
Animal Models of Attention-Deficit Hyperkinetic Disorder (ADHD)

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1 Introduction

ADHD (attention-deficit hyperkinetic disorder) is a common childhood-onset neuropsychiatric disorder characterized by attention deficit, hyperactivity, and impulsiveness, which employs to both children and adults. ADHD is a behavioral disorder and believed to affect 1 in 20 children in the USA, 3–7% of school-aged children, and persists in 30–50% of adults. ADHD was first illustrated 100 years ago as a childhood disorder, which affects boys more often than girls. In the 1930s and 1940s, children with the ADHD were called “brain damaged” or “brain injured” because their behaviors were identical with persons having brain damage. In 1960s, “hyperactive” or “hyperkinetic” became the term of choice for describing ADHD children, and in the year 1980s, the disorder was renamed to “attention deficit disorder” (ADD). This disorder was mainly divided into two types: with hyperactivity (ADD + H) and without hyperactivity (ADD-H). The first example of a disorder that appears to be related to ADHD was given by Sir Alexander Crichton in 1798. Children diagnosed with ADHD without exhibiting any signs of hyperactivity had eight of the inattention and impulsivity characteristics. In 1955, FDA

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approved methylphenidate (Ritalin) for the treatment of hyperactivity disorder. In 1987, DSM-III-R (revised) changed “attention deficit disorder” to “attention deficit hyperactivity disorder” (ADHD) and it accounts 14 symptoms, in which any eight symptoms were sufficient for diagnosis of ADHD.

1.1 Clinical Signs and Symptoms

Inattention, hyperactivity, and impulsivity are the major symptoms of ADHD. According to DSM-IV and ICD-10, inattention problems include: failure to give close attention to work or other activities like difficulty in sustaining attention in tasks, failure to finish schoolwork, chores, duties in the workplace, and non-willingness to engage in tasks that require sustained mental effort. Hyperactivity problems include: often fidgets or squirms on seat, often leaving seat when expected to sit, excessive inappropriate running or climbing, persistent over activity not modulated by request or context. Impulsiveness includes: often blurts out answers before the question is complete, often fails to wait in groups, games, or queues, and often talks excessively without response to social appropriateness. In adults, inattention and impulsivity seem to be more reliable markers of ADHD than hyperactivity. Daydreaming, poor concentration in work, and forgetfulness recommend inattention, although frustration with tasks, easy loss of temper, and impatience recommend inattention and impulsivity. Children and adults with ADHD frequently have comorbid antisocial, drug abuse, mood, anxiety, or learning disorders. Children with ADHD may be quite intelligent, but their lack of attention frequently results in low grades and problems in schooling. Children with ADHD continually move, run, climb, squirm, and fidget, but often have trouble with fine motor skills.

Crichton also reports that the disorder can be born with a person, and when born with a person, it becomes evident at a very early period of life. ADHD is reported to coexist with a variety of disorders. These disorders include oppositional defiant disorder 40%, developmental reading disorder, substance use disorder, anxiety disorder, mood disorder 10–20%, epilepsy, antisocial behavior, tics or tourette syndrome, 7%, obsessive compulsive disorder etc.

1.2 Pathophysiology

The exact causes of ADHD are not absolutely known. However, 80% of the disease prevalence is dependent on genetic factors, but it is not the only reason. The midbrain dopamine (DA) system, including the ventral tegmental area and the substantia nigra, is thought to play a pivotal role in the pathogenesis of ADHD and is relatively similar in different mammals. Chronic intake of dopamine agonists such as cocaine and amphetamines will produce a down-regulation of dopamine synthesis. Mesocortical DA is thought to be involved in the pathophysiology of ADHD because of its pivotal role in selective attention and working memory.

In some studies, it is well documented that the incidence of ADHD is high during short or long labor, hypoxia, fetal distress, eclampsia birth weight, and birth injuries. In 1999, various studies looked at pre- and perinatal striatal injury as a possible cause of ADHD and found that those perinatal adverse events may play a major role in the pathogenesis of some cases of ADHD. September births are significantly associated with ADHD and learning disability because, during this time, the seasonally mediated viral infections affect the both mothers and their fetus. The dysfunctioning of basal ganglia inhibitory circuits and also the shorter size on either the left or right side of caudate are make the peoples more prone to ADHD. The risk of ADHD is 2.47 times increased in the group of mothers who currently smoke than in the group who did not smoke. During pregnancy, if the mother smokes, the risk of ADHD is approximately 2.64 times higher. Organophosphate insecticide chlorpyrifos used in some fruits and vegetables causes delay in learning rates, decreases physical coordination, and causes behavioral abnormalities in children, especially ADHD. Research on several hundred children in the UK showed that 20–62.4 mg of food dyes and preservative pushes ordinary children about 10% closer to an ADHD diagnosis (Fig. 1).

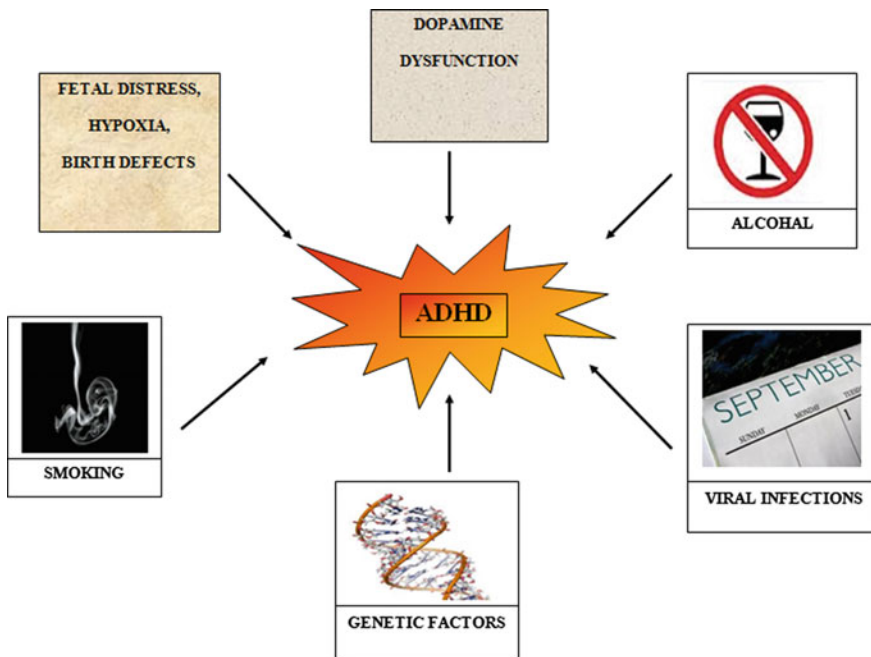


Fig. 1 Pathophysiology of ADHD

1.3 Need of Animal Model

Animal models are the best tools to evaluate any therapeutic agent. Animal models help to advance our understanding of the underlying mechanisms of disease and have proven to be invaluable in the preclinical evaluation of potential therapeutic interventions. Animal models of ADHD provide clear advantages over human studies. Animal models for ADHD with good predictive validity would allow the assessment of novel treatments and also provide tools for the study of underlying neurochemical and neuropathological alterations that lead to ADHD.

2 Classification of Animal Models of ADHD (Fig. 2)

2.1 Toxin-Induced Models

2.1.1 6-Hydroxydopamine

6-hydroxydopamine (6-OHDA) is a synthetic organic compound widely used by researchers due to its selectivity toward dopaminergic and noradrenergic neurons in the brain. 6-OHDA significantly decreases the endogenous levels of DA in the striatum, frontal cortex, and other limbic areas including the nucleus accumbens, septum, and olfactory tubercles in rats. Among these regions, striatum is mostly affected and thus dopamine dysfunction is involved in the pathogenesis of ADHD. Previous data also suggest that there is abnormality in dopamine-modulated frontal-striatal circuits, reflected by size (smaller-than-average components) and function (hypoactivation) in ADHD.

2.1.2 DSP4

DSP4, also known as N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine, can easily cross the blood–brain barrier and produce selective degeneration of noradrenergic neurons. The prefrontal cortex has been shown to be involved in the memory functions, and deficits in working memory have been observed in patients with ADHD. Literature data suggest that NA and alpha 2 agonists enhance working memory functions, whereas alpha 2 antagonists impair the memory functions. These findings suggest that depletion of NA can impair prefrontal functions, particularly in regard to memory functions.

Different doses and routes of administration of 6-hydroxydopamine

S. no.	Dose	Route	Species	Reference
1.	Single-dose administration of 6-OHDA (100 µg, 488 nmol) to 5-day male pups	Intracisternal injection	Wistar rats	Masuo et al. (2004a, b)
2.	Single-dose administration of 6-OHDA (16 mg/1 ml). Coordinates-A -1.8, L 2.4, V -7 mm	Medial forebrain bundle	Wistar rats	Erlj et al. (2012)
3.	Bilaterally injected 6-OHDA with 0.5 µg/0.5 µl at PND 11. Coordinates-AP: +2.8, ML: ±0.5, DV: 2.6	Medial prefrontal cortex (PFC)	Sprague–Dawley rats	Freund et al. (2014)

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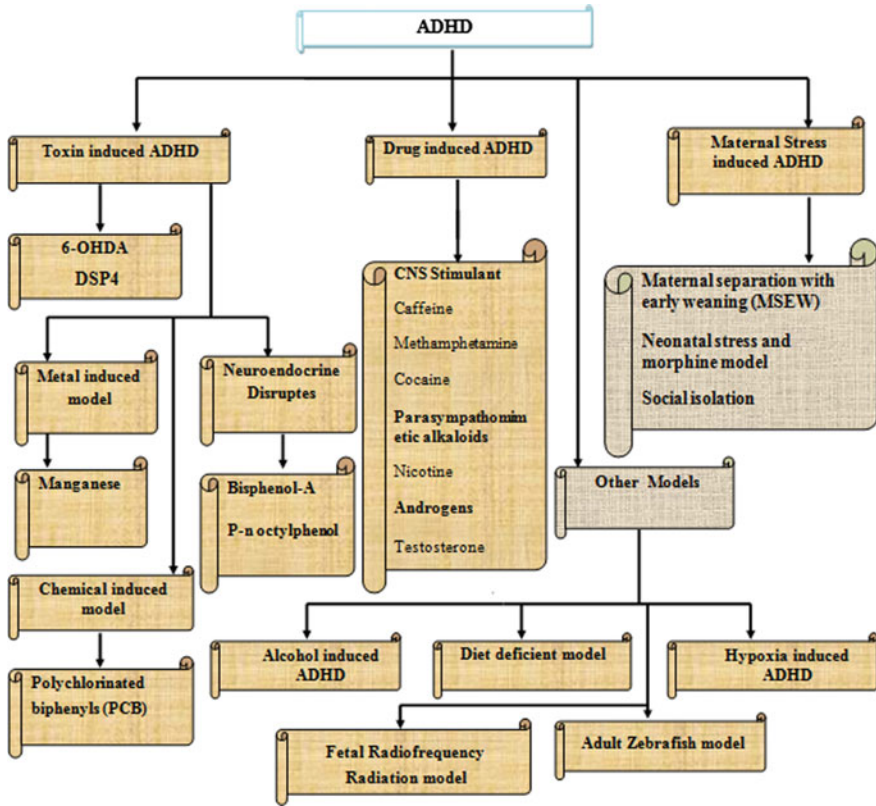


Fig. 2 Classification of animal model of ADHD

(continued)

S. no.	Dose	Route	Species	Reference
4.	Unilaterally lesion of 6-OHDA (8 µg/4 µl) on PND 7 coordinates-AP: +0.7 mm, ML: +2.2 mm, DV: -3.2 mm	Left striatum	Sprague–Dawley rats	Caballero et al. (2011)
5.	DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) (10, 20 or 50 mg/kg/body weight) for 5 weeks	i.p.	Wistar rats	Hauser et al. (2012)

Clinical relevance

6-OHDA produces alterations in the levels of DA in the striatum, frontal cortex, and other limbic brain regions. Similarly, disturbances in dopaminergic signaling have been reported to occur clinically in ADHD patients.

2.2 Metal-Induced Model

2.2.1 Manganese

Exposure to manganese (Mn) during neurodevelopment stage significantly alters the dopaminergic synaptic environments in the brain nuclei that control the executive functions, such as hyperactivity, impulsivity, and cognitive flexibility Kern et al. (2010).

Different doses and routes of administration of MnCl₂

S. no.	Dose	Route	Species	Reference
1.	MnCl ₂ (25 µl of providing 0, 50, 145, 250, or 500 µg/day)	Orally gavaged	Sprague–Dawley rats	Tran et al. (2002)
2.	Mn doses of (0, 25, and 50 mg Mn/kg/d)	Orally	Sprague–Dawley rats	Kern et al. (2010)

Clinical relevance

Manganese toxicity has been known to be associated with hyperactivity, impulsivity, and violent behavior. Therefore, this model mimics all the behavioral symptoms as seen in ADHD patients.

2.3 Endocrine Disruptors

2.3.1 Bisphenol-A

Bisphenol-A (BPA) is an estrogenic chemical used in the production of polycarbonate and beverage cans and in dental sealants. During the development of fetal life, small changes in the levels of hormones (like estradiol) can lead to changes in the functions of brain and the behavior. Long-term exposure of BPA to female rats induces modifications in β -estrogen receptor immunoreactivity in various brain areas regulating reproductive and maternal behavior. Bisphenol-A exposure to rats has been reported to exhibit motor hyperactivity

Different doses and routes of administration of Bisphenol-A and p-n-octylphenol

S. no.	Dose	Route	Species	Reference
1.	Single-dose administration of Bisphenol-A (10, 20 and 40 µg/rat) into 5-day-old male pups	Intracisternal	Wistar rats	Kiguchi et al. (2007)
2.	Bisphenol-A (10 mg/kg/day) GD 11–PND 7	Electronic micropipette (as opposed to gavaged)	CD-1 mice	Palanza et al. (2008)

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S. no.	Dose	Route	Species	Reference
3.	Bisphenol A (0.1 and 50 mg/l) in drinking water (unchlorinated purified water) from GD 11 to PND 21	Orally	Sprague–Dawley rats	Xu et al. (2007)
4.	Bisphenol-A(87 nmol/10 µl/rat) injected into 5-day-old pups	Intracisternal	Wistar rats	Ishido et al. (2005)
5.	Low doses of four EDCs, atrazine (10 mg/kg), perfluorooctanoic acid (PFOA 0.1 mg/kg), Bisphenol-A (50 mg/kg), 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD-0.25 mg/kg) alone, or combined in a mixture from GD 7 until weaning	Orally	C57BL/6 mice	Sobolewski et al. (2014)
6.	Single-dose administration of p-n-octylphenol (87 nmol) into 5-day-old male pups	Intracisternal	Wistar rats	Ishido et al. (2004)

Clinical relevance

According to the recent study reports, the increased level of bisphenol during early pregnancy is mainly associated with higher hyperactivity and aggression in 2-year-old girls and stayed in line with rodents study.

2.4 Chemical-Induced Model

2.4.1 Polychlorinated Biphenyls

PCBs belong to a broad family of man-made organic chemicals also known as chlorinated hydrocarbons. PCBs induce hyperactivity and motor impulsiveness and work via monoaminergic pathway. PCBs also interfere with the thyroid hormone signaling during brain development. Many of the neurodegenerative deficits linked to PCB exposures are similar to those associated with pre- and postnatal thyroid hormone insufficiency. These PCB congeners affect the intracellular regulation of calcium in rodent brain which is very important in nerve cell development and functioning. Dopamine and serotonin levels are decreased possibly via inhibition of dopamine synthesis and deficient vesicular storage or release.

Different doses and routes of administration of PCBs

S. no.	Dose	Route	Species	Reference
1.	Polychlorinated biphenyls (PCB) (1, 3 or 6 mg/kg) three times between PND 8 and 20	Orally	Spontaneously hypertensive rats (SHRs) and Wistar-Kyoto rats (WKY)	Baerland (2011)

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S. no.	Dose	Route	Species	Reference
2.	Low combination of (1 mg/kg PCBs + 1.5 ppm MeHg) or the high combination (3 mg/kg PCBs + 4.5 ppm MeHg) throughout gestation and lactation	Orally	Nulliparous female Long-Evans rats	Sable et al. (2009)
3.	Aroclor 1254 (18 mg/kg/day) administered daily from gestational day (GD) 6–PND 21 (total 35 days)	Maternal injection	Male and female ICR mice	Nam et al. (2014)
4.	PCB 153 (5 mg/kg) or PCB 126 (2 mg/kg) of every 2 days from day 3 to 13 after delivery	Orally	DA:OLA:HSD females	Holene et al. (1998)

Clinical relevance

The number of reports in human populations represents that chronic exposures to PCBs during fetal development are linked to neurodegenerative disorders. In these reports, the populations include: the Yu-Cheng children in Taiwan (born to mothers exposed to thermally degraded PCBs between 1978 and 1979) and the Lake Michigan cohort children born to mothers who ate PCB contaminated fish. In the rodent studies, PCB exposures consistently showed the decline in serum thyroid hormone levels which are important factor for neurodevelopment.

2.5 Drug-Induced Models

2.5.1 CNS Stimulants

Cocaine is a tropane alkaloid obtained from the leaves of the coca plant. Caffeine is a bitter substance also found in coffee, tea, soft drinks, chocolate, kola nuts, and certain medicines. Dopamine agonists such cocaine and amphetamines produce down-regulation of dopamine synthesis.

Different doses and routes of administration

S. no.	Dose	Route	Species	Reference
1.	Caffeine (0.3 g/l in drinking water) to for 6 weeks	Orally	WT mice	Björklund et al. (2008)
2.	Single-dose administration of methamphetamine hydrochloride (1.5 mg/kg)	i.p.	SHR, WKY and SD rats	Womersley (2014)
3.	Single-dose administration of cocaine (20 mg/kg) during development	i.p.	SHR and WKY rats	Womersley (2014)

Clinical relevance

Dopamine agonists such as cocaine and amphetamines alter the synthesis of dopamine. The midbrain dopamine (DA) system, which includes the ventral tegmental area and the substantia nigra, is thought to play an important role in the pathogenesis of ADHD.

2.5.2 Parasympathomimetic Alkaloids

During pregnancy, if mother smokes, the risk of ADHD is approximately 2.64 times increased. Some animal experiments have shown that exposure to nicotine during the pregnancy results in hyperactivity in the child and the possible mechanisms for this may be the increase in number of nicotine receptors and an abnormality in the dopaminergic system. Smoking may reduce the amount of blood sent to the fetus which retards the supply of oxygen and nutrition to the fetus.

Different doses and routes of administration of nicotine

S. no.	Dose	Route	Species	Reference
1.	Nicotine (1.5 mg/kg) from the day 11 to 20 of gestation	s.c.	Female albino rats	Abdu (2013)
2.	Nicotine (2 mg/kg) from PND 1 to 7	Orally	Sprague–Dawley rat	Huang et al. (2006)
3	Nicotine (66 µg/kg) twice daily for 5 days starting at the age of either 3, 10, or 19 days	s.c.	Male NMRI mice	Ankarberg (2003)
4.	Nicotine (200 µg/ml) drinking solution for 30 days prior to and during pregnancy	Orally	C57Bl/6 mice	Pauly et al. (2004)

Clinical relevance

The risk of ADHD is 2.5 times higher in smoking mothers than who did not. Twenty-four studies on maternal tobacco smoking published between 1973 and 2002, indicated that there is an increased risk of ADHD in the offspring. The smoking-induced ADHD symptoms could be due to nicotine causing hypoxia to the fetus in uterus.

2.5.3 Androgens

Testosterone (a type of androgen) is a steroid hormone found in mammals, reptiles, birds, and other vertebrates. The role of androgens in the ADHD pathogenesis suggested that the increased levels of testosterone can diminish the brain–blood flow in the frontal cortex, via lowering of the level of estrogen receptor alpha and the vascular endothelial growth factor (VEGF)

Different doses and routes of administration of testosterone

S. no.	Dose	Route	Species	Reference
1.	Testosterone (10 ng) on PND 10 coordinates: 4.8 mm anterior to bregma, 2.3 mm lateral to the midsagittal suture, and 2.2 mm below dura	Intracranial	SHR and WKY rats	King et al. (2000)
2.	Testosterone (7.5 mg/kg) for 5 days/week	s.c.	Male Long-Evans rats	Wood et al. (2013)

Clinical relevance

It is well demonstrated that the early androgen treatment significantly reduced tyrosine hydroxylase (TH) in the specific area of brain-like caudate-putamen. This area of brain is important for behavioral inhibition, motor control, and habit teaching. Therefore, this model mimics all the behavioral symptoms of ADHD.

2.5.4 Ethanol-Induced ADHD

Alcohol consumption during pregnancy is reported to increase the risk of ADHD. Exposure to alcohol consumption during pregnancy is associated with neurobehavioral disorders. The caudate has been reported to be one of the most sensitive brain areas to prenatal alcohol insult. Abnormalities in the caudate region are hypothesized to be related to defects in executive functioning, attention, and response inhibition. It is also reported that moderate levels of prenatal and postnatal alcohol exposure can have long-lasting effects on IQ and learning problems in young school children.

Different doses and routes of administration of ethanol

S. no.	Dose	Route	Species	Reference
1.	Ethanol (5 g/kg) every other day from PND 2 to 8 Ethanol (3 g/kg/day) from PND 1 to 7	i.p. oral intubation	Swiss mice Sprague–Dawley rats	Nunes et al. (2011), Smith et al. (2012)
2.	Ethanol (6 g/kg/day) from GD 7 to 16	Intragastric intubation	Sprague–Dawley rats	Choi et al. (2012)

Clinical relevance

The prevalence of ADHD is 1.55 times higher in children of mothers who consumed alcohol than in those whose mothers did not consume alcohol. The necropsy reports of an infant with fetal alcohol syndrome showed abnormalities in the basal ganglia, hippocampus, cerebellum, CNS disorganization, abnormalities in pituitary gland, and optic nerve.

2.6 Maternal Stress-Induced ADHD

2.6.1 Maternal Separation with Early Weaning (MSEW)

Early life stress has been shown to reduce glutamate and GABA transmission, particularly the GABA_A receptor expression.

Species SHR and Control WKY Rats

Procedure

The day of birth is designated as PND 0. The maternal separation includes removal of the dam from the pups for 3 h daily from PNDs 2 to 14. Pups are transferred in the home cage to a different room to prevent communication with the dam by ultrasound vocalization. The temperature is maintained at 31–33 °C by infrared heating lamps so to prevent the risk of possible hypothermia. After 3 h, the pups are returned to the animal facility and the dams returned to their home cages.

Species: C57Bl/6 J Mice

Procedure

The day of birth is designated as PND 0. The maternal separation includes removal of the dam from the pups for 4 h daily from PNDs 2 to 5, and pups are transferred in a clean cage with access to food and water. The temperature is maintained by heating blanket set at 32 °C, and it is placed underneath the pups to assist with thermoregulation. From PNDs 6 to 16, the period of separation is increased to 8 h. On PND 17, MSEW animals are weaned and then left undisturbed aside from daily brief checks for dehydration on PNDs 18 to 19. They are housed in a single cage till PND 30.

2.6.2 Neonatal Stress and Morphine Model

The prolonged inescapable stress can have harmful effects on learning and development and on the immune system. This also increases the susceptibility to neurodegenerative diseases.

Species: Adult Wild-Type C57BL/6 Mice

Procedure

Morphine (2 mg/kg) is given by subcutaneous route twice daily between PNDs 5 and 9. Stressed pups are separated from the dam and isolated in individual containers within a veterinary warmer at 32 °C. To induce the stress, the pups are exposed to hypoxia (100% nitrogen 1 min followed by hyperoxia (100% oxygen 5 min) twice daily.

Clinical relevance

The study shows the absolute relation between the stress exposures during the pregnancy and ADHD symptoms in the child. Children of mothers who experienced moderate or severe stress have higher Child Behavior Checklist (CBCL)

scores than the no-stressor group. This ensures that prenatal stress does cause more severe symptoms of ADHD and also shows that during the third trimester, increased stress levels correlate with greater CBCL scores.

2.6.3 Social Isolation-Induced ADHD

Species Male ICR Mice

Procedure

Male mice are socially isolated for 3–70 days before experiments in (24 cm × 17 cm × 12 cm) sized cages.

2.7 Other Models

2.7.1 Hypoxia-Induced ADHD

Due to hypoxia, extracellular DA in medial PFC is reduced and transporter in PFC is increased in animals.

Species: Sprague–Dawley Rats

Procedure

Firstly unilateral ligation is performed with uterine artery supplying one uterine horn on embryonic day 17 (ED17) under the deep anesthesia (350 mg/kg chloral hydrate i.p.). Uterine artery ligation is made at the cervical level and generates the prenatal ischemia in pups located close to the site of ligation. Possibly the animals are exposed to hypoxia followed by a reperfusion phase during delivery. Prenatal ischemia at ED 17 caused a gradient of growth restriction along the uterine horn that we used to differentiate pups after delivery.

Species: Pregnant Albino Sprague–Dawley Rats

Procedure

The day of birth was designated as PND 0. Sprague–Dawley rat pups from three different litters are exposed to repeated hypoxia on PND 1–3. The rat brain during PND 1–3 is generally considered to be developmentally equivalent to the fetal human brain from mid-gestation to late in the second trimester (i.e., at 22–28) weeks of gestation. The repeated hypoxic pups are exposed to humidify 1.5% oxygen, 5% carbon dioxide, and 93.5% nitrogen at 37 °C every 2 h for 12 h daily from PND 1 to 3. The hypoxic exposure was 15 min on PND 1 and 14 min on PND 2 and PND 3.

Species: Sprague–Dawley Rats*Procedure*

On the day of parturition, Sprague–Dawley dams are decapitated and hysterectomized. After this, quickly isolate entire uterus from the fetus and immediately immerse in a 37 °C saline bath for 15 min for the induction of birth hypoxia. The pups are removed from the uterus and stimulated by gentle rubs to initiate breathing.

Species: Male Wistar Rats*Procedure*

In this experiment, the pups are exposed repeatedly to hypoxia during the first week of life. The mother is gently removed or separated, and the home cage containing the pups is then placed in an incubator connected to a gas supply. The temperature inside the chamber is maintained to 30 °C. Litters are assigned to be exposed to 100% nitrogen. The gas outflow is set at 40 L/min (1 bar). Exposure is to be given for 20 min on PND 1, –3, and –5 and for 10 min on PND 7.

2.7.2 N-3 PUFA-Deficient Diet Model of ADHD

The rise in consumption of processed foods and vegetable oils has markedly increased the intake of omega-6 PUFAs in Western diets, while the consumption of n-3 PUFAs in fish, nuts, and seeds has declined. Some studies have reported that children with ADHD have reduced levels of blood n-3 PUFAs as compared to healthy subjects.

S. no.	Dose	Route	Species	Reference
1.	n-3 PUFA-deficient diet to SHR for 6 weeks	Orally	Male SHR	Lange et al. (2013)

Clinical relevance

Some studies have reported that children with ADHD have reduced levels of blood n-3 PUFAs as compared to healthy subjects.

2.7.3 Fetal Radiofrequency Radiation Model

In utero cellular telephone, radiation exposure may lead to impairment of glutamatergic synaptic transmission onto pyramidal cells in the prefrontal cortex associated with behavioral changes.

S. no.	Dose	Route	Species	Reference
1.	Radiation from muted and silenced (800–1900 MHz) cellular phones with a SAR of 1.6 W/kg throughout GD 1–17	Radiation	Female mice	Aldad et al. (2012)

Clinical relevance

In utero exposure to radiofrequency is a potential cause of neurobehavioral disorders.

2.7.4 Adult Zebra Fish Model

The biological half-life of PCBs is long and has high liposolubility which leads to their bioaccumulation and biomagnifications through food chains over a wide range of trophic levels.

Species: Wild-Type Zebra Fish

Procedure

Eight-week-old fish are exposed to commercial food for 8 months at 26–28 °C. Commercial food consists of PCB mixtures composed of the seven indicator PCB congeners (CB-28, CB-52, CB-101, CB-118, CB-138, CB-153, and CB-180). For this, two doses are selected, i.e., intermediate and high doses. Fish exposed to an intermediate dose (equivalent to that found in the Loire Estuary, $\Sigma\text{CB} = 515$ ng/g dry weight in food) display behavioral abnormality in exploration capacities. Similarly, the fish exposed to the highest dose (equivalent to that found in the Seine Estuary, $\Sigma\text{CB} = 2302$ ng/g dry weight in food) display an increased swimming activity and larval activity.

Clinical relevance

The transparency of the zebra fish embryo allows direct and continuous visualization of tissue morphogenesis in vivo. The zebra fish also possess powerful cognitive abilities, including learning and memory. Zebra fish have been developed to be a useful tool for explaining not just the structural and chemical effects of neurotoxins, but also for assessing the behavioral impairment associated with such neurotoxins exposure.

3 Ethical Statement

All institutional guidelines, national guidelines, state and local laws, and regulations with professional standards for the care and use of laboratory animals should be followed. Studies involving animals must state that the institutional animal ethical committee has approved the protocol. For authors using experimental animals, a statement should be made that the animals' care is in accordance with institutional guidelines and animals used have been treated humanely and with regard to the alleviation of suffering. Researchers should treat animals as sentient and must consider their proper care and use and the avoidance or minimization of discomfort, distress, or pain as imperatives. Animal experiments should be designed only after due consideration of animal health. It should be ensured that all researchers who are using animals have received instruction in research methods and in the care,

maintenance, and handling of the species being used. All the surgical procedures should be performed under appropriate anesthesia and follow only those procedures which avoid infection and minimize pain during and after surgery.

References

- Abdu F (2013) Effect of *Moringa Oleifera* extract on nicotine induced neurotoxicity in female rat and their embryos
- Aldad T, Gan G, Gao X et al (2012) Fetal radiofrequency radiation exposure from 800 to 1900 mhz-rated cellular telephones affects neurodevelopment and behavior in mice. *Sci Rep* 2
- Ankarberg E (2003) Neurotoxic effects of nicotine during neonatal brain development: critical period and adult susceptibility
- Baerland N (2011) Behavioral effects of PCB exposure in an animal model of attention-deficit/hyperactivity disorder: the spontaneously hypertensive rat
- Björklund O, Kahlström J, Salmi P et al (2008) Perinatal caffeine, acting on maternal adenosine A₁ receptors, causes long-lasting behavioral changes in mouse offspring. *PLoS ONE* 3:3977
- Caballero M, Núñez F, Cuffi M et al (2011) Caffeine improves attention deficit in neonatal 6-OHDA lesioned rats, an animal model of attention deficit hyperactivity disorder (ADHD). *Neuroscience Lett* 494:44–48
- Choi I, Kim P, Joo H et al (2012) Effects of preconceptional ethanol consumption on ADHD-like symptoms in Sprague-Dawley rat offsprings. *Biomol Therap* 20:226
- Erlj D, Acosta-García J, Floran B et al (2012) Dopamine D4 receptor stimulation in GABAergic projections of the globus pallidus to the reticular thalamic nucleus and the substantia nigra reticulata of the rat decreases locomotor activity. *Neuropharmacology* 62:1111–1118
- Freund N, MacGillivray H, Andersen S et al (2014) Sex-dependent changes in ADHD-like behaviors in juvenile rats following cortical dopamine depletion. *Behav Brain Res* 270:357–363
- Hauser J, Sontag T, Tucha O et al (2012) The effects of the neurotoxin DSP4 on spatial learning and memory in Wistar rats. *ADHD Atten Def Hyp Disord* 4:93–99
- Holene E, Nafstad I, Skaare J et al (1998) Behavioural hyperactivity in rats following postnatal exposure to sub-toxic doses of polychlorinated biphenyl congeners 153 and 126. *Behav Brain Res* 94:213–224
- Huang L, Hsiao S, Frye G et al (2006) Chronic nicotine induces growth retardation in neonatal rat pups. *Life Sci* 78:1483–1493
- Ishido M, Masuo Y, Oka S et al (2004) Intracisternal administration of p-n-octylphenol into neonatal rats causes hyperactivity concomitantly with the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL)-positive cells in the mesencephalon where immunoreactivity for tyrosine hydroxylase is reduced by the chemical. *J Health Sci* 50:407–412
- Ishido M, Morita M, Oka S et al (2005) Alteration of gene expression of G protein-coupled receptors in endocrine disruptors-caused hyperactive rats. *Regul Pept* 126:145–153
- Kern C, Stanwood G, Smith D et al (2010) Prewaning manganese exposure causes hyperactivity, disinhibition, and spatial learning and memory deficits associated with altered dopamine receptor and transporter levels. *Synapse* 64:363–378
- Kiguchi M, Fujita S, Lee J et al (2007) Behavioral responses to methylphenidate and apomorphine in rats exposed neonatally to bisphenol-A. *J Oral Sci* 49:311–318
- King JA, Barkley RA, Delville Y, Ferris CF (2000). Early androgen treatment decreases cognitive function and catecholamine innervation in an animal model of ADHD. *Behav brain res* 107:35–43
- Lange K, Reisinger J, Sontag T et al (2013) Dietary omega-3 fatty acids and locomotor activity in an animal model of attention deficit hyperactivity disorder (ADHD). *Funct Foods Health Dis* 3:223–229

- Masuo Y, Ishido M, Oka S et al (2004a) Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats. *Neural Plast* 11:59–76
- Masuo Y, Morita M, Oka S et al (2004b) Motor hyperactivity caused by a deficit in dopaminergic neurons and the effects of endocrine disruptors: a study inspired by the physiological roles of PACAP in the brain. *Regul Pept* 123:225–234
- Nam Y, Shin E, Lim Y et al (2014) YY162 prevents ADHD-like behavioral side effects and cytotoxicity induced by Aroclor1254 via interactive signaling between antioxidant potential, BDNF/TrkB, DAT and NET. *Food Chem Toxicol* 65:280–292
- Nunes F, Kubrusly R, Manhaes A et al (2011) Acute administration of vinpocetine, a phosphodiesterase type 1 inhibitor, ameliorates hyperactivity in a mice model of fetal alcohol spectrum disorder. *Drug Alcohol Depend* 119:81–87
- Palanza P, Gioiosa L, Saal F et al (2008) Effects of developmental exposure to bisphenol A on brain and behavior in mice. *Environ Res* 108:150–157
- Pauly JR, Sparks JA, Hauser KF, Pauly TH (2004). In utero nicotine exposure causes persistent, gender dependant changes in locomotor activity and sensitivity to nicotine in C57Bl/6 mice. *Int J Dev Neurosci* 22:329–337
- Sable H, Eubig P, Powers B et al (2009) Developmental exposure to PCBs and/or MeHg: effects on a differential reinforcement of low rates (DRL) operant task before and after amphetamine drug challenge. *Neurotoxicol Teratol* 31:149–158
- Smith A, Wellmann K, Carter M et al (2012) Lobeline attenuates neonatal ethanol-mediated changes in hyperactivity and dopamine transporter function in the prefrontal cortex in rats. *Neuroscience* 206:245–254
- Sobolewski M, Conrad K, Allen J et al (2014) Sex-specific enhanced behavioral toxicity induced by maternal exposure to a mixture of low dose endocrine-disrupting chemicals. *Neurotoxicology* 45:121–130
- Tran T, Chowanadisai W, Lonnerdal B et al (2002) Effects of neonatal dietary manganese exposure on brain dopamine levels and neurocognitive functions. *Neurotoxicology* 23:645–651
- Womersley J (2014) Methamphetamine and cocaine effects on dopamine neurons in a rat model of developmental stress and attention-deficit/hyperactivity disorder. University of Cape Town, Dept of Human Biology
- Wood R, Armstrong A, Shah V et al (2013) ‘Roid rage in rats? testosterone effects on aggressive motivation, impulsivity and tyrosine hydroxylase. *Physiol Behav* 110:6–12
- Xu X, Liu Y, Kato N et al (2007) Perinatal bisphenol A affects the behavior and SRC-1 expression of male pups but does not influence on the thyroid hormone receptors and its responsive gene. *Neurosci Res* 58:149–155