF and RD-L: contributed reagents/materials/analysis tools. MC-B, EL-A, CAC, PO, EM, VG-S, RF and RD-L: contributed to the writing of the manuscript.

## FUNDING

EL-A and PO were supported by a post-doctoral fellowship from Consejo Nacional de Ciencia y Tecnología (CONACYT; CB-2012/179294 to VG-S, RF and RD-L; CB-0864-Q to RD-L and 229866 to EM). EL-A, MC-B and CAC were supported by fellowships from CONACYT.

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