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Comments on the article by Jensen et al. (2020)

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In their recent article, Jensen et al. (2020) found that spinal motoneurons from SOD1(G93A) mice recorded at P70 and P110 were seemingly hyperexcitable: they fired at lower currents and at higher frequencies than controls. However, we believe there are substantial issues with this paper that bring into question their conclusions.

Firstly, we argue that the authors misused the Discontinuous Current Clamp (DCC) recording mode, resulting in a dramatic misrepresentation of the motoneurons firing pattern. We recently demonstrated that the choice of the DCC switching rate is critical, as a sub-optimal rate can lead to an overestimation of the excitability of the recorded cells: they fire at lower current and higher frequency than what would be observed with a higher DCC rate (Manuel, 2021). The optimal DCC rate depends on the cell's membrane time constant, and needs to be at least 15× faster than the membrane time constant to match with the passive and firing properties recorded in bridge mode. Over this value, excitability parameters (e.g. conductance, recruitment current, F-I gain) should be insensitive to the DCC rate. Unfortunately, the authors have not provided values of their membrane time constants. However, our data indicate that FF motoneurons have an average time constant of 2.1 ± 0.9 ms, FR motoneurons 2.9 ± 0.9 ms, while S motoneurons have a time constant of 4.0 ± 0.7 ms (Manuel, 2021). Therefore, FF motoneurons, the most vulnerable ones in ALS and the cells that lose their capacity for repetitive firing the earliest, should be recorded with a DCC rate of at least 7 kHz. Several lines of evidence suggest that the DCC rate used by Jensen et al. (3 kHz in most cases) is

indeed too low and that the physiological firing properties of the cells are obfuscated by the DCC: i) careful examination of the traces in Figure 4B–C reveals that the rate of rise of the membrane potential before the onset of firing is lower at 3kHz than at 8kHz, meaning that the apparent conductance of the cell measured at 3kHz is incorrect. ii) The current onset for firing is lower at 3kHz than at 8kHz (Figure 4B) as anticipated with a sub-optimal DCC rate. iii) Most of the frequencygrams shown in this paper, and in many of the other papers by this group (Meehan et al., 2010; Bonnevie et al., 2020; Jørgensen et al., 2020), exhibit a clear step-like pattern. This pattern is never seen when recording with sufficiently high DCC rates and is suggestive that the firing is driven by the membrane potential ripples generated by a low-frequency DCC. These ripples directly drive firing at discrete frequency steps, multiples of the fundamental DCC rate, and at higher frequencies than would be observed with appropriate DCC rates. iv) Finally, a suboptimal DCC rate can even force a silent motoneuron to fire because, at low switching rates, the ripples generated by the DCC are large enough to trigger artifactual spiking (Manuel, 2021). Figure 4C shows a cell that did not fire repetitively when recorded with an 8kHz DCC (as seen in Delestrée et al., 2014; Martínez-Silva et al., 2018), but which was firing when recorded at 3kHz. The fact that the behavior of the cell changes based on the DCC rate is a strong indication that 3kHz is too low and that the firing at low switching rates is an artifact.

Even if we were to accept their recordings, the authors' conclusions remain debatable due to the time points they have investigated (P70–110). The authors claim that, at P70, “there is not significant motoneurone loss in this model”. However, there is mounting evidence that motoneuron death is, in fact, already started at this age (Kong and Xu, 1998; Martin et al., 2007; Kanning et al., 2009; Wootz et al., 2012; Vinsant et al., 2013; Lalancette-Hebert et al., 2016), even though mice perform normally on the grip test. Moreover, there is a significant loss of intramuscular axons at this stage, particularly in compartments containing a large number of FF and FR motor units (Frey et al., 2000; Pun et al., 2006), as well as a dramatic reduction in the number of large axons in ventral

50 roots (Kong and Xu, 1998; Fischer et al., 2004). Therefore, it is plausible that, by performing their
51 recordings at this age, the authors skewed the distribution of the properties they were measuring
52 by not being able to record from the large, high threshold motoneurons. This holds true at P110
53 when mice are symptomatic indicating that a substantial fraction of the vulnerable motoneurons
54 has degenerated. Many of the changes that they observe in mutant motoneurons are compatible
55 with this hypothesis: i) shift towards higher resistances, with a parallel decrease in the amount of
56 sag; ii) shift towards longer AHPs; iii) lower rheobase; and iv) larger ΔI (which is characteristic of
57 small motoneurons (Iglesias et al., 2011)). Overall, these phenomena are consistent with the
58 hypothesis that the largest motoneurons have already degenerated, truncating the distributions
59 and shifting the average values towards the typical values for the smallest cells. In this context, we
60 believe it is erroneous to state that the cells are more excitable, they are just as excitable as they
61 normally are, but there are just fewer of them to be recruited, particularly among the largest, least
62 excitable cells.

63 Altogether, technical problems with the use of the DCC recording mode and the choice of the age
64 of the animals cast doubts on the author's conclusions that spinal motoneurons are intrinsically
65 more responsive in adult SOD1(G93A) mice

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