META-ANALYSIS OF SINGLE UNIT NEURAL RECORDINGS TO ASSESS SPIKE TRAIN VARIABILITY ACROSS LEVELS OF PROCESSING AND ANESTHESIA CONDITION

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ABSTRACT

The process of neuronal information transfer involves the propagation of electric signals known as action potentials, or spike trains, across nerve fibers in the central nervous system. Under controlled experimental conditions, it has been observed that even when presented with identical stimuli, there is a high level of trial-to-trial variability in single-unit (single neuron) spike trains. Even when comparing functionally similar neurons, the response reliability can differ considerably. It was hypothesized that the visual pathway neurons closer to photoreceptors would display greater response reliability, and vice versa. Furthermore, it was hypothesized that neurons from subjects under anesthesia would also display greater response reliability compared to non-anesthetized neurons. This meta-analysis examines single-unit recordings from different species across several major sensory processing regions of the brain. The data sets were analyzed through calculation of the Fano factor in MATLAB 2020a and statistical analysis tests. It was determined that there was a correlation between anesthesia and response reliability, while the results for the relationship between level of processing and response reliability were more inconclusive. These conclusions serve to further knowledge of spike train variability and its influencing factors.

Abbreviations: ISIs, interspike intervals; CLM, caudolateral mesopallium; CMM, caudomedial mesopallium; LGN, lateral geniculate nucleus; MS, medial septum; NCM, caudomedial nidopallium; PFC, prefrontal cortex; SI, primary somatosensory cortex; VI, primary visual cortex; V2, secondary visual cortex; VP, ventral pallidum

Keywords: action potentials, Fano factor, interspike intervals, single-unit recordings, spike trains, variability
INTRODUCTION

The most basic unit of the nervous system is the neuron, a specialized cell that sends signals through the brain. Neurons transmit information through sequences of electric pulses. These pulses are called action potentials, which travel along the body of the neuron from one end to the other. When transferring information between neurons, the axon terminal of one cell will release chemical signals, called neurotransmitters, to the dendrites of another. Electrophysiological tools are used to monitor the brain’s activity. For example, the firing of action potentials can be recorded by electrodes implanted in the brain, and then graphically represented (Harris, 2000). These electrophysiological recordings are of considerable interest to researchers because they give an accurate glimpse into the workings of the brain.

A specific type of electrophysiological recording is the single-unit study, which involves monitoring single neurons responding to stimuli. Action potentials are often referred to as “spikes,” based on the distinctive shape of their graphical representations. Likewise, a series of action potentials is referred to as a “spike train.” These spike trains are a useful tool for researchers to visualize the electrical response of neurons and collect data about response times; as such, they are commonly used in neurological experiments (Amarasingham, 2006). Spike trains for data sets incorporated in this study are represented in raster plots (Figure 1-A). The x-axis represents the amount of time the test subject was exposed to the stimulus, typically a few milliseconds, while the y-axis represents which trial the spike train was recorded from. A raster plot is an accurate portrayal of individual spike times, as opposed to a peristimulus time histogram (Figure 1-B), which shows a broader distribution of times with higher neuronal activity levels.

Theoretically, a neuron should respond the same way to the same stimulus, to an acceptable degree of variation. Under certain circumstances, however, a sensory neuron will respond unpredictably, leading to a lower level of response reliability. In many experiments, neurons had a much greater degree of variation than expected. These neurons were responding unpredictably to the same stimulus. In particular, the duration between two consecutive spikes (the interspike interval, or ISI) often differs arbitrarily (Steimer & Schindler, 2015). Spike trains also tend to vary in their peak times and peak intensities, even when the stimuli are nearly identical. This change in response reliability of a single unit is referred to as spike train variability (Gur, 2005).
Spike train variability in electrophysiological data is a source of considerable interest, since the origins of these irregularities remain poorly understood (Brette, 2015). Previous research used various neuron models, such as the Hodgkin-Huxley and integrate-and-fire models, to analyze spike train variability. In these models, a regular sequence of spikes is observed. However, when recording in the living brain, there is much more variability than predicted (Davies, 2006). Some research has indicated that spike train variability could be caused by noise from neighboring signaling pathways. With this spontaneous activity, neuron signals may become less reliable. This mechanism is also unclear, though (Gabbiani & Cox, 2010). Essentially, while previous studies offer some insight into spike train variability, its origin and purpose is largely unknown. This study aims to assess spike train variability under different conditions in order to examine its potential sources, primarily focusing on two factors which may influence response reliability.

First, it was predicted that the fewer levels of processing between the photoreceptor and the neuron, the less of a chance for the signal to vary. For this purpose, the study focuses on the neuronal pathways of vision, due to the large volume of data available for spike trains caused by visual stimuli. Visual processing begins when incoming light strikes small areas on the retina of the eye, activating the retina’s receptive field ganglion cells. From there, information travels through the optic nerve pathways to the optic chiasm, where it is then further relayed through the lateral geniculate nucleus (LGN) in the thalamus and towards the primary visual cortex at the occipital lobe of the brain (Figure 2). The relay of information through multiple levels of processing to the brain involves the transmission of many signals. The farther the signals have to travel, the more likely that they will fluctuate to some extent, due to the highly branching nature of neurons and greater possibility of divergence (Nawrot, 2009). Accordingly, the first hypothesis of this study is that cells recorded from brain regions fewer levels of processing away from sensory receptor cells will show more reliable neural responses to stimuli, and that those that are more levels of processing away from receptor cells will show less reliable neural responses.

**Figure 2: Visual Processing Pathways**
Light bouncing off an object hits the retina. Receptive fields transform the light into electrical signals that are propagated via optic nerves to the optic chiasm cross junction. Visual information is then relayed through the lateral geniculate nucleus and then to the primary visual cortex.

Secondly, it was hypothesized that anesthetizing the subjects may also affect spike train variability. Many spike train experiments are conducted under anesthesia, which acts to suppress the activity of pain-related neurons by strengthening neuronal inhibitors (Forman, 2013; Steinmetz, 2013). This alters the subject’s level of awareness, and reduces the amount of potential distractions which could interfere with the stimuli being presented (Kelland, 1991). Under anesthetized conditions, the neurons should then experience a smaller amount of “interference” while being excited by the stimuli, resulting in decreased spike train variability and a more reliable response (Antkowiak, 1998; Antkowiak, 1999). Accordingly, the second hypothesis was that anesthetized samples will display greater response reliability and less spike train variability compared to non-anesthetized samples.

This study is a meta-analysis where multiple data sets were compiled and analyzed to derive conclusions about the above hypotheses. The benefit of utilizing this study design includes a synthesized, empirical review of a large pool of resources. The sizable body of data now generated may produce more reproducible research as compared to an individual study contributing to a larger set of analysis. Furthermore, the meta-analytic approach allows for data that fits the hypothesis to be selected, in contrast to an experimental design where experiments are carried out in order to obtain data. The integration of findings from many data sets thus ensures that the study’s research is desirable and enables researchers to make more varied hypotheses.

MATERIALS AND METHODS

Overview

Data sets analyzed in this paper were taken from online data banks (see below). They were chosen based on several factors, such as the inclusion of single-unit recordings, types of stimuli they used, and number of neurons observed, among other factors. After excluding certain data sets the number of viable sets had decreased significantly. Therefore, more data sets were procured and a second screening process was conducted. The chosen data sets were then run through a MATLAB program and converted into a form more usable for the purposes of the analyses.

Search Strategy

After understanding that neurons, when stimulated, produce different spike times despite having the same strength and duration of stimuli, data sets across multiple electronic repositories (Dryad, Zenodo, Figshare, G-Node Open Data, Open Science Framework, and Buzsaki Lab), found on Github.com, were obtained and analyzed. To narrow down the search, more data sets were accessed from CRCNS (Collaborative Research in Computational Neuroscience). Research for these data sets used advanced searches included keywords/phrases: single-unit*, single neuron*, spike train*, spike times*, matfile*, spontaneous activity*, stimuli activity*, spike coding*, and recorded action potentials*. 
Inclusion/Exclusion Factors

In order to be included in the study, data sets needed to have both spike and stimulus times available. Additionally, as data sets were acquired, factors including the brain regions that were studied, the species of the subjects, the use (or lack thereof) of anesthesia, and the type of stimuli were examined. The formats of data files and the number of neurons tested in each study were also documented. This information was gathered from abstracts, as well as data descriptions provided in the corresponding research paper(s). In the case of op.cousens, the information was directly provided by the researcher.

Data sets were excluded if: i) they did not include single-unit recordings; ii) stimulus was not present; iii) they did not include spike or stimuli timestamps; iv) they were either too large or not formatted in a file compatible with MATLAB 2020a; v) fewer than five neurons were tested. When choosing the final data sets to be used, the stimuli were limited to only being olfactory, auditory, visual, or tactile. Spontaneous recording and stimuli, such as behavioral tasks dealing with maze runs, were excluded.

Quality Assessment Process

The initial selection process across the original 31 data sets using the aforementioned search factors revealed the need for more in-depth research across the repositories to make a thorough meta-analysis, which led to the addition of 116 more data sets predominantly from CRCNS. While reviewing the 147 data sets gathered, the only determining factors were the presence of single-unit electrophysiology data with spike times, stimulus times, permission granted from authors, and compatibility with MATLAB 2020a. This process resulted in 26 data sets. After a second review requiring that there be discrete stimuli and at least five neurons tested in each experiment for substantial comparisons, 15 of the 26 data sets were recognized to be viable options. Ultimately, 11 of the 15 data sets were picked to formulate histograms and raster graphs based on how well they fit the hypotheses.

Data Extraction

After notifying the lead researchers from the data sets selected, the 15 viable data sets were downloaded for further analysis of the raw data. Data sets from the mentioned repositories were found to be in a variety of file types (.mat files, .txt files, .wav files). One data set, pfc-1, was in a form incompatible with MATLAB 2020a and was thus discarded. The remaining file types were converted into a standardized cell file format based on the information provided by the source’s data documentation.

Using an individualized program, a separate standardized cell file name was generated for each neuron in the data file. This was based on the unit number and defined as the neuron whose data was stored in the data. The program then stored the spike time data again based on the unit and trial number. Next, the program retrieved data regarding the type of stimulus for each trial. Finally, the session duration was recorded based on the final value of stimtime,
calculated as the cumulative sum of each trial’s duration. Ultimately, all the spikes, recorded from the one second after the stimulus/stimuli, were extracted to be further analyzed.

**Statistical Analysis**

Once the data was extracted, it was fed into another researcher-written program to determine the Fano factors and $p$-scores of a neuron. The Fano factor, commonly used in neurobiology, is the measure of the variability in the number of spikes to the mean number of spikes. Many experiments have demonstrated that Fano factors differ considerably depending on the brain region, with cortical neurons of the visual system being particularly variable (Gabbiani and Cox, 2010). Another output of this program is the $p$-score, also known as the probability value; this is defined as the largest probability of obtaining test results at least as extreme as the results observed under the assumption that the null hypothesis is correct (Halsey, 2019). The $p$-scores with the lowest values were then used to determine which neurons in each data set were the most responsive to stimuli. The Fano factors’ frequency and the number of spikes from those neurons were then used to formulate the peri-stimulus diagrams and raster plots.

Following this, the data for the Fano factors, organized by neuron and stimulus, were transferred to Google Sheets, where they were organized into bins based on brain region: cortical (1) or pre-cortical (2) for hypothesis one, and alertness: anesthetized (1) or non-anesthetized (2) for hypothesis two. Next, the Fano factors were assigned a random $x$-coordinate value equal to their bin number $\pm 0.5$, spaced 0.001 apart, to prevent clumping of the data when graphed. Then, the Fano factors were plotted on a logarithmic scale based on bin assignment. Separate graphs were made for Fano factors from specific trials following selection for trials with $p$-scores of less than or equal to 0.05.

To test if a comparison was statistically significant, the Mann-Whitney $U$-test was used to find $N$, $U$, and $P$. In this test, $P$ represents the asymptotic significance; a number under 0.05 suggests that the difference between the outcomes of each scenario is statistically significant, i.e. not solely subject to random chance. $N$ represents how many neurons were analyzed, thus showing how big the sample size was. $U$ represents how much each observation or neuron exceeds one another; a higher $U$ suggests a greater effect size and more statistical significance.

When analyzing the histograms, the distribution of the Fano factors of each data set was interpreted in two ways: response reliability and spike variability. Response reliability was determined by looking at the median of a data set’s Fano factors. The closer the median was to one, the greater the response reliability a neuron had, meaning the neuron would respond more consistently in each trial when presented with the same stimulus. Meanwhile, spike variability was determined by how clustered the Fano factors were to the median, regardless of the median. If the median was far from one, but the distribution of the Fano factors was closely clustered to it, the spike variability would be low. The lower the spike variability, the more similar the levels of variability of the different neurons were relative to each other in responses to stimuli.
RESULTS

Overall Results

The data used for this meta-analysis came from multiple sources. 31 data sets from a multitude of different repositories found on Github.com and 116 data sets from repositories from the data-sharing website CRCNS were initially gathered, totaling 147 data sets (Figure 3). Through the exclusion process described in section 2.4, only eleven data sets (v1, v2, ssc9a, ssc9na, ret-1, lgn, pfc-4, cb-1, aa-1, aa-2, and aa-4) were selected to support the hypotheses; these data sets are listed below (Table I).

Figure 3: Flowchart of Research Process.
Depicts which factors were used to narrow down the data sets to the final eleven. Reasons are given for why each data set was chosen for their respective hypothesis.
<table>
<thead>
<tr>
<th>Data Sets*</th>
<th>Author (Year)</th>
<th>Species</th>
<th>Neuron Region</th>
<th>Stimulus Type</th>
<th>Anesthesia</th>
<th>No. of Neurons</th>
<th>Used For Which Hypothesis</th>
<th>No. of Fano Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>v1v2-1 (v1)</td>
<td>Zandvakili &amp; Kohn (2019)</td>
<td>Macaque Monkey</td>
<td>Primary Visual Area</td>
<td>Visual (sets of oriented gratings)</td>
<td>Yes</td>
<td>111</td>
<td>Both</td>
<td>887</td>
</tr>
<tr>
<td>pfc-4</td>
<td>Romo, Brody, Hernández, &amp; Lemus (2016)</td>
<td>Macaque Monkey</td>
<td>Prefrontal Cortex</td>
<td>Auditory (varying frequencies)</td>
<td>No</td>
<td>4</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>aa-1</td>
<td>Theunissen, Hauber, Woolley, Gill, Shaevitz, Amin, Hsu, Singh, Grace, Fremouw, Zhang, Cassey, Doupe, &amp; David (2009)</td>
<td>Zebra Finch</td>
<td>Forebrain</td>
<td>Auditory (pure tones, zebra finch songs, synthetic songs, modulation-limited noise, and white noise)</td>
<td>Yes</td>
<td>10</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>aa-2</td>
<td>Theunissen, Gill, Noopur, Zhang, Woolley, &amp; Fremouw (2011)</td>
<td>Zebra Finch</td>
<td>Forebrain, Midbrain</td>
<td>Auditory (modulation-limited noise, ripple noise, zebra finch songs)</td>
<td>Yes</td>
<td>5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>aa-4</td>
<td>Elie &amp; Theunissen (2019)</td>
<td>Zebra Finch</td>
<td>Caudolateral /Caudomedial Mesopallium, Caudomedial Neopallium, Field L (L1, L2, L3)</td>
<td>Auditory (ripple noise, zebra finch songs, vocalizations from the repertoire)</td>
<td>Yes</td>
<td>76</td>
<td>2</td>
<td>1367</td>
</tr>
<tr>
<td>ret-1</td>
<td>Zhang, Asari, &amp; Meister (2014)</td>
<td>Mouse</td>
<td>Ganglion Cell Layer</td>
<td>Visual (images projected on the photoreceptor layer of the retina)</td>
<td>No</td>
<td>43</td>
<td>Both</td>
<td>43</td>
</tr>
<tr>
<td>lgn-1</td>
<td>Scholl, Tan, Corey, &amp; Priebe (2013)</td>
<td>Mouse</td>
<td>Lateral Geniculate Nucleus</td>
<td>Visual (mouse to drifting gratings)</td>
<td>Yes</td>
<td>72</td>
<td>Both</td>
<td>921</td>
</tr>
<tr>
<td>ssc9 anesthesia (ssc9a)</td>
<td>Laboy-Juárez, Langberg, Ahn, &amp; Feldman (2019)</td>
<td>Mouse</td>
<td>Primary Somatosensory Cortex</td>
<td>Tactile (stimulated whiskers)</td>
<td>Yes</td>
<td>58</td>
<td>2</td>
<td>409</td>
</tr>
</tbody>
</table>

*All data sets are from CRCNS.
Figure 4-A displays a tuning graph displaying the number of spikes for each stimulus for cell 52 from the data set v1v2-1_v1. The tuning graph shows how cell 52 was selectively responsive to the stimulus. The cell had an extremely high number of spikes for stimulus 6 compared to all the other stimuli. Even though it is clear that the cell responds selectively to one certain stimulus, there is still variability within that stimulus. To highlight the selectivity of the cell, a raster plot was made for stimuli 2 which the cell did not respond to heavily, as shown by Figure 4-B, and a raster plot was also made for stimulus 6 as shown by Figure 4-C. It is clear how the raster plots differ; the raster plot from 4-C is much more populated than the raster plot from 4-B. Therefore, it can be assumed that cell 52 has been tuned to selectively respond to stimulus 6. However, even within this tuning, there is a large amount of variability. As seen by the raster plot of 4-C, the different trials have different amounts of spikes. Furthermore, the histogram from figure 4-C also shows that there is a large variability within the firing rates. The firing rates of cells, even when it is tuned to respond to that stimulus, greatly varied.

Data Organization

The Fano factors from four data sets (v1, v2, lgn-1, and ret-1) were chosen for broad analysis in the first hypothesis, segregated by whether they were pre-cortical or cortical neurons. The 2235 Fano factors from these data sets—964 pre-cortical and 1183 cortical—were plotted on a point distribution graph (Figure 5-A). The 964 Fano factors from the pre-cortical data sets—43 from ret-1, 921 from lgn—were chosen for further analysis because both came from pre-cortical neurons and thus could be useful for a comparison (Figure 5-B). The 1183 Fano factors from the cortical data sets—887 from v1, 296 from v2—were also chosen for further analysis because both data sets came from the same original study on macaque monkeys, where neurons were tested in the primary visual cortex and the secondary visual cortex (Zandvakili, 2019). With all variables constant except for the region of the brain, this data set up the ideal comparison in terms of finding a correlation between change in variability and distance (Figure 5-C). The last level of processing involved selecting only the 1064 Fano factors from statistically significant cell-stimulus pairs—810 from v1, 268 from v2 (Figure 5-D).

On the other hand, the Fano factors from eleven data sets (v1, v2, pcf-4, aa-1, aa-2, aa-4, ret-1, lgn-1, cb-1, ssc9a, and ssc9na) were chosen for analysis in the second hypothesis, segregated by whether they were recorded from non-anesthetized or anesthesia neurons. The 4868 Fano factors from these data sets were plotted on a point distribution graph (Figure 5-E). The 751 Fano factors from the somatosensory cortex—409 non-anesthetized, 342 anesthetized—were chosen for further analysis because both data sets from the same original study on somatosensory cortex neurons from mice whose whiskers were stimulated under either anesthetized or non-anesthetized conditions (Laboy-Juárez, 2019). These comparisons provide an environment where confounding variables are likely avoided and can suggest relationships between anesthetization and variability (Figure 5-F). The last level of processing involved selecting only the 67 Fano factors from statistically significant cell-stimulus pairs—25 from non-anesthetized somatosensory cortex neurons, 42 from anesthetized somatosensory cortex neurons (Figure 5-G).
Figure 4: Tuning of Cell 52 (Primary Visual Cortex).

Note: Arrow indicates the time at which the stimulus was presented (0 seconds).

Tuning Graph of Cell 52, which was a statistically significant cell with a p value of \(4.2 \times 10^{-13}\), showing Total Number of Spikes Per Stimulus (Figure 4-A); Spike Raster of Stimulus 2 from Figure 4-A (Figure 4-B); Spike Raster and Histogram of Stimulus 6 from Figure 4-A (Figure 4-C);
Figure 5: Point Distribution of Fano Factors.

Note: ✽ indicates $p \leq 0.05$

Point distribution of Fano factors for pre-cortical or cortical Neurons (Figure 5-A); Point distribution of Fano factors for the ret-1 and lgn data sets (Figure 5-B); Point distribution of Fano factors for the v1 and v2 data sets (Figure 5-C); Point distribution of Fano factors for the v1 and v2 data sets, $p \leq 0.05$ (Figure 5-D); Point distribution of Fano factors for non-anesthetized or anesthetized Neurons (Figure 5-E); Point distribution of Fano factors for the ssc9na and ssc9a data sets (Figure 5-F); Point distribution of Fano factors for the ssc9na and ssc9a data sets, $p \leq 0.05$ (Figure 5-G).
Variability Among Neurons Based on Level of Processing

Figure 5-A displays the distribution of all the Fano factors for all the cell-stimulus pairs from four data sets (lgn-1, ret-1, v1, and v2), segregated by sample location in or outside of the cortex. The median calculated for the pre-cortical Fano factors was 1.011, whereas the median calculated for the cortical Fano factors was 1.431. As can be seen in Figure 5-A, the point distribution for the pre-cortical Fano factors has a more loosely correlated distribution about the median than the cortical distribution. Meanwhile, the cortical Fano factors showed a more tightly correlated distribution around the median, with fewer outliers. Using the Mann-Whitney U test to compare the Fano factors of pre-cortical and cortical regions, the null hypothesis was rejected ($N = 2231; U = 790390; p = 0.000$), indicating statistical significance.

Figure 5-B displays the distribution of Fano factors for cell-stimulus pairs for the ret-1 and lgn-1 data sets. The median Fano factor for the former data set was found to be 0.738, and the median for the latter was 1.013. As shown in the image, there was a substantially smaller amount of neurons in the ret-1 data set compared to lgn-1. Additionally, the Fano factors for the lgn-1 data set are slightly more closely clustered about the median than they are for ret-1, but the general distributions appear of similar size and shape. According to the results obtained from the Mann Whitney U test, there is no significant difference between the two distributions pictured ($N = 1035; U = 24418.5; p = 0.107$).

Figure 5-C displays the distribution of Fano factors for cell-stimulus pairs for the v1 and v2 data sets. The median calculated for the v1 Fano factors was 1.398, while the median calculated for the v2 Fano factors was 1.556. As shown above, the point distribution for the v1 Fano factors proportionately has a slightly more tightly correlated distribution around the median than v2; however, in terms of outliers, there is a stark contrast between the data sets with v2 having a larger spread of outliers that even reach 100. According to the results obtained from the Mann Whitney U test, the null hypothesis should be rejected ($N = 1183; U = 150749; p = 0.000$), indicating statistical significance.

Figure 5-D displays the distribution of the Fano factors for cell-stimulus pairs that were statistically significant according to the Kruskal-Wallis test (having a $p \leq 0.05$) from the v1 and v2 data sets. The median of the subset of the v1 Fano factors was 1.409, whereas the median for the subset of v2’s Fano factors was 1.556. From the graph above, it is observed that the point distribution for the Fano factors of the v1 subset proportionately has a slightly more compacted distribution around the median than subset v2’s Fano factors. In terms of outliers, however, the v2 subset contains a larger amount of outliers that even reach 100. According to the results obtained from the Mann Whitney U test, the null hypothesis should be rejected ($N = 1063; U = 117890; p = 0.004$), indicating statistical significance.

Variability Among Neurons Based on Anesthesia Condition

Figure 5-E displays the distribution of all the Fano factors for all cell-stimulus pairs from ten data sets (v1, v2, aa-1, aa-2, aa-4, pfc-4, ret-1, lgn-1, cb-1, ssc9a, and ssc9na), segregated by
whether they were from an anesthetized or a non-anesthetized sample. The median calculated for the non-anesthetized Fano factors was 1.077, whereas the median calculated for the anesthetized Fano factors was 1.111. As can be seen above, the point distribution for the non-anesthetized Fano factors has a more loosely correlated distribution around the median, and also demonstrates many more outliers, with some Fano factor values reaching as high as 100. In contrast, the anesthetized Fano factors showed a more tightly correlated distribution around the median, with fewer outliers. In comparing the Fano factors of ssc9a vs ssc9na using the Mann-Whitney U test, no significant difference in their Fano factor scores between 2 groups was found (N = 4439; U = 2415312; p = 0.778).

Figure 5-F displays the distribution of all the Fano factors for cell-stimulus pairs segregated from the two data subsets from Figure 4-E: ssc9a, and ssc9na. The median of the ssc9na Fano factors was 1.29 whereas the median for the Fano factors of ssc9a was 1.222. The Fano factor point distribution for ssc9na has a loosely correlated point distribution around the median and also has many outliers even reaching 100. Dissimilarly, the Fano factor point distribution for ssc9a has a more tightly correlated point distribution around the median and has fewer outliers than ssc9na. In comparing the Fano factors of ssc9a vs ssc9na using the Mann-Whitney U test, no significant difference in their Fano factor scores between 2 groups was found (N = 750; U = 73539; p = 0.202).

Figure 5-G displays the distribution of all the Fano factors for cell-stimulus pairs that were statistically significant according to the Kruskal-Wallis test (having a p ≤ 0.05) from ssc9na and ssc9a. The median of the subset of the ssc9na Fano factors was 0.971, whereas the median for the Fano factors for ssc9a was 1.292. The Fano factor point distribution for both subsets are loosely correlated around the median with the same ranges of outliers. Meaningfully, the median for anesthetized was significantly higher than non-anesthetized. The Mann Whitney U test was conducted, and the results indicated significantly lower values for data set 1 than data set 2 (N = 67; U = 240.5; p = 0.000).

DISCUSSION

Variability Among Neurons Based on Level of Processing

The first hypothesis, which predicted that neurons recorded in early stages of visual processing, such as the retina and thalamus, would exhibit more reliable responses compared to those in later visual processing areas, was not conclusively supported. From the broadest sense, in comparing neuronal activity in the retina and lateral geniculate nucleus as opposed to activity in the visual cortex, which can be seen in Figure 5-A, more variability was found in the results from the cortex than the other regions, in contrast to the first hypothesis. Within the pre-cortical region, however, there was not found to be a significant difference in distribution between the ret-1 data set and the lgn-1 set, which is shown in Figure 5-B. Meanwhile, in the cortical region comparison in Figure 5-C, the primary and secondary visual cortices reveal very similar distributions, without much significant difference, aside from the median of the primary cortex being closer to one.
Looking at the summarized results, it can be easy to become confused over whether the data supports the hypothesis—much of this was due to the somewhat contradicting results, which could have stemmed from multiple factors. Starting from the broadest perspective, different types of species and different types of visual stimuli were used across the various data sets, which could have impacted the Fano factor distribution. Similarly, the difference in spike train variability between the overall pre-cortical and cortical data could have occurred due to other factors besides neuron area, as these experiments were conducted by different researchers in different labs, meaning that some aspects, such as use of anesthesia, were not kept constant across all experiments. Regardless of whether or not these factors influenced the results, the hypothesis was not supported by the analysis of these data sets.

Using Figure 5-C to compare two sets of data which had been collected under similar conditions, with the only difference being location, should be a better comparison to use when figuring out whether distance truly played a role in spike train variability. However, in contrast to the first hypothesis, the Fano factor distributions were proportionally the same, without many significant differences. Thus, a deeper study, as pictured in Figure 5-D, was conducted using only the most selective response neurons of each data set in the primary and secondary visual cortex. This data comparison, which was the most consistent in terms of variables, did not support the hypothesis, with the secondary visual cortex showing greater response reliability. Ultimately, there was not enough clear support for the hypothesis for it to be proven unconditionally.

Variability Among Neurons Based on Anesthesia Condition

The second hypothesis, that anesthetized samples would display better response reliability compared to non-anesthetized samples, was supported by the data. In examining Figure 5-E, it can be seen that there is a clear difference in the distribution of the anesthetized and the non-anesthetized data sets, as the anesthetized data sets are much more tightly correlated around the median and also have fewer outliers. When looking at the ssc9a and ssc9na subsets of this broad data set, a similar pattern is seen, with the anesthetized ssc9a also being more tightly correlated around the median. These two data sets suggest that anesthetized cells yield a higher response reliability and a lower level of spike variability. However, when looking at the most specific level of analysis which contains only the statistically significant cells, there is not a marginal difference in the distributions. This statistical anomaly could be explained by the relatively low sample size available for this smaller subset. Even the two data sets from the same source showed some disparities (ssc9a had a total of 15,865,695 trials and 58 cells, whereas, in ssc9na, there were a total of 2,257,632 trials and 39 cells), and more research should be done before an conclusive statement can be reached.

From a theoretical standpoint, this could be because anesthesia typically suppresses central nervous system activity. The mechanism of anesthesia usually works to suppress the activity of pain-related neurons by increasing the activity of inhibitory receptors (Zhao, 2019; Forman, 2008). This means that in anesthetized samples, the inhibitory neurons can more effectively suppress “noise” from the brain, preventing the action potential threshold from being triggered as easily from random fluctuations. Moreover, this would allow the neurons to “focus” more on the stimulus and produce more reliable responses. This would create a lower level of
spike train variability in anesthetized neurons when trying to respond to a controlled stimulus. On the other hand, non-anesthetized neurons have poorer response reliability because their inhibitory neurons cannot refine the signal as well.

While the documentation for ssc9a did not state the particular type of anesthesia that the mice were undergoing, the mechanism and results are largely the same, regardless of which chemical was used (Nishikawa, 2000; Kajiwara, 2020). Since ssc9a and ssc9na were both given stimuli relating to physical sensations (whisker stimulation), and both neurons were sampled from the somatosensory cortex—a region crucial in pain and tactile sensation—in the ssc9a neurons, there would likely be effects from the anesthesia which would work to enhance the activity of the inhibitory neurons. This would then positively impact the response reliability of those neurons, as its ability to focus on stimuli would be refined.

By conducting this experiment again with a much larger set of neurons, statistical anomalies could be eliminated as outliers in the Fano factor so as to not affect the median, and therefore the effect sizes, the correlation around the median, and the p-scores as much. Additional research may include conducting a study where the non-anesthetized trials and the anesthetized trials have the same species experiencing the same types of stimuli, number of trials, and ISI’s. Two clear differences between the two data sets that were analyzed were the number of cells analyzed within each data set and the number of trials used in each data set. Using the same experimental setup could result in a similar level of habituation and sensitization within the cells, and also help make the data more comparable by reducing the isolated differences that are present in the two experiments.

Data Sharing

Throughout the research of multiple repositories (Dryad, Zenodo, Figshare, G-Node Open Data, Open Science Framework, and Buzsaki Lab), it was discovered that many of the data sets found were reported in differing formats (e.g., mat files, .txt files, .wav files, .h5 files, and .csv files), creating a need to reformat the files into MATLAB. Additionally, the format within MATLAB files varied greatly from one another. It was therefore necessary to change the structure further to match the written code. This also resulted in difficulty when trying to use certain data sets that needed the use of Python code to format, specifically those formatted in .csv files; these data sets were deemed unsuitable. Other files such as those coded in Python were also rendered unusable. Upon efforts to open certain data files, it was also found that several files were corrupted and restricting access to the information. The neuroscience network recognizes the global issue of the need to establish a universally standard organizational structure and type of file. Organizations, like CRCNS (Collaborative Research in Computational Neuroscience), have taken steps towards standardization to make data sharing more efficient and accessible between professionals.

Numerous other limiting factors presented themselves in the research process. One of the greatest problems was the lack of publicly available data sets in a downloadable format. The information included in such data sets also proved to be restricting; a large quantity of data sets, although containing other relevant information, did not record important factors for this meta-analysis, such as stimulus times (see Figure 3). As previously mentioned, the readability of files
was also a limiting factor. Many files did not have a clear documentation. For example, some files did not document in detail what each of the files contained, which hindered the ability to clearly understand each of the files. In order to solve this problem, stricter guidelines must be established about the types of documentation that are associated with the data files. By having stricter guidelines, it would ensure that the reader fully understands all aspects of the data. Ultimately, by improving the levels of readability and also ensuring that there is a universal file type that is clearly established, the process of data sharing can become much more accessible and universal.

**Error and Reliability**

The information outlined in this meta-analysis is only as good as that of the primary source data sets that were analyzed. Even in the most carefully regulated laboratory settings, sources of error are present. In the research examined in this analysis, several possibilities of error are present, including but not limited to: background stimulation and accidental inputs from surroundings arising from an incompletely controlled setting/possible previous exposure to stimuli. Additionally, data used may not be representative of an entire population under the same circumstances as the result of age, sex/gender, sensory/processing disorder, and/or physical disability (such as a decreased sense of smell, hearing loss, or reduced eyesight). The different formats of the data sets included in the meta-analysis may have also led to the possibility of misinterpretation. As with any experimental study including primary data collection, one must remember the limitations of equipment upon the results, altering precision and accuracy; because each data set was procured from a different laboratory (for the most part), discrepancies between such factors vary between sets.

**Future Direction**

This study demonstrates and examines many key characteristics of spike train variability, such as how the use of anesthesia results in more stable firing patterns. These findings could potentially serve as the basis for future studies in the field of neuroscience, such as an analysis of whether spike train variability correlates with spontaneous action potentials. This can be accomplished by using non-anesthetized neurons, thus increasing spike train variability, and subsequently measuring the rate at which spontaneous firings occur. Whether the amount of spike train variability differs between spontaneous neuronal activity and controlled-stimuli neuronal activity can also be determined. Additionally, while it was determined in the meta-analysis that the presence of anesthesia increases the uniformity of the spike trains, there was not enough data to conclusively state the rate at which this occurs. Future experimentation with anesthesia levels as an independent variable, as well as controls of species and stimuli type, should be conducted before a definitive answer can be stated. As for the potential role that distance from sensory inputs can have in neural activity, it may also be worth doing further experimentation to conclusively confirm or reject the first hypothesis.

**CONCLUSION**

Spike train variability in single-unit neuronal recordings is an ongoing challenge for neuroscience researchers. Through a meta-analysis of the spike train variabilities from single-
unit neural recordings sourced from varying species, stimuli, and conditions, several interesting results were found. The first hypothesis, that neurons which were fewer levels of processing away from sensory receptor stimuli will display greater response reliability, was inconclusively resolved. On the other hand, the second hypothesis, that anesthetized samples will display greater response reliability compared to non-anesthetized samples, was confirmed. Another insight is the importance of implementing a universal data-sharing system so that data can be viewed, understood, and expanded on for future applications and experiments without inconsistencies. Overall, the results of this study could have considerable implications in generalizing experimental factors for the improvement of neuronal response reliability.

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APPENDIX

Additional Raster Plots

Raster plot for ssc9a cellfile 52 featuring first 100 action potentials after stimulus onset

Raster plot for ssc9na cellfile 29 featuring first 100 action potentials after stimulus onset

Raster plot for v1 cellfile 82 featuring first 100 action potentials after stimulus onset

Raster plot for v2 cellfile 22 featuring first 100 action potentials after stimulus onset

Equations

EQUATION 1, Fano factor

\[ F = \frac{\sigma^2_W}{\mu_W}, \]

(Where \( F \) is the Fano factor, \( \sigma \) is the standard deviation, and \( \mu \) is the mean of the population.)

Additional Sources

Note that these sources were initially examined as potential data sets to be analyzed, but were ultimately eliminated through the process described in Figure 3.


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