Impact of Distinct Cortical Inputs on Striatal Circuitry and Behavior

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Abstract

The striatum is the input nucleus of the basal ganglia and is a key site in facilitating sensorimotor integration. It receives input from the primary somatosensory (S1) and primary motor cortex (M1), which are responsible for processing touch sensation and driving motor movements, respectively. It was found that corticostriatal inputs from S1 and M1 produced different patterns of innervation in projection neurons and interneurons. However, it is unknown how these results affect behavior. To investigate this, mice were trained to perform a go/no-go task using their whiskers to discriminate between textures for a water reward. In order to analyze the results of the behavioral task, an algorithm in MATLAB was developed to analyze how performance in this task was impacted by optogenetic stimulation of S1 and M1. This was accomplished by analyzing data accumulated from lick traces and outputting data that allows us to discern minute behavioral patterns. Our results show that activation of S1 projections decreased responding, whereas activation of M1 projections increased responding during both trial types.

In this study, we show that S1 corticostriatal innervation causes behavioral suppression and M1 corticostriatal innervation causes behavioral facilitation. This phenomenon of behavioral suppression and activation is likely due to the differences in effect of innervation of striatal circuitry by different cortical regions in the brain.

Introduction

The basal ganglia are a conglomeration of interconnected neural structures which are significant in the facilitation of motor and cognitive controls, such as sensorimotor integration and voluntary movement control (Assous et al. 2017; Alloway et al., 2017). The striatum is the primary input nucleus of the basal ganglia, which plays a role in modulating motor behavior Striatal neurons play an important role in the facilitation and subsequent termination of movement (Diaz-Hernandez et al., 2018; Hooks et al., 2017; Klug et al., 2018; Melzer et al., 2017). The role of sensory areas of the neocortex in control of movement is not understood. It is possible that primary sensory areas influence striatal circuitry through their cortostriatal projections. (Reig and Silberg, 2014; Slippy et al., 2015).

Excitatory impulses to the striatum innervate D1 and D2 expressing spiny projection neurons (SPNs) as well as parvalbumin (PV)-expressing GABAergic fast spiking interneurons. Activation of D1-SPNs (the direct pathway) promotes movement whereas activation of D2-SPNs (indirect pathway) suppresses movement (Tecuapetla et al., 2016). Striatal interneurons such as PV-expressing interneurons inhibit D1 and D2-SPNs which has varied effects on behavior such as modulating experience-dependent behavior (Gittis et al., 2010, 2011; Lee et al., 2017; O'Hare et al., 2017; Tepper et al., 2010). Most of these neuron types receive excitatory inputs from different areas of the neocortex (Assous and Tepper, 2018). The primary sensory areas of the neocortex have become the centerpiece for much research surrounding its role in sensorimotor integration and motor control. The primary somatosensory barrel cortex (S1) of the mouse vibrissal system receives whisker related tactile signals via the thalamus. Its function serves as an important vehicle for investigating sensorimotor integration, in particular, sensory guided

behavior in decision making (Diamond et al., 2008). The primary motor cortex aids in the process of executing motor plans generated by premotor areas. The linkage between S1 and M1 through their projections is well documented (Chen et al., 2013a, 2015). However, it is largely unknown how projections from these sensory areas impact striatal circuitry and whether they play a role in modulating behavior.

To identify whether M1 and S1 exerted different effects on behavior, mice were trained to perform a go/no-go task using their whiskers to discriminate between textures for a water reward. In order to reach a satisfactory conclusion regarding the influence of these sensory and motor areas in modulating behavior, it was important to effectively interpret and visualize experimental outcomes. An algorithm in MATLAB was designed to analyze how performance in this task was impacted by optogenetic stimulation of S1 and M1. This was accomplished by analyzing data accumulated from lick traces and outputting data that allows us to discern minute behavioral patterns.

Ultimately, S1 corticostriatal innervation causes behavioral suppression and M1 corticostriatal innervation causes behavioral facilitation. This phenomenon of behavioral suppression and activation is likely due to the differences in effect of innervation of striatal circuitry by different cortical regions in the brain.

Problem and motivation:

It was uncertain how S1 and M1-corticostriatal inputs effected sensorimotor behavior. In order to address this, performance in a whisker-depended texture discrimination task in head-fixed mice during optogenetic stimulation of specific corticostriatal inputs was interrogated. This Go/NoGo task rewarded mice with water for correct licking response to a rough texture (Hit trial) and withheld water when mice licked in response to the smooth texture (False Alarm trial). A motorized stage presented texture pads to whiskers during the experiment (Figure 1). In addition, an optical fiber was surgically implanted above the dorsal striatum in mice expressing ChR2 (channelrhodopsin-2) in either S1 or M1 (Figure 1B). Prior to my involvement in the project, effects of optogenetic stimulation were measured through changes in Hit Rate, False Alarm Rate, Sensitivity, and Bias compared to the start of a session. In each session consisting of 127 trials, the first 50 were without stimulation (baseline) and the next 77 with stimulation (above baseline). While this information demonstrates that activation of striatal inputs affected decision making, it was unclear if this activation impacted performance in the learned behavioral task. Previous measures only take into account the first lick in each trial. This is limiting because it does not illustrate licking behavior throughout the tactile discrimination task. In order to attack this question, it was necessary to generate graphs that represented performance during a tactile discrimination task that incorporate the entire licking response in each trial so that behavioral trends could be visualized.

Results/ troubleshooting measures:

Data conversion:

Our experimental rig, which is run by custom built software coded in National Instrument's LabVIEW not only operates the physical rig during experiments, it also collects all data accumulated during experimentation and stores data in LabVIEW's proprietary tdms file type. However, this file format is not readable by default in MATLAB. Therefore, in order to perform any kind of analysis on data accumulated during experimentation in MATLAB, each tdms file had to be converted to a mat file so that MATLAB could read them. Therefore, it was necessary to find a way to read into MATLAB all data stored in the tdms files so that the information contained within could be analyzed. A pre-made program called readTDMS was found on matlabcentral.com that was able to convert tdms files to mat files. It is very common when programming to use scripts that have already been made in tandem with one's own. Ultimately, readTDMS creates a new matrix variable called lick_data that is the composite of all trials combined to form a session (Figure 2A). Each trial of a session stored in lick_data can be graphically represented (Figure 2B). Doing this reveals that each trial is a composite of small noisy signals and brief spikes.

Results of initial Peak Analysis:

Following the generation of the matrices, it became obvious that the piezo pressure sensor which recorded licks during experiment was, in addition to capturing actual sensor activation or licks, tremendously noisy. The piezo sensor is subject to electrical and physical interference, so noise appearing in data is just a reflection of that. It was therefore important to filter out the noise in our analysis and only operate on actual sensor activation. In order to do this, a peak analysis was performed on each trial contained within the lick_data file. A peak analysis program is one that finds the local maxima and/or minima of a dataset or function. These maxima and minima represent instances of sensor activation that with an amplitude higher than the experimental thresholds specified during the initial data accumulation. Thresholds are the values above which a sensor activation can be considered a lick. In each session, there was an upper and a lower threshold specified because spikes in lick_data could also appear below average noise readings. While MATLAB has a default peak analysis function, this turned out to be insufficient because it only collected the local maxima in a given dataset and I was unable to factor in either the upper or lower thresholds. In order to circumvent this issue, it was necessary to write some code that would only record spikes above the upper threshold and below the lower threshold. This was done iteratively. Each element of the matrix was scanned and compared to the upper and lower thresholds. If the element was either above the upper threshold or below the lower threshold it was counted as a lick. If an element was determined to be a lick, the trial number, time of occurrence (calculated by multiplying the col number by 0.01 to convert from milliseconds to seconds), and amplitude of the lick recorded were respectively placed in a matrix called peaks_and_vallies_above_and_below_noise. By parsing and organizing the data in this way we were able to separate true signals from the noise. Without doing this, any further analysis would have been impossible.

Binarization of lick signals and the problem and the solution

While the peak analysis was successful in capturing instances where sensor activation was above or below the specified thresholds, these do not necessarily parse licks. Some recorded spikes are simply too close together to be considered licks individually (Figure 3). Some studies suggest that the fastest rate a mouse can lick is 6.87 licks per second or one lick every 0.146 seconds (Murakami, 1977). However, many readings were reporting quicker response times than this. Because of this, it was impossible to determine where one lick ended and the next began. In order to rectify this, an algorithm was written to binarize sensor readings based on time in order to group readings that occurred too close to one another to be distinct. A new column vector is created called Times which is simply the second column of peaks_and_vallies_above_and_below_noise (Figure 4A). The algorithm checks if the difference of two adjacent times are less than or equal to 0.5 seconds. The difference threshold of 0.5 was arbitrarily chosen to be less than the average difference between confirmed licks in our datasets. Let the variable k be an element in the vector with index greater than 2. If the difference between the kth and k-1th reading is less than 0.5, then they are considered to be part of the same lick and each is assigned a value of 1 which will be inserted at the kth and k-1th position in a new column vector B. If the difference between the kth and k-1th element is greater than 0.5 then only the kth term is assigned a value of 0 which is placed at the kth position in B. This process was performed twice, first beginning at index 2 then beginning at index 3. This is because if the comparison was only made starting at index 2, then the k+1th term would never be compared to the kth term. Since it is possible that the distance between the k+1th term and the kth term is greater than 0.5, which would mark the beginning of a new lick, it was essential to perform both iterations. When this algorithm was complete, the final form of vector B was concatenated onto the matrix peaks_and_vallies_above_and_below_noise and made into the fourth column (Figure 4B).

In all sessions, there existed a “grace period” or period of time during each trial where activation of the sensor was not counted. This grace period was variable, and as such, a new grace period must be input by the user each time the program is run. This grace period is compared iteratively to all times in column 2 of peaks_and_vallies_above_and_below_noise. If an element was found to be less than the grace period, then its respective value in column 4 would be changed to a 0, signifying that no lick took place.

While this script was successful in marking the beginning of licks, often times the string of ones would trail between trials. Since licks cannot transcend trials, it was important to mark the end of trials. Another block of code was written to insert a zero row between trials so that the end of a lick in a trial could be differentiated from the beginning of one in the following trial.

Finally, trial number and start times of each lick were accumulated and stored in a new matrix called Lick_log (Figure 4C).

Graph generation:

It was uncertain what the information organized and stored by the program would yield regarding performance in the tactile discrimination task. In order to find out, a method was written to take the data accumulated in the Lick_log matrix and output several graphs interpreting data.

It was useful to identify how general licking activity in a session differed before and after optogenetic stimulation of M1 and S1. For this, scatterplots were generated by plotting times of licks per trial. They were created by inputting all lick times and their respective trial number into

the scatterplot function in MATLAB. When comparing raster plots of M1 and S1 sessions, it was revealed that M1 stimulation increased licking response, whereas S1 stimulation seemed to have the opposite effect (Figures 5A and 5B).

It was also useful to investigate how licking rate varied between below baseline (or no stimulation) and above baseline (stimulation occurring) conditions. To do this, several histograms are generated and plot reaction times over time. The first of which was created using all trials that were below baseline, the second used all trials that were above baseline, and the final histogram used all trials (this one turned out not to be useful). From these, a difference of rate could be inferred in licking rate between stimulated above baseline and non-stimulated below baseline conditions.

From these sets of graphs, it was easy to conclude that S1 corticostriatal innervation causes behavioral suppression and M1 corticostriatal innervation causes behavioral facilitation.

Proof:

Validation of results of program:

In order to verify that the program was capturing the timing of licks to a high degree of accuracy, statistics were performed on both a small and large constructed sample set. First a small session of 15 trials was created manually by triggering the sensor on the experimental rig and recording reaction time on paper as well as accumulating tdms files through LABVIEW. Manually and electronically collected lick times were input into excel for the sample set, and two statistical tests were performed. A Pearson correlation coefficient was found, which serves as a measure of the linear correlation between two variables and has a value between -1 and 1, where 1 is total positive correlation, 0 being no linear correlation, and -1 being total negative linear correlation. The correlation coefficient from resulting from the comparison between the manually and electronically gathered datasets was 0.998142. This implies a very strong linear correlation between the two sample sets. The second statistical test performed on the datasets was a student's paired ttest. This test finds if there is a significate difference between the means of two groups and ultimately determines if two sets of data are significantly different from each other. The sample sets when compared statistically received scores of 0.998142 for the Pearson correlation coefficient and 0.967304 (which is the resulting probability from the excel function for ttest) for the paired student's ttest. These numbers suggest a high correlation between known values and electronically gathered values. This suggests that in both cases, the manually recorded and electronically gathered data were statistically similar. Therefore, it was concluded that the program operated with a high degree of accuracy.

Discussion:

It was observed that corticostriatal inputs form S1 and M1 produced different patterns of innervation in projection neurons and interneurons within the striatum. It was initially unclear if this lead to any discernable behavioral changes. This was largely due to the fact that prior analysis of licking behavior during tactile discrimination task did not track entire licking response during each trial. Overall behavior during the experiment was therefore incomplete. Through effective storage and translation of experimental data into readable and interpretable graphs that tracked entire the licking response, it was revealed that S1-corticostriatal inputs suppress response during a tactile discrimination task, whereas M1-corticostriatal input has the

opposite effect. Thus, it is shown that differential activation of striatal neurons by certain neocortical areas yields differing effects on sensory driven behavior.

References

Alloway, K.D., Smith, J.B., Mowery, T.M., and Watson, G.D.R. (2017). Sensory Processing in the Dorsolateral Striatum: The Contribution of Thalamostriatal Pathways. Front. Syst. Neurosci. *11,* 53.

Assous, M., and Tepper, J.M. (2018). Excitatory extrinsic afferents to striatal interneurons and interactions with striatal microcircuitry. Eur. J. Neurosci.

Chen, J.L., Pfäffli, O.A., Voigt, F.F., Margolis, D.J., and Helmchen, F. (2013b). Online correction of licking-induced brain motion during two-photon imaging with a tunable lens. J. Physiol. *591,* 4689–4698.

Diamond, M.E., von Heimendahl, M., Knutsen, P.M., Kleinfeld, D., and Ahissar, E. (2008). "Where" and "what" in the whisker sensorimotor system. Nat. Rev. Neurosci. *9,* 601–612.

Díaz-Hernández, E., Contreras-López, R., Sánchez-Fuentes, A., Rodríguez-Sibrían, L., Ramírez-Jarquín, J.O., and Tecuapetla, F. (2018). The Thalamostriatal Projections Contribute to the Initiation and Execution of a Sequence of Movements. Neuron.

Gittis, A.H., Leventhal, D.K., Fensterheim, B.A., Pettibone, J.R., Berke, J.D., and Kreitzer, A.C. (2011). Selective inhibition of striatal fast-spiking interneurons causes dyskinesias. J. Neurosci. : Off. J. Soc. Neurosci. *31,* 15727–15731.

Gittis, A.H., Nelson, A.B., Thwin, M.T., Palop, J.J., and Kreitzer, A.C. (2010). Distinct roles of GABAergic interneurons in the regulation of striatal output pathways. J. Neurosci. Off. J. Soc. Neurosci. *30,* 2223–2234.

H.Murakami, H. (december 1977). Rhythmometry on licking rate of the mouse. *Science Direct,19*(6), 735-738.

Hooks, B.M., Papale, A.E., Paletzki, R.F., Feroze, M.W., Eastwood, B.S., Couey, J.J., Winnubst, J., Chandrashekar, J., and Gerfen, C.R. (2018). Topographic precision in sensory and motor corticostriatal projections varies across cell type and cortical area. Nat. Commun. *9,* 3549.

Klug, J.R., Engelhardt, M.D., Cadman, C.N., Li, H., Smith, J.B., Ayala, S., Williams, E.W., Hoffman, H., and Jin, X. (2018). Differential inputs to striatal cholinergic and parvalbumin interneurons imply functional distinctions. ELife *7.*

Lee, K., Holley, S.M., Shobe, J.L., Chong, N.C., Cepeda, C., Levine, M.S., and Masmanidis, S.C. (2017). Parvalbumin Interneurons Modulate Striatal Output and Enhance Performance during Associative Learning. Neuron *93,* 1451–1463.e4.

Melzer, S., Gil, M., Koser, D.E., Michael, M., Huang, K.W., and Monyer, H. (2017). Distinct Corticostriatal GABAergic Neurons Modulate Striatal Output Neurons and Motor Activity. Cell Reports *19,* 1045–1055.

O'Hare, J.K., Li, H., Kim, N., Gaidis, E., Ade, K., Beck, J., Yin, H., and Calakos, N. (2017). Striatal fast-spiking interneurons selectively modulate circuit output and are required for habitual behavior. ELife *6.*

Reig, R., and Silberberg, G. (2014). Multisensory integration in the mouse striatum. Neuron *83,* 1200–1212.

Sippy, T., Lapray, D., Crochet, S., and Petersen, C.C.H. (2015). Cell-Type-Specific Sensorimotor Processing in Striatal Projection Neurons during Goal-Directed Behavior. Neuron *88,* 298–305.

Tecuapetla, F., Jin, X., Lima, S.Q., and Costa, R.M. (2016). Complementary Contributions of Striatal Projection Pathways to Action Initiation and Execution. Cell *166,* 703–715.

Tepper, J.M., Tecuapetla, F., Koós, T., and Ibáñez-Sandoval, O. (2010). Heterogeneity and diversity of striatal GABAergic interneurons. Front. Neuroanat. *4,* 150.

Figures

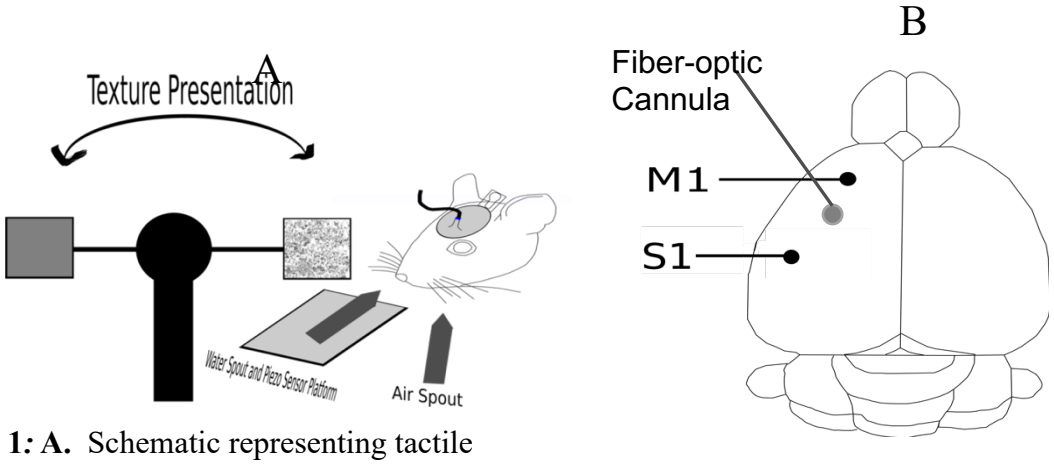
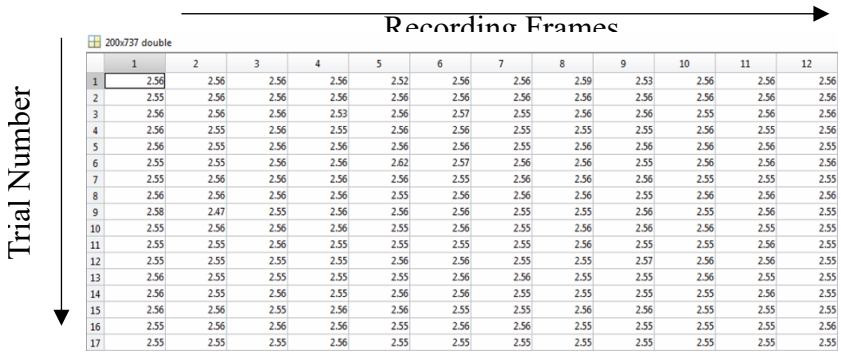


Figure 1*: A.* Schematic representing tactile discrimination task.
B. Shows injection sites and placement of fiber-optic cannula in mouse brain.

2A



2B

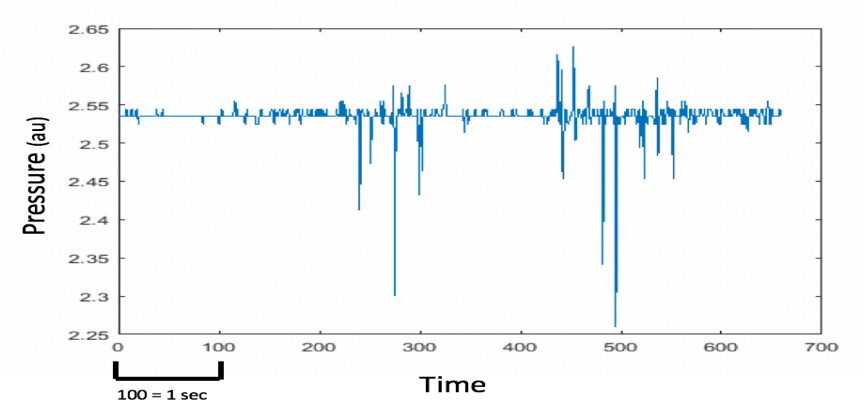
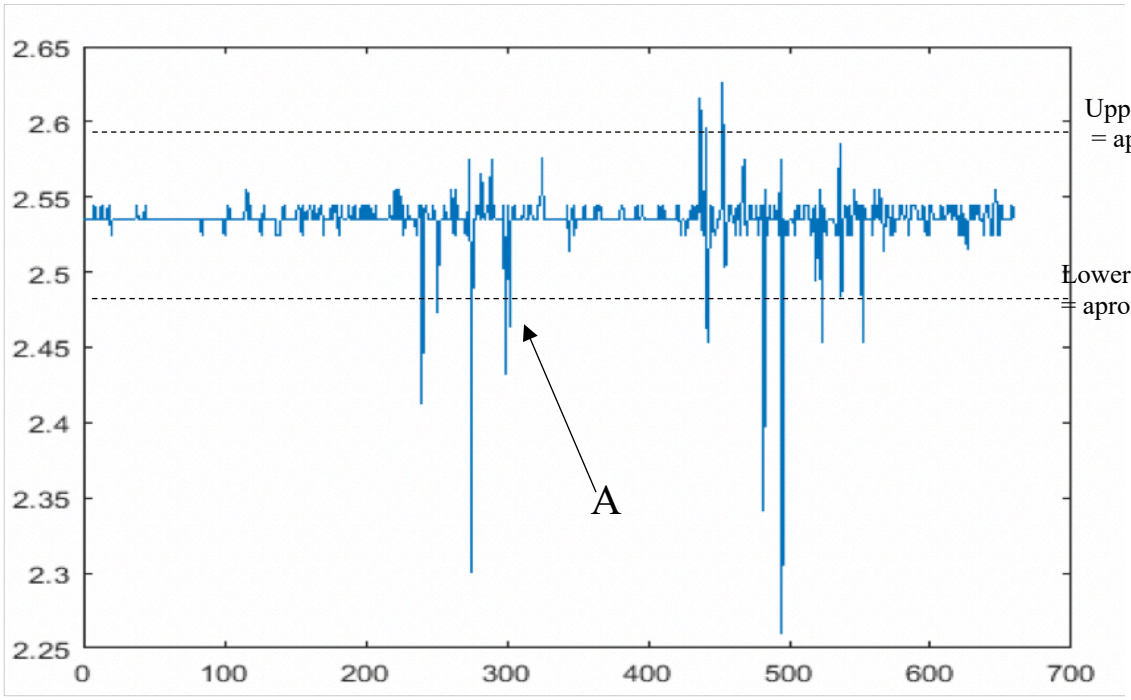


Figure 2: A. The above matrix is a sample Lick_data matrix. The numbers on the y axis represent trial numbers. The numbers on the x axis represent the individual frames of the piezo sensor recording. The recording was done at 100 Hz, therefore each unit is 1 millisecond.

B. This figure is an example of a lick trace. Larger spikes represent licks. Spikes that are



extremely close to one another are grouped together as individual licks.

Figure 3: The arrow A in the above figure demonstrates an instance where two spikes exist too close to one another to be distinct licks. The program will group these two together as one lick. Please note that thresholds indicated on this figure are representative and are subject to change on other datasets.

A

450x3 double

	1	2	3
1	1.00	4.00	2.65
2	1.00	4.17	2.62
3	1.00	4.18	2.45
4	1.00	4.68	2.62
5	1.00	5.66	2.46
6	1.00	5.83	2.45
7	2.00	2.64	2.70
8	2.00	2.80	2.45
9	2.00	2.81	2.44
10	2.00	3.71	2.61
11	2.00	3.72	2.64
12	2.00	4.10	2.40
13	4.00	5.50	2.46
14	4.00	5.89	2.61
15	4.00	5.90	2.47
16	5.00	4.60	2.45
17	5.00	4.64	2.40
18	5.00	4.65	2.46
19	5.00	6.35	2.62
20	5.00	6.71	2.64
21	6.00	3.43	2.62
22	6.00	3.44	2.44
23	6.00	3.63	2.47
24	6.00	3.64	2.61
25	6.00	4.00	2.47
26	8.00	4.32	2.47
27	8.00	5.15	2.45
28	8.00	5.31	2.46
29	8.00	5.81	2.42
30	9.00	3.59	2.46

B

450x4 double

	1	2	3	4
1	1.00	4.00	2.65	1.00
2	1.00	4.17	2.62	1.00
3	1.00	4.18	2.45	1.00
4	1.00	4.68	2.62	1.00
5	1.00	5.66	2.46	0.00
6	1.00	5.83	2.45	1.00
7	2.00	2.64	2.70	1.00
8	2.00	2.80	2.45	1.00
9	2.00	2.81	2.44	1.00
10	2.00	3.71	2.61	1.00
11	2.00	3.72	2.64	1.00
12	2.00	4.10	2.40	1.00
13	4.00	5.50	2.46	0.00
14	4.00	5.89	2.61	1.00
15	4.00	5.90	2.47	1.00
16	5.00	4.60	2.45	1.00
17	5.00	4.64	2.40	1.00
18	5.00	4.65	2.46	1.00
19	5.00	6.35	2.62	0.00
20	5.00	6.71	2.64	1.00
21	6.00	3.43	2.62	1.00
22	6.00	3.44	2.44	1.00
23	6.00	3.63	2.47	1.00
24	6.00	3.64	2.61	1.00
25	6.00	4.00	2.47	1.00
26	8.00	4.32	2.47	1.00
27	8.00	5.15	2.45	0.00
28	8.00	5.31	2.46	1.00
29	8.00	5.81	2.42	1.00
30	9.00	3.59	2.46	1.00

C

391x2 double

	1	2
1	1.00	4.00
2	1.00	4.17
3	1.00	4.18
4	1.00	4.68
5	1.00	5.83
6	2.00	2.64
7	2.00	2.80
8	2.00	2.81
9	2.00	3.71
10	2.00	3.72
11	2.00	4.10
12	4.00	5.89
13	4.00	5.90
14	5.00	4.60
15	5.00	4.64
16	5.00	4.65
17	5.00	6.71
18	6.00	3.43
19	6.00	3.44
20	6.00	3.63
21	6.00	3.64
22	6.00	4.00
23	8.00	4.32
24	8.00	5.31
25	8.00	5.81
26	9.00	3.59
27	9.00	3.78
28	9.00	3.79
29	9.00	3.99
30	10.00	3.65

Figure 4: A: Representative data from peaks_and_vallies_above_and_below_noise. The first column represents the trial numbers each recording was discovered in. The second column contains the reaction times and is highlighted because this was the column which was significant in performing the binarization step. The third column represents the amplitude of each spike.
B: Figure B is the same as figure A however it contains the new column grouping close together spikes into one lick. Strings of 1s represent one and the same lick, and 0s indicate the termination of that lick.
C: Figure C shows the final product of binerization and sorting. The first column represents trial number and the second column represents time in trial where beginning of lick occurred.

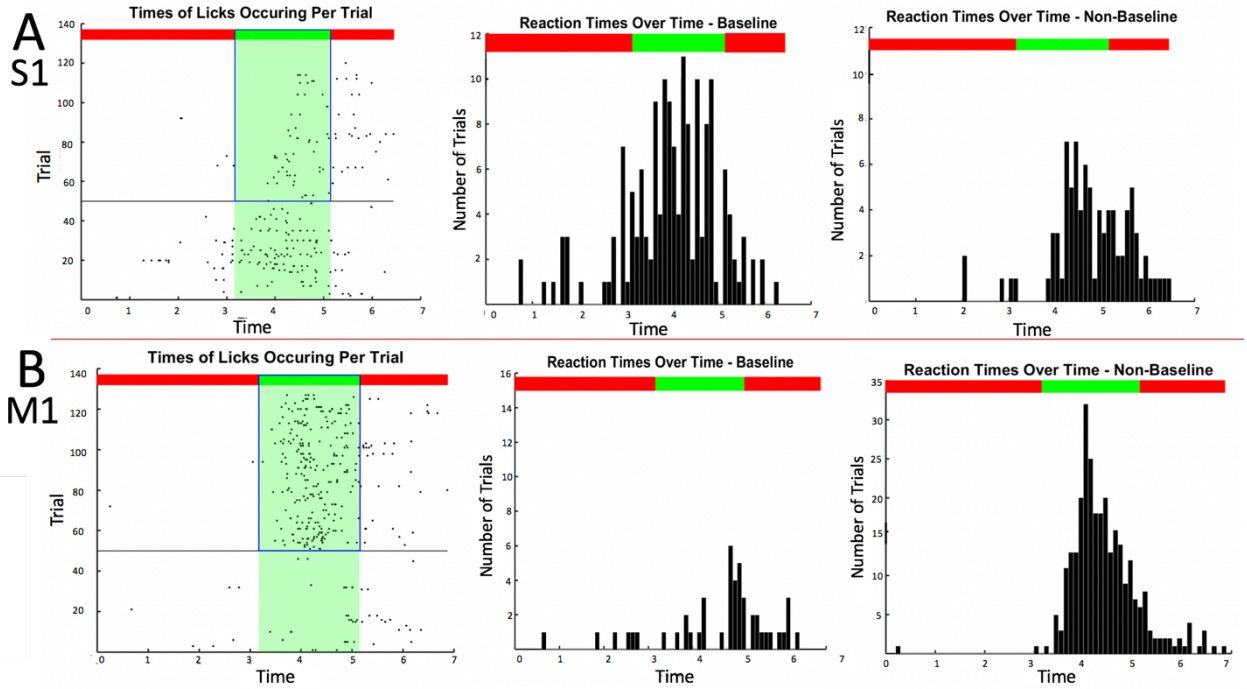


Figure 5: **A**. (Left to Right) S1 stimulation during discrimination task revealed a behavioral inhibition, as seen in first image. Reaction times during S1 stimulation were also impacted as can be seen in scatter plots. Histograms represent performance overtime below an above baseline. What is found in this circumstance is that below baseline licking behavior is exaggerated and above baseline shows a steep decline in licking behavior.
B. (Left to Right) M1 stimulation increased performance in behavioral task during presentation window, as seen in first image. Reaction times during M1 stimulation honed and increased performance. Histograms represent performance overtime below an above baseline. What is found in this circumstance is that below baseline licking behavior is very minimal and above baseline shows a steep increasing licking behavior.