AOP ID and Title:

SNAPSHOT

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AOP 218: Inhibition of CYP7B activity leads to decreased reproductive success via decreased locomotor activity

Short Title: Inhibition of CYP7B leads to decreased locomotor activity

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Abstract

This AOP details the downstream events of CYP7B inhibition leading to a decreased locomotor activity 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 locomotor activity induction. 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. Exposure to one of these molecules induces a decrease in 7α-hydroxypregnenolone synthesis which, in turn, reduces dopamine release in the telencephalon and limits locomotor activity. Since locomotor activity is closely associated to reproductive success through courtship enhancement (newt), expansion of territory (bird) and homing migration (salmon), 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 in salmon, quail and newt. At present, it is believed that the function of this neurosteroid differs in mammals, which suggest that this AOP is only applicable to non-mammalian vertebrates. Also, the sex applicability of the AOP varies according to species.

Background

The stressor identified for this AOP is used as fungicide both in the field for crop protection and in animal against fungus infection. Because it can inhibit various cytochrome P450 enzymes activity, a family of enzymes involved in a plethora of pathways including steroidogenesis, it has the potential to induce many different side effects for animal exposed indirectly through the environment or directly through medical treatment. This AOP targets one of these side effects.

Summary of the AOP

Stressors

Name	Evidence
Ketoconazole	Strong

Ketoconazole

Conazole is a class of fungicide that inhibits CYP51 14α-lanosterol demethylase activity in yeats and moults, thereby preventing ergosterol synthesis (Hof et al., 2006). In animals, conazoles are known to be less specific than in fungi since they can interfere with various cytochromes P450 activity. For instance, it is clearly demonstrated that ketoconazole directly inhibits CYP7B activity which induces a decrease in 7α-hydroxypregnenolone (Matsunaga et al., 2004, Tsutsui et al., 2008; Toyoda et al., 2012; Ogura et al., 2016).

Molecular Initiating Event

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

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).

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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
Locomotor activity, decreased (https://aopwiki.org/events/1389)	Locomotor activity, 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)
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).

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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).

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1389: Locomotor activity, decreased (https://aopwiki.org/events/1389)

Short Name: Locomotor activity, 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

Biological Organization

Level of Biological Organization
Individual

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)

Sex Applicability

Sex	Evidence
Mixed	Strong

Measurement of locomotor activity can be performed on any motile animal.

How this Key Event Works

Vertebrate move for a variety of reasons including reproduction, search for food or suitable microhabitat, and escape predator. In birds, newt, and other vertebrates, locomotor activity is cyclic and follows the circadian and/or seasonal rhythm (Saper et al., 2005; Binkley et al., 1971; Chabot and Menaker, 1992).

- Locomotor activity is elevated in quail under daylight and decreases at night, following a circadian cycle. It was shown in bird that locomotor activity was mainly related to maintenance of territory (Wada, 1981; Watson, 1970).
- In newt, locomotor activity is high during breeding season and night time (Nagai et al., 1998).
- In salmon, the maximum locomotor activity is observed during homing migration where fishes swim against the water flow (Gowans et al., 2003).

How it is Measured or Detected

Locomotor activity is a measurement of distance per unit of time. Experiment design should take into account the normal seasonal and daily variation of locomotor activity.

To measure locomotor activity, animals can be placed individually in a water-filled aquarium (newts) marked with parallel lines to define sectors. Quantification of total number of lines crossed during a certain amount of time is then measured (Lowry et al., 2001; Moore et al., 1984).

Birds can be put in a soundproof box with a telemetry system implanted to calculate their total distance during the experiment (or in a box with wire-mesh floor and ceilings and photobeams activated when the animal break the beam (Levens et al., 2001; Tsutsui et al., 2008).

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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	Strong	Moderate
CYP7B activity, inhibition	indirectly leads to	Locomotor activity, decreased	Weak	Weak
7α-hydroxypregnenolone synthesis in the brain, decreased	indirectly leads to	Locomotor activity, decreased	Strong	Moderate

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
7α-hydroxypregnenolone synthesis in the brain, decreased	directly leads to	Dopamine release in the brain, decreased	Weak	Weak
Dopamine release in the brain, decreased	directly leads to	Locomotor activity, decreased	Strong	Moderate
Locomotor activity, decreased	directly leads to	Decreased, Reproductive Success		
Decreased, Reproductive Success	directly leads to	Decreased, Population trajectory		

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.

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CYP7B activity, inhibition leads to Locomotor activity, decreased (https://aopwiki.org/relationships/1494)

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)	**indirectly leads to**	**Weak**	**Weak**

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	

CYP7B is expressed in the mammalian brain where it synthesizes, among others, 7α-hydroxypregnenolone. However, it governs spatial memory and learning rather than locomotor activity. Thus, although CYP7B and 7α-hydroxypregnenolone are present in the brain of vertebrates, there functions are different in mammals and non-mammals.

How Does This Key Event Relationship Work

CYP7B is expressed in the mammalian brain where it catalyzes synthesis of 7α-hydroxypregnenolone, among other neurosteroids. However, it governs spatial memory and learning rather than locomotor activity. Thus, although CYP7B and 7α-hydroxypregnenolone are present in the brain of vertebrates, there functions are different in mammals and non-mammals.

The importance of CYP7B neurosteroid synthesis is sex dependent in bird and newt. In these species, only male locomotor activity is influenced by CYP7B expression. However, both male and female are affected by CYP7B activity in salmon.

Weight of Evidence

Biological Plausibility

The relationship between CYP7B and locomotor activity is clearly established is quail, newt and salmon. However, the regulation of CYP7B differs in these species.

In diurnal bird such as quail, melatonin secretion during nighttime inhibits CYP7B activity which is reflected by the decreased locomotor activity. Under daylight condition, melatonin secretion is abolished which induces an upregulation of CYP7B and an increase in locomotor activity (Tsutsui et al., 2008).

Oppositely, newt is a nocturnal animal and melatonin secretion acts as an inducer of CYP7B activity. Consequently, CYP7B activity is elevated at night and drives locomotor activity (Koyama et al., 2009).

CYP7B activity is also dependent on the peptide hormone prolactin secreted by the adenohypophysis, at least in male newt. Prolactin is a neuropeptide which secretion varies according to season. In newt, breeding season is characterized by an elevation of locomotor activity which correlates with a peak in brain prolactin concentration.

It is plausible that prolactin induces the same increase in locomotor activity in salmon during homing migration. During this period, both prolactin and CYP7B (7α-hydroxypregnenolone) are known to peak (Haraguchi et al., 2015; Onuma et al., 2010).

Empirical Support for Linkage

- Conazoles are known to cross the blood brain barrier.
- The activity of CYP7B is inhibited by conazoles (Matsunaga et al., 2004; Tsutsui et al., 2008).
- Exposure to ketoconazole inhibited CYP7B activity (decreased 7α-hydroxypregnenolone concentration) and decreased locomotor activity in male quail and newt.
- Depletion of CYP7B substrate (pregnenolone) with intracranial injection of aminoglutethimide (CYPscc inhibitor) decreased locomotor activity in salmon (Haraguchi et al., 2015).
- Penguins treated with voriconazole (6 µg/ml of blood) became lethargic and weak. The side effects dissipated or resolved with discontinuation or dose reduction of voriconazole (Hyatt et al., 2015).

Uncertainties or Inconsistencies

Conazoles are known to inhibit a variety of CYPs. Thus, when an animal is exposed to a chemical of this family, multiple enzymatic targets are likely to be affected. It is plausible that the impacts of the exposure are the result of multiple CYPs inhibition that all converge toward the same phenotype.

Quantitative Understanding of the Linkage

No information is available at this moment.

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7α-hydroxypregnenolone synthesis in the brain, decreased leads to Locomotor activity, decreased (https://aopwiki.org/relationships/1495)

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)	**indirectly leads to**	**Strong**	**Moderate**

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)

Term	Scientific Term	Evidence	Links
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
Adult, reproductively mature	Strong

Sex Applicability

Sex	Evidence
Male	

How Does This Key Event Relationship Work

The presence of 7α-hydroxypregnenolone in the brain is associated with locomotor activity in the salmon and in the male bird and newt. 7α-hydroxypregnenolone is a neurosteroid synthesized from pregnenolone by CYP7B in vertebrates including bird, newt, and fish. When 7α-hydroxypregnenolone concentration increases in the brain (endogenous or exogenous), these animals become active. Oppositely, decreased synthesis of 7α-hydroxypregnenolone limits locomotor activity (Matsunaga et al., 2004).

The importance of 7α-hydroxypregnenolone synthesis is sex dependent in bird and newt. In these species, only male locomotor activity is influenced by the neurosteroid (Matsunaga et al., 2004, Tsutsui et al., 2008). However, both male and female are affected by 7α-hydroxypregnenolone in salmon (haraguchi et al., 2015).

It was known before that locomotor activity in vertebrates fluctuated over a circadian and/or seasonal cycle, although the full mechanism was elusive (Saper et al., 2005). The discovery of 7α-hydroxypregnenolone activity in the brain allowed a better understanding of the locomotor activity regulation in the context of cyclic variations of the environment.

Weight of Evidence

Biological Plausibility

The relationship between 7α-hydroxypregnenolone and locomotor activity is clearly established in quail, newt and salmon. However, the regulation of its synthesis differs in these species.

In diurnal bird such as quail, melatonin secretion during nighttime inhibits 7α-hydroxypregnenolone synthesis which is reflected by the decreased locomotor activity. Under daylight condition, melatonin secretion is abolished which induces an increase in 7α-hydroxypregnenolone and stimulates locomotor activity (Tsutsui et al., 2008).

Oppositely, newt is a nocturnal animal and melatonin secretion acts as an inducer of 7α-hydroxypregnenolone synthesis. Consequently, 7α-hydroxypregnenolone is elevated at night and drives locomotor activity (Koyama et al., 2009).

7α-hydroxypregnenolone concentration is also dependent on the peptide hormone prolactin secreted by the adenohypophysis, at least in male newt. Prolactin is a neuropeptide which secretion varies according to season. In newt, breeding season is characterized by an elevation of locomotor activity which correlates with a peak in brain prolactin concentration.

It is plausible that prolactin induces the same increase in locomotor activity in salmon during homing migration. During this period, both prolactin and CYP7B (7α-hydroxypregnenolone) are known to peak (Haraguchi et al., 2015; Onuma et al., 2010).

Empirical Support for Linkage

- Intracranial injection of 7α-hydroxypregnenolone induced a significant increase in salmon, male quail and newt locomotor activity. The same injection had no effect on female quail and newt (Haraguchi et al., 2015).
- Intracranial injection of ketoconazole, an inhibitor of 7α-hydroxypregnenolone synthesis, in male quail and newt decreases locomotor activity. The same injection had no effect on female quail and newt (Matsunaga et al., 2004, Tsutsui et al., 2008).
- Intracranial delivery of melatonin, an inhibitor of 7α-hydroxypregnenolone synthesis, decreases locomotor activity in male quail (Tsutsui et al., 2008).
- The concentration of 7α-hydroxypregnenolone in the male quail diencephalon is high between 7 AM and 1 PM and peaks at 11 AM. The locomotor activity follows the same pattern. However, the concentration of 7α-hydroxypregnenolone in the female brain is constantly low which correlates with their low locomotor activity.
- Decreased 7α-hydroxypregnenolone in the salmon brain induced by aminoglutethimide (an inhibitor of CYP11A which induces a depletion of pregnenolone and a concurrent decline in 7α-hydroxypregnenolone concentration) abolishes salmon homing migration (Haraguchi et al., 2015).

Uncertainties or Inconsistencies

No inconsistency was reported so far.

Quantitative Understanding of the Linkage

- 7α-hydroxypregnenolone injected in the quail brain (0, 10, or 100 ng) induced a dose-dependent increase of locomotor activity (Tsutsui et al., 2008).
- The same experiment was conducted in the newt using 0.1, 0.5, or 1 ng. A dose-dependent increase of locomotor activity was observed

(Matsunaga et al., 2004).
- The same experiment was conducted in the chicken using 0.10, or 200 ng. A dose-dependent increase of locomotor activity was observed (Hatori et al., 2011).

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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).

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Dopamine release in the brain, decreased leads to Locomotor activity, decreased (https://aopwiki.org/relationships/1497)

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**

Locomotor activity, decreased leads to Decreased, Reproductive Success (https://aopwiki.org/relationships/1500)

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**		

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

A decrease in locomotor activity can be detrimental for the animal since it can limit exploration of territory, search for mating partner, and food consumption. It can also increase vulnerability to predation. Thus, a decrease in locomotor activity can have multiple effects that synergetically contribute to decreasing reproductive success.

Weight of Evidence

Biological Plausibility

Locomotor performance measured in the laboratory has frequently been used as a surrogate for fitness in animals (Bennett and Huey, 1990). In an environment with easily accessible food, the impact of a decreased locomotor activity are minimal. However, in a hostile environment that requires extensive foraging, insufficient locomotor activity can limit food intake and induce energetic deficit which, in turn, affects the energy available for reproduction. Similarly, a decreased locomotor activity is likely to limit the ability to escape predation and, consequently, to impair reproduction.

In a context of high competition between males for sexually-matured females, a decreased locomotor activity can limit the reproductive success.

Empirical Support for Linkage

In nature, locomotion, feeding and mate searching are interrelated behaviors.

- In a behavioral experiment, it was concluded that locomotor activity was correlated with the chemo-investigative behavior of nose tapping, a behavior used in both foraging and mate searching in the plethodontid salamanders (Schubert et al., 2006).
- It is predicted that suppression of locomotor activity by an acute stressor likely incurs costs to foraging and reproduction in salamender (Desmognathus ochrophaeus) (Ricciardella et al., 2010).
- In lizards, male behaviour (including social interactions and general locomotion) had a positive correlation that explained 81% of fertilization success. More active males sired offspring from more clutches (R2=0.9, $F_{1,7}=56.12$; $P=0.002$). This correlation could be the result of an increased probability of encountering receptive female and thus reproductive success when male are active and traverse their territory (Keogh et al., 2012).
- The same observation was made in bird and newt. Indeed, increased locomotory activity in breeding male is believed to contribute to the rapid encounter of the male with a sexually mature female (Jones et al., 2001; Tsutsui et al., 2013).
- Lizards exposed to pesticides had decreased fitness caused by a decrease in locomotor performance. This sublethal effect is believed to decrease individual's ability to avoid predators, capture prey, and defend territories (DuRant et al., 2007).

Quantitative Understanding of the Linkage

It is reasonnable to believe that all mobile animals using sexual reproduction could experience a decline in reproductive success following a decreased locomotor activity.

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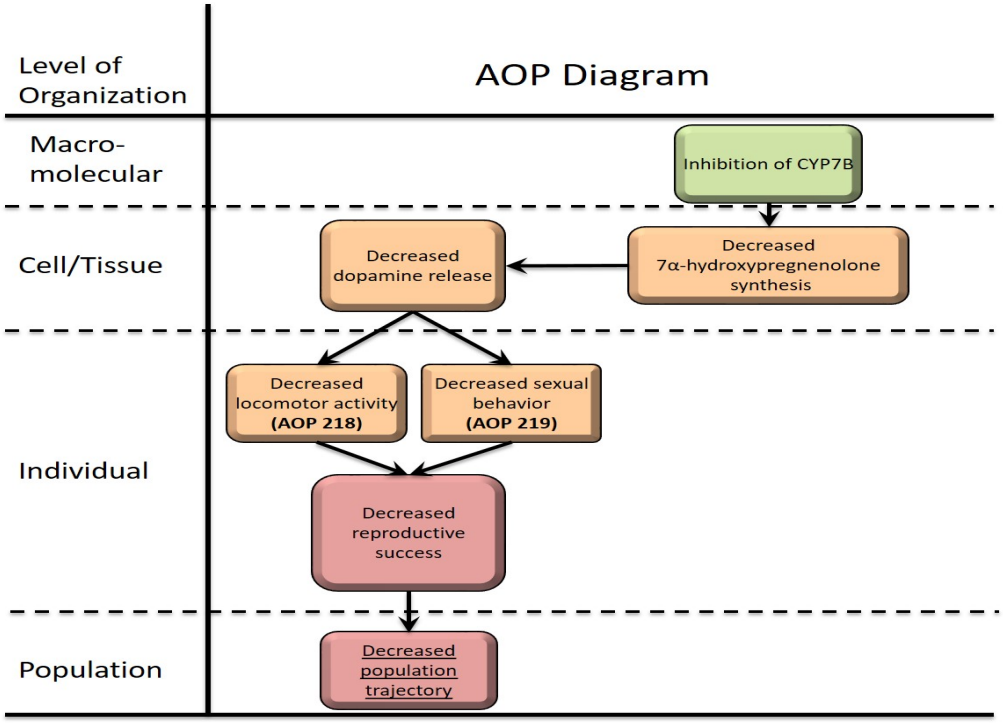
Decreased, Reproductive Success leads to Decreased, Population trajectory (https://aopwiki.org/relationships/1501)

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**		

Graphical Representation



Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult, reproductively mature	Strong

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	Strong

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

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: The sex applicability of this AOP is species-specific. Female quail and newt are insensitive to this MIE in regard to locomotor activity whereas male are highly sensitive. In salmon, both male and female exhibit a decreased locomotor activity with induction of the MIE.

Life Stage: This AOP applies to sexually mature animals since the endpoint is related to reproduction. However, all the key events except “reproductive success, decreased” (event 1141) and and the adverse outcome (event 442) are known to occur in juveniles which suggest that an AOP connecting CYP7B inhibition and decreased locomotor activity in juvenile to an endpoint not sexually-oriented could be built.

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, quail, and salmon.

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 prevented locomotor activity in newt and quail (Tsutsui et al., 2008, 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*. ◦ In salmon, decreased locomotor activity was observed following a depletion of CYP7B substrate (pregnenolone) with intracranial injection of aminoglutethimide, an inhibitor of cytochrome P450 ssc (Haraguchi et al., 2015). ◦ Penguins exposed orally to voriconazole, an azole molecule with the same effects as ketoconazole on CYPs activity were lethargic and weak. The side effects dissipated or resolved with discontinuation or dose reduction of voriconazole (Hyatt et al., 2015).
KE1 7α-hydroxypregnenolone, decreased	Strong	Numerous direct evidences connecting this neurosteroid to locomotor activity were described. ◦ Intracerebroventricular injection of 7α-hydroxypregnenolone in male chick, salmon, quail, and newt induced spontaneous locomotor activity in a dose-dependent manner. The same treatment had no effect on female. The experiments were conducted during the season (salmon, newt) or the time of the day (chick, quail) with the lowest endogenous locomotor activity (Matsunaga et al., 2004; Tsutsui et al., 2008; Hatori et al., 2011; Toyoda et al., 2012; Haraguchi et al., 2015). ◦ Salmon treated with aminoglutethimide to deplete pregnenolone concentration in the brain exhibited increased locomotor activity following intracerebroventricular injection of 7α-hydroxypregnenolone (Haraguchi et al., 2015).

KE2 Dopamine release, decreased	Moderate	There is strong evidence demonstrating the involvement of dopamine in locomotor activity 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. ○ Locomotor activity 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 locomotor activity. ○ Inhibition of 7α-hydroxypregnenolone synthesis with aminoglutethimide (pregnenolone depletion) decreases dopamine concentration in the salmon brain. Supplementation with physiological concentration of 7α-hydroxypregnenolone restored dopamine concentration to normal (Haraguchi et al., 2015).
KE3 Locomotor activity, decreased	Strong	All the previous key events can decrease locomotor activity in salmon and male quail, chicken, 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 locomotor activity that in turn impacts on reproductive success is well described and undisputed (Bardo M.T. et al., 1999; Levens et al., 2000). 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 inconsistencies

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