Effects of optogenetic and visual stimulation on gamma activity in the visual cortex

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Abstract:

Studying brain functions and activity during gamma oscillations can be a challenge because it requires careful planning to create the necessary conditions for a controlled experiment. Such an experiment consists of placing the brain into a gamma state and investigating cognitive processing with a careful design. Cortical oscillations in the gamma frequency range (30-80 Hz) play an essential role in a variety of cognitive processes, including visual processing and cognition. The present study aims to investigate the effects of a visual stimulus on the primary visual cortex under gamma oscillations. Specifically, we sought to explore the behavior of gamma oscillations triggered by optogenetic stimulation in the II and IV layers of the visual cortex, both with and without concurrent visual stimulation. Our results show that optogenetic stimulation increases the power of gamma oscillation in both layers of the visual cortex. However, the combined stimuli resulted in a reduction of gamma power in layer II and an increase and reinforcement in gamma power in layer IV. Modelling the results with the Wilson-Cowan model suggests changes in the input of the excitatory population due to the combined stimuli. In addition, our analysis of the data using the Lempel-Ziv complexity method supports our interpretations from the modeling. Thus, our results suggest that optogenetic stimulation enhances low gamma power in both layers of the visual cortex, while simultaneous visual stimulation has differing effects on the two layers, reducing gamma power in layer II and increasing it in layer IV.

Keywords:


1. Introduction
Cortical oscillations in the gamma frequency range (30-80 Hz) play an essential role for a large range of cognitive processes[1-6], including visual processing and cognition. For example, gamma-band activity correlates with the speed of visual change detection in humans[7]. The interaction between gamma oscillations and visual stimulation has been the subject of numerous studies[8-10]. The visual dependence of the power spectrum of the local field potential (LFP) in the primary visual cortex (V1) was measured by a network model for the visual cortex and it was shown that the gamma oscillation amplitude correlated positively with the stimulus power[11]. It has been shown that laminar-specific recurrent circuits and/or feedback significantly impact visual responses of gamma-band activity in cortico-cortical output layers 2, 3 and 4B[12]. We hypothesized that this describes the difference in the behaviour of the gamma power in layers II and IV.

Optogenetics is a neuromodulation method that uses a combination of techniques from optics and genetics to control and monitor individual neural activities in living tissues while being able to measure these manipulation effects in real-time[13-15]. Optogenetic stimulation has been used as a method in many studies to induce and restore gamma oscillations[16-17]. Recent studies have used optogenetic stimulation to probe the mechanisms and neural circuits underlying gamma oscillations' generation in the neo-cortex[18 - 22]. It has been predicted that gamma oscillations are generated by the synchronous activity of fast-spiking inhibitory interneurons, which was confirmed in the barrel cortex by employing targeted optogenetic manipulation and showing that light-driven activation of fast-spiking interneurons at various frequencies (80-200 Hz) selectively amplified gamma oscillations[23].

The effect of concurrent optogenetic stimulation and other stimuli has been studied in order to investigate neural activity and behaviour. For example, Lu et al[24], performed optogenetic stimulation on two macaque monkeys during their awake resting and reach and grasp states in the primary motor (M1) and ventral premotor (PMv) cortices, while Nassi et al[25], used optogenetic activation of excitatory neurons in alert macaque's primary visual cortex with and without simultaneous visual stimulation. Optogenetic depolarization of excitatory neurons was shown to facilitate or suppress baseline activity, which was consistent with the prediction of the normalisation model. In this study, we investigated (i) gamma oscillations induced by optogenetic stimulation in two different layers in the visual cortex, and (ii) the response of the LFP to simultaneous optogenetic and visual stimulation.

2. Materials and Methods

A schema of the timeline of the experiment is shown in Figure 1. The general setup of the experiment is shown in Figure 2.
Figure 1 Timeline of the experiment: The animal was anaesthetized with Ketamine/xylazine. After craniotomy, injections of the lentiviruses carrying hchR2(H134R)-mCherry gene under the control of the CaMKII promoter were performed at two different depths (200 microns and 500 microns). Two weeks later the animal was anaesthetized with urethane (see the Appendix), a craniotomy was performed and LFP recording from layer II (200 microns) of V1 was performed under four different conditions: control, visual, laser, visual+laser groups. There were three consecutive trials for each condition. Each trial lasted one minute. After a five minute refractory period, the experiment was repeated with LFP recordings taken from layer IV (500 microns).

Figure 2. Schematic representation of set up for stereotaxic surgery and local field potential recording from layer II and IV of primary visual cortex. Recording was performed in the left hemisphere while the
anesthetized rat was subjected to optogenetic stimulation of the exposed visual cortex or a drifting grating stimulus presented on a monitor on the right side of the animal.

2.1. Animals

Ten naive rats of either sex housed under a constant 12h dark/light cycle and standard temperature conditions (20 ± 2°C) with food and water ad libitum were used in our study. All experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the ethics committee, Shahid Beheshti University of Medical Sciences, ethical code IR.SBMU.PHNS.REC. 1398.024.

In order to prepare for the experiment, the following procedure was performed: the animals were anaesthetized with ketamine/xylazine (ip, 80/2.5 mg/kg). During the procedure, their body temperature was maintained at 36.0-36.5°C by a rectal thermos probe feeding back to a heating pad (ATC-402, Unique Medical). A small custom-made head plate to stabilize the animal's head under virus injection and LFP recording was attached over the occipital region of the left hemisphere. After implanting the cranial window over the primary visual cortex (V1) (3-9 mm posterior to Bregma; 1-5 mm lateral), a small part of the skull and dura mater were removed and the exposed cortex was covered by Ringer’s solution. We injected the lentiviruses carrying hchR2(H134R)-mCherry gene under the control of the CaMKII promoter (1.5-2 µl) in layers II-IV (200 - 500 µm) of V1 by applying positive pressure to the glass electrode (pipette).

Subjects were in deep anesthesia for the entirety of the experiment. To ensure this, we periodically checked the degree of anesthesia by pinching the rat's leg or touching the cornea of its eyes. If the rat reacted, we would inject a booster dose of anesthesia. In this way the rat was kept in a state of deep anaesthesia.

2.2. Visual Stimulation, optogenetic stimulation and experimental paradigm

The visual stimulus consisted of a square-wave drifting grating (0.08 cycles/deg at 100 mW/mm² contrast) with one of 12 possible orientations differing by 30 degree steps. The 12 patterns were presented in a randomly shuffled order. The stimulus was shown on an LCD monitor and covered 80*50 degrees of the visual field at a viewing distance of 28 cm[26]. Exposure time was 3 seconds with an inter stimulus interval (ISI) of 2 seconds. See Figure 3. To perform optogenetic stimulation we expressed channelrhodopsin-2 (ChR2) using the lentivirus method [27]. Injections of the lentiviruses carrying hchR2(H134R)-mCherry gene under the control of the CaMKII promoter were performed at two different depths in the visual cortex (200 µm and 500 µm) two weeks before performing LFP recordings at each layer during laser and visual stimulation.

A blue laser light (470 nm) was directed into an optical fiber (700 µm diameter), mounted on a holder for positioning in the recording chamber. The laser's output function was tested with the optical power meter at the tip of the optical fiber. Only a low total output power may be needed to achieve ChR2 activation. We used three different laser intensities in each trial (input: 50 mW/mm² and output: 5 mW/mm²; input: 75 mW/mm² and output: 31 mW/mm²; input: 100 mW/mm² and output 80 mW/mm²) The laser was pulsed at a frequency of 20 Hz (5 milliseconds off and 45 milliseconds stimulus on). Exposure time to the stimulus was 3 seconds with an ISI of 2 seconds (See Figure 3).
Figure 3 Schematic depiction of visual and laser stimuli: The visual stimulus is a square-wave drifting grating with a randomly selected orientation. The laser stimulus consists of a blue laser. The laser was pulsed at a frequency of 20 Hz (5 milliseconds off and 45 milliseconds laser pulse on). Each trial takes one minute. It is composed of 12 cycles, each cycle taking 5 seconds. A stimulus was presented for a duration of 3 seconds with an ISI of 2 seconds. The stimulus was either a visual stimulus of only, a laser stimulus only or concurrent visual and laser stimuli.

In order to analyse the effects of simultaneous optogenetic and visual stimulation on the LFP in these layers, recordings were made for the following four of experiments: (i) control group with both laser and visual stimuli switched off, (ii) visual stimulation group with only the visual stimulus switched on, (ii) laser stimulation group with only the laser stimulus switched on, and (iv) the visual and laser stimulation group with both the visual and laser stimulation switched on.

LFPs were recorded in the rat’s visual cortex during their comatose states using intra-cortical one-channel extracellular techniques. Data were analyzed by comparing the gamma power across different conditions and groups. In addition, modelling of the results were performed using the Wilson-Cowan model[28] which has previously been used to explain enhanced gamma oscillations[29].

2.3. Recording of local field potentials

Animals were anaesthetized with urethane (ip, 1.5 mg/g body weight) and then placed in a stereotaxic frame (craniotomy). We recorded the LFPs in layer II (200 µm) and layer IV (500 µm) of V1. Recording electrodes consisted of a borosilicate glass capillary with filaments (0.86 mm inner diameter, 1.5 mm outer diameter) filled by Ringer’s solution, in order to cause less damage to the tissue. The resistance of these electrodes was 500 kΩ. LFPs were recorded for the following four groups of experiments: 1) no stimulation, 2) with optogenetic stimulation, 3) with visual stimulation, 4) with combined visual and optogenetic stimulation. Using a molecular device amplifier (Axopatch 200B, Molecular Devices),
recorded data was sampled at 20 kHz, filtered at 2-5 KHz, digitized at 20 KHz, and fed into a personal computer with an NI-DAQ board (PCI-MIO-16E-4, National Instruments).

After draining the Ringer's solution on the surface of the exposed cortex the recording electrode was brought to the chosen area of the visual cortex (chosen for ease of access when recording). In order to locate layer II and IV of the visual cortex we relied on a (Sutter) manipulator controlling the electrode. According to the literature the thickness of layers in the mouse cerebral cortex are as follows: layer I=123±4.9µm, layers II/III=357±9.4µm and layer IV=152±7.0µm. Thus, after detecting the surface of the cortex by observing a change in the test pulse produced by the oscilloscope, we moved the electrode to the appropriate depth (200 µm Layer II and a further 300 µm for layer IV) [30].

2.3.1. Data analysis

Recorded LFP data were band-pass filtered (5-300 Hz, FIR) and then the gamma power for the range of 30-150 Hz calculated. Signal power in gamma range from 30 - 150 Hz was calculated for different trials.

To find the pure effect of optogenetic laser stimulation at different layers, we defined the biological response (BR) to this type of stimulus according to:

\[ BR = (P_{\text{Laser+Visual}} - P_{\text{Laser}}) - (P_{\text{Visual}} - P_{\text{Control}}) \]  

where \( P_X \) is the power of the gamma oscillations when exposed to stimulus \( x \). \( P_{\text{Visual}} - P_{\text{Control}} \) then represents change in the power of gamma oscillations when a visual stimulus is applied when compared to the control trial.

\( P_{\text{Laser+Visual}} - P_{\text{Laser}} \) then defines changes caused by the application of a visual stimulus in the case of a system which is undergoing optogenetic stimulation. If the biological response is equal to zero, there is no effect from optogenetic stimulation of visual responses.

We used the ELAN package based on the EEGLAB toolbox to analyze the data[31 - 32].

3. Results

3.1. Experimental results

Figure 4A shows an LFP sample recorded during simultaneous optogenetic and visual stimuli. A comparison of gamma power during the different experiments can be seen in Figure 4B.

To assess the statistical significance of the performance differences between conditions, a t-test was performed for each condition. The statistical significance of the observed performance differences was determined using the p-values obtained from these tests. Applying optogenetic stimulation greatly increases gamma power at both depths compared to the control group. In layer II the addition of a visual stimulus decreases gamma power in comparison to just optogenetic stimulation alone. In layer IV, however, gamma power did not decrease with the addition of a visual stimulus. In fact, gamma power increases significantly with the addition of a visual stimulus when the laser is at full intensity.

In order to analyse the effect of combined optogenetic and visual stimuli, \( P_{\text{Visual}} - P_{\text{Control}} \) and \( P_{\text{Laser+Visual}} - P_{\text{Laser}} \) were compared. The results for the 500 µm and 200 µm conditions are summarized in table 1. A negative biological response at 200 µm could be found while an increase and reinforcement at 500 µm was evident.
Table 1 Biological response at two depths, 500 and 200µ, against different laser intensities (50, 75 and 100 mW/mm²)

<table>
<thead>
<tr>
<th>Laser Intensity mW/mm²</th>
<th>P Visual - P Control</th>
<th>P Laser+Visual - P Laser</th>
<th>(P Laser+Visual - P Laser) - (P Visual - P Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>Coefficient (× 10⁻⁵)</td>
<td>(× 10⁻⁴)</td>
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<tr>
<td>Depths</td>
<td></td>
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<tr>
<td>500 µ</td>
<td>0.63±8</td>
<td>1±9</td>
<td>20±23</td>
</tr>
<tr>
<td>200 µ</td>
<td>-0.13±1</td>
<td>-18±2.2</td>
<td>54±6.7</td>
</tr>
</tbody>
</table>

Figure 4 A) LFP was recorded under laser and visual stimulation in two different layers of the visual cortex. B) Mean gamma power (blue for depth 200µ (Layer II) and red for depth 500µ, normalized by dividing by the maximum gamma power across experiments). Green bars give the standard error; p-value < 0.02. C) Non-monotonic behaviour of gamma power resulting from increasing input current to the
excitatory population. For both layers, we use the same weights and hypothesize that the difference in gamma power between the two layers, could be connected to the quantity of input current to the excitatory neurons ($J_e$) to the neurons in that layer. D) Variation of gamma power in response to different amounts of excitatory and inhibitory time constants and input. The first 1.5 seconds: Control + Visual. From 1.5 seconds to 4 seconds: Control+ Visual+Laser. a: The fourth trial in Laser+Visual in 500 microns (There is no optogenetic stimulus in the first 1.5 seconds.) b: Laser stimulus has a period similar to the experiment and the amplitude of 0.5, $\tau_i = 0.0016$ and $\tau_e = 0.0032$ (There is no optogenetic stimulus in the first 1.5 seconds, i.e.,$J_e$ is only from the visual stimulus and control Figure8C.).

To clarify the differences between visual and control trials, we calculated power spectrum density (PSD). As it is shown in figure 5 we can see gamma power in visual trials are slightly more than the control trial (error bars are standard error). The power spectrum densities for control, visual, laser, and visual+laser stimulation conditions are in the appendix (Figure A1 and A2).

The analysis of the gamma power data with a Bayesian ANOVA revealed that the data could be best described by a model with the terms “laser intensity”, “condition” (comprising “control, “laser”, “visual” and “laser + visual”), “tissue layer” and an interaction of the terms “condition” and “tissue layer” (Bayes Factor (BF)) = 121.492 (see Figure 6). With regard to differences in gamma power between the two tissue layers, post-hoc analysis (Bayesian Mann-Whitney U test) showed highly statistically significant differences (FB > 100) for the conditions “control” (BF = 1.368E+6, W = 37437, R2 = 1.022), “visual” (BF = 2.708E+6, W= 16562, R2= 1.017) and laser 50%” (BF = 222.346, W = 853, R2= 1.047).

A difference of gamma power between the two tissue layers was not evident (BF < 10) for the conditions “laser 75%” (BF = 1.497, W = 982, R2 = 1.155), “laser 100%” (BF = 1.598, W = 1915, R2 = 1.020), “laser 50% + visual” (BF = 0.232, W = 1272, R2 = 1.049), “laser 75% + visual” (BF= 0.226, W = 1308, R2 = 1.018) and “laser 100% + visual” (BF = 0.440, W = 576, R2 = 1.049).

Comparing the gamma power data for the two conditions “laser” and “laser + visual” (Bayesian ANOVA with the fixed factor “tissue layer” and “laser intensity”) revealed that (i) statistically significantly different (BF > 5) gamma powers for the two layers (condition “laser”: BF = 10.531; condition “laser + visual”: BF= 8.109) with (i) a weaker increase in gamma power in layer IV compared to layer II for the condition “laser”, but (ii) a stronger increase in gamma power in layer IV compared to layer II for the condition “laser + visual”.
Figure 5: Average power spectrum density for the gamma activity in control and visual trials. 

- **A**: Layer 200 µm.
- **B**: Layer 500 µm.

(Additional figures, Figs. A2-A3, can be seen in the appendix.)

Figure 6: Normalized gamma power as a function of the experimental conditions, tissue layer, and laser intensities. An opposite trend of the gamma power of the two layers can be seen: an increase (decrease, respectively) in the power upon the laser + visual stimulation (compared to only laser stimulation) in layer II (layer IV, respectively).
Figure 7 Analysis of the Permutation Lempel-Ziv complexity (PLZC) in the low 30-80Hz (right) and high 80-150Hz (left) gamma band, for the layer at 200 microns (top) and 500 microns (bottom). Each graph shows the complexity comparison for the Controls, Visual, Laser (L) and Laser + Visual (LV) states. The asterisks represent the statistical significance using the Mann-Whitney test (*<p=0.05) between the state and the control group. The ordinal patterns’ parameter using here were d=4 and r=1.

4. Discussion and Conclusions

Gamma oscillations are critical for cortical function and have been extensively studied in relation to cognitive processes. Our study investigated the effect of optogenetic stimulation on gamma oscillations in two layers of the visual cortex and the response to simultaneous visual stimulation. Our results show that optogenetic stimulation increases low gamma power in both layers of the visual cortex. Simultaneous visual stimulation had different effects in the two layers, reducing gamma power in layer II and increasing it in layer IV. Our simulations using the Wilson-Cowan model suggest that differing currents injected to the excitatory population can play a crucial role in modeling the stimulus. The Lempel-Ziv complexity method revealed an increase in complexity in the low gamma range (30-80 Hz) for both layers in response to optogenetic stimulation and laser + visual stimulus, while no significant changes were observed in the high gamma range, 80-150 Hz.

These findings suggest a specific role for low gamma oscillations in response to optogenetic stimulation and may indicate a more specific role for high gamma oscillations in the visual cortex. Our study found that optogenetic stimulation induced gamma oscillations in both depths of the visual cortex for all laser intensities. The nonmonotonic response of gamma oscillations suggests that changes in gamma power might be due to the number of activated inhibitory interneurons or the visual stimulus size. Our experiment activated excitatory neurons with the opsin, which may have caused inhibitory neurons to
activate, leading to a reduction or insignificant changes in gamma power. In future studies, different stimulation frequencies and investigating the tuning curve based on gamma power for preferred and anti-preferred stimuli could be explored. Further investigations are necessary to fully understand the contributions of low and high gamma oscillations to visual processing.

**Conflict of Interest**
The authors declare no competing interests.

**Author Contributions**
FA, MDH, and MSS proposed the idea, FA, Sa.R performed the experiments in the lab of MSS with getting help from the lab of MIZ. MDH and FA discussed the model with VS, FS, Se.R, DMM, and RB, and then MDH, FA, and DMM developed the models for experimental data under the supervision of VS and SeR. All authors contributed to discussions, and wrote the manuscript.

**Funding**
SR and RB are grateful for the financial support from the Basque Government through the BERC 2022-2025 program and by the Ministry of Science and Innovation. Moreover, SR and RB are grateful for the support from BCAM Severo Ochoa accreditation CEX2021-001142-S / MICIN / AEI / 10.13039/501100011033. SR further acknowledges Elkartek project SILICON BURMUIN no. KK-2023/00090. All authors gratefully acknowledge financial support from their affiliated institution.

**Ethics Approval**
All experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the ethics committee, Shahid Beheshti University of Medical Sciences, ethical code IR.SBMU.PHNS.REC. 1398.024. The study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

**Acknowledgments**
Authors are very thankful for valuable discussions with Dr. Leila Dargahi, and Dr. Udo Ernst for their critical and constructive comments, and thank Mehdi Aslani for graphical representation of the experimental setup under the supervision of VS.

**Data Availability Statement**
The datasets for this study are available based on reasonable requests.

**References:**


Park, K. *et al.* Optogenetic activation of parvalbumin and somatostatin interneurons selectively restores theta-nested gamma oscillations and oscillation-induced spike timing-dependent


Supplementary Material

Effects of optogenetic and visual stimulation on gamma activity in the visual cortex

- The effect of anesthesia

In our experiments, we expect that urethane as an anesthetic would not alter the dynamics of the visual cortex. Sorrenti et al. [A1] highlights the advantages of urethane for investigations of neuronal function in visual, somatosensory, and hippocampal cortical regions. According to their findings, urethane appears to have a lesser impact on sensory-evoked responses and neuronal discharge patterns compared to volatile or barbiturate anesthetics [A2-A4]. Urethane offers several benefits in animal anesthesia, including the ability to administer it through various parenteral routes, producing long-lasting surgical anesthesia while exerting minimal effects on the autonomic and cardiovascular systems. Furthermore, it is assumed that animals anesthetized with urethane exhibit physiological and pharmacological behaviors similar to those observed in unanesthetized animals [A5]. However, on the other side, Mondino et. al [A6] focused on comparing awake states with subanesthetic levels of urethane and found out that the effect of urethane might be significant and should not be underestimated. It is important to note that their results are not directly comparable to ours due to the use of different urethane doses in our respective works and different nature of experiments. Our analysis involved studying raw signals rather than specific frequency bands, which significantly impacts the interpretation of results. However, the certainty in this context is still challenging. Here, we have used urethane based on previous studies that emphasize the advantages of urethane in specific neuronal investigations, its minimal impact on sensory responses and neuronal discharge patterns compared to other anesthetics.

- Statistical analysis:

The p-values obtained from the tests were used to determine the statistical significance of the observed performance differences. Here, the Mann-Whitney test is a non-parametric statistical test used to compare the medians of two independent groups. It is often employed when the data do not meet the assumptions required for parametric tests such as the t-test. It was first proposed in 1945 by Frank Wilcoxon for samples of equal sizes and extended to samples of arbitrary size by Henry B. Mann and D. R. Whitney in 1947 [A7-A8]. The Mann-Whitney test assumes that the two groups being compared have independent observations, and the data are at least ordinal in nature. It does not require the assumption of normality, making it useful for analyzing skewed or non-normally distributed data.

Null hypothesis (H₀) and alternative hypothesis (H₁):

The null hypothesis states that there is no difference between the two groups being compared, while the alternative hypothesis suggests that there is a significant difference.

Ranking the data:

Combine the data from both groups and assign ranks to the observations, regardless of the group they belong to. If there are ties (i.e., multiple observations with the same value), assign them the average rank.

Calculating the U statistic:

The U statistic is calculated by summing up the ranks of one group and comparing it to the sum of the ranks of the other group. The formula for calculating the U statistic varies depending on the sample sizes and ties in the data.

Comparison to critical values:
Determine the critical values for the U statistic based on the significance level (e.g., $\alpha = 0.05$).

**Decision:**

Compare the calculated U statistic to the critical values. If the calculated U value is less than or equal to the critical value, then there is no significant difference between the groups (fail to reject the null hypothesis). If the calculated U value is greater than the critical value, then there is a significant difference between the groups (reject the null hypothesis).

**Interpretation:**

If the null hypothesis is rejected, it means that there is evidence to suggest that the two groups have different medians. However, the Mann-Whitney test does not indicate the direction or magnitude of the difference.

- **Lempel-Ziv complexity**

The Lempel-Ziv complexity (LZC) is an information measure based on the Kolmogorov complexity that attempts to find the minimal “information" contained in the sequence[A19]. This complexity has been used in the analysis of different types of neurophysiological signals, in particular LFP has been used in studying the effects of anesthesia, seizures, Parkinson’s disease and sleep, among others[A10 - A13].

To estimate the complexity of a time series $X(t) = \{ x_t ; t=1, ..., T \}$ we will use the Lempel and Ziv method proposed in 1976[A14]. In this approach, a sequence $X(t)$ is parsed into a number $W$ of words by considering any subsequence that has not yet been encountered as a new word. The Lempel-Ziv complexity $c_{LZ}$ is defined as the minimum number of words $W$ required to reconstruct the information contained in the original time series. For example, the sequence 10011011001010001011 can be parsed in 7 words: 1 . 0 . 01 . 101 . 1100 . 1010 . 001011, giving a complexity $c_{LZ}$=7. Kaspar and Schuster develop a easy algorithm to calculate the Lempel-Ziv complexity[A15]. The LZC can be normalized based in the length T of the discrete sequence and the alphabet length (α) as:

$$G_{LZ} = \frac{c_{LZ} \log \alpha}{T}$$

[2]

Although Lempel and Ziv first developed the method for binary sequences, the length of the alphabet could be used for any finite-length alphabet. For continuous sequences (as an electrophysiological signal), it is necessary to discretize the signal first. There are many methods for signal discretization such as histogram, binarization for mean values or multiscale method. Particularly, in this work we use a method introduced by Band and Pompe *ordinals patterns* [A16], which is based on comparison of neighboring values of the time series. The use of the ordinal patterns with the Lempel-Ziv Complexity is known as *Permutation Lempel-Ziv Complexity* (PLZC)[A17]. The ordinal patterns have two parameters embedding dimension D and time delay $\tau$, in our analysis we used the parameters D=3,4,5 and $\tau$=1 obtaining similar results.

- **Our method based on Lempel-Ziv complexity**

First, the data was preprocessed. A band-pass filter butterwood was applied for the bands’ low gamma [30 80 ] Hz and high gamma [80 150] Hz. A notch filter was applied to 50, 100 and 150 Hz. PLZC was analyzed for each signal in each group for both low and high gamma frequency signals and in both layers
(200 microns and 500 microns). The statistical analysis between each stimulation state and the control group was calculated using a non-parametric Mann-Whitney test.

Figure 7 shows the signal's analysis coming from the 200 microns layer analyzed for low gamma bands. We can observe there is a marked increase in complexity for laser stimulation in all the three intensities. There is also an increase in the Laser + visual stimulus for the intensity of 50 and 75. We observed no significant changes in the visual stimulus. For the analysis of high gamma frequency over the same layer, non-significant changes are present between stimulation experiments and control.

For the signals coming from the 500 microns layer, low gamma laser and laser + visual stimuli present a significant increase in complexity in comparison with controls. For high gamma range in the same layer, non-significant changes can be seen between experiments.

- Modelling

To gain further insights into our empirically obtained results, we investigated our experimental results by modelling them with Wilson-Cowan model[A18] following the approach of Onslow[A19]. Our primary objective was to determine how different currents influence the resulting local field potential. We considered the laser stimulus as a source of an external current into the neuronal populations ( \( J_e \) and \( J_i \) in Figure 8). During the laser onset, the outward current, \( J_e \) and \( J_i \), was applied to the neuronal population. In developing this model, we emphasized a descriptive rather than a quantitative focus. Our goal was to provide a general representation of our empirical findings, focusing on the variables that contribute to the observed power variations in gamma oscillations. The model was constructed to account for the patterns seen in the experimental data. In particular, it accounts for the varying power of the gamma oscillations.

It is known that the specific range of injected currents leads to gamma oscillations in population activity[A19]. Because of the type of opsins, the optogenetic laser excites only excitatory neurons. We, therefore, considered a pulse input to the excitatory population in the modeling and investigated the changing gamma power (Figure 8: laser input).

Since, in the model, gamma power is a concave function of excitatory population input (see Figure 4C), we hypothesized that this describes the difference in the behaviour of the gamma power in layers II and IV. Although the laser stimulation increased the activity of the excitatory neurons in layer II, as we have already surpassed the maximum of the graph in Figure 4C, further increases in the excitatory population lead to a decrease in gamma power. The dynamics of the excitatory and inhibitory populations are given in the model by:

\[
\tau_e \frac{dE}{dt} = -E + f(\omega_{ee} E - \omega_{ei} I + \Theta_e + J_e) \quad [3]
\]
\[
\tau_i \frac{dI}{dt} = -I + f(\omega_{ie} E - \omega_{ii} I + \Theta_i + J_i)
\]

where \( E \) and \( I \) represent inhibitory and excitatory population activity, respectively, and each population has a unique membrane time constant \( \tau \). \( \Theta \) is a current coming from linked populations or an applied stimulus to the patch site. \( J_e \) and \( J_i \) are inputs generated by the optogenetic laser and visual stimulation to the excitatory and inhibitory populations, respectively. \( \omega_{\cdot e} \), \( \omega_{\cdot i} \) \( \in \{ ii, ie, ei, ee \} \) are weights giving the synaptic effectiveness between excitatory (e) and inhibitory (i) neuronal populations. Our simulation's initial parameters are \( \omega_{ee} = 2.4, \omega_{ei} = 2.4, \omega_{ie} = 2.4 \) and \( \omega_{ii} = 0 \)[A19]. The threshold function, denoted by \( f \) in equation 3, is expressed as follows:
\[ f(x) = \frac{1}{1 + \exp(-4(x-1))} \]  \[4\]

The LFP is expressed as a function of the excitatory and inhibitory populations[A19].

\[ LFP = 0.8E + 0.2I \]  \[5\]

Figure SA shows the laser stimulus in blue and the resulting LFP is shown in black. As seen in the figure, when the laser is switched on, there is a large jump in the LFP magnitude which induces a high frequency fluctuation in the analysis. Therefore, we analysed LFP only between these spikes. By removing these spikes and focusing on time between two pulses, artifacts do not produce high frequencies. Figure SB illustrates our model based on the hypothesis that the main difference between layer II and layer IV is a difference in the external currents arriving to the excitatory population (i.e., the term \( J_e \)).

Generating oscillations depend on the inputs to the populations. Figure SC shows the inputs to the populations in different trials. There is no input to inhibitory neurons in the control trials, and there is a constant input to the excitatory population. This implementation ensures there are gamma oscillations in the LFP[A19]. The visual stimulus is modelled as a constant input to both populations (inhibitory and excitatory). While the input current in the laser stimulus has the same time and a period similar to the laser stimulus in the experiment, the input's amplitude depends on laser intensity. Because opsin mainly affects the excitatory population, laser stimulation is only considered for excitatory populations. Figure 8D shows LFPs in different conditions of the control group, laser and laser + visual stimulation.
Figure S A) The blue line shows the amplitude of the laser in time. The black line is the corresponding LFP recorded during laser stimulus. B) An illustrative figure of excitatory and inhibitory populations with their connections and induced currents. Our simulation's initial parameters are $\omega_{ee} = 2.4$, $\omega_{ei} = 2$, $\omega_{ii} = 2.4$, $\omega_{i} = 0$, $\Omega_{e} = 0.5$, and $\Omega_{i} = 0$. C) Different types of inputs to the population. Input during the "control" (or no stimulus state) was chosen to simulate baseline gamma activity. The laser and control current inputs are to the excitatory population only. The visual stimulation results in an input to both the inhibitory and excitatory populations. D) Simulated LFP for different stimuli.

Our simulations propose that changing input currents plays the most crucial role in modelling the optogenetic behaviour. Although the model provided a descriptive explanation of the data, the outcome did not depend on the choice of parameters, particularly the excitatory time constant, as shown in Figure A1.
Figure A1. The gamma power, when normalized, shows a non-monotonic pattern that is unaffected by the choice of the excitation time constant, where the given \( \tau_i \) is 0.0032. Our simulation's initial parameters are \( \omega_{ee} = 2.4, \omega_{ei} = 2, \omega_{ii} = 0, \theta_e = 0.5, \) and \( \theta_i = 0 \)

- **Power spectrum**

The power spectrum densities for the control, visual, laser, and visual+laser stimulation conditions are shown in Figure A2 and A3.
Figure A2 200 microns: average power spectrum density for the gamma activity in control, Laser, Visual, and Laser+ Visual trials. Average power spectrum density difference between control and visual trials (a), control and laser 50 % (b), control and laser 75 % (c), control and laser 100% (d), control and laser+visual 50% (e), control and laser+visual 75% (f) and control and laser+visual 100%.
Figure A3 500 microns: average power spectrum density for the gamma activity in control, Laser, Visual, and Laser+ Visual trials. Average power spectrum density difference between control and visual trials (a), control and laser 50% (b), control and laser 75% (c), control and laser 100% (d), control and laser+visual 50% (e), control and laser+visual 75% (f) and control and laser+visual 100%.
References (Appendix)


[A8] - Mann, H. B., & Whitney, D. R. (1947). On a test of whether one of two random variables is stochastically larger than the other. The annals of mathematical statistics, 50-60


