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Developing a High-Dimensional Dataset of the Changes in Ion Channels Expression Level at the Cholinergic Boutons in Amyotrophic Lateral Sclerosis

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Abstract
ALS, or Amyotrophic Lateral Sclerosis, is a neurodegenerative disease that causes motor neuron degeneration. Affecting nerve cells in the brain and spinal cord, this disease eventually leads to respiratory muscle failure and death. Although ALS is fairly uncommon, our lack of information on how ALS develops drives the need for further study. In the Neuro Engineering, Rehabilitation, and Degeneration laboratory at Wright State University, immunohistochemical data has been collected on a mouse model (SOD1-G93A) throughout their lifespan to better examine the neurological effects of ALS. To better organize and interpret the data, a high-dimensional dataset was created in this project using Microsoft Access. This was performed by rearranging the current data in Excel and importing it into Access. In Access, forms with buttons were created to streamline the process of viewing or editing data. By creating this database, the NERD laboratory will be able to review data and investigate trends that weren't immediately evident during data collection. Furthermore, this database will serve as a tool to both add current data and view its relation to past data. To test the accuracy and usability of the database, points of data were compared between current storage techniques and those stored and retrieved within the database. Using the database, available data on multiple proteins of interest were also compared to test its power as an investigative tool. Graphs testing the compared points of data show the same values between preliminary data and data taken from the database, verifying its reliability as an accurate and efficient storage platform. Graphs produced through its use also show its effectiveness and potential as a relational tool.

Introduction
Our scant information on Amyotrophic Lateral Sclerosis drives one of the research aims of the NERD laboratory. Starting a few years ago, immunohistochemistry data has been collected on a mouse model (SOD1-G93A) to investigate potential trends and relationships between its subgroups. This investigation is expected to provide new avenues of research to pursue, as well as improve our mechanistic understanding of ALS. The data is collected from the Vacht, Kv2.1, SK2, and SK3 proteins. Parameters of interest include cross-sectional area, cluster area, and number of clusters. To serve as a control, data from the same parameters have been collected on WT, or Wild Type mice. To organize, store, and investigate this data, a database was created using Microsoft Access.

Databases are powerful tools that allow researchers to view large amounts of various types of information in one central place. The key power in Access databases is its ability to compare and relate different sets of data. Containing its own coding language as well as built-in help tools, Access provides a database as simple or complex as necessary. Its relatively low learning curve allows creators to design working databases quickly, while its ease of use allows even the most novice users to access it. With these functions, Access is an ideal database tool for busy laboratory environments where many people with different skill levels will be accessing and using it.

Method
In order to create the database, the relevant data within Excel was first organized. Figure 1 shows the data in its raw form, and Figure 2 shows the data after organization. This process was applied to each Excel file to ensure uniformity and accuracy. After initial organization, relevant data was combined and organized into larger tables. Here, data collected for the same animals was

presented together for the first time. At this stage, even before import into Access, these Excel "Master Tables" provided greater and more efficient use as a relational tool than the initial, singular Excel files. A "Master Table" is shown in Figure 3.

L5_Slcie2_Left	Cell1				**1001.124**	519.067	**Large**
		1	4.499	829.39	388.979	449.17	473.995
		2	4.242	772.04	388.979	449.17	473.995
		3	4.499	867.4	388.979	449.17	473.995
		4	4.028	610.83	388.979	449.17	473.995
		5	4.713	746.745	388.979	449.17	473.995
		6	5.27	636.902	388.979	449.17	473.995
		7	4.456	601.452	388.979	449.17	473.995
			4.529571				
			0.056044				
L5_Slice1_left	Cell 2				**925.324**	113.008	**Large**
		1	4.499	692.162	638.425	672.155	644.464
		2	5.013	869.556	638.425	672.155	644.464
		3	7.07	777.309	638.425	672.155	644.464
		4	7.627	811.382	638.425	672.155	644.464
		5	7.13	772.837	638.425	672.155	644.464
		6	6.085	833.641	638.425	672.155	644.464
		7	5.27	795.569	638.425	672.155	644.464
			6.099143				
			0.172688				
L5_Slice1_left	Cell 3				**977.386**	115.307	**Large**

Figure 1

Animal_ID	Slice_Number	Cell_Number	Cluster_Number
109.1 SK2	L5_Slice2_Left_Avgs	1	333
109.1 SK2	L5_Slice2_Left	1	1
109.1 SK2	L5_Slice2_Left	1	2
109.1 SK2	L5_Slice2_Left	1	3
109.1 SK2	L5_Slice2_Left	1	4
109.1 SK2	L5_Slice2_Left	1	5
109.1 SK2	L5_Slice2_Left	1	6
109.1 SK2	L5_Slice2_Left	1	7
109.1 SK2	L5_Slice1_left_Avgs	2	333
109.1 SK2	L5_Slice1_left	2	1
109.1 SK2	L5_Slice1_left	2	2
109.1 SK2	L5_Slice1_left	2	3
109.1 SK2	L5_Slice1_left	2	4
109.1 SK2	L5_Slice1_left	2	5
109.1 SK2	L5_Slice1_left	2	6
109.1 SK2	L5_Slice1_left	2	7
109.1 SK2	L5_Slice1_left_Avgs	3	333
109.1 SK2	L5_Slice1_left	3	1
109.1 SK2	L5_Slice1_left	3	2
109.1 SK2	L5_Slice1_left	3	3
109.1 SK2	L5_Slice1_left	3	4
109.1 SK2	L5_Slice1_left	3	5
109.1 SK2	L5_Slice1_left	3	6

Figure 2

Age	Animal_ID	Slice_Number	Cell_Number	Cluster_Number	SK_Cluster_Area	SK_Intensity	SK_Cell_CS_Area	SK_Cell_Perimeter
P10	109.1 SK2	L5_Slice2_Left_Avgs	1	333	**4.529571429**	**723.537**	**1001.124**	
P10	109.1 SK2	L5_Slice2_Left	1	1	4.499	829.39	**1001.124**	
P10	109.1 SK2	L5_Slice2_Left	1	2	4.242	772.04	**1001.124**	
P10	109.1 SK2	L5_Slice2_Left	1	3	4.499	867.4	**1001.124**	
P10	109.1 SK2	L5_Slice2_Left	1	4	4.028	610.83	**1001.124**	
P10	109.1 SK2	L5_Slice2_Left	1	5	4.713	746.745	**1001.124**	
P10	109.1 SK2	L5_Slice2_Left	1	6	5.27	636.902	**1001.124**	
P10	109.1 SK2	L5_Slice2_Left	1	7	4.456	601.452	**1001.124**	
P10	109.1 SK2	L5_Slice1_left_Avgs	2	333	**6.099142857**	**793.208**	**925.324**	113.008
P10	109.1 SK2	L5_Slice1_left	2	1	4.499	692.162	**925.324**	113.008
P10	109.1 SK2	L5_Slice1_left	2	2	5.013	869.556	**925.324**	113.008
P10	109.1 SK2	L5_Slice1_left	2	3	7.07	777.309	**925.324**	113.008
P10	109.1 SK2	L5_Slice1_left	2	4	7.627	811.382	**925.324**	113.008
P10	109.1 SK2	L5_Slice1_left	2	5	7.13	772.837	**925.324**	113.008
P10	109.1 SK2	L5_Slice1_left	2	6	6.085	833.641	**925.324**	113.008
P10	109.1 SK2	L5_Slice1_left	2	7	5.27	795.569	**925.324**	113.008
P10	109.1 SK2	L5_Slice1_left_Avgs	3	333	**5.827666667**	**764.1716667**	**977.386**	115.307
P10	109.1 SK2	L5_Slice1_left	3	1	5.442	661.787	**977.386**	115.307
P10	109.1 SK2	L5_Slice1_left	3	2	7.584	743.373	**977.386**	115.307
P10	109.1 SK2	L5_Slice1_left	3	3	5.785	864.378	**977.386**	115.307
P10	109.1 SK2	L5_Slice1_left	3	4	5.528	784.798	**977.386**	115.307
P10	109.1 SK2	L5_Slice1_left	3	5	4.928	778.183	**977.386**	115.307
P10	109.1 SK2	L5_Slice1_left	3	6	5.699	752.511	**977.386**	115.307
P10	109.1 SK2	L5_Slice2_right_Avgs	4	333	**5.833571429**	**697.8754286**	**1028.162**	128.566
P10	109.1 SK2	L5_Slice2_right	4	1	7.884	611.016	**1028.162**	128.566
P10	109.1 SK2	L5_Slice2_right	4	2	4.713	796.091	**1028.162**	128.566
P10	109.1 SK2	L5_Slice2_right	4	3	4.542	750.17	**1028.162**	128.566
P10	109.1 SK2	L5_Slice2_right	4	4	5.656	743.136	**1028.162**	128.566
P10	109.1 SK2	L5_Slice2_right	4	5	5.528	591.132	**1028.162**	128.566
P10	109.1 SK2	L5_Slice2_right	4	6	6.213	654.855	**1028.162**	128.566

Figure 3

As a last step before import, all data was organized into one Master Table. Besides serving as a point of reference for the Access database, this file also allows for easier, more efficient import. After removing any less important parameters, a copy of the Master Table was imported into Access.

In Access, the data was placed in a table, which serves the same function as an Excel sheet. Each row of data was given a Primary Key, or unique identifier, which marks the row of data as a unique set and prevents it from being modified. This becomes particularly useful when individual parameters within each set are being examined or compared.

At this point the database was tested to ensure its correct import of the data. Looking at the P30 age group of our preliminary SK2 and SK3 data, average values for cell cross-sectional area were found for each animal. Then, using the same parameters, average values were collected from the database data. As shown in Figure 4 below, there is no variation between the data. This shows there was no error in modifying the data or importing it into Access.

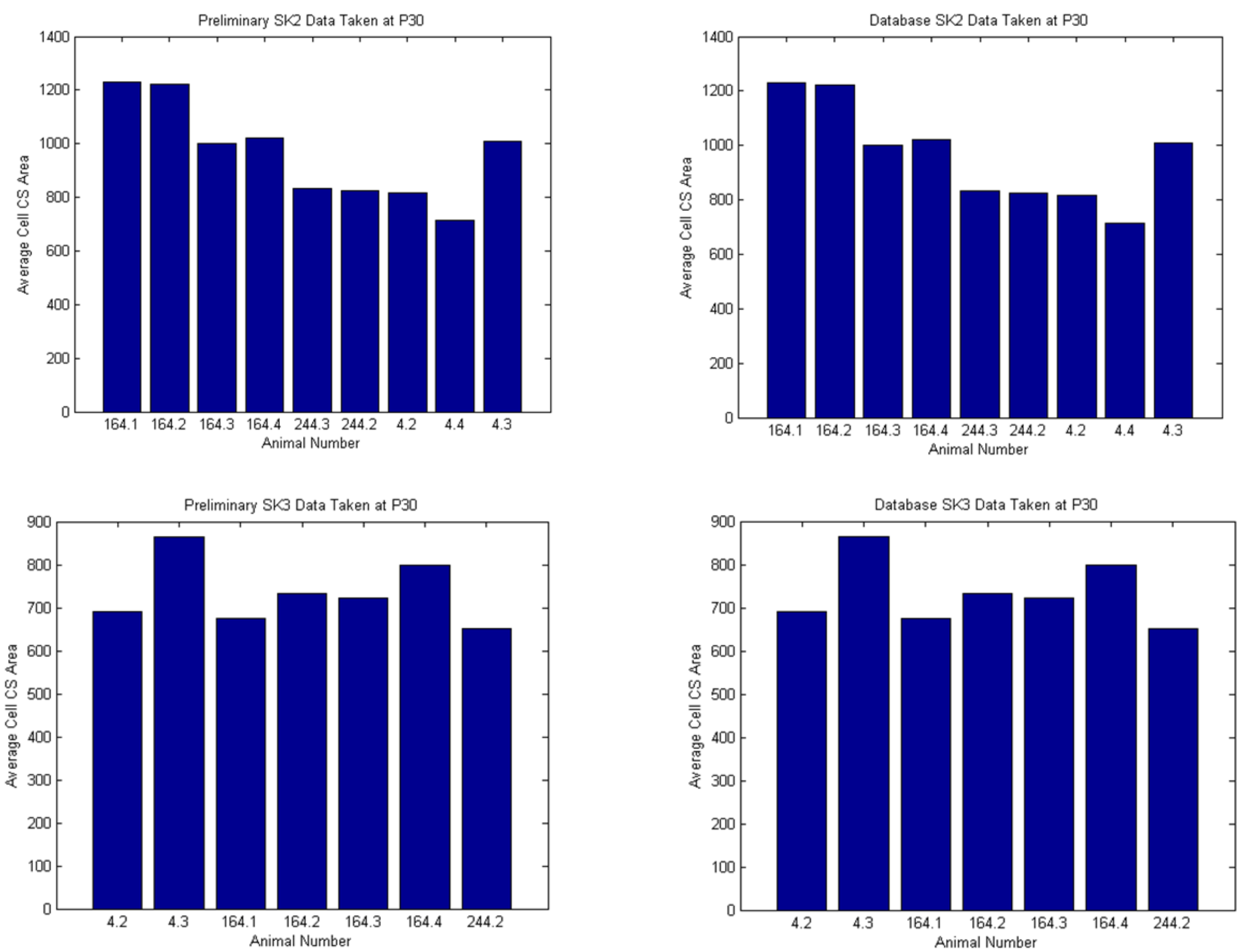

Figure 4

After validating the accuracy of the imported data, a form was created to express it. Forms are a feature of Access that allow for a cleaner, more organized view of the data. Along with the other main features of Access, forms are built off of a table that houses the data they will express. Unlike tables, forms can be coded or automated to showcase only the data searched for. This allows for higher efficiency, greater usability as a relational tool, and a smoother interface for the user.

To provide the best interface for users, correlated combo boxes were integrated into the form's design. Correlated combo boxes are linked drop-down boxes where a selection made in the first box limits the choices of the second box. To showcase the selected data, a sub-form was added to the form. This appears as a miniature table below the combo boxes. This sub-form is linked to the main form through the two parameters chosen within the drop-down boxes, and is intrinsically linked back to the table. An example of this form including its sub-form and drop-down boxes is shown in Figure 5 below. Although users don't see the data in its raw form, they have direct access to it and its potential relationships.

To further increase its use as a relational tool, buttons were coded into the form to add, edit, and delete data. Through the use of these buttons, users can directly modify the main table to add to the collected knowledge of the database. With these features, the database will not just relate data but serve as an ever-growing, centrally located storage platform for the lab's experimental data. Buttons can be seen implemented in Figure 5 as well.

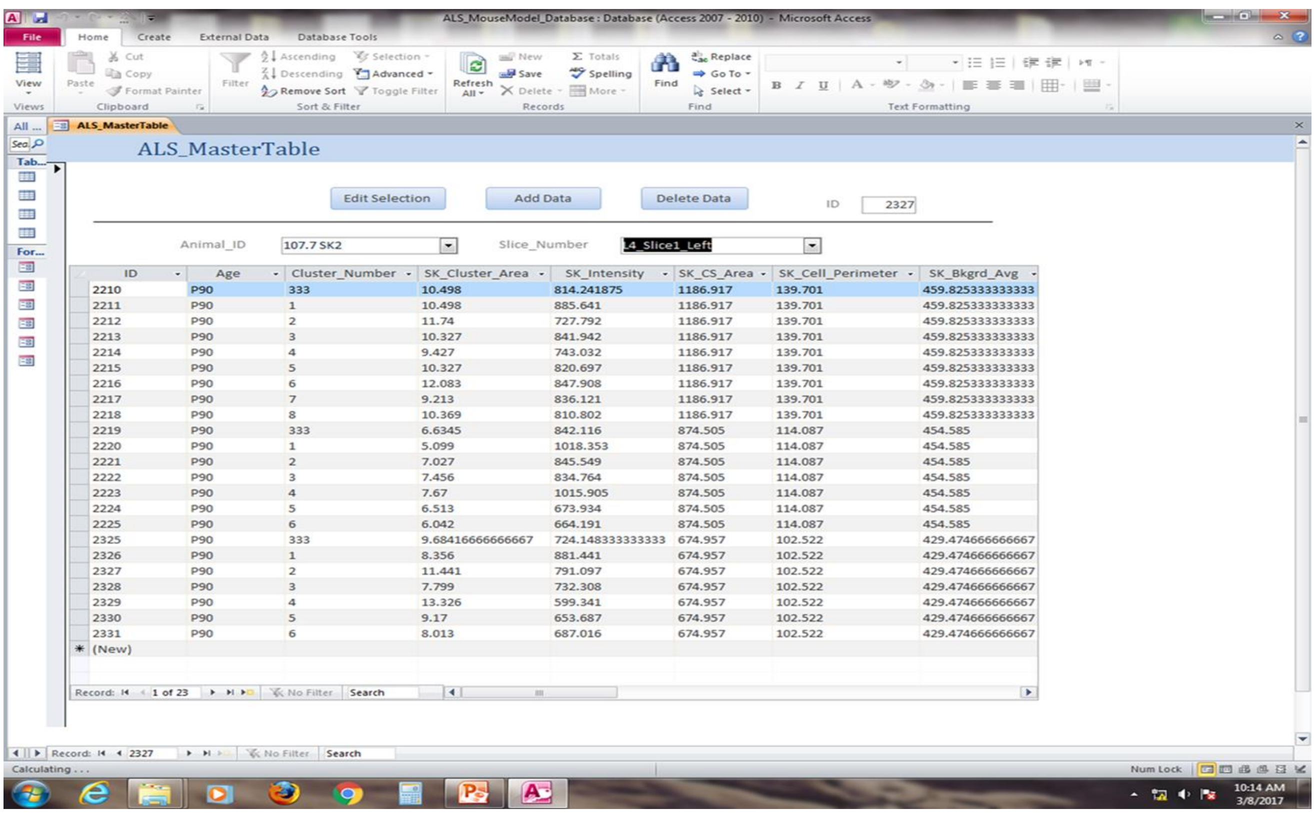

Figure 5

Results
Using the database, average cluster area was compared between Vacht and SK3 proteins. This data was expressed as either WT or SOD, and was represented over all age groups. A graph of the results is shown in Figure 6. Using this approach, 6 variations in the parameters of interest can be studied quickly and efficiently. Because all the data is centrally located, preliminary investigations can be made before the data is graphed, saving time and effort. Furthermore, a singular storage platform allows for quicker data interpretation over a larger pool of data. Techniques applied to current data and experimental parameters can be applied to future data to investigate trends, establish more meaningful relationships, and provide more confident avenues of research. Based on its successful use in producing the graphs in Figure 6, Access can be used as an effective relational tool to compare large amounts of data.

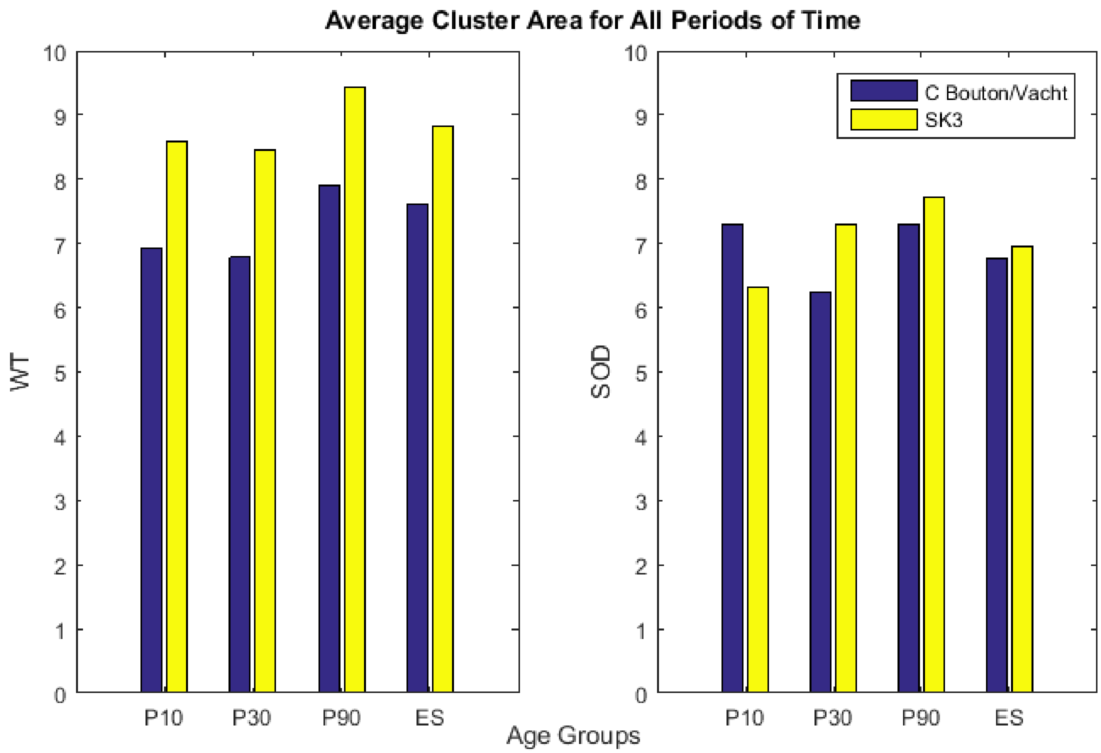

Figure 6

Conclusion
Based on the validity of the data imported into Access compared to preliminary data, this program can be used as a reliable and efficient storage platform. Because it can be used to produce graphs and investigate trends more quickly than singular Excel files, Microsoft Access is a useful and effective relational tool.