1) Registration Number: 23-R-0016

2) Species (common name) used in study: Rabbit

3) Number of animals used in this study: 10

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

Protocol # 1

Justification for Category E designation from Protocol/ PI: (type or copy here):
Occlusion splints have been studied in both rats and rabbits to determine the effect of malocclusion on such things as the gross and histological appearance of the mandibular condyle, muscle activity, and indicators of pain. Using similar methods, metal bite-raising splints will be attached on the lower molar teeth on one side of the jaw. As per published methods, the rabbits will be anaesthetized by intramuscular injection of ketamine chloride (20 mg/kg) and xylazine (2 mg/kg).

Since pain will be an outcome measurement, it will not be controlled with anesthetics or analgesics. The purpose of this study is to identify mechanisms underlying pain associated with temporomandibular joint disorder. In order to study pain associated with this disorder, it is necessary to recreate the disorder. We have proposed to do so with 3 different approaches, all of which are believed to be associated with the initiation of inflammation in the joint and the subsequent break-down of joint tissue.

Analgesics will not be administered to relieve discomfort associated with the induction of inflammation in this study phase of the study. This decision is based on what is known about the impact of all major classes of analgesic, anti-inflammatory and anesthetics on both the inflammatory response and the nervous system. Opioid based compounds are the most commonly used analgesics for moderate to severe pain. Use of these compounds in our study is contraindicated because activation of opioid receptors in both the peripheral (Aley, Green et al. 1995) and central (Lim, Wang et al. 2005) nervous system, that are necessary for the analgesic effect, may produce long lasting changes in neuronal function. More problematic is the fact that opioids may influence the inflammatory response (Green and Levine 1992; Shakhanbeh and Lynn 1993), thereby masking the impact of inflammation on the nervous system. Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin or ibuprofen are useful for relief from mild to moderate pain, but as the name implies, these compounds are also very effective inhibitors of inflammatory responses (Vane 1971; Miller 2006; Morris, Stables et al. 2006) and are, therefore, subject to the same problems as those associated with opioids. Steroids may also be employed for treatment of more severe and persistent inflammatory responses (Cameron 2005), and are reported to produce pain relief. However, these compounds are even more effective at suppressing inflammatory responses than NSAIDs, and are, therefore, also contraindicated for use in the present study. General anesthetics are used to prevent pain, but because we are interested in the effects of persistent inflammation, developing over
several days, it would not be feasible to employ general anesthetics in the study we have proposed. Finally, local anesthetics can be used for pain relief. There are relatively long acting local anesthetics, like bupivacaine, which can provide up to 6 hours of anesthesia. Even bupivacaine, however, would require multiple injections increasing the risk of local toxicity and nerve damage {Mather, Copeland et al. 2005}. Alternatively, local anesthetics, in combination with GABA-B agonists (baclofen) and α2-adrenergic agonists (clonidine) are administered spinally for pain relief {Brogan 2006} and rats, could, in theory, be fitted with chronic catheters and osmotic pumps to provide continuous infusions of spinal anesthetics. Unfortunately, given increasing evidence of a critical role for spinal circuitry in mediating the response to inflammation {Willis 1999}, even these spinal interventions would be contraindicated for the present study. This is particularly true for the present study given the focus on inflammation-induced changes in spinal circuitry.

In short, because administration of analgesic and/or anti-inflammatory may mask changes in the nervous system that develop in the presence of persistent inflammation, these interventions will not be employed in the present study.
1) Registration Number: 23-R-0016

2) Species (common name) used in study: Rhesus Macaque

3) Number of animals used in this study: 3

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

Protocol # 2

Vestibular labyrinthectomy surgery is performed. Once the animal regains consciousness it is expected that the animal’s balance and head stability will be compromised. This may cause distress in the animal, but the animal is expected to compensate for the balance loss by the next day.

Justification for Category E designation from Protocol/ PI: (type or copy here):

Anesthetics will be employed during every surgery, and analgesia will be delivered after every surgery. Nonetheless, deep sedation would be required to assure that animals are not distressed by the postural instability and spatial disorientation that they experience immediately following removal of vestibular inputs. Such level of analgesia would be impractical because it would impact the behavioral responses we seek to characterize the time course of rehabilitation following a vestibular lesion. In fact, human literature indicates that recovery is delayed in patients that remain sedentary following a vestibular lesion surgery. Thus, it is possible that continued analgesic treatment would prolong the period of distress because it will extend the duration of recovery from vestibular deficits.
1) Registration Number: 23-R-0016

2) Species (common name) used in study:
   Musk shrew

3) Number of animals used in this study:
   32

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

   Protocol # 3

   Justification for Category E designation from Protocol/ PI: (type or copy here):

   **Class E: includes animals used in behavioral testing after emetic treatments and restraint stress**

   The purpose of these studies is to determine the neurobiological pathways responsible for nausea and emesis, produced by cancer chemotherapy. The present Class E studies involve measurement emesis, feeding and stress responses. In order to activate emesis and other related neural circuits, e.g., anorexia, animals were injected with chemotherapy agents or gut toxins. Restraint stress (up to 120 min; a common stressor used in rodent studies) is used to determine the effects of distress on emesis, which is a major problem for cancer patients.
1) Registration Number: 23-R-0016

2) Species (common name) used in study:
   Musk shrew

3) Number of animals used in this study:
   11

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

   Protocol # 4

   Justification for Category E designation from Protocol/ PI: (type or copy here):

   Animals under category E will experience brief electric shock (via the test chamber floor).

   The purpose of these studies is to develop a model of stress in the musk shrew so that the relationship between stress and vomiting can be tested in future experiments.
1) Registration Number: 23-R-0016

2) Species (common name) used in study:
   Ferret

3) Number of animals used in this study:
   24

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

Protocol # 5

Justification for Category E designation from Protocol/ PI: (type or copy here):

This study is the first necessary step to establish an in vivo postoperative vomiting model. This model will enable the development of new anti-emetic therapy strategies for postoperative nausea and vomiting in the clinic. The present study is a step towards this goal and is designed to examine the influence of morphine and isoflurane on the emetic response and identify the optimal combination necessary to induce a reliable emetic response in the ferret.

We cannot use anesthetics, analgesics, sedative, or tranquilizers to alleviate emesis because this is the endpoint of the study. All of these drugs would affect the primary experimental measure, emesis, and would make the experimental result impossible to interpret.

The ferret is used as the gold standard in developing anti-emetics by the pharmaceutical industry. It also has a reported vomiting response to morphine treatment.

References:


1) Registration Number: 23-R-0016

2) Species (common name) used in study: Rabbits

3) Number of animals used in this study: 28

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

Protocol # 6

Justification for Category E designation from Protocol/ PI: (type or copy here):
The goals of this project are to establish rabbits as a model for aerosol exposure to Francisella tularensis and to determine the protection conferred by attenuated mutants of a virulent strain.

Chemical compounds that alleviate pain or distress can mask or alter clinical signs of infection; it is important to the interpretation of the data to know if animals become sick after challenge. These same chemical compounds can also dampen the immune response to infection, particularly inflammation, which could alter the outcome of disease. One of the goals of this study is to evaluate the ability of potential vaccine candidates to protect against aerosol exposure to Francisella tularensis. Animals will be closely monitored for development and progression of clinical signs of illness using a scoresheet to track fever, weight loss and changes in appearance and behavior. Animals that become moribund will be euthanized promptly to prevent further pain and distress. We are evaluating other clinical signs (anorexia, dehydration, pulse oximetry) to determine their utility in setting experimental endpoints.
1) Registration Number: 23-R-0016

2) Species (common name) used in study:
   Ferrets

3) Number of animals used in this study:
   17

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

   Protocol #7

   Justification for Category E designation from Protocol/PI: (type or copy here):

   Unvaccinated control animals may be infected with influenza viruses. The use of analgesics would interfere with the immune response elicited by the vaccine and virus infection.

   Opioids can produce several well known adverse events, and, as has recently been recognized, can interfere with the immune response. The immunomodulatory activities of morphine have been characterized in animal and human studies. Morphine can decrease the effectiveness of several functions of both natural and adaptive immunity, and significantly reduces cellular immunity (Palliative Medicine, Vol. 20, No. 8 suppl, 9-15 (2006).

   In addition to that common adverse drug reactions associated with the use of buprenorphine are similar to those of other opioids and include: nausea and vomiting, drowsiness, dizziness, headache, itch, dry mouth, miosis, orthostatic hypotension, urinary retention. Hepatic necrosis and hepatitis with jaundice have been reported with the use of buprenorphine.
1) Registration Number: 23-R-0016

2) Species (common name) used in study:
   Ferrets

3) Number of animals used in this study:
   131

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

   Protocol # 8

   Justification for Category E designation from Protocol/ PI: (type or copy here):
   Unvaccinated control animals may be infected with influenza viruses

   The use of analgesics would interfere with the immune response elicited by the vaccine and virus infection.
   Unvaccinated animals that are infected will lose weight and display clinical signs. We will not use analgesics, etc. due to: Non-steroidal, anti-inflammatory drugs that act by inhibiting the release of prostaglandins by inflammatory cells...i.e. such as aspirin, ibuprofen... or other analgesic act to reduce immune reactions. The point of the study is to determine the effectiveness of our vaccine and compare to non-vaccinated animals.
   The animals in category E will be non-vaccinated or vaccinated animals, where the vaccine was ineffective. Therefore, these are the controls for the experiment. We cannot observe the reduced/dampened inflammatory response to compare “wild-type” inflammatory response to infection to reassortant viruses infections, if we artificially reduce the inflammatory response to infection using analgesics.
   Opioids can produce several well known adverse events, and, as has recently been recognized, can interfere with the immune response. The immunomodulatory activities of morphine have been characterized in animal and human studies. Morphine can decrease the effectiveness of several functions of both natural and adaptive immunity, and significantly reduces cellular immunity (Palliative Medicine, Vol. 20, No. 8 suppl. 9-15 (2006).
   In addition to that common adverse drug reactions associated with the use of buprenorphine are similar to those of other opioids and include: nausea and vomiting, drowsiness, dizziness, headache, itch, dry mouth, miosis, orthostatic hypotension, urinary retention. Hepatic necrosis and hepatitis with jaundice have been reported with the use of buprenorphine.
1) Registration Number: 23-R-0016

2) Species (common name) used in study:
   Cat

3) Number of animals used in this study: 3

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

Protocol # 9

Vestibular neurectomy (removal of vestibular inputs)

Justification for Category E designation from Protocol/ PI: (type or copy here):

Anesthetics were employed during every surgery, and analgesia was delivered after every surgery. Nonetheless, deep sedation would be required to assure that animals are not distressed by the postural instability and balance deficits that they experience immediately following removal of vestibular inputs. Such level of analgesia would not be prudent because it would impact on the data collected after the surgery and would also interfere with the animal’s compensation for the effects of the lesion. Vestibular rehabilitation is based on the notion that improvement can only occur following vestibular lesions if subjects make frequent head and body movements. Thus, even if we were to sedate animals for several days following surgery, they would likely experience distress after the sedation is discontinued (as they did not compensate for the lesion after surgery). We thus deem it most beneficial both scientifically and for the long-term condition of the animal to refrain from providing sedation following removal of vestibular inputs.
1) Registration Number: 23-R-0016

2) Species (common name) used in study: Ferrets

3) Number of animals used in this study: 22

4) Explanation of procedures producing pain and/or distress and scientific justification why pain and/or distress could not be relieved:

Protocol # 10

Justification for Category E designation from Protocol/PI: (type or copy here):
The objectives of the study are fourfold:
1. Identify differences in the pathogenesis of infection.
2. The infectious dose to be used in vaccine challenge studies for both routes,
3. Characterize clinical disease for both routes.
4. Select the best route for vaccine challenge studies.
The endpoints of the study are longitudinal measurements of clinical disease (fever, weight loss, activity), pathology (virus titers and pathology in the lungs and other tissues), and virus burden in nasal washes and BAL, relating these to the timing and peak of virus in the upper respiratory tract.
Animals in this study are infected with influenza A/Sydney virus via aerosol and intranasal route. The exposure to virus will vary in concentration from stock virus to various dilutions. Animals will be serially sacrificed at predetermined timepoints. Animals infected with the virus will be monitored using a scoring system that is based on the one described by Reuman et al. It will be used to assess the activity level as follows:
0, alert and playful and responsive to touch;
1, alert but playful only when stimulated;
2, alert but not playful when stimulated;
3, neither alert nor playful when stimulated, unresponsive to touch.
Animals with mild influenza symptoms will possibly ride out the illness. The body temperature will be monitored twice daily and compared to pre-infection temperature values. Animals scored between 0 and 1 will be closely monitored. Animals scoring 2 will be observed at least 4 times daily and if their condition deteriorates, they will be humanely euthanized. Animals scoring 3 will be humanely euthanized. Because disease is the endpoint, and fever is one of the primary indicators of disease, we cannot provide additional therapy. We will, however, humanely euthanize animals that become nonresponsive to stimuli (score 3).