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Acclimatization Plan for Rats – Daily Protocol (3 Weeks)

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We use this protocol and it's working

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Abstract

We developed a 3-week acclimatization protocol combining gradual habituation, environmental enrichment. The protocol improves data quality and animal welfare and is compatible with randomized, blinded, and transparent study designs.



Introduction

- 1 General Considerations for a 3-Week Acclimatization Period
 - Purpose: Ensure animals adapt to new housing, handling, and study-related procedures.
 - Regulatory Compliance: Follow FELASA, ARRIVE guidelines, and institutional animal welfare regulations.
 - Environmental Familiarization: Gradual exposure to handling, noise levels, and experimental procedures to reduce stress responses.



Week 1 – Basic Adaptation & Health Monitoring

- 2 Goal: Allow rats to adapt to their new environment, minimize transport stress.



A	B	C
Day 1	Arrival & Housing	• Group housing (7–8 per cage) with bedding, nesting material, tunnels. • Health check: fur, eyes, activity, hydration, respiration. • Introduce minimal human contact by keeping hands in the cage, no active engagement, let rats approach. • Offer peanuts as a treat in the end. • Observe social behavior, document findings.
Day 2	Behavioral Observation	• Observed social behavior, aggression, grooming, and activity levels. • Brief (10–15 sec) cupping with rats showing interest in human interaction. • Treat: One teaspoon of oats offered per cage.



A	B	C
Day 3	Behavioral Observation	• Performed partial bedding changes in preparation for the weekend. • Brief (10–15 sec) cupping and rat tickling with two hands. • Each rat was transferred to a new cage after tickling. • Treat: Peanuts offered at the end.
Day 4	Behavioral Observation	• Increased handling duration to ~1 minute per rat. • Minimal restraint to allow voluntary interaction.
Day 5	Behavioral Observation	
Day 6	Behavioral Observation	• Increased handling duration to 1–2 minutes per rat, including cup cuddling and tickling. • Treat: One teaspoon of oats per cage.



A	B	C
Day 7	Behavioral Observation	- Planned exposure to routine facility sounds (e.g., door opening, cage transport on roller cargo, equipment beeping). - Observed activity levels, weight stability, and signs of stress

Week 2 – Handling and Refinement

- 3 **Goal:** Familiarize rats with handling, introduce a homemade small playground for social play in a larger group, and begin non-invasive procedure acclimatization (weight measurement, exposure to anesthesia induction box without anesthesia).

Day 8	Extended Handling & Social Play	Increase handling duration to 2–3 min per rat. Introduce rats to the homemade small playground (allowing larger group interaction outside cages). Observe social interactions.	
Day 9	Weight Measurement Introduction	Place each rat briefly on a digital scale, allowing voluntary exploration. Reward calm behavior with treats.	
Day 10	Acclimatization to Anesthesia Induction Box	Introduce the anesthesia induction box without administering anesthesia. Allow exploration inside to reduce stress for future procedures.	
Day 11	Handling & Second Playground Session	Repeat extended handling (2–3 min per rat), followed by another social play session in the larger playground. Observe interactions.	



	Da y 12	Rest Day	No handling, only routine health monitoring.
	Da y 13	Handling Refinement & Enrichment	Increase handling time if tolerated, continue interaction training. Introduce a new enrichment object.
	Da y 14	Weight Training & Repeat Box Acclimation	Repeat weight measurement, ensuring rats voluntarily enter the scale. Second exposure to the anesthesia induction box .

Week 3 – Experimental Procedure Acclimatization

- 4 **Goal:** Familiarize rats with noise exposure to the echocardiography room, metabolic cage acclimation, and final health monitoring (blood sample collection on the last day).

	Da y 15	Noise Acclimatization – Echocardiography Room	Expose rats to the echocardiography room sounds (machine beeping, probe setup, researcher presence) for 10–15 min. Observe stress responses.
	Da y 16	Metabolic Cage Acclimation (Short Exposure)	Place rats in metabolic cages for 30 min . Observe behaviors, assess stress levels.
	Da y 17	Second Noise Acclimatization & Handling	Repeat echocardiography room exposure , increasing duration. Continue handling.
	Da y 18	Extended Metabolic Cage Exposure	Increase time in metabolic cages to 1 hour . Monitor food/water intake and activity.
	Da y 19	Rest Day	No handling, only routine health checks.
	Da y 20	Final Echocardiography Room Visit	Ensure all rats tolerate the environment well. Observe behavior.
	Da y 21	Final Monitoring & Blood Sample Collection	Weigh all rats and collect blood samples for baseline assessment before the study begins.

Protocol references

Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, et al. (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLOS Biology 18(7): e3000411.