**Background**

The ultimate goal of this preliminary study is to test the effectiveness of a previous protocol that was successfully used to create changes in urine output by doing 6-OHDA microinjections into the caudate nuclei. These experiments focus on applying the toxin to different areas of the body such as the Periaqueductal Grey Area (PAG) and the Cerebrospinal Fluid (CSF) in the spinal cord in order to create changes in bladder function. The PAG possesses sensory-motor integration for lower urinary tract symtoms, including some autonomic issues. Motor symptoms occur early in the disease, and are described as tremors, muscle rigidity and slowness of movement. A common autonomic issue is bladder dysfunction, typically caused by detrusor overactivity, leading to nocturia, increased urgency and increased frequency.

This project aims to recreate these urinary symptoms in laboratory rats by injecting 6-OHDA into the periaqueductal grey area of the midbrain and the lumbosacral spinal cord. The purpose of the injection is to prove that the loss of dopaminergic neurons are in fact the cause of these bladder issues. The current protocol begins with a 6-OHDA injection in the intended area (either into the midbrain or the CSF in the lumbosacral spinal cord) and requires the periodic monitoring of micturition patterns in awake rats with the use of diuresis chambers. After this period of observation, the animals are anesthetized for a terminal procedure that examines and records bladder-sphincter reflexes. Lastly, immunohistochemistry is used to test for tyrosine hydroxylase (TH), a dopamine marker, once the brain and spinal cord are removed. It is anticipated that these manipulations will result in changes in LUT function similar to those that occur in PD.

**Abstract**

Parkinson’s Disease (PD) is a neurodegenerative disease associated with a loss of dopaminergic neurons in the basal ganglia, located in the midbrain. PD patients are typically over the age of 50 because dopamine production slows due to continuous degeneration of dopamine cells as age progresses. Individuals with the disease typically exhibit both motor and non-motor symptoms, including some autonomic issues. Motor symptoms occur early in the disease, and are described as tremors, muscle rigidity and slowness of movement. A common autonomic issue is bladder dysfunction, typically caused by detrusor overactivity, leading to nocturia, increased urgency and increased frequency. The purpose of the injection is to prove that the loss of dopaminergic neurons are in fact the cause of these bladder issues. The current protocol begins with a 6-OHDA injection in the intended area (either into the midbrain or the CSF in the lumbosacral spinal cord) and requires the periodic monitoring of micturition patterns in awake rats with the use of diuresis chambers. After this period of observation, the animals are anesthetized for a terminal procedure that examines and records bladder-sphincter reflexes. Lastly, immunohistochemistry is used to test for tyrosine hydroxylase (TH), a dopamine marker, once the brain and spinal cord are removed. It is anticipated that these manipulations will result in changes in LUT function similar to those that occur in PD.

**Methods**

1. 6-OHDA Lesion Application
   - Female Sprague Dolly rats (270-300g).
   - Pre-administration of desipramine to protect noradrenergic neurons.
   - Microinjection into PAG/L4-5 of spinal cord
   - 3. Terminal Procedure: Bladder-Sphincter Reflexes
      - Anesthetized with urethane (1.25g/kg sc.)
      - IV line in Jugular vein
      - Catheter in bladder dome, electrodes in external urethral sphincter
      - Collect data for a series of hours as saline is infused through syringe pump
      - Transectional Perfusion with 4% Paraformaldehyde
      - Remove brain/spinal cord

2. Micturition pattern in awake rat

3. Immunohistochemical processing to visualize Tyrosine Hydroxylase

**Conclusions**

Terminal experiments have been completed for all of the animals that received injections.
- We observed micturition events after the bladder catheter was placed and found that all animals are slightly different in their urine output, void length and frequency.
- Data analysis is in progress and results currently suggest that while the PAG lesions alter micturition patterns, the intrathecal spinal injections have little to no effect on micturition.
- Tissue that has gone through the immunohistochemical process must be further analyzed before any changes can be made to the current experimental protocol.

**Future Studies**

- Slides that were prepared using brain and spinal cord tissue from the rats used in this study will be further analyzed and compared.
- These procedures will be repeated in order to compile more data in hopes of finding trends in micturition events.
- 6-OHDA lesions can be done in different areas of the spinal cord and brain in order to compare frequency and severity of symptoms.
- Different animals can be used as subjects for the lesions.
- Animals can be kept alive longer to observe long term changes and even be treated with drugs similar to levodopa if the desired symptoms are exhibited.
- A higher volume or concentration of 6-OHDA can be used for more significant changes in bladder function.

**References**


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