NAFTA Developmental Neurotoxicity Guidance Document

Motor Activity Testing

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Outline

- Lecture Series on NAFTA Guidance Continues
  - Last time – NAFTA Background and FOB & Clinical Observations
  - This time – Motor Activity Introduction
- Methods
  - Common methods used
  - Important features that influence motor activity and ontogeny
- Interpretation:
  - Examples of control data
  - Examples of test data – the good, the bad, and the ugly
- Summary

Goals of the NAFTA Document

- Improve understanding of DNT guideline studies
  - Appropriate conduct of behavioral tests
  - Biological significance of endpoints
- Increase consistency in interpretation and assessment of outcomes
- Focus on behavioral tests
  - Observations
  - **Motor activity**
  - Auditory startle response
  - Learning and memory
- Neuropathology/morphometrics not included
  - Several excellent peer-reviewed publications already available

Courtesy V. Moser

What is “motor activity”

- US EPA Test Guidelines
  - “any movement of the experimental animal”.
  - Although this is a very broad definition, motor activity is more typically considered to be locomotor movements in a horizontal direction (ambulation) as well as other directions (e.g., vertical -rearing)
  - Smaller movements such as grooming, sniffing, stereotypies are not normally considered locomotor – but may be measured by some apparatus (more below)
  - Motor activity is an apical behavior that reflects a number of underlying processes including motor capacity, sensory functioning, emotional processing, non-associative learning

Why measure motor activity

- Motor function is a critical part of normal behavior
- IN DNT testing:
  - Motor activity levels provide a sensitive measure of apical nervous system function.
  - The ontogeny of motor activity follows a developmental pattern that reflects the development and maturation of the nervous system.
  - Motor activity studies provide a simple measure of non-associative learning (habituation), a basic form of learning essential to adaptive behavior and critical for normal interaction of animals with their environment.
- Decades of research show it to be sensitive to chemical stressors

DNT Guidelines

Testing Requirements for Motor Activity

	EPA 870.6300	OECD 426	OECD 443
Test species	Rat	Rat	Rat
Exposure	GD6 to weaning	GD6 to weaning	2 weeks pre-mating to weaning
Motor activity	**Preweaning ontogeny and adult**	**Preweaning and adult**	**Preweaning and adult**
Neuromotor ontogeny	None	Preweaning	None
Functional/Clinical observations	Throughout	Throughout	Adult
Auditory startle response	Weaning and adult	Weaning and adult	Weaning
Learning and memory	Weaning and adult	Weaning and adult	None
Neuropathology and morphology	Weaning and adult	Weaning and adult	Adult

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Requirements (con't)

- EPA 870.6300
  - Multiple ages - PND13, 17, 21 and 60 (±2)
    - Same animals repeated tested
  - Must use automated apparatus
  - Must be capable of detecting increases and decreases
  - Session length must be long enough to achieve asymptotic activity levels by last 20% of session (i.e., habituation)
  - Activity recorded in 10 min bins
  - Treatment groups should be:
    - Counter-balanced across test devices
    - Counter-balanced across test times

- *MUST control confounding variables, e.g.:*
  - sound levels, size and shape of the test cage, temperature, relative humidity, light conditions, odors, use of home cage or novel test cage, and environmental distractions.

Requirements (con't)

OECD 426

- Multiple ages – 1 to 3 times preweaning, and young adult (PND60-70)
  - Ontogeny 'strongly suggested"*
- Same animals tested across days
- Session length must be long enough to achieve asymptotic activity levels (i.e., habituation)
  - (no % or time bins, and note
  - If PND 13 or 17 only there will be no habitation
- Must be capable of detecting increases and decreases
- Must use automated apparatus
- Standard procedures for reliable operation
- Treatment groups should be:
  - Counter-balanced across test devices
  - Counter-balanced across test time to control for circadian rhythms

- *MUST control confounding variables, e.g.:*
  - sound levels, size and shape of the test cage, temperature, relative humidity, light conditions, odors, use of home cage or novel test cage, and environmental distractions.

* Testing on PND13, 17, 21 fulfills OECD's requirement for behavioral ontogeny evaluation

Requirements (con’t)

OECD 443 Extended One Gen

- DNT cohort is optional
- If used motor activity testing:
  - Only once between PND63 and 75
  - Methods must be consistent with OECD 424 and 426

Factors that may influence motor activity measurements

- Organismal Factors
  - Age
  - Sex
  - Species and Strain
  - Biological Rhythms
  - Previous Experience
  - Food Deprivation
- Experimental Factors
  - Test chamber type
  - Detection method
  - Social environment
  - Environmental conditions
  - (lighting, noise, temperature, humidity, odor, housing)
  - Length of test session

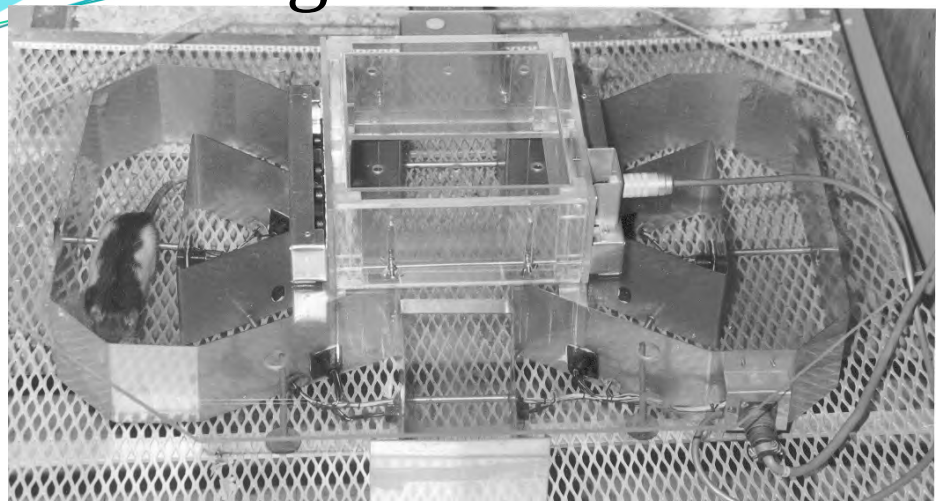
From: Crofton and MacPhail, 1996

Factors (con’t_

- A critical aspect in the interpretation of motor activity testing is understanding how the testing apparatus works and how an animal moves in the device
- Common measurement Technologies
  - Photobeam, inductive field, stabli-meters, video tracking
  - All detect movement
- Device shape
  - Circular, square, rectangular, maze-like
  - Shape will influence behavior

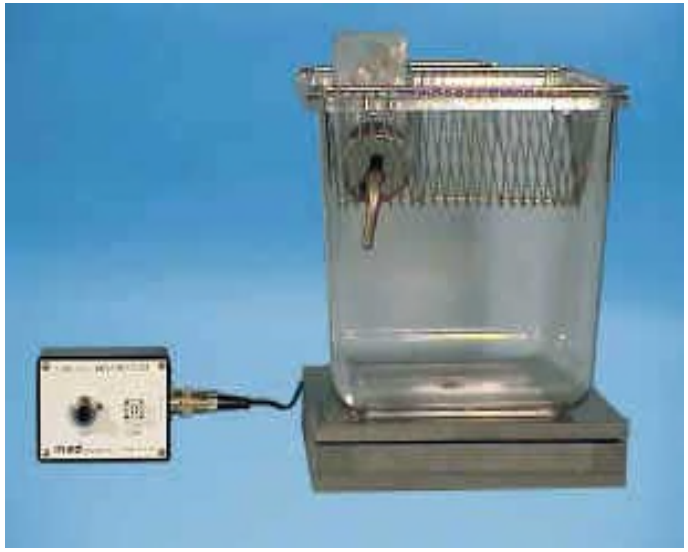
For more details see Reiter an MacPhail, 1982

Figure 8 Maze



Open Field

Inductive Field



Circular ‘donut”

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Examples of Common Devices

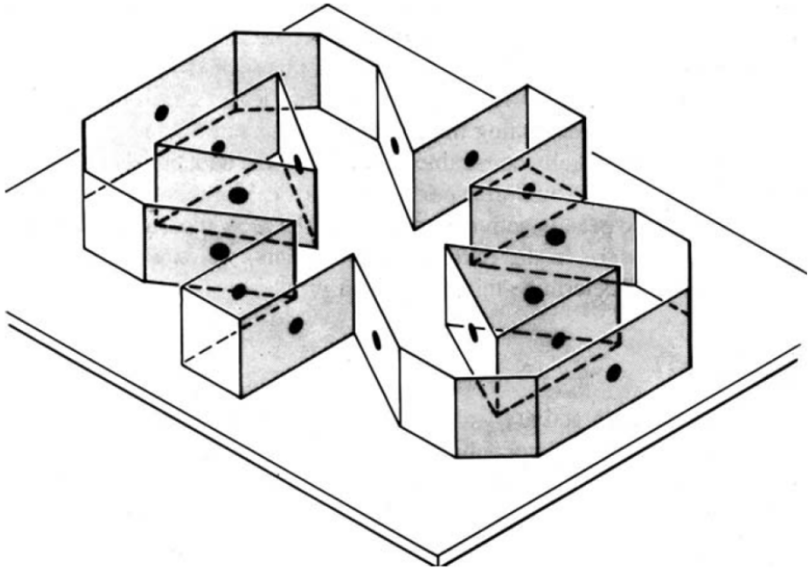


Figure-8 mazes

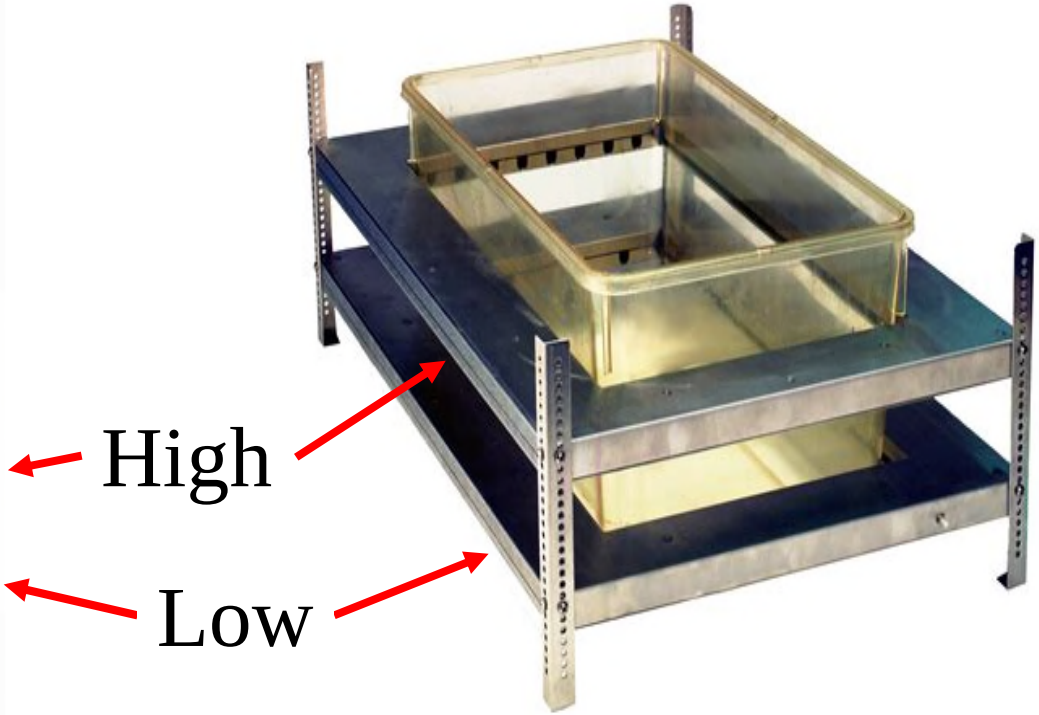
- Dots = 8 beams widely spaced
- Size based on wild rat burrows

Open Field systems

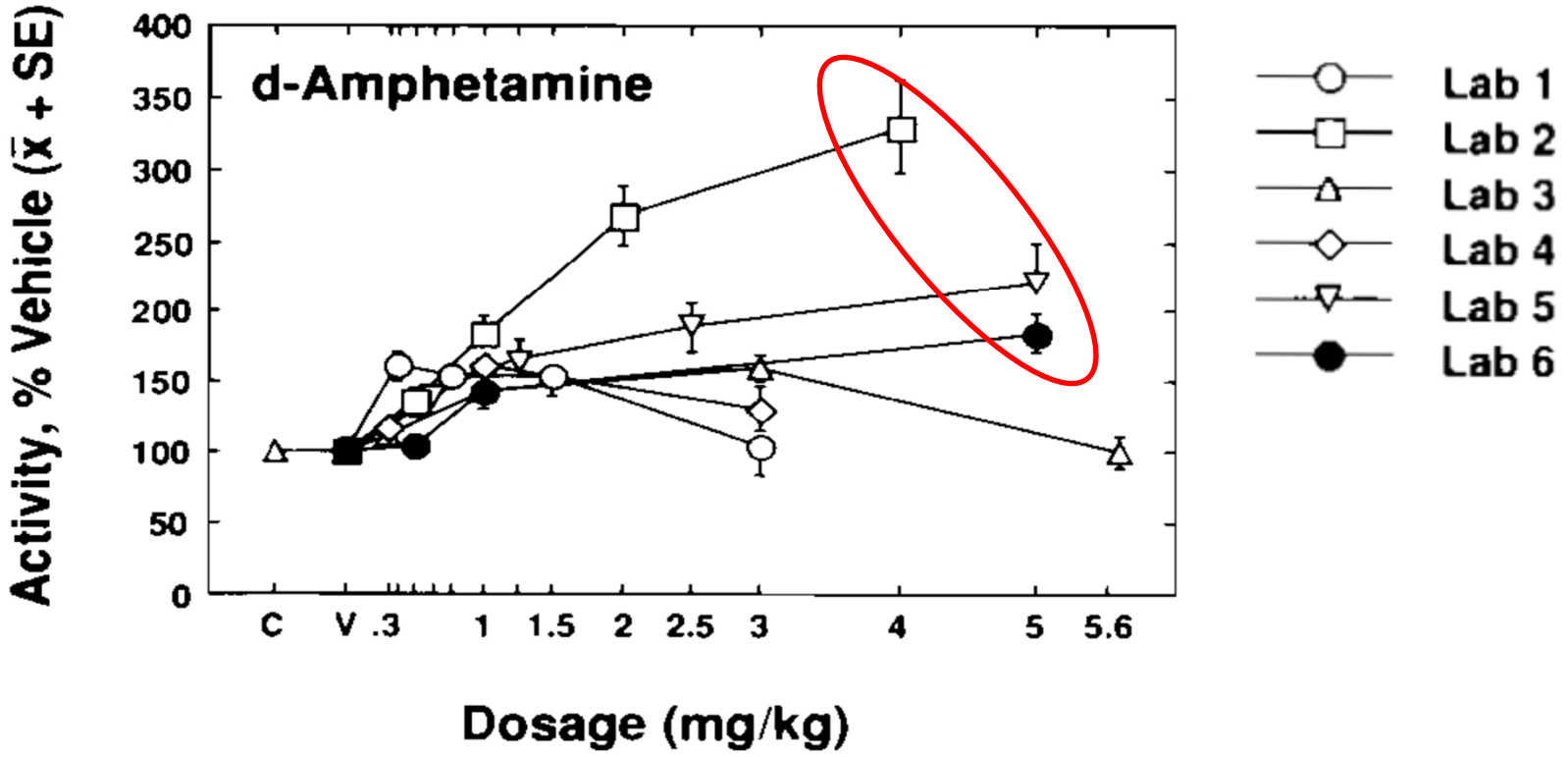
- Can have lots of beams (e.g., 16 each on x- and y-axes closely spaced)

Low vs High Photobeams

- Low can be set for small and large animals
- High can be set for rearing behavior



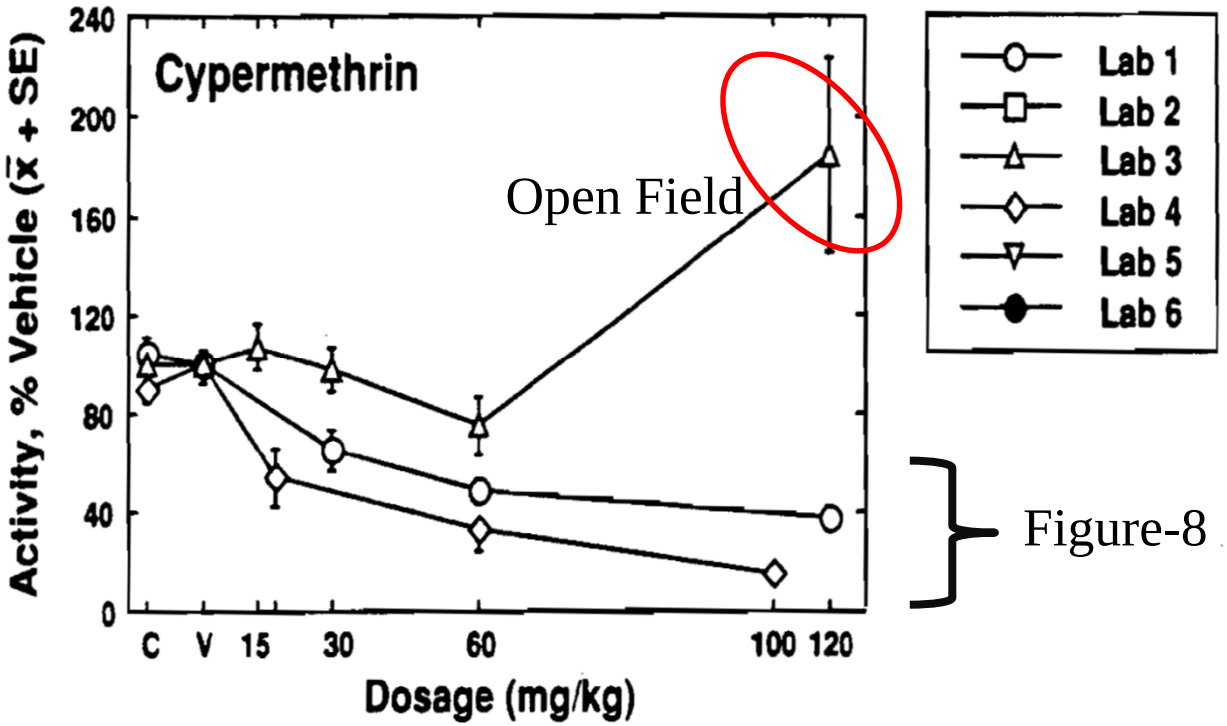
How does Test Device Impact Results?



- d-Amp will cause increased locomotion at lower dose followed by stereotyped behaviors at higher doses
- Devices with widely spaced beams will show a U-shaped curve because they are not designed to detect small movements
- Devices with closely space beams detect both large and small movements

Crofton et al, 1991

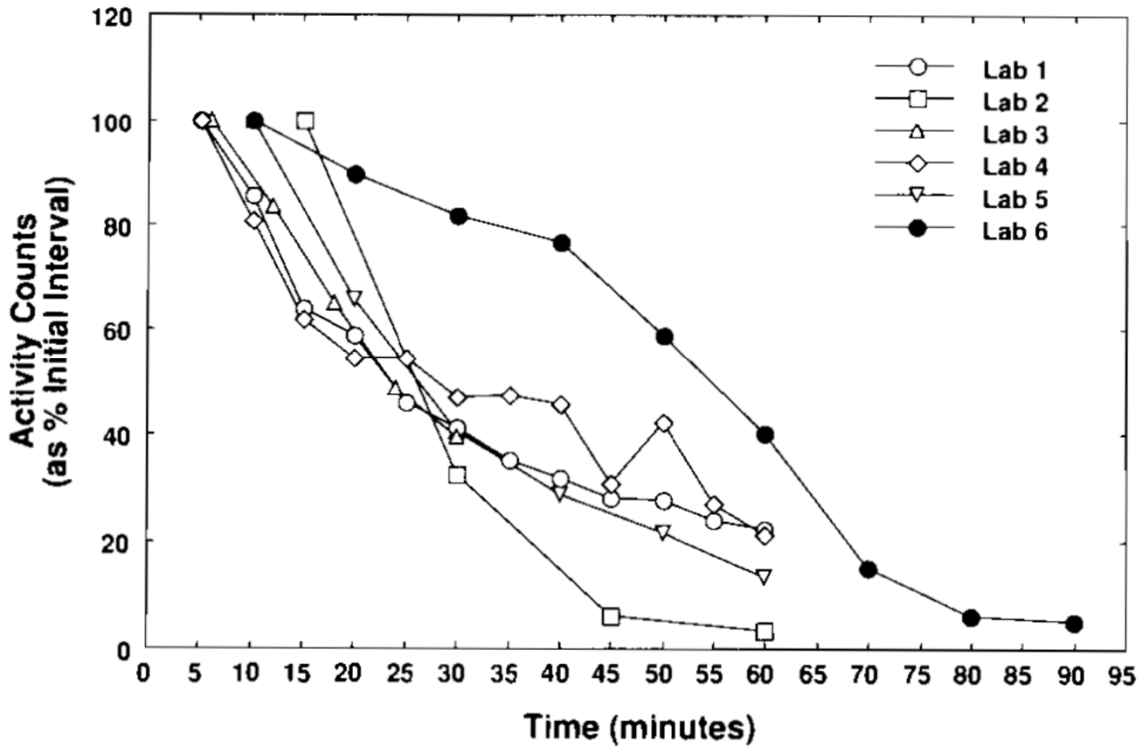
How does Test Device Impact Results?



- Cypermethrin will depresses motor activity at the lower doses but higher doses induce the Type II syndrome which include small movements (e.g.,pawing, burrowing, coarse tremors, choreoathetosis) that can be detected as increased photobeam counts

Crofton et al, 1991

Test Session Duration – Adults



- Data from 4 different devices from 6 different labs
- Most reach asymptote within about 60 min

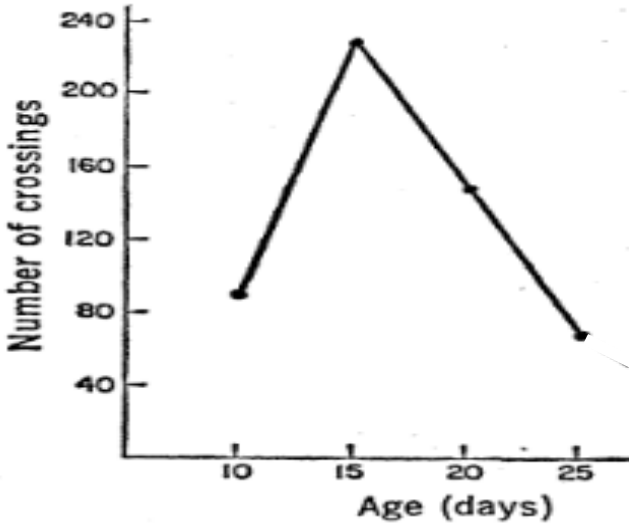
Crofton et al, 1991

Developmental Testing - Preweaning

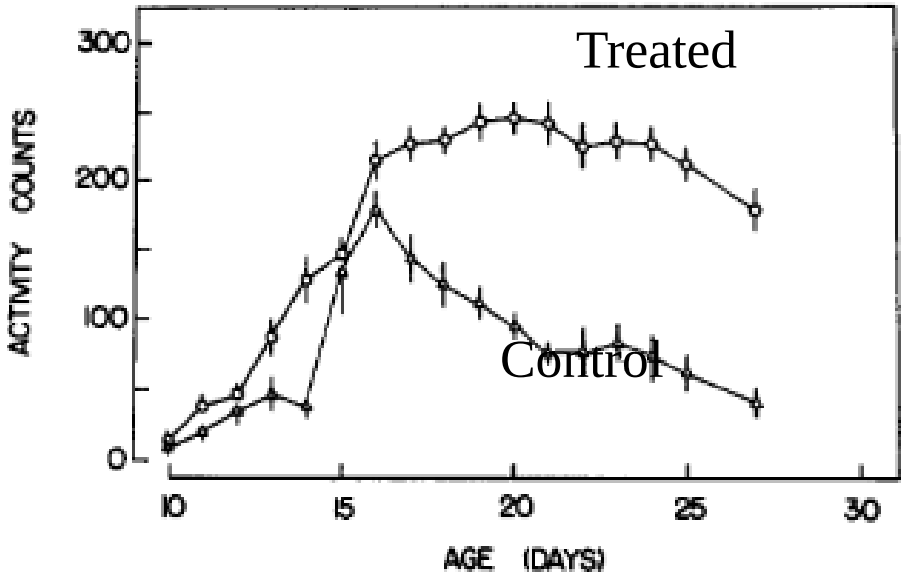
- Why do the Guidelines require/suggest motor activity testing on PND13, 17 and 21.
  - It is the only automated test of functional ontogeny
  - Development of motor activity has two important components that interact:
    - Activity levels
    - Habituation (simple form of learning)

Motor Activity Development

- Motor activity peaks at the end of the second postnatal week – multiple reports from different labs and different devices*
- Repeated testing is critical



Campbell et al, 1969



Erinoff et al, 1979

* *When testing on PND13, 17 and 21 activity may be similar between 17 and 21. It can be device and lab dependent. Check historical control data!*

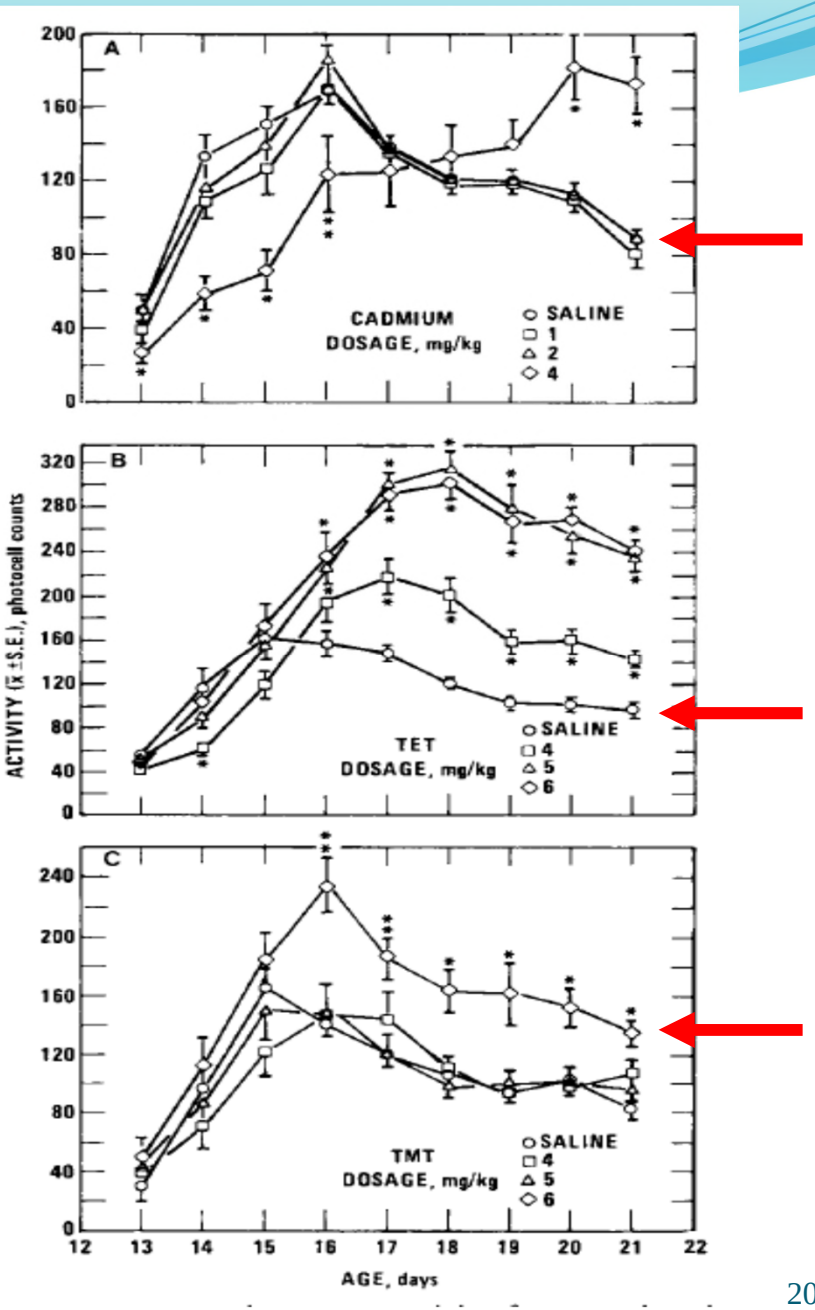
Motor Activity Development

Chemicals can cause disrupt classical patterns in motor activity ontogeny

Delay

Overshoot

Suppression



Ruppert et al 1985a; Ruppert et al., 1985b)

→ = control group

Reminder: Important Information in Study Protocol and/or Report

- Look for
  - Type of device used (and calibration)
  - Treatment balanced across time of day and test boxes
  - Good environmental control (e.g., noise, lighting, smell, experience)
  - Training and experience of technical staff
  - Whether same animal is tested each time
  - Experimenter blind with respect to treatment
    - *(not as important with automated equipment compared to FOB)*

Data Checks and Interpretations

- **Control group**
  - Activity levels should be age-appropriate
    - e.g., activity levels should follow expected ontogeny
  - Variability should not be excessive and decline with age (see Raffaele et al. 2008)
    - Need to look at raw data for outliers
  - Habituation should develop at correct age (by ~PND21)

Data Checks and Interpretations (con't)

- **Historical control and positive control data**
  - Historical control data
    - demonstrate reliability of techniques
    - Allows comparison to identify possible "outliers"
  - Positive control data should be available & helps in interpretation
    - Able to detect chemical induced changes (competency)
    - Show sufficiency of technical personnel
    - Defines the dynamic range
      - e.g., amphetamine: does the device report an increase of 30% or 200%

Data Checks and Interpretations (con't)

- Look at both intrasession (habituation) and total session data

- Statistics (briefly)
  - Within session testing (habituation) is a repeated measure!
  - Multiple testing of the same animal is a repeated measure!
  - SEX – must be included in the stats model
    - If not, there should be no conclusions of sex-dependent effects

Data Checks and Interpretations (con't)

- Statistical vs biological significance
  - There needs to be balance in determination of effects
  - From NAFTA Guidance:

    "guideline DNT studies are conducted to screen chemicals for possible adverse effects on the developing nervous system and are often the only study examining all of these endpoints. Thus, consideration of a higher false positive rate rather than a lower false negative rate may be a more conservative approach in some cases.
- Large or excessive variance can occur due to poor control over testing and possible false negatives
  - Compare to within lab results (historical control) or other labs using similar equipment

Examples – Good Report

- Company X submits DNT with the following
  1. Extensive methods section
  2. Positive control data showing ability to detect increases and decreases (in adults)
  3. Positive control data shows appropriate age-dependent activity in controls and statistically significant changes due to treatment
  4. Historical controls from other DNT guideline studies

Good data – Pesticide Y

Test Day	Dietary concentration (ppm)			
	0	Low	Middle	High
Males				
PND 13	8 ± 9	15 ± 22	10 ± 10	10 ± 13
PND 17	45 ± 27	47 ± 35	55 ± 28	50 ± 32
PND 21	93 ± 36	73 ± 27	92 ± 28	75 ± 38
PND 60	344 ± 71	384 ± 101	381 ± 102	383 ± 89
PND 120	255 ± 56	257 ± 72	281 ± 99	270 ± 61
Females				
PND 13	7 ± 10	4 ± 6	9 ± 14	7 ± 10
PND 17	57 ± 49	50 ± 38	50 ± 34	46 ± 36
PND 21	91 ± 38	84 ± 31	79 ± 33	80 ± 36
PND 60	471 ± 112	438 ± 164	456 ± 132	482 ± 185
PND 120	350 ± 90	369 ± 179	335 ± 110	373 ± 156

- Variance - not excessive
- Ontogeny – Peak on PND21? Consistent with historical control data from this lab
- Adult females – higher counts than males? Also consistent with historical control data

Examples – not great

- Chemical X (pesticide)

TABLE 9. Mean (±S.D.) motor activity data[a]

Test day	0 ppm	Low	Middle	High
Males				
PND 13	5.6 ± 3.9	14.1 ± 7.0	20.3* ± 8.9	7.6 ± 3.6
PND 17	13.3 ± 10.0	19.9 ± 7.2	23.9 ± 18.1	42.8* ± 11.9
PND 21	21.2 ± 15.2	21.1 ± 18.1	18.6 ± 18.7	28.4 ± 16.1
PND 60±2	99.7 ± 51.9	118.6 ± 35.8	107.9 ± 49.7	91.5 ± 43.8
Females				
PND 13	13.3 ± 6.3	16.3 ± 6.0	14.3 ± 6.6	25.2 ± 8.9
PND 17	23.3 ± 14.0	17.7 ± 13.4	22.1 ± 13.7	22.9 ± 7.1
PND 21	21.4 ± 21.9	23.9 ± 20.5	17.0 ± 12.3	16.2 ± 13.4
PND 60±2	97.0 ± 37.8	84.9 ± 37.8	78.9 ± 42.1	97.4 ± 32.9

- Controls: Males peak at PND21 – females at PND17??
  - No historical control data supplied and other guideline studies from this lab show both males and females peak on PND17
- Treatment: Effect only in males at middle dose – may not be significant if sex included as a dependent variable in the stats model)

Examples – Stats vs Biology

DNT for Compound Z (industrial chemical)

- Report states no significant findings for motor activity at any age
- Brief review of the data:
  - Age-related ontogeny and activity level are normal
  - No effects or trends early ages
  - Excessive variance in adult age testing: CVs >100% in male high dose group
  - Dose-related decreases up to 41%
  - Same trend in both males and females

Table XX. Mean (±SD) for total session activity counts in F1 adults (PND62-66)

	Age	Control	Low	Midde	High
Males	62	597.1±394.7	564.3±332.6	445.5±201.6	353.4±402.3
Females	62	606.1±224.9	636.3±349.2	459.5±352.8	375.6±301.1

Next steps: Need in depth review. Why high variance, need to look at HC and PC from this lab, and why testing across 5 days?

Take Home Messages

- The test apparatus is important
  - What does the apparatus actually measure?
- Critical to control of external variables to prevent excessive variation
- Sex effects – statistical effect or an opinion?
- Statistical vs biological significance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