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Active Avoidance protocol 01282020

Forked from a private protocol

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Teresa Macheda[1], Henry Snider[1], James B. Watson[1], Kelly N. Roberts[1], Adam D Bachstetter[2,3]

[1]Spinal Cord & Brain Injury Research Center, University of Kentucky, Lexington KY and Department of Neuroscience, University of Kentucky, Lexington KY;
[2]Spinal Cord & Brain Injury Research Center, University of Kentucky, Lexington KY;
[3]Department of Neuroscience, University of Kentucky, Lexington KY and Sanders-Brown Center on Aging, University of Kentucky, Lexington KY

Teresa Macheda
University of Kentucky

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An Active Avoidance Behavioral Paradigm for Use in a Mild Closed Head Model of Traumatic Brain Injury in Mice

Protocol status: Working
We use this protocol and it's working

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Abstract

Active Avoidance Test Using Shock as unconditioned stimulus

Active avoidance is a widely-used paradigm to evaluate fear-motivated associative learning and memory.

The Gemini shuttle box was used to perform the active avoidance test. In the active avoidance behavior, a subject is trained to avoid an aversive unconditioned stimulus (US), by associating the conditioned stimulus (CS: light and/or auditory tone) with the foot-shock (US). Over repeated trials, the subject learns that to avoid the foot-shock (US) it must shuttle between chambers when the warning cue (CS) is presented. Learning performance is evaluated by analysis of avoidance behavior during the test.

Five days test:
The mouse will be placed in the dark box with the guillotine door open, free to explore both sides of the chamber for 5 minutes. Then a ***cue -house light (1864 lux)-*** will be presented for 10 seconds. The mouse could step into the safe side (no shock delivered), otherwise a foot shock would be delivered **(e.g. 0.2 mA, 2 sec**). The mouse will be exposed to 50 trials/day (ITT 30±5 sec) for a total of 5 days. CS opposite side of US side.

Habituation=300 sec
CS= housing light, 10 sec
US= 0.2 mA (e.g.) shock, 2 sec
ITT=30±5 sec
 # Trials= 50
 # days=5

Materials

GEMINI *active and passive avoidance* system (https://sandiegoinstruments.com/product/gemini/)
MB-10 solution (100 ppm Chlorine dioxide; C.A.S. #: 10049-04-4) or 70% Ethanol
Bedding to be placed in the removable tray
Clean cages to retrieve the mouse after the test session

Before start

Move the animals in the testing room and allow them to acclimate for at least 30 minutes before beginning testing.

1

Experiment Design and Recommendations:

Housing: Behavior is affected by the housing mode. Singly housed mice could perform differently than group housed mice.

Blinding: Experimental groups of mice should be coded while the operator is testing the mice.

Test order: Mice should be randomized in test order, to avoid confounded results if all the mice from the same experimental group are tested before all the mice from a different group. The randomization should be applied in all the cohorts tested across time.

Time of testing: Time of day strongly affects performance. Testing should be conducted during the same time between days and experiments. If a study requires more animals, several cohorts should be tested. It is recommended that each cohort of mice is tested starting the same day of the week and by the same personnel to reduce the introduction of new variability in the task.

Sex: Males and females should be tested in separate groups, in particular in different cohorts. If this is not possible and more than one active avoidance chamber is available make sure to dedicate one chamber for each sex.

2 **Setting up the gemini box**

Active Avoidance Maintenance:

- Each testing day, the lights and shock should be tested before the start of each testing session.
- Active Avoidance box should be cleaned between subjects, in particular the chamber floor. The presence of urine will increase the shock intensity experienced by the animal.

- Turn on the box

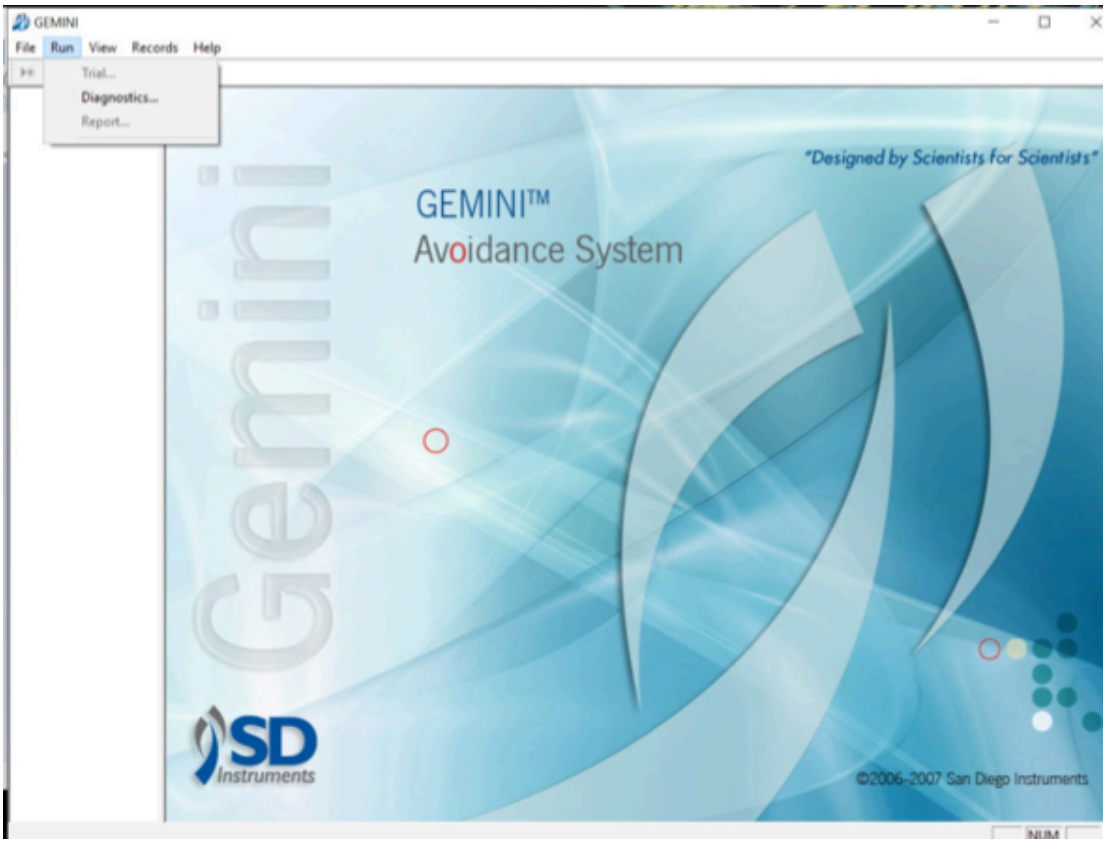
- Set up the shock intensity to use on the box → put the switcher PROGRAM/ MANUAL on MANUAL → put the switcher RUN/ADJUST on Adjust → adjust current using the CURRENT ADJUST knob to the shock to use during the test (you can read the value on the SHOCK INTENSITY (mA) display) → Switch the switcher RUN/ADJUST in the center

A. Based on the color the location of the shock can be determined
B. This knob sets the shock amplitude when "C" is positioned in manual mode
C. The position of this switch determines the source of the shock (it should be positioned on Manual the entire time: when you adjust the shock intensity or when you test mice)
D. Range switch, this switch determines the total range of the shocker output: 0-2 mA or 0-5 mA
E. The switch on Adjust is used when the shock intensity needs to be adjusted, you can read the the shock intensity value on the shock intensity display.

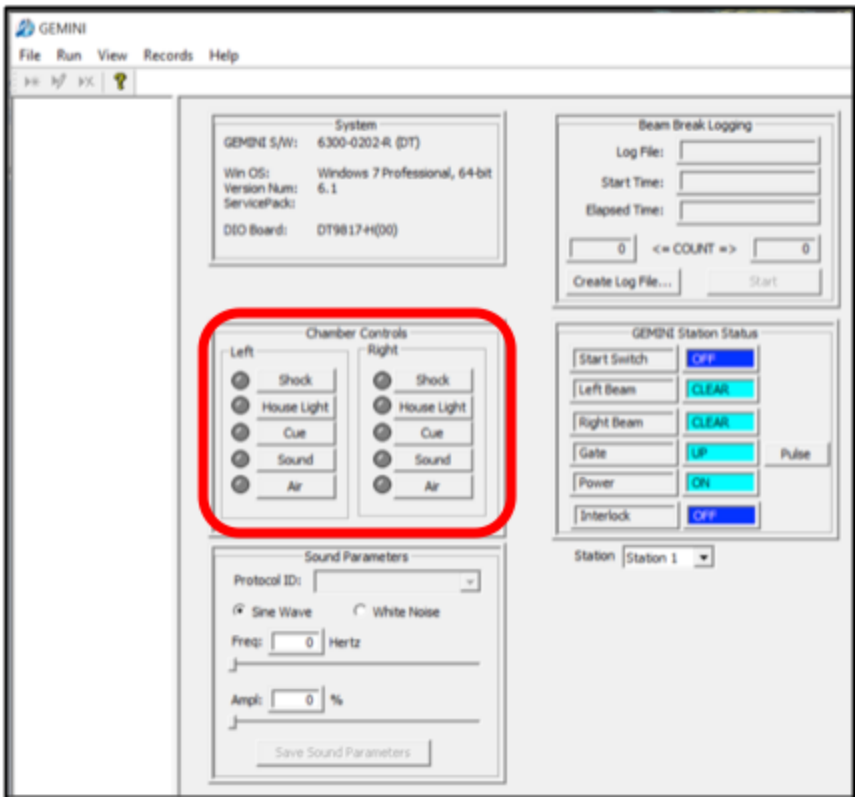
3 How to check if the chamber works properly

Computer setup

- Turn on the computer
- Make sure that the Avoidance system is plugged in and turned on
- Open the avoidance system program (***GEMINI.exe***)
- Click Run → click Diagnostics



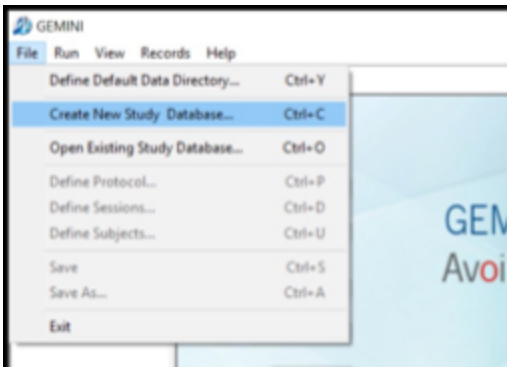
- Click on the chamber controls to evaluate if the chamber is working properly



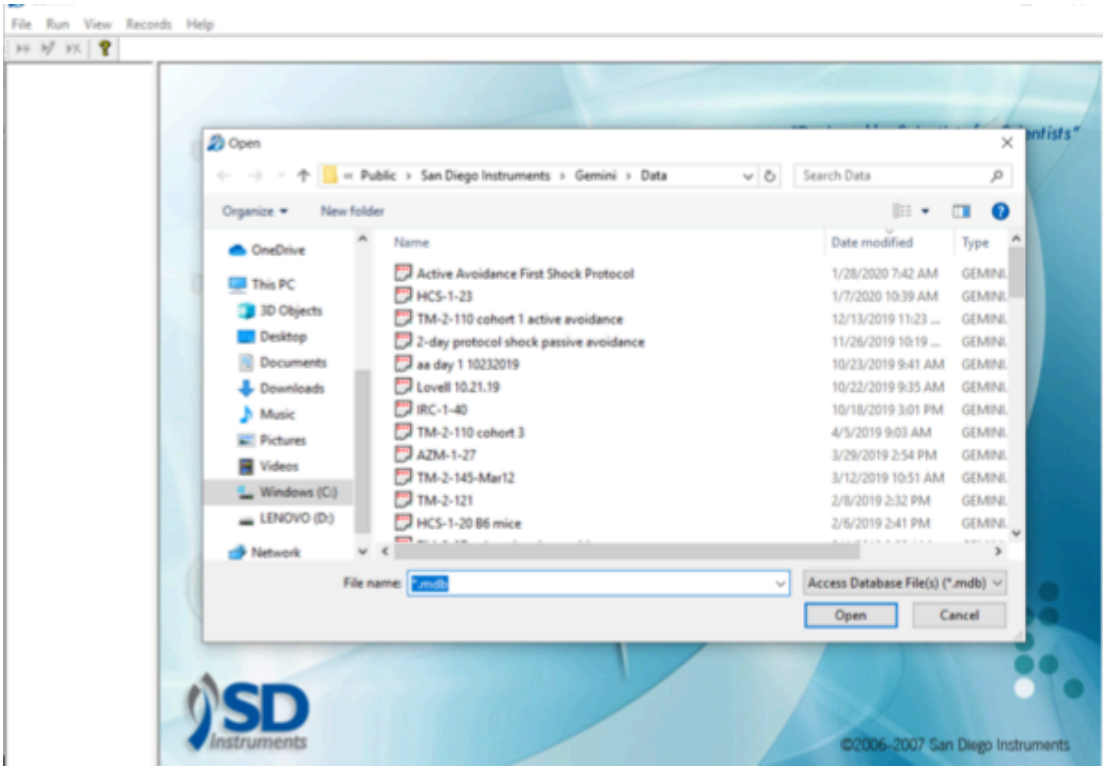
4 Set up

Computer setup

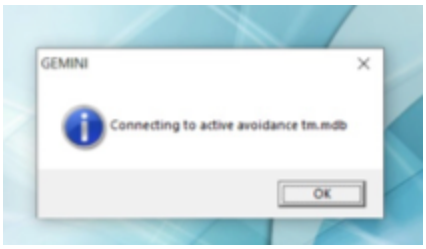
- Turn on the computer
- Make sure that the Avoidance system is plugged in and turned on
- Open the avoidance system program (GEMINI.exe)
- **Click file→ click create a new study database**



Name your DATABASE making sure to leave the m-defined extension *.mdb* and click Open

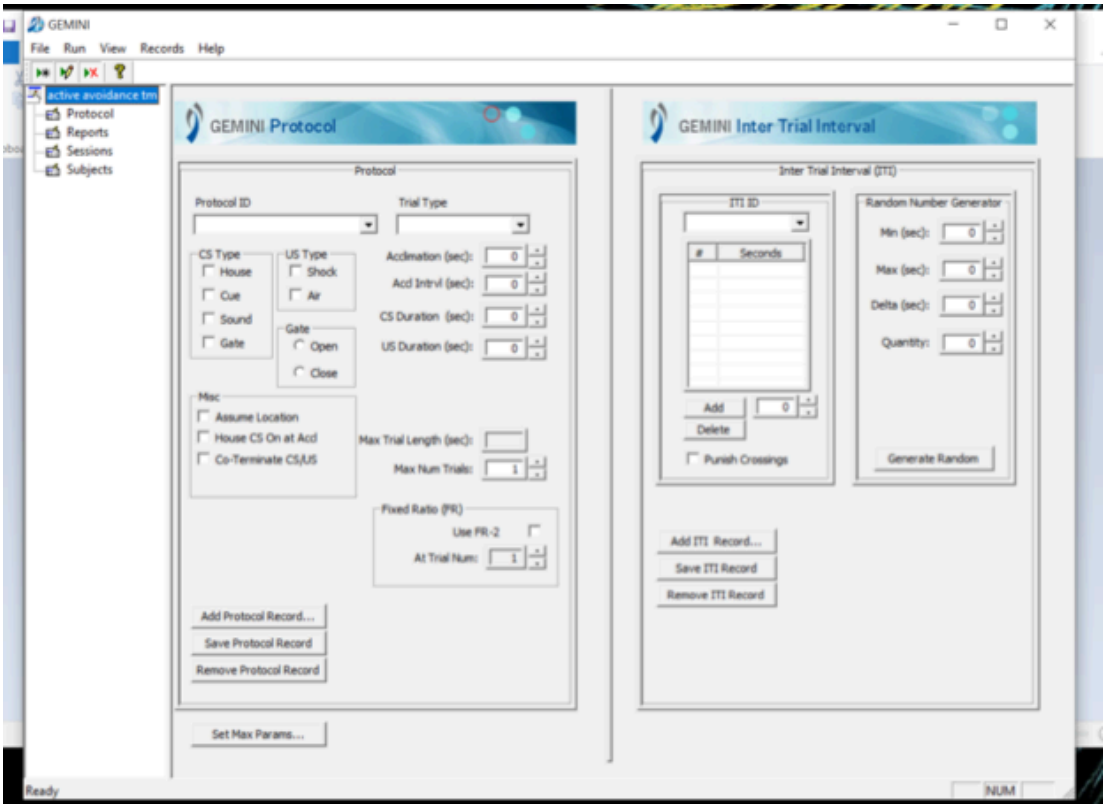


- A "connecting" window will pop pup, click OK



Defining a Protocol

- Make your protocol, click "Add Protocol Record"



e.g.

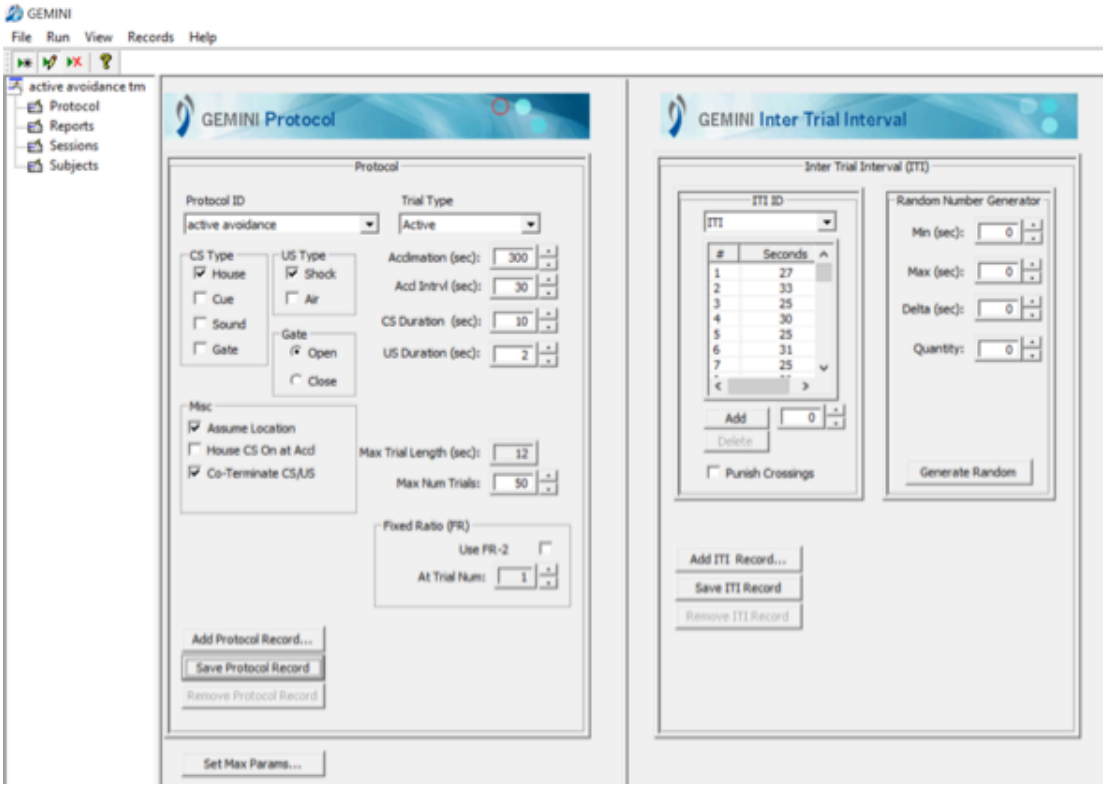
Protocol ID: active avoidance
CS Type: House
US Type: Shock
Gate: Open
Misc.: Assume location/ Co-Terminate CS/US

Trial type: Active
Acclimation (sec): 300
Accl Intrvl (sec): 30
CS Duration (sec): 10
US Duration (sec): 2

Max trial Length (sec): 12 -this will be calculated automatically
Max Num Trial: 50

ITI= 30±5 sec will be generated by the software following this steps:
click "Add ITI Record → name it
Min (sec): 25
Max (sec): 35
Delta (sec): 5
Quantity: 49
-click Generate Random

- Save protocol record

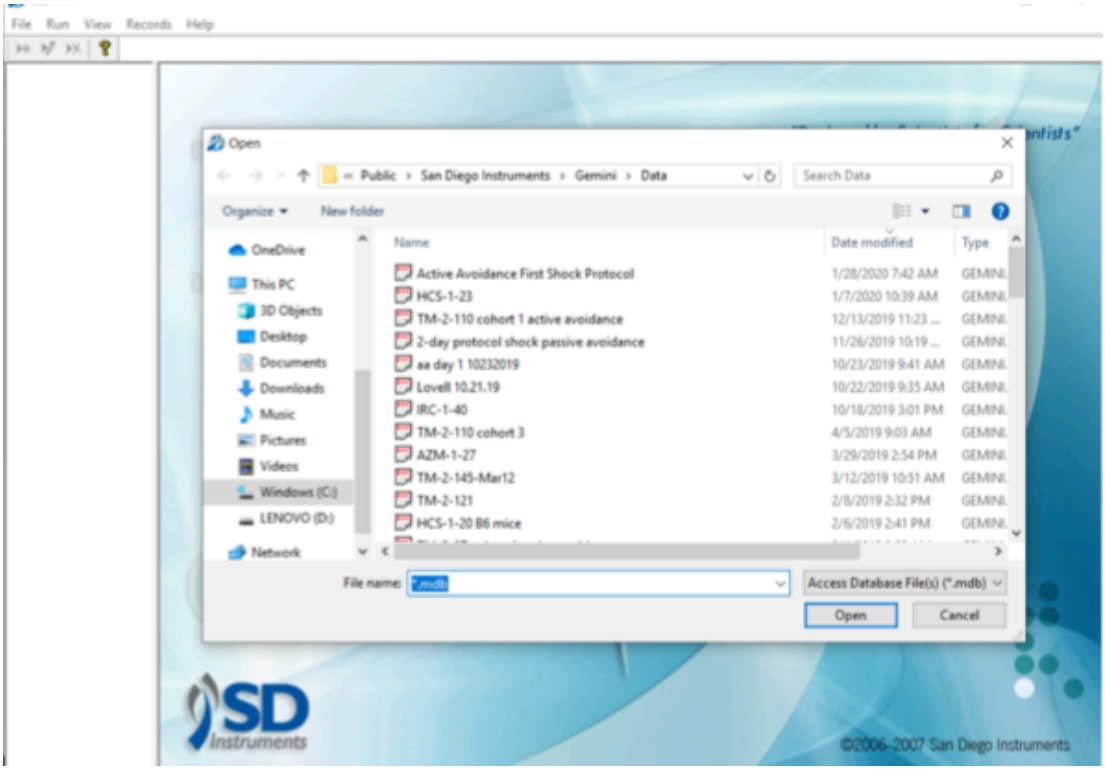


Example of Active avoidance protocol

How to open study database/ protocol

- **Click file→ click open existing study database**

- Choose your database and click open. The database contains any previously defined protocols. The protocol of your choice will be ready to be applied to your study

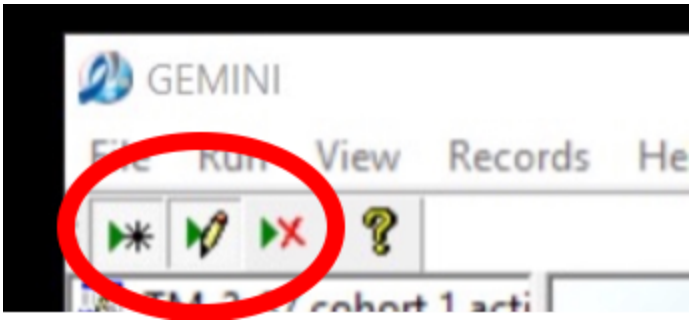


How to save your file

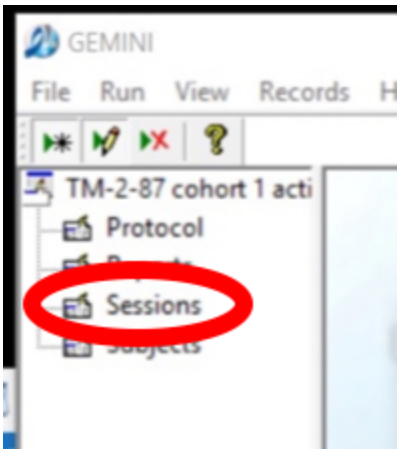
- Click file, then “save as”

How to add subjects to the experiment
Once the protocol is defined, you can add subjects to your experiment.

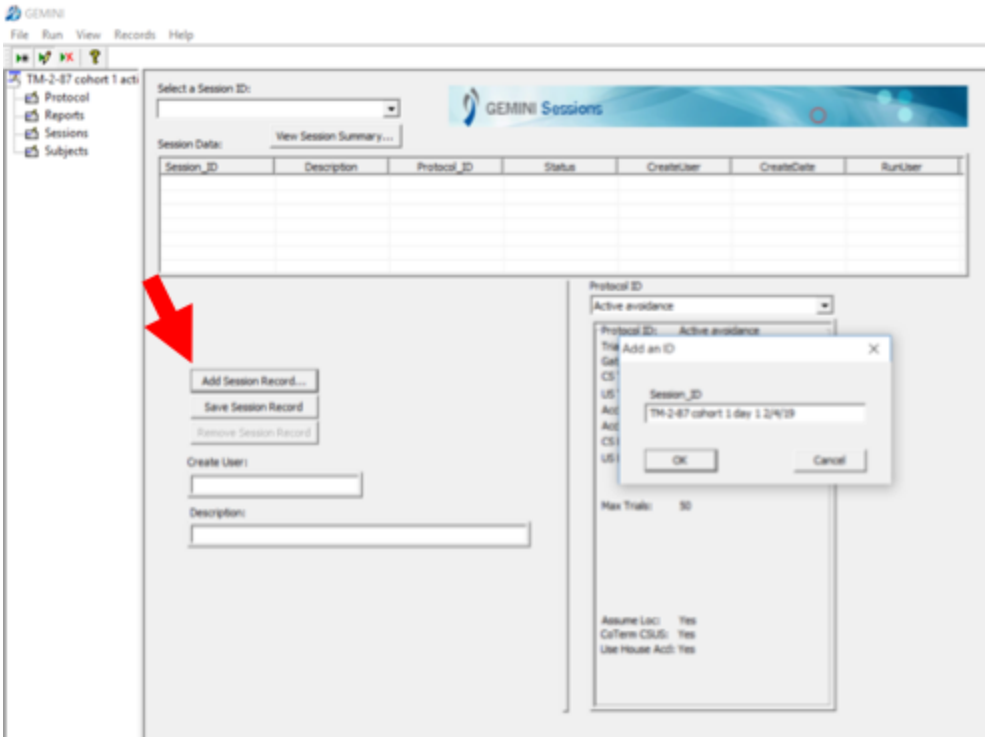
- Click triangle-star and pen button on top left side (you can create a session and add subjects)



- Click Session

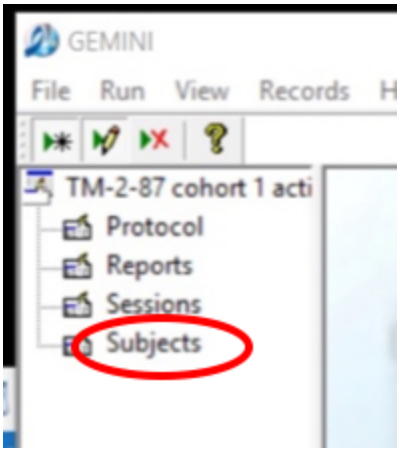


- Click Add session record, Fill in Session ID (e.g. name , date)

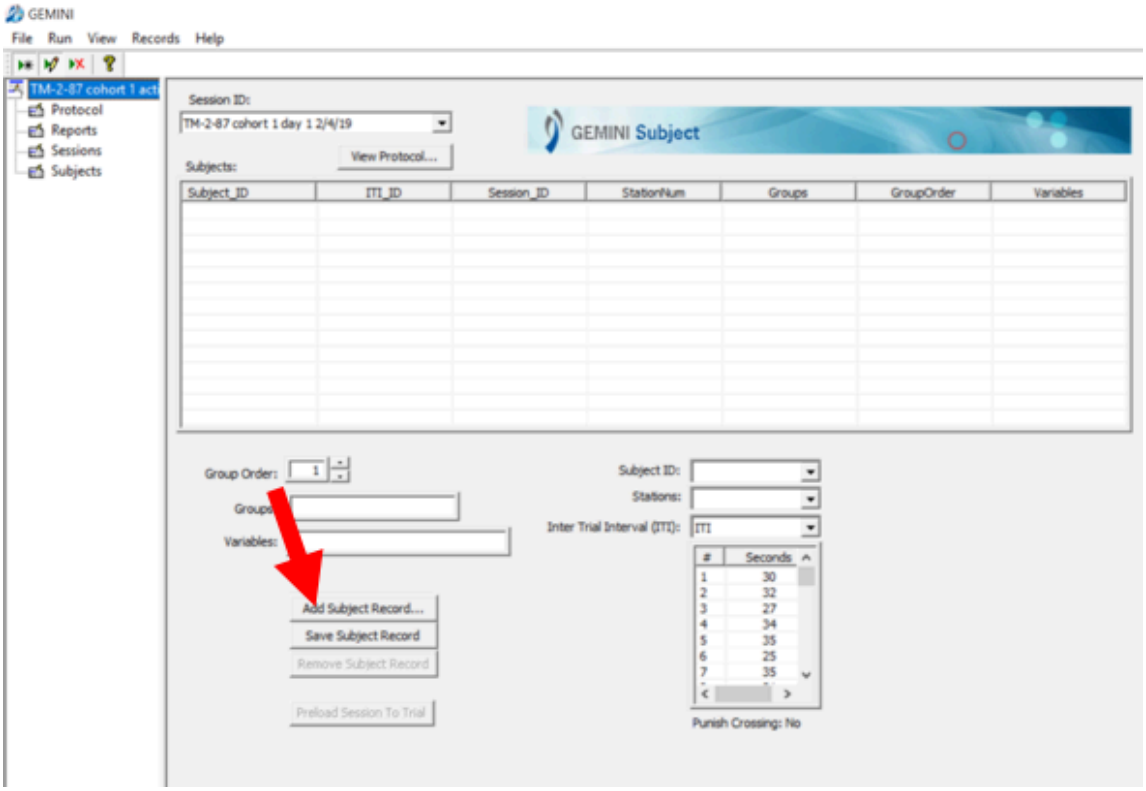


- Click OK
- Under “Protocol ID,” select the appropriate protocol

- Click Subjects

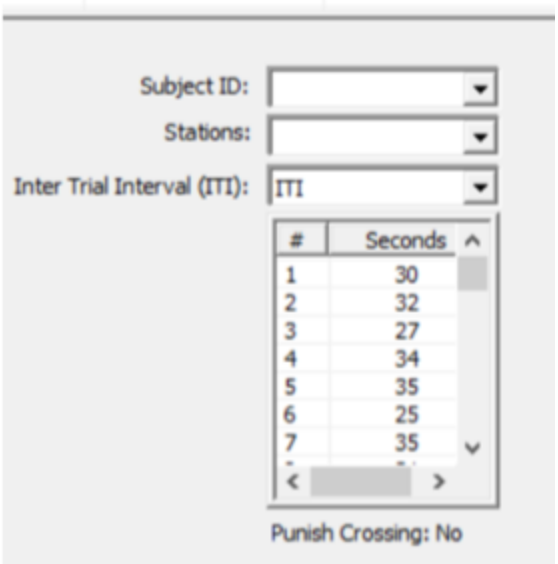


- Fill in group ID, and group order (you can just put a random #), then click add subject record

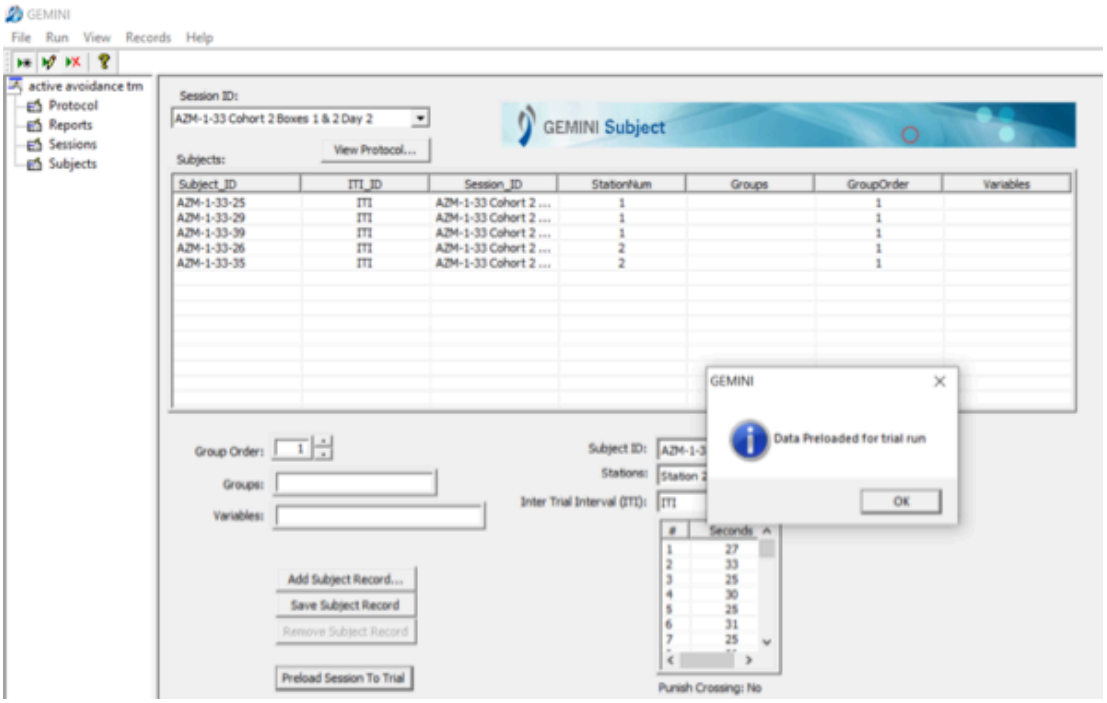


- If the animal ID is already saved, click Subject ID and select your subject

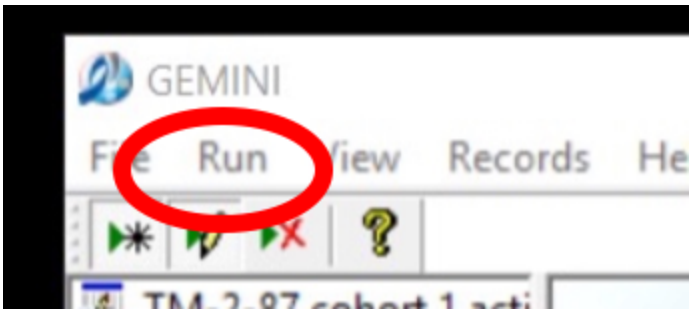
- Select the station (it is important to assign the subject to the correct station/chamber when you use more than one box)



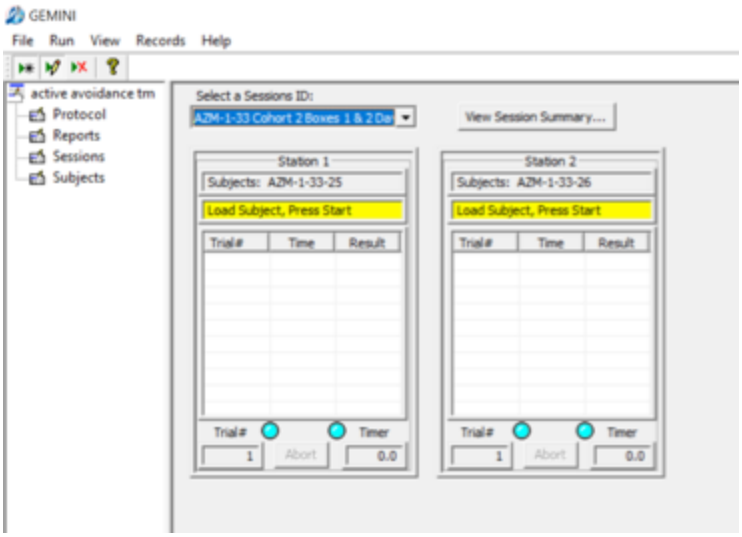
- Click Preload session to trial, data will be preloaded and experiment will be able to start



- Click Run → click Trial → Type in your name in the “user login” box that pops up



- Now mice can be loaded in the gemini box, push the "start" button on the box, trial will start and data will be recorded



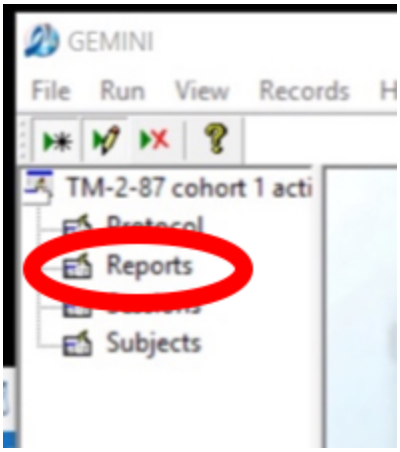
4.1 Experimental Procedure

1. Before beginning, clean the chamber floor and walls
2. Place bedding in the tray beneath the grid floor
3. Place the mouse in one of the two compartments (Right or left side)
4. Shut the door
5. Press start on the Gemini box: after 5 minutes of habituation, the mouse will be tested in a total of 50 trials
6. When the mouse is finished it may be returned to its home cage
7. Clean the chamber floor and walls between each mouse in order to maintain a consistent intensity of shock released from the floor

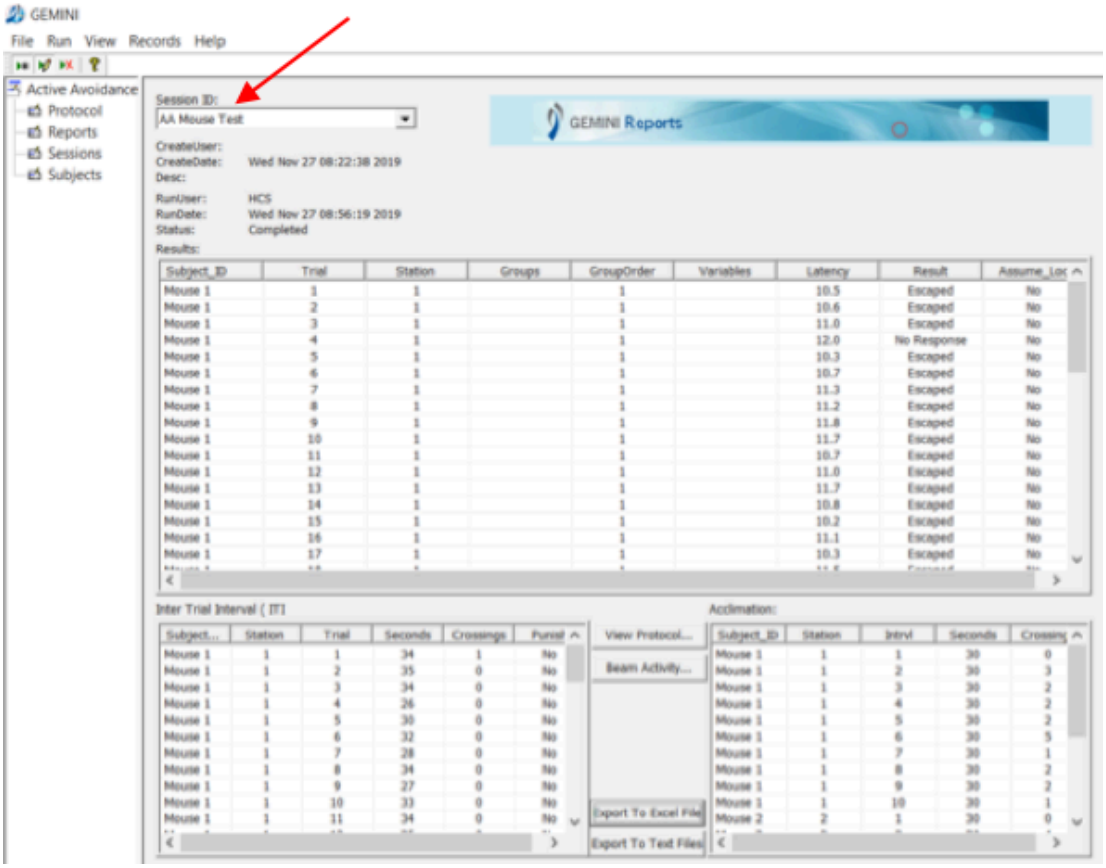
8. Place next mouse in the chamber and continue with steps 1-7
9. Repeat 1-7 every day for 5 days

5 **Export data**

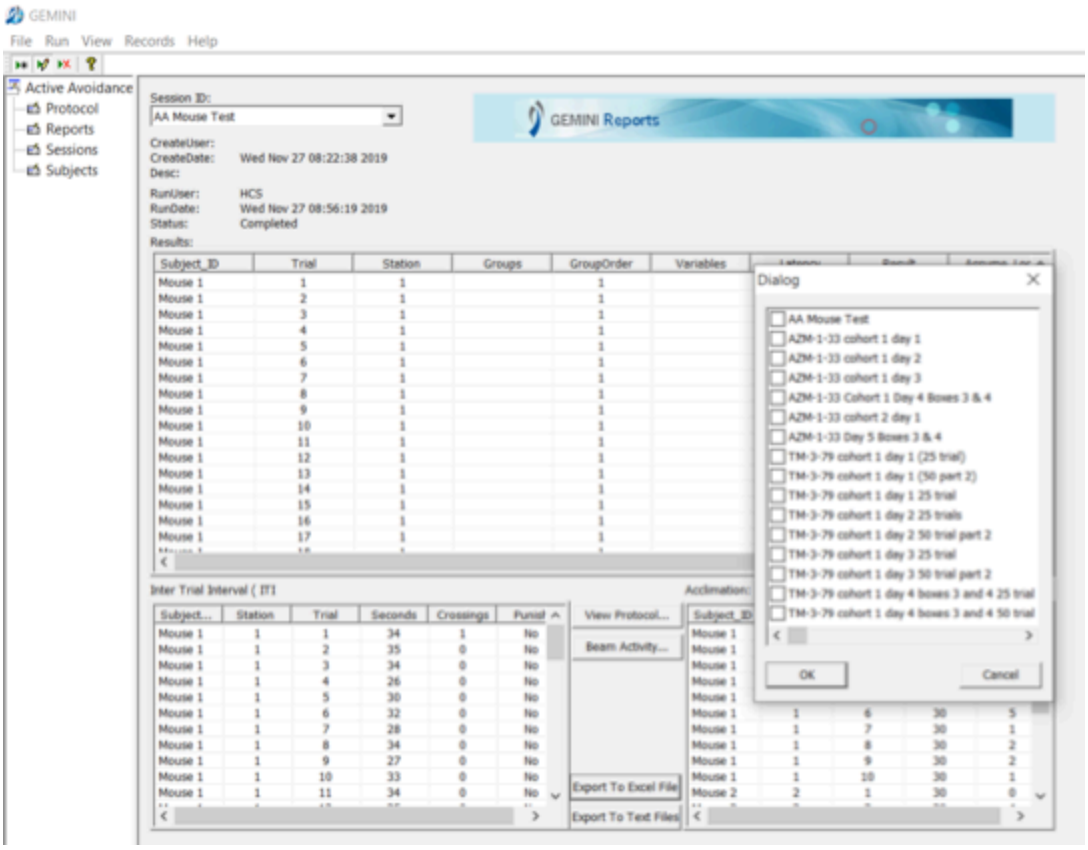
- Click ***Reports***



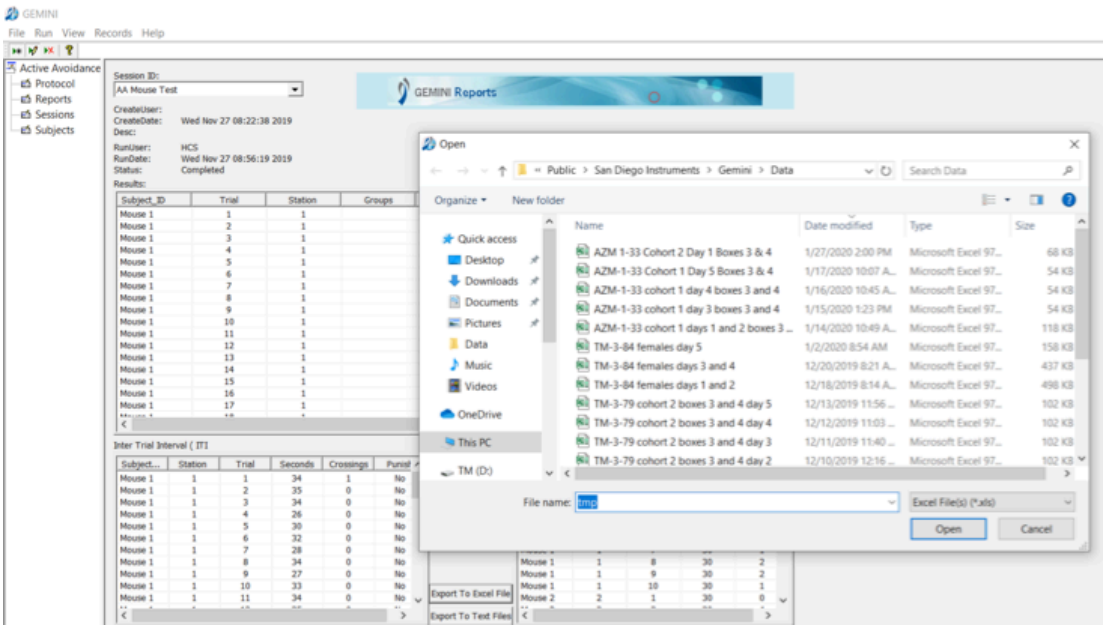
- Select "Session ID"



- Click "***Export to Excel File***" and select on the ***Dialog*** window the file you want to export and click ***OK***

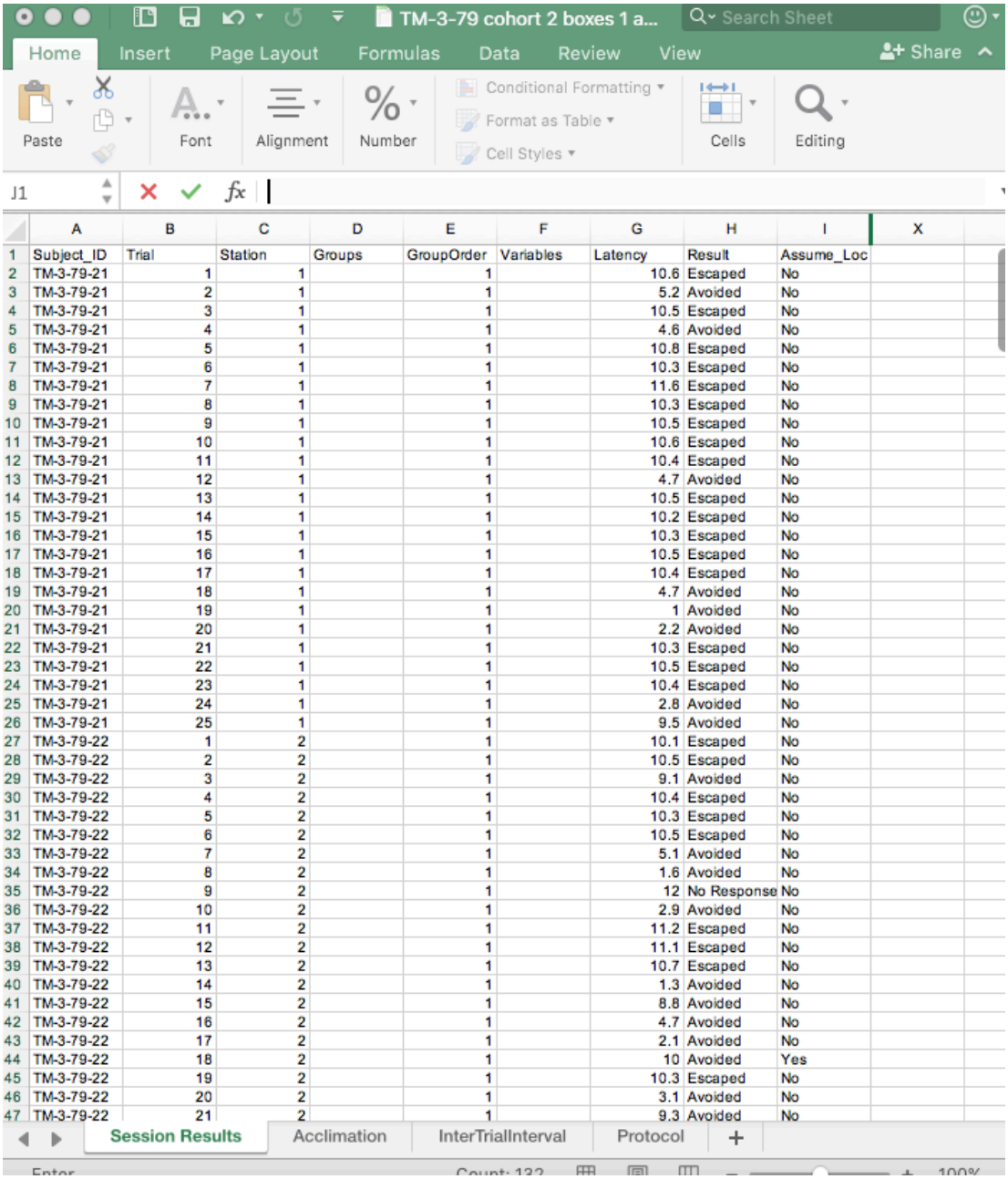


- Select the folder where you want to save your file, name the file and click "Open"



- **Example of excel file, 4-worksheet:**

1. Session Results → Subject ID, trials, latency and results (avoided, escaped or No Response) are reported in this worksheet.
2. Acclimation → number of crossing during acclimation time.
3. InterTrialInterval → inter trial time (seconds) and number of crossing during this interval.
4. Protocol → info about your session and protocol are saved in this worksheet.



	A	B	C	D	E	F	G	H	I	X
1	Subject_ID	Trial	Station	Groups	GroupOrder	Variables	Latency	Result	Assume_Loc	
2	TM-3-79-21	1	1		1		10.6	Escaped	No	
3	TM-3-79-21	2	1		1		5.2	Avoided	No	
4	TM-3-79-21	3	1		1		10.5	Escaped	No	
5	TM-3-79-21	4	1		1		4.6	Avoided	No	
6	TM-3-79-21	5	1		1		10.8	Escaped	No	
7	TM-3-79-21	6	1		1		10.3	Escaped	No	
8	TM-3-79-21	7	1		1		11.6	Escaped	No	
9	TM-3-79-21	8	1		1		10.3	Escaped	No	
10	TM-3-79-21	9	1		1		10.5	Escaped	No	
11	TM-3-79-21	10	1		1		10.6	Escaped	No	
12	TM-3-79-21	11	1		1		10.4	Escaped	No	
13	TM-3-79-21	12	1		1		4.7	Avoided	No	
14	TM-3-79-21	13	1		1		10.5	Escaped	No	
15	TM-3-79-21	14	1		1		10.2	Escaped	No	
16	TM-3-79-21	15	1		1		10.3	Escaped	No	
17	TM-3-79-21	16	1		1		10.5	Escaped	No	
18	TM-3-79-21	17	1		1		10.4	Escaped	No	
19	TM-3-79-21	18	1		1		4.7	Avoided	No	
20	TM-3-79-21	19	1		1		1	Avoided	No	
21	TM-3-79-21	20	1		1		2.2	Avoided	No	
22	TM-3-79-21	21	1		1		10.3	Escaped	No	
23	TM-3-79-21	22	1		1		10.5	Escaped	No	
24	TM-3-79-21	23	1		1		10.4	Escaped	No	
25	TM-3-79-21	24	1		1		2.8	Avoided	No	
26	TM-3-79-21	25	1		1		9.5	Avoided	No	
27	TM-3-79-22	1	2		1		10.1	Escaped	No	
28	TM-3-79-22	2	2		1		10.5	Escaped	No	
29	TM-3-79-22	3	2		1		9.1	Avoided	No	
30	TM-3-79-22	4	2		1		10.4	Escaped	No	
31	TM-3-79-22	5	2		1		10.3	Escaped	No	
32	TM-3-79-22	6	2		1		10.5	Escaped	No	
33	TM-3-79-22	7	2		1		5.1	Avoided	No	
34	TM-3-79-22	8	2		1		1.6	Avoided	No	
35	TM-3-79-22	9	2		1		12	No Response	No	
36	TM-3-79-22	10	2		1		2.9	Avoided	No	
37	TM-3-79-22	11	2		1		11.2	Escaped	No	
38	TM-3-79-22	12	2		1		11.1	Escaped	No	
39	TM-3-79-22	13	2		1		10.7	Escaped	No	
40	TM-3-79-22	14	2		1		1.3	Avoided	No	
41	TM-3-79-22	15	2		1		8.8	Avoided	No	
42	TM-3-79-22	16	2		1		4.7	Avoided	No	
43	TM-3-79-22	17	2		1		2.1	Avoided	No	
44	TM-3-79-22	18	2		1		10	Avoided	Yes	
45	TM-3-79-22	19	2		1		10.3	Escaped	No	
46	TM-3-79-22	20	2		1		3.1	Avoided	No	
47	TM-3-79-22	21	2		1		9.3	Avoided	No	