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Basal forebrain's role in procedural learning

In this paper, we aim to understand how the basal forebrain is involved in procedural learning, given that we know that without the basal forebrain's cortical projection system, learning proficiency decreases. To answer this question, we studied the basal forebrains of four long-evans male rats on a neuronal level by inserting microwires surgically. We found that the basal forebrain's role in procedural learning is to act as a teaching signal to the motor cortex.

Key words: basal forebrain, procedural learning, "reach-to-grasp", motor skill learning

Introduction

One form of procedural learning involves development of a motor skill. For example, when you learn how to play golf, swinging the golf club to successfully hit the golf ball requires a very complex learned pattern of muscle activity that involves the arm, wrist, back, and leg muscles. Another example of procedural learning is when a baby learns to reach and grab something. This act also involves a complex set of muscle activity that coordinates to successfully grab the desired object.

In order to study procedural learning on a neuronal level, we used four male long-evans rats. By using single cell recordings, we are able to see what areas of the brain are involved in procedural learning by examining their electrical activity patterns. In order to study procedural learning in rats, they must learn a new motor skill. A common motor skill rats learn is called a "reach-to-grasp" task. In teaching rats this task, they learn to reach and grab a desired object, often a sucrose pellet, with the arm and paw. This distinction of the use of the arm and paw is important because rats normally pick up objects with their mouth. By restricting the use of their mouth to pick up an object, it encourages the rat to learn to use its arm and paw. Learning in a reach to grasp task is measured by the number of successful reaches compared to the number of failed reaches. Over time, the percent of successful reaches increases, and by extension procedural learning occurs.

We know of at least three areas of the rat that are involved in procedural learning: the muscles, the motor cortex, and the basal forebrain. During procedural learning the complex patterns of muscle coordination change as the animal becomes more proficient at the task (Kargo, Nitz 2003). In the motor cortex there is improved signal to noise ratio of its reach related activity as the rat becomes more proficient at the task, as shown in figure 0 (Kargo, Nitz 2004). And, we know that the motor cortex regions associated with forelimb movement expand. We also know that the basal forebrain plays a critical role. In order to understand, there are a few characteristics of the basal forebrain that must be understood.

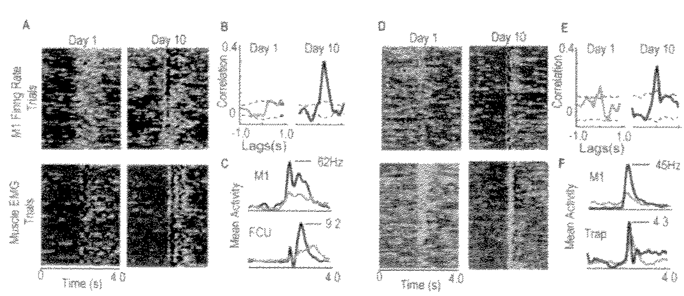

Figure 0. From day 1 to day 10, there is an increased signal relative to noise, showing evidence for learning on a neurophysiological level.

The basal forebrain is located rostrally and ventrally to the striatum in a human brain. The basal forebrain is a complex region that is made up of mainly three neurochemical types: gaba, glutamate, and acetylcholine (Zaborszky, 2013). These different cell types project from the basal forebrain to different areas of the brain as shown in Figure 1. This cortical projection system allows the basal forebrain to have localized input to different regions of the brain. For example, the basal forebrain's projections to the motor cortex can be activated while the projections to other regions of the brain remain inactive. This gives the basal forebrain a powerful ability to control which regions of the brain it stimulates. As shown in figure 2, learning proficiency decreases without the basal forebrain acetylcholine projection (Conner, Chiba 2003)

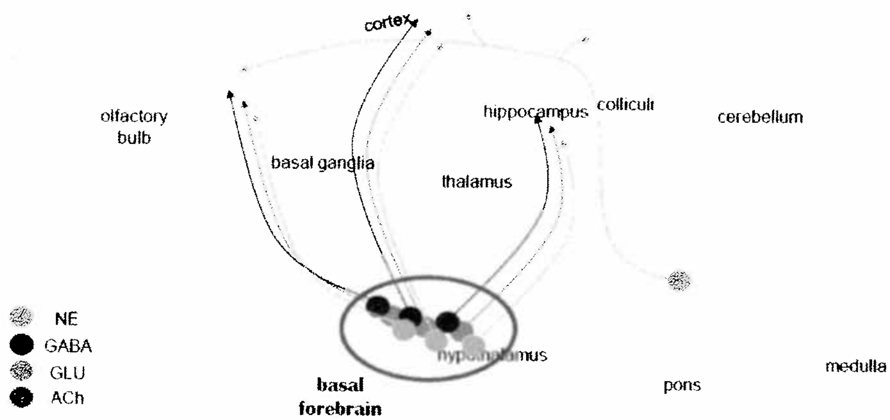

Figure 1. schematic of the basal forebrain's position in a rat brain

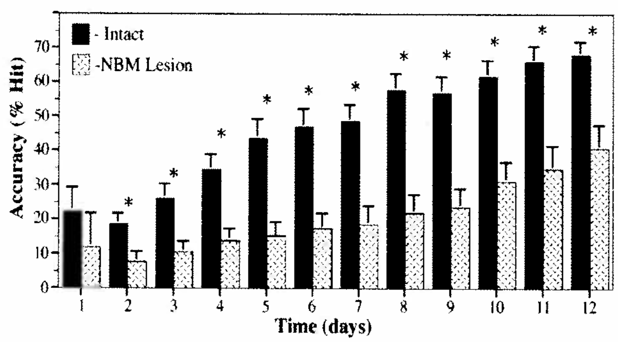

Figure 2. If the basal forebrain's acetylcholine projections to the motor cortex are extinguished then procedural learning is weakened drastically. This is indicated by the bar graph. On the x-axis is time, and on the y-axis is accuracy. The white bars represent a rat with acetylcholine projections and the amount of accuracy increases over time, indicating learning. The grey bars represent a rat without acetylcholine projections, who doesn't learn as well over time.

Methods and Measures

In this experiment, four rats were trained the reach to grasp task. Each rat was recorded for 8 days. During each recording, the rat performed 100 reaches. The rat was kept on a restrictive food diet during this time, in order to incentivize him to learn to reach. All rats reached for a sucrose pellet. In order to answer our question we focused our attention on three things: the firing rates of basal forebrain neurons, the time surrounding the reach, and the outcome, whether the reach was a success or fail. In order to record the activity in the basal forebrain, we inserted recording devices into the basal forebrain. The sucrose pellet rests on a piezo electric recording device that is extremely sensitive to touch. When the rat reaches for the sugar pellet, his touch is registered with this device, which allows us to pinpoint, the exact time that the rat made contact with the pellet. This is important because all of our analysis is focused around the moment of the pellet touch. After extracting the moment of the pellet touch, we also looked at the 5 seconds before and after the pellet touch, so 10 seconds total. To you, 10 seconds may not seem like a long time, but when the actual reach takes on average .65 seconds, 5 seconds on either side is a long time period to neurons. In addition, there is a lab technician in the recording room that records the outcome (success or failure) of the each reach, directly after it occurs.

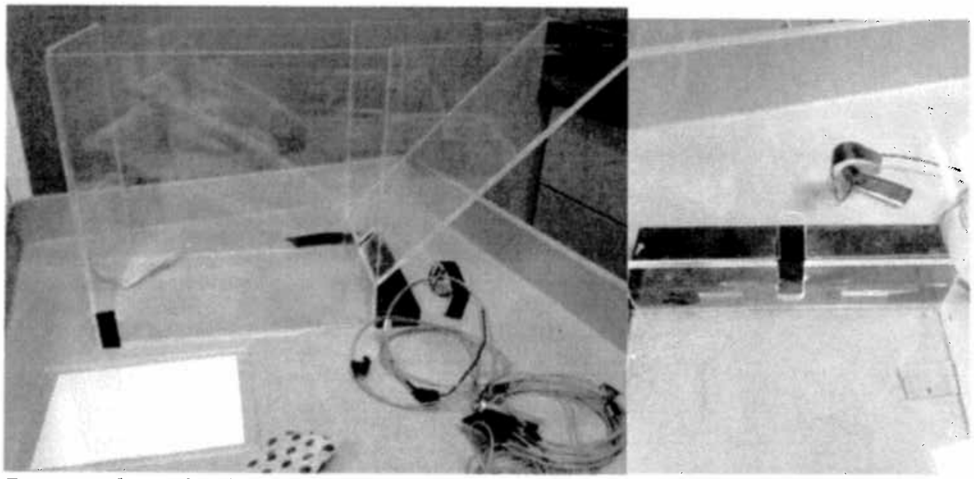

Figure 3: This is the plastic container that was used to conduct the reach to grasp task. The black bar is the piezo electric recording device.

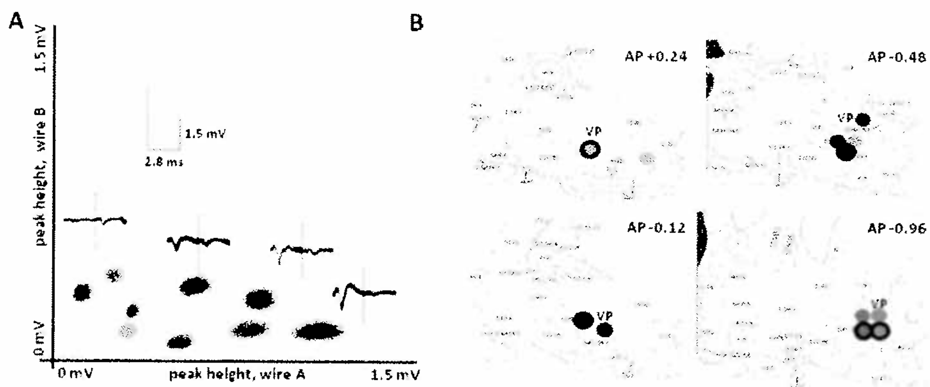

Figure 4: This shows where the microwires we inserted in one of the animals.

Possible Outcomes

There are four possible outcome models that we came up with as show in figure 5. The overall arousal model is indicated by the straight line where differences in successful and failed reaches are high throughout the reach, pre-reach and post-reach periods. This result would indicate that the basal forebrain's role in procedural learning to generally cause arousal in the animal. The preparation model begins at high differences of firing rate between successful and failed reaches, but drops to low differences of firing rates around the pellet touch. This could indicate that the basal forebrain is preparing the animal to reach, perhaps increasing attention, or stimulating the motor cortex to set up the position of the rat correctly. The action model starts with low differences of firing rate, and then peaks during the actual reach, and then returns to low differences. This could mean that the basal forebrain's role in procedural learning is to instruct the motor cortex at all stages of the reach, but only the reach. The outcome model is indicated with the greatest differences in activity after the reach. This could indicate that the basal forebrain acts as a teaching signal, signaling, "Yes you did it correctly, do that again"

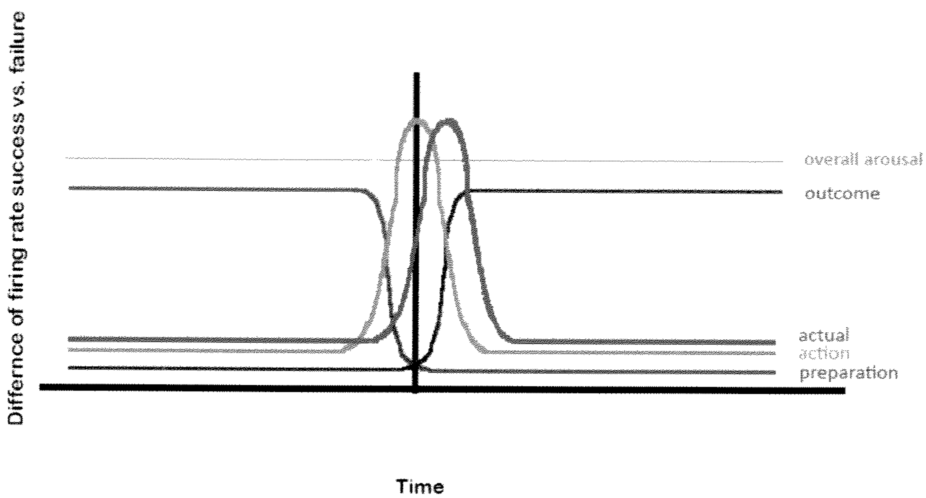

Figure 5: On the x-axis is time surrounding the moment the animal touches the pellet. On the y-axis is the difference in basal forebrain firing rates between successful and failed reaches. Indicated by the grey bar, is the total reach time, is a very short period of time. The center of the graph, indicated by the white arrow, is the moment that the rat touches the pellet.

Results

Figure 6 shows how 3 of the 4 rats showed a positive relationship between training day and success rate. Two of the rats had, for unknown reasons, reduced performance on day 8. In addition, rat AG1 showed reasonable proficiency, but a lack of improvement across days.

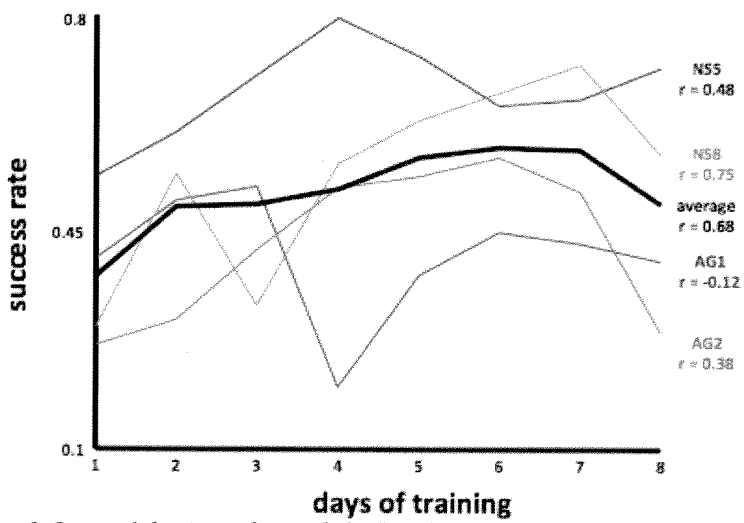

Figure 6: 3 out of the 4 rats learned the "reach-to-grasp" task. On the x-axis are days one through 8 of training and on the y axis is success rate. The thick black line shows a positive correlation of the average success rates across all 4 rats.

We started to analyze the data on a cell by cell basis, starting with 4 different cells from one of the animals, NS8 on his eighth and last recording day. The four images in figure 7 are the perievent histograms, which is a measure of activity around a point of interest, in this case the pellet touch. In figure 8, which shows the means of figure 7, all four cells are doing something different. The first cell has its peak firing rate just before the pellet touch. The second cell has its peak firing rate just after the pellet touch. The third cell has a wider peak, and fires at a high rate throughout the entire reach. The fourth cell has its peak firing rate way before the pellet touch, and even has a large depression of activity leading up to the reach. It may look like it's not really involved in any specific way, but we'll see differently in a moment.

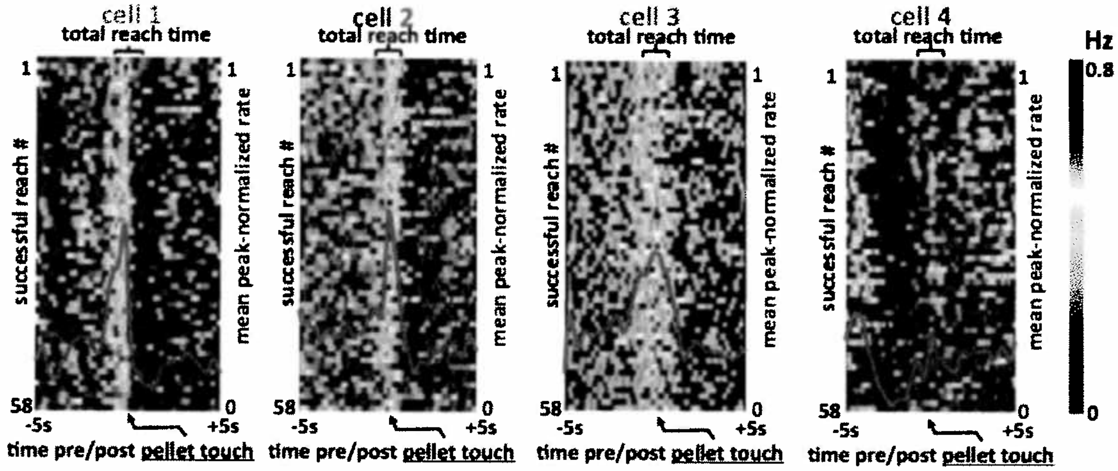

Figure 7: In these graphs, activity is colormapped from low, blue, to high, red. On their x axis is time starting with 5 seconds before the pellet touch and ending 5 seconds after the pellet touch. At the center of the x-axis is the pellet touch, indicated by the arrow. The x-axis is represented by 100 times bins, each time bin being one tenth of a second. On the left y-axis is the successful reach number. So this animal on this recording day had 58 successful reaches. Each row is the peak normalized firing rate specific to that reach. For example, the first row is the peak normalized firing rate for this cell during the animal's first successful reach. On the right y-axis, indicated in red, is the mean peak normalized firing rate of all 58 successful reaches. This mean is drawn on top of the perievent histogram in red. Indicated at the top of each perievent histogram is the total reach time, which makes up a fraction of the time plotted on the axis. On average, a reach takes about .65 seconds.

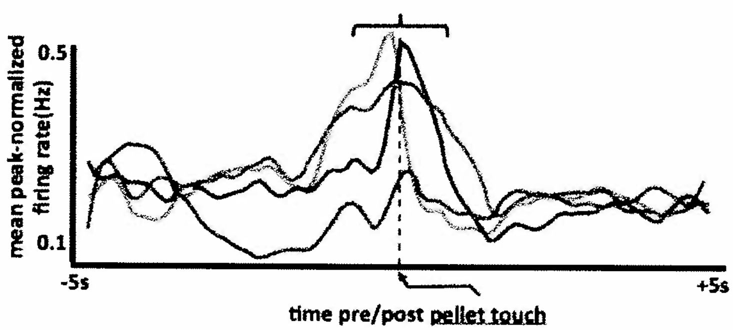

Figure 8:
The mean peak normalized firing rate for all four cells. Again, on the x-axis is 10 seconds surrounding the pellet touch and total reach time indicated with the bracket on the top.

Next using the same rat, NS8, on the same recording day, day 8, we looked at the comparison of successes and failures of the same four cells. From figure 9, we noticed that the statistically significant differences that occurred between the successful and failed reaches surrounded the time just after the pellet touch. Upon further analysis, cells two through four showed that during this time, the differences were significant at a 0.01 p value. Cell no. 4, which looked uninvolved when examining success rates only, can now be seen to provide a 'you failed' signal at the moment the animal touches the pellet, presumably in the wrong way on fail trials.

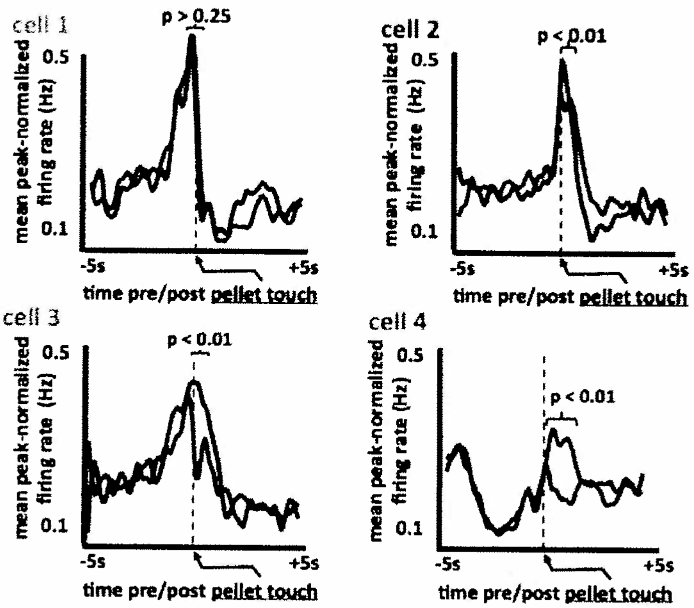

Figure 9: Here, the x-axes are time and the y-axes are the mean peak normalized firing rates or the success in blue and of the failures in red. The mean peak normalized firing rates of the failures were peak normalized to the firing rate successes, in order to compare the two data sets.

The next step we took was to look at the mean peak-normalized firing rates of all 20 cells from NS8 recording day 8, and look at the comparison of successes and failures, shown in figure 10. Figure 11 shows the means of the values in figure 10. From figure 10 and figure 11, the main difference between successes and failures was located at the moment just after the pellet touch. If we statistically compare the values seen during the reach but prior to pellet touch with those just after pellet touch, also during the reach, we find that they are significantly different. We can even see a significant difference if we simply compare the 100 milliseconds periods just before and just after pellet touch.

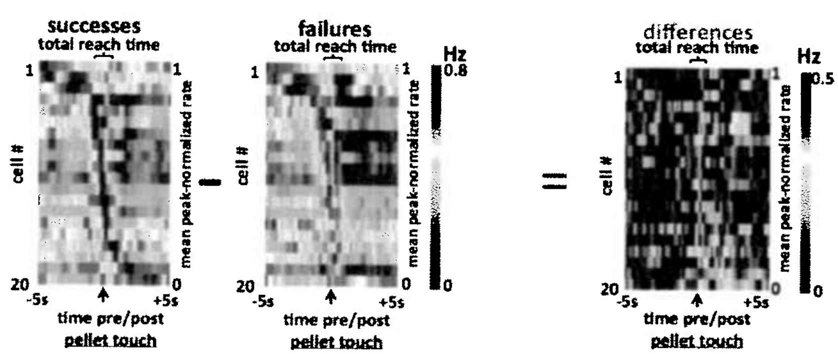

Figure 10: Looking at the perievent histograms: on the x-axis, again we have time surrounding the pellet touch. And on the left y-axis is the cell number; each row corresponds to one cell. The first perievent histogram represents the mean peak-normalized firing rate during the successful reaches for all 20 cells. The second perievent histogram represents the mean peak-normalized firing rate during the failed reaches for all 20 cells. And the third perievent histogram represents the absolute differences between successes and failures, which we found by simply subtracting the failures from the successes.

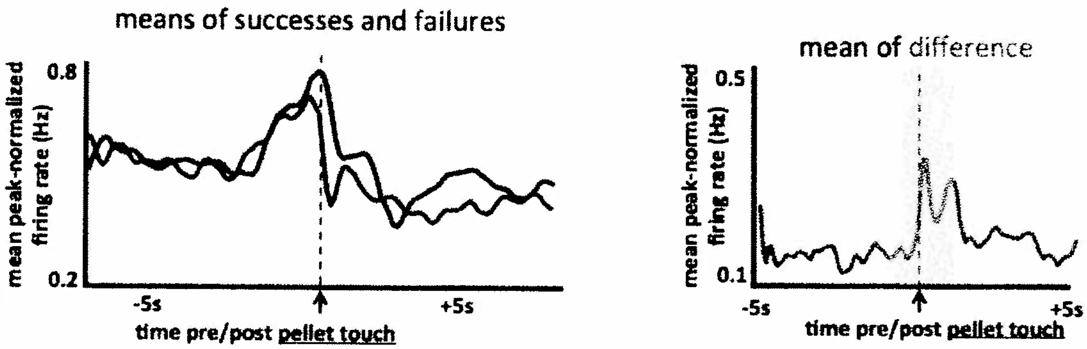

Figure 11: These are the means for the successes, failures and success-failure differences are illustrated using blue to represent successes and red to represent failures. In green, the mean of the absolute difference is illustrated.

Lastly, we wanted to see if these differences of firing rate of the basal forebrain neurons between successes failed reaches surrounding the moment of pellet touch held true across all four animals on all 8 recording days

In figure 12 is the mean of differences for just the one animal, NS8, on his eighth recording day from the previous slide, spread out in order to be able to see it in more detail. We first started our population level analysis looking at this time, so the time highlighted in pink, which is approximately half of a second before and after the pellet touch. We found that if you compare mean differences between successful and failed reaches for all animals from the first half of this time frame with the second half, these differences are statistically significant, shown in figure 12.

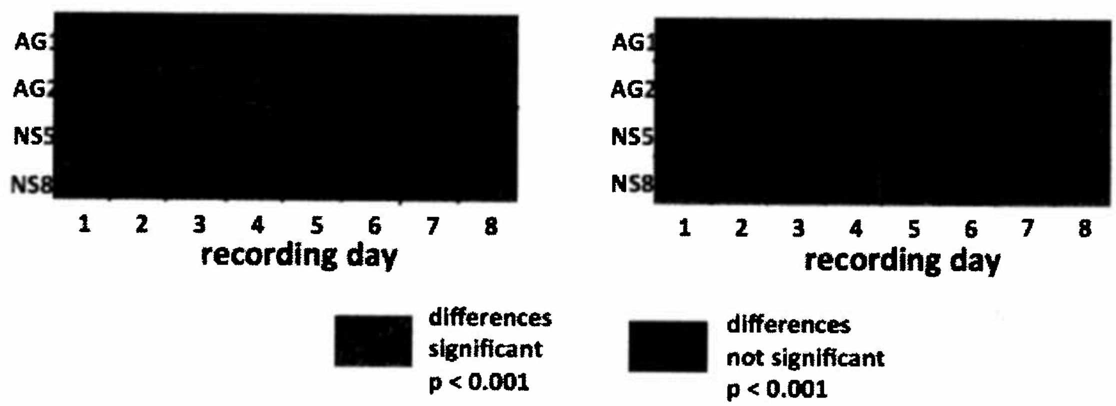

Figure 12: Here, on the x-axis are recording days one through 8, and on the y-axis are all four rats. A red square means the differences are statistically significant at a p value of 0.001, while a blue means the differences are not statistically significant. For example, on AG2's first recording day, the mean differences of basal forebrain neurons firing rate between successful and failed reaches during this time frame, have a statistical significance at a p value of 0.001.

All animals show this significance on all recording days except for AG1, strangely enough is the one animal that didn't learn. However, half a second is a lot of time when it comes to neuronal firing rate. We then reduced the amount of time where the looked at the differences to one tenth of a second before and after the pellet touch, shown here in orange. Running the same analysis, we found that even with such a small time frame, most of the statistically significant differences hold true. Even within such a small time frame, one tenth of a second, the differences between successes and failures for most rats on most recording days still holds a statistically significant p value of 0.001. That is, there is on almost every day, a sudden difference in basal forebrain neuron activity at the moment that the rat touches the pellet. Again, the one rat that doesn't show this same trend, is the one rat who didn't learn the reach to grasp task across the 8 days.

In Figure 13, our results, depicted in cyan, do not exactly match up with any of our possible outcome This model, with the peak of differences between successful and failed reaches just after the moment the reach touches the pellet, was a combination of the action and outcome model. This suggests that the rat knows the moment that his paw touches the pellet whether he will be successful or not.

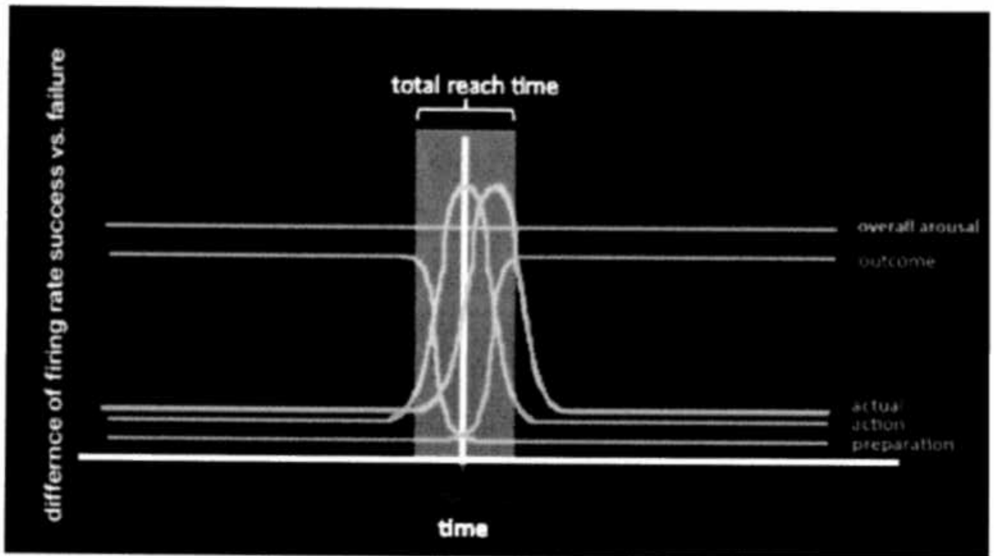

Figure 13: Possible outcomes model and the actual outcome

Conclusions and discussion

We suggest that the basal forebrain acts as a teaching signal to the cortex, indicating, "yes you did that correctly, do that again" and this signal is extremely precise, down to the hundredths of milliseconds after the reach.

In short, our findings lead us to believe that the basal forebrains role in procedural learning is a teaching signal that occurs within tenths of milliseconds after the reach. Next steps with this experiment would be to train more rats the reach-to-grasp task and study the firing rates of their basal forebrains in order to make this finding more robust. Another step would be to reduce the amount we look at, and by extension increase the definition of the perievent histograms, honing in on the time of interest – the moment just after the pellet touch. These steps, however, are just a few that would be necessary to show a robust finding with this experiment.

Citations

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