

Dear Editor,

We read with interest the report by Badawy et al (Badawy et al., 2013a) highlighting changes in cortical excitability in people with epilepsy and their siblings. It adds to the body of work of this group consistently showing that cortical excitability, measured by transcranial magnetic stimulation (TMS) may have potential as an epilepsy biomarker (Badawy and Jackson, 2012a; Badawy et al., 2013b, 2014, 2015). Responses to TMS were shown to have a relatively large inter-individual variability (see for example Valls-Solé et al., 1992; Du et al., 2014). Badawy's et al. reports do not provide clear information on inter-individual variability. It proved impossible to obtain it from the authors, so to estimate the variability across these reports, we extracted the data from some of their figures and compared these.

Several questions arose:

1) The long-interval intracortical inhibition (LICI) curves of several groups of people with epilepsy in this report (Badawy et al., 2013a) seem to overlap with LICI curves in other reports from the same authors.

- For example, the curve of the new-onset juvenile myoclonic epilepsy group, based on 7 subjects, appears the same as the one from a report based on 10 subjects (Badawy et al., 2013b), the re-digitalised curves are shown in figure A.
- The curve of the new-onset temporal lobe epilepsy group, based on 6 subjects (Badawy et al., 2013a), appears the same as the one from a report based on 10 subjects (Badawy et al., 2015), see figure B.
- The curve from the group with new onset generalised epilepsy with tonic-clonic seizures only (N=7) (Badawy et al., 2013a), appears the same as the curve of the same patient group with a different sample size (N=12) (Badawy et al., 2013b) and as the curve of the new-onset generalised epilepsy group with tonic-clonic, myoclonic and/or absence seizures (N=20) (Badawy et al., 2014), see figure C.

Were the results of the epilepsy groups reported by the authors obtained from overlapping subject groups? Given the large inter-individual variability, we would not expect such similar curves for groups with such a different sample size (N=7 and N=20) and different pathologies (generalised epilepsy with tonic-clonic seizures only, and generalised epilepsy with tonic-clonic, myoclonic and/or absence seizures).

2) Within the report, there is a difference between the LICI curve of non-epilepsy controls shown in figure 1 (the red line) and the LICI curve of the non-epilepsy controls shown in the other figures as a grey shaded area (Badawy et al., 2013a). In the middle panel of figure 2, ("Refractory"), the grey shaded area appears to be shifted down, below the x-axis in the first figure on the left (JME). In figure 1, the response ratio at an inter stimulus interval of 200ms appears to be greater than 100%, but in figure 2, the curve does not seem to reach 100%, see figure D. Do those figures show data from the same group of control subjects?

3) The effect sizes reported do not seem to fit with the error bars shown in the figures, leading us to wonder what the "error bars" in the figures represent.

For example in figure 2, 2nd frame from the top, 1st on the left (juvenile myoclonic epilepsy drug naive new onset) (Badawy et al., 2013a). The accompanying text on p.1182 says: *In the drug naïve-new onset groups, cortical excitability was higher in patients compared with their siblings at the 150, 250 and 300ms interstimulus intervals ($P<0.01$, effect sizes ranging 0.5–0.7; maximum in juvenile myoclonic epilepsy). [...] (Fig. 2).*

The effect size was calculated as: (mean of epilepsy) – (mean of siblings) / standard deviation of siblings (p.1181). The sample size of the group of siblings of people with new-onset juvenile myoclonic epilepsy was 11.

- For the 150ms interstimulus interval, based on the effect size of 0.5-0.7, the standard deviation should be between ± 164 and $\pm 230\%$, and the standard error of the mean between ± 49 and $\pm 69\%$. The "error bar" in the figure shows $\pm 25\%$.
- For the 250ms interstimulus interval, based on the effect size of 0.5-0.7, the standard deviation should be between ± 250 and $\pm 350\%$, and the standard error of the mean between ± 75 and $\pm 105\%$. The "error bar" in the figure shows $\pm 30\%$.
- For the 300ms interstimulus interval, based on the effect size of 0.5-0.7, the standard deviation should be between ± 192 and $\pm 270\%$, and the standard error of the mean between ± 58 and $\pm 81\%$. The "error bar" in the figure shows $\pm 40\%$.

4) Lastly, the mean resting motor threshold of the control group reported $55.4\pm 5.7\%$ (Badawy et al., 2013a), is similar to the motor threshold of $55.2\pm 5.6\%$ repeatedly reported by the same authors (summary in table). Were these results obtained from the same groups of participants?

It is essential to clarify these questions, as the promise of any clinical biomarker critically depends on its inter-individual variability, which ultimately influences its specificity and sensitivity.

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Table: Control group characteristics and resting motor threshold (rMT).

Publication (journal, year)	Group characteristics				Result
	# of controls	# of females	Mean age	Age range	rMT (mean±SD)
Int J Neural Syst 2014	20	11	-	16-40 ^a	55.2 ± 5.6
Clin Neurophysiol 2015	20	11	27	18-40	55.2 ± 5.6
Epilepsia 2013b	20	11	27	18-40	55.2 ± 5.6
Epilepsia 2013c	20	11	27	18-40	55.2 ± 5.6
Epilepsia 2012b	20	11	27	18-40	55.2 ± 5.2 ^b
J Clin Neurophysiol 2012a	19 ^c	13 ^c	20 ^c	16-28 ^c	55.2 ± 8.3 ^c

^a group characteristics and rMT (mean±SD) are the same as in publications 2015, 2013b and 2013c, while the age range differs slightly.

^b group characteristics and mean rMT are the same as in publications 2015, 2013b and 2013c while the SD rMT differs slightly.

^c mean rMT is the same as in all the other publications, while the group characteristics and SD rMT differ.

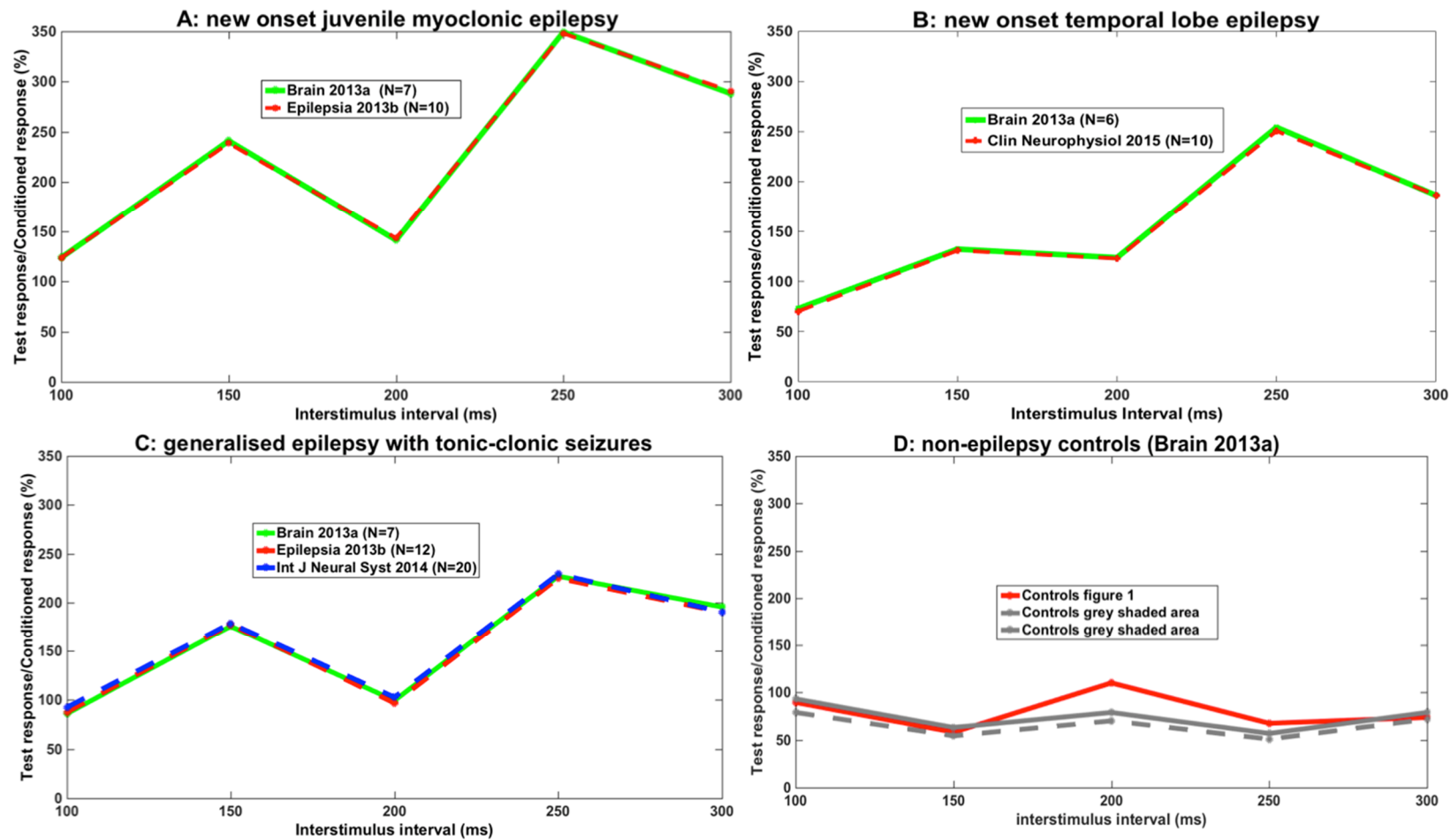


Figure: Re-digitalised long-interval intracortical inhibition (LICI) recovery curves of A: juvenile myoclonic epilepsy groups from two different studies with different sample sizes (shown in brackets). The curves overlap completely. B: new onset temporal lobe epilepsy groups from two different studies with different sample sizes (shown in brackets). The curves overlap completely. C: idiopathic generalised epilepsy groups from three different studies with different sample sizes (shown in brackets). The curves overlap completely. D: non-epilepsy control groups shown in figure 1 and figure 2 in Brain 2013a. The article text indicates that both curves are obtained from the same control group, yet they show a different pattern.