

# Limited influence of running speed on neuronal activity in mice visual cortex subregions: a comparative study using linear regression and binned analyses

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## Introduction

As mice undergo physical exertion, such as running, the patterns of neural activity experience significant changes. It has been established that neural activity in the primary visual cortex (V1) increases substantially as mice transition from standing still to running, without significant changes in selectivity (Niell and Stryker, 2010). Additional studies have demonstrated correlations, both positive, negative, and non-monotonic, between running speed and V1 subregion activity, including the lateral visual cortex (VISl), posterior medial visual cortex (VISpm), anterior lateral visual cortex (VISal), anterior medial visual cortex (VISam), and rostromedial visual cortex (VISrl) (Christensen and Pillow, 2022). Still, the activity in higher-order visual cortex areas at varying running speeds (as opposed to stationary vs running) are less explored, more so at the neuronal level.

Considering these previous findings and the gap at the neuronal level, we investigate the impact of mouse running speed on individual neuron firing rate responses to visual stimuli in three regions: primary visual area (VISp), anteromedial visual area (VISam), and the rostromedial visual area (VISrl). By studying these areas, we aim to develop a better understanding of the interplay between physical activity and neuronal responses to visual stimuli in the mouse brain.

We found no evidence that running speed has a significant impact on neuronal activity within the VISp and VISam regions. These results suggest that neuronal activity is independent of running speed, is influenced more complexly, or that this escalation in activity is through increased neuronal population activity rather than an increase in firing rate. Alternatively, we found that running speed has a statistically significant influence on VISrl neuronal activity. Although significant, we found the relationship between variables to be extremely weak. Furthermore, after binning speed groups, we failed to find significance between all binned groups, leading to the conclusion that running speed is a poor predictor of VISrl neuronal activity in mice.

## Results

In order to answer how running speed influences the neuronal response to visual stimuli within the VISp, VISam, and VISrl areas in mice, experimental data from the Allen Institute for Brain Science, originally published in 2019 by de Vries et al., was analyzed. The data consists of the cortical activity of almost 60,000 neurons from six visual areas, 4 layers, and 12 transgenic mouse lines in a total of nearly 250 adult mice, in response to multiple sets of visual stimuli.

For this analysis, we focused on the data gathered from the presentation of small patches of black and white gratings, or Gabor patches, which were visually presented in different locations with each trial being 2 seconds in duration. Relevant data consisted of arrays of start and stop times for Gabor presentation, times at which a particular neuron spikes, the running speed of the mouse throughout the trial, time points at which the running speed was measured, index of neurons recorded, and an index of regions in which specific neurons exist.

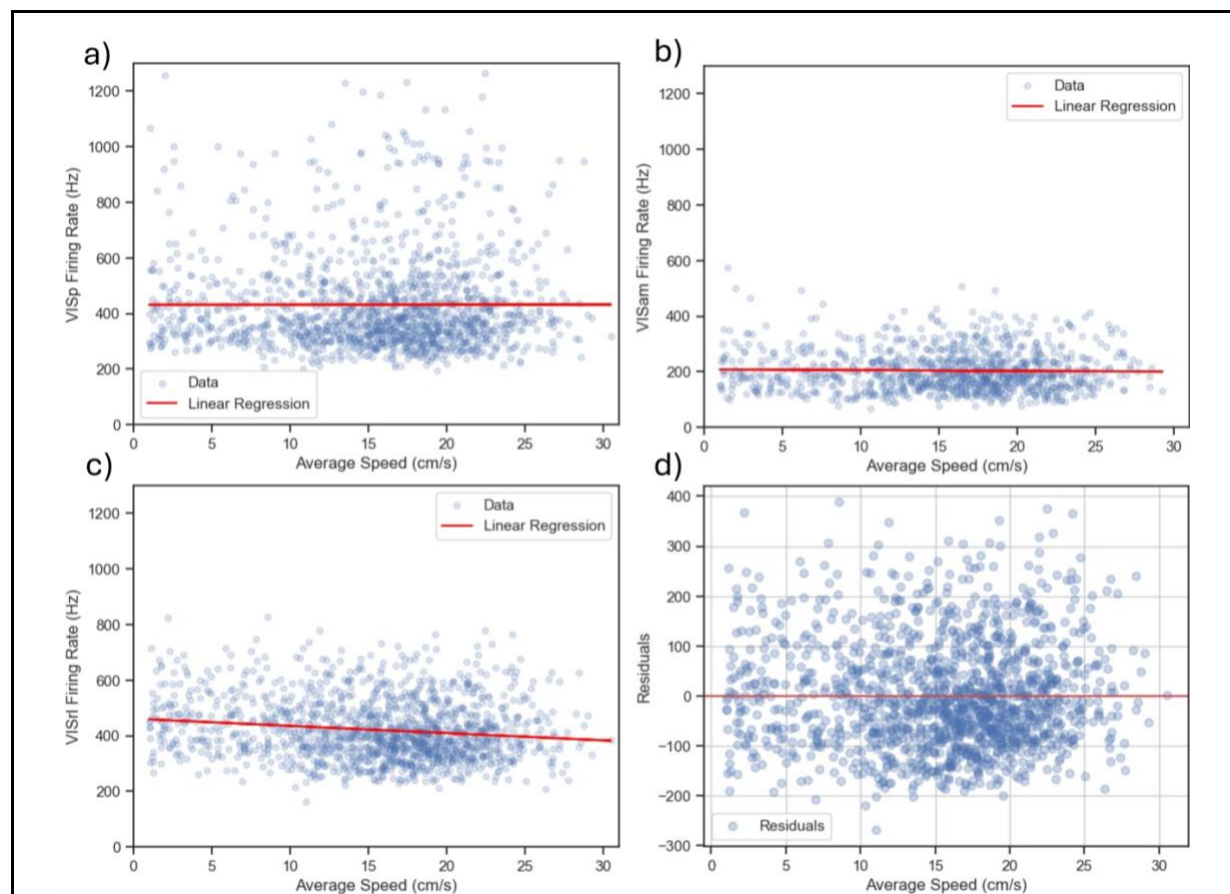
### Regression analyses of neuronal activity in VIS regions with respect to running speed

Here, we asked: How do neuronal responses in the VISp, VISam, and VISrl areas vary as a function of mouse running speed? The potential conclusion forming our null hypothesis was that running speed has no statistically significant relationship with or impact on neuronal activity within the respective VIS area. Here, one of two outcomes were expected. It was possible that our null hypothesis was supported, and there is no relationship between neuronal activity and running speed, or we reject our null hypothesis and accept our alternative hypothesis, that running speed has a statistically significant relationship with or influence on the respective VIS area neuronal activity.

To answer this question, we first calculated the mean speed in cm/s for each segment or Gabor patch presentation period. Segments in which the average speed was less than 1 cm/s were dropped, as we are focused on differences in active running or movement, rather than comparisons between stationary and running periods. Following this, we calculated the firing rate of the neuron(s) of interest from each region by dividing the number of neuron spikes within the segment by the duration of the segment, generating neuronal activity in spikes per second, or Hz. Neuronal activity was then plotted against its paired average speed and linear regressions were run. The results from the analyses are shown in Figure 1. In panel a, we see the data and associated linear regression for VISp ( $p = 0.97$ ,  $r^2 = 8.1 \times 10^{-9}$ ,  $SEM = 0.76$ ), while in panel b, we see the VISam data and its regression ( $p = 0.47$ ,  $r^2 = 5.1 \times 10^{-4}$ ,  $SEM = 0.37$ ). These panels demonstrate our failure to reject our null hypothesis and demonstrate no statistically significant relationship between running speed and neuronal activity within these regions, assuming a standard alpha ( $\alpha$ ) of 0.05. Panel c shows the plotted data and associated regression for VISrl ( $p = 3.3 \times 10^{-8}$ ,  $r^2 = 0.02$ ,  $SEM = 0.47$ ). The results shown in panel c allow us to reject our null hypothesis and demonstrate that running speed has a statistically significant relationship with or influence on VISrl neuronal activity, supporting our alternative hypothesis. However, within the VISrl region, running speed explains only 2% of the variance in neuronal activity. From the observed relationship within the VISrl region, panel d demonstrates the

residuals or the difference between our VISrl regression predicted and observed firing rates ( $x - x_0$ ) with the line of best fit.

From these analyses, we can reach multiple conclusions. First, we find no evidence that mouse running speed affects individual neuron activity within the VISp and VISam areas. It is possible that running speed does not influence neuronal activity in these regions, that these region's neuronal activity is influenced by running speed in a much more complex way, that multiple variables contribute, or that the previously discovered increase in activity was via raised neuronal population activity, rather than firing rates of individual neurons. Secondly, we can conclude that the speed at which mice run has a statistically significant relationship with or influence on VISrl neuron activity. However, as only 2% of the total variance in firing rate is explained by running speed, running speed is a poor or weak predictor. Similarly to the VISp and VISam regions, it is possible that running speed has a more complex effect on VISrl neuron firing rate than modeled, or that it also depends on numerous other factors or influences.



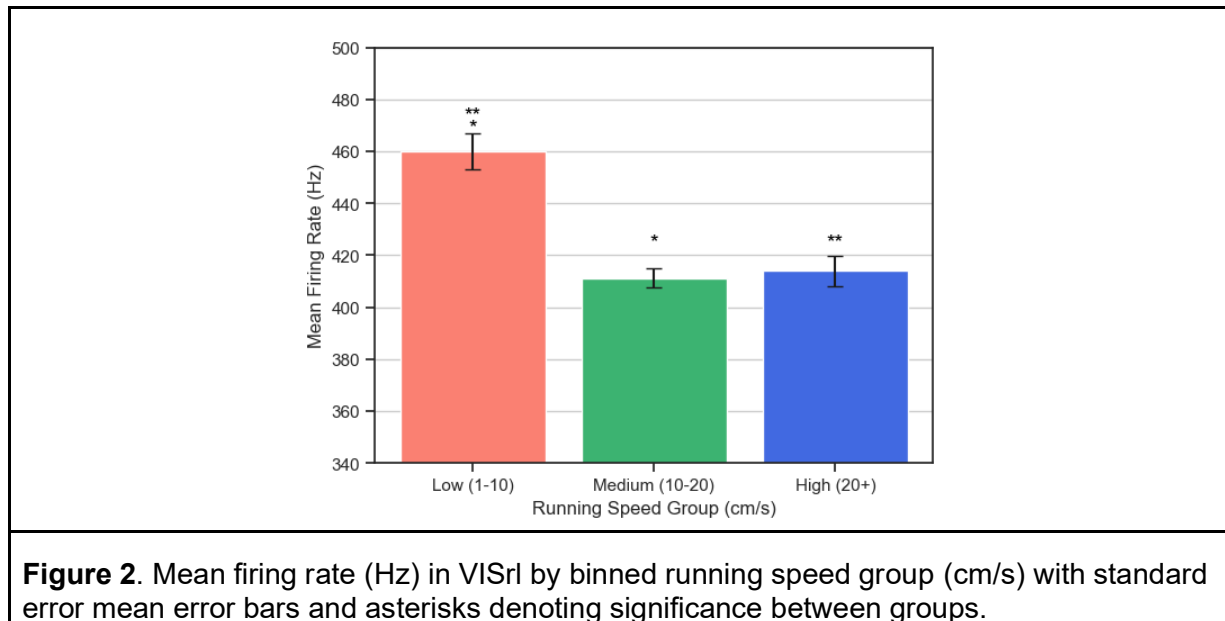
**Figure 1. a)** VISp firing rate (Hz) by average speed (cm/s) data points (blue) with linear regression (red). **b)** VISam firing rate (Hz) by average speed (cm/s) data points (blue) with linear regression (red). **c)** VISrl firing rate (Hz) by average speed (cm/s) data points (blue) with linear regression (red). **d)** Scatter plot of VISrl regression residuals ( $x - x_0$ ) against average speed (cm/s) with red center line representing line of best fit ( $x = x_0$ ).

## Statistical analysis of neuronal activity in VISrl across binned running speed groups

Next, we asked how the neural responses in the VISrl or rostralateral area in mice differ between binned running speed groups. Also, are the differences in neuronal responses between each of these groups statistically significant? Here, our null hypothesis was that there is no significant difference between neuronal activity by running group, with the alternative hypothesis being that there exists a significant difference between neuronal activity within these groups. If the null hypothesis was rejected, we continue statistical testing between each combination of groups. In this continued testing, similarly, the null hypothesis was that there is no statistically significant difference between neuronal activity in the respective VISrl groups being tested, with the alternative hypothesis being that a statistically significant difference between the compared groups is present.

To address this question, we first had to bin our data. However, as the distribution of running speeds is non or abnormal, this binning had to be done arbitrarily, as extremely right-skewed running speeds prevented binning by percentile or symmetrical bin width. Furthermore, because frequency is irrelevant when categorizing by magnitude of speed, arbitrary binning was necessary. Using the same data collected above during the linear regression analyses, still excluding average running speeds less than 1 cm/s, groups were arbitrarily binned into Low (1-10 cm/s,  $n = 254$ ), Medium (10-20 cm/s,  $n = 811$ ), and High (20+ cm/s,  $n = 344$ ) running speed groups. After binning this data and its paired neuronal activity, an ANOVA was run before the mean and standard error neuronal activity for each of these running speed groups was calculated (Low =  $460.02 \pm 6.97$  Hz, Medium =  $411.29 \pm 3.72$  Hz, High =  $413.97 \pm 5.70$  Hz). From this ANOVA, we rejected our null hypothesis and accepted that neuronal activity is significant within these groups ( $p = 1.0 \times 10^{-9}$ , F-stat = 21.03). Continuing with a Tukey's Test (HSD), we found significant differences between the low and medium, and low and high speed groups ( $p < 0.05$ ), but not the medium and high speed groups ( $p = 0.92$ ). The results from this analysis can be seen in Figure 2, with each bar representing a group mean firing rate, error bars showing the standard error mean, and asterisks denoting significance between the groups.

Similarly to the regression analysis, we find that running speed has a significant relationship with VISrl neuronal activity in mice and that there is a significant difference between low and medium and low and high-speed groups, but not between medium and high speed groups. These results support our previous conclusion that running speed is a predictor of VISrl neuron firing rate but is relatively weak. This suggests that the relationship between VISrl neuron activity and running speed in mice is complex, influenced by additional variables, or occurs primarily, but not solely, at the population level.



## Discussion

In this study, we found no evidence that running speed influences the neuronal response to visual stimuli within the VISp and VISam areas in mice. Additionally, we discovered that running speed has a weak influence on mouse neuron firing rate within the VISrl region in response to visual stimuli. These results suggest that within VISp and VISam areas, neuronal activity is influenced by running speed more elaborately, uninfluenced entirely, that multiple variables contribute, or that the previously discovered increase in activity was via raised neuronal population activity, rather than firing rates of individual neurons. Within the VISrl area, running speed is a poor or weak predictor of neuronal activity with effects likely influenced by other variables, or is one of many factors contributing to neuronal activity. In addition, it is possible that within these mice VIS regions, specifically VISrl, only the shift from the mice being stationary to running or running to stationary evoke consistent changes in neuronal activity, potentially explaining the significant difference in mean for the low speed group. This analysis demands future investigation on neuronal population activity and its relationship to running speed, further analyses of individual neuron firing rates between stationary and running groups, and analyses of neuronal activity with respect to running speed in other V1/ VIS regions, such as VISpm or VISl, contributing to a comprehensive understanding of neuronal activity in mice.

## References

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