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Commentary: Synaptic Excitation in Spinal Motoneurons Alternates with Synaptic Inhibition and Is Balanced by Outward Rectification during Rhythmic Motor Network Activity

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A commentary on

Synaptic Excitation in Spinal Motoneurons Alternates with Synaptic Inhibition and Is Balanced by Outward Rectification during Rhythmic Motor Network Activity


In a recent study, Guzulaitis and Hounsgaard (2017) (GH2017) used whole cell voltage clamp (VC) to assess their synaptic currents (Johnston and Wu, 1995; Brette and Destexhe, 2012). GH2017 concluded that inhibition and excitation alternated during rhythmic scratching, and a voltage-dependent intrinsic conductance was masking this input such that it appeared as balanced excitation and inhibition in previous published work (Berg et al., 2007; Petersen et al., 2014). Nevertheless, this reasoning relies entirely on the validity of the clamp and, as we will see below, there is a clamp error, which complicates the interpretation of their data. Errors associated with voltage-clamp is a common problem as noted in previous reports (Spruston et al., 1993; Williams and Mitchell, 2008; Petersen, 2017).

The membrane current (I) is composed of intrinsic, leak, excitatory and inhibitory currents with individual conductances and reversal potentials, which collectively form a membrane resistance ($R_m$) and an equilibrium potential ($E_m$). When recording these using a pipette electrode, its resistance ($R_s$), sometimes called access or series resistance, is in series with $R_m$ (Figure 1A). When there is no electrode current the membrane potential $V_m = E_m$. However, during VC, a non-zero current introduces a drop in potential over $R_s$, which can only be partially compensated with the amplifier electronics (Brette and Destexhe, 2012). $R_s$ therefore has an uncompensated part (blue, $R_{us}$, Figures 1A,B), which generates an unaccounted drop in potential from the clamp potential ($V_c$) proportional to the pipette current:

$$V_m = V_c - I \cdot R_{us}$$

GH2017 report: “Voltage clamp (VC) experiments were performed on motoneurones when access resistance was low ($R_a < 20 \, \Omega$) and possible to compensate by 60-80%.” This means that $R_{us} = 20 - 40\% \cdot 20 \, \Omega = 4 - 8 \, \Omega$. When clamping at 0 mV the applied current is likely large. The authors do not report I for their clamp experiments (Figures 8–9), but their IV-plots suggest up to 10 nA (Figures 5E, 6). Hence, when trying to clamp at 0 mV, $V_m$ is really $-10nA \cdot 4\Omega = -40mV$ with 80% $R_s$-compensation.
To better understand the issue, we consider steady-state where all current passes through the resistors. From Ohm’s law the voltage drop over $R_{us}$ is $V_m - V_c = I \cdot R_{us}$. Similarly, the voltage drop over the membrane is $E_m - V_m = I \cdot R_m$. Combining these we can eliminate $I$ and isolate $V_m$:

$$V_m = \frac{V_c R_m + E_m R_{us}}{R_m + R_{us}}$$

(2)

Hence, for a good clamp ($V_m \approx V_c$) it is required that $R_m \gg R_{us}$. GH2017 report a membrane conductance of 49.2 nS (Figure 5B), which gives $R_m = 20 \text{M} \Omega$. With these values ($E_m = -70 \text{mV}$) clamping at 0 mV gives

$$V_m = \frac{0 - 70 \text{mV} \cdot 8 \text{M} \Omega}{28 \text{M} \Omega} = -20 \text{mV}$$

(3)

Whereas $R_{us}$ is assumed constant, $R_m$ may change dramatically due to synaptic and intrinsic conductance. GH2017 nicely document a nonlinearity starting at $-30 \text{mV}$ (Figures 5, 6), and a conductance of 314 nS ($R_m = 3.2 \text{M} \Omega$). Here, the low $R_m$ even becomes smaller than $R_{us}$ and therefore the clamp deteriorates further:

$$V_m = \frac{0 - 70 \text{mV} \cdot 8 \text{M} \Omega}{11.2 \text{M} \Omega} = -50 \text{mV}$$

(4)

The clamp is unlikely to be this bad, since the reduction in $R_m$ occurs above $-50 \text{mV}$. Also, $E_m$, which we assume constant, may depolarize due to change in the weighted average (Figure 1B), which mitigates the effect. The exact level of clamping of $V_m$ with ($V_c = 0 \text{mV}$) is difficult to estimate and may change in time. A reasonable guess is around $V_m = -30 \text{mV}$.

What is the consequence of this clamping error? To address this question, we use a one-compartment model receiving either reciprocal (Figure 1C) or concurrent (balanced) excitation and inhibition (E/I) (Figure 1D), which are the schemes that GH2017 intended to distinguish between. Both result in rhythmic $V_m$, although the effect of balanced E/I may seem counter-intuitive (Kolind et al., 2012; Petersen et al., 2014). The problem appears when presuming the outward current is inhibition, when setting $V_c = 0 \text{mV}$ (assumed clamp, black line Figures 1E,F). From
the above, we know that the actual clamp is likely at $-30$ mV (red traces). Here, the phase of the outward current reverses making the actual clamp in the balanced scheme (red, F) appear qualitatively similar to the assumed clamp in the reciprocal (black, E). Therefore, the VC experiments by GH2017 are difficult to interpret and ill-suited to discriminate between these schemes.

Although reciprocal E/I is a widely held belief in the literature, there is remarkably little experimental support in tetrapod vertebrates. The Ia-inhibitory interneuron has reciprocal activity (Geertsen et al., 2011), whereas the Renshaw interneuron has recurrent inhibition, both connected to motoneurons. Nevertheless the action of the remaining inhibitory population is largely unexplored. The scarcity in experimental reports that resolve E/I input is likely due to nonlinear properties and difficulties in separating synaptic current, although methods have been proposed (Berg and Ditl Aspen, 2013; Vich et al., 2017). Space clamp issues also confound the separation of E/I (Chadderton et al., 2014). Previous observations in turtles based on current-clamp indicated concurrent E/I. Here, voltage-activated conductances were circumvented by injecting negative current to hyperpolarize $V_m$ below the onset of the IV-nonlinearity. Therefore the disparity between reports cannot be attributed to outward rectification, as otherwise suggested by GH2017, see e.g., Figure 3A in Berg et al. (2007) and Figures 2–4 in Berg et al. (2008). Further, VC experiments were performed using sharp electrodes where spikes were blocked by pharmacology (QX314). QX314 likely also has the advantage of increasing $R_m$, thus improving the $R_m \gg R_{on}$ requirement (Monier et al., 2008). A current-reversal was observed in accordance with the balanced scheme (Figure 1G).

Other experiments confirm that when blocking excitation and inhibition pharmacologically, the high conductance vanish even at the same $V_m$, suggesting that conductance increase is caused by synaptic input rather than voltage-activated conductances (Figure 8 in Berg and Ditlesen, 2013). Application of strychnine had a strong depolarizing effect (Figure 1H) especially in the on-phase, which is also difficult to reconcile with the reciprocal E/I scheme (Berg et al., 2007; Vestergaard and Berg, 2015).

Contrary to the conclusions of GH2017, these observations suggest that a substantial fraction of the spinal neurons receive concurrent E/I, which may not exclude that others receive reciprocal. In fact, the neuronal population is divided between irregular and regular spiking, suggesting some receive reciprocal and others receive balanced input most likely on a spectrum between the two (Petersen and Berg, 2016; Berg, 2017). Notice in addition to the in-phase E/I there is also a weaker out-of-phase inhibition (Figure 1G). Spinal motor pattern generation may therefore be more complex and not exclusively conform to either of the schemes (Kishore et al., 2014).

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The author confirms being the sole contributor of this work and approved it for publication.

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**REFERENCES**


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