AOP ID and Title:

SNAPSHOT

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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

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Author status	OECD status	OECD project	SAAOP 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 7α-hydroxypregnenolone, a neurosteroid that stimulates the release of dopamine in the telencephalon. When released through this pathway, dopamine binds D_2 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, 7α-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.

7α-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

Stressors

Name	Evidence
Ketoconazole	

Molecular Initiating Event

Title	Short name
CYP7B activity, inhibition (https://aopwiki.org/events/1386)	CYP7B activity, inhibition

Title	Short name

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
218: Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	MolecularInitiatingEvent
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

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).

Evidence for Perturbation of this Molecular Initiating Event by Stressor

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 thightly 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 µm), propiconazole (0.13 µm) and the antifungal drugs tioconazole (0.15 µm) and miconazole (0.23 µm). Voriconazole and metyrapone (non-azole compound) also interacted with CYP7B (Yantsevich et al., 2014).

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

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)

How this Key Event Works

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

References

Dulos, J., van der Vleuten, M.A., Kavelaars, A., Heijnen, C.J., and Boots, A.M. (2005). CYP7B expression and activity in fibroblast-like synoviocytes from patients with rheumatoid arthritis: regulation by proinflammatory cytokines. Arthritis Rheum 52, 770-778.

Handschin C., Gnerre C., Fraser DJ., Martinez-Jimenez C., Jover R., Mever UA., (2005) Species-specific mechanisms for cholesterol 7α-hydroxylase (CYP7A1) regulation by drugs and bile acids, Archives of Biochemistry and Biophysics, Vol 434-1, pp75-85

Haraguchi, S., Koyama, T., Hasunuma, I., Okuyama, S., Ubuka, T., Kikuyama, S., Do Rego, J.L., Vaudry, H., and Tsutsui, K. (2012). Acute stress increases the synthesis of 7alpha-hydroxypregnenolone, a new key neurosteroid stimulating locomotor activity, through corticosterone action in newts. Endocrinology 153, 794-805.

Haraguchi, S., Yamamoto, Y., Suzuki, Y., Hyung Chang, J., Koyama, T., Sato, M., Mita, M., Ueda, H., and Tsutsui, K. (2015). 7alpha-Hydroxypregnenolone, a key neuronal modulator of locomotion, stimulates upstream migration by means of the dopaminergic system in salmon. Sci Rep 5, 12546.

Li-Hawkins, J., Lund, E.G., Turley, S.D., and Russell, D.W. (2000). Disruption of the oxysterol 7alpha-hydroxylase gene in mice. J Biol Chem 275, 16536-16542.

Liu, C., Yang, X.V., Wu, J., Kuei, C., Mani, N.S., Zhang, L., Yu, J., Sutton, S.W., Qin, N., Banie, H., et al. (2011). Oxysterols direct B-cell migration through EBI2. Nature 475, 519-523.

Martin, C., Bean, R., Rose, K., Habib, F., and Seckl, J. (2001). cyp7b1 catalyses the 7alpha-hydroxylation of dehydroepiandrosterone and 25-hydroxycholesterol in rat prostate. Biochem J 355, 509-515.

Martin, C., Ross, M., Chapman, K.E., Andrew, R., Bollina, P., Seckl, J.R., and Habib, F.K. (2004). CYP7B generates a selective estrogen receptor beta agonist in human prostate. J Clin Endocrinol Metab 89, 2928-2935.

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.

Rose, K., Allan, A., Gauldie, S., Stapleton, G., Dobbie, L., Dott, K., Martin, C., Wang, L., Hedlund, E., Seckl, J.R., et al. (2001). Neurosteroid hydroxylase CYP7B: vivid reporter activity in dentate gyrus of gene-targeted mice and abolition of a widespread pathway of steroid and oxysterol hydroxylation. J Biol Chem 276, 23937-23944.

Rose, K.A., Stapleton, G., Dott, K., Kieny, M.P., Best, R., Schwarz, M., Russell, D.W., Bjorkhem, I., Seckl, J., and Lathe, R. (1997). Cyp7b, a novel brain cytochrome P450, catalyzes the synthesis of neurosteroids 7alpha-hydroxy dehydroepiandrosterone and 7alpha-hydroxy pregnenolone. Proc Natl Acad Sci U S A 94, 4925-4930.

Souidi, M., Parquet, M., Dubrac, S., Audas, O., Becue, T., and Lutton, C. (2000). Assay of microsomal oxysterol 7alpha-hydroxylase activity in the hamster liver by a sensitive method: in vitro modulation by oxysterols. Biochim Biophys Acta 1487, 74-81.

Toll, A., Wikvall, K., Sudjana-Sugiaman, E., Kondo, K.H., and Bjorkhem, I. (1994). 7 alpha hydroxylation of 25-hydroxycholesterol in liver microsomes. Evidence that the enzyme involved is different from cholesterol 7 alpha-hydroxylase. Eur J Biochem 224, 309-316.

Tsutsui, K., Inoue, K., Miyabara, H., Suzuki, S., Ogura, Y., and Haraguchi, S. (2008). 7Alpha-hydroxypregnenolone mediates melatonin action underlying diurnal locomotor rhythms. J Neurosci 28, 2158-2167.

Weihua, Z., Lathe, R., Warner, M., and Gustafsson, J.A. (2002). An endocrine pathway in the prostate, ERbeta, AR, 5alpha-androstane-3beta,17beta-diol, and CYP7B1, regulates prostate growth. Proc Natl Acad Sci U S A 99, 13589-13594.

Yantsevich, A.V., Dichenko, Y.V., Mackenzie, F., Mukha, D.V., Baranovsky, A.V., Gilep, A.A., Usanov, S.A., and Strushkevich, N.V. (2014). Human steroid and oxysterol 7alpha-hydroxylase CYP7B1: substrate specificity, azole binding and misfolding of clinically relevant mutants. FEBS J 281, 1700-1713.

Key Events

Title	Short name
7α-hydroxypregnenolone synthesis in the brain, decreased (https://aopwiki.org/events/1387)	7α-hydroxypregnenolone synthesis in the brain, decreased
Dopamine release in the brain, decreased (https://aopwiki.org/events/1388)	Dopamine release in the brain, decreased
Sexual behavior, decreased (https://aopwiki.org/events/1390)	Sexual behavior, decreased
Decreased, Reproductive Success (https://aopwiki.org/events/1141)	Decreased, Reproductive Success

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

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

AOPs Including This Key Event

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

Stressors

Name
Ketoconazole

Biological Organization

Level of Biological Organization
Cellular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

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

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
salmonid fish	salmonid fish	Strong	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 7α-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)

How this Key Event Works

7α-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, 7α-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).

7α-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 7α-hydroxypregnenolone concentration peaks during the breeding period in response to prolactin signal (Matsunaga et al., 2004).
- In salmon, 7α-hydroxypregnenolone stays high during homing migration (Haraguchi et al., 2015). The endogenous factor regulating its synthesis has yet to be determined.

Thus, 7α-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 7α-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 7α-hydroxypregnenolone can be measured by HPLC analysis (Haraguchi et al., 2015).
- To distinguish 7α- and 7β-hydroxypregnenolone, HPLC analysis was performed (Tsutsui et al., 2008). Brain homogenates can be incubated in presence of pregnenolone and NADPH. Concentration of 7α-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 a;., 2008).

References

Haraguchi, S., Koyama, T., Hasunuma, I., Vaudry, H., and Tsutsui, K. (2010). Prolactin increases the synthesis of 7alpha-hydroxypregnenolone, a key factor for induction of locomotor activity, in breeding male Newts. Endocrinology 151, 2211-2222.

Haraguchi, S., Yamamoto, Y., Suzuki, Y., Hyung Chang, J., Koyama, T., Sato, M., Mita, M., Ueda, H., and Tsutsui, K. (2015). 7alpha-Hydroxypregnenolone, a key neuronal modulator of locomotion, stimulates upstream migration by means of the dopaminergic system in salmon. Sci Rep 5, 12546.

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.

Petkam, R., Renaud, R.L., Freitas, A.M., Canario, A.V., Raeside, J.I., Kime, D.E., and Leatherland, J.F. (2003). In vitro metabolism of pregnenolone to 7alpha-hydroxypregnenolone by rainbow trout embryos. Gen Comp Endocrinol 131, 241-249.

Tsutsui, K., Inoue, K., Miyabara, H., Suzuki, S., Ogura, Y., and Haraguchi, S. (2008). 7Alpha-hydroxypregnenolone mediates melatonin action underlying diurnal locomotor rhythms. J Neurosci 28, 2158-2167.

Tsutsui, K., and Yamazaki, T. (1995). Avian neurosteroids. I. Pregnenolone biosynthesis in the quail brain. Brain Res 678, 1-9.

Yau, J.L., Noble, J., Graham, M., and Seckl, J.R. (2006). Central administration of a cytochrome P450-7B product 7 alpha-hydroxypregnenolone improves spatial memory retention in cognitively impaired aged rats. J Neurosci 26, 11034-11040.

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
218: Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	KeyEvent
219: Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	KeyEvent

Biological Organization

Level of Biological Organization
Tissue

Evidence Supporting Applicability of this Event

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	Strong

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.

How this Key Event Works

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 vesicules nearby the synaps, and is released into the synaptic cleft after excitation of the neuron. Once released, it can bind D_1-like or D_2-like G protein receptor which have different effects (Stoof and Kebabia, 1984; Vallender et al., 2010).

It is conserved among vertabrates 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 D_1 and D_2 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. 7α-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).

References

Barron, A.B., Sovik, E., and Cornish, J.L. (2010). The roles of dopamine and related compounds in reward-seeking behavior across animal phyla. Front Behav Neurosci 4, 163.

Carlsson, A., Lindqvist, M., and Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180, 1200.

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.

Stoof, J.C., and Kebabian, J.W. (1984). Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci 35, 2281-2296.

Vallender, E.J., Xie, Z., Westmoreland, S.V., and Miller, G.M. (2010). Functional evolution of the trace amine associated receptors in mammals and the loss of TAAR1 in dogs. BMC Evol Biol 10, 51.

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
219: Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	KeyEvent

Biological Organization

Level of Biological Organization
Individual

Evidence Supporting Applicability of this Event

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.

How this Key Event Works

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).

References

Adkins, E. K. and N. T. Adler. 1972. Hormonal control of behavior in the Japanese quail. J. Comp. Physiol. Psychol.81:27-36.

Halldin, K., Berg, C., Brandt, I., and Brunstrom, B. (1999). Sexual behavior in Japanese quail as a test end point for endocrine disruption: effects of in ovo exposure to ethinylestradiol and diethylstilbestrol. Environ Health Perspect 107, 861-866.

Hutchison, R.E. (1978). Hormonal differentiation of sexual behavior in Japanese quail. Horm Behav 11, 363-387.

Ogura, Y., Haraguchi, S., Nagino, K., Ishikawa, K., Fukahori, Y., and Tsutsui, K. (2016). 7alpha-Hydroxypregnenolone regulates diurnal changes in sexual behavior of male quail. Gen Comp Endocrinol 227, 130-135.

Sachs, B.D. (1967). Photoperiodic control of the cloacal gland of the Japanese quail. Science 157, 201-203.

Toyoda, F., Ito, M., Tanaka, S., and Kikuyama, S. (1993). Hormonal induction of male courtship behavior in the Japanese newt, Cynops pyrrhogaster. Horm Behav 27, 511-522.

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

Short Name: Decreased, Reproductive Success

AOPs Including This Key Event

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

Biological Organization

Level of Biological Organization
Individual

Adverse Outcomes

Title	Short name
Decreased, Population trajectory (https://aopwiki.org/events/442)	Decreased, Population trajectory

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

Short Name: Decreased, Population trajectory

AOPs Including This Key Event

AOP ID and Name	Event Type
16: Acetylcholinesterase inhibition leading to acute mortality (https://aopwiki.org/aops/16)	AdverseOutcome
218: Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	AdverseOutcome
219: Inhibition of CYP7B activity leads to decreased reproductive success via decreased sexual behavior (https://aopwiki.org/aops/219)	AdverseOutcome
21: AhR activation leading to early life stage mortality (https://aopwiki.org/aops/21)	AdverseOutcome

Biological Organization

Level of Biological Organization
Population

Scientific evidence supporting the linkages in the AOP

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
CYP7B activity, inhibition	directly leads to	7α-hydroxypregnenolone synthesis in the brain, decreased		
7α-hydroxypregnenolone synthesis in the brain, decreased	indirectly leads to	Sexual behavior, decreased		
7α-hydroxypregnenolone synthesis in the brain, decreased	directly leads to	Dopamine release in the brain, decreased		
Sexual behavior, decreased	directly leads to	Decreased, Reproductive Success		
Decreased, Reproductive Success	directly leads to	Decreased, Population trajectory		
Dopamine release in the brain, decreased	directly leads to	Sexual behavior, decreased		

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

AOPs Referencing Relationship

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

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 7α-hydroxypregnenolone is more understood in birds, newts, and rats than in human. However, the direct causal effect between CYP7B inhibition and the decrease in 7α-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.

How Does This Key Event Relationship Work

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 7α-hydroxypregnenolone. CYP7B is the only enzyme responsible for the synthesis of this neurosteroid. Therefore, its inhibition induces a decrease in 7α-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 7α-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 7α-hydroxypregnenolone synthesis. These results indicate that CYP7B expression and therefore 7α-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.

Weight of Evidence

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 7α-hydroxypregnenolone Therefore, the brain possesses both the molecular precursor and the enzyme required to synthesized 7α-hydroxypregnenolone. Since CYP7B is the only enzyme known to synthesize 7α-hydroxypregnenolone, its inhibition is assumed to decrease 7α-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 ventrodedialis 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 pallium, the suprachiasmatic nucleus, the ventral hypothalamic nucleus, and the tectum mesencephali (Haraguchi et al., 2010).

Empirical Support for Linkage

- CYP7B inhibitor (ketoconazole, 10^{-4} M) decreased the synthesis of 7α-hydroxypregnenolone *in vitro* (Tsutsui et al., 2008; Matsunaga et al., 2008; Toyoda et al., 2012).
- CYP7B inhibitor (intracerebroventricular injection of ketoconazole, 5 µg, from 5 AM to 6 AM) decreased the synthesis of 7α-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 7α-hydroxypregnenolone significantly increased (Tsutsui et al., 2008). The opposite effect was observed on newt where melatonin stimulated 7α-hydroxypregnenolone synthesis (Koyama et al., 2009).
- Similarly, orbital enucleation and pinealectomy performed on male quail, which abolished melatonin synthesis, induces a significant increase in 7α-hydroxypregnenolone concentration (Tsutsui et al., 2008).

Uncertainties or Inconsistencies

Quantitative Understanding of the Linkage

Little is known about the dose-response of CYP7B inhibitors. This information is lacking in the literature.

One experiment conducted *in vitro* with mouse recombinant enzyme showed that 1 µM clotriconazole significantly decreased CYP7B activity while 10 µM abolished it (Rose et al., 1997). However, the subtrate used in the experiment was DHEA meaning that the measured product was 7α-hydroxyDHEA. It is highly plausible that the same result would have been observed with pregnenolone as a substrate for CYP7B and 7α-hydroxypregnenolone as a product.

References

do Rego, J.L., and Vaudry, H. (2016). Comparative aspects of neurosteroidogenesis: From fish to mammals. Gen Comp Endocrinol 227, 120-129.

Haraguchi, S., Matsunaga, M., Koyama, T., Do Rego, J.L., and Tsutsui, K. (2009). Seasonal changes in the synthesis of the neurosteroid 7alpha-hydroxypregnenolone stimulating locomotor activity in newts. Ann N Y Acad Sci 1163, 410-413.

Haraguchi, S., Koyama, T., Hasunuma, I., Vaudry, H., and Tsutsui, K. (2010). Prolactin increases the synthesis of 7α-hydroxypregnenolone, a key factor for induction of locomotor activity, in breeding male newts. *Endocrinology* 151, 2211–2222.

Haraguchi, S., Yamamoto, Y., Suzuki, Y., Hyung Chang, J., Koyama, T., Sato, M., Mita, M., Ueda, H., and Tsutsui, K. (2015). 7alpha-Hydroxypregnenolone, a key neuronal modulator of locomotion, stimulates upstream migration by means of the dopaminergic system in salmon. Sci Rep 5, 12546.

Koyama, T., Haraguchi, S., Vaudry, H., and Tsutsui, K. (2009). Diurnal changes in the synthesis of the neurosteroid 7alpha-hydroxypregnenolone stimulating locomotor activity in newts. Ann N Y Acad Sci 1163, 444-447.

Paul, S.M., and Purdy, R.H. (1992). Neuroactive steroids. FASEB J 6, 2311-2322.

Tsutsui, K., Inoue, K., Miyabara, H., Suzuki, S., Ogura, Y., and Haraguchi, S. (2008). 7Alpha-hydroxypregnenolone mediates melatonin action underlying diurnal locomotor rhythms. J Neurosci 28, 2158-2167.

Tsutsui, K., Ukena, K., Takase, M., Kohchi, C., and Lea, R.W. (1999). Neurosteroid biosynthesis in vertebrate brains. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 124, 121-129.

Tsutsui, K., and Yamazaki, T. (1995). Avian neurosteroids. I. Pregnenolone biosynthesis in the quail brain. Brain Res 678, 1-9.

Yantsevich, A.V., Dichenko, Y.V., Mackenzie, F., Mukha, D.V., Baranovsky, A.V., Gilep, A.A., Usanov, S.A., and Strushkevich, N.V. (2014). Human steroid and oxysterol 7alpha-hydroxylase CYP7B1: substrate specificity, azole binding and misfolding of clinically relevant mutants. FEBS J 281, 1700-1713.

Yau, J.L., Noble, J., Graham, M., and Seckl, J.R. (2006). Central administration of a cytochrome P450-7B product 7 alpha-hydroxypregnenolone improves spatial memory retention in cognitively impaired aged rats. J Neurosci 26, 11034-11040.

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

AOPs Referencing Relationship

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

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	

How Does This Key Event Relationship Work

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 7α-hydroxypregnenolone in the brain, a neurosteroid that acts as a trigger on sexual behavior.

7α-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 7α-hydroxypregnenolone concentration is the variation in sexual behavior frequency in male. Indeed, it was previously noted that 7α-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 7α-hydroxypregnenolone in sexually immature newt has no effect on this specific parameter. However, no sexual behavior can be elicited in absence of 7α-hydroxypregnenolone.

Weight of Evidence

Biological Plausibility

It is known that 7α-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 synthetized 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 7α-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 7α-hydroxypregnenolone concentration. Following the same pattern, sexual behavior in male quail is high during the day and significiantly 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 Support for Linkage

- In male newt, 7α-hydroxypregnenolone concentration in the brain peaks in April and October, similarly to their sexual behavior (Haraguchi et al., 2010).
- Intracranial injection of 7α-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 7α-hydroxypregnenolone injection (Ogura et al., 2016).
- Ketoconazole, a CYP7B inhibitor, prevented 7α-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 7α-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 or Inconsistencies

Courtship and sexual behavior is due to the synergistic effect of multiple hormones (Iwata et al., 2000). 7α-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).

References

Kikuyama, S., Yamamoto, K., Seki T. (1980). Prolactin and its role in growth, metamorphosis and reproduction in amphibians, Gunma Symp. Endocrinol., 17, 3–13

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.

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

AOPs Referencing Relationship

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

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

How Does This Key Event Relationship Work

7α-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.

Weight of Evidence

Biological Plausibility

7α-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 7α-hydroxypregnenolone release induces dopamine secretion in the brain.

Empirical Support for Linkage

- 7α-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 7α-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).
- 7α-hydroxypregnenolone (1 ng) was injected in non-breeding male newt and several monoamines concentration were measured using HPLC-electrochemical detection. 7α-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 7α-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 or Inconsistencies
Since the neurosteroid receptor has yet to be identified, no direct interaction between 7α-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.

Quantitative Understanding of the Linkage
One study specifically performed a dose-reponse experiment for 7α-hydroxypregnenolone in relation to dopamine concentration.

- Newt brain incubated with 7α-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).

References

Haraguchi, S., Yamamoto, Y., Suzuki, Y., Hyung Chang, J., Koyama, T., Sato, M., Mita, M., Ueda, H., and Tsutsui, K. (2015). 7alpha-Hydroxypregnenolone, a key neuronal modulator of locomotion, stimulates upstream migration by means of the dopaminergic system in salmon. Sci Rep 5, 12546.

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.

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

AOPs Referencing Relationship

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

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

AOPs Referencing Relationship

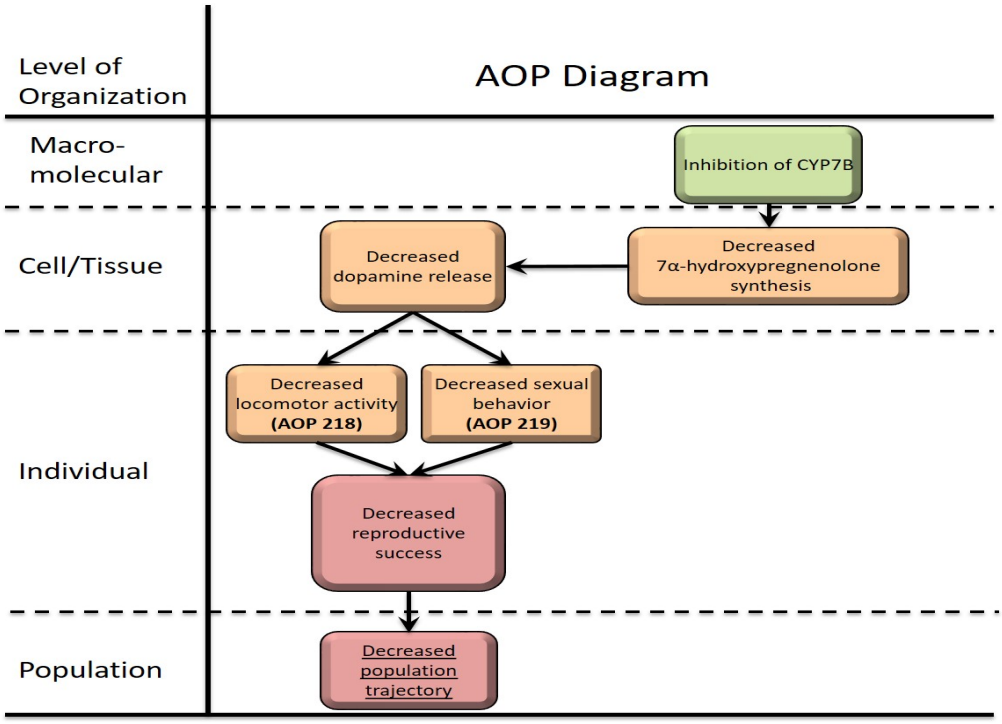
AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity (https://aopwiki.org/aops/218)	**directly leads to**		
Inhibition of CYP7B activity leads to decreased reproductive success via decreased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

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

AOPs Referencing Relationship

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

Graphical Representation



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
MIE Inhibition of CYP7B	Moderate	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. • 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 *in vitro* and *in vivo*.
KE1 7α-hydroxypregnenolone, decreased	Strong	Direct evidences connecting this neurosteroid to sexual behavior were described. • 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).
KE2 Dopamine release, decreased	Moderate	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. • 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).
KE3 Locomotor activity, decreased	Strong	All the previous key events can decrease sexual behavior in male quail and newt.

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 7α-hydroxypregnenolone and dopamine release. Since the neurosteroid receptor has yet to be identified, no direct interaction between 7α-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 7α-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 D_1- and D_2-like dopamine receptor and control motor activity (Orgen S. et al., 1986; Mezey S. et al., 2002; Callier S. et al., 2003).

Uncertainties or inconsistencie

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

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

In addition, mammalian CYP7B not only catalyzes the 7α-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 7α-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 7α-hydroxypregnenolone since its concentration cannot be specifically decreased.

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

Quantitative Consideration

This information is not available for the moment.

References