

## Attachment 4

## Column E Explanation Form for Regulated Species

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 72-R-0003
2. Number of animals used under Column E conditions in this study. 27
3. Species (common name) of animals used in this study. New Zealand White Rabbits
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbits are used in this study focusing on enhancing regeneration of corneal nerves damaged after corneal surgery. In this study rabbits undergo an 8 mm corneal dissection to sever sensory nerves. The surgery is performed under ketamine/ xylazine, proparacaine anesthesia. Moxifloxacin ophthalmic drops are given after surgery for infection prophylaxis. Rabbits are used for because they are a good model for corneal wound healing studies and the principal investigator has extensive experience with this model.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Ketamine/ xylazine and proparacaine are used intra-op for anesthesia and analgesia. Post op analgesics cannot be used because they will interfere with the action of the experimental drug in wound healing and nerve regeneration. The animals are checked twice daily and are also closely observed by the clinical veterinarian. Standard veterinary nursing care is provided, and if infection or excessive pain occurs the animal is treated or euthanized if necessary.

LSU Health Sciences Center New Orleans

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1. Registration Number:
2. Number of animals used under Column E conditions in this study. 2
3. Species (common name) of animals used in this study: Rhesus Macaque
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rhesus macaques are used because their brains are similar to humans and have been shown to express depressive like behavior to different stimuli. The animal is implanted with brain electrodes to study a potential treatment for depression. Brain electrodes are placed under ketamine/xylazine and isoflurane anesthesia. Analgesia is produced with Buprenorphine, and bupivacaine. The animals receive buprenorphine and for 3 days after the procedure. Every effort is taken to ensure that unrelieved pain doesn't occur as a result of the surgeries.

Distress is produced in cycles where the electrodes are turned on then off for stimulating targets in the brain and producing behavioral changes similar to depression. During these ON-stimulation (when electrodes are turned on, thus actively stimulating targets of the brain) cycles the animals experience a depressive state and in their stress exhibit huddling and certain vocalizations. Neither of these behaviors, however, will cause physical harm to the subjects and the stimulation is not painful in any way. The ON stimulations are applied once a day for 3-4 hours for two weeks and then the stimulations site is changed and the stimulation is performed for 2 weeks. During OFF-stimulation (when electrodes are turned off, with no stimulation) the animal subjects do not experience any pain or new distress.

5. Provide scientific justification why pain and/or distress could not be relieved.  
State methods or means used to determine that pain and/or distress relief would interfere with test results.

To be able to measure the occurrence, intensity, and frequency of depressive behavior, animal subjects cannot be given anything beyond what is considered standard for primate housing and environmental enrichment to alleviate depression. The ability to induce depression with deep brain stimulation is one of the specific aims of this study thus to try to relieve any produced depression (with medication) would directly interfere with test results.

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