

Introduction

- Regulatory developmental/reproductive toxicology require large numbers of animals and multiple endpoints evaluated.
- We present our training and validation plan following remodeling of our Neurotoxicology Suite and training new staff in preparation for an OECD 443 Extended One-Generation Reproductive Toxicity Study.
- OECD 443 overview on next slide

Methods & Objectives

- Equipment Validation
- Training
- Performance Validation with Positive Control Substances

FOB

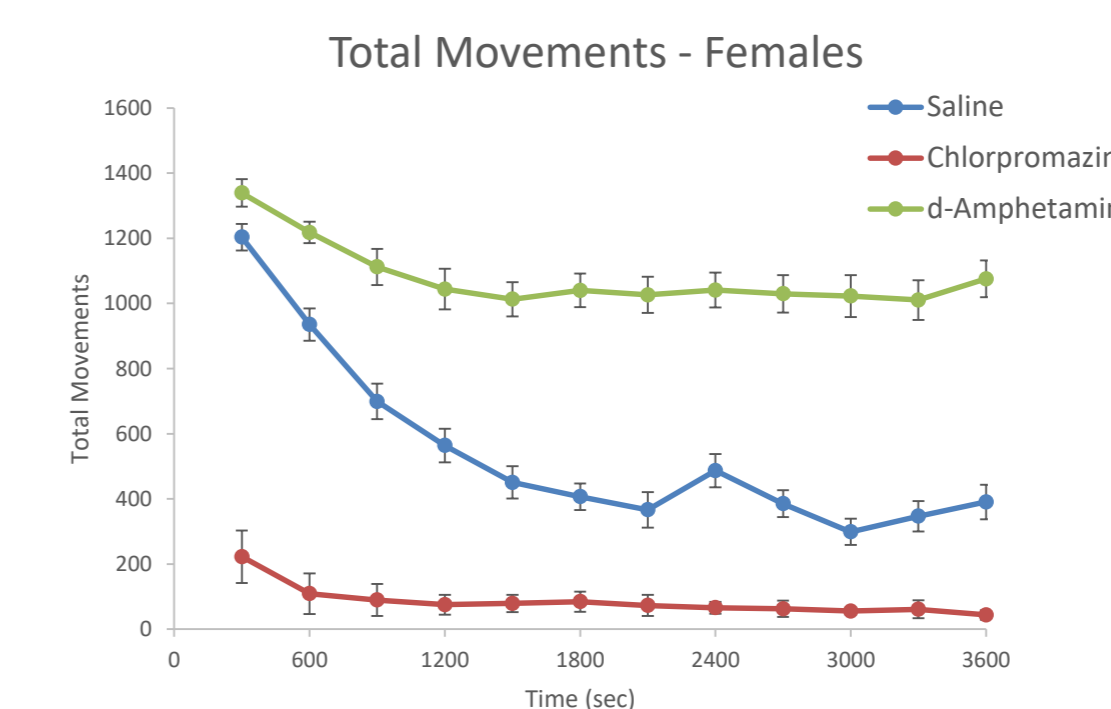
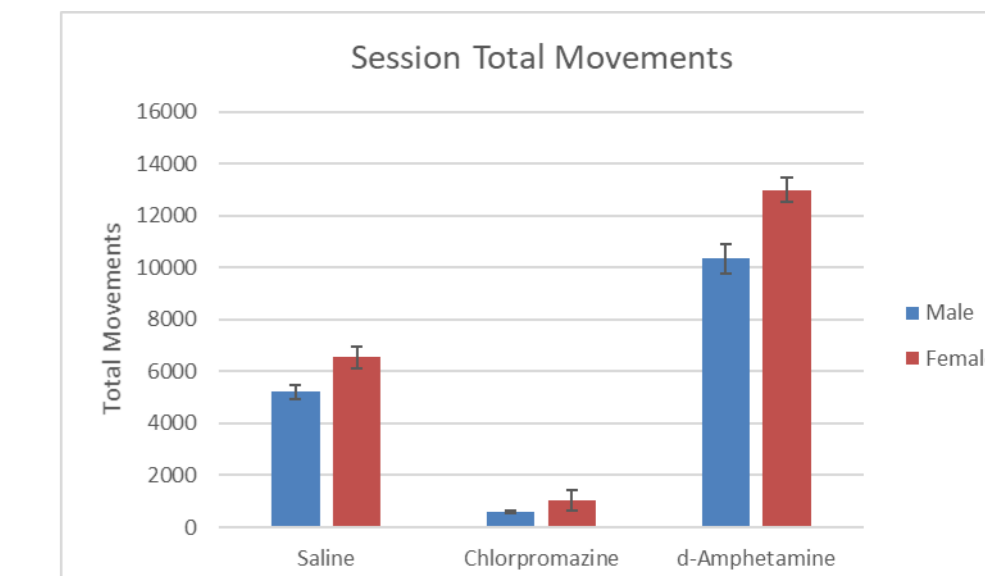
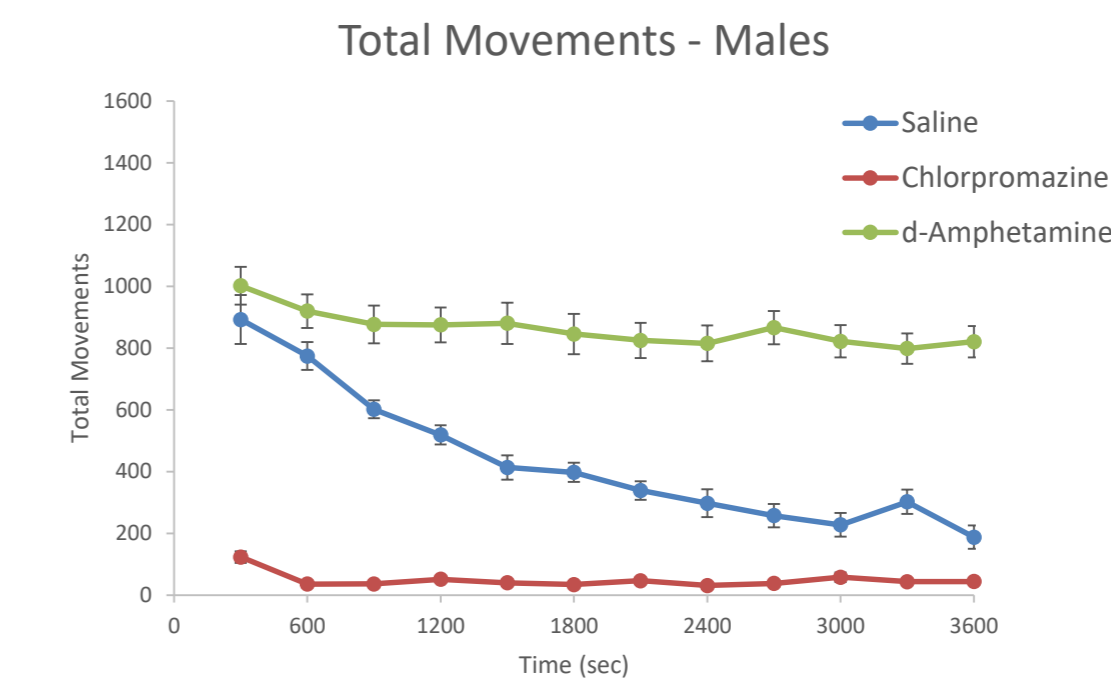
Classroom Training with Video

15 training sessions with control animals (165 observations)

4 training sessions with positive control-treated rats

Blinded Certification Test with positive control-treated rats (15/sex/group)

Motor Activity

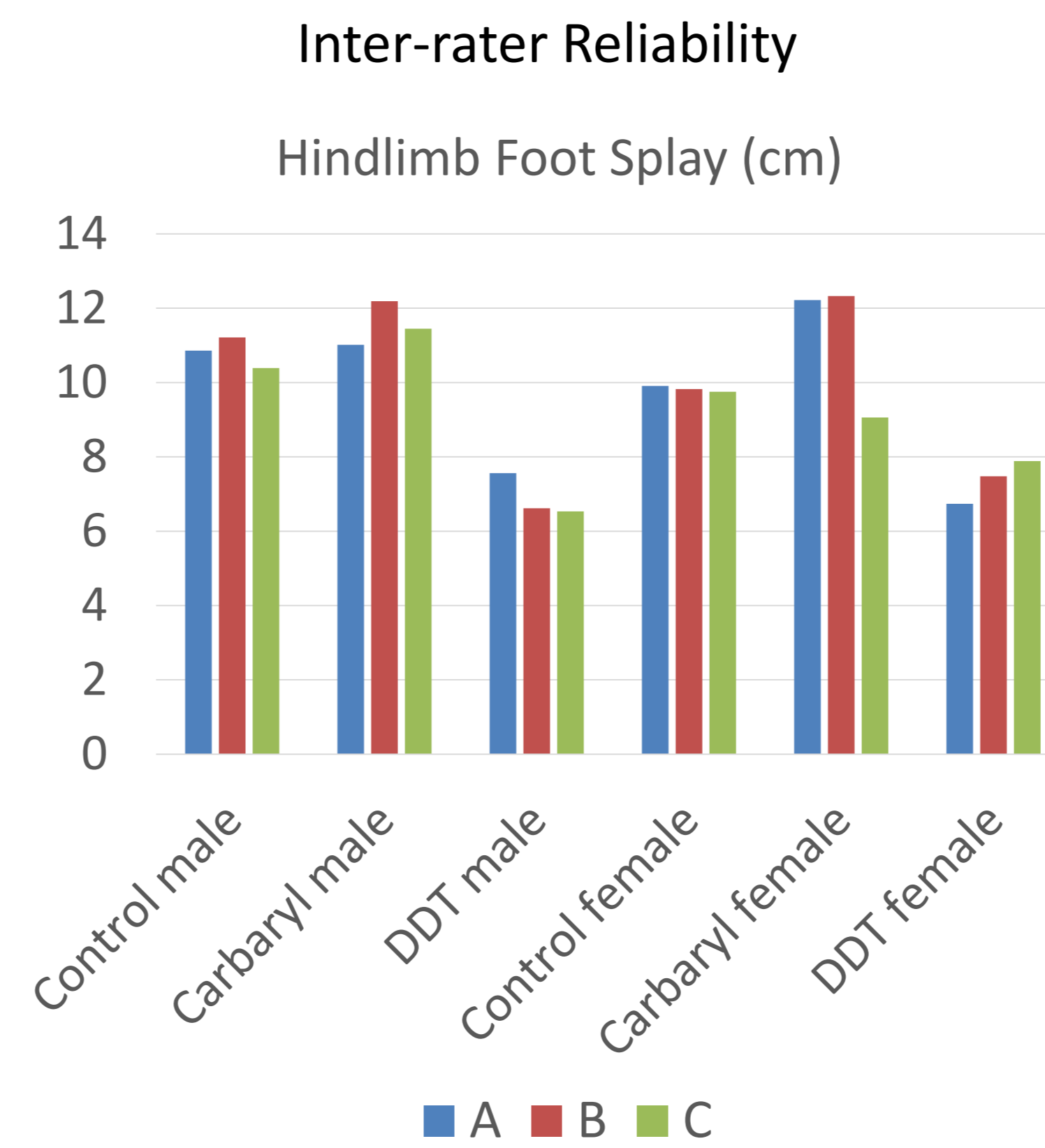
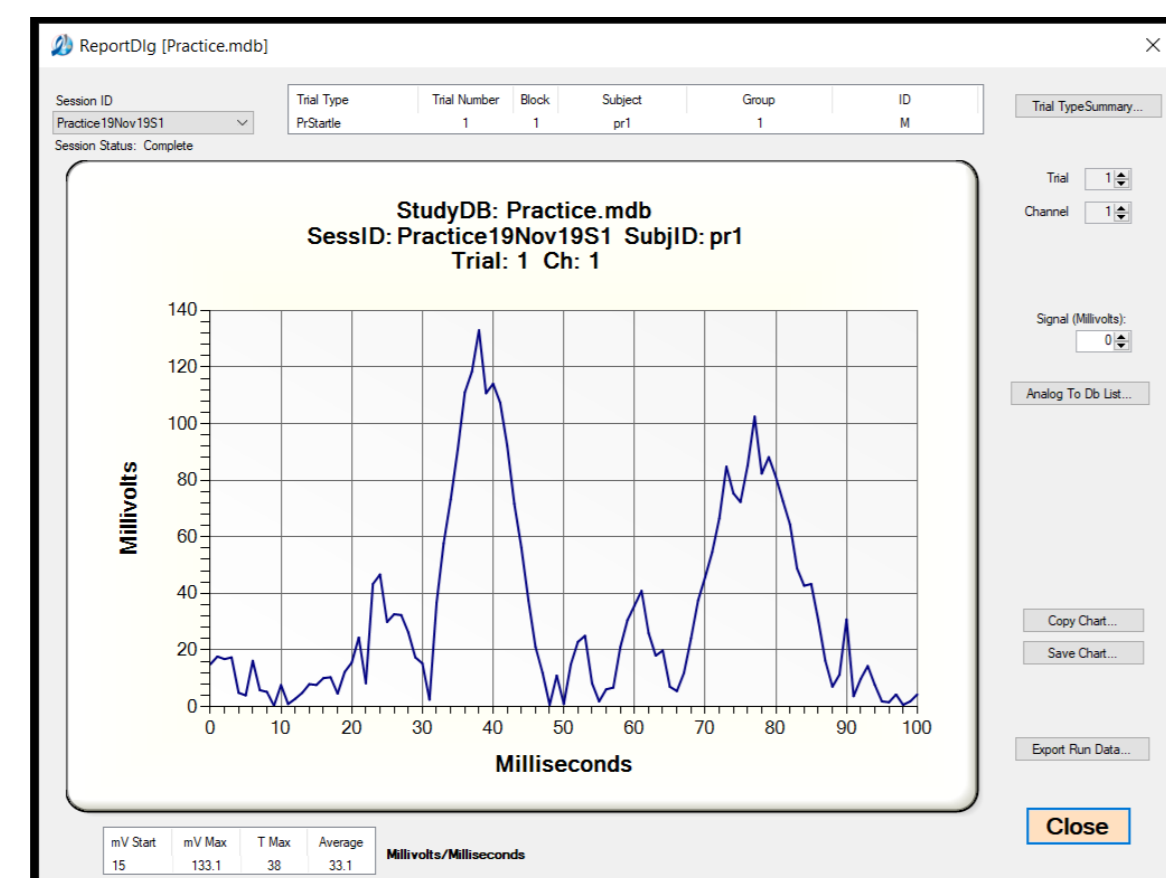


Animals dosed with Chlorpromazine or d-Amphetamine showed persistent decreased or increased motor activity, respectively

Equipment Validation

Installation, Operational, and Performance Qualifications for FDA CFR Part 11 Compliance

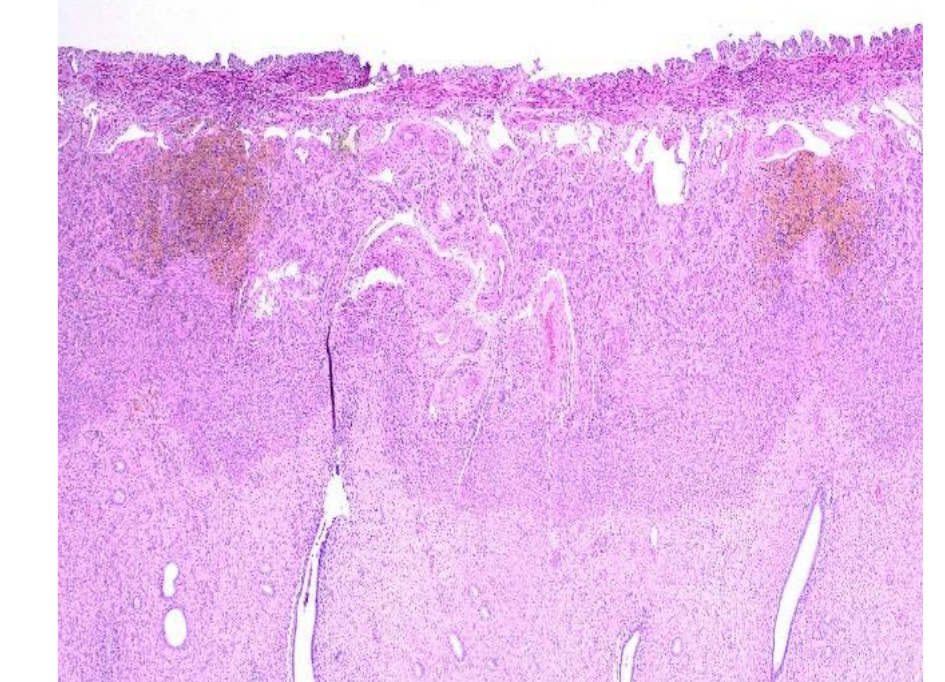
- San Diego Instruments Photobeam Activity System
- San Diego Instruments SR-LAB Acoustic Startle System
- Bioseb Grip Strength Gauge
- Micro-Therma 2T Thermometer
- Hamilton Thorne CASA IVOS Sperm Analysis System



Pathology



Brain Morphometry



Uterus Stained with Potassium Ferricyanide and H&E

Conclusions

Conduct of a regulatory developmental/reproductive study such as an OECD 443 requires a considerable amount of time and resources to fully validate a laboratory's ability to perform all aspects of this complex study design.

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Reference & Acknowledgements

OECD (Organisation for Economic Co-operation and Development). (2018). Extended One-Generation Reproductive Toxicity Study. OECD Guideline for the Testing of Chemicals, No. 443, OECD, Paris.

We want to thank the ILS Research Assistants and Tom Earp at ILS for their support in getting our validation complete.

So You Want To Do Regulatory Developmental Neurotoxicology Studies: A Training and Validation Plan

Foster, Melanie L., Donahue, Douglas A., Mahapatra, Debabrata, Owigho, Pamela, Henry, Joyce

OECD 443 Extended One-Generation Reproductive Toxicity Study

Overview

- Objective is to evaluate effects of exposure to a substance during all phases of the reproductive cycle and on development, growth, and functional endpoints of offspring up to PND 90.
- Parental (P₀) animals exposed 2 weeks pre-mating, through mating, gestation, and lactation. P₀ males exposed at least 70 days.
- At least 20 litters/dose group
- Offspring (F₁) exposed via gestation and lactation, and throughout life.
- General and **reproductive** toxicity assessed in P₀ animals.
- Offspring (F₁) from each litter allocated to 1 of 5 cohorts:
 - 1A and 1B – **Reproductive Developmental** endpoints
 - 2A and 2B – **Developmental Neurotoxicity** at weaning and up to adulthood
 - 3 – **Developmental Immunotoxicity**
- Combined study reduces total number of animals used compared to separate studies for the same endpoints.

Endpoints

- Body Weight, Clinical Observations, Food Consumption
- **Estrus Cyclicity, Mating Success, Gestation Length, Litter Size, Sex Ratio**
- **Anogenital Distance, Male Nipple Retention, Vaginal Patency, Balano-Preputial Separation**
- **Auditory Startle, Automated Test of Motor Activity, Functional Observational Battery**
- **T-cell Dependent Antibody Response Assay**
- Hematology, Urinalysis, Clinical Chemistry including Thyroid Hormones
- Necropsy with Gross Observations, Organ Weights, and Histopathology
- **Perfusion Fixation for Neurohistopathology, including Brain Morphometry**
- **Splenic Lymphocyte Subpopulation Analysis**
- **Ovarian Follicle, Corpora Lutea, and Implantation Sites Examination**
- **Sperm Motility, Concentration, and Morphology**

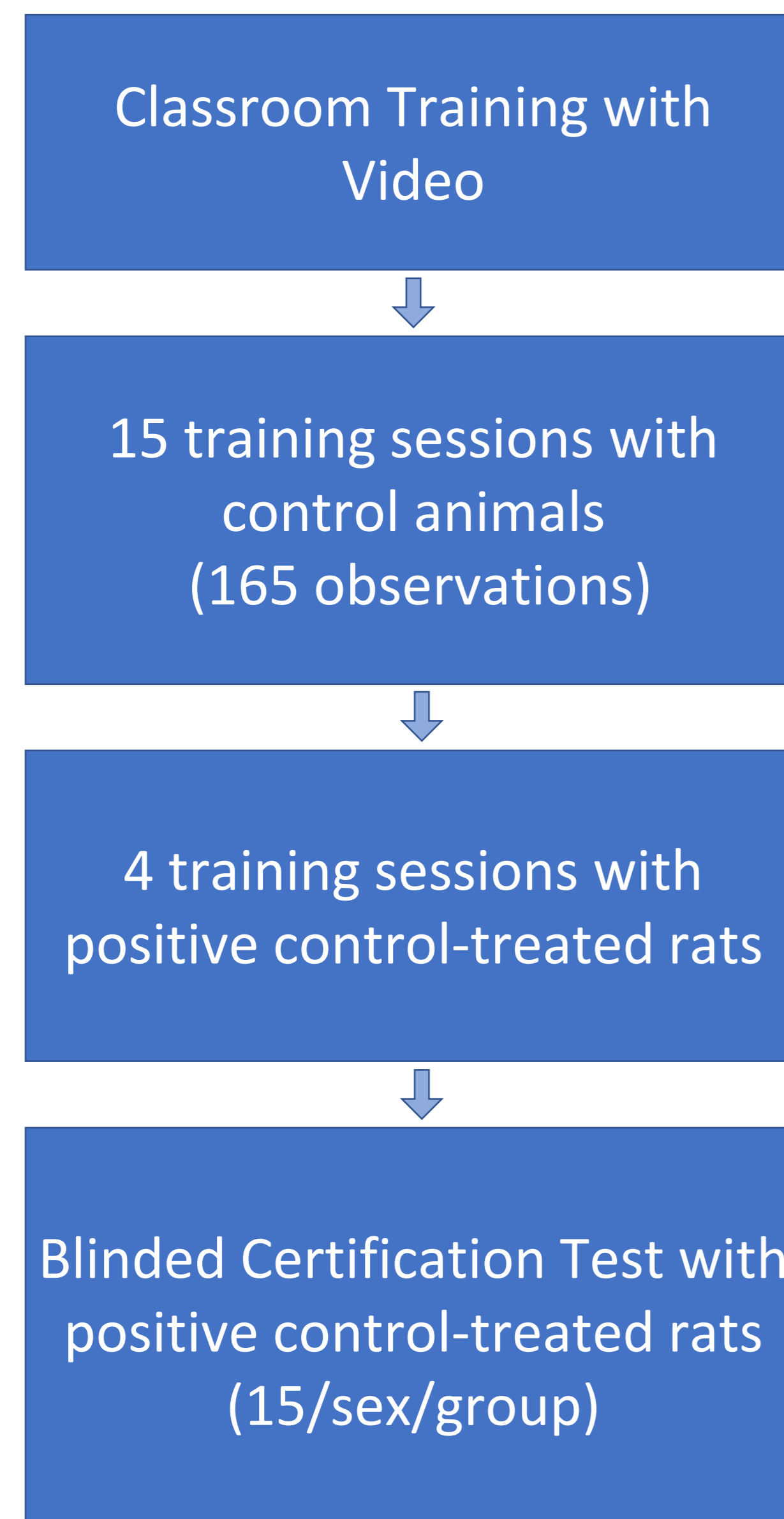
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Functional Observational Battery (FOB)

Training Plan

- Research Assistants were trained in pairs to facilitate standardization of methods and observations
- Data was collected directly into Provantis, eliminating need for transcribing written data



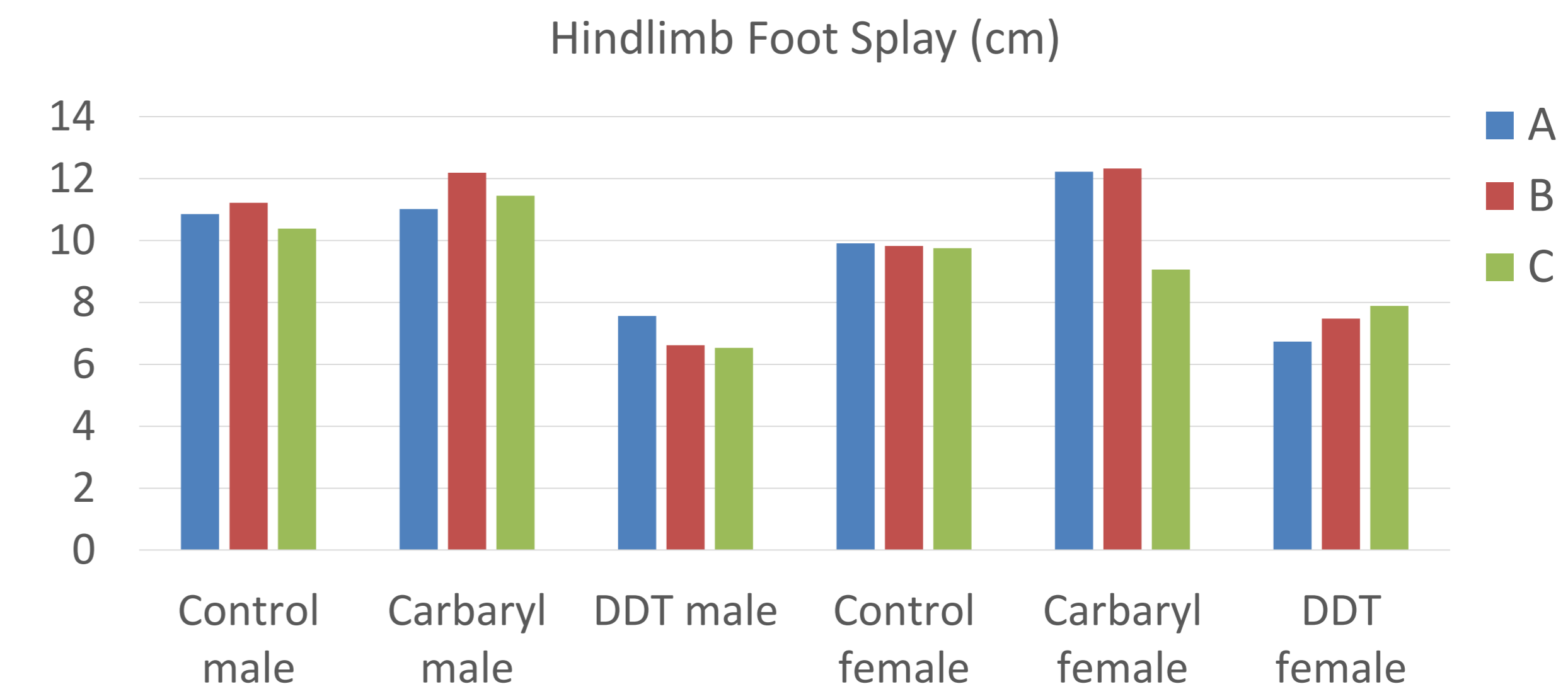
Positive Control Certification Test

Hsd: Sprague Dawley rats n=15/sex/group

- Corn oil
- 4,4'-DDT 75 mg/kg
- Carbaryl 100 mg/kg
- Blind ID number and randomized order used to prevent bias

Results

- Research Assistants were able to confidently, accurately identify changes in sensory, motor, and autonomic nervous system function.
- Concordance between the trainer and 2 Research Assistants was 98% for animals in the same dose groups.

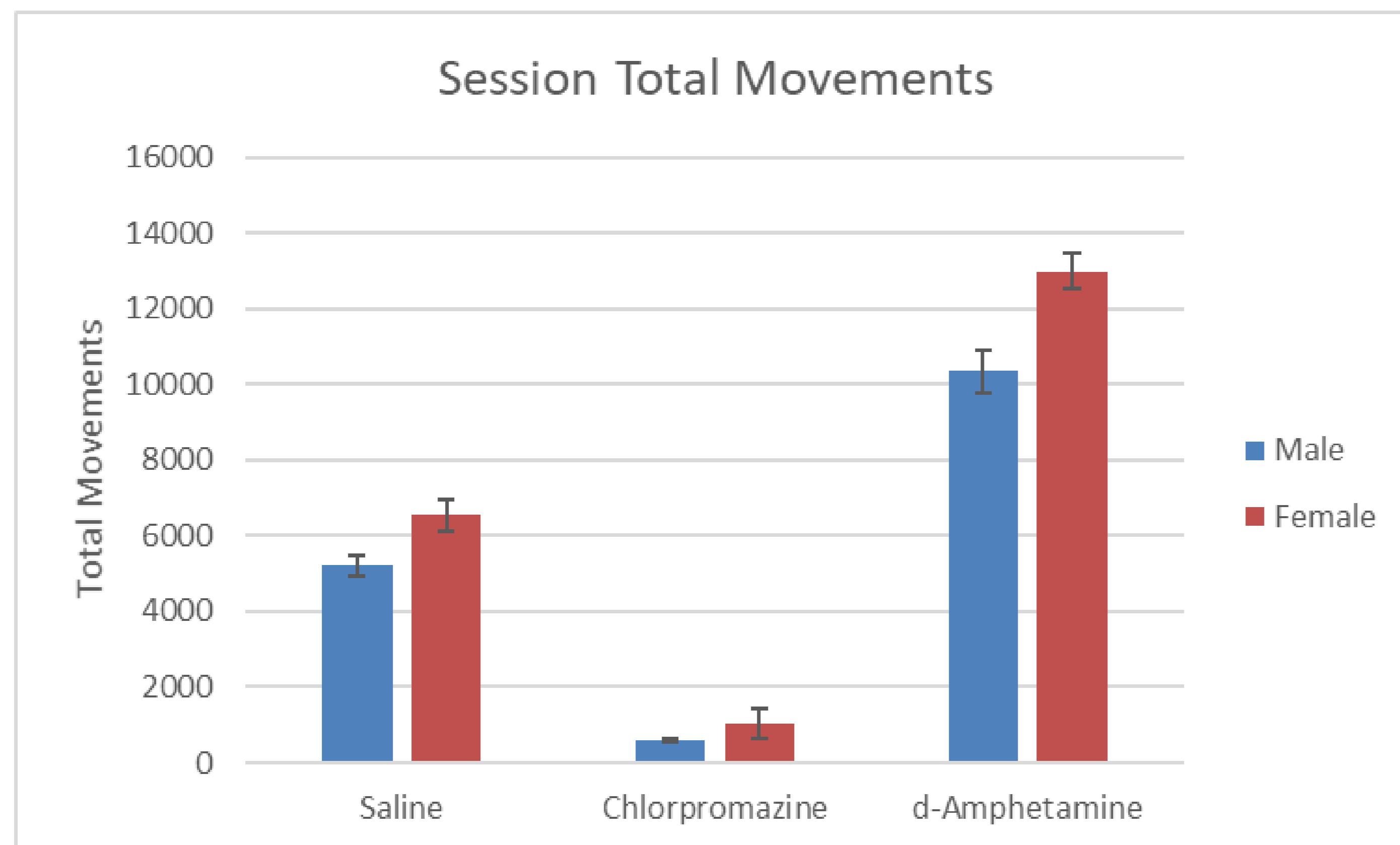


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Positive Control Motor Activity Data

While Total Movements in a session shows overall difference between dose groups, the pattern of activity over time is important to reveal differences in effects.

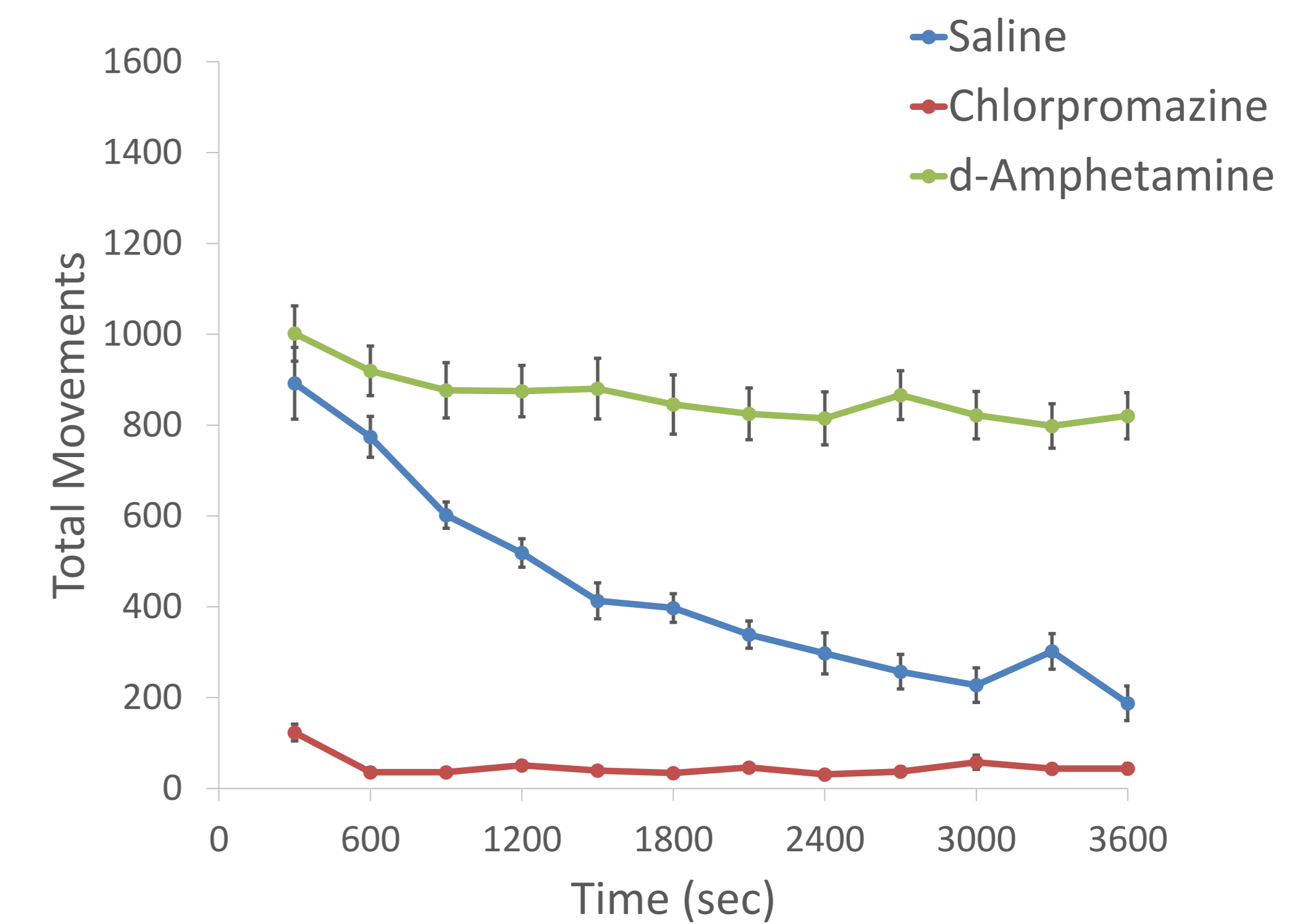


Hsd: Sprague Dawley rats n=15/sex/group

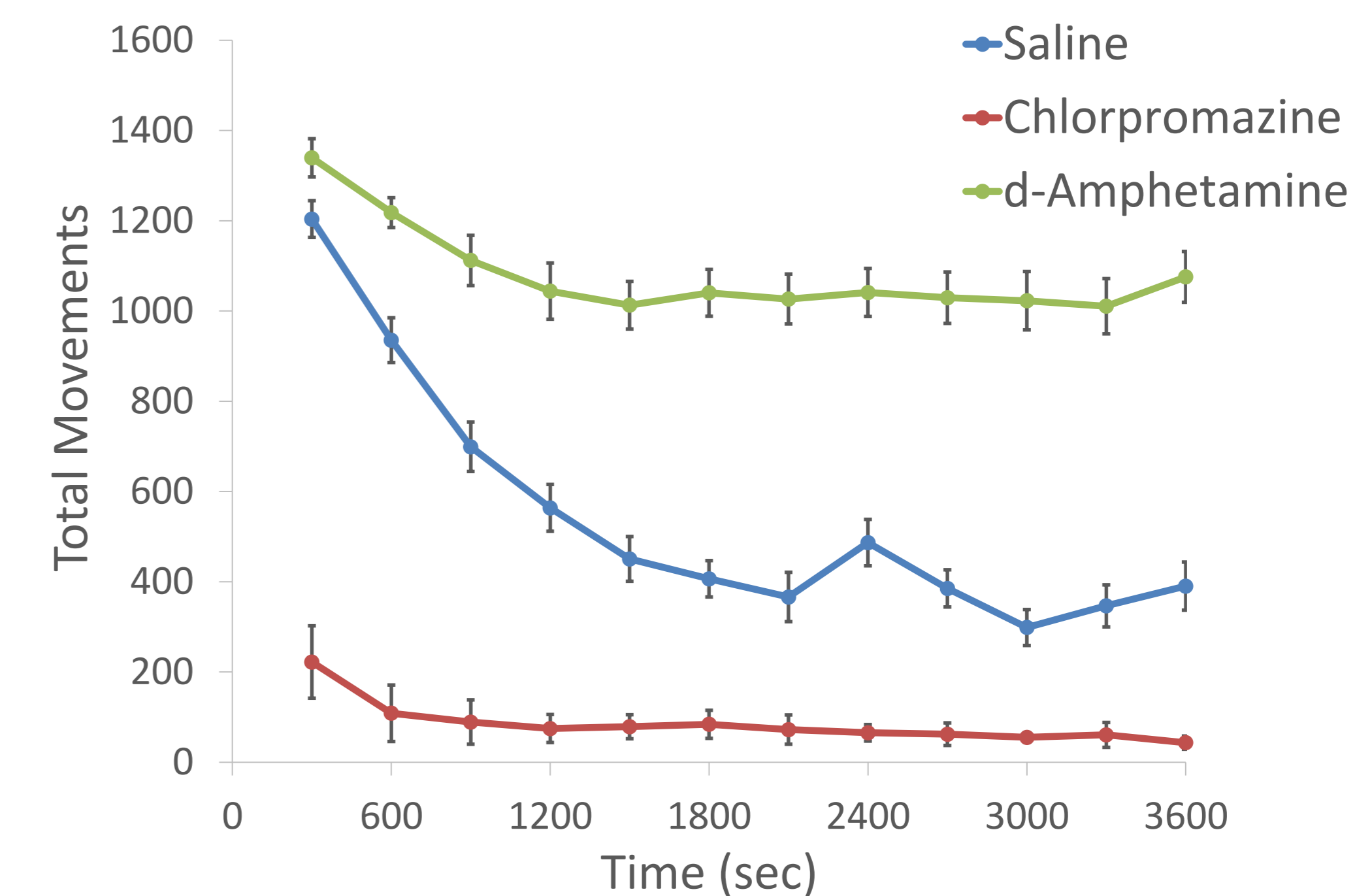
- Saline
- Chlorpromazine 6 mg/kg
- D-Amphetamine 4 mg/kg

San Diego Instruments Open Field Photobeam Activity System (PAS)

Total Movements - Males



Total Movements - Females



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Neuropathology

- *In situ* perfusion of the brain and peripheral nervous system in PND 22 and PND 77 F₁ pups
- Brain morphometry as well as traditional neuropathological examination of CNS and PNS

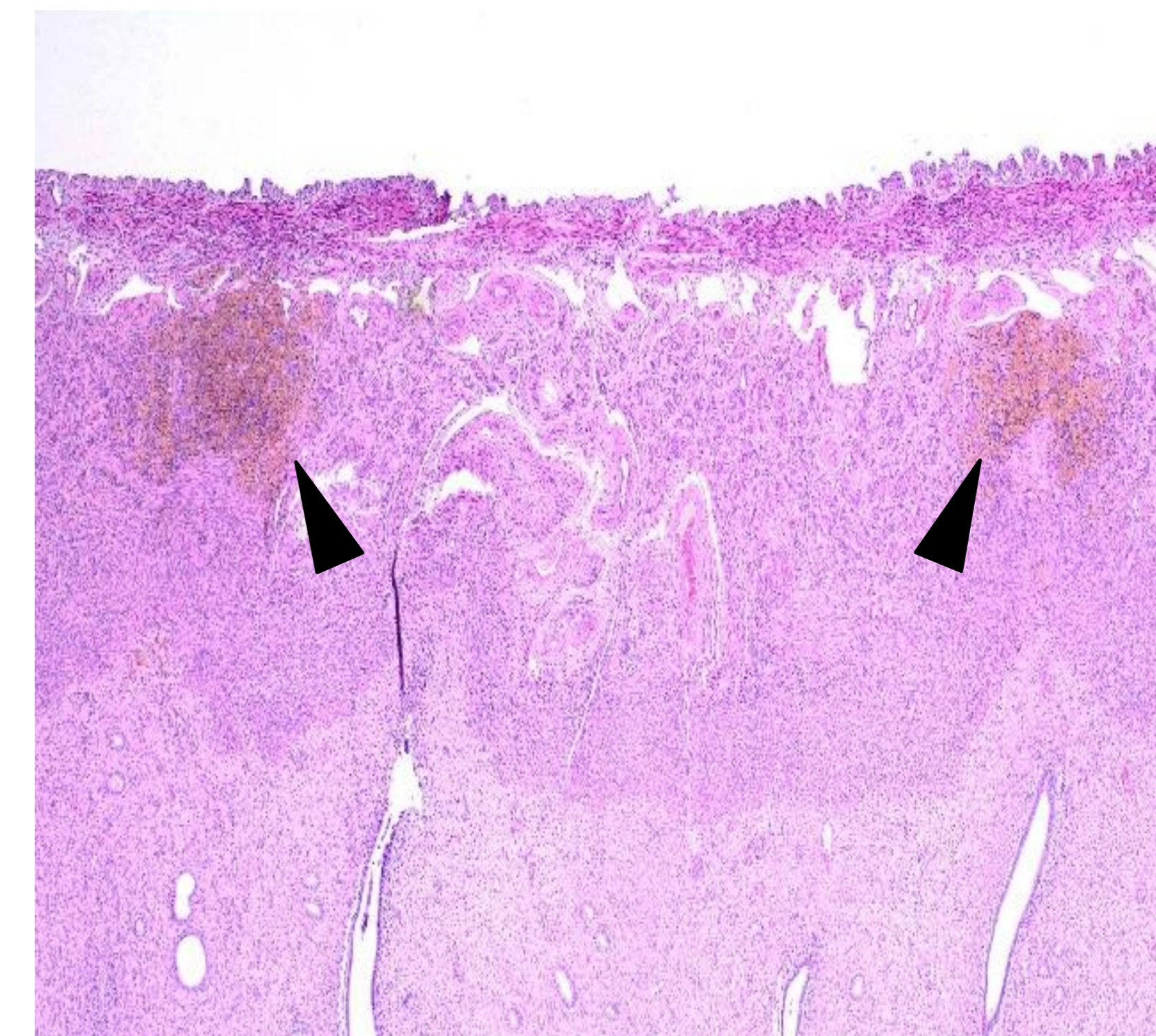


Hsd: Sprague Dawley rats n=3/sex/group

- Corn oil
- 4,4'-DDT 75 mg/kg
- Carbaryl 100 mg/kg

Reproductive Pathology

- Staining uterus to count implantation sites as well as histopathology
- Vaginal smears for estrus cycle determination
- Ovarian follicle and corpora lutea examination
- Sperm motility, concentration and morphology



Uterus stained with 10% Potassium Ferricyanide for identification of implantation sites (arrows) at necropsy, then processed and stained with H&E for histopathology