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Active Avoidance protocol 01282020

Forked from a private protocol

DOI

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Manuscript citation:

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

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Protocol status: Working

We use this protocol and it's working

Created: January 28, 2020

Last Modified: July 06, 2020

Protocol Integer ID: 32382

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.

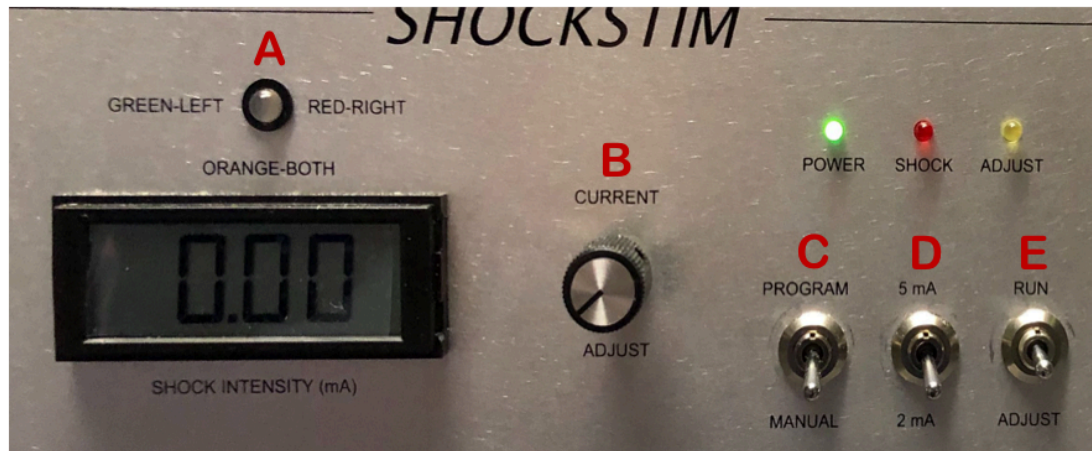
Sex: Males and females should be tested in separate groups, in particular in different cohorts. If this is not possible and more than one active avoidance chamber is available make sure to dedicate one chamber for each sex.

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

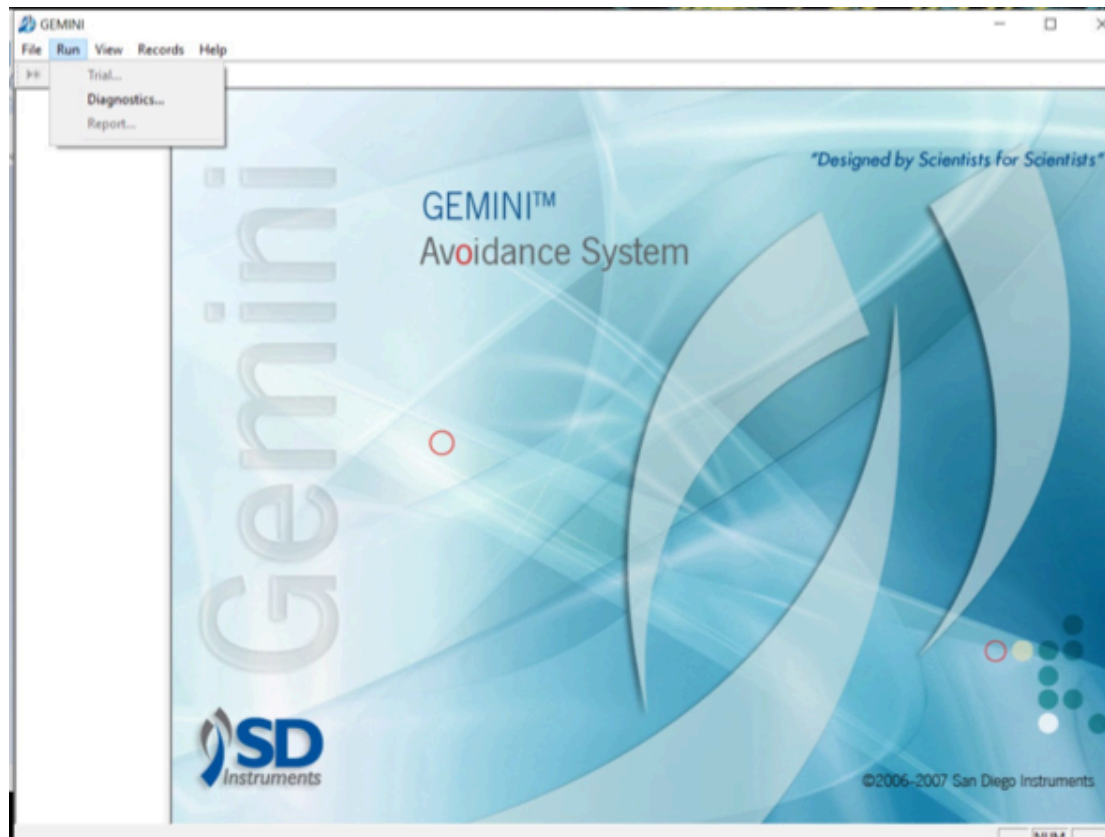


- A.** Based on the color the location of the shock can be determined
- B.** This knob sets the shock amplitude when “C” is positioned in manual mode
- C.** The position of this switch determines the source of the shock (it should be positioned on Manual the entire time: when you adjust the shock intensity 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.

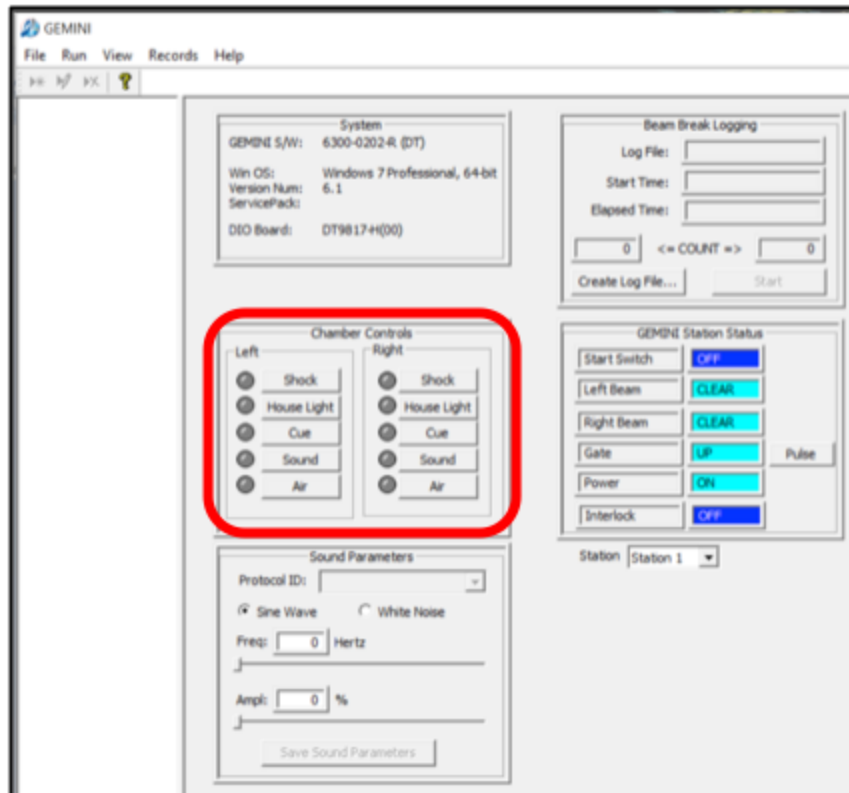
3 How to check if the chamber works properly

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



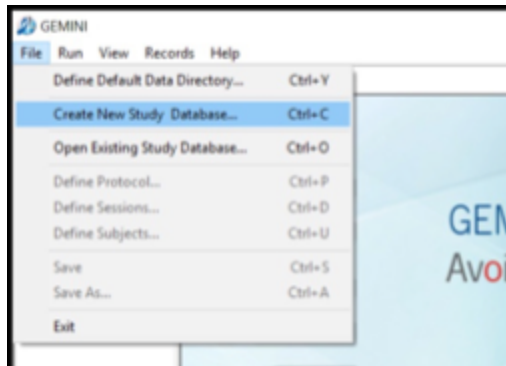
- Click on the chamber controls to evaluate if the chamber is working properly



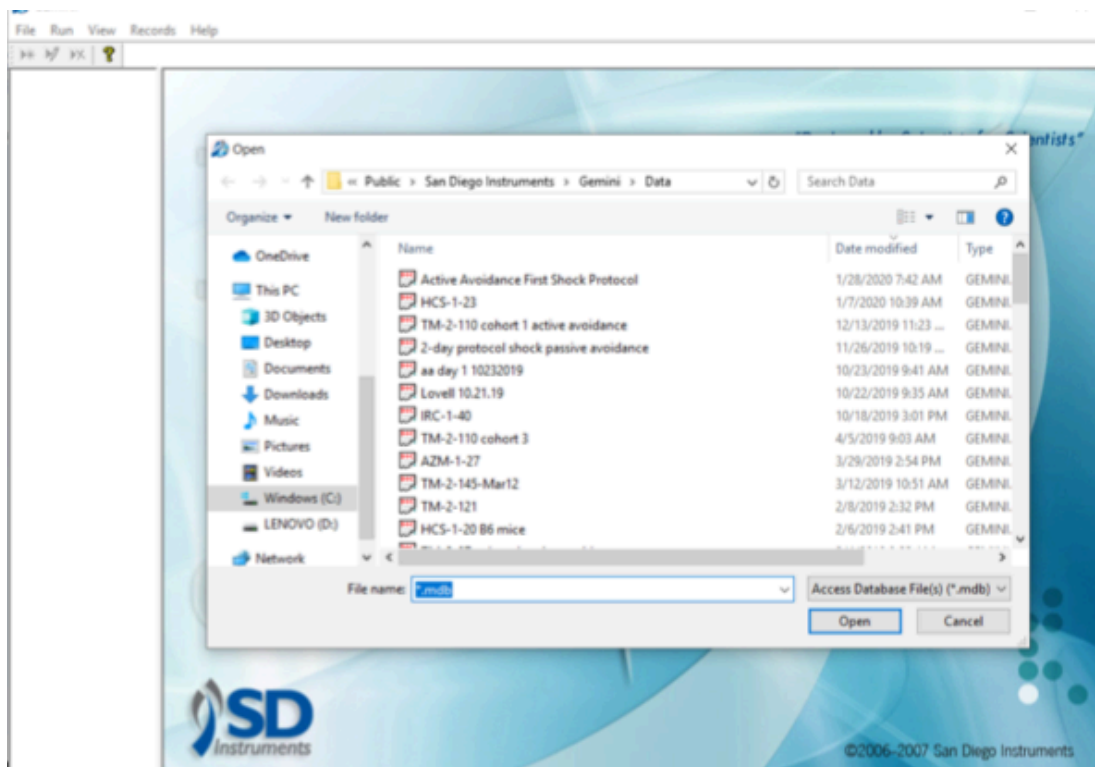
4 Set up

Computer setup

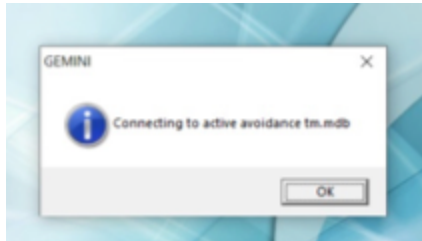
- Turn on the computer
- Make sure that the Avoidance system is plugged in and turned on
- Open the avoidance system program (GEMINI.exe)
- **Click file → click create a new study database**



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

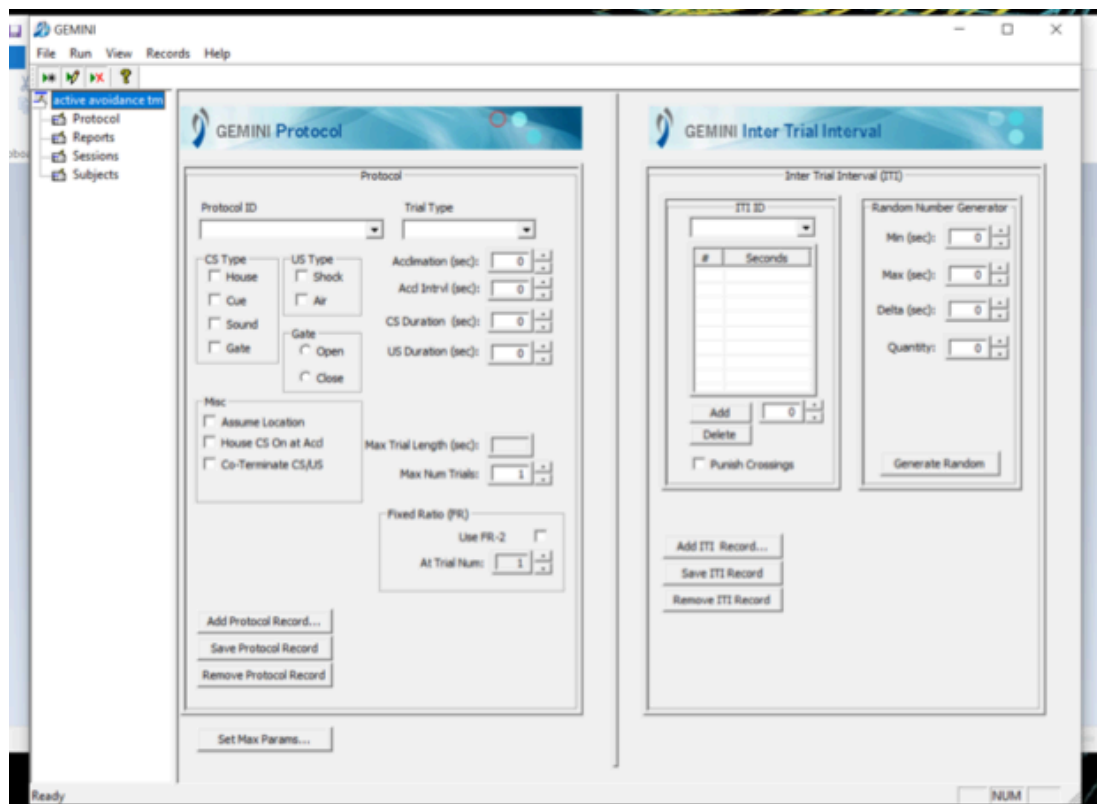


- A "connecting" window will pop up, click OK



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

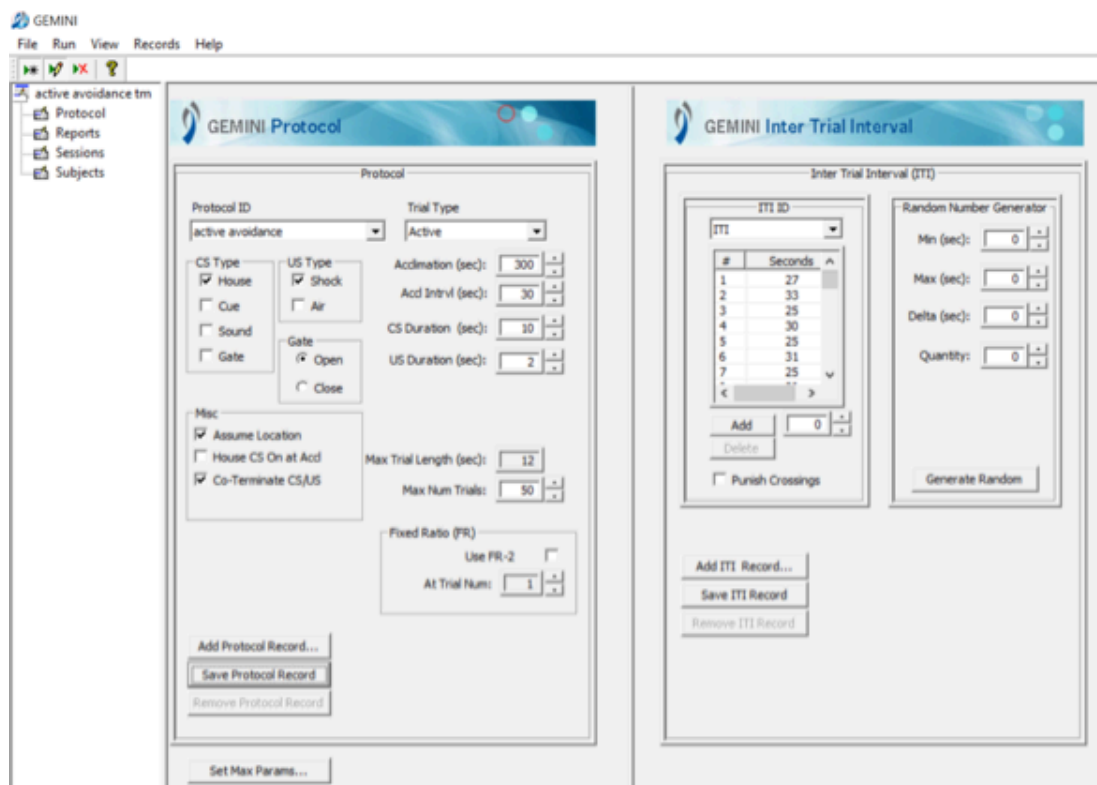
Max (sec): 35

Delta (sec): 5

Quantity: 49

-click Generate Random

- Save protocol record



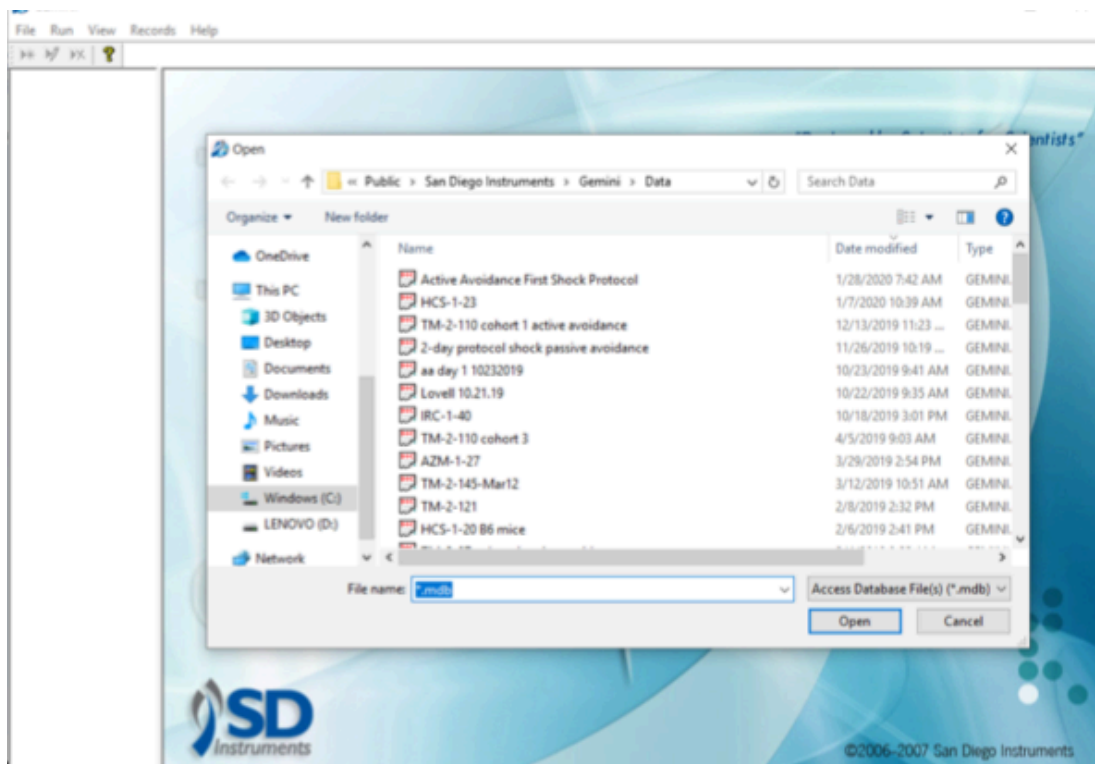
Example of Active avoidance protocol

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



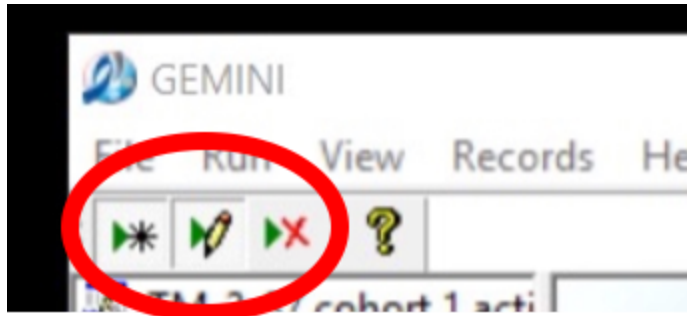
How to save your file

- Click file, then "save as"

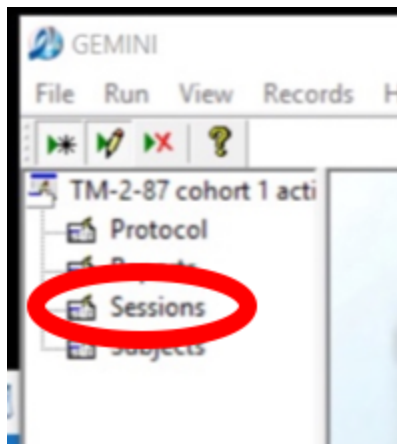
How to add subjects to the experiment

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

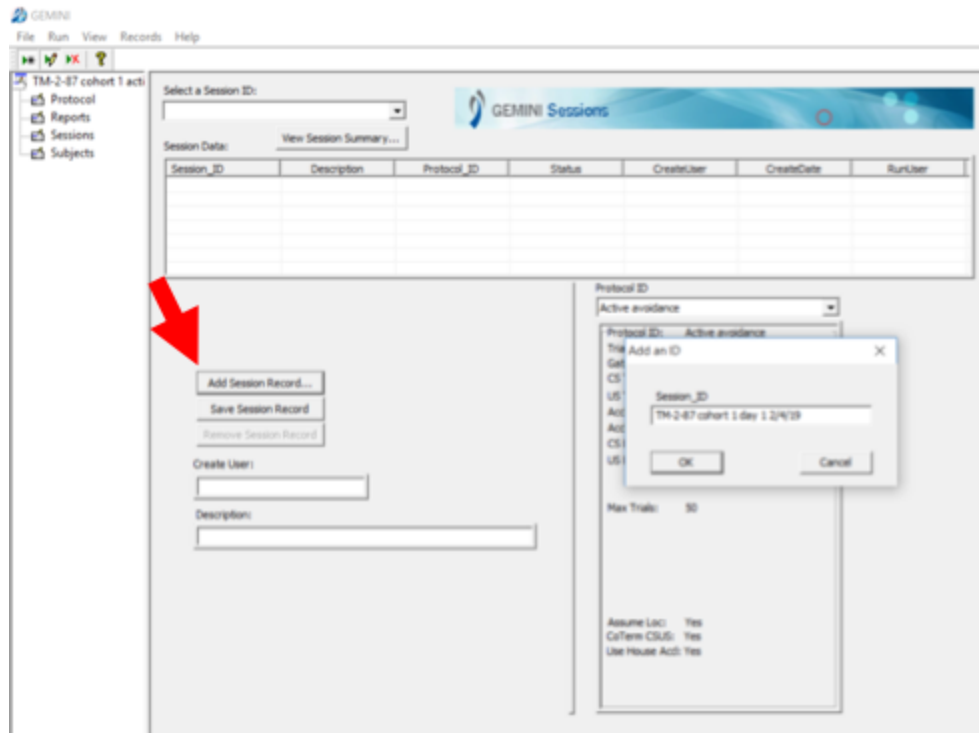
- Click triangle-star and pen button on top left side (you can create a session and add subjects)



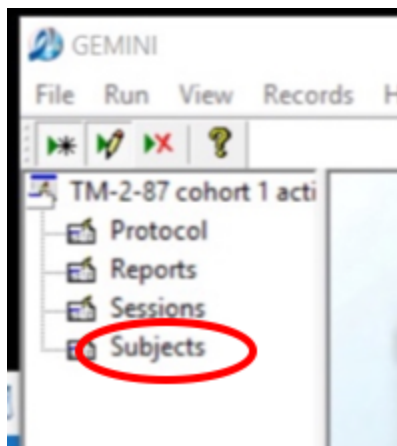
- Click Session



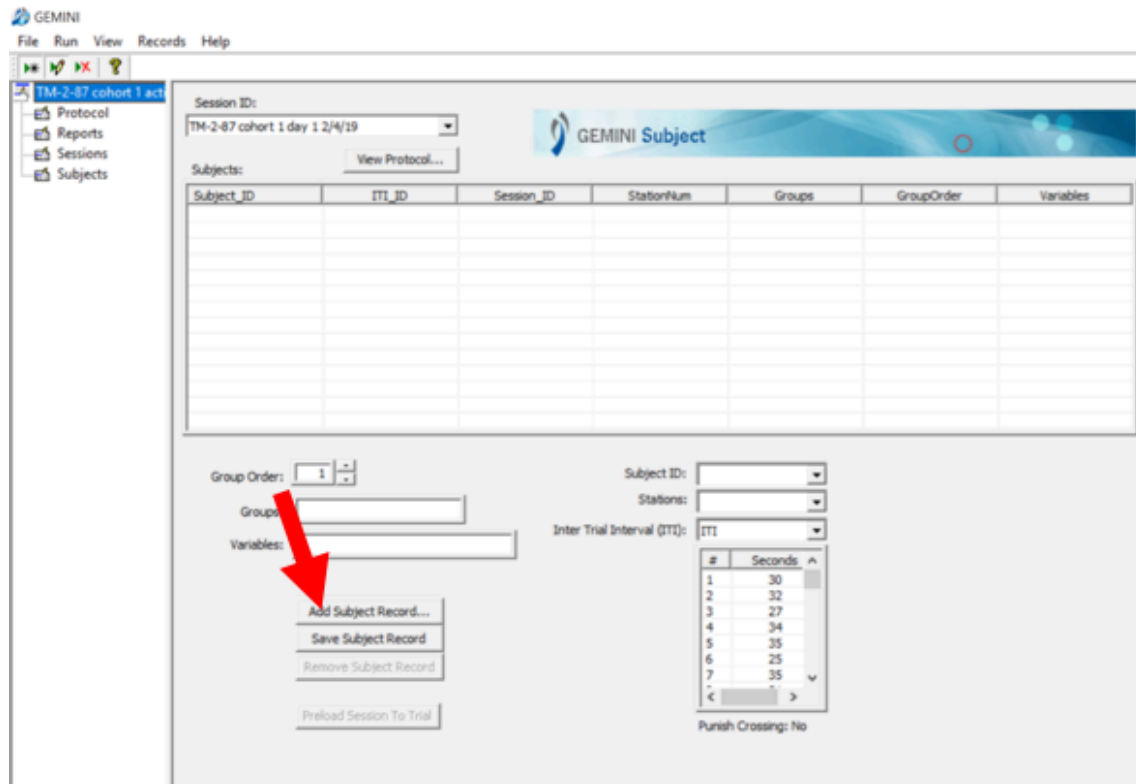
- Click Add session record, Fill in Session ID (e.g. name , date)



- 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)

Subject ID:

Stations:

Inter Trial Interval (ITI):

#	Seconds
1	30
2	32
3	27
4	34
5	35
6	25
7	35

Punish Crossing: No

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

GEMINI

File Run View Records Help

active avoidance tm

Protocol Reports Sessions Subjects

Session ID: AZM-1-33 Cohort 2 Boxes 1 & 2 Day 2

Subjects: View Protocol...

Subject ID	ITI ID	Session ID	StationNum	Groups	GroupOrder	Variables
AZM-1-33-25	ITI	AZM-1-33 Cohort 2 ...	1		1	
AZM-1-33-29	ITI	AZM-1-33 Cohort 2 ...	1		1	
AZM-1-33-39	ITI	AZM-1-33 Cohort 2 ...	1		1	
AZM-1-33-26	ITI	AZM-1-33 Cohort 2 ...	2		1	
AZM-1-33-35	ITI	AZM-1-33 Cohort 2 ...	2		1	

Group Order: 1

Groups:

Variables:

Add Subject Record...
Save Subject Record
Remove Subject Record
Preload Session To Trial

Subject ID: AZM-1-33
Stations: Station 2
Inter Trial Interval (ITI): ITI

GEMINI Subject

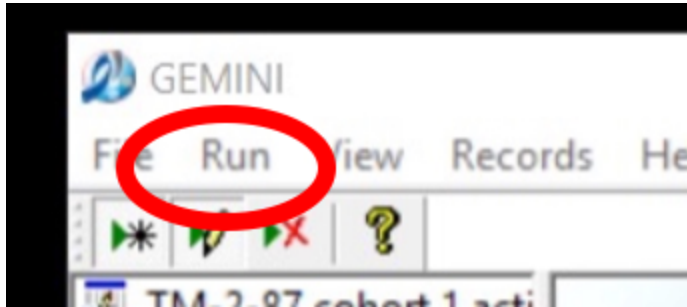
GEMINI Data Preloaded for trial run

OK

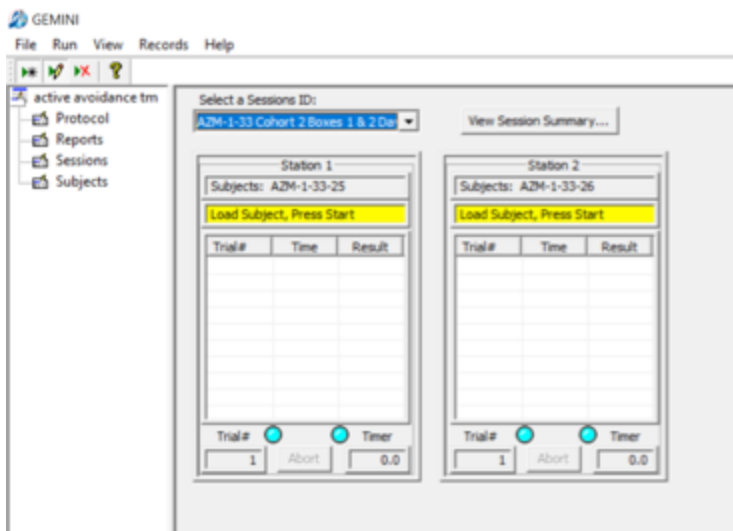
#	Seconds
1	27
2	33
3	25
4	30
5	25
6	31
7	25

Punish Crossing: No

- 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

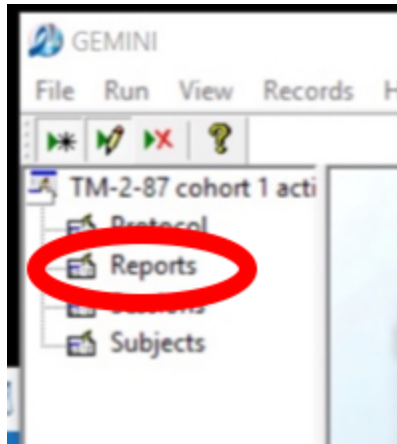
1. Before beginning, clean the chamber floor and walls
2. Place bedding in the tray beneath the grid floor
3. Place the mouse in one of the two compartments (Right or left side)
4. Shut the door
5. Press start on the Gemini box: after 5 minutes of habituation, the mouse will be tested in a total of 50 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"

The screenshot displays the GEMINI software interface. At the top, a menu bar includes 'File', 'Run', 'View', 'Records', and 'Help'. A sidebar on the left contains 'Active Avoidance', 'Protocol', 'Reports', 'Sessions', and 'Subjects'. The main window shows session details for 'AA Mouse Test', including 'CreateUser: HCS', 'CreateDate: Wed Nov 27 08:22:38 2019', 'RunDate: Wed Nov 27 08:56:19 2019', and 'Status: Completed'. A red arrow points to the 'Session ID' dropdown menu. Below this, a large table lists results for 17 trials, with columns for Subject_ID, Trial, Station, Groups, GroupOrder, Variables, Latency, Result, and Assume_Loc. The bottom section contains two smaller tables: 'Inter Trial Interval (ITI)' and 'Accimation:'. The ITI table has columns for Subject..., Station, Trial, Seconds, Crossings, and Pursuit. The Accimation table has columns for Subject_ID, Station, Intvl, Seconds, and Crossing. On the right side of the bottom section, there are buttons for 'View Protocol...', 'Beam Activity...', 'Export To Excel File', and 'Export To Text File'.

Subject_ID	Trial	Station	Groups	GroupOrder	Variables	Latency	Result	Assume_Loc
Mouse 1	1	1		1		10.5	Escaped	No
Mouse 1	2	1		1		10.6	Escaped	No
Mouse 1	3	1		1		11.0	Escaped	No
Mouse 1	4	1		1		12.0	No Response	No
Mouse 1	5	1		1		10.3	Escaped	No
Mouse 1	6	1		1		10.7	Escaped	No
Mouse 1	7	1		1		11.3	Escaped	No
Mouse 1	8	1		1		11.2	Escaped	No
Mouse 1	9	1		1		11.8	Escaped	No
Mouse 1	10	1		1		11.7	Escaped	No
Mouse 1	11	1		1		10.7	Escaped	No
Mouse 1	12	1		1		11.0	Escaped	No
Mouse 1	13	1		1		11.7	Escaped	No
Mouse 1	14	1		1		10.8	Escaped	No
Mouse 1	15	1		1		10.2	Escaped	No
Mouse 1	16	1		1		11.1	Escaped	No
Mouse 1	17	1		1		10.3	Escaped	No

Subject...	Station	Trial	Seconds	Crossings	Pursuit
Mouse 1	1	1	34	1	No
Mouse 1	1	2	35	0	No
Mouse 1	1	3	34	0	No
Mouse 1	1	4	26	0	No
Mouse 1	1	5	30	0	No
Mouse 1	1	6	32	0	No
Mouse 1	1	7	28	0	No
Mouse 1	1	8	34	0	No
Mouse 1	1	9	27	0	No
Mouse 1	1	10	33	0	No
Mouse 1	1	11	34	0	No

Subject_ID	Station	Intvl	Seconds	Crossing
Mouse 1	1	1	30	0
Mouse 1	1	2	30	3
Mouse 1	1	3	30	2
Mouse 1	1	4	30	2
Mouse 1	1	5	30	2
Mouse 1	1	6	30	5
Mouse 1	1	7	30	1
Mouse 1	1	8	30	2
Mouse 1	1	9	30	2
Mouse 1	1	10	30	1
Mouse 2	2	1	30	0

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

GEMINI

File Run View Records Help

Active Avoidance

Protocol Reports Sessions Subjects

Session ID: AA Mouse Test

Create User: HCS

Create Date: Wed Nov 27 08:22:38 2019

Desc:

Run User: HCS

Run Date: Wed Nov 27 08:56:19 2019

Status: Completed

Results:

Subject_ID	Trial	Station	Groups	GroupOrder	Variables
Mouse 1	1	1		1	
Mouse 1	2	1		1	
Mouse 1	3	1		1	
Mouse 1	4	1		1	
Mouse 1	5	1		1	
Mouse 1	6	1		1	
Mouse 1	7	1		1	
Mouse 1	8	1		1	
Mouse 1	9	1		1	
Mouse 1	10	1		1	
Mouse 1	11	1		1	
Mouse 1	12	1		1	
Mouse 1	13	1		1	
Mouse 1	14	1		1	
Mouse 1	15	1		1	
Mouse 1	16	1		1	
Mouse 1	17	1		1	

Inter Trial Interval (ITI)

Subject...	Station	Trial	Seconds	Crossings	Funnel
Mouse 1	1	1	34	1	No
Mouse 1	1	2	35	0	No
Mouse 1	1	3	34	0	No
Mouse 1	1	4	26	0	No
Mouse 1	1	5	30	0	No
Mouse 1	1	6	32	0	No
Mouse 1	1	7	28	0	No
Mouse 1	1	8	34	0	No
Mouse 1	1	9	27	0	No
Mouse 1	1	10	33	0	No
Mouse 1	1	11	34	0	No

Acclimation:

View Protocol...

Beam Activity...

Export To Excel File

Export To Text File

Dialog

AA Mouse Test

AZM-1-33 cohort 1 day 1

AZM-1-33 cohort 1 day 2

AZM-1-33 cohort 1 day 3

AZM-1-33 Cohort 1 Day 4 Boxes 3 & 4

AZM-1-33 cohort 2 day 1

AZM-1-33 Day 5 Boxes 3 & 4

TM-3-79 cohort 1 day 1 (25 trial)

TM-3-79 cohort 1 day 1 (50 part 2)

TM-3-79 cohort 1 day 1 25 trial

TM-3-79 cohort 1 day 2 25 trials

TM-3-79 cohort 1 day 2 50 trial part 2

TM-3-79 cohort 1 day 3 25 trial

TM-3-79 cohort 1 day 3 50 trial part 2

TM-3-79 cohort 1 day 4 boxes 3 and 4 25 trial

TM-3-79 cohort 1 day 4 boxes 3 and 4 50 trial

OK Cancel

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

The screenshot displays the GEMINI Reports application window. On the left, a sidebar shows navigation options: Protocol, Reports, Sessions, and Subjects. The main window is titled 'Active Avoidance' and shows session details for 'AA Mouse Test'. Below this, a table lists results for multiple mice, including Subject ID, Trial, Station, and Groups. An 'Open' file dialog is overlaid on the right, showing the file explorer for 'Public > San Diego Instruments > Gemini > Data'. The dialog lists several Excel files, with 'TM (D)' selected. The file name field shows 'TM (D)' and the file type is set to 'Excel File(s) (*.xls)'. The 'Open' button is highlighted.

Subject ID	Trial	Station	Groups
Mouse 1	1	1	
Mouse 1	2	1	
Mouse 1	3	1	
Mouse 1	4	1	
Mouse 1	5	1	
Mouse 1	6	1	
Mouse 1	7	1	
Mouse 1	8	1	
Mouse 1	9	1	
Mouse 1	10	1	
Mouse 1	11	1	
Mouse 1	12	1	
Mouse 1	13	1	
Mouse 1	14	1	
Mouse 1	15	1	
Mouse 1	16	1	
Mouse 1	17	1	

Subject	Station	Trial	Seconds	Crossings	Latency
Mouse 1	1	1	34	1	No
Mouse 1	1	2	35	0	No
Mouse 1	1	3	34	0	No
Mouse 1	1	4	26	0	No
Mouse 1	1	5	30	0	No
Mouse 1	1	6	32	0	No
Mouse 1	1	7	28	0	No
Mouse 1	1	8	34	0	No
Mouse 1	1	9	27	0	No
Mouse 1	1	10	33	0	No
Mouse 1	1	11	34	0	No

■ 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.



TM-3-79 cohort 2 boxes 1 a... Search Sheet

Home Insert Page Layout Formulas Data Review View Share

Paste Font Alignment Number Conditional Formatting Format as Table Cell Styles Cells Editing

J1

	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	

Session Results Acclimation InterTrialInterval Protocol +

Enter Count: 123 100%