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Treatment with the flavonoid 7,8-Dihydroxyflavone: a promising strategy for a constellation of body and brain disorders

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**Title: Treatment with the Flavonoid 7,8-Dihydroxyflavone: a promising Strategy for a Constellation of Body and Brain Disorders**

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## ABSTRACT

Flavonoids have long been known to exert benefits in various health problems. Among them, the BDNF mimetic 7,8-Dihydroxyflavone (7,8-DHF) is emerging as a potential treatment for a constellation of brain and body pathologies. During the past 10 years, more than 180 preclinical studies have explored the efficacy of 7,8-DHF in animal models of different pathologies. The current review intends to be an exhaustive survey of these studies. By providing detailed information on the rationale of the experimental design and outcome of treatment, we will give the reader tools to critically interpret the achievement obtained so far. If we put together each individual piece of this complex mosaic, a picture emerges that is full of promise regarding the potential usefulness of 7,8-DHF for human treatment. Much has been done so far and we believe that the time is now ripe to move from the bench to the bedside, in order to establish whether supplementation with 7,8-DHF may serve as therapy or, at least, as adjuvant for the treatment of pathologies affecting brain and body functioning.

**Key words:** Nutraceuticals; 7,8-Dihydroxyflavone; BDNF-TrkB system; Antioxidants; Neuroprotection; Body pathophysiology

## LIST OF ABBREVIATIONS

7M8H-flavone: 7-methoxy-8-hydroxyflavone  
7,8-DHF: 7,8-Dihydroxyflavone  
A $\beta$ 40 and A $\beta$ 42: Beta amyloid 40, Beta amyloid 42  
AD: Alzheimer's disease  
ALS: Amyotrophic lateral sclerosis  
AMPK: Adenosine Monophosphate-Activated Protein Kinase  
APP: Amyloid precursor protein  
ASD: Autism Spectrum Disorder  
BACE1:  $\beta$ -secretase 1  
BBB: Blood-brain barrier  
Bcl-2: B-cell lymphoma-2  
 $\beta$ -CTF:  $\beta$ -secretase-cleaved C-terminal fragment  
BDNF: Brain-derived Neurotrophic Factor  
CA1, CA3: Cornu Ammonis 1, Cornu Ammonis 3  
CaMKII: Ca<sup>2+</sup>/calmodulin-dependent protein kinase II  
CAT: Catalase  
CCI: Controlled Cortical Impact Model  
CFC: Contextual Fear Conditioning  
COII: Mitochondrial cytochrome oxidase II  
CREB: c-AMP response element-binding protein  
CTX-B: Cyclotraxin-B  
DAG: Diacylglycerol  
DG: dentate gyrus  
DOX: Doxorubicin  
DS: Down syndrome  
ER- $\alpha$ : Estrogen receptor  $\alpha$   
FMRP: Fragile X mental retardation protein  
FPI: Fluid Percussion Injury  
FST: Forced swim test  
FXS: Fraxile X syndrome  
GAP-43: Growth Associated Protein 43  
GSH: Glutathione  
HC: Hair cells  
HD: Huntington's disease  
HI: Hypoxia-ischemia  
HO-1: Heme oxygenase 1  
iNOS: Inducible nitric oxide synthase  
IP3: inositol-1,4,5-triphosphate  
I/R: Ischemia/Reperfusion  
L&M: Learning and memory  
LPS: Lipopolysaccharide  
LTP: Long-term potentiation  
MAPK: Mitogen-activated protein kinase  
MCAO: Middle cerebral artery occlusion  
MECP2: methyl-CpG binding protein 2  
METH: Methamphetamine  
MDA: Malondialdehyde  
MDD: Major depressive disorder  
MMPs: Matrix metalloproteinases  
Mn-SOD: Mn-superoxide dismutase  
mTOR: mammalian target of rapamycin  
MWM: Morris Water Maze  
NO: nitric oxide

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3 NGF: Nerve growth factor  
4 NOR: Novel Object Recognition  
5 Nrf2: Nuclear factor erythroid 2-related factor 2  
6 OA: Osteoarthritis  
7 OGD: Oxygen-glucose deprivation  
8 OPA1: Optic atrophy 1  
9 PD: Parkinson's disease  
10 PFC: Prefrontal cortex  
11 PGC-1 $\alpha$ : Peroxisome proliferator-activated receptor- $\gamma$  coactivator 1-alpha  
12 PI3K: Phosphatidylinositol 3-kinase  
13 PLC: Phospholipase C  
14 PPI: Prepulse Inhibition  
15 Rb: Retinoblastoma protein  
16 RGC: Retinal ganglion cells  
17 ROS: Reactive oxygen species  
18 RTT: Rett syndrome  
19 SGN: Spiral ganglion neurons  
20 SOD: Superoxide dismutase  
21 Shc: Src-homology 2-domain  
22 Sp1: Specificity protein 1  
23 SPT: Sucrose Preference Test  
24 TBI: Traumatic brain injury  
25 Trk: Tropomyosin receptor kinase  
26 TFAM: Mitochondrial transcription factor 1  
27 TST: Tail suspension test  
28 UCP1: Uncoupling protein 1  
29 VrK3: Vaccinia-related kinase 3  
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## 1. INTRODUCTION

Flavonoids are low molecular weight compound that belong to a class of plant secondary metabolites having a polyphenolic structure. They are not synthesized by animals but can be assumed with the diet through fruits, vegetables and certain beverages (Panche, Diwan, and Chandra 2016). Flavonoids are categorized into 6 subgroups (Flavones, Flavonols, Isoflavonoids, Flavanones, Chalcones, Anthocyanins), based on the carbon of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring (Panche, Diwan, and Chandra 2016). In addition to their role in plants, flavonoids appear to play a number of protective functions in the human body due to their antioxidant, cytotoxic, anticancer, anti-inflammatory, antiviral, antiallergic, cardioprotective, and hepatoprotective properties (Nijveldt et al. 2001; Panche, Diwan, and Chandra 2016; Tapas, Sakarkar, and Kakde 2008). For this reason, flavonoids are now considered as an indispensable component in a variety of nutraceutical, and pharmaceutical applications.

7,8-Dihydroxyflavone (7,8-DHF) is a flavonoid belonging to the subclass of flavones that, similarly to other flavonoids, was known to exert anticancer action (Le Bail et al. 1998) and prevention of oxidative stress (Zhang et al. 2009). The discovery ten years ago that 7,8-DHF is a bioactive high-affinity agonist of the tropomyosin-related kinase B (TrkB) receptor for the brain-derived neurotrophic factor (BDNF) that crosses the blood brain barrier (BBB) and provokes TrkB autophosphorylation and activation of downstream signaling pathways represented a milestone in the history of this flavonoid (Jang et al. 2010). Various brain disorders that are related to deficits in BDNF-TrkB signaling cannot be “cured” with exogenous BDNF due to its unfavorable pharmacokinetic properties. 7,8-DHF, however, is a BDNF mimetic that crosses the BBB and, therefore, can be pharmacologically exploited to treat BDNF-related brain disorders. Indeed, the pioneering study by Jang et al. provided preliminary evidence for a positive effect of treatment with 7,8-DHF *in vivo* for stroke and Parkinson disease (Jang et al. 2010). This evidence heavily drew the attention of the scientific community and prompted a number of studies aimed at establishing the therapeutic potential of 7,8-DHF in different brain diseases. The growing interest for 7,8-DHF as potential medicine is documented by results of a search in Pub Med using the keyword “7,8-Dihydroxyflavone”. This search yielded a total 203 publications, 16 of which were in the period 1992-2009 and 187 in the period 2010-2020. Moreover, t

publication rate in the last decade has grown exponentially, as shown in Fig. 1. This is not surprising considering the therapeutic potential of BDNF and, hence, 7,8-DHF in a variety of neurological disorders. In addition, 7,8-DHF being a flavonoid has the added value of being an antioxidant, which may be of relevance by itself and because BDNF-related pathologies may also be associated with metabolic dysregulation and oxidative stress.

The available reviews regarding the therapeutic potential of 7,8-DHF were mainly focused on brain disorders (Du, and Hill 2015; Liu, Chan, and Ye 2016). It must be noted, however, that 7,8-DHF exerts important effect on numerous body organs and functions, which anticipates a wide scope of action of this molecule in the framework of human health. For this reason, we thought it may be important to include in this review studies that have examined the effects of 7,8-DHF both on the brain and body. This review was additionally prompted by the idea to give the reader detailed information of most of the preclinical studies on 7,8-DHF published so far. Our description will be analytical in terms of i) categorization of the effects of 7,8-DHF, disease by diseases and ii) abundance of experimental details (e.g., doses, duration of treatment, utilized strain, age, etc.). Each section is organized in a brief premise, that provides propaedeutic information, the description of the actual studies on a given topic, and a brief comment on the potential significance of the experimental evidence. In addition, we have summarized in two tables (Table 1 and Table 2) the animal models, doses, route of administration used in all the reviewed studies and in a third table (Table 3) the studies that have investigated a given brain parameter (e.g., neurogenesis, spine density etc.) to help the reader quickly gather available evidence on the topic/s of interest. We hope that this effort may provide a clear and exhaustive view of the state of the art of preclinical research regarding the benefits of 7,8-DHF. This, in turn, may help to design new targeted studies founded on the existing body of knowledge and to individuate possible gaps to be addressed in future research aimed at expanding the characterization of this very promising molecule.

## **2. BDNF AND TRKB SIGNALING**

### **2.1 Brief overview on neurotrophins**

Neurotrophins are a family of protein regulators of neural survival, development, function, and plasticity in the central and peripheral nervous system (see (Huang, and Reichardt 2001)). Nerve growth factor (NGF)

was the first identified and best characterized member of this family (Levi-Montalcini 1987). There are four neurotrophins characterized in mammals: NGF, BDNF, neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). Neurotrophins bind two types of receptors: the tropomyosin receptor kinase (Trk) and the so called low-affinity NGF receptor p75 (p75NTR). The Trk receptors mediate specificity of the neurotrophic functions of neurotrophins. There are three main types of Trk receptors: TrkA is a receptor for NGF, NT-6 and NT-7; TrkB is a receptor for BDNF and NT-4/5; and TrkC is a receptor for NT-3. Since 7,8-DHF specifically binds to the TrkB receptor for BDNF, we will focus here on the BDNF-TrkB system.

**2.2 Synthesis of BDNF** The human *BDNF* gene (~70 kb) consists of 11 exons, located on chromosome 11, and its different splicing enables formation of transcripts specific to various tissues and responsive to different stimuli (see(Cattaneo et al. 2016)). These transcripts, however, are translated and processed into identical pro-BDNF protein. The BDNF protein is synthesized and folded in the endoplasmic reticulum as a precursor form, pre-pro-BDNF (~ 27 kDa). The pre-region is then cleaved, resulting in formation of pro-BDNF. The pro-BDNF is then further processed via the Golgi apparatus where the pro-domain is proteolytically cleaved off to form the pro-domain (of 129 amino acids) and the mature domain m-BDNF (of 118 amino acids) also simply named BDNF. The ratio of pro-BDNF to m-BDNF varies between particular stages of brain development and regions. While in the early postnatal period higher concentration of pro-BDNF is reported, m-BDNF prevails in adulthood (Yang et al. 2014a). While pro-BDNF interacts preferentially with the p75NTR, m-BDNF binds the TrkB receptor.

### 2.3 The TrkB receptor

The TrkB receptor is encoded by a single *TrkB* gene, the *NTRK2* gene encoding 24 exons (350 kbp) located on humans chromosome 9q22 (see (Pradhan, Noakes, and Bellingham 2019)). The TrkB protein consists of three different domains: an extracellular ligand binding domain (encoded by exons 5-14), a transmembrane domain (encoded by exon 15) and an intracellular tyrosine kinase domain (encoded by exons 20-24). Exon 12 encodes an IG-like domain that has been postulated to be the region responsible for binding specificity to its ligand, BDNF (Middlemas, Lindberg, and Hunter 1991). The extracellular transmembrane domain and the intracellular cytoplasmic domain of the TrkB protein full length (TrkB F.L.) act as phosphorylation-dependent docking sites and rapidly transmits the effects of neurotrophin binding to downstream network.



Alternative splicing at exon 18 of the primary transcript produces truncated TrkB isoforms (TrkB-T1 and TrkB-T-shc in humans; T1 and T2 in rat) that lack the intracellular tyrosine kinase domain (Fig. 2) and cannot elicit rapid intracellular signaling (Stoilov, Castren, and Stamm 2002). BDNF activation of TrkB-T1 and TrkB-T2 has been shown to increase the rate of acidic metabolites release from the cell (Baxter et al. 1997). TrkB-T1 can behave as a dominant negative isoform by competing with neurotrophins and forming heterodimers with the TrkB F.L. In human, the isoform TrkB-T1 is predominantly expressed in the brain but also detected in heart, kidney and pancreas (see (Gupta et al. 2013a)).

## 2.4 BDNF-TrkB signaling

The binding of BDNF to the TrkB receptor, causes receptor dimerization and autophosphorylation of intracellular tyrosine residues (Y705/6), which results in the formation of the phosphorylated-TrkB receptor (see (Rantamaki et al. 2007)). This, in turn, launches autophosphorylation of other tyrosine residues situated outside the activation loop (Y515 and Y816) that serve as docking sites for adaptor proteins. Phosphorylated TrkB sets in motion three interconnected signaling transduction pathway with specific cellular functions (Fig. 3): a) Phosphatidylinositol 3-kinase (PI3K), b) mitogen-activated protein kinase (MAPK) and c) phospholipase C $\gamma$  (PLC $\gamma$ ) (see (Chao 2003; Rantamaki et al. 2007)).

a) Activation of the PI3K pathway incorporates combined actions of Ras at the Tyr515 residue, which activates the PI3K/Akt and MEK/MAPK pathways. The **PI3K/Akt-related pathway** results in the activation of B-cell lymphoma 2 (Bcl-2)-associated agonist of cell death (BAD) and inhibition of the kinase glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ), which in turn leads to a reduction in the phosphorylation of collapsing response mediator protein 2 (CRMP-2), a protein that is highly expressed in the developing of nervous system and that regulates axonal outgrowth and dendritic branching. Activation of mammalian target of rapamycin (mTOR) by PI3K/Akt pathway enhances local BDNF translation to dendrites at active synapses, enhances dendritic growth and branching.

b) Activation of the TrkB receptor at Tyr490 and Tyr515 residue causes the docking of Src-homology 2-domain (Shc) adaptor protein at these tyrosine sites and recruits growth factor receptor bound protein 2 (grb2) which binds with GTPase Ras to form a complex, and initiates activation of the **MAPK/ERK pathway** that is an important regulator of cell survival and neuronal differentiation. The MAPK/ERK signaling cascade is able to phosphorylate and activate the transcription factor cAMP response element-

binding protein (CREB). Phosphorylated CREB is then translocated into the nucleus, where it induces BDNF transcription by binding to BDNF promoters. Binding to BDNF promoters drives BDNF expression to regulate neuronal survival, differentiation and synaptic plasticity. Moreover, ERK/CREB regulates the transcription of genes required for dendritic growth including proteins that regulate cytoskeleton dynamics (e.g., Arc and cypin).

c) The phosphorylation of the TrkB receptor at its Tyr816 residue activates the **PLC $\gamma$  pathway** with consequent intracellular generation of inositol-1,4,5-triphosphate (IP<sub>3</sub>) and 1,2-diacylglycerol (DAG). The PLC $\gamma$ /IP<sub>3</sub> pathway induces the release of calcium from intracellular stores. Calcium activates Ca<sup>2+</sup>/calmodulin-dependent protein kinase (CAMKII) which in turn induces CREB phosphorylation. The generation of DAG activates PKC that is translocated to the membrane for further activation and phosphorylation of ERK leading to survival, neuritic outgrowth, and synaptic plasticity. Activation of PLC $\gamma$  appears to be dispensable for increasing dendritic branching but is required to induce hippocampal long-term potentiation (LTP).

## 2.5 TrkB activation without neurotrophins

The TrkB receptor can execute autophosphorylation activity and downstream signaling without stimulation by its ligand. BDNF-independent TrkB activation occurs *via* a mechanism known as trans-activation (see (Chao 2003; Sasi et al. 2017)). For instance, activation of TrkB receptors in the absence of neurotrophins can be mediated by the G-protein-coupled adenosine 2A receptor (A2A-R) or the dopamine D1 receptor. This effect is mediated by Src family of protein tyrosine kinases. Transactivating agents of TrkB include pituitary adenylate cyclase-activating polypeptide (PACAP), epidermal growth factor (EGF), zinc, and H<sub>2</sub>O<sub>2</sub> (Huang, and McNamara 2012).

## 2.6 TrkB inhibitors

K252a is an alkaloid (467.48 Da) that is able to penetrate the cell and act as a potent Trk receptor inhibitor (Boulle et al. 2012). However, the lack of specificity for TrkB, as compared to TrkA and TrkC, constitutes a major limitation for the use of K252a. More recently, two specific and potent TrkB antagonists have been developed, ANA-12 and cyclotraxin-B. ANA-12 (407.49 Da), which has been identified by an *in silico* screening approach, crosses the BBB, binds the extracellular domain of TrkB, prevents BDNF-induced TrkB activation, and abolishes the biological effects of BDNF but not those of NGF or NT-3 on TrkA- and TrkC-

expressing cells (Cazorla et al. 2011). Cyclotraxin-B (CTX-B), a cyclic peptide (1200 Da), is a TrkB antagonist that crosses the BBB and allosterically alters the conformation of the receptor, leading to long-lasting inhibition of BDNF activity. The action of this molecule is mediated by the link with the TrkB binding extracellular domains, that are not critical for the interaction with BDNF but rather involved in its activation capacity (Cazorla et al. 2010). CTX-B inhibits the two main signaling pathways downstream of TrkB, the MAPK- and PLC $\gamma$ -pathway, with similar amplitude of inhibitory effects (Cazorla et al. 2010). Very recently, a novel inhibitor has been discovered, GZD2202, that has a moderate selectivity between Trk B/C and TrkA (Zou et al. 2019). GZD2202 dose-dependently inhibits the phosphorylation of TrkB and its downstream signaling proteins Akt, ERK, PLC $\gamma$ .

### 3. PHARMACOLOGY AND SIGNALING OF 7,8-DHF

#### 3.1 Pharmacology of 7,8-DHF and its prodrugs

##### 3.1.1 Sources and Chemistry

7,8-DHF is a flavone substituted by hydroxy groups at positions 7 and 8 (Fig. 4). It is naturally present in *Godmania aesculifolia*, *Tridax procumbens* and various species of *Primula*. *Godmania aesculifolia* is a species of flowering plant belonging to the Bignoniaceae family. Bignonia plants are widely used in traditional medicine as remedy for skin ailments, postpartum hemorrhage, malaria, diabetes and pneumonia (Mahmoud et al. 2019). Flavonoids are the most abundant class in different *Bignonia* plants and are contained in the roots and leaves. *Tridax procumbens* L. (*Tridax*) is a flowering plant belonging to the Asteraceae family that has been traditionally in use from ancient times (Beck et al. 2018). *Tridax* has various pharmacological properties including immunomodulatory, antioxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antimicrobial activities. Flavonoids are found in the leaves and other organs. Different substances such as powdered leaves, decoctions, oils, teas, leaf juice, skin cataplasms have been produced using this species (Beck et al. 2018). Flowers, leaves and roots of various *Primula* species contain different flavonoids (Colombo et al. 2014; Jäger et al. 2006). *Primula* flower and root preparations, which are usually available as herbal tea to be drunk and in liquid forms to be taken by mouth are approved by EMA and can be used for coughs associated with colds (Web reference 1; Web reference 2). Flowers, leaves and roots are used in Danish folk medicine against convulsions (Jäger et al.

2006). 7,8-DHF was identified from 2,000 biologically active compounds through the screening of the Spectrum Collection Library as a high affinity TrkB agonist, that provokes dimerization and phosphorylation of TrkB, and activation of downstream signaling cascades (Jang et al. 2010). A structure-activity relationship study suggests that the catechol group (7,8-dihydroxy on the A ring) is indispensable for the agonistic activity, in particular the 8-hydroxy group in A ring is essential for the TrkB stimulatory effect (Liu et al. 2010).

### 3.1.2 Pharmacokinetics

The *in vivo* investigation of 7,8-DHF pharmacokinetics profile determined that the plasma half-life of 7,8-DHF in mice, after oral gavage of 50 mg/kg, was about 134 min. The plasma concentration peaked at 10 min with 70 ng/ml and was still detectable after 8 h (5 ng/ml). 7,8-DHF was able to cross the BBB and peaked at 10 min with concentration of 50 ng/g of brain, at 4 h its concentration was 7 ng/g and at 6 h was below the quantification limits (Liu et al. 2013). Catechol-containing compounds are usually cleared in the circulatory system and undergo oxidation and conjugation (glucuronidation, sulfation and methylation). From a chromatographic analysis of plasma samples, metabolites were detected in addition to the parent drug, including 7-methoxy-8-hydroxyflavone (7M8H-flavone), 7-hydroxy-8-methoxyflavone (7H8M-flavone) and the glucuronidated product of 7,8-DHF (Liu et al. 2013). In plasma, 7M8H-flavone was detected after 3 min, its concentration peaked at 10 min (1.0 ng/ml), decreased to 0.2 ng/ml at 4 h and was below the quantification limits at 6 h (Liu et al. 2013). In brain samples, 7M8H-flavone was below the limit of quantification, whereas the 8M7H-flavone was observable 3 min after oral administration and still detectable at 240 min (Liu et al. 2013). The *in vivo* agonistic effects exerted by 7,8-DHF are thought to mainly result from the activity of 7,8-DHF and its metabolite 8M7H-flavone (Liu et al. 2013). Indeed, Catechol-O-methyltransferase (COMT) inhibition reduced the TrkB activation exerted by 7,8-DHF, indicating that the methylated metabolite contributes to TrkB receptor activation after oral administration (Liu et al. 2013). A study in monkey plasma showed that 7,8-DHF has longer half-life in monkeys compared to mice. The half-life of 7,8-DHF was approximately 4-8 h during chronic oral treatment with 30 mg/kg/day (He et al., 2016). The study of 7,8-DHF metabolism in monkeys confirmed the methylation, glucuronidation and sulfation clearance mechanisms observed in mice (He et al. 2016; Sun et al. 2017).

### 3.1.3 Bioavailability

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3 Oral administration of 7,8-DHF in mice elicits robust TrkB activation in the brain, suggesting that this  
4 compound is orally-bioavailable (Liu et al. 2010). Both the methylated metabolites of 7,8-DHF are able to  
5 activate TrkB in mouse brain and in primary neurons (Liu et al. 2013). However, the catechol group makes 7,8-  
6 DHF labile for fast metabolism, including sulfation, glucuronidation, methylation. Growing evidence suggests  
7 that intestinal microbiota increase the bioavailability and physiological function of dietary polyphenols.  
8 These metabolites may have larger bioavailability and exert larger biological effect than the parent compound  
9 (Murota, Nakamura, and Uehara 2018). No specific evidence exists regarding the role of gut microbiota in the  
10 metabolism of 7,8-DHF. However, it is conceivable that bioactive phenolic acids derived from the  
11 catabolism of 7,8-DHF elicited by gut microbiota may increase the biological activity of 7,8-DHF and that  
12 interpersonal variability in the microbiota composition may underlie different biological responses following  
13 oral administration of 7,8-DHF. Moreover, an O-methyltransferase SpOMT2884, originating from  
14 *Streptomyces peucetius* ATCC 27952 has been shown to convert 7,8-DHF into 7-hydroxy-8-methoxyflavone in  
15 an *in vitro* system (Koirala et al. 2014). This metabolite is more stable than 7,8-DHF and exhibits potent  
16 antioxidant effects without inducing cytotoxicity. Interestingly, *Streptomyces* has been shown to be present in  
17 gut microbiome (Bolourian, and Mojtahedi 2018), suggesting that these and possibly other gut bacteria may  
18 impact on the bioavailability and activity of 7,8-DHF. Several prodrugs of 7,8-DHF have been synthesized and  
19 tested in order to improve the bioavailability of 7,8-DHF. A recent study identified the compounds R13  
20 and R7 as the prodrugs with the best pharmacokinetic profile (Chen et al. 2018). R13 improved the oral  
21 bioavailability from 4.6% to 10.5% (Chen et al. 2018) and R7 from 4.6% to 82.2% (Liu et al. 2013). R13  
22 appeared to be the prodrug with the best pharmacokinetics profile. Accordingly, it was found to be readily  
23 hydrolyzed into 7,8-DHF in liver microsomes and following oral administration of 78 mg/kg of R13 (equal to  
24 50 mg/kg of 7,8-DHF) the concentration of 7,8-DHF in the mouse brain was significantly greater (>8 ng/gr  
25 even after 4 h) than the parent compound DHF (below the quantification limit at 6 h) (Chen et al. 2018).

### 3.1.4 Toxicity

Exposure to 7,8-DHF did not impair viability of the human kidney cell line HK-2 up to a concentration of 200  $\mu$ M (Ma et al. 2016). Accordingly, an  $IC_{50} > 100 \mu$ M has been established for cytotoxicity in HepG2 cells exposed for 24 h to 7,8-DHF (Kozics, Valovicova, and Slamenova 2011). Neuronal cell death assay revealed

that 7,8-DHF is non-toxic for primary cortical neurons up to 5  $\mu$ M (Liu et al. 2010). In mice treated with 5 mg/kg/day of 7,8-DHF for 3 weeks no pathological changes were found in the kidney, liver, lung, muscle, spleen, cortex, hippocampus, heart, intestine and testis in comparison with the vehicle treated counterparts (Liu et al. 2013). Moreover, from complete blood count analysis no significant difference appeared between 7,8-DHF-treated and control mice. This is consistent with another long-term experiment where female mice receiving 7,8-DHF (~2.4 mg/kg/day) for 20 weeks displayed normal complete blood count values (Chan et al. 2015). In addition, a study in monkeys showed that chronic treatment with 7,8-DHF for 7 months with a dose of 30 mg/kg/day, a dose higher than the one commonly used in rodents (5 mg/kg), did not cause toxicity (assessed by evaluating hematology and liver, heart, muscle, kidney and pancreas histology) and health problems (He et al. 2016). Taken together, these data suggest that chronic treatment with 7,8-DHF does not exert toxic effects.

### **3.2 7,8-DHF signaling**

#### **3.2.1 TrkB-dependent signaling**

7,8-DHF specifically binds the TrkB (but not TrkA or TrkC) receptor extracellular domain with a binding constant  $K_d = 320$  nM, acting as a selective TrkB agonist and mimicking the physiological actions of BDNF (Jang et al. 2010; Liu et al. 2014). 7,8-DHF appears, however, to bind to a different site of TrkB extracellular domain in comparison with BDNF (Liu et al. 2013). Similarly to BDNF, 7,8-DHF induces phosphorylation of TrkB at Tyr515, Tyr706, and Tyr816 although 7,8-DHF-induced phosphorylation of Tyr515 and Tyr816 is less robust in comparison with that induced by BDNF (Liu et al. 2014). 7,8-DHF-mediated TrkB phosphorylation leads to activation of the MAPK, PI3K/Akt, and ERK1/2 pathways in a time frame that is comparable to BDNF and in a dose-dependent manner (Jang et al. 2010; Liu, Chan, and Ye 2016; Liu et al. 2010; Liu et al. 2014; Liu et al. 2013). Internalization of the neurotrophin-Trk complex, leading to formation of signaling endosomes and ubiquitination/degradation, plays an important role in signal transduction. While BDNF is more potent than 7,8-DHF in stimulating TrkB internalization and early endosomes delivery in the first 10 min, 7,8-DHF produces a more robust endocytic response than BDNF at 60 min after stimulation. BDNF induces TrkB ubiquitination 10 min after stimulation and its ubiquitination signals correlates with its phosphorylation pattern. While BDNF-triggered TrkB phosphorylation peaks at 10 min, decreases at 60 min and fades away at 180 min, 7,8-DHF-triggered TrkB phosphorylation lasts for more than 3 h, without

inducing TrkB ubiquitination or degradation (Liu et al. 2014). These findings support the idea that 7,8-DHF and BDNF activate TrkB with different mechanisms and with a different time-course.

### 3.2.2 TrkB-independent signaling

A recent study in cell assays failed to confirm that 7,8-DHF activates the TrkB receptor (Boltaev et al. 2017). Further studies are needed to explain this discrepancy which may be due to the used cell model, culture condition and duration of treatment. In any case, the possibility must be taken into account that 7,8-DHF may induce transactivation of the TrkB receptor without the need to binding to it. Indeed, some studies show that some effects (in particular, antioxidant effects) of 7,8-DHF do not require the TrkB receptor as they are generated in conditions in which the receptor is pharmacologically inhibited or in cells that do not express the TrkB receptor (Garcia-Diaz Barriga et al. 2017; He et al. 2018; Huai et al. 2014; Ryu et al. 2014a). In addition, there is evidence for uptake of various flavonoids and their metabolites into the cytosol of several cell types, including cells of the central nervous system (CNS) (Ferrara, and Thompson 2019; Mukai et al. 2011; Spencer, Abd-el-Mohsen, and Rice-Evans 2004). If this also holds for 7,8-DHF, its cell uptake will provide the possibility of a TrkB-independent direct modulation of signaling pathways such as the MAPK or PI3K/Akt pathway (Williams, Spencer, and Rice-Evans 2004). In addition, being an antioxidant, 7,8-DHF might exert a direct antioxidant action within the cell which may contribute to its powerful antioxidant effects (Han et al. 2014).

## 4. ACTIONS OF 7,8-DHF IN THE CENTRAL NERVOUS SYSTEM

BDNF is present in almost all brain regions and is involved in a number of biological functions that include regulation of neurogenesis, gliogenesis, synaptogenesis, neuroprotection, synaptic plasticity, memory and cognition (see (Kowianski et al. 2018)). In view of its multifaceted roles, it is not surprising that BDNF dysregulation is involved in the pathogenesis of a plethora of brain disorders. Although BDNF may represent a beneficial therapeutic agent against these disorders, its poor brain delivery and short half-life represent a serious issue. In view of its chemical and functional properties (Liu, Chan, and Ye 2016), however, 7,8-DHF may represent a key therapeutic alternative to the use of BDNF. In this section we will review preclinical studies showing that 7,8-DHF exerts a myriad of benefits in the CNS.

### 4.1 Genetic disorders

#### 4.1.1 Down syndrome

**Brief premise.** Down syndrome (DS) is a genetic pathology due to triplication of chromosome 21. The brain of individuals with DS is hypotrophic starting from fetal life stages due to neurodevelopmental defects that include neurogenesis reduction, dendritic pathology, and altered brain wiring (Stagni et al. 2018). These defects underlie the typical cognitive impairment in DS. In spite of numerous preclinical studies (Stagni et al. 2015), no effective treatment is currently available for DS. Reduced BDNF levels have been found in the DS brain (see (Stagni et al. 2017)), suggesting that this defect may concur to alter brain development in DS. Based on this premise, three studies have explored the effects of 7,8-DHF in the Ts65Dn mouse model of DS.

**The evidence.** Stagni et al. (Stagni et al. 2017) treated Ts65Dn mice with 7,8-DHF in the early postnatal period, a critical time window for hippocampal development. Pups treated with 7,8-DHF from P3 to P15 underwent an improvement in hippocampal neurogenesis, rescue of total granule cell number, granule neuron dendritic spine density, and hippocampal synaptophysin levels. These effects were accompanied by an increase in hippocampal p-TrkB (Y816) and p-ERK1/2 levels. Mice treated with 7,8-DHF from infancy to adolescence (P3-P45) exhibited full rescue of learning and memory (L&M) (Morris Water Maze test, MWM). A second study (Giacomini et al. 2019) showed that the positive effects of treatment during infancy on hippocampal development did not translate into a behavioral benefit in adulthood if treatment was discontinued, indicating the necessity of chronic administration of 7,8-DHF. This study additionally shows that in 4-month-old mice treatment with 7,8-DHF for 40 days with the same dose that was effective in pups had no effect on neurogenesis, L&M (MWM) and hippocampal p-TrkB (Y816) levels. At variance with this latter study, Parrini et al. (Parrini et al. 2017) showed that adult Ts65Dn mice treated with 7,8-DHF for 4 weeks underwent restoration of hippocampal neurogenesis, LTP in field CA1, memory (contextual fear conditioning, CFC; Novel Object recognition, NOR) and an increase in hippocampal p-TrkB levels. This discrepancy may be explained by the higher dose of 7,8-DHF (~ 22 mg/kg) used by Parrini et al., while Giacomini et al. used a dose of 5 mg/kg.

**Significance.** These studies show that 7,8-DHF fully restores hippocampal development and cognitive performance if administered during early life stages. Its effects, however, are ephemeral suggesting that continuous administration is needed. This may be feasible in view of the lack of toxicity of 7,8-DHF. Thus,



in view of its safe profile, treatment with 7,8-DHF may represent a promising therapeutic opportunity for children with DS.

#### 4.1.2 Rett syndrome

**Brief premise.** Rett syndrome (RTT), is a rare disease characterized by intellectual disability and a host of neurological hallmarks that include stereotypic hand movements, impaired motor coordination, autonomic dysfunction, seizure and loss of language skills. RTT is due to loss of function of a gene encoding the transcription factor methyl-CpG binding protein 2 (MECP2). MECP2 is a transcription regulator of several genes, including BDNF (Li, and Pozzo-Miller 2014). Accordingly, strategies for improving BDNF signaling appear to exert some benefit in RTT mouse models (Li, and Pozzo-Miller 2014).

**The evidence.** A single study examined the effect of treatment with 7,8-DHF in a model of RTT (Johnson et al. 2012). *Mecp2* mutant mice that were treated with 7,8-DHF for one month lived significantly longer in comparison with untreated mice, displayed a delay in weight loss, an improvement in the size of hippocampal CA1 neuronal nuclei, in voluntary locomotor distance (wheel-running activity) and in the breathing irregularity that is typical of RTT.

**Significance.** The effects of 7,8-DHF in the *Mecp2* mutant mouse mimic some of the effects obtained by manipulating BDNF levels in RTT models (Li, and Pozzo-Miller 2014). This predicts the potential usefulness of treatment with the BDNF mimetic 7,8-DHF in RTT patients to compensate for the loss of BDNF expression. Further evidence, however, is necessary to confirm and better characterize the efficacy of 7,8-DHF in RTT.

#### 4.1.3 Fragile X syndrome

Fragile X syndrome (FXS) is characterized by moderate-to-severe intellectual disability. It is caused by silencing of the fragile X mental retardation 1 (*FMR1*) gene with consequent absence/reduction of the fragile X mental retardation protein (FMRP), an RNA-binding protein that regulates the translation of neuronal mRNAs at synapses (Kumari, and Gazy 2019). The absence of FMRP causes abnormal dendritic spine formation and altered synaptic plasticity. Absence of FMRP reduces BDNF expression and hampers TrkB-mediated protein synthesis (see (Tian et al. 2015)) suggesting that BDNF-TrkB signaling may play a role in the pathophysiology of FXS.

**The evidence.** Tian et al. (Tian et al. 2015) tested the effects of 7,8-DHF in the *Fmr1* KO mouse model of FXS. Weaning mice that received 7,8-DHF for 4 weeks exhibited restoration of L&M (MWM, CFC), and of the aberrantly high spine density in neurons of field CA1, and reinstatement of p-TrkB (Y816) levels and downstream targets (p-CaMKII and p-PKC $\alpha$ / $\beta$ II) in synaptosomes of the hippocampus and amygdala. Moreover, treatment enhanced the phosphorylation of GluA1- and GluA2-containing AMPARs. In a recent study, Seese et al. (Seese et al. 2020) examined the effect of 7,8-DHF in adult *Fmr1* KO mice. In hippocampal slices from *Fmr1* KO mice exposure to 7,8-DHF normalized the reduced magnitude of LTP. In *in vivo* experiments, mice were acutely (one injection), semi-chronically (4 days) or chronically (one month) treated with 7,8-DHF. Irrespective of its duration, treatment rescued spatial memory (object location memory). In addition, a single injection of 7,8-DHF prior to training was sufficient to rescue visual recognition memory (NOR). Finally, acute treatment with 7,8-DHF increased activation of TrkB (Y515) in stratum radiatum of field CA1.

**Significance.** These two studies demonstrate that 7,8-DHF improves synaptic structure, hippocampal synaptic plasticity and memory in a model of FXS. Interestingly, a single administration of 7,8-DHF is sufficient to restore LTP and spatial/object memory, which suggests a fast restoring effect of the synaptic plasticity alterations that characterize FXS. While these results are encouraging in the framework of potential therapies for individuals with FXS, the duration of the beneficial effects of treatment on synaptic functioning and cognitive performance remains a key unanswered issue.

## 4.2 Psychiatric disorders

### 4.2.1 Autism

**Brief premise.** Autism Spectrum Disorder (ASD) is a developmental disorder characterized by deficits in social behavior, such as inability to initiate social interactions or develop relationships, lack of social or emotional reciprocity, lack of interest in the emotions of others and communication deficits (Lai, Lombardo, and Baron-Cohen 2014). Various animal models incorporating ASD-relevant behavioral phenotypes have been developed, to identify the alterations underlying this disorder and to test possible pharmacotherapies.

**The evidence.** Rhine et al. (Rhine et al. 2019) studied the effects of different drugs, including 7,8-DHF, on the behavioral phenotype of two autism mouse models, BTBR and Shank3B. Mice received 7,8-DHF 5 or 7 days before a battery of behavioral tests and on each testing days (60 min before the start). BTBR mice

showed restoration of sociability (three-chamber test and social sniffing) and Shank3B mice showed an improvement in learning but not memory (MWM). The effect of 7,8-DHF was tested in the vaccinia related kinase 3 (*Vrk3*) KO mouse model generated by Kang et al. (Kang et al. 2017). The *Vrk3*-KO mouse shows unusual social interactions and repetitive behaviors similarly to autism subjects. Acute treatment with 7,8-DHF restored hippocampal levels of p-TrkB (Y706) and social interaction (three-chamber test) but the latter effect was not retained at 7 days after treatment cessation. Chronic treatment restored spine density in CA1 pyramidal neurons and suppressed elevated grooming activity, improved abnormal social activity and L&M (passive avoidance and NOR). These behavioral effects were retained for several weeks after treatment cessation. Lee (Lee, and Han 2019) induced an autistic-like behavior in a heterozygous mouse for the D2 receptor by administering an early-life stress (through maternal separation) to the pups. A three-day treatment with 7,8-DHF increased social interactions (three-chamber test), suppressed increased grooming and increased the expression of *TrkB*, *Bdnf*, *Mecp2* and *Hdac2* genes in the dorsal striatum. Inhibition of TrkB in the dorsal striatum during treatment with 7,8-DHF by injections of TrkB-siRNA prevented the treatment-induced behavioral rescue.

**Significance.** ASD is heterogeneous in etiology (interaction between environmental factors and genetic variants) and symptoms. Thus, ASD models can only approximate the complexity of the human condition. Yet, the studies reviewed above suggest that 7,8-DHF could be a pharmacological strategy to ameliorate the defects in social behavior, a common central feature of ASD.

#### 4.2.2 Schizophrenia

**Brief premise.** Schizophrenia, a complex psychiatric disorder that arises from genetic susceptibility and environmental factors, is characterized by a combination of psychotic symptoms and cognitive deficits such as impairments in L&M, attention, and executive functions. Reduced BDNF levels in serum (Toyooka et al. 2002) and reduced expression of BDNF in multiple cortical areas (Ray, Shannon Weickert, and Webster 2014) have been found in subjects with schizophrenia suggesting that impairment of BDNF-TrkB signaling may contribute to the cognitive deficits that characterize this pathology.

**The evidence.** Yang et al. examined the effects of 7,8-DHF in a rat model of schizophrenia obtained by blocking NMDA receptors through administration of MK-801 during the neonatal period (P7-P10) (Yang et al. 2014b). At 2 months of age these rats were treated with 7,8-DHF for 14 days. Treatment restored LTP in

field CA1, learning performance (MWM), hippocampal levels of p-TrkB (Y515 and 816), p-ERK1/2, p-CaMKII, p-CREB, and p-GluR1. Since gestational exposure to infection appears to be involved in the etiology of schizophrenia, maternal immune activation can be used to obtain animal models of schizophrenia. In the first of three studies, Han et al. treated pregnant mice with polyriboinosinic-polyribocytidylic acid [poly(I:C)], a potent inducer of immune response (Han et al. 2016). At 4 weeks of age, the offspring was treated with 7,8-DHF for 4 weeks and then behaviorally tested. Mice exposed to poly(I:C) exhibited cognitive deficits (NOR), PPI deficits of the acoustic startle response, reduced levels of BDNF and p-TrkB (Y706) in the prefrontal cortex (PFC) and field CA1, and reduced parvalbumin and peroxisome proliferator-activated receptor- $\gamma$  coactivator 1- $\alpha$  (PGC-1 $\alpha$ ) immunoreactivity in the PFC and CA1. All these defects were completely restored in mice treated with 7,8-DHF. In view of the association between increased circulating levels of complement protein C1q and schizophrenia, a subsequent study investigated the effect of the same treatment schedule on C1q levels in adult offspring (Han, Zhang, and Hashimoto 2017). Treatment with 7,8-DHF restored the excessive C1q levels in the PFC, suggesting a positive impact of 7,8-DHF on the immune system. A further study was aimed at investigating whether supplementation of 7,8-DHF from pregnancy to weaning can prevent the onset of cognitive deficit (Han et al. 2017). Pregnant mice, treated with poly(I:C) from E12 to E17, received 7,8-DHF in drinking water from embryonic day E12 to 3-weeks after delivery. An evaluation of the effects of 7,8-DHF in offspring aged 12 weeks showed that 7,8-DHF prevented the onset of cognitive defects (NOR) and restored the reduced levels of BDNF and p-TrkB (Y706) in the PFC.

**Significance.** These studies show that 7,8-DHF improves the cognitive defects associated with schizophrenia through activation of TrkB signaling. One explanation for the development of schizophrenia is that the disorders begins in utero following infections, obstetric complications or excess stress levels. These preclinical studies suggest that supplementation with 7,8-DHF during pregnancy may prevent the subsequent transition to schizophrenia in adulthood. Further studies are needed to confirm the preventive effect of 7,8-DHF in other models of fetal disturbances and to better characterize the molecular mechanisms whereby 7,8-DHF exerts its positive effects.

#### 4.2.3 Major depressive disorder

**Brief premise.** Major depressive disorder (MDD) represents the most common and debilitating of psychiatric disorder, affecting 17% of the population at some point in their lifetime. Despite the current plethora of antidepressant therapies, approximately two third of patient with depression fail to respond to first-line antidepressant treatments. Depressed patients exhibit low circulating levels of BDNF (Sen, Duman, and Sanacora 2008) and a postmortem study found that patients with a history of major depression who had committed suicide had reduced levels of BDNF and TrkB in the hippocampus and PFC, two brain regions essential for mood regulation and cognitive function (Dwivedi et al. 2003). Accumulating evidence suggests that enhancement of BDNF-TrkB signaling mediates the therapeutic effects of antidepressant drugs and that a BDNF-dependent increase in hippocampal neurogenesis plays a key role in the beneficial effects of antidepressants. Accordingly, ablation of TrkB in hippocampal neural progenitor cells prevented antidepressant-induced neurogenesis and rendered mice behaviorally insensitive to chronic antidepressant treatment (Li et al. 2008). Moreover, in accordance with this hypothesis, intracerebral administration of BDNF has been shown to induce long-lasting antidepressant-like effects in models of depression (Hoshaw, Malberg, and Lucki 2005).

**The evidence.** Based on these lines of evidence, Liu et al. sought to establish whether 7,8-DHF elicits potential antidepressant effects, similarly to BDNF (Liu et al. 2010). In mice treated with 7,8-DHF for 21 days, the swimming immobility time during the forced swim test (FST) was significantly decreased, suggesting an antidepressant effect. This effect was associated with an increase in neurogenesis in the dentate gyrus (DG). This study also used the *TrkB*<sup>F616A</sup> knockin mouse because this mouse harbors a *TrkB* F616A mutation which allows blocking of TrkB receptor kinase activity by the inhibitor 1NMPP1. *TrkB*<sup>F616A</sup> mice treated with 1NMPP1 one day before administration of 7,8-DHF for 5 days, did not show any reduction in the swimming immobility in the FST, suggesting that the antidepressant effects of 7,8-DHF are mediated by TrkB-dependent signaling. A subsequent study showed that the 7,8-DHF methylated metabolite 7M8H-flavone promoted hippocampal neurogenesis and displayed antidepressant effect (FST and tail suspension test, TST) by activating the TrkB receptor (Liu et al. 2013). Based on evidence that inflammatory processes have a role in the pathophysiology of MDD, a study examined potential antidepressant effects of 7,8-DHF in an inflammation-related mouse model of depression (Zhang et al. 2014a). In this model, systemic administration of the bacterial endotoxin lipopolysaccharide (LPS) induced depression-like behavior

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3 resulting from activation of pro-inflammatory cytokine signaling and decreased BDNF and p-TrkB (Y706)  
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5 levels in CA3, DG and PFC. These mice were injected with 7,8-DHF 23 h after LPS administration. 7,8-  
6  
7 DHF restored the LPS-induced reduction in spine density of pyramidal neurons in CA3, DG, and PFC,  
8  
9 normalized p-TrkB levels in all these regions and improved depression-like behavior in the FST and TST.  
10  
11 The effects of 7,8-DHF on LPS-induced depressive behavior were blocked by pretreatment with the TrkB  
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13 antagonist ANA-12. The antidepressant effect of 7,8-DHF was also examined in the social defeat stress  
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15 model of depression (Zhang et al. 2015). Mice that were identified as susceptible to social defeat stress,  
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17 received a single injection of 7,8-DHF. Treatment elicited antidepressant effects immediately after 7,8-DHF  
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19 administration (FST and TST). In addition, 7,8-DHF restored the reduced levels of the synaptic markers  
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21 PSD-95 and GluA1 in CA3, DG, and PFC at 4 but not 8 days after the injection, suggesting that a single dose  
22  
23 of 7,8-DHF elicits a rapid but not sustained antidepressant response. The effects of a single bilateral infusion  
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25 of 7,8-DHF into specific brain regions (medial PFC, CA3 or DG) were investigated in a learned helplessness  
26  
27 (LH) rat model of depression (Shirayama et al. 2015). LH rats that received a microinjection of 1.0 pmol 7,8-  
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29 DHF into the PFC, CA3 or DG underwent an improvement in the conditioned avoidance test, whereas  
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31 injections of 0.1 pmol did not exert any antidepressant effects, suggesting that the dose of 7,8-DHF is critical  
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33 for inducing antidepressant effects. Zhang et al. investigated the effects of 7,8-DHF in a chronic mild stress  
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35 model of depression (Zhang et al. 2016). In this model, depressive-like state develops gradually over time in  
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37 response to stressful conditions, recapitulating the human condition. Mice were exposed to various mild  
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39 stresses (e.g. food or water deprivation) before and during treatment with 7,8-DHF for 28 days. 7,8-DHF  
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41 reverted depressive-like behavior assessed with sucrose preference test (SPT) and novelty suppressed  
42  
43 feeding test, restored levels of synaptophysin and PSD95 in the PFC and normalized the reduced levels of  
44  
45 BDNF and p-TrkB (Y515). These effects were abolished by pretreatment with the TrkB antagonist K252a.  
46  
47 Consistently with the study in mice (Zhang et al. 2016), 7,8-DHF reverted depressive-like behaviors in rats  
48  
49 exposed to chronic mild stress for 8 weeks and treated with 7,8-DHF during the last 4 weeks (Chang et al.  
50  
51 2016). The therapeutic impact of 7,8-DHF alone or in combination with the antidepressant fluoxetine was  
52  
53 investigated in a model of chronic unpredictable mild stress during the perimenopausal period, when the  
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55 possibility to develop depression increases due to a reduction in levels of the estrogen hormone (Amin et al.  
56  
57 2020). Adult C57BL/6J female mice were first subjected to bilateral ovariectomy and then were exposed to a  
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battery of stressors in combination with drugs administration (7,8-DHF or 7,8-DHF+fluoxetine 18/mg/kg/day) for 28 days. 7,8-DHF alone or in combination with fluoxetine improved in a similar manner depressive-like behavior (FST and SPT). The combined treatment, however, was more effective in reducing NF-kB and inducible nitric oxide synthase (iNOS) levels (markers of brain inflammation), increasing the reduced number of neurons and microglia and reverting the downregulation of BDNF/TrkB/ERK1/2 and PI3K/Akt/mTOR signaling in comparison with treatment with 7,8-DHF alone.

**Significance.** These studies show that 7,8-DHF has beneficial effects in different models of depression suggesting that it may be considered as a new therapeutic agent for MDD of different etiology and may represent an effective treatment for patients resistant to first-line antidepressant treatments. These findings additionally indicate that activation of TrkB and its downstream signaling cascade in the hippocampus and PFC play a crucial role in mediating the antidepressant effects of 7,8-DHF.

#### 4.2.4 Anxiety and Stress

**Brief premise.** Anxiety and stress disorders are among the most prevalent neuropsychiatric disorders. Anxiety disorders may fall along a continuum that ranges from specific fear-based reactivity to more diffuse and prolonged stress (Duval, Javanbakht, and Liberzon 2015). Based on the notion that BDNF expression is affected by stress, a few studies have explored the effect of 7,8-DHF in anxiety/stress-models.

**The evidence.** In a first study, Andero et al. (Andero et al. 2011) examined the effects of 7,8-DHF on the acquisition and extinction of cue-dependent conditioned fear. Mice were subjected to cued fear conditioning (a tone followed by a foot shock) and the expression of fear (mean percentage of time spent freezing) was assessed after 24 h. Mice injected with 7,8-DHF 1 h prior to fear conditioning showed increased freezing, indicating an enhancement of fear learning. On the next day, mice were given 7,8-DHF 1 h prior to extinction training. These mice froze less than did the vehicle group indicating enhancement of extinction. A similar treatment-induced enhancement of extinction was also obtained in mice subjected to immobilization stress (a putative model of post-traumatic stress disorder) before the cued-fear conditioning protocol. In a second study, Andero et al. (Andero et al. 2012) analyzed the effects of 7,8-DHF on spatial memory in rats exposed to a traumatic stressor event consisting in immobilization for 2 h. Rats received 7,8-DHF 2 h prior or 8 h after termination immobilization and L&M were assessed after three days with the MWM test. Treatment with 7,8-DHF either before or after the stressor event prevented the spatial memory deficit caused

by stress. Choi et al. (Choi, Gourley, and Ressler 2012) used BDNF-floxed mice to knockdown *Bdnf* bilaterally in the prelimbic cortex and examined the outcome in a cue-dependent fear conditioning protocol. While untreated *Bdnf* knockdown mice exhibited a reduced expression of learned fear, mice injected with 7,8-DHF 24 h after fear conditioning training expressed fear similarly to the control mice. TrkB<sup>F616A</sup> mice that received an infusion of the TrkB inhibitor 1NMPP1 in the prelimbic cortex before fear conditioning training failed to show fear learning, confirming the role of TrkB signaling in this region in this behavior. Sanz-Garcia et al. (Sanz-Garcia et al. 2016) examined the effect of 7,8-DHF in rats exposed to an immobilization stress for 2 h. At 2 days after the stressor, rats that had received a single dose of 7,8-DHF 8 h after the stress exhibited an improvement in the stress-induced impairment of spatial memory (MWM) and normalization of the stress-induced increase in LTP in field CA1. Barfield et al. (Barfield et al. 2017) exposed adolescent mice to the stress hormone cortisol from P31 to P47. As adults, these mice exhibited reduced spine density in the medial PFC, increased habit behavior (i.e. lack of goal-directed behavior) in a test of instrumental contingency degradation, and increased immobility in the FST, a typical stress-related depressive behavior. In cortisol-treated mice, co-treatment with 7,8-DHF (from P39 to P47) blocked cortisol-induced habit biases and reduced the immobility time in the FST. In addition, 7,8-DHF blocked the p-ERK42 deficit due to cortisol exposure and increased the levels of PSD95 in the ventral hippocampus. The same group studied the effects of 7,8-DHF on behavioral flexibility after adolescence exposure to cortisol (Barfield, and Gourley 2017). Male and female mice treated with cortisol from P31 to P42 were tested at P56 with an instrumental reversal learning task, a task commonly used to model cognitive stress related deficits. Cortisol-exposed females, but not males, exhibited perseverative errors. Co-treatment with 7,8-DHF in the period P39-47 eliminated cortisol-induced errors in females and improved the performance in males.

**Significance.** Although the issue as to whether animal species can really be used as “models” of anxiety and stress, given the highly subjective nature of these emotions, they may help predict the efficacy of pharmacological treatments. These studies suggest that 7,8-DHF may represent a potential treatment for the prevention/improvement of stress-related memory alterations.

### 4.3 Addiction

#### 4.3.1 Drug abuse



**Brief premise.** Abuse of substances such as cannabis, cocaine, and amphetamine-type stimulants has severe effects on brain and behavior. Both reward seeking and inhibitory control (that requires behavioral flexibility), contribute to drug abuse vulnerability (Perry et al. 2011). All addictive drugs have in common that they enhance dopaminergic reward synaptic function in the nucleus accumbens (Gardner 2011). Preclinical and clinical studies have attempted to cure drug addiction by pharmacologically targeting addiction-related systems in the brain (Liu, and Li 2018). BDNF plays roles in many types of plasticity including drug addiction. Physical exercise, a condition that is known to increase BDNF levels, appears to exert some benefit (Lynch et al. 2013), However, it must be underlined that mixed results are reported regarding the role of BDNF in drug-seeking behavior in animals, as BDNF can either enhance reinstatement or have protective properties, being stage-dependent (Li, and Wolf 2015).

#### ***The evidence.***

**4.3.1.1 Cocaine.** Choi et al. (Choi, Gourley, and Ressler 2012) trained prelimbic cortex-specific *Bdnf* knockdown mice in a cocaine-conditioned place preference paradigm. These mice had attenuated preference for the cocaine-associated chamber. In contrast, mice receiving 7,8-DHF immediately post-training underwent restoration of cocaine-associated place preference suggesting a role of TrkB signaling in drug seeking behavior. A study in rats examined the effect of 7,8-DHF on extinction of drug seeking in a cocaine-induced conditioned place preference task (Otis, Fitzgerald, and Mueller 2014). Systemic injections of 7,8-DHF or infralimbic infusions of BDNF strengthened extinction of drug seeking whereas blockade of infralimbic TrkB receptors with the inhibitor ANA-12 disrupted consolidation of extinction. A recent study (DePoy, Allen, and Gourley 2016) sought to establish whether adolescent cocaine exposure has an effect on decision-making strategies in adulthood. Since males but not females with a history of escalating self-administration of cocaine developed a bias towards habit-based behaviors the rest of the study focused on females. In females, low, ‘stable’ cocaine-reinforced response rates during adolescence were associated with cocaine-conditioned context preference in adulthood. Administration of 7,8-DHF 60 min before the test mitigated context-elicited reward-seeking responses, suggesting a role of TrkB signaling in this behavior.

**4.3.1.2 3,4-Methylenedioxypyrovalerone.** A very recent study has examined the effects of 7,8-DHF on behavioral sensitization (progressive and enduring increase in the motor stimulant response to drugs) induced by MDPV, a popular and potent psychostimulant, or cocaine (Duart-Castells et al. 2019). Mice received

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3 either MDPV or cocaine for 5 days and their locomotor activity was examined during and 10 days after the  
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5 last drug administration. Both drugs enhanced locomotor activity and induced a higher locomotor response  
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7 after withdrawal (behavioral sensitization). Pretreatment with 7,8-DHF 30 min prior to MDPV injections  
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9 blocked MDPV behavioral sensitization and this effect was prevented by co-administration of the TrkB  
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11 receptor inhibitor ANA-12. In contrast, 7,8-DHF had no effect on cocaine-dependent behavioral  
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13 sensitization, suggesting involvement of a different signaling pathway.  
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16 *4.3.1.2 Methamphetamine.* Ren et al. examined the effect of 7,8-DHF on the prepulse inhibition (PPI) deficit  
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18 of the acoustic startle response caused by methamphetamine (METH) (Ren et al. 2013). While a single dose  
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20 of METH impaired PPI, administration of 7,8-DHF 30 min before METH improved PPI. This improvement  
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22 was prevented by co-administration of the TrkB inhibitor ANA-12. In addition, 7,8-DHF attenuated the  
23  
24 METH-induced increase in dopamine levels in the striatum, suggesting that 7,8-DHF may improve PPI  
25  
26 through inhibition of dopamine release. In a subsequent study, the same group examined the effects of 7,8-  
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28 DHF on the hyperlocomotion induced by METH (Ren et al. 2014). Pretreatment with 7,8-DHF, reduced the  
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30 hyperlocomotion induced by a single administration of METH, in a dose-dependent manner. Repeated  
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32 administration of METH (once daily for 5 days) caused an increase in hyperlocomotion after 7 days  
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34 (behavioral sensitization) that was attenuated by injection of 7,8-DHF before METH. In addition, 7,8-DHF  
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36 mitigated the METH-induced reduction in the expression of the dopamine transporter in the striatum and this  
37  
38 effect was prevented by the TrkB inhibitor ANA-12. In a third study the same group examined the role of  
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40 TrkB signaling on METH withdrawal symptoms (Ren et al. 2015). Mice were subjected to repeated  
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42 administration of METH (once daily for 5 days). During withdrawal (from day 12 to 25) mice received either  
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44 ANA-12 or 7,8-DHF. Depressive-like behavior (TST and FST) due to METH withdrawal on day 28, was  
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46 improved by ANA-12 but not by 7,8-DHF suggesting that increased BDNF–TrkB signaling may play a role  
47  
48 in the behavioral abnormalities during withdrawal and, consequently, that a BDNF mimetic such as 7,8-DHF  
49  
50 is not a suitable treatment for METH-induced depressive-like-behavior.  
51

52  
53 **Significance.** These studies show that 7,8-DHF may have a positive impact on some (e.g. extinction) but not  
54  
55 all addiction-related behaviors, which emphasizes the complexity of the BDNF-dependent mechanisms  
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57 underlying drug addiction and poses some caveats for the use of 7,8-DHF as pharmacological adjunct for  
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59 therapies against drug abuse.  
60

### 4.3.2 Alcohol abuse

**Brief premise.** Alcoholism is a brain disorder associated with compulsive and escalating consumption of alcohol. BDNF, in addition to being implicated in the neurobiology of drug addiction, appears to play a role in alcohol use disorders (Olsen et al. 2019; Pandey 2016). Decreased levels of BDNF in some brain areas, such as the medial PFC, striatum, hippocampus, and amygdala, are associated with excessive alcohol drinking behaviors and correction of the deficits in BDNF levels attenuates these behaviors, suggesting that BDNF is a negative regulator of alcohol consumption (Pandey 2016).

**The evidence.** Pandey et al. have examined the possible neuroprotective effect of 7,8-DHF on behavioral and neurochemical changes induced by alcohol and high fat diet (Pandey et al. 2020). Results of alcohol-treated rats only will be described here. Rats received alcohol in the drinking water for 12 weeks. Another group received in addition to alcohol 7,8-DHF during the last 4 weeks. The alcohol-induced impairment in hippocampus-dependent L&M (MWM) was improved by co-treatment with 7,8-DHF. In addition, 7,8-DHF restored the alcohol-induced increase in malondialdehyde (MDa) and nitrite hippocampal levels and the reduction in glutathione (GSH) levels, indicating amelioration of oxido-nitrosative stress. Finally, the alcohol-upregulated mRNA levels of NF- $\kappa$ B, iNOS, and caspase-3 (indicative of apoptosis) and downregulated levels of nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase 1 (HO-1; indicative of cellular resistance to oxidants) were normalized by 7,8-DHF. Hogarth et al. have explored the possibility that activation of the TrkB receptor *via* 7,8-DHF may modify voluntary alcohol intake reinstatement (Hogarth, Djouma, and van den Buuse 2020). Sprague Dawley rats were trained for about 7 weeks to self-administer through lever presses a 10% ethanol solution (acquisition). During extinction (removal of ethanol reward) animals were treated for two weeks with 7,8-DHF. Following extinction, both male and female rats exhibited alcohol-primed reinstatement (alcohol consumption). While this effect was not altered in male rats by pretreatment with 7,8-DHF, in female rats reinstatement was significantly greater following 7,8-DHF treatment compared to vehicle. Thus, in contrast with the hypothesis of this work, i.e. that 7,8-DHF-mediated TrkB activation would reduce alcohol reinstatement, 7,8-DHF had an opposite effect, at least in females.

**Significance.** These studies show that while 7,8-DHF may be of benefit for the protection of the brain from the anatomical and functional damages caused by alcohol, it appears to have a negative effect, at least

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3 regarding reinstatement of alcohol abuse in females. Further studies are necessary in order to delineate the  
4 potential benefits of 7,8-DHF as treatment for alcohol use disorders.  
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#### 7 **4.4 Brain injury**

##### 8 **4.4.1 Traumatic brain injury**

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11 **Brief premise.** Traumatic brain injury (TBI) results from a violent blow to the head or when an object pierces  
12 the skull and enters the brain. The pathogenesis of TBI results from primary and secondary injuries that  
13 induce temporary or permanent neurological deficits (Galgano et al. 2017; Prins et al. 2013). The  
14 primary injury is due to the mechanical impact of the brain. The secondary injury consists in  
15 neurochemical alterations that include changes in glucose cerebral metabolism, energy crisis  
16 with mitochondrial impairments, accumulation of free radicals, and inflammation (Galgano et al.  
17 2017; Prins et al. 2013). Numerous studies conducted in animal models of TBI sought to discover  
18 treatments that prevent further brain damage due to the secondary injury and promote functional recovery  
19 (Galgano et al. 2017). Commonly used model of TBI are the Fluid Percussion Injury (FPI) model based  
20 on hydraulically induced pressure transients on the brain and the Controlled Cortical Impact Model (CCI)  
21 that uses a rigid impactor to deliver mechanical energy. Various studies, reviewed below, have  
22 examined the potential benefit of 7,8-DHF in these models.  
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37 **The evidence.** Agrawal et al. examined the effects of 7,8-DHF on memory retention and brain energy  
38 homeostasis in a rat model of FPI (Agrawal et al. 2015). Rats were first trained (learning) on the Barnes  
39 maze test for 5 days and then subjected to FPI. In the following 7 days rats received 7,8-DHF or vehicle and  
40 then were tested by the Barnes maze for memory retention, their brains were then removed and used for  
41 various analyses. In rats treated with 7,8-DHF the memory impairment caused by TBI was fully rescued.  
42 Administration of the TrkB receptor inhibitor K252a prior to TBI prevented the memory rescue. Treatment  
43 with 7,8-DHF improved the TBI-induced reduction in the hippocampal expression of p-TrkB (Y816),  
44 counteracted the reduction of molecules associated with brain plasticity as p-CREB, growth associated-  
45 protein 43 (GAP-43), and syntaxin-3. In addition, 7,8-DHF normalized the levels of molecules involved in  
46 mitochondrial biogenesis as PGC-1 $\alpha$ , mitochondrial transcription factor 1 (TFAM), mitochondrial  
47 cytochrome oxidase II (COII) and brain energy metabolism as adenosine monophosphate-activated protein  
48 kinase (AMPK) and sirtuin 1 (SIRT1). A second study from the same group (Krishna et al. 2017) used a  
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substantially similar protocol but examined the effect of exercise and treatment with 7,8-DHF alone or in combination. Sprague-Dawley rats were subjected to FPI after a training of 5 days on the Barnes maze (learning). Thereafter rats received 7,8-DHF with or without voluntary wheel running for 7 days. The TBI-induced memory impairment and reduction in hippocampal p-TrkB levels were increased by treatment with either 7,8-DHF or exercise but were further increased by the combined application of 7,8-DHF and exercise. Likewise, the reduction in the levels of p-CREB, GAP-43, syntaxin-1, ~~PGC-1 $\alpha$~~  and AMPK was restored by treatment with either 7,8-DHF or exercise and this effect was enhanced by their combination.

Wu et. al. (Wu et al. 2014) examined the effects of 7,8-DHF in a mouse CCI model. Mice received 7,8-DHF 10 min after TBI injury and in the following 3 days. Treatment caused an improvement in various neurological tests, reduced the ipsilateral contusion volume, the number of degenerating neurons and apoptotic cells, the expression levels of apoptotic markers and counteracted the TBI-induced reduction in p-TrkB (Y705) and p-Akt, with no effect on p-ERK1/2 (suggesting activation of TrkB and downstream PI3K/Akt signaling). Intracerebroventricular administration of the TrkB receptor inhibitor K252a and the PI3K inhibitor LY294002 before TBI abolished the reduction in contusion volume induced by 7,8-DHF. Chen et al. (Chen et al. 2015) examined the effect of 7,8-DHF on the survival of hippocampal immature neurons in a mouse CCI model. Mice received 7,8-DHF 1 h prior to TBI injury and their brain was collected after 24 h. Treatment with 7,8-DHF reduced the number of dying neurons, increased the number of new granule neurons in the DG, and increased hippocampal levels of p-TrkB (Y816). Treatment with the TrkB inhibitor ANA12 before 7,8-DHF administration prevented the positive effects of 7,8-DHF on the number of dying neurons and of new granule neurons. Zaho et al. (Zhao et al. 2016b) examined the effect of 7,8-DHF on neurogenesis and dendritogenesis in the DG of a CCI mouse model. Mice received 7,8-DHF 1 h after TBI and in the following two weeks. Treated mice showed an increase in the number of new granule neurons, and an improvement in length and number of dendritic branches of the granule neurons. In a subsequent study (Zhao et al. 2016a), the same group analyzed the effect of 7,8-DHF on neocortical neurons of layer II/III in a CCI mouse model. Mice received 7,8-DHF 1 h after TBI and in the following 3 days. Treatment with 7,8-DHF did not ameliorate the TBI-induced cortical lesion. Treatment however, reduced dendritic swelling (indicative of degeneration) of layer II/III neurons, restored the number and length of apical and basal dendritic branches and the density of dendritic spines. Treated mice showed no improvement in L&M

(MWM) and a partial improvement in the rotarod test, indicating that the protective effects of 7,8-DHF on cortical neurons did not translate into a behavioral improvement.

**Significance.** Experimental studies in animal models of TBI have not been successfully translated into clinical therapies so far. The results obtained following treatment with 7,8-DHF in rat and mouse models of TBI show that treatment may be beneficial in terms of brain plasticity, energy homeostasis, protection from apoptosis and dendritic degeneration although its effects on behavior are very limited. It must be underlined that these studies used a relatively short period of treatment with 7,8-DHF (maximum 14 days). It remains to be established whether optimization of the treatment schedule (duration and doses) may have a larger impact on the damages caused by TBI.

#### 4.4.2 Stroke/Ischemia

**Brief premise.** Cerebral ischemia consists in a reduction in the flow to the brain causing reduction in the supply of glucose and oxygen. Ischemic stroke is due to the block of brain blood vessels by a clot formation, and hemorrhagic stroke is caused by the rupture of blood vessels and blood leakage. Stroke causes oxidative stress, excitotoxicity, inflammation and apoptosis. Various models of perinatal and adult ischemic stroke have been developed (Northington 2006; Sommer 2017). A widely used animal model of stroke consists in the permanent occlusion of the middle cerebral artery (MCAO). Another model is obtained with transient occlusion (ischemia) followed by reperfusion (I/R). In this model, the absence of oxygen during ischemia favors inflammation and oxidative stress when circulation is restored (reoxygenation injury). Another model that mimics neonatal hypoxia-ischemia (HI) is obtained by exposing pups to hypoxia after ligation of the common carotid artery which results in reproducible brain injury ipsilateral to the carotid ligation. Numerous preclinical studies in animal models of stroke have shown protective effects of various therapies, although most of the clinical trials do not have shown any success so far (Kaur, Prakash, and Medhi 2013).

**The evidence.** The first evidence regarding the effect of 7,8-DHF in stroke was obtained by Jang et al. (Jang et al. 2010) in the TrkB<sup>F616A</sup> knockin mouse. Mice were injected with 7,8-DHF 2 h before occlusion of the middle cerebral artery and their brains were removed after 2 days. Pretreatment with 7,8-DHF largely reduced the infarct volume and this effect was prevented in mice that had received 1NMPP1 1 day before MCAO. Based on the notion that perinatal ischemia in preterm infants mainly targets the oligodendrocyte lineage, Hung et al. (Hung et al. 2013) explored in a model of HI the effects of thyroxine (T4), a key hormone

for oligodendrocyte maturation and that is known to upregulate BDNF levels, and of 7,8-DHF. Rat pups underwent HI at P7 and received on P7, P9, and P11 either T4 or 7,8-DHF. T4 attenuated axonal injury, astrogliosis, and microgliosis, and increased preoligodendrocyte survival. 7,8-DHF, similarly to T4, improved the hypomyelination caused by ischemic injury. In the study by Uluc et al. (Uluc et al. 2013) mice pups subjected to HI received 7,8-DHF at 10 min after HI and for the following 1 or 6 days. At 72 h after HI mice exhibited an increase in the number of degenerating neurons in hippocampal field CA1. While treatment with 7,8-DHF had no effect in males, it reduced the number of degenerating cells in females. In both sexes, 7,8-DHF enhanced hippocampal p-TrkB levels but this increase was more pronounced in females. At 21 days after HI, motor learning was improved by 7,8-DHF in males and females in a similar manner. At 51 days after HI, spatial learning (MWM) was improved by treatment with 7,8-DHF in females but not in males. The study by Cikla et al. (Cikla et al. 2016) was prompted by evidence that the brains of male pups are more susceptible to perinatal hypoxia and ischemia. Pups underwent permanent occlusion of the left carotid, subjected to hypoxia and were treated with 7,8-DHF at 10 min after HI and in the following 2 days. While no sex differences were found in p-TrkB (Y705) levels in the hippocampus of sham mice, at 3 days post HI there was a higher hippocampal TrkB phosphorylation in 7,8-DHF-treated females compared with males, suggesting that this difference may account for the larger neuroprotection observed in females. Interestingly, following HI there was an increase in hippocampal ER $\alpha$  expression that was more pronounced in females. Estrogen receptor  $\alpha$  (ER $\alpha$ ) is coupled to Src family kinase (SFK) activation which, in turn, enhances the activation of the TrkB receptor, in part by direct phosphorylation of TrkB (Y705/Y706) and in response to ligands such as 7,8-DHF. Thus, the sexually differentiated phosphorylation of TrkB following HI and treatment with the TrkB agonist 7,8-DHF appears to be due to sex-related differences in the expression of ER $\alpha$ . In a study by Wang et al. (Wang et al. 2014) rats were subjected to MCAO for 90 min followed by reperfusion (ischemia/reperfusion, I/R) for 24 h and received 7,8-DHF immediately after MCAO. Treatment with 7,8-DHF improved the I/R-induced neurological deficits and the infarct volume, counteracted the increase in the cortical expression levels of cleaved caspase-3 and Bcl-2 homologous antagonist/killer (Bax) and the reduction in Bcl-2 levels, reduced oxidative stress, evaluated based on the content of MDA and the activity of antioxidant enzymes as GSH, glutathione peroxidase (GSH-PX), superoxide dismutase (SOD). Finally, 7,8-DHF inhibited nitric oxide (NO) production and iNOS expression, and suppressed the NF-kB

pathway, indicating reduction of inflammation. These effects were accompanied by an increase in p-TrkB levels. In an *in vitro* study, Tecuatl et al. (Tecuatl et al. 2018) investigated the effect of 7,8-DHF on the ischemic damage in hippocampal fields CA1 and CA3. Rat hippocampal slices were exposed to an Oxygen-Glucose Deprivation (OGD) solution for 10 min and then re-perfused with normal artificial cerebrospinal fluid. OGD caused a permanent suppression of CA1 but not CA3 field potentials. The basal levels of p-TrkB (Y816) were higher in CA3 than in CA1 and OGD caused a larger increase in p-TrkB in CA3 in comparison with CA1. Pre-incubation with 7,8-DHF (or BDNF) enhanced the recovery of CA3 but not CA1 response after OGD, suggesting that the TrkB receptor plays a central role in the different vulnerability of CA3 and CA1.

**Significance.** These studies show that 7,8-DHF can protect against cerebral I/R injury by reducing apoptotic cell death, neuronal degeneration, oxidative stress and neuroinflammation. These effects translate into amelioration of hippocampal evoked potentials and behavior. Similarly to models of addiction, treatment with 7,8-DHF appears to exert sexually dimorphic effects in models of ischemia.

#### 4.4.3 Brain radiation

**Brief premise.** Brain radiation, used to treat brain tumors, may be associated with brain injury and cognitive impairment that can occur in up to 50-90% of patients (Greene-Schloesser et al. 2012). Preclinical studies in rodents have shown that irradiation damages hippocampal neurogenesis and causes neuroinflammation and cognitive impairment (Greene-Schloesser et al. 2012), suggesting that treatments that counteracts these effects may ameliorate radiation-induced brain injury.

**The evidence.** In the study by Yang et al. (Yang et al. 2016), mice received 5 Gy of cranial irradiation and after one week were treated with 7,8-DHF for 3 or 5 weeks. At 4 weeks after completion of 7,8-DHF administration mice showed restoration of spatial (Barnes Maze), contextual (CFC), and working memory (Y maze), of hippocampal neurogenesis and granule cell dendritic spine loss. Treatment, in addition, increased the levels of p-TrkB (Y816) and its downstream targets ERK and Akt.

**Significance.** This study shows that 7,8-DHF exerts remarkably positive effects on the brain subjected radiation. These effects span from restoration of hippocampal neurogenesis to prevention of dendritic and dendritic spine regression. Importantly, thanks to these neuroanatomical effects, 7,8-DHF restores cognitive performance.



## 4.5 Neurodegeneration

### 4.5.1 Alzheimer's disease

**Brief premise.** Alzheimer's disease (AD) is the leading cause of dementia in individuals over 65. This progressive neurodegenerative disease is characterized by brain extracellular neuritic plaques of amyloid beta ( $A\beta$ ) and intracellular neurofibrillary tangles consisting of hyperphosphorylated microtubule-associated Tau protein, resulting in synaptic deficit and neuronal death at advanced stage of the disease. In addition to these main neuropathological hallmarks, the levels of BDNF and TrkB receptor are reduced in the hippocampus and some cortical areas of AD patients, even at the early AD stages (Ferrer et al. 1999; Zhang et al. 2012), suggesting that impairment of BDNF-TrkB signaling may contribute to the pathogenesis of AD. Accordingly, central BDNF administration or gene delivery to AD animal models exert beneficial effects, including restoration of L&M (Nagahara et al. 2009). Based on evidence of the efficacy of BDNF against AD pathology, 7,8-DHF was tested in the preclinical field of AD in order to overcome the problems related to the delivery and scarce half-life of BDNF *in vivo*.

**The evidence.** A first study investigated the effects of 7,8-DHF in the 5XFAD mouse model of AD (Devi, and Ohno 2012). This model develops cerebral amyloid plaques at 2 months of age and shows synaptic dysfunction and memory impairment by 4-5 months of age. Mice aged 12-15 months received 7,8-DHF for 14 days. Treatment completely restored the reduced hippocampal levels both of the phosphorylated (Y705) and total form of TrkB receptor, without affecting the reduced BDNF levels. The activation of TrkB receptor was accompanied by rescue of spatial working memory (Y maze) and by reduction of the excessive levels of ( $\beta$ -secretase) BACE1,  $\beta$ -CTF ( $\beta$ -secretase-cleaved C-terminal fragment), and of  $A\beta$ 40 and  $A\beta$ 42. A subsequent study in the 5XFAD model tested the effects of 7,8-DHF at an earlier stage of disease progression (Zhang et al. 2014b). Mice received 7,8-DHF for 4 months starting from 2 months of age, at the beginning of plaque deposition. Administration of 7,8-DHF reversed the loss of dendritic spines and synapses in the hippocampal field CA1 and restored of hippocampal LTP and hippocampus-dependent L&M (MWM). At the molecular levels, 7,8-DHF elicited robust brain TrkB phosphorylation (Y816) and also increased p-Akt and p-ERK1/2 levels. Treatment also reduced the density of hippocampal  $A\beta$  plaques but, in contrast with the previous study, did not affect total  $A\beta$ 42 levels. Exploration of the effects of 7,8-DHF *in vitro* showed that 7,8-DHF (500 nM) promoted dendritic arborization of primary cortical neurons and

reduced the apoptotic rate induced by pre-aggregated A $\beta$ , both in cortical and locus coeruleus neurons. The anti-apoptotic effect was abolished by the TrkB receptor inhibitor K252. In a further study, one-month-old 5XFAD mice were treated with 7,8-DHF for 2 months in order to establish whether 7,8-DHF can prevent the AD-related phenotypes (Aytan et al. 2018). 5XFAD mice showed, similarly to individuals with AD, a reduction in hippocampal glutamate levels and an increase in choline and phosphocholine levels. These alterations were restored by treatment. In addition, treatment prevented the pruning of the dendritic tree of cortical neurons of layer V although it had a very modest effect on spine density. Finally, 7,8-DHF reduced the A $\beta$  plaque burden and A $\beta$ 42 levels, although this latter effect was not statistically significant. Chen et al. investigated the effects of R13, a prodrug of 7,8-DHF, in 2-month-old 5XFAD mice (Chen et al. 2018). All tested doses of R13 prevented the loss of dendritic spines and synapses in field CA1, improved LTP, and restored L&M (MWM). These positive effects were associated with increased hippocampal levels of p-TrkB (Y816), p-Akt, and p-ERK. In addition, treatment reduced amyloid plaques in the hippocampus and frontal cortex and the levels of A $\beta$ 40, but not A $\beta$ 42. Furthermore, R13 reduced the enzymatic activity of delta-secretase (AEP; asparagine endopeptidase) with a consequently reduction in the levels of APP/Tau pathological fragments (APP N373, APP N585, and Tau N368) and pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ). Gao et al. (Gao et al. 2016) investigated the effects of 7,8-DHF in the Tg2576 mouse, one of the most widely used AD models that overexpresses the human APP with the Swedish mutation, resulting in elevated A $\beta$  levels and amyloid plaques. In 10-month-old Tg2576 mice a single injection of 7,8-DHF was ineffective on field CA1 spine loss and L&M impairment (MWM). Conversely, chronic administration of 7,8-DHF for 4 weeks fully rescued spine density of CA1 pyramidal neurons and L&M. In addition, in hippocampal synaptoneurosomal fractions, chronic treatment increased the reduced protein levels of both GluA1 and GluA2, two subunits of the glutamate AMPA receptor. This effect was mediated by activation of TrkB (Y816) and its downstream PI3K/Akt, Ras/ERK, and PLC $\gamma$ /CaMKII signaling pathways because it was prevented by the TrkB inhibitor cyclotraxin B, U0126 (a Ras-ERK pathway inhibitor), Wortmannin (an Akt phosphorylation inhibitor), and KN-93 (a CaMKII inhibitor). No treatment effects, however, were found in the excessive hippocampal levels of APP and A $\beta$ 42. The effect of a single injection of 7,8-DHF on spatial memory (object location memory test) was investigated in the APP<sup>swe</sup>/PS1<sup>dE9</sup> mouse model of AD at 7

months of age, when hippocampal functional deficits start to be present (Bollen et al. 2013). Mice that received 7,8-DHF 3 h after the first exploration trial of the test underwent an improvement in their discrimination performance following a retention period of 24 h, suggesting an acute effect of 7,8-DHF on spatial memory. An improvement of memory (CFC test) was found in APPswe/PS1dE9 male mice aged 9 months, following chronic administration of 7,8-DHF for 10 days (Hsiao et al. 2014). The effects of 7,8-DHF were also examined in the CaM/Tet-DT<sub>A</sub> mouse, an inducible model of neuronal loss in field CA1 (but not CA3), DG and entorhinal cortex that replicates the neurodegenerative feature of the AD brain (Castello et al. 2014). Administration of 7,8-DHF to 3.5-5-month-old mice for 2 weeks increased the density of thin immature dendritic spines of CA1 pyramidal neurons, with no effect on the mushroom and stubby spines, and caused an improvement in L&M performance (MWM). In contrast with all above studies, 7,8-DHF failed to affect AD-related phenotypes in the APP23/PS45 mouse model of AD (Zhou et al. 2015). Treatment with 7,8-DHF for 4 weeks starting from 6 weeks of age had no effect on the levels of APP, CTFs, BACE1, A $\beta$  42 and A $\beta$ 40, and A $\beta$  plaque formation. Furthermore, 7,8-DHF did not improve L&M deficits (MWM). Positive effects of treatment with 7,8-DHF on AD-like pathological dysfunction were observed in rats treated with scopolamine, a non-selective muscarinic receptor antagonist that, by blocking cholinergic signaling, induces A $\beta$  deposition, oxidative stress, synaptic dysfunction and L&M impairment (Chen et al. 2014). Administration of 7,8-DHF restored LTP in the DG and decreased the scopolamine-induced excessive levels of hippocampal A $\beta$ 42 and A $\beta$ 40. In addition, 7,8-DHF reduced hippocampal lipid peroxidation, caused an increase in MDA levels, a reduction in GSH levels and in the activity of SOD, revealing an antioxidant effect. These effects were accompanied by restoration of L&M (MWM) and hippocampal levels of p-TrkB (Y516) and its downstream molecules p-Akt and p-ERK1.

**Significance.** This evidence in different AD models suggests that 7,8-DHF may represent a promising therapeutic strategy not only for the prevention/delay of AD but also for the amelioration of pathophysiological processes associated with the late stage of disease progression. Further studies are needed in order to establish the optimum effective dose of 7,8-DHF and the duration of its effects. While the positive effects of 7,8-DHF on synaptic dysfunction and memory impairments seem to be directly mediated by the activation of TrkB and its downstream signaling pathways, the molecular mechanisms underlying the effects of 7,8-DHF on APP processing and A $\beta$  aggregation remain to be elucidated.

#### 4.5.2 Parkinson's disease

**Brief premise.** Parkinson's disease (PD), which is the second-most common neurodegenerative disorder, is characterized by loss of dopaminergic neurons in the substantia nigra of the midbrain - possibly due to intracellular overexpression of  $\alpha$ -synuclein and/or oxidative injury - and diminished content of dopamine in the striatum. These alterations cause impairment of dopamine-regulated motor behavior (bradykinesia, postural instability, rigidity and tremor) (Poewe et al. 2017). Although various preclinical studies have shown that the delivery of neurotrophic factors, including BDNF, to the brain protects dopaminergic neurons and alleviates PD symptoms, the problem of the delivery of trophic factors in humans remains a challenge (Tome et al. 2017).

**The evidence.** The first evidence that 7,8-DHF may be of benefit for PD derives from a model of PD obtained by administering the dopaminergic toxicant MPTP (Jang et al. 2010). Eight-week-old mice were treated with 7,8-DHF for 14 days. On day 7, mice were treated with MPTP and were killed on day 14. 7,8-DHF reduced the neurotoxic effects of MPTP, as revealed by preservation of the expression of tyrosine hydroxylase (a marker of dopaminergic neurons) in the striatum and reduced the levels of activated caspase-3 in the substantia nigra. In a study by Sconce et al. (Sconce et al. 2015), mice received 7,8-DHF starting at midpoint (week 3) of a 4-week-MTPT administration and for an additional 2 weeks. Mice treated with 7,8-DHF maintained about 50% of tyrosine hydroxylase levels in the dorsolateral striatum, with no effect, however, in the substantia nigra underwent restoration of motor deficits, as assessed through gait dynamics. In addition, treated mice underwent an increase in the levels of p-TrkB (Y817), and p-ERK1/2 (but not of p-Akt) in the dorsolateral striatum and substantia nigra compared to untreated mice. In a third study in the MPTP model (Li et al. 2016), mice received 7,8-DHF from the first day of a 5-day-long MPTP treatment until 9 days after discontinuation of MPTP treatment. Mice that were treated MTPT alone were impaired in motor performance (rotarod test, pole test and wire suspensions test), exhibited loss of dopaminergic neurons in the substantia nigra and of dopaminergic terminals in the striatum, increased levels of  $\alpha$ -synuclein, and reduced levels of p-TrkB in the substantia nigra and striatum. All these defects were improved/restored in mice that had received 7,8-DHF. In addition, 7,8-DHF restored the levels of the endogenous antioxidants GSH and SOD in the substantia nigra and striatum. The protective effects of 7,8-DHF have been also examined in a rotenone-induced animal model of PD (Nie et al. 2019). Rats were injected with rotenone

daily for 5 weeks in order to induce a PD phenotype. Administration of 7,8-DHF during treatment with rotenone improved behavioral performance (distance travelled and speed in an open field test), reduced the loss of dopaminergic neurons in the substantia nigra and of dopaminergic terminals in the striatum, and reduced the rotenone-induced increase in p-MAPK, p-Tau and p- $\alpha$ -synuclein. The effects of 7,8-DHF were associated with increased levels of p-TrkB (Y816) and p-Akt. The study by He et al. (He et al. 2016) examined the effect of 7,8-DHF in a primate model (Macaca Fascicularis; 8-year-old) of PD obtained with intracerebroventricular injection of MPP<sup>+</sup>, the toxic metabolite of MPTP, delivered over a period of seven months. 7,8-DHF was administered 2 weeks earlier than MPP<sup>+</sup> injections and during the following 7 months. An evaluation of dopaminergic neurons in the substantia nigra showed loss of neurons in the monkey that had not received 7,8-DHF. In contrast, 7,8-DHF-treated monkeys had a similar number of neurons as wild-type monkey indicating a powerful neuroprotective effect of treatment. No effect of either MPP<sup>+</sup> or 7,8-DHF were found on astrogliosis, microglia activation and neurogenesis in the substantia nigra.

**Significance.** Taken together, these studies show that 7,8-DHF has a positive impact in different models of PD through TrkB-dependent mechanisms. The differences in the activated pathways downstream of the TrkB receptor may be due to differences in the employed model of PD and/or duration of treatment. Whatever the mechanism, all these studies show that 7,8-DHF has beneficial effects in PD models, suggesting that it may represent a possible treatment for alleviating the symptoms of PD.

#### 4.5.3 Huntington's disease

**Brief premise.** Huntington's disease (HD) is a neurodegenerative disorder caused by an expanded CAG repeat in the huntingtin gene. This mutation causes widespread neurodegeneration, with massive loss of the medium spiny neurons in the striatum, motor impairment and cognitive decline (Vonsattel, and DiFiglia 1998). It has been suggested that a reduction in brain BDNF levels due to mutant huntingtin underlies neurodegeneration in HD (Zuccato, and Cattaneo 2007). The reduced BDNF levels are paralleled by a reduction in TrkB receptor. In the R6/1 mouse model of HD, administration of exogenous BDNF can improve the HD-related neurological phenotype, indicating that the remaining TrkB receptors are sufficient to mediate BDNF-dependent signaling (Zuccato, and Cattaneo 2007). Accordingly, various studies have suggested the potential benefits of BDNF in HD which, in turn, has prompted studies that have exploited the BDNF mimetic 7,8-DHF.

**The evidence.** A study in the HD mouse model N171-82Q has examined the effect of 7,8-DHF on HD-related phenotypes (Jiang et al. 2013). Mice received 7,8-DHF from 6 weeks of age until they were 20-week-old. Treated mice displayed an improvement in motor function (balance beam), an increase in their lifespan, and attenuation of brain atrophy (MRI scans *in vivo*), and an increase in p-TrkB (Y706) and p-MAPK levels. A study in the mouse R6/1 model of HD examined the effects of 7,8-DHF *in vitro* and *in vivo* (Garcia-Diaz Barriga et al. 2017). In cultures of striatal neurons from R6/1 mice, 7,8-DHF was found to phosphorylate the TrkB receptor at Y816 (but not Y515), leading to activation of PLC $\gamma$ 1 downstream signaling. These effects were associated with an increase in the number and length of neurites and improvement of neuronal excitability. R6/1 mice chronically treated with 7,8-DHF, starting at pre-symptomatic stages (8 weeks) and continuing up to 20 weeks of age, partially improved motor performance in the rotarod test, completely reversed the deficit in the NOR test, partially recovered the soma size of the medium spiny neurons, and underwent a reduction in mutant huntingtin aggregation in the striatum. At the molecular level, *in vivo* treatment with 7,8-DHF reverted the reduction in p-TrkB (Y816) and increased the levels of p-PLC $\gamma$ 1. In addition, treatment restored the reduced levels of neuronal nitric oxide synthase (nNOS) and reduced the excessive levels of iNOS, indicating an effect on NO metabolism and, thus, on neuroinflammation. Finally, in the Hdh(Q7) striatal immortalized cell line exposed to H<sub>2</sub>O<sub>2</sub>, 7,8-DHF was able to prevent cell death in the presence of the TrkB inhibitor K252a, indicating that 7,8-DHF protects against oxidative stress with a mechanism independent from TrkB activation.

**Significance.** These studies show that 7,8-DHF has a positive impact in models of HD through TrkB-dependent and independent mechanisms. Although 7,8-DHF does not restore the phenotype of HD, it may have a therapeutic potential for the amelioration of the motor and cognitive deficits in this pathology.

#### 4.5.4 Amyotrophic lateral sclerosis

**Brief premise.** Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by degeneration of motor neurons, progressive motor weakness and, ultimately, death due to respiratory failure. Various neurotrophic factors have been investigated pre-clinically but with disappointing results in clinical trials (Henriques, Pitzer, and Schneider 2010).

**The evidence.** The possible neuroprotective effect of 7,8-DHF has been investigated in the SOD1<sup>G93A</sup> mouse, a transgenic ALS mouse model (Korkmaz et al. 2014). Mice aged 30 days received 7,8-DHF until 105 days

of age. Treatment prevented the motor decline (rotarod test) that started to appear at 60 days of age. An evaluation of the number of motor neurons in the ventral horn of the spinal cord and of dendritic spine density at the end of treatment showed that untreated SOD1<sup>G93A</sup> mice had a reduced number of motor neurons and a reduced dendritic spine density. Treatment prevented neuron loss and fully restored dendritic spine density.

**Significance.** Since the only available study in a model of ALS did not examine the TrkB receptor and its downstream signaling, it remains to be established whether the beneficial effects of 7,8-DHF were mediated by the TrkB receptor or rather by antioxidant actions. Nevertheless, this study shows that 7,8-DHF may be a possible tool to mitigate the outcome of ALS.

#### 4.6 Actions of 7,8-DHF in the healthy brain

**Brief premise.** The studies mentioned above were aimed at establishing the therapeutic potential of 7,8-DHF in a variety of brain diseases. A few studies show that 7,8-DHF also improves the anatomy and function in the healthy brain.

**The evidence.** Zimmerman et al. (Zimmermann et al. 2017) examined the effect of 7,8-DHF on goal directed action selection in normal mice using a test of instrumental contingency degradation. Mice are trained to generate two food-reinforced responses, then the likelihood that one response will be reinforced is reduced (action-outcome contingency degradation). During a subsequent probe test, preferential engagement of the response that is likely to be reinforced is considered “goal-directed”. While control mice failed to differentiate between the responses that were likely to be reinforced, mice injected with 7,8-DHF immediately following the training session exhibited an increase in the preference for the response likely to be reinforced, indicating that 7,8-DHF enhances action-selection. In addition, treatment increased dendritic spine density on neurons in the orbitofrontal cortex. Bollen et al. examined the effects of 7,8-DHF on memory consolidation in normal healthy rats (Bollen et al. 2013). Rats were subjected to a NOR test and the effects of different doses of 7,8-DHF administered either immediately after or 3 h after the learning trial carried out after 24 h were tested. Animals that had received 7,8-DHF exhibited a better discrimination index (time of exploration of the new object in comparison with the old object) in comparison with vehicle treated rats. Perez-Rando et al. (Perez-Rando et al. 2018) examined the effects of 7,8-DHF in Thy1-YFP transgenic mice, a strain in which layer V cortical pyramidal neurons are labeled with the fluorescent protein YFP,

which allows observation of dendritic spine dynamics *in vivo* with 2-photon microscopy. Mice received 7,8-DHF for 14 days and were behaviorally examined before and at the end of treatment. 7,8-DHF did not modify locomotion or anxiety related behavior (Open Field Test) but improved object recognition (modified version of the NOR) in comparison with the control group. Regarding spine dynamics, treatment improved the gain rate, loss rate, and stability rate of spines of neocortical pyramidal neurons, providing the first *in vivo* evidence of how 7,8-DHF modifies spine dynamics in healthy animals. Even in the absence of specific pathologies, memory undergoes a “physiological” decline with aging. The study by Zeng et al. (Zeng et al. 2012b) was aimed to establish whether treatment with 7,8-DHF has a protective effect on age-related memory decline. This study used aged rats (20- or 30-month-old) that revealed memory impairment in comparison with young rats (assessed by pretesting them with the MWM test). These rats were treated with 7,8-DHF for 34 days and tested with the MWM 10 days after beginning of treatment and in the last week of treatment. Treated rats of either age showed full restoration of L&M, although the older group showed a restoration in the second retest only. An evaluation of LTP in hippocampal slices from some of the behaviorally tested rats showed that LTP in field CA1 was restored by 7,8-DHF in the 20-month group and improved in the 30-month group. Likewise, dendritic spine density of CA1 pyramidal neurons was restored by 7,8-DHF in the 20-month group and improved in the 30-month group. In both age groups, treatment with 7,8-DHF increased the age-related reduction in the hippocampal levels of p-TrkB (Y816), p-ERK1/2, p-CREB, p-CaMKII, and p-GluR1. In a subsequent study (Zeng et al. 2012a) the same group demonstrated that 25-month-old rats exhibited impairment for contextual and cued fear conditioning. Treatment with 7,8-DHF for 4 weeks prevented the decline in fear conditioning, restored LTP in the amygdala, spine density, p-TrkB (Y515 and Y816), p-CaMKII, p-ERK1/2, p-CREB, and p-GluR1 levels in the amygdala, hippocampus, and PFC.

**Significance.** These studies show that 7,8-DHF may be used in normal subjects to favor development of dendritic spines, which are the substrate of synaptic plasticity associated with L&M and, consequently, to boost memory performance. Importantly, treatment with 7,8-DHF may represent a suitable treatment to combat and prevent the age-related memory decline.

## 5. ACTIONS OF 7,8-DHF IN THE PERIPHERAL NERVOUS SYSTEM



## 5.1 Retina

**Brief premise.** In the retina BDNF-TrkB signaling has a key role in the development of inner retinal neuronal circuits but not in the rod function and synaptic transmission to bipolar cells (Grishanin et al. 2008). Retinal ganglion cells (RGCs) express high levels of both full-length and truncated forms of TrkB and while TrkB signaling is not required for survival of RGCs during the period of target-dependent survival, it reduces degeneration of RGCs in adult animals (Rohrer et al. 2001). BDNF and TrkB are also important for the differentiation and survival of retinal pigment epithelium (Liu, Zhu, and Eide 1997).

**The evidence.** In cultures of primary rat RGCs and the retinal neuronal precursor RGC-5 cell line, treatment with 7,8-DHF activated the TrkB receptor, Akt and ERK1/2 (Gupta et al. 2013b). Pretreatment with 7,8 DHF protected cells against the injury caused by glutamate-induced excitotoxicity and H<sub>2</sub>O<sub>2</sub>-induced oxidative stress and prevented the activation of the apoptotic protein caspase-3. While exposure to glutamate or H<sub>2</sub>O<sub>2</sub> decreased by 50% TrkB kinase activity, pretreatment with 7,8 DHF caused a substantial protection of TrkB activity. Recent evidence shows that 7,8-DHF also has a positive impact on retinal pigment epithelial cells (Yu et al. 2018). ARPE-19 cells (a human retinal pigment epithelial cell line) were treated with D-glucose in order to induce apoptosis. Pretreatment with 7,8-DHF prior to glucose, increased the percentage of non-apoptotic cells. Treatment with the TrkB antagonist K252a abrogated this effect and caused downregulation of p-TrkB. A study *in vivo* sought to establish whether 7,8-DHF can protect the immature retina from early HI injury (Huang et al. 2018). Rat pups were treated with 7,8-DHF 2 h and 18 h after induction of HI injury and their retinas were examined at different time points. Treatment with 7,8-DHF improved the functionality of the retina (assessed with electroretinography), counteracted gliosis and reduction in the number of RGCs and favored the survival of proliferating inner retinal cells, including Müller glia, enhancing their trans-differentiation to bipolar cells. The positive effect of 7,8-DHF was prevented by inhibition of the ERK pathway with the ERK inhibitor PD98059, suggesting that these effects were mediated by TrkB-ERK signaling. A recent study (Daly et al. 2017) used a zebrafish model of inherited blindness (*dye<sup>ucd6</sup>*) in order to establish whether it is possible to pharmacologically improve visual function. Treatment of the mutant larvae with HDAC inhibitors restored retinal morphology and visual function (assessed with functional tests and electroretinography). Treatment with 7,8-DHF alone was sufficient to rescue retinal morphology, reduce the number of dying cells and rescue visual function. The latter effect was greater than that elicited by the most

effective among the HDAC inhibitors. The 7,8-DHF-induced rescue of the visual function was prevented by co-treatment with the TrkB inhibitor ANA-12.

**Significance.** This evidence envisages the possibility that systemic or local administration of 7,8-DHF may be a useful therapy for disorders, such as glaucoma, associated with degeneration of RGCs or with degeneration of retinal pigment epithelial cells, as well for inherited blindness. In addition, systemic administration of 7,8-DHF may be a potential strategy to improve retinal development and function following perinatal ischemic injury.

## 5.2 Cochlea

**Brief premise.** Spiral ganglion neurons (SGN) and cochlear hair cells (HC) express the TrkB receptor (Knipper et al. 1996; Liu et al. 2011) and development and maintenance of SGN is critically modulated by BDNF (and NT3) (Leake et al. 2011). Degeneration of the HC and SGN underlies age-related hearing loss. Following damage of the HC, the peripheral neurites of the SGN gradually degenerate but their cell bodies persist for months after degeneration of the HC. Although the delivery of BDNF to the inner ear has been shown to exert beneficial effect, its use is hampered by a poor pharmacokinetics.

**The evidence.** An approach *in vitro* and *in vivo* explored the effect of both 7,8-DHF and another agonist of the TrkB receptor, 7,8,3'-trihydroxyflavone (7,8,3'-THF), in organotypic cultures of cochlea from C57BL/6 and TrkB<sup>F616A</sup> mice and an *in vivo* model of degeneration of the SGN, the conditional connexin26 (cCx26) null mouse (Yu et al. 2013). We will mention here the effects of 7,8-DHF only. In the *in vitro* experiments, exposure to 7,8-DHF increased the density of SGN and neurite density and length in comparison with the no treatment condition, with a potency like that of BDNF. The surviving SGN were functional because they could generate inward Na<sup>+</sup> currents and action potentials. In *in vivo* experiments in cCx26 null mice, 7,8-DHF was delivered locally to the cochlea at P2 and P30 and its effects were examined at P60. Mice exhibited a greater density of SGN in the treated in comparison with the untreated cochlea and a reduced threshold of the electrically evoked auditory brain stem response, which is expression of the function of SGN. A more recent study in mice (Kempfle et al. 2018) sought to improve the local delivery of 7,8-DHF to the inner ear through the round window membrane by conjugating it with a bisphosphonate (Ris-DHF), based on the rationale that bisphosphonates have strong affinity to bone mineral and may prolong the permanence of 7,8-DHF in the inner ear. Treatment with either Ris-DHF or 7,8-DHF caused an increase in neurite length of

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2  
3 SGN. This effect was abrogated by co-treatment with the TrkB inhibitor ANA-12. In explants of the organ of  
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5 Corti with attached synapses and exposed to kainate, in order to cause excitotoxic damage of the synapses  
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7 between the HC and SGN (ribbon synapses), treatment with either Ris-DHF or 7,8-DHF caused an increase  
8  
9 in the number of synapses, indicating a regenerating effect.  
10

11 **Significance.** These two studies provide proof-of-principle evidence that 7,8-DHF locally delivered to the  
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13 cochlea may be a suitable therapy for improving damage to the HC and SGN and hearing loss. It remains to  
14  
15 be established whether systemic administration of 7,8-DHF elicits comparable effects.  
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### 17 **5.3 Peripheral nerves**

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19 **Brief premise.** Although axons in injured peripheral nerves have the capacity to regenerate and reinnervate  
20  
21 their targets, functional recovery is very poor. In the context of nerve regeneration, BDNF appears to play a  
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23 key role by binding to the TrkB receptor in the regenerating axons. Local treatment with BDNF promotes  
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25 enhanced early axon regeneration in mice (Wilhelm et al. 2012).  
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27  
28 **The evidence.** English et al. (English et al. 2013) examined the effects of 7,8-DHF on axon regeneration in  
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30 wild type and TrkB knockout (*SLICK::trkB<sup>fl/fl</sup>*) mice. In experiments *in vitro* they found that axon  
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32 regeneration of the cut sciatic nerve was enhanced by topical treatment with 7,8-DHF. This effect did not  
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34 take place in mice in which the neurotrophin receptor TrkB was selectively knocked out in neurons,  
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36 indicating requirement of TrkB signaling. In *in vivo* experiments, mice received 7,8-DHF for 2 weeks after  
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38 sciatic nerve transection. Systemic treatment was as effective as topical application in enhancing the  
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40 elongation of regenerating axons. Moreover, treatment increased muscle reinnervation (assessed as  
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42 amplitude of the response evoked in the gastrocnemius by proximal stimulation of the sciatic nerve) for at  
43  
44 least 6 weeks after the end of treatment.  
45

46  
47 **Significance.** Despite many advances in the field, the problem of axon regeneration in the peripheral (as well  
48  
49 as central) nervous system remains a challenge and long-lasting denervation may lead to progressive atrophy  
50  
51 of the target organs (Fenrich, and Gordon 2004). Systemic or local administration of BDNF as treatment for  
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53 axon regeneration poses several practical problems. The study by English et al. provides new hopes for the  
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55 development of effective strategy to treat peripheral nerve injury with systemic administration of 7,8-DHF.  
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## 60 **6. ACTIONS OF 7,8-DHF ON PERIPHERAL TISSUES**

Although BDNF is widely expressed in the CNS and is important for neuronal survival and differentiation it also plays important roles in non-neuronal peripheral tissue.

## 6.1 Cardiovascular system

### 6.1.1 Heart

**Brief premise.** BDNF is expressed in the heart (Nakagomi et al. 2015) and plays a fundamental role in heart development (Donovan et al. 2000) and angiogenesis of the heart vascular beds (Kermani, and Hempstead 2007). TrkB receptor have been found in the myocardium (Okada et al. 2012) and BDNF-TrkB signaling represents an important pathway in the heart mechanics, being required for the heart both to fully contract and relax (Feng et al. 2015). Two recent studies provide novel evidence for a pharmacological effect of 7,8-DHF on cardiac damage.

**The evidence.** Wang et al. showed that treatment with 7,8-DHF improved cardiac dysfunction (ejection fraction, fractional shortening, diastolic and systolic ventricular volume) and attenuated cardiac tissue injury in a myocardial ischemic mouse model (Wang et al. 2019). In the h9c2 cell line of cardiomyocytes exposed to H<sub>2</sub>O<sub>2</sub>, treatment with 7,8-DHF increased cell viability and inhibited excessive mitochondrial fission through Akt activation. This activation inhibited the proteolytic cleavage of optic atrophy 1 (OPA1), a mitochondrial inner membrane protein that regulates fusion and cristae structure. These effects were mediated by the TrkB receptor, as they were prevented by the TrkB inhibitor ANA-12. A second study investigated the effect of 7,8-DHF on cardiotoxicity induced by Doxorubicin (DOX), an anthracycline used as chemotherapy drug in cancer patients (Zhao et al. 2019). In a DOX mouse model, treatment with 7,8-DHF improved cardiac function (ejection fraction, fractional shortening) and attenuated cardiac tissue injury. In h9c2 cells exposed to DOX, 7,8-DHF increased cell viability, mitochondrial respiration and membrane potential, and the expression of OPA1. The beneficial effects of 7,8-DHF on cardiomyocytes were prevented by the antagonist of the TrkB receptor ANA-12.

**Significance.** These two studies provide novel evidence for a pharmacological effect of 7,8-DHF on cardiac mitochondrial dynamics and ischemia/toxicity-induced heart damage, suggesting a potential therapeutic role of 7,8-DHF in ischemic and metabolic heart diseases.

### 6.1.2 Vessels and blood pressure

**Brief premise.** TrkB receptors exist in human and rat vascular endothelia and smooth muscles (Donovan et al. 2000; Donovan et al. 1995) and neurotrophins appear to play an important role in regulating the response of vascular smooth muscle cells to injury. Very few studies have examined the effects of 7,8-DHF on vascular dynamics.

**The evidence.** One of these studies shows that 7,8-DHF induced relaxation of pre-constricted rat aortic rings and that this effect was mediated by an action of 7,8-DHF on both endothelia and smooth muscle cells (Huai et al. 2014). The dilating effect was not mediated by the TrkB receptor because it was not prevented by its inhibitor ANA-12. Rather it appeared to be mediated by the NO/cGMP pathway and through a blocking (in a manner not identified) of both intracellular  $\text{Ca}^{++}$  release and extracellular  $\text{Ca}^{++}$  influx in the smooth muscle cells. Consistently with the dilating effects observed *in vitro*, intravenous injection of 7,8-DHF significantly reduced the blood pressure of spontaneously hypertensive rats, with no effect on heart rate. This effect occurred within 20 min and disappeared at 60 min after the injection. In addition of being involved in vasodilation by acting on vascular endothelia, 7,8-DHF appears to exert a protective role on vascular endothelial cells. A study in a human umbilical vein endothelial cell line EA.hy926 (Wang et al. 2015) showed that 7, 8-DHF protected the cells from the damage caused by exposure to  $\text{H}_2\text{O}_2$  by counteracting apoptosis, mitigating release of inflammatory factors (IL-1 $\beta$ , ICAM-1, and TNF), and inhibiting the generation of reactive oxygen species (ROS). These effects were abrogated by the TrkB antagonist ANA-12. **Significance.** Dysfunction of vascular endothelia favors development of vascular diseases (such as hypertension or atherosclerosis). These two studies suggest that intake of 7,8-DHF may represent a possible prophylaxis against vascular diseases.

## 6.2 Pulmonary system

**Brief premise.** BDNF and its receptors, including the TrkB receptor, are expressed in different lung structures such as nerves, immune cells, bronchial and alveolar epithelium, smooth muscle, fibroblasts and vascular endothelium (Nakagomi et al. 2015; Prakash, and Martin 2014). BDNF is emerging as a particularly important player in the lung and airways physiology and pathophysiology (Prakash, and Martin 2014).

**The evidence.** Cultures of airway smooth muscle exposed to BDNF undergo an increase in  $\text{Ca}^{++}$  response to acetylcholine (ACh) and histamine and this effect is mimicked by 7,8-DHF, suggesting that 7,8-DHF, similarly to BDNF, may enhance airway muscle cell contractility (Abcejo et al. 2012). A study by Helan et

al. (Helan et al. 2014) showed that human pulmonary artery endothelial cells secrete BDNF in response to hypoxia which, in turn, induces an increase in TrkB-FL/TrkB-T1 ratio, upregulates the expression of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ), that is known to regulate expression of nitric oxide synthase genes and increase iNOS levels and the production of NO. The same effects were elicited by exposure of 7,8-DHF, suggesting a role of BDNF-TrkB (and, hence, 7,8-DHF) signaling in vascular diseases involving hypoxia, such as pulmonary hypertension. A study in Chinese hamster lung fibroblast cells examined the effects of 7,8-DHF on H<sub>2</sub>O<sub>2</sub>-induced cell damage (Zhang et al. 2009). 7,8-DHF protected lung fibroblasts by scavenging intracellular ROS, inhibiting apoptosis and activating the Akt signaling pathway. A study in the same cell model examined the mechanisms whereby 7,8-DHF exerts cytoprotective effects against oxidative stress, focusing in particular on the antioxidant enzyme HO-1 (Ryu et al. 2014b). The results show that 7,8-DHF protected the cells by increasing the expression of HO-1 and Nrf2, a transcription factor that plays a critical role in the transcriptional regulation of HO-1. Activation of Nrf2 was mediated *via* ERK phosphorylation. Thus, the ERK/Nrf2/HO-1 pathway appears to play an important role in the effects of 7,8-DHF against oxidative stress in lung fibroblasts.

**Significance.** These studies indicate that 7,8-DHF affects the function of pulmonary airway smooth muscle cells, vascular endothelial cells and fibroblasts. 7,8-DHF appears to enhance airway muscle cells contractility, thereby increasing airway resistance. On the other hand, 7,8-DHF may have a positive impact on pulmonary hypertension by enhancing the production of NO by endothelial cells. These two opposite effects must be taken into account when administering 7,8-DHF to patients with airway or vascular lung diseases. 7,8-DHF, in addition, appears to ameliorate pulmonary fibroblast function by reducing oxidative stress. Since oxidative stress is implicated in the pathogenesis of pulmonary fibrosis, treatment with 7,8-DHF may positively impact lung function in pathologies involving the lung parenchyma.

## 6.3 Gastrointestinal system

### 6.3.1 Intestine

**Brief premise.** BDNF is present in the intestine where it is released from mucosal enteroendocrine cells and from intrinsic primary afferent neurons innervating the mucosa. In the intestine, TrkB receptors have been identified in enteric neurons and glia, epithelial cells of the mucosa and smooth muscle cells (see (Al-Qudah et al. 2014)).

**The evidence.** Al-Qudah et al (Al-Qudah et al. 2014) by using strips of intestinal longitudinal smooth muscle demonstrated that BDNF alone does not enhance smooth muscle contraction but it enhances the contraction induced by muscarinic receptor stimulation with charbacol (a commonly used cholinomimetic activator of muscle strip contractions) and that this effect was mediated by activation of the TrkB receptors and the phosphorylation of PLC, most likely PLC $\gamma$ . Exposure to 7,8-DHF enhanced the contraction induced by muscarinic receptor stimulation, similarly to BDNF. A more recent study has examined the effects of 7,8-DHF on gastric motility *in vitro* and *in vivo* (He et al. 2018). 7,8-DHF alone, did not induce contraction of gastric strips but enhanced charbacol-induced motility. The inhibitor of the TrkB receptor ANA-12 partially blocked this effect. Adult rats orally fed with 7,8-DHF for 7 days exhibited an increase in the velocity of gastric emptying, indicating that *in vivo* 7,8-DHF alone can affect gastric motility.

**Significance.** The increased contractility of smooth muscle associated with gut inflammation has been attributed to enhancement of BDNF-TrkB signaling. Therefore, treatment with 7,8-DHF may have adverse effects on intestinal motility in pathologies associated with gut inflammation. On the other hand, 7,8-DHF may be a useful agent for enhancing gastric motility in diseases causing alterations in stomach emptying dynamics.

### 6.3.2 Liver

**Brief premise.** The TrkB receptor is present in the liver of newborn and adult mice (Garcia-Suarez et al. 2006b). In neonate mice (0 to 15 days) the TrkB receptor is present in monocyte-macrophage-dendritic cells scattered throughout the organ. In adult mice (3- and 6-months-old) it is restricted to nerve fibers. Detectable levels of the two main TrkB ligands, the BDNF and NT-4/5 (Lommatzsch et al. 2005) are also present in the liver at different ages.

**The evidence.** High-fat diet and alcohol intake induce liver inflammation and injury. A study in rats subjected to high fat diet and/or alcohol intake for twelve weeks examined the possibility that concomitant treatment with 7,8-DHF during the last 4 weeks may attenuate liver injury (Kumar et al. 2019). This study shows that treatment with 7,8-DHF had a beneficial effect on liver histopathology, restored serum levels of biomarkers of hepatic damage (aspartate aminotransferase and alanine aminotransferase), oxido-nitrosative markers, and the inflammatory cytokine IL-1 $\beta$ . The diet-induced down-regulation of Nrf-2 and HO-1 and up-regulation of NF-kB and iNOS mRNA expression resulted ameliorated by treatment with 7,8-DHF.

**Significance.** This study shows that 7,8-DHF has an hepatoprotective action against liver toxicity induced by alcohol and high-fat diet *via* a reduction of oxido-nitrosative stress and NF-kB activation, suggesting a potential therapeutic role of 7,8-DHF supplementation in diseases causing liver hepatotoxicity.

## 6.4 Genitourinary system

### 6.4.1 Oocytes

**Brief premise.** In the ovary, BDNF secreted by granulosa and cumulus cells acts on TrkB receptors expressed in oocytes and enhances first polar body extrusion and promotes the *in vitro* development of zygotes (Kawamura et al. 2005). Oxidative stress is heavily involved in the physiology of the female reproductive tract and influences the outcomes of assisted reproductive technology in human and animal reproduction (Agarwal et al. 2012; Choi et al. 2013). Since 7,8-DHF in addition to be a BDNF mimetic has antioxidant properties, it is conceivable that exposure to 7,8-DHF favors oocyte maturation in a dual manner. **The evidence.** Choi et al. (Choi et al. 2013) cultured porcine oocytes in media supplemented with 7,8-DHF during maturation and culture *in vitro*, after parthenogenic activation. Treatment with 7,8-DHF (1  $\mu$ M) caused increased oocyte cytoplasmic maturation and accelerated attainment of the blastocysts stage. This effect was accompanied by an increase in GSH levels, reduction of ROS generation, and reduction of Bcl-2 homologous antagonist/killer (BAK1, pro-apoptotic) and increase of Bcl-2-like 1 (BCL2L1, antiapoptotic) protein expression. The contribution of the TrkB receptor in these effects remains to be established. **Significance.** In human reproduction, increased implantation and clinical pregnancy rates are reported when antioxidant-supplemented medium is used in assisted reproductive technology (Agarwal et al. 2012). However, although low concentration of 7,8-DHF improves oocyte maturation, higher concentrations have negative effects (Choi et al. 2013). Oxidative stress is known to affects female reproductive abilities (Agarwal et al. 2012). It remains to be established whether supplementation with 7,8-DHF may have a positive effect on reproduction *in vivo*.

### 6.4.2 Kidney tubular cells

**Brief premise.** BDNF is expressed in the kidney (Nakagomi et al. 2015) and the kidney possesses TrkB receptors identical to those in the brain (Garcia-Suarez et al. 2006a), suggesting a role of TrkB and its ligands in the control of renal function.



**The evidence.** The possible protective function of 7,8-DHF against hypoxia has been examined in the human proximal tubular cell line HK-2 (Ma et al. 2016). This study shows that HK-2 cells express the TrkB receptor and that 7,8-DHF increased their proliferation rate at concentrations of 50-200  $\mu$ M but had adverse effects at a higher concentration (250  $\mu$ M). In cells exposed to hypoxia 7,8-DHF improved cell viability, possibly through an increase in the expression of cysteine-rich protein 61(CYR61), an extracellular matrix-associated signaling protein that regulates inflammation (Lau 2011). In addition, 7,8-DHF reduced the hypoxia-induced increase in the expression of CCAAT/enhancer-binding protein homologous protein (CHOP), a key regulator of endoplasmatic reticulum (ER) stress. This study did not investigate the pathways involved in the effects of 7,8-DHF on HK-2 cells.

**Significance.** Acute kidney injury, which is generated in most cases by hypoxia, is characterized by severe renal damage, in particular of the cells of the proximal tubule. This study suggests that treatment with of 7,8-DHF may protect the tubular cells from the hypoxic damage thereby preserving their key role in the renal function.

## **6.5 Musculoskeletal system**

### **6.5.1 Muscle**

**Brief premise.** Muscle cells produce BDNF that by acting in an autocrine/paracrine manner regulates muscle physiology. TrkB-mediated signaling is relevant to the maintenance of function and structure at the neuromuscular junction, but not muscle fibers, even though muscle fibers also may express TrkB receptors (Mantilla et al. 2014). Recent data show that BDNF is essential for specification of glycolytic muscle fiber type (Delezie et al. 2019). In addition, BDNF produced locally by muscle cells in response to contraction enhances fat oxidation in skeletal muscle (Matthews et al. 2015).

**The evidence.** A study using a diaphragm-phrenic nerve preparation from TrkB<sup>F616A</sup> mice (sensitive to inhibition by 1NMPP1) examined the effect of 7,8-DHF on muscle fiber properties and neuromuscular transmission (Mantilla, and Ermilov 2012). 7,8-DHF did not directly affect either the twitch force or the fatigability. Treatment with 7,8-DHF, however, reduced the contribution of neuromuscular transmission failure to muscle fatigue which is in agreement with the role of neurotrophins in neuromuscular transmission (Mantilla et al. 2014). This effect was prevented by inhibition of the TrkB receptor with 1NMPP1. A subsequent study explored the possibility that chronic treatment with 7,8-DHF may mitigate the age-related

diaphragm neuromuscular transmission failure and sarcopenia (Greising et al. 2017). TrkB<sup>F616A</sup> mice aged 18 months received 7,8-DHF for 6 months and a diaphragm-phrenic nerve preparation from these mice was used to evaluate the effects of treatment. An evaluation of neuromuscular transmission failure, muscle force, and fiber cross-sectional areas at the end of treatment showed no beneficial effect of 7,8-DHF, suggesting that treatment with 7,8-DHF is not useful in order to mitigate age-related neuromuscular functional alterations.

**Significance.** Although 7,8-DHF appears to enhance neuromuscular transmission *via* activation of the TrkB receptor in a diaphragm-phrenic nerve preparation, it fails to exert any benefit in age related neuromuscular transmission failure. Further studies should address the potential benefit of 7,8-DHF in neuromuscular junction diseases, such as myasthenia gravis, that are characterized by failure of the neuromuscular synapse due to autoimmune or toxic events.

#### 6.5.2 Articular Chondrocytes

**Brief premise.** Articular chondrocytes express neurotrophins and various neurotrophin receptors, including the TrkB receptor and neurotrophins are considered to be of relevance for the articular cartilage and the inflammation process in arthritis (Grimsholm et al. 2008).

**The evidence.** Cai et al. (Cai et al. 2019) investigated whether 7,8-DHF can protect cartilage from oxidative stress and delay the progression of articular cartilage destruction in a mouse model of osteoarthritis (OA), induced by sectioning the medial meniscotibial ligament. In cultures of primary chondrocytes, 7,8-DHF activated the Nrf2 signaling pathway and attenuated H<sub>2</sub>O<sub>2</sub>-induced oxidative stress. OA mice received 7,8-DHF once a week postoperatively and their joints were harvested 4 and 8 weeks postoperatively. While vehicle-treated mice exhibited a loss of knee joint cartilage, mice treated with 7,8-DHF exhibited a reduced loss both at 4 and 8 weeks. In the cartilage of the joints, 7,8-DHF suppressed the increase in mRNA levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and MMP-1, MMP-3 and MMP-13 that occurred in OA mice in comparison with the sham group and increased the expression of HO-1 and Nrf2. This suggests that 7,8-DHF protects the damage cartilage through an antioxidant action.

**Significance.** No cure exists for osteoarthritis, a well-known cause of disability around the globe that consumes a substantial amount of healthcare resources. Treatment for OA includes pain relievers and anti-inflammatory drugs and, ultimately, surgery. The study reported above suggests that treatment with 7,8-DHF

may represent a suitable therapy that by preserving the articular cartilage may delay/reduce the necessity of symptomatic and/or surgical interventions.

## 6.6 Skin

**Brief premise.** Neurotrophins and their receptors play a role in the control of skin homeostasis (Botchkarev et al. 1998; Botchkarev et al. 2006), which is not surprising considering the common ectodermal origin of skin and nervous system. In addition, development and maintenance of cutaneous mechanoreceptors has also been shown to depend on BDNF and its TrkB receptor (Garcia-Piqueras et al. 2019). Most of the cells of the epidermis are represented by keratinocytes and most of the cells of the dermis are represent by fibroblasts. Human keratinocytes secrete all neurotrophins and express TrkA and TrkC but do not express the TrkB F.L., but express the truncated isoform of TrkB (Marconi et al. 2003). In contrast, mouse keratinocytes express the TrkB receptor (Botchkarev et al. 1998). Human dermal fibroblasts secrete all neurotrophins and express all neurotrophin receptors, including the TrkB receptor (Palazzo et al. 2019).

**The evidence.** Skin aging is characterized by destruction of collagen fibrils due to increased activity of matrix metalloproteinases (MMPs; enzymes that degrade the extracellular matrix) secreted by epidermal keratinocytes and dermal fibroblasts. A study by Choi et al. (Choi, Lee, and Park 2017) examined the effects of 7,8-DHF on Hs68 cells (human dermal fibroblasts) treated with TNF- $\alpha$ , an inflammatory cytokine that mediates skin aging and activates MMPs. 7,8-DHF restored the reduction in procollagen caused by exposure to TNF- $\alpha$  and lowered the levels of MMP1, indicating that 7,8-DHF counteracts the reduction in collagen *via* an induction in the synthesis of collagen and a reduction of MMP levels. In addition, 7,8-DHF reduced the increase in ROS production due to exposure to TNF- $\alpha$ . This effect was associated with an increase in the expression levels of the antioxidant enzymes catalase (CAT), Mn-superoxide dismutase (Mn-SOD), and HO-1. These effects were accompanied by suppression of TNF- $\alpha$ -induced activation of the MAPKs and Akt pathways. The effect of 7,8-DHF against oxidative stress has been investigated in human keratinocytes (HaCaT cells), with a focus on HO-1, in view of its powerful cytoprotective properties against oxidative damage (Ryu et al. 2014a). HaCAT cells treated with 7,8-DHF exhibited an increased expression of HO-1 and Nrf2. In cells exposed to H<sub>2</sub>O<sub>2</sub> or ultraviolet B (UVB) radiation to cause oxidative stress, treatment with 7,8-DHF improved cell viability and increased the levels of p-ERK and p-PI3K/Akt. ERK and PI3K inhibitors reduced but not abrogated the effects of 7,8-DHF. Since the HaCaT cells do not express functional

TrkB receptors (Rossler, and Thiel 2004), it ensues that 7,8-DHF must exert its effects *via* a TrkB-independent mechanism.

**Significance.** Skin aging, which is due to intrinsic and environmental factors, ultimately promotes skin inflammation, impaired wound repair, and increased risk of skin cancer. Slowing down skin aging may be important in order to preserve the role of the skin, i.e. to protect the underlying tissues, and to prevent the occurrence of skin cancer. The results of the two studies reported above, that were obtained *in vitro*, show that skin cells exposed to 7,8-DHF result protected from oxidative and radiation damage. It may be interesting to establish whether a similar effect is elicited *in vivo* by systemic or topic administration of 7,8-DHF.

## 6.7 Body mass and energy metabolism

**Brief premise.** BDNF and the TrkB receptor are expressed in brain centers involved in energy homeostasis and BDNF plays a key role in the regulation of body weight and energy balance (see (Nakagomi et al. 2015)). BDNF is expressed in the adipose tissue and the TrkB receptor is expressed in the adipose tissue at higher levels in comparison with other peripheral tissues (Nakagomi et al. 2015). In mice, a high-caloric diet increases BDNF expression and reduces TrkB receptor expression and adipocyte-specific BDNF or TrkB deficiency causes obesity and hyperphagia, suggesting an important role of BDNF in the peripheral control of energy homeostasis (Nakagomi et al. 2015).

**The evidence.** Based on evidence that oxidative stress induces lipid accumulation and the suppressive effects of antioxidants on adipogenesis, a study *in vitro* has examined the anti-obesity effects of 7,8-DHF in 3T3-L1 preadipocyte cells (Choi et al. 2016). Low concentrations of 7,8-DHF did not affect cell viability, whereas high concentrations ( $>20\mu\text{M}$ ) induced apoptotic cell death. During differentiation of 3T3-L1 preadipocytes to mature adipocytes, concentrations of 7,8-DHF  $<20\mu\text{M}$  reduced the accumulation of lipid droplets and the expression of CCAAT/enhancer-binding protein- $\alpha$  (C/EBP- $\alpha$ ), C/EBP- $\beta$ , and peroxisome proliferator activated receptor- $\gamma$  (PPAR- $\gamma$ ), factors that regulate fat storage. In addition, 7,8-DHF reduced the ROS accumulation that takes place in adipocytes during adipogenesis and increased the expression of antioxidant enzymes such as Mn-SOD, CAT, heme HO-1. MAPK proteins such as ERK, and p38 are activated by ROS and this accelerates the differentiation of preadipocyte cells. Treatment with 7,8-DHF reduced the increase in p-ERK and p-p38 levels in differentiated cells, suggesting involvement of these pathways in its antioxidant

actions. The anti-obesity action of 7,8-DHF has been examined *in vivo* in adult female mice (Chan et al. 2015). This study was conducted in females because in males 7,8-DHF did not reduce the body weight. Chronic administration of 7,8-DHF for 22 weeks attenuated the obesity induced by high fat diet by activating the TrkB receptor in muscle. Specifically, treatment reduced the increase in white adipose tissue and the hepatic concentration of cholesterol in the liver and triglyceride and free fatty acids in the liver and muscle, improved insulin sensitivity and glucose tolerance, reduced the percentage of body fat mass, and increased the metabolic rate. At the molecular level, these effects were accompanied by enhanced expression of uncoupling protein 1 (UCP1) and AMPK activity in skeletal muscle, p-ERK (liver and muscle) and p-CREB (liver, muscle, and white adipose tissue). In muscle-specific TrkB knockout mice, treatment with 7,8-DHF failed to mitigate the diet-induced obesity, indicating that the anti-obesity activity of 7,8-DHF was muscular TrkB-dependent. A subsequent study (Wood et al. 2018) sought to establish whether treatment with 7,8-DHF reverses the metabolic impairment in mice that are already obese. Mouse females aged 8 weeks were fed with high fat diet for 13 weeks. Thereafter mice received 7,8-DHF for 9 weeks. Treated mice exhibited a reduction in body weight in comparison with control animal, due to a reduction in white adipose tissue, and a reduction in hyperlipidemia. In the muscle of treated mice there was an increase in the levels of p-AMPK, p-ERK, p-CREB and PGC- $\alpha$ . All these factors play a key role in mitochondrial biogenesis. Accordingly, in the muscle of treated mice there was an increase in various mitochondrial markers, including UCP1, and in the expressions of TFAM and nuclear respiratory factor 1(Nrf1), two master transcriptional factors of genes underlying mitochondrial biogenesis. In line with an increase in mitochondrial biogenesis, treated mice exhibited a higher O<sub>2</sub> consumption and an increase in total energy expenditure. Interestingly, treatment also improved insulin sensitivity in obese mice.

**Significance.** These studies suggest that chronic activation of the muscular TrkB receptor by 7,8-DHF may represent a potential strategy against development of obesity. Obesity is a major risk factor for numerous diseases including diabetes, and cardiovascular diseases, Therefore, treatment with 7,8-DHF may have an impact that is not limited to obesity but may involve serious health issues of the modern society. Interestingly, the study by Chan et al. shows that 7,8-DHF has an anti-obesity effect in females but not in males (Chan et al. 2015). This issue opens the way to further studies aimed at identifying the causes of the sexually dimorphic response to the anti-obesity (and possibly other) effects of 7,8-DHF.

## 7. ANTICANCER ACTION OF 7,8-DHF

**Brief premise.** Flavonoids have been shown to be potent therapeutic candidates for the treatment of various types of cancer due to their promotion of apoptotic cell death (Abotaleb et al. 2018). Their effects have been documented so far in a variety of carcinomas, which are tumors of epithelial origin. A few studies have specifically examined the potential of 7,8-DHF as anticancer molecule in cell lines of different types of carcinoma.

### ***The evidence.***

**Breast cancer cell line.** The initial evidence for an anticancer action of 7,8-DHF was obtained in a human breast cancer cell line (MCF-7), which is a cell line estrogen hormone-dependent (Le Bail et al. 1998). The goal of the study was to evaluate the anti-proliferative activity of several flavonoids (tested at doses of 10-50,000 nM) in the presence or absence of estradiol. In the absence of estradiol, 7,8-DHF induced a small increment in cell proliferation and had no anti-proliferative activity even at the highest concentration. However, in the presence of estradiol it inhibited cell proliferation at the concentration of 50,000 nM suggesting that it could serve as anti-breast cancer molecule.

**Hepatocellular carcinoma cell line.** A study in the human hepatocellular carcinoma cell line (HepG2) (Kozics, Valovicova, and Slamenova 2011) examined the protective effects of ten flavonoids, including 7,8-DHF, against the genotoxic effects induced by B(a)P, a potent mutagen and carcinogenic agent that results from incomplete combustion of organic matter at high temperature (e.g. tobacco smoke, grilled meats). A cytotoxicity assay of different concentration of flavonoid (1-100  $\mu$ M) showed that 7,8-DHF had a cytotoxicity lower in comparison with most of the other tested flavonoids ( $IC_{50} > 100 \mu M$ ). Cells exposed to B(a)P exhibited micronuclei, which is indicative of carcinogenicity, and DNA lesions. 7,8-DHF inhibited the increase in cells with micronuclei at all tested concentrations (2.5-25  $\mu$ M) and DNA lesions at concentrations of 5-25  $\mu$ M. This study thus shows that 7,8-DHF can exert protective effects against genotoxicity at concentration well below those inducing cytotoxicity.

**Monocytic leukemia cell line.** Park et al. (Park et al. 2012) tested the anti-proliferative effects of 7,8-DHF in U937 human monocytic leukemia cells. Cultures exposed to 7,8-DHF (70  $\mu$ M) exhibited a reduced viability due to increased apoptosis. FACS analysis showed that most of the cells exposed to 7,8-DHF were in the G1

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3 phase of the cell cycle. This effect was associated with reduction in the protein levels of cyclin E,  
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5 upregulation of p27, and reduced phosphorylation of retinoblastoma protein (Rb). The protein p27 prevents  
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7 the activation of the cyclin E/Cdk2 complex which by phosphorylating Rb promotes G1 progression. These  
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9 findings provide a mechanistic link between exposure to 7,8-DHF and arrest of the cells in G1 and  
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11 consequent apoptosis. The cellular pathways that underlie the effects of 7,8-DHF on the cell cycle, however,  
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13 remain to be established. In a second study (Park et al. 2013), the same group investigated the mechanisms  
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15 underlying the apoptotic effect of 7,8-DHF in U937 human monocytic leukemia cells. Cultures exposed to  
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17 7,8-DHF (5-100  $\mu$ M) exhibited a reduced viability at concentrations  $\geq 20 \mu$ M and in cultures exposed to 70  
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19  $\mu$ M concentration of 7,8-DHF there was an increase in apoptotic cell death. These cultures exhibited an  
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21 increase in various death membrane receptor-related protein and a reduction in inhibitor of apoptosis proteins  
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23 (IAP; a group of negative regulators of both caspases and cell death). In addition, 7,8-DHF modulated the  
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25 expression of members of the Bcl-2 family (that acts on the mitochondrion) and released cytochrome c from  
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27 mitochondria, indicating an involvement of the mitochondrial pathway in its apoptotic effects. The effects of  
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29 7,8-DHF were mediated by activation of ERK and JNK because inhibitors of ERK and JNK counteracted the  
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31 7,8-DHF-induced apoptosis.

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34 *Oral squamous cell carcinoma cell lines.* Lee et al. (Lee et al. 2015) examined the anticancer effect of 7,8-  
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36 DHF in the oral squamous cell carcinoma cell lines HN22 and HSC4. Cultures exposed to 7,8-DHF (5-40  
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38  $\mu$ M) exhibited a reduced viability at concentrations  $\geq 5 \mu$ M and an increase in apoptotic cell death at  
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40 concentrations of 10-40  $\mu$ M. Treatment with 7,8-DHF (10-40  $\mu$ M) reduced the expression of specificity  
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42 protein 1 (Sp1), a transcription factor that is involved in many cellular processes and plays pivotal roles in  
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44 cell proliferation and metastasis of various tumors. The reduced expression of Sp1 was accompanied by  
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46 increased expression of cleaved caspase-3 levels, suggesting, that the reduction in Sp1-levels may take part  
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48 in the treatment-induced apoptosis increase.

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51 *Malignant melanoma cell line.* Sim et al. (Sim, Sohng, and Jung 2016) examined the chemotherapeutic  
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53 potential and underlying mechanisms of action of 7,8-DHF on malignant melanoma cells using B16F10  
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55 melanoma cells. Cells were treated with  $\alpha$ -MSH, a hormone that stimulates skin and hair melanogenesis and  
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57 favors melanoma development, and co-exposed to 7,8-DHF. Treatment with 7,8-DHF (0.4-100  $\mu$ M)  
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59 inhibited cell proliferation ( $IC_{50} = 9.04 \mu$ M) and colony-forming ability and reduced cell migration capacity.  
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Treatment, in addition, reduced the formation of melanin and tyrosinase activity (the enzyme controlling the production of melanin), suggesting inhibition of differentiation. These effects were associated with downregulation of microphthalmia-associated transcription factor (MITF), a transcription factor associated with melanoma development, and its main downstream transcription target, *MITF* and tyrosine-protein kinase MET (c-MET), through a reduction in cAMP level.

**Significance.** These studies clearly show that 7,8-DHF has an anticancer activity *via* inhibition of cell proliferation and enhancement of apoptosis at concentrations higher than those that enhance proliferation. While they provide a hope that a natural compound such as 7,8-DHF may represent a weapon against cancer, the issues of efficacy, toxicity, and safety of large doses of 7,8-DHF *in vivo* still remains to be addressed. In this connection, a recent study must be mentioned that has created 7,8-DHF conjugated to nanoparticles as a tool for targeted antitumoral nanomedicine (Choi et al. 2018) which may provide a tool to overcome the problem of administration of large doses of 7,8-DHF.

## 8. CONCLUSIONS

The over 100 preclinical studies reviewed here show that treatment with 7,8-DHF has beneficial effects in a number of brain (Fig. 5) and body (Fig. 6) diseases. In most cases the effects consist in a full functional rescue or at least in an improvement. In very few cases (six studies) treatment exerted no benefit (Tables 1 and 2) or had a negative effect (one study, Table 1). It must be noted that the majority of the *in vivo* studies used a dose of 5.0 mg/kg/day of 7,8-DHF administered either i.v. or i.p. and showed positive outcome of such a treatment in most cases. Very few studies administered 7,8-DHF *per os* (see Tables 1 and 2) using doses equal to or higher than 5.0 mg/kg/day. It remains to be established whether or not the lack of effects in some of the latter studies is attributable to the scarce absorption rate of 7,8-DHF through the gut epithelium (Chen et al. 2019). Thus, the issue of the appropriate dose of 7,8-DHF when administered *per os* needs still to be better elucidated. Most of the effects of 7,8-DHF appear to be mediated through the TrkB receptor, i.e. *via* a BDNF mimetic action, although some of its effects may exploit additional pathways. The doses exerting positive effects did not cause toxicity, suggesting that 7,8-DHF may represent a safe treatment. Since most of the preclinical studies were carried out in males (see Tables 1 and 2), the question as to whether comparable effects are elicited in females remains open. In addition, a sexually dimorphic response



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3 to treatment with 7,8-DHF has been found in 6 studies (see Table1 and 2), emphasizing the need to further  
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5 explore this issue. All *in vivo* studies reviewed here did not report any side effect of even high doses of 7,8-  
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7 DHF. Yet, despite its enormous benefits, possible adverse effects of 7,8-DHF on brain activity cannot be  
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9 ignored. For instance, it has been shown that in rat hippocampal-entorhinal cortex slices although 7,8-DHF  
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11 did not initiate any epileptiform activity it increased the frequency of ictal and interictal events induced by  
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13 the convulsant 4 aminopyridine (Aydin-Abidin, and Abidin 2019). This effect needs to be better elucidated  
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15 in the framework of chronic treatments with 7,8-DHF.  
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18 We must be aware that the experimental evidence showing extremely positive effects of 7,8-DHF in a  
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20 constellation of disorders has been obtained in animal disease models (in most cases mouse models) and/or in  
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22 the simplified conditions of *in vitro* experiments. Yet, the large body of coherent evidence accumulated so far  
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24 in a diversity of models of the same pathology and in different experimental settings encourages us to  
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26 believe that 7,8-DHF may be proposed as a natural medicine to be tested in future clinical trials for a number  
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28 of brain and body diseases.  
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### 41 **Disclosure statement**

42  
43 The authors declare no potential conflict of interest.  
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### 47 **Author contributions**

48  
49 R. Bartesaghi and F. Stagni conceived, wrote, revised the manuscript and created the tables. M. Emili  
50  
51 contributed to write the manuscript and created the figures. S. Guidi, A. Giacomini and B. Uguagliati  
52  
53 contributed to write the manuscript.  
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## LEGEND TO FIGURES

Fig. 1. Number of articles by year of publication that have examined the effects of 7,8-DHF in animal or cellular models in the period January 1990-June 2020. Source: PubMed using keywords “7,8-Dihydroxyflavone” or “7,8-DHF”.

Fig. 2. TrkB receptor isoforms. TrkB full length (TrkB F.L.) bears a tyrosine kinase domain that autophosphorylates upon BDNF-triggered receptor dimerization. TrkB autophosphorylation, in turn, elicits activation of the cellular pathways summarized in Fig. 3. The truncated form of TrkB (TrkB-T1) lacks the tyrosine kinase domain and does not activate these pathways. Dimerization of TrkB-T1 with TrkB F.L. has a dominant negative effect.

Fig. 3. TrkB signaling pathways. Dimerization of the TrkB receptor leads to transphosphorylation of the autophosphorylation loops (Tyr705/6; not shown here) and phosphorylation of tyrosine residues 490, 515 and 816. Phosphorylation and recruitment of adaptors to Tyr515 and 490 launches the MAPK signaling cascade (Ras/Raf/MEK/ERK) and Akt signaling cascade. Phosphorylation of Tyr 816 launches the PLC $\gamma$  signaling cascade. Abbreviations: Akt, protein kinase B; CaM, Calmodulin; CaMKII, Ca<sup>2+</sup>/calmodulin-dependent protein kinases; CREB, cAMP response element binding protein; DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; IP3, inositol triphosphate; PI3K, phosphoinositide 3-kinases; PKC, protein kinase C; PLC $\gamma$ , phospholipase C $\gamma$ ; Shc, Src-homology 2- domain.

Fig 4. Chemical structure of 7,8-dihydroxyflavone (7,8-Dihydroxy-2-phenyl-4*H*-1-benzopyran-4-one).

Fig. 5. Brain disorders positively affected by treatment with 7,8-DHF. 7,8-DHF also positively impacts memory in the healthy brain. Abbreviation: MDPV, 3,4-Methylenedioxypyrovalerone.

Fig. 6. Body organs positively affected by treatment 7,8-DHF.

**Table 1. Effect of treatment with 7,8-dihydroxyflavone on the central and peripheral nervous system.**

Condition/Tissue/Function	Model	Gender	Age	Treatment Duration	Dose and route of administration	Notes	Reference
<b>GENETIC DISORDERS</b>							
<b>Down syndrome</b>	Ts65Dn mice	M & F	P3	13 D or 45 D	5 mg/kg/day s.c.		(Stagni et al. 2017)
	Ts65Dn mice	M	16-20 W	4 W	~ 22 mg/kg/day p.o.		(Parrini et al. 2017)
	Ts65Dn mice	M & F	P3	15 D	5 mg/kg/day s.c.	NB	(Giacomini et al. 2019)
		M	4 Mo	40 D	5 mg/kg/day i.p.	NB	(Giacomini et al. 2019)
<b>Rett</b>	Mecp2 mutant mice	N.A.	4 W	4 W	80 mg/l p.o. dose N.A		(Johnson et al. 2012)
<b>Fragile X</b>	<i>Fmr1</i> KO mice	M	21 D	4 W	~ 345 mg/kg/day p.o.		(Tian et al. 2015)
	<i>Fmr1</i> KO mice	M	3-5 Mo	1 D or 4 D	5 mg/kg/twice day i.p.		(Seese et al. 2020)
	<i>Fmr1</i> KO mice	M	3-5 Mo	1 Mo	0.08 mg/ml p.o. dose N.A.		(Seese et al. 2020)
	<i>Fmr1</i> KO mice (slices)	M	3-4 Mo	30 min	1 µM		(Seese et al. 2020)
<b>PSYCHIATRIC DISORDERS</b>							
<b>Autism</b>	Shank3B mouse	M & F	6-16 W	5 D *	5 mg/kg/day i.p.		(Rhine et al. 2019)
	BTBR mice	M & F	6-16 W	7 D *	2.5, 5.0, 7.5, 15.0 mg/kg/day i.p.		(Rhine et al. 2019)
	VRK3-KO mice	M & F	12 W	3 D	10 mg/kg/day i.p.		(Kang et al. 2017)
<b>Schizophrenia</b>	VRK3-KO mice	M & F	4 W	8 W	10 mg/kg/week i.p.		(Kang et al. 2017)
	D2 <sup>+/-</sup> C57BL/6 mice	M & F	8-10 W	3 D	10 mg/kg/day i.p.		(Lee, and Han 2019)
	Sprague-Dawley rats	M	2 Mo	14 D	5 mg/kg/day i.p		(Yang et al. 2014)
	ddY mice	M	4 W	4 W	1 mg/ml p.o. dose N.A.		(Han et al. 2016)
	ddY mice	M	4 W	4 W	1 mg/ml p.o. dose N.A.		(Han, Zhang, and Hashimoto 2017)
	ddY mice	M	E10	E10 to 3 W	1 mg/ml p.o. dose N.A.		(Han et al. 2017)
<b>Major depressive disorder</b>	C57BL/6J mice	M	2-3 Mo	21 D	5 mg/kg/day p.o.		(Liu et al. 2010)
	C57BL/6 mice	M	8 W	1 injection	1, 3, 10 mg/kg, i.p.		(Zhang et al. 2014a)
	C57Bl/6 mice	M	8 W	1 injection	10 mg/kg, i.p		(Zhang et al. 2015)
	Sprague-Dawley rats	M	7 W	1 infusion	0.1, 1.0 pmol b.i.		(Shirayama et al. 2015)
	C57BL/6 mice	M	8 W	28 D	10, 20 mg/kg/day i.p.		(Zhang et al. 2016)

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<b>Anxiety and stress disorder</b>	Sprague-Dawley rats	M	8 W	2 W	5, 20 mg/kg i.p.		(Chang et al. 2016)
	C57BL/6J mice	F	Adult	28 D	5 mg/kg/day i.p		(Amin et al. 2020)
	Sprague-Dawley rats	M	2 Mo	1 injection	5 mg/kg s.c.		(Andero et al. 2012)
	C57BL/6J mice	M	2-4 Mo	1 injection	5 mg/kg i.p.		(Andero et al. 2011)
	Sprague-Dawley rats	M	60 D	1 injection	5 mg/kg s.c.		(Sanz-Garcia et al. 2016)
	<i>bdnf</i> -floxed and TrkB <sup>F616A</sup> mice	M	2-4 Mo	1 injection	5 mg/kg i.p.		(Choi, Gourley, and Ressler 2012)
<b>ADDICTION</b>	C57BL/6 mice	M	31 D	8 D	3, 10 mg/kg/day i.p.		(Barfield et al. 2017)
	C57BL/6 mice	M & F	31 D	8 D	3 mg/kg/day i.p.	SD	(Barfield, and Gourley 2017)
	<i>bdnf</i> -floxed mice	M	2-4 Mo	1 injection	5 mg/kg i.p.		(Choi, Gourley, and Ressler 2012)
	Rats	M	3-6 Mo	1 injection	5 mg/kg i.p.		(Otis, Fitzgerald, and Mueller 2014)
	C57BL/6 mice	M & F	Adult	1 injection	5 mg/kg i.p.		(DePoy, Allen, and Gourley 2016)
	Swiss CD-1 mice	F	41-44 D	5 D	10 mg/kg/day i.p.		(Duart-Castells et al. 2019)
<b>MDPV</b>	ddy mice	M	8 W	1 injection	3.0, 10, 30 mg/kg, i.p.		(Ren et al. 2013)
	BALB/cAnCrlCrlj	M	10 W	1 injection	3.0, 10, 30 mg/kg i.p.		(Ren et al. 2014)
	C57BL/6 mice	M	8 W	14 D	10 mg/kg/day i.p.	NB	(Ren et al. 2015)
<b>Alcohol</b>	Wistar rats	M	8-10 W	4 W	5 mg/kg/day i.p.		(Pandey et al. 2020)
	Sprague Dawley rats	M & F	6 W	2 W	5 mg/kg/day i.p.	NB, AE, SD	(Hogarth, Djouma, and van den Buuse 2020)
<b>BRAIN INJURY</b>							
<b>Traumatic Brain Injury</b>	Sprague–Dawley rats	M	2 Mo	7 D	5 mg/kg/day i.p.		(Agrawal et al. 2015)
	Sprague–Dawley rats	M	8-10 W	7 D	5 mg/kg/day i.p.		(Krishna et al. 2017)
	C57BL/6J mice	M	8-10 W	4 D	20, 50 mg/kg/day i.p.		(Wu et al. 2014)
	C57BL/6J mice	M	8-10 W	1 injection	5 mg/kg i.p.		(Chen et al. 2015)
	C57BL/6J mice	M	8-10 W	15 D	5 mg/kg/day i.p.		(Zhao et al. 2016b)
	C57BL/6J mice	M	8-10 W	4 D	5 mg/kg/day i.p.		(Zhao et al. 2016a)
<b>Stroke/Ischemia</b>	TrkB <sup>F616A</sup> mice	M	N.A.	1 injection	5 mg/kg i.p.		(Jang et al. 2010)
	Rats	M	7 D	5 D	5 mg/kg/every other day i.p		(Hung et al. 2013)
	C57BL/6J mice	M & F	9 D	1 or 7 D	5 mg/kg/day i.p	SD	(Uluc et al. 2013)

	C57BL/6J mice	M & F	9 D	3 D	5 mg/kg/day i.p.	SD	(Cikla et al. 2016)
	Sprague–Dawley rats	M	Adult	1 injection	5 mg/kg i.p.		(Wang et al. 2014)
	Sprague-Dawley rats (slices)	N.A.	35-45 D	–	25 µM		(Tecuatl et al. 2018)
<b>Irradiation</b>	C57BL/6J mice	M	2 Mo	3 or 5 W	5 mg/kg/day s.c. or o.p.		(Yang et al. 2016)
<b>NEURODEGENERATION</b>							
<b>Alzheimer Disease</b>	5XFAD mice	M & F	12-15 Mo	10 D	5 mg/kg/day i.p.		(Devi, and Ohno 2012)
	5XFAD mice	N.A.	2 Mo	2 Mo	5 mg/kg/day p.o.		(Zhang et al. 2014b)
	5XFAD mice	F	1 Mo	2 Mo	5 mg/kg/day i.p.		(Aytan et al. 2018)
	5XFAD mice	N.A.	2 Mo	3 Mo	§ 7.2, 21.8, 43.6 mg/kg/day p.o.		(Chen et al. 2018)
	Tg2576 mice	M	10 Mo	4 W	~ 200 mg/kg/day p.o.		(Gao et al. 2016)
	APPswe/PS1dE9 mice	M	7 Mo	1 injection	0.1 mg/kg i.p.		(Bollen et al. 2013)
	APPswe/PS1dE9 mice	M	9 Mo	10 D	5 mg/kg/day i.p.		(Hsiao et al. 2014)
	CaM/Tet-DT <sub>A</sub> mice	M & F	3.5-5 Mo	10 D	5 mg/kg/day i.p.		(Castello et al. 2014)
	APP23/PS45 mice	N.A.	6 W	4 W	5 mg/kg/day i.p.	NB	(Zhou et al. 2015)
	Sprague-Dawley rats	M	Adult	4 W	1 mg/kg/day i.p.		(Chen et al. 2014)
<b>Parkinson Disease</b>	C57BL/6 mice+MPTP	N.A.	8 W	14 D	5 mg/kg/day p.o.		(Jang et al. 2010)
	C57BL/6 mice+MPTP	M	8 W	4 W	5 mg/kg/day i.p.		(Sconce et al. 2015)
	C57BL/6 mice+MPTP	N.A.	Adult	14 D	5 mg/kg/day i.p.		(Li et al. 2016)
	Sprague–Dawley rats+Rot	M	Adult	5 W	5 mg/kg/day i.p.		(Nie et al. 2019)
	Macaca Fascicularis+ MPP+	M	8 Y	7 Mo	30 mg/kg/day po.		(He et al. 2016)
<b>Huntington’s Disease</b>	N171-82Q mice	M	6 W	14 W	5 mg/kg/day p.o.		(Jiang et al. 2013)
	R6/1 mice	M	8 W	12 W	5 mg/kg/day p.o.		(Garcia-Diaz Barriga et al. 2017)
<b>Amyotrophic Lateral Sclerosis</b>	SOD1 <sup>G93A</sup> mice	M	30 D	75 D	5 mg/kg/day i.p.		(Korkmaz et al. 2014)
<b>HEALTHY BRAIN</b>							
	Wistar rats	M	3 Mo	1 injection	0.1, 0.3, 1, 3 mg/kg p.o.		(Bollen et al. 2013)
	Thy1-YFP mice	N.A.	2 Mo	14 D	5 mg/kg/day p.o.		(Perez-Rando et al. 2018)
	Sprague-Dawley rats	M	22&30 Mo	34 D	5 mg/kg/day i.p.		(Zeng et al. 2012b)
	Sprague-Dawley rats	M	24 Mo	4 W	5 mg/kg/day i.p.		(Zeng et al. 2012a)
	C57BL/6 mice	M	8 W	1 injection	5 mg/kg		(Zimmermann et al. 2017)



## PERIPHERAL NERVOUS SYSTEM

Retina	Rat RGCs, RGC-5 cells	—	—	10 min-16 h	100 nM	(Gupta et al. 2013)
	ARPE-19 cells	—	—	48 h	0.01-20 µM	(Yu et al. 2018)
	Sprague-Dawley rats	N.A.	7 D	2 injections	5 mg/kg i.p	(Huang et al. 2018)
Cochlea	Zebrafish dye <sup>ucd6</sup>	N.A.	Embryos	2 D	10 µM	(Daly et al. 2017)
	Cochlear cultures from C57BL/6 and TrkB <sup>F616A</sup> mice	M & F		72 h	300 nM	(Yu et al. 2013)
	cCx26 null mice	M & F	2 D	at 2 D and 30 D	200 µM local application	(Yu et al. 2013)
Nerves	SGN cultures CBA/CaJ mice	N.A.	4 D	48 h	400 nM	(Kempfle et al. 2018)
	C57B6 and <i>SLICK::trkB<sup>fl/fl</sup></i> mice	M & F	N.A.	2 W	500 nM local application	(English et al. 2013)
	C57B6 and <i>SLICK::trkB<sup>fl/fl</sup></i> mice	M & F	N.A.	2 W	5 mg/kg/day, i.p	(English et al. 2013)

Summary of the treatment methodology of the studies that have examined the effects of 7,8-DHF on the central and peripheral nervous system. Abbreviations: AE, adverse effects; b.i., brain infusion; D, day; F, female; h, hour; i.p., intraperitoneal; M, male; min, minutes; Mo, month; MDPV, 3,4-Methylenedioxypropylvalerone; MPP+, 1-methyl-4-phenylpyridinium; MTPP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; N.A., not available; NB, no benefit; o.p., osmotic pump; P, postnatal day; p.o., per os; s.c., subcutaneous; Rot, Rotenone; SD, sex differences; W, week; Y, year. \* plus one injection before behavioral testing; § prodrug R13.

**Table 2. Effect of treatment with 7,8-Dihydroxyflavone on peripheral tissues.**

Organ	Model	Gender	Age	Treatment Duration	Dose and route of administration	Notes	Reference
<b>Heart</b>	Kunming mice	N.A.	Adult	4 W	5 mg/kg/day i.p.		(Wang et al. 2019)
	H9c2 cells	—	—	24 h	100 $\mu$ M		(Wang et al. 2019)
	Kunming mice	N.A.	Adult	3 W	5 mg/kg/day i.p.		(Zhao et al. 2019)
	H9c2 cells	—	—	24 h	1-600 $\mu$ M		(Zhao et al. 2019)
<b>Blood Vessels</b>	Rat aortic rings from Wistar rats	M	16 W	acute (minutes)	1-500 $\mu$ M		(Huai et al. 2014)
	Wistar rats	M	16 W	1 injection	2.5 mg/kg i.v.		(Huai et al. 2014)
	EA.hy926 cells	—	—	24 h	1-100 $\mu$ M		(Wang et al. 2015)
<b>Lung</b>	Human ASM	—	—	24 h	500 nM		(Abcejo et al. 2012)
	Human PAEC	—	—	24 h	N.A.		(Helan et al. 2014)
	Chinese hamster V79-4 lung fibroblasts	—	—	24 h	10 $\mu$ g/ml		(Zhang et al. 2009)
	Chinese hamster V79-4 lung fibroblasts	—	—	24 h	0.1, 1, 10 $\mu$ g/ml		(Ryu et al. 2014b)
<b>Intestine</b>	Muscle strips from the intestine of New Zeland rabbits	N.A.	N.A.	60 min	1 $\mu$ M		(Al-Qudah et al. 2014)
	Muscle strip from the stomach of rats	M	N.A.	5-60 min	1- 100 $\mu$ M		(He et al. 2018)
	Rats	M	N.A.	7 D	0.2, 0.8, 3.2 mM in 1 ml/day p.o.		(He et al. 2018)
<b>Liver</b>	Wistar rats	M	Adult	4 W	5 mg/kg/day i.p.		(Kumar et al. 2019)
<b>Oocytes</b>	Porcine oocyte cultures	—	—	7 D	0, 1, 5, 10 $\mu$ M		(Choi et al. 2013)
<b>Kidney</b>	HK-2 cells	—	—	1-12 h	50-250 $\mu$ M		(Ma et al. 2016)
<b>Muscle</b>	Diaphragm muscle preparations from TrkB <sup>F616A</sup> mice	M	Adult	30 min	10 $\mu$ M		(Mantilla, and Ermilov 2012)
	TrkB <sup>F616A</sup> mice	M	18 Mo	6 Mo	5 mg/kg/day p.o.	NB	(Greising et al. 2017)
<b>Articular Chondrocytes</b>	Primary chondrocytes from B6 mice	N.A.	5 D	8 h	0-10 $\mu$ M		(Cai et al. 2019)
	C57BL/6 mice	N.A.	8-10 W	4 or 8 W	5 mg/kg/week i.p.		(Cai et al. 2019)

<b>Skin</b>	Hs68 cells	—	—	24 h	0-20 µM	(Choi, Lee, and Park 2017)
	HaCaT cells	—	—	24 h	0-5 µg/ml	(Ryu et al. 2014a)
<b>Metabolism</b>	3T3-L1 cells	—	—	24 h	0-100 µM	(Choi et al. 2016)
	C57BL/6J mice	F	8 W	22 W	0.16 mg/ml (2.26 mg/kg/day) p.o.	SD (Chan et al. 2015).
	C57BL/6J mice	F	8 W	9 W	0.16 mg/ml (2.50 mg/kg/day) p.o.	SD (Wood et al. 2018)
<b>Anticancer action</b>						
Breast cancer	MCF-7 cells	—	—	6 D	1-50,000 nM	(Le Bail et al. 1998)
Hepatocellular carcinoma	HepG2 cells	—	—	24 h	1-100 µM	(Kozics, Valovicova, and Slamenova 2011)
Monocytic leukemia	U937 cells	—	—	24 h	70 µM	(Park et al. 2012)
Monocytic leukemia	U937 cells	—	—	1-24 h	5-100 µM	(Park et al. 2013)
Oral squamous cell carcinoma	HN22 and HSC4 cells	—	—	24 or 48 h	5-40 µM	(Lee et al. 2015)
Malignant melanoma	B16F10 cells	—	—	72 h	0.4-100 µM	(Sim, Sohng, and Jung 2016)

Summary of the treatment methodology of the studies that have examined the effects of 7,8-DHF on peripheral tissues. Abbreviations: ASM, airway smooth muscle; D, day; F, female; h, hour; i.p., intraperitoneal; i.v. intravenous; M, male; min, minutes; Mo, month; N.A., not available; NB, no benefit; PAEC, pulmonary artery endothelial cells; p.o., per os; SD, sex differences; W, week.

**Table 3. Studies grouped by examined brain parameter.**

PARAMETER	REFERENCE
<b>Neurogenesis/Differentiation</b>	(Chen et al. 2015; Giacomini et al. 2019; He et al. 2016; Huang et al. 2018; Hung et al. 2013; Liu et al. 2010; Parrini et al. 2017; Stagni et al. 2017; Yang et al. 2016; Zhao et al. 2016b)
<b>Apoptosis/Neurodegeneration</b>	(Amin et al. 2020; Chen et al. 2015; Daly et al. 2017; Gupta et al. 2013; He et al. 2016; Huang et al. 2018; Jang et al. 2010; Korkmaz et al. 2014; Li et al. 2016; Nie et al. 2019; Pandey et al. 2020; Sconce et al. 2015; Uluc et al. 2013; Wang et al. 2014; Wu et al. 2014; Yu et al. 2013; Yu et al. 2018; Zhang et al. 2014b; Zhao et al. 2016a)
<b>Dendritic arbor/Neurites</b>	(Aytan et al. 2018; Garcia-Diaz Barriga et al. 2017; Kempfle et al. 2018; Yu et al. 2013; Zhang et al. 2014b; Zhao et al. 2016a; Zhao et al. 2016b)
<b>Spine density</b>	(Aytan et al. 2018; Barfield et al. 2017; Castello et al. 2014; Gao et al. 2016; Kang et al. 2017; Korkmaz et al. 2014; Perez-Rando et al. 2018; Stagni et al. 2017; Tian et al. 2015; Yang et al. 2016; Zeng et al. 2012a; Zeng et al. 2012b; Zhang et al. 2014a; Zhang et al. 2014b; Zhao et al. 2016a; Zimmermann et al. 2017)
<b>Synaptic proteins</b>	(Agrawal et al. 2015; Barfield et al. 2017; Gao et al. 2016; Krishna et al. 2017; Stagni et al. 2017; Tian et al. 2015; Zeng et al. 2012a; Zhang et al. 2015; Zhang et al. 2016)
<b>Inflammation</b>	(Amin et al. 2020; Garcia-Diaz Barriga et al. 2017; Wang et al. 2014).
<b>Oxidative stress</b>	(Chen et al. 2014; Garcia-Diaz Barriga et al. 2017; Gupta et al. 2013; Li et al. 2016; Pandey et al. 2020; Wang et al. 2014; Yang et al. 2014)
<b>Cell bioenergetics</b>	(Agrawal et al. 2015; Han et al. 2016; Krishna et al. 2017)
<b>LTP</b>	(Chen et al. 2014; Parrini et al. 2017; Sanz-Garcia et al. 2016; Seese et al. 2020; Yang et al. 2014; Zeng et al. 2012a; Zeng et al. 2012b; Zhang et al. 2014b)
<b>Hippocampus-dependent memory (MWM, NOR, CFC)</b>	(Agrawal et al. 2015; Andero et al. 2012; Andero et al. 2011; Bollen et al. 2013; Castello et al. 2014; Chen et al. 2014; Devi, and Ohno 2012; Gao et al. 2016; Garcia-Diaz Barriga et al. 2017; Giacomini et al. 2019; Han et al. 2016; Hsiao et al. 2014; Kang et al. 2017; Krishna et al. 2017; Pandey et al. 2020; Parrini et al. 2017; Perez-Rando et al. 2018; Sanz-Garcia et al. 2016; Seese et al. 2020; Stagni et al. 2017; Tian et al. 2015; Uluc et al. 2013; Yang et al. 2016; Yang et al. 2014; Zeng et al. 2012b; Zhang et al. 2014b; Zhao et al. 2016a; Zhou et al. 2015)
<b>TrkB signaling</b>	(Agrawal et al. 2015; Amin et al. 2020; Andero et al. 2011; Chen et al. 2014; Chen et al. 2015; Cikla et al. 2016; Devi, and Ohno 2012; Garcia-Diaz Barriga et al. 2017; Giacomini et al. 2019; Gupta et al. 2013; Han et al. 2017; Han et al. 2016; Jiang et al. 2013; Krishna et al. 2017; Li et al. 2016; Nie et al. 2019; Parrini et al. 2017; Sconce et al. 2015; Seese et al. 2020; Stagni et al. 2017; Uluc et al. 2013; Wang et al. 2014; Wu et al. 2014; Yang et al. 2016; Yang et al. 2014; Yu et al. 2018; Zeng et al. 2012a; Zeng et al. 2012b; Zhang et al. 2014a; Zhang et al. 2016; Zhang et al. 2014b)
<b>TrkB inhibition</b>	(Agrawal et al. 2015; Chen et al. 2015; Choi, Gourley, and Ressler 2012; Daly et al. 2017; Duarte-Castells et al. 2019; Gao et al. 2016; Garcia-Diaz Barriga et al. 2017; Jang et al. 2010; Kang et al. 2017; Kempfle et al. 2018; Liu et al. 2010; Otis, Fitzgerald, and Mueller 2014; Ren et al. 2015; Ren et al. 2013; Ren et al. 2014; Wu et al. 2014; Yu et al. 2018; Zhang et al. 2014a; Zhang et al. 2016; Zhang et al. 2014b; Zimmermann et al. 2017)

The reported studies have examined the effects of 7,8-DHF on the indicated brain parameters. The parameter “TrkB inhibition” refers to studies that have used TrkB inhibitors in order to demonstrate whether the effects of 7,8-DHF were mediated by the TrkB receptor. Abbreviations; CFC, contextual fear conditioning; LTP, long-term potentiation; MWM, Morris water maze; NOR, novel object recognition.

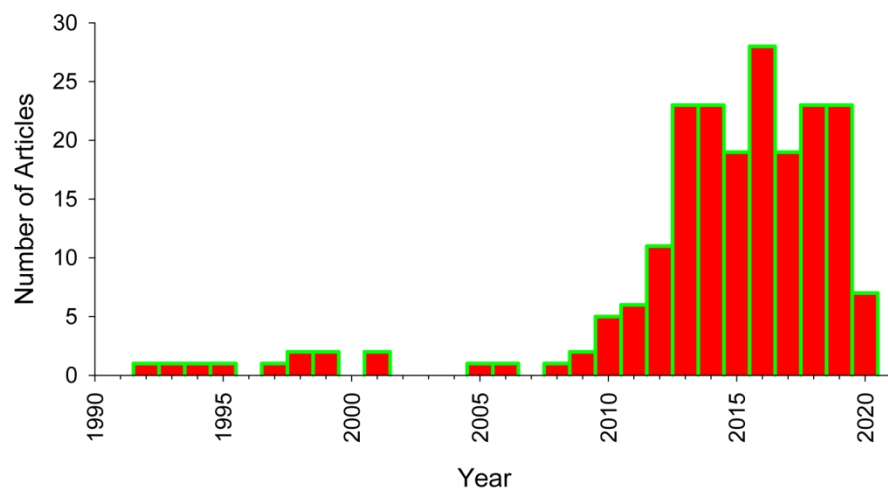


Fig. 1

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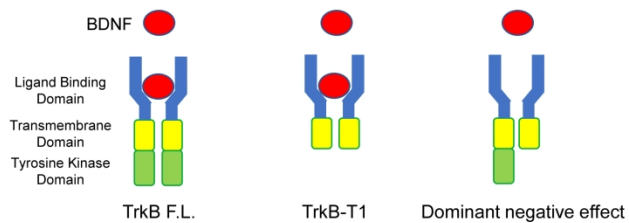


Fig. 2

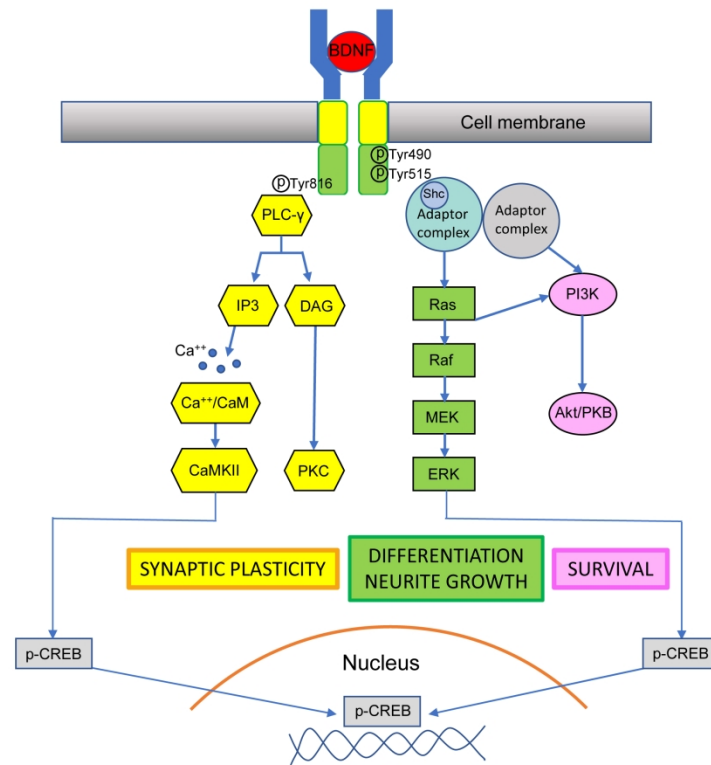


Fig. 3

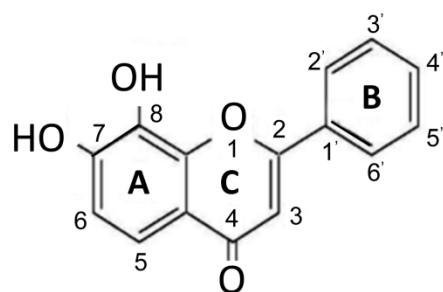


Fig. 4



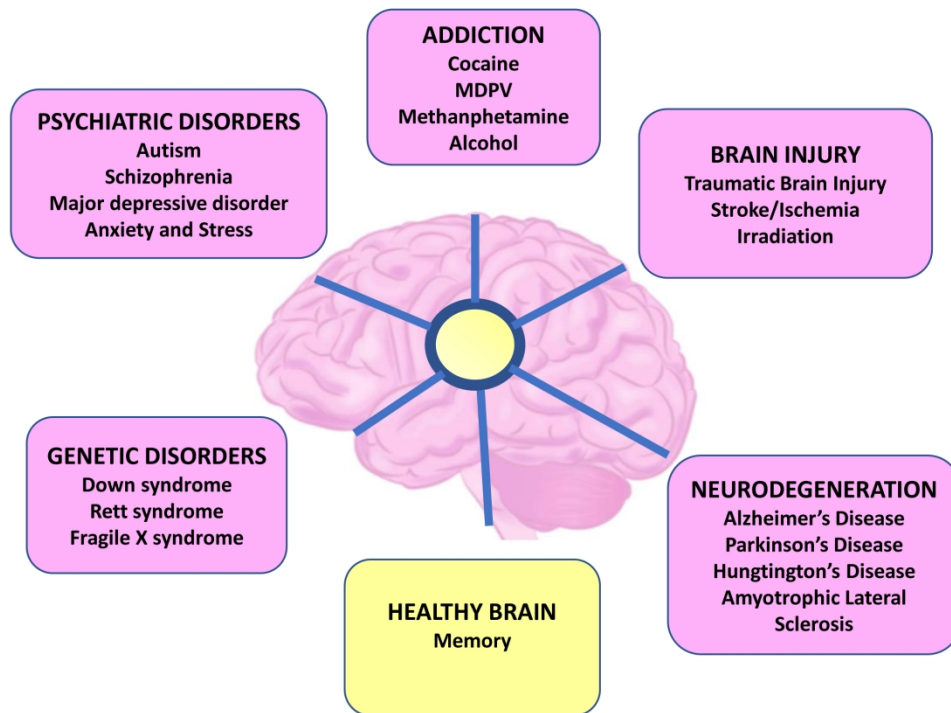


Fig. 5

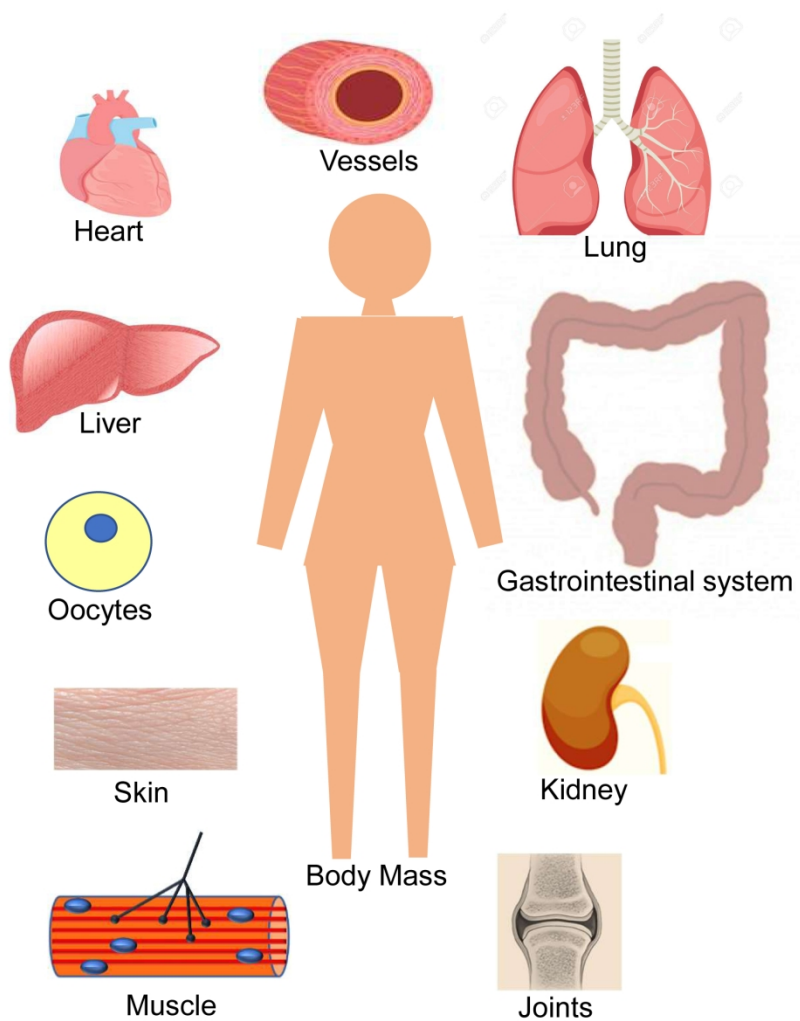


Fig. 6