Does the cortical response to electroacupuncture or TEAS
depend on stimulation frequency?
Results of a pilot EEG study first proposed at the AACP Conference in 2001

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"Not everything that can be counted counts, and
not everything that counts can be counted"[1]

William Bruce Cameron (1920-?)
(often attributed to Albert Einstein)

INTRODUCTION

EEG (electroencephalography) is a low-cost, accessible method of investigating variations in electrical brain activity that is sensitive to rapid changes (unlike fMRI). The activity is recorded from scalp electrodes in the form of electrical power or amplitude [**Figs 1, 2**], and then analysed into frequency bands, here either standard bands such as Delta (1.5-4 Hz), Alpha (8-12 Hz) and Gamma (35-45 Hz) [**Table 1**], or narrow bands, 3-4 Hz wide [**Table 2**].

These intrinsic cortical oscillations involve processes of thalamo-cortical resonance that are fundamental to our 'rhythmic' senses – auditory, visual and somatic.

Fig 1. International 10-20 system of electrode placement

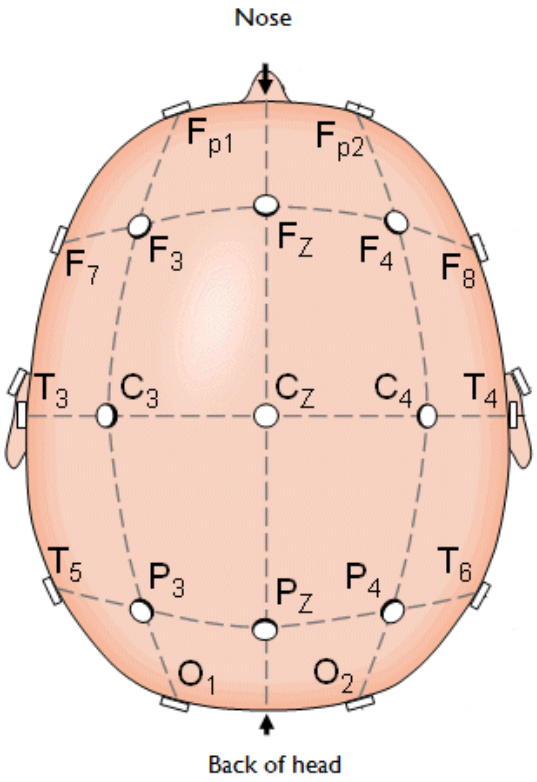


Fig 2. Experimental set-up.

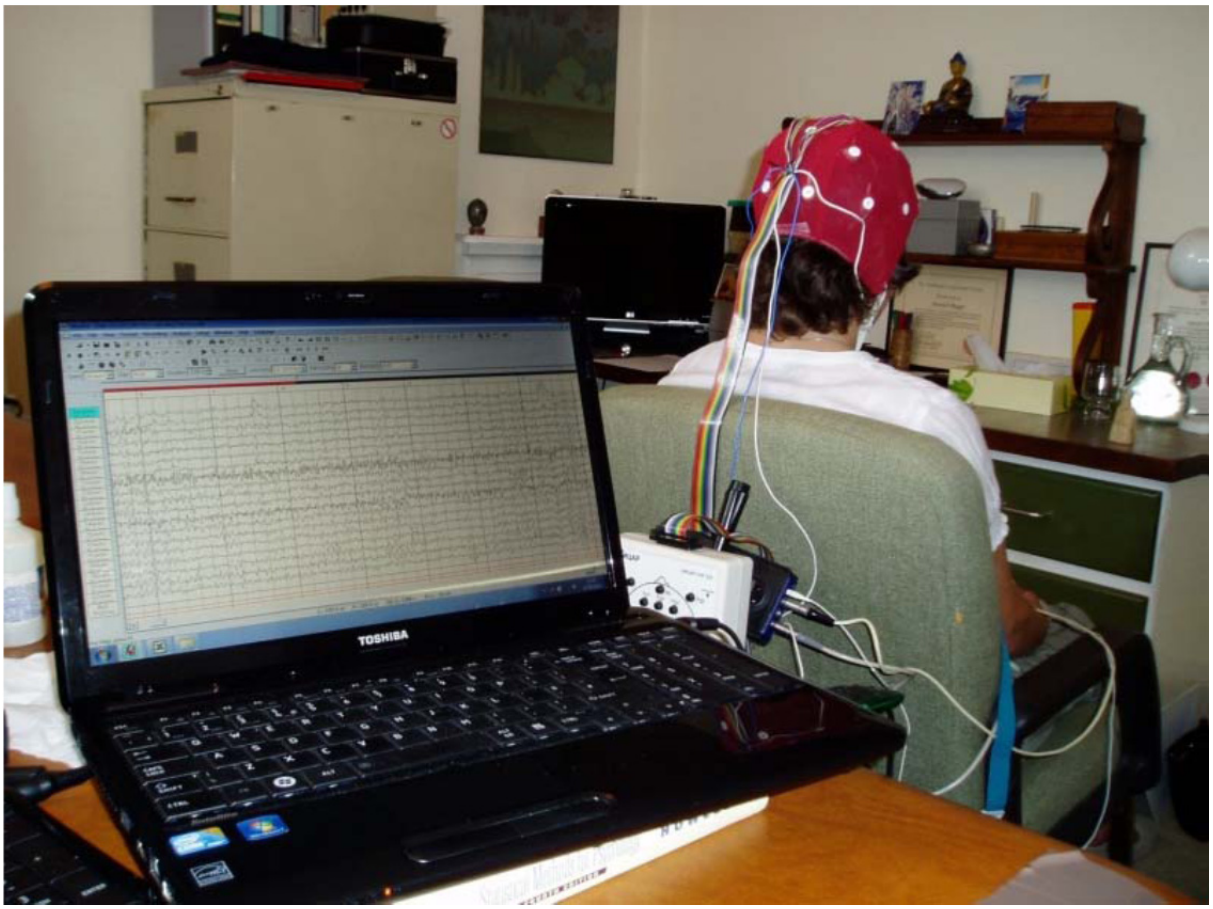


Table 1. Standard EEG frequency bands used in this study

Name	Frequency range
Delta	1.5-4 Hz
Theta	4-8 Hz
Alpha1	8-10 Hz
Alpha2	10-12 Hz
Alpha (All)	8-12 Hz
SMR (sensorimotor rhythm)	12-15 Hz
Beta1	15-18 Hz
Beta2	19-25 Hz
Beta3	25-35 Hz
Beta ('All')	13-21 Hz
Gamma	35-45 Hz

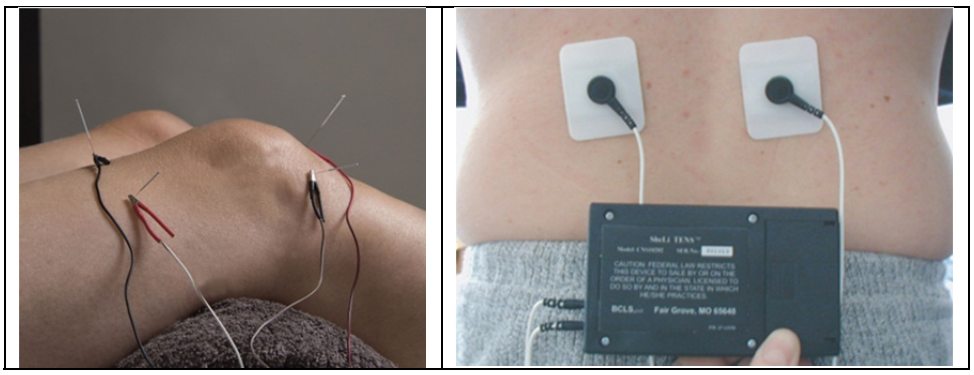
Table 2. Narrow bands, 3-4 Hz wide, centred on 2.5 Hz, 10 Hz, their harmonics, and some non-harmonics ('controls')

Name	Frequency range
Ctr2.5	1-4 Hz
Ctr5	3.5-6.5 Hz
Ctr7	5.5-8.5 Hz
Ctr10	8.5-11.5 Hz
Ctr13	11.5-14.5 Hz
Ctr17	15.5-18.5 Hz
Ctr20	18-22 Hz*
Ctr23	21-25 Hz
Ctr27	25-29 Hz
Ctr30	28-32 Hz
Ctr33	31-35 Hz
Ctr37	35-39 Hz
Ctr40	38-42 Hz

* Above 20 Hz, data could only be exported from WinEEG software in 1-Hz steps, so that 4-Hz rather than 3-Hz bins had to be used.

Electroacupuncture (EA) and Transcutaneous Electrical Acupoint Stimulation (TEAS) are methods of acupuncture-like stimulation [**Fig 3**]. This pilot crossover study, first proposed at the 2001 AACP Conference,[2,3] explores the relationship between frequency of TEAS applied peripherally and frequencies of cortical electrical activity detected centrally.

Fig 3. Electroacupuncture (Left), Transcutaneous Electrical Acupoint Stimulation (TEAS) (Right).



OBJECTIVE

To determine whether there is a central 'frequency following response' (FFR) to peripheral stimulation, as has been claimed for auditory and photic stimulation,[4-9] although not always supported by research.[10] In other words, if TEAS is applied at 2.5 Hz or 10 Hz, is more Delta or Alpha activity likely to appear in the EEG, either generally or at particular electrodes.

In principle, changes in a number of EEG measures could support the FFR hypothesis for TEAS. For instance:

- Absolute spectral power, ASP, the amount of electrical power, at a scalp measurement electrode in a particular EEG band (measured in μV^2)
- Relative spectral power, RSP, the ASP in a band divided by the total ASP for all ranges
- Amplitude, A (measured in μV)
- Derivations of spectral power, such as Ratios of spectral power in different bands, or left/right Asymmetry
- Average frequency within bands, AvHz, and its standard deviation (AvHz SD)
- Frequency with maximum power within bands, PkHz
- Coherence, Coh, a measure of phase synchronisation or coupling between signals at different electrodes[9]
- Cross-correlation, XC, used to assess time delays between signals at different scalp electrodes
- Phase delay, PD, another measure of the temporal 'lead' or 'lag' of spectra between electrodes[9]
- Autocorrelation, AC, a measure of the self-similarity of the EEG signal over time, or how often it repeats at a single electrode.

A FFR could result in increases in some of these measures in Delta-related bands in response to 2.5 Hz stimulation, or an increase in Alpha-related bands in response to 10 Hz stimulation.

More precisely, if some 'entrainment' effect occurs in the somatosensory (or sensorimotor) cortex which then dissipates at higher levels of cortical processing, changes would be expected more at the central than frontal, parietal, temporal or occipital scalp electrodes.

METHODS

In each 2-hour session, TEAS (Equinox, Liverpool) was applied at a 'strong but comfortable' intensity for five minutes at six different 'Locations', or combinations of LI4 and ST36 (in balanced order):

LI4[2]	Bilateral LI4	**ST36**[2]	Bilateral ST36
Left	Left LI4 & ST36	**Right**	Right LI4 & ST36
Bilateral	Left & Right	**LLSS**	LI4[2] & ST36[2]

Five participants attended for two sessions (2.5 Hz or 10 Hz TEAS, pulse duration fixed at 256 μs), two for one session each. EEG was monitored for five minutes before and after each 5-minute stimulation following standard EEG procedures (frequency bands and filtering, average reference montage), using the 10/20 system of electrode location (19 electrodes with linked ears as reference).[11-13] The Mitsar EEG-202 amplifier and WinEEG software (v2.91.54) were used (Mitsar Ltd, St Petersburg). Following artefact processing, data for various standard EEG measures were exported for analysis, when appropriate in the standard or narrow bands described above [**Tables 1 & 2**]. Statistical computation was carried out in SPSS (v 20) and Microsoft Excel (v 14).

Differences in measures for the two stimulation frequencies were assessed in two ways:

(1) By subtracting its value at 10Hz from that at 2.5Hz and then counting the number of resulting positive and negative ('countertrend') differences. Subtracting the 2nd (negative) number from the 1st provided a 'plus – minus' (PmM) count, which was then plotted against EEG band. Greater PmM in the EEG band centred on 2.5Hz (ctr2.5) than in that centred on 10Hz (ctr10) would support the FFR hypothesis. Significance was computed using the Binomial test for the proportion of positive and countertrend counts (assuming a 50/50 proportion as the likely outcome).

(2) Mean (sometimes median) values of the measure were compared using standard statistical methods such as Student's t-test or the Mann-Whitney U testNon-parametric correlation tests were conducted where appropriate. Bootstrap is planned for further analysis.

RESULTS

Absolute spectral power (ASP)

1. Counts♒♋ [For explanation of symbols, see **General notes** p.26 below]

(a) Standard bands

The numbers of decreases (-) in ASP over all bands following stimulation are identical for 2.5 Hz and 10 Hz stimulation, although there are more increases (+) for 10 Hz (p=0.02) [**Table 3**].[s1]

In particular, Delta and Alpha2 (10-12 Hz) ASP changes are similar for both frequencies [**Table 3**], whereas there are more increases than decreases for 10 Hz, and fewer for 2.5 Hz, in Alpha1 (8-10 Hz) and Alpha-All (8-12 Hz) (p=0.019 and p=0.013, respectively[s4[3]]), as would be expected if there is a FFR.

Table 3. Changes in ASP in the different EEG bands following 10 Hz and 2.5 Hz stimulation.

Band	10 Hz increases	10 Hz decreases	2.5 Hz increases	2.5 Hz decreases
A (Delta)	11	14	13	17
B (Theta)	2	2	11	2
C (Alpha1)	12	3	2	1
D (Alpha2)	9	0	7	1
E (Alpha-All)	15	1	4	0
F (SMR)	0	2	2	2
G (Beta1)	2	6	1	10
H (Beta2)	4	8	2	10
I (beta3)	6	10	1	9
J (Beta_All)	3	7	0	6
K (Gamma)	7	8	2	3
Totals	**71**	**61**	**45**	**61**

Out of 209 possible electrode-band combinations, only 27 showed significant differences in ASP for the two frequencies.[s2] Of these, 23 were positive (greater ASP at 2.5 Hz than 10 Hz) (p<0.001).[s1] However, with 3 out of 4 differences in Delta being positive, and 6 out of 10

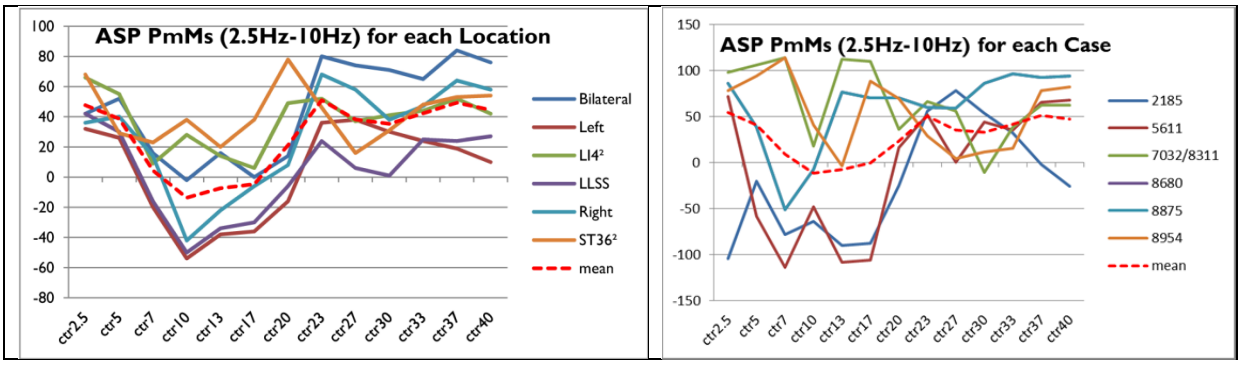
positive in the three Alpha bands considered, this does not provide evidence either way for a FFR.

(b) 3-4 Hz bands

Plotting the difference between PmMs for 2.5 Hz and 10 Hz stimulation shows that ASP counts for the group are higher in the band centred on 2.5 Hz (1-4 Hz, ctr2.5) than in the band centred at 10 Hz (8.5-11.5 Hz, ctr10).

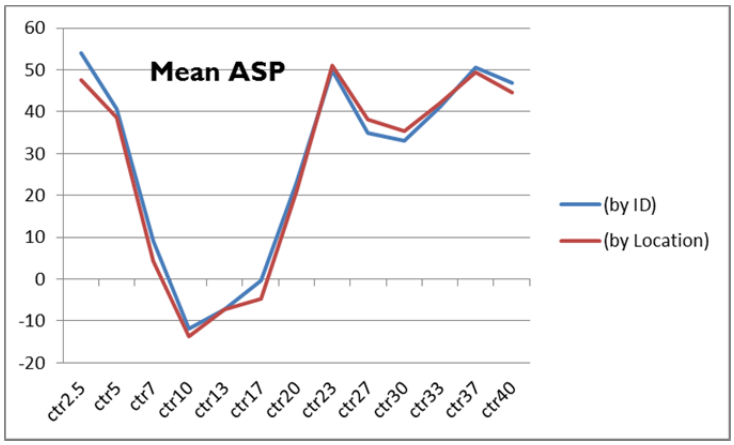
This pattern of more increases around 2.5 Hz with 2.5 Hz stimulation and more around 10 Hz with 10 Hz stimulation is supportive of the FFR hypothesis. However, while found for the group as a whole, and for each Location, it is not followed in all Cases [**Fig 4**].

Fig 4. Subtracting numbers of decreases from numbers of increases in ASP ('plus – minus,' or PmM), showing results for the group split by Location (Left) and Case (Right).



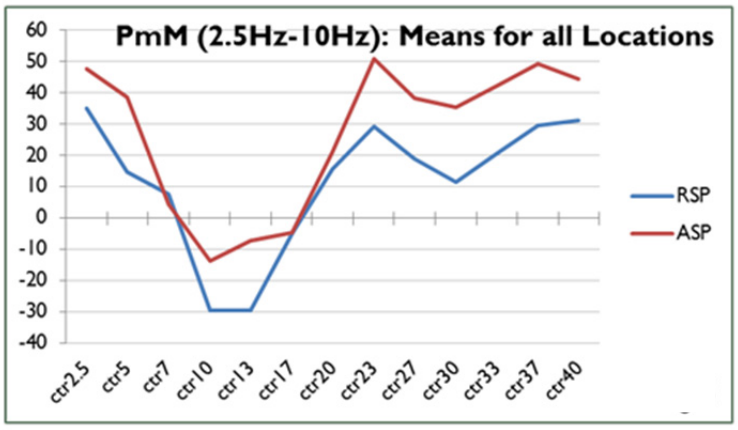
However, the means are virtually identical, whichever way the group is divided [**Fig 5**].

Fig 5. Mean PmM for ASP, comparing results for the group split by Location and Case.



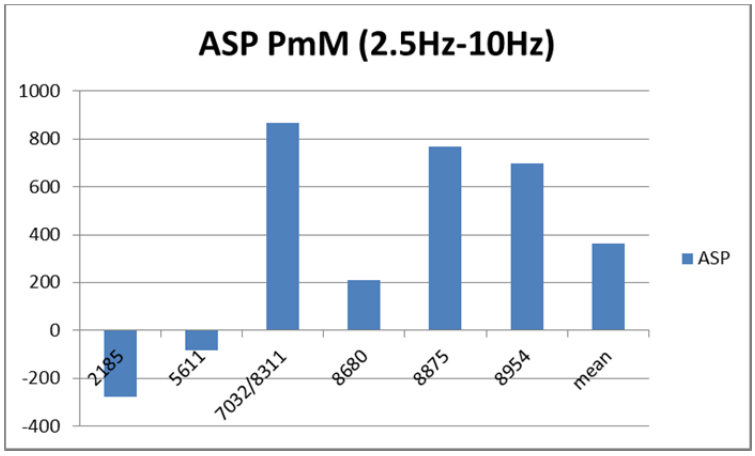
A similar pattern is evident for RSP [**Fig 6**].

Fig 6. Mean PmM for RSP, comparing results for the group split by Location.



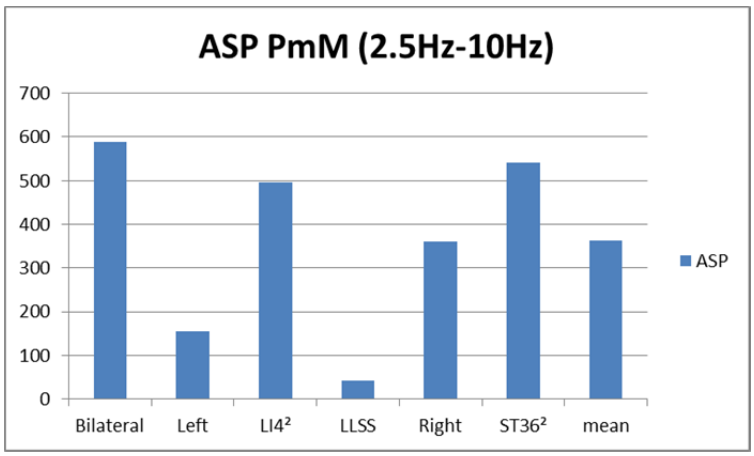
Summed over all bands, PmMs for ASP were positive for four out of six Cases [**Fig 7**].

Fig 7. PmMs for ASP, by Case.



There were also positive for *all* Locations [**Fig 8**].

Fig 8. PmMs for ASP, by Location.



2. Values♋

(b) 3-4 Hz bands

[No further findings]

Results for ASP, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
ASP	6	14	5	1	8

Relative spectral power (RSP)

1. Counts

(a) Standard bands

For the group, there is a preponderance of decreases (-) in RSP over all bands following stimulation for both 2.5 Hz and 10 Hz stimulation ($p<0.001$), without any major difference between them.[s1]

When the various Locations are considered separately, there are no significant differences in preponderance of increases or decreases for the two frequencies. Nor are there particular differences in preponderance of increases or decreases for Pre-EO and Post-EO for the two frequencies. However, overall Pre-Post EO differences differ at the two frequencies, with more decreases (increased cortical activity) over the course of the sessions at 10 Hz, and more increases (decreased cortical activity) at 2.5 Hz [**Table 4**].

Table 4. Numbers of increases and decreases of RSP from baseline to post-stimulation.

Pre-EO to Pre-EO	Increases	Decreases
2.5 Hz	148	61
10 Hz	75	134

Taking numbers of increases following stimulation as a percentage of all difference scores for that Location (+, - or 0) shows little variation between 2.5 Hz and 10 Hz across Locs, with most variation for $LI4^2$ and least for $ST36^2$ stimulation.

There were 150 counts for which RSP (averaged over electrodes or bands) was greater for 2.5 Hz, but only 38 for which it was greater for 10 Hz ($p<0.001$),[s1] with 49 of the 150 proportions and only 3 of the 38 proportions being themselves significant. The proportion of these counts was significant too for each Location.

Most instances for which RSP was greater at 10 Hz than 2.5 Hz ('countertrends') occurred in the Alpha2 and Alpha-All bands, conforming to the FFR hypothesis.

However, there were marginally more increase counts in Alpha1 at 2.5 Hz, and marginally more decreases in Delta (and possibly Alpha2) at 10 Hz, which is equivocal as regards the hypothesis.

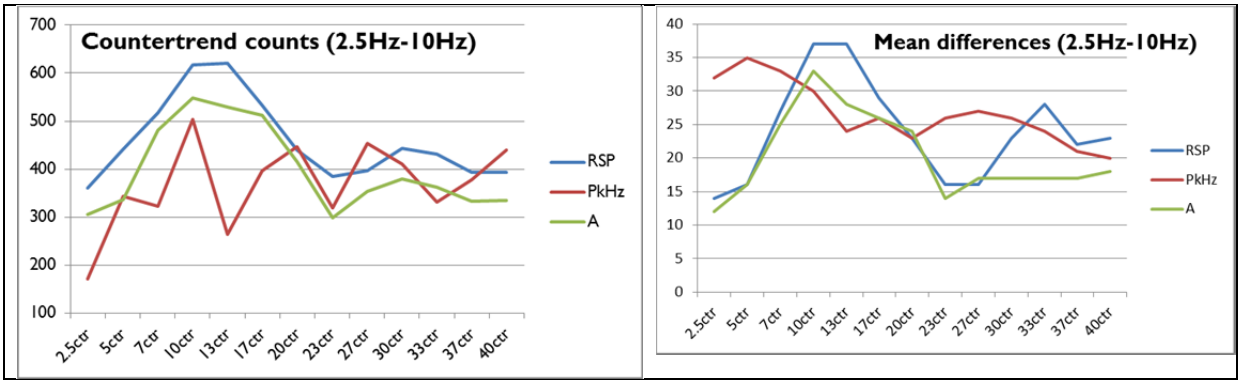
For two cases (2185 and 5611), there were marginally more Locations where RSP was greater at 10 Hz than 2.5 Hz ('countertrends').

Increase and decrease counts for the individual scalp electrodes are insufficiently consistent to support the hypothesis that a FFR occurs in the somatosensory cortex. Analysis of further data would be required to confirm this.

(b) 3-4 Hz bands

'Countertrend' counts show that RSP for the group is higher for 10 Hz stimulation in the ctr10 band, but for 2.5 Hz stimulation in the ctr2.5 band, supporting the FFR hypothesis. This is the case whether the difference between measures at the same electrode are simply counted [**Fig 9-Left**], or if averages are taken over all 19 electrodes for each intervention in turn, and then the difference between these averages calculated and counted [**Fig 9-Right**].

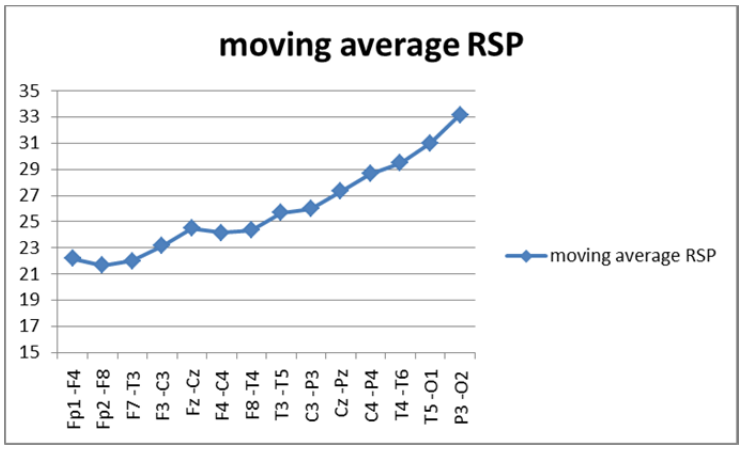
Fig 9. Countertrend counts supporting the FFR hypothesis, using two different methods of calculation.



This pattern persisted if Cases were removed and replaced in turn. However, it was not evident for any individual Case!

RSP is more frequently higher for 10 Hz than 2.5 Hz when moving from frontal to occipital electrodes. **Fig 10**, showing moving average 'of countertrend' counts, demonstrates this.

Fig 10. Countertrend counts of RSP, higher for 10 Hz than 2.5 Hz occipitally.

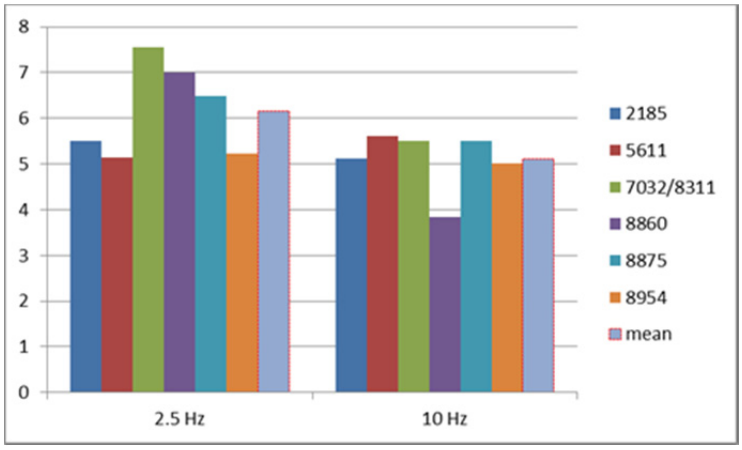


2. Values♒♋

(a) Standard bands

Mean RSP is a little less for 10 Hz than 2.5 Hz stimulation (for all Cases except one) [**Fig 11**].

Fig 11. Comparison of mean RSP for the two stimulation frequencies, by Case.



This is also the case for *all* stimulation Locations, although mean RSP is *greater* for 10 Hz than 2.5 Hz in the Pre-EO slots [**Fig 12**].

Fig 12. Comparison of mean RSP for the two stimulation frequencies, by Location.

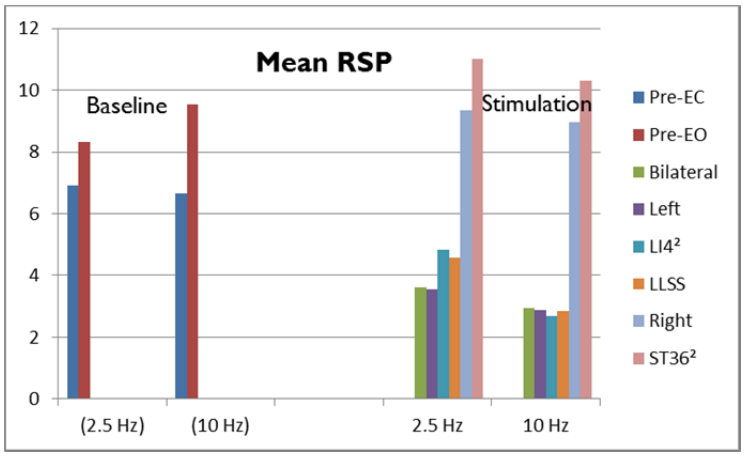


Fig 12 shows that mean RSP is a little less for 10 Hz than 2.5 Hz stimulation in the Pre-EC slot as well.

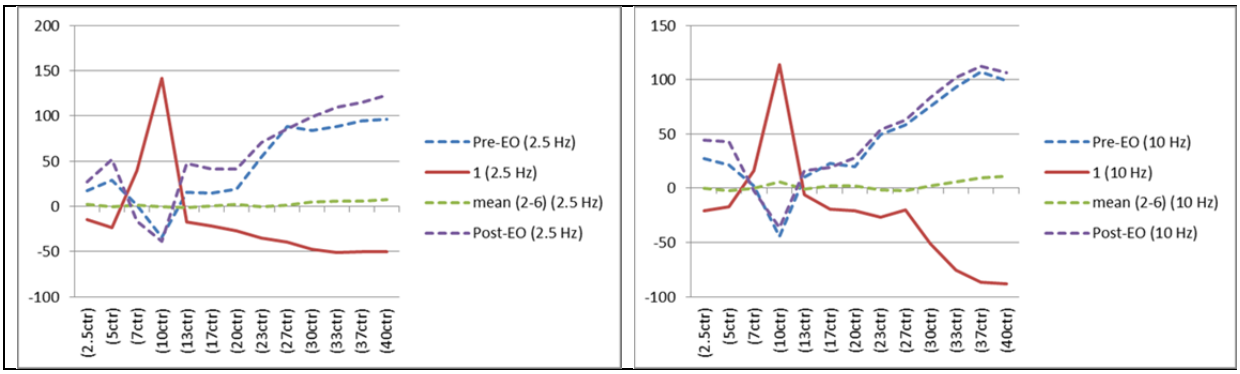
However, for the group there was no significant difference in RSP for the two stimulation frequencies.[s3]

(b) 3-4 Hz bands

For the group as a whole, the only significant difference in RSP between 2.5 Hz and 10 Hz stimulation occurred in ctr2.5.[s2]

There appears to be a difference in mean RSP in the first stimulation slot of each session between 2.5 Hz and 10 Hz stimulation, but little difference between them for the other slots (the maxima/minima at ctr10 reflect the difference between EC and EO states) [**Fig 13**].

Fig 13. Mean RSP before (Pre-EO) and after (Post-EO) stimulation, as well as during stimulation (consecutive 'slots' 1 to 6): 2.5 Hz (Left); 10 Hz (Right).



Results for RSP, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
RSP	9	19	4	1	14

Amplitude (A)♒♋

1. Counts

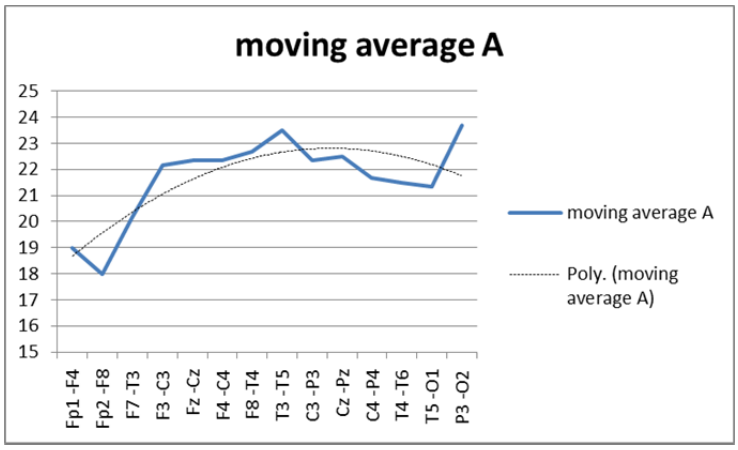
(b) 3-4 Hz bands

Amplitude (averaged over all electrodes) showed only 264 ‘countertrends’ (i.e. for which amplitude greater at 10 Hz than 2.5 Hz) out of a possible 702), significantly different from the 50% expected by chance ($p<0.001$).[s1]

As for RSP, two Cases (2185 and 5611) contributed high numbers of these.

Again as for RSP, A is more frequently higher for 10 Hz than 2.5 Hz when moving from frontal to occipital electrodes – although less markedly so than for RSP [**Fig 14**].

Fig 14. Countertrend counts of A, higher for 10 Hz than 2.5 Hz occipitally.



As for RSP (Fig A/B above),‘countertrend’ counts show that A for the group is higher for 10 Hz stimulation in the ctr10 band, but for 2.5 Hz stimulation in the ctr2.5 band, supporting the FFR hypothesis. Again this is the case whether averages are taken over all 19 electrodes for each intervention in turn, and then the difference between these averages calculated [Fig A], or if the difference between measures at the same electrode are taken over all the interventions, and then these differences averaged [Fig B]. And, again, this pattern was not evident for any individual Case.

Distribution of positive and negative slot changes in A for the two stimulation frequencies over the 6 stimulation Locations (and Pre-EO, Post-EO) is very similar, with no significant difference between the ratios of numbers of positive to all slot changes for the two frequencies.

2. Values

(b) 3-4 Hz bands

For the group as a whole, the only significant difference in A between 2.5 Hz and 10 Hz stimulation occurred in ctr2.5.[s2]

Results for A, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

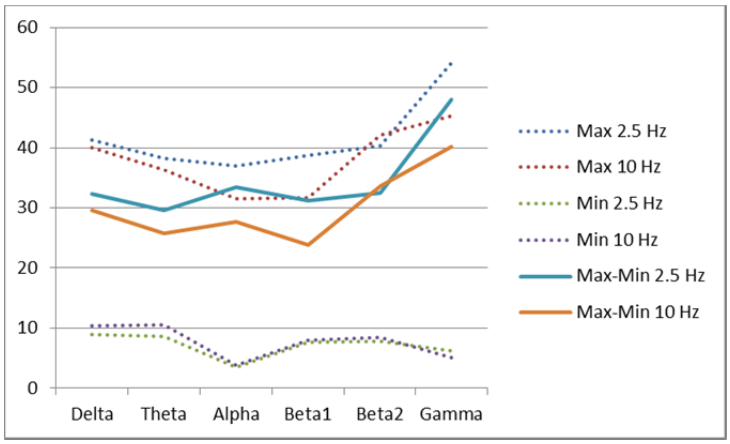
Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
A	5	8	2	1	5

Asymmetry (and Asymmetry SD)♋

Asymmetry, defined in WinEEG as [ASP(L) – ASP(R)] / [ASP(L) + ASP(R)], showed only minor differences for the two stimulation frequencies.

However, the range for Asymmetry SD was consistently greater for 2.5 Hz than for 10 Hz, except in the Beta2 band (simplified bands were used when exporting Asymmetry from WinEEG) [**Fig 15**].

Fig 15. Asymmetry SD**:** ranges across EEG bands (simplified) for 2.5 Hz and 10 Hz stimulation.



This may be the result of chance.

A more intriguing result is that the numbers of bilaterally symmetrical electrode pairs for each intervention that show positive or negative changes in ASP in the same direction appear to differentiate strongly between the two stimulation frequencies [**Fig 16**, **Table 5**].

Fig 16. Bilaterally symmetrical electrode pairs for each intervention that show positive or negative changes in ASP in the same direction

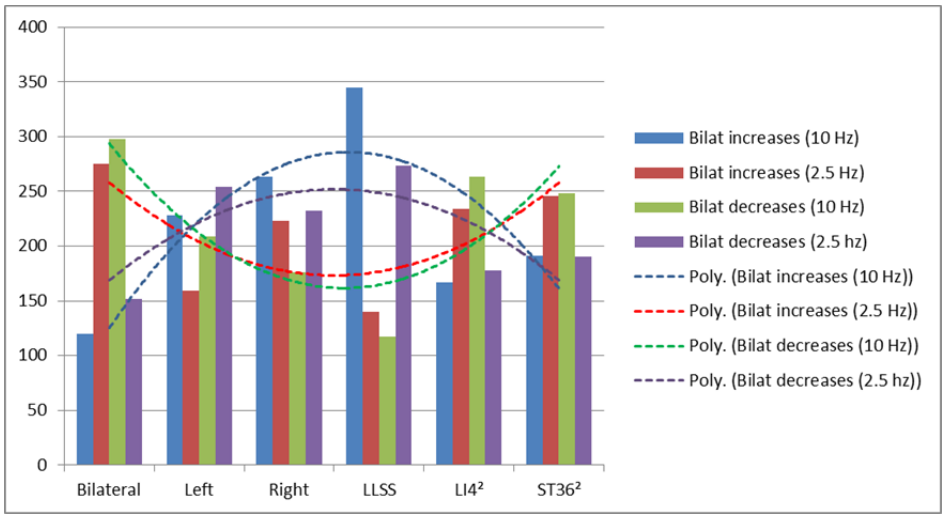


Table 5. Numbers of bilateral electrode pairs for each intervention that show positive or negative changes in absolute spectral power in the same direction, or changes in opposite directions.

Location	**Increases at both**		**Decreases at both**		**Opposite changes**	
	10 Hz	2.5 Hz	10 Hz	2.5 Hz	10 Hz	2.5 Hz
Bilateral	120	**275****	**298**	152**	110	101
Left	**228**	159*	209	**254***	91	115
Right	263	223	176	***232***	89	73
LLSS	**345**	140**	117	**273****	66	115**
LI4	167	**234***	**263**	178$^{(*)}$	98	116
ST36	191	**246$^{(*)}$**	**248**	190$^{(*)}$	89	92

** p<0.001; * p<0.01; $^{(*)}$ p<0.05.

As a result, numbers of positive and negative differences in (Bilat – Left), (Bilat – Right), (LLSS – LI4^2) and (LLSS – ST36^2) scores also appear to differentiate between 2.5 Hz and 10 Hz stimulation [**Table 6**], although it is hard to interpret this finding, if indeed it is not due to chance.

Table 6. Numbers of positive and negative differences if the Left and Right scores are subtracted from the Bilateral scores, and similarly for LI4, ST6 and LLSS.

Locations	**Increases at both**		**Decreases at both**		**Opposite changes**	
	10 Hz	2.5 Hz	10 Hz	2.5 Hz	10 Hz	2.5 Hz
Bilat – Left	0 +; 8 -	7 +; 1 -	7 +; 0 -	0 +; 8 -	*5 +; 3 -*	*3 +; 4 -*
Bilat – Right	1 +; 7 -	7 +; 1 -	7 +; 1-	1 +; 7-	*5 +; 3 -*	7 +; 1 -
	10 Hz	2.5 Hz	10 Hz	2.5 Hz	10 Hz	2.5 Hz
LLSS – LI4	8 +; 0 -	1 +; 7 -	0 +; 8 -	7 +; 1 -	0 +; 8 -	*3 +; 5 -*
LLSS – ST36	8 +; 0 -	2 +; 6 -	0 +; 8 -	6 +; 2 -	1 +; 6 -	5 +; 2 -

Results for Asymmetry and its SD, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
Asymmetry & SD	5	6	0	0	6

Ratios of spectral power♒

2. Values

(a) Standard bands

Changes in Delta/Theta and Delta/Alpha ratios did not support the FFR hypothesis.

Theta/Beta ratio (at Cz a marker for ADHD) varied considerably for different participants, with little to differentiate 2.5 Hz and 10 Hz interventions.

Results for Ratios of SP, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
Ratios of SP	2	2	0	2	0

Average frequency within bands (AvHz), and its standard deviation (AvHz SD)♒♋

1. Counts

(b) 3-4 Hz bands

The proportion of positive to negative differences in AvHz (for 2.5 Hz – 10 Hz) was significant only in ctr2.5 (11:25) and ctr13 (25:11) (p=0.029).[s1] This finding does not support a FFR.

'Countertrends' (for which AvHz at 10 Hz > at 2.5 Hz) did not conform to the pattern of low for ctr2.5 and high for ctr10 shown by RSP and A (above).

However, within most 3-4 Hz bins, AvHz SD showed more increases with 10 Hz than 2.5 Hz stimulation. This suggests greater variation at the higher frequency, but does not support the FFR hypothesis.

For Total AvHz (AvHz for the full range of 3-4 Hz bands), the proportion of positive to negative differences (2.5 Hz – 10 Hz), was significant for all Locations taken together (the whole group) (p=0.008), but for the individual Locations only Bilat (p<0.001) and ST36[2] (p=006).[s1] This suggests that Total AvHz was less for 10 Hz than 2.5 Hz stimulation, a finding that does not support the FFR hypothesis.

2. Values

(b) 3-4 Hz bands

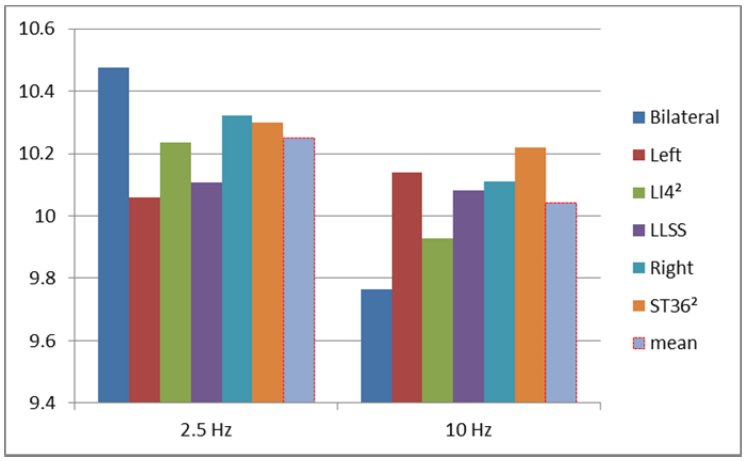
Differences in AvHz for the two stimulation frequencies were least in ctr10. Significant differences in AvHz were only found in ctr13.[s2, s3] These findings do not support the FFR hypothesis.

Significant differences in AvHz SD were found in a number of 3-4 Hz bands, not just ctr2.5 and ctr10 or related (sub)harmonic bands.[s2]

Differences in AvHz SD over the whole session (between Pre-EO and Post-EO were) greater for 2.5 Hz in the band centred on 10 Hz, and less for 2.5 Hz in the band centred on 2.5 Hz, both for the whole group and for some participants. These differences were very small, although supportive of the FFR hypothesis

Total AvHz (AvHz for the full range of 3-4 Hz bands) was less for 10 Hz than 2.5 Hz stimulation (apart from Left Location). Again, this does not support the FFR hypothesis [**Fig 17**].

Fig 17. Total AvHz for 10 Hz and 2.5 Hz stimulation



In contrast, differences in AvHz and AvHz SD were not consistent between the two stimulation frequencies, although both *increased* more with 10 Hz than 2.5 Hz stimulation in *both* ctr2.5 and ctr10. This is an equivocal results as regards the FFR hypothesis.

The FFR hypothesis would in principle result in AvHz SD being less in the 2.5 Hz band when stimulation is at 2.5 Hz (than at 10 Hz), or in the 10 Hz band when stimulation is at 10 Hz (rather than at 2.5 Hz). Results do not support the hypothesis.

Results for AvHz and AvHz SD, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
AvHz & SD	7	9	3	3	3

Frequency with maximum power within bands, PkHz♒♋

This measure is quite unstable and so not very reliable.

1. Counts

(b) 3-4 Hz bands

As for RSP and A [Figs A/B above], 'countertrend' counts show that PkHz for the group was higher for 10 Hz stimulation in the ctr10 band, but for 2.5 Hz stimulation in the ctr2.5 band, supporting the FFR hypothesis when averages are taken over all 19 electrodes for each intervention in turn, and then the difference between these averages calculated [Fig A]. in contrast, if the difference between measures at the same electrode are taken over all the interventions, and then these differences averaged [Fig B], this pattern is lost.

Furthermore (as for RSP and A), this pattern was not evident for any individual Case.

Results for PkHz, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
PkHz	2	3	2	1	0

Coherence, Coh ♋

1. Counts

(a) Standard bands

There appear to be differential effects of stimulation frequency for *some* of the Locations. At Left, for example, there were more increases than decreases in Coh with 2.5 Hz (and more decreases with10 Hz), whereas at LLSS and ST36[2] there were more decreases with 2.5 Hz (and more increases with 10 Hz).

In addition, at 10 Hz, Alpha2 predominantly showed decreases with Bilat stimulation, but not with Left or Right, whereas at 2.5 Hz, Alpha2 showed more increases with Bilat stimulation, but not with Left or Right stimulation.

Overall, most changes were found in Alpha1 and Alpha-All.

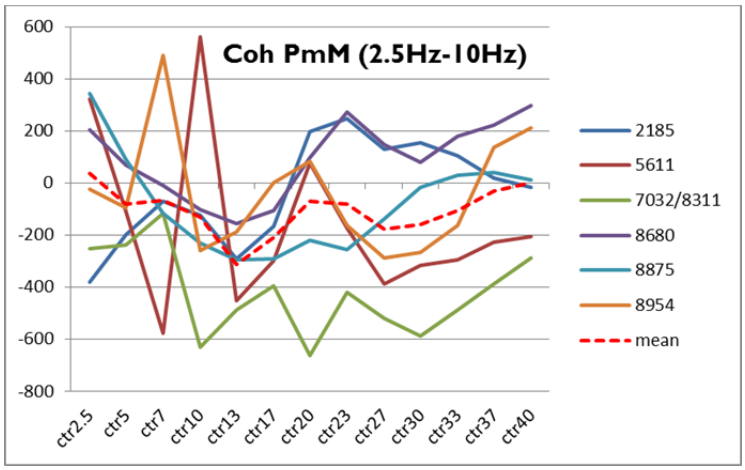
If there were a FFR, more increases than decreases would possibly be expected in the Alpha bands at 10 Hz, with fewer in these bands at 2.5 Hz (with correspondingly more decreases). This does not appear to be the case, but the numbers involved do not really provide any evidence either way, given the uncertainty of the supposition that coherence might increase in the presence of FFR.

(b) 3-4 Hz bands

Summing Coherence plus-minus differences (PmMs) (for 2.5 Hz – 10 Hz) shows a maximum in ctr2.5 for the group (with further peaks in Beta and Gamma), as would be expected from

the FFR hypothesis, but a minimum in ctr13 (rather than ctr10) [**Fig 18**]. (This pattern did not hold for all Cases.)

Fig 18. Coherence PmMs, showing a peak in the narrow band centred on 2.5 Hz (ctr2.5).



PmMs (for 2.5 Hz – 10 Hz) showed most maxima and minima at the frontal electrodes.

Five cases showed Coh greater for 10 Hz, with only one case (8680) showing Coh greater for 2.5 Hz. Coh tended to show more changes with 10 Hz than 2.5 Hz stimulation at all Locations.

Results for Coh, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
Coh	2	5	1	0	4

Cross-correlation, XC

XC data from WinEEG is tabulated in milliseconds (zero delay corresponding to cross-correlation) and coefficient rho (ρ), together with visual schematics of the correlated electrodes (numbers of inter-electrode links counted).

1. Counts

(a) Standard bands

From the schematics, it appears there are no significant differences in numbers of links between 2.5 Hz and 10 Hz for any Case, or for the group as a whole ($p>0.05$).[s1]

However, XC (counts) appear to be less for all Locations at 10 Hz than at 2.5 Hz, suggesting the greater possibility of a FFR at 2.5 Hz rather than at 10 Hz. In contrast, a greater number of *significant* cross-correlations occurred with 10 Hz stimulation ($p=0.015$).[s1]

These findings should be viewed with caution, given a likely dependence on (Pre-EO) baseline disparities (see below). Nonetheless, XC responses to the two stimulation

frequencies at F7 and Fz, and at Pz and P4, appear not to co-vary with these baseline differences.

Further analysis of XC is required to assess whether it increases or decreases in relation to Pre-EC baseline.

Results for XC, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
XC	2	3	0	0	3

Phase delay, PD

1. Counts

(b) 3-4 Hz bands

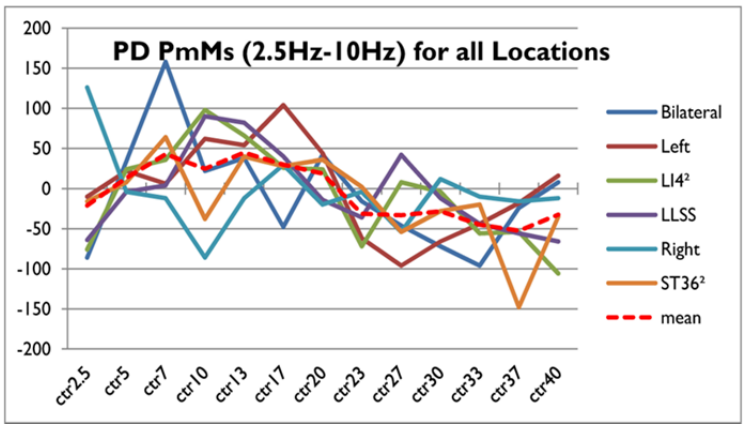
Plus-minus (PmM) difference scores (2.5 Hz – 10 Hz) showed that in 5 of 6 cases overall PD was slightly greater for 2.5 Hz than 10 Hz, with only one case (2185) showing the opposite (although mean differences were very small).

However, for Location, PD tended to show more changes with 10 Hz than 2.5 Hz stimulation.

PmMs (for 2.5 Hz – 10 Hz) showed most maxima and minima at the temporal electrodes.

Maxima and minima obtained by summing PD PmMs (for 2.5 Hz – 10 Hz) do not support the FFR hypothesis, with maxima in ctr7 and ctr13, and mean PD diminishing in the higher bands. However, the mean PmM is still lower in ctr2.5 than ctr10, which does support the hypothesis [**Fig 19**].

Fig 19. Phase delay PmMs, showing mean PmM as lower in ctr2.5 than ctr10.



Results for PD, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
PD	1	5	2	2	1

Autocorrelation, AC

Autocorrelation is calculated prior to separation of data into individual bands (whether standard or 3-4 Hz), so no meaningful results are obtainable for different bands. Greater AC (lower equivalent frequency) would indicate that it takes longer for a signal to repeat.

1. Counts

The number of positive counts for $AC_{2.5Hz} - AC_{10Hz}$ is greater than the number of negative counts, suggesting greater AC at 2.5 Hz.

2. Values

Comparing all 2.5 Hz and 10 Hz sessions, four of five Cases showed greater mean AC at 10 Hz than at 2.5 Hz.

These contradictory results do not throw light on whether or not there is a FFR.

Mean values of AC found at the different electrodes varied from 0.0022 to 0.0543 Hz, and so bear no relationship to stimulation frequency.

Results for AC, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	2.5Hz≈10Hz	2.5Hz≠10Hz	Supports FFR hypothesis	Contradicts FFR hypothesis	Neither
AC	1	2	0	0	2

Possible confounding factors

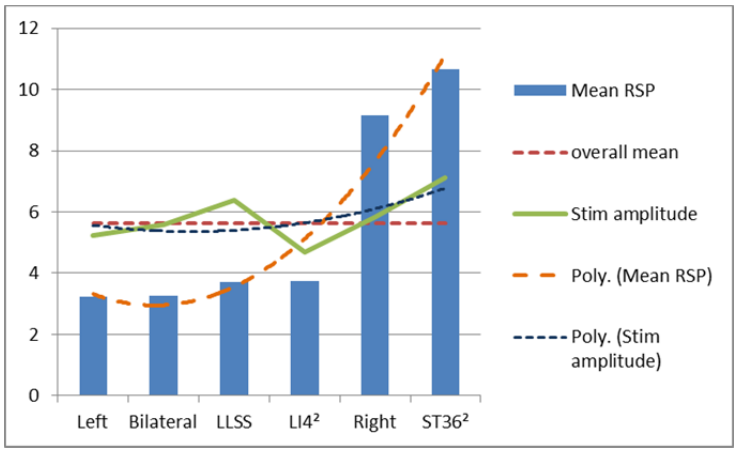
Stimulation amplitude, StAmp

Although for the whole sample StAmp was not well correlated with stimulation frequency (η=0.203),[s4] it was greater at 10 Hz than 2.5 Hz ($p<0.001$),[s3] indicating a need for caution in interpreting results.

Out of 209 possible electrode-band combinations, 57 showed significant Spearman correlations between ASP and StAmp (including 13 in Delta, 5 in Alpha1, 6 in Alpha2, 5 in Alpha-All). All were negative (except for some correlations at occipital electrodes in higher bands).

There is no significant relationship between mean RSP and StAmp, either for the group as a whole, or split by ID or Loc [**Fig 20**].[s5]

Fig 20. Comparing stimulation amplitude and EEG mean RSP.



However, StAmp is more likely to correlate positively than negatively with RSP when averaged over scalp electrodes or different bands (in contrast to ASP), and more such correlations are significant for 'minimum' amplitude (taking lower values if two stimuli are applied simultaneously) than for 'mean' or 'maximum' amplitude.[s6]

More data is required to confirm a lack of relationship between StAmp and RSP.

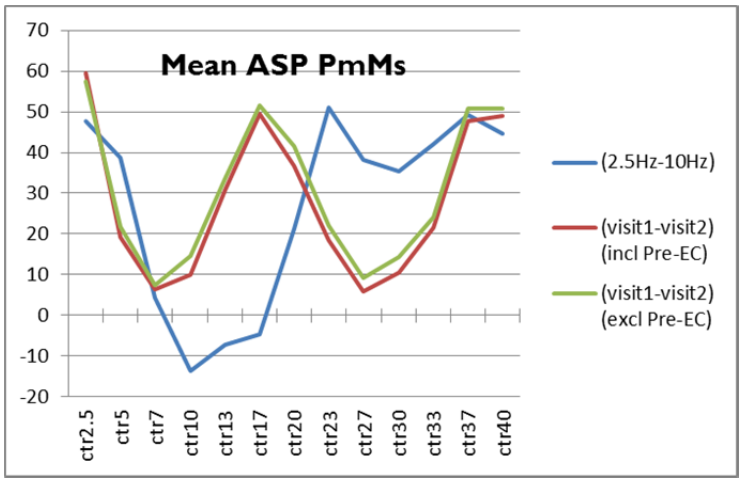
Correlations between StAmp and A, Asymmetry, Asymmetry SD, AvHz, AvHz SD and PkHz were small. There were no significant correlations between AtAmp and Total AvHz.[s5] For Coh, a few correlations (3.6% of the total possible) were both significant and >0.5.

Visit order

Comparing results by Visit rather than Stimulation frequency is equivalent to 'shuffling' the data. If patterns of high PmM in ctr2.5 and low PmM in ctr10 persist after shuffling, this would suggest that it is not the result of a difference in stimulation frequency.

For ASP, for example, this is not the case [**Fig 21**].

Fig 21. Shift in maxima and minima of ASP PmMs when comparing by Visit as opposed to stimulation frequency.

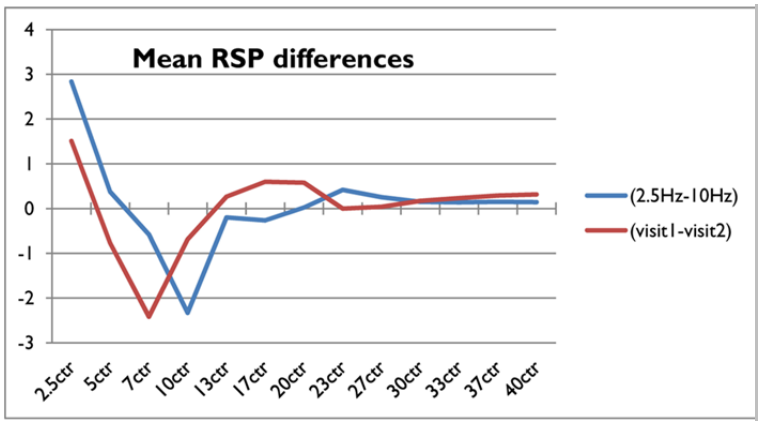


Here the difference in the two plots (Hz/Visit) at ctr10 is marked: $PmM_{v1\text{-}v2}$ remains positive, while $PmM_{2.5\text{-}10\ Hz}$ is negative. At ctr2.5, both are positive.

Whereas mean RSP is less for 10 Hz than 2.5 Hz stimulation for all Cases except one, mean RSP may increase or decrease between visit1 and visit2 (three Cases each). This suggests that the difference in RSP with stimulation frequency is likely not to be a random finding.

As for ASP, comparing RSP results by Visit rather than Stimulation frequency results in a shift in the pattern of low PmM in ctr10 (shifting to ctr7), although not for high PmM in ctr2.5, suggesting that the difference in RSP with stimulation frequency may not altogether be due to chance [**Fig 22**]. This pattern persists if single Cases are removed and replaced in turn from the whole group.

Fig 22. Shift in maxima and minima of RSP PmMs when comparing by Visit as opposed to stimulation frequency.



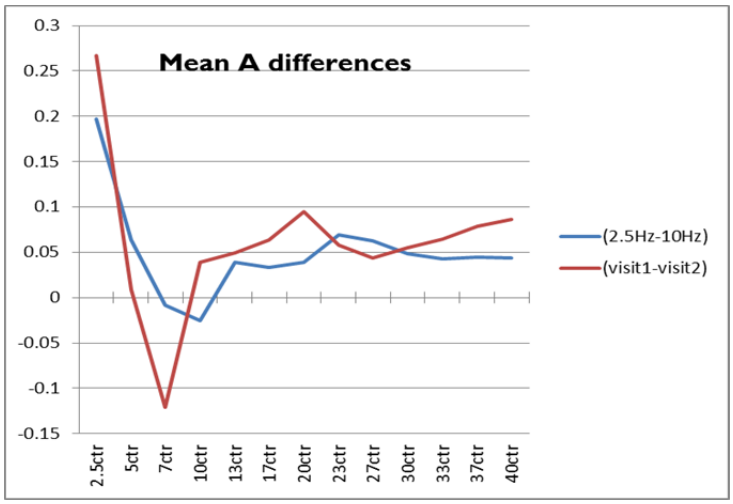
Furthermore, numbers of RSP, A and PkHz differences are less for visit1 – visit2 than for 2.5 Hz – 10 Hz, suggesting that Hz has a greater differentiating effect than visit. [But for RSP one test resulted in a contradictory finding suggesting a greater effect of visit than of stimulation frequency.[s2]]

However, for RSP, both PmM plots become negative, and $PmM_{2.5\text{-}10\ Hz}$ *less* so than for ASP, so this is in several respects a less definite differentiation.

There was no consistent pattern of *eta* being lower or higher for RSP or A vs visit rather than for RSP vs stimulation frequency.

As for RSP (above), comparing A results by Visit rather than Stimulation frequency results in a shift in the pattern of low PmM in ctr10 (shifting to ctr7), although not for high PmM in ctr2.5. $PmM_{2.5\text{-}10\ Hz}$ is negative at ctr10, but $PmM_{v1\text{-}v2}$ positive [**Fig 23**].

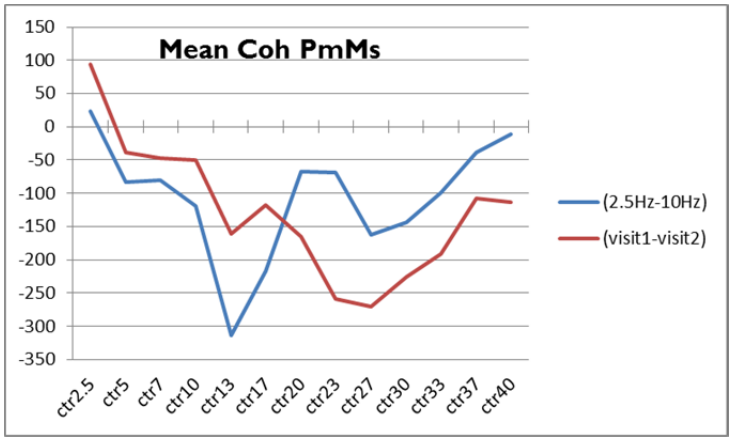
Fig 23. Shift in maxima and minima of A PmMs when comparing by Visit as opposed to stimulation frequency.



Overall mean differences in A for the two frequencies were greater for Visit than stimulation Frequency.[s2]

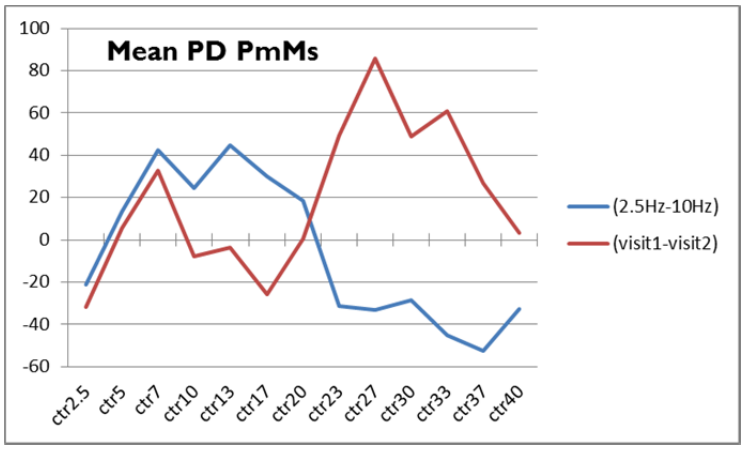
Differences in Coh means for the two frequencies were greater for Visit than for stimulation Frequency in the lower bands, although less in Beta and Gamma [**Fig 24**].

Fig 24. Shift in maxima and minima of Coh PmMs when comparing by Visit as opposed to stimulation frequency.



Differences in PD means for the two frequencies show an opposite pattern [**Fig 25**].

Fig 25. Shift in maxima and minima of PD PmMs when comparing by Visit as opposed to stimulation frequency.



‘Slot’ order (time during session)

As stated above, RSP may change differently during the first stimulation ‘slot’ in a session than in subsequent slots in the same session.

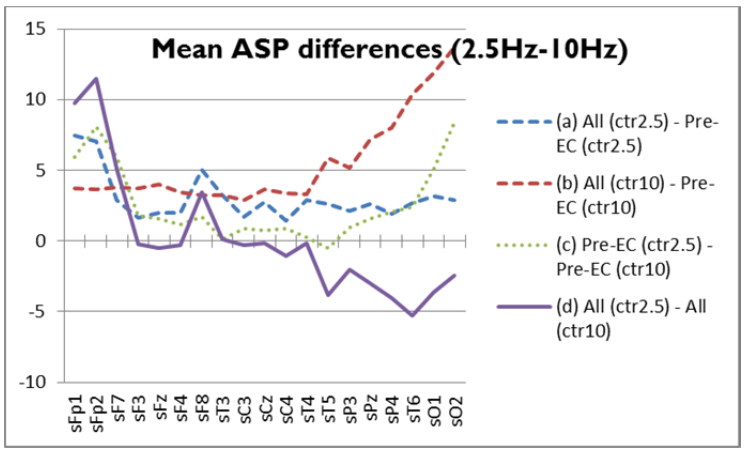
Another pattern is that AC tends to be greatest at the beginning of a session (Pre-EO or slot 1), and least at the end (slot 6 or Post-EO).

Pre-existing baseline differences

If differences between measures for 2.5 Hz and 10 Hz stimulation are already present at *pre*-stimulation baseline (in the ‘Pre-EC’ slot), then they cannot be the result of stimulation.

This does not appear to be the case, for instance, for ASP [**Fig 26**].

Fig 26. Comparing mean ASP for all intervention slots and at baseline, for 2.5 Hz and 10 Hz stimulation.

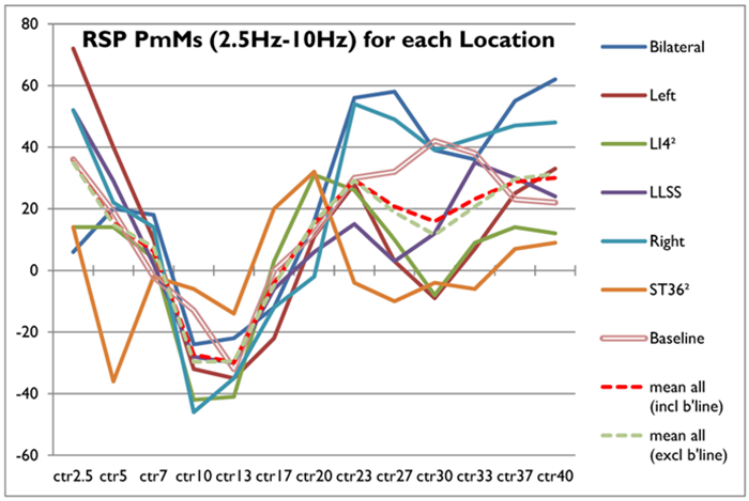


Here differences in mean ASP (ASP at 2.5Hz – ASP at 10Hz) are compared for the two narrow bands ctr2.5 and ctr10: (a, b) for all stimulation time ‘slots’ taken together vs the baseline (‘Pre-EC’) slot; (c) for the two narrow bands at baseline; (d) for the two narrow bands for all ‘slots’. Patterns of difference between all stimulation ‘slots’ and the Pre-EC ‘slot’ in both ctr2.5 and ctr10 are small but positive – for 10 Hz becoming larger posteriorly,

and for 2.5 Hz larger frontally (a,b). The differences in mean ASP between ctr2.5 and ctr10 are also different at baseline (c), and for all stimulation slots taken together (d).

For RSP, some of the difference in mean RSP for the two frequencies may be a carry-over effect from baseline (Fig 7, above). This is particularly clear when the difference is plotted for the various stimulation locations [**Fig 27**].

Fig 27. Comparing mean RSP for all intervention Locations and at baseline, for 2.5 Hz and 10 Hz stimulation.



However, RSP *counts* show that while the proportion of positive to negative differences for the two frequencies (2.5 Hz – 10 Hz) was significant for all interventions (with more positive differences for the interventions taken together), it was not for Pre-EC, for which there were more negative differences. In contrast, this suggests lack of a carry-over effect from baseline.

As for RSP, apparent patterns of difference in A in 2.5ctr and 10ctr for the two stimulation frequencies were – to some extent – present *before* stimulation (during Pre-EC). Similarly, a pattern of difference in the numbers of XC links (and numbers of *significant* XC links) between 2.5 Hz and 10 Hz during stimulation were also apparent at baseline (Pre-EO). Although dependence on baseline values for PkHz appears to be less than for RSP, A or XC, this is probably due to its inherent instability.

Countertrend counts for RSP, PkHz and A were only marginally different between stimulation and baseline.

Size of effects

For RSP, for example, slot difference percentage scores are greater between Pre-EC and Pre-EO than between two successive interventions. Differences in measures due to stimulation location or frequency are likely to be lower than those caused by variations in individual Case response, or basic experimental conditions such as whether eyes are open or closed).

Individual responses♒

Responses are very idiosyncratic, and while there may be differences in various measures in response to the two frequencies (e.g. RSP, A), these frequently appear to be specific to the individual. For a given Case, for example, responses in AvHz SD may be similar, whatever the stimulation Location or Frequency.

The effect of individual differences in baseline characteristics (e.g. whether an individual is a 'High Beta' or 'High Alpha' type) remain to be investigated.

CONCLUSION/CLINICAL RELEVANCE

Compiling the results for all measures [**Table 7**] shows that a considerable number of them (c. 75) indicate a difference between the effects of 2.5 Hz and 10 Hz stimulation. However, of these, only 19 support the FFR hypothesis, and 11 do not, with 46 giving an equivocal result. At this stage, only ASP and RSP provide markedly more support than non-support for the hypothesis. (This difference is not significant if a 50/50 result is expected, although for ASP it would be significant for an expected 40/60 result.[s1])

Table 7. Results for all measures, showing numbers of findings that are: (a) similar for both stimulation frequencies; (b) different; (c) supportive of the FFR hypothesis; (d) contradictory of the hypothesis; (e) neither supportive nor contradictory of the hypothesis.*

Measure	(a) 2.5Hz≈10Hz	(b) 2.5Hz≠10Hz	(c) Supports FFR hypothesis	(d) Contradicts FFR hypothesis	(e) Neither
ASP	6	14	**5**	1	8
RSP	9	19	**4**	1	14
A	5	8	2	1	5
Asymmetry & SD	5	6	0	0	6
Ratios of SP	2	2	0	2	0
AvHz & SD	7	9	3	3	3
PkHz	2	3	2	1	0
Coh	2	5	1	0	4
XC	2	3	0	0	3
PD	1	5	2	2	1
AC	1	2	0	0	2
Totals	**42**	**75**	**19**	**11**	**46**

Using this rough and ready way of assessing the EEG data, a central frequency following response to peripheral rhythmic electrical stimulation remains possible, although – so far – there is little strong evidence that it occurs.

However, individual response is very variable and may mask a FFR. Further research is justified, to clarify the possible role of confounding factors, and also explore issues such as whether a FFR occurs in response to some frequencies and not others, or in some individuals and not others.

If evidence for a FFR is found, this would throw new light on the effects of different stimulation frequencies on the brain and, potentially, different mental states, with potential for immediate clinical applications.

In further analysis:

- The effects at particular electrodes, or clusters of electrodes (perhaps central, or temporal) should be investigated in more detail.
- RSP, AC and perhaps other measures should be assessed for possible changes during the course of monitoring.
- The effect of the various confounding factors mentioned, such as StAmp, Visit order, baseline differences and individual response, has to be carefully considered (e.g. by partialling them out when checking correlations or differences).
- Some measures may need to be given more weight than others
- The question of whether a FFR occurs in response to some frequencies and not others needs to be addressed.

Data remains to be analysed from further completed pilot studies using longer stimulation periods, concurrent rather than subsequent monitoring, and comparing EA and TEAS.

Further research using different acupoints and more participants may then be justified.

General notes

♒ This symbol indicates greater variability of a measure by Case than by stimulation Frequency or Location.

♋ This symbol indicates significant association of a measure with Cases (η>0.75), but not with stimulation Frequency, Location or Visit. The association with Cases was least marked for AvHz SD.

* The tabulated figures shown for each measure are in some cases only approximate. (Inclusion of a finding in a particular category may be subject to interpretation, and some findings have multiple parts, some contradictory.) However, they do provide a rough overall impression of the usefulness of the measure in assessing whether data conforms to the FFR hypothesis or not. For most measures, a number of equivocal ('Neither') results were found, counted in the Tables but not reported in detail.

Statistics notes

S1. Binomial (proportion) test.
S2. T-test for independent samples. Pending Bootrapping, t-tests were conducted in some cases rather than the more correct Mann-Whitney U test.
S3. Mann-Whitney U test for independent samples.
S4. Correlation coefficient *eta* (η) test of association
S5. Assessed using Spearman's rank correlation coefficient. If significant correlations between stimulation amplitude were found, there were none with *rho* > 0.5.
S6. Partial non-parametric correlation test.

References

1. Cameron B.C. (1963) *Informal Sociology: A casual introduction to sociological thinking*. NY: Random House, New York, p 13.

2. Mayor D.F. (2001) CNS resonances to peripheral stimulation: is frequency important? *Journal of the AACP* **Nov**, 29-63.

3. Mayor D.F. (ed) (2007) *Electroacupuncture: A practical manual and resource.* Churchill Livingstone (Elsevier), Edinburgh.

4. Brainwave Entrainment to External Rhythmic Stimuli: Interdisciplinary research and clinical perspectives. Saturday, May 13, 2006. Stanford University's Center for Computer Research in Music and Acoustics (CCRMA). http://www.stanford.edu/group/brainwaves/2006/research.html [accessed 24 April 2013].

5. Entrainment Network. http://ethnomusicology.osu.edu/EMW/EntrainNetwork.html [accessed 23 April 2013].

6. Neher A. (1961) Auditory driving observed with scalp electrodes in normal subjects. *Electroencephalogry and Clinical Neurophysiology* **13** (3), 449-51.

7. Schwab K., Ligges C., Jungmann T., Hilgenfeld B., Haueisen J., Witte H. (2006) Alpha entrainment in human electroencephalogram and magnetoencephalogram recordings. *Neuroreport* **17** (17), 1829-33.

8. Cvetkovic D., Cosic I. (2009) EEG inter/intra-hemispheric coherence and asymmetric responses to visual stimulations. *Medical and Biological Engineering and Computing* **47** (10), 1023-34.

9. Koenig T., van Swam C., Dierks T., Hubl D. (2102) Is gamma band EEG synchronization reduced during auditory driving in schizophrenia patients with auditory verbal hallucinations? *Schizophrenia Research* **141** (2-3), 266-70.

10. Goodin P., Ciorciari J., Baker K., Carrey A-M., Harper M, Kaufman J. (2012) A high-density EEG investigation into steady state binaural beat stimulation. *PLoS ONE* **7** (4), e34789. doi:10.1371/journal.pone.0034789

11. Kropotov J.D. (2009) *Quantitative EEG, Event-related Potentials and Neurotherapy*. Academic Press (Elsevier), London.

12. Thompson T., Steffert T., Ros T., Leach J., Gruzelier J. (2008) EEG applications for sport and performance. Methods **45** (4), 279-88.

13. Chen M., Gruzelier J., Steffert T., Thompson T., Leach J. (2008) *Combining transcutaneous electrical acupuncture with neurofeedback training: effects on cognitive performance and EEG spectrum*. Society of Applied Neuroscience, second biennial meeting, Seville, 7-11 May. Revista Española de Neuropsicología **10**, 140. URL http://www.manu.edu.mk/costb27/Abstracts/Seville.pdf.

14. Thatcher R. W., Biver C. J., North D. (2004) EEG coherence and phase delays: comparisons between single reference, average reference and current source density. http://www.appliedneuroscience.com/COMPARISONS-COMMONREF-AVE-LAPLACIAN.pdf [accessed 22 April 2013].

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