**VA IACUC Training Exercise #1 – 2019 (Which Category – Part 1)**



The following exercise may be useful in stimulating discussion regarding compliance with PHS Policy and VA Handbook 1200.07. To facilitate discussion, pages 1 and 2 of the exercise may be distributed to the IACUC members prior to a meeting. After a few minutes of discussion about the exercise during the meeting, the remainder of the exercise may be distributed to provide ideas for the committee’s consideration.

Dr. Patrick O’Keefe, a physician-scientist, studies the relationship between sleep and memory/cognitive function in guinea pigs at the Hometown VAMC. He and a number of his colleagues prefer guinea pigs over mice or rats for neurobehavioral studies because:

* “Guinea pig brains are a miniature copy of the human brain at many levels including a complete Circle of Willis” (Lee et al 2011).
* Guinea pigs like humans are born “precocial” which means they are physically well-developed, have open eyes and ears, and have relatively advanced brain development (Lee et al 2011).
* “Many behaviors overlap between mice, rats, and guinea pigs, which facilitates scoring of guinea pig behavior during standard mouse or rat assays” (Sferrazza 2018).

Dr. O’Keefe has two new protocols that he plans to submit to the IACUC office for review so he scheduled an appointment for veterinary consultation with Dr. Diaz, the Attending Veterinarian. In advance of their meeting, he sent drafts of his protocols (see the summaries provided below):

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| --- | --- | --- |
| Protocol # | Summary | Pain & Distress category |
| 91-2019 | This study will investigate if acute sleep deprivation impairs transient memory and learning. Forty female\* guinea pigs will be divided into two groups – sleep deprived or controls; the animals will be group housed. The sleep deprived guinea pigs will be kept awake for six hours by handling during the light cycle; while the control guinea pigs will be allowed to sleep undisturbed. Both groups of guinea pigs will initially undergo novel object recognition (NOR) and Y-maze testing to assess memory and cognitive function. Testing will be performed again after sleep deprivation to determine its impact on the guinea pigs’ performance in the NOR and Y-maze tests.\*Same experiment will also be conducted with male guinea pigs to determine if there are gender differences. |  C |
| 92-2019 | This study will determine if a brief period of sleep interruption, 30 seconds of sleep interruption caused by tapping on the side of the cage, every 3 hours) during the 12 hour light phase will affect NOR recognition and Y-maze testing. Forty female\* guinea pigs will be divided into two groups – sleep deprived or controls; the animals will be group housed. Control animals will undergo the same procedures with the exception that no sleep disturbances will occur. All groups of guinea pigs will initially undergo NOR and Y-maze testing to assess memory and cognitive function, followed by MRI of the head; these procedures will be repeated after sleep interruption. Guinea pigs are easily startled by loud noises so Dr. O’Keefe trained the guinea pigs using food rewards to be quiet and still in small bed-like devices that hold their heads in place during MRI imaging. Training to be still during imaging accomplishes two goals; the guinea pigs were relaxed as well as immobile and there was no need for sedation.\*Same experiment will also be conducted with male guinea pigs to determine if there are gender differences. | C |

When Dr. O’Keefe arrived for the veterinary consultation, Dr. Diaz asked why Dr. O’Keefe thought the guineas used in ACORP 91-2019 (light phase six-hour sleep deprivation) should be assigned to category C. He confidently explained that the six-hour sleep deprivation was relatively brief, was not repeated, and no clinical signs of stress were apparent. Guinea pigs that had undergone six hours of sleep deprivation were expected to be indistinguishable from control guinea pigs in terms of food and water consumption, body weight, and general behavior. The two neurobehavioral tests he selected to detect changes in short term memory and cognition were not stressful to guinea pigs (Machatschke et al 2011) unlike the Morris Water Maze and were also short in duration. Similarly, he thought ACORP 92-2019 was even more benign because the guineas were allowed to sleep a full 12 hours but were only very briefly interrupted four times during the light phase. The sleep interrupted guinea pigs were also grossly indistinguishable from the control guinea pigs.

Do you agree with Dr. O’Keefe that the guinea pigs used in both of his protocols should be assigned to category C?

After Dr. O’Keefe had presented his justification for assigning the guinea pigs used in the six-hour sleep deprivation (protocol 91-2019) to category C, Dr. Diaz showed him a paper that stated “When comparing “mild” stimulation, such as tapping on the cage, to “stressful” direct handling of the animals at 30 min intervals, it was found that corticosterone levels in the former group were comparable to those of control animals, but were three times as high in handled animals” (Kopp et al., 2006). If sleep deprivation involving handling to keep the animals awake was stressful as indicated by three times higher corticosterone levels, clearly category C was not appropriate. Next, Dr. Diaz asked if Dr. O’Keefe could give an anxiolytic drug to make the guinea pigs less anxious and presumably lower their corticosterone response. As expected, Dr. O’Keefe replied that he could not use diazepam or similar drugs because they are associated with sedation and memory impairment (Juárez-Portilla et al., 2017). He went on say, I can’t use antidepressants because they may improve cognitive function and NSAIDS may indirectly have a negative effect on cognition (Juárez-Portilla et al., 2017). Dr. Diaz was of the opinion the guineas used in ACORP 91-2019 should be assigned to Category E because drugs could not be administered to relieve distress without adversely affecting the procedures, results, or interpretation.

In regard to protocol 92-2019 where the guinea pigs experienced mild but brief intermittent sleep interruption due to tapping on the side of the cage every 3 hours during the 12-hour light, she agreed that these sleep interrupted guinea pigs could be assigned to category C for the following reasons:

* sleep deprivation per say did not occur.
* corticosterone levels had been shown to be comparable in the intermittent sleep interrupted animals kept awake by cage tapping and the control animals.
* guinea pigs were acclimated to the MRI and even took naps inside the MRI.

Which pain and distress category is appropriate depends on the application of USDA-AWAR Section 2.36 descriptions of each category:

* Category C – “animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain relieving drugs”
* Category D – “animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, of tranquilizing drugs were used”
* Category E – “animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretations of the teaching, research, experimentation, surgery, or tests.”

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