University of Zurich[UZH]

Institute of Laboratory Animal Science[LTK]

Humane research to improve science and education for the benefit of animals and humans

Set of slides for the Preclinical Research

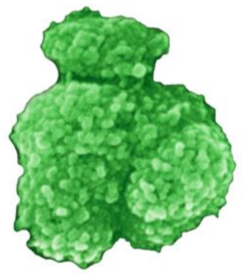
Authors: Dr. Ivana Jaric and Prof. Dr. Thorsten Buch

Biology (sex) and socio-cultural factors (gender) influence health

Sex - biological determinants Genes, hormones and phenotypes

X: ca 1500 Genes
Heart-, Brain-, Immune function

Y: ca 78 Genes,
Sexual function



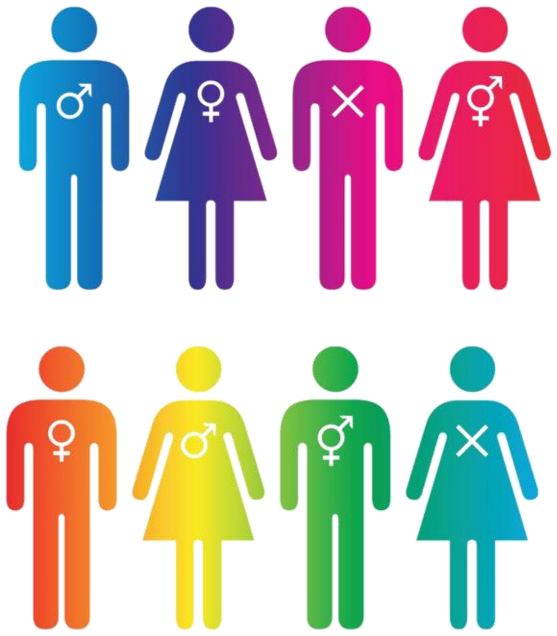
Estrogens: regenerative
Testosterone: growth, aggression

Gender-modified behavior

Environment modified

DNA packaging "Epigenetics"

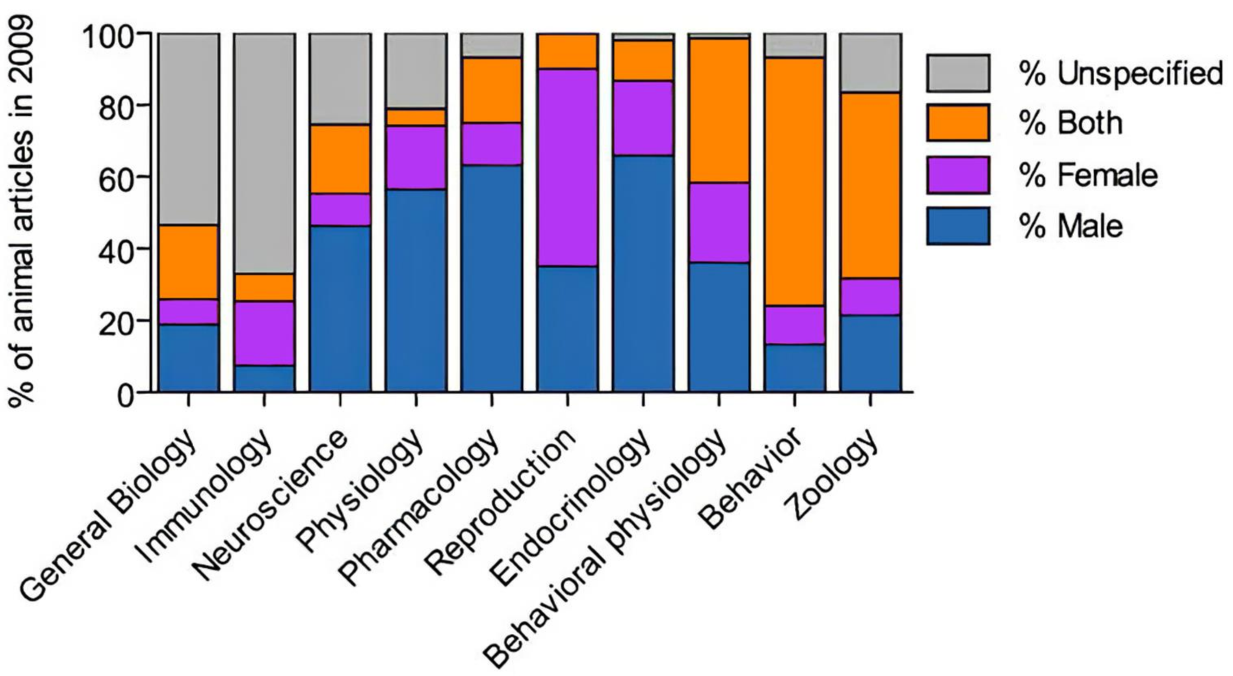
Gender: sociocultural construct



The neglect of sex within *in-vivo* preclinical research

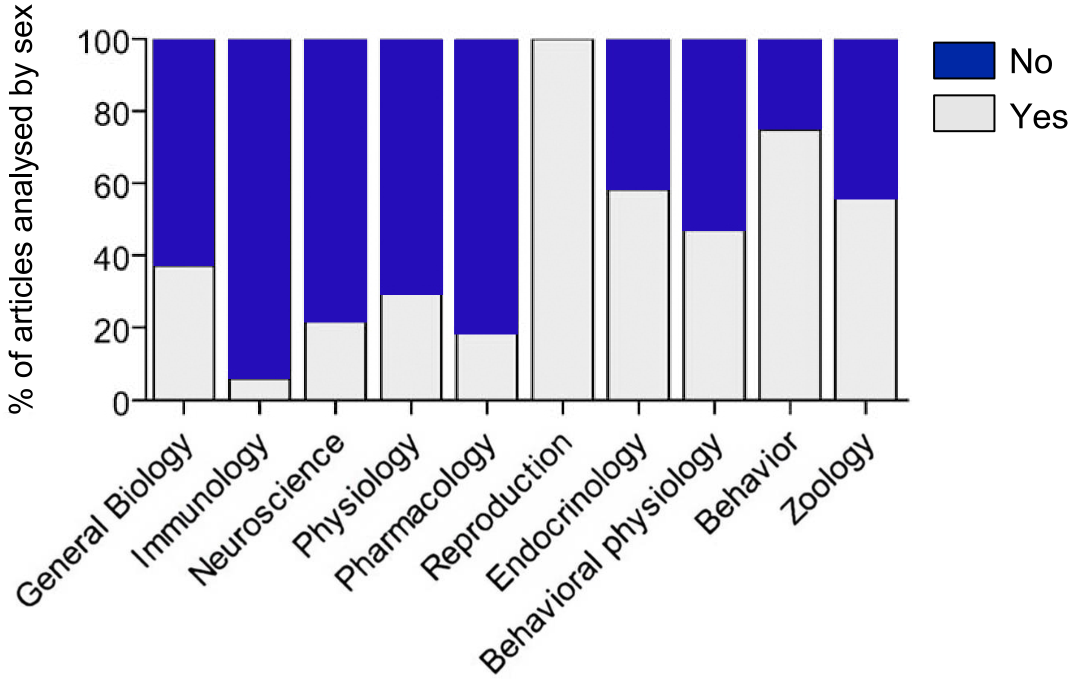
Experimental design

Field-specific sex bias across disciplines



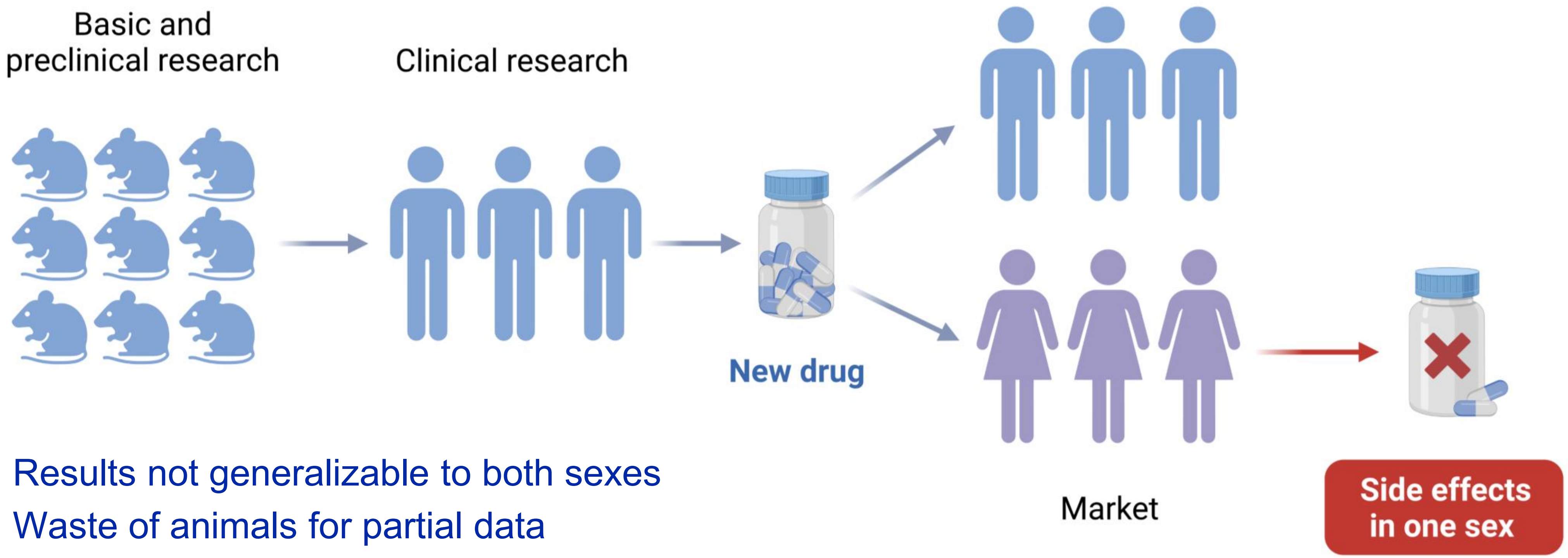
Analysis

Only 33% analysed by sex (when both sexes are included)



Beery and Zucker 2011 *Neurosi Biobehav Rev*

Consequences of sex (male) bias in preclinical research and drug development



- Results not generalizable to both sexes
- Waste of animals for partial data
- Hinders understanding of sex-specific disorders
- Limits development of effective therapies for both sexes

Adapted from Lee 2018 *BMB Rep.* Created with BioRender.com

Sexual dimorphism in mouse phenotypic traits


nature COMMUNICATIONS

ARTICLE

Received 27 Oct 2016 | Accepted 30 Mar 2017 | Published 26 Jun 2017 DOI: 10.1038/ncomms15475 OPEN

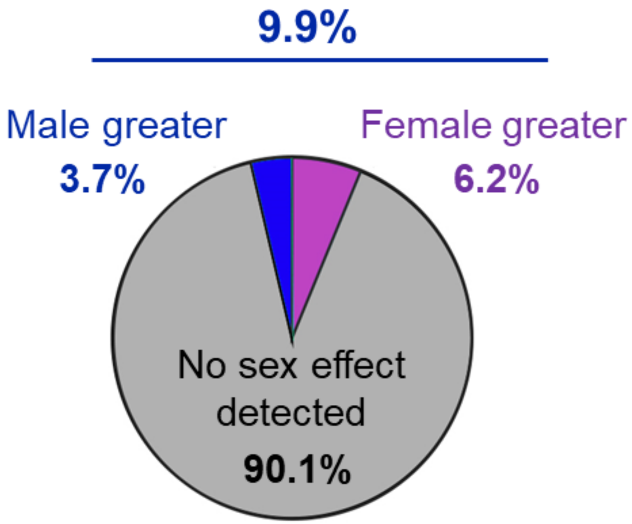
Prevalence of sexual dimorphism in mammalian phenotypic traits

14250 WT and 40192 mutant mice
2186 knockout lines
up to 234 traits

Sex differences are common in traits previously assumed to be identical between males and females.

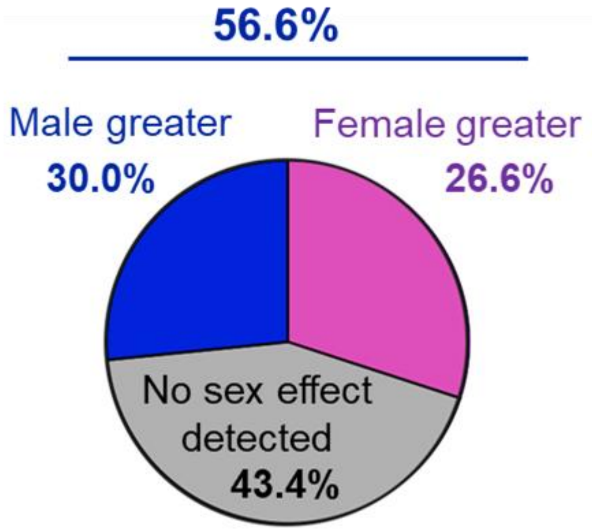
Genetic modifications can affect males and females differently.

The proportion of experiments where sex had a significant role in wildtype phenotype



Categorical data

(head shape, whisker shape, paw shape, coat color)



Continous Data

(metabolic, cardiovascular, bone, behavioral, hematological, blood clinical chemistry parameters)

Misconception 1

Including both sexes doubles the number of animals

Clarification →

PLOS BIOLOGY

META-RESEARCH ARTICLE

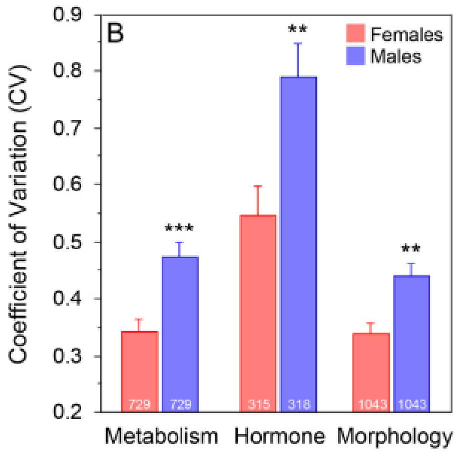
Statistical simulations show that scientists need not increase overall sample size by default when including both sexes in in vivo studies

Benjamin Phillips[1], Timo N. Haschler[2], Natasha A. Karp[1]*

Misconception 2

Female hormone fluctuations increase data variability

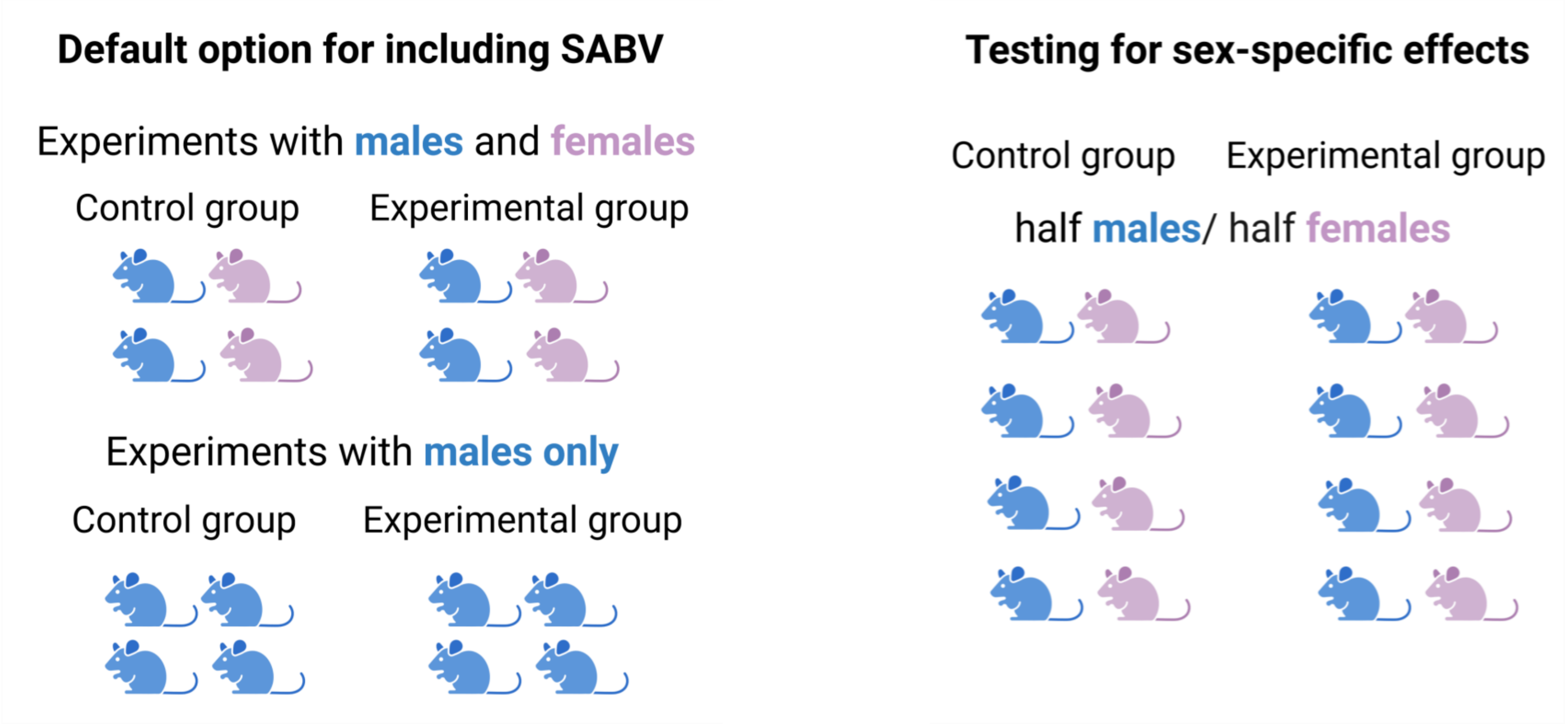
Clarification →



Females have **less variability** than males

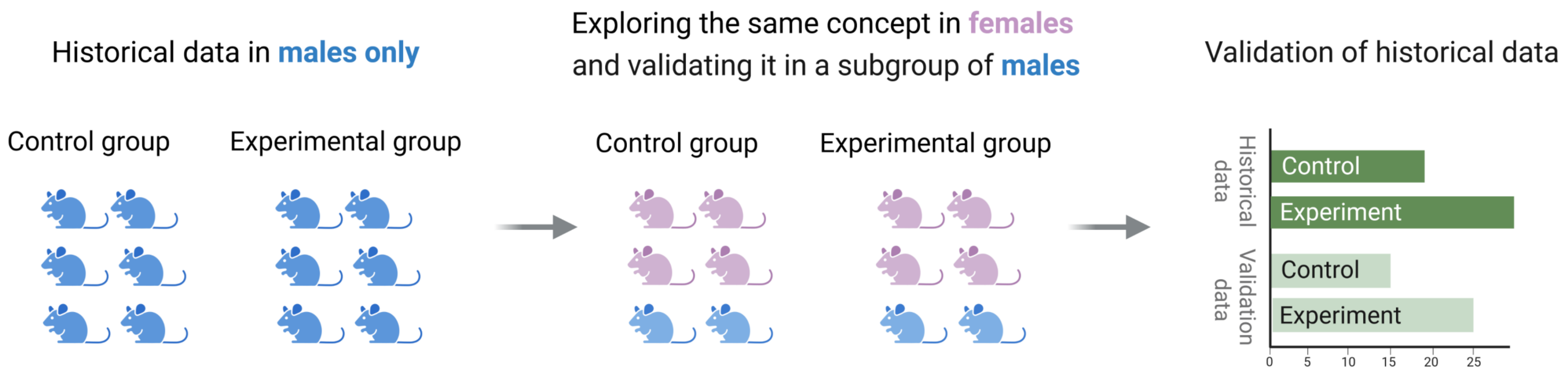
Prendergast 2014
Neurosi Biobehav Rev

Integration of SABV into study design:
default option *vs* testing for sex-specific effects



Adapted from Dalla, Jaric et al. 2024 *J Neurosci Methods*

Integration of SABV into study design:
how to relate to existing single-sex knowledge



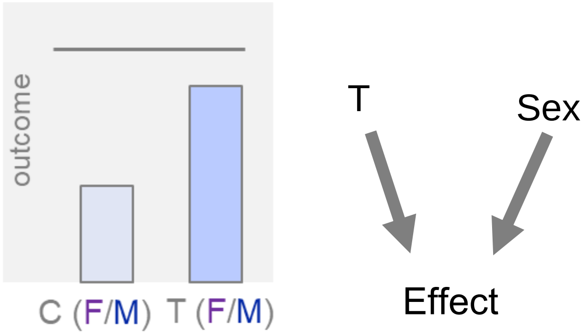
Dalla, Jaric et al. 2024 *J Neurosci Methods*

Inclusion of SABV in a single experiment: statistical design and analysis

Sex as a confounding variable

Block design

	Female (F)	Male (M)
Control (C)	Group 1a	Group 1a
Treatment (T)	Group 2a	Group 2a

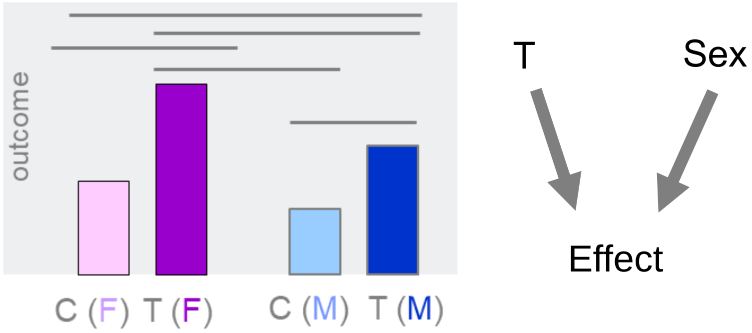


Only interested in the treatment outcome and do not want to know the effect size of the influence of sex

Sex as an outcome variable

Factorial design

	Female (F)	Male (M)
Control (C)	Group 1	Group 2
Treatment (T)	Group 3	Group 4

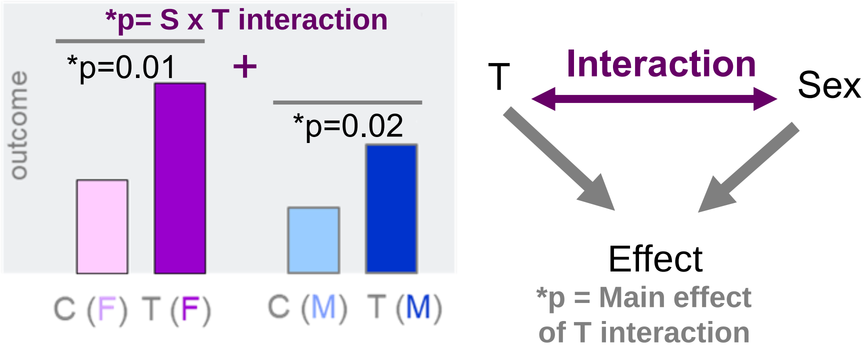


Direct comparison between groups and sexes, but no information if sex influences treatment

Interaction between sex and treatment

Factorial design, interaction and main effect

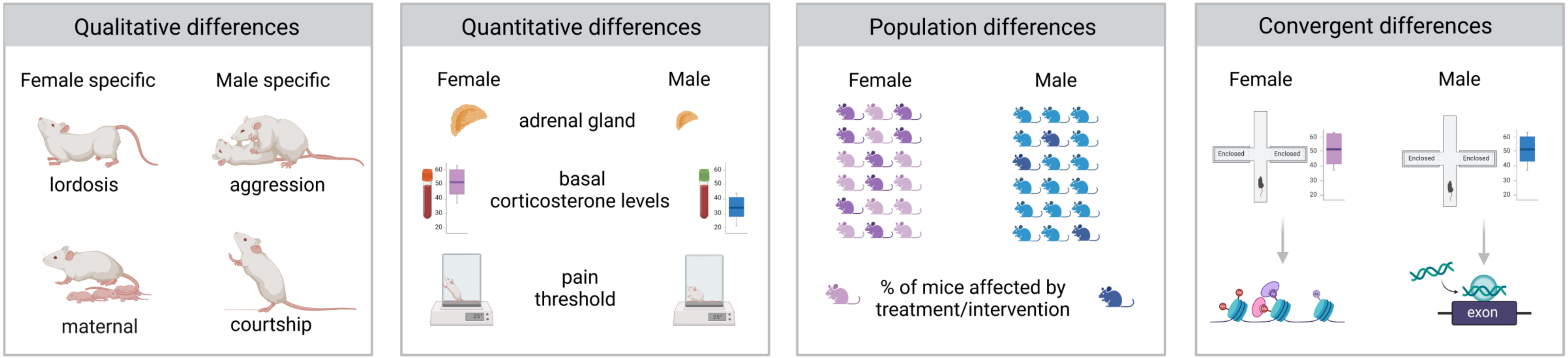
	Female (F)	Male (M)
Control (C)	Group 1	Group 2
Treatment (T)	Group 3	Group 4



Direct comparison between groups and sexes; also gives the interaction (sex x treatment) p-value and a treatment p-value.

Buch et al. 2019 *J Mol Med (Berl)*

Four types of sex differences



Sexually dimorphic traits	Traits vary along a continuum in both sexes	Incidence differs between sexes	The endpoint is similar, but the mechanisms differ between sexes

Dalla, Jaric et al. 2024 *J Neurosci Methods*

Biological sources of sex differences

Sex effects stem from two different biological mechanisms

Sex hormones

Experimental models for detecting sex hormone effects

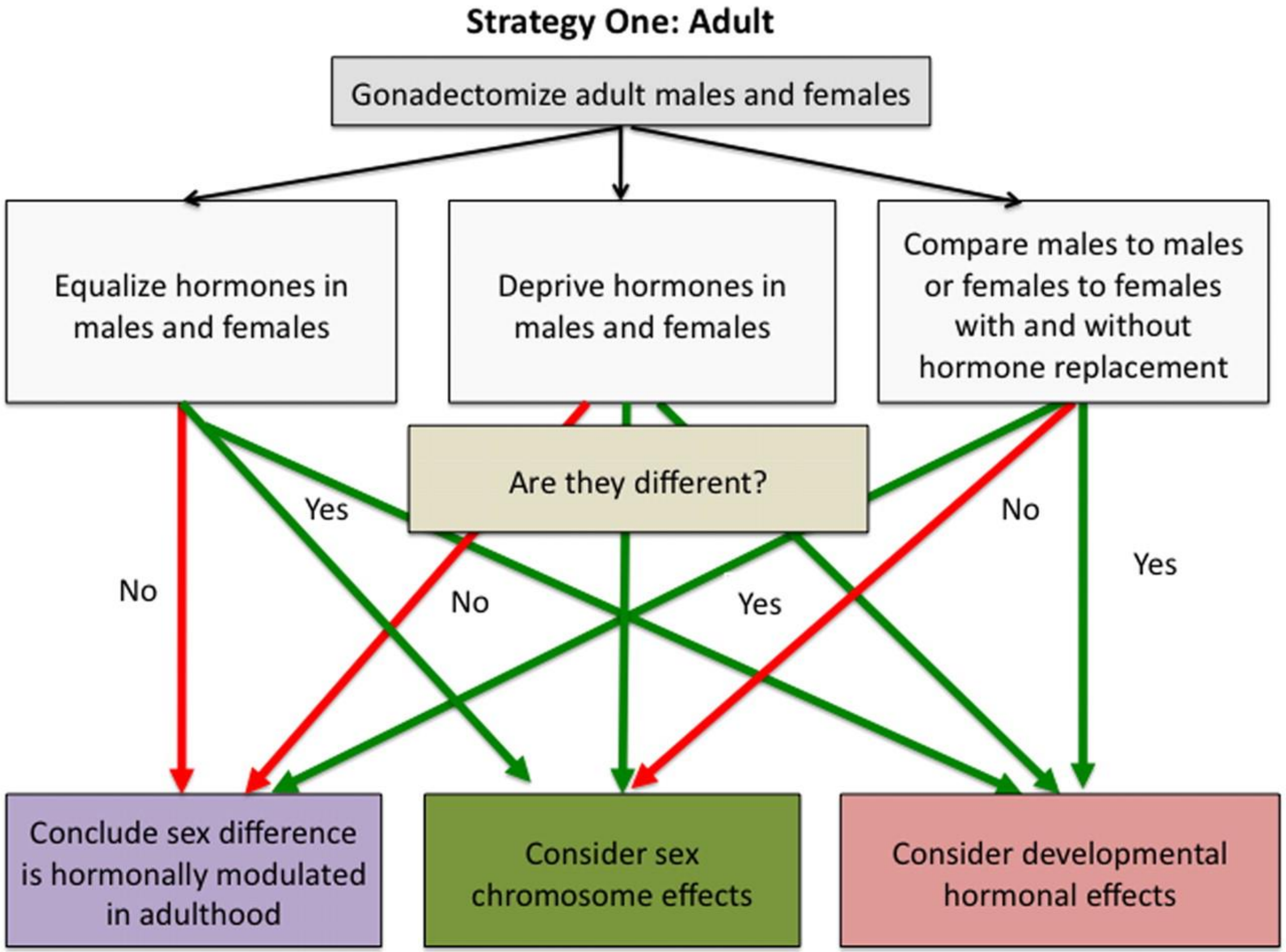
- Adult origins
- Developmental origins

Sex chromosomes

Experimental models for detecting sex chromosome effects

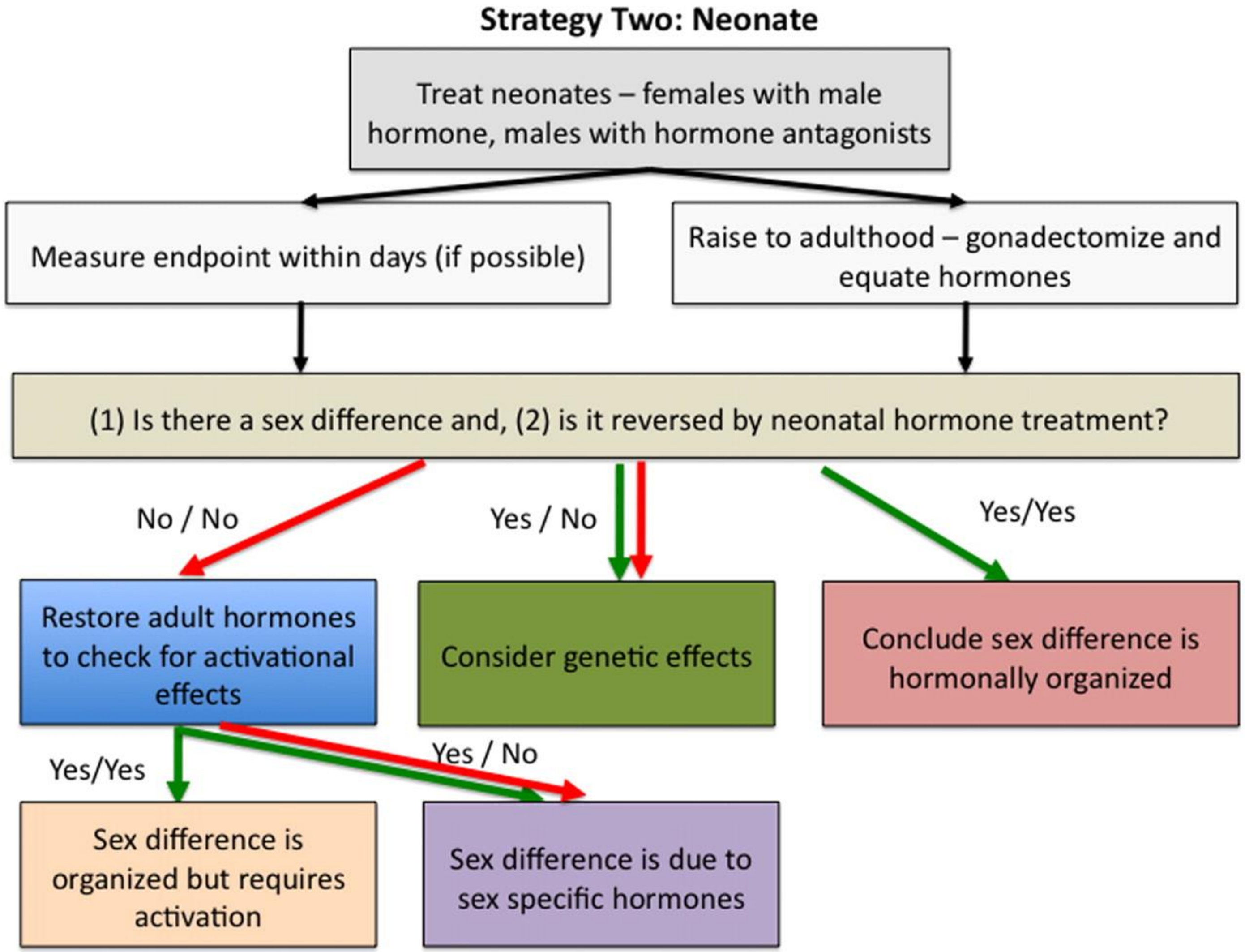
- Four Core Genotypes
- XY* model

Sex differences *via* sex hormones:
experimental designs: adult origins



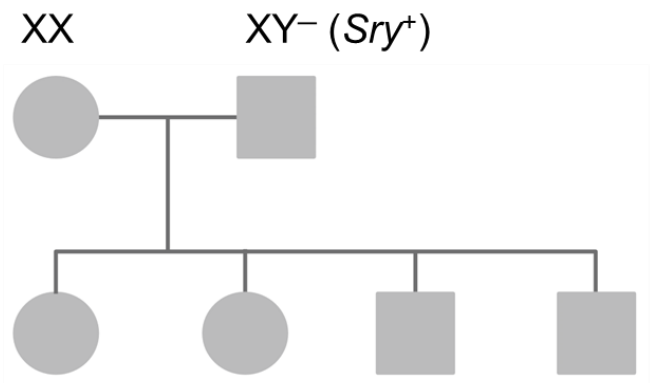
McCarthy et al. 2012 *J Neurosci*

Sex differences *via* sex hormones: experimental designs: developmental origins



McCarthy et al. 2012 *J Neurosci*

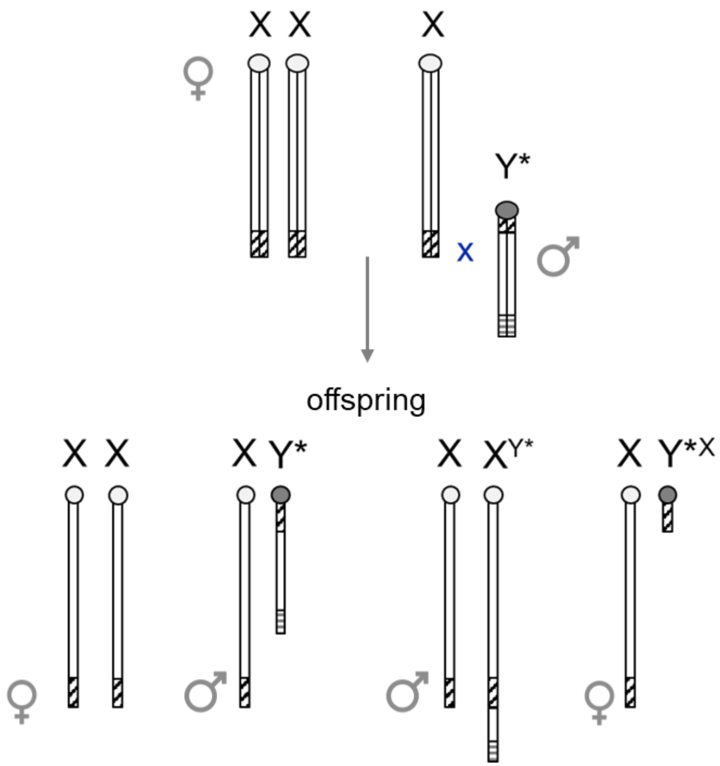
Sex differences *via* sex chromosome: Four Core Genotypes (FCG) mouse model



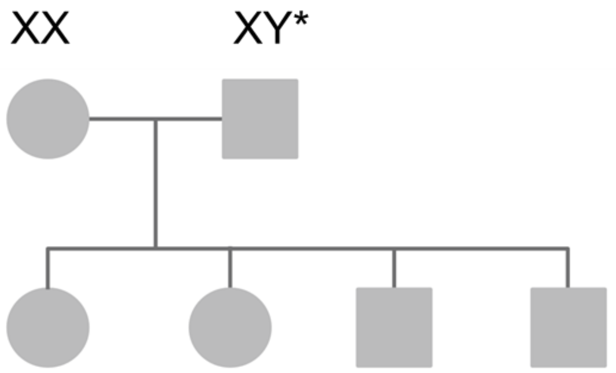
Genotype	XX	XY⁻	XX (*Sry*⁺)	XY⁻ (*Sry*⁺)
Abbreviation	XXF	XYF	XXM	XYF
Sry transgene	–	–	+	+
Gonads	ovaries	ovaries	testes	testes
Y chromosome	–	+	–	+
X chromosome	two	one	two	one

Arnold 2020 *Neurosci Biobehav Rev.*

Sex differences *via* sex chromosome: XY* mouse models



Eicher et al. 1991 *Cytogenetics and Cell Genetics*

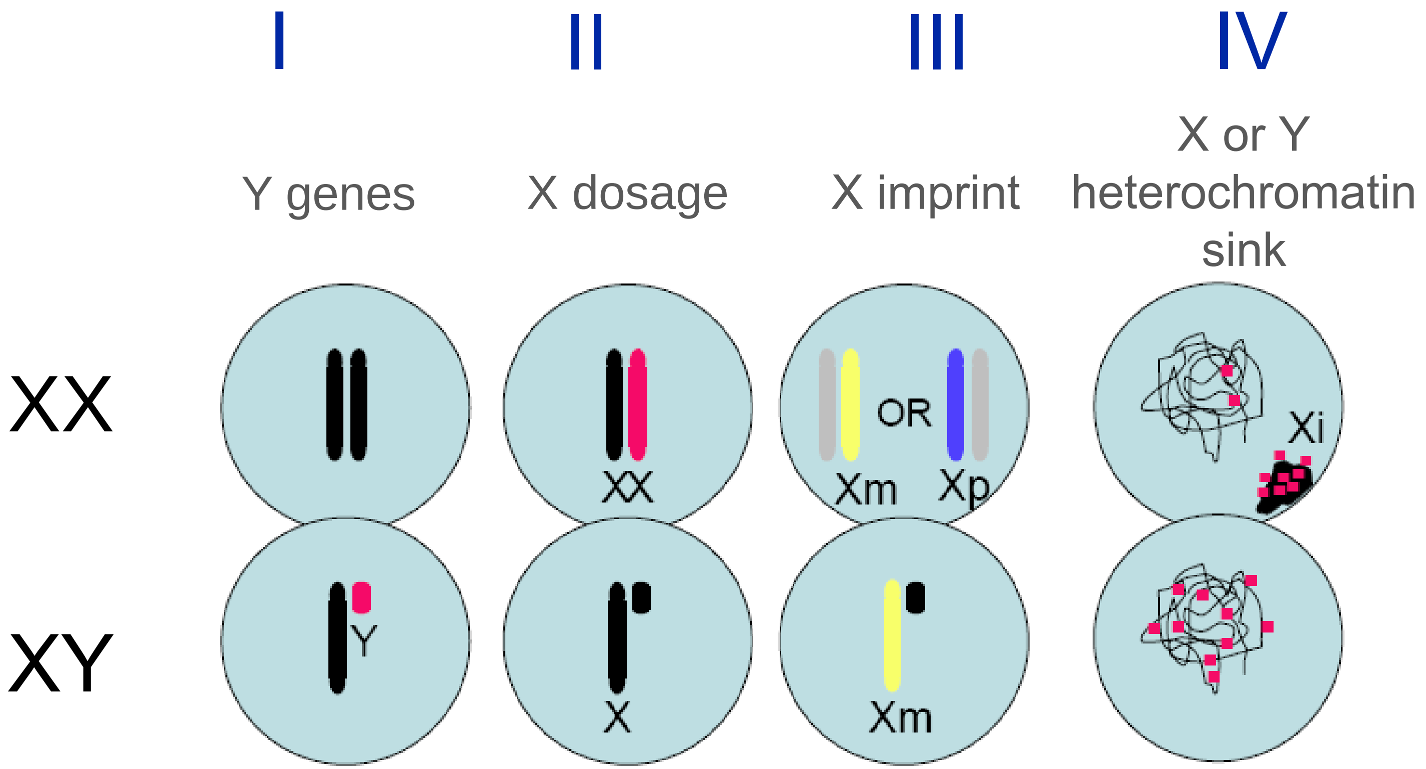


Genotype	XY^{*X}	XX	XY*	XX^{Y*}
Shorthand	XO	XX	XY	XXY
Gonads	ovaries	ovaries	testes	testes
Sry	–	–	+	+
Y chromosome	–	–	+	+
X chromosome	one	two	one	two

Arnold 2020 *Neurosci Biobehav Rev.*

Sex chromosome-based mechanisms of sex differences

Classes of primary determining genes/factors



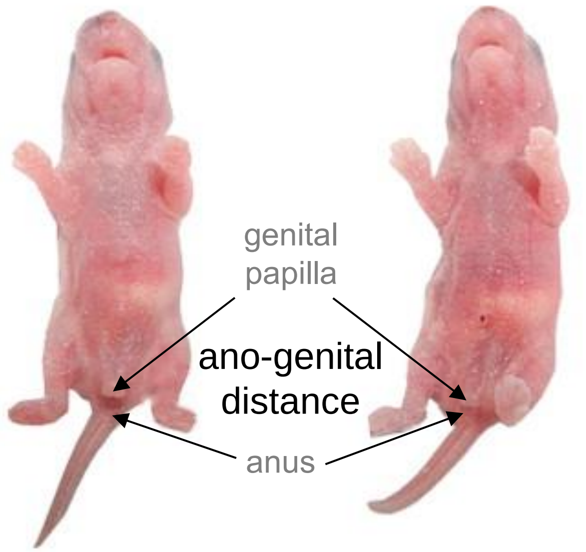
Arnold 2011 *Trends in Genetics.*

Operationalizing sex

Sex determination in neonatal mice

Anogenital distance

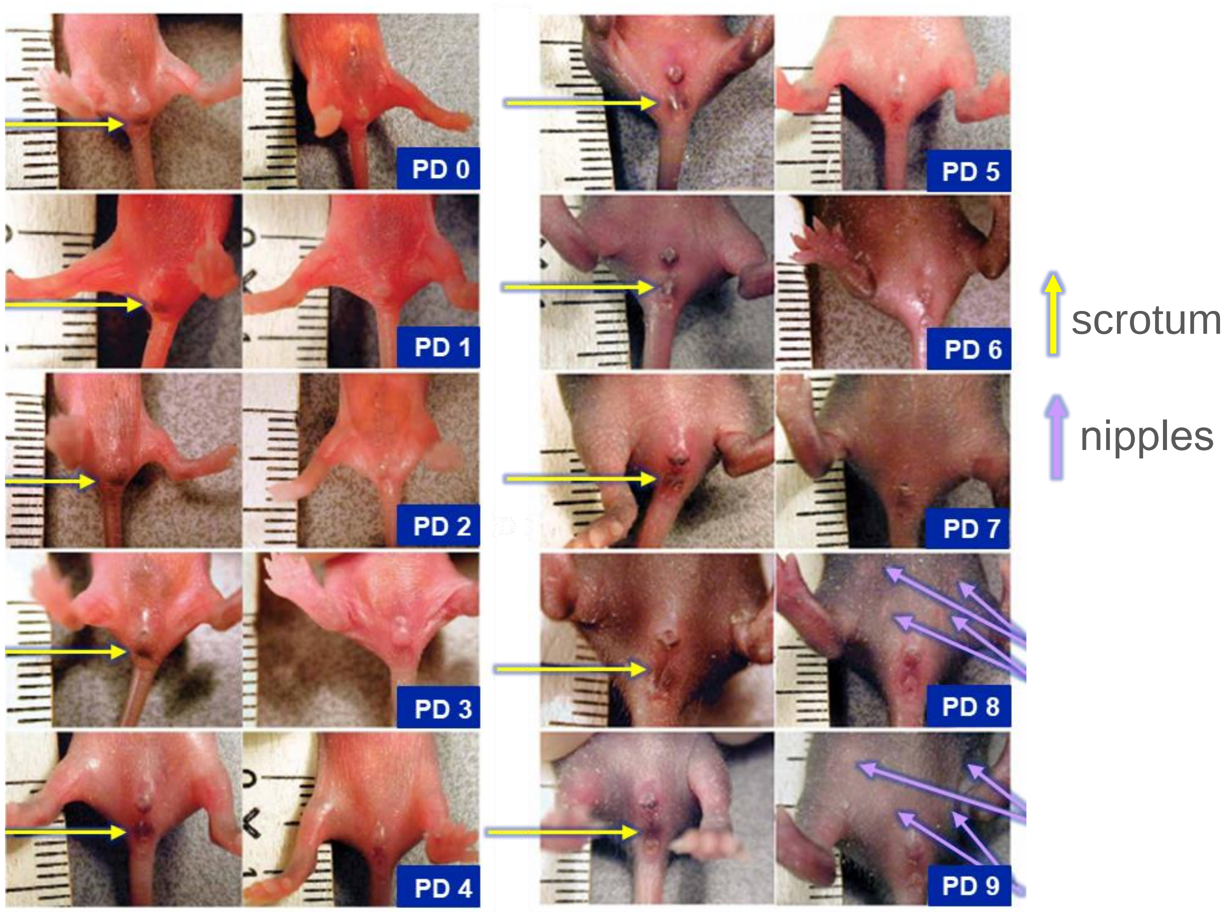
MALE **FEMALE**



Liu M et al. 2008 *J Neurosci Methods*

Anogenital pigmentation of neonatal mice

MALE **FEMALE** **MALE** **FEMALE**

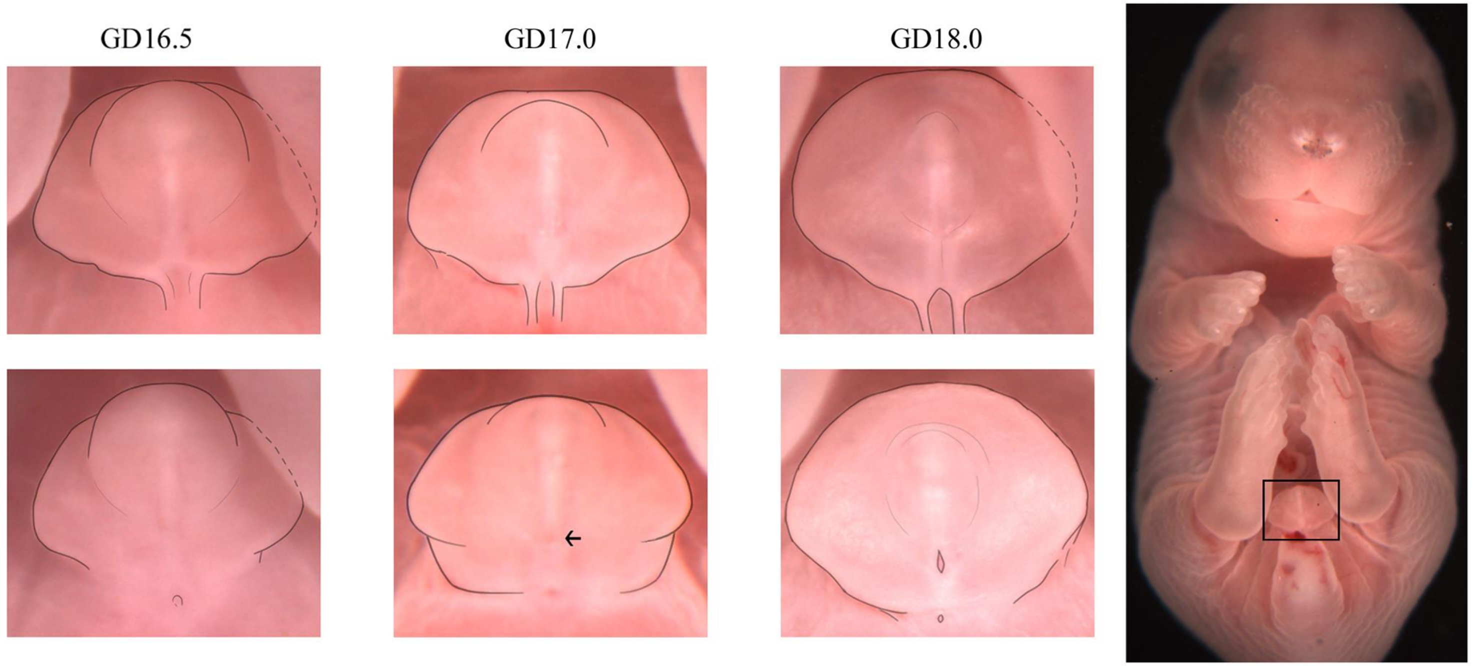


Wolterink-Donselaar et al. 2009 *Lab Anim*

Operationalizing sex

Sex determination in fetal mice

The method for determining sex in late-term gestational mice based on the external genitalia

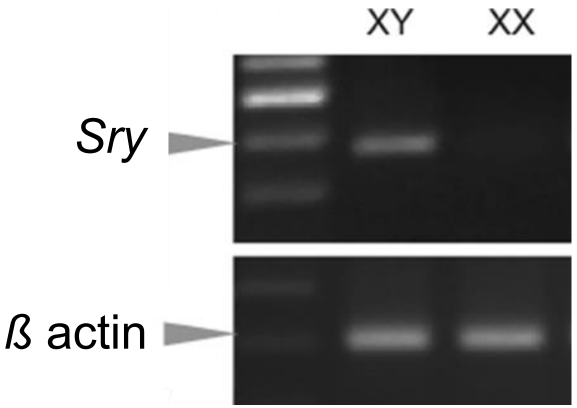


Murdaugh et al. 2018 *PLoS One.*

Operationalizing sex

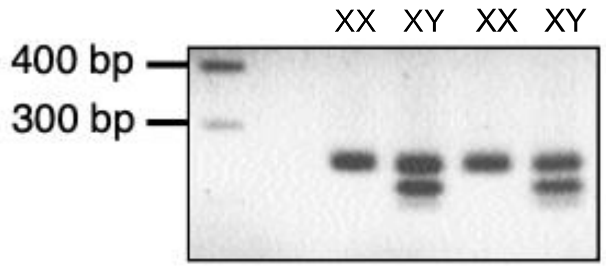
Sex determination in mice using the PCR

Sry PCR



Zushi H et al. 2017 *Chromosoma*

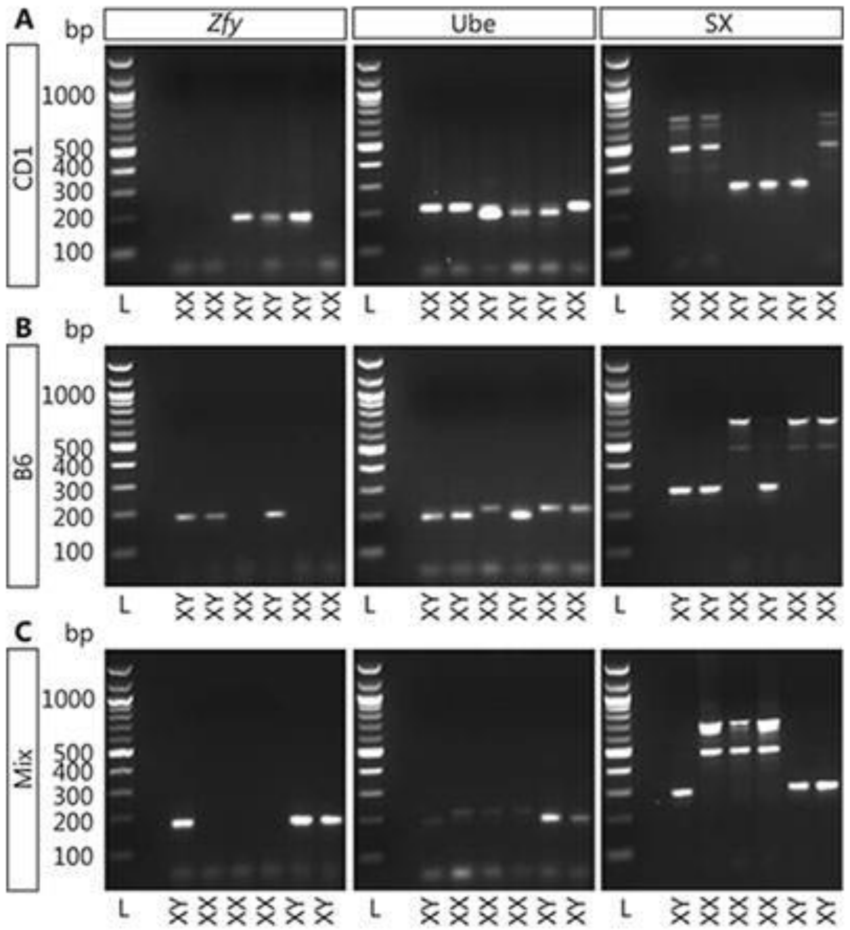
Nds 3/4 and *Zfy11/12* PCR



Nds3/4 amplifies an X-chromosomal DNA fragment (upper band). *Zfy11/12* amplifies a Y-chromosomal DNA fragment (lower band).

Buch 2000; *PhD thesis, University of Cologne*

Sly/Xlr (SX) PCR



McFarlane et al. 2013 *Sex Dev*

Rodent models of gender-affirming hormone therapies (GAHT)

GAHT with Testosterone (T-GAHT)

Study	Sex, species, strains, age	Hormone treatment	Exposure Duration
Kinner et al. 2019 *Hum. Reprod.*	Female, mouse, C57BL/6N (8-9 weeks)	T enanthate	6 weeks
Kinner et al. 2021 *F S Sci.*	Female, mouse,C57BL/6N (8-9weeks)	T enanthate	6 weeks
Battels et al. 2021 *Hum. Reprod.*	Female ,mouse, CF-1 (6 weeks)	T cypionate	6 weeks

GAHT with Estradiol (E-GAHT)

Study	Sex, species, strains, age	Hormone treatment	Exposure Duration
Alexander et al. 2022 *FASEB J.*	Male, rat, Sprague Dawley (13 weeks)	17-beta E2 benzoate	3 weeks
Pfau et al. 2023 *Adv. Biol.*	Male, mouse, C57BL/6NHsd (8 weeks)	Estradiol powder	6 weeks
Tassarini et al. 2023 *Cells*	Male, rat, Sprague Dawley (9–10 weeks)	17-beta E2 valerate + CPA	2 months
Gusmão-Silva et al. 2022 *J. Endocrinol. Invest.*	Male, rat, Wistar (2 months)	E2 enanthate + DHPA	5 months

GAHT in the peripubertal population

Study	Sex, species, strains, age	Hormone suppression and treatment	Suppression and treatment durations
Dela Cruz et al. 2023 *Hum Reprod.*	Female, mouse, C56BL/6N (26 days)	Depot-GnRHa and T enanthate	3 weeks + 6 weeks
Dela Cruz et al. 2024 *F S Sci.*	Female, mouse, C56BL/6N (26 days)	Depot-GnRHa and T enanthate	3 weeks + 6 weeks
Godiwala et al. 2023 Endocrinology.	Female, mouse, CF-1 (3 weeks)	Depot-LA and T cypionate	12 weeks + 8 weeks

CPA: cyproterone acetate; DHPA: dihydroxyprogesterone acetophenide ; LA: leuprolide acetate.

Hormonal influence on sexual behaviors and preferences

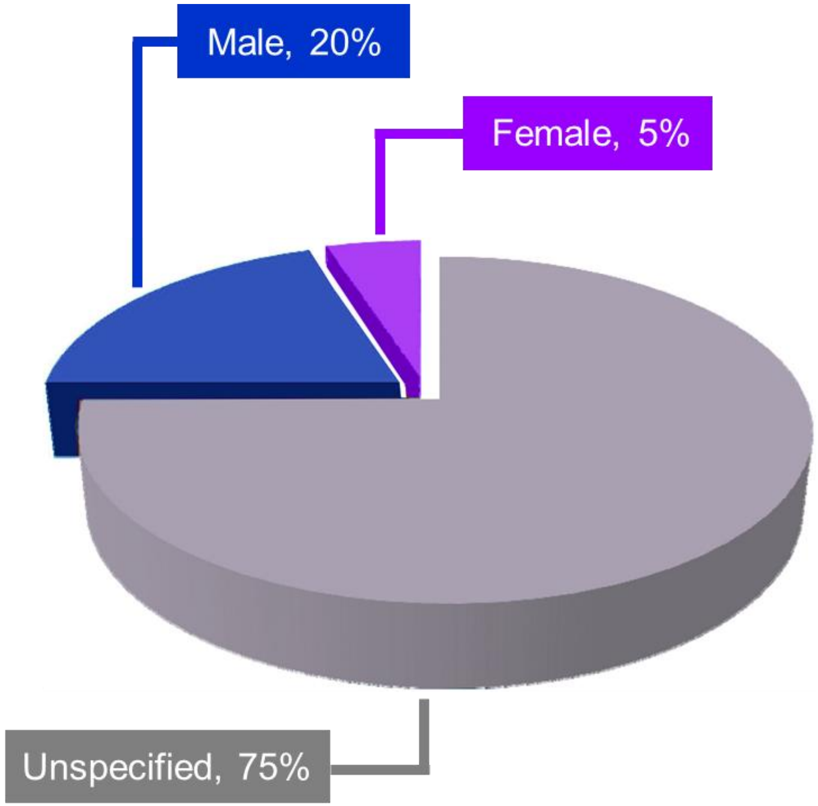
Endocrine mediation of male-typical and female-typical sexual behaviors and preferences.

Endocrine manipulation	Male-typical sexual behavior	Female-typical sexual behavior	Gynephilia	Androphilia	References
Neonatal decrease in T in males	↓	↑	↓	↑	For review, see Cooke et al. (1998)
Neonatal increase in T in males	=	=[a]	↓	↑	e.g., Henley et al. (2010) and Cruz and Pereira (2012)
Estrogenic manipulations					
Global ERα KO in males	↓	=	↓	=	Ogawa et al. (1998) and Wersinger and Rissman (2000)
Global ERβ KO in males	=	↑	=	=	Kudwa et al. (2005)
Global AFP KO in females	↑	↓	=	=	Bakker et al. (2006, 2007)
Global Arom KO in males	↓	=[a]	↓	=	Honda et al. (1998) and Bakker et al. (2002)
Androgenic manipulation					
Global AR KO in males	↓	=	↓	↑	Reviewed in Zuloaga et al. (2008), Bodo and Rissman (2007) and Sato et al. (2004)
Neural AR KO in males	↓	=[a]	=	=	Juntti et al. (2010) and Raskin et al. (2009)
Global AR overexpression in males	↑	=[a]	=	↑	Swift-Gallant et al. (2016a, 2016b)
Neural AR overexpression in males	=	=[a]	=	=	Swift-Gallant et al. (2016a, 2016b)

Swift-Gallant 2019 *Horm Behav.*

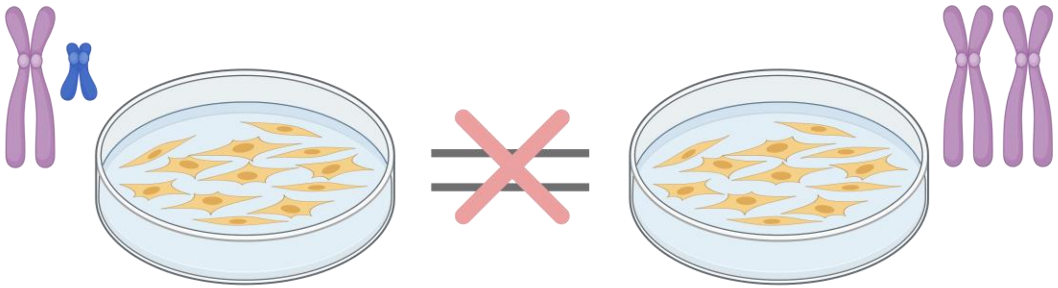
The neglect of sex within *in vitro* studies

Reporting



Shah et al. 2014 *AJP Cell Physiol*

Cells demonstrate sex-specific gene expression and responses to stimuli



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Considerations and guidelines for incorporating SABV in *in-vitro* experiments

Primary cells and organoids

Taken directly from tissue

Multiple donors: **possibility to include both sexes**

↓

Include multiple individuals of both sexes

Immortalized cell lines

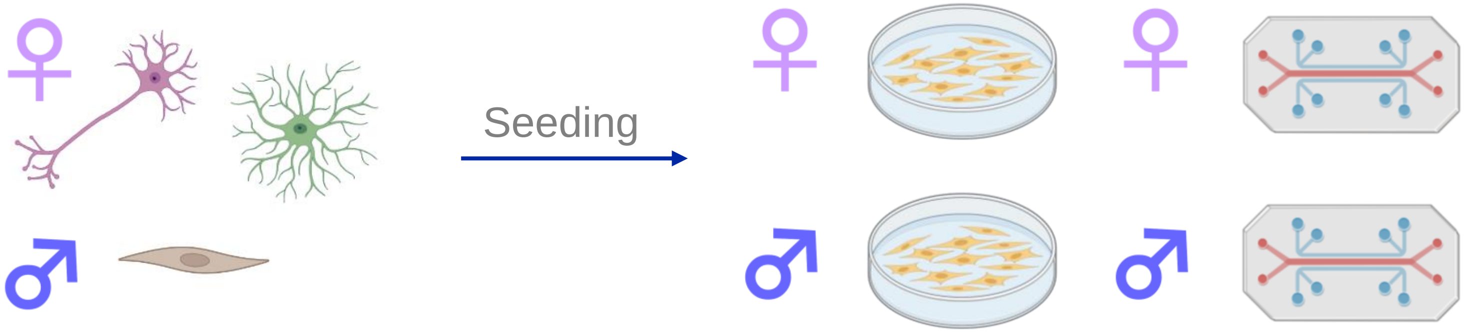
Commercially available

Chromosomal instability: **sex must be confirmed**

Single donor source: single sexes

↓

Check the sex of the cells and buy cells of both sexes



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Take home messages and useful educational material

Consideration of SABV in basic and preclinical research:

- Increases scientific rigor and reproducibility
- Increases validity and generalizability of research findings
- Saves the euthanasia of supposedly useless surplus animals

SABV in basic and preclinical research is relevant when the research:

- Uses human tissues, cells, or bodily fluids
- Uses animal tissues, cells, or bodily fluids
- Uses animal models of human physiology or disease
- May impact diagnosis or treatment
- Leads to the development of products for human use

Integration of SABV into animal study design

- Does not (usually) double total group size
- Saves the euthanasia of supposedly useless surplus animals
- Is not the same as studying sex differences

- **Video training series on SABV in preclinical research**

Cohen Veterans Bioscience — GLOBAL PRECLINICAL DATA FORUM

A link is provided: here

- **NIH: SABV Primer course instructor guide**

NIH National Institutes of Health — Office of Research on Women's Health

A link is provided: here

- **Sex Inclusive Research Framework (SIRF)**

A link is provided: here