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Behavioral Core Protocols and Training

Estrous Cycle Staging

The Rodent Estrous Cycle

Rats and mice are spontaneous ovulators – i.e. they do not need the presence of males to induce ovulation. There is some evidence the stimulation from male rodents can induce and or/hasten ovulation, but it is not obligatory. Rats and mice typically have a 4-6 day cycle which consists of 4 stages – estrus, proestrus, diestrus I (a.k.a metestrus) and diestrus II.

Terminology

You will notice that there are 2 spellings for each of the terms for the cycle stages – one ending in “us” and one ending in “ous”. The “us” form (as for other similar English vocabulary) is a noun. The “ous” form is an adjective.

Estrus can refer to a stage of the cycle or to the behavioral manifestation of behavioral receptivity (also known as “heat”). With rodents, the most highly receptive time is actually proestrus, although many females will still mate the following day, when they are in estrus

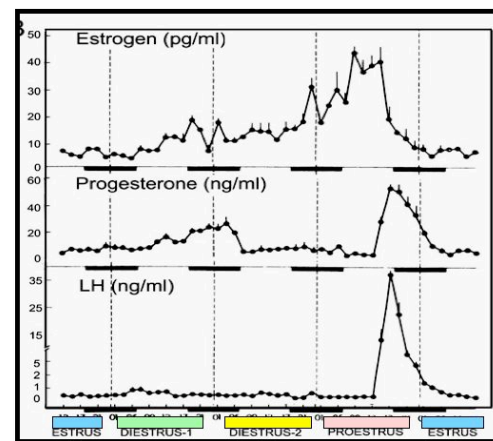
Difference between rats and mice

Mice are not just little rats. With regards to the regulation of hormonal cycles and reproduction, this is especially true. In general mice are more olfactory than rats. Mice are also typically more sensitive to introduction of new males (see article regarding the Whitten Effect, Bruce Effect etc)

Differences from Human Menstrual Cycle

One of the main differences between the rodent and the human cycle, other than the overall time it takes for a full cycle, is that the peaks of estrogens and progesterone are typically separated in humans, whereas these overlap in rodents. Rodents are also more sensitive to estrous cycle disruption from light zeitgebers than are humans.

THE RAT ESTROUS CYCLE



From: Smith MS, Freeman ME,
Neill JD. Endocrinology
1075;96:219-226.

A simple method for determining estrus cycle stage is known as a vaginal lavage or vaginal smear. The 4 stages can be distinguished by noting characteristic cell types that are visible during each stage, in addition differences in cell density. A small amount of a physiological saline solution is inserted into a rats vagina with a disposable pipette, removed, placed on a slide and examined under a microscope.

Approximately 200-500 microliters of saline are used for vaginal lavage in rats and 50 – 200 microliters for mice. Animals are either held by the tail in the home cage or picked up and held in a similar manner to what one would use for i.p injections.

Label an eyedropper with colour-coordinated tape for use with each rat; avoid cross-contamination. Alternatively, use disposable plastic pipettes or a gilson-type pipettor (cut the tips slightly to prevent clogging) Label a slide with the date, time, and the rat's name and colour. Introduce a few drops of 0.9% saline into the eyedropper and gently insert about 0.5 cm into the vagina. Express the saline back and forth two or three times by quickly squeezing and releasing the dropper bulb, then remove the resultant fluid and express it onto the labelled slide. Keep the slide flat and do not let it dry.

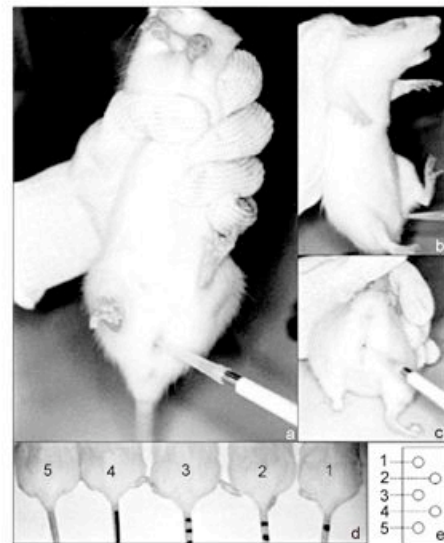
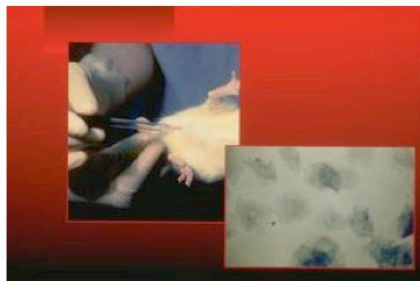







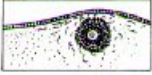


Fig. 1—Ways to holding the female rat for the collection of the vaginal lavage (a-c), individual tail marks of five female rats from the same cage (d) and way to distribute the drops of vaginal lavage from each female rats in a glass slide (e). During a month, every morning between 8:00 and 9:00 a.m., vaginal secretion was collected from female rats. It was done by using a plastic pipette filled with 10 μ L of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply (1a). If the animals were aggressive ways 1b and 1c were used. Vaginal fluid was placed on glass slides and a different glass slide was used for each cage of animals (1e). One drop was collected with a clean tip from each rat (1d, 1e).

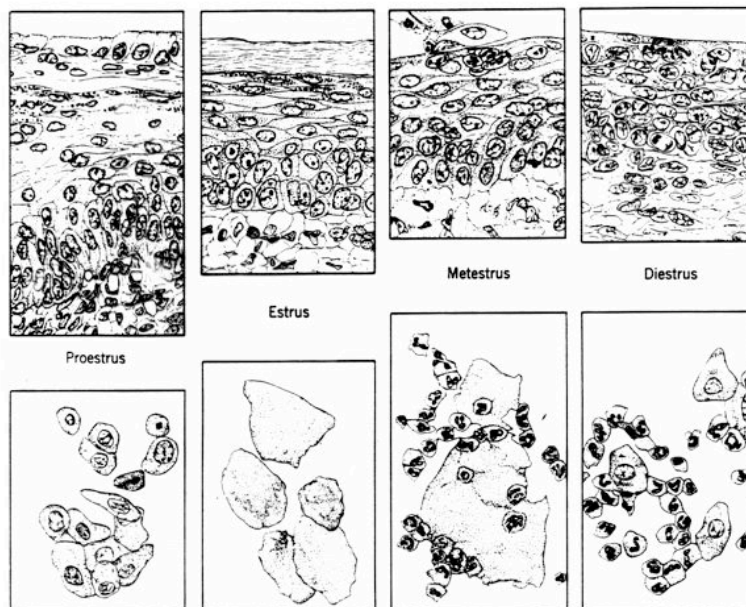
Criteria for Stage Determination

The fluctuation in hormone levels during the cycle results in changes in the number and types of cells present in the vagina. This is mostly due to the cyclical hypertrophy and subsequent sloughing of the uterine epithelium and also the concomitant invasion of white blood cells that scavenge the dead and dying cells. If you recall the process holistically, you are likely to make better decisions about stages based on inconclusive cell types.

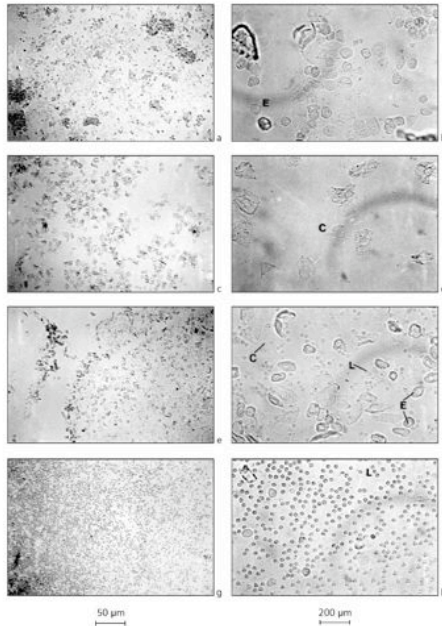
Cell density is not usually an accurate way to determine stage in isolation, however it is true that estrus and proestrus are typified by a very high cell density.

Proestrus is defined by the presence of nucleated epithelial cells that are round. However, sometimes these cornify rapidly, especially in mice. If you are having trouble distinguishing between P and E, you should probably consider any slide in which nucleated cell predominate to be P, even if these cells are not the smooth round shape you are expecting. **Estrus** is characterized by non-nucleated, cornified epithelial cells. **Metestrus** (or Diestrus I) typically has a low cell number, often with a lot of cell debris. **Diestrus II** contains mostly lymphocytes.

Time of day	1400	1400	1400	1400-1800
Hours after ovulation	12	36	60	84-88
	Estrus	Diestrus I	Diestrus II	Proestrus
Vaginal smear				
	Primary corpus luteum	Primary follicle	Secondary follicle	Graafian follicle
Follicular development				
Sexual behavior	—	—	—	+



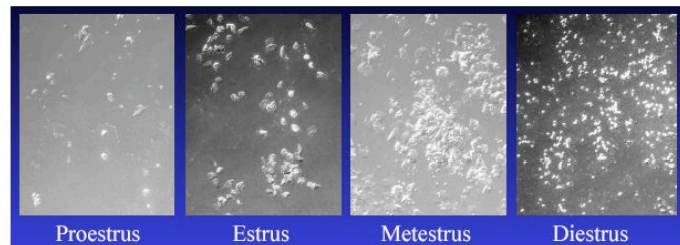
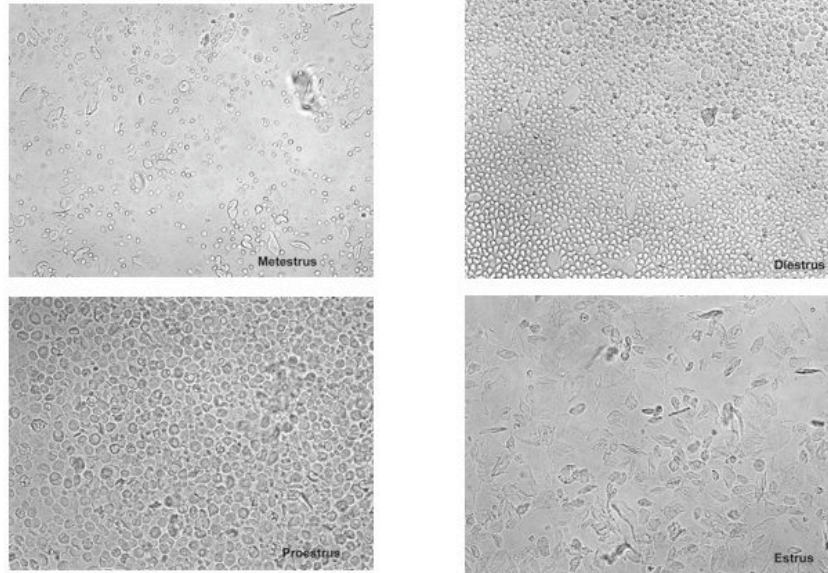
Stage	Smear	Vagina	Ovary	Uterus
Diestrus	Stringy mucous in which are entangled many leucocytes and a few nucleated epithelial cells.	Epithelium thin, mitotic figures infrequent. Leucocytes abundant in stroma, migrate through the epithelium into vaginal lumen.	Small follicles only are present with large corpora lutea from the previous ovulation. These secrete for only a very short time unless pregnancy or pseudopregnancy intervene.	Small and anaemic, low motility, lumen small and slit-like. Cells of the uterine mucosa columnar; polymorphonuclear leucocytes in stroma; endometrial glands collapsed, atrophic.
Proestrus	Largely small, round, nucleated epithelial cells, singly or in sheets. None to few leucocytes.	Epithelium thickens, numerous mitoses in inner layers. Old layers of epithelium line the lumen. Leucocytes no longer migrate through the epithelium. Superficial epithelial cells slough off into lumen.	Some follicles grow rapidly.	Become more vascular, water content increases, organ distends. Contractility more pronounced. Epithelial cells become higher (continuing into estrus). Leucocytes disappear from mucosa. Endometrial glands hypertrophy.
Estrus	Contains hundreds of large cornified cells (squames) with degenerate nuclei. Towards the end of estrus the smear becomes "cheesy" - masses of adherent cornified cells.	Outer layer of epithelial cells become cornified and sloughed into the lumen. In early estrus these cells retain their nuclei, but in later stages no nuclei visible and the cells are irregular, flat, cornified plates. The skin around the vaginal orifice becomes swollen.	Ovulation in the rat is spontaneous and occurs about 10 hours after the beginning of estrus. "Heat" (receptivity) lasts about 13 hours. Usually 10-20 eggs ovulated each time.	gains maximum vascularisation. Epithelial cells reach maximum development. No leucocytes.
Metestrus	Many leucocytes and a few cornified cells.	Deeper layers of the estrous epithelium now line the lumen, the older, superficial layers having become cornified and sloughed off. Reduction of mitotic activity in epithelium. Leucocytes in stroma and migrating through the epithelium into the lumen.	Many corpora lutea, which secrete only for a very short time, and small follicles.	Epithelium continues vacuolar degeneration and replacement. Leucocytes in stroma. Decrease in size and vascularity.



Photomicrographs of unstained vaginal smear from female rats at proestrus (a, b), estrus (c, d), metestrus (e, f) and diestrus (g, h). Leucocytes (L), epithelial (E) and cornified (C) cells are indicated. 50 x (left panel) and 200 x (right panel). Unstained native vaginal smear from female rats was observed under a light microscope, without the use of the condenser lens, with 10 and 40 x objective lenses. The proportion of the three types of cells was used for the determination of the estrous cycle phases. Round and nucleated ones are epithelial cells (E); irregular ones without nucleus are the cornified cells (C); and the little round ones are the leucocytes (L). A proestrus smear consists of a predominance of nucleated epithelial cells (Figs. 2a, 2b); an estrous smear primarily consists of anucleated cornified cells (Figs. 2c, 2d); a metestrus smear consists of the same proportion among leucocytes, cornified, and nucleated epithelial cells (Figs. 2e, 2f); and a diestrus smear primarily consists of a predominance of leucocytes (Figs. 2g, 2h).

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MARCONDES, F. K., BIANCHI, F. J. and TANNO, A. P.



The appearance the of external genitalia can also help to establish the stage of the cycle.



Vaginal Impedance

Vaginal impedance measurements are also useful, though mainly to distinguish proestrus from estrus. Monitors are available from Fine Science Tools.

Estrous Cycle –Dependent Phenomena

Below is an abridged version of some phenomena that are estrous cycle dependent.

Learning

[Berry, 1997 #211][Diaz-Veliz, 1989 #184][Mora, 1996 #302][Roberts, 1998 #178]

Other Hormones

[Atkinson, 1997 #312][Audrain, 1978 #341][Carey, 1995 #217][Guo, 1995 #351]

Pain Threshold

This study examined sex and estrous differences in vocalization thresholds of rats to hindpaw and tail pressure stimulation tested daily throughout at least 3 weeks. Thresholds of female rats in proestrus and estrus were lower than those of rats in metestrus and diestrus for both tail and hindpaw stimulation

Anxiety

[Fernandez-Guasti, 1992 #236] [Bitran, 1991 #235][Nomikos, 1988 #120][Bitran, 1996 #231][Mora, 1996 #302]

Acoustic Startle and PPI

[Farr, 1995 #231] [Koch, 1998 #1164]

Other Behavior

[Sell, 2000 #217]

Sensitivity to Neurosteroid

[Finn, 1993 #21][Finn, 1994 #22]

BDZ Insensitivity to Anxiolysis

[Carey, 1992 #244][Fernandez-Guasti, 1990 #300][Nomikos, 1988 #120]

BDZ Binding

[Martin, 1995 #131]

GABAAR Subunits

[Clark, 1998 #296]

Neuronal Activity / Neuronal Excitation / LTP / LTD

afternoon of proestrus= greatest degree of LTP

Seizure Susceptibility During Estrous Cycle

[Finn, 1994 #22] [Wahnschaffe, 1992 #148][Wilson, 1992 #118] [Frye, 1998 #489][Edwards, 1999 #198][Frye, 1998 #352]

NT Release

[Belmar, 1998 #350]

Synapse Number and Morphology

[Crispino, 1999 #134][Prevot, 2000 #205] [Woolley, 1990 #270][Frankfurt, 1990 #274] [Gould, 1990 #271]

GABAB R

[al-Dahan, 1994 #124][al-Dahan, 1996 #282]