
Enforced Restraint of Rodents: A Discussion by the Refinement & Enrichment Forum

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"Rodents are often restrained for data collection procedures, such as blood collection and injection, by coaxing them into tubes, for example syringe cylinders or perspex tubes. Such enforced restraint/immobilization presumably exposes the animal subject to considerable stress. Published reports show that stress-sensitive parameters respond significantly to enforced restraint, suggesting that the subject experiences intense fear.¹⁻⁵ There is some published evidence that the animals do not fully habituate to this stressor.⁶⁻⁸ What can be done to buffer the stress response of rodents to enforced restraint in tubes?" (Reinhardt)

"We had a large group of visiting scientists who used tube-restraint as classical stressor for experimental purposes in their research facility. When they saw us working with our rats they could not believe their eyes: our rats were quite happy to crawl into the tubes, go to sleep and show no apparent signs that they had become stressed by the procedure. It may well be that our rats were particularly good natured and laid back and/or were so well habituated to being 30-minute tube-restrained that they calmly accepted the situation. I should perhaps add, that being restrained did not imply any additional procedure for the animals." (Francis).

"I endorse Roger's remarks. We find that rats, mice and guinea pigs will enter restraint tubes quite happily, provided we are patient and gentle-and-firm the first couple of times when we prompt the animals to crawl into a tube. The initial experience associated with the tube is probably the determining factor of the restrained subject's response during subsequent restraint sessions. We encourage our researchers to handle their animals daily during the week prior to the study. On these occasions they will also tube-restrain their animals. This preliminary routine assures that the subjects will be familiar with the researcher and well acclimatized to the tube already at the beginning of the study. I think this provides a good condition for the animals to experience little or no restraint-related stress during the experiment.

We use ordinary transparent restraining tubes that we either cover with a paper towel, or we use opaque tubes made of red Perspex so that they become a dark, 'safe' hiding place. With a bit of initial 'training' rodents do enter such tubes without appearing to be stressed, and as Roger already pointed out, they will fall asleep given half the chance if being restrained for a few minutes or half an hour." (Barley)

"It may well be that the animals fall asleep in the tubes as long as no hands-on procedure is done with them, but how do you actually know that they sleep? It is my experience as clinical vet that animals are often very stoical, even though you know from clinical evidence that they are experiencing a lot of pain. This, actually, can make the work of a vet quite a challenge because the animal gives 'wrong' signals. Could it not be that a rat or a mouse who does not appear to be stressed while being immobilized in the tube, is in a state of anxiety and fear rather than sleeping? Telemetrically assessed cardiovascular stress parameters would probably be the best answer to this question." (Reinhardt)

"Yes, it is difficult to know for sure if the animals actually sleep. But I can say that they do give the impression of being relaxed even though we take tail-cuff blood pressure readings or withdraw blood from previously implanted cannulae at various time points. When I take the animals out of the tube after a procedure, they do not appear to be agitated and disturbed but usually resume their routine business such as exploring the environment and grooming themselves. I really believe that gently habituated rodents do not experience undue stress or any stress at all while they are restrained in dark tubes during noninvasive procedures. I should perhaps emphasize that it is very important that the animals do not get too hot while they are restrained in the tubes. They become hyperthermic easily in the tubes and this seems to stress them, especially mice. In this condition the animals come out of the tubes in a state that I can only describe as 'prostrated' reluctant to move, panting, semi-conscious, damp or moist. This must be avoided, and it can be avoided by keeping the animals in the restrainer for only short periods at a time." (Barley)

"As project for my Master in Laboratory Animal Science I restrained rats three hours daily, from 9 am to 12 am, for ten consecutive days in a plastic tube and a Bollmann metal cage. I measured blood pressure, heart rate and body temperature by telemetry when the animals were restrained and when they were unrestrained in their home cages the day before and the day after the ten restraint periods. There were no differences between the two restraint methods, but cardiovascular stress responses were significantly increased approximately 15% with consistency on all ten days while the rats were restrained. The first hour of restraint was probably the most stressful period for the animals as cardiovascular responses were highest during that time approximately 30% increase. At the end of the repeated, three-hour restraint blood pressure and heart rate had decreased, but values were still significantly elevated compared to non-restraint, baseline values." (Mikkelsen)

"That's really interesting. We also measure blood pressure telemetrically in tube-restrained rats but have to raise the room temperature to get the animals stressed enough for the blood pressure to increase. We are working with outbred Wistar and Sprague Dawleys who are fairly laid back and, therefore, perhaps less sensitive to this kind of stress." (Barley) "Well, I also worked with Sprague Dawleys, which suggests that the strain of the rats does not necessarily determine the cardiovascular stress response to enforced restraint." (Mikkelsen)

"Would you recommend to always keep the restraint tube-environment dark (opaque tube material, tube covered with paper), or has your experience shown that it does not make a noticeable difference whether a rodent is restrained in a transparent tube or a dark tube?" (Reinhardt)

"We use the typical plastic tube restrainers for short term restraint of rats during tail bleeding. I always cover the tube with a surgical drape to darken it so that the animals might feel more secure. After all, their natural instinct is to seek a dark shelter in the event of danger." (Johnston)

"It is my experience that if the tubes are red Perspex or covered with paper the rodents seem to be and remain relaxed throughout the procedure, even if I am doing venipuncture which must cause some discomfort despite the use of topical analgesics. When the tubes are transparent and uncovered, the animals will often wriggle about in what I presume is an attempt to get out our tubes open at both ends so the animals don't have to come out backwards unless they want to!"

Since odor/scent of a stranger adhering to the tube is likely to induce a negative reaction in rodents, we thoroughly rinse the tubes between cages. We did notice that the animals are more reluctant to crawl into the tube and tend to be restless whilst in the tube when we skipped the rinsing after animals from a different cage had been restrained in the same tube. For PK studies we always use one and the same tube per animal so that each subject is restrained always in the same tube." (Barley)

"In summary, enforced restraint is not necessarily an intrinsic stressor for rodents. If an animal has been well familiarized with the handling personnel and with the restraint tube, the tube kept dark and the duration of the restraint session kept short enough to forestall overheating, behavioral signs of stress can be avoided. It needs to be demonstrated if physiological stress parameters reflect baseline values in animals who seem to accept tube-restraint." (Reinhardt)

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Published in *Animal Technology and Welfare* 6 (1), 11-13 (April 2007).

