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In Vitro Study of Neurochemical Changes Following Low-Intensity Magnetic Stimulation

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ABSTRACT Given its ability to modulate neuronal excitability, low-intensity magnetic stimulation (LIMS) has therapeutic potential in the treatment of neurological disorders. However, the underlying of LIMS effects remain poorly understood because LIMS does not directly generate action potentials. We aimed to elucidate these mechanisms by studying and systematically comparing the neurochemical changes induced in vitro by LIMS. To this end, we developed a simple in vitro magnetic stimulation device that allowed delivery of a range of stimulation parameters in order to generate sufficient field intensity for the subthreshold. In characterizing our custom-built system, we conducted computational simulations to determine the electromagnetic field exposure to a cell culture dish. Subsequently, using the custom-built LIMS system, we applied three different stimulation protocols to differentiated neuroblastoma cells for 30 min and then assessed the resultant neurochemical changes. We found that high-frequency (HF: 10 Hz) stimulation increased levels of the excitatory neurotransmitter, glutamate, while low-frequency (LF: 1 Hz) stimulation increased levels of the inhibitory neurotransmitter, GABA. These results suggest that LIMS effects are frequency-dependent: suppression of neuroexcitability occurs at LF and facilitation occurs at HF. Furthermore, we observed pattern-dependent changes when comparing repetitive high-frequency (rHF) and intermittent high-frequency (iHF) stimulations: iHF took more time to induce neurochemical change than rHF. In addition, we found that calcium changes were closely associated with glutamate changes in response to different stimulation parameters. Our experimental findings indicate that LIMS induces the release of neurotransmitters and affects neuronal excitability at magnetic field intensities far lower than suprathreshold, and that this in turn induces action potentials. Therefore, this study provides a cellular framework for understanding how low-intensity magnetic stimulation could affect clinical outcomes.

INDEX TERMS Calcium, GABA, glutamate, neurotransmitter, subthreshold.

I. INTRODUCTION

Magnetic stimulation of body tissues and organs was first described by Barker, 1985 [1]. The operating principle of magnetic stimulation is based on electromagnetic induction, which is described on Faraday's law: a time-varying magnetic field produces an electric field in conductive materials such as human body. In recent years, magnetic stimulation technique has shown significant experimental and therapeutic potential in the treatment of neurological and psychiatric

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disorders by stimulating the central nervous system [2]–[4] or pain relief and in bone generation [5] by stimulating the peripheral nervous system [6]. Currently, the standard clinical application of magnetic stimulation is primarily based on suprathreshold stimulation, in which high-intensity fields (i.e. magnetic field range: 1-2 T; electric field: $>$100 V/m) are used to directly trigger action potentials and depolarize the cell membrane. Despite its significant therapeutic potential, the classical high-intensity magnetic stimulation system is composed of a very large, heavy, and complex system structure: it requires a cooling system as well as thick coils to withstand the heat generated by the strong electromagnetic field.

As an effort to advance magnetic stimulation, low-intensity magnetic stimulation (LIMS; magnetic field range: μT-mT; electric field: $<$1 V/m) and its therapeutic effects have been studied in recent decades [7]–[12]. In comparison to the classical high-intensity stimulation system, LIMS has the advantage of scaled-down system size as well as simple and cost-effective production [13]. In addition, LIMS is a subthreshold stimulation technique because it does not generate a sufficiently high electric field to directly evoke an action potential and depolarize the neuron [14], [15], but rather it modulates neuronal output [16], [17]. Previous experimental studies suggest that low-intensity magnetic exposure could indirectly affect almost all cerebral functions including motor control [18], sensory perception [19], cognitive activities [20], and mood [10]. These findings are supported by the results of neurophysiological studies that have revealed measurable changes in brain electrical activity following low-intensity magnetic exposure [21]–[24].

There is evidence that LIMS can modulate brain function in humans [12] and in animal models [24]; however, the cellular and molecular mechanisms underlying the therapeutic effects of LIMS remain poorly understood. A few *in vitro* and *ex vivo* studies have demonstrated that LIMS alters gene expression [25], intracellular calcium concentrations in non-neuronal cells [26]–[28] and neuronal cells [29], neurobiological changes [30], neuronal excitability [31], and cellular metabolic and biochemical profiles [32]. However, it is still unknown whether LIMS provides facilitation or suppression effects depending on the stimulation protocol. In clinical applications of high-intensity magnetic stimulation (HIMS), the effects are critically dependent on stimulation parameters, including frequency and intensity of stimulation [2]–[4], [33], [34]. For instance, high-frequency (HF: $>$5 Hz) stimulation results in facilitation of neuronal activities, whereas low-frequency (LF: $<$1 Hz) stimulation reduces neuronal activities [35], [36]. In a previous study, Hong *et al.* [32] measured the depletion of metabolites, which may involve an increase in GABA release, and showed that stimulation at 1 Hz is stronger with respect to these effects than 10-Hz stimulation, but did not identify the frequency-dependent effect correlated with neuronal excitability. In addition, the low-intensity fields are too weak to directly trigger action potential and the neurochemical changes may thus not be immediate but rather cause an increase in excitability by reducing action potential threshold and increasing spike firing potential [31]. In a recent study, Poh *et al.* [37] investigated changes in neurochemicals in brain homogenates immediately after low-intensity (12 mT) and high-intensity (1.2 T) repetitive magnetic stimulation at 10 Hz for 10 min; however, they found statistically significant changes in the levels of compounds following HIMS but not after LIMS (relative to the sham control group). To better understand the mechanisms underlying LIMS and increase its utilization, it is necessary to conduct a parametric study in a controlled environment and analyze the data quantitatively not only immediately after stimulation for a longer time but also for longer periods of time.

In the present study, we aimed to identify the neurochemical changes over time after stimulation and to reveal the protocol-dependent effect of LIMS. Specifically, we aimed to examine the changes in the levels of excitatory and inhibitory neurotransmitters, glutamate and γ-aminobutyric acid (GABA), respectively, which are most likely to produce neuromodulation effects [38]. We expected that LF stimulation would be associated with increased GABA concentrations and HF stimulation would be associated with increased glutamate concentrations, consistent with the HIMS frequency-dependent effect. In order to conduct an *in vitro* experiment, we designed and fabricated a simple magnetic stimulation device. Our custom-built system can be easily duplicated and adapted to specific culture conditions for future research. Furthermore, the field levels created by our system were fully characterized using a computational electromagnetic modeling technique. To thoroughly investigate the neurochemical changes induced by LIMS, we used three different stimulation LIMS protocols and assessed the resultant metabolic changes that occurred with each one.

II. MATERIALS AND METHODS

A. CELL CULTURE

A neuroblastoma cell line (SH-SY5Y; Korean Cell Line Bank, 22266) was plated on 10 μg/ml laminin-coated 48-well plates at a density of 5×10^4 cells per well. First, cells were cultured in MEM medium (Gibco) supplemented with 10% fetal bovine serum (Sigma Aldrich), 25 mM HEPES (Gibco), and 1% penicillin and streptomycin (Gibco) for proliferation at 37°C within a CO_2 incubator (5% CO_2+ 95% air). To differentiate cells into neuronal cells, we changed the media to serum free MEM containing 50 nM IGF- 1 and incubated the cells for at least 72 h [39]. Although primary cultures of neurons from the cerebellum and cortex of rats have previously been used to study the release of glutamate and GABA, it is difficult to obtain a sufficient number of cells and characterize protein-protein interactions of neuronal type. Neuronal-like cell lines are also commonly employed for various research purposes instead of primary neurons, and

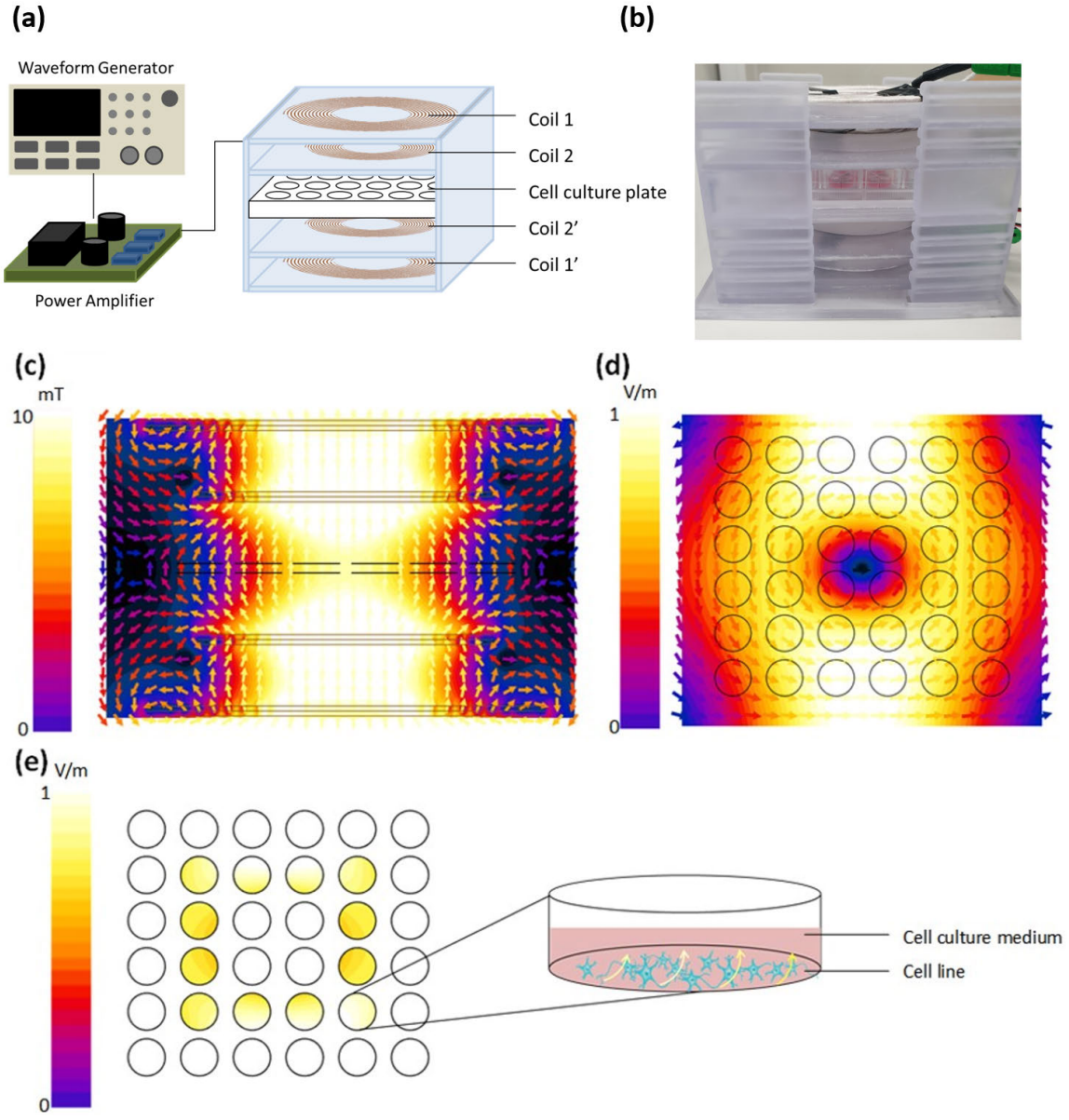

FIGURE 1. (a) A schematic diagram of the in vitro low-intensity magnetic stimulation system. (b) Side-view of the coils. (c) Computationally modeled YZ plane (sagittal) of the magnetic field strength (mT; colors) and vector (arrow) from the coil. (d) Computationally modeled XY plane (axial) of the electric field strength (V/m; colors) and vector (arrow). The cycliners indicate the position of the 48-well cell culture plate and each cylinders is filled with a conductivity of 1.8 S/m. (e) The axial view of the electric field distribution in 12 wells selected to seed cells (left) and example of a zoom-view of a cell-seeding well (right).

thus, we used SH-SY5Y cells, which express several neuronal phenotypes [40] and possess a number of endogenous neuronal receptors [42].

B. DEVELOPMENT OF THE MAGNETIC STIMULATION SYSTEM

We developed an *in vitro* magnetic stimulation system that could deliver constant current to the stimulation coil and allow for the adjustment of parameters such as pulse width, frequency, and amplitude (Fig. 1). Such parameters could be easily adjusted using the integrated waveform generator (33600A Series; Keysight). The circuit of the power amplifier (EVAL127; PowerAmp Design), which was based on the improved Howland current pump, converted the waveforms into a constant current source to the coil. During stimulation, the output waves to the coil were monitored using an oscilloscope (DPO 3032; Tektronix).

The coils were constructed with litz wire (USTC Wire, 0.12 mm/200) to improve coil efficiency [41]. As shown in Fig. 1, there are four parallel coils in two pair configurations, and each pair is organized similar to a Helmholtz coil for

generating the required intensity of an electromagnetic field. When active, the electric current flowed through each coil in the same direction to form a strong magnetic field along the z axis. A pair of outer coils was constructed to a size that fits the cell culture plate's width (outer diameter: 80 mm; inner diameter: 40 mm; number of turns: 30) for covering most wells of the plate. A pair of inner coils, which were smaller than the outer coils (outer diameter: 60 mm; inner diameter: 20 mm; number of turns: 30), was inserted closer to the cell culture plate to form a stronger field. The coil holder was created by a 3D printer. The distance between Coil 1 and Coil 2 was 15 mm, and that between cell culture plate and Coil 2 was also 15 mm. The height, width, and length of the total fixture were 91 mm, 130 mm, and 120 mm, respectively.

Using a computational electromagnetic simulation run by the Magneto Quasi-static Solver of Sim4life software (ver. 4.0), we confirmed that our fabricated coil could generate the required field intensity. Subthreshold studies were conducted in the next field range: an electric field $\leq$ 1 V/m; a magnetic field $\leq$10 mT. Additionally, we aimed to create a magnetic field perpendicular to our culture cell; thus, the electric field would be parallel to the cell. According to previous studies, excitation is much more likely if the electric field is in the direction of the primary axis of the neuron [42], [43]. The cells were seeded into only 12 wells of the 48-well plate (Fig. 1e) to ensure that the electric field was as strong and uniform as possible (this was necessary because neuron excitability is ultimately modulated by the electric field induced by the magnetic field). The conductivity of cell culture medium was measured as 1.8 S/m using a conductivity meter (CP-50N; Istek, Inc.), and this value was used as a simulation parameter. In addition, we ensured that coil temperature did not rise above 1°C, ruling out the confounding effect of temperature change using Sim4life, a thermal solver.

C. EXPERIMENT PROTOCOL

The stimulation was applied with the coils and cells inside a clean bench. The control group (unstimulated) was left on a clean bench for 30 min without stimulation. Three stimulation protocols were used: repetitive high-frequency stimulation (10 Hz, 18,000 pulses), intermittent high-frequency (10 Hz, 1800 pulses), and low-frequency (1 Hz, 1800 pulses), as described in Table 1. The applied stimulation parameters matched those routinely used in clinical high-intensity

TABLE 1. Parameters of three stimulation protocols (LF: low-frequency; rHF: repetitive high-frequency; iHF: intermittent high-frequency).

	Frequency	Pulse width	Inter-pulse interval	Train duration	Inter-train interval	Total duration	Total pulse number
LF	1 Hz	500 us	999.5 ms	30 min	0 s	30 min	1800
rHF	10 Hz	500 us	99.5 ms	30 min	0 s	30 min	18000
iHF	10 Hz	500 us	99.5 ms	1 s	9 s	30 min	1800
Control							

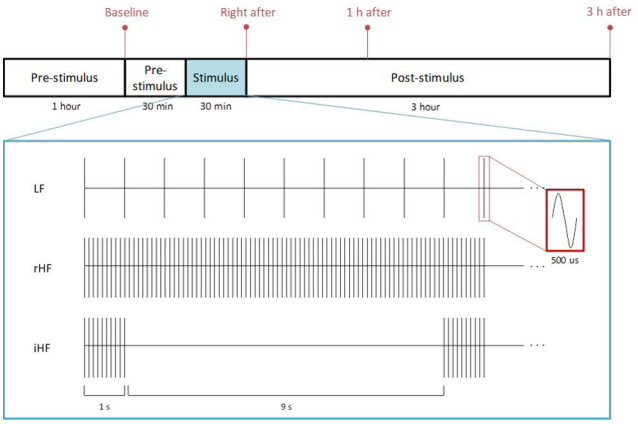

FIGURE 2. Schematic diagram of the experimental protocol and stimulation waveforms (LF: low-frequency; rHF: repetitive high-frequency; iHF: intermittent high-frequency).

stimulations [34], [44]. In all protocols, the current applied to the coils was 10 A, peak to peak.

We evaluated the expression of cells at a total of four time points: baseline, right after stimulation, 1 h after, and 3 h after (Fig. 2). As mentioned previously, we stimulated the 12 wells at once (Fig. 1e). The medium was replaced at baseline point for every well. We then collected the cell culture medium for each four time points at randomly selected 3 wells of 12 sample wells described in Fig. 1e, respectively. We repeated this experiment twice; therefore, the sample size is six for each time points of stimulus condition or control. To stabilize the cell conditions and assess changes over 1 h (similar to the baseline measurement), the cells were left untouched in the medium for 30 min before stimulation (pre-stimulus after baseline in Fig. 2) and then the stimulus was applied for the next 30 min (stimulus: Fig. 2). After removing the medium from each well, the cells were immediately stained with Trypan Blue for measuring the change in cell number.

D. NEUROCHEMICAL ASSESSMENT

The concentration of glutamate in the collected medium was quantified by colorimetric assays (MAK004; Sigma Aldrich). The concentration of GABA in the collected medium was evaluated by ELISA assay (OKEH02564; Aiva Systems Biology). Data of glutamate and GABA were collected by measuring the absorbance at 450 nm using a microplate spectrophotometer (Multiskan GO; Thermo Scientific) and analyzed using a standard curve. Similarly, the concentration of calcium in the collected medium was evaluated by colorimetric assays (MAK022; Sigma Aldrich). However, data were collected by measuring absorbance at 575 nm using a microplate spectrophotometer. A blank sample was included, and all samples and standards were duplicated.

E. QUANTIFICATION OF CELLS

Trypan Blue staining was performed to count live cells following stimulation and at each time point after collecting

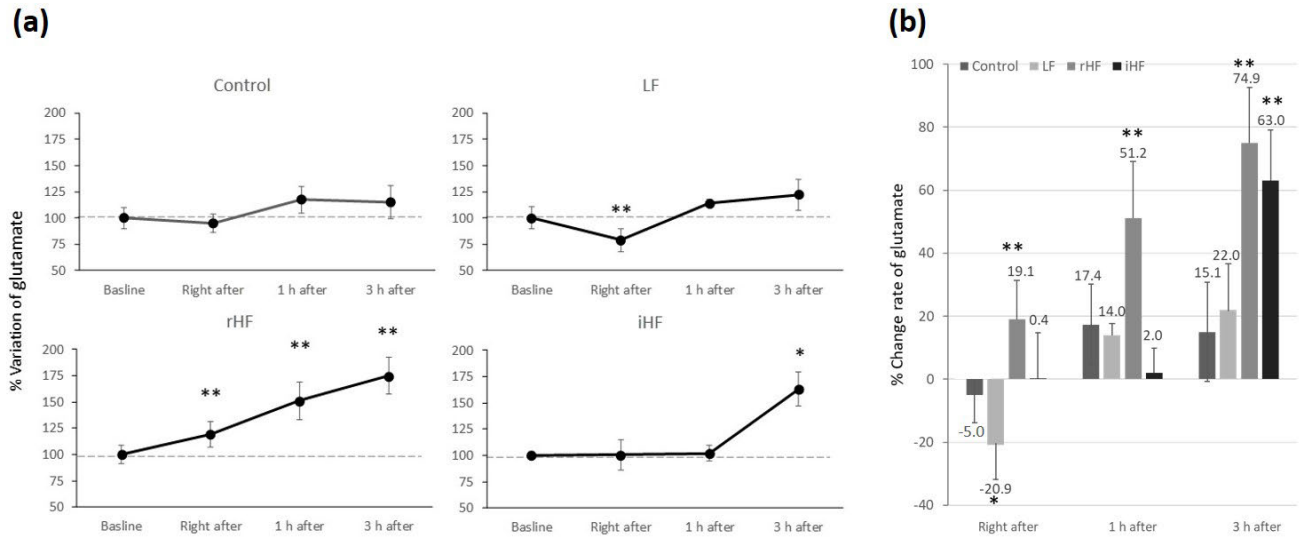

FIGURE 3. Mean variations ± SD (n = 6) in glutamate concentrations. (a) Trends in glutamate levels following stimulation. Mann–Whitney tests were performed to compare levels against the baseline for each group (LF: low-frequency; rHF: repetitive high-frequency; iHF: intermittent high-frequency; ∗p < 0.05, ∗∗p < 0.01). (b) Change rates of glutamate. Mann–Whitney tests were performed to compare the sham control against each stimulus group.

the medium. For staining, we detached the cells using trypsine/EDTA (0.25%) just after collecting the medium for other chemical assay assessments. Subsequently, we dyed the cells with Trypan Blue and counted the live cells using a hemocytometer.

F. STATISTICAL ANALYSIS

All data are given as the mean ± SD. The average of pre-stimulus data was set to 100% as the baseline for each group (i.e. control, LF, rHF, and iHF) per each experiment. The statistical difference in results was confirmed using the Mann–Whitney test. P values <0.05 were considered to be significant.

III. RESULTS

A. CHANGE IN GLUTAMATE LEVEL

HF-LIMS induced significantly increased glutamate concentrations, whereas LF-LIMS induced decreased glutamate concentrations (Fig. 3). Following LF stimulation, glutamate decreased immediately after stimulation and recovered to baseline over time. Following rHF stimulation, glutamate concentration tended to increase steadily and significantly in a continuous manner from immediately after to 3 h after stimulation. Following iHF stimulation, the amount of glutamate did not show any change, but it increased 3 h after stimulation.

In order to exclude any effects of being outside the incubator for 30 min, the change rates of the stimulation groups were compared to that of the control group (Fig. 3b). Following LF stimulation, glutamate concentration decreased immediately after stimulation (20.9% ± 10.9% decrease) and then increased to levels that were not significantly different from the control. After rHF stimulation, glutamate increased by 19.1% ± 12.2% right after stimulation, 51.2% ± 18.0% 1 h after, and 74.9% ± 17.7% 3 h. Following iHF stimulation, glutamate concentration first remained constant and then increased rapidly 3 h after stimulation (63.0% ± 16.1% increase).

B. CHANGE IN GABA LEVEL

Remarkably, GABA concentrations tended to increase after LF stimulation, whereas they tended to decrease after HF stimulation (Fig. 4). Following LF stimulation, GABA concentrations began to increase immediately after stimulation and showed a significant change 1 h after and continued to significantly increase thereafter compared to baseline. In contrast, GABA values decreased 1 h after rHF stimulation and 3 h after stimulation.

We also compared the change rate between the control group and stimulated group (Fig. 4b). Specifically, after LF stimulation, the change rate in GABA showed statistically significant increase of 1.6% ± 3.0% immediately after, 10.5% ± 8.5% 1 h after, and 15.9% ± 4.7% 3 h after stimulation. However, there were no statistically significant differences between the control and both HF groups, with one exception: 1 h after rHF stimulation (15.6% ± 7.0% decrease).

C. CHANGE IN CALCIUM LEVEL

The concentrations of Ca^{2+} decreased in the LF group and increased in the HF groups compared to the control (Fig. 5). With LF stimulation, Ca^{2+} levels gradually decreased and reached levels that were statistically different to the control after 3 h. In the rHF group, Ca^{2+} levels significantly increased immediately after stimulation; in the iHF group, Ca^{2+} levels significantly increased after 1 h of stimulation.

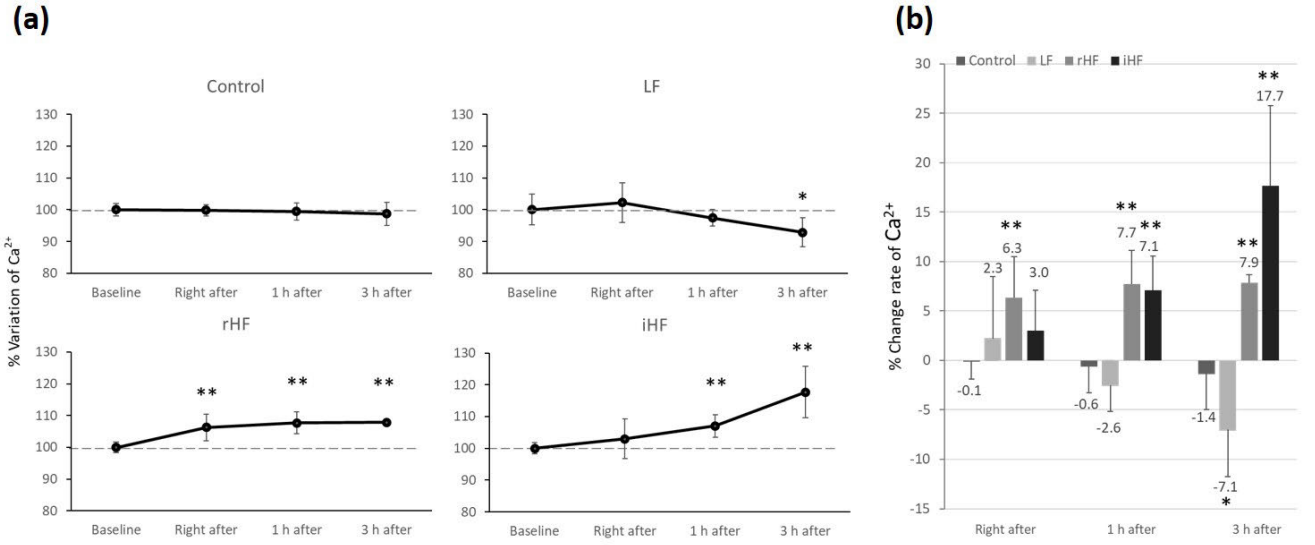

FIGURE 4. Mean variations ± SD (n = 6) in calcium concentrations. (a) Trends in calcium levels following stimulation. Mann–Whitney tests were performed to compare levels against the baseline for each group (LF: low-frequency; rHF: repetitive high-frequency; iHF: intermittent high-frequency; $*p < 0.05$, $**p < 0.01$). (b) Change rates of calcium. Mann–Whitney tests were performed to compare the sham control against each stimulus group.

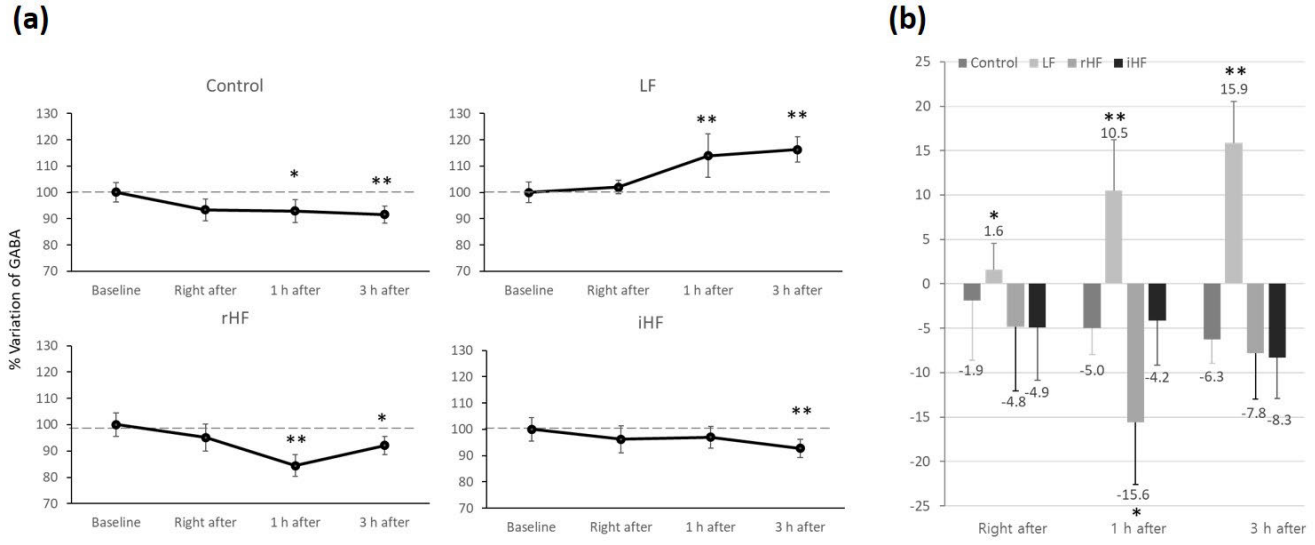

FIGURE 5. Mean variations ± SD (n = 6) in GABA concentrations. (a) Trends in GABA levels following stimulation. Mann–Whitney tests were performed to compare levels against baseline for each group (LF: low-frequency; rHF: repetitive high-frequency; iHF: intermittent high-frequency; $*p < 0.05$, $**p < 0.01$). (b) Change rates of GABA. Mann–Whitney tests were performed to compare the sham control against each stimulus group.

Compared to the change rate of the control group, Ca^{2+} concentrations decreased significantly 3 h after LF stimulation (7.1% ± 4.6% decrease). However, with rHF stimulation, the Ca^{2+} concentrations significantly increased right after (6.3% ± 4.2%), 1 h after (7.7% ± 3.4%), and 3 h after (7.9% ± 0.8%) stimulation relative to the baseline. With iHF stimulation, the Ca^{2+} concentrations showed a significant increase 1 h after (7.1% ± 3.5%) and 3 h after (17.7% ± 8.1%) stimulation compared to the baseline.

D. CHANGE IN THE NUMBER OF CELLS

There were no significant differences in the number of cells counted pre-stimulation and post-stimulation (Fig. 6). Therefore, the low-intensity electromagnetic field apparently did not significantly induce cell death or proliferation during our experiment.

IV. DISCUSSION

Magnetic stimulation has been rapidly accepted as a non-invasive and easy-to-use therapeutic modality, but its

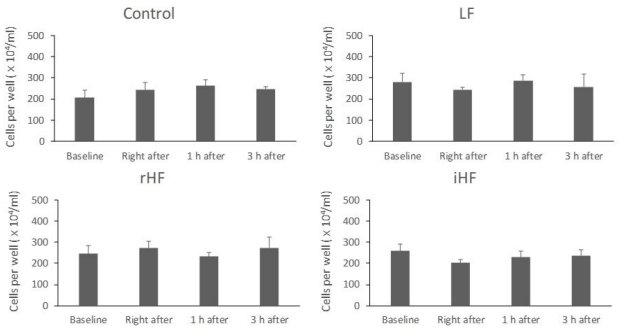

FIGURE 6. Mean variations ± SD (n = 6) in number of cells at each time point (baseline, right after, 1 h after, 3 h after). Mann–Whitney tests were performed to compare between the pre-stimulus and post-stimulus data for each group (LF: low-frequency; rHF: repetitive high-frequency; iHF: intermittent high-frequency).

biological effects remain poorly characterized owing to most studies of magnetic stimulation having been performed in humans in which the cellular and molecular changes could not be adequately measured. While LIMS has recently received interest as a therapeutic method, the cellular and neurochemical mechanisms underlying its therapeutic effects are yet to be documented. Furthermore, the protocol of high-intensity stimulation is restricted according to its purpose and safety consideration. To our knowledge, this study is the first to demonstrate the protocol specific neurochemical changes of LIMS over time.

To conduct this study, we designed and constructed a scaled-down stimulation system suitable for *in vitro* experiments. This system can be tailored to meet the requirements of subthreshold stimulation intensity and it enables easy alteration of the important stimulation parameters. The main advantage of our system is that it can be easily built in a lab environment because of its simple structure. As previously stated, it permits relatively easy and fast changes to stimulation parameters, and, to achieve this, the basic design of the electronic circuit does not need to be altered; thus, the system has increased applicability to a range of experimental requirements.

Our *in vitro* experiments with the custom-built LIMS system confirmed that subthreshold magnetic stimulation could induce changes in neurochemical metabolites, namely glutamate, GABA, and Ca^{2+}. In particular, glutamate and GABA were the main excitatory and inhibitory neurotransmitters, respectively. The alternation of neuronal excitability may be influenced by changes in neurotransmitter turnover favoring (1) an increase in release, (2) reduced efficiency of the uptake system, or (3) both. As shown by previous studies of TMS, which is a commonly used high-intensity stimulation technique in clinics, LF stimulation induces inhibitory effects, whereas HF stimulation is associated with excitatory effects [34], [35]. The results of our study are in agreement with the data from TMS studies in which neurochemical change was measured.

Our results confirmed that LIMS also has a frequency-dependent effect. We demonstrated that LF stimulation induced the higher levels of the inhibitory neurotransmitter, GABA, and lower levels of the excitatory neurotransmitter, glutamate, detected compared to the control. Conversely, HF stimulation increased the level of excitatory neurotransmitter and decreased the level of inhibitory neurotransmitter. The observed changes to Ca^{2+} concentration showed a similar trend to that shown by glutamate and contrary to that shown by GABA. The possibility that the effect of LIMS is determined by the stimulation parameter, such as frequency, rhythm, and number of pulses, was also discussed by Zhang *et al.* [28], but they did not match the number of pulses with those used in a different protocol. However, although iHF (10 Hz; 18000 pulses) and LF (1 Hz; 18000 pulses) delivered the same number of pulses, we showed that the neurochemical change in iHF showed a trend similar to that in rHF (10Hz; 180000 pulses) and opposite to that in LF. Our results showed that LF-LIMS has inhibitory effects and HF-LIMS has excitatory effects, which is consistent with the study by Tang *et al.* [31], who found that 1 Hz has a stronger effect in neuronal cells with an inhibitory phenotype than 10 Hz.

We also found that the response time to stimulation depended on the stimulation pattern at the same frequency. Overall, the neurochemical profile changes observed for iHF took longer than those of rHF. LIMS induces subthreshold changes in membrane potential and thereby influences the level of excitability instead of eliciting an action potential [30]. Therefore, one possible explanation is that the changes induced by iHF are slower than those induced by other stimulus groups that involve continuous stimulation because the membrane potential is likely to slightly recover during the intertrain interval of iHF. This delay due to intertrain agreed with the results of Vlachos *et al.* [45] although they used a much higher intensity, a significant increase in miniature excitatory postsynaptic potential amplitude was detected after 2 h of stimulation and the amplitude returned to baseline after 6 h of stimulation when a stimulation protocol with 30 s intertrain was used. On the other hand, it may have a faster response because rHF delivers more pulses per hour. Zhang *et al.* [28] also suggested that the rhythm with which the pulses are delivered is fundamental to LIMS effect. In summary, our study confirms the hypothesis that LIMS has various effects depending on the parameters. Therefore, it means that appropriately designed LIMS protocols may generate highly adaptable therapies to treat a wide range of neurological conditions by modulating the levels of biochemical substances that play a role in neuronal processes [46], [47].

One limitation of this study is that we focused only on extracellular neurochemical concentrations in the cell culture medium. That being so, it remains unclear whether the neurochemical changes we reported arose as a direct consequence of the release of the substances or as secondary effect related to re-uptake or both. We can speculate that our results, i.e., the

chemical changes in intracellular stores rather than an influx from the extracellular medium, are based on the assumption that Ca^{2+} and other chemicals behave similarly [28]. Another limitation is that our experiment did not assess changes that occurred >3 h after stimulation; thus, we cannot confirm how long the neurochemical changes last. Vlachos *et al*. [45] showed that the repetitive magnetic stimulation-induced increase in excitatory synaptic strength of CA1 pyramidal neurons returns back to baseline levels around 6-8 h after stimulation. Additionally, experimental evidence indicates that repetitive TMS can change the excitability of the human cortex for hours after the stimulation period [48]. Therefore, and extended investigation, in which neurochemical changes are measured until their levels return to the baseline value, is warranted.

Despite these limitations, our study provides valuable insights into the functional aspects of neurochemical change following LIMS. Our finding of the protocol-dependent effects of LIMS, suppression, or facilitation in neuronal excitability suggest the possibility of it clinical use because the frequency of stimulation has always been considered as the main determinant of the direction of excitability modulation. This study may provide a guide for choosing LIMS protocol including frequency, pattern, duration, and number of pulses. We also confirm that LIMS can alter levels of neurochemicals that function in neuronal processes immediately after stimulation although they have an intensity lower than the threshold that causes the action potential. Moreover, this modulation of neurochemicals provides a potential trigger for a wide range of changes in neuronal biochemistry, which suggests that LIMS may have more as yet undefined clinical applications. Especially, because the two neurotransmitter, glutamate and GABA, systems have been regarded as an important target for many central nervous system diseases [49], [50], LIMS may be responsible for a potential therapeutic use in a variety of psychiatric diseases.

V. CONCLUSION

Here, we constructed a novel *in vitro* LIMS system that could be applied in a variety of future lab-based experiments because of its size, ease-of-use, and adaptability. Using this system, we assessed the changes to glutamate, GABA and calcium concentrations from cell culture media at three different time points after a single 30-min stimulation session and at pre-stimulation. Our results showed that LIMS induces a protocol-dependent effect in that HF stimulation facilitates neuroexcitability while LF stimulation suppresses neuroexcitability. Our findings provide useful insights that improve our understanding of the cellular mechanisms underlying the therapeutic effects of LIMS

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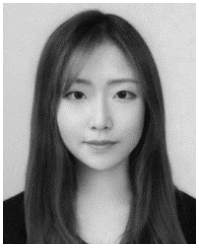

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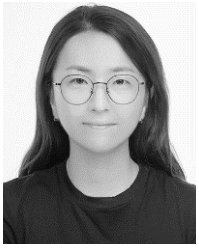

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