Society for Birth Defects Research & Prevention

EST. 1960 AS THE TERATOLOGY SOCIETY

Reactive Oxygen Species and DNA Damage/Repair in Developmental Disorders

Warkany Lecture

Peter G. Wells, Pharm.D.
Division of Biomolecular Sciences
Faculty of Pharmacy
University of Toronto

Society for Birth Defects Research and Prevention's **2021 Virtual 61st Annual Meeting**

Former Graduate Students & PDFs

Their published research provided the basis for this award!

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Richard Arlen
Yadvinder Bhuller
Connie Chen
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Luisa Goncalves
Zhuojan Hu
Deqi Huang
Winnie Jeng
Grazyna Kalabis
Julia Kennedy
Perry Kim
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Alan Miranda
Christopher Nicol
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Terence Ozolins
Toufan Parman
Thomas Preston
Annmarie Ramkissoon
Aaron Shapiro
Michelle Siu
Nicole Sweeting
Louise Winn
Andrea Wong
Mayme Wong

+ > 120 undergraduate research students

Current & Recent Graduate Students

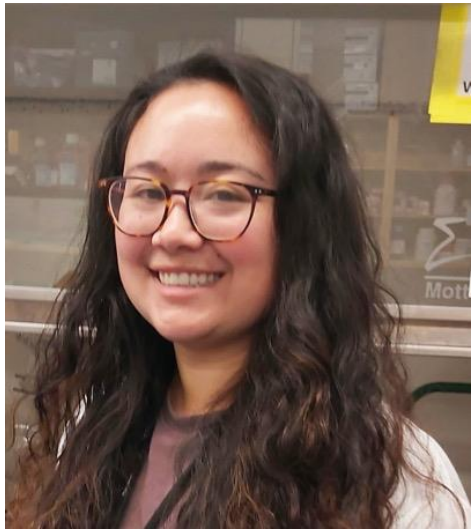
Their unpublished research is included in and/or informs this presentation

Shama Bhatia,
Ph.D.

Danielle Drake,
Ph.D.

Kian Afsharian,
B.Sc.

Ashley Cheng,
M.Sc.



Disclosure

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- NIEHS
- Toronto Hospital for Sick Children Foundation
- University of Toronto Faculty of Pharmacy

"Take-home Messages"

- Embryonic/fetal **DNA damage** caused by reactive oxygen species (**ROS**) can initiate developmental disorders
- **Risk** is determined by the balance of **fetal pathways** for ROS formation vs. ROS detoxification and DNA repair
- **Physiological levels** of ROS formation can be pathogenic in biochemically predisposed fetuses
- **Fetal brain** is highly susceptible to ROS damage
- Many **xenobiotics** enhance ROS formation

Reactive Oxygen Species

- **Physiological** pathways

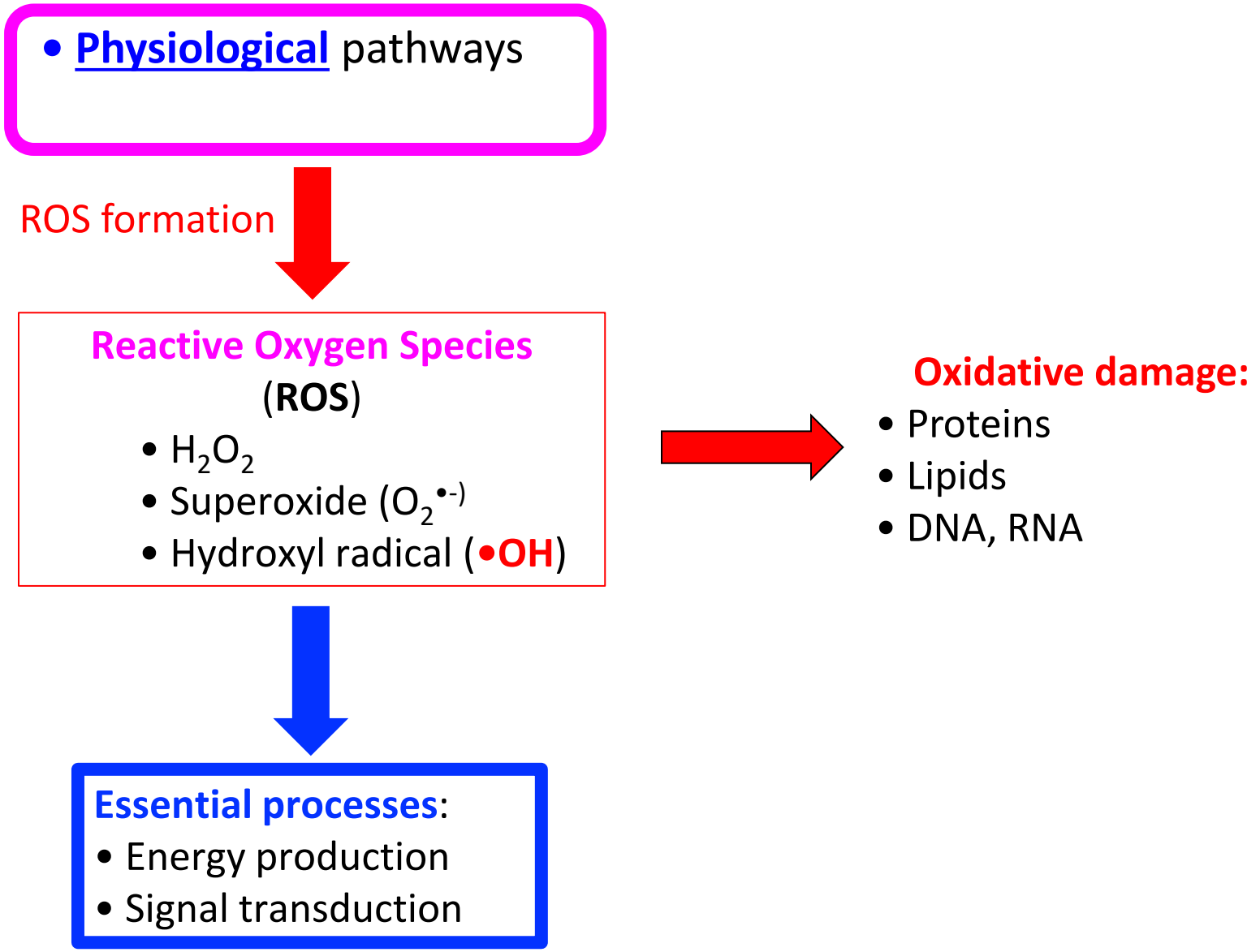
ROS formation O_2

Reactive Oxygen Species
(**ROS**)

- H_2O_2
- Superoxide ($O_2^{\bullet -}$)
- Hydroxyl radical (•OH)

Essential processes:

- Energy production
- Signal transduction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ROS formation
Reactive Oxygen Species
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Oxidative damage:
• Proteins
• Lipids
• DNA, RNA
Essential processes:
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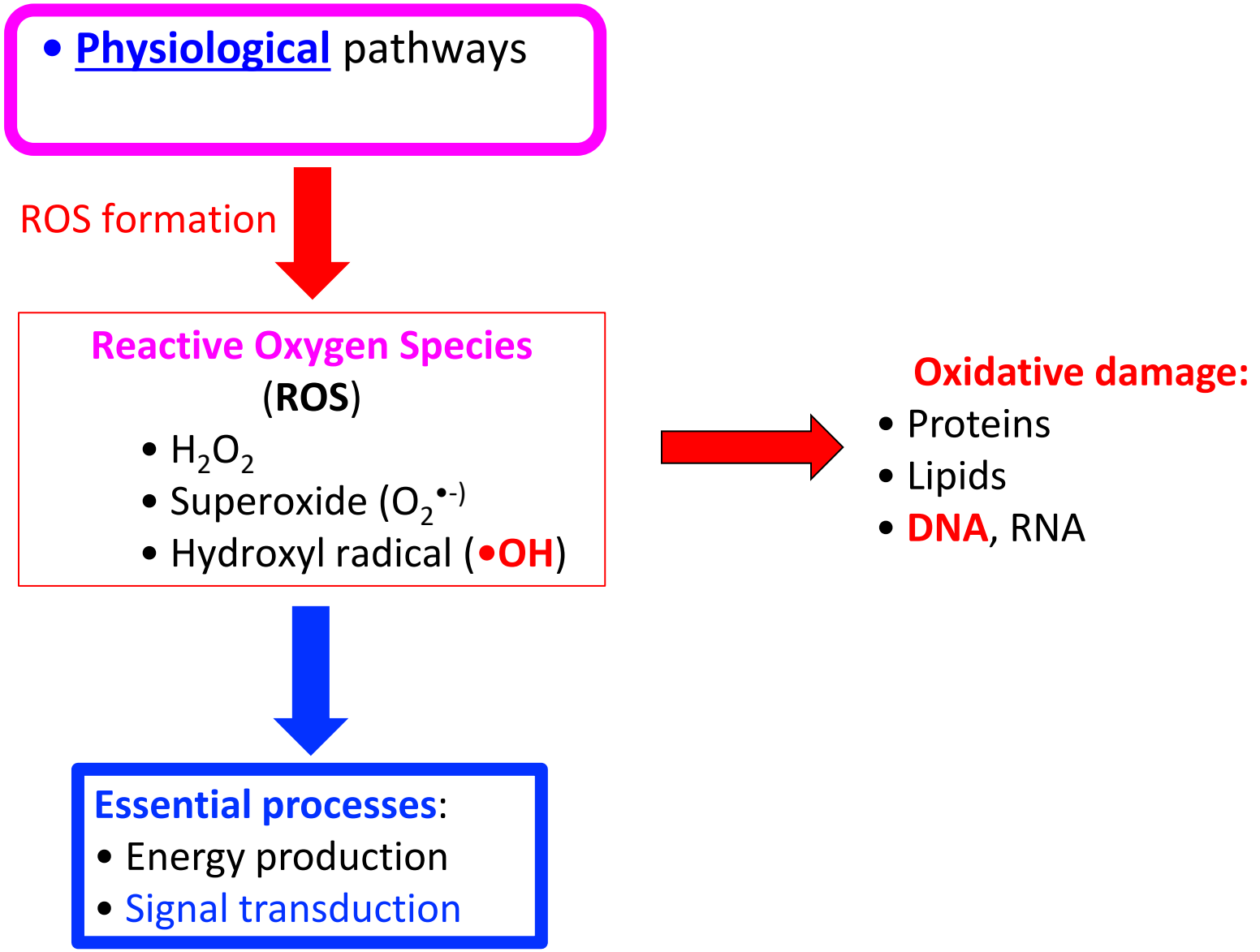
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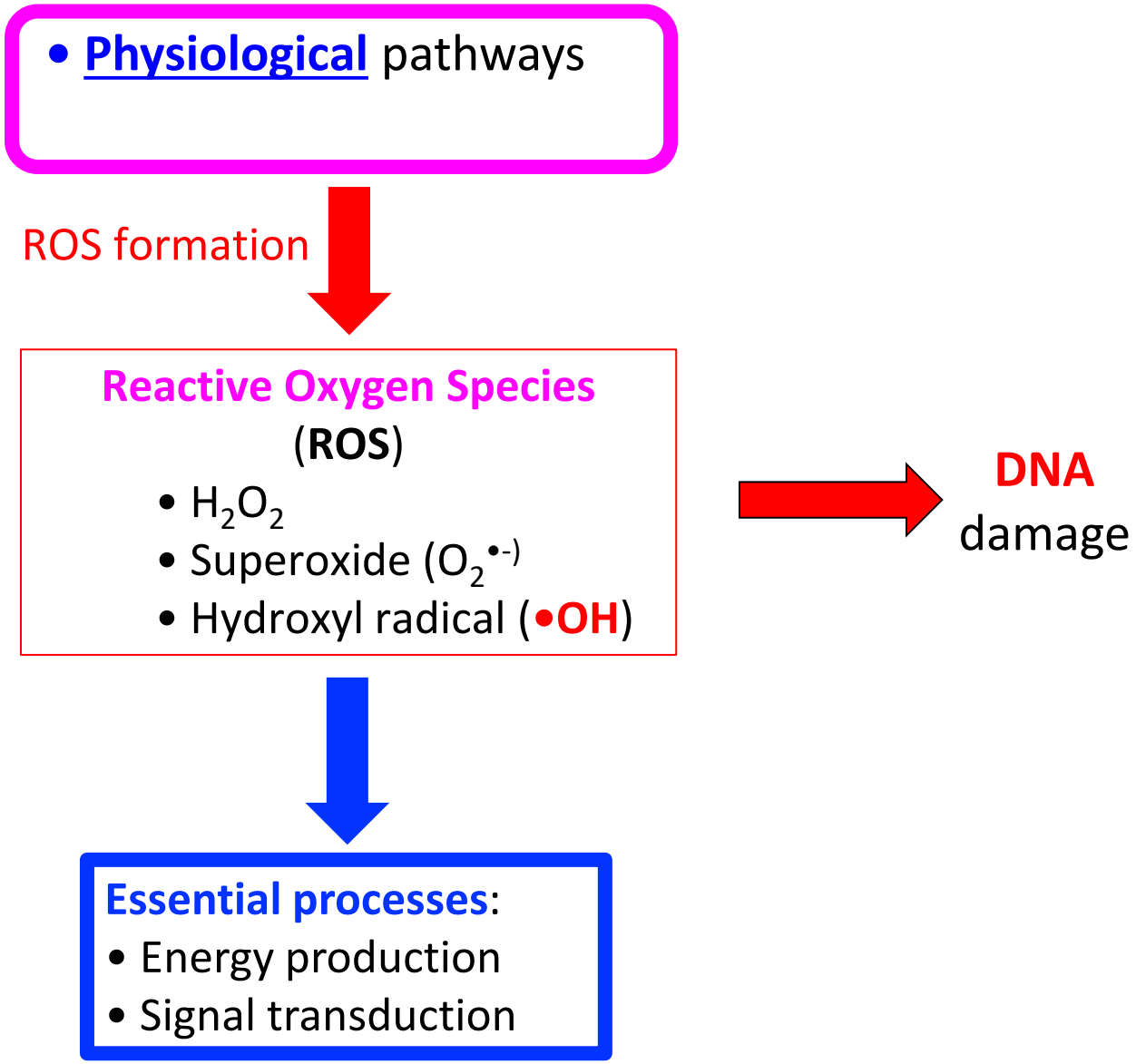
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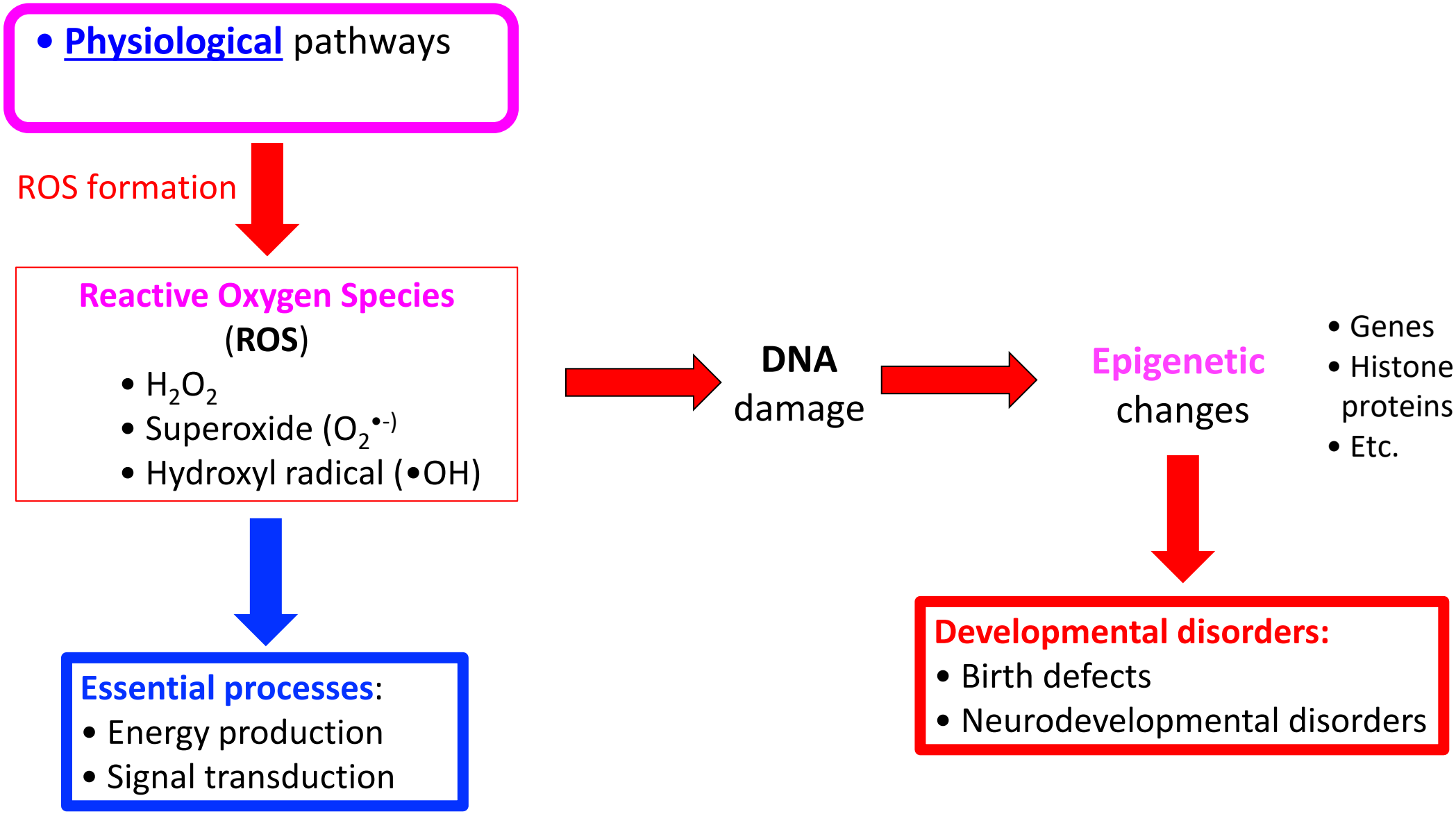
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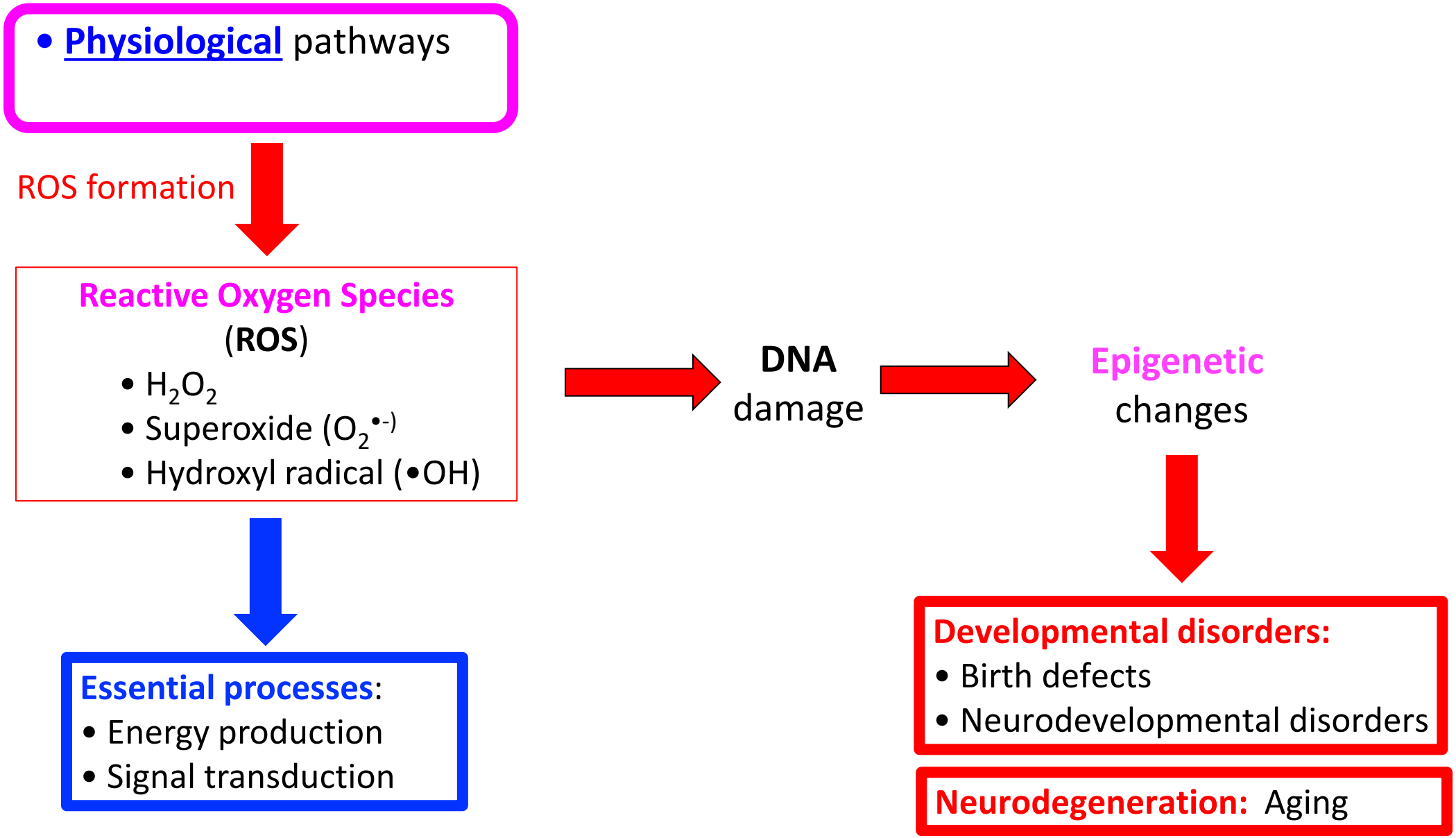
DNA
damage
Epigenetic
changes
• Genes
• Histone proteins
• Etc.
Essential processes:
• Energy production
• Signal transduction
Developmental disorders:
• Birth defects
• Neurodevelopmental disorders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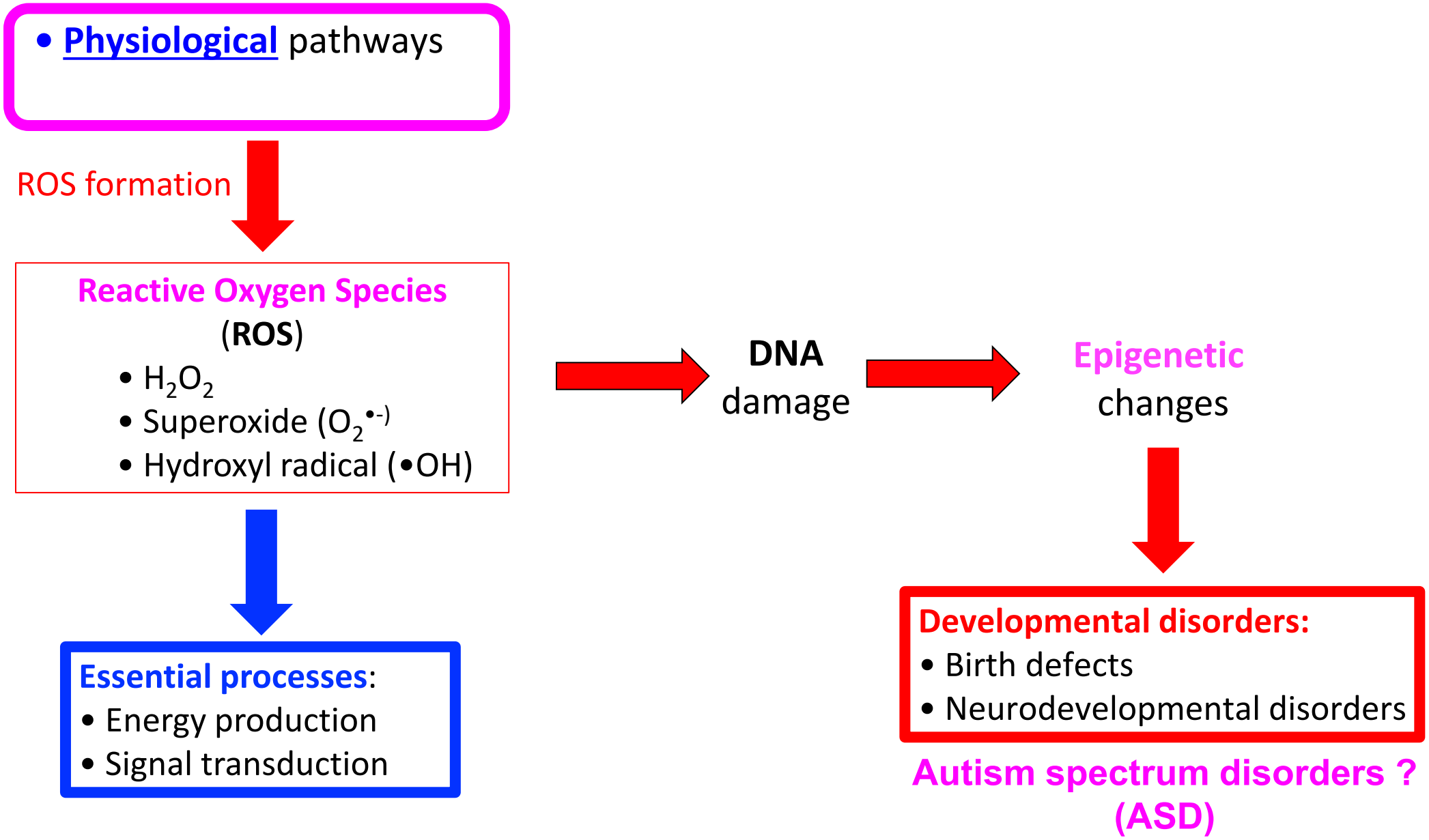
Neurodegeneration: Aging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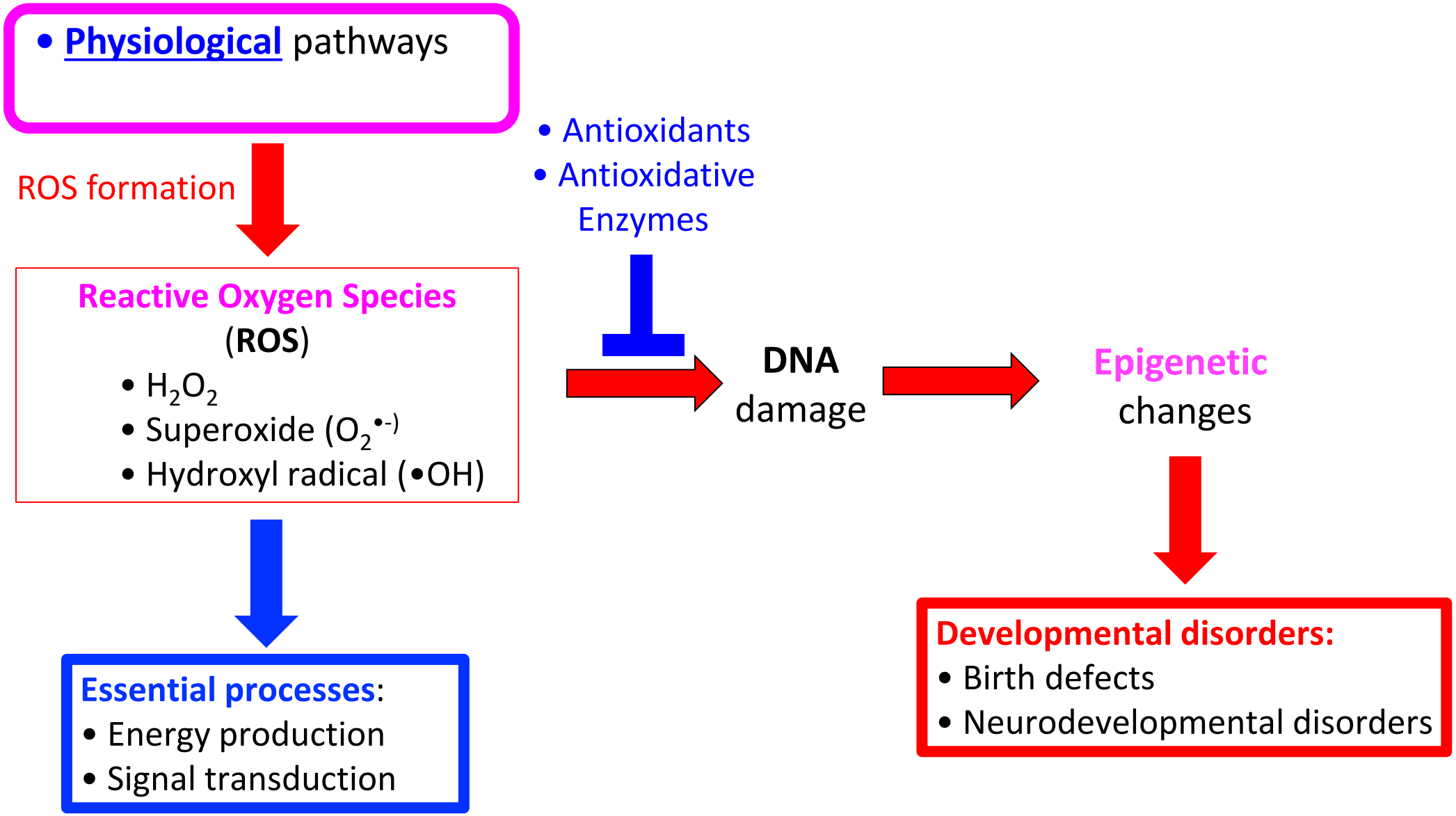
Autism spectrum disorders ?
(ASD)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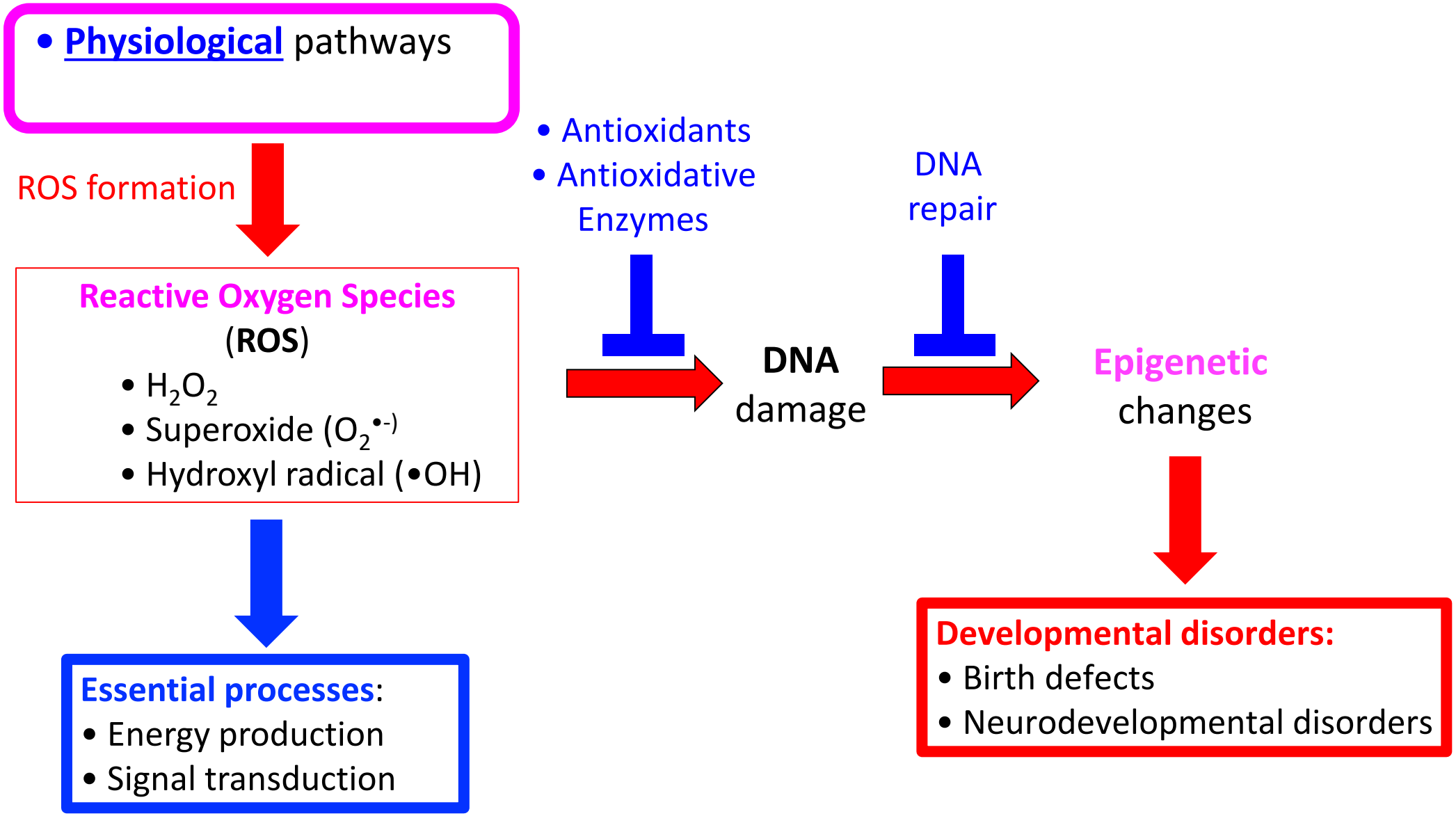
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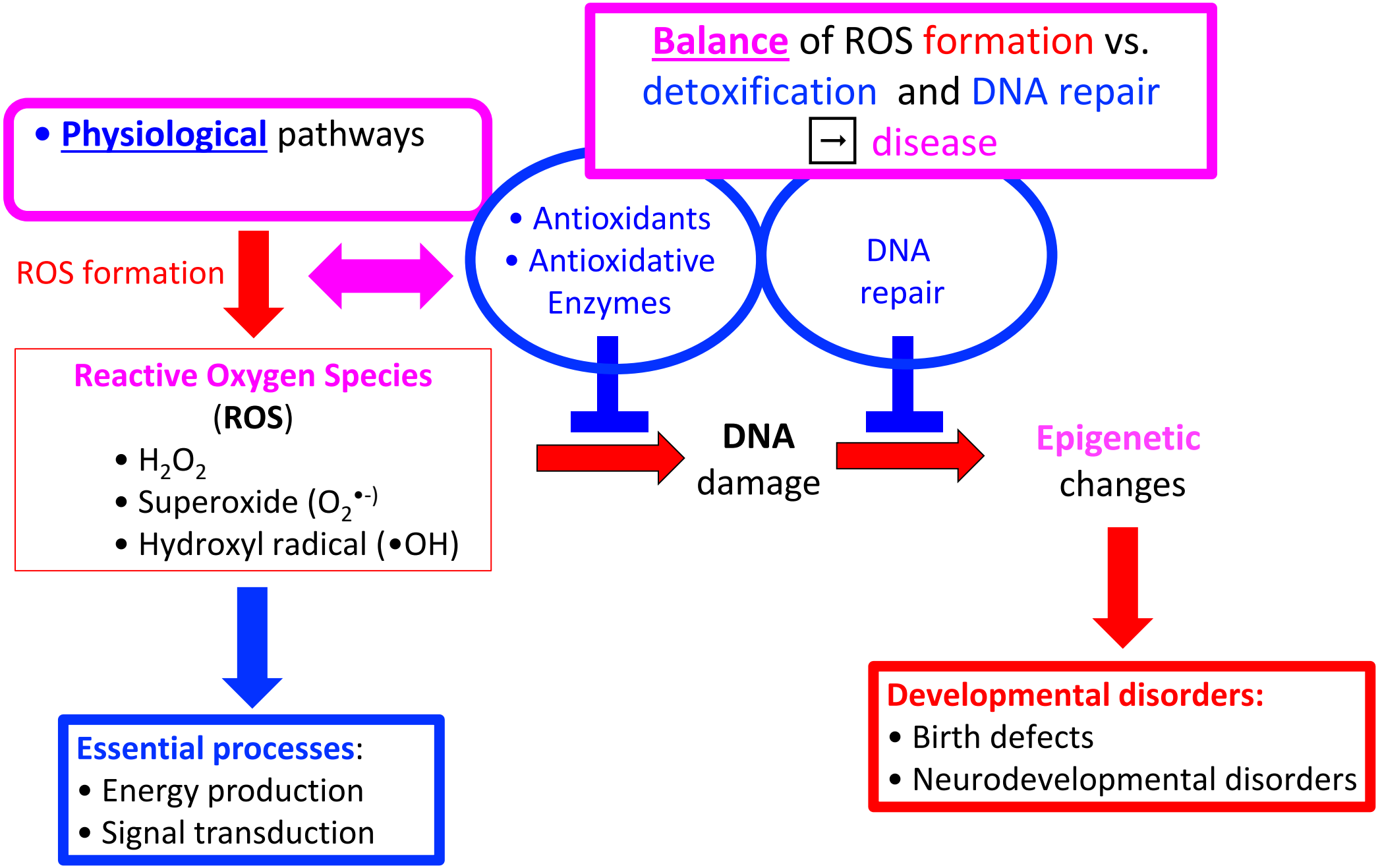
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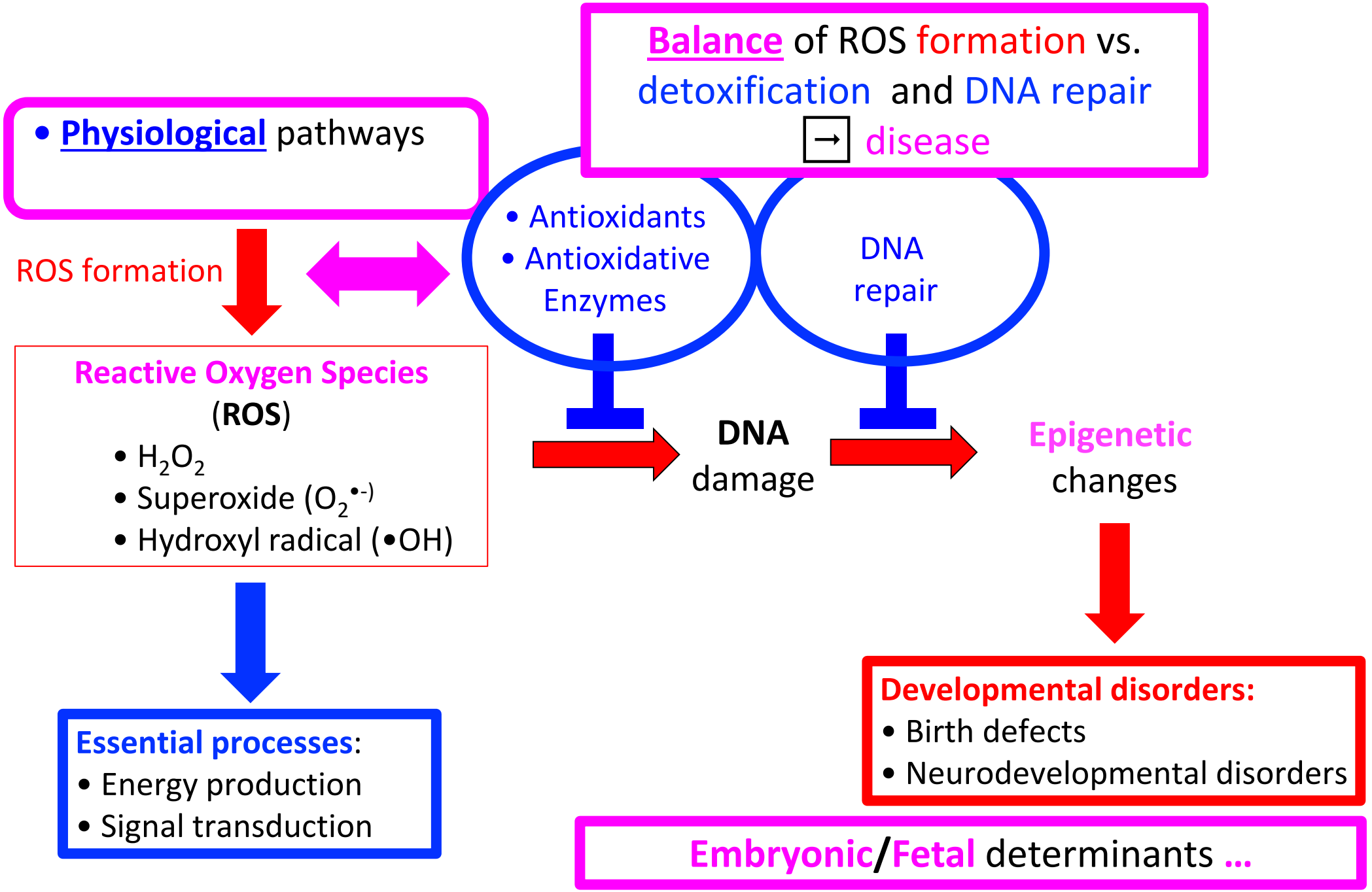
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Embryonic/Fetal determinants …

Embryonic/Fetal pathways
determine risk of
developmental disorders

- **ROS (particularly •OH) are highly unstable**

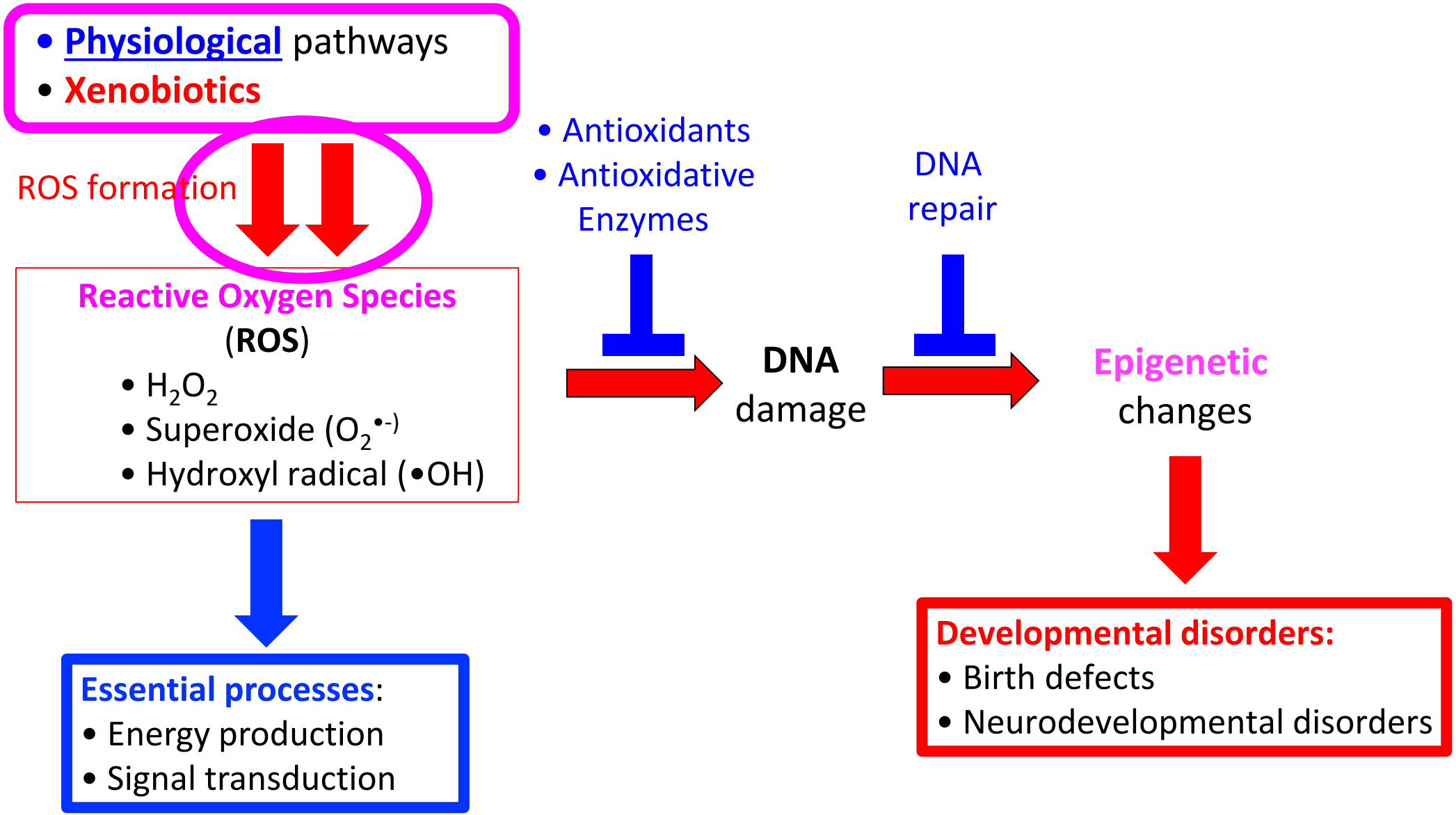
→ must be formed within the embryo

→ embryonic pathways are the key determinants of risk

- **All pathways reflected in embryo culture**

- **Maternal measurements do not reflect embryonic risk**

Xenobiotics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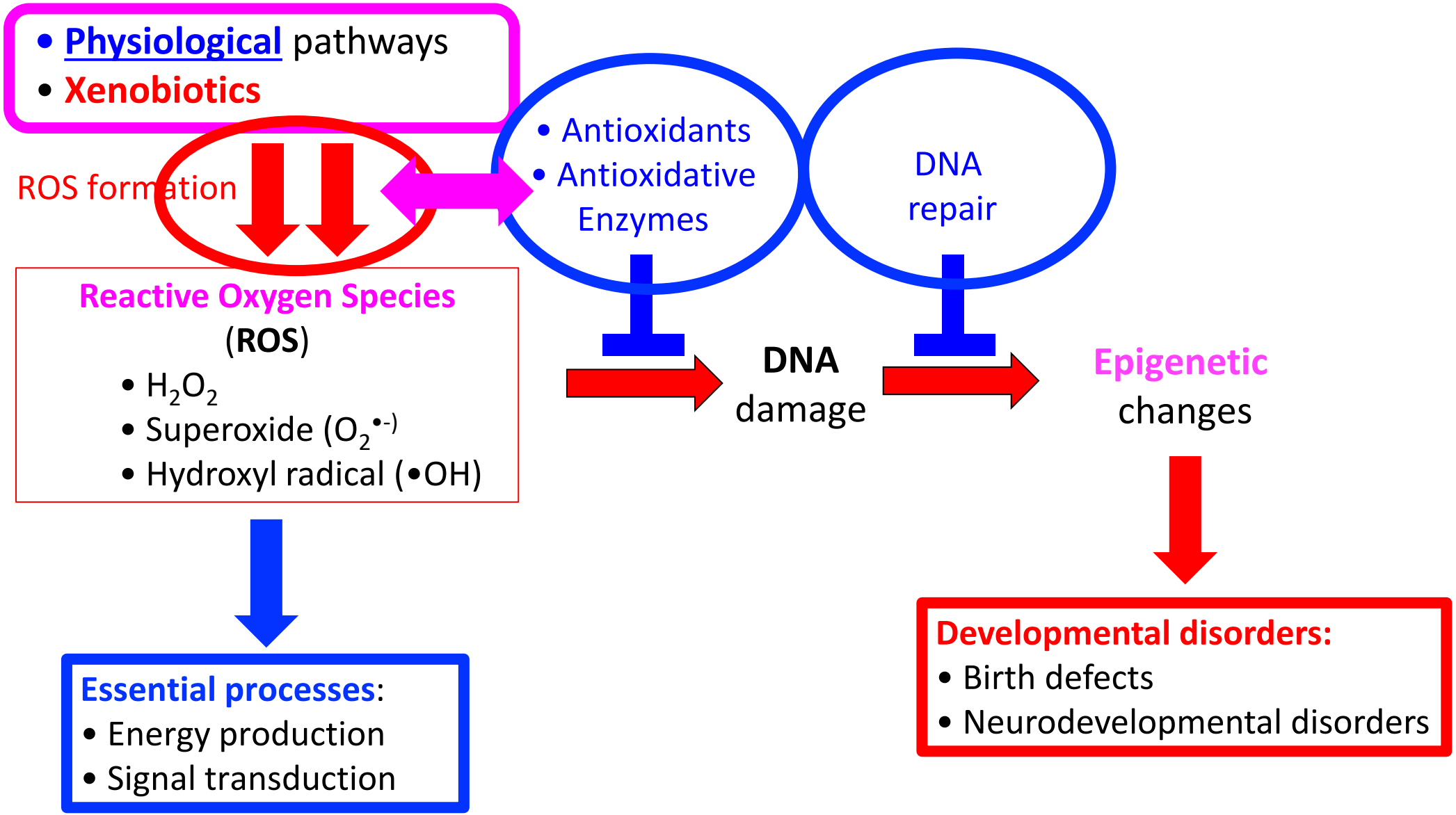
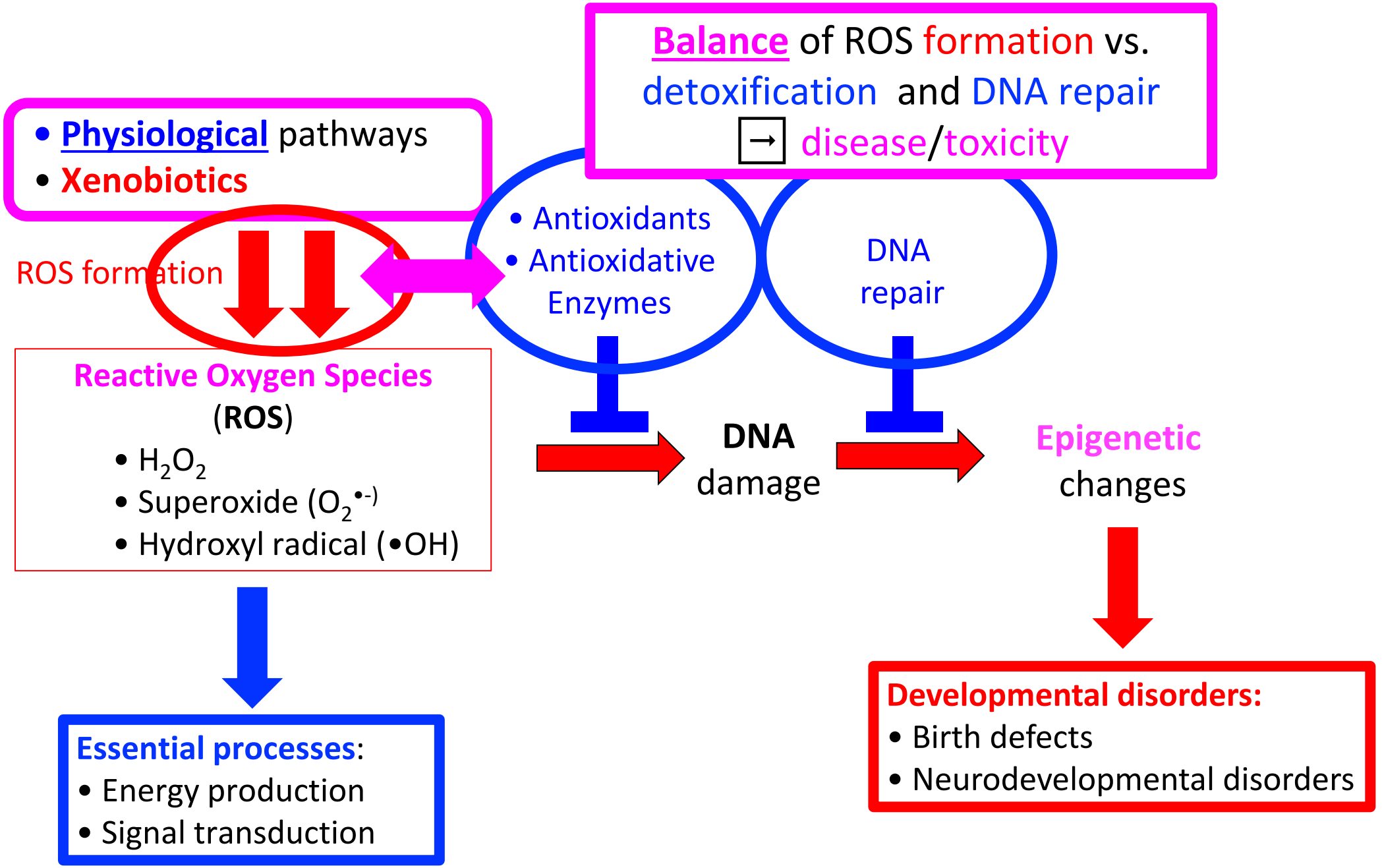
Difference from most drug toxicity …

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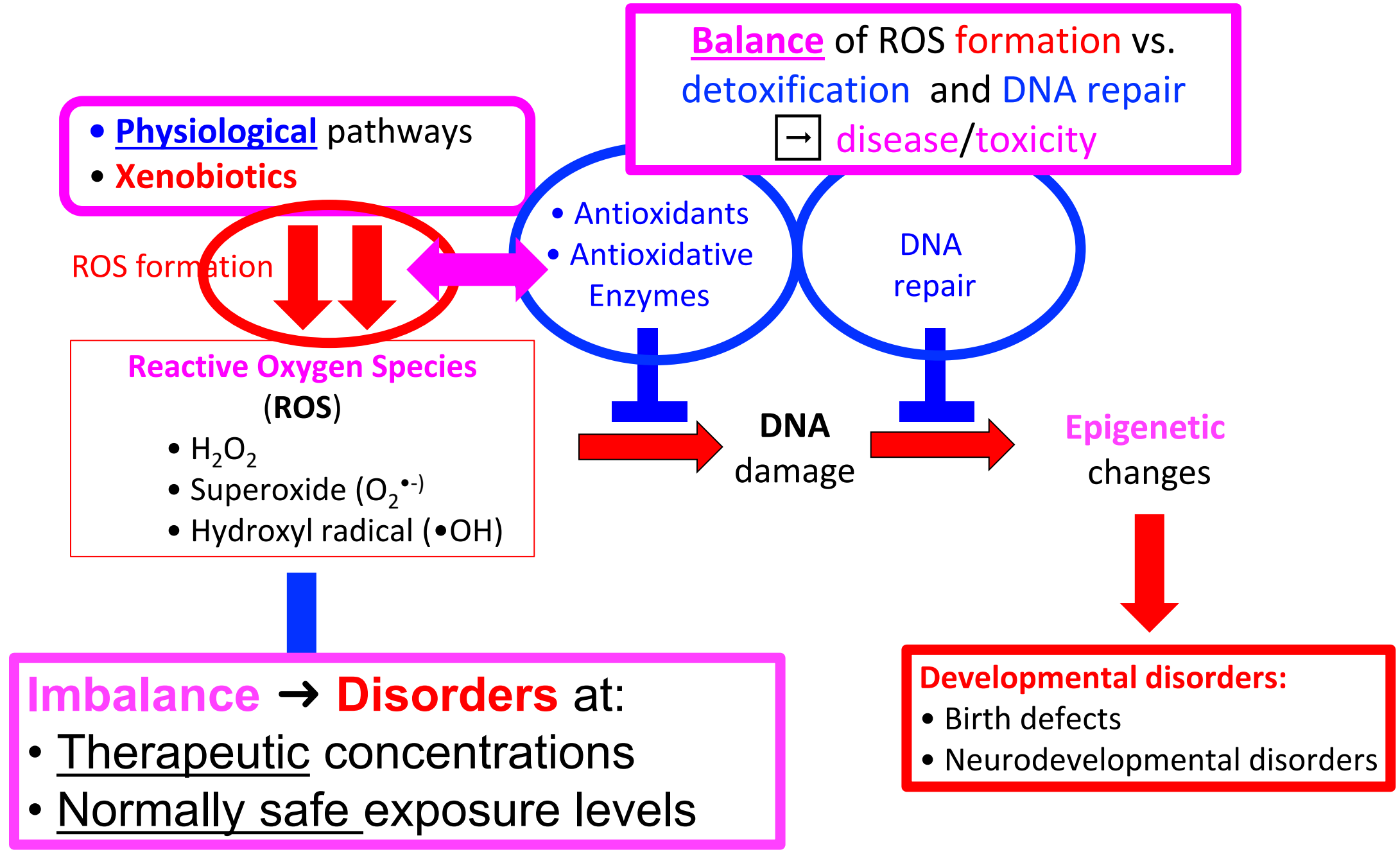
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DNA damage
Epigenetic changes
Developmental disorders:
• Birth defects
• Neurodevelopmental disorders
Imbalance → Disorders at:
• Therapeutic concentrations
• Normally safe exposure levels

Focus of today's talk ...

- **Physiological** pathways
- **Xenobiotics**

ROS formation

Reactive Oxygen Species
(**ROS**)
- H_2O_2
- Superoxide ($O_2^{\bullet -}$)
- Hydroxyl radical (•OH)

Essential processes:
- Energy production
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Antioxidative Enzymes

DNA repair

DNA damage

Epigenetic changes

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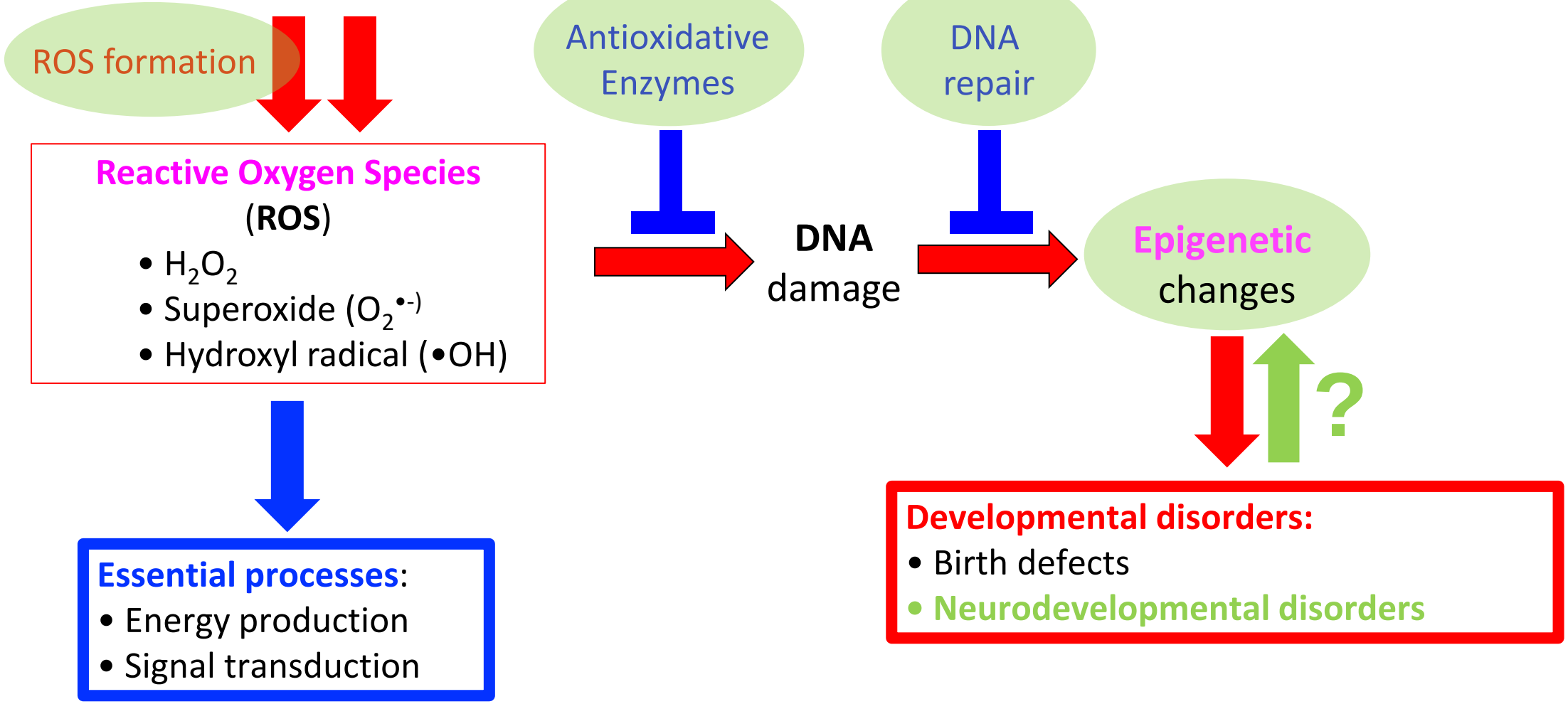
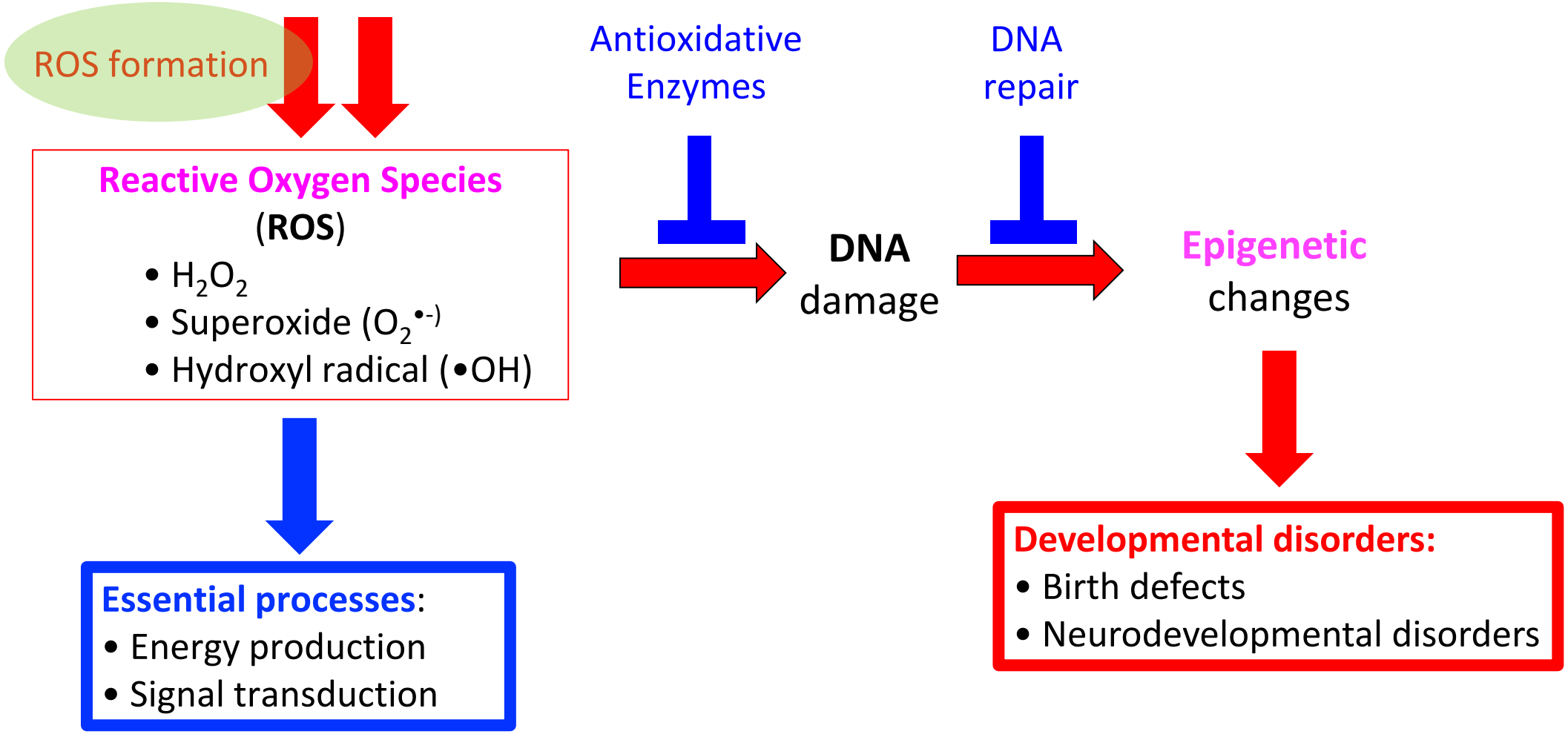
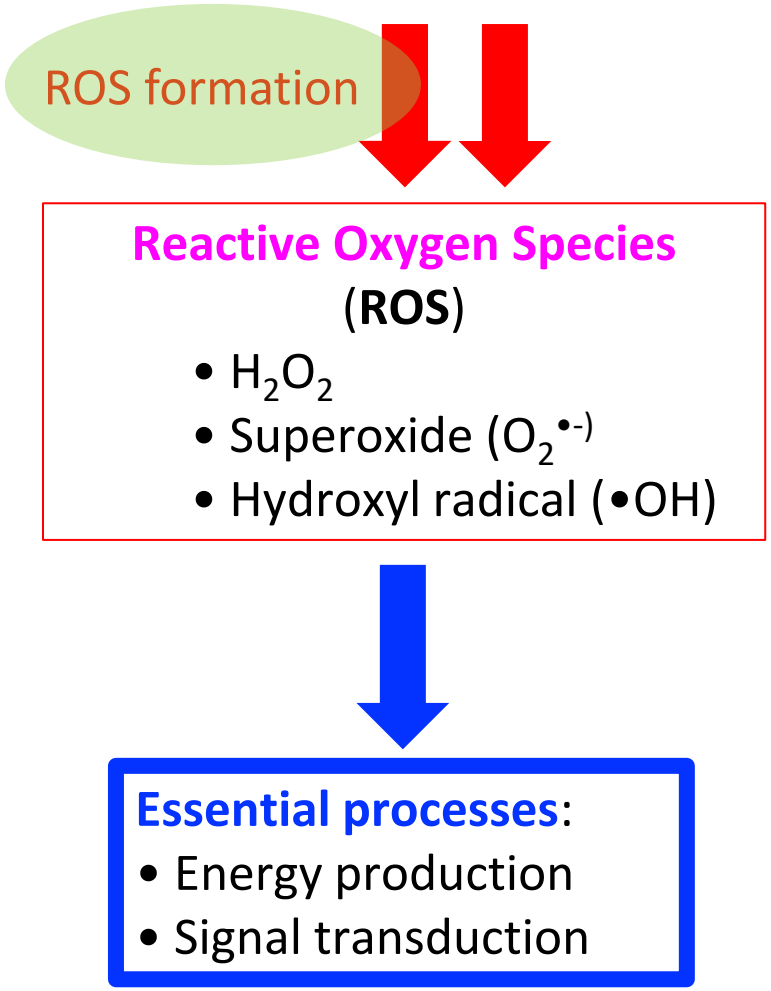


ROS formation

- **Physiological** pathways
- **Xenobiotics**



- **Physiological** pathways
- **Xenobiotics**



Fetal enzymes catalyzing ROS formation

Prostaglandin H synthases (PHS)

- Phenytoin
- Methamphetamine
- Benzo[a]pyrene
- Thalidomide

NADPH oxidases (NOX)

- Ethanol
- Methanol
- Methamphetamine

(see FASD symposium, Wednesday)

Nitric oxide synthases (NOS)

- Phenytoin
- Benzo[a]pyrene

- **Physiological** pathways
- **Xenobiotics**

ROS formation

Reactive Oxygen Species
(**ROS**)
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Essential processes:
- Energy production
- Signal transduction

Fetal enzymes catalyzing ROS formation

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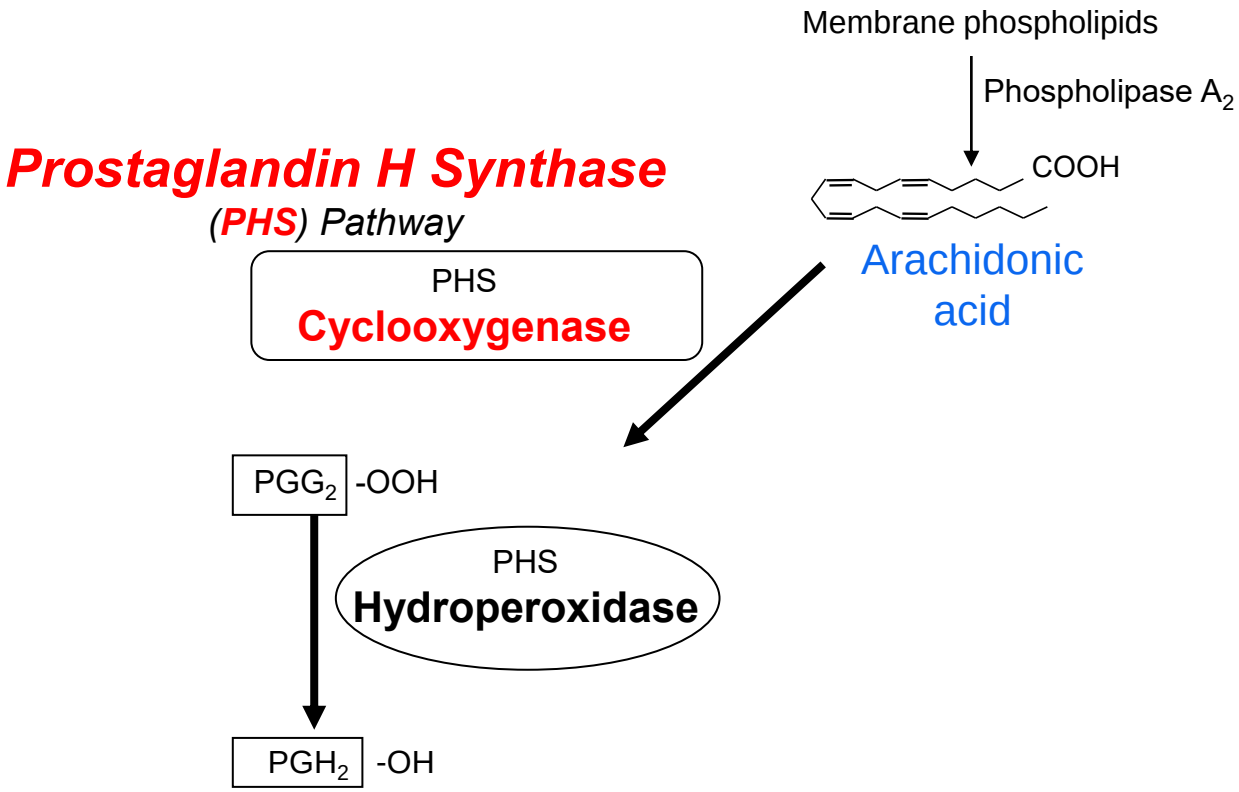
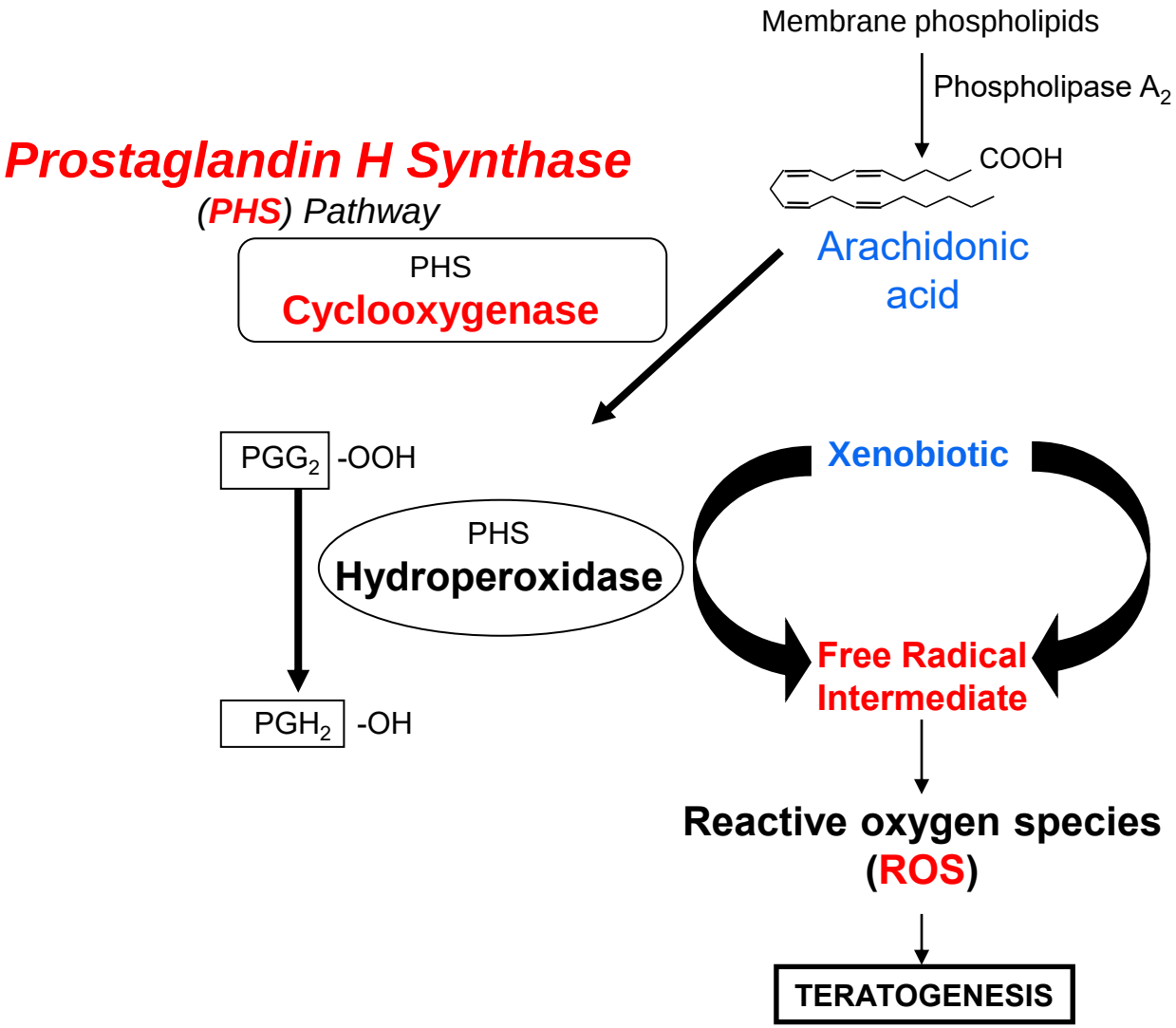
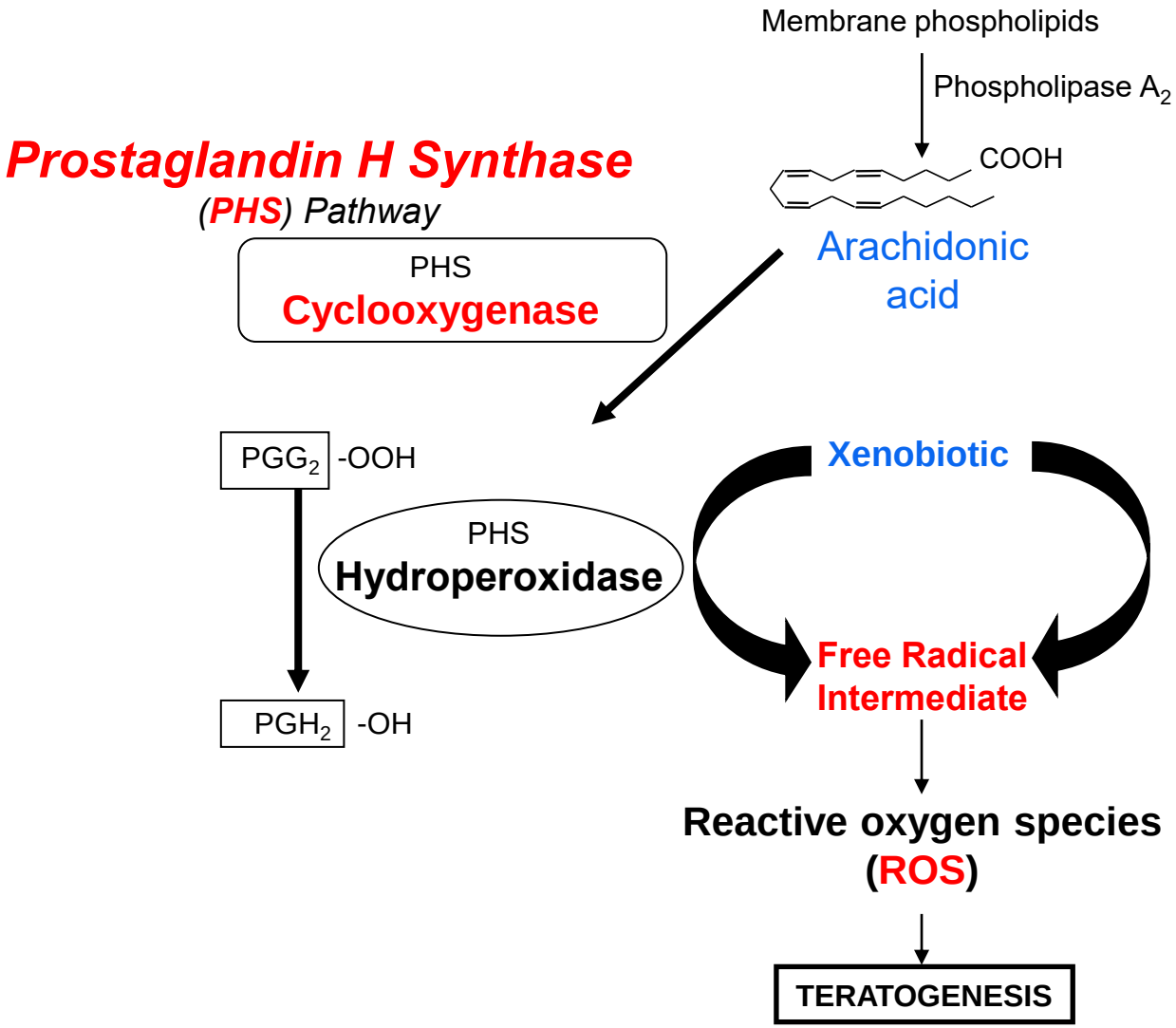


Prostaglandin synthesis


Prostaglandin H Synthase
(PHS) Pathway
Membrane phospholipids
Phospholipase A2
COOH
Arachidonic acid
PHS
Cyclooxygenase
PGG2 -OOH
PHS
Hydroperoxidase
PGH2 -OH
Xenobiotic
Free Radical Intermediate
Reactive oxygen species (ROS)
TERATOGENESIS



Teratogens bioactivated by PHS

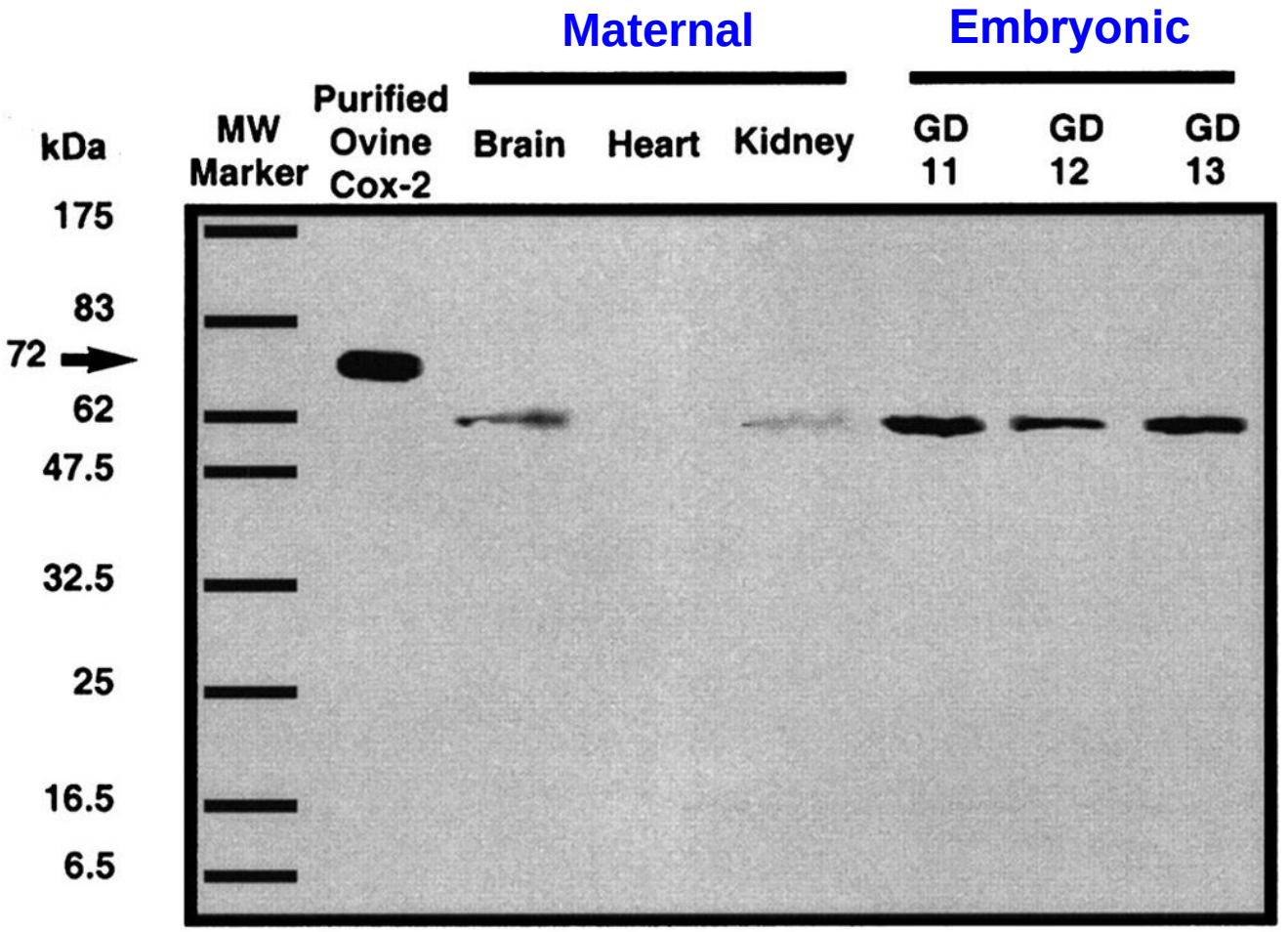
- Phenytoin & structurally related antiepileptic drugs
- Benzo[a]pyrene
- Methamphetamine
- Thalidomide

PHS-2 (COX-2)

- “Non-constitutive” (low) in most adult tissues

Western blot analysis

Prostaglandin H Synthase-2 (PHS-2/Cox-2)



(Parman and Wells, FASEB J 16: 1001-1009, 2002)

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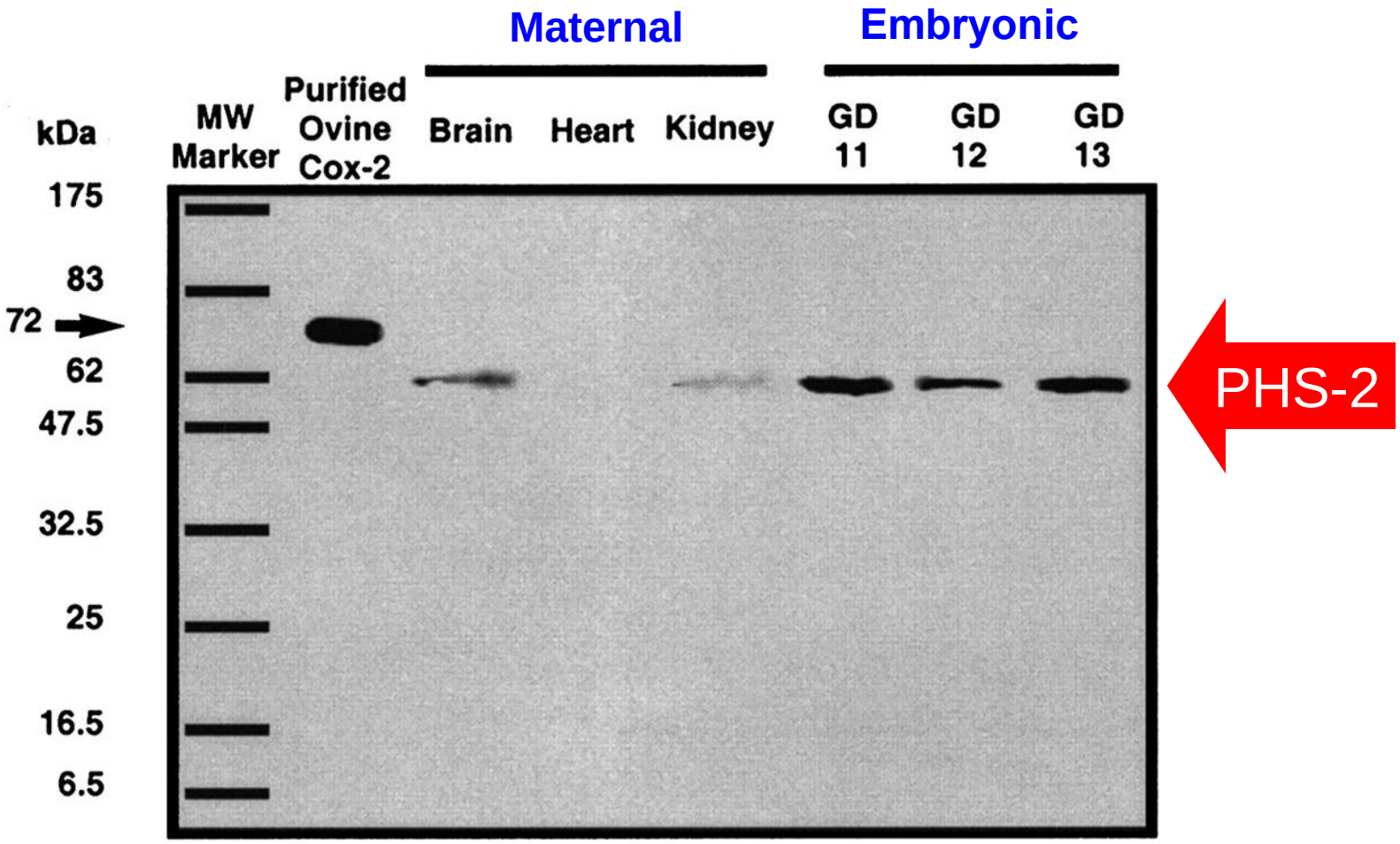

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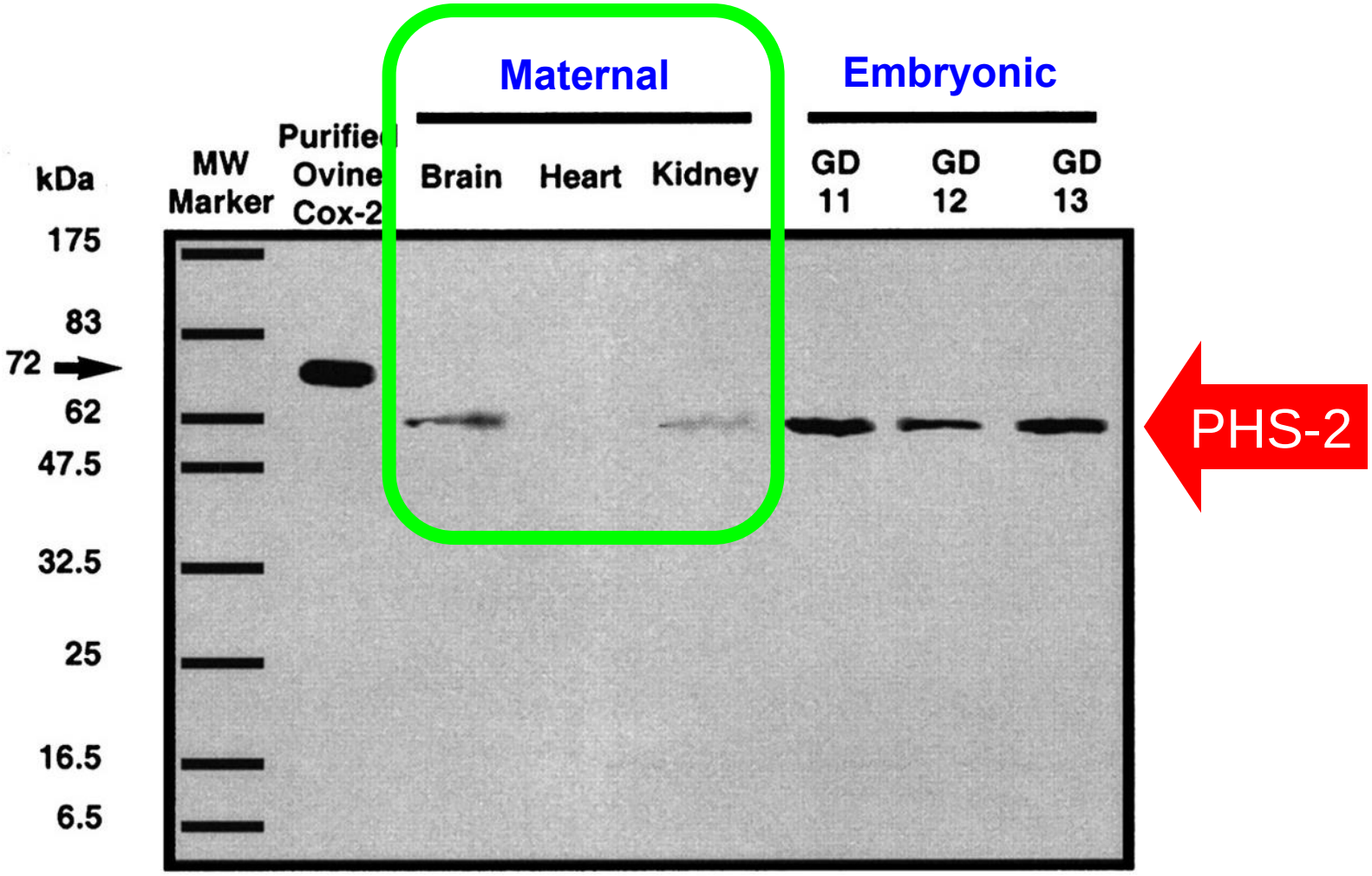

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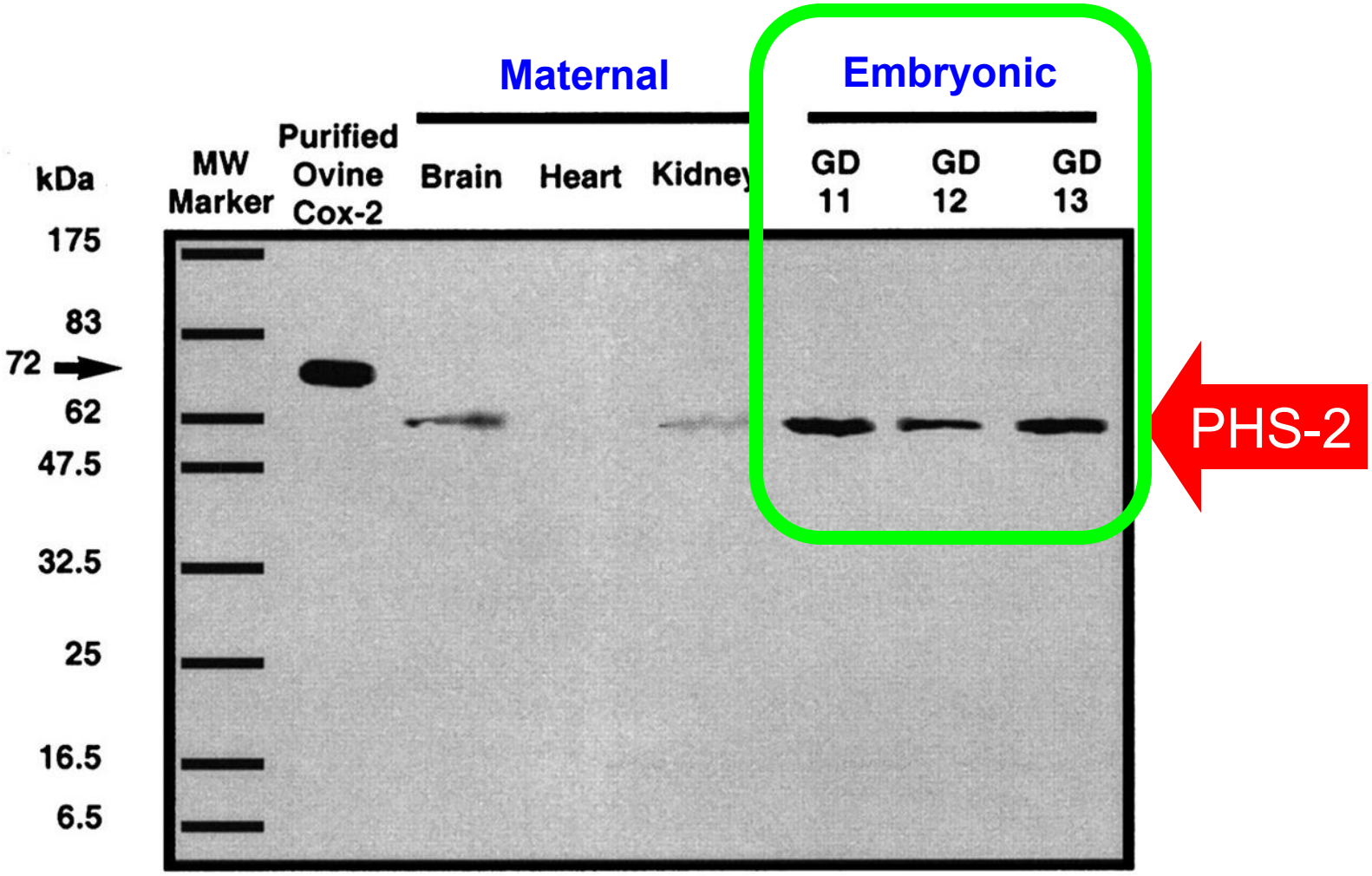


(Parman and Wells, FASEB J 16: 1001-1009, 2002)

Oxidative DNA Damage & Teratogenesis

Thalidomide

Teratogenicity

- **Rabbits** susceptible
- **Mice** resistant

Relevance of Oxidative DNA Damage

Thalidomide

- **Rabbits** vs. **Mice**
- **DNA oxidation** and **birth defects**
- Free radical spin trapping and
  ROS blocking agent
  - Phenylbutylnitrone [**PBN**])

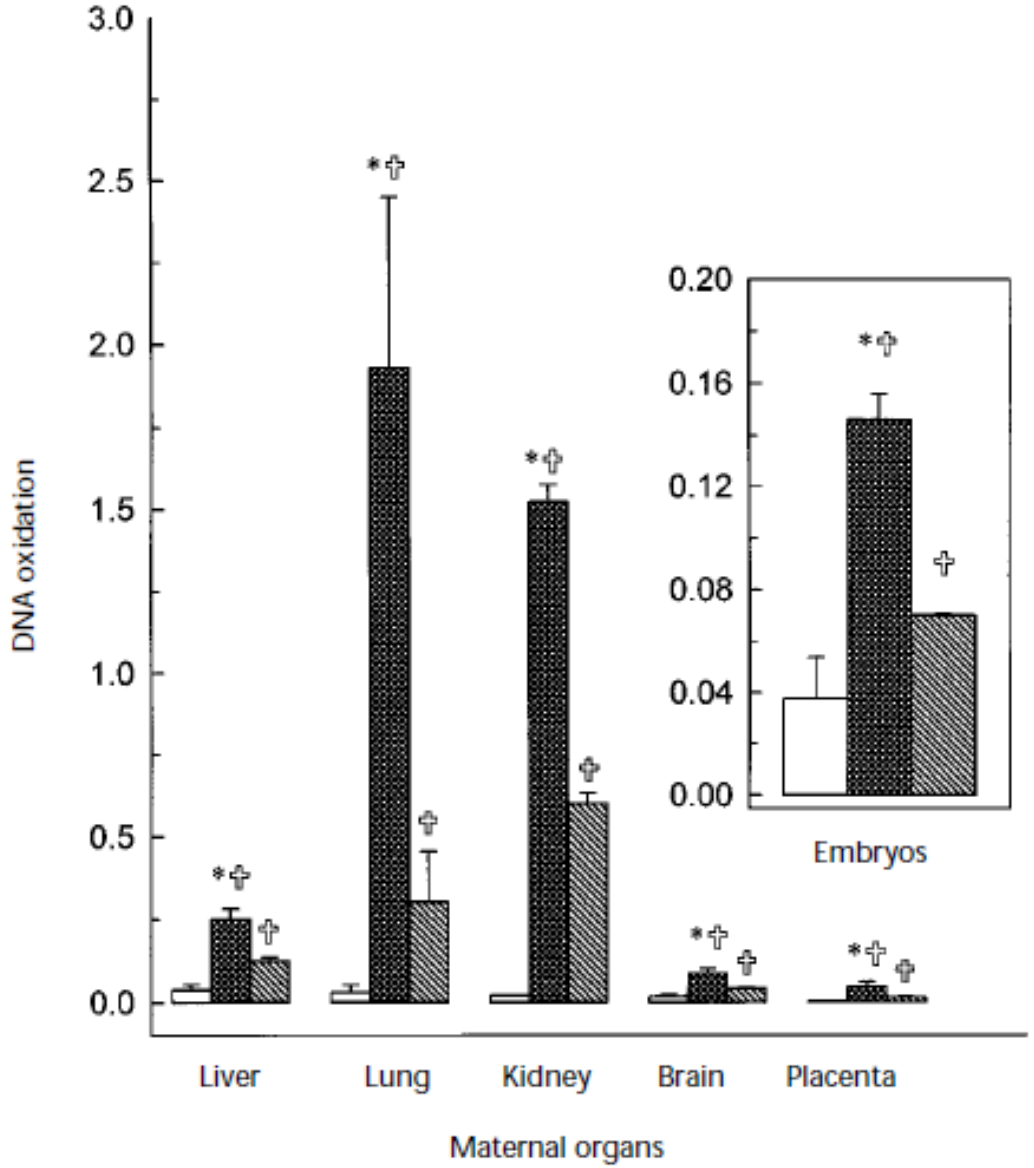
Fetal bioactivation: Prostaglandin H Synthase (PHS)

[Parman et al., Nat. Med. 1999]

DNA oxidation

(8-oxoG)

T, TD = thalidomide



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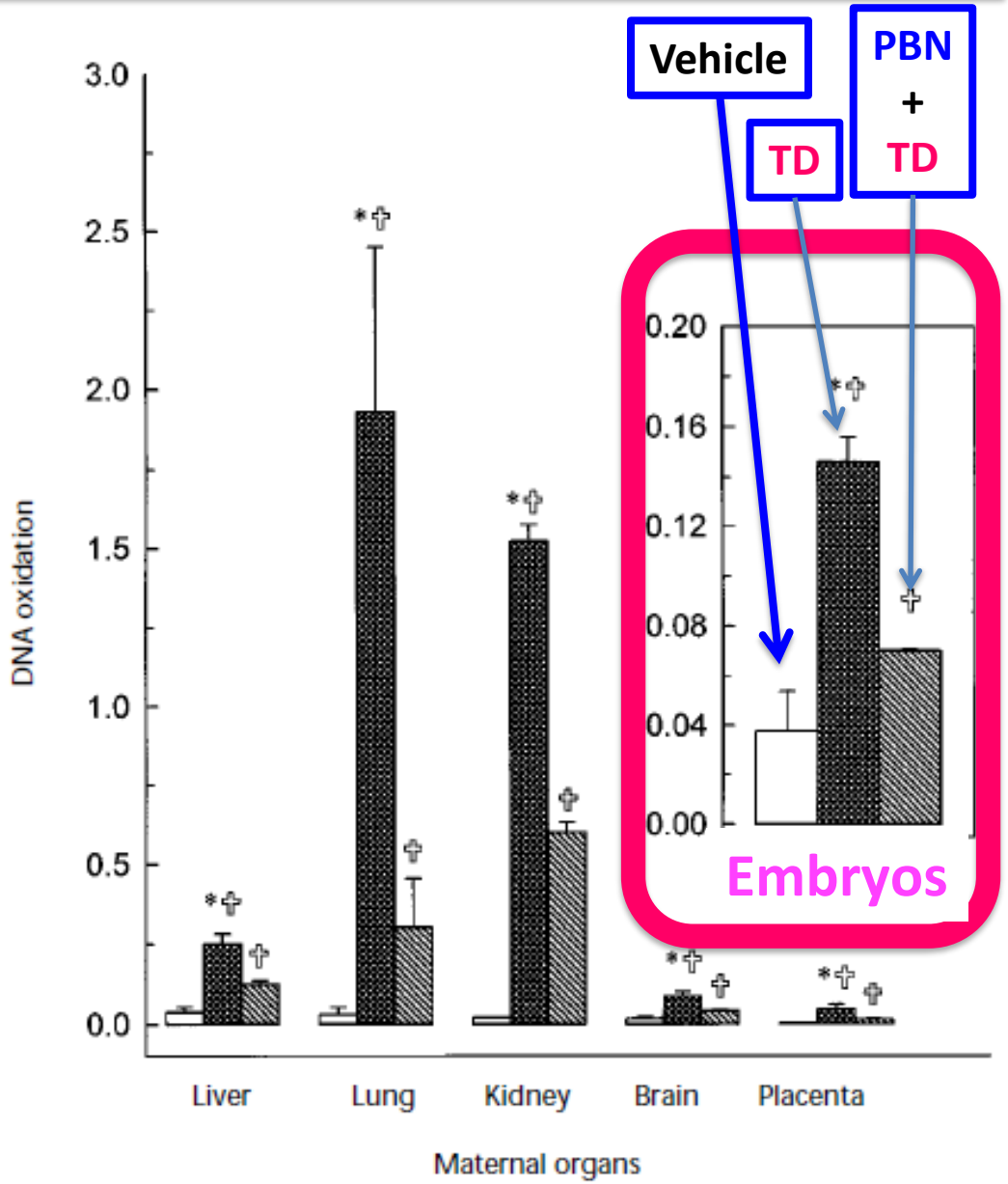


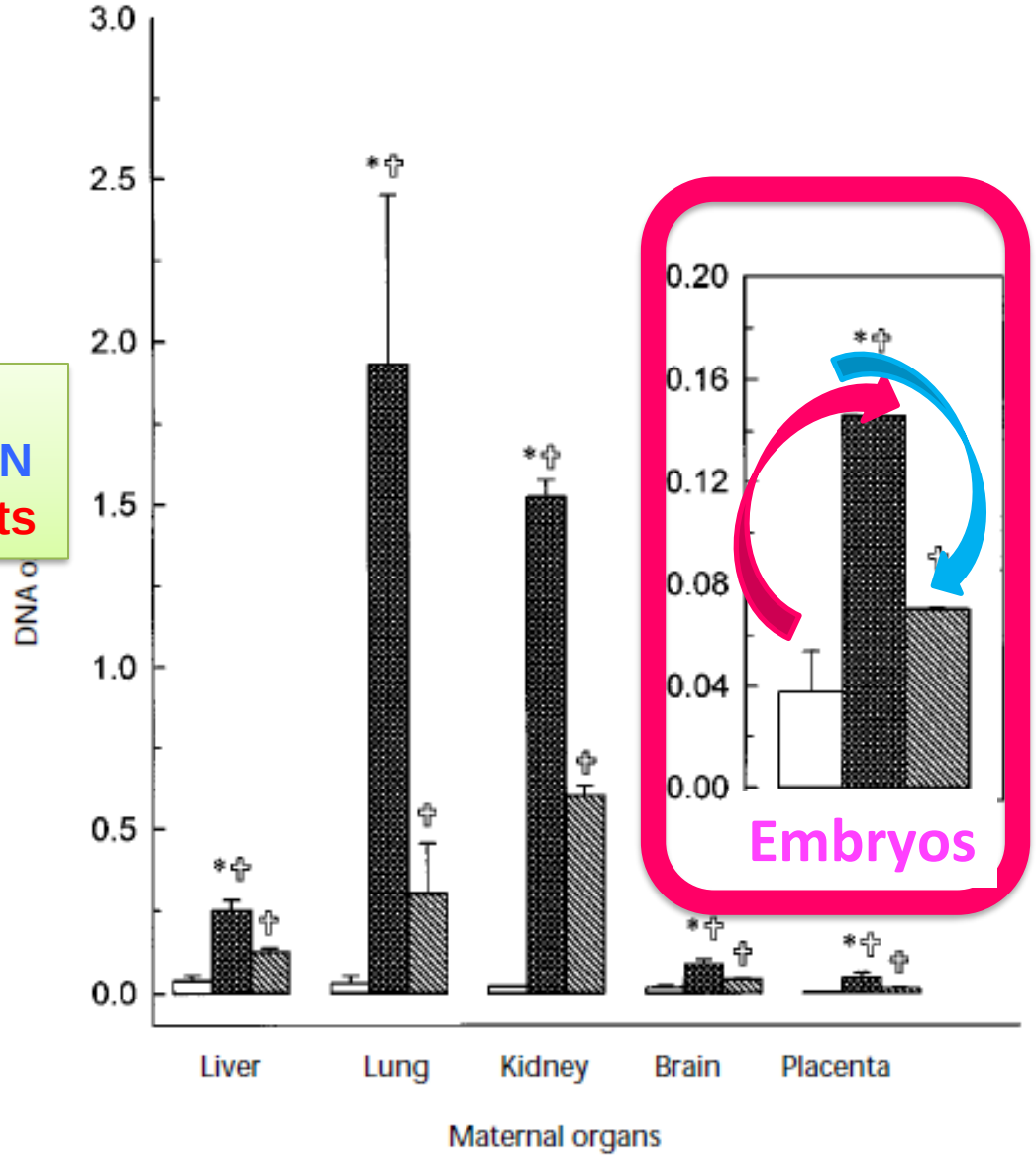
Fetal bioactivation: Prostaglandin H Synthase (PHS)

[Parman et al., Nat. Med. 1999]

- TD ↑ DNA oxidation
- Blocked by PBN

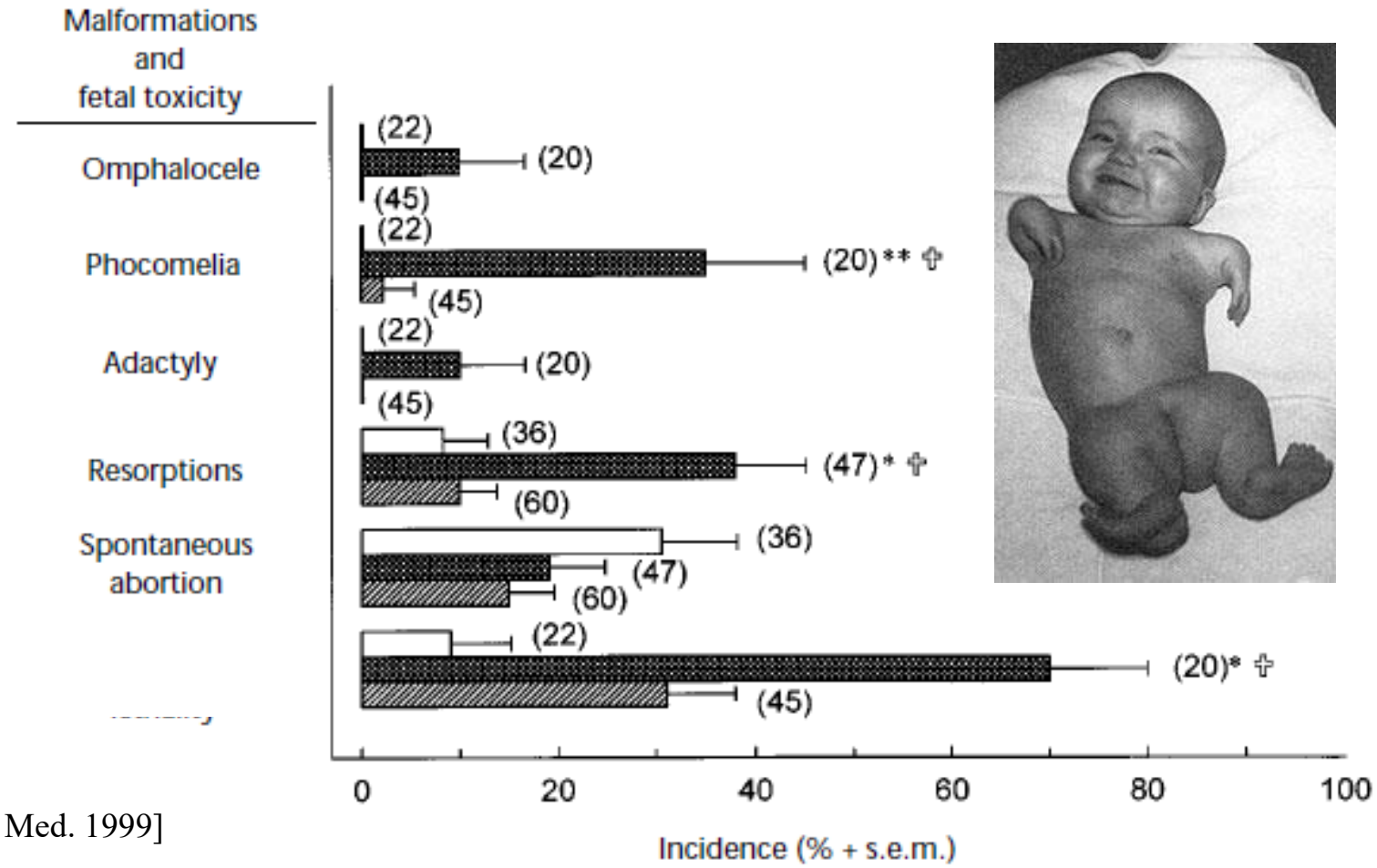
If ROS and DNA oxidation are important in TD teratogenesis, PBN should protect against birth defects

T, TD = thalidomide



Fetal bioactivation: Prostaglandin H Synthase (PHS)

Malformations & Fetal Toxicity



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Fetal bioactivation: Prostaglandin H Synthase (PHS)

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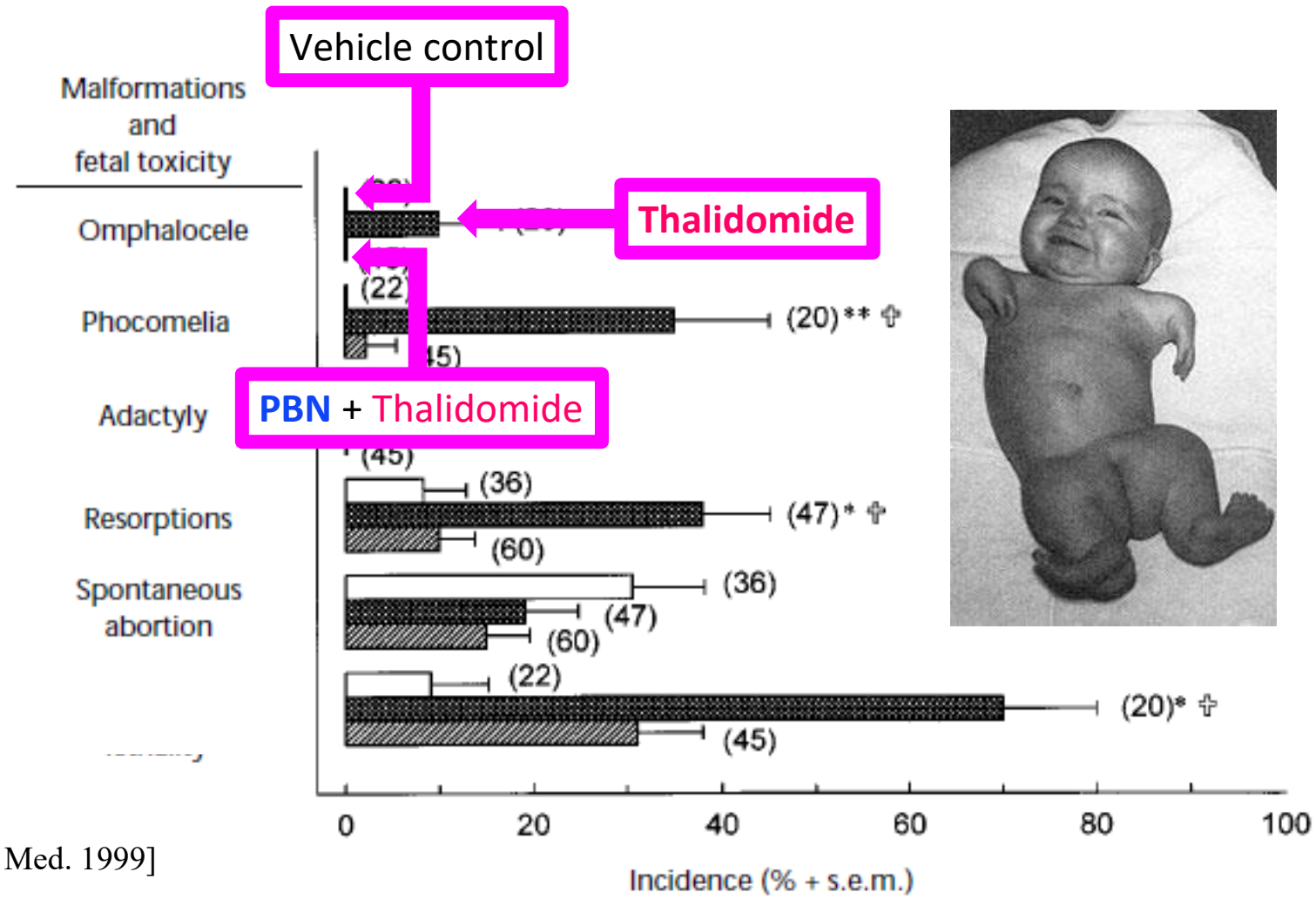
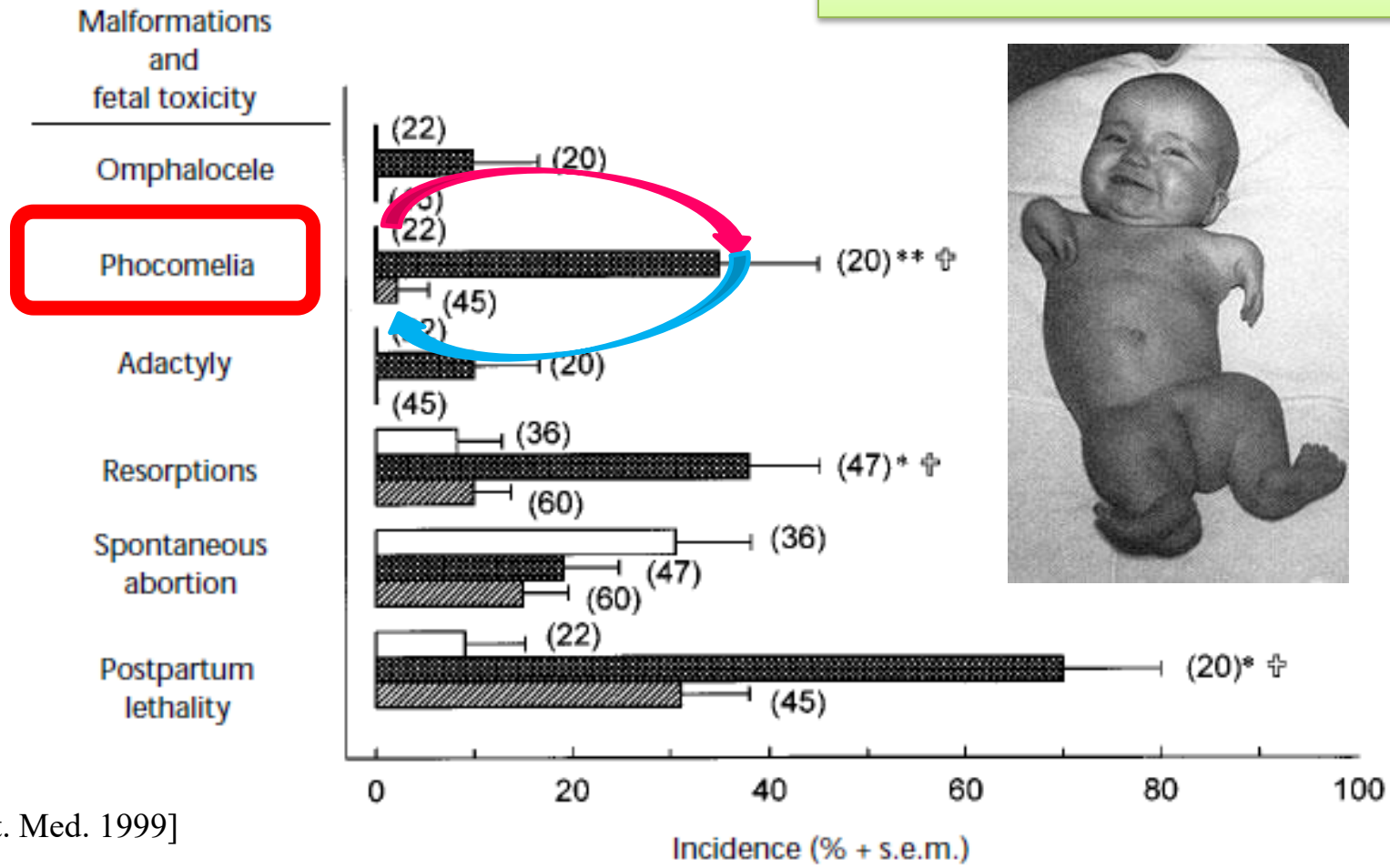


[Parman et al., Nat. Med. 1999]

Fetal bioactivation: Prostaglandin H Synthase (PHS)

- TD ↑ phocomelia to 40% vs. 0% in controls
- Blocked by PBN

ROS and DNA oxidation are important in the mechanism of TD teratogenesis



[Parman et al., Nat. Med. 1999]

Fetal bioactivation: Prostaglandin H Synthase (PHS)

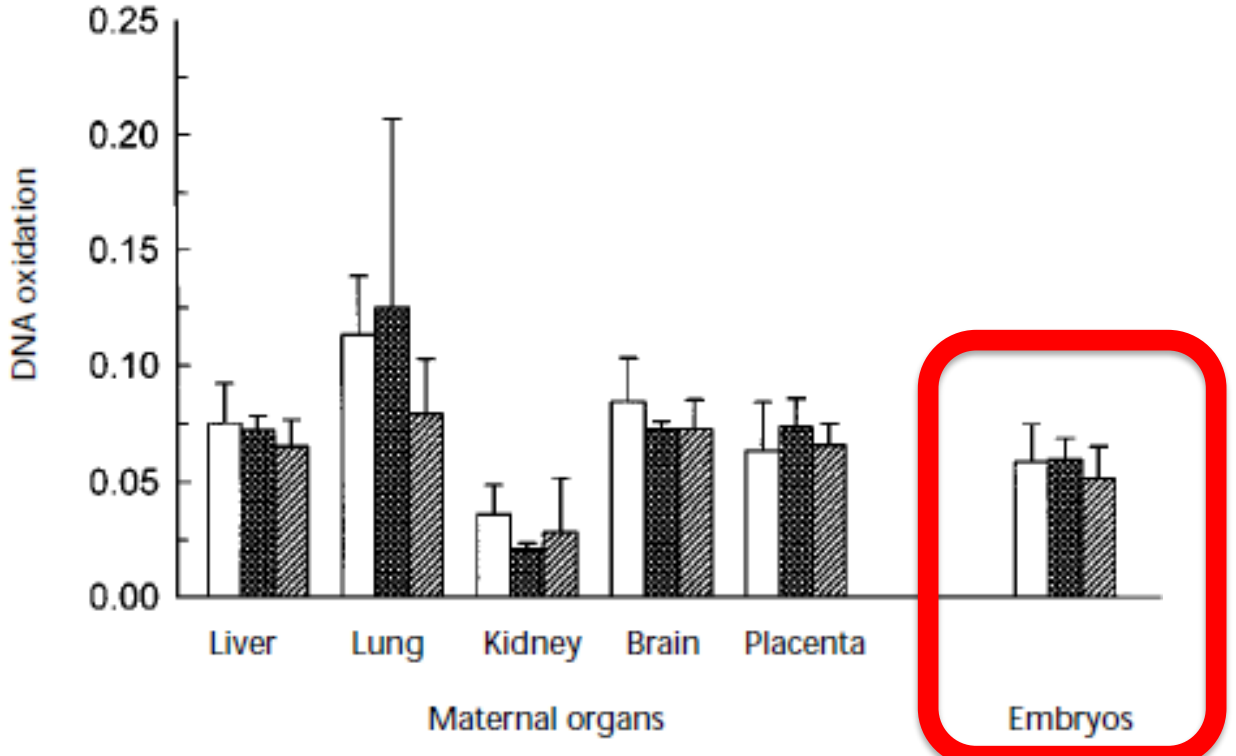
- **To corroborate the mechanism:**
  - **Compare across species!**

Rodents are resistant to TD teratogenesis

Fetal bioactivation: Prostaglandin H Synthase (PHS)

- No DNA oxidation in mouse embryos
- Consistent with resistance to TD birth defects

Suggests:
TD → ROS formation → DNA oxidation → Birth defects



[Parman et al., Nat. Med. 1999]

Corroborating PHS studies

PHS inhibitors

- In vivo – Thalidomide **teratogenicity** in rabbits inhibited by pretreatment with the PHS-1/2 inhibitor acetylsalicylic acid (ASA)

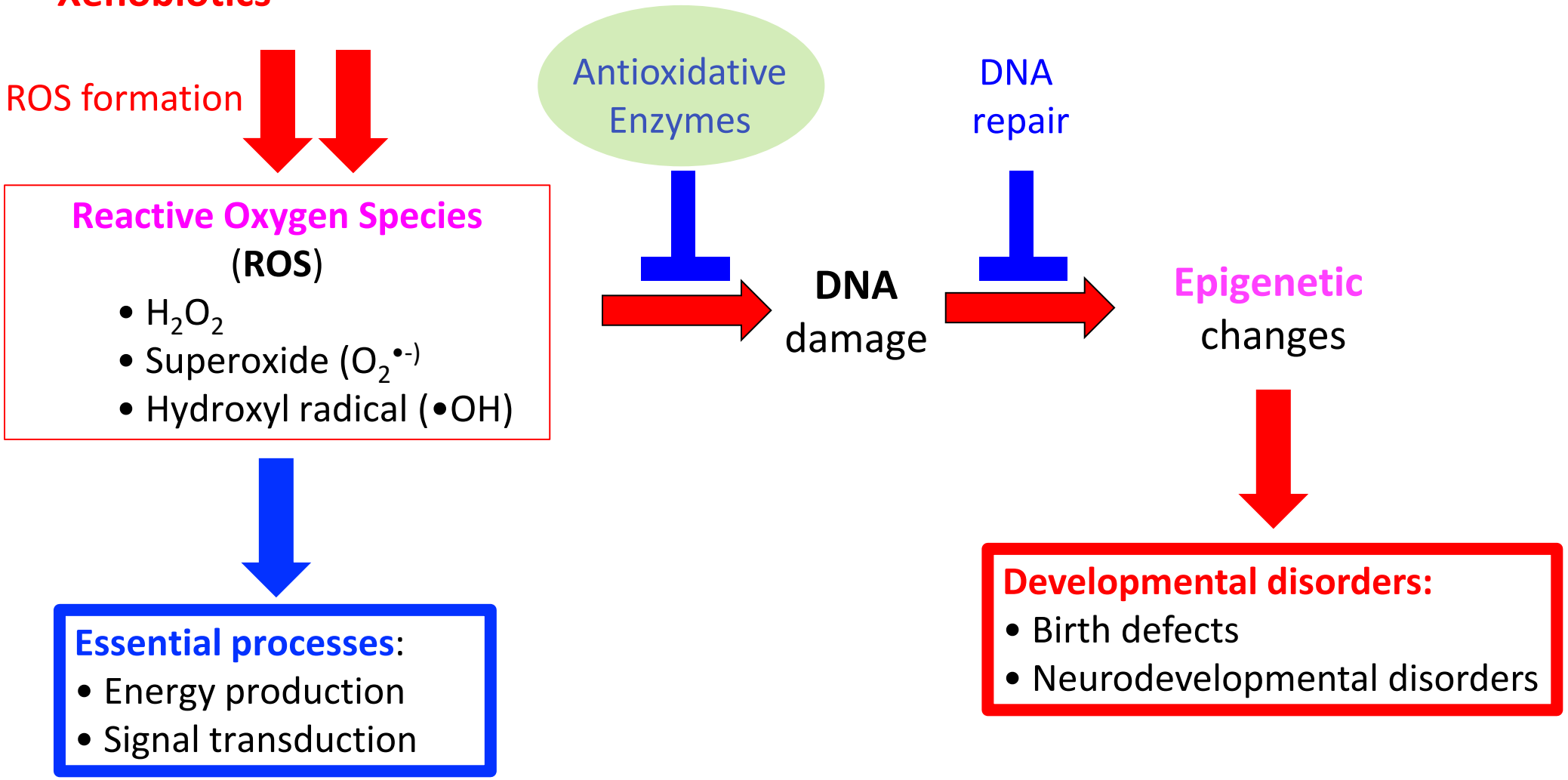
Corroborating PHS studies

PHS inhibitors

- In vivo – Thalidomide **teratogenicity** in rabbits inhibited by pretreatment with the PHS-1/2 inhibitor acetylsalicylic acid (ASA)
- In rabbit embryo culture:
  - **DNA damage** and **limb & ocular anomalies** caused by thalidomide & 2 phthalimido hydrolysis products
  - All blocked by:
    - PHS inhibitors eicosatetraynoic acid (ETYA) and ASA
    - ROS blocker phenylbutylnitrone (PBN)

Antioxidative Enzymes

- **Physiological** pathways
- **Xenobiotics**



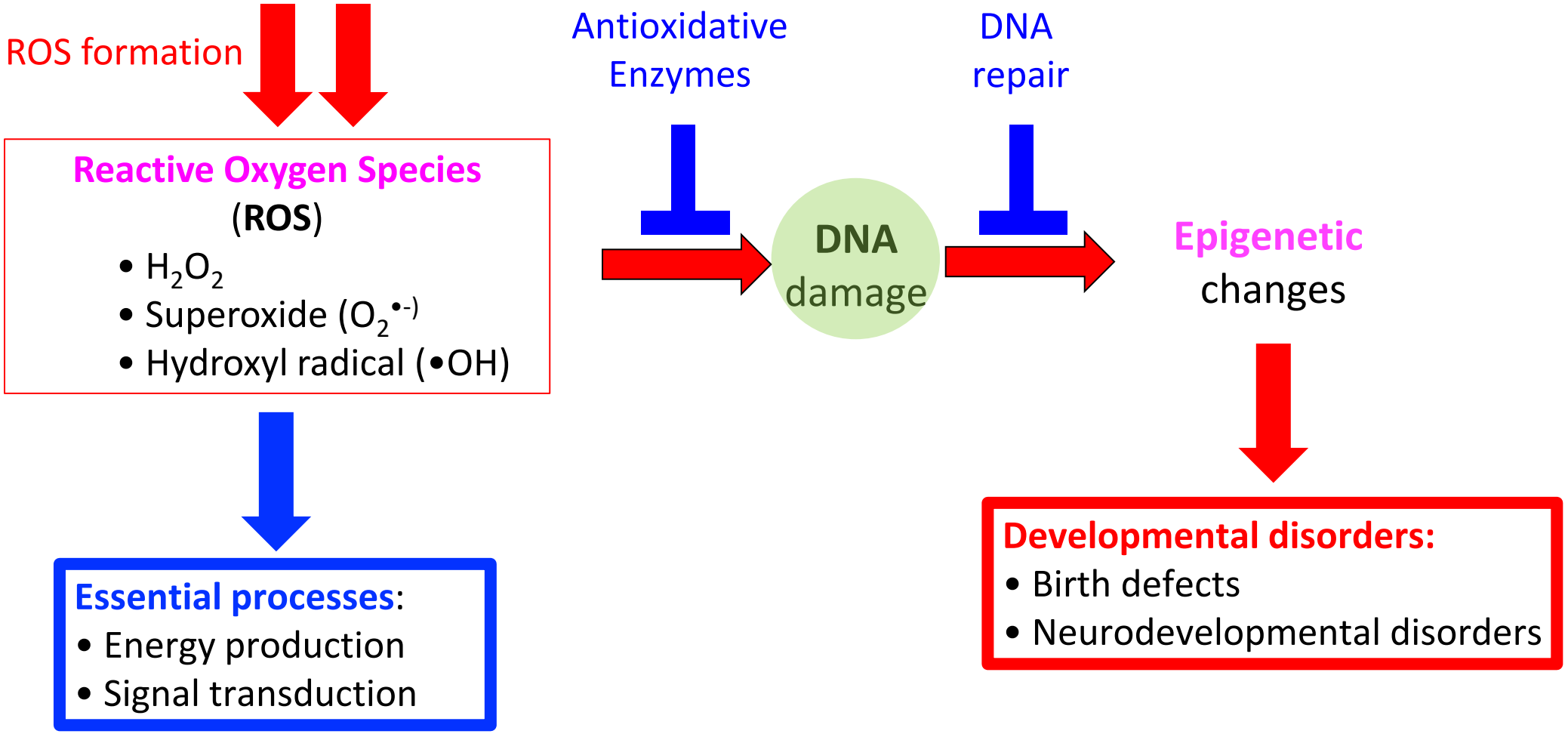
Antioxidants & antioxidative enzymes protecting the embryo and fetus

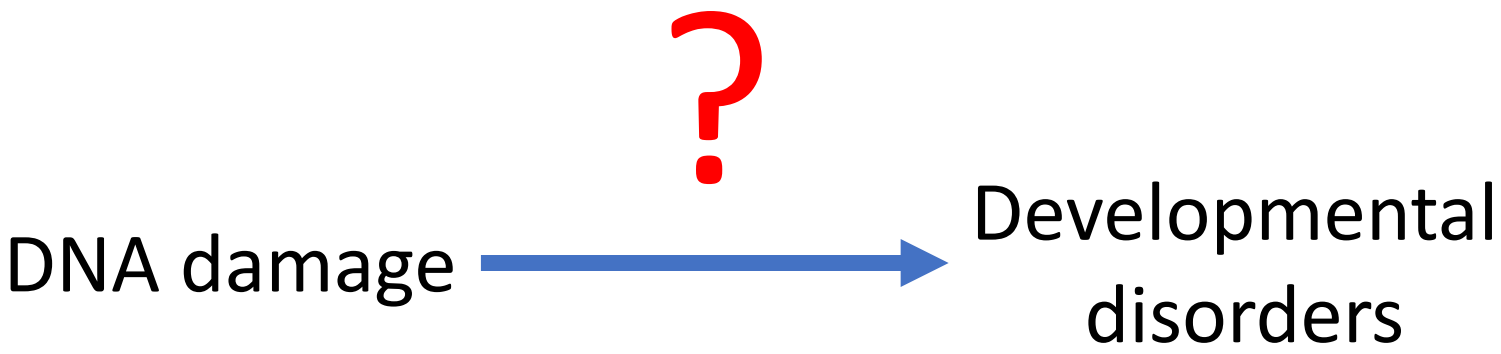
- Glutathione (GSH)
- Glucose-6-phosphate dehydrogenase (G6PD)
- Superoxide dismutase (SOD)
- Glutathione reductase
- Glutathione peroxidase
- **Catalase** (see FASD symposium on Wednesday)

Review: Bhatia, Drake, Miller and Wells. Birth Defects Research 111(12): 714-748, 2019.

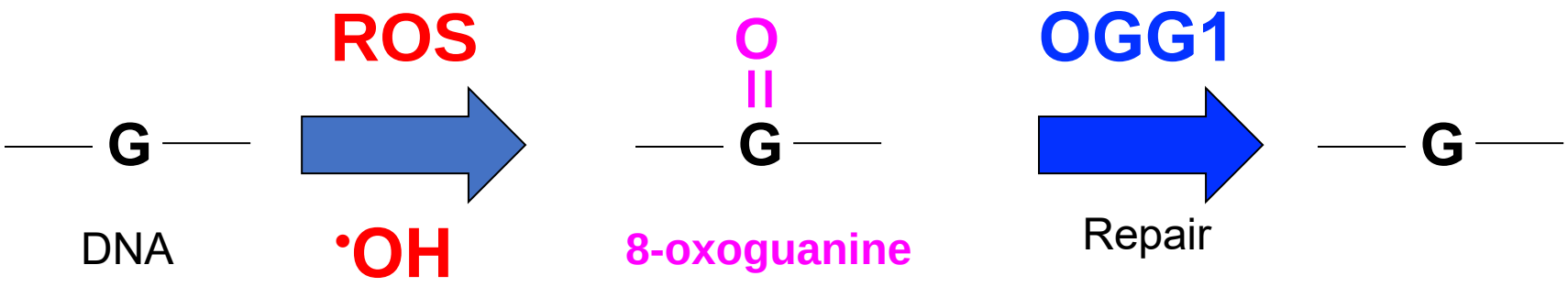

DNA damage

- **Physiological** pathways
- **Xenobiotics**



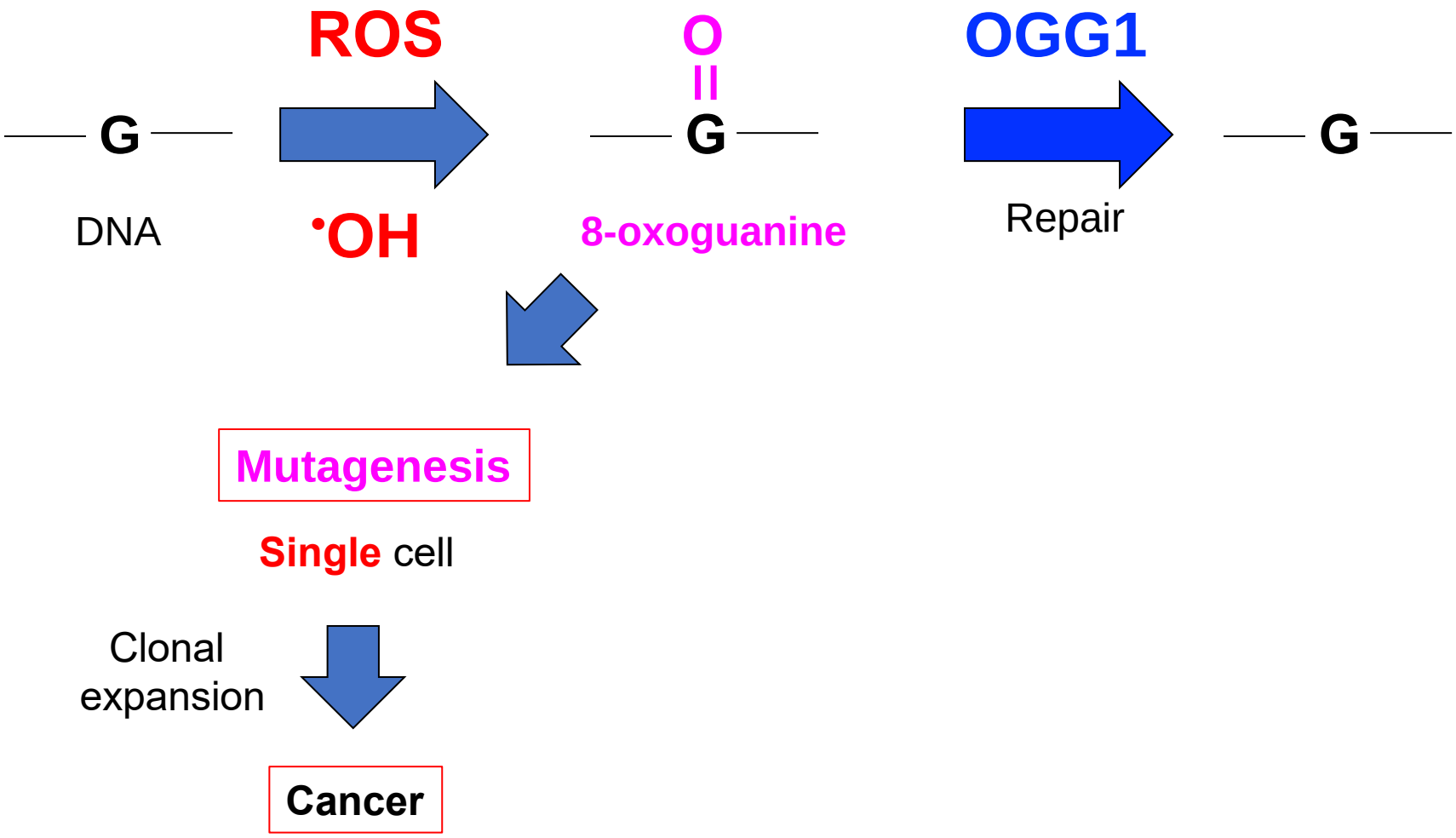

?
DNA damage
Developmental
disorders

Role of OGG1



(**G** = guanine, **OGG1** = oxoguanine glycosylase 1)

Role of OGG1



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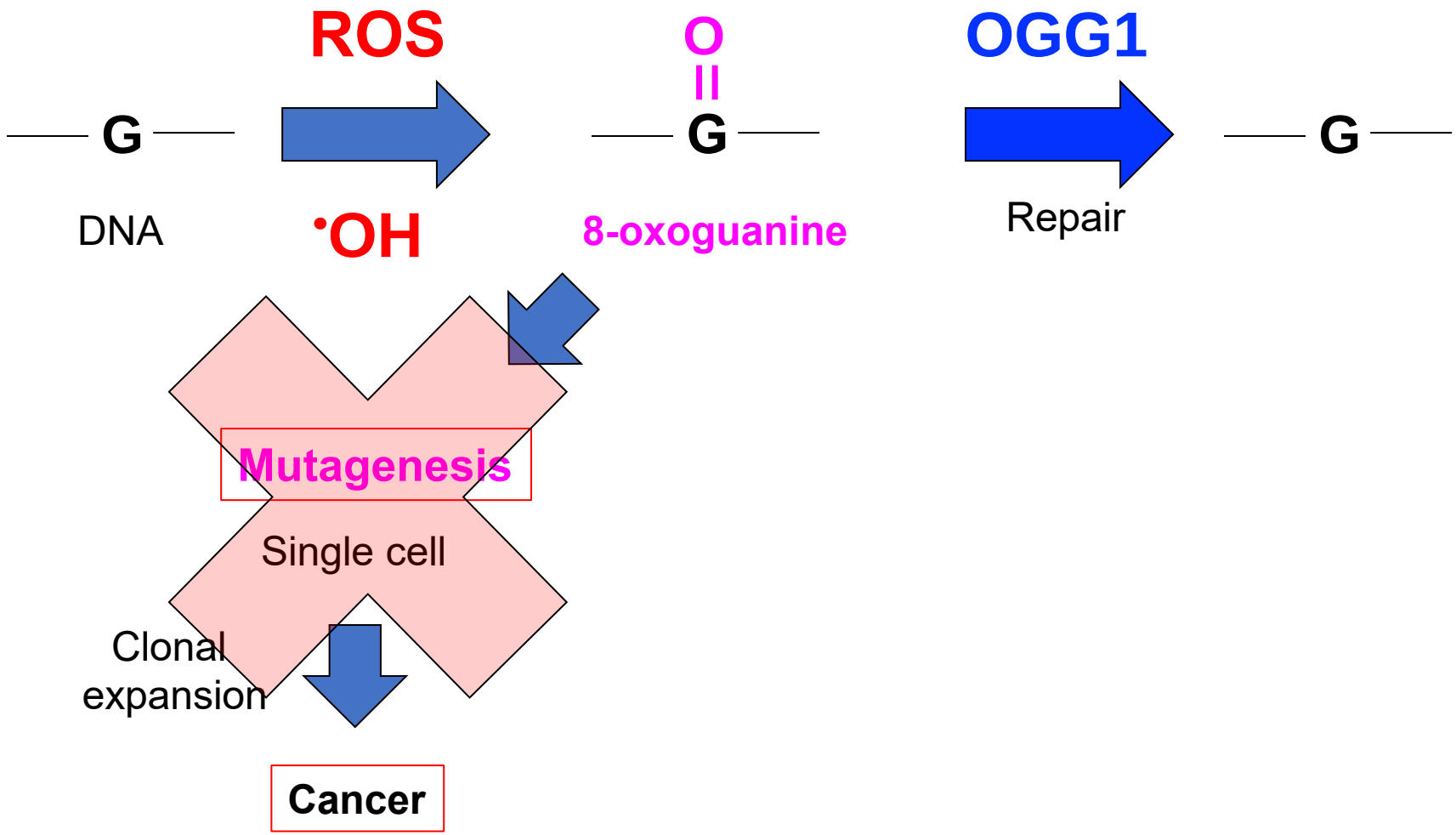

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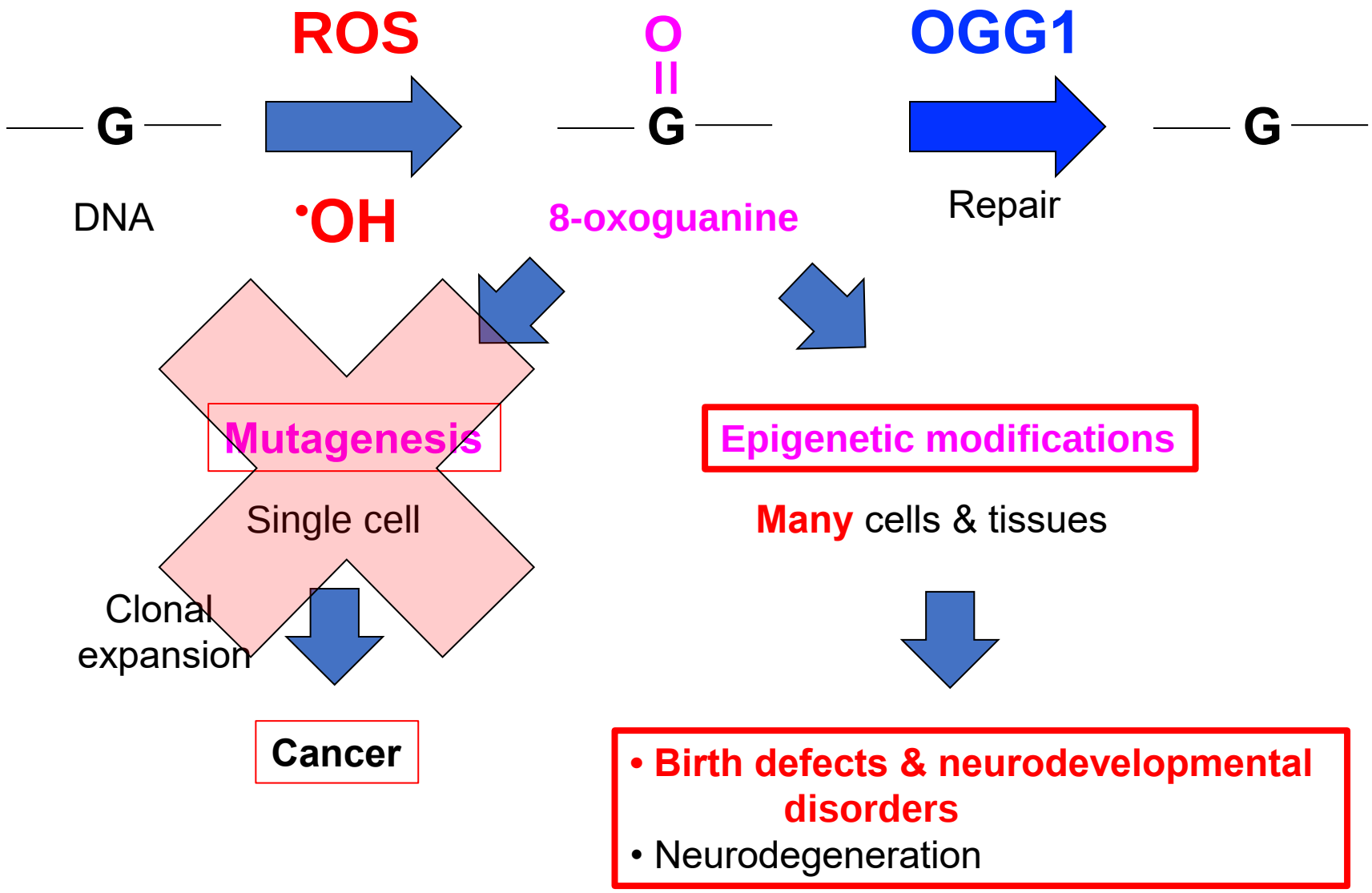

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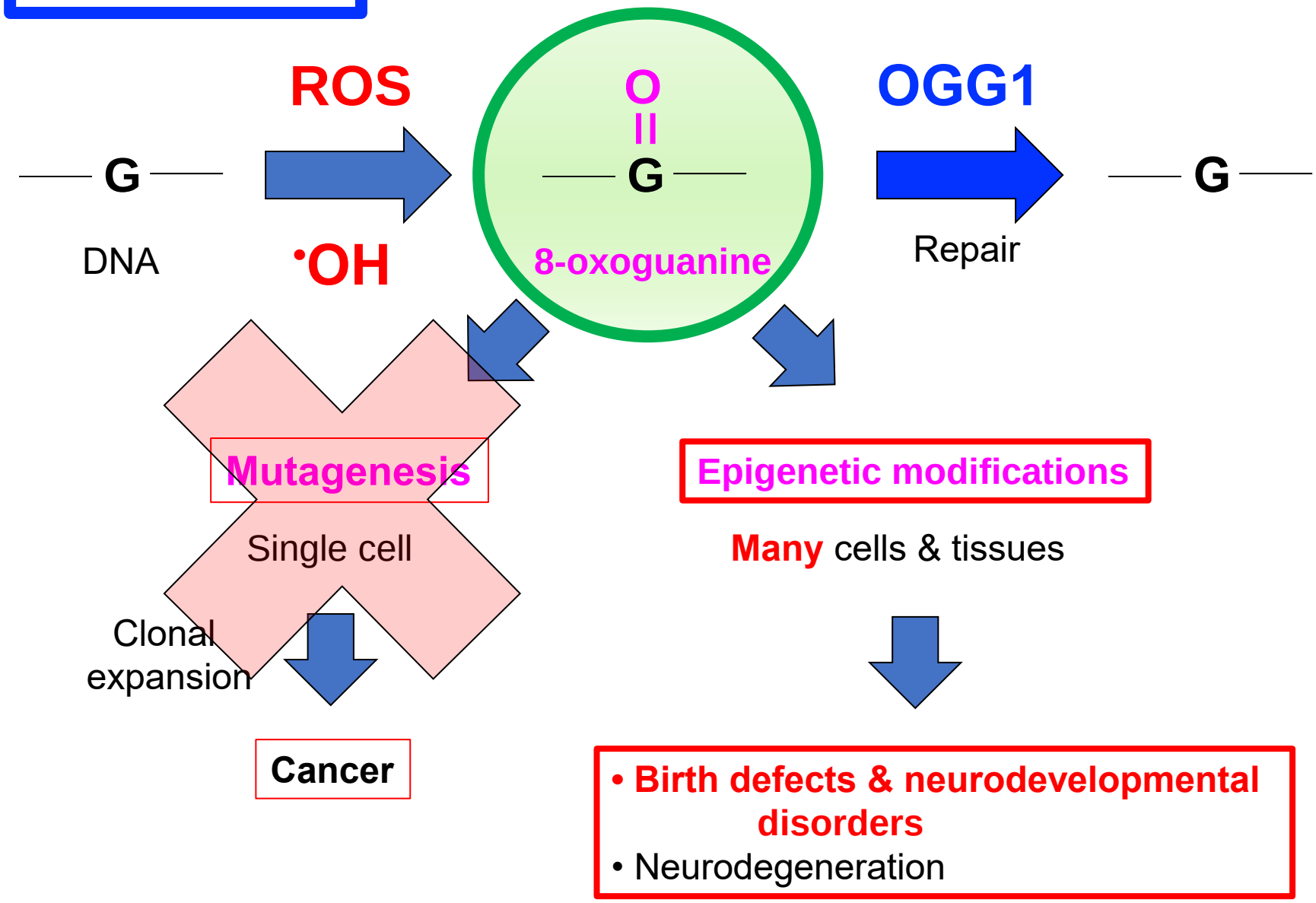
Role of OGG1



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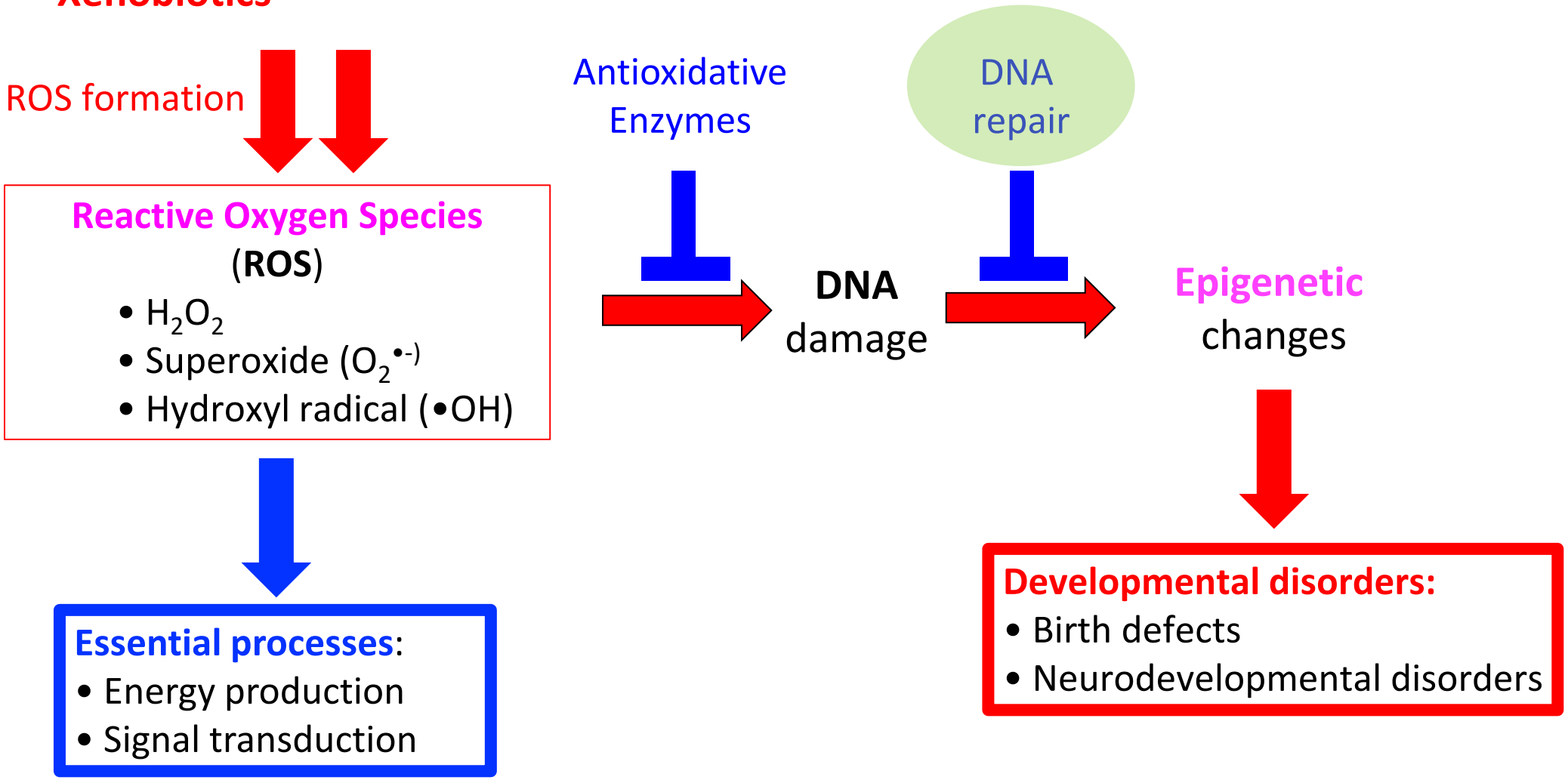
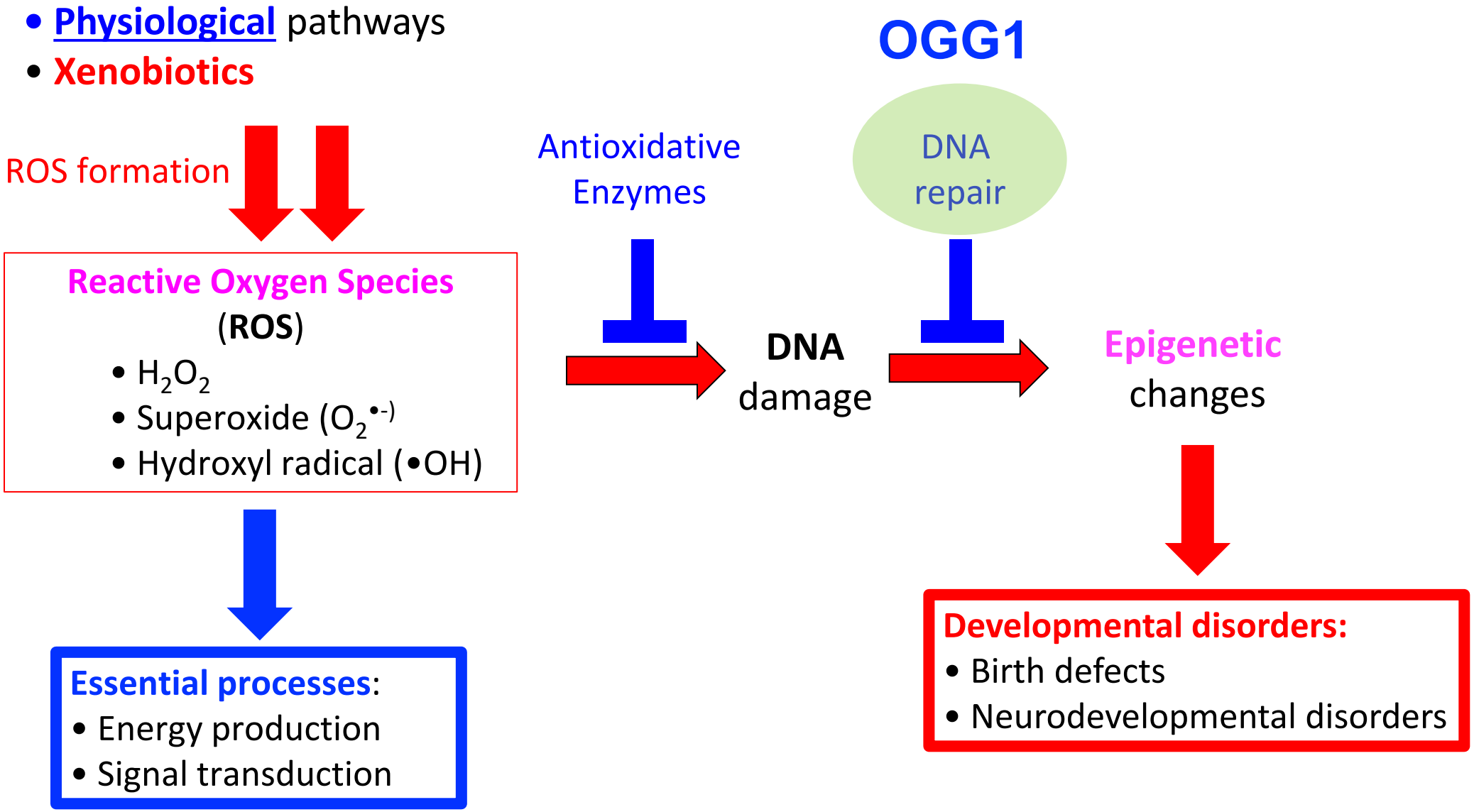
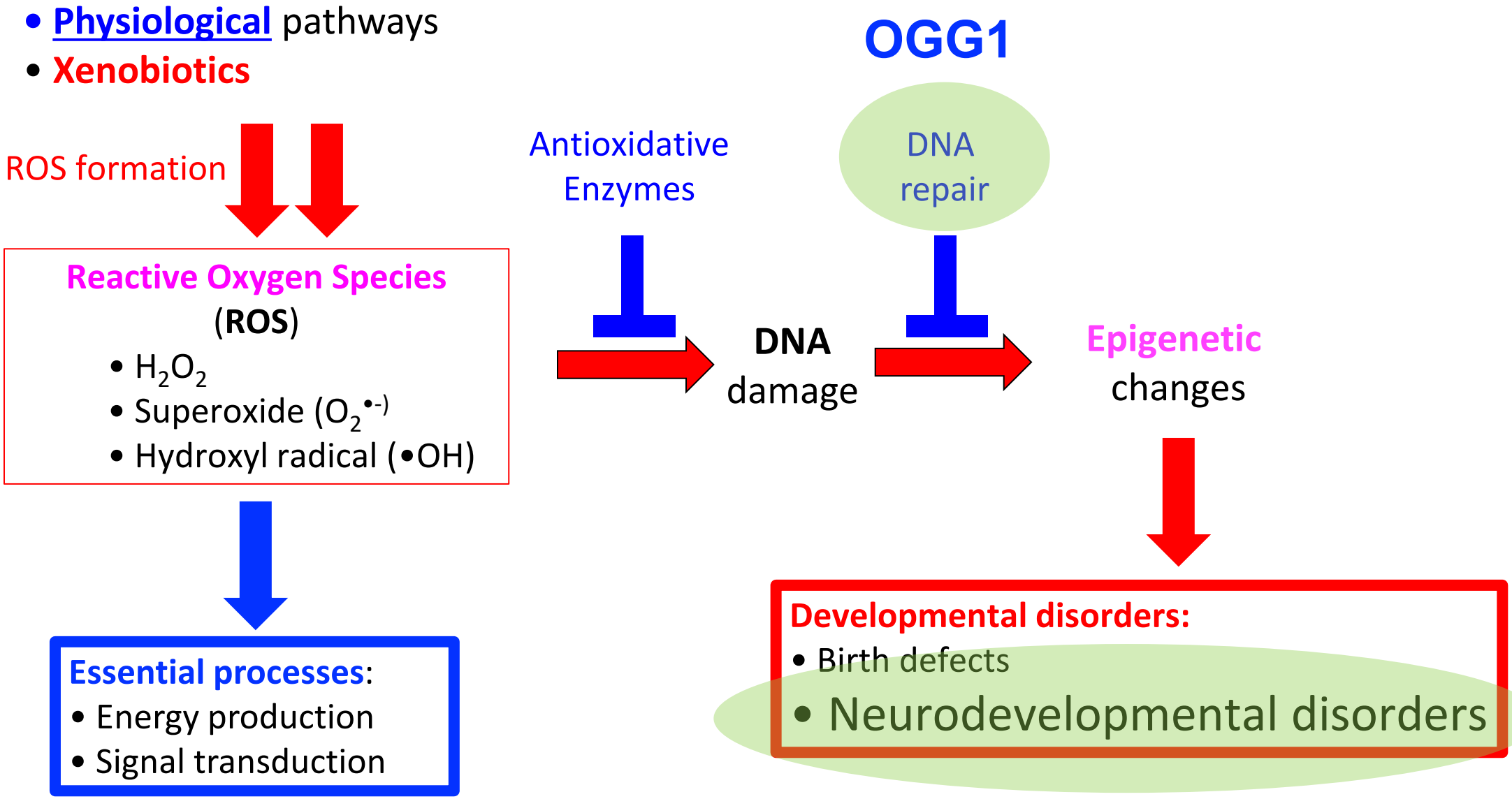
DNA repair

- **Physiological** pathways
- **Xenobiotics**




• Physiological pathways
• Xenobiotics
ROS formation
Reactive Oxygen Species
(ROS)
• H_2O_2
• Superoxide ($O_2^{\bullet -}$)
• Hydroxyl radical (•OH)
Essential processes:
• Energy production
• Signal transduction
Antioxidative Enzymes
DNA damage
OGG1
DNA repair
Epigenetic changes
Developmental disorders:
• Birth defects
• Neurodevelopmental disorders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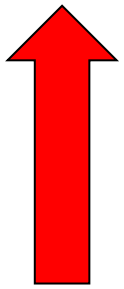
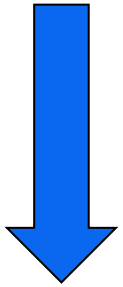
Fetus and **fetal brain**

highly susceptible to ROS-initiated damage



ROS-forming enzymes
(PHS, NOX)

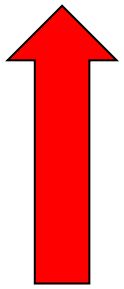


- **Antioxidants** (GSH)
- **Antioxidative enzymes**

(catalase, SOD, GSH reductase)

Fetus and fetal brain

highly susceptible to ROS-initiated damage



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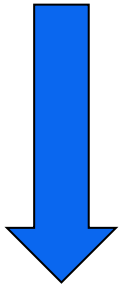

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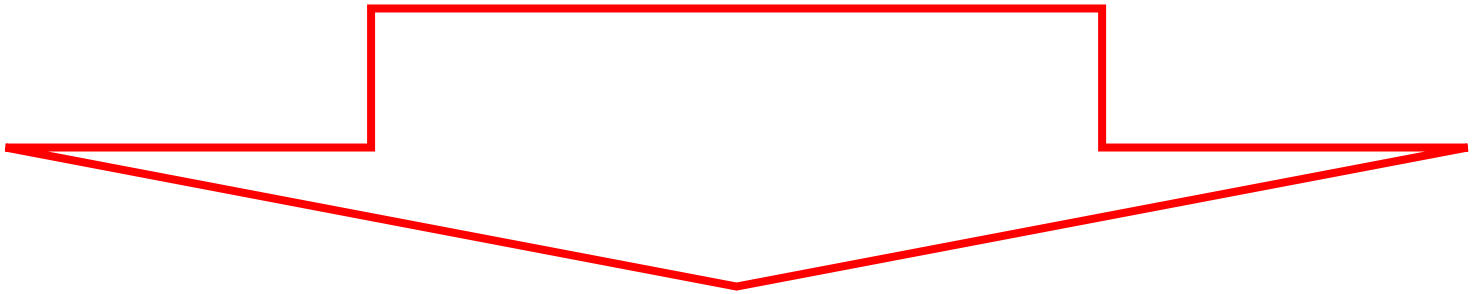


Neurodevelopmental Disorders >> Morphological birth defects

DNA Repair

OGG1

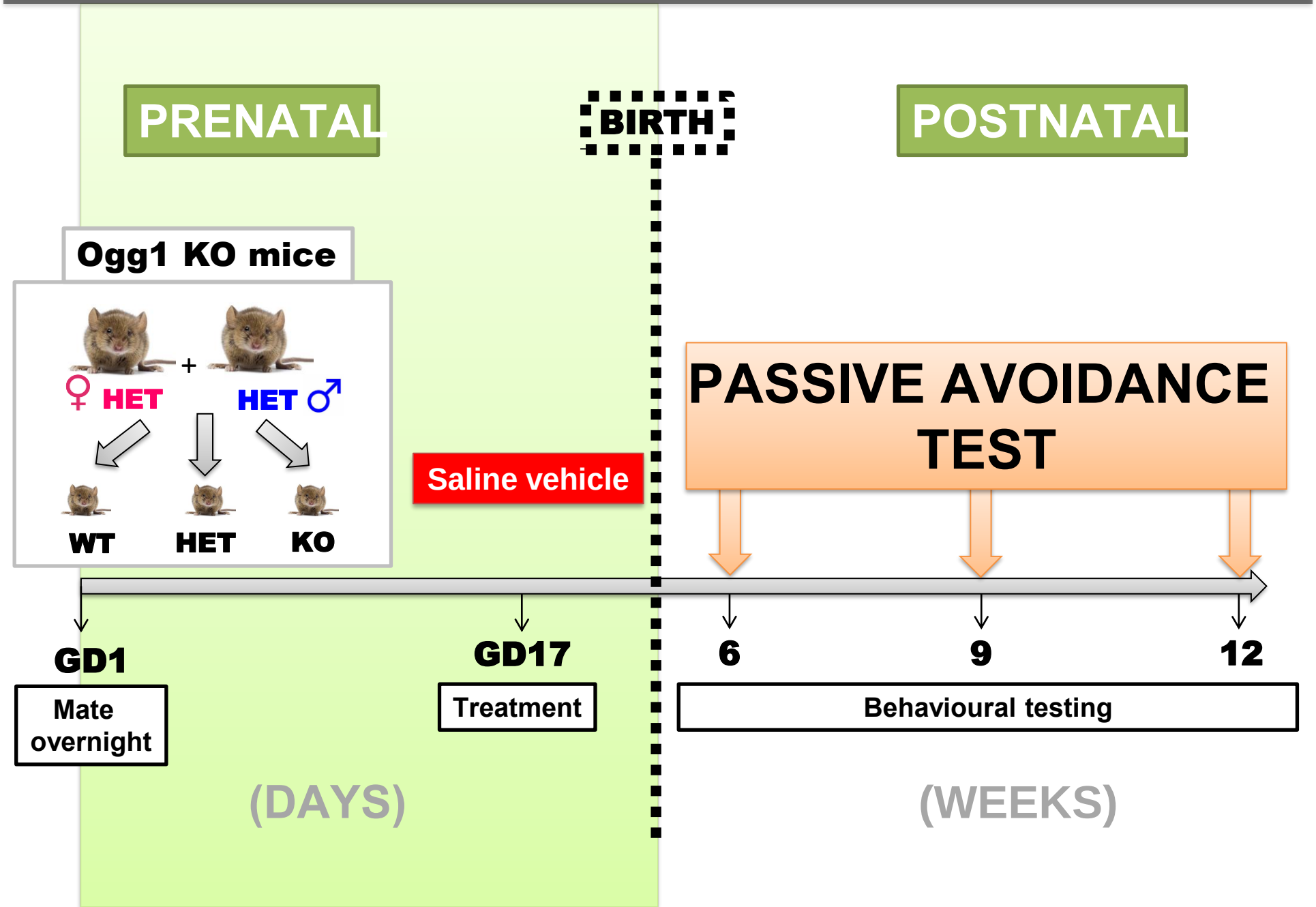
(**Oxoguanine glycosylase 1**)

- Major enzyme in the base excision pathway for **repair** of **oxidative DNA damage**
- Specifically repairs 8-oxoguanine lesion

Approach: ***Ogg1*** **knockout mice**

- untreated (physiological ROS levels)
- ethanol

Methods: Functional Deficits



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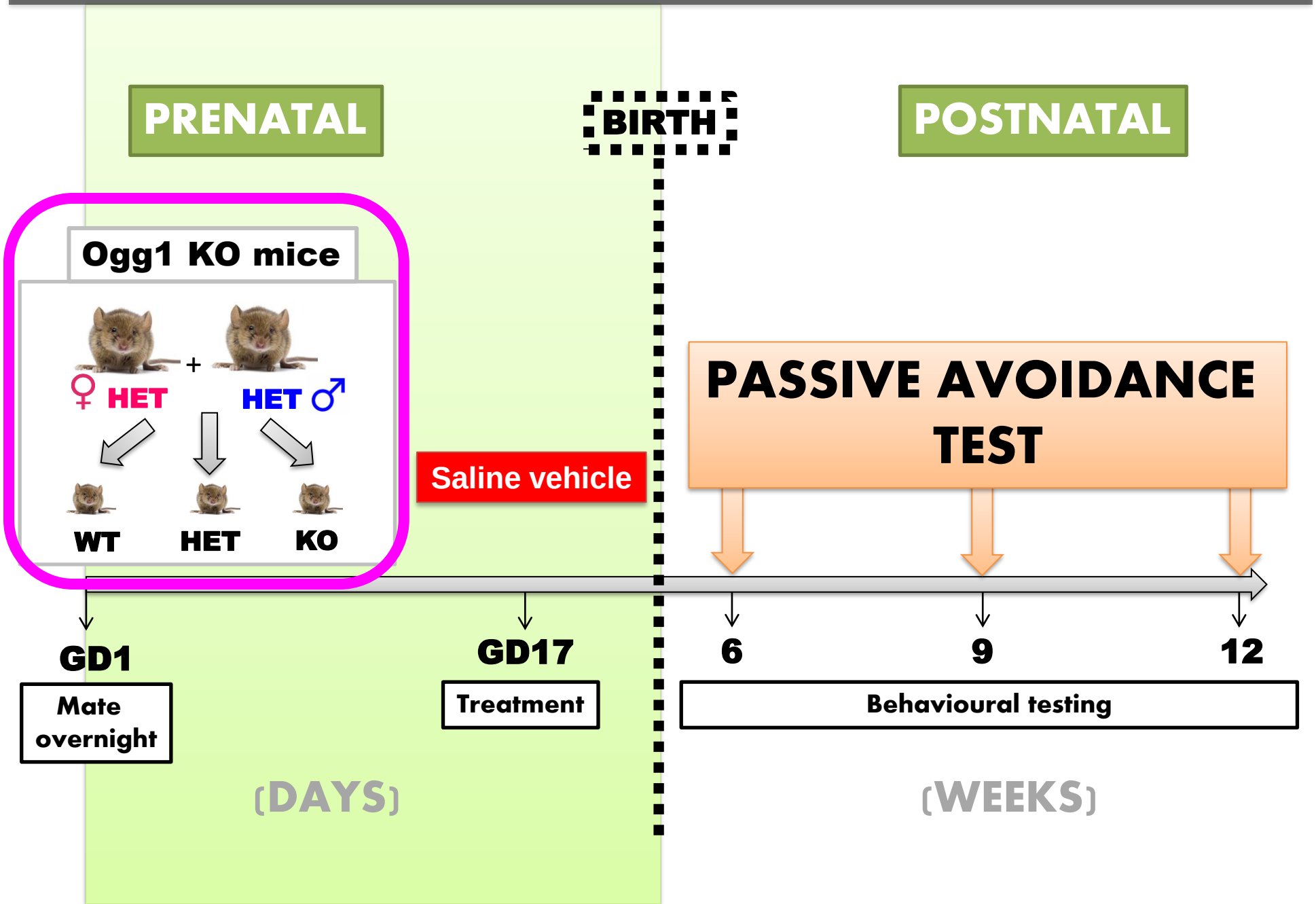

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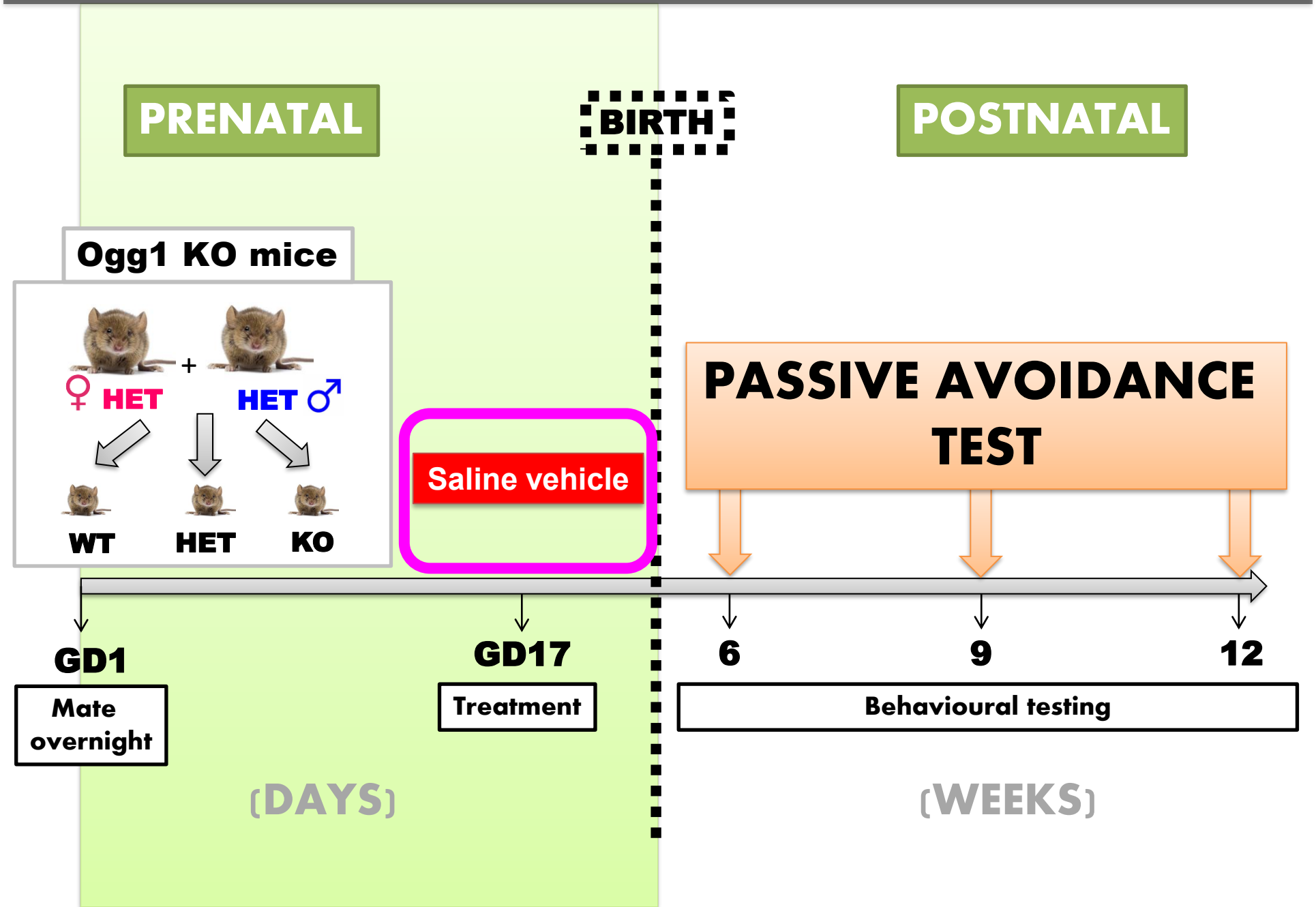

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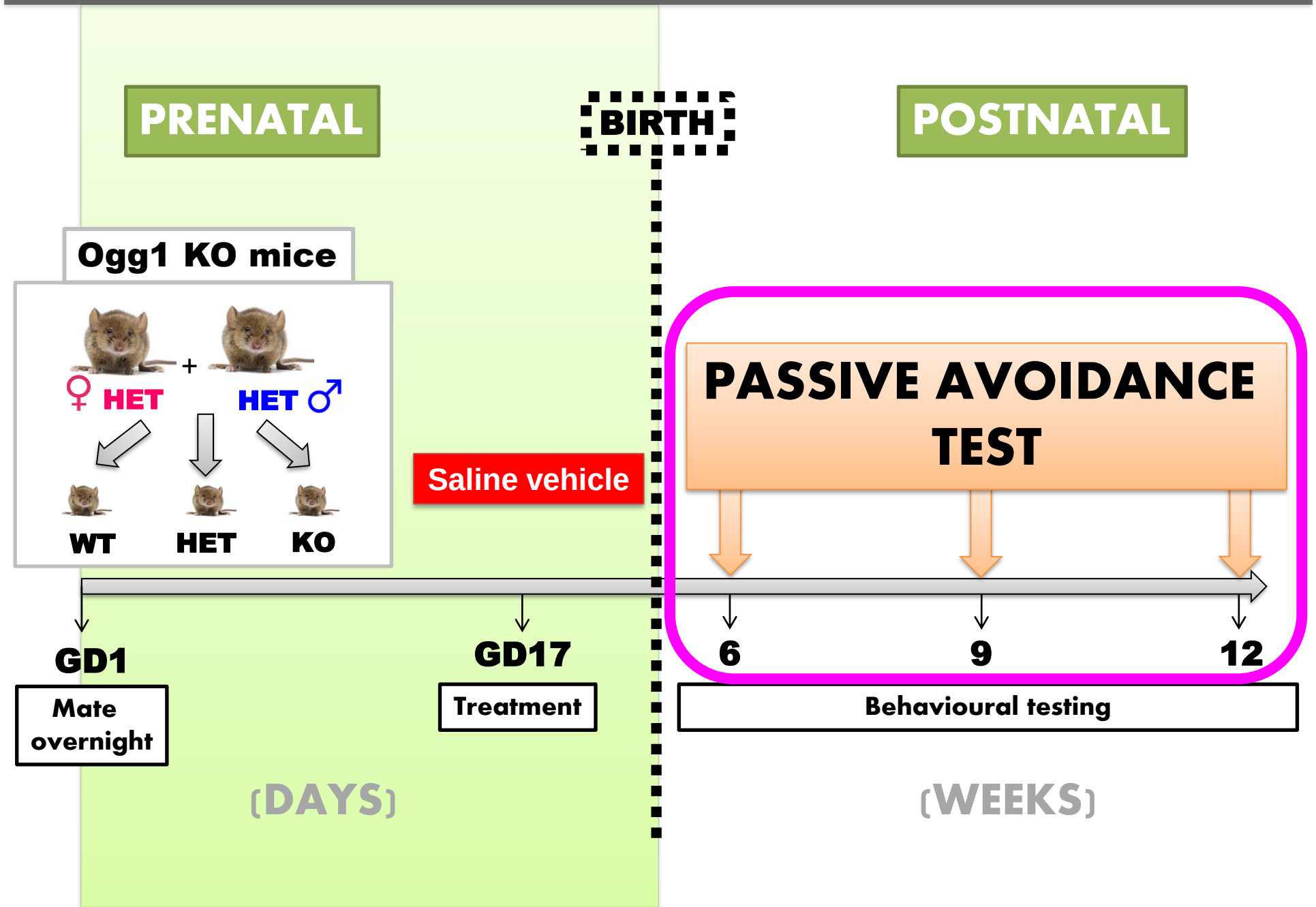
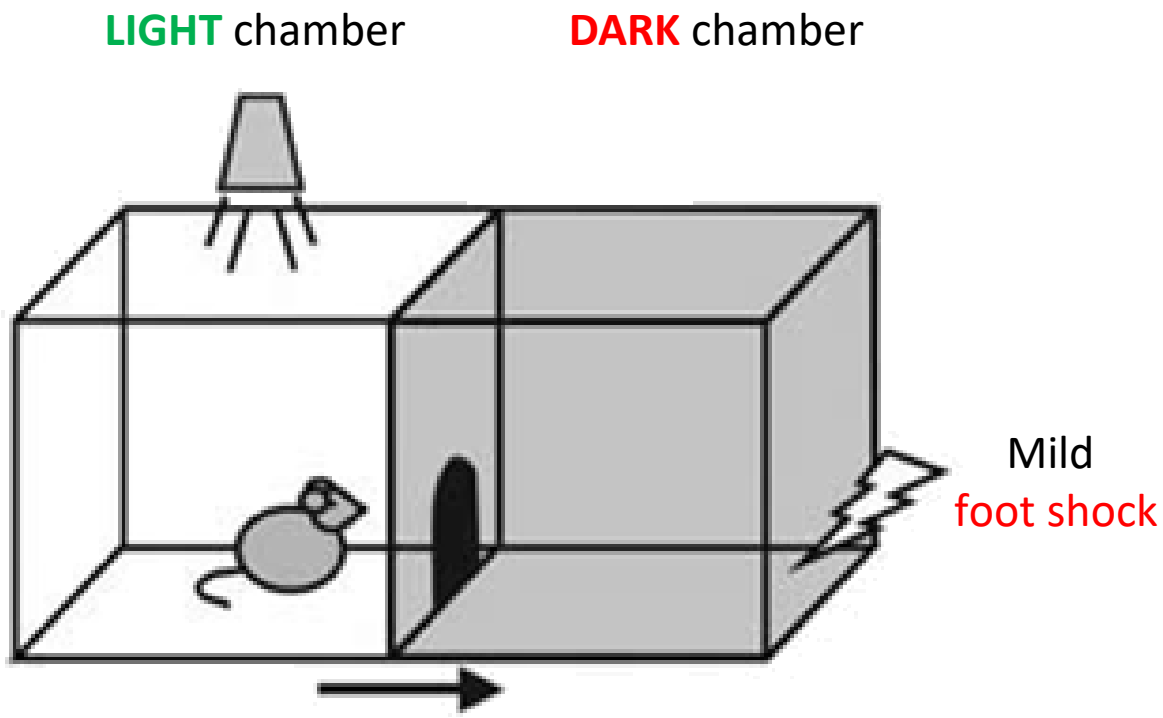


Postnatal **learning and memory**

Passive avoidance test

(up to 3 months after birth)



Smart animals learn to avoid the dark chamber with the foot shock

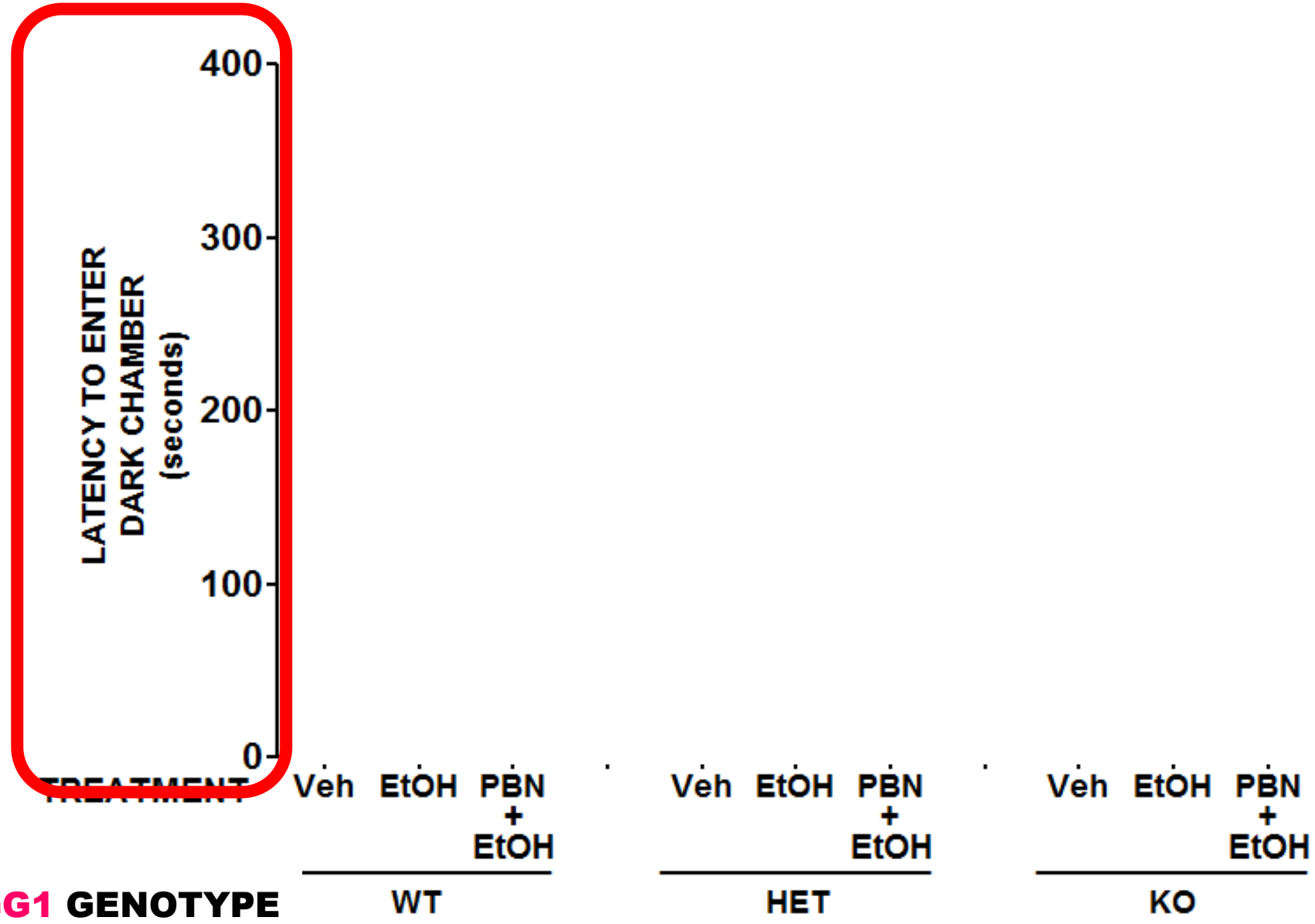
Drawing modified from: Zhao Y, et al. Biomedical Research (India) **28**: 8022-8026, 2017.

OGG1 DNA REPAIR-DEFICIENT MICE

UNTREATED

Postnatal learning & memory

EtOH-initiated behavioural deficits in Ogg1 mice



(Miller-Pinsler et al., Free Radic. Biol. Med. 78(C): 23-29, 2015)

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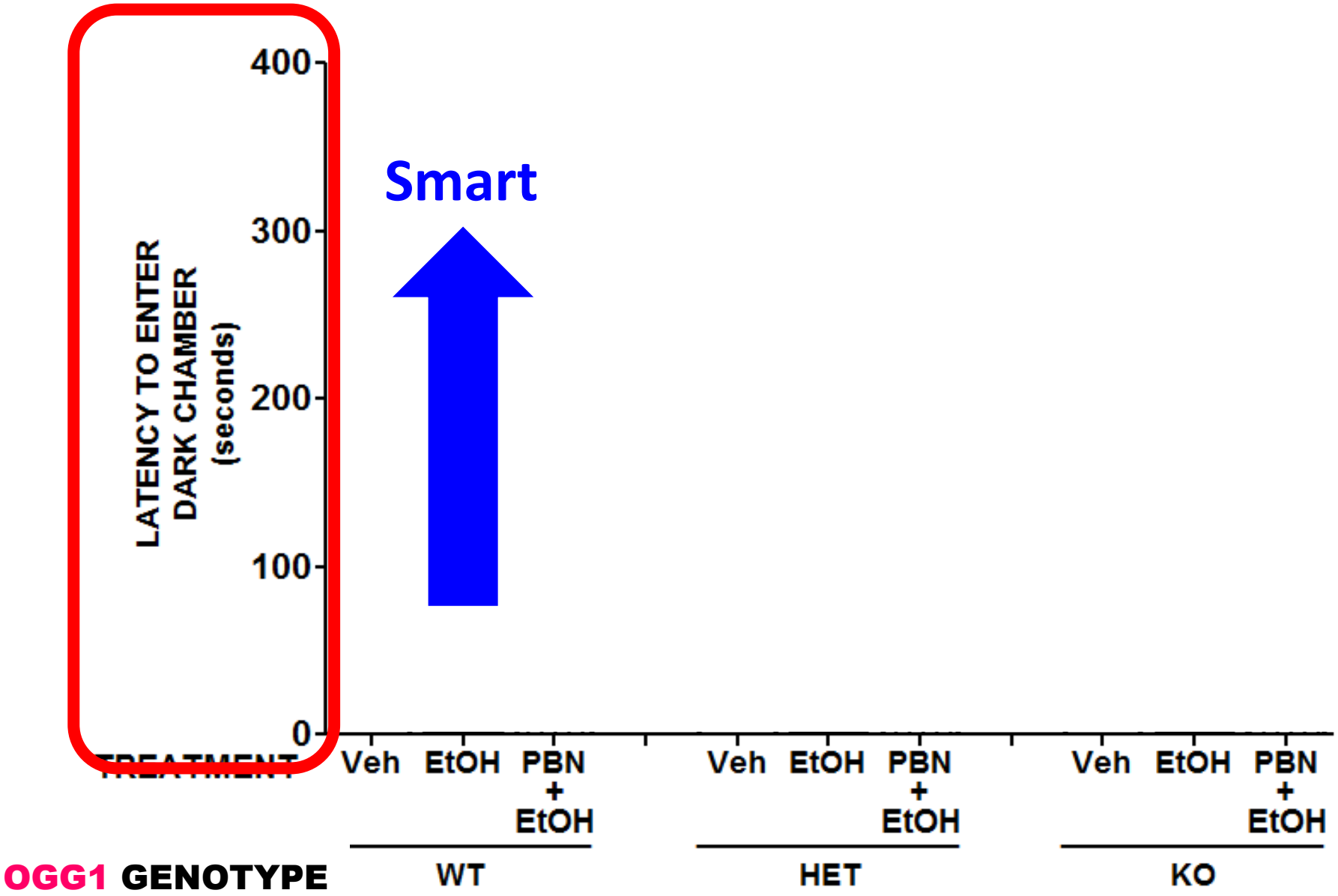

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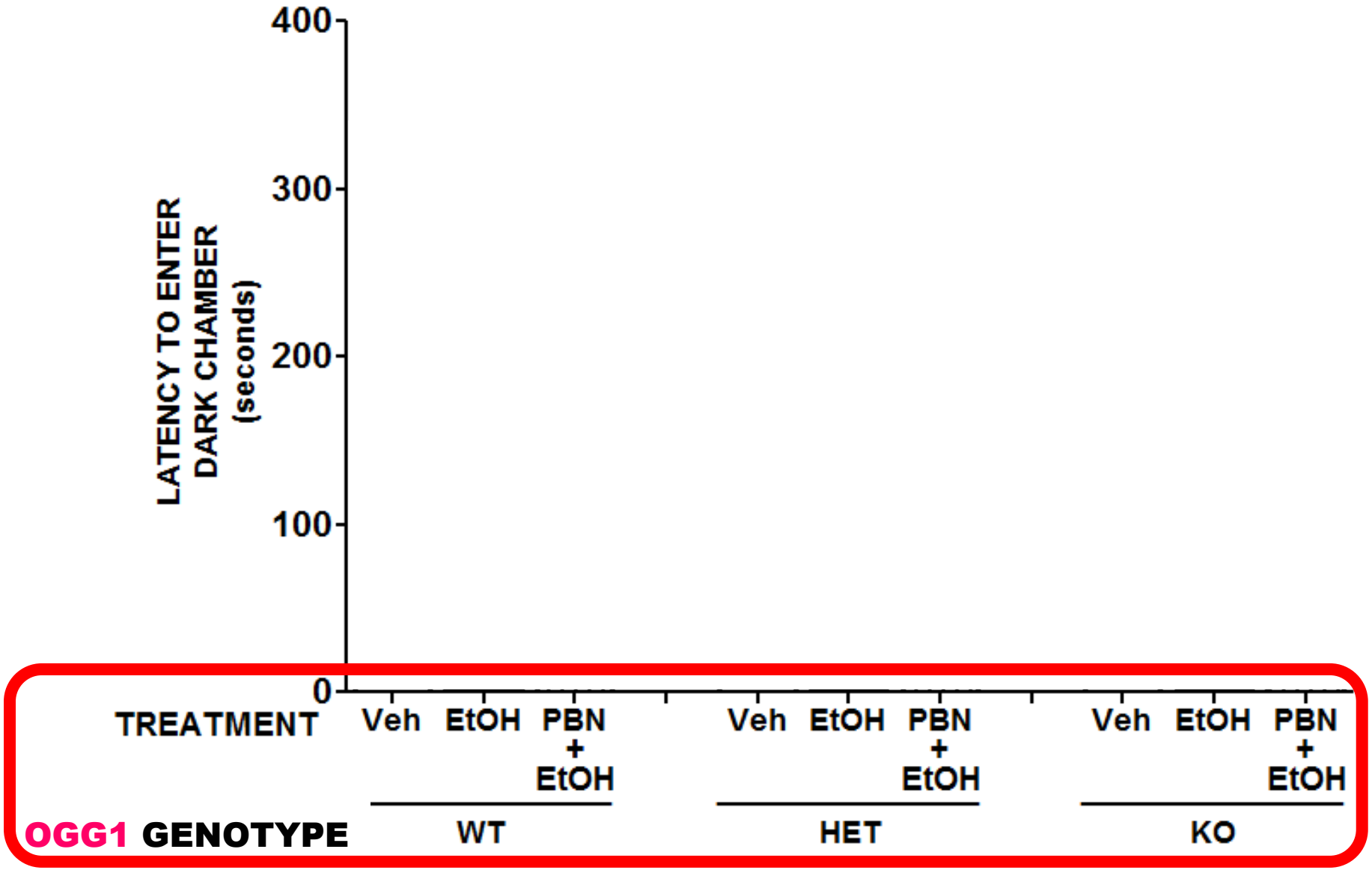

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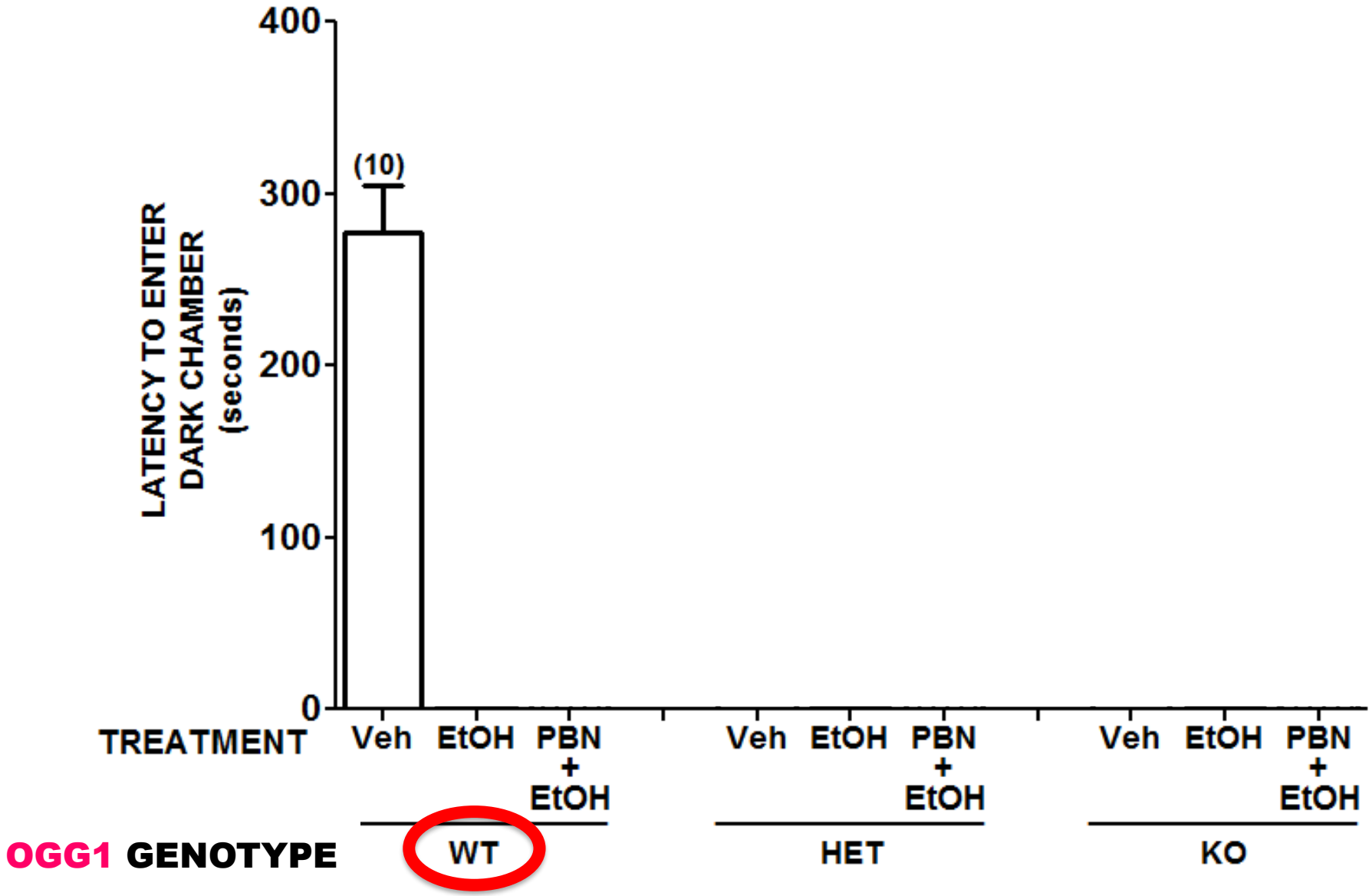

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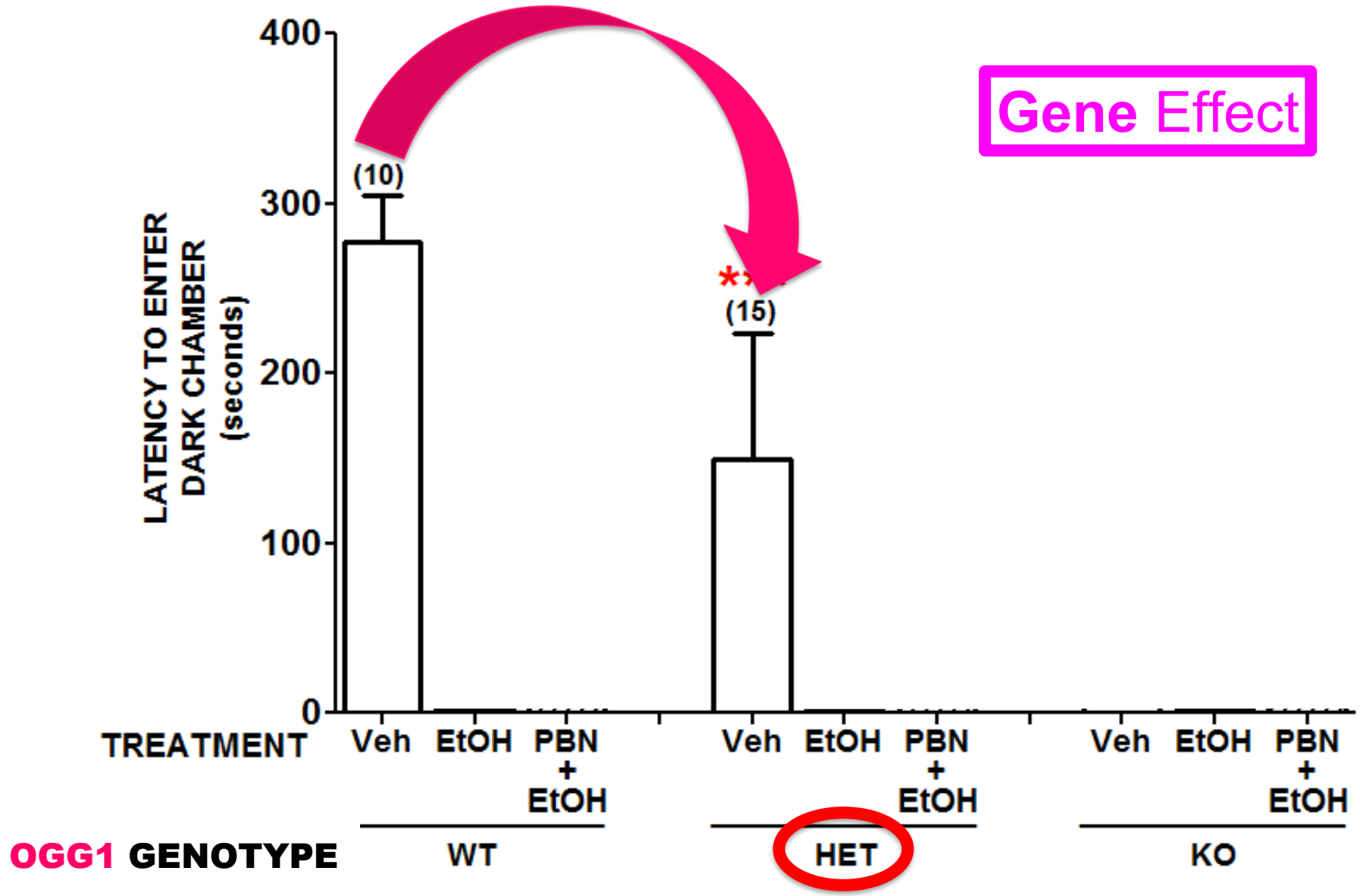

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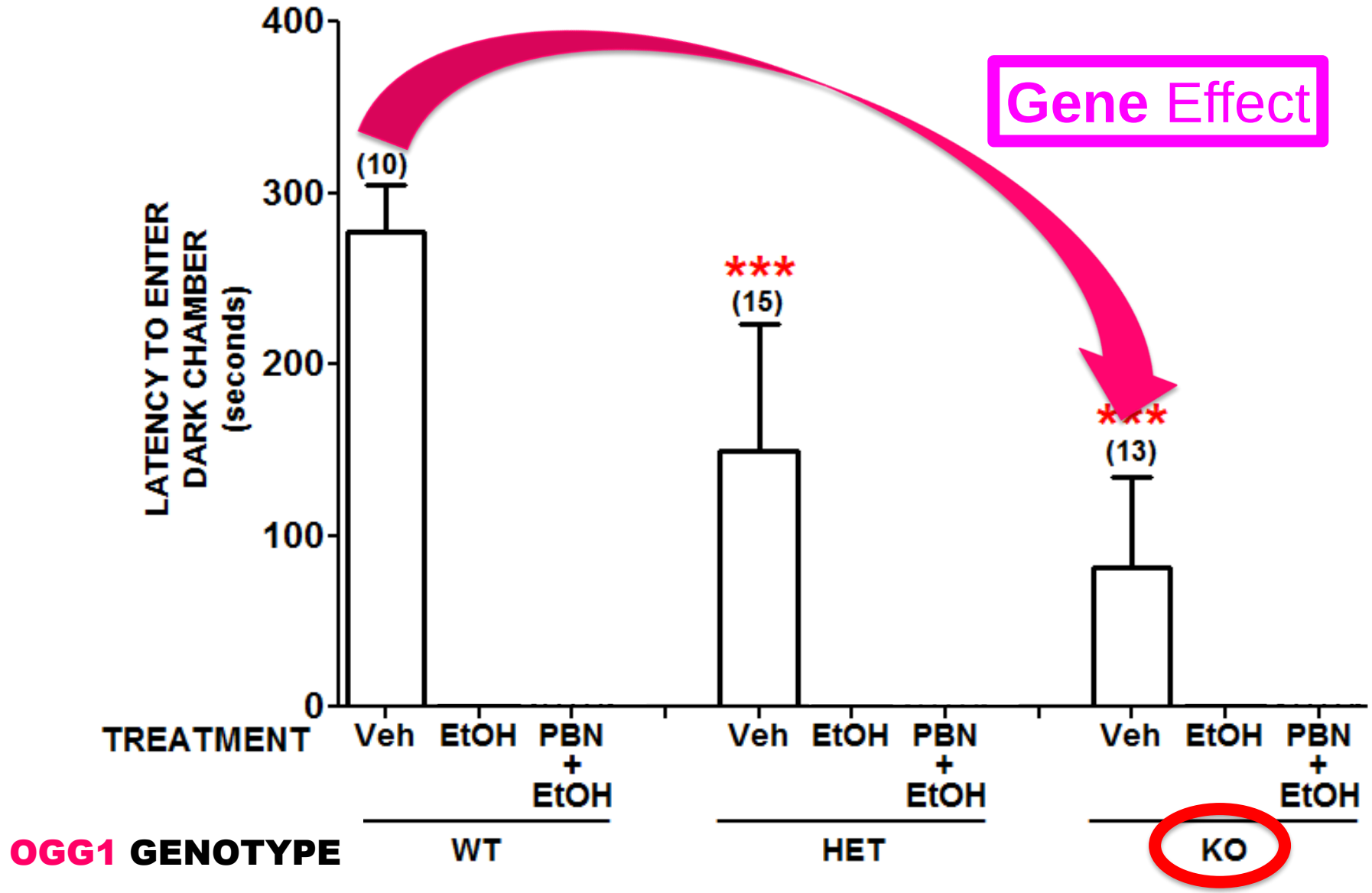

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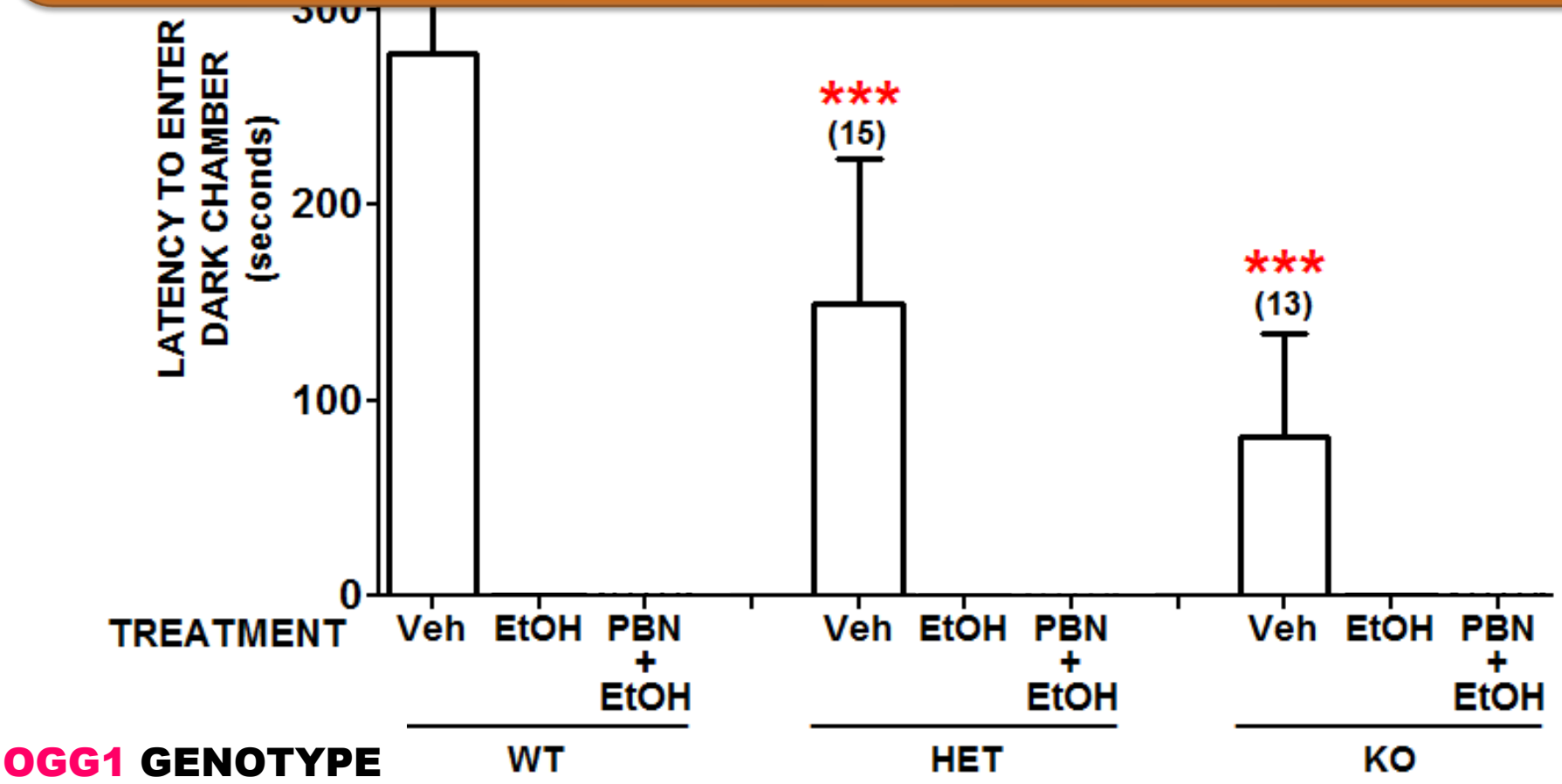
EtOH-initiated behavioural deficits in Ogg1 mice

- **FIRST EVIDENCE: OGG1 cognitive phenotype**



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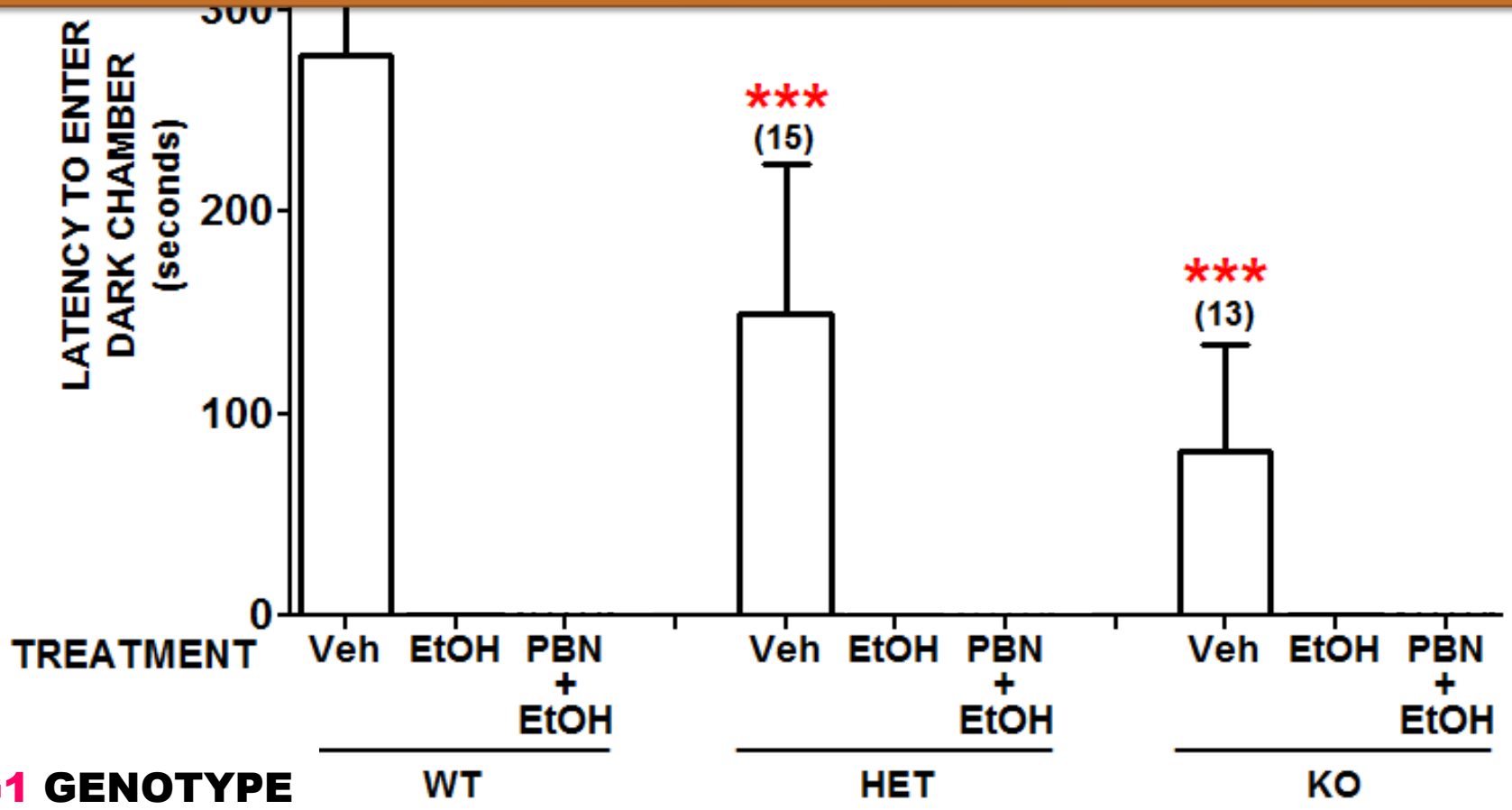
EtOH-initiated behavioural deficits in Ogg1 mice

- **FIRST EVIDENCE: OGG1 cognitive phenotype**
- **toxic potential of physiological ROS levels**



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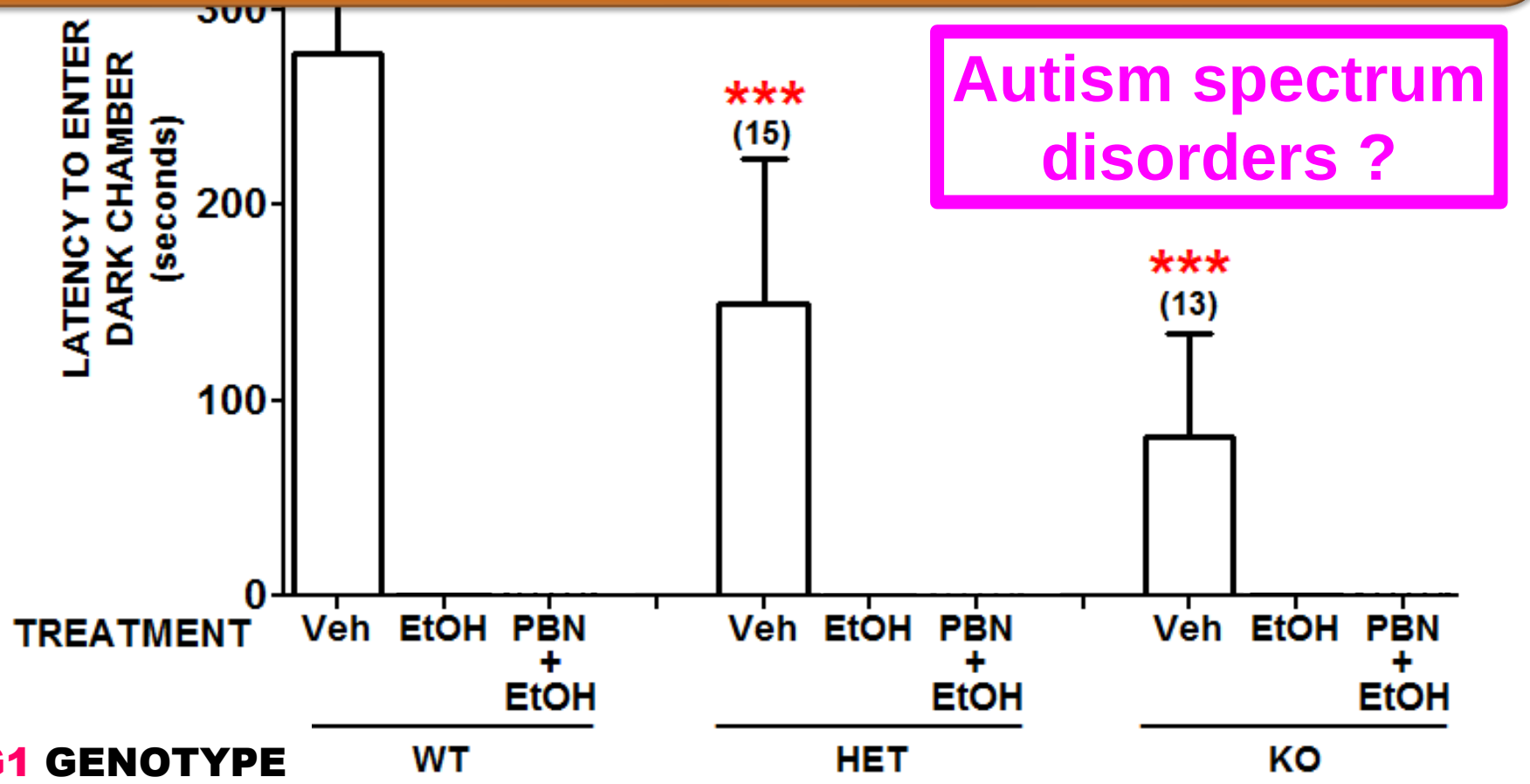

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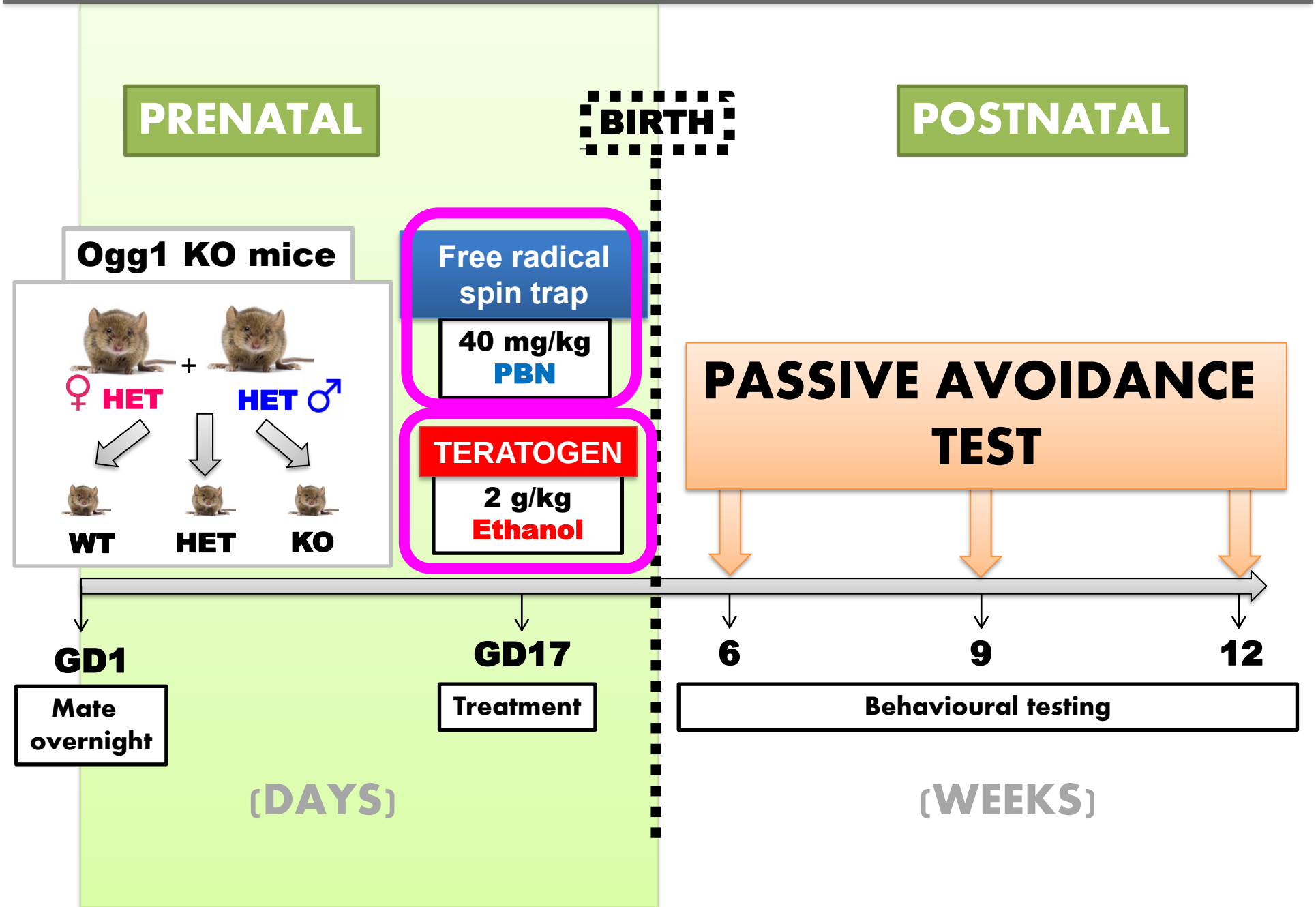
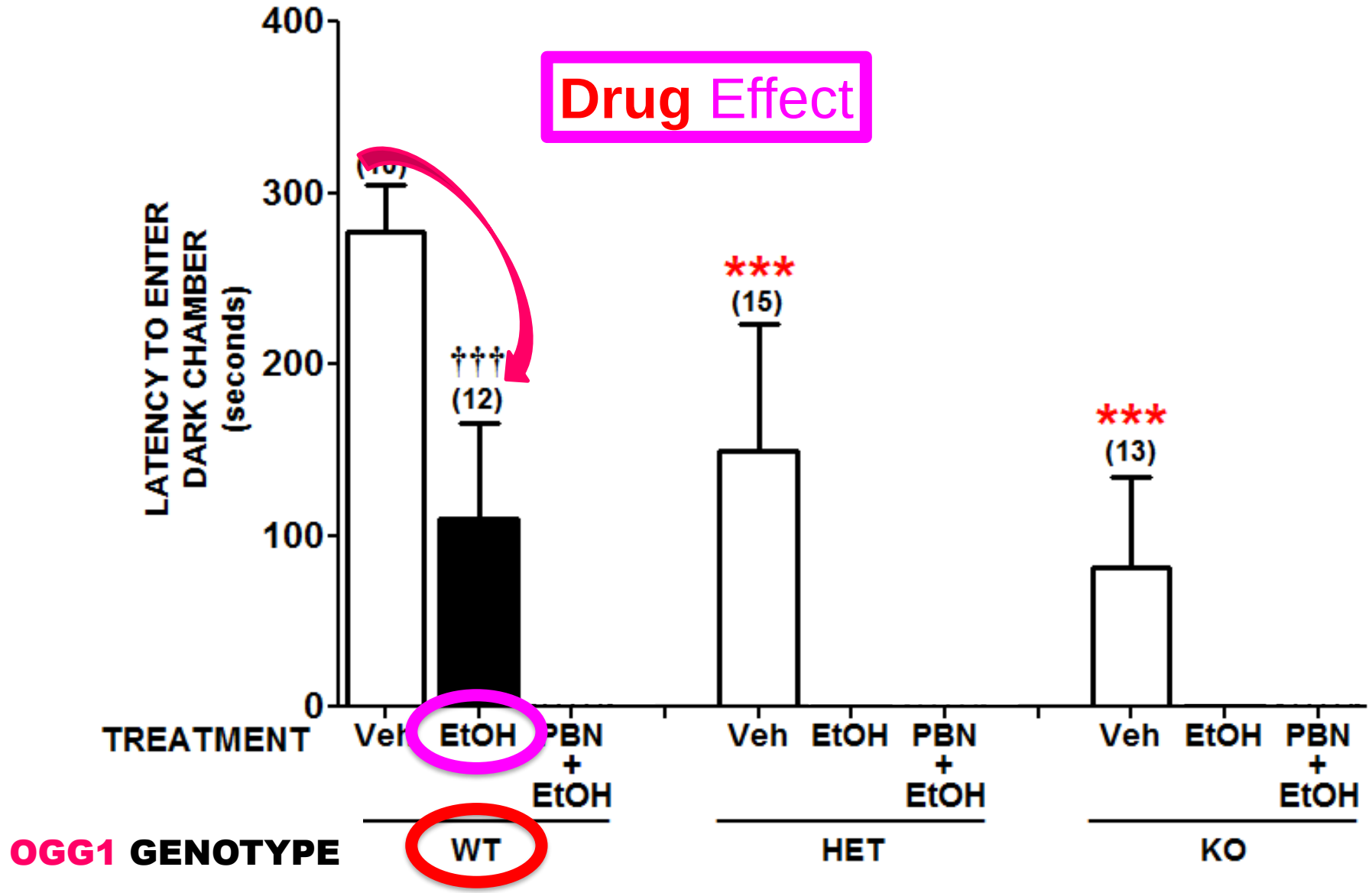
OGG1 DNA REPAIR-DEFICIENT MICE

EtOH TREATMENT

Methods: Functional Deficits



EtOH-initiated behavioural deficits in Ogg1 mice



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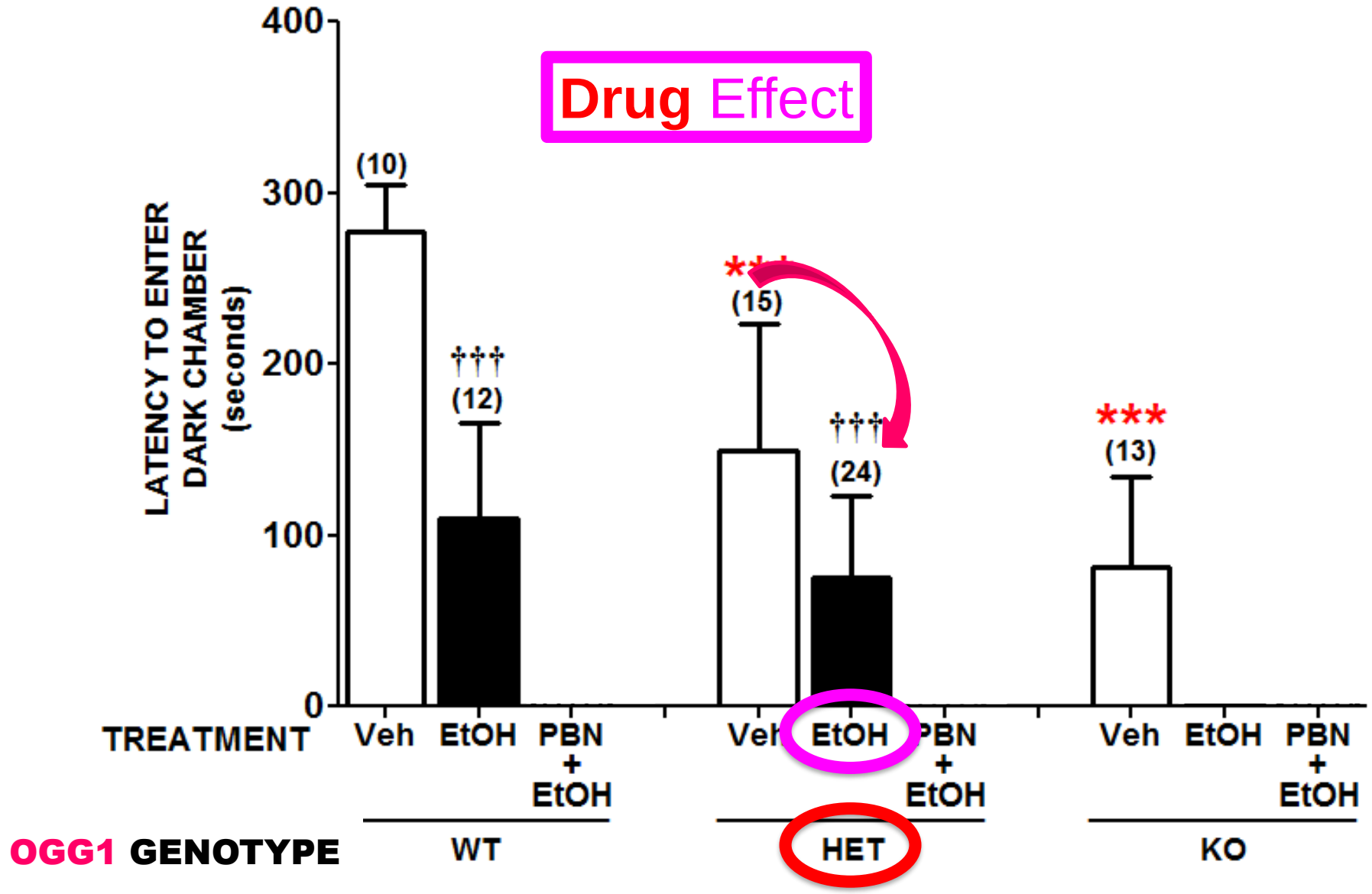

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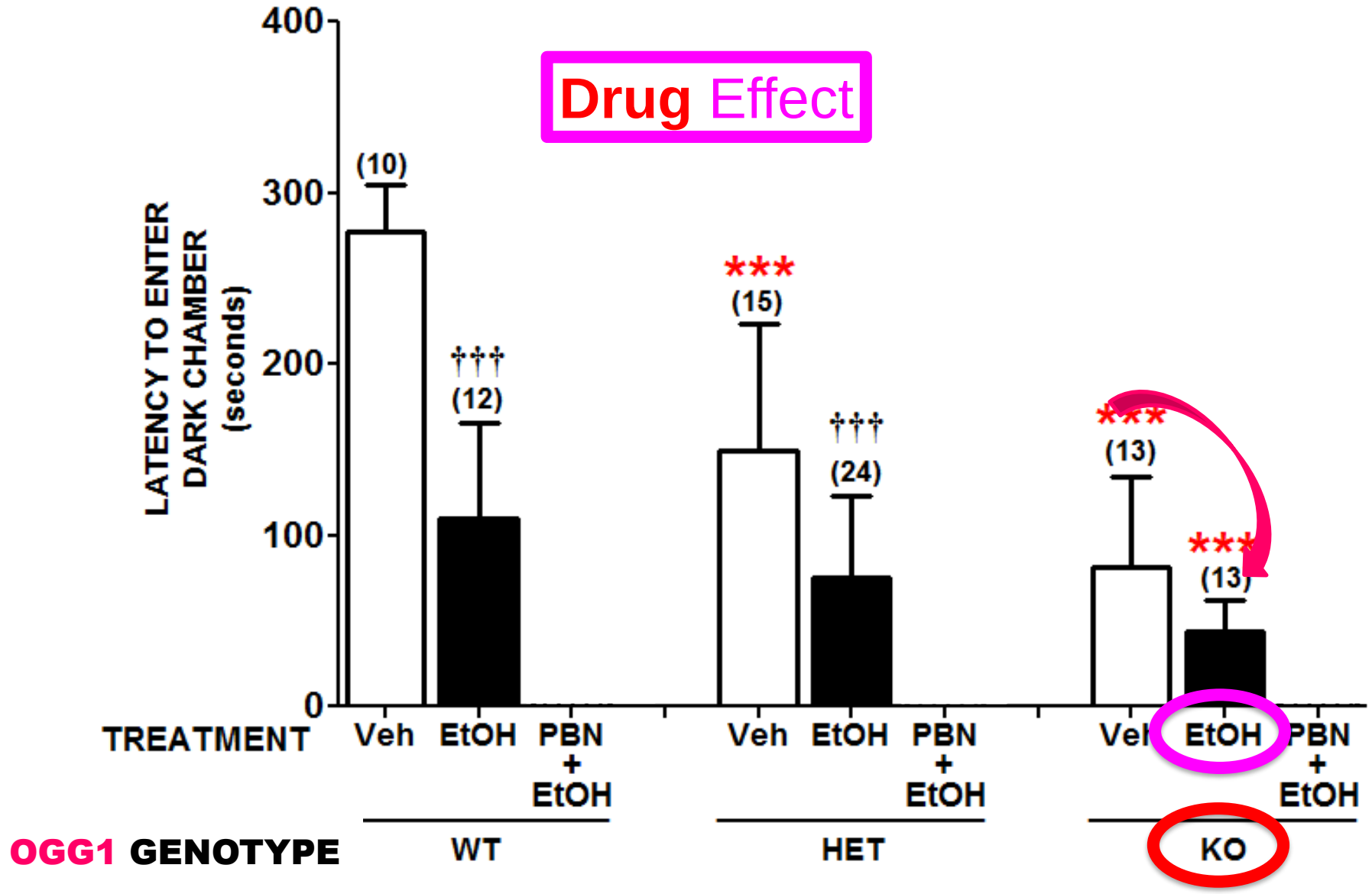

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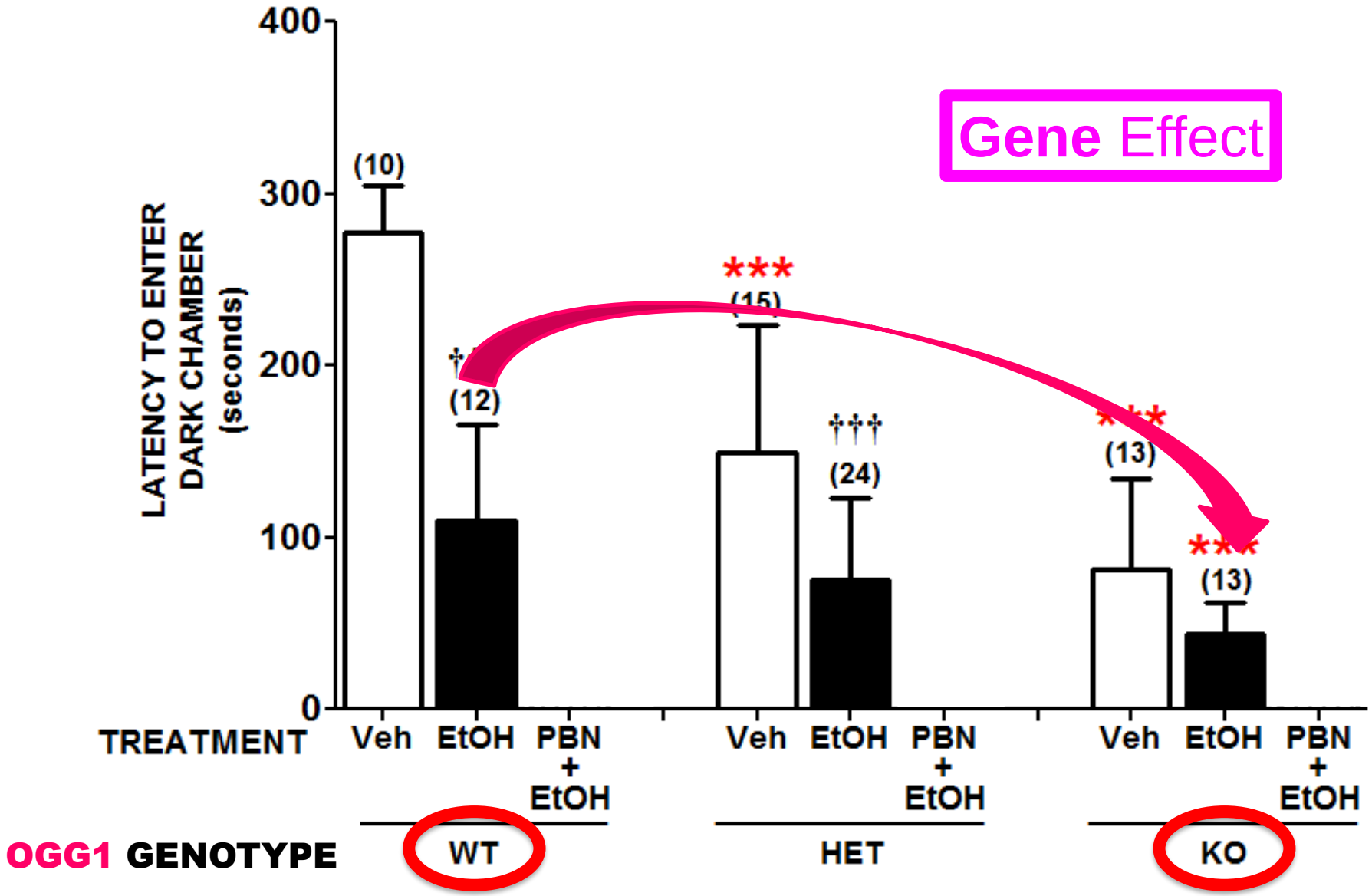
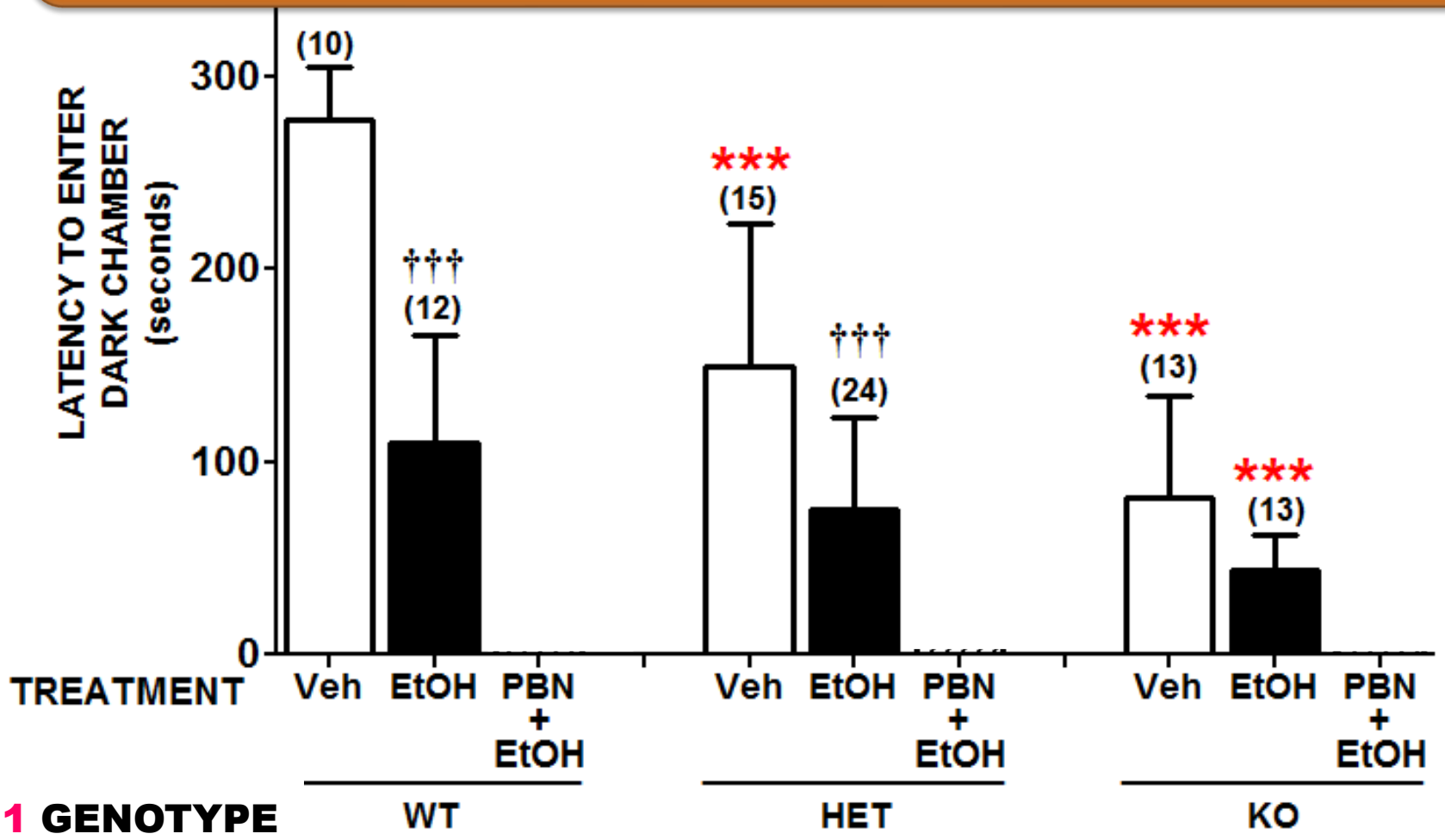


(Miller-Pinsler et al., Free Radic. Biol. Med. 78(C): 23-29, 2015)

EtOH-initiated behavioural deficits in Ogg1 mice

- **Developmental** importance of **DNA repair**
- **8-oxoG** is a developmentally **pathogenic** lesion



(Miller-Pinsler et al., Free Radic. Biol. Med. 78(C): 23-29, 2015)

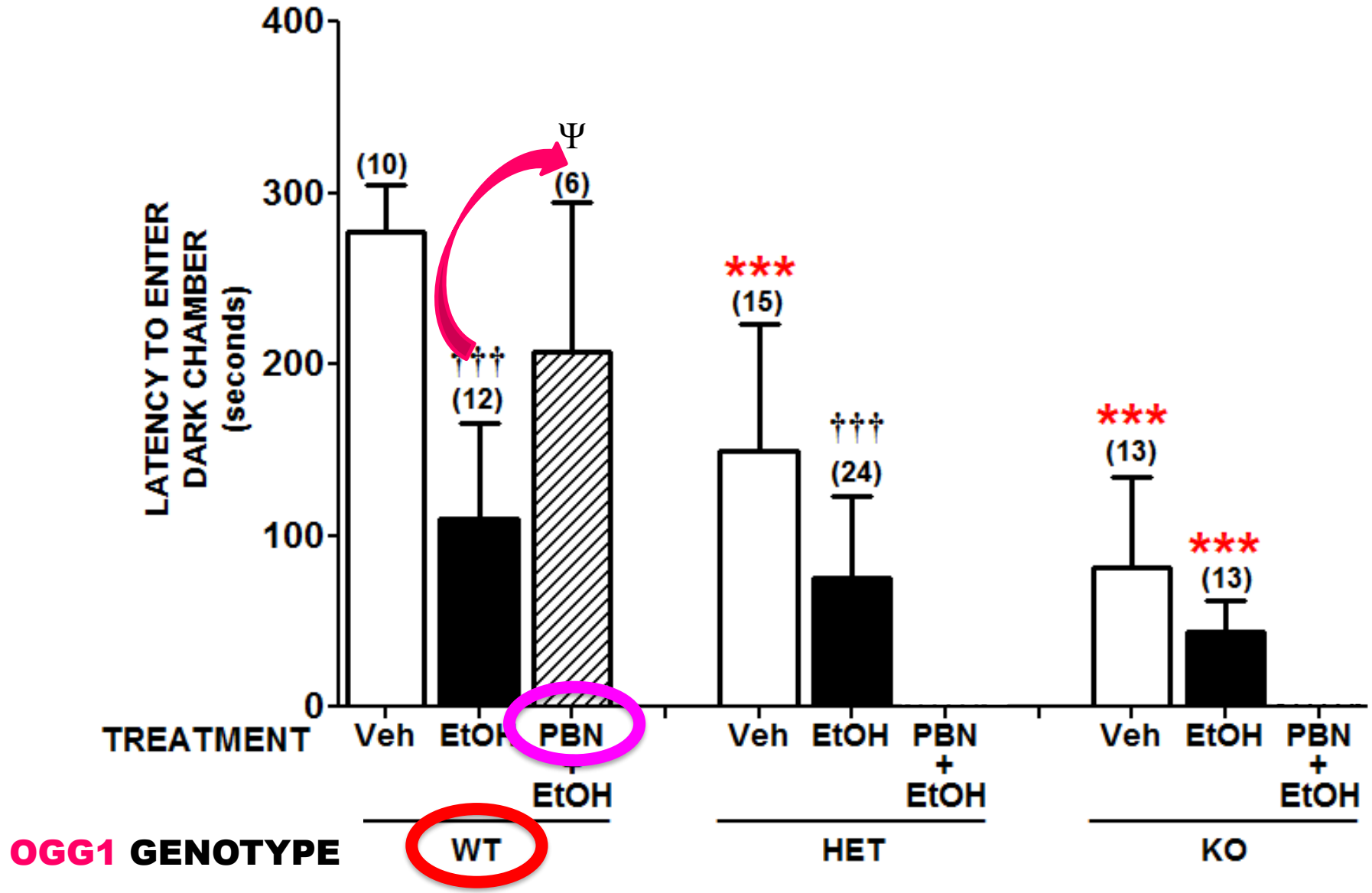
OGG1 DNA REPAIR-DEFICIENT MICE

PBN Pretreatment

Free radical spin trapping agent
and
inhibitor of ROS formation

(PBN = phenylbutylnitrone)

EtOH-initiated behavioural deficits in Ogg1 mice



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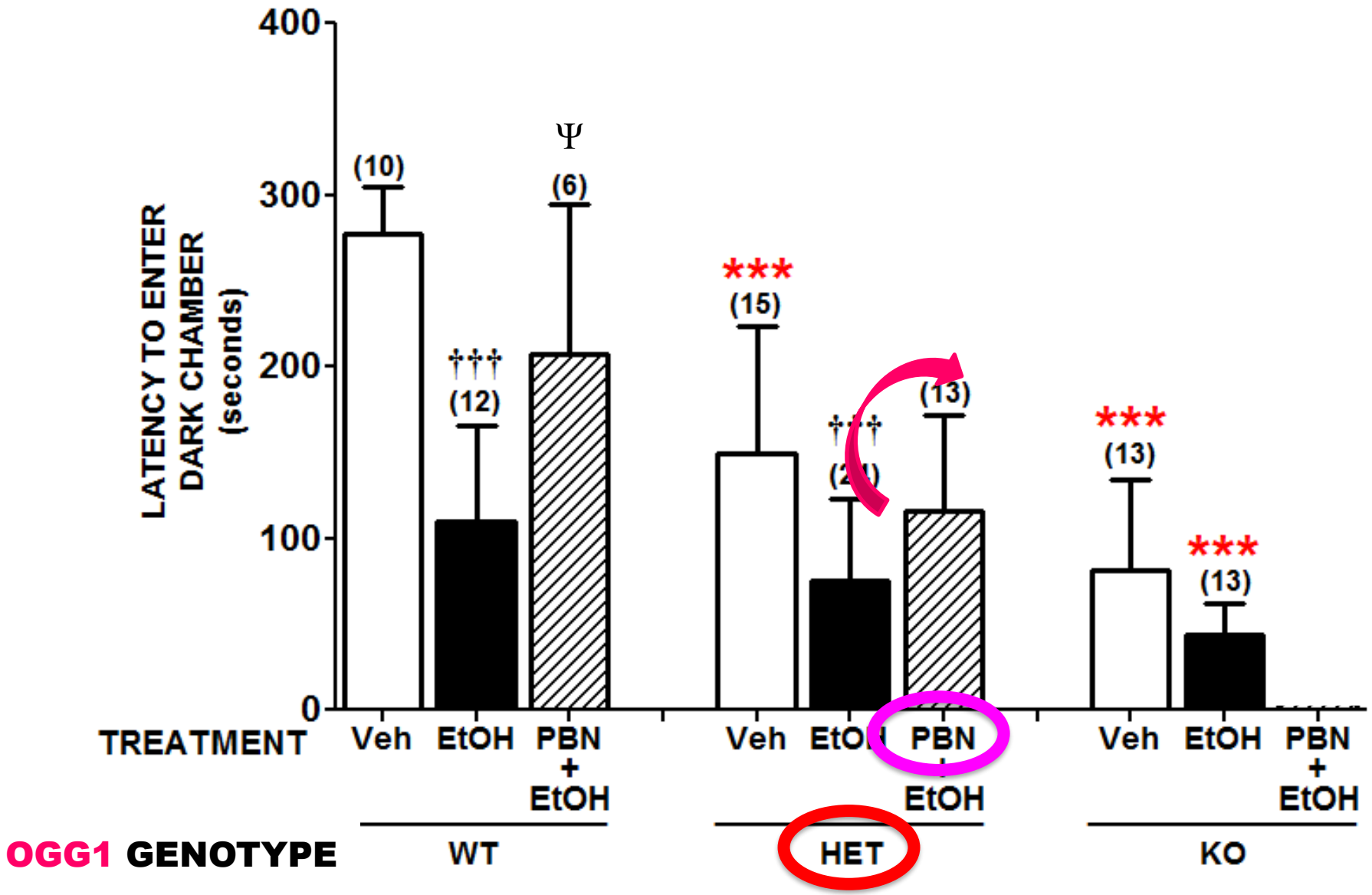

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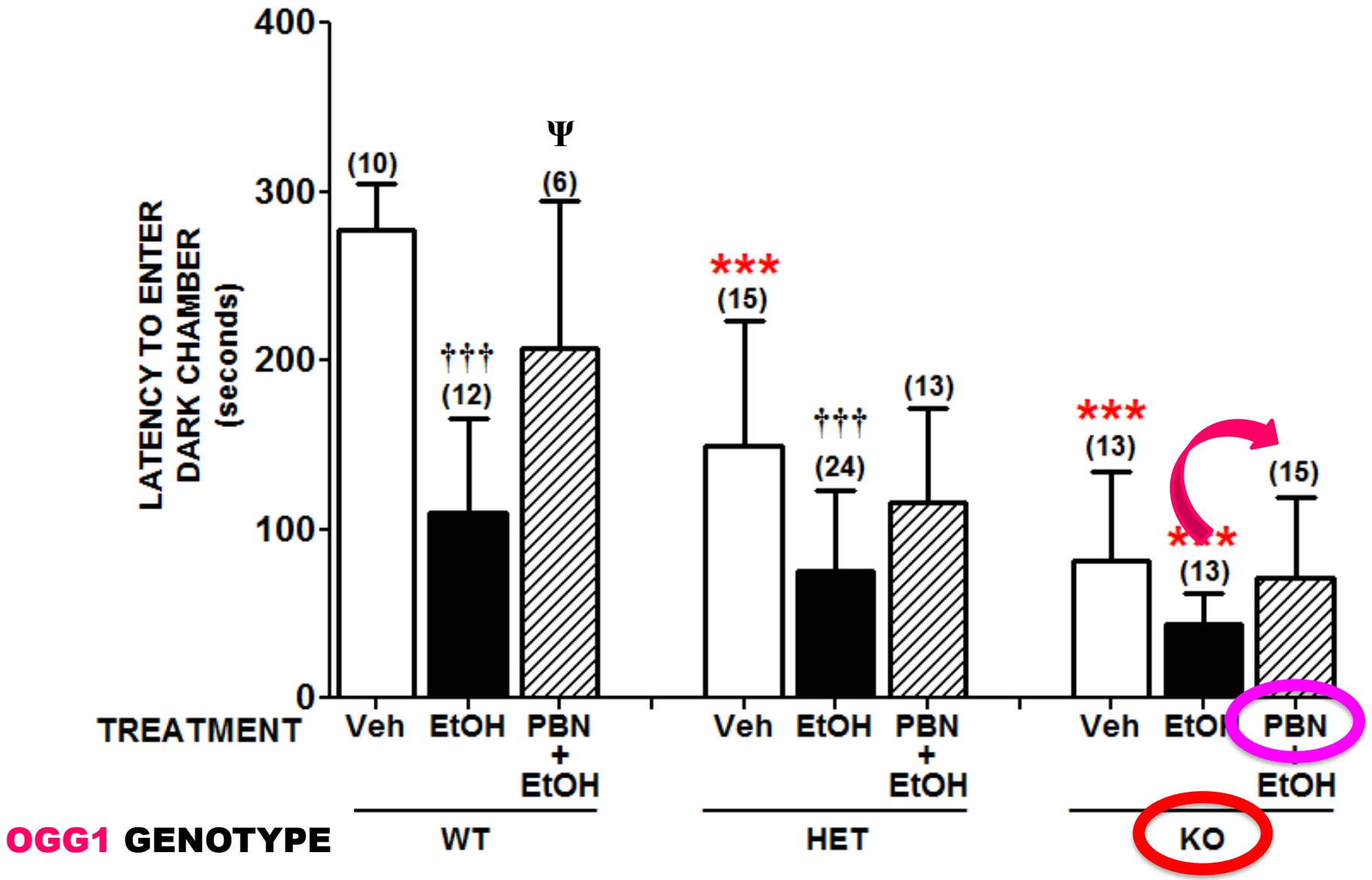

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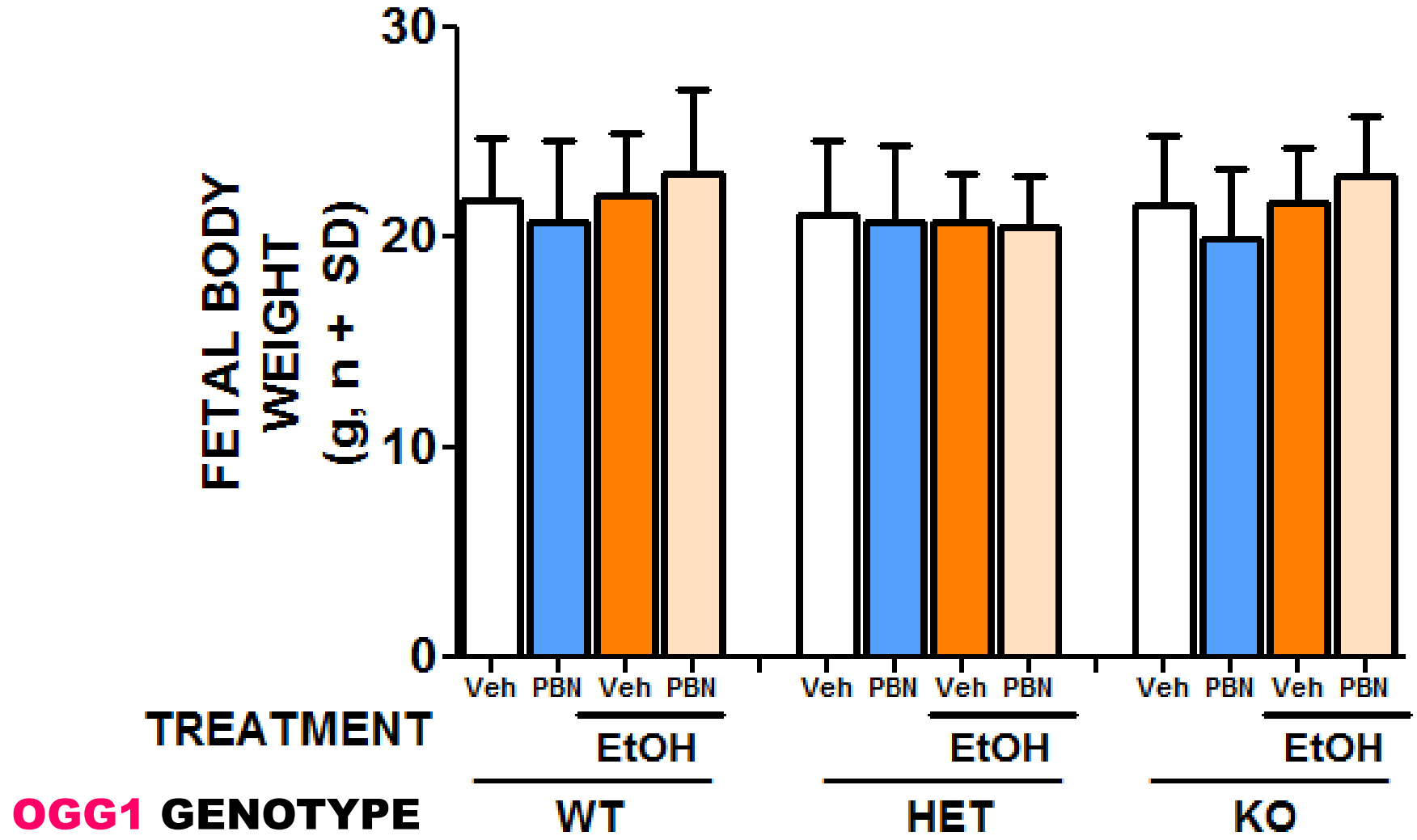
OGG1 DNA REPAIR-DEFICIENT MICE

Fetal Body Weight

General Fetal Health

Fetal Weight in EtOH-treated Ogg1 mice



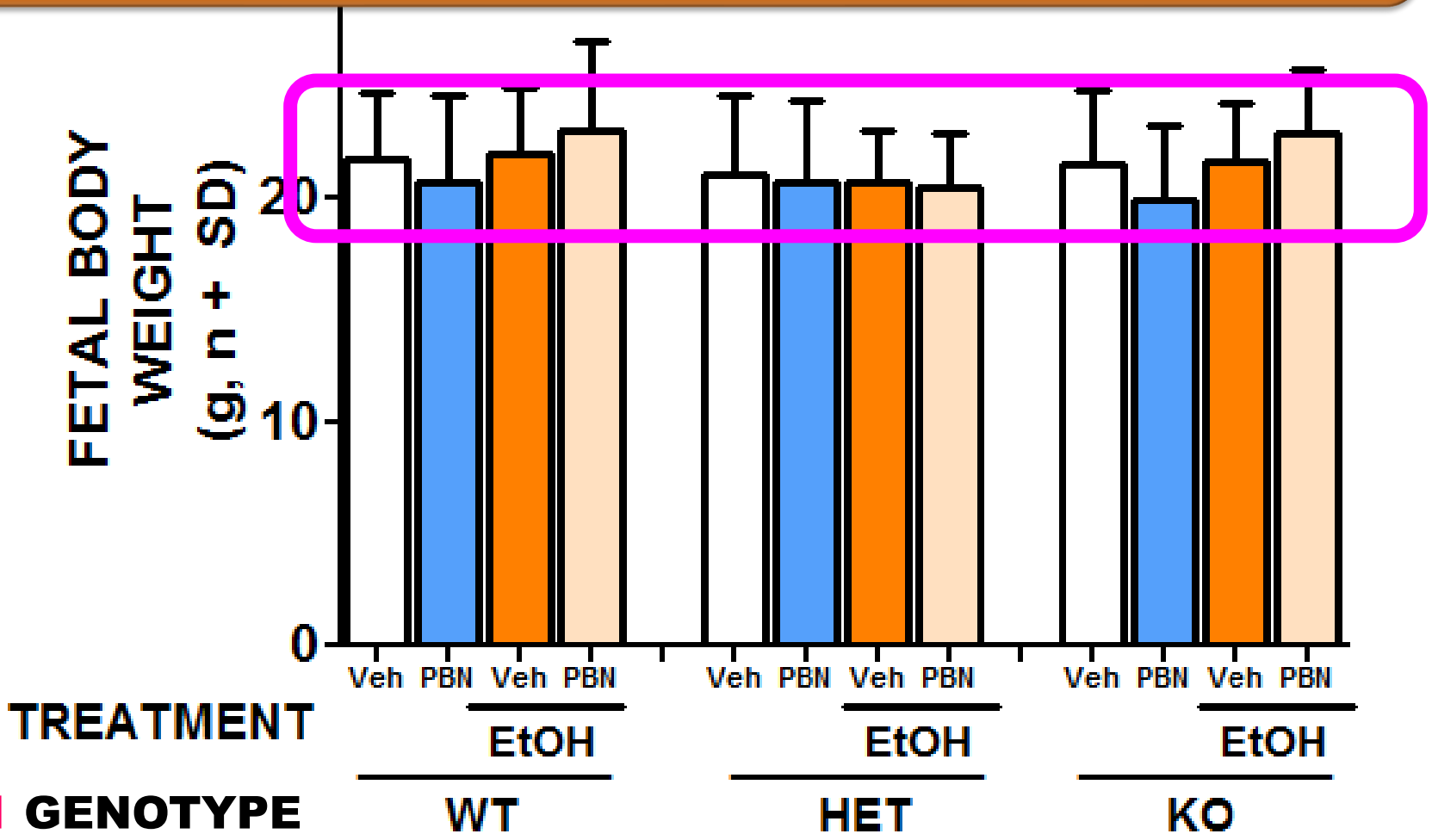
(Miller-Pinsler et al., Free Radic. Biol. Med. 78(C): 23-29, 2015)

Fetal Weight in EtOH-treated Ogg1 mice

Threshold dose

- **No differences in fetal body weight (or birth defects)**

➔ **Exquisite sensitivity of the developing brain**



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OGG1 DNA Repair Studies

Conclusions

- Oxidative DNA damage (8-oxoguanine) is an **embryopathic** molecular lesion

OGG1 DNA Repair Studies

Conclusions

- Oxidative DNA damage (8-oxoguanine) is an **embryopathic** molecular lesion
- Deficiencies in DNA repair may constitute a risk factor for:
  - neurodevelopmental deficits
  - neurodegeneration

DNA Repair

Similarly developmentally protective:

Other DNA repair proteins (knockout mice):

p53 protein (Nicol et al., Nat. Genet., 1995)
- Initiates DNA repair and/or apoptosis
- **Protection**: - benzo[a]pyrene
  - cyclophosphamide (Moallem & Hales, Development, 1998)

Ataxia telangiectasia mutated protein (**ATM**) (Laposa et al., FASEB J., 2004)
- Initiates DNA repair
- **Protection**: - ionizing radiation
  - phenytoin (Bhuller & Wells, Toxicol. Sci., 2006)

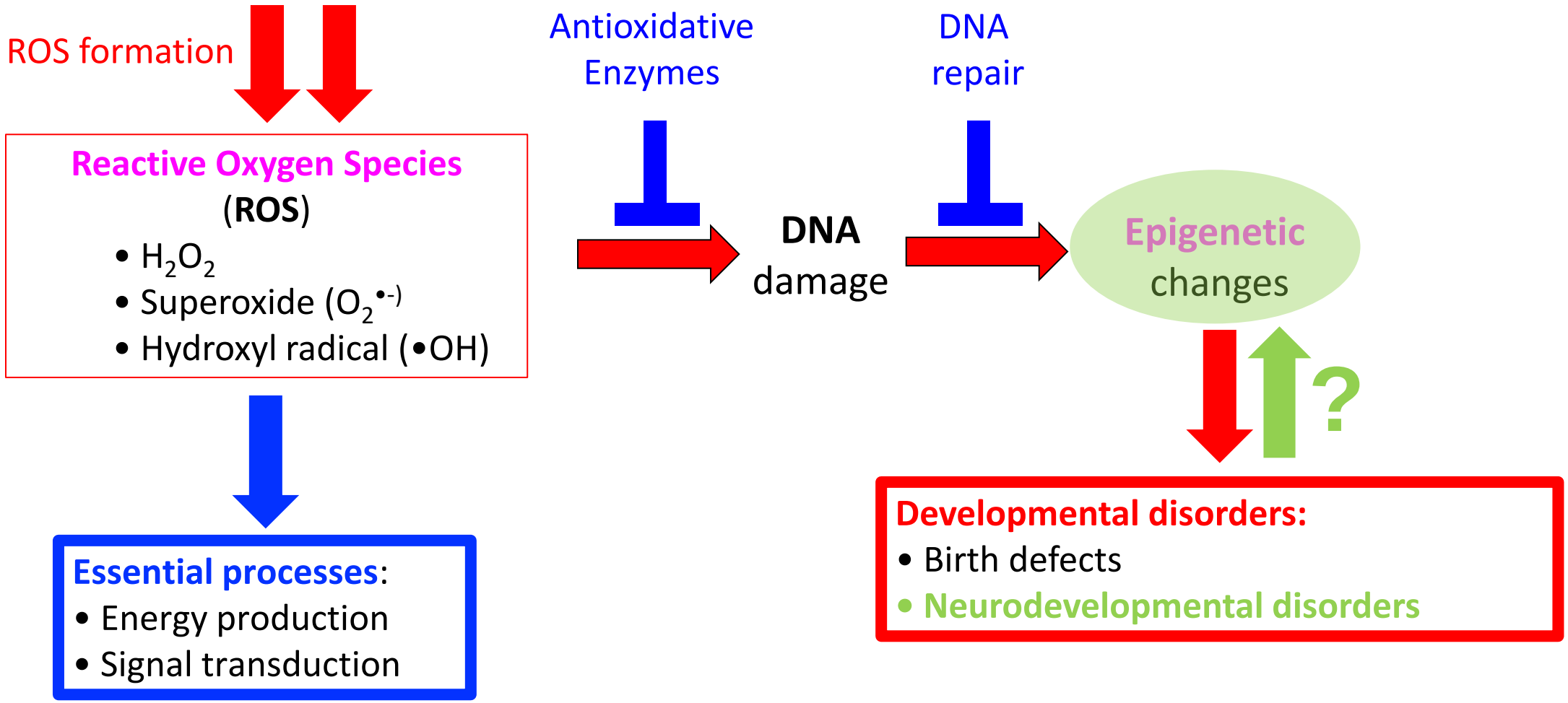
Cockayne syndrome B protein (**CSB**) (McCallum et al., Antioxid. Redox Signal., 2011)
- Contributes to the repair of the **8-oxoguanine** lesion
- **Protection**: - methamphetamine

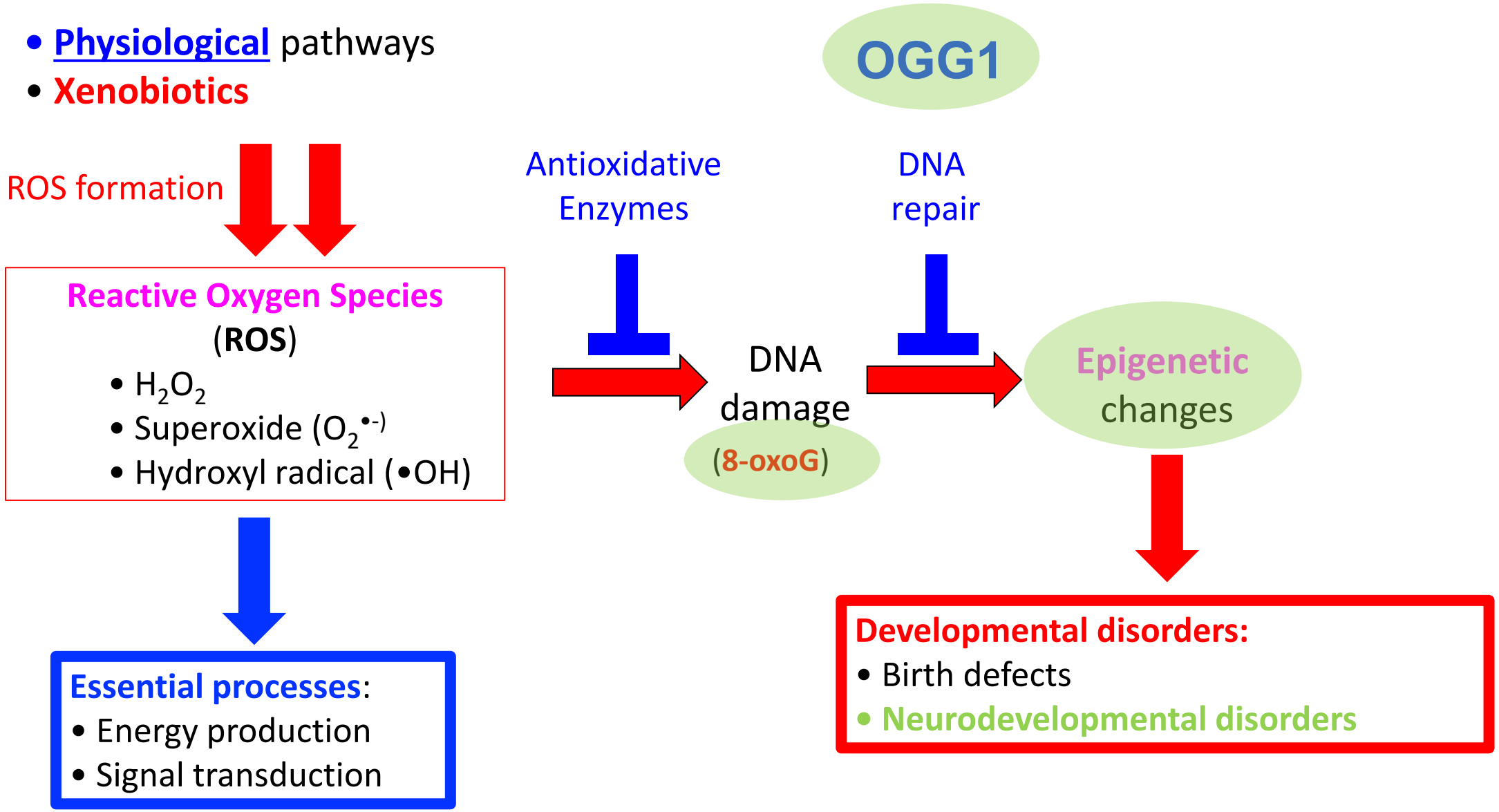
Breast cancer 1 protein (**BRCA1**) (Shapiro et al. Redox Biol., 2016)
- **Protection**: - ethanol
- See Danielle Drake & Kian Afsharian today at 1:00 p.m. and Wednesday FASD symposium

(Review: Wells et al., Birth Defects Res. Part C: Embryo Today: Reviews 90(2): 103-109, 2010)

Epigenetic changes

- **Physiological** pathways
- **Xenobiotics**

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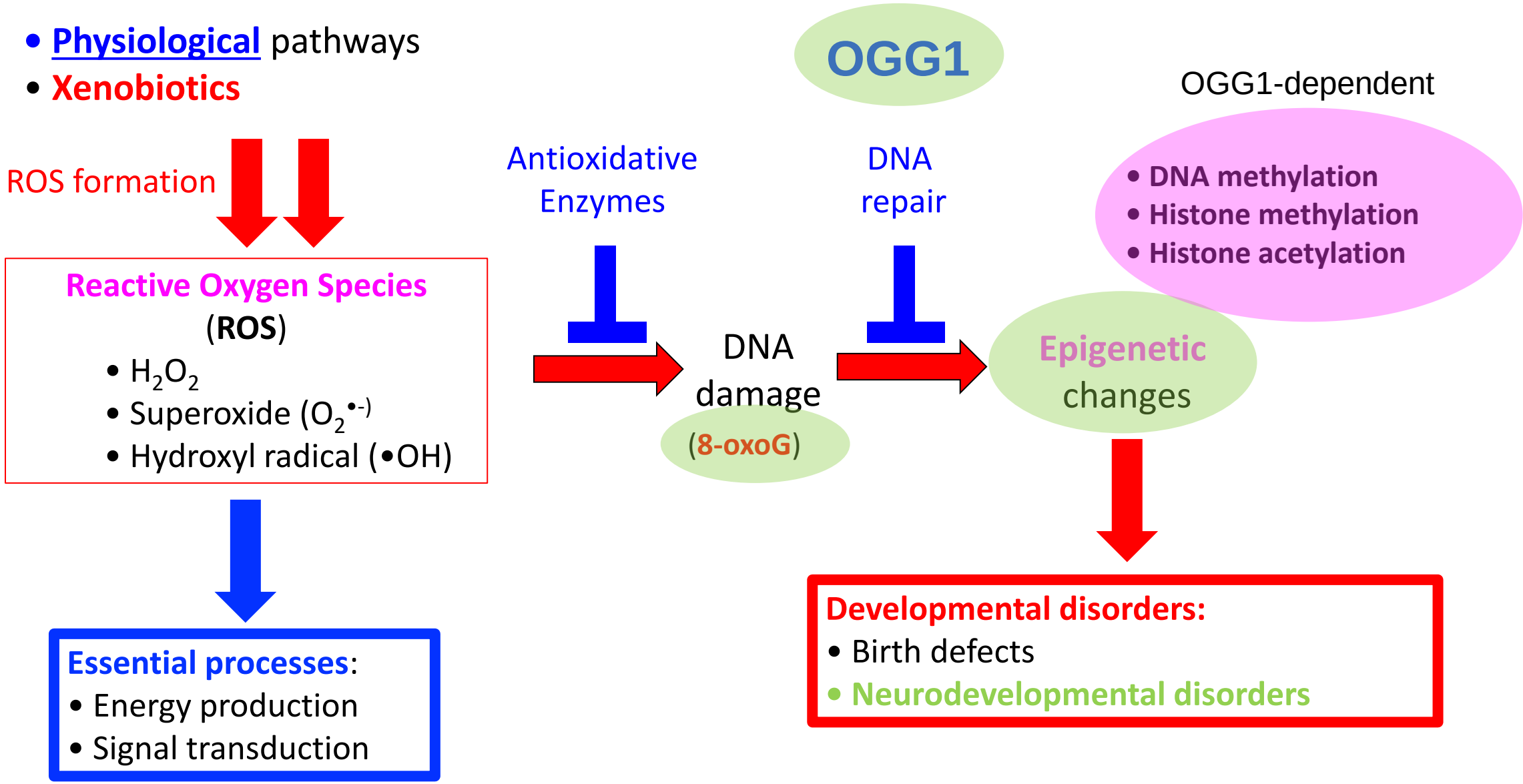
• Xenobiotics
ROS formation
Reactive Oxygen Species
(ROS)
• H_2O_2
• Superoxide ($O_2^{\bullet -}$)
• Hydroxyl radical (•OH)
Essential processes:
• Energy production
• Signal transduction
Antioxidative Enzymes
DNA damage
(8-oxoG)
OGG1
DNA repair
Epigenetic changes
Developmental disorders:
• Birth defects
• Neurodevelopmental disorders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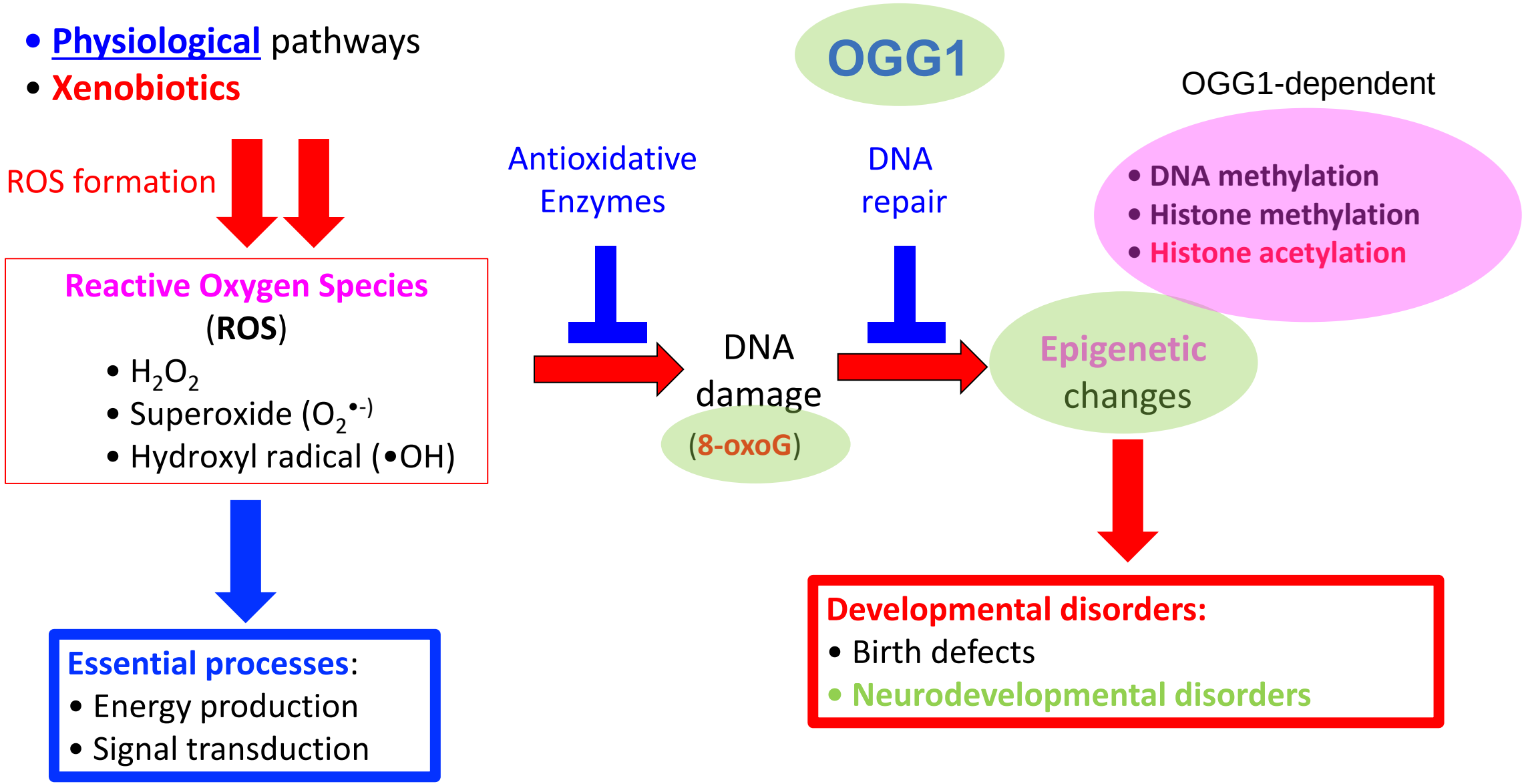
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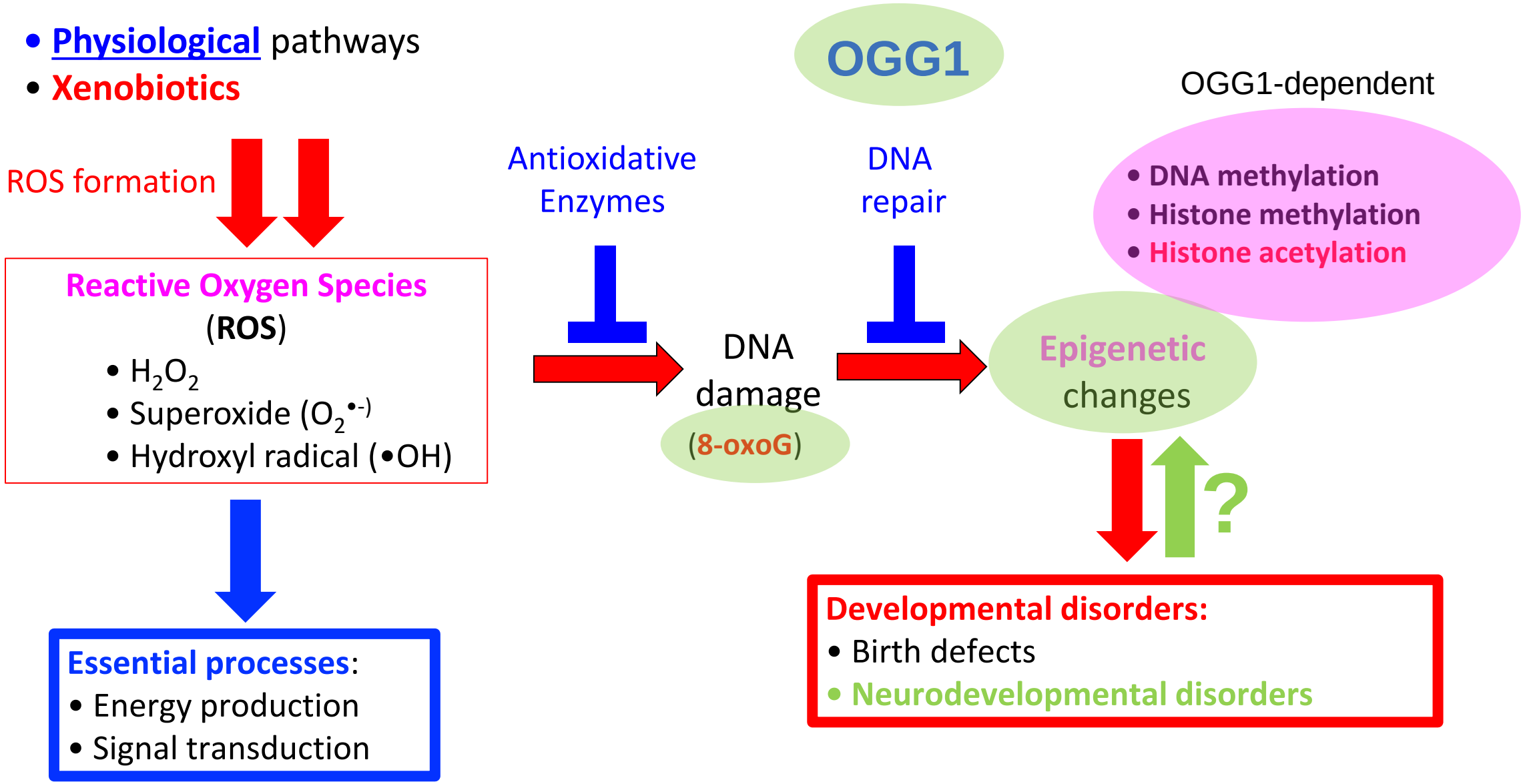
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?
Developmental disorders:
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- **Physiological** pathways
- **Xenobiotics**

ROS formation

Reactive Oxygen Species
(ROS)
- H_2O_2
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Essential processes:
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DNA damage
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DNA repair

OGG1

OGG1-dependent
- **DNA methylation**
- **Histone methylation**
- **Histone acetylation**

Epigenetic changes

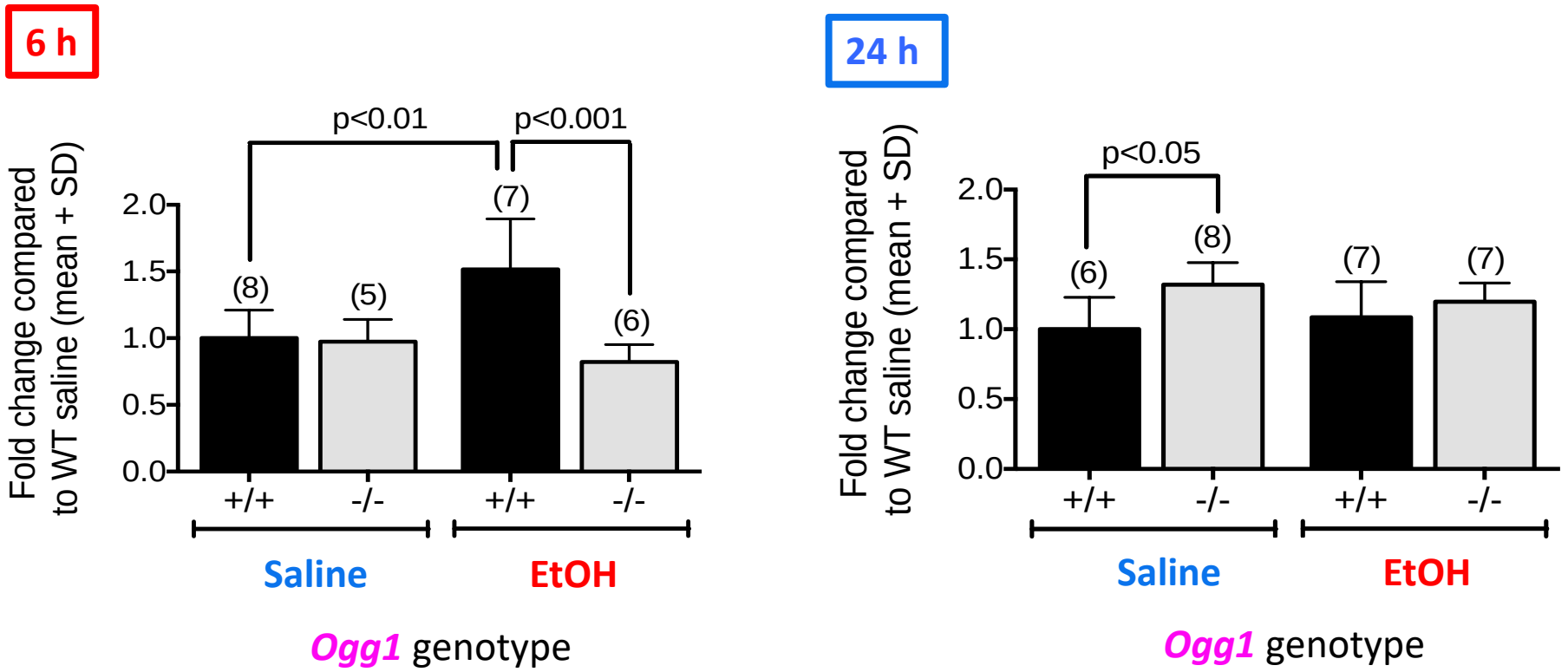
HDAC inhibitor
Trichostatin (**TSA**)

Developmental disorders:
- Birth defects
- **Neurodevelopmental disorders**

(HDAC – histone deacetylase)

Histone Acetylation in Fetal Brains

H3K9ac in GD 17 *Ogg1* fetal brains

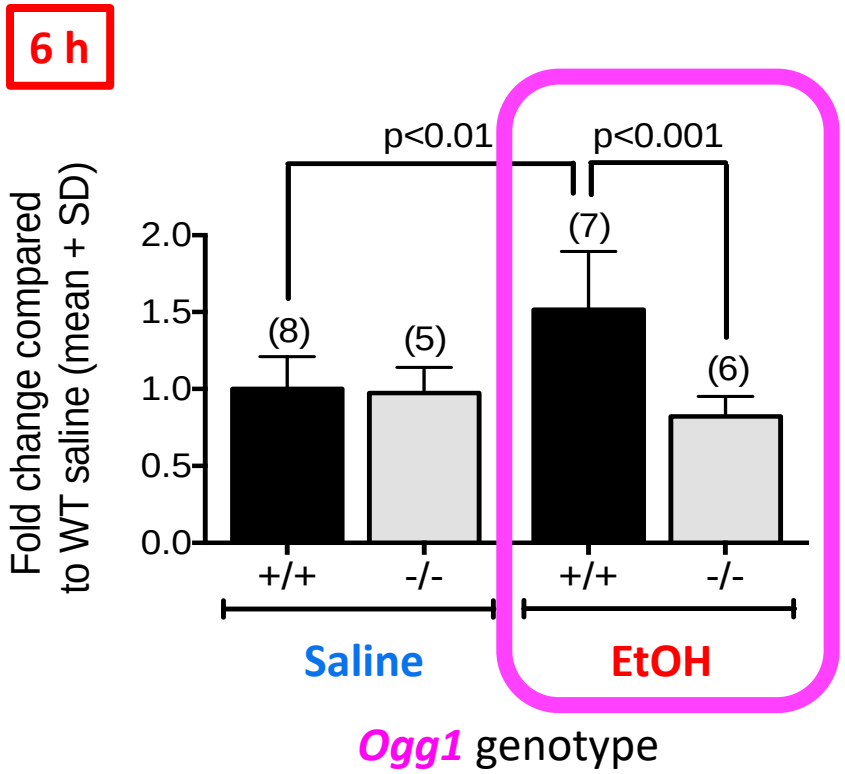


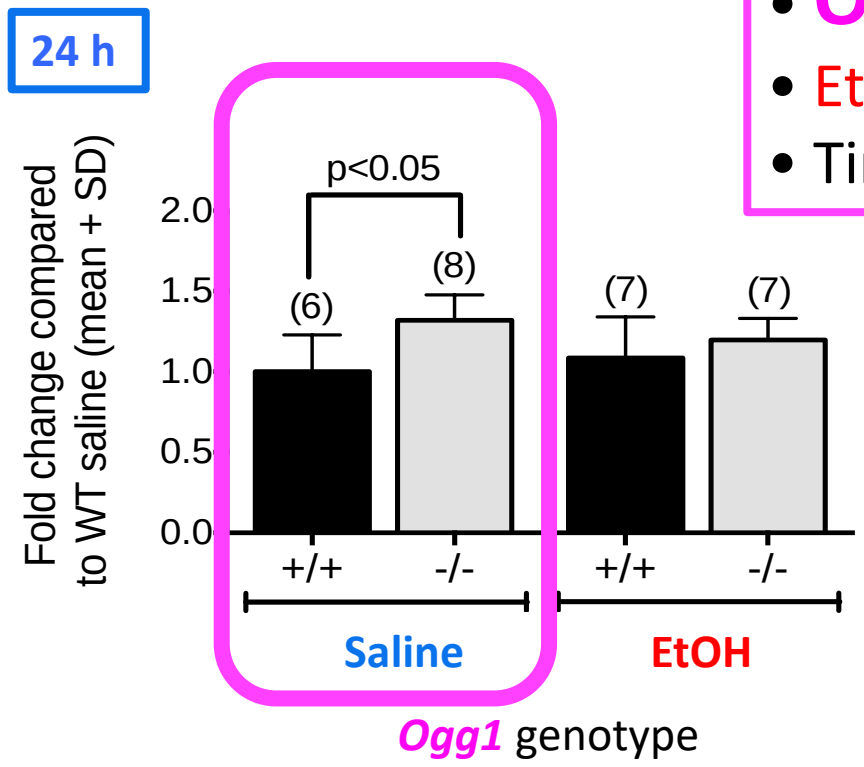
(Bhatia et al., submitted)

Histone Acetylation in Fetal Brains

H3K9ac in GD 17 *Ogg1* fetal brains

- **OGG1-dependent**
- EtOH-dependent
- Time-dependent





(Bhatia et al., submitted)

Postnatal **reversal** of neurodevelopmental disorders caused by *in utero* ETOH exposure ?

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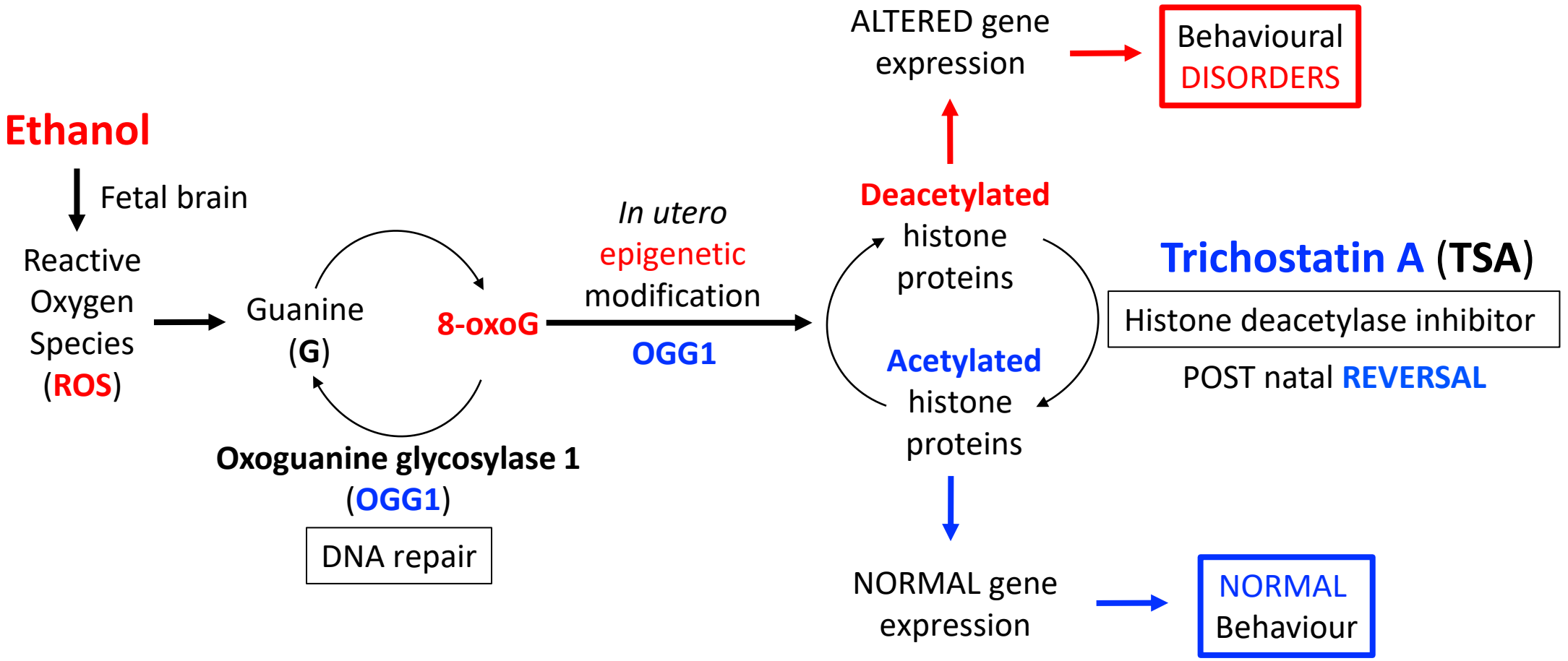

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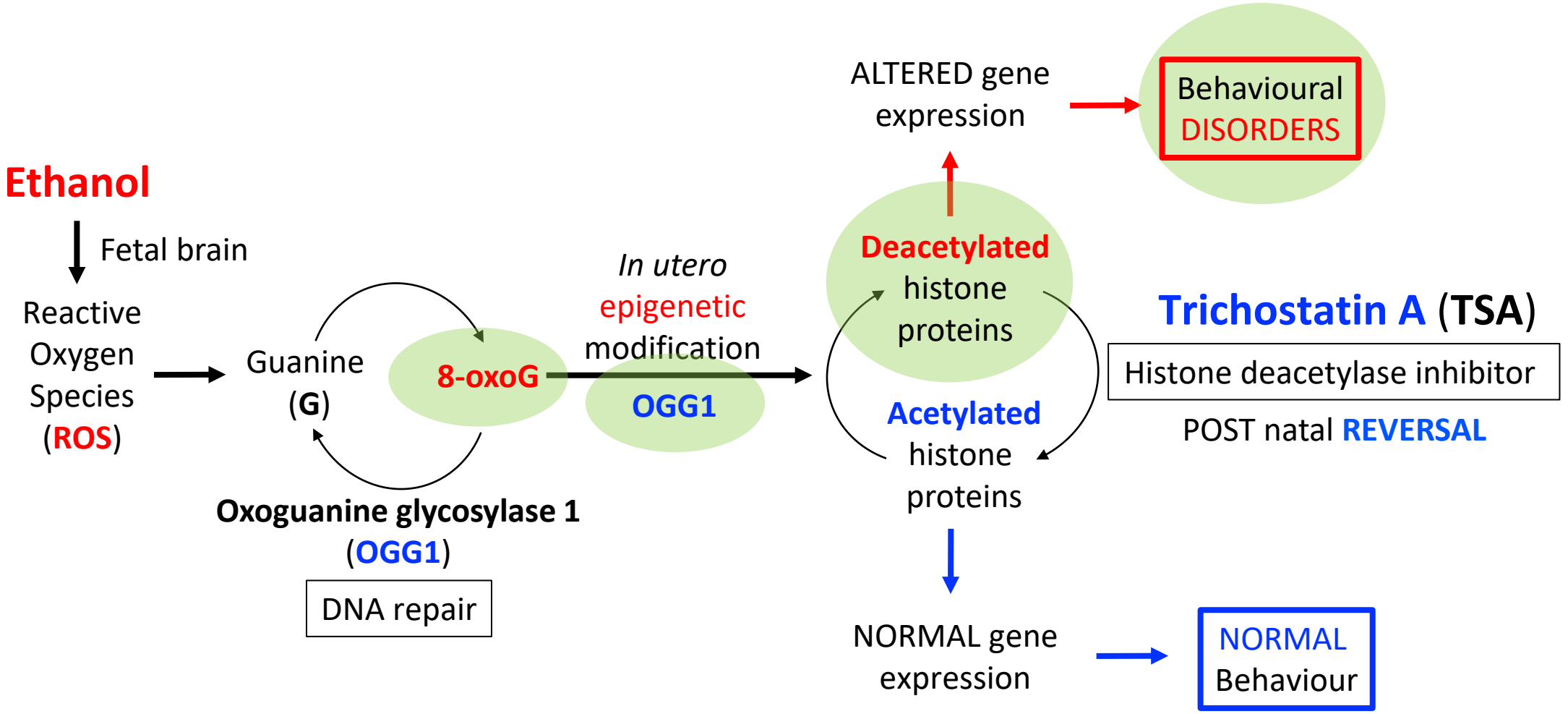

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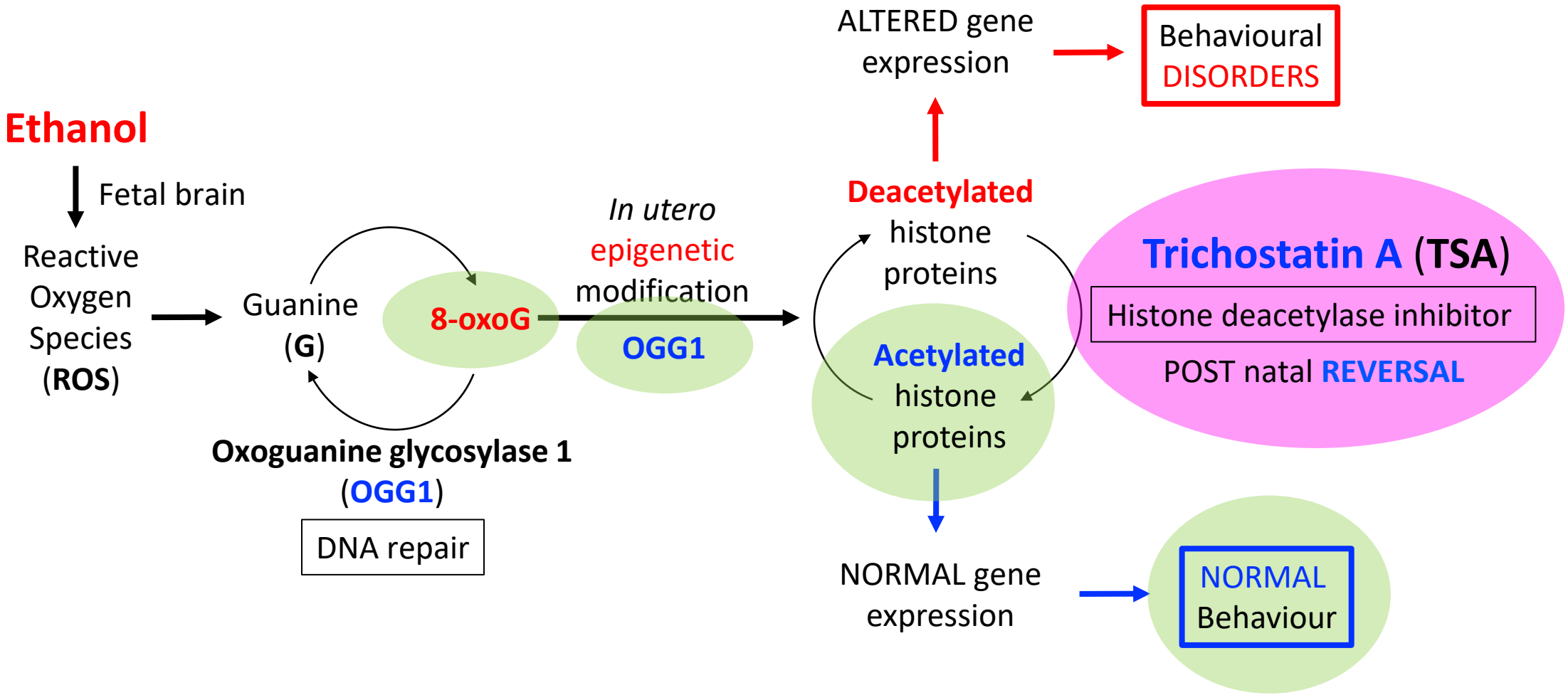

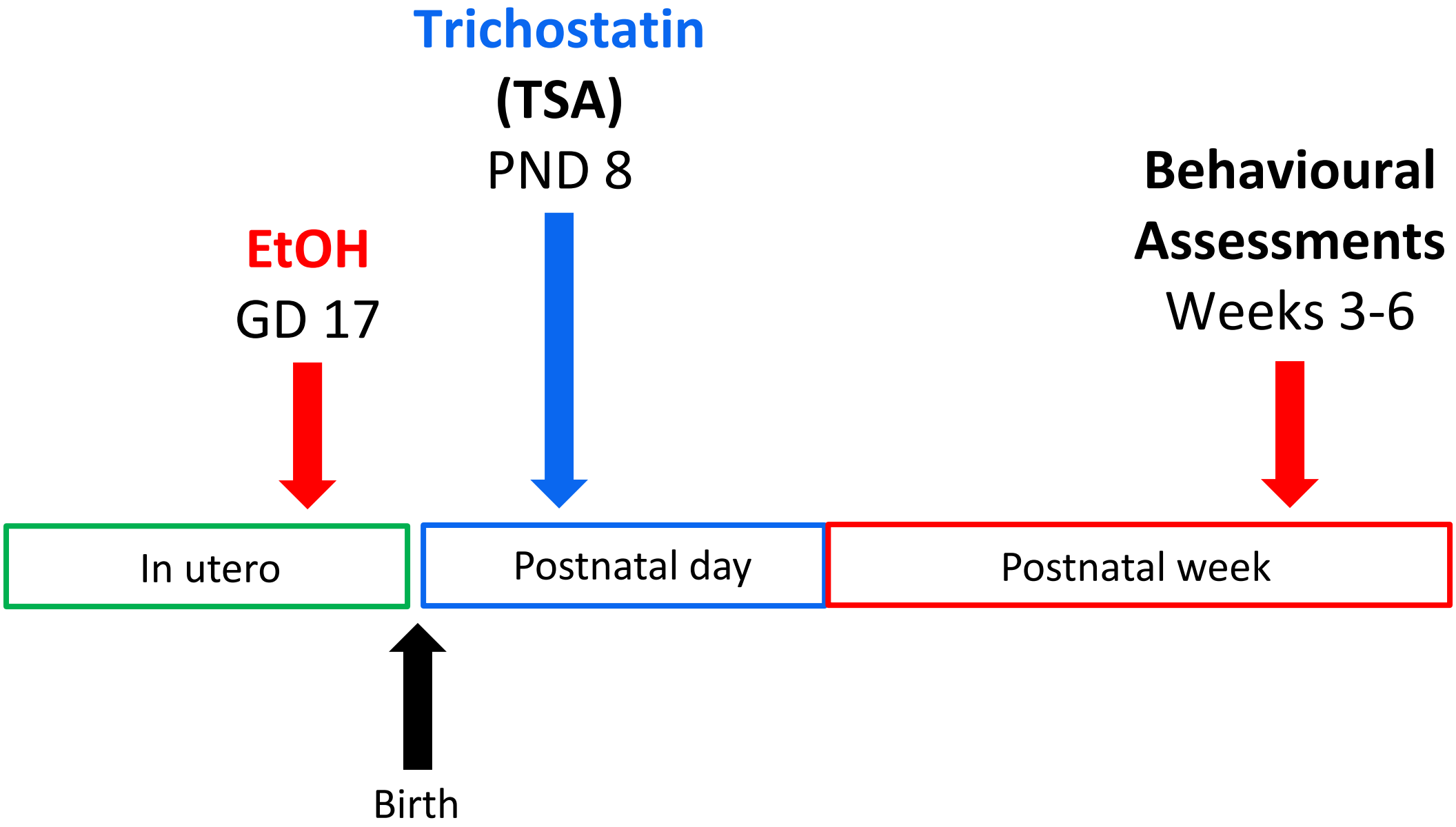
Trichostatin
(TSA)
PND 8
EtOH
GD 17
Behavioural
Assessments
Weeks 3-6
In utero
Postnatal day
Postnatal week
Birth

Behavioural Disorders in *Ogg1* KO Mice

Tests	Measures	Age Group
Nesting Material Shredding	Goal-directing, nurturing, repetitive behaviour	3.7 weeks
Novel Location Recognition	Spatial memory	4 weeks
Novel Object Recognition	Recognition memory	5 weeks
Rotarod	Motor coordination	5.5 weeks

(Bhatia et al., submitted)

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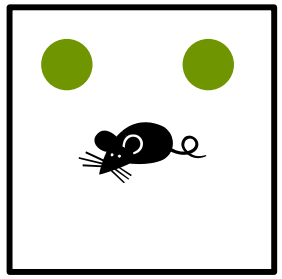
Novel Location Recognition in *Ogg1* KO : Spatial Memory

Test	Measures
Novel Location Recognition	Spatial memory

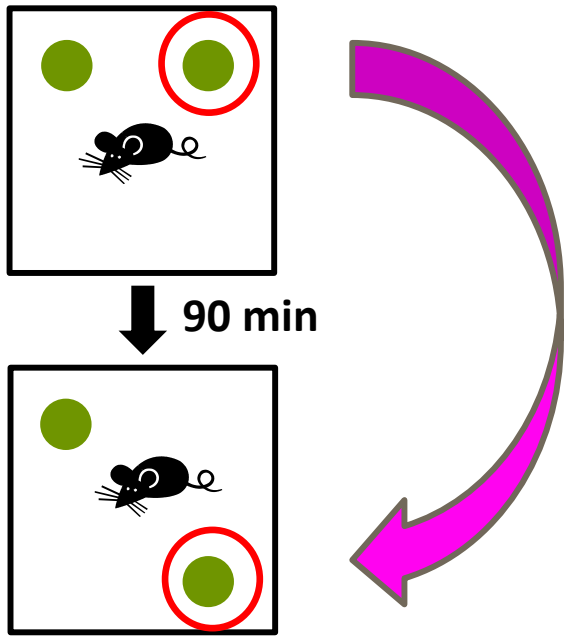


Two objects in a box

(Bhatia et al., submitted)

Novel Location Recognition in *Ogg1* KO : Spatial Memory

Test	Measures
Novel Location Recognition	Spatial memory



(Bhatia et al., submitted)

One object moved to novel location

↓

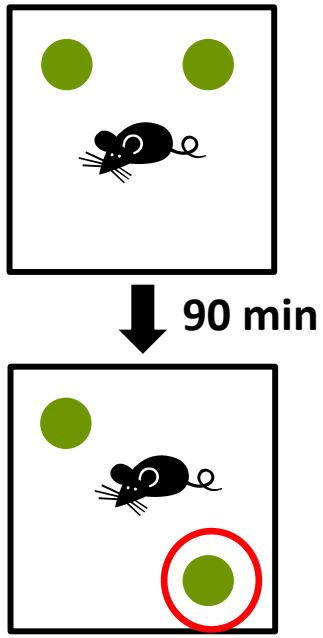
↑ time spent at the novel location ("preference")

↓

Better spatial memory

Novel Location Recognition in *Ogg1* KO : Spatial Memory

Test	Measures
Novel Location Recognition	Spatial memory



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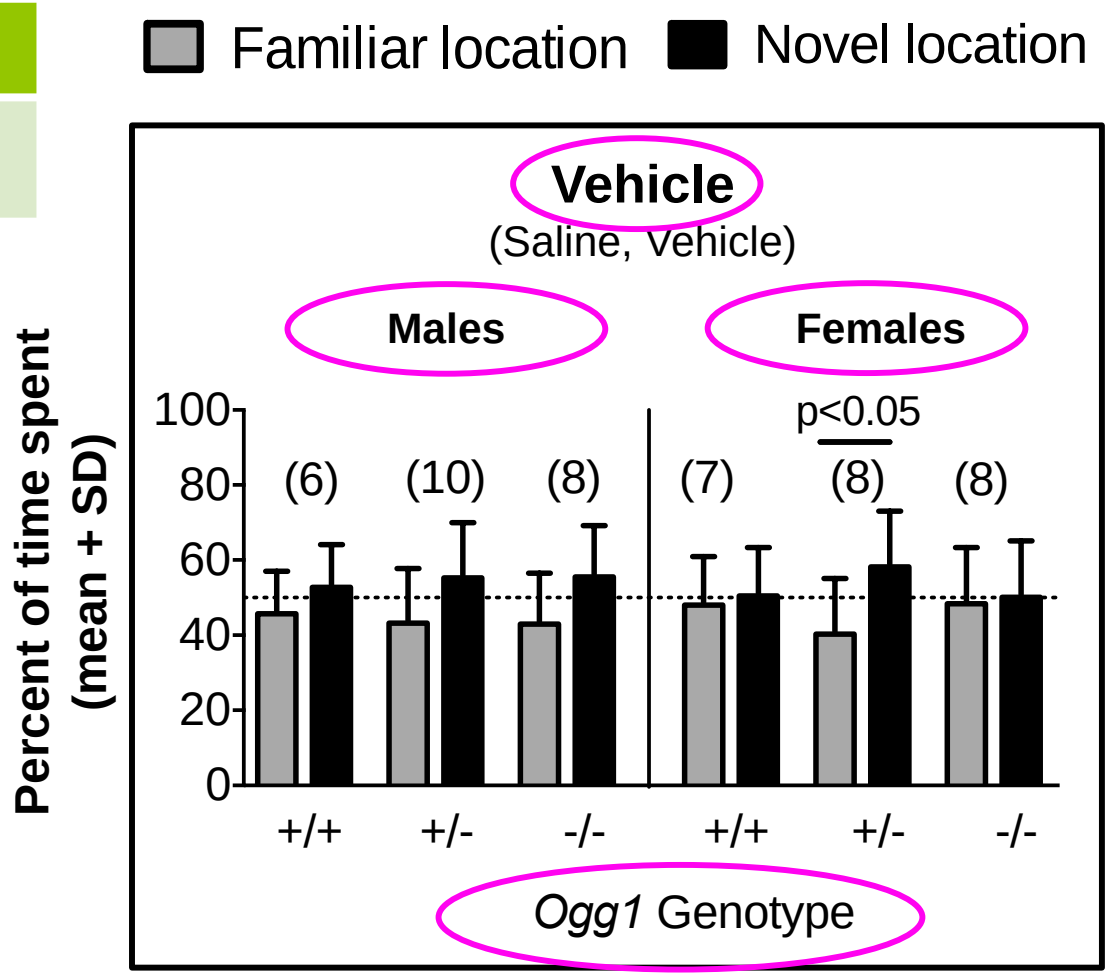

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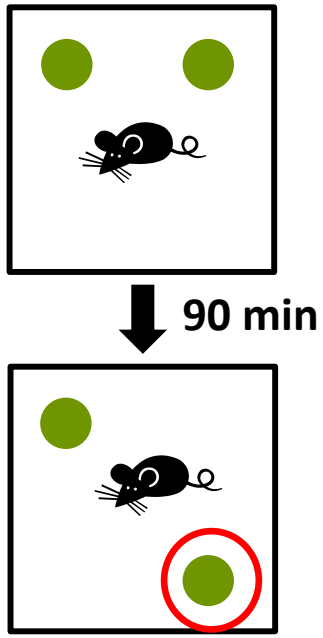

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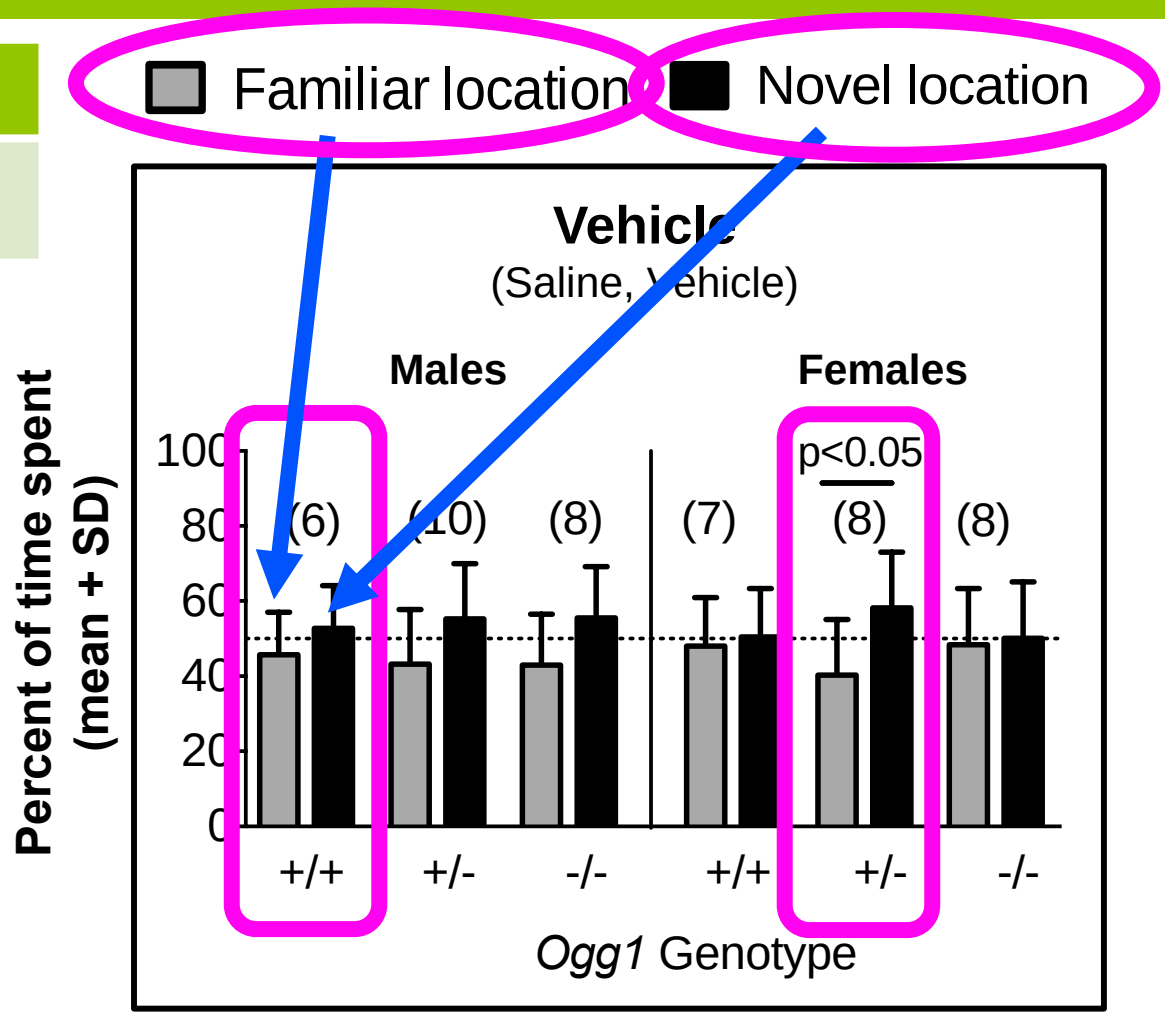

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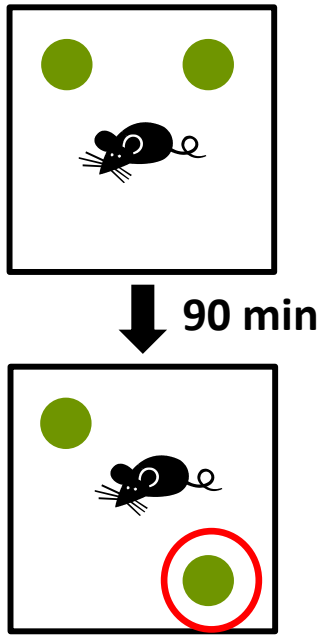

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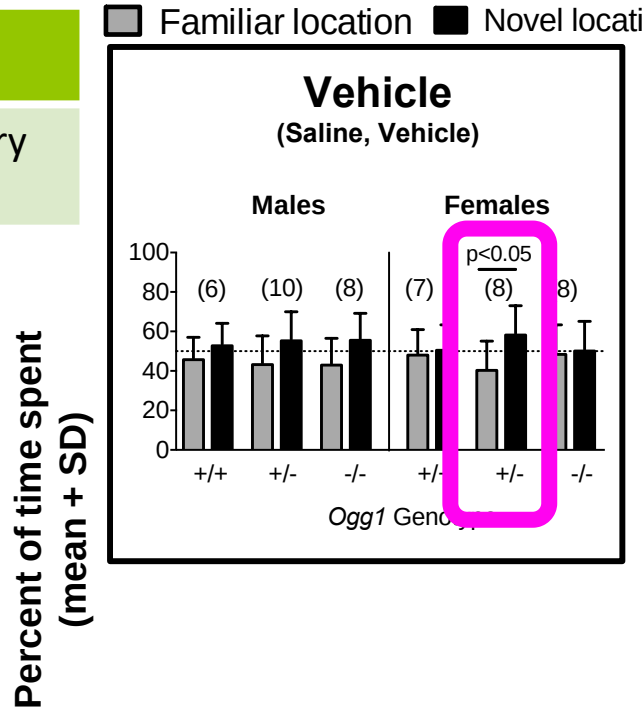


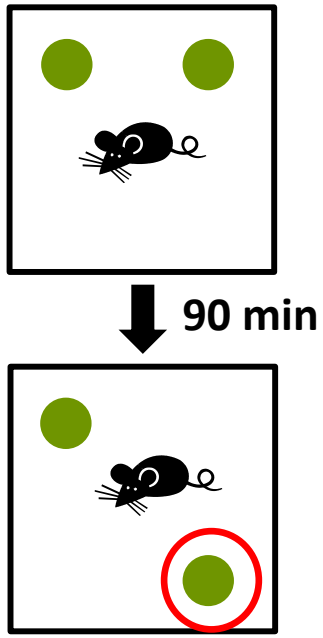
Pattern

Limited spatial memory

(only in +/- *Ogg1* female progeny)

Novel Location Recognition in *Ogg1* KO : Spatial Memory

Test	Measures
Novel Location Recognition	Spatial memory



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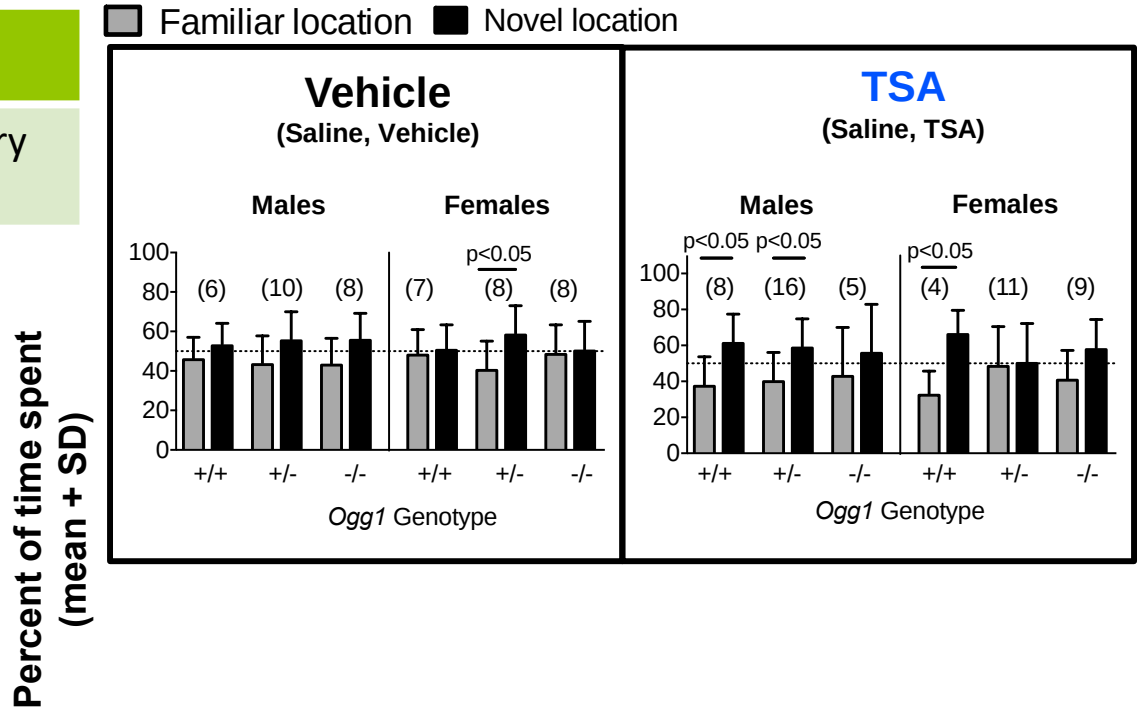

TSA = Trichostatin

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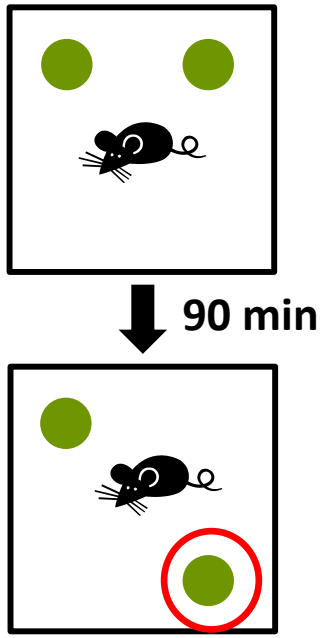

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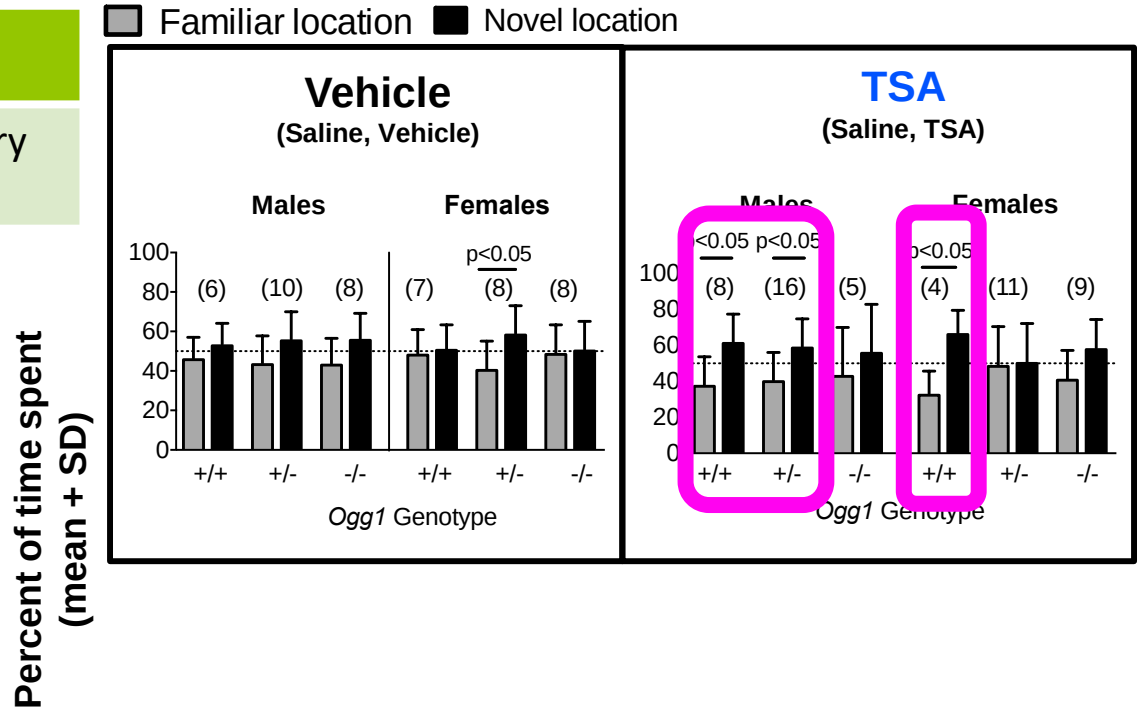

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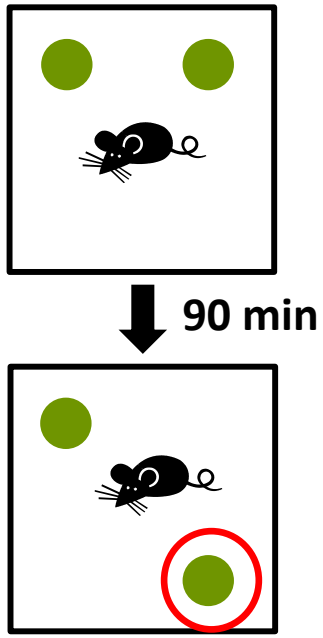

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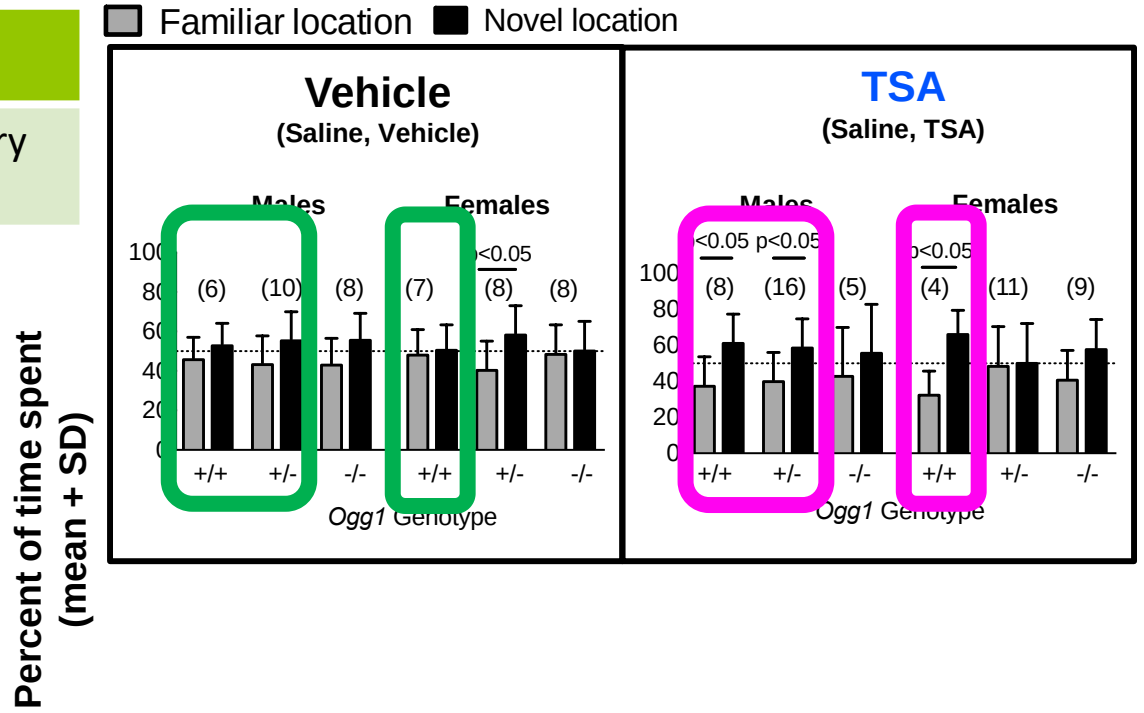

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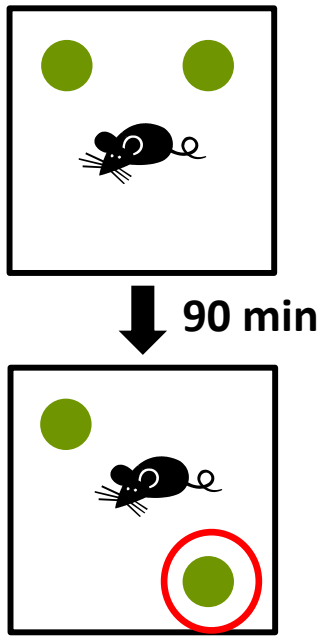

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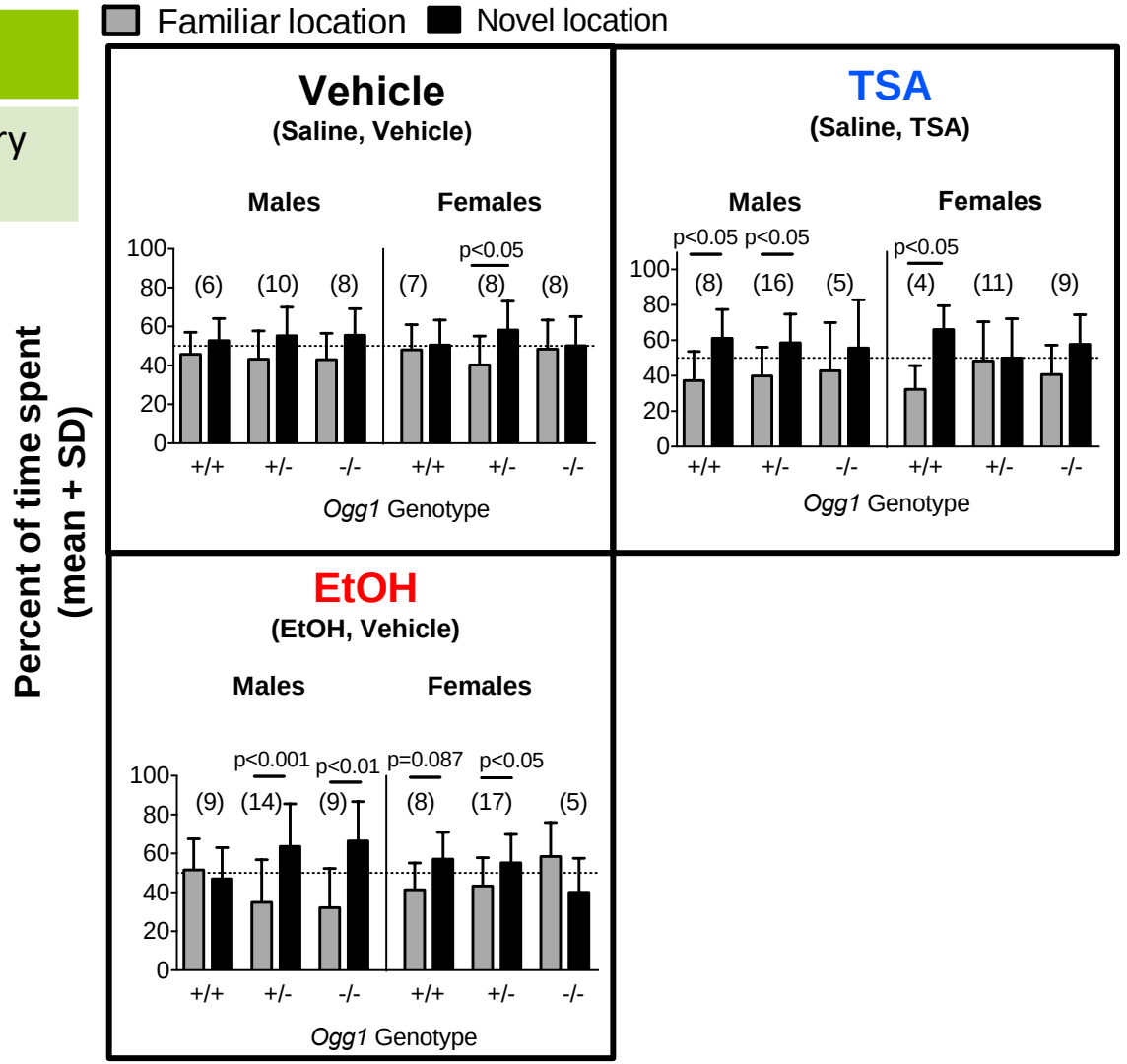

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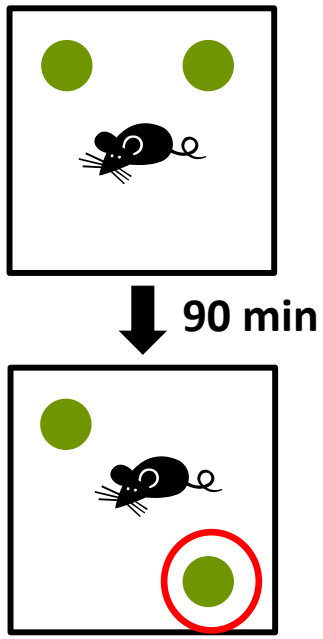

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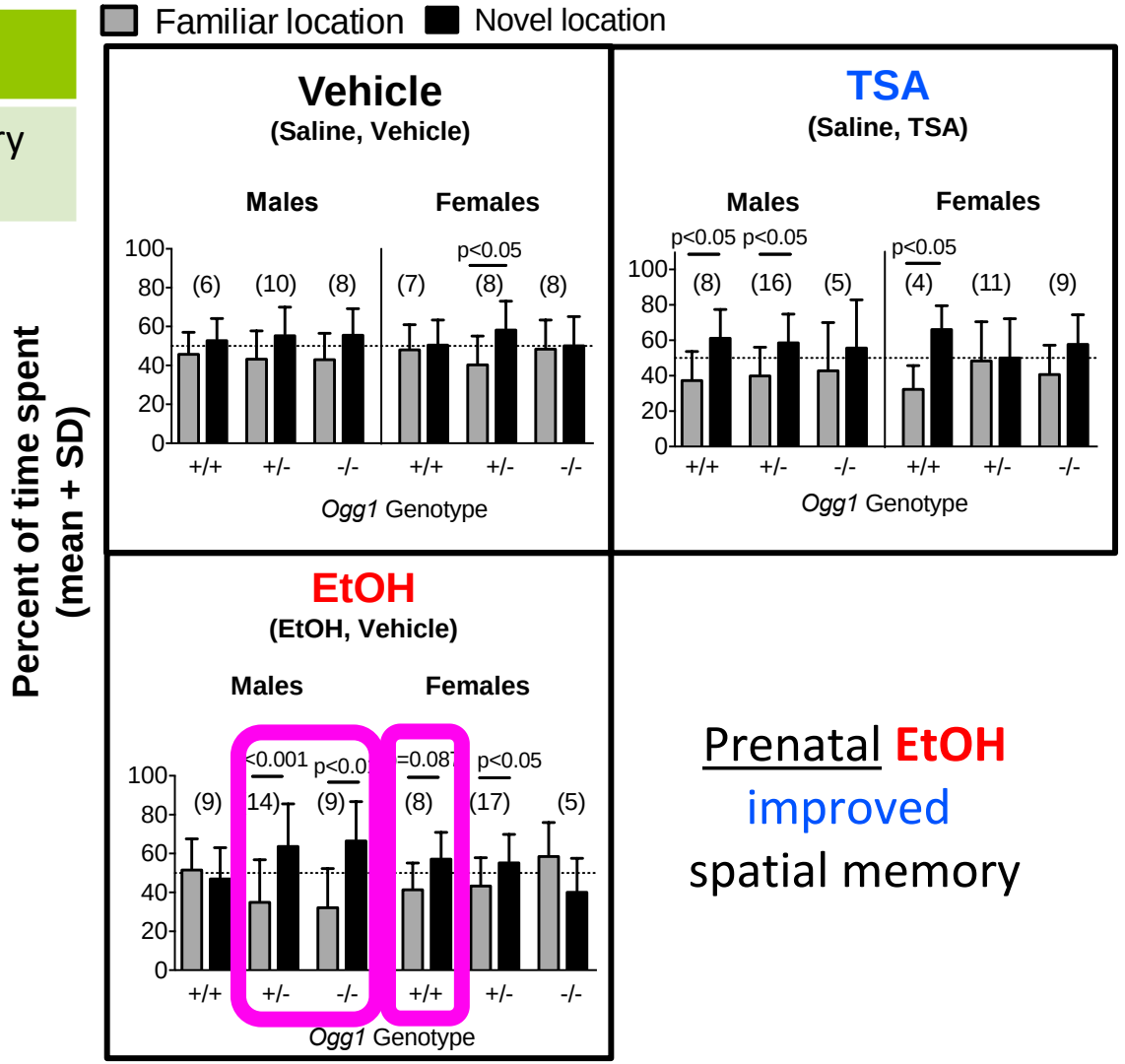

Prenatal **EtOH** improved spatial memory

Novel Location Recognition in *Ogg1* KO : Spatial Memory

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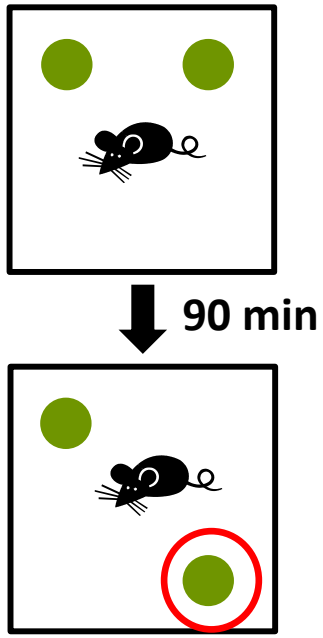

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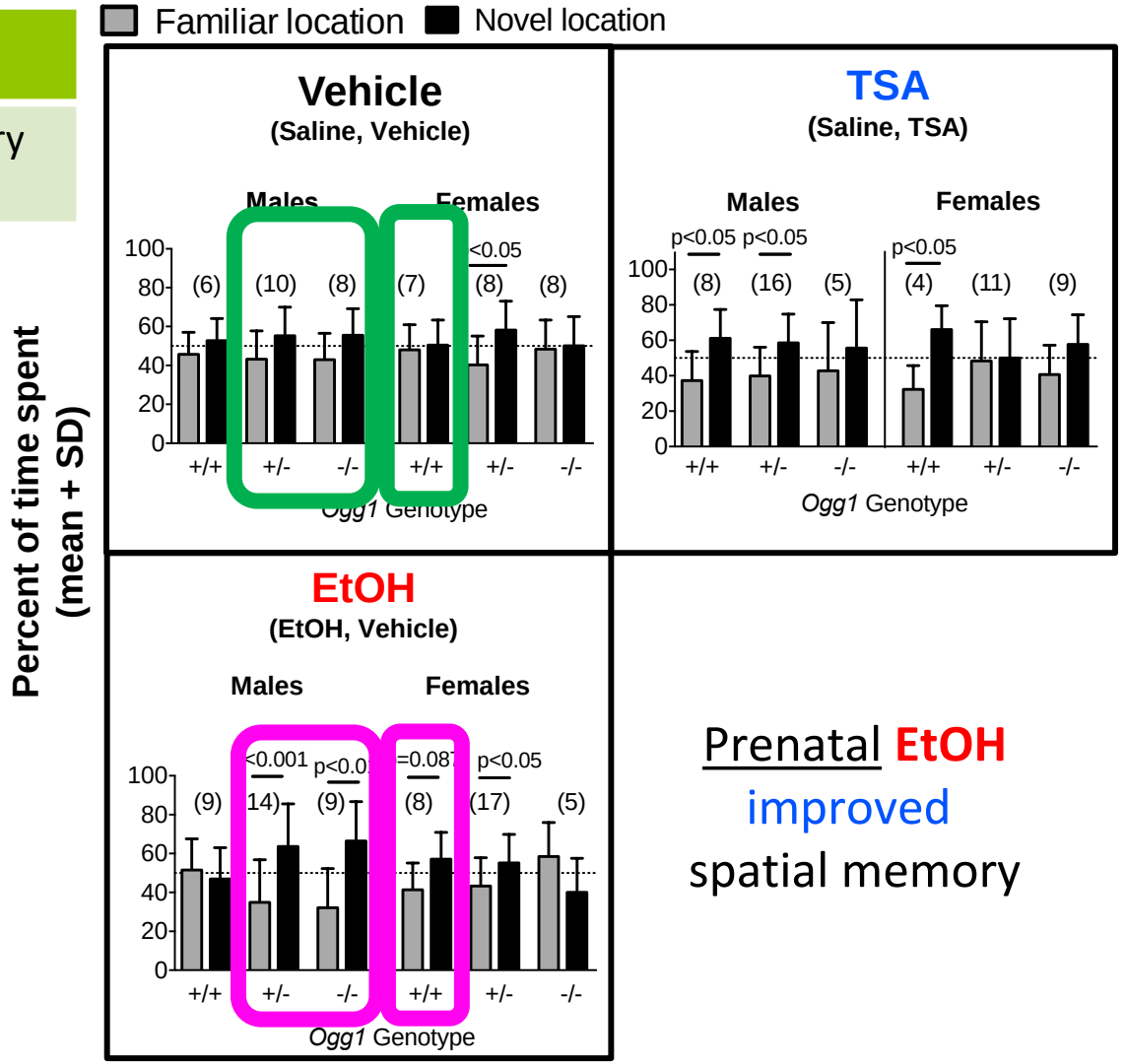

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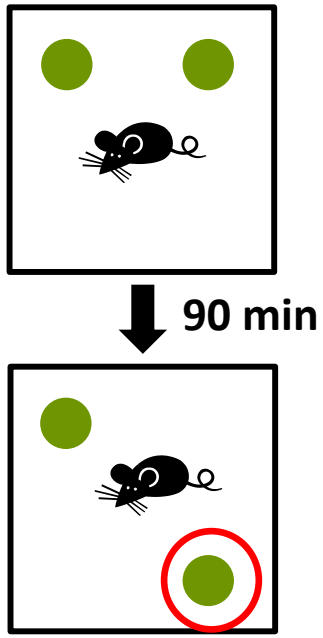

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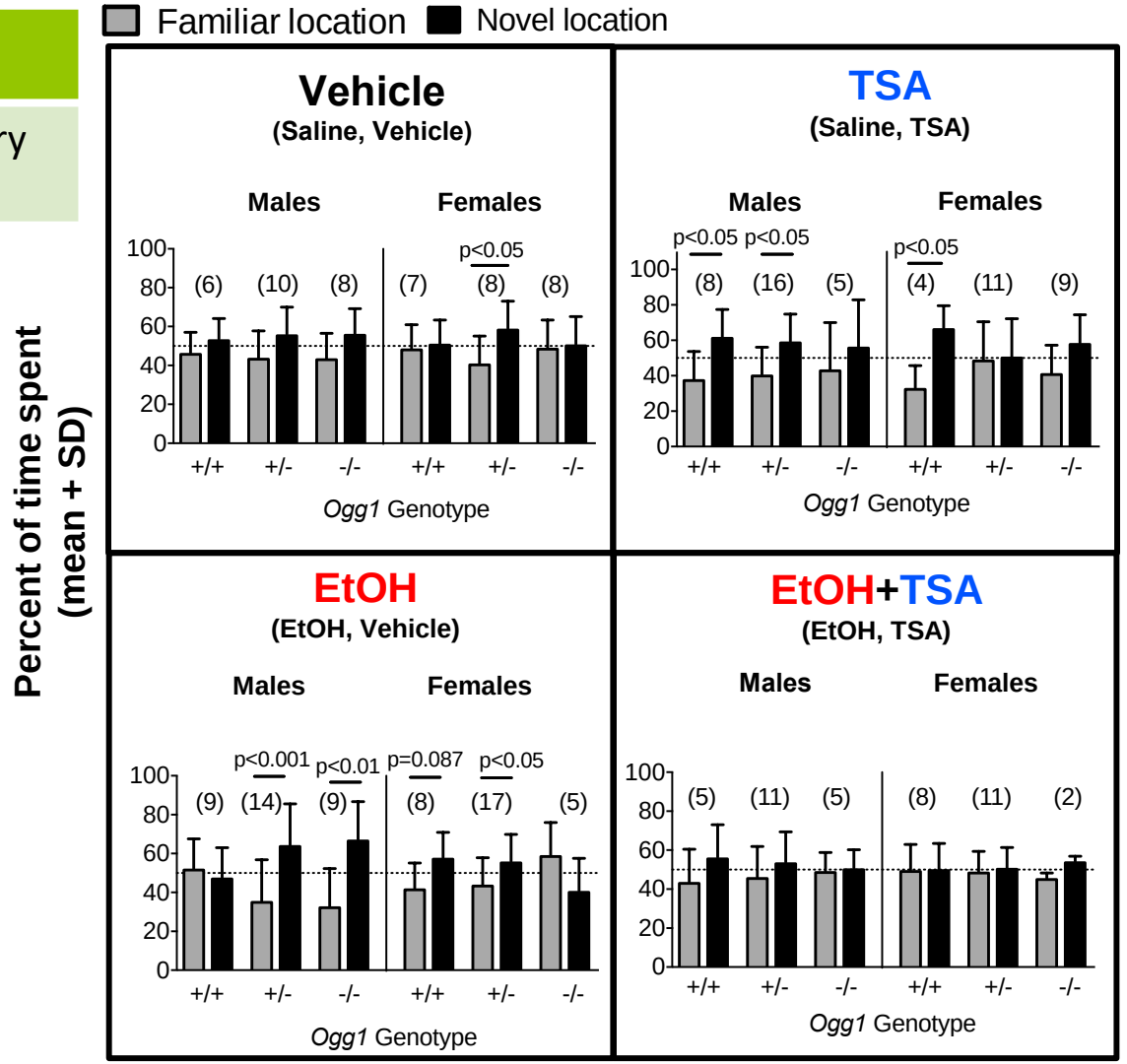

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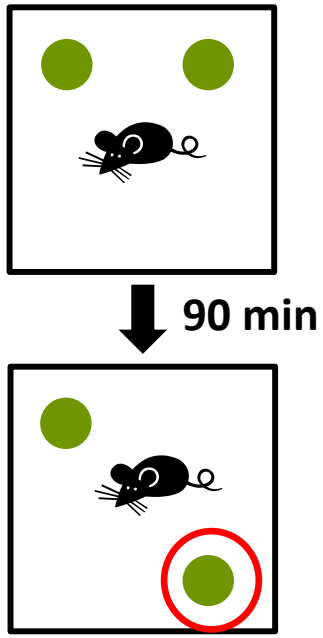

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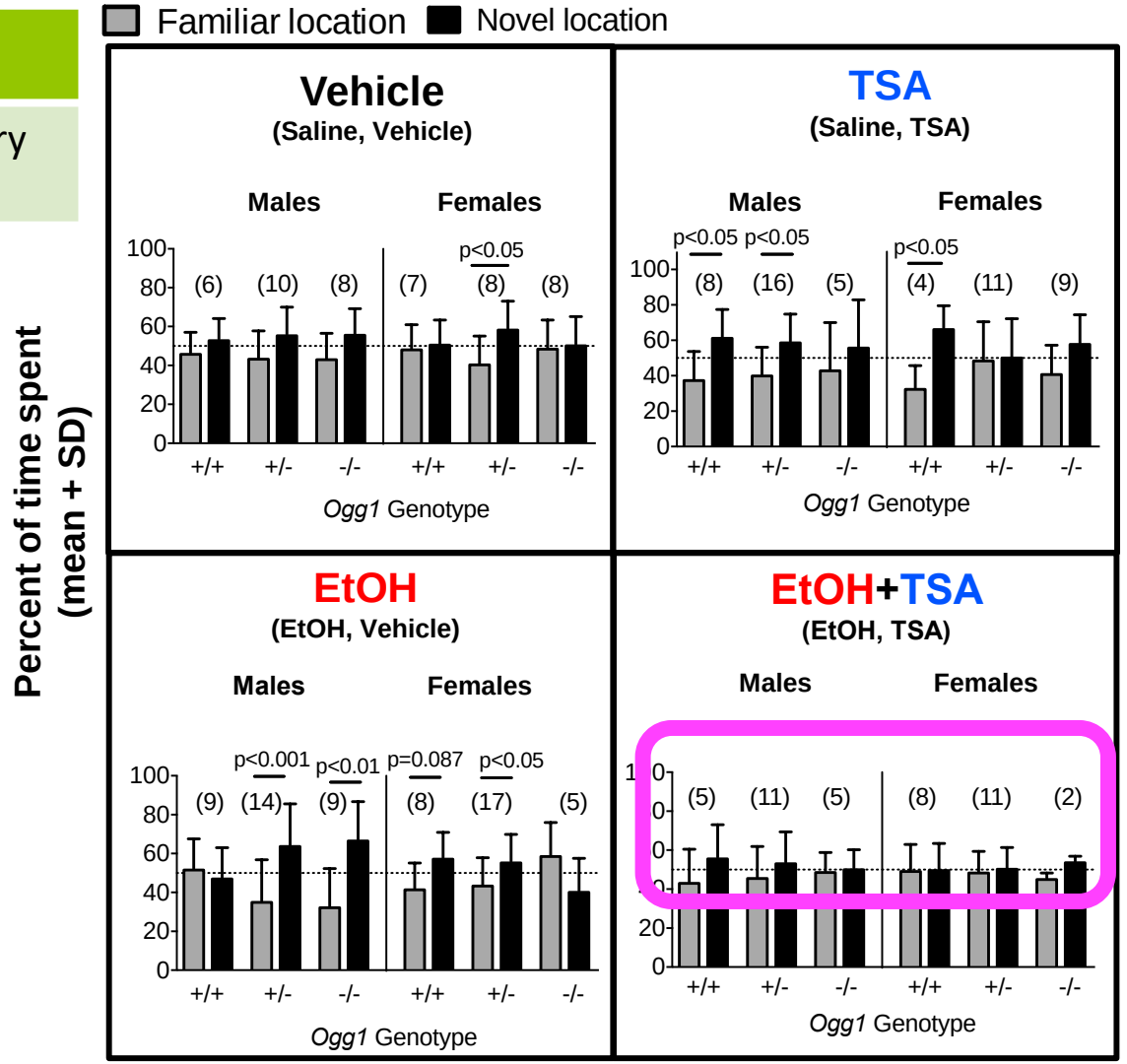

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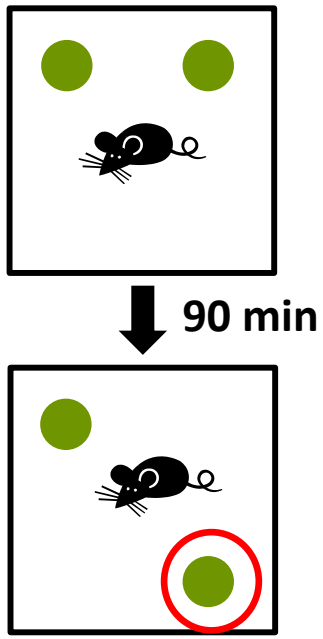

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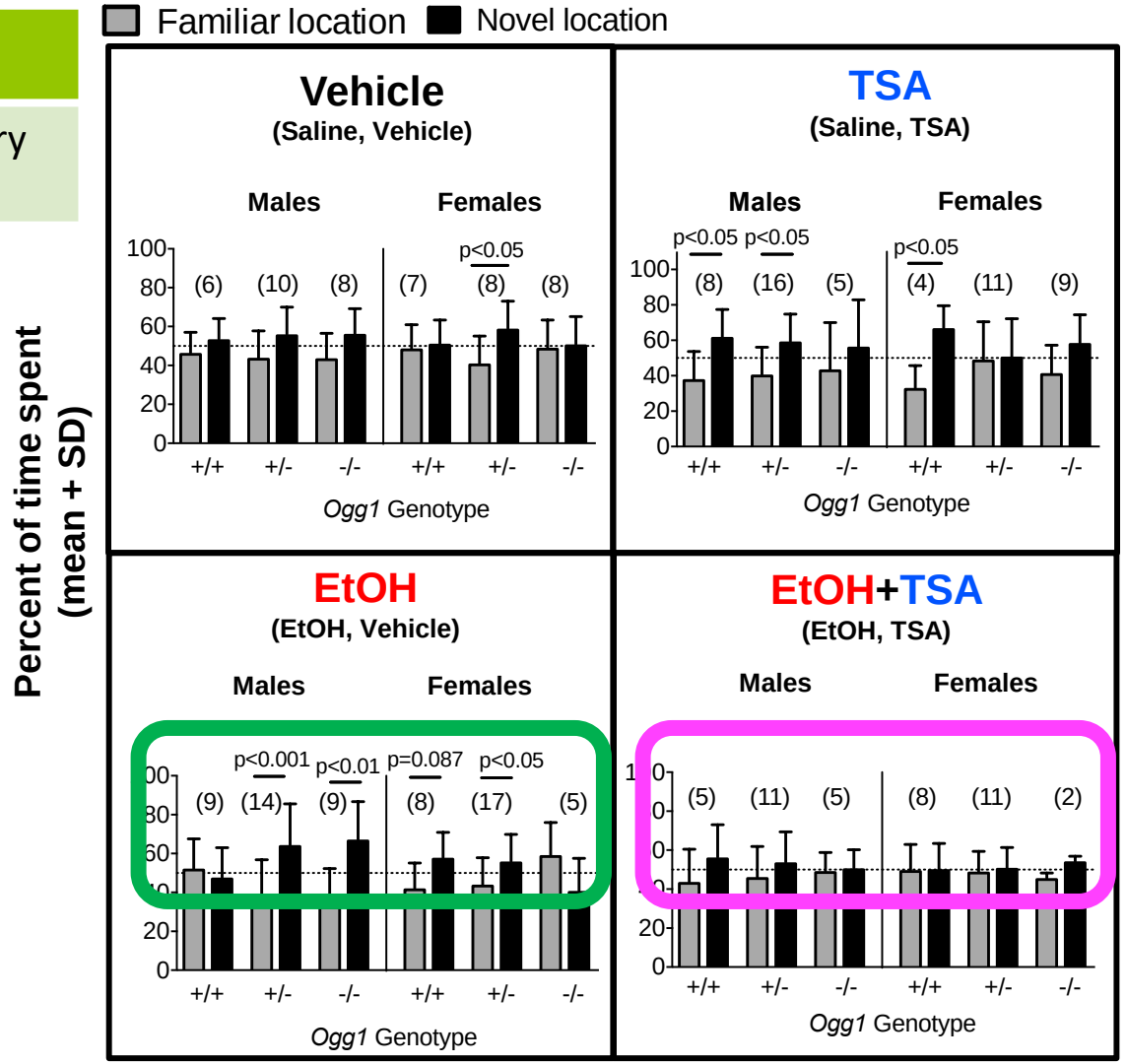
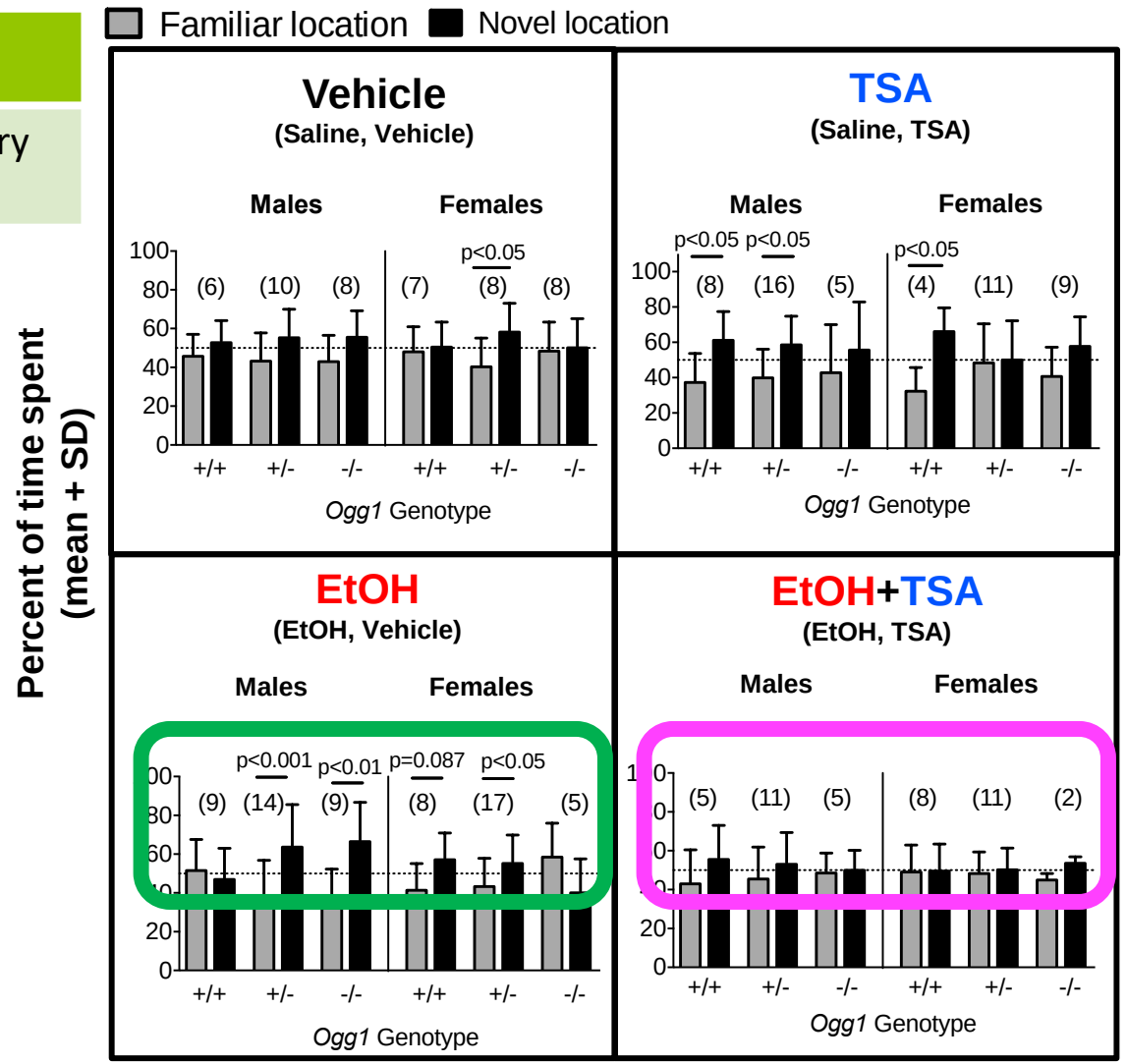


TSA reversed the effects of **EtOH**

Novel Location Recognition in *Ogg1* KO : Spatial Memory

Preliminary studies suggest:

May be possible to reverse some neurodevelopmental components of FASD by postnatal treatment with epigenetic modifiers



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In summary …

- **Physiological** pathways
- **Xenobiotics**

ROS formation

Reactive Oxygen Species
(**ROS**)
- H_2O_2
- Superoxide ($O_2^{\bullet -}$)
- Hydroxyl radical (•OH)

Essential processes:
- Energy production
- Signal transduction

Antioxidative Enzymes

DNA damage
(**8-oxoG**)

OGG1

DNA repair

Epigenetic changes

HDAC inhibitor
Trichostatin (**TSA**)

Developmental disorders:
- Birth defects
- **Neurodevelopmental disorders**

(**8-oxoG** = 8oxoguanine; **OGG1** = oxoguanine glycosylase 1; **HDAC** = histone deacetylase)

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- **Xenobiotics**

OGG1

ROS formation

Antioxidative Enzymes

DNA repair

Fetal balance determines risk

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"Take-home Messages"

- Embryonic/fetal **DNA damage** caused by reactive oxygen species (**ROS**) can initiate developmental disorders
- **Risk** is determined by the balance of **fetal pathways** for ROS formation vs. ROS detoxification and DNA repair
- **Physiological levels** of ROS formation can be pathogenic in biochemically predisposed fetuses
- **Fetal brain** is highly susceptible to ROS damage
- Many **xenobiotics** enhance ROS formation

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The End