NTP
National Toxicology Program

Environmental Enrichment in NTP Studies

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Presentation overview

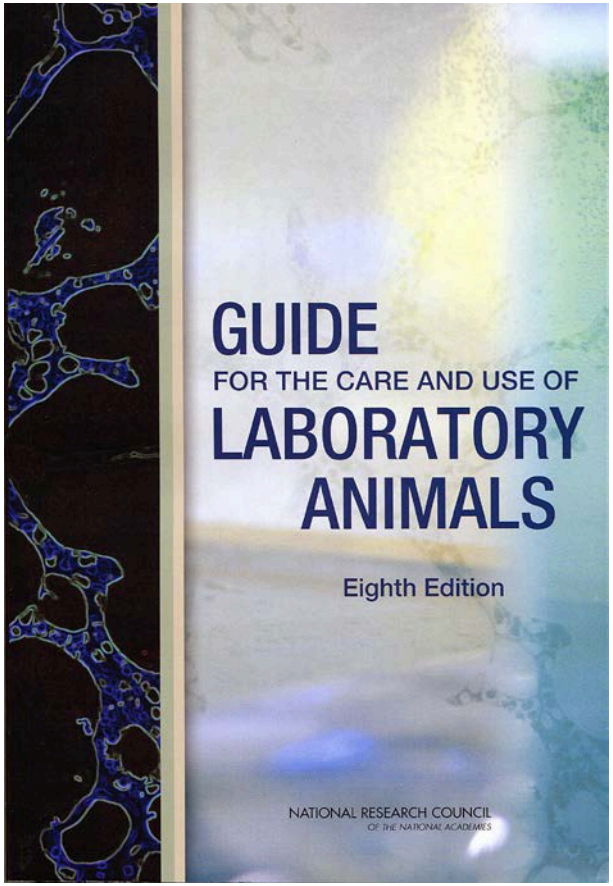
- Definition of environmental enrichment
- Rationale for use of environmental enrichment
- Considerations when using enrichment items
- The NTP Enrichment Program
- Implementation of environmental enrichment strategies in NTP studies

Environmental enrichment: any measure which promotes expression of natural species specific behavior and a decrease in (or disappearance of) abnormal behaviors.

Brinkman C (1996). Toys for the boys: environmental enrichment for singly housed adult male macaques (*Macaca fascicularis*). Laboratory Primate Newsletter 35, 4-9.

- The *Guide for the Care and Use of Laboratory Animals,* 8th edition National Academies Press, Washington DC, 2011 (The *Guide*)

- The *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (PHS Policy) requires that Assured Institutions base their animal care and use programs on the *Guide*



The *Guide* – NIH adoption and implementation plan

- The NIH adopted the 8th edition of the *Guide* effective January 1, 2012
- Evaluation of the animal care and use program, and the plan and implementation schedule for the 8th edition of the *Guide* must be developed by December 31, 2012.
  - Applies to all institutes that receive NIH funding
- Major changes found in the new *Guide*:
  - Inclusion on a section on environmental enrichment for all species
  - Changes in housing standards
  - Inclusion of guidelines for aquatic species
- The NTP is compliant with many changes from the new *Guide* (*e.g.*, currently use larger cage sizes for dams with litters)

Environmental enrichment rationale, The *Guide*, 8th ed.

- Primary aim of environmental enrichment is to enhance animal well-being by providing animals with sensory and motor stimulation.
- House animals with enough space with supplementary structures and resources to meet physical, physiologic, and behavioral needs.
- Social animals should be housed in stable groups or pairs unless experimental reasons or social incompatibilites necessitate single housing.
- Environments that fail to meet the animals' needs may result in abnormal brain development, physiologic dysfunction, and behavioral disorders. The primary enclosure may need to be enriched to prevent such effects.

Office of Animal Care and Use (OACU) of NIH

The Animal Research Advisory Committee (ARAC) suggested guidelines for housing rats and mice in the laboratory:

- Rats and mice benefit from being socially housed whenever possible.
- Mice benefit from being housed on nestable bedding or being provided with a suitable substrate with which to build a nest.
- Rats benefit from being provided with increased structural complexity, i.e., nestbox, platforms or paper towels.

Enrichment Strategies for Rodents in the Laboratory, ARAC 2004

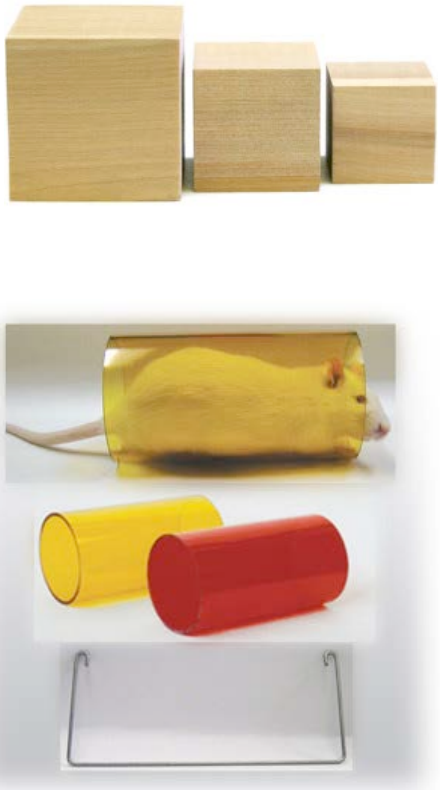
Considerations for enrichment

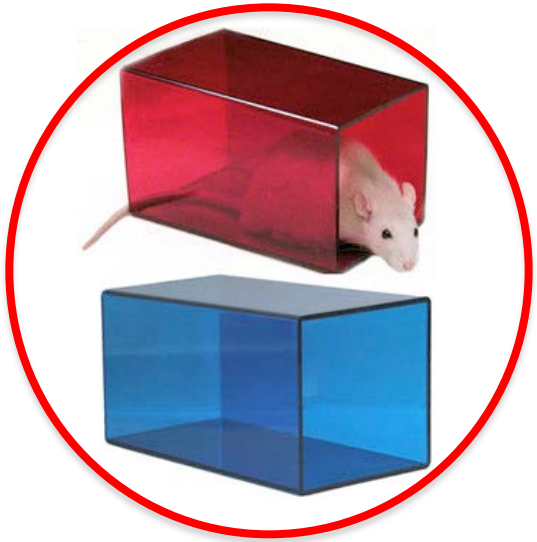
- Enrichment program strategies should be based on natural behaviors of the animals under consideration.
  - Rodents (mice and rats)
    - Nocturnal, nest building, burrowing, foraging, gnawing
    - Thigmotaxic - dislike open spaces
    - Prefer shelters (nest or other types of shelters)
  - Mice are nest builders
  - Rats prefer gnawing but will build a loosely constructed nest with the “right” materials

Considerations for enrichment, cont.

- Social housing allows animals to perform social behaviors such as grooming, vocalization, and play.
  - Animals that cannot be group housed should at least have visual contact with conspecifics
- Physical enrichment items should be biologically relevant items that have intrinsic value.
  - Allows animal to display species appropriate behavior
- Enrichment should provide animals with choices and a degree of control over their environment allowing them to better cope with environmental stressors

Enrichment items for rats and mice





http://www.bio-serv.com/category/Rodent_Enrichment_Devices/18.html

Environmental enrichment at other animal facilities

Facility	Socialization	Physical device	Other	Comments
SRI	Group housed rats and mice	Rodents are given: Nestlets or plastic shelter/tube	All animals are given a consumable treat 2 x/week unless exempted	
BNW – non-NTP studies	All animals singly housed	Rats: nylabones to chew on Mice: polypropylene tubes	NA	Prefer not to use nestlets. Want to try bedding with twisty paper.
BC – non-NTP studies	Group housed as appropriate but male mice are singly housed	Rats: Nylabones, polycarbonate huts Mice: Sheppard shacks	Bedded cages are also considered enrichment	
NIEHS - CMB	Single or group housed	Mice: Autoclaved nestlets or Sheppard shack Autoclaved paper towels or crinkled paper	NA	Type of enrichment device changed if needed
NIAID/CMB	Not provided	Mice: at least one nestlet per cage except in nude mice. Rats: provide to each cage either a plastic hut, igloo, PVC pipe or plastic tubes	NA	All rodents are provided with enrichment unless PI requests otherwise or for medical reasons
NCTR – non NTP studies	Not provided	Rats: Plastic tunnels, nylabones Mice: plastic igloos, nestlets	NA	
UNC CH	Group housed for compatible animals	Breeding mice: nesting material Mice: PVC tunnel or nyla-rings Rats: crinkled paper	NA	

Current NTP practices incorporating enrichment

- Rats and female mice are group housed in solid bottom polycarbonate cages
- Male mice, and rats and mice in whole body inhalation studies are individually housed but have visual, auditory and olfactory contact with conspecifics.
  - Wire caging in whole body inhalation studies allows tactile contact with adjacent conspecifics
- Contact bedding is used in polycarbonate cages for dosed feed, dosed water, dermal, and gavage studies
  - Irradiated hardwood chips

Enhancement of NTP's current strategies

- Routine consideration of inclusion of enrichment items
  - All individual study protocols shall address enrichment
  - The agent being studied will be considered when addressing enrichment
- All enrichment items shall be contaminant-screened.
- Nesting material added to bedding shall be irradiated.
- All study animals and sentinels shall be given the enrichment device.
  - Animals shall have the same type of enrichment device for the same amount of time

Enhancement of NTP's current strategies, cont.

- Device shall be provided upon animals arrival during acclimation until study termination.
- Testing laboratories shall provide SOPs for environmental enrichment and personnel shall be appropriately trained.
- Enrichment will be phased in starting with short-term studies, then subchronic studies and lastly chronic studies.
  - This allows the NTP to assess the impact of enrichment of the study/agent

Enrichment items shall be included in:

- Dose range finding (DRF), subchronic and chronic toxicology/carcinogenicity studies
  - Dosed Water Studies
  - Dosed Feed Studies
  - Gavage Studies
  - Nose-only Inhalation Studies
- Reproduction and Developmental studies
- Chemistry characterization studies
  - ADME (except metabolism cages)
  - Toxicokinetics
  - Palatability
- Dermal Studies
  - Need to determine the effect of enrichment items on the agent being studied

Enrichment items for NTP studies - Rats & Mice

Natural (unbleached) crinkled kraft paper

- **Mice:**
  - Encourages nesting behavior in male and female mice
  - Shall be added to the cages of single and group housed mice
  - 6-8 grams shall be added to each clean cage set-up
- **Rats:**
  - Allows rats dams to better care for their offspring
  - Shall be added to the cages of pregnant dams and dams with litters
  - 12-16 grams shall be added to each clean cage set-up

Photo courtesy N. Flagler

Enrichment items for NTP studies - Rats & Mice

Natural crinkled kraft paper

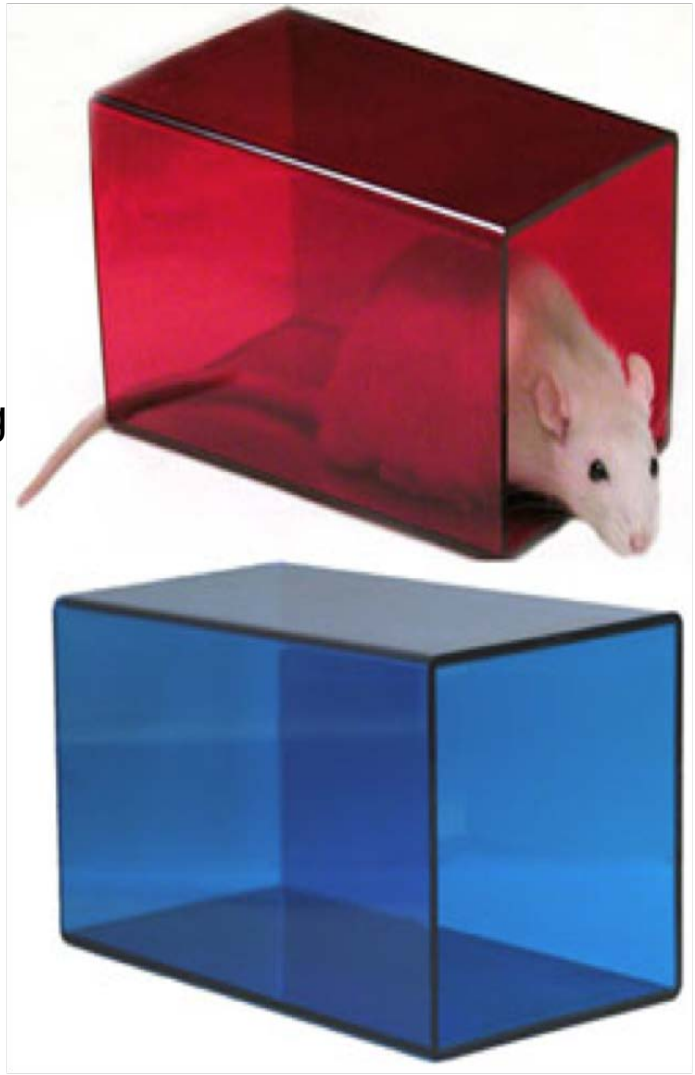
- Unbleached crinkled kraft paper shall be purchased from a NTP specified vendor that specializes in bedding products for laboratory animals
- Crinkled kraft paper shall be irradiated prior to use
- Crinkled kraft paper shall be analyzed by the vendor for contaminants prior to use:
  - Pesticide residues, microbial contaminants, heavy metals, aflatoxins

http://www.ssponline.com/enviro_dri.htm

Enrichment items for NTP studies - Rats only

Polycarbonate rectangular shelters

- Shall be used for weanling to adult rats and breeding pairs
- Allows rats to perch on top and go inside
- Shelters shall be 3.75“ wide X 3.75” high X 6” long
- Vendor must provide contaminant screening information prior to use
- Shelters will be agent and dose specific
  - Shelters for control groups can be used in any study
- Shelters shall be sanitized at each cage change
  - Cloudy/damaged shelters are replaced immediately
  - Shelter are made of high temperature polycarbonate plastic so that potential damage is minimized



Implementation of the enrichment program

- Enrichment use will be phased
  - The NTP will start with short-term studies and then 90 day studies and finally 2 year studies.
  - This approach will allow the NTP to assess the impact of enrichment

- Testing laboratory's SOP must be in place prior to use; personnel will be trained on the use of enrichment

Practical considerations for inhalation studies

- House animals in solid bottom cages when they are not being exposed
  - Highly labor intense
  - Will increase the cost of inhalation studies significantly
- Devices in cage during exposure may impact air flow to the animal
  - Consider the use of specialized shelving
  - Pilot studies?

Photos courtesy of N. Flagler & B. Mahler

NTP Animal Welfare Committee

Dr. Raj Chhabra

Dr. Jean Harry

Dr. Angela King-Herbert

Dr. Bill Stokes

Charge

To review and comment on the information presented on NTP's guidelines for using environmental enrichment in NTP rodent studies.

1. What other suggestions might you have for the NTP as it moves forward in using enrichment devices for rodents in NTP studies?
2. Please identify any caveats that should be considered when using enrichment devices in NTP studies.
3. Provide any other comments you feel NTP staff should consider as the NTP moves forward in incorporating environmental enrichment into studies.