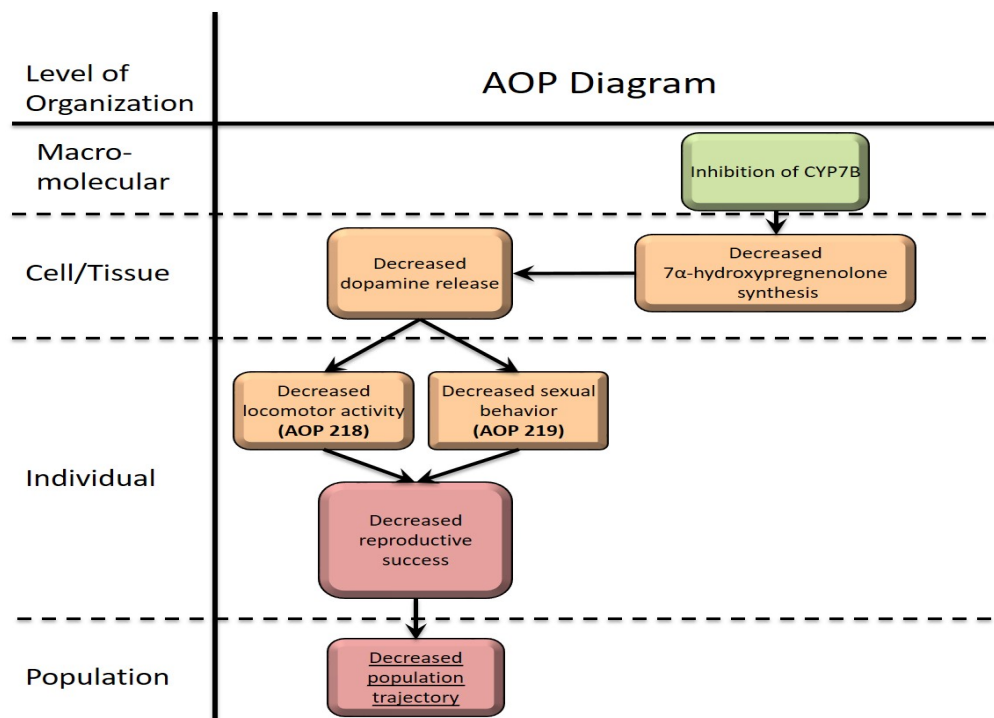


AOP 219: Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior

Short Title: Inhibition of CYP7B activity leads to decreased sexual behavior

Graphical Representation



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Status

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Abstract

This AOP details the linkage between CYP7B inhibition and decreased sexual behavior that adversely impacts reproductive success. CYP7B is expressed in the brain and catalyzes the conversion of pregnenolone to 7 α -hydroxypregnenolone, a neurosteroid that stimulates the release of dopamine in the telencephalon. When released through this pathway, dopamine binds D₂ receptor which is involved in induction of sexual behaviors, among other effects. Ketoconazole and other azole fungicides are potent inhibitor of cytochrome P450s, including CYP7B. They bind to the heme site of the enzyme preventing its catalytic activity. When exposed to one of these molecules, 7 α -hydroxypregnenolone synthesis decreases which, in turn, reduces dopamine release in the telencephalon and limits sexual behavior. Since sexual behaviors are closely associated to reproductive success, its inhibition negatively affects the fitness of animals.

7 α -hydroxypregnenolone was recently discovered and its function and regulation remain unclear. The few studies that focused on this neurosteroid and that were used for this AOP are based on *in vitro* and *in vivo* experiments quail and newt. Since the function of this neurosteroid differs in mammals, this AOP is only applicable to non-mammalian vertebrates. It is also limited to male.

Background

This AOP shares most of its key events with AOP 218, with the exception of *Locomotor activity, decreased* (Event 1389). Due to this difference, the domain of applicability of the two AOPs differs and limits their compatibility. For that reason, two similar AOPs with different domain of applicability were created.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	1386	CYP7B activity, inhibition (https://aopwiki.org/events/1386)	CYP7B activity, inhibition
2	KE	1387	7 α -hydroxypregnenolone synthesis in the brain, decreased (https://aopwiki.org/events/1387)	7 α -hydroxypregnenolone synthesis in the brain, decreased
3	KE	1388	Dopamine release in the brain, decreased (https://aopwiki.org/events/1388)	Dopamine release in the brain, decreased
4	KE	1390	Sexual behavior, decreased (https://aopwiki.org/events/1390)	Sexual behavior, decreased
5	KE	1141	Decreased, Reproductive Success (https://aopwiki.org/events/1141)	Decreased, Reproductive Success
6	AO	442	Decreased, Population trajectory (https://aopwiki.org/events/442)	Decreased, Population trajectory

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
CYP7B activity, inhibition (https://aopwiki.org/relationships/1493)	adjacent	7 α -hydroxypregnenolone synthesis in the brain, decreased		
7 α -hydroxypregnenolone synthesis in the brain, decreased (https://aopwiki.org/relationships/1496)	adjacent	Dopamine release in the brain, decreased		
Sexual behavior, decreased (https://aopwiki.org/relationships/1499)	adjacent	Decreased, Reproductive Success		
Decreased, Reproductive Success (https://aopwiki.org/relationships/1501)	adjacent	Decreased, Population trajectory		
Dopamine release in the brain, decreased (https://aopwiki.org/relationships/1502)	adjacent	Sexual behavior, decreased		

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
7 α -hydroxypregnenolone synthesis in the brain, decreased (https://aopwiki.org/relationships/1498)	non-adjacent	Sexual behavior, decreased		

Stressors

Name	Evidence
Ketoconazole	

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Japanese quail	Coturnix japonica		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=93934)
Cynops pyrrhogaster	Cynops pyrrhogaster		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8330)

Sex Applicability

Sex	Evidence
Male	

Taxons: This AOP is supported with evidence from studies conducted with newt and quail. Based on anticipated conservation of the biology associated with the KEs and KERs described, it is presumed to be applicable to all amphibian and bird.

Previous evidence suggest that this AOP is not applicable to mammal. All the key events of this AOP are described or are biologically plausible in mammal, but the relationship between them might differ, as suggested by Yau et al. (2006).

Sex: This AOP is applicable to male only.

Life Stage: This AOP applies to sexually mature animals since the endpoint is related to reproduction.

Essentiality of the Key Events

Few studies measured multiple key events of this AOP. For this reason, the evidence for essentiality of the key events is mainly indirect and provided by a series of antagonist/exogenous supplementation experiments. The animal models used for these investigations were newt and quail.

Key event	Essentiality	Rational
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<u>MIE</u> Inhibition of CYP7B	Moderate	<p>At present, no CYP7B knock-out experiments were conducted in species of interest. However, several indirect evidences linking CYP7B inhibition to a decreased locomotor activity suggest an important correlation between the two events.</p> <ul style="list-style-type: none"> • CYP7B is the only enzyme able to synthesize 7α-hydroxypregnenolone and this neurosteroid is strongly related to locomotor activity http://www.genome.jp/dbget-bin/www_bget?rn:R08943 (http://www.genome.jp/dbget-bin/www_bget?rn:R08943). • Inhibition of CYP7B with intracranial injection of ketoconazole decreased 7α-hydroxypregnenolone synthesis and decreased sexual behavior in newt and quail (Ogura et al., 2016, Toyoda et al., 2012). Ketoconazole is a non-specific inhibitor of cytochromes P450 activity known to bind to and inhibit CYP7B both <i>in vitro</i> and <i>in vivo</i>.
<u>KE1</u> 7 α -hydroxypregnenolone, decreased	Strong	<p>Direct evidences connecting this neurosteroid to sexual behavior were described.</p> <ul style="list-style-type: none"> • Intracerebroventricular injection of 7α-hydroxypregnenolone in male quail and newt induced spontaneous sexual behavior in a dose-dependent manner. The same treatment had no effect on female (Toyoda et al., 2012; Ogura et al., 2016).
<u>KE2</u> Dopamine release, decreased	Moderate	<p>There is strong evidence demonstrating the involvement of dopamine in sexual behavior among all vertebrates. However, only indirect evidence relates CYP7B inhibition to a decreased dopamine release. The rational is stronger for 7α-hydroxypregnenolone in relation to dopamine release, although this neurosteroid receptor remains to be identified.</p> <ul style="list-style-type: none"> • Sexual behavior was stimulated in male newt with intracerebroventricular injection of 7α-hydroxypregnenolone. Newt treated with a dopamine D2-like receptor antagonist (haloperidol or sulpiride) prior to receiving 7α-hydroxypregnenolone exhibited no increase in sexual behavior (Toyoda et al., 2012).
<u>KE3</u> Locomotor activity, decreased	Strong	<p>All the previous key events can decrease sexual behavior in male quail and newt.</p>

Weight of Evidence Summary

Biological plausibility

This AOP connects the CYP7B catalyzed synthesis on an important neurosteroid to a well characterized sequence of events. For instance, the involvement of dopamine in sexual behavior that in turn impacts on reproductive success is well described and undisputed (Melis et al., 1995; Hull et al., 2004). What is less characterized is the relation between 7 α -hydroxypregnenolone and dopamine release. Since the neurosteroid receptor has yet to be identified, no direct interaction between 7 α -hydroxypregnenolone and dopaminergic neuron has been demonstrated. It is thus possible that an intermediate event takes place in between to indirectly connect the neurosteroid to dopamine release.

In terms of structural plausibility, the brain expresses the steroidogenic enzymes required for pregnenolone synthesis, the main substrate of CYP7B. It also expresses CYP7B which synthesizes high concentration of 7 α -hydroxypregnenolone in the diencephalon. This region of the brain is populated by neurons projecting into the striatum which is known to express a high quantity of D₁- and D₂-like dopamine receptor and control motor activity (Orgen S. et al., 1986; Mezey S. et al., 2002; Callier S. et al., 2003).

Uncertainties or inconsistencies

At present, there are no inconsistencies reported in the literature, but some gaps remain to be filled.

The most important ones are 7 α -hydroxypregnenolone receptor localization and the connection between 7 α -hydroxypregnenolone and dopamine release discussed in the previous section.

In addition, mammalian CYP7B not only catalyzes the 7 α -hydroxylation of pregnenolone but also that of dehydroepiandrosterone (DHEA). Although no clear information reported this enzymatic reaction in the bird, it is plausible that CYP7B catalyzes the hydroxylation of DHEA. Thus, the phenotypic effect of CYP7B inhibition in the brain cannot be uniquely attributed to a depletion in 7 α -hydroxypregnenolone. Additionally, ketoconazole is known to inhibit a variety of CYPs, which suggest that animal exposed to it are likely to have several other enzymes inhibited. It is plausible that the impacts of ketoconazole are the result of multiple CYPs inhibition that all converge towards the same phenotype. These off target effects greatly limit the investigations on 7 α -hydroxypregnenolone since its concentration cannot be specifically decreased.

If a CYP7B knock-out in the brain was to be performed in an animal species, 7 α -hydroxyDHEA supplementation would be required to properly study 7 α -hydroxypregnenolone function.

Quantitative Consideration

This information is not available for the moment.

References

Appendix 1

List of MIEs in this AOP

Event: 1386: CYP7B activity, inhibition (<https://aopwiki.org/events/1386>)

Short Name: CYP7B activity, inhibition

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:218 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	MolecularInitiatingEvent
Aop:219 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	MolecularInitiatingEvent

Stressors

Name
Ketoconazole
Tebuconazole
Propiconazole
Tioconazole
Miconazole
Fluconazole
Voriconazole
Clotrimazole

Biological Context

Level of Biological Organization
Molecular

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

The binding of inhibitors to CYP7B is demonstrated *in vitro* with purified recombinant protein in presence of the inhibitor. Ligand-induced spectral changes is analyzed using spectrophotometric titration as a shift of the heme (Yantsevich et al., 2014).

Ketoconazole and other conazole are known to bind to CYPs preventing its enzymatic activity.

- CYP7B inhibitor (ketoconazole, 10^{-4} M) decreased the synthesis of 7α -hydroxypregnenolone
- CYP7B inhibitor (intracerebroventricular injection of ketoconazole) decreased the synthesis of 7α -hydroxypregnenolone in the male quail and newt brain, *in vivo* (Matsunaga et al., 2004; Rose et al., 1997; Tsutsui et al., 2008).
- The heme prosthetic group (catalytic site) of human recombinant CYP7B tightly bound to various imidazole- and triazole-based drugs in an *in vitro* spectrometric titration assay. The drugs with the highest affinities were the industrial pesticides tebuconazole (0.11 μ m), propiconazole (0.13 μ m) and the antifungal drugs tioconazole (0.15 μ m) and miconazole (0.23 μ m). Voriconazole and metyrapone (non-azole compound) also interacted with CYP7B (Yantsevich et al., 2014).

Ketoconazole

It is clearly demonstrated that ketoconazole directly inhibits CYP7B (Matsunaga et al., 2004). It is expected for the other members of the conazole family to have the same effect.

Some other azoles such as clotrimazole can also inhibit CYP7B activity (Liu et al., 2011; Rose et al., 1997).

Tebuconazole

In vitro, tebuconazole was shown to bind to the catalytic site of the human recombinant CYP7B and to inhibit its catalytic activity (Yantsevich et al., 2014).

Propiconazole

In vitro, propiconazole was shown to bind to the catalytic site of the human recombinant CYP7B and to inhibit its activity (Yantsevich et al., 2014).

Tioconazole

In vitro, tioconazole was shown to bind to the catalytic site of the human recombinant CYP7B and to inhibit its activity (Yantsevich et al., 2014).

Miconazole

In vitro, miconazole was shown to bind to the catalytic site of the human recombinant CYP7B and to inhibit its activity (Yantsevich et al., 2014).

Fluconazole

In vitro, fluconazole was shown to bind to the catalytic site of the human recombinant CYP7B and to inhibit its activity (Yantsevich et al., 2014).

Voriconazole

In vitro, voriconazole was shown to bind to the catalytic site of the human recombinant CYP7B and to inhibit its activity (Yantsevich et al., 2014).

Clotrimazole

Clotrimazoles can inhibit CYP7B activity (Liu et al., 2011; Rose et al., 1997).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Japanese quail	Coturnix japonica		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=93934)
Cynops pyrrhogaster	Cynops pyrrhogaster		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8330)
Oncorhynchus keta	Oncorhynchus keta		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8018)

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

CYP7B is known to be conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. <https://www.ncbi.nlm.nih.gov/homologene/3544> (<https://www.ncbi.nlm.nih.gov/homologene/3544>)

Key Event Description

Site of action:

CYP7B is expressed in different organs including liver, prostate and brain.

How does it work :

CYP7B is a member of the cytochrome P450 family of enzymes. It is involved in steroidogenic pathways as well as in the synthesis of bile acids. In the brain, it is involved in neurosteroids synthesis.

In the brain, the reactions catalyzed by CYP7B are :

- Probably in all vertebrates: Pregnenolone into 7 α -hydroxypregnenolone and its stereoisomer 7 β -hydroxypregnenolone (bird only) (R08943) (Matsunaga et al., 2004; Rose et al., 1997; Tsutsui et al., 2008)
- Proven in mouse and human: Dehydroepiandrosterone (DHEA) to 7 α -hydroxy-DHEA and its stereoisomer 7 β -hydroxy-DHEA (Martin et al., 2004; Weihua et al., 2002).

In the human and mouse liver, CYP7B is responsible for (Toll et al., 1994):

- 5-cholesten-3-beta, 25(S)-diol into Cholest-5-ene-3 beta-7 alpha, 25-thiol (R07209 R08723),
- Cholest-5-ene-3 beta, 26-diol into 7 alpha, 27-dihydroxycholesterol (R07372 R08724),
- 3 beta-hydroxy-5-cholestenoate into 3 beta, 7 alpha-dihydroxy-5-cholestenoate (R08727 R08728).
- It is expressed in the chicken liver and is probably involved in the same reactions (Handschin et al., 2005).

In the prostate:

- Proven for human and rat: Dehydroepiandrosterone (DHEA) to 7 α -hydroxy-DHEA and 7 β -hydroxy-DHEA (Martin et al., 2001; Martin et al., 2004).

Inhibitors prevent the metabolism of pregnenolone into 7-alpha-hydroxypregnenolone, thereby decreasing the concentration of the neurosteroid.

How it is Measured or Detected

In vitro

To measure CYP7B activity *in vitro*, different experiments based on HPLC and GS-MS analysis can be performed.

- An assay in liver microsome followed by HPLC analysis of the metabolites (Souidi et al., 2000).
- Labeled steroid conversion *in vitro* with cell or tissue extract in presence of NADPH followed by GS-MS analysis (Rose et al., 1997; Tsutsui et al., 2008).
- CYP7B can be cloned in bacteria to produce an active protein *in vitro*. In presence of adequate precursor and cofactors, the enzymatic activity of the protein can be measured and analyzed using HPLC.
- CYP7B can be transfected in a cell line unable to synthesize 7 α -hydroxypregnenolone in order to measure with HPLC the ability of the protein to catalyze the enzymatic reaction in presence of the appropriate substrate and cofactor (Tsutsui et al., 2008)

In vivo

Experiments may include knock-out of mice (followed by RNA, protein blotting and enzymatic activity to confirm knock-out) (Li-Hawkins et al., 2000) followed by the measurement of substrate and metabolites of CYP7B in plasma and tissues (Rose., 2001).

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List of Key Events in the AOP

Event: 1387: 7 α -hydroxypregnenolone synthesis in the brain, decreased (<https://aopwiki.org/events/1387>)

Short Name: 7 α -hydroxypregnenolone synthesis in the brain, decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:218 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	KeyEvent
Aop:219 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	KeyEvent

Stressors

Name
Ketoconazole

Biological Context

Level of Biological Organization
Cellular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

Term	Scientific Term	Evidence	Links
Japanese quail	Coturnix japonica	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=93934)
Cynops pyrrhogaster	Cynops pyrrhogaster	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8330)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
salmonid fish	salmonid fish	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=36500)

Life Stage Applicability

Life Stage	Evidence
During development and at adulthood	

Sex Applicability

Sex	Evidence
Unspecific	

The enzyme synthesizing 7 α -hydroxypregnenolone is known to be conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. <https://www.ncbi.nlm.nih.gov/homologene/3544> (<https://www.ncbi.nlm.nih.gov/homologene/3544>)

Key Event Description

7 α -hydroxypregnenolone is an active neurosteroid synthesized in the brain from pregnenolone via a reaction catalyzed by CYP7B (R08943). Pregnenolone can also be synthesized in most vertebrate brain by CYP11A from cholesterol (Tsutsui and Yamazaki, 1995; do Rego et al., 2016).

Compared to other brain regions of the male quail and newt, 7 α -hydroxypregnenolone concentration is higher in the diencephalon. In the brain of both salmon and newt, the peak concentrations are measured in the hypothalamus and optic tectum (Matsunaga et al., 2004; Tsutsui et al., 2008; Haraguchi et al., 2015).

7 α -hydroxypregnenolone synthesis in the brain is cyclic and driven by a different mechanism according to the specie.

- In male quail, a diurnal animal, it is inhibited by a melatonin-receptor mechanism after melatonin secretion from the pineal gland (Tsutsui et al., 2008).
- In male newt, a nocturnal animal, melatonin secretion stimulates its synthesis in the brain.
- Another regulating mechanism is observed in male newt where 7 α -hydroxypregnenolone concentration peaks during the breeding period in response to prolactin signal (Matsunaga et al., 2004).
- In salmon, 7 α -hydroxypregnenolone stays high during homing migration (Haraguchi et al., 2015). The endogenous factor regulating its synthesis has yet to be determined.

Thus, 7 α -hydroxypregnenolone synthesis is regulated by the circadian cycle and/or by seasonal factors such as breeding and migration.

How it is Measured or Detected

Detection and quantification of 7 α -hydroxypregnenolone can be performed using GC-MS and/or HPLC analysis.

In vitro

- Cell not expressing CYP7B can be transfected with CYP7B cDNA and incubated in presence of pregnenolone and NADPH. Concentration of 7 α -hydroxypregnenolone can be measured by HPLC analysis (Haraguchi et al., 2015).
- To distinguish 7 α - and 7 β -hydroxypregnenolone, HPLC analysis was performed (Tsutsui et al., 2008). Brain homogenates can be incubated in presence of pregnenolone and NADPH. Concentration of 7 α -hydroxypregnenolone can be measured by HPLC analysis Haraguchi et al., 2015).

In vivo

The extracted steroids derived from brain homogenates and plasma can be measured using GC-MS analysis (Tsutsui et al., 2008).

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Event: 1388: Dopamine release in the brain, decreased (<https://aopwiki.org/events/1388>)

Short Name: Dopamine release in the brain, decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:218 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	KeyEvent
Aop:219 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	KeyEvent

Biological Context

Level of Biological Organization
Tissue

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	High

Dopamine is used as a neurotransmitter in multicellular animals (Barron et al., 2010). Across a wide range of vertebrates, dopamine has an "activating" effect on behavior-switching and response selection, comparable to its effect in mammals.

Key Event Description

Dopamine is a monoamine, catecholaminergic neurotransmitter synthesized in the brain and the kidney from precursor L-DOPA (Carlsson et al., 1957). It is synthesized in neuron cells, stored in vesicles nearby the synaps, and is released into the synaptic cleft after excitation of the neuron. Once released, it can bind D₁-like or D₂-like G protein receptor which have different effects (Stoof and Kebabian, 1984; Vallender et al., 2010).

It is conserved among vertebrates and regulates neural activity, behavior and gene expression. The main impacts are related to voluntary movement, feeding, and reward.

In birds, fish, and other vertebrates, dopaminergic neurons located in mesencephalic region (VTA, SN) project to the telencephalon, a region of the brain rich in D₁ and D₂ receptors (Hara et al., 2007; Ball et al., 1995; Levens et al., 2000).

How it is Measured or Detected

In vitro

To measure the ability of a molecule to stimulate dopamine release, brain can be incubated in physiological saline in presence of a presumptive activator (e.g. 7 α -hydroxypregnenolone, a neurosteroid) and dopamine concentration in saline is measured by HPLC-ECD (Matsunaga et al., 2004).

In vivo

To measure the concentration of dopamine in the brain *in vivo*, freshly collected brain can be homogenized and dopamine concentration can be analyzed using HPLC-ECD (ECD-300, Eicom).

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Event: 1390: Sexual behavior, decreased (<https://aopwiki.org/events/1390>)

Short Name: Sexual behavior, decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:219 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	KeyEvent

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Japanese quail	Coturnix japonica		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=93934)
Cynops pyrrhogaster	Cynops pyrrhogaster		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8330)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	

Sex Applicability

Sex	Evidence
Male	

This key event can be applied to any animal having sexual reproduction. It does not apply to asexual animals.

Key Event Description

Sexual behavior in male bird is characterized by components such as crowing, strutting, and mounting, whereas the newt exhibits a tail-vibrating behavior (Hutchison, 1978). In both species, sexual behavior varies on a daily (photoperiod) and seasonal (breeding) basis. A decrease in sexual behavior is defined by a reduction in the frequency of these typical behaviors.

How it is Measured or Detected

Since sexual behavior varies along the day and the season, timing is an important component of the measurement. Light exposure, endocrine disruptors and season should all be considered in the protocol design in order to limit the bias in the measurement.

Sexual behavior in male is measured in presence of a sexually receptive female. To limit the risk of bias induced by differences in female receptivity, it is important to repeat the experiment later/the day after with a different female for each male (Halldin et al., 1999). In bird, the frequency of chasing, pecking, head grabbing, and mounting for a X minutes observation can be measured (Halldin et al., 1999; Ogura et al., 2016).

For newt, sexual behavior is characterized by a tail-vibrating behavior and can be measured by counting the frequency and incidence of this behavior during X minutes. Incidence and frequency are expressed as the percentage of animals exhibiting the behavior and the mean number of times the behavior was recorded per test animal over the test period, respectively (Toyoda et al., 1983).

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Event: 1141: Decreased, Reproductive Success (<https://aopwiki.org/events/1141>)

Short Name: Decreased, Reproductive Success

AOPs Including This Key Event

AOP219

AOP ID and Name	Event Type
Aop:203 - 5-hydroxytryptamine transporter inhibition leading to decreased reproductive success and population decline (https://aopwiki.org/aops/203)	AdverseOutcome
Aop:218 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	KeyEvent
Aop:219 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	KeyEvent

Biological Context

Level of Biological Organization
Individual

List of Adverse Outcomes in this AOP

Event: 442: Decreased, Population trajectory (<https://aopwiki.org/events/442>)

Short Name: Decreased, Population trajectory

Key Event Component

Process	Object	Action
population growth rate		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:16 - Acetylcholinesterase inhibition leading to acute mortality (https://aopwiki.org/aops/16)	AdverseOutcome
Aop:218 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	AdverseOutcome
Aop:219 - Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	AdverseOutcome

Biological Context

Level of Biological Organization
Population

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 1493: CYP7B activity, inhibition leads to 7 α -hydroxypregnenolone synthesis in the brain, decreased (<https://aopwiki.org/relationships/1493>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	adjacent	High	Moderate
Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Japanese quail	Coturnix japonica		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=93934)

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

The vertebrate brain expresses all the enzymes involved in the different steroidogenic pathways (do Rego and Vaudry, 2016; Tsutsui et al., 1999).

The physiological function of 7 α -hydroxypregnenolone is more understood in birds, newts, and rats than in human. However, the direct causal effect between CYP7B inhibition and the decrease in 7 α -hydroxyPREG was demonstrated in human, fish and other vertebrates (Haraguchi et al., 2015; Yantsevich et al., 2014; Yau et al., 2006).

Therefore, it is plausible that this KER is applicable to all vertebrates.

Key Event Relationship Description

Neurosteroids are steroids synthesized in the brain that interact with cell surface receptors or ligand-gated ion channels in order to modify the neuronal excitability (Paul and Purdy, 1992). They are involved in numerous biological functions including locomotor activity, memory, learning, sexually-dimorphic behaviors and anxiety.

Neurosteroids are synthesized from pregnenolone or its derivatives by different cytochromes P450. Among these CYPs is CYP7B hydroxylase which synthesizes the neurosteroid 7 α -hydroxypregnenolone. CYP7B is the only enzyme responsible for the synthesis of this neurosteroid. Therefore, its inhibition induces a decrease in 7 α -hydroxypregnenolone concentration in the brain.

The expression of CYP7B and the synthesis of its molecular product vary cyclically on a daily and/or seasonal basis. In male quail, a diurnal animal, CYP7B expression and 7 α -hydroxypregnenolone are inhibited by melatonin secretion, a hormone involved in circadian rhythm and sleep regulation. Oppositely, in a nocturnal animal such a newt, melatonin acts as an inducer of CYP7B expression and 7 α -hydroxypregnenolone synthesis. These results indicate that CYP7B expression and therefore 7 α -hydroxypregnenolone synthesis follow a circadian rhythm regulation.

In addition to this daily variation, CYP7B and its product are regulated by seasons in salmon and male newt where it peaks during homing migration (salmon) and breeding (newt) period (Haraguchi et al., 2009). It is plausible that the same seasonal variation occurs in avian.

Evidence Supporting this KER

Biological Plausibility

The vertebrate brain expresses all the enzymes involved in the different steroidogenic pathways, including CYP7B (review do Rego and Vaudry, 2016; Tsutsui and Yamazaki, 1995). These enzymes in the brain are known to convert cholesterol into pregnenolone, the precursor of 7 α -hydroxypregnenolone. Therefore, the brain possesses both the molecular precursor and the enzyme required to synthesize 7 α -hydroxypregnenolone. Since CYP7B is the only enzyme known to synthesize 7 α -hydroxypregnenolone, its inhibition is assumed to decrease 7 α -hydroxypregnenolone concentration in the brain.

In the quail brain, the precise localization of CYP7B protein was explored and the results were as followed: nucleus preopticus medialis (POM), the nucleus paraventricularis magnocellularis (PVN), the nucleus ventrodorsalis hypothalami (VMN), the nucleus dorsolateralis anterior thalami (DLA) and the nucleus lateralis anterior thalami (LA) (Tsutsui et al., 2008).

In the salmon, cells expressing CYP7B are mainly localized in the magnocellular preoptic nucleus, oculomotor nucleus, nucleus lateralis valvulae, and nucleus lateralis valvulae (Haraguchi et al., 2015).

In the newt brain, CYP7B cells are mainly localized in the anterior preoptic area, the magnocellular preoptic nucleus, and the tegmental area. It was also detected in the lateral and dorsal pallidum, the suprachiasmatic nucleus, the ventral hypothalamic nucleus, and the tectum mesencephali (Haraguchi et al., 2010).

Empirical Evidence

- CYP7B inhibitor (ketoconazole, 10^{-4} M) decreased the synthesis of 7 α -hydroxypregnenolone *in vitro* (Tsutsui et al., 2008; Matsunaga et al., 2008; Toyoda et al., 2012).
- CYP7B inhibitor (intracerebroventricular injection of ketoconazole, 5 μ g, from 5 AM to 6 AM) decreased the synthesis of 7 α -hydroxypregnenolone in the brain (quail) *in vivo* (Tsutsui et al., 2008).
- CYP7B activity is regulated (inhibited) by melatonin in male quail. When male quail brains were injected (intracerebroventricular) with a melatonin receptor antagonist (luzindole), the production of 7 α -hydroxypregnenolone significantly increased (Tsutsui et al., 2008). The opposite effect was observed on newt where melatonin stimulated 7 α -hydroxypregnenolone synthesis (Koyama et al., 2009).
- Similarly, orbital enucleation and pinealectomy performed on male quail, which abolished melatonin synthesis, induces a significant increase in 7 α -hydroxypregnenolone concentration (Tsutsui et al., 2008).

Uncertainties and Inconsistencies

References

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Relationship: 1496: 7 α -hydroxypregnenolone synthesis in the brain, decreased leads to Dopamine release in the brain, decreased (<https://aopwiki.org/relationships/1496>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	adjacent	Low	Low

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Cynops pyrrhogaster	Cynops pyrrhogaster		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8330)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	

Sex Applicability

Sex	Evidence
Male	Moderate

Key Event Relationship Description

7 α -hydroxypregnenolone is synthesized in the diencephalon and the rhombencephalon (newt only) and has a paracrine effect on dopaminergic neurons that project into the telencephalon including the striatum.

Evidence Supporting this KER

Biological Plausibility

7 α -hydroxypregnenolone cannot be directly related to dopamine release since it has no known receptor and the cells that synthesize it are not in direct contact with the dopaminergic neurons. However, it was shown that 7 α -hydroxypregnenolone release induces dopamine secretion in the brain.

Empirical Evidence

- 7 α -hydroxypregnenolone concentration in the brain of salmon prior to upstream migration was decreased using aminoglutethimide (AG) injection (CYP11A inhibitor) and lead to a decreased dopamine concentration in the telencephalon compared to control. This effect was rescued with intracerebroventricular injection of 7 α -hydroxypregnenolone. Simultaneous measurements of hypothalamic dopamine concentration showed an absence of variation after AG-injection or AG+7 PREG (Haraguchi et al., 2015)
- Tyrosine hydroxylase (TH) is a marker of dopamine neurons. Immunolabelling of CYP7B and TH revealed that these enzymes are expressed in two different cell populations in the salmon magnocellular preoptic nucleus and that they are in close proximity to each other (Haraguchi et al., 2015).
- 7 α -hydroxypregnenolone (1 ng) was injected in non-breeding male newt and several monoamines concentration were measured using HPLC-electrochemical detection. 7 α -hydroxypregnenolone increased dopamine concentration in the rostral brain region including striatum. No change was observed in the concentration of any other monoamine (Matsunaga et al., 2004).
- Newt brain incubated *in vitro* with 7 α -hydroxypregnenolone (0, 10⁻⁸, 10⁻⁷, or 10⁻⁶ M) induced a dose-dependent increase of dopamine concentration after 10 minutes (Matsunaga et al., 2004).

Uncertainties and Inconsistencies

Since the neurosteroid receptor has yet to be identified, no direct interaction between 7 α -hydroxypregnenolone and dopaminergic neuron has been demonstrated. It is thus possible that an intermediate event takes place in between to indirectly connect the neurosteroid to dopamine release.

References

AOP219

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Matsunaga, M., Ukena, K., Baulieu, E.E., and Tsutsui, K. (2004). 7alpha-Hydroxypregnenolone acts as a neuronal activator to stimulate locomotor activity of breeding newts by means of the dopaminergic system. *Proc Natl Acad Sci U S A* 101, 17282-17287.

Relationship: 1499: Sexual behavior, decreased leads to Decreased, Reproductive Success
(<https://aopwiki.org/relationships/1499>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	adjacent		

Relationship: 1501: Decreased, Reproductive Success leads to Decreased, Population trajectory
(<https://aopwiki.org/relationships/1501>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	adjacent		
Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	adjacent		

Relationship: 1502: Dopamine release in the brain, decreased leads to Sexual behavior, decreased
(<https://aopwiki.org/relationships/1502>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	adjacent		

List of Non Adjacent Key Event Relationships

Relationship: 1498: 7α-hydroxypregnenolone synthesis in the brain, decreased leads to Sexual behavior, decreased
(<https://aopwiki.org/relationships/1498>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	non-adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Japanese quail	Coturnix japonica		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=93934)
Cynops pyrrhogaster	Cynops pyrrhogaster		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8330)

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	

Sex Applicability

Sex	Evidence
Male	

Key Event Relationship Description

Gonadal steroid and prolactin are required to initiate a cascade of molecular events leading to sexual behavior. The cumulation of these reactions leads to the secretion of 7 α -hydroxypregnenolone in the brain, a neurosteroid that acts as a trigger on sexual behavior.

7 α -hydroxypregnenolone is synthesized by CYP7B in the telencephalon of both male and female. Its concentration fluctuates in the male brain according to season and light exposure, whereas it remains low in female. In agreement with the variation in 7 α -hydroxypregnenolone concentration is the variation in sexual behavior frequency in male. Indeed, it was previously noted that 7 α -hydroxypregnenolone peaked during the breeding period, increasing locomotor activity and frequency of tail-vibrating behavior (newt) or chasing, crowing, strutting, and mounting (bird).

Other components are required to induce sexual behavior since injection of 7 α -hydroxypregnenolone in sexually immature newt has no effect on this specific parameter. However, no sexual behavior can be elicited in absence of 7 α -hydroxypregnenolone.

Evidence Supporting this KER**Biological Plausibility**

It is known that 7 α -hydroxypregnenolone is secreted in the diencephalon. However, the fine-tuning mechanism of its regulation is still undetermined.

In newt, the neurosteroid secretion is driven by melatonin and prolactin, an important circadian and breeding regulator, respectively (Matsunaga et al., 2004; Tsutsui et al., 2008). Prolactin is synthesized in the hypophysis and is one of the molecules required to initiate the cascade of molecular events leading to sexual behavior in newt (Kikuyama et al., 1980). In male newt, it induces a dose-dependent activation of sexual behavior. Immunofluorescence experiments conducted on newt brain sections revealed the presence of prolactin receptors on neurons expressing CYP7B, which could explain the direct correlation between prolactin and 7 α -hydroxypregnenolone secretion in relation to sexual behavior.

Quail are highly sensitive to light exposure and their behavior mostly relies on circadian rhythm. Melatonin secretion, high during the night, is known to inhibit CYP7B activity in male brain, which, in turn, decreases 7 α -hydroxypregnenolone concentration. Following the same pattern, sexual behavior in male quail is high during the day and significantly lower at night.

A decrease in sexual behavior can be induced by steroid hormones deregulation since their involvement in the regulation of sexual behavior is prominent. Indeed, castration of male bird induces a decrease/loss of reproductive behavior, which can be rescued by a testosterone therapy (Adkins and Adler, 1972). The same effect can be induced in bird by transferring them from a 16-hr.-light 8-hr.-dark cycle to an 8-hr.-light 16-hr.-dark cycle, which demonstrates the photoperiodic regulation of sexual behavior in bird (Sach, 1967).

Empirical Evidence

- In male newt, 7 α -hydroxypregnenolone concentration in the brain peaks in April and October, similarly to their sexual behavior (Haraguchi et al., 2010).
- Intracranial injection of 7 α -hydroxypregnenolone in male newt increased tail-vibration (the newt sexual behavior) incidence and frequency in a dose-dependent manner (Toyoda et al., 2012). In male quail, sexual behavior (frequency of chasing, pecking, head grabbing, mounting, and cloacal contact movement) was also increased in a dose-dependent manner following 7 α -hydroxypregnenolone injection (Ogura et al., 2016).
- Ketoconazole, a CYP7B inhibitor, prevented 7 α -hydroxypregnenolone synthesis *in vivo* and abolished sexual behavior when given to male quail and newt. The behavior in ketoconazole-treated animals was successfully retrieved with 7 α -hydroxypregnenolone supplementation or

after ketoconazole treatment was stopped (Toyoda et al., 2012; Ogura et al., 2016).

A decrease in sexual behavior can be induced by steroid hormones deregulation since their involvement in the regulation of sexual behavior is prominent. Indeed, castration of male bird induces a decrease/loss of reproductive behavior, which can be rescued by a testosterone therapy (Adkins and Adler, 1972). The same effect can be induced in bird by transferring them from a 16-hr.-light 8-hr.-dark cycle to an 8-hr.-light 16-hr.-dark cycle, which demonstrates the photoperiodic regulation of sexual behavior in bird (Sach, 1967).

Uncertainties and Inconsistencies

Courtship and sexual behavior is due to the synergistic effect of multiple hormones (Iwata et al., 2000). 7 α -hydroxypregnenolone is one of them and it was shown to be essential for sexual behavior, but it is not sufficient in itself to trigger sexual behavior in absence of prolactin (secreted in sexually mature animals during the breeding season).

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