

PROTOCOL FOR DECAPITATION WITHOUT ANESTHESIA

This procedure applies to rodents only (rats and mice), 15 days of age or greater.

Rationale and Justification:

When conducting biochemical analyses in rodents, it is sometimes necessary to examine samples of the tissues and fluids (organs, blood, cells, etc.) in the absence of anesthetics, analgesics, or other drugs that would modify the biochemical activity of these tissues and biological fluids. These drugs would contaminate the samples, and obscure the effects of the experimental manipulations. See for example, papers by Carney and Walker (1973), Barna et al. (1993), Carlberg et al. (1995), and Illera et al. (2000). When these circumstances arise, the most rapid and efficient method for terminating the life of the rodent and collecting the samples is decapitation. This method provides only momentary distress to the animal. It is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association, and it is conditionally acceptable in a laboratory setting when performed by trained professionals.

The justification to avoid the use of anesthetics or analgesics must be scientifically justified on an experiment-by-experiment basis.

Training Requirements:

Any researcher who conducts live decapitation without anesthetics or analgesics must be trained and authorized by an IACUC-approved trainer. This IACUC –approved trainer must demonstrate competence to a designated member of the veterinarian staff of ACS in order to receive approval from the IACUC. A department or unit may have an IACUC-approved trainer, failing which; the researcher must be trained and authorized by an IACUC-designated veterinarian. Personnel training will consist of the following steps:

- 1) The trainer will demonstrate the decapitation procedure to one or more researchers.
- 2) The researcher(s) will (each) practice the procedure on anesthetized or dead rodents until proficient. The trainer will be present for each of these practice decapitations.
- 3) The researcher will then perform a live decapitation under the supervision of the trainer. This will be repeated (including additional anesthetized/dead decapitations, at the discretion of the trainer) until the researcher demonstrates proficiency.
- 4) Proficiency will be determined by the trainer, and will be based upon one or more demonstrations that the researcher conducts the decapitation quickly and smoothly, without any overt signs of distress in the animal.
- 5) If animals are required for training, the Principal Investigator will request those animals on the relevant protocol.
- 6) Upon completion of training / demonstration of proficiency, the trainer will document the proficiency on the certificate of completion of training. A copy of the certificate of completion will be kept in the IACUC office (see attached certificate).

Researchers who are approved to perform live decapitations must be listed on the appropriate IACUC protocol.

Decapitation Procedure:

- 1) Each decapitation will be performed in a room that is isolated from all other rodents.
- 2) The guillotine will be placed upon a clean and stable benchtop or other stable surface, and the sharpness and smooth operation of the guillotine will be verified before introducing any rodent.
- 3) The rodent will be removed from its home cage, or experimental environment, and carried to the guillotine. The researcher will make every effort to adjust the transport of the rodent until it appears calm (note that the affective state of the animal may be determined by the experimental conditions). Although not required, use of de-capi-cones is suggested.
- 4) The researcher will hold the rodent securely, and place the rodent on the stage at the entrance to the guillotine.
- 5) In most cases the rodent will move its head forward. Whether this occurs or not, the researcher will gently, but assertively push forward until the rodent's head is securely in the guillotine apparatus.
- 6) When the head is in position, the researcher will pause momentarily and verify the head is completely through the opening of the guillotine, and the researcher's hand is clear of the blade path. Then, the researcher will smoothly and quickly depress the guillotine lever, decapitating the rodent.

Care of the Guillotine:

- 1) After each decapitation, the researcher will rinse and/or wipe down the guillotine and surrounding area to remove all blood and tissues.
- 2) At the end of each day of use, the researcher will thoroughly wash the guillotine with detergent and water, and dry it. After drying, the researcher will oil the moving parts with light machine oil (e.g. 3-in-1 oil), and run the blade up and down several times to spread the oil.
- 3) The researcher will sharpen or replace the blades whenever they are dull. Sharpening may be performed by passing a flat sharpening stone or file that is suitable for knife sharpening across the bevel of the blades a few times until they are sharp to the touch. Never hone the flat side of either blade because it will destroy the shear by opening up a space between the top and bottom blades.

Reminders:

- DO NOT** perform this procedure unless properly trained and authorized.
- DO NOT** perform this procedure unless approved as part of an IACUC protocol.
- DO NOT** depress the guillotine lever unless the rodent's head is fully engaged in the guillotine.
- DO NOT** depress the guillotine lever unless the rodent's head is immobile.
- DO NOT** depress the guillotine lever unless your fingers are out the way.
- DO NOT** depress the guillotine lever unless confident that the rodent's head will be removed in one clean stroke.
- DO NOT** allow any distractions in the room during this procedure.

References:

- Carney, J.A. and Walker, B.L. (1973). Mode of killing and plasma corticosterone concentrations in the rat, *Lab. Anim. Sci.* **23**: 675-676.
- Barna, I., Acs, Z., and Koenig, J.I. (1993). Effects of Hypnorm (fentanyl) on ACTH/beta-endorphin levels in plasma, pituitary and brain of 10-day old rats, *Life Sci.* **52**: 1417-1424.
- Carlberg, K.A., Gwosdow, A.R., and Alvin, B.L. (1995). Effects of anesthesia with halothane and methoxyflurane on plasma corticosterone concentration in rats at rest and after exercise, *Lab. Anim. Sci.* **45**: 584-587.
- Illera, J.C., Gonzalez, G.A., Silvan, G., and Illera, M. (2000). The effects of different anaesthetic treatments on the adreno-cortical functions and glucose levels in NZW rabbits, *J. Physiol. Biochem.* **56**: 329-336.
- Report of the AVMA Panel on Euthanasia. (2001). *J. Am. Vet. Med. Assoc.* **218 (5)**: 669-696.

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SUBJECT: Documentation of Training

This memorandum serves to document that INSERT NAME OF RESEARCHER has been formally trained by the undersigned and has demonstrated proficiency at performing decapitation of MICE/RATS without anesthesia. The undersigned is authorized by the University of Florida IACUC to conduct this training and to certify individual proficiency.

INSERT NAME OF TRAINER

NAME OF TRAINER

INSERT DATE TRAINING COMPLETED

DATE TRAINING COMPLETED

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