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Repetitive low intensity magnetic field stimulation in a neuronal cell line: A metabolomics study

Ivan Hong¹, Andrew Garrett², Garth Maker¹, Ian Mullaney¹, Jennifer Rodger^{Corresp., 2,3}, Sarah J Etherington¹

¹ School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, Australia

² School of Biological Sciences, Experimental and Regenerative Neuroscience, The University of Western Australia, Crawley, WA, Australia

³ Brain Plasticity laboratory, Perron Institute for Translational Neuroscience, Perth, WA, Australia

Corresponding Author: Jennifer Rodger
Email address: jennifer.rodger@uwa.edu.au

Low intensity repetitive magnetic stimulation of neural tissue modulates neuronal excitability and has promising therapeutic potential in the treatment of neurological disorders. However, the underpinning cellular and biochemical mechanisms remain poorly understood. This study investigates the behavioural effects of low intensity repetitive magnetic stimulation (LI-rMS) at a cellular and biochemical level. We delivered LI-rMS (10 mT) at 1 Hz and 10 Hz (n=5 wells per group) to B50 rat neuroblastoma cells *in vitro* for 10 minutes and measured levels of selected metabolites immediately after stimulation. LI-rMS at both frequencies depleted selected tricarboxylic acid (TCA) cycle metabolites without affecting the main energy supplies. Furthermore, LI-rMS effects were frequency-specific with 1 Hz stimulation having stronger effects than 10 Hz. The observed depletion of metabolites was consistent with an increase in GABA release as a result of higher spontaneous activity. Although the absence of organised neural circuits and other cellular contributors (e.g. excitatory neurons and glia) in the B50 cell line limits the degree to which our results can be extrapolated to the human brain, the changes we describe provide novel insights into how LI-rMS modulates neural tissue.

2 **Repetitive Low intensity magnetic field stimulation in a neuronal cell line: a**
3 **metabolomics study**

4 **Ivan Hong**^{1,2}, **Andrew Garrett**², **Garth Maker**¹, **Ian Mullaney**¹, **Jennifer Rodger**^{2,3}, **Sarah**
5 **J. Etherington**¹

6 1. School of Veterinary and Life Sciences, Murdoch University, Murdoch WA.

7 2. Experimental and Regenerative Neuroscience, School of Animal Biology, University of
8 Western Australia, Crawley WA.

9 3. Perron Institute for Neurological and Translational Science, Nedlands, WA

10 **Corresponding author:**

11 Dr Jennifer Rodger
12 Experimental and Regenerative Neuroscience,
13 School of Animal Biology, University of Western Australia.
14 35 Stirling Highway
15 Crawley 6009
16 Australia
17 Email: Jennifer.rodger@uwa.edu.au
18 Tel: (+61) 8 6488 2245

19 **Abstract**

20 Low intensity repetitive magnetic stimulation of neural tissue modulates neuronal excitability and
21 has promising therapeutic potential in the treatment of neurological disorders. However, the
22 underpinning cellular and biochemical mechanisms remain poorly understood. This study
23 investigates the behavioural effects of low intensity repetitive magnetic stimulation (LI-rMS) at a
24 cellular and biochemical level. We delivered LI-rMS (10 mT) at 1 Hz and 10 Hz (n=5 wells per
25 group) to B50 rat neuroblastoma cells *in vitro* for 10 minutes and measured levels of selected
26 metabolites immediately after stimulation. LI-rMS at both frequencies depleted selected
27 tricarboxylic acid (TCA) cycle metabolites without affecting the main energy supplies.
28 Furthermore, LI-rMS effects were frequency-specific with 1 Hz stimulation having stronger
29 effects than 10 Hz. The observed depletion of metabolites was consistent with an increase in
30 GABA release as a result of higher spontaneous activity. Although the absence of organised
31 neural circuits and other cellular contributors (e.g. excitatory neurons and glia) in the B50 cell
32 line limits the degree to which our results can be extrapolated to the human brain, the changes we
33 describe provide novel insights into how LI-rMS modulates neural tissue.

34 Key words: rTMS, ELF-MF, GABA, neuron

35 Introduction

36 Faraday's discovery that a changing magnetic field induces a current in a conductor has
37 contributed to many applications, such as the electromagnetic stimulation of body tissues and
38 organs [Barker et al., 1985]. Specifically, electromagnetic stimulation of brain tissue has
39 significant experimental and therapeutic potential because the induction of electric currents
40 within neurons can modulate neuronal excitability, allowing non-invasive investigation and
41 manipulation of brain circuit function and connectivity. Although clinical applications of
42 magnetic fields most commonly involve high intensity fields that trigger action potentials and
43 activate neural circuits [Müller-Dahlhaus and Vlachos, 2013], therapeutic effects are also
44 observed during low intensity magnetic stimulation [Di Lazzaro et al., 2013; Rohan et al., 2014].
45 For example in humans, low intensity repetitive transcranial magnetic stimulation (LI-rTMS;
46 pulse amplitude <100 mT) is beneficial in treating depression [Martiny et al., 2010] and pain
47 [Shupak et al., 2004] and influences memory [Navarro et al., 2016].

48 It is apparent, however, that cellular mechanisms underpinning behavioural effects of LI-rTMS
49 remain poorly characterised. Many studies in non-neuronal tissues and cells have focussed on the
50 potentially negative effects of low intensity electromagnetic fields in the context of safety
51 concerns surrounding the use of equipment emitting extremely low-frequency magnetic fields
52 (ELF-MF; e.g. [ICNIRP, 1998]). However minimal investigation into low intensity stimulation in
53 neuronal systems has been performed, despite demonstrated evidence of modulation of brain
54 excitability in humans [Capone et al., 2009] and in animal models [Yang et al., 2015; Balassa et
55 al., 2013]. It has been established that the intensity of the induced electric fields in LI-rTMS is
56 not sufficient to depolarise neurons to action potential firing threshold [Davey and Riehl, 2006;
57 Rudiak and Marg, 1994]. In contrast, *in vitro* experiments have consistently shown that low
58 intensity repetitive magnetic stimulation (LI-rMS– no cranium) modulates intracellular calcium

59 levels in non-neuronal [Aldinucci et al., 2000; Walleczek and Budinger, 1992; Zhang et al., 2010]
60 and neuronal cells [Grehl et al., 2015].

61 We recently demonstrated that LI-rMS of dissociated cortical neurons rapidly increases levels of
62 intracellular calcium (within 10 minutes of the onset of stimulation), with higher levels of
63 intracellular calcium detected following 10 Hz compared to 1 Hz stimulation [Grehl et al., 2015].
64 Such modulation of intracellular calcium alters NMDA receptor function [Manikonda et al.,
65 2007] and provides a potential trigger for a wide range of changes in neuronal biochemistry
66 which may underpin the LI-rTMS effects observed clinically [Martiny et al., 2010; Shupak et al.,
67 2004]. Further, there is also evidence that low intensity magnetic fields alter levels of
68 biochemicals that function in neuronal processes, for example, low intensity magnetic fields
69 modulate the level of the primary metabolite of serotonin, 5-HIAA, in rat brain in a dose (time)-
70 dependent manner [Shahbazi-Gahrouei et al., 2016]. In light of these findings, further
71 investigation of biochemical and metabolic changes induced by LI-rMS in neuronal cells is
72 warranted.

73 We hypothesize that changes in biochemical pathways due to LI-rTMS, will modify levels of a
74 range of small molecule metabolites, including amino acids, carbohydrates and organic acids,
75 which can be profiled using metabolomic techniques. Metabolomic analysis that profiles as many
76 metabolites as possible in a single analysis is known as non-targeted screening. We performed
77 such screening of a neuronal cell line immediately following 10 minutes of LI-rMS at 1 Hz or 10
78 Hz *in vitro*. We describe changes in the levels of 12 metabolites, 3 of which changed in a
79 frequency-dependent manner.

80 **METHODS**

81 Cell culture

82 Rat neuroblastoma cells from the B50 cell line were seeded directly onto 6-well plates and grown
83 for 24 hours in media containing DMEM with 5% (v/v) heat-inactivated foetal calf serum, 2mM
84 L-glutamine, 100U/ml streptomycin and 100U/ml penicillin. Cells were grown at 37°C within a
85 CO₂ incubator (5% CO₂ + 95% air). Cells from each 6-well plate were later pooled during
86 extraction to make one replicate. Each stimulus condition or control had 6 replicates in total.

87 LI-rMS stimulation

88 We used LI-rMS parameters that have previously been shown to increase intracellular calcium in
89 primary cultured neurons *in vitro* [Grehl et al., 2015]. Stimulation was delivered to cells in the
90 incubator using custom built round coils (34 mm diameter, 17.1 mm height, 0.812 mm thickness,
91 138 turns). In order to deliver reproducible stimulation to each well, coils were designed to fit
92 within a single well of a 6-well plate so that a plate containing cells could be placed on top of a
93 plate containing 5 coils, resulting in reliable and reproducible placement at a distance of 2.8 mm
94 from the base of each well (Figure. 1A,B). As the stimulator could only accommodate 5 coils,
95 only 5 wells were stimulated on each plate. The coils were driven by a 12 V magnetic pulse
96 generator under control of a programmable micro-controller card (CardLogix, USA), which
97 delivered monophasic pulses (rise time of 0.725 ms). To approximate the stimulation dose in the
98 6-well plate, we measured the magnitude of the magnetic field in the x and y axes with a Hall
99 Effect probe (Honeywell SS94A2D, USA). Magnetic field measurements were made at a
100 distance of 0.5 cm from the base of the coil surface. Magnetic field strengths were converted to
101 dB/dT, as a measure of the change in magnetic field over time of each delivered pulse. A map of
102 dB/dT across the 6-well plate was generated with Matlab 2013a (Figure 1C). Peak magnetic field
103 and dB/dT inside each well ranged between ~2.2 to ~2.4 mT and ~3 to ~3.2 T/s respectively. Coil

104 temperature did not rise above 37°C, ruling out confounding effects of temperature change.
105 Vibration of the coil was assumed to be within vibration amplitude of background (bench
106 surface), as shown previously in Grehl et al.[2015]. For the experiments, 6-well plates were
107 assigned to one of 3 conditions: control (pulse generator switched off, no LI-rMS), 1 Hz
108 (continuous stimulation for 10 minutes at 1 Hz) and 10 Hz (continuous stimulation for 10 minutes
109 at 10 Hz). Stimulation and sham (control) stimulations were performed at 37°C within the CO₂
110 incubator.

111 **Metabolite extraction and derivatisation**

112 Cells were immediately quenched and washed twice post-stimulation with 2 ml of ice-cold
113 phosphate-buffered saline. Cells were then scraped, aggregated and frozen in liquid nitrogen
114 before being freeze-dried. 500 µl of extraction solution consisting of 2.6µg/ml of ¹³C₆-sorbitol, as
115 an internal standard, was dissolved in methanol and added to the dried cells. Cells were lysed
116 with a Precellys 24 tissue lyser (Bertin Technologies, France) at 6500rpm for 40 seconds. The
117 supernatant was evaporated before adding 500 µl of water and freeze-drying again. Samples were
118 derivatised according to the protocol described in Abbiset al.[2012]. 20µl of methoxyamine HCl
119 solution (20mg/ml in pyridine) was added to each extract and agitated at 1200rpm and 30°C for
120 90 minutes. 40µl of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was subsequently
121 added to each extract and agitated at 300rpm and 37°C for 30 minutes. The derivatised samples
122 were transferred to GC vials and 5 µl of alkanes(C₁₀, C₁₂, C₁₅, C₁₉, C₂₂, C₂₈; 0.156 mg.mL⁻¹; C₃₂, C₃₆;
123 0.313 mg/ml) in hexane were added to each sample for calculation of a Kovat's retention index,
124 which aids comparison of data between samples.

125 **Sample analysis**

126 An Agilent 6890 series gas chromatograph with an Agilent 7863 autosampler coupled to an
127 Agilent 5973N single quadrupole gas chromatography-mass spectrometer(GC-MS) (Agilent
128 Technologies, Australia, Mulgrave, Australia) was used along with a Varian Factor Four-fused
129 silica capillary column VF-5ms (30mx0.25mm x 0.25 μ m + 10m EZ-Guard). The analytical
130 method was also as described in Abbisset al.[2012].1 μ l of the sample was injected splitless into
131 the inlet that was set to 230°C. The oventemperature was initially set to 70°C with an initial
132 temperature ramp of 1°C/min for 5 minutes and was subsequently set to 5.63°C/min, to a final
133 temperature of 330°C and held for 10 minutes. The ion source was set to 70 eV and 230°C while
134 the transfer line to the mass spectrometer was set to 330°C. The detector, set to full scan,
135 monitored a mass range of m/z 45-600 at 1 scan per second. The carrier gas, helium, was set at a
136 flow rate of 1ml/min.

137 **Data Analysis**

138 Due to co-elution, the peaks of the $^{13}\text{C}_6$ -sorbitol internal standard and glutamate could not be
139 deconvoluted. Results were therefore normalized using the total ion chromatogram (TIC). The
140 presence of aberrant peaks suggested that two of the 6-well plates (1 Hz stimulation condition)
141 were not adequately washed during the extraction process and these were therefore excluded
142 from the final analysis.

143 GC-MS data were viewed with AnalyzerPro v2.70 (SpectralWorks, Runcorn, UK). The mass
144 spectra of peaks from the chromatogram were matched against the NIST (National Institute of
145 Standards and Technology) mass spectral library. Metabolites with a similarity index of more
146 than 60% were tentatively identified as metabolites. Metabolites with multiple derivative peaks
147 were summed and treated as a single metabolite. Peak areas were normalised to the total ion

148 chromatogram (TIC) and imported into Unscrambler X version 10.1 (CAMO Software, Oslo,
149 Norway). The data was log transformed [$X=\log(x+1)$] and a principal component analysis (PCA)
150 was performed using a non-iterative partial least squares algorithm, cross validation and no
151 rotation. Statistical comparisons between treatment samples were conducted with SPSS v21
152 (IBM, Corporation, Armonk, USA) using a one-way ANOVA with Tukey's post-hoc test.

153 **RESULTS**

154 Based on GC-MS profiling, a total of 18 reproducible intracellular metabolites were identified
155 from the total ion chromatograms (TIC) (Table 1). At 1 Hz, significant decreases were observed
156 in a total of 12 metabolites. This included 7 amino acids, namely alanine, glycine, isoleucine,
157 phenylalanine, serine, threonine (all $p < 0.01$) and aspartate (all $p < 0.05$). Significant decreases
158 were also observed in cholesterol, glycyglutamic acid, inositol, pyroglutamate and succinate (all
159 $p < 0.01$). Non-significant decreases were observed in three carbohydrates: fructose, galactose
160 and glucose.

161 At 10 Hz, significant decreases were observed in a total of 9 metabolites, all of which were also
162 significantly decreased at 1 Hz. These were the amino acids glycine, phenylalanine (both $p <$
163 0.01), isoleucine, serine and threonine (all $p < 0.05$), as well as glycyglutamic acid ($p < 0.01$),
164 cholesterol, pyroglutamate and succinate (all $p < 0.05$). Non-significant decreases were also
165 observed in the same three carbohydrates as for 1 Hz. Measured as a fold change, the decrease
166 observed at 1 Hz was greater than at 10 Hz for all 12 significantly different metabolites.

167 In order to visualise the metabolomic data, relative peak areas (as measured by GC-MS) were
168 subjected to principal component analysis (PCA). The PCA scores plot could be separated into 3
169 groups based on the frequency at which the cells were stimulated (Figure 2). The majority of the
170 variation between control, 1 Hz and 10 Hz could be attributed to principal component 1 (PC-1),
171 which accounted for 63% of the variance.

172 **DISCUSSION**

173 This study demonstrates for the first time that *in vitro* application of LI-rTMS depletes selected
174 metabolites in B50 cells, including numerous amino acids. Furthermore, we confirm that LI-rMS
175 effects are frequency-specific with 1 Hz stimulation having stronger effects than 10 Hz.

176 *LI-rTMS induces specific metabolic changes associated with GABA synthesis*

177 Following LI-rTMS, we did not detect any significant change in the levels of glucose, fructose or
178 galactose, which are key energy supplies to the cell. This is in contrast with outcomes of high
179 intensity rTMS in human and in cat studies, where changes in glucose levels were detected
180 during and immediately after stimulation [Valero-Cabr e et al., 2007]. In the latter studies,
181 glucose uptake was significantly reduced during stimulation, suggesting a local suppression of
182 neuronal firing [Valero-Cabr e et al., 2007]. However, immediately after high frequency, high
183 intensity rTMS, the same regions had increased metabolism [Valero-Cabr e et al., 2005],
184 presumably due to long lasting plastic changes in intracortical circuitry [Walsh and Pascual-
185 Leone, 2003]. Together, these findings suggest that the intensity and frequency of stimulation
186 contribute to changes in metabolic response.

187 However, in contrast to the stability of major carbohydrates, the metabolic profiles of metabolites
188 implicated in GABA synthesis were reduced following LI-rMS. GABA is synthesised via
189 glutamate, from α -ketoglutarate, a principal component of the TCA cycle (Figure 3, box a). We
190 observed depletion of three amino acids involved in *de novo* synthesis of TCA cycle
191 intermediates, aspartate, phenylalanine and isoleucine (see Figure 3, boxes b, c and d,
192 respectively). A possible explanation is that LI-rMS caused increased spontaneous
193 neurotransmitter release in B50 cells, requiring the use of α -ketoglutarate to replenish GABA
194 pools and depleting the downstream TCA cycle intermediates and their substrates (Table 1 and

195 yellow boxes, Figure 3). Similarly, increased GABA synthesis could explain the LI-rTMS
196 dependent reduction in pyroglutamate and alanine [Kumar and Bachhawat, 2012; Westergaard et
197 al., 1993], both of which can be converted to glutamate and subsequently to GABA. In support of
198 this, spontaneous transmitter release is increased following LI-rMS in excitatory neurons [Ahmed
199 and Wieraszko, 2008], and our results suggest a similar increase in spontaneous GABA release
200 from B50 cells.

201 *Reduction in other amino acids and metabolites*

202 The amino acids serine and glycine were also significantly decreased following 1 Hz and 10Hz
203 LI-rMS. The technique used in our study could not differentiate between L- and D-serine.
204 Although both stereoisomers are found in the brain, only L-serine is incorporated into proteins,
205 while D-serine acts as a neuromodulator by co-activating NMDA receptors [Wolosker, 2006].
206 The reduction in serine could be due to increased protein synthesis, which has been shown for
207 specific proteins such as BDNF, c-fos and various neurotransmitter receptors following rTMS
208 [Chervyakov et al., 2015; Rodger et al., 2012]. Glycine is mostly synthesized *de novo* in the brain
209 from serine, rather than taken up via the blood-brain barrier [Shank and Aprison, 1970], and
210 accumulation of glycine in neurons requires the activity of the glycine transporter GlyT2
211 [Gomez et al., 2003]. Thus, disruption of L-serine transport and/or diminished conversion of L-
212 serine into glycine may contribute to the observed reduction in glycine. A key function for serine
213 and glycine is to act as co-agonists for the NMDA receptor, which plays a central role in the long
214 term plastic changes induced by high intensity rTMS[Vlachos et al., 2012], and may also
215 contribute to LI-rTMS effects [Makowiecki et al., 2014; Rodger et al., 2012].

216 *LI-rMS downregulates inositol and cholesterol - implications for calcium signalling and*
217 *exocytosis*

218 Inositol plays a key role as a precursor for inositol lipid and inositol phosphate syntheses, which
219 are vital for signal transduction and intracellular calcium homeostasis [Wen et al., 2011]. A
220 previous study showed that LI-rMS increases the levels of intracellular calcium in cortical
221 neurons by release from intracellular stores, possibly via inositol-1,4,5-triphosphate (IP3)
222 signalling [Grehl et al., 2015]. Inositol depletion as detected here may thus reflect the changes in
223 intracellular signalling events induced by LI-rMS. Cholesterol was also decreased in our study.
224 This lipid is not only important as a structural constituent of lipid rafts and cell membranes, but
225 also modulates vesicle trafficking and exocytosis [Churchward et al., 2005; Lang et al., 2001;
226 Zhang et al., 2009]. A recent study in ageing mice found that rTMS reduced cholesterol that
227 accumulated with age [Wang et al., 2013]. This is important because cholesterol is associated
228 with oxidative stress during normal aging [Cutler et al., 2004], and decreasing cholesterol levels
229 can improve cognition in rats [Wang et al., 2013]. A decrease of cholesterol following rTMS and
230 LI-rTMS, as suggested by our data, may thus contribute to the cognitive improvements observed
231 following rTMS treatment of Alzheimer's patients [Hsu et al., 2015], and also in healthy
232 volunteers [Miniussi and Ruzzoli, 2013].

233 *Frequency-dependent metabolic changes*

234 High and low frequency rTMS have different effects on neuronal circuits, with functional and
235 genetic studies identifying distinct mechanisms in different neuronal cell types [Grehl et al.,
236 2015; Pell et al., 2011]. Previous work using high intensity rTMS in anaesthetised cats
237 demonstrated that metabolic changes were also frequency specific with increased glucose
238 metabolism following high, but not low frequency stimulation [Valero-Cabr e et al., 2007]. Our
239 study provides a different approach, because we examined metabolic changes in a uniform

240 population of neuronal cells with an inhibitory phenotype. Thus our finding that the effects of LI-
241 rMS are stronger with 1 Hz than with 10 Hz matches the inhibitory effect of 1 Hz that is
242 consistently reported in animal and human literature [Chen et al., 1997; Maeda et al., 2000; Pell
243 et al., 2011; Trippe et al., 2009]. However, because LI-rTMS does not induce action potentials, it
244 remains unclear whether the metabolic changes we report are a direct consequence of the
245 electromagnetic field, or whether they are secondary to the changes in excitability induced by
246 rTMS.

247 CONCLUSION

248 Our study suggests that LI-rMS induces a depletion of metabolites consistent with an increase in
249 GABA release as a result of higher spontaneous activity. While the B50 cell line is composed of
250 cells that are electrically excitable and homogenous thus allowing for a targeted examination of
251 the effects of LI-rMS on a defined cell population, the absence of defined neural circuits and
252 other cellular contributors (e.g. excitatory neurons and glia) limits the degree to which our results
253 can be extrapolated to the human brain. Nonetheless, the changes we describe provide novel
254 insights into how LI-rTMS may modulate neural tissue and contribute to our understanding of the
255 therapeutic application of electromagnetic brain stimulation.

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402 **FIGURE LEGENDS**

403 **Figure 1.** Stimulation equipment. A,B: Photographs of the *in vitro* stimulation coils used in this
404 study. Views are from the side (A) and top (B). The coils were situated at a distance of 2.8 mm
405 from the bottom of the culture well because of the thickness of the coverplate and base of the
406 culture dish. C: heatmap showing the measured change in magnetic field for a single pulse
407 (dB/dT) when 6 coils are placed in a 6-well plate arrangement. Note the lack of overlap of
408 magnetic field between the coils/wells of the plates.

409 **Figure 2.** Principal component analysis score plot of intracellular metabolites from GC-MS
410 analysis of cells stimulated with LI-rMS at 1 Hz (n=4), 10 Hz (n=6) and unstimulated controls
411 (n=6).

412 **Figure 3.** TCA cycle. Modified TCA cycle showing effects of LI-rMS on metabolic profile of
413 B50 cells. Metabolites highlighted in yellow were significantly reduced in B50 cells following 1
414 and/or 10 Hz LI-rMS, compared to control B50 cells. The level of glucose (highlighted in green)
415 was measured in our experiments and found to be unchanged by LI-rMS. The observed changes
416 are proposed to result from increased *de novo* GABA synthesis (pathway a) that results in
417 depletion of TCA cycle intermediates and precursors (pathways b, c, d).

418 **Table 1.** Intracellular metabolites identified by GC-MS and PCA as contributing the most to the
419 variance between unstimulated controls and cells stimulated at 1 or 10 Hz and the fold change
420 observed between stimulated cells and controls 1 Hz (n=4), 10 Hz (n=6) and unstimulated
421 controls (n=6). Statistical significance was determined using a one-way ANOVA with Tukey's
422 post-hoc test and is indicated as * = $p \leq 0.05$; ** = $p \leq 0.01$.

Table 1 (on next page)

Fold change of metabolites following magnetic stimulation

Intracellular metabolites identified by GC-MS and PCA as contributing the most to the variance between unstimulated controls and cells stimulated at 1 or 10 Hz and the fold change observed between stimulated cells and controls 1 Hz (n=4), 10 Hz (n=6) and unstimulated controls (n=6). Statistical significance was determined using a one-way ANOVA with Tukey's post-hoc test and is indicated as * = $p \leq 0.05$; ** = $p \leq 0.01$.

Metabolite	Fold change (1Hz)	Fold change (10 Hz)	F-statistic
Amino acids			
Alanine	0.625**	0.873	6.477
Aspartate	0.322*	0.619	3.846
Glycine	0.570**	0.761**	19.162
Isoleucine	0.599**	0.753*	7.835
Phenylalanine	0.379**	0.477**	10.710
Serine	0.389**	0.656*	15.784
Threonine	0.606**	0.764*	11.286
Valine	0.942	0.956	0.064
Carbohydrates			
Fructose	0.638	0.958	1.788
Galactose	0.629	0.948	1.149
Glucose	0.858	0.938	0.161
Other metabolites			
Carbonic acid, 4-methylphenyl phenyl ester	1.268	1.267	2.021
Cholesterol	0.713**	0.831*	8.909
Glycerol-3-phosphate	0.801	0.798	4.155
Glycylglutamic acid	0.526**	0.693**	26.740
Inositol	0.563**	0.742	7.881
Pyroglutamate	0.531**	0.727*	9.603
Succinate	0.640**	0.803*	11.461

Table 1: Fold change between intracellular metabolites of cells stimulated at 1 and 10 Hz vs control. *

p≤0.05, ** p≤0.01

Figure 1

Photographs showing equipment used for magnetic stimulation of cells

Stimulation equipment. A,B: Photographs of the *in vitro* stimulation coils used in this study. Views are from the side (A) and top (B). The coils were situated at a distance of 2.8 mm from the bottom of the culture well because of the thickness of the coverplate and base of the culture dish. C: heatmap showing the measured change in magnetic field for a single pulse (dB/dT) when 6 coils are placed in a 6-well plate arrangement. Note the lack of overlap of magnetic field between the coils/wells of the plates.

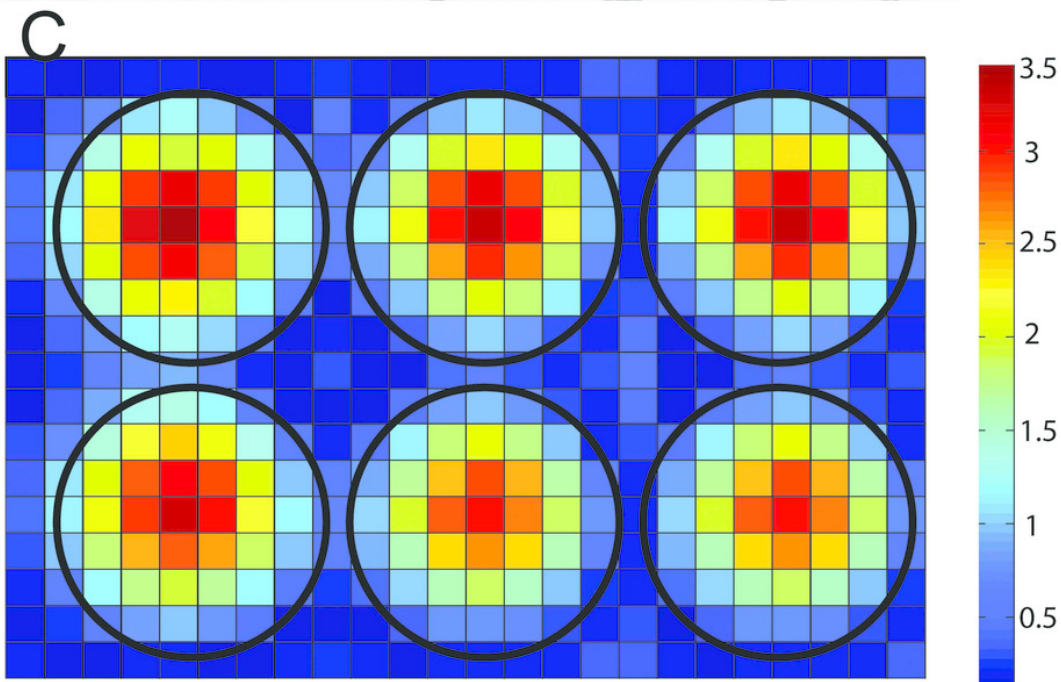
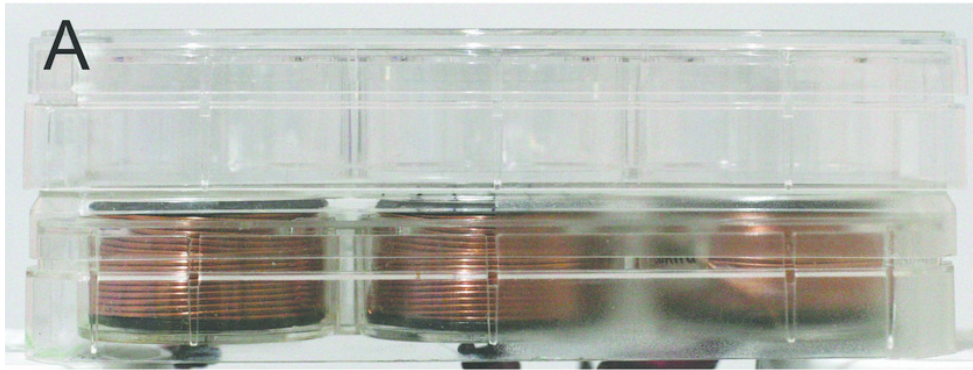


Figure 2

Principal components analysis of metabolites

Principal component analysis score plot of intracellular metabolites from GC-MS analysis of cells stimulated with LI-rMS at 1 Hz (n=4), 10 Hz (n=6) and unstimulated controls (n=6).

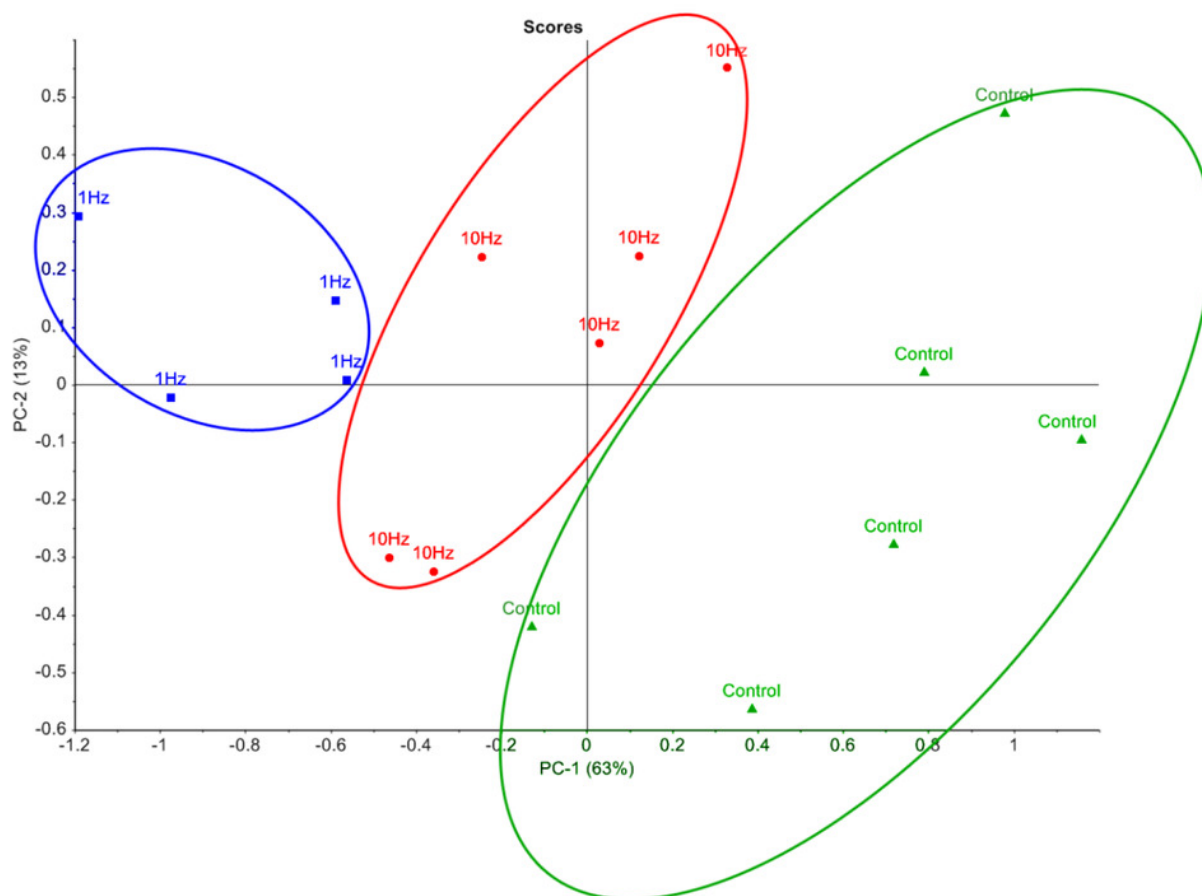


Figure 3

Diagram of TCA cycle highlighting changes observed in our study

TCA cycle. Modified TCA cycle showing effects of LI-rMS on metabolic profile of B50 cells. Metabolites highlighted in yellow were significantly reduced in B50 cells following 1 and/or 10 Hz LI-rMS, compared to control B50 cells. The level of glucose (highlighted in green) was measured in our experiments and found to be unchanged by LI-rMS. The observed changes are proposed to result from increased *de novo* GABA synthesis (pathway a) that results in depletion of TCA cycle intermediates and precursors (pathways b, c, d).

