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EtOH Self-Administration

Brent Boomhower[1], Lani Tieu[1], Olivier George[1]

[1]University of California, San Diego

1 *Works for me* This protocol is published without a DOI.

George Lab
Tech. support email: olgeorge@ucsd.edu

Brent Boomhower

ABSTRACT

This protocol outlines in detail the procedure for conducting ethanol self-administration studies (e.g., Pharm) including the use of passive vapor to induce dependence.

EXTERNAL LINK

https://docs.google.com/document/d/1-qOz9Jg1-_hng69KBvdNTm0EwCLRTWS7XeP46eBfe88/edit?usp=sharing

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GUIDELINES

Note: Monitor the health of all the animals by keeping an eye on their weights in comparison to preceding weeks and by assessing their activity level during handling. Often the water system in at least a couple home cages malfunction, leading to animal dehydration and eventual death.

MATERIALS

NAME	CATALOG #	VENDOR
190 Proof Ethanol		

STEPS MATERIALS

NAME	CATALOG #	VENDOR
190 Proof Ethanol		

BEFORE STARTING

For Pharm grant cohorts, see previous lab notebooks and Dropbox .xls files for data collection template.

After animal arrival and quarantine, handle and tail-mark all animals. Handle animals at least a few times per week before the experiment and weigh them once a week throughout the entire experiment.

(TIP: Mark the first animal in each home cage at the base of the tail and the second toward the middle of the tail. This will prevent losing track of which animal is which as the tail-marks may fade beyond recognition.)

Begin front lever training (Water only) 2d

1 Run 24 animals in an overnight session with only the front lever connected to water and a handful of food added directly into the chamber (*see "**Operant Chamber Set-up**" below for specific session instructions*). 1d

2 Run the next 24 animals the following day for their overnight session in the same manner. 1d

Operant Chamber Set-up

3 Fill syringes with water to maximum capacity (~33 mL).

4 Connect syringes to the EtOH pump and tubing, which operates via the front lever and fills the front well, respectively.

5 Push through 1-2 ml water to flush out air bubbles from the line.

6 Start a test session on both computers to test the levers and flush out any remaining air bubbles from the lines. Do so by repeatedly pressing the lever to activate the pump.

6.1 Open ETOHi12 application to set up session on each computer (switch between computers by pressing *Scroll Lock* twice).

6.2 Set levers used from 'Both Levers' to '**Front Lever**'.

6.3 Start test session for each chamber by pressing '**Start Group**'.

7 Use paper towels to soak up all the flushed water.

8 End the test session.

9 Now, set-up the computers for the overnight session.

9.1 Set Max Rewards to **300** to prevent animals from pressing to an empty syringe.

9.2 Set Levers Used from 'Both Levers' to '**Front Lever**'.

9.3 Load subject names to proper **animal IDs** under *Group Settings* tab based on chamber placement for data collection.

9.4 Set session length to **1140 minutes** to allow the program to run all night.

In future sessions, the session length will be much shorter and progressively shorten until reaching 30 minutes.

9.5 Change **File Name**.

File names should be simple yet descriptive (e.g., “pharm7.30min2F1” for the first female 2-lever, 30 min session of the 7th cohort for the pharm grant).

9.6 Place each animal into their respective chamber and start the overnight session.

9.7 Before the session ends the next day, check if any animals have responded to the maximum rewards. Take out all animals that have pressed to the maximum rewards.

9.8 Allow all other animals to continue until they reach the maximum rewards OR the session ends.

9.9 Clean the bedding trays and operant chambers.

9.10 Only animals that did not reach at least 150 lever responses need to be run in a joint session after both 24 animal groups have done one overnight session. Use same parameters as outlined above.

Typically, there will be less than 24 animals that have significantly low pressing, therefore one joint session is possible.

Front lever training (EtOH only) 6h

10 Prepare a solution of [M]**10 % volume** ethanol (EtOH) in the wetlab area.

10.1 For 1 liter volume solution, pipet **105 mL** ***190 Proof EtOH*** (95%) into a 1000 mL capacity graduated cylinder.

190 Proof Ethanol

10.2 Add **895 mL** water from the sink to the graduated cylinder.

10.3 Pour contents of the graduated cylinder into a 1000 mL capacity glass bottle with a screwable cap and shake.

10.4 Label contents as "10% EtOH" including the date and your initials.

11 Run the first group of 24 animals in a **3-hour session** with [M]**10 % volume** EtOH in the front lever. 3h

Begin this first session earlier in the day so the next 3-hour session will not run too late.

All session parameters will remain the same as the overnight sessions, ***except*** the session length (180 min) and the file name.

12 Run the second group in the same manner as above. 3h

13 After each session ends, record lever presses (physically and digitally).

14 Thoroughly clean chambers after both sessions are finished.

Overnight training sessions, continued

15 Run animals with < 50 lever responses in another overnight session with **water** connected to the front lever and following all other guidelines as in the first overnight sessions.

15.1 Take out animals from their overnight session the next day.

All animals should reach significantly high lever responses during this overnight session. If a few animals do not respond significantly, you should likely move forward in the protocol despite this, but first ask for further guidance from your mentor.

Front lever training (EtOH only), continued

16 After the overnight session finishes, run each set of animals in another **3-hour session** with [M]**10 % volume** EtOH connected to the front lever as outlined previously.

17 After the session, check the average lever responses for each group. The average responses should be close to **50 or more** for these 3-hour sessions.

If this is not the case, once again ask for further guidance from your mentor before continuing in the protocol.

18 Continue to run animals each day as described above, ***except*** decrease the session lengths to **2 hours**.
Run **2-hour sessions** for 2-3 days.

19 Next, run animals in **1-hour sessions** for 2-3 days.

Always keep an eye on average lever responses and decrease subsequent session lengths as lever responses stabilize.

20 Finally, run animals in **30-minute sessions** for at least 2-3 days.
These sessions should start close to the beginning of the animals' dark cycle.

Consult your mentor before moving forward in the protocol to the 2-lever paradigm.

2-lever paradigm (EtOH & water)

21 Upon reaching stable responses for [M]**10 % volume** EtOH, begin **30-minute sessions** with **2-lever access**.

These sessions will be run under the same parameters as the previous 30-minute sessions, ***except*** the lever option will be left as '**Both Levers**', rather than 'Front Lever'.

21.1 Attach the [M]**10 % volume** EtOH syringe to the **front lever pump/line**.

Attach the water syringe to the **back lever pump/line**.

21.2

22 Run animals with 2-lever access for 2-3 weeks.

At any point, if there are days that are overwhelmingly busy, talk to your mentor and up to 1-2 consecutive days may be skipped.

Passive vapor, or chronic intermittent ethanol (CIE) exposure

23 **Consult your mentor before proceeding.**

Split animals into two groups (dependent & nondependent), with the dependent group defined as the group to be housed in the passive EtOH vapor chambers.

Dependent and nondependent groups should be split so each group has 1/2 the male and 1/2 the female animals.

24 For at least 2-3 weeks, all animals will not undergo self-administration sessions.
The next few weeks are designated for the modulation of the dependent group's blood alcohol levels (BALs).

*See "**Blood collection for BALs**" protocol in "Biological Sampling" folder to begin the process of reaching animal BALs of ~150-250 mg/dL blood alcohol for the dependent group.*

25 Upon reaching or nearing the desired BAL range for the dependent animals, begin **2-lever sessions** again.
These sessions will be run ONLY on ***Monday***, ***Wednesday***, and ***Friday*** for both nondependent and dependent groups.

25.1 Run the **nondependent group** within 1-2 hours of their light cycle switching to dark.

One operant chamber rack should be designated for males and the other females, since males and females will now be run simultaneously.

25.2 Next, run the **dependent group** 4-5 hours into passive vapor withdrawal (i.e., the pump turning OFF and ethanol beakers empty).

26 Continue to run both groups of animals and begin pharmacological treatments or another experimental paradigm.

Treatment groups

27 Both the nondependent and dependent groups will be split into a vehicle group and a treatment group.

27.1 These groups will be formed by analyzing the last several sessions.

High, **moderate**, and **low** responders must be distributed evenly into each group (*see example*

below).

NONDEPENDENT

	RAT ID						avg ETOH		RAT ID						avg EtOH
treatment	**40**	32	34	55	29	41	38.2	vehicle	**38**	19	37	53	46	24	35.8
treatment	**28**	13	10	10	10	21	12.8	vehicle	**43**	15	9	22	25	5	15.2
treatment	**33**	32	32	27	33	53	35.4	vehicle	**37**	39	36	31	27	40	34.6
treatment	**47**	41	15	10	13	10	17.8	vehicle	**25**	22	14	11	10	39	19.2
treatment	**44**	33	37	44	31	23	33.6	vehicle	**29**	33	51	12	29	42	33.4
treatment	**34**	14	43	18	16	11	20.4	vehicle	**36**	18	18	40	3	28	21.4
treatment	**30**	28	30	29	30	30	29.4	vehicle	**27**	24	11	32	22	42	26.2
treatment	**45**	31	30	2	17	28	21.6	vehicle	**41**	20	27	19	24	34	24.8
						group avg	26.15							group avg	26.325

DEPENDENT

	RAT ID						avg ETOH		RAT ID						avg ETOH
treatment	**4**	87	88	71	90	94	86	vehicle	**22**	87	95	77	51	104	82.8
treatment	**13**	51	17	19	47	4	27.6	vehicle	**24**	25	31	32	43	30	32.2
treatment	**3**	69	61	67	60	70	65.4	vehicle	**6**	20	43	41	64	98	53.2
treatment	**18**	49	27	21	23	41	32.2	vehicle	**11**	23	35	36	36	42	34.4
treatment	**7**	62	58	48	47	43	51.6	vehicle	**16**	47	55	49	51	46	49.6
treatment	**5**	40	29	18	52	46	37	vehicle	**1**	27	55	49	31	25	37.4
treatment	**2**	47	37	53	48	50	47	vehicle	**21**	40	29	48	53	60	46
treatment	**20**	21	41	68	37	33	40	vehicle	**14**	29	40	50	43	43	41
						group avg	48.35							group avg	47.075

28 Continue 2-lever sessions with applied treatments.