Chapter 7

Novelty-Suppressed Feeding in the Mouse

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Abstract

The use of hyponeophagia, in which exposure to a novel environment suppresses feeding behavior, has been used to assess anxiety-related behavior in animals for over seven decades. More recent work has shown that variations of hyponeophagia, such as the novelty-suppressed feeding test, have become effective paradigms for testing treatment with drugs such as anxiolytics and antidepressants. Most interestingly, unlike many other behavioral paradigms, novelty-suppressed feeding is sensitive to chronic, but not acute, antidepressant treatment, which mirrors the effects of antidepressant treatment in human patients. Here we provide a brief historical overview of novelty-suppressed feeding and provide a protocol for running the test with mice.

Key words: Anxiety, Depression, Mood, Mouse, Antidepressant, Neurogenesis

1. Background and Historical Overview

Novelty-suppressed feeding (hyponeophagia) is a conflict-based test in which an animal that has been deprived of food for a full day faces a choice of approaching and consuming a piece of food in the center of a brightly lit, novel open arena or staying to the side and avoiding the center of this anxiogenic environment. Subjects participating in this test do not require any previous complex training, are not exposed to painful stimuli, and are usually deprived of food for 24 h, less than an animal would face in a normal foraging situation. The main measure of the test is latency to eat (defined as the amount of time it takes for the animal to enter the center of the arena and bite the food pellet with use of forepaws while sitting on its haunches). This is a test of anxiety-related behavior, and therefore, experimental animals with a significantly longer latency to eat than control animals are usually described as more anxious.

Todd D. Gould (ed.), *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests, Volume II*, Neuromethods, vol. 63, DOI 10.1007/978-1-61779-313-4_7,

Many genetic manipulations resulting in anxiety-related phenotypes in mice, such as the 5-HT1A receptor knockout (1), glucocorticoid receptor knockdown by transgenic antisense (2), and substance P receptor knockout (3), exhibit increased hyponeophagia (4). Furthermore, many drugs with anxiolytic properties, such as benzodiazepines, barbituates, azapirones, and β-adrenergic antagonists, decrease hyponeophagia in rodents (4–6). For a detailed review of pharmacological effects on hyponeophagia, please see Dulawa and Hen (4).

Historically, various precursors of novelty-suppressed feeding have long been used to assess anxiety behavior. Hall first observed a negative correlation between feeding and defecation when animals were exposed to a novel environment in 1934 (7). Classic studies in the 1970s tested the effects of many different anxiolytics in both rats and mice (4, 8). What has become clear is that there are a few essential components of the test that, when altered, can lead to very distinct results. Both hyponeophagia and defecation attenuate with repeated exposures to the environment, suggesting that novelty is a key component of the anxiogenic paradigm. In addition, the lighting used to illuminate the arena can have large effects on anxiety. The greater the intensity of the lighting, the more anxiogenic the environment will be. Finally, when novelty-suppressed feeding is performed in mice, different inbred strains have large baseline differences in hyponeophagia (9), making strain choice critical.

In addition to usefulness in testing anxiety and the effects of anxiolytics, novelty-suppressed feeding has become a popular test for assessing antidepressant efficacy. Commonly used tests for assessing predictive validity for antidepressants, such as forced swim test or tail suspension test, require only a single acute treatment for positive effects. Therefore, from a behavioral perspective, these tests have always lacked face validity as most antidepressants require chronic treatment to yield beneficial effects in humans (10). Importantly, positive effects of antidepressants in novelty-suppressed feeding are only seen after chronic, but not acute or subchronic, treatment in rats (11) and mice (12). Chronic treatment with various antidepressants have been shown to decrease latency to eat, including selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (prozac) (12–14) and tricyclics (TCAs) such as imipramine (12–14) and desipramine (15).

Anxiety and depression have generally been conceived of as distinct psychiatric disorders, believed to be caused by alterations of different brain circuits (10). However, in reality, anxiety and depression have a high comorbidity with cooccurrence rates up to 60% (16, 17). Therefore, the use of novelty-suppressed feeding to study the effects of chronic antidepressant treatment in various mouse models and the various mechanisms mediating the antidepressant response will shed light on the neurobiology of both anxiety and depression.

One critical mechanism mediating the antidepressant response has already been uncovered using novelty-suppressed feeding as the readout. In addition to decreasing latency to eat in novelty-suppressed feeding, chronic, but not acute or subchronic, treatment of rats and most mouse strains with antidepressants results in an increase in proliferation of adult neural progenitor cells in the dentate gyrus of the hippocampus (12, 18). Interestingly, ablation of this adult neurogenesis niche with a focal radiologic procedure eliminates the antidepressant-induced decrease in latency to eat in novelty-suppressed feeding (12–14), suggesting a requirement of adult neurogenesis to mediate the beneficial effects of antidepressants. Furthermore, multiple stress-induced animal models of depression, such as unpredictable chronic mild stress (UCMS) and chronic corticosterone treatment, yield an increase in latency to eat in novelty-suppressed feeding that can be reversed by chronic antidepressant treatment (13, 14).

Another potentially interesting aspect of novelty-suppressed feeding that has not yet been fully investigated is that animals subjected to chronic antidepressant treatment tend to show a bimodal distribution where some animals show a clear decrease in latency to eat and others do not (Fig. 1). In this example, mice were given fluoxetine (18 mg/kg/day) or saline by oral gavage for 25 days. One of the major drawbacks of antidepressants in psychiatry is that many patients do not respond to treatment. As an example, only 47% of patients respond and only 33% of patients achieve remission

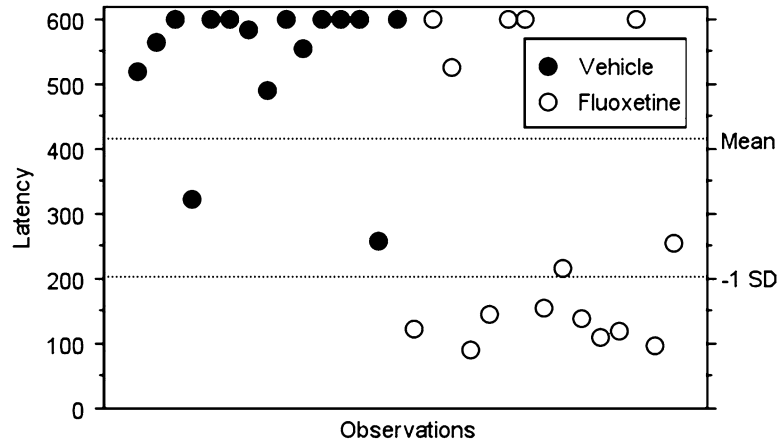

Fig. 1. A univariate scattergram showing response times of individual mouse subjects in novelty-suppressed feeding. In this test, 15 animals were chronically treated (25 days) with the antidepressant fluoxetine (18 mg/kg/day; white circles) and another 15 were treated with saline (*black circles*) by oral gavage. The test ran for 600 s (10 min). Each *circle* represents one animal, with latency to eat plotted along the *y* axis. The mean and standard deviation of all points are denoted by dashed lines. Fluoxetine significantly decreases the latency to eat in most, but not all, subjects. Note the bimodal distribution within the fluoxetine group in which five subjects are at or near the ceiling of the test. It is possible that these five subjects are models of nonresponders to antidepressant treatment.

in the first line of treatment with a commonly used SSRI (19). Therefore, it is possible that the bimodal distribution of animals in novelty-suppressed feeding following chronic antidepressants may present a model of responders and nonresponders to treatment, and animals could theoretically be separated based on their behavior to study potential mechanisms underlying this divide in responsiveness to treatment.

Finally, when performing novelty-suppressed feeding, it is critical to control for any potential effects of the independent variable of appetite on feeding behavior (4). Many classic anxiolytics, such as benzodiazepines, can stimulate appetite (4). The two most commonly used controls are therefore home cage feeding and percentage weight lost during the deprivation. Ideally, latency to eat in the home cage can also be assessed.

2. Equipment, Materials, and Setup

1. Fifteen to 20 adult mice per group. Mice can be group-housed prior to experiment. All mice should be the same gender and approximately the same age.
2. Experimental arena: Any large container can be used. We use standard mouse shipping containers, available from Taconic Farms. Containers should be cleaned prior to experiment (see Fig. 2 for a picture of animals in the testing arena).

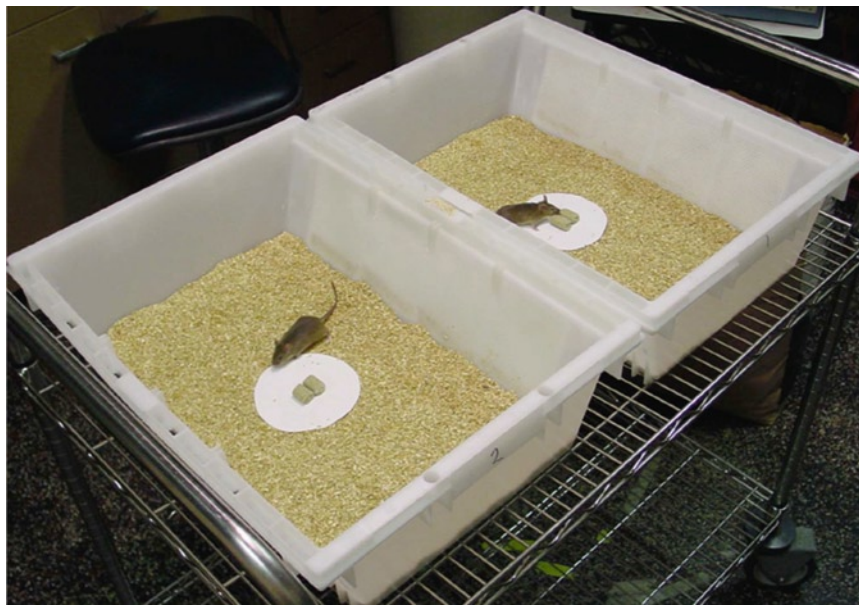

Fig. 2. Animals participating in the novelty-suppressed feeding test. Here, the testing apparatus consists of food pellets attached to a food platform and placed in the center of a large arena (here it is a mouse shipping container).

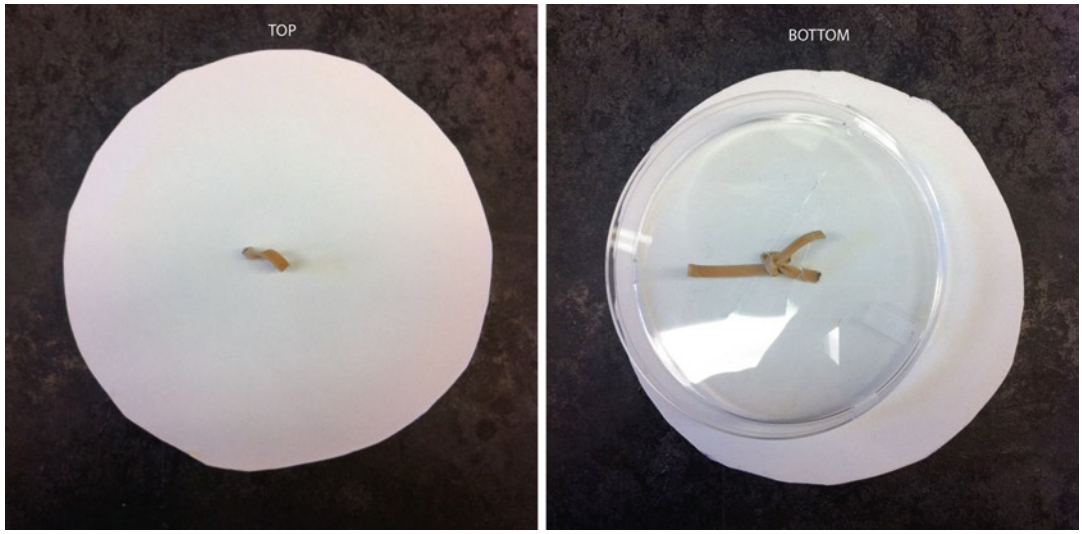

Fig. 3. The food platform. Construction of the platform is simple and requires only a few commonly found lab items. As described in the text, small holes are made in a petri dish and a circle cut from a fresh piece of white Whatman paper. A rubber band is cut, fed through the holes and tied at the bottom. This provides a small loop on the top of the platform to hold the food pellet in place. The walls of the petri dish are then used to hold the platform in place by digging into the bedding of the testing arena.

3. Traceable Daul-Range Light Meter (Luxmeter). Available from VWR International.
4. Standard bedding used in housing cages.
5. Two balances: one with minimum accuracy of 0.5 g for weighing animals, another with minimum accuracy of 0.01 g for weighing food pellets.
6. Platform for food: A petri dish with white circle cut from Whatman paper, tied together with rubber band (Fig. 3). To make the platform: Cut two small holes near the center of a petri dish bottom, approximately 0.5 in. apart. Cut a circle out of a white piece of Whatman paper, diameter approximately 4.5 in. Cut a rubber band to straighten out, then feed through both holes of Whatman paper and both holes of petri dish bottom. Whatman paper should be flush with bottom of petri dish, with dish walls facing away from Whatman paper. Insert a pellet of food into the rubber band loop on top of the Whatman paper. Tighten rubber band and tie on petri dish bottom. These platforms are reusable, but Whatman paper should be replaced regularly. These platforms are necessary to ensure animal does not transport food pellet to the side of arena.
7. Four lamps with 75–100 W bulbs to increase lighting of arena as necessary.
8. A second experimenter to record latencies that is preferably blind to treatment and/or genotype of animals.

9. Two timers and two stopwatches.
10. Clean new cages. Will need 2× new cages for the number that are being run. Therefore, if running eight cages, need 16 clean new cages and 16 lids. Will also need water bottles, food trays, and food for half of the new cages (8 in this example).
11. Plan ahead for order of animal testing and prepare sheets to record latencies, pellet weights, and animal weights (see Fig. 4 and Tables 1 and 2). Can run up to four animals at a time, so plan that groups are distributed evenly across testing. So, for example, if running 40 animals total (8 cages of 5 animals each, one vehicle group of 20 and one treatment group of 20 – see Fig. 4), each run will consist of two animals from the vehicle group and two from the treatment group. This is critical so that across groups animals have been deprived of food for equal

VEHICLE GROUP	TREATMENT GROUP
Cage 1	Cage 5
1	21
2	22
3	23
4	24
5	25
Cage 2	Cage 6
6	26
7	27
8	28
9	29
10	30
Cage 3	Cage 7
11	31
12	32
13	33
14	34
15	35
Cage 4	Cage 8
16	36
17	37
18	38
19	39
20	40

Fig. 4. Designing a new experiment. Here we are outlining the mice, cages, and treatment groups for a new experiment. In this experiment, there are five mice housed per cage and eight cages total. Four cages (cages #1–4; mice #1–20) are the vehicle group, while the other four cages (cages #5–8; mice #21–40) were given a treatment.

Table 1
The log sheet for home cage feeding for the new experiment outlined in the protocol using the mouse groups in Fig. 4

Home cage feeding

Cage	Animal	Pellet weight start	Pellet weight end	Animal weight predep	Animal weight postdep	Home cage latency
1	1					
1	2					
1	3					
1	4					
1	5					
2	6					
2	7					
2	8					
2	9					
2	10					
3	11					
3	12					
3	13					
3	14					
3	15					
4	16					
4	17					
4	8					
4	19					
4	20					
5	21					
5	22					
5	23					
5	24					
5	25					
6	26					
6	27					
6	28					

(continued)

Table 1 (continued)

Home cage feeding

Cage	Animal	Pellet weight start	Pellet weight end	Animal weight predep	Animal weight postdep	Home cage latency
6	29					
6	30					
7	31					
7	32					
7	33					
7	34					
7	35					
8	36					
8	37					
8	38					
8	39					
8	40					

periods of time. Also, it is critical that only one animal per home cage is run at a time. This is so that at the end of the novelty-suppressed feeding test, individual animals can be placed back in their home cage and food consumption over a set time period can be measured. A log sheet for this example experiment is shown in Tables 1 and 2.

12. Statistical analysis software capable of generating Kaplan–Meier curves (usually considered a type of survival analysis).

3. Procedure

Day 1:

Weigh all animals. Record weight in home cage feeding log sheet (animal weight predep). A sample log sheet is shown in Table 1.

Mark tails with sharpie for quick and easy identification on testing day. This is to avoid stressing animals during behavioral testing by checking toes or ears.

Table 2
The log sheet for novelty-suppressed feeding for the new experiment outlined in the protocol using the mouse groups in Fig. 4

Run #	Cage #	Animal #	Latency	Notes
1	1	1		
	2	6		
	5	21		
	6	26		
2	1	2		
	2	7		
	5	22		
	6	27		
3	1	3		
	2	8		
	5	23		
	6	28		
4	1	4		
	2	9		
	5	24		
	6	29		
5	1	5		
	2	10		
	5	25		
	6	30		
Change bedding in testing arenas				
6	3	11		
	4	16		
	7	31		
	8	36		
7	3	12		
	4	17		
	7	32		
	8	37		
8	3	13		
	4	18		
	7	33		
	8	38		
9	3	14		
	4	19		
	7	34		
	8	39		
10	3	15		
	4	20		
	7	35		
	8	40		

Transfer animals to new, clean home cage. If group-housed, retain all cage mates.

Add new water bottle and food tray, but do not add food. If same water bottles must be used, wipe clean to remove any food particles.

If possible, transfer to testing room for holding overnight during deprivation to acclimatize.

Determine order of testing and prepare log sheets to record latency and home cage feeding. Sample log sheets are shown in Tables 1 and 2.

Day 2:

Testing should begin approximately 24 h after start of food deprivation.

If animals were not held overnight in testing room, transfer to testing room at least 1 h prior to start of testing.

Setup for testing:

Cover bottom of arena with fresh bedding.

Attach 1–2 food pellets to food platform using the rubber band.

Place food platform in center of arena using the walls of the petri dish as a foundation to stabilize location in the bedding.

Ensure lighting is accurate for strain and experimental aim by measuring with luxmeter. To increase lighting, can place lamps with 75–100 W bulbs above arena.

Can run up to four mice at a time, all in different arenas. If running multiple animals at once, ensure even lighting across all arenas by measuring with luxmeter.

If running four mice at once, place all animals from those four home cages into new, clean holding cages with lids only to clear the home cages.

Prepare area for home cage feeding experiment by placing the original four home cages onto a table in a dimly lit area of the room. Weigh a pellet of food for each cage, record the weight (pellet weight start in Table 1), and place it on food tray for animal to have access when returned to home cage. Remove water bottles.

Test can run for 5, 8 or 10 min. In this example, we will assume 10 min.

When ready to begin testing, simultaneously place all subjects in a corner of the arena and then immediately start timer (10 min) and stopwatch simultaneously.

Ensure that the testing room is quiet during the testing period.

Animals will likely first approach the food pellet and sniff without biting. This does not count as eating.

When animal enters center, grasps food pellet with forepaws, and bites, use lap feature to pause the time on the stopwatch.

Immediately remove food platform from testing arena.

Record latency to eat in seconds (latency).

Press lap on stopwatch, which should then revert to the time from when the experiment began.

Repeat until all four animals have eaten or the timer indicates 10 min have passed.

Assign a latency of 600 s to animals that did not eat in the testing period.

Place animals into their original home cage with a single food pellet of known weight in the food tray.

Run timer #2 for 5 min.

If possible, also use stopwatch #2 to also record latency for animals to eat in the home cage. This is usually very quick, within the first 30 s. Record as home cage latency on home cage feeding log sheet (Table 1).

At the end of 5 min, weigh each animal. Record all weights (animal weight postdep in Table 1).

Weigh the food pellet from the home cage and record (pellet weight end in Table 1).

Place each animal into a new home cage with free access to food and water.

Return food pellet that had been weighed to original home cage for next run of testing (pellet weight end of cage 1 animal 1 will be the same as pellet weight start for cage 1 animal 2).

Remove any defecation from the testing arena, and shake the bedding.

When starting second run, ensure original cage mates are placed in the same testing arena.

After five runs when all animals from first set of original home cages have been run through the test, replace bedding and food pellet in testing arena. Also prepare next set of home cages for home cage feeding by transferring animals to a holding cage.

Tip:
If there are two experimenters in the room, it is possible to start the second run of testing while animals from the first run are feeding in the home cage. One experimenter will record latencies in the novelty-suppressed feeding, while the other records animal and pellet weights from the home cage feeding.

4. Data Analysis and Anticipated Results

Data Analysis for novelty-suppressed feeding can be somewhat tricky for two reasons: (1) there will usually be animals that do not eat during the test and (2) the latencies usually do not adhere to a normal distribution. Therefore, standard statistical tests such as ANOVA are inappropriate for analyzing novelty-suppressed feeding latencies. Animals that are assigned 600 s (or the ceiling of the test) are not actually eating at 600 s and thus need to be censored during the statistical analysis. Nonparametric statistics are therefore required. The Kaplan–Meier estimator is particularly useful, because it allows for censoring animals that do not eat during the test. Standard statistical programs such as StatView, SAS, and SPSS are capable of this analysis. It is often found under survival analysis in these programs. Using the same data points shown in the scattergram in Fig. 1, an example of Kaplan–Meier curves is shown in Fig. 5.

To perform statistical analysis, use only total seconds for latencies when performing the Kaplan–Meier estimator to generate a curve. Censor the animals that did not eat in the allotted time. When dealing with multiple variables (e.g., genotype and

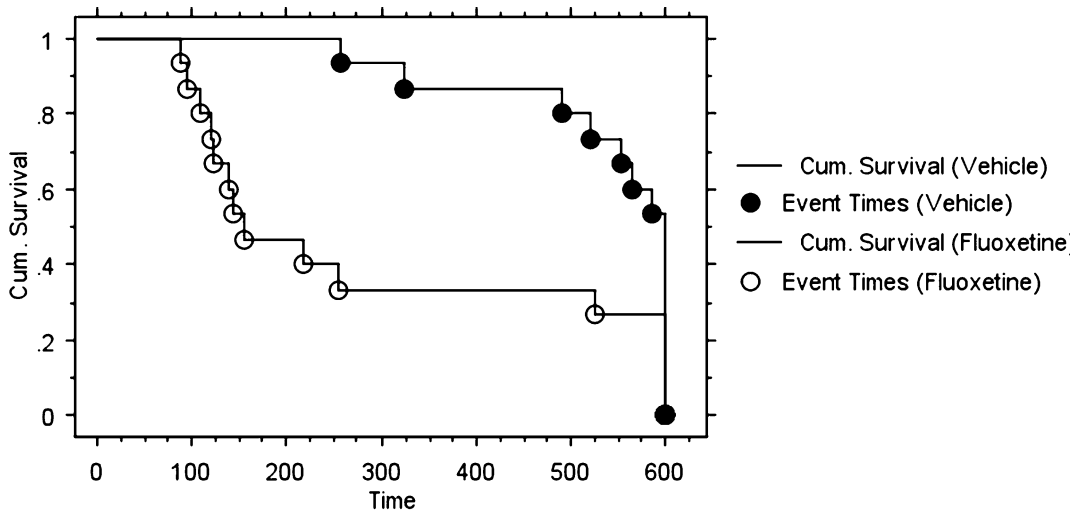

Fig. 5. Data analysis. These are the Kaplan–Meier curves for the animals given fluoxetine and for which the scattergram was shown in Fig. 1. The fluoxetine-treated animals are again denoted by *white circles* while the vehicle-treated are denoted by *black circles*. As an example of how to read this graph, looking at the responders to fluoxetine, approximately 70% ate within the first 250 s of the test (cumulative survival is at approximately 0.3 at 250 s).

Rank Tests for Latency
Censor Variable: Censor
Grouping Variable: Treatment

	Chi-Square	DF	P-Value
Logrank (Mantel-Cox)	5.554	1	.0184
Breslow-Gehan-Wilcoxon	8.238	1	.0041
Tarone-Ware	7.014	1	.0081
Peto-Peto-Wilcoxon	8.238	1	.0041
Harrington-Fleming (rho = .5)	7.014	1	.0081

Fig. 6. Statistical analysis. Multiple tests can be used to compare Kaplan–Meier curves. The most common is the logrank test (Mantel–Cox). For the data shown in Figs. 1 and 5, fluoxetine significantly decreases latency to eat in the novelty-suppressed feeding test ($p = 0.0184$).

drug treatment), group the animals with genotype as the strata and the drug treatment as the treatment (sample curves are shown in Fig. 5). The logrank test (often called Mantel–Cox) is used to compare two Kaplan–Meier curves (log rank results for the example experiment are shown in Fig. 6). For home cage feeding, subtract pellet weight at the end of home cage feeding from pellet weight at the start of home cage feeding. Divide the amount eaten (in mg) by the animal's weight. Plot the results and perform analysis of variance (ANOVA) to statistically compare different genotypes and/or treatments. For animal weight lost, subtract weight prior to deprivation from weight following the novelty-suppressed feeding test. Calculate the percentage of weight lost from the weight prior to deprivation. Most mouse strains lose about 10% of their body weight with a 24-h deprivation.

5. Experimental Variables and Troubleshooting

Several variables can lead to dramatically different results in the NSF test. Mouse strain and lighting are the most obvious variables. For mouse strains that are generally anxious, such as 129/SvEv or BALB/c, it is recommended to use relatively lower lighting (approximately 800 lux) or most animals will be on the ceiling of the test (they will not eat). Vice versa, for less anxious strains, such as C57BL/6 or CD-1, it is recommended to use relatively higher lighting (approximately 1,000 lux). For strains of mixed backgrounds, it is highly recommended that extra animals are produced

to pilot the lighting conditions. The key is to adjust the anxiogenic properties of the test so that there is a distribution of latencies. If absolutely necessary, it is possible to rerun a cohort of animals through the test, but it is highly recommended that there be a minimum of 10 days between experiments.

Potential confounds of the test are manipulations that will affect feeding behavior. This should be controlled for by comparing both the home cage feeding and weight lost across genotype and/or treatment groups, and if possible, the latency to eat in the home cage.

Though less likely to affect results than alterations in lighting, we have also had some success altering the deprivation period. This only really works if all animals are eating (on the floor of the test) after 24 h. Variations on the deprivation period to 12 h have worked. It is not recommended to increase the deprivation period. However, there are other variations on hyponeophagia that test satiated animals. One example is the novelty-induced hypophagia test (4), in which mice are trained over 3 days to drink sweetened condensed milk. On the fourth day, mice are then presented the sweet milk in a highly anxiogenic environment.

Additionally, an important aspect of the test is the addition of bedding to the arena. The bedding in the testing arena should be the same type of bedding as in the animal's home cage. The bedding is important as it makes the testing arena less aversive around the outside and enhances the contrast with the platform holding the food pellet. Using bedding that is different from the animal's home cage will increase the anxiogenic properties of the test (it will increase latency to eat). Though less common than altering the lighting, using different bedding is also another way to affect results and can be useful if all animals are eating (on the floor of the test) after 24 h.

It is also commonly asked whether the test can be automated. Unfortunately, this has not been sufficiently worked out yet. It is certainly possible to videotape animals participating in the test and measure latency to eat by watching the videos at a later time, but standard video tracking systems have not proved sufficient because they only track an animal's location, not the actual behavior. Animals participating in this test will often enter the center of the arena and sniff the food pellet and then quickly run away, but will not actually take a bite of the food until several minutes later. Currently, standard video tracking software cannot differentiate the sniffing of the food from the actual biting, as in both instances the animal enters the center of the arena and remains for a brief period of time. One way to increase throughput may be to videotape animals in conjunction with video tracking software, and then watch the timepoints when the animal has approached the pellet.

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