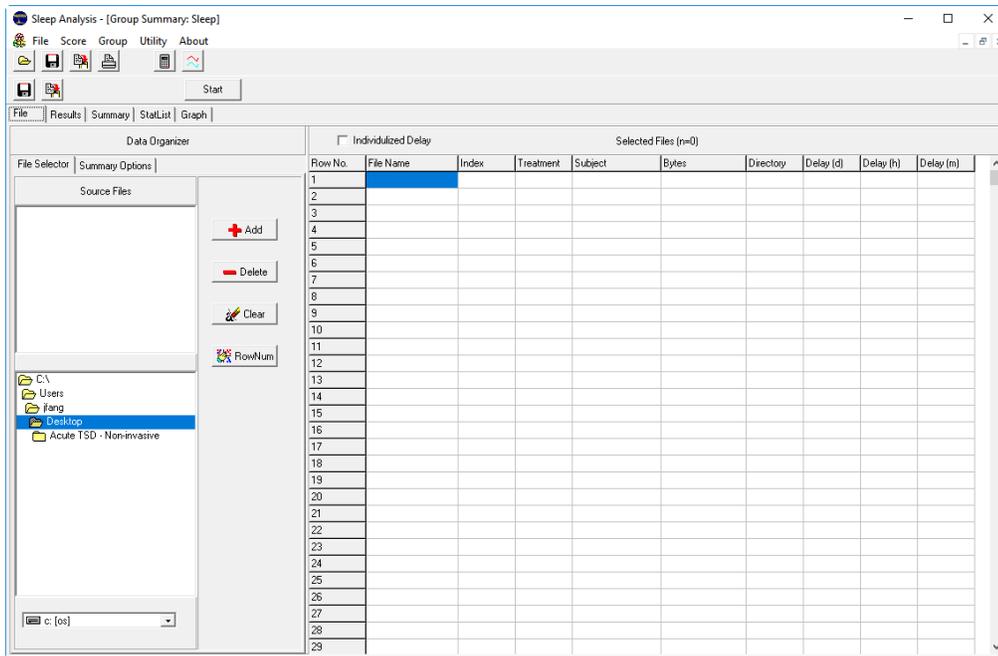


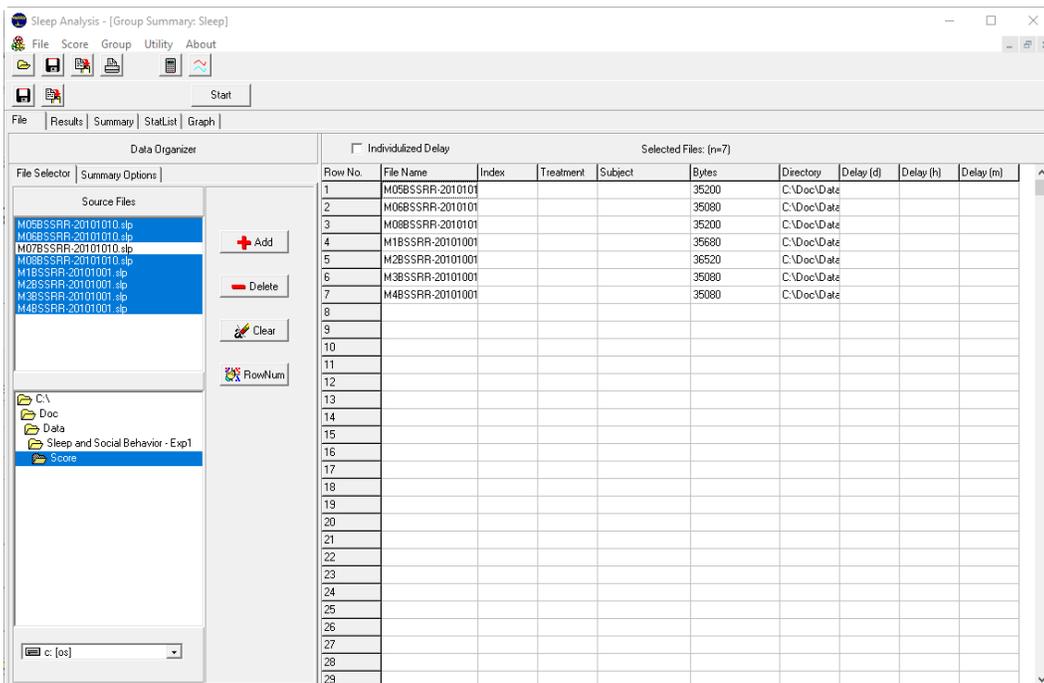
Procedures for Group Analysis of Sleep Data

After scoring sleep of set of animals, the scored data can be analyzed by group analysis. It will be convenient to place all SLP files from one experiment into the same folder. Here, assume we have a small group of mice, recorded for multiple days continuously, and each mouse received different treatment on different days, but the treatment will be the same for all animals on the same recording days. Please notice that all data should be scored continuously, and should have enough length for data analysis.

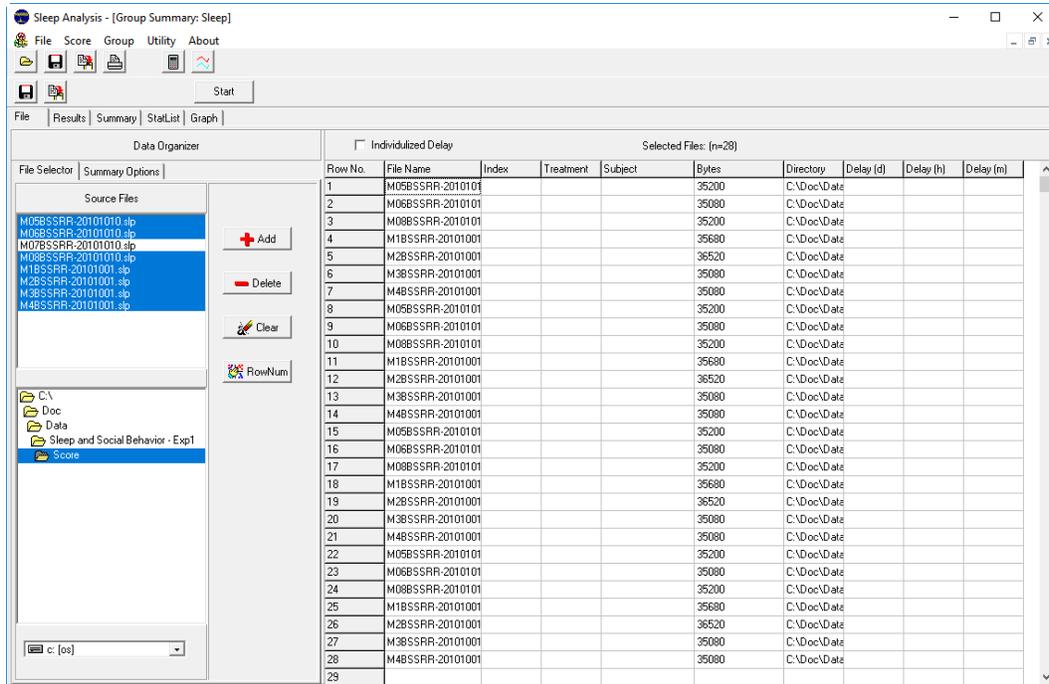
1. **Start SleepAnalysis.exe**, click on Group on the menu bar, and click on Sleep in the dropdown list. The following window will be shown.



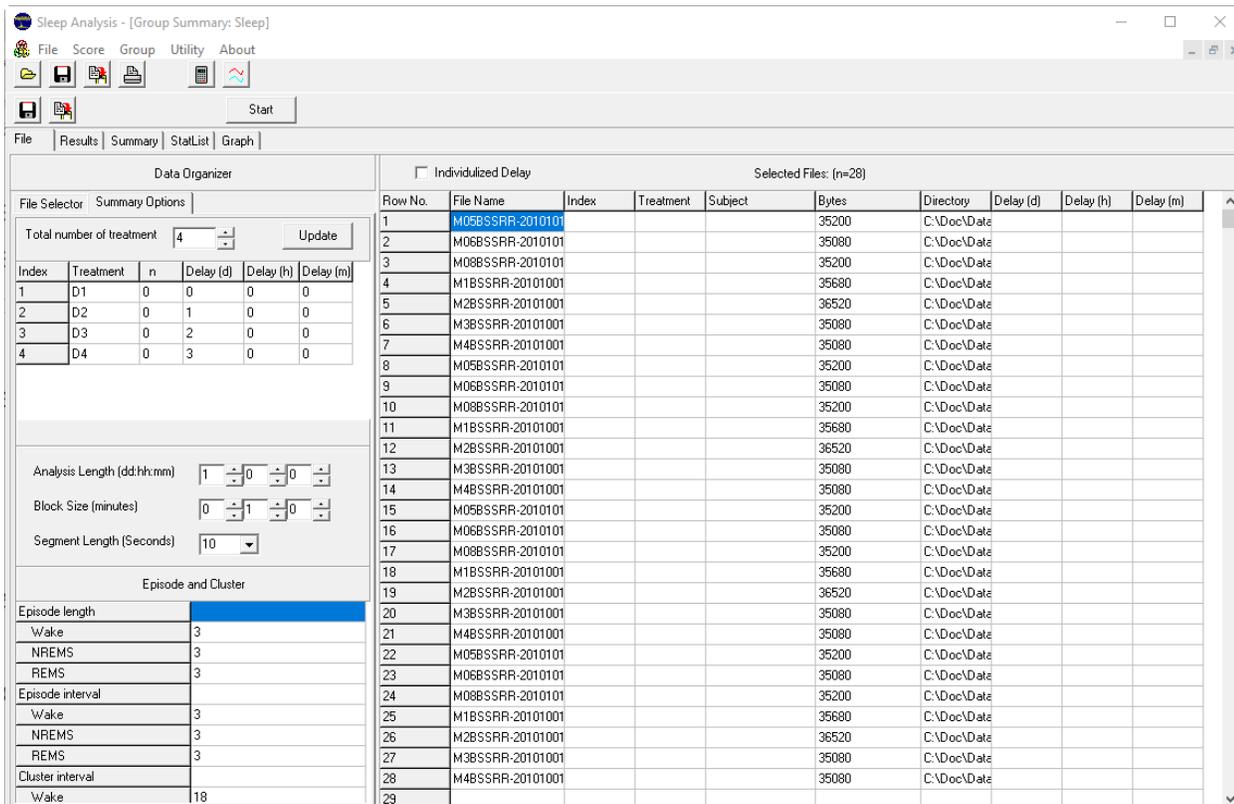
2. **Select data files.** In the File Selector tab, search and select for the files to be analyzed, and click on the Add button. The selected files will be added to the list of the Selected Files on the right.



In our example, each mouse was recorded for 4 days, and we will compare among the data from multiple days. To do this, click on Add 3 more times, so that each selected file will be added 4 times.



- Set up the summary options.** Click on the Summary Option tab, type in 4 treatments since we have 4 recording days. In this case, we simply type in D1, D2, D3, and D4 corresponding to the Index number (you can type in other short names for the treatment). We also need to type in the Delay time. The Delay time is the time from the start of the recordings. This will tell where the program should start to read the data. Obviously, the delay time will be 0 day, 0 hour and 0 minute for D1; and the 1 day, 0 hour and 0 minute for D2; and the delays for D3 and D4 will be 2 days and 3 days, respectively.



Next, in the list of Selected Files, we need to type in the treatment index for each animal, and shown below. As we typing through the files, the treatment will be displayed next to the treatment index. The Delay time will also be shown on the right.

The screenshot shows the 'Data Organizer' window in Sleep Analysis software. The 'Selected Files: (n=28)' table is as follows:

Row No.	File Name	Index	Treatment	Subject	Bytes	Directory	Delay (d)	Delay (h)	Delay (m)
1	M05SSRR-20101011	D1			35200	C:\doc\Date 0	0	0	0
2	M06SSRR-20101011	D1			35080	C:\doc\Date 0	0	0	0
3	M08SSRR-20101011	D1			35200	C:\doc\Date 0	0	0	0
4	M18SSRR-201010011	D1			35680	C:\doc\Date 0	0	0	0
5	M28SSRR-201010011	D1			36520	C:\doc\Date 0	0	0	0
6	M38SSRR-201010011	D1			35080	C:\doc\Date 0	0	0	0
7	M48SSRR-201010011	D1			35080	C:\doc\Date 0	0	0	0
8	M05SSRR-20101012	D2			35200	C:\doc\Date 1	0	0	0
9	M06SSRR-20101012	D2			35080	C:\doc\Date 1	0	0	0
10	M08SSRR-20101012	D2			35200	C:\doc\Date 1	0	0	0
11	M18SSRR-201010012	D2			35680	C:\doc\Date 1	0	0	0
12	M28SSRR-201010012	D2			36520	C:\doc\Date 1	0	0	0
13	M38SSRR-201010012	D2			35080	C:\doc\Date 1	0	0	0
14	M48SSRR-201010012	D2			35080	C:\doc\Date 1	0	0	0
15	M05SSRR-20101013	D3			35200	C:\doc\Date 2	0	0	0
16	M06SSRR-20101013	D3			35080	C:\doc\Date 2	0	0	0
17	M08SSRR-20101013	D3			35200	C:\doc\Date 2	0	0	0
18	M18SSRR-201010013	D3			35680	C:\doc\Date 2	0	0	0
19	M28SSRR-201010013	D3			36520	C:\doc\Date 2	0	0	0
20	M38SSRR-201010013	D3			35080	C:\doc\Date 2	0	0	0
21	M48SSRR-201010013	D3			35080	C:\doc\Date 2	0	0	0
22	M05SSRR-20101013	D3			35200	C:\doc\Date 2	0	0	0
23	M06SSRR-20101013	D3			35080	C:\doc\Date 2	0	0	0
24	M08SSRR-20101013	D3			35200	C:\doc\Date 2	0	0	0
25	M18SSRR-20101001		Treatment		35680	C:\doc\Date Delay (d)	Delay (h)	Delay (m)	
26	M28SSRR-20101001		Treatment		36520	C:\doc\Date Delay (d)	Delay (h)	Delay (m)	
27	M38SSRR-20101001		Treatment		35080	C:\doc\Date Delay (d)	Delay (h)	Delay (m)	
28	M48SSRR-20101001		Treatment		35080	C:\doc\Date Delay (d)	Delay (h)	Delay (m)	
29									

We can also assign each animal with a unique number. In our example, the number will be repeated 4 times for each animal since each file is added to the list 4 times.

The screenshot shows the 'Data Organizer' window in Sleep Analysis software. The 'Selected Files: (n=28)' table is as follows:

Row No.	File Name	Index	Treatment	Subject	Bytes	Directory	Delay (d)	Delay (h)	Delay (m)
1	M05SSRR-20101011	D1		1	35200	C:\doc\Date 0	0	0	0
2	M06SSRR-20101011	D1		2	35080	C:\doc\Date 0	0	0	0
3	M08SSRR-20101011	D1		3	35200	C:\doc\Date 0	0	0	0
4	M18SSRR-201010011	D1		4	35680	C:\doc\Date 0	0	0	0
5	M28SSRR-201010011	D1		5	36520	C:\doc\Date 0	0	0	0
6	M38SSRR-201010011	D1		6	35080	C:\doc\Date 0	0	0	0
7	M48SSRR-201010011	D1		7	35080	C:\doc\Date 0	0	0	0
8	M05SSRR-20101012	D2		1	35200	C:\doc\Date 1	0	0	0
9	M06SSRR-20101012	D2		2	35080	C:\doc\Date 1	0	0	0
10	M08SSRR-20101012	D2		3	35200	C:\doc\Date 1	0	0	0
11	M18SSRR-201010012	D2		4	35680	C:\doc\Date 1	0	0	0
12	M28SSRR-201010012	D2		5	36520	C:\doc\Date 1	0	0	0
13	M38SSRR-201010012	D2		6	35080	C:\doc\Date 1	0	0	0
14	M48SSRR-201010012	D2		7	35080	C:\doc\Date 1	0	0	0
15	M05SSRR-20101013	D3		1	35200	C:\doc\Date 2	0	0	0
16	M06SSRR-20101013	D3		2	35080	C:\doc\Date 2	0	0	0
17	M08SSRR-20101013	D3		3	35200	C:\doc\Date 2	0	0	0
18	M18SSRR-201010013	D3		4	35680	C:\doc\Date 2	0	0	0
19	M28SSRR-201010013	D3		5	36520	C:\doc\Date 2	0	0	0
20	M38SSRR-201010013	D3		6	35080	C:\doc\Date 2	0	0	0
21	M48SSRR-201010013	D3		7	35080	C:\doc\Date 2	0	0	0
22	M05SSRR-20101014	D4		1	35200	C:\doc\Date 3	0	0	0
23	M06SSRR-20101014	D4		2	35080	C:\doc\Date 3	0	0	0
24	M08SSRR-20101014	D4		3	35200	C:\doc\Date 3	0	0	0
25	M18SSRR-201010014	D4		4	35680	C:\doc\Date 3	0	0	0
26	M28SSRR-201010014	D4		5	36520	C:\doc\Date 3	0	0	0
27	M38SSRR-201010014	D4		6	35080	C:\doc\Date 3	0	0	0
28	M48SSRR-201010014	D4		7	35080	C:\doc\Date 3	0	0	0
29									

The default Analysis Length is 1 day (24 hours); and the default Block Size is 1h. We simply leave it unchanged here. But in practice, it is often better to use 2 or 3 hour block size for displaying the changes to get smoother curves and to increase statistical power.

4. **Start Analysis.** Click on the Start button, the Group Summary will be shown almost instantaneously.

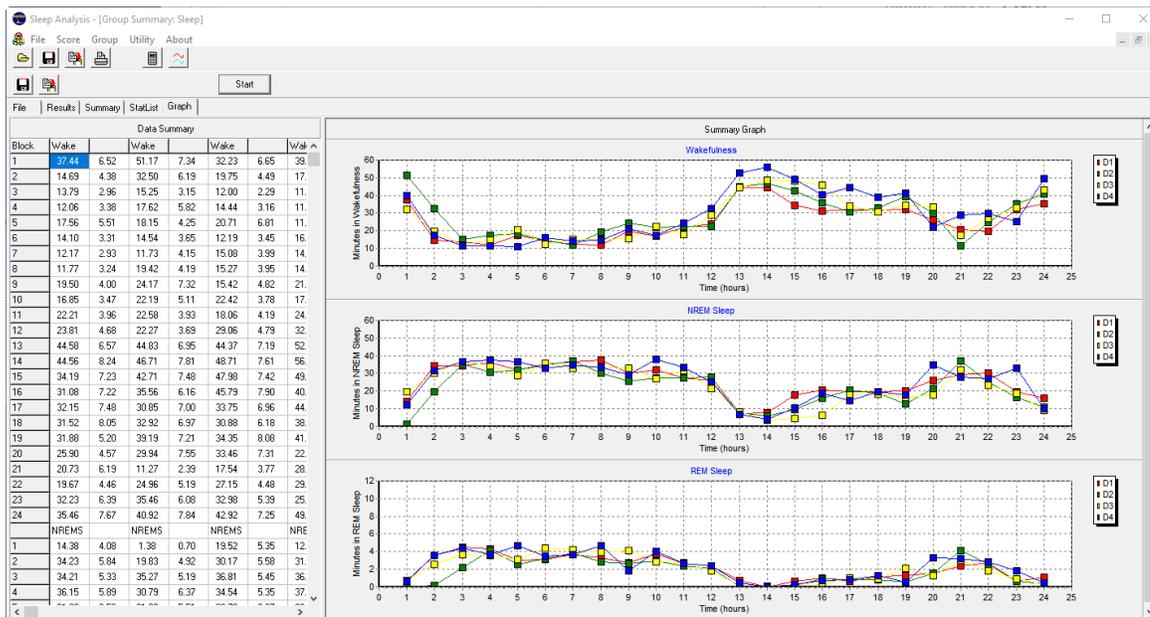
The screenshot shows the 'Group Summary' window in the Sleep Analysis software. The window title is 'Sleep Analysis - [Group Summary: Sleep]'. It features a menu bar (File, Score, Group, Utility, About) and a toolbar with icons for file operations and a 'Start' button. Below the toolbar is a tabbed interface with 'File', 'Results', 'Summary', 'StatList', and 'Graph' tabs. The 'Summary' tab is active, displaying a large table with the following columns: Group, Treatmen, n, Block, Wake, NREMS, REMS, Undef, nWake, nNREMS, nREMS, nUndef, dWake, dNREMS, dREMS, and dUndef. The table contains 28 rows of data, with the first row (row 1) labeled 'Regular' and subsequent rows numbered 2 through 28. Each row provides mean and standard error values for various sleep states across different treatment conditions and blocks.

The data are listed in sequence. The first treatment goes first (with 24 rows of data for 24 1-h blocks). For each state (Wake, NREMS and REMS), the mean and standard error are shown in two columns (the time units is minute). Next, the bout numbers are displayed (nWake, nNREMS and nREMS). Again, both means and standard errors are displayed. Further right, the bout durations for each state (dWake, dNREMS and dREMS) are displayed (The time unit is second instead of minute). These data can be copied to the clipboard and pasted to other program for plotting purpose.

To look at individual animal's data, click on the Stat List tab page, the following data will be displayed:

The screenshot shows the 'Statistics Work Sheet' window in the Sleep Analysis software. The window title is 'Sleep Analysis - [Group Summary: Sleep]'. It features a menu bar (File, Score, Group, Utility, About) and a toolbar with icons for file operations and a 'Start' button. Below the toolbar is a tabbed interface with 'File', 'Results', 'Summary', 'StatList', and 'Graph' tabs. The 'StatList' tab is active, displaying a table with the following columns: Row, No., Name, Subject, Group, Block, Hour, WAKE, NREMS, REMS, UNDEF, nWAKE, nNREMS, nREMS, nUNDEF, dWAKE, dNREMS, dREMS, and dUNDEF. The table contains 24 rows of data, with the first row (row 1) labeled 'Regular' and subsequent rows numbered 2 through 24. Each row provides detailed data for individual animals, including their names, subjects, groups, and various sleep state metrics.

Clicking on the Graph tab, the sleep patterns will be shown for different treatment conditions. This graph display is certainly not for publication purpose, but the users can quick get an idea about the trend of the experiment.



One problem is that the 1 hour time block is not suitable for calculating the bout number and duration. First, the rodents often stay awake continuously for more than 1 hour. There will be many missing data when the bout duration of NREMS or REMS is computed. The calculation will not be incorrect if the duration is simply set to zero (this is what the current program does). This error can be fixed in the future, but it is still undesirable to have many missing data. Second, the calculation is inaccurate when the block size is too small and unnecessary to have huge amounts of data. Therefore, we selected 12-h block size. Unfortunately, there is a bug in the program. Occasionally, we need to restart the program and only change this parameter as shown below.

After doing so, click on the Start button, we will get the following results as shown below.

The screenshot displays the 'Group Summary' table in the Sleep Analysis software. The table has columns for 'Group', 'Treatment', 'n', 'Block', 'Wake', 'NREMS', 'REMS', 'nWake', 'nNREMS', 'nREMS', 'nUndef', 'dWake', 'dNREMS', and 'dREMS'. The data is organized into rows for different groups and treatments. The 'Wake' column contains mean and SE values for 'Wake' and 'nWake'. The 'NREMS' and 'REMS' columns contain mean and SE values for 'NREMS' and 'nNREMS'. The 'dWake', 'dNREMS', and 'dREMS' columns contain mean and SE values for 'dWake', 'dNREMS', and 'dREMS'. The table shows that the bout numbers of Wake, NREMS, and REMS are similar across light and dark periods, while the bout duration of Wake is longer during the light period compared to the dark period.

In the above display, the data from each day (treatment) are displayed in 2 rows. Since we started recording from light onset, we can see that the bout numbers of Wake are similar between light and dark period, the bout numbers of NREMS and REMS are greater during the light period compared to the dark period. In contrast, the bout duration of Wake is longer during the light period compared to the dark, whereas the bout duration of NREMS and REMS are similar across the light-dark period. This example indicates that the bout number and duration may be different across the light-dark cycle. The differences are certainly dependent on the species and experimental conditions.

Definition of bout. A bout for any state

The screenshot shows the 'Group Summary' window in the Sleep Analysis software. The table displays summary statistics for various groups and treatments. The columns include Group, Treatment, n, Block, Wake, nREMS, REMS, Undef, nWake, nNREMS, nREMS, nUndef, dWake, dnREMS, and dREMS. The data is organized into rows for different groups and treatments, with mean and SE values for each parameter.

has to be at

least 30 seconds long.

Cluster analysis. The program also performs cluster analysis. For instance, REM sleep bouts may occur with short intervals. Technically, we set the default interval to be 18 segments (3 minutes). Therefore, if the interval between two sequential bouts for a particular state is less than this interval, they are considered within the same cluster.

Clicking on the Stat List table, the data for individual animals will be displayed. This will be useful for statistical programs such as SigmaPlot.

The screenshot shows the 'Statistics Work Sheet' window in the Sleep Analysis software. The table displays detailed data for individual animals, including their names, subjects, groups, blocks, hours, and various sleep state parameters (WAKE, nREMS, REMS, UNDEF, nWAKE, nNREMS, nREMS, nUNDEF, dWAKE, dnREMS, dREMS, dUNDEF). The data is organized into rows for each animal, with columns for Row, No., Name, Subject, Group, Block, Hour, WAKE, nREMS, REMS, UNDEF, nWAKE, nNREMS, nREMS, nUNDEF, dWAKE, dnREMS, dREMS, and dUNDEF.

- Between group comparisons.** If you only need to compare between groups, then each animal from each group should be added to the Selected Files once. The number of treatments will be the same as the number of groups. Typically, the Delay time will be the same for all groups, 0 day, 0 hour and 0 minute.

Another situation is that you may need to compare the data from the first day of recording in one group with the data from the second day of recording in another group. In this case, just adjust the Delay to 1 day for the second group.

Sometimes, you may need to both within subject and between subjects comparisons. Group analysis can also be performed, just make sure you have correct number of treatments, and assigned the group index and latency correctly.

