Maternal and sexual phenotypes

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Maternal behavior

- Covers a wide range of behaviors
- An important factor that increases survival

Includes:

- Nest building
- Nursing,
- Licking
- Ano-genital grooming
- lifting or moving pups,
- Retrieving pups,
- protection of the pups

Michael Forster Rothbart

Weber, 2005

Maternal behavior

- Commonly, observations of maternal behavior are made on mice housed in barren cages
- These cages contain nothing but sawdust and do not provide the animals with the opportunity to hide, to build nests or to make burrows
- Mice are highly explorative animals and the barren environment thus restrains the animals from performing many natural behavior patterns

Weber, 2005

Maternal behavior

- Most studies concentrate on a specific item in the complexity of the behaviors, such as **maternal** aggression, communal nesting, infanticide etc.

- Pup retrieval tests are often used to measure **maternal behavior** and it is considered to be highly characteristic for the rodents to retrieve pups if they are absent from the nest

Weber, 2005

Procedure

- All females were mated in pairs at the age of approximately 10 weeks
- They were separated from the male and housed singly when visibly pregnant (around two weeks after mating)
- The cages were kept in the racks (for side view filming only) or on the floor (when filmed from above) during recording
- When moved out of the rack, females were habituated in the new location for one hour prior to filming

Weber, 2005

Behaviors and definitions

Behavior, location	**Definition**
Cage top (CT)	One or more paws on cage top but no paw touching nest, nest box or floor
Floor (FL)	Two or more paws on the cage floor except the nest, the tube and the cage box
Nest (NE)	>50% of the body in nest area. Nest area defined as a structure made from paper and other loose parts from the cage and organized into a cluster in different shapes. When paper is missing, area defined by a.cavity in the sawdust where the animal spends most time resting
Chewblock (CB)	Sitting or standing on top of the chew block
Nest box (NB)	Animal on or in the nest box (red PVC nest box) with >50% of the body
Tube (TU)	Animal inside or on the paper tube with >50% of the body, does not include if the tube is a part of the nest
Tube in nest (TN)	Animal on or in tube with >50% of the body, when tube is a part of the nest

Weber, 2005

Behaviors and definitions

Digging (DG)	Digging in the sawdust litter
Bar circling (BC)	Repeatedly tracing a circle on the cage bars (movement repeated more than twice)
Circling (CC)	Chasing own tail in circle movements
Eat/drink (ED)	Intake of food or water (from feeding site or elsewhere)
Exploring (XX)	Sniffing the components of the cage e.g. sawdust substrate, cage equipment, cage walls, airborne odours or the environment external locomotion without obviously exploring
Hidden (HI)	Animal is behind any structure or out of camera view, and not seen. Does not include when animal is hidden in the nest by nest structure (see Nest activity and Nest still)

Weber, 2005

Protocol: Observation of dam behavior

1) Assessed dam behavior from day 5 to 14
2) Observations of dam behavior were recorded over a 30-min period using 10 spot checks at 3-min intervals
3) Each observation was instantaneous and a note was made whether dams were in or out of the nest
4) A dam was considered to be in the nest only if she was engaged in nursing

Rocha et al., 2002

Pup retrieval test

- On day 4, the female was removed from the home cage and placed in a cage containing nothing but sawdust
- The pups were then gently moved from the nest, weighed and placed back in the home cage in a definite position from the nest

Weber, 2005

Pup retrieval test

- Always place the pups with the same distance from the nest
- After placing the pups in their position, return the female to the home cage and placed in the nest
- The test ends when all pups had been retrieved, or, if this did not happen, after 15 minutes
- Video record the test
- Measure the time for start and end of pup retrieval

Weber, 2005

Definition of behaviors

Direct complete (DC)	Female retrieves pup from original (primary) position and places it in the nest, also includes if the female leaves the pup but retrieves the same pup again within 30s and places it in the nest
Indirect complete (IC)	Female retrieves pup from other than original position and places it in the nest
Incomplete (I)	Female retrieves pup from the original position or elsewhere but does not place it in the nest, also includes if the female leaves the pup but retrieves the same pup again within 30s and still does not place it in the nest
Manipulate nest (MN)	Female manipulates pup within the nest
Sniff young (SY)	Female sniffs at young without retrieving or manipulating it
Manipulate outside nest (MO)	Female manipulates young outside nest without retrieving

Weber, 2005

Summary of merged behaviors

Merged behavior	**Behavior category**
Nest building	Manipulate Paper, Manipulate Tube, Manipulate Chewblock
Pup Activities	Pup activity, Groom pup, Pup retrieval
Nest behaviors	Pup activity, Groom pup, Nest active, Nest still, Nurse pup, Pup retrieval
Locomotion	Exploring, Circling,Bar circling
Stereotypies	Circling, Bar circling
Tube	Tube in, Tube on
Totalnest	Nest, Tube in nest

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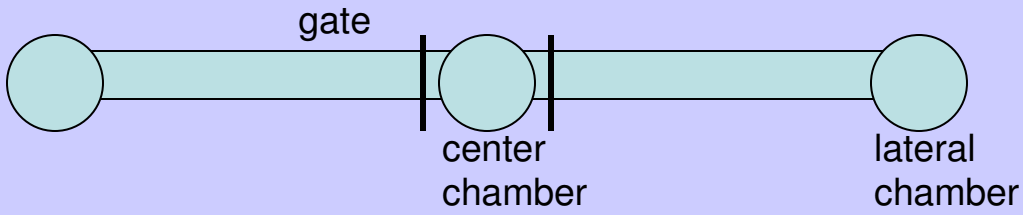
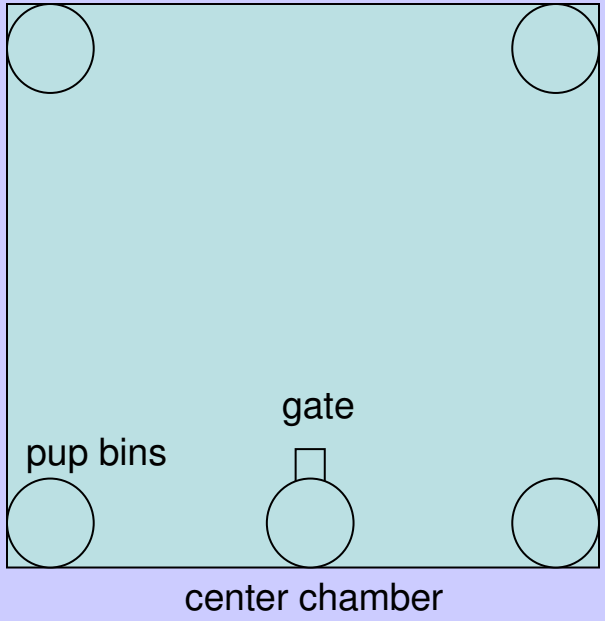
Pup retrieval apparatus

Two-choice apparatus



Open Field Apparatus



Hess et al., 2002

Practical considerations

- Observing maternal behavior can be a difficult task
- All females built complex nests and most often the pups were never seen
- It was often impossible to tell the difference between self-grooming, licking, lifting or moving pups, or manipulating material
- It was also difficult to see if the female was nursing or sleeping, since small movements were difficult to detect

Weber, 2005

Protocol: Modified pup retrieval test

1) On day 7 after delivery, move the home cage from the colony room to an adjacent anteroom, where the pups are separated from the dam and kept in a plastic container
2) Position each pup one-by-one in the cage on the side opposite to the nest, regardless of dam position
3) Record the latency to retrieve each pup, presented individually
4) If the mother has not retrieved within 120 s, the pup must be returned to the nest by the experimenter and the next pup is immediately positioned in the cage on the side opposite to the nest, regardless of dam position

Rocha et al., 2002

Protocol: Little disturbed retrieval test

1) On day 10 after delivery, the home cage was removed from the colony room to an adjacent anteroom
2) After transportation of the cage, a short period of time (5 to 10 min) was given to the mother to readapt to the new position of the cage
3) The dam was then gently moved to one side of the nest and one pup was selected at random and placed on the side opposite to the nest.
4) The latency to retrieve this single pup was recorded
5) If the dam had not retrieved within 180 s, the test was ended

Rocha et al., 2002

Protocol: Pup-retrieval test

- Is accurate marker of maternal care. Some endpoints show a strong correlation with pup survival

- The following endpoints can be measured:

  a) the time elapsed between the first contact and the picking up of the first pup

  b) the number of times the female moved away from one of her pups, positioned outside the nest, without transporting it

  c) the percentage of females remaining in the nest with all their pups for 2 consecutive minutes during the 15-min observation period ("nest with all pups")

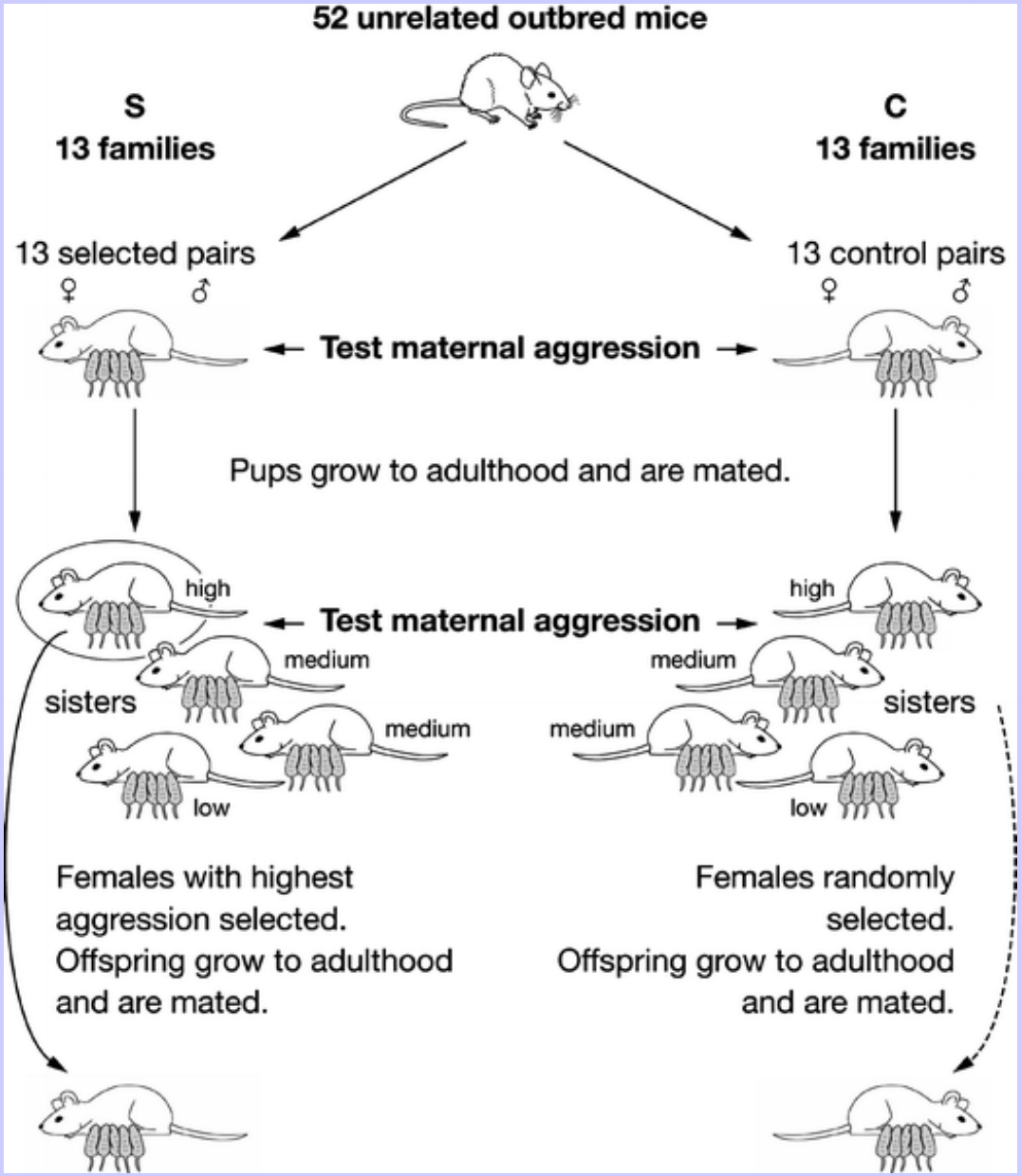
- 3rd pup retrieval test [latency to retrieve the 3rd pup]

Pardon et al., 2000

Protocol: Maternal aggression test

1) All females tested for maternal aggression on PD 8, the first day after the peak display of maternal aggression
2) Sexually naive adult male wad used as intruders, placed in the home cage of the female in the presence of her litter for 10 min
3) Each test session was video-recorded and subsequently analyzed. Each intruder was used only once
4) Behavioral features for both the intruder and the female:
    - number of aggressive moves (rapid thrusts toward the intruder, falling short of physical contact)
    - number of attacks (bouts of fighting characterized by biting or intense body contact)
    - Latency of the first attack
    - Total duration of attacks on intruder
    - the amount of time attacking different regions of the male (including head/neck, flank/back, or a combination of these two general regions)
    - time spent in the nest
    - number of tail-rattling episodes
    - number of pup retrievals (by the female)
    - pup survival at the end of the test

Pardon et al., 2000; Gamie et al., 2006



Gammie et al., 2006

Ultrasonic Vocalization in rat pups

- Commonly used as a measure of anxiety behavior, but clearly has a social component as well
- More social species of rodents often emit more vocalizations
- When removed from their nest, mouse pups emit ultra-sonic vocalizations at 50-80 kHz in protest and these calls can be detected
- Vocalization in mouse pups is highly dependent on both temperature and age of the pups

Young, 2003

Protocol: Ultrasonic vocalization in rat pups

1. Place the home cage with pups 2-14 days of age in a room with a stable temperature, or on a heating pad to maintain pup temperatures above 30ºC
2. Remove the mother for 15 min before testing. The tests should be carried out in a room with no adult mice
3. After placing the mouse pup in a clean mouse cage, place the ultrasound detector (e.g., Pettersson Elektronik AB) over the pup so that the calls are audible
4. The locomotor activity of the pup during the test may also be recorded
5. The calls should be recorded for 2-5 min, and the number of calls during the tests is recorded

Endocrine phenotypes: Prolactin (PRL)

Peptide hormone associated with lactation

Synthesized and secreted by lactotrope cells in the pituitary gland

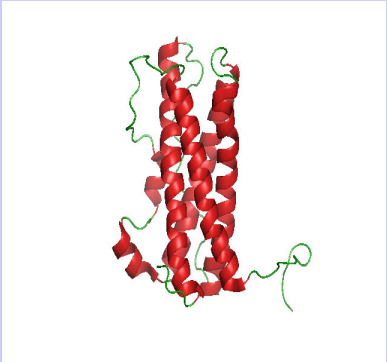
Regulated by hypothalamic neurons

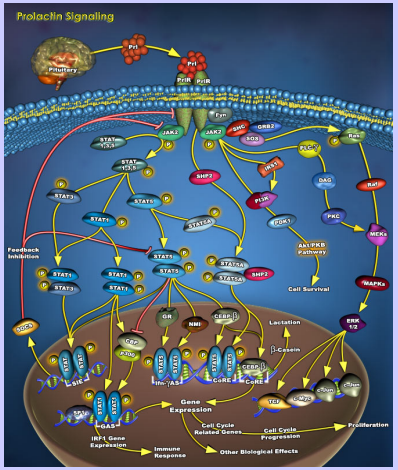
Activates multiple signaling pathways

PRL receptors are highly expressed in anterior pituitary

Regulates maternal behavior in females

Plays role in the regulation of nest building in male and female mice





Impaired nest-building

Wild type male

VDR KO male

Abnormal maternal behavior:
100% pup-eating

Kalueff et al., 2006, J Neurosci Res

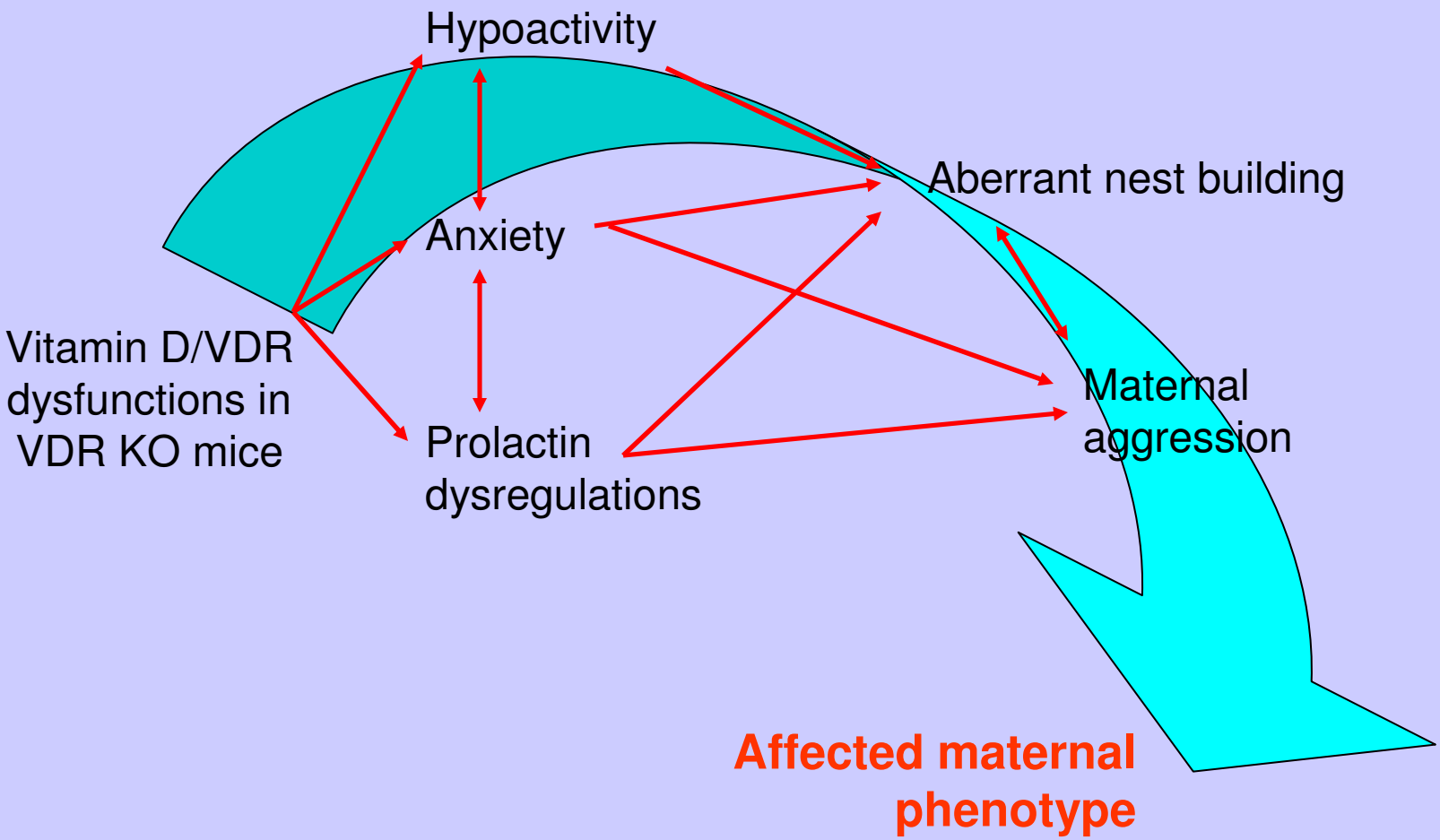
VDR -/- mice

Prolactin?

Keisala et al., 2007:

Increased plasma prolactin
Abnormal rythmicity of prolactin secretion in VDR-/- mice

From behavioral phenotypes to endocrine abnormalities



Sexual phenotypes

http://www.flyfishingdevon.co.uk

Sexual Behavior

- Highly regulated by gonadal steroid secretion
- Testing often performed on gonadectomized, hormonally replaced experimental animals

Sexual Behavior

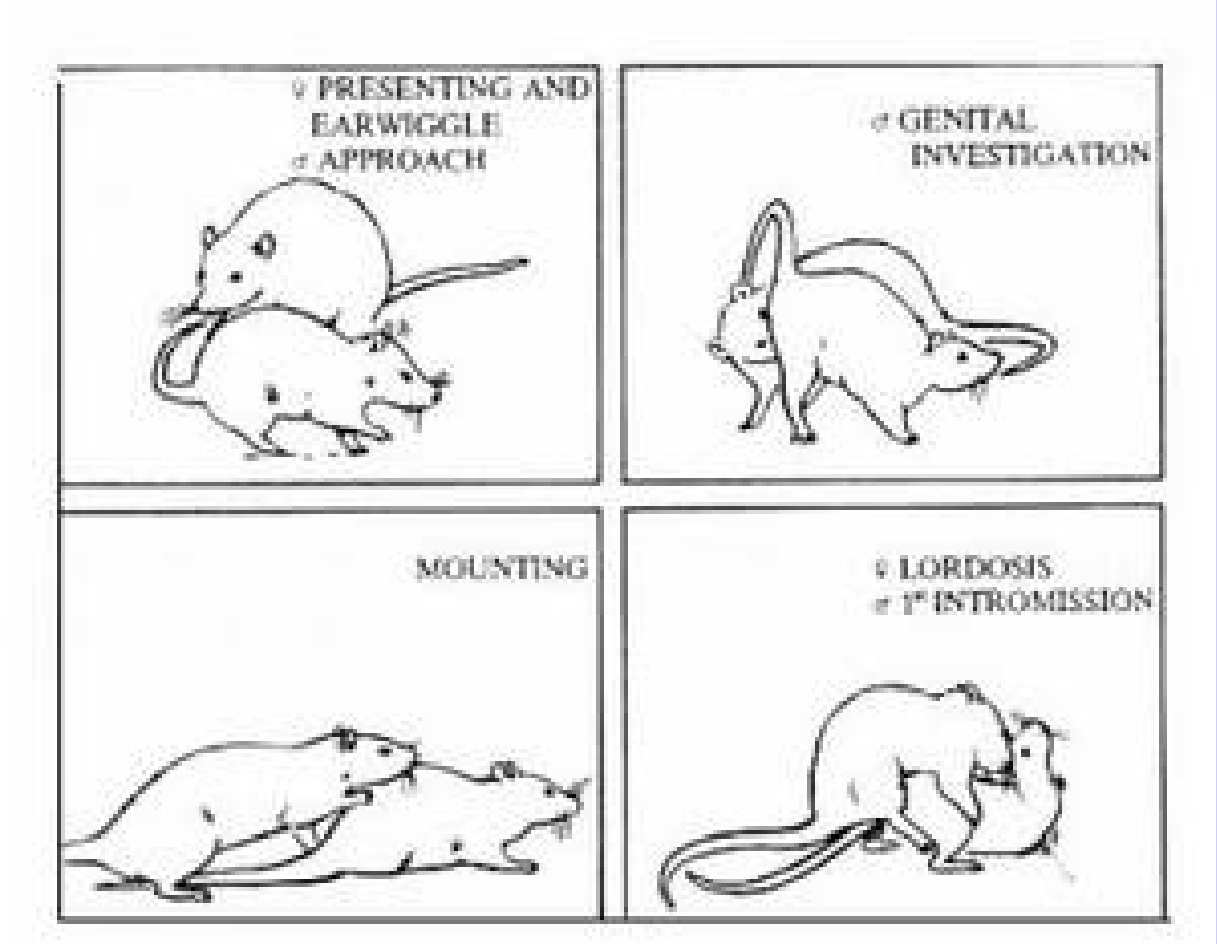
Females:

- exhibit receptive behaviors such as darting, hopping, and ear wiggling
- Assumes “lordosis” position (arches back, elevates head and rump, extends back feet, deflects tail to one side) when mounted

Males:

- Follow a fixed sequence of motor patterns starting with anogenital investigation, then mounting, pelvic thrusts, intromissions (vaginal penetration), and ejaculation

Sexual behaviors



www.pelvipharm.com

Sexual motivation

- Males quantified in presence of female in adjacent chamber with audio, visual, and olfactory cues available but no physical contact
- Latency and number of penile erections

Other tests:

- Males measured through operant lever tasks reinforced with access to receptive females
- Place preference tests for locations previously shared with a sexual partner

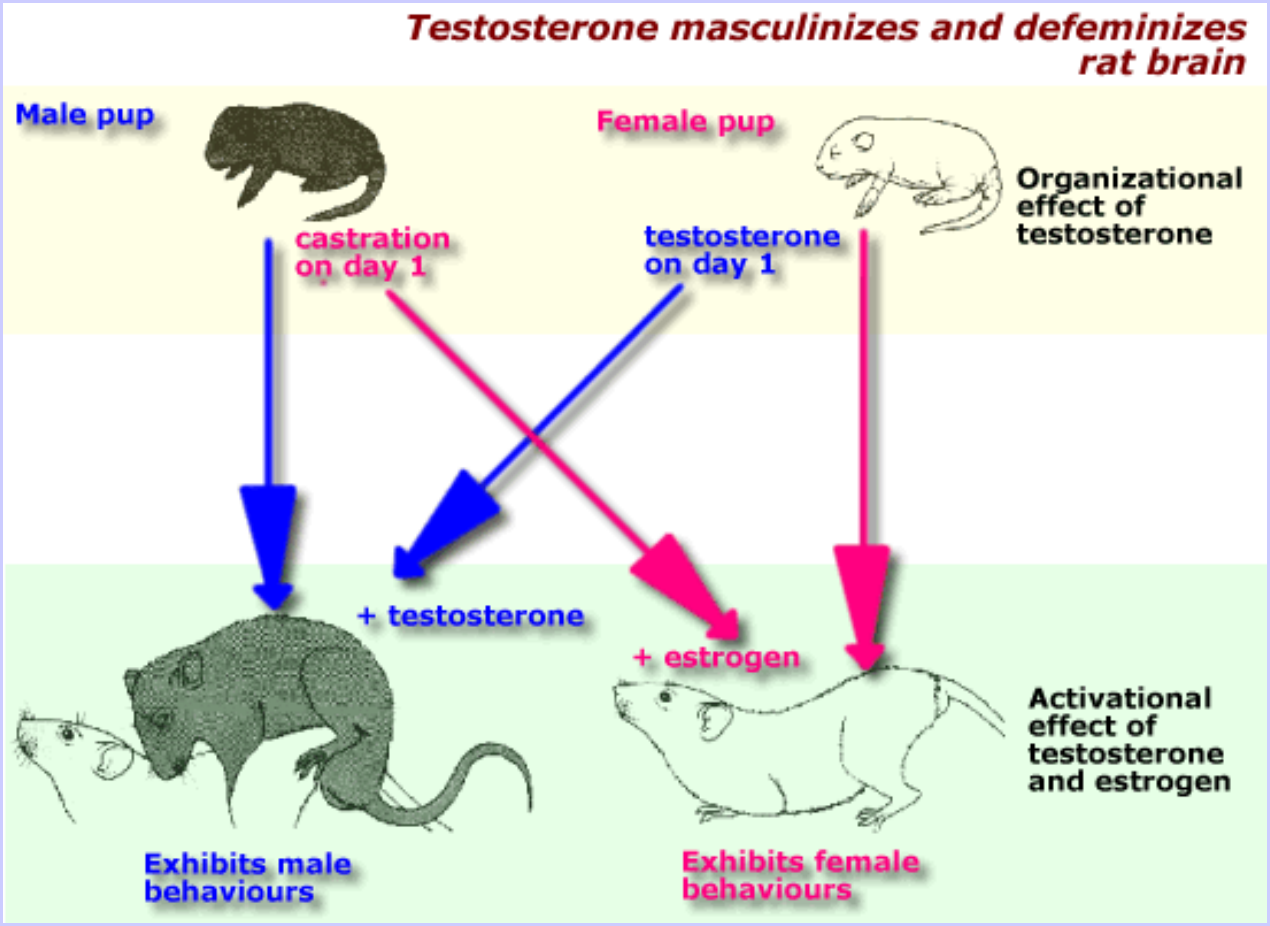
Crawley, 2000

Protocol: Sexual behavior

- Female is placed in a sexually experienced male's home cage for 30 min
- Female mice display receptive behavior by allowing the male to mount without resistance and exhibiting a lordosis
- Total number of attempted mounts, successful mounts, intromissions, and the number of mounts in which the female displays lordosis is recorded
- The number of attempted mounts is a good indicator of the attractivity of the female to the stimulus male

Young, 2003

Effects of male castration and female testosterone treatment



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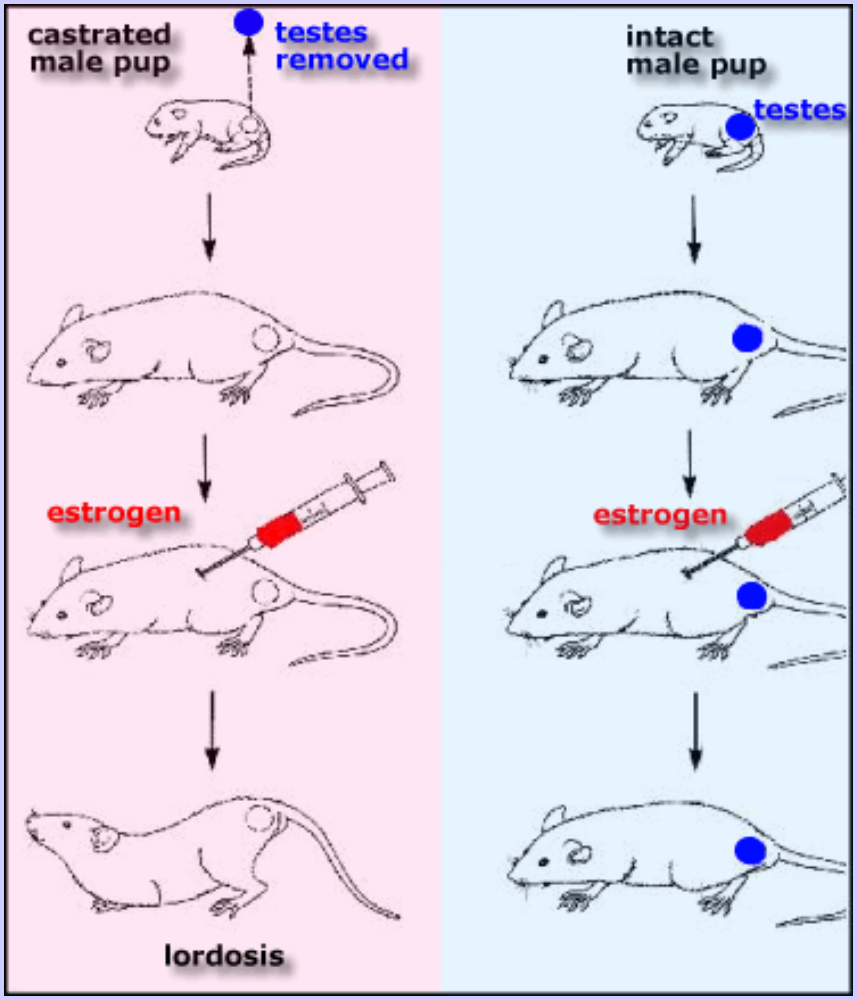
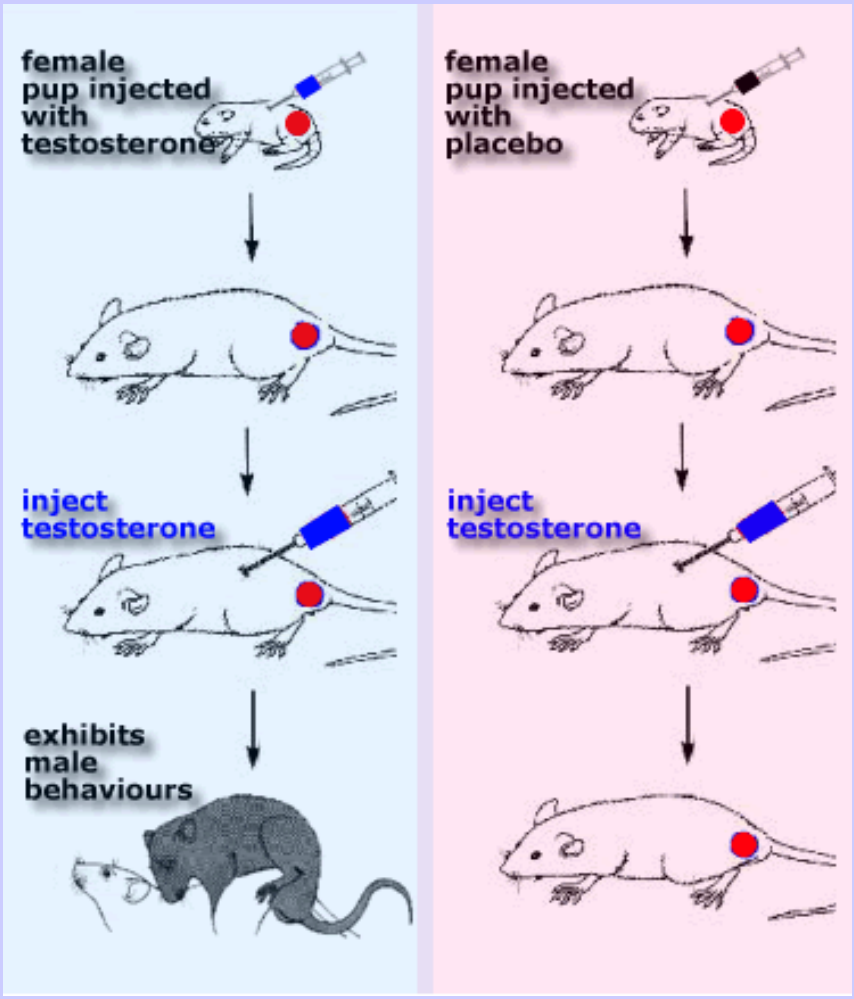
Feminization of male rat by castration in infancy



Masculinization of female pups by injection of testosterone in infancy



http://www.flyfishingdevon.co.uk

Protocol: Male sexual behavior

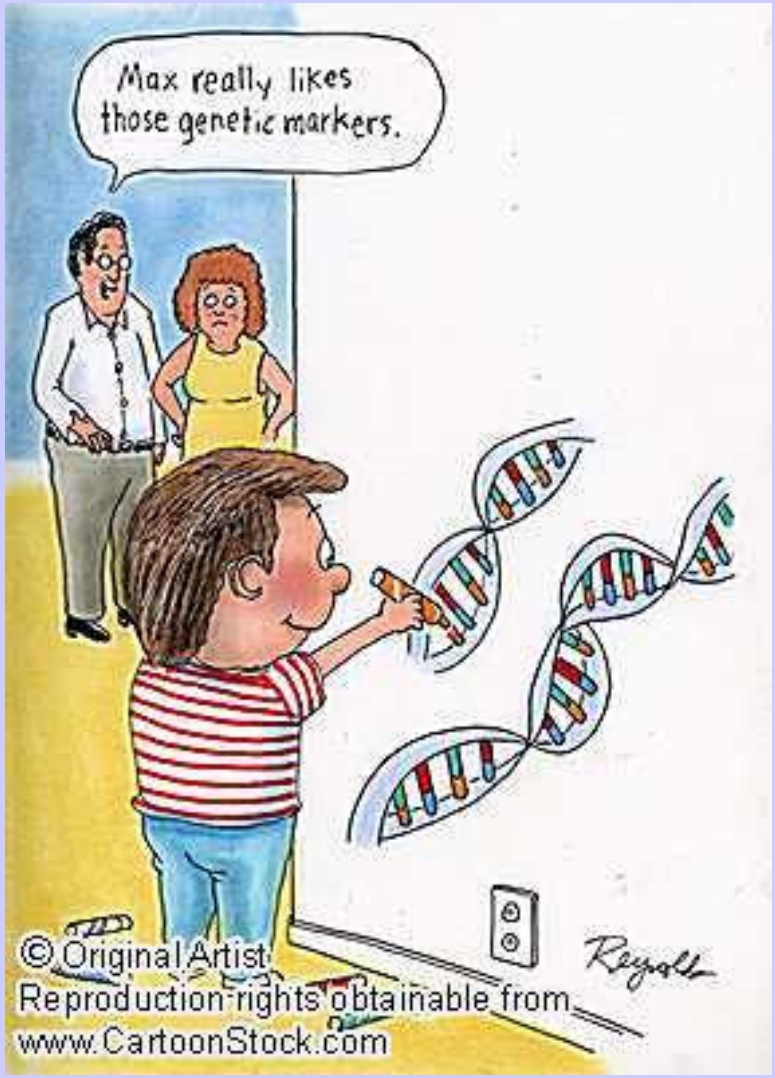
- Ovariectomized, hormonally-primed females used as stimulus animals
- Males are singly housed for at least 1 week and the tests are carried out in male's home cage
- The female is placed in the cage and the behavior is recorded for 30 minutes
- The number of mounts, intromissions, and ejaculations, as well as the inter-ejaculation interval should be recorded

Young, 2003

Patterning of male sexual behavior

- Sexual behavior is a strictly patterned activity
- Investigation/sniffing -> Follows -> attempted mounts -> mounts -> ejaculations
- Patterning can be affected by different drugs or mutations
- Assess all patterns in their sequences
- Analyze “correct” vs. “incorrect” transitions between different stages
- Assess correctly initiated vs. incorrectly initiated bouts
- Assess completed vs. incomplete bouts (%)
- Build ethograms

Kalueff and Tuohimaa, 2005


Max really likes those genetic markers.
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