BPC Welcome Packet

4/20/2022

FEINBERG SCHOOL OF MEDICINE

BEHAVIORAL PHENOTYPING CORE

Notable Quotes:

"Novices to animal behavior research are encouraged to collaborate with a reputable behavioral neuroscience laboratory to learn the many methodological details that comprise rigorous animal behavioral testing. Rizzo & Crawley (2017) Annu. Rev. Anim. Biosci. 5:371-89.

"For complex behavioral tests or extensive phenotyping goals, collaboration with an established rodent behavioral core facility or investigators currently using the specific phenotyping approaches can be the most efficient strategy." McCarson, KE. Strategies for Behaviorally Phenotyping the Transgenic Mouse. In: Larson, MA (Ed)Transgenic Mouse: Methods and Protocols, Methods in Molecular Biology, vol. 2066, 2020.

A. Overview

History: The Behavioral Phenotyping Core (BPC) was founded in 2006 in response to an NIH request for Facilities Improvement Applications (FIA) and an increased demand for behavioral testing services for mice and rats from colleagues of Dr. John Disterhoft, now Executive Director of the BPC, and Dr. Craig Weiss, now Director of the BPC. While the FIA was not funded, the need for the BPC was apparent. The benefits of such a facility were presented to the Feinberg School of Medicine Dean for Research and the Northwestern University Vice President for Research, and funding was approved.

The BPC became operational by using Dr. Disterhoft's equipment in a suite of rooms in the Lurie basement. New equipment was acquired through successful applications to Shared Facilities competitions. The BPC began charging for services in 2008 with rates established by the Office of Cost Studies, and it began billing through NUCore in 2014. Demand for Core services increased beyond the limits of the original half-time technician so a full-time technician was requested and approved by the Dean for Research. Utilization of the BPC increased in the last few years such that another room (Ward 15W-124) was requested (and approved) in order to meet the demands of BPC users. The BPC now operates mostly in the West wing of the 15th floor of the Ward Building. A procedure room in Ward 15 East (15-178) is being used for procedures on rats (in order to maintain separation of mice and rats).

Mission*:* The mission of the BPC is to: **1.** Provide equipment and services to test behavioral effects of genetic manipulations, pharmaceuticals, aging and other manipulations upon normal behavior or learning capacities for rodent-based model systems. **2.** Provide training in acquisition of behavioral data, study designs, data analysis, and loan of portable equipment (when available) to laboratories that require short-term loans. **3.** Work with the Institutional Animal Care and Use Committee (IACUC) to provide Approved Animal Protocols (AAPs) that can be readily added by a Principal Investigator to their approved Animal Study Protocol (ASP). AAPs allow for much more rapid approval by the IACUC of a PI's animal protocol that is being modified to include behavioral testing. **4.** Provide NU investigators with information needed for the Resources and Methods sections of their NIH grant applications, and to provide data and testing information that can help make their applications more competitive and translational in nature. Dr. Weiss continues to write letters of support for grant applications from PIs to the NIH.

The BPC provides the NU research community with high-quality behavioral phenotyping at a significantly lower expense than if each PI had to secure equipment individually, and the shared facility reduces the total space that would otherwise be required for duplication of equipment. The BPC offers testing of learning and memory, gait analysis, motor coordination, and anxiety on a self-service or full-service basis according to the research needs of the user. The equipment and services coupled with careful attention to user satisfaction makes the BPC at Northwestern University competitive with facilities at other leading Research I Universities like Baylor, Johns Hopkins, Medical College of Georgia, Univ. of Cincinnati, Univ. of North Carolina, Vanderbilt and Brown (for whom Dr. Weiss served as a consultant to Dr. Kevin Bath during the inception of their facility).

B. Academic Governance

CJ Heckman, Ph.D. is the Chair of the Advisory committee for the BPC. Dr. Heckman contributes his service as Chair.

John Disterhoft, Ph.D., Magerstadt Memorial Research Professor, Department of Physiology is the Executive Director of the Behavioral Phenotyping Core (BPC).

C. Administrative structure

Dr. Craig Weiss, PhD, Research Professor of Neuroscience, serves as Director of the BPC and is responsible for the day-to-day operation of the Core. He is a behavioral neuroscientist with extensive experience using rodent models. He designs behavioral experiments, maintains the computer systems that control the experiments (in conjunction with NUIT), develops data analysis templates for BPC users, writes Approved Animal Protocols for incorporation into investigators' Animal Study Protocols, writes letters of support for grant applications, brings new tests on-line, supervises the BPC technician, and trains new users on proper equipment use and data collection. Dr. Weiss provides 50% effort to the BPC.

The BPC recharge account is part of the Neuroscience Department (160-5108200). Billing for BPC services is handled by NUCORE with the assistance of the Core Technician for the BPC. Journaling the NUCORE information is done by the Business Administrators of the Basic Science Division (Laurie Daniels and Colleen De Luca). Supplies for the BPC are ordered through the PO Tool and the Basic Science Division. Laurie Daniels of the Basic Science Division assisted in the updating of the three-year budget spreadsheet.

Dr. Weiss and the Core technician have completed their required Research Safety training through the myHR Learn website, and a Business Continuity Plan is uploaded to CFA website.

D. Personnel

John Disterhoft, Ph.D.- Executive Director

Craig Weiss, Ph.D.- Director

TBD- Core Technician

E. Facilities and Location

Describe locations where services are provided:

Campus	***Building***	***Room #***	***Sq. Ft.***	***Space Assignee***
Chicago	Ward	15-105 (mouse behavior room 1)	248	FSM (BPC)
Chicago	Ward	15-124 (mouse behavior room 2)	171	FSM (BPC)
Chicago	Ward	15-030 (rat behavior room)	205	FSM (BPC)

Chicago	Ward	15-007 (mouse water maze)	100	FSM (BPC)
Chicago	Ward	15-132 (rat water maze)	132	FSM (BPC)
Chicago	Ward	15-011 (mouse holding room, normal light)	145	CCM (multi-PI)
Chicago	Ward	15-019 (mouse holding room, reverse light)	179	CCM (multi-PI)
Chicago	Ward	15-029 (rat holding room, reverse light)	160	CCM (multi-PI)
Chicago	Ward	15-023 (rat holding room, normal light)	163	CCM (multi-PI)
Chicago	Ward	15-126 (surgery, mouse)	153	CCM (multi-PI)
Chicago	Ward	15-178 (surgery, west)	136	CCM (multi-PI)

Note that the mouse and rat holding rooms (15- 011, 019, 023, 029), as well as the surgery/procedure room (15-126), are CCM common rooms that are shared among several PIs. Also note that the BPC has separate rooms for normal and reverse lighting for both mice and rats.

The main mouse behavior room (15-105) holds equipment for Startle/PPI, fear conditioning, Y maze, zero maze and open field / social behaviors. The rat behavior room (15-030) houses equipment for eyeblink conditioning, fear conditioning, and DigiGait. At this time, the BPC has not seen many conflicts regarding scheduling of the rat behavior room, therefore, housing multiple instruments there proves to be an efficient use of space.

A second mouse behavior room (Ward 15-124) was acquired in 2021 in order to relieve congestion in 15-105. The rotarod and DigiGait systems were moved into the new room and increased the bandwidth of the BPC.

F. Equipment

Instruments and Services in the BPC are intricately linked to each other. Computers running the acquisition and analysis software were updated within the last three years, and software is updated at least twice per year (if updates are available).

Equipment Name	
Rat IAC Isolation Chambers (N=4)	NeuroStar Motorized Stereotaxic
Mouse IAC Isolation Chambers (N=4)	Actimetrics LimeLight System
Rat DigiGait	Actimetrics FreezeFrame System
Mouse Rotarod	Ugo Basile Thermal Plantar Analgesia System
Mouse DigiGait	Mouse watermaze system
Med Associates Startle System	
Rat watermaze system	

G. Services

BPC equipment can be utilized in either full-service or self-service modes unless otherwise noted. Full-service uses BPC staff to perform all testing on behalf of a PI, while during self-service the PI or other researcher performs all testing independently (after training with BPC staff). BPC users can also customize a combination of these two modes if they require additional assistance, but do not necessarily need the BPC staff to do everything. These two modes (and combinations thereof) provide BPC users with flexible options to fit their budget and schedule, and increases the bandwidth of the BPC.

Self-service reservations should be made using the NUCORE reservation system:
https://nucore.northwestern.edu/facilities/bpc

Full-service reservations should be scheduled in consultation with the BPC technician or Dr. Weiss.

Services are billed according to rates established as a result of the annual Cost Study analysis. A table of rates is published on the BPC website, and the table includes full cost (unsubsidized) rates that should be used when justifying NIH budgets.

Morris Water Maze is the gold standard behavioral assay to test spatial memory. The BPC has two computerized systems (Actimetrics WaterMaze) to control the experiment and collect data. The mouse and rat systems require different sized pools, and each pool is optimized for the room in which it is housed. The software tracks the position of the animal in the pool and measures latency, swimming distance, and proximity to a hidden platform submerged in a pool of opaque water.

DigiGait (Mouse Specifics) collects and analyzes gait data by digitally recording paw prints on a transparent treadmill belt that can be set to move at speeds from 1 to 99 cm/sec. A new belt for the mouse DigiGait was purchased this year to improve image quality.

LimeLight (Actimetrics**)** is the software for recording data from open field based tests such as the zero maze test for anxiety, Y maze test for spontaneous alternation (spatial memory), activity during social exploration (social memory), and of course the open field test for general activity. Testing is done inside sound attenuating chambers manufactured by Industrial Acoustics Corporation (IAC), the Gold Standard of isolation chambers.

Fear Conditioning (Actimetrics) is a well-understood learning paradigm involving the association of a context and tone with a mild footshock. Fear is assayed by the presence of behavioral freezing, or immobility. Testing is also done in the IAC chambers.

Prepulse Inhibition (PPI) of Startle Response (Med Associates) detects the reduction of a sound induced startle response due to the presentation of a brief, more quiet prepulse prior to the startle stimulus. This brainstem-mediated sensory gating is impaired in schizophrenia and is used to test models of the disease.

RotaRod (TSE) is used to test motor coordination and motor learning by measuring the latency to fall off a rotating rod at varying speeds with or without constant acceleration.

Surgery service includes use of a stereotaxic frame with head-holder and anesthetic vaporizer. A robotic stereotaxic is also available and is suited for multiple injection sites that can be programmed and repeated on successive animals.

Eyeblink conditioning is a well-understood associative learning paradigm that pairs a conditioning stimulus such as a tone with a mild shock or airpuff to the periorbital region. Variations of the paradigm are used to assay forebrain-cerebellar interactions. Dr. Disterhoft's custom equipment is used.

Thermal Plantar Pain Test (Ugo Basille) measures the latency to withdraw a paw away from increasing thermal stimulation. This device is located within the new behavioral room (Ward 15-124).

Data analysis: The software packages selected by the BPC include good analysis options that create Excel based files. A single session with Dr. Weiss is usually sufficient to teach the user how to analyze their data and get their results. Several laboratories have been taught to use the routines. The BPC encourages laboratories to analyze their own data so that they better understand the significance of the tests. Additionally, the BPC does not currently have the resources to analyze data for all the users.

Recharge Rates are indicated in Appendix III. The BPC attempts to maintain the same recharge rates from year to year in order to balance its budget. This decision was made in consultation with the BPC Advisory Committee after surveying BPC rates at similar institutions. See Appendix IV for more detail.

DigiGait Key rental: We rent one of the analysis dongles (software license) to laboratories that want to do analyses in their own laboratory overnight or during the weekend for a cost that is equivalent to three hours of data acquisition. This provides considerable savings to PIs and leaves the DigiGait for mice more available for data collection. This scheme has proven to be a good use of the second DigiGait license.

Services Without Charge:

Consulting is provided free of charge, as part of "University Citizenship," in recognition of the support the BPC receives as an FSM Core Facility, and as a way for PIs to discover if the BPC will be a good fit for the intended project. We view this service as an investment in future use of the BPC.

Instruction in data analysis is often provided free of charge. The software packages selected by the BPC include good analysis options that create Excel based files. A single session with Dr. Weiss is usually sufficient to teach the user how to analyze their data and get their results. Several laboratories have been taught to use the routines. The BPC encourages laboratories to analyze their own data so that they better understand the significance of the tests (and the BPC does not have the resources to analyze data for all the users).

Letters of Support for NIH grants (and for other funding agencies).

Description of Facilities and Resources, and Description of Equipment

Equipment Loans: The BPC (and Disterhoft laboratory) has several stereotaxic frames and head holders for rats and mice. We have loaned a stereotaxic device to a laboratory for a day or weekend for them to use in their own laboratory.

Alternatives to using the Behavioral Core:

There is at least one commercial alternative for Behavioral Testing, i.e. PsychoGenics (Tarrytown, NY). The company is not likely to be as interactive as having an on-site facility.

Laboratories that do much behavioral testing use their own equipment. It would not be cost effective for those laboratories to utilize the BPC, and the BPC does not have the throughput to support a laboratory doing intensive behavioral testing on a continuous basis.

Animal Protocols and per diem Charges:

All animals stay on the PI's protocol and the PI is responsible for their inventory and per diem charges.

Many tests have Approved Animal Protocols (AAPs) that can be inserted into a PI's animal study protocol relatively quickly and easily. The AAP includes permission for the BPC to test the PI's animals. Procedures that are not part of an AAP should be written to include BPC personnel so that the Core remains compliant with IACUC and USDA regulations. A list of AAPs can be found at: https://iacuc.northwestern.edu/resources/approved-animal-procedures-aaps.html

The following AAPs are on the IACUC website:

Examination of Gait using the Rodent Specifics DigiGait in BPC

Examination of Open Field and Zero Maze Behaviors AAP in BPC

Rodent Fear Conditioning in BPC

Rotarod Testing

Water Maze Test for Spatial Memory in BPC

Y Maze Test of Spontaneous Alternation (1 Day, 5 min, 22 entries) in BPC

Example Publications and Grants

1. Gregory S. McElroy, Colleen R. Reczek, Paul A. Reyfman, Divakar S. Mithal, Craig M. Horbinski, Navdeep S. Chandel (2020) NAD+ Regeneration Rescues Lifespan, but Not Ataxia, in a Mouse Model of Brain Mitochondrial Complex I Dysfunction, Cell Metabolism, 32 (2): 301-308.e6.

doi.org/10.1016/j.cmet.2020.06.003.

The BPC enabled the collection of rotarod and open field activity, as shown in their Figure 2A/B and 2D.

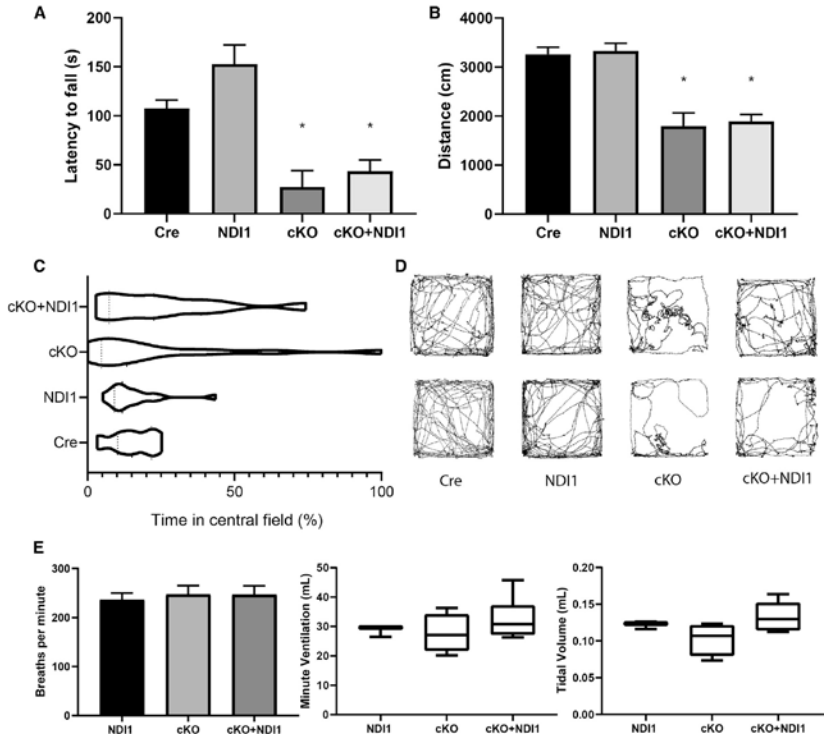

Figure 2. NDI1 Expression in the Brain Does Not Rescue Progressive Ataxia or Alter Breathing Parameters in a Mouse Model of Leigh Syndrome

(A) Latency to fall in an accelerating rotarod challenge in seconds (s) (n = 8–22, ANOVA, $p < 0.0001$, Dunnett's test to Cre control, $^*p < 0.01$).
(B) Distance traveled in 300 s in a novel open field environment (n = 12–22, ANOVA, $p < 0.0001$, Dunnett's test to Cre control, $^*p < 0.0001$).
(C) Percent time spent in the central zone of a novel open field environment (Bartlett's test, $p < 0.0001$).
(D) Two representative path images of the open field test for each mouse genotype.
(E) Whole-body plethysmography breathing parameters: frequency, minute ventilation, and tidal volume (n = 3–6, ANOVA, not significant). Bar graphs represent mean ± SEM.

2. Maneshi, Toth, Ishii, Hori, Tsujikawa, Shum, Shrestha, Yamashita, RJ Miller, Radulovic, Swanson, Prakriya (2020) Orai1 Channels Are Essential for Amplification of Glutamate-Evoked Ca2+ Signals in Dendritic Spines to Regulate Working and Associative Memory, Cell Reports 33 (9): 108464, https://doi.org/10.1016/j.celrep.2020.108464.

The BPC enabled the collection of data (spontaneous alternation in the Y maze, and fear conditioning) for their Figure 6.

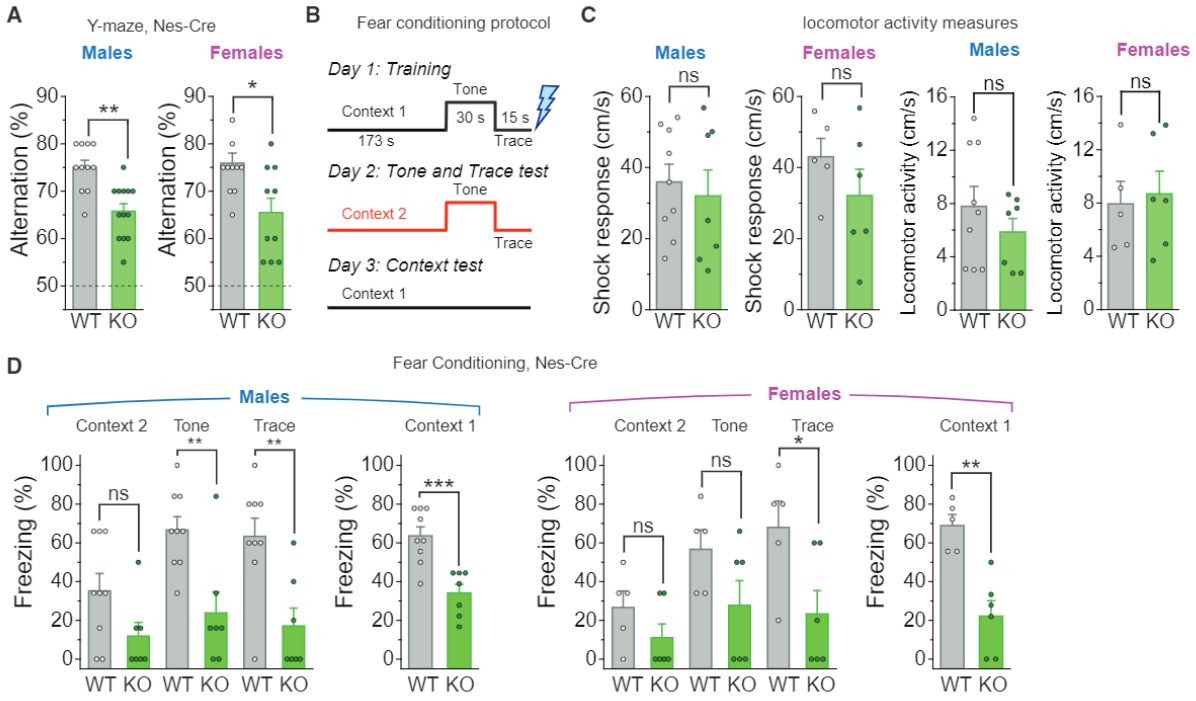

Figure 6. Orai1 KO Mice Are Impaired in Spatial Working and Associative Memory

(A) Summary of spontaneous alternations in the Y-maze for male and female WT (Orai1$^{fl/fl}$) and Orai1 KO (Orai1$^{fl/fl\ Nes\text{-}Cre}$) mice. Male mice (n = 11 WT; n = 13 Orai1 KO, p = 0.0018), female mice (n = 11 WT; n = 10 Orai1 KO, p = 0.0109). Dashed line denotes chance (50% alternation).

(B) Schematic of the fear-conditioning protocol. On day 1, mice in a conditioning chamber (context 1) were exposed to a conditioned stimulus (tone and trace interval), followed by an unconditioned stimulus (foot shock, in blue). On day 2, freezing response to the conditioned tone and trace (in a neutral context 2) was measured. On day 3, freezing response to the conditioned context 1 was measured.

(C) Shock responses and average locomotor activity in the indicated genotypes on day 1.

(D) Percentage of freezing to context 2, tone, trace, and context 1 in WT and Orai1 KO mice. Males: n = 9 WT, 7 KO. p values are as follows: context2, p = 0.0717; tone, p = 0.0035; trace, p = 0.0039; context1, p = 0.00051. Females: n = 5 WT, n = 6 KO. p values are as follows: context2, p = 0.1875; tone, p = 0.11725; trace, p = 0.03544; context1, p = 0.00134). ns, not significant.

3. Makinde, Just, Gadhvi, Winter, Schwulst (2020) Microglia Adopt Longitudinal Transcriptional Changes After Traumatic Brain Injury. Journal of Surgical Research. 246:113-122. https://doi.org/10.1016/j.jss.2019.08.024

The BPC enabled the collection of data (Watermaze, fear conditioning, startle response, prepulse inhibition of the startle response, and rotarod activity) for their Figure 1.

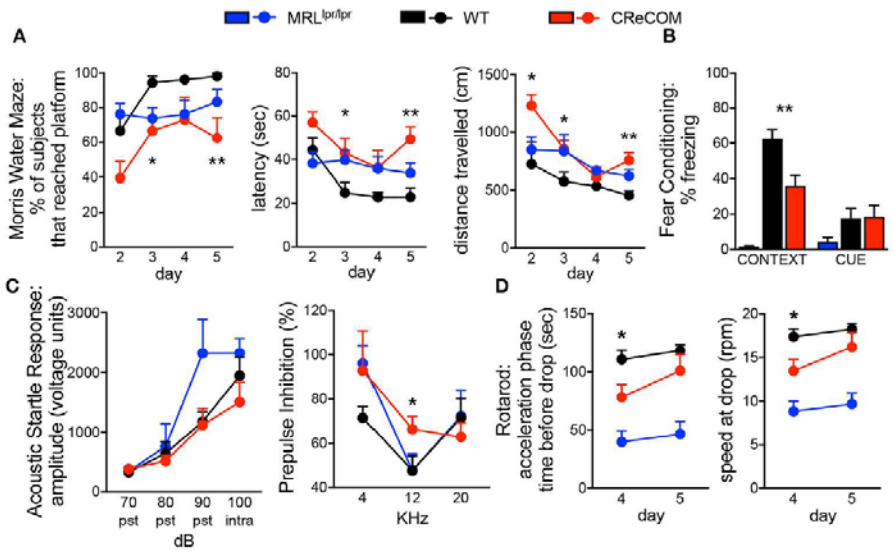

FIGURE 1 | Behavioral deficits occur in young CReCOM mice. 3-4-month-old female MRL$^{lpr/lpr}$ ($n = 7$), WT ($n = 9$), and CReCOM ($n = 8$) mice underwent behavioral testing. Data are combined from 3 independent experiments. **(A)** Morris water maze: % of mice that reached the hidden platform, latency to platform, and distance traveled. **(B)** Fear conditioning: % freezing following reintroduction of "shock" environment (CONTEXT) or tone associated with "shock" (CUE). **(C)** Prepulse inhibition: amplitude of acoustic startle response at 70, 80, 90, and 100 dB and % prepulse inhibition at 4, 12, and 20 KHz frequencies. **(D)** Rotarod: time spent on rod prior to drop and speed at drop during acceleration phase. (*$p < 0.05$; **$p < 0.005$).

- Awards or proposals that contain work produced in the core.

John F Disterhoft	SP0032916	60043387	Synaptic substrates of age-dependent memory deficits	Department of Health and Human Services	2RF1AG017139-15
Congcong He	SP0042114	60047929	Mechanism of autophagy activation in the prevention of type 2 diabetes	Department of Health and Human Services	5R01DK113170-04
Robert J Vassar	SP0045606	60050195	Molecular and cellular mechanisms of the UNC5C netrin receptor in Alzheimer's disease pathogenesis	Department of Health and Human Services	1 RF1 AG057727-01A1

Advisory Committee

CJ Heckman, Ph.D., Professor, Physiology, Physical Medicine & Rehabilitation, Physical Therapy & Human Movement Sci., Chair of the committee, c-heckman@northwestern.edu

David Johnson, Ph.D., Assoc. Dean Res. Operations-Med, davej@northwestern.edu

Jennifer Kearney, Ph.D., Assoc. Professor Pharmacology, Jennifer.kearney@northwestern.edu

Robert Vassar, Ph.D., Professor, Neurology and Cell & Developmental Biology, r-vassar@northwestern.edu

Martha Vitaterna, Ph.D., Res. Professor, Center for Sleep and Circadian Biology, m-vitaterna@northwestern.edu

Appendix III: Recharge Rates

Service	FY22 Hourly Rate ($)*			
	NIH Full Service	NIH Self Service	NU Full Service	NU Self Service
Watermaze	116	60	100	50
DigiGait	136	52	100	50
DigiGait Key Rental (per day)		166		150
LimeLight**	104	64	100	50
Fear Conditioning**	116	96	100	50
Startle/Prepulse Inhibition (PPI)***	114	52	100	50
Rotarod**	104	52	100	50
Surgery			100	50
Eyeblink Conditioning	124	52	100	50
General Use		192		100
Data Analysis	154		150	
Robotic Stereotaxic	104	64	100	50

*Rates are subject to change without notice.
NIH rates should be used when projecting budgets for grant applications. NU rates are used for billing.
**4 mice can be tested simultaneously
***2 mice can be tested simultaneously

Appendix IV: User Fees at Other Universities

Instituation	Full Serve	Self Serve	Training Rate	Statistical Analysis	Funding	Director	Website
UCLA	$115/hr	$55/hr	$90/hr	$75/hr	UCLA Service Fund	Lindsay Lueptow	https://btc.psych.ucla.edu/rates/
Stanford	$138/hr (FY20)	$63/hr (FY20)	x	x	x	Mehrdad Shamloo, PhD	http://med.stanford.edu/sbfnl/services/rates.html
Univ. Colorado	x	x	x	x	x	Michael Mesches, PhD	https://medschool.cuanschutz.edu/neurotechnologycenter/Cores/animal-behavior/about
Salk Institute	$60/hr	$25/hr	Free	$95/hr	NINDS Core	Nick Andrews, PhD	https://www.salk.edu/science/core-facilities/behavior-testing-core/scheduling-and-rates
Univ of Houston	$40/hr	$20/hr	$20/hr	$70/hr	x	Colin N. Haile	http://uh.edu/animal-behavior-core/about/
Univ of Rochester	$40/hr + technician time of $40/hr	$40/hr	x	$75/hr	x	Katherine Bachmann, M.S.	https://www.urmc.rochester.edu/environmental-health-sciences/cores-facilities/behavioral-sciences-facilty/animal-facilities/pricing.aspx
Univ of Kentucky	$59/hr + $24 lab reservation fee	$25/hr	$60/hr	x	x	Bruce O'Hara, PhD	https://www.research.uky.edu/rodent-behavior-core/rodent-behavior-core-fees
UC Davis IDDRC	x	x	x	x	x	Jacqueline Crawley	https://health.ucdavis.edu/mindinstitute/centers/iddrc/cores/rodent_behavior.html
Univ. Penn	x	x	x	x	x	W. Timothy O'Brien Ph.D.	https://www.itmat.upenn.edu/nbtc.html
Univ. Washington	x	x	x	x	x	Thomas M. Burbacher	https://depts.washington.edu/chdd/iddrc/cores/abc.html

An example AAP is on the following two pages.

TITLE	:	**Examination of Open Field and Zero Maze Behaviors AAP in BPC**
SCOPE	:	This Approved Animal Procedure (AAP) is applicable to all Center for Comparative Medicine (CCM) and research personnel involved in the care and maintenance of laboratory mice and rats.
PURPOSE	:	The open field arena is used to evaluate ambulation patterns and exploration of objects or another subject within the arena. It does not involve punishment or reward.
RELATED DOCUMENTS	:	None

Background

Approved Animal Procedures (AAPs) have been approved by the IACUC. If you use an AAP in your protocol, you must cite the AAP title and agree to follow it exactly with no modifications.

- In the PI Library; create a new AAP procedure type titled "Examination of Open Field and Zero Maze Behaviors AAP in BPC."
- On the Experimental Groups page, add the new AAP procedure in the applicable experimental group(s).
- Update the sequence and timing sections in each experimental group to include the time point(s) of when the AAP will be performed.
- On the Define Procedure Personnel page, select "Core Personnel" for this AAP and include any lab members from your own lab who will perform the procedure.
- On the Animal Housing and Use page in 1.0 Animal Housing, under "Housing Type" select "Conventional" and under "Building" select "Chicago : Ward." This is CCM decentralized housing space.
- On the Animal Housing and Use page in 3.0 Animal Use, under "Building" select "Chicago: Ward," under "Facility Type" select "Lab Space" and include "BPC procedure room." Check the "Use Duration" box for <12 hours.
- On the Animal Care Exceptions page, indicate you require an exception to the Pair/Group Social Housing program if applicable.
- Users of this AAP must be trained by the staff of the Behavioral Phenotyping Core.

Staffing

Open field behaviors will be tested by the personnel listed in the ASP into which this AAP is placed, and by the staff of the BPC. .

Examination of Open Field Behaviors

Purpose: The open field test is designed to measure behavioral responses such as locomotion, hyperactivity, and exploratory behaviors. Open field is also used as a measure of anxiety. Rats and mice tend to avoid brightly illuminated, novel, open spaces, so the open field environment acts as an anxiogenic stimulus and allows for measurement of anxiety-induced locomotor activity and exploratory behaviors.

Materials:

- Video camera and Limelight software system (This computer system is recommended but optional.)
- Open Field or Zero Maze

- Disinfectants and odor que Cleaning supplies (e.g., Windex, Sparkle, Clidox, or 70% Ethanol)
  Odor cues such as food grade vanilla or banana extract, lemon oil
- Kimwipes
- Data Sheet

Experiment:

Bring the animals to be tested into the testing room for acclimation.

Note if there are any health related concerns.

Turn on the lights inside the isolation chamber.

Turn on the t software (it should default to 4 cameras and 4 arenas)

Align the maze in the chamber so that it is centered when viewed with the software system.

Set the analysis grid for a 5 x 5 array of cells. Confirm the arena width to be 56 cm.

Set the duration of the experiment (typically 5 minutes).

Create a data folder and name the files:

File: Open Data File: Make folder with users name then make folder with user's initials and date.

Assign a file name to each of the four chambers.

Click the "Reference" button to collect a baseline image for each of the four chambers.

Place each animal in a corner of the field and click the "Start" button to record its behavior for five minutes. Measures such as total distance, average speed, rearing/elongation behavior, and time spent in various parts of the field (e.g. the border areas vs. the open, middle area) are calculated.

At the end of the five minute period return the animal to its home cage. Clean the arena with ethanol then spray the arena with C-Dox. After a minimum of five minutes, wipe away the disinfectant with a known odor. Repeat the process with additional animals to be tested.