

# The molecular neurobiology and neuropathology of opioid use disorder

Christopher A. Blackwood<sup>\*\*</sup>, Jean Lud Cadet<sup>\*</sup>

Molecular Neuropsychiatry Research Branch, NIH/NIDA Intramural Research Program, 251 Bayview Boulevard, Baltimore, MD, 21224, USA

## ARTICLE INFO

### Keywords:

Heroin  
Oxycodone  
Fentanyl  
Methadone  
Neuroimaging  
Postmortem

## ABSTRACT

The number of people diagnosed with opioid use disorder has skyrocketed as a consequence of the opioid epidemic and the increased prescribing of opioid drugs for chronic pain relief. Opioid use disorder is characterized by loss of control of drug taking, continued drug use in the presence of adverse consequences, and repeated relapses to drug taking even after long periods of abstinence. Patients who suffer from opioid use disorder often present with cognitive deficits that are potentially secondary to structural brain abnormalities that vary according to the chemical composition of the abused opioid. This review details the neurobiological effects of oxycodone, morphine, heroin, methadone, and fentanyl on brain neurocircuitries by presenting the acute and chronic effects of these drugs on the human brain. In addition, we review results of neuroimaging in opioid use disorder patients and/or histological studies from brains of patients who had expired after acute intoxication following long-term use of these drugs. Moreover, we include relevant discussions of the neurobiological mechanisms involved in promoting abnormalities in the brains of opioid-exposed patients. Finally, we discuss how novel strategies could be used to provide pharmacological treatment against opioid use disorder.

## 1. Introduction

The prolific (mis)use of opioids in the United States (U.S.) and many other countries has created a large number of individuals suffering from opioid use disorder (OUD). OUD is a chronic relapsing disorder driven by neurocircuits that modulate adverse emotional states and trigger relapses (Strang et al., 2020). OUD is also characterized by compulsive drug taking, craving for opioids, and continued opioid intake despite adverse life consequences (Table S1). Although much of the initial study of the neurobiology of drug addiction focused on the acute impact of opioids on the brain using preclinical models, key issues that still need more attention are the long-lasting and potentially toxic effects of opioids on brain structures and their structural consequences on the neurocircuitry that drives the vicious cycle of addiction in people suffering from OUD.

The cost of OUD has led to global health and financial crises, fueled by over-prescription of opioids, increases in recreational opioid use, and expansion of opium cultivation and supplies in various world markets (Banta-Green et al., 2009; UNODC, 2019). In 2018, the U.S. reported that 3.7% of the population over the age of 12 had experimented with opioids (SAMHSA, 2019). That same year, the U.S. reported 46,802 opioid-related fatalities (Hedegaard, 2020). Furthermore, the projected

cost of OUD-related problems is approximately \$78.5 billion, a number that includes expenditures in healthcare, the criminal justice system, and substance abuse treatment programs (Florence et al., 2016).

Physicians commonly prescribe opioids to manage moderate to severe pain. However, some patients consume opioids to experience euphoria, tranquility, and sedation (Schuckit, 2016). The (mis)use of opioids causes adverse effects such as psychological/physical dependency, continuous relapses, and withdrawal symptoms (Schuckit, 2016) and can lead to overdose (Schiller et al., 2021). Furthermore, compulsive drug takers often develop tolerance (Morrone et al., 2017), which is associated with a need to consume greater amount of drugs to experience the desired effects, thus perpetuating the vicious cycle of addiction.

The behavioral effects of opioids are mediated by their binding to opioid receptors that are located in various regions of the peripheral nervous system (PNS) (Liu et al., 2020) and central nervous system (CNS) (Cumming et al., 2019). Mu, delta and kappa opioid receptors are G-protein-coupled receptors (GPCR) expressed throughout the brain (Hirvonen et al., 2009; Naganawa et al., 2015; Weerts et al., 2008) and the periphery (Mansour and Watson, 1993). Upon stimulation by an agonist, these GPCRs form homo- and heterodimeric complexes to activate G proteins and transduce intercellular signals via a wide range

\* Corresponding author. Molecular Neuropsychiatry Research Branch NIH/NIDA Intramural Research Program 251 Bayview Boulevard Baltimore, MD, USA

\*\* Co-Corresponding author.

E-mail addresses: [Christopher.blackwood@nih.gov](mailto:Christopher.blackwood@nih.gov) (C.A. Blackwood), [jcadet@intra.nida.nih.gov](mailto:jcadet@intra.nida.nih.gov) (J.L. Cadet).

<https://doi.org/10.1016/j.crneur.2021.100023>

Received 13 May 2021; Received in revised form 2 October 2021; Accepted 11 October 2021

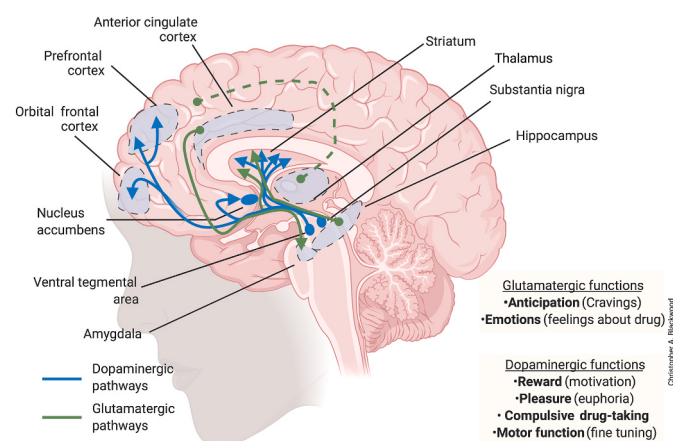
Available online 14 October 2021

2665-945X/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

of intracellular pathways in the PNS and CNS. These include the mitogen-activated protein kinase phosphorylation (MAPK) cascade (Blackwood et al., 2021; Deng et al., 2019). Opioid receptors can signal through Gai/o subunits, inhibit activity of adenylyl cyclase, and reduce cyclic AMP production. Stimulation of opioid receptors can also result in the activation of potassium ion channels (Christie et al., 1987), which have been shown to have inhibitory effects on neurons (Torrecilla et al., 2002). Opioid receptor stimulation can also regulate calcium activity at various nerve terminals (Rusin et al., 1997).

Several synthetic opioids are agonists at the mu opioid receptor that is encoded by the gene OPRM1 (Y. Chen, Mestek, Liu, Hurley and Yu, 1993). Mu opioid receptors are located in various regions of the human body including vascular system, gastrointestinal tract, immune cells, the PNS, and the CNS (Brejchova et al., 2020; Hirvonen et al., 2009; Kuhar et al., 1973; Mosinska et al., 2016). In humans, mu opioid receptors have very high levels of expression in the amygdala, anterior cingulate cortex, cerebellum, nucleus accumbens, hippocampus, thalamus, and amygdala (Hirvonen et al., 2009; Johansson et al., 2019), with the highest densities observed in the striatum, thalamus, and cingulate cortex (Nummenmaa et al., 2018). Mu opioid receptors modulate various behavioral functions including reward, mood, anxiety, neuroendocrine function, and gastrointestinal motility (Nummenmaa and Tuominen, 2018; Pecina et al., 2019). Mu opioid receptors have also been implicated in various aspects of substance use disorders (Blackwood et al., 2019; Blackwood et al., 2019; Gorelick et al., 2008; Meye et al., 2012), in part, via interactions with glutamatergic and dopaminergic pathways (Chartoff and Connery, 2014; Hearing, 2018). Specifically, administration of opioids induces dopamine release in dopaminergic reward-related structures (Di Chiara and Imperato, 1988; Johnson and North, 1992). However, there are reports that dopamine in the thalamus performs a limited role in rodents compared to primate and human brain (Garcia-Cabezas et al., 2009; Garcia-Cabezas et al., 2007). Nevertheless, repeated exposure to drugs may lead to alterations in these structures, with these molecular and cellular neuroadaptations being responsible for the various manifestations of OUD including compulsive drug taking and craving (Fig. 1).

In what follows, we summarize neurobiological and neuropathological changes associated with exposure to several opioid drugs including oxycodone, morphine, heroin, methadone and fentanyl.



**Fig. 1. A model of the brain reward system.** Sagittal illustration of a human brain depicting the dopaminergic pathways (blue) including the ventral tegmental area to the nucleus accumbens, striatum or frontal cortex; and the substantia nigra to the striatum. Glutamatergic pathways (green) are shown from the anterior cingulate cortex to the amygdala and striatum; hippocampus to the striatum; and the cortico-thalamo-cortical interactions. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

## 2. Oxycodone use disorder

In 2018, 9.9 million people aged 12 older were found to (mis)use prescription opioids, including oxycodone (SAMHSA, 2019). In the same year, prescription opioids were responsible for 14,975 overdose deaths (Wilson et al., 2020). Oxycodone is a semisynthetic opioid that is synthesized mainly from thebaine, an alkaloid found in opium poppy (Berenyi et al., 2009). Physicians prescribe oxycodone to treat moderate to severe pain largely from cancer and postsurgical operations. The short-term effects of oxycodone include euphoria and relaxation (Remillard et al., 2019). Long-term use can result in confusion, drowsiness, and respiratory complications (Fox et al., 2018). The repeated use of oxycodone increases tolerance and is associated with severe withdrawal and repeated relapses even during treatment for addiction (Schuckit, 2016). Oxycodone is structurally similar to morphine, but contains a methyl functional group enabling its faster penetration into the blood brain barrier (Bostrom et al., 2008). Oxycodone is metabolized into nor-oxycodone and oxymorphone (Z. R. Chen, Irvine, Somogyi and Bochner, 1991; Lalovic et al., 2006; Lalovic et al., 2004). In addition to binding of mu opioid receptors, oxycodone and oxymorphone appear to have lower binding affinities for delta opioid receptors (Gendron et al., 2016; Thompson et al., 2004). In addition to its biochemical effects, long-term use is also accompanied with brain abnormalities (Duran et al., 2017; Morales Odia et al., 2010; Odia, Jinka and Ziai, 2010; Wheaton et al., 2019). In what follows, we summarize some of these defects and how they disrupt critical neurocircuits as well as their clinical ramifications.

### 2.1. Biochemical and structural abnormalities

Several investigators have described lesions of the brain caused by toxic levels of oxycodone in children and adults (Duran et al., 2017; Morales Odia et al., 2010; Odia et al., 2010; Wheaton et al., 2019). Magnetic resonance imaging (MRI) of a 4-year old and a 46-year old brains of patients with opioid intoxication revealed leukoencephalopathy in cerebellum (Odia, 2010; Wheaton et al., 2019). Several clinical findings showed that toxic levels of oxycodone caused structural abnormalities in the cerebellum (Duran et al., 2017; Morales Odia et al., 2010; Odia et al., 2010; Wheaton et al., 2019) and in the globus pallidus (Odia, 2010). Radiological findings in postmortem brains from oxycodone addicts showed edema (Wheaton et al., 2019), hypoattenuation (Duran et al., 2017), and hydrocephalus (Wheaton et al., 2019) in the cerebellar regions. Postmortem brains from oxycodone addicts with a history of polydrug use with stimulants showed gray matter and white matter defects in the frontal and temporal cortices as well as neuronal axon damage (Alturkustani et al., 2017).

### 2.2. Oxycodone effects on neurocircuits

Structural abnormalities in the brain may in part contribute to defects in the neural activity found in oxycodone abusers. Brain electrical recording studies in oxycodone abusers have shown altered neural activity in the cortex and basal ganglia (Hansen et al., 2018; Lelic et al., 2016; Lelic et al., 2017). Electroencephalography from patients administered oxycodone showed a decrease in neural activity in the insula and frontal gyrus of the cortex (Lelic et al., 2017). Neural circuits connected to the cingulate cortex showed altered electrical stimulation after exposure to oxycodone (Lelic et al., 2016). Oxycodone users showed decreased functional connectivity between the frontal cortex and structures of the basal ganglia region (Hansen et al., 2018). Decreased neural activity may impact neurocircuits between the prefrontal cortex and nucleus accumbens or within the basal ganglia regions (Fig. S1) that are associated with the process of decision-making and habit learning/reward as illustrated in Fig. S2. These changes in neurocircuits are postulated to cause the progression of chronic relapses.

### 3. Morphine use disorder

Morphine is an organic alkaloid substance produced from opium poppy plants. According to the *World Drug Report* during 2014–2018, more than 89% of opium production is largely concentrated in Afghanistan, Myanmar and Mexico. Moreover, the recent expansion of opium cultivation in Mexico is partially responsible for the increase of illicit use in the U.S. (UNODC, 2019). Unauthorized use of morphine along with improper injection hygiene has largely contributed to high rates of hepatitis, human immunodeficiency viruses and other blood borne illnesses. Morphine has been used in clinical settings to treat pain for several decades. The adverse effects of morphine include respiratory depression and physical or psychological dependence (Cooper et al., 2017). The affects of morphine are based on its ability to be metabolized. Morphine is metabolized into morphine-6-glucuronide and morphine-3-glucuronide (Yoshimura et al., 1973). Morphine-6-glucuronide penetrates the blood-barrier and has a high affinity to mu opioid receptors (De Gregori et al., 2012). Moreover, morphine-6-glucuronide is more potent than morphine-3-glucuronide in its analgesic effects (Frances et al., 1990; Frances et al., 1992) on the CNS.

#### 3.1. Biochemical and structural abnormalities

Clinical reports have shown that morphine intoxication leads to brain defects in corpus callosum, globus pallidus and cerebrum (Duran et al., 2017; Nanan et al., 2000). T1- and T2-weighted images from postmortem brain found enlarged corpus callosum and atrophied cerebellum and cerebrum tissues (Duran et al., 2017; Nanan et al., 2000). Postmortem tissue from morphine users also showed lesions in the spinal root ganglia, which contains the respiratory neurons that play a role in breathing (Martin et al., 1987). Clinical investigations found that morphine users showed hypoxic leukoencephalopathy 2–40 days after an overdose (Nanan et al., 2000; Reisner et al., 2015; Salazar and Dubow, 2012). Computed tomography scans from morphine users showed hypodensities in globus pallidus (Salazar and Dubow, 2012).

Other brain defects in morphine users include cerebellar edema, hydrocephalus and cerebellar hypoattenuation (Duran et al., 2017; Reisner et al., 2015) (Fig. 2). Morphine intoxication in infants showed decreased brain volume and cerebral depression (Norman et al., 2013; Tataranno et al., 2020).

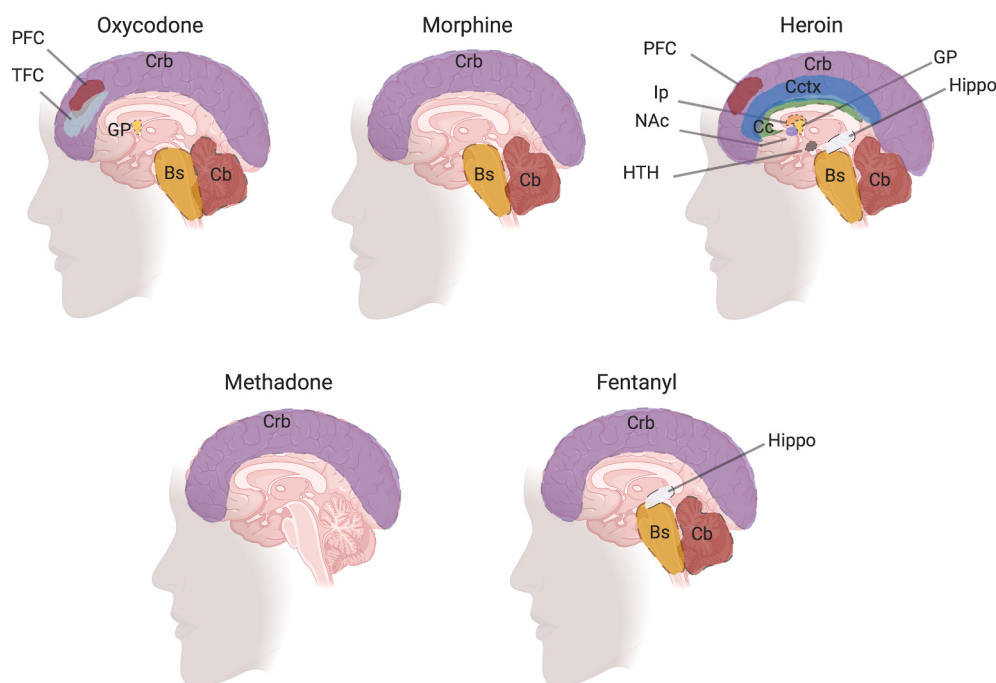
#### 3.2. Morphine effects on neurocircuits

Administration of morphine in volunteers showed increased neural activity in the frontal, anterior cingulate and insula cortices (Jones et al., 1991). In resting-state functional MRI of patients who were given morphine, were found to have altered brain connectivity in the posterior cingulate and frontal cortices, basal ganglia, and cerebellum (Khalili-Mahani et al., 2012; Kleinloog et al., 2015). Moreover, patients taking morphine showed deficits in brain connectivity that caused defects in perception that were associated with hallucinations (Kleinloog et al., 2015).

### 4. Heroin use disorder

Heroin is a highly addictive natural substance derived from the seedpod of various opium plants largely cultivated in Asia, Mexico and Colombia. In 2018, over 800,000 people that consumed heroin were above 12 years of age (SAMHSA, 2019). During 1999–2018, heroin caused approximately 115,000 overdose deaths (Wilson et al., 2020). 24–36% of all heroin addicts experienced the criminal justice system because of illegal drug use and criminal activities to obtain the drug (Boutwell et al., 2007). Because heroin is an illegal substance many users often reuse needles, which increases the incidence of blood-borne infections, including hepatitis and human immunodeficiency viruses from needle sharing (Reardon, 2019). Heroin mixed with a cutting reagent is also consumed through snorting or smoking.

The acetyl functional groups allow heroin to rapidly pass through the blood brain barrier. In the brain, heroin is metabolized into 6-monacetylmorphine, which has a high affinity for mu opioid receptors. The psychopharmacological effects of heroin include euphoria and the sense



**Fig. 2. Brain deficits in human opioid use disorders.** Cartoon illustration of human brain showing areas affected by acute or chronic intoxication of heroin, morphine, oxycodone, methadone and fentanyl. Abbreviations: Bs, Brain stem; Cb, Cerebellum; Crb, Cerebrum; Cctx, Cingulate cortex; Cc, Corpus Callosum; GP, Globus Pallidus; Hippo, Hippocampus; HTH, Hypothalamus; Ip, Insula and putamen; NAC, Nucleus Accumbens; PFC, Prefrontal Cortex.

of relaxation as well as sleep (NIDA, 2019). Short-term effects are clouded mental functioning and back-and-forth state of being conscious (Louria et al., 1967). Long-term effects are depression and antisocial personality disorder (Louria et al., 1967). Other effects of heroin are tolerance and withdrawal symptoms that occur after long period of abstinence. Furthermore, heroin use causes respiratory depression, which can lead to permanent neurological damage and death (Jolley et al., 2015; Ngai, 1961).

#### 4.1. Biochemical and structural abnormalities

Heroin use is linked to several adverse consequences in the human brain (Buttner et al., 2000; Pandria et al., 2018) (Table 1 and Fig. 2). Studies from postmortem brain tissues revealed decrease in volume of hypothalamus (Muller et al., 2018), nucleus accumbens (Muller et al., 2015; Seifert et al., 2015; Tolomeo et al., 2018) and putamen (Bach et al., 2019) structures. Heroin use causes neuronal depletion (Pearson et al., 1976) and structural defects in the globus pallidus (Muller et al., 2019; Tolomeo et al., 2018; Vila and Chamorro, 1997). Heroin addicts showed reduce gray matter density in the frontal, cingulate and occipital cortical regions. The gray matter defects were visible after three days of abstinence, but disappeared after one month, suggesting that this abnormality is recoverable over time (X. Wang et al., 2012).

It has also been shown that heroin users sustained brain lesions from drug toxicity (Chang et al., 1997) or oxygen deprivation (Oehmichen et al., 1996). Drug users that inhaled heroin vapors form lesions in the white matter called spongiform leukoencephalopathy (Chang et al., 1997). Spongiform leukoencephalopathy is often caused by lipophilic toxin contaminants released from the heating of heroin (Kass-Hout et al., 2011; Pirompanich and Chankrachang, 2015; Rizzuto et al., 1997; Wolters et al., 1982). Lesions observed in heroin users are mainly secondary to hypoxic-ischemic damage and secondary encephalopathic pathological changes in the brain. These pathological changes occur as a result of complete or partial reduction of cerebral oxygen supply (Adamides et al., 2009; Fitzgerald et al., 2010). Hypoxic-ischemic encephalopathy contributes to the swelling of the cerebrum causing cerebral edema (Oehmichen et al., 1996; Richter et al., 1973), neuronal damage (Wolters et al., 1982) and neuronal depletion (Bayer et al., 2015).

Immunohistopathological studies revealed that leukoencephalopathy and hypoxic-ischemic encephalopathy activate cell types involved in the immune responses to neuronal damage, further indicative of brain injury (Chistiakov et al., 2017; Oehmichen et al., 1996). Furthermore, brain tissue from heroin users showed an increase in the presence of hippocampal *Glial Fibrillary Acid Protein*, a marker for microglial cells, which are activated in response to inflammation and/or local injury to cells (Oehmichen et al., 1996; Rizzuto et al., 1997). Postmortem hippocampal tissue from heroin addicts show increased *Cluster of Differentiation 68* (CD68)-positive monocytes/macrophages (Chistiakov et al., 2017), supporting the notion that heroin intoxication causes severe brain damage.

#### 4.2. Neurobiological mechanisms in heroin addicts

Several studies from heroin users have revealed biochemical alterations in the striatum and prefrontal cortex (Table 2 and summarized in Fig. 3) of heroin abusers (Ferrer-Alcon et al., 2004; Sullivan et al., 2013). Biochemical analyses of postmortem brains from heroin users showed abnormal protein levels of mu opioid receptors in the putamen (Sullivan et al., 2013) and prefrontal cortex (Ferrer-Alcon et al., 2004). Tissue from prefrontal cortex of heroin abusers also showed increased protein expression of G protein-coupled receptor kinase (GRK) GRK2, GRK6 and  $\beta$ -arrestin-2 (Ferrer-Alcon et al., 2004). Several reports from postmortem cortical tissue found decreased protein expression of Protein Kinase C- $\alpha$  (PKC $\alpha$ ) (Busquets et al., 1995; Garcia-Sevilla et al., 1997) and adenylyl cyclase-I (Shichinohe et al., 1998, 2001). Postmortem tissue from heroin users showed increased abundance of guanine nucleotide binding

**Table 1**

Summary of data from human postmortem brains.

| Opioid           | Brain region                     | Brain abnormalities                                                                                       | References                   |
|------------------|----------------------------------|-----------------------------------------------------------------------------------------------------------|------------------------------|
| <b>Oxycodone</b> | Cb and GP                        | Leukoencephalopathy, cerebellar and GP lesions.                                                           | Odia et al. (2010)           |
|                  | Cb                               | Cerebellar edema, cerebellar hypoattenuation                                                              | Duran et al. (2017)          |
|                  | Cerebellum, white matter and Crb | Cerebellar edema, hydrocephalus, cerebellar lesions, white matter hyperintensity, and leukoencephalopathy | Wheaton et al. (2019)        |
| <b>Morphine</b>  | Neuronal defects                 | Loss of neurons in the spinal root ganglia.                                                               | Martin et al. (1987)         |
|                  | Cb and white matter              | Leukoencephalopathy, white matter hyperintensity                                                          | Nanan et al. (2000)          |
|                  | Crb                              | Cerebellar edema, hydrocephalus and cerebellar hypoattenuation                                            | Duran et al. (2017)          |
| <b>Heroin</b>    | GP                               | Neuronal depletion in the GP                                                                              | Pearson et al. (1976)        |
|                  | Cb and Crb                       | Leukoencephalopathy, Cb white mater defects and crb edema                                                 | Wolters et al. (1982)        |
|                  | Cb                               | Leukoencephalopathy, Cb edema, gray and white mater cerebral defects                                      | Rizzuto et al. (1997)        |
|                  | GP, Hippo, Crb                   | bilateral lesions in GP, defects in hippocampus and Crb                                                   | Andersen et al., 1999        |
|                  | Gray matter                      | Gray matter defects                                                                                       | Wang et al. (2010)           |
|                  | Cerebellum and Cc                | White matter edema, defects in the corpus callosum, and small ventricles                                  | Krinsky and Reichard, 2012   |
|                  | Cortex and gray matter           | Reduced gray matter density in frontal, cingulate and occipital cortices                                  | Wang et al. (2012)           |
|                  | NAc                              | Reduced NAc                                                                                               | Muller et al. (2015)         |
|                  | NAc                              | Reduced NAc                                                                                               | Seifert et al. (2015)        |
|                  | HTH                              | Lesions in HTH                                                                                            | Montoya-Filardi et al., 2016 |
|                  | White matter                     | White matter hyperintensity                                                                               | Short et al., 2017           |
|                  | HTH                              | Reduced HTH                                                                                               | Muller et al. (2018)         |
| <b>Methadone</b> | GP and NAc                       | Reduced NAc and GP                                                                                        | Tolomeo et al. (2018)        |
|                  | Insula and putamen GP            | Reduced gray matter volume in putamen and smaller insula                                                  | Bach et al. (2019)           |
| <b>Fentanyl</b>  | Crb                              | Reduced GP                                                                                                | Muller et al. (2019)         |
|                  | Crb                              | Crb edema                                                                                                 | Palmiere et al. (2011)       |
| <b>Fentanyl</b>  | Crb                              | Crb edema                                                                                                 | Winkhofer et al. (2014)      |
|                  | Crb                              | Crb edema                                                                                                 | Foy et al. (2011)            |
| <b>Fentanyl</b>  | Crb                              | Crb edema                                                                                                 | Helander et al., 2016        |
|                  | Crb                              | Crb edema                                                                                                 | Guerrieri et al. (2017)      |
| <b>Fentanyl</b>  | Crb                              | Crb edema                                                                                                 | Geile et al. (2019)          |
|                  | Crb                              | Crb edema                                                                                                 | Freni et al. (2019)          |

Cb, Cerebellum; Cc, Corpus Callosum; Crb, Cerebrum; GP, Globus Pallidus; Hippo, Hippocampus.

HTH; Hypothalamus; NAc, Nucleus Accumbens; and PFC, Prefrontal Cortex.

proteins subunits:  $G\alpha_{i1/2}$ ,  $G\alpha_o$ ,  $G\alpha_s$  and  $G\alpha\beta$  (Busquets et al., 1995; Escriba et al., 1994; Garcia-Sevilla et al., 1997; Hashimoto et al., 1996). These findings support the notion that heroin intoxication causes several neurobiological abnormalities in the prefrontal cortex (Ferrer-Alcon

**Table 2**  
Postmortem brains of heroin addicts.

| Region                  | Biochemical alterations                                                                       | References                   |
|-------------------------|-----------------------------------------------------------------------------------------------|------------------------------|
| Prefrontal Cortex       | Decrease PKC- $\alpha$ , increase Gai1/2                                                      | Garcia-Sevilla et al. (1997) |
| Striatum                | Decreased ERK and ELK-1                                                                       | Sullivan et al. (2013)       |
| Temporal Frontal Cortex | Decreased adenylyl cyclase                                                                    | Shichinohe et al. (2001)     |
| Nucleus Accumbens       | Decrease Gai1/2                                                                               | McLeman et al., 2000         |
| Prefrontal Cortex       | GRK 2 (membrane associated)                                                                   | Ozaita et al., 1998          |
| Prefrontal Cortex       | Decrease mu opioid receptors, increase GRK 2, increase GRK 6 and increase $\beta$ -arrestin-2 | Ferrer-Alcon et al. (2004)   |
| Prefrontal Cortex       | Increase in Gai1/2, G $\alpha$ O, G $\alpha$ S, and Gai                                       | Escriba et al. (1994)        |
| Prefrontal Cortex       | Decrease PKC $\alpha$ , increase in Gai1/2                                                    | Busquest et al. (1995)       |
| Temporal Frontal Cortex | Decrease adenylyl cyclase-I                                                                   | Shichinohe et al. (1998)     |
| Temporal Frontal Cortex | Increases G $\beta$ subunits                                                                  | Hashimoto et al. (1996)      |
| Orbital Frontal Cortex  | Altered DNA methylation                                                                       | Kozlenkov et al., 2017       |

et al., 2004) and striatum (Sullivan et al., 2013).

4.3. Heroin effects on neurocircuits

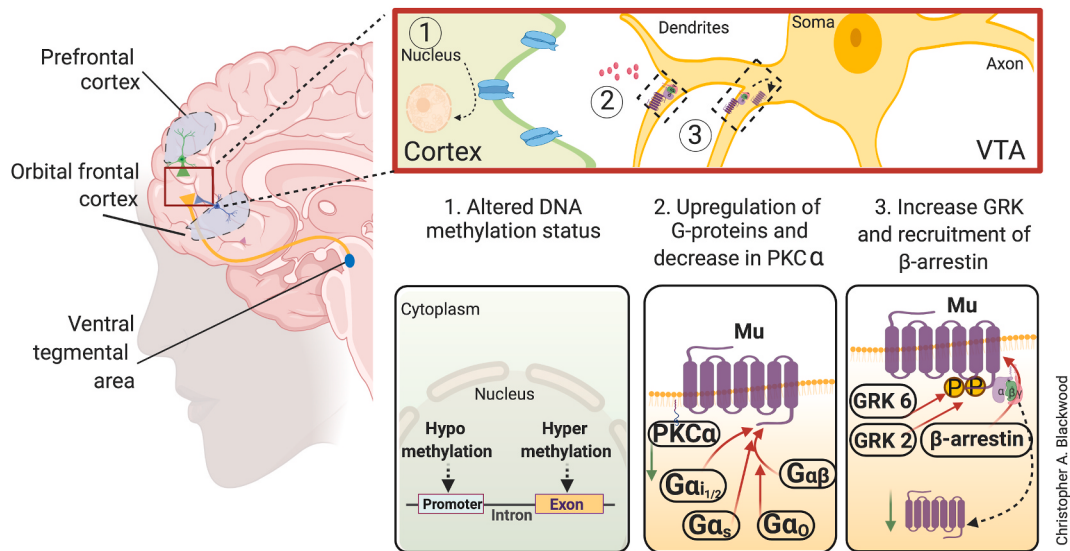
Heroin dependency is associated with defects in the temporal, frontal, and anterior cingulate regions of the cortex, which has negative consequences to glutamatergic efferents, from the frontal cortex to the nucleus accumbens (Kalivas, 2004; W. Wang et al., 2010). Clinical evidence from heroin abusers found decreased functional connectivity between the frontal cortex and nucleus accumbens (W. Wang et al., 2010; Zou et al., 2015). Resting-state functional MRI from heroin-dependent patients showed defects in connectivity between the prefrontal cortex and nucleus accumbens (Zou et al., 2015). Furthermore, heroin addicts

showed abnormal functional connectivity in the basal ganglia and amygdala (Ma et al., 2010; Upadhyay et al., 2010). In patients who had abstained from heroin were found to have abnormal connectivity between the nucleus accumbens and amygdala (Xie et al., 2014), suggesting this regions may be related to withdrawal symptoms. In the same study, neural connectivity defects were also observed between the thalamus and the prefrontal cortex, which neurocircuits have been implicated in the process of decision-making and habit learning/reward as depicted in Fig. S3.

The data reviewed here indicate that heroin intoxication is accompanied by biochemical changes in the abundance of mu opioid receptor. Although the underlying mechanisms of heroin addiction are still unknown, this review revealed a potential mechanism that involves signaling through mu opioid receptor and intracellular proteins downstream of this receptor (see Fig. 3 for summary). This statement is consistent with preclinical models of opioid self-administration (Blackwood et al., 2021). The involvement of mu opioid receptor in addiction is supported by reports that genetic mutations in OPRM1 increased vulnerability of heroin addiction in humans (Drakenberg et al., 2006) and in preclinical models (Sullivan et al., 2013). Moreover, the role of this receptor in addictive processes is supported by observations that an A118G single nucleotide polymorphism of mu opioid receptor is linked to opioid dependence (Bart et al., 2004) and alcoholism (Bart et al., 2005). In addition, a single nucleotide polymorphism found in the CREB gene was reported to correlate with heroin use (Pal et al., 2014). Also relevant to this discussion are findings that epigenetic factors that regulate the expression of mu opioid receptor are associated with heroin addiction. Specifically, there was increased methylation of cytosine residues in cytosine:guanine (CpG) dinucleotides clusters located in the OPRM1 promoter of former heroin addicts (Nielsen et al., 2009). Together, these observations suggest that mu opioid receptor-associated mechanisms may involve changes in signaling mechanisms downstream of CREB (Kumar et al., 2011; Lane-Ladd et al., 1997).

5. Methadone use disorder

In the 1960's, methadone was introduced as a treatment for opioid



**Fig. 3. Potential neurobiological mechanisms associated with craving in heroin addicts.** Decision-making is part of the progression to chronic relapse and is associated with the prefrontal cortex. In postmortem brain heroin causes the (1) altered DNA methylation status in glutamatergic neurons. (2) Increased levels of G-protein subunits: Gai1/2, G $\alpha$ O, G $\alpha$ S, and G $\alpha$  $\beta$ . (3) Another possible mechanism involves the upregulation of GRK 2 and GRK 6, which can lead to the phosphorylation of Mu opioid receptors. Moreover, GRKs and receptor phosphorylation leads to the recruitment of  $\beta$ -arrestin 2 causing desensitization of mu opioid receptors through internalization and degradation. Abbreviations: Mu, mu opioid receptor; G-proteins, guanine nucleotide-binding proteins; red and green arrows, increase and levels, respectively; GRK, G protein-coupled receptor kinase. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

withdrawal symptoms (Dole and Nyswander, 1965). Subsequently, these methadone clinics were helpful in the treatment of opioid users infected with the human acquired immunodeficiency (HIV). Methadone is a synthetic opioid that is commonly administered orally as a racemic mixture of two enantiomers: R-methadone (R-Met) and S-methadone (S-Met) (Foster et al., 1999; Inturrisi, 2005). Methadone is one the most long-lasting opioid medication with a half-life of approximately 13–50 hours. The drug also has an affinity for the NMDA (N-methyl-D-aspartate) receptor, an ionotropic glutamate receptor, which plays a critical role in modulating long-term and memory formation in addiction (Bauer et al., 2002). Methadol, the active metabolite of methadone, causes analgesia, sedation, and euphoria. The negative side effects include itching, nausea, and respiratory depression.

Methadone usage has increased, in large part, due to growing numbers of treatment programs that offer methadone maintenance therapy (MMT) (Kreek et al., 2019). MMT is one of the few effective treatments for people suffering from OUD (D'Aunno et al., 2014). In fact, several studies have reported that MMT correlated with a reduction in heroin-related deaths (Capehorn et al., 1994; Gronbladh et al., 1990). Successful use of MMT requires trained medical staff because methadone remains a controlled substance. Furthermore, unsupervised usage of methadone has caused overdose deaths and serious neurological damage (Drummer et al., 1992; Hedegaard et al., 2020; Wu and Henry, 1990).

### 5.1. Biochemical and structural abnormalities

In children, acute methadone toxicity can cause brain abnormalities. T2-weighted images from magnetic resonance imaging (MRI) found lesions in the cerebellar hemispheres (Hosseini, 2017). This finding is consistent with reports of leukoencephalopathy (Anselmo et al., 2006; Metkees et al., 2015; Tiong et al., 2019) and cerebellar edema (Hosseini, 2017) in methadone users. Other defects in cerebellar hemispheres include hyperintensities in white matter (Metkees et al., 2015) and hydrocephalus (Anselmo et al., 2006; Metkees et al., 2015). Structural abnormalities were also found in the U-fibers of the cortex (Metkees et al., 2015). Moreover, brain MRI detected lesions in the hippocampus (Anselmo et al., 2006). In contrast to children, adults show very little severe neurological conditions. However, computed tomographic scans showed cerebellar edema in adult patients that inhaled methadone (Palmieri et al., 2011; Winkelhofer et al., 2014).

In children and young adults, methadone exposure causes widespread defects in the cortex (Metkees et al., 2015; Tiong et al., 2019), subcortical U-fibers (Metkees et al., 2015), cerebellum (Hosseini, 2017; Metkees et al., 2015; Reisner et al., 2015), hippocampus (Anselmo et al., 2006), and other white matter regions (Metkees et al., 2015). Hence, special consideration should be taken to limit the exposure of methadone to children, young adults, and pregnant women. Methadone-induced lesions in the cerebrum and cerebellum were found to be reversible, suggesting that patients may recover over time (Cerese et al., 2011). The etiology of methadone-induced lesions remains unknown. However, these are thought to be secondary to hypoxia-induced leukoencephalopathy or atrophy. Long-term use of methadone has indeed been reported to cause damage to white matter in patients maintained on the drug for therapeutic purposes (Li et al., 2016). Additional studies examining the neurological consequences of long-term use of opioids for maintenance therapy may be necessary in order to develop recommendation for possible monitoring of these patients in medical settings.

## 6. Fentanyl use disorder

Fentanyl or its derivatives have been used by physicians for pain management and for treatment in opioid-tolerant patients (Schifano et al., 2019). However, in recent years the U.S. spike in opioid-related deaths are due to illicit use of fentanyl and fentanyl-related analogs

(Blackwood and Cadet, 2021; Rudd et al., 2016). The effects of fentanyl include sedation, nausea, and respiratory depression. Fentanyl also causes neurotoxic psychological symptoms in the form of visual hallucinations, insomnia, and anxiety (Ostwal et al., 2015). Furthermore, these compounds have the potential to adversely impact the respiratory and immune systems (Bidlack et al., 2006; Tabatabai et al., 1989). Human studies have reported that high doses of fentanyl lead to respiratory depression (Mildh et al., 2001), likely from its effect on respiratory neurons located in the brainstem (Yuge et al., 1985).

Fentanyl and fentanyl analogs are potent mu opioid receptor agonists. For example, fentanyl is approximately 80–100 times more potent than morphine. A fentanyl-derived drug, carfentanyl, has approximately 10,000 more affinity to mu opioid receptor than morphine (Meert et al., 1988; Van Bever, Niemegeers, Schellekens and Janssen, 1976). Fentanyl and fentanyl analogs undergo biotransformation by CYP enzymes into inactive metabolites (Feasel et al., 2016; Tateishi et al., 1996).

### 6.1. Biochemical and structural abnormalities

Acute fentanyl intoxication cause spongiform leukoencephalopathy in toddlers (Foy et al., 2011) and adult patients (Switzer et al., 2020). MRI and histological findings showed cerebral hemorrhage (Helander et al., 2017) and cerebral edema (Freni et al., 2019; Geile et al., 2019; Guerrieri et al., 2017; Schonfeld et al., 2019) in the majority of fentanyl and fentanyl-related clinical cases (Fomin et al., 2018). Fentanyl users also had hyperintensities in the hippocampus (Switzer et al., 2020). MRI in preterm infants found cerebellar injury and cerebellar hemorrhage that correlated with high dosages of fentanyl (McPherson et al., 2015).

## 7. Conclusions

In this review, we summarized the structural abnormalities reported after acute and chronic opioid exposure. The accumulated evidence supports the notion of potential opioid-induced neuropathological damage in discrete brain regions that serve as substrates for the cycle of opioid use disorder. The further identification of potentially vulnerable brain regions in these patients might serve as guides for optimal implantation sites for deep brain stimulation technology, one of the latest tools being explored to treat opioid addiction (Krauss et al., 2021) because abnormalities in these areas might regulate the vicious addiction process.

Finally, Fig. 3 represents a schematic rendering for the potential involvement of signaling factors downstream mu opioid receptors in various brain circuits implicated in substance use disorders. Activation or inhibition of these signaling cascades might lead to drug-induced epigenetic alterations that include DNA methylation and histone post-translational modifications. Indeed, DNA samples taken from patients suffering from heroin use disorder were found to have increased levels of DNA methylation (Nielsen et al., 2008; Xu et al., 2018). Aberrant alterations in DNA methylation are associated with various neuropsychiatric diseases, including heroin use disorder (Cadet and Jayanthi, 2021; Nielsen et al., 2008; Wang et al., 2016).

### Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

The authors declare no other relevant competing interests that could have appeared to influence the work reported in this review.

### Funding

Work in Dr. Jean Lud Cadet's laboratory is supported by funds of the Intramural Research Program of the DHHS/NIH/NIDA.

## CRediT authorship contribution statement

**Christopher A. Blackwood:** Data curation, Visualization, Writing –original and revised manuscript. **Jean Lud Cadet:** Conceptualization, preformed writing –review and editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We would like to thank Michael T. McCoy, Mark L. Hudak and NIH Fellows Editorial Board for editorial assistance. Due to reference limitations, we have not been able to cite all authors whose research has supported the advancement of this area of interest. We apologize to any authors whose relevant findings were left out during the preparation of this review.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crneur.2021.100023>.

## References

- Adamides, A.A., Cooper, D.J., Rosenfeldt, F.L., Bailey, M.J., Pratt, N., Tippett, N., Rosenfeld, J.V., 2009. Focal cerebral oxygenation and neurological outcome with or without brain tissue oxygen-guided therapy in patients with traumatic brain injury. *Acta Neurochir.* 151 (11), 1399–1409. <https://doi.org/10.1007/s00701-009-0398-y>.
- Alturkustani, M., Ang, L.C., Ramsay, D., 2017. Pathology of toxic leukoencephalopathy in drug abuse supports hypoxic-ischemic pathophysiology/etiology. *Neuropathology* 37 (4), 321–328. <https://doi.org/10.1111/neup.12377>.
- Anselmo, M., Campos Rainho, A., do Carmo Vale, M., Estrada, J., Valente, R., Correia, M., Barata, D., 2006. Methadone intoxication in a child: toxic encephalopathy? *J. Child Neurol.* 21 (7), 618–620. <https://doi.org/10.1177/08830738060210071101>.
- Bach, P., Frischknecht, U., Reinhard, I., Bekier, N., Demirakca, T., Ende, G., Hermann, D., 2019. Impaired working memory performance in opioid-dependent patients is related to reduced insula gray matter volume: a voxel-based morphometric study. *Eur. Arch. Psychiatr. Clin. Neurosci.* <https://doi.org/10.1007/s00406-019-01052-7>.
- Banta-Green, C.J., Merrill, J.O., Doyle, S.R., Boudreau, D.M., Calsyn, D.A., 2009. Measurement of opioid problems among chronic pain patients in a general medical population. *Drug Alcohol Depend.* 104 (1–2), 43–49. <https://doi.org/10.1016/j.drugalcdep.2009.03.022>.
- Bart, G., Hellig, M., LaForge, K.S., Pollak, L., Leal, S.M., Ott, J., Kreek, M.J., 2004. Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. *Mol. Psychiatr.* 9 (6), 547–549. <https://doi.org/10.1038/sj.mp.4001504>.
- Bart, G., Kreek, M.J., Ott, J., LaForge, K.S., Proudnikov, D., Pollak, L., Heilig, M., 2005. Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. *Neuropsychopharmacology* 30 (2), 417–422. <https://doi.org/10.1038/sj.npp.1300598>.
- Bauer, E.P., Schafe, G.E., LeDoux, J.E., 2002. NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. *J. Neurosci.* 22 (12), 5239–5249. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/12077219>.
- Bayer, R., Franke, H., Ficker, C., Richter, M., Lessig, R., Buttner, A., Weber, M., 2015. Alterations of neuronal precursor cells in stages of human adult neurogenesis in heroin addicts. *Drug Alcohol Depend.* 156, 139–149. <https://doi.org/10.1016/j.drugalcdep.2015.09.005>.
- Berenyi, S., Csutoras, C., Sipos, A., 2009. Recent developments in the chemistry of the opiate and its transformation products as pharmacological targets. *Curr. Med. Chem.* 16 (25), 3215–3242. <https://doi.org/10.2174/092986709788803295>.
- Bidlack, J.M., Khimich, M., Parkhill, A.L., Sumagin, S., Sun, B., Tipton, C.M., 2006. Opioid receptors and signaling on cells from the immune system. *J. Neuroimmune Pharmacol.* 1 (3), 260–269. <https://doi.org/10.1007/s11481-006-9026-2>.
- Blackwood, C.A., Cadet, J., 2021. COVID-19 pandemic and fentanyl use disorder in african Americans. *Front. Neurosci.* 15 <https://doi.org/10.3389/fnins.2021.707386>.
- Blackwood, C.A., Leary, M., Salisbury, A., McCoy, M.T., Cadet, J., 2019. Escalated oxycodone self-administration causes differential striatal mRNA expression of FGFs and IEGs following abstinence-associated incubation of oxycodone craving. *Neuroscience* 415, 173–183. <https://doi.org/10.1016/j.neuroscience.2019.07.030>.
- Blackwood, C.A., McCoy, M.T., Ladenheim, B., Cadet, J., 2021. Oxycodone self-administration activates the mitogen-activated protein kinase/mitogen- and stress-activated protein kinase (MAPK-MSK) signaling pathway in the rat dorsal striatum. *Sci. Rep.* 11 (1), 2567. <https://doi.org/10.1038/s41598-021-82206-3>.
- Bostrom, E., Hammarlund-Udenaes, M., Simonsson, U.S., 2008. Blood-brain barrier transport helps to explain discrepancies in in vivo potency between oxycodone and morphine. *Anesthesiology* 108 (3), 495–505. <https://doi.org/10.1097/ALN.0b013e318164cf9e>.
- Boutwell, A.E., Nijhawan, A., Zaller, N., Rich, J.D., 2007. Arrested on heroin: a national opportunity. *J. Opioid Manag.* 3 (6), 328–332. <https://doi.org/10.5055/jom.2007.0021>.
- Brejchova, J., Holan, V., Svoboda, P., 2020. Expression of opioid receptors in cells of the immune system. *Int. J. Mol. Sci.* 22 (1) <https://doi.org/10.3390/ijms22010315>.
- Busquets, X., Escriba, P.V., Sastre, M., Garcia-Sevilla, J.A., 1995. Loss of protein kinase C-alpha beta in brain of heroin addicts and morphine-dependent rats. *J. Neurochem.* 64 (1), 247–252. <https://doi.org/10.1046/j.1471-4159.1995.64010247.x>.
- Buttner, A., Mall, G., Penning, R., Weis, S., 2000. The neuropathology of heroin abuse. *Forensic Sci. Int.* 113 (1–3), 435–442. [https://doi.org/10.1016/s0379-0738\(00\)00204-8](https://doi.org/10.1016/s0379-0738(00)00204-8).
- Cadet, J.L., Jayanthi, S., 2021. Epigenetics of addiction. *Neurochem. Int.* 147, 105069. <https://doi.org/10.1016/j.neuint.2021.105069>.
- Caplehorn, J.R., Dalton, M.S., Cluff, M.C., Petrenas, A.M., 1994. Retention in methadone maintenance and heroin addicts' risk of death. *Addiction* 89 (2), 203–209. <https://doi.org/10.1111/j.1360-0443.1994.tb00879.x>.
- Cerase, A., Leonini, S., Bellini, M., Chianese, G., Venturi, C., 2011. Methadone-induced toxic leukoencephalopathy: diagnosis and follow-up by magnetic resonance imaging including diffusion-weighted imaging and apparent diffusion coefficient maps. *J. Neuroimaging* 21 (3), 283–286. <https://doi.org/10.1111/j.1552-6569.2010.00530.x>.
- Chang, Y.J., Tsai, C.H., Chen, C.J., 1997. Leukoencephalopathy after inhalation of heroin vapor. *J. Formos. Med. Assoc.* 96 (9), 758–760. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/9308333>.
- Chartoff, E.H., Connery, H.S., 2014. It's MORE exciting than mu: crosstalk between mu opioid receptors and glutamatergic transmission in the mesolimbic dopamine system. *Front. Pharmacol.* 5, 116. <https://doi.org/10.3389/fphar.2014.00116>.
- Chen, Y., Mestek, A., Liu, J., Hurley, J.A., Yu, L., 1993. Molecular cloning and functional expression of a mu-opioid receptor from rat brain. *Mol. Pharmacol.* 44 (1), 8–12. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/8393525>.
- Chen, Z.R., Irvine, R.J., Somogyi, A.A., Bochner, F., 1991. Mu receptor binding of some commonly used opioids and their metabolites. *Life Sci.* 48 (22), 2165–2171. [https://doi.org/10.1016/0024-3205\(91\)90150-a](https://doi.org/10.1016/0024-3205(91)90150-a).
- Chistiakov, D.A., Killingsworth, M.C., Myasoedova, V.A., Orekhov, A.N., Bobryshev, Y. V., 2017. CD68/macrosialin: not just a histochemical marker. *Lab. Invest.* 97 (1), 4–13. <https://doi.org/10.1038/labinvest.2016.116>.
- Christie, M.J., Williams, J.T., North, R.A., 1987. Cellular mechanisms of opioid tolerance: studies in single brain neurons. *Mol. Pharmacol.* 32 (5), 633–638. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/2824980>.
- Cooper, T.E., Chen, J., Wiffen, P.J., Derry, S., Carr, D.B., Aldington, D., Moore, R.A., 2017. Morphine for chronic neuropathic pain in adults. *Cochrane Database Syst. Rev.* 5, CD011669. <https://doi.org/10.1002/14651858.CD011669.pub2>.
- Cumming, P., Marton, J., Lilius, T.O., Olberg, D.E., Rominger, A., 2019. A survey of molecular imaging of opioid receptors. *Molecules* 24 (22). <https://doi.org/10.3390/molecules24224190>.
- D'Aunno, T., Pollack, H.A., Frimpong, J.A., Wutchiett, D., 2014. Evidence-based treatment for opioid disorders: a 23-year national study of methadone dose levels. *J. Subst. Abuse Treat.* 47 (4), 245–250. <https://doi.org/10.1016/j.jsat.2014.06.001>.
- De Gregori, S., De Gregori, M., Ranzani, G.N., Allegri, M., Minella, C., Regazzi, M., 2012. Morphine metabolism, transport and brain disposition. *Metab. Brain Dis.* 27 (1), 1–5. <https://doi.org/10.1007/s11011-011-9274-6>.
- Deng, M., Chen, S.R., Chen, H., Luo, Y., Dong, Y., Pan, H.L., 2019. Mitogen-activated protein kinase signaling mediates opioid-induced presynaptic NMDA receptor activation and analgesic tolerance. *J. Neurochem.* 148 (2), 275–290. <https://doi.org/10.1111/jnc.14628>.
- Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U. S. A.* 85 (14), 5274–5278. <https://doi.org/10.1073/pnas.85.14.5274>.
- Dole, V.P., Nyswander, M., 1965. A medical treatment for diacetylmorphine (heroin) addiction. A clinical trial with methadone hydrochloride. *J. Am. Med. Assoc.* 193, 646–650. <https://doi.org/10.1001/jama.1965.03090080008002>.
- Drakenberg, K., Nikoshkov, A., Horvath, M.C., Fagergren, P., Gharibyan, A., Saarelainen, K., Hurd, Y.L., 2006. Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proc. Natl. Acad. Sci. U. S. A.* 103 (20), 7883–7888. <https://doi.org/10.1073/pnas.0600871103>.
- Drummer, O.H., Opeskin, K., Syrjanen, M., Corder, S.M., 1992. Methadone toxicity causing death in ten subjects starting on a methadone maintenance program. *Am. J. Forensic Med. Pathol.* 13 (4), 346–350. <https://doi.org/10.1097/00000433-199212000-00017>.
- Duran, D., Messina, R.D., Beslow, L.A., Montejó, J.D., Karim, J.K., Gavankar Furey, C., Kahle, K.T., 2017. Malignant cerebellar edema subsequent to accidental prescription opioid intoxication in children. *Front. Neurol.* 8, 362. <https://doi.org/10.3389/fneur.2017.00362>.
- Escriba, P.V., Sastre, M., Garcia-Sevilla, J.A., 1994. Increased density of guanine nucleotide-binding proteins in the postmortem brains of heroin addicts. *Arch. Gen.*

- Psychiatr. 51 (6), 494–501. <https://doi.org/10.1001/archpsyc.1994.03950060058006>.
- Feasel, M.G., Wohlfarth, A., Nilles, J.M., Pang, S., Kristovich, R.L., Huestis, M.A., 2016. Metabolism of carfentanyl, an ultra-potent opioid, in human liver microsomes and human hepatocytes by high-resolution mass spectrometry. *AAPS J.* 18 (6), 1489–1499. <https://doi.org/10.1208/s12248-016-9963-5>.
- Ferrer-Alcon, M., La Harpe, R., Garcia-Sevilla, J.A., 2004. Decreased immunodensities of micro-opioid receptors, receptor kinases GRK 2/6 and beta-arrestin-2 in postmortem brains of opiate addicts. *Brain Res Mol Brain Res* 121 (1–2), 114–122. <https://doi.org/10.1016/j.molbrainres.2003.11.009>.
- Fitzgerald, A., Aditya, H., Prior, A., McNeill, E., Pentland, B., 2010. Anoxic brain injury: clinical patterns and functional outcomes. A study of 93 cases. *Brain Inj.* 24 (11), 1311–1323. <https://doi.org/10.3109/02699052.2010.506864>.
- Florence, C.S., Zhou, C., Luo, F., Xu, L., 2016. The economic burden of prescription opioid overdose, abuse, and dependence in the United States, 2013. *Med. Care* 54 (10), 901–906. <https://doi.org/10.1097/MLR.0000000000000625>.
- Fomin, D., Baranauskaitė, V., Usaviciene, E., Sumkovskaja, A., Laima, S., Jasulaitis, A., Stasiuniene, J., 2018. Human deaths from drug overdoses with carfentanyl involvement-new rising problem in forensic medicine: a STROBE-compliant retrospective study. *Medicine (Baltim.)* 97 (48), e13449. <https://doi.org/10.1097/MD.00000000000013449>.
- Foster, D.J., Somogyi, A.A., Bochner, F., 1999. Methadone N-demethylation in human liver microsomes: lack of stereoselectivity and involvement of CYP3A4. *Br. J. Clin. Pharmacol.* 47 (4), 403–412. <https://doi.org/10.1046/j.1365-2125.1999.00921.x>.
- Fox, L.M., Hoffman, R.S., Vlahov, D., Manini, A.F., 2018. Risk factors for severe respiratory depression from prescription opioid overdose. *Addiction* 113 (1), 59–66. <https://doi.org/10.1111/add.13925>.
- Foy, L., Seeyave, D.M., Bradin, S.A., 2011. Toxic leukoencephalopathy due to transdermal fentanyl overdose. *Pediatr. Emerg. Care* 27 (9), 854–856. <https://doi.org/10.1097/PEC.0b013e31822c281f>.
- Frances, B., Gout, R., Campistron, G., Panconi, E., Cros, J., 1990. Morphine-6-glucuronide is more mu-selective and potent in analgesic tests than morphine. *Prog. Clin. Biol. Res.* 328, 477–480. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/2154808>.
- Frances, B., Gout, R., Monsarrat, B., Cros, J., Zajac, J.M., 1992. Further evidence that morphine-6 beta-glucuronide is a more potent opioid agonist than morphine. *J. Pharmacol. Exp. Therapeut.* 262 (1), 25–31. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/1320685>.
- Freni, F., Pezzella, S., Vignali, C., Moretti, M., Cisini, S., Rossetti, C., Morini, L., 2019. A case report on potential postmortem redistribution of fentanyl fentanyl and 4-ANPP. *Forensic Sci. Int.* 304, 109915. <https://doi.org/10.1016/j.forsciint.2019.109915>.
- García-Cabezas, M.A., Martínez-Sánchez, P., Sánchez-González, M.A., Garzon, M., Cavada, C., 2009. Dopamine innervation in the thalamus: monkey versus rat. *Cerebr. Cortex* 19 (2), 424–434. <https://doi.org/10.1093/cercor/bhn093>.
- García-Cabezas, M.A., Rico, B., Sánchez-González, M.A., Cavada, C., 2007. Distribution of the dopamine innervation in the macaque and human thalamus. *Neuroimage* 34 (3), 965–984. <https://doi.org/10.1016/j.neuroimage.2006.07.032>.
- García-Sevilla, J.A., Ventayol, P., Busquets, X., La Harpe, R., Walzer, C., Guimon, J., 1997. Regulation of immunolabelled mu-opioid receptors and protein kinase C- $\alpha$  and zeta isoforms in the frontal cortex of human opiate addicts. *Neurosci. Lett.* 226 (1), 29–32. [https://doi.org/10.1016/S0304-3940\(97\)00227-9](https://doi.org/10.1016/S0304-3940(97)00227-9).
- Geile, J., Maas, A., Kraemer, M., Doberentz, E., Madea, B., 2019. Fatal misuse of transdermal fentanyl patches. *Forensic Sci. Int.* 302, 109858. <https://doi.org/10.1016/j.forsciint.2019.06.016>.
- Gendron, L., Cahill, C.M., von Zastrow, M., Schiller, P.W., Pineyro, G., 2016. Molecular pharmacology of delta-opioid receptors. *Pharmacol. Rev.* 68 (3), 631–700. <https://doi.org/10.1124/pr.114.008979>.
- Gorelick, D.A., Kim, Y.K., Bencherif, B., Boyd, S.J., Nelson, R., Copersino, M.L., Frost, J. J., 2008. Brain mu-opioid receptor binding: relationship to relapse to cocaine use after monitored abstinence. *Psychopharmacology (Berlin)* 200 (4), 475–486. <https://doi.org/10.1007/s00213-008-1225-5>.
- Gronblad, L., Ohlund, L.S., Gunne, L.M., 1990. Mortality in heroin addiction: impact of methadone treatment. *Acta Psychiatr. Scand.* 82 (3), 223–227. <https://doi.org/10.1111/j.1600-0447.1990.tb03057.x>.
- Guerrieri, D., Rapp, E., Roman, M., Druid, H., Kronstrand, R., 2017. Postmortem and toxicological findings in a series of fentanyl-related deaths. *J. Anal. Toxicol.* 41 (3), 242–249. <https://doi.org/10.1093/jat/bkw129>.
- Hansen, T.M., Lelic, D., Olesen, A.E., Drewes, A.M., Frokjaer, J.B., 2018. Differential effects of oxycodone and venlafaxine on resting state functional connectivity-A randomized placebo-controlled magnetic resonance imaging study. *CNS Neurosci. Ther.* 24 (9), 820–827. <https://doi.org/10.1111/cns.12827>.
- Hashimoto, E., Frolich, L., Ozawa, H., Saito, T., Shichinohe, S., Takahata, N., Riederer, P., 1996. Alteration of glutamyltranspeptidase binding proteins in postmortem brains of heroin addicts. *Alcohol Clin. Exp. Res.* 20 (9 Suppl. 1), 301A–304A. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/8986227>.
- Hearing, M., 2018. Prefrontal-accumbens opioid plasticity: implications for relapse and dependence. *Pharmacol. Res.* 139, 158–165. <https://doi.org/10.1016/j.phrs.2018.11.012>.
- Hedgegaard, H.M., Warner, M., 2020. Drug Overdose Deaths in the United States, 1999–2018. *NCHS Data Brief*, p. 356. <https://www.cdc.gov/nchs/data/databriefs/db356-h.pdf>.
- Helander, A., Backberg, M., Signell, P., Beck, O., 2017. Intoxications involving acrylfentanyl and other novel designer fentanyls - results from the Swedish STRIDA project. *Clin. Toxicol.* 55 (6), 589–599. <https://doi.org/10.1080/15563650.2017.1303141>.
- Hirvonen, J., Aalto, S., Hagelberg, N., Maksimow, A., Ingman, K., Oikonen, V., Scheinin, H., 2009. Measurement of central mu-opioid receptor binding in vivo with PET and [<sup>11</sup>C]carfentanyl: a test-retest study in healthy subjects. *Eur. J. Nucl. Med. Mol. Imag.* 36 (2), 275–286. <https://doi.org/10.1007/s00259-008-0935-6>.
- Hosseini, F.N., Ali, 2017. Acute cerebellitis following opium intoxication: a case report and literature review. *J. Pediatr. Rev.* 5 (1), 1. <https://doi.org/10.17795/jpr-8803>.
- Inturrisi, C.E., 2005. Pharmacology of methadone and its isomers. *Minerva Anestesiol.* 71 (7–8), 435–437. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/16012416>.
- Johansson, J., Hirvonen, J., Lovro, Z., Ekblad, L., Kaasinen, V., Rajasilta, O., Scheinin, M., 2019. Intranasal naloxone rapidly occupies brain mu-opioid receptors in human subjects. *Neuropsychopharmacology* 44 (9), 1667–1673. <https://doi.org/10.1038/s41386-019-0368-x>.
- Johnson, S.W., North, R.A., 1992. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci.* 12 (2), 483–488. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/1346804>.
- Jolley, C.J., Bell, J., Rafferty, G.F., Moxham, J., Strang, J., 2015. Understanding heroin overdose: a study of the acute respiratory depressant effects of injected pharmaceutical heroin. *PLoS One* 10 (10), e0140995. <https://doi.org/10.1371/journal.pone.0140995>.
- Jones, A.K., Friston, K.J., Qi, L.Y., Harris, M., Cunningham, V.J., Jones, T., Frackowiak, R.S., 1991. Sites of action of morphine in the brain. *Lancet* 338 (8770), 825. [https://doi.org/10.1016/0140-6736\(91\)90717-4](https://doi.org/10.1016/0140-6736(91)90717-4).
- Kalivas, P.W., 2004. Glutamate systems in cocaine addiction. *Curr. Opin. Pharmacol.* 4 (1), 23–29. <https://doi.org/10.1016/j.coph.2003.11.002>.
- Kass-Hout, T., Kass-Hout, O., Darkhabani, M.Z., Mokin, M., Mehta, B., Radovic, V., 2011. Chasing the dragon—heroin-associated spongiform leukoencephalopathy. *J. Med. Toxicol.* 7 (3), 240–242. <https://doi.org/10.1007/s13181-011-0139-5>.
- Khalili-Mahani, N., Zoethout, R.M., Beckmann, C.F., Baerends, E., de Kam, M.L., Soeter, R.P., Rombouts, S.A., 2012. Effects of morphine and alcohol on functional brain connectivity during "resting state": a placebo-controlled crossover study in healthy young men. *Hum. Brain Mapp.* 33 (5), 1003–1018. <https://doi.org/10.1002/hbm.21265>.
- Kleinlog, D., Rombouts, S., Zoethout, R., Klumpers, L., Niesters, M., Khalili-Mahani, N., van Gerven, J., 2015. Subjective effects of ethanol, morphine, delta(9)-tetrahydrocannabinol, and ketamine following a pharmacological challenge are related to functional brain connectivity. *Brain Connect.* 5 (10), 641–648. <https://doi.org/10.1089/brain.2014.0314>.
- Krauss, J.K., Lipsman, N., Aziz, T., Boutet, A., Brown, P., Chang, J.W., Lozano, A.M., 2021. Technology of deep brain stimulation: current status and future directions. *Nat. Rev. Neurol.* 17 (2), 75–87. <https://doi.org/10.1038/s41582-020-00426-z>.
- Kreek, M.J., Reed, B., Butelman, E.R., 2019. Current status of opioid addiction treatment and related preclinical research. *Sci Adv* 5 (10), eaax9140. <https://doi.org/10.1126/sciadv.aax9140>.
- Kuhar, M.J., Pert, C.B., Snyder, S.H., 1973. Regional distribution of opiate receptor binding in monkey and human brain. *Nature* 245 (5426), 447–450. <https://doi.org/10.1038/245447a0>.
- Kumar, D., Deb, I., Chakraborty, J., Mukhopadhyay, S., Das, S., 2011. A polymorphism of the CREB binding protein (CREBBP) gene is a risk factor for addiction. *Brain Res.* 1406, 59–64. <https://doi.org/10.1016/j.brainres.2011.05.048>.
- Lalovic, B., Kharasch, E., Hoffer, C., Risler, L., Liu-Chen, L.Y., Shen, D.D., 2006. Pharmacokinetics and pharmacodynamics of oral oxycodone in healthy human subjects: role of circulating active metabolites. *Clin. Pharmacol. Ther.* 79 (5), 461–479. <https://doi.org/10.1016/j.clpt.2006.01.009>.
- Lalovic, B., Phillips, B., Risler, L.L., Howald, W., Shen, D.D., 2004. Quantitative contribution of CYP2D6 and CYP3A to oxycodone metabolism in human liver and intestinal microsomes. *Drug Metab. Dispos.* 32 (4), 447–454. <https://doi.org/10.1124/dmd.32.4.447>.
- Lane-Ladd, S.B., Pineda, J., Boundy, V.A., Pfeuffer, T., Krupinski, J., Aghajanian, G.K., Nestler, E.J., 1997. CREB (cAMP response element-binding protein) in the locus coeruleus: biochemical, physiological, and behavioral evidence for a role in opiate dependence. *J. Neurosci.* 17 (20), 7890–7901. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/9315909>.
- Lelic, D., Fischer, I.W., Olesen, A.E., Mørch, C.D., Arguissain, F.G., Manresa, J.A., Drewes, A.M., 2016. Venlafaxine and oxycodone effects on human spinal and supraspinal pain processing: a randomized cross-over trial. *Eur. J. Neurosci.* 44 (11), 2966–2974. <https://doi.org/10.1111/ejn.13443>.
- Lelic, D., Hansen, T.M., Mark, E.B., Olesen, A.E., Drewes, A.M., 2017. The effects of analgesics on central processing of tonic pain: a cross-over placebo controlled study. *Neuropharmacology* 123, 455–464. <https://doi.org/10.1016/j.neuropharm.2017.06.022>.
- Li, W., Li, Q., Wang, Y., Zhu, J., Ye, J., Yan, X., Liu, Y., 2016. Methadone-induced damage to white matter integrity in methadone maintenance patients: a longitudinal self-control dti study. *Sci. Rep.* 6, 19662. <https://doi.org/10.1038/srep19662>.
- Liu, Q., Fan, W., He, H., Huang, F., 2020. The role of peripheral opioid receptors in orofacial pain. *Oral Dis.* <https://doi.org/10.1111/odi.13435>.
- Louria, D.B., Hensle, T., Rose, J., 1967. The major medical complications of heroin addiction. *Ann. Intern. Med.* 67 (1), 1–22. <https://doi.org/10.7326/0003-4819-67-1-1>.
- Ma, N., Liu, Y., Li, N., Wang, C.X., Zhang, H., Jiang, X.F., Zhang, D.R., 2010. Addiction related alteration in resting-state brain connectivity. *Neuroimage* 49 (1), 738–744. <https://doi.org/10.1016/j.neuroimage.2009.08.037>.
- Mansour, A., Watson, S.J., 1993. Anatomical distribution of opioid receptors in mammals: an overview. In: Herz, A., Akil, H., Simon, E.J. (Eds.), *Opioids*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 79–105.

- Martin, J.J., Cras, P., De Schutter, E., 1987. Spinal neuroaxonal dystrophy and angioneuromatosis. *Acta Neuropathol.* 73 (1), 19–24. <https://doi.org/10.1007/BF00695497>.
- McPherson, C., Haslam, M., Pineda, R., Rogers, C., Neil, J.J., Inder, T.E., 2015. Brain injury and development in preterm infants exposed to fentanyl. *Ann. Pharmacother.* 49 (12), 1291–1297. <https://doi.org/10.1177/1060028015606732>.
- Meert, T.F., Lu, H.R., van Craenendonck, H., Janssen, P.A., 1988. Comparison between epidural fentanyl, sufentanil, carfentanil, lofentanil and alfentanil in the rat: analgesia and other in vivo effects. *Eur. J. Anaesthesiol.* 5 (5), 313–321.
- Metkees, M., Meesa, I.R., Srinivasan, A., 2015. Methadone-induced acute toxic leukoencephalopathy. *Pediatr. Neurol.* 52 (2), 256–257. <https://doi.org/10.1016/j.pediatrneurol.2014.10.021>.
- Meye, F.J., van Zessen, R., Smidt, M.P., Adan, R.A., Ramakers, G.M., 2012. Morphine withdrawal enhances constitutive mu-opioid receptor activity in the ventral tegmental area. *J. Neurosci.* 32 (46), 16120–16128. <https://doi.org/10.1523/JNEUROSCI.1572-12.2012>.
- Mildh, L.H., Scheinin, H., Kirvela, O.A., 2001. The concentration-effect relationship of the respiratory depressant effects of alfentanil and fentanyl. *Anesth. Analg.* 93 (4), 939–946. <https://doi.org/10.1097/0000539-200110000-00028>.
- Morales Oda, Y., Jinka, M., Ziai, W.C., 2010. Severe leukoencephalopathy following acute oxycodone intoxication. *Neurocritical Care* 13 (1), 93–97. <https://doi.org/10.1007/s12028-010-9373-y>.
- Morrone, L.A., Scuteri, D., Rombola, L., Mizoguchi, H., Bagetta, G., 2017. Opioids resistance in chronic pain management. *Curr. Neuropharmacol.* 15 (3), 444–456. <https://doi.org/10.2174/1570159X14666161101092822>.
- Mosinska, P., Zielinska, M., Fichna, J., 2016. Expression and physiology of opioid receptors in the gastrointestinal tract. *Curr. Opin. Endocrinol. Diabetes Obes.* 23 (1), 3–10. <https://doi.org/10.1097/MED.0000000000000219>.
- Muller, U.J., Mawrin, C., Frodl, T., Dobrowolny, H., Busse, S., Bernstein, H.G., Steiner, J., 2019. Reduced volumes of the external and internal globus pallidus in male heroin addicts: a postmortem study. *Eur. Arch. Psychiatr. Clin. Neurosci.* 269 (3), 317–324. <https://doi.org/10.1007/s00406-018-0939-6>.
- Muller, U.J., Schiltz, K., Mawrin, C., Dobrowolny, H., Frodl, T., Bernstein, H.G., Steiner, J., 2018. Total hypothalamic volume is reduced in postmortem brains of male heroin addicts. *Eur. Arch. Psychiatr. Clin. Neurosci.* 268 (3), 243–248. <https://doi.org/10.1007/s00406-017-0809-7>.
- Muller, U.J., Truebner, K., Schiltz, K., Kuhn, J., Mawrin, C., Dobrowolny, H., Steiner, J., 2015. Postmortem volumetric analysis of the nucleus accumbens in male heroin addicts: implications for deep brain stimulation. *Eur. Arch. Psychiatr. Clin. Neurosci.* 265 (8), 647–653. <https://doi.org/10.1007/s00406-015-0617-x>.
- Naganawa, M., Zheng, M.Q., Henry, S., Nabulsi, N., Lin, S.F., Ropchan, J., Huang, Y., 2015. Test-retest reproducibility of binding parameters in humans with 11C-LY2795050, an antagonist PET radiotracer for the kappa opioid receptor. *J. Nucl. Med.* 56 (2), 243–248. <https://doi.org/10.2967/jnumed.114.147975>.
- Nanan, R., von Stockhausen, H.B., Petersen, B., Solymosi, L., Warmuth-Metz, M., 2000. Unusual pattern of leukoencephalopathy after morphine sulphate intoxication. *Neuroradiology* 42 (11), 845–848. <https://doi.org/10.1007/s002340000442>.
- Ngai, S.H., 1961. Effects of morphine and meperidine on the central respiratory mechanisms in the cat; the action of levallorphan in antagonizing these effects. *J. Pharmacol. Exp. Therapeut.* 131, 91–99. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/13728720>.
- NIDA, 2019. Drug Facts - Heroin. National Institutes of Health; Department of Health and Human Services. <https://d14rmgtwz75a.cloudfront.net/sites/default/files/drugfacts-heroin.pdf>.
- Nielsen, D.A., Ji, F., Yuferov, V., Ho, A., Chen, A., Levran, O., Kreek, M.J., 2008. Genotype patterns that contribute to increased risk for or protection from developing heroin addiction. *Mol. Psychiatr.* 13 (4), 417–428. <https://doi.org/10.1038/sj.mp.4002147>.
- Nielsen, D.A., Yuferov, V., Hamon, S., Jackson, C., Ho, A., Ott, J., Kreek, M.J., 2009. Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. *Neuropsychopharmacology* 34 (4), 867–873. <https://doi.org/10.1038/npp.2008.108>.
- Norman, E., Wikstrom, S., Rosen, I., Fellman, V., Hellstrom-Westas, L., 2013. Premedication for intubation with morphine causes prolonged depression of electrocortical background activity in preterm infants. *Pediatr. Res.* 73 (1), 87–94. <https://doi.org/10.1038/pr.2012.153>.
- Nummenmaa, L., Saanijoki, T., Tuominen, L., Hirvonen, J., Tuulari, J.J., Nuutila, P., Kallioikoski, K., 2018. mu-opioid receptor system mediates reward processing in humans. *Nat. Commun.* 9 (1), 1500. <https://doi.org/10.1038/s41467-018-03848-y>.
- Nummenmaa, L., Tuominen, L., 2018. Opioid system and human emotions. *Br. J. Pharmacol.* 175 (14), 2737–2749. <https://doi.org/10.1111/bph.13812>.
- Oda, Y., Ziai, W.C., 2010. Severe leukoencephalopathy following acute oxycodone intoxication. *Neurocritical Care* 13, 93–97.
- Oehmichen, M., Meissner, C., Reiter, A., Birkholz, M., 1996. Neuropathology in non-human immunodeficiency virus-infected drug addicts: hypoxic brain damage after chronic intravenous drug abuse. *Acta Neuropathol.* 91 (6), 642–646. <https://doi.org/10.1007/s004010050478>.
- Ostwal, S., Salins, N., Deodhar, J., Muckaden, M.A., 2015. Fentanyl-induced neurotoxicity in children. *J. Pain Palliat. Care Pharmacother.* 29 (4), 385–387. <https://doi.org/10.3109/15360288.2015.1101639>.
- Pal, A., Chakraborty, J., Das, S., 2014. Association of CREB1 gene polymorphism with drug seeking behaviour in eastern Indian addicts. *Neurosci. Lett.* 570, 53–57. <https://doi.org/10.1016/j.neulet.2014.03.064>.
- Palmiere, C., Brunel, C., Sporkert, F., Augsburger, M., 2011. An unusual case of accidental poisoning: fatal methadone inhalation. *J. Forensic Sci.* 56 (4), 1072–1075. <https://doi.org/10.1111/j.1556-4029.2011.01730.x>.
- Pandria, N., Kovatsi, L., Vivas, A.B., Bamidis, P.D., 2018. Resting-state abnormalities in heroin-dependent individuals. *Neuroscience* 378, 113–145. <https://doi.org/10.1016/j.neuroscience.2016.11.018>.
- Pearson, J., Baden, M.B., Richter, R.W., 1976. Neuronal depletion in the globus pallidus of heroin addicts. *Drug Alcohol Depend.* 1 (5), 349–356. [https://doi.org/10.1016/0376-8716\(76\)90037-5](https://doi.org/10.1016/0376-8716(76)90037-5).
- Pecina, M., Karp, J.F., Mathew, S., Todtenkopf, M.S., Ehrlich, E.W., Zubieta, J.K., 2019. Endogenous opioid system dysregulation in depression: implications for new therapeutic approaches. *Mol. Psychiatr.* 24 (4), 576–587. <https://doi.org/10.1038/s41380-018-0117-2>.
- Pirompanich, P., Chankrachang, S., 2015. Intravenous heroin-associated delayed spongiform leukoencephalopathy: case report and reviews of the literature. *J. Med. Assoc. Thai.* 98 (7), 703–708. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/26267994>.
- Reardon, S., 2019. The US opioid epidemic is driving a spike in infectious diseases. *Nature* 571 (7763), 15–16. <https://doi.org/10.1038/d41586-019-02019-3>.
- Reisner, A., Hayes, L.L., Holland, C.M., Wrubel, D.M., Kebriaei, M.A., Geller, R.J., Chern, J.J., 2015. Opioid overdose in a child: case report and discussion with emphasis on neurosurgical implications. *J. Neurosurg. Pediatr.* 16 (6), 752–757. <https://doi.org/10.3171/2015.4.PEDS14667>.
- Remillard, D., Kaye, A.D., McAnally, H., 2019. Oxycodone's unparalleled addictive potential: is it time for a moratorium? *Curr. Pain Headache Rep.* 23 (2), 15. <https://doi.org/10.1007/s11916-019-0751-7>.
- Richter, R.W., Pearson, J., Bruun, B., Challenor, Y.B., Brust, J.C., Baden, M.M., 1973. Neurological complications of addiction to heroin. *Bull. N. Y. Acad. Med.* 49 (1), 3–21. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/4344820>.
- Rizzuto, N., Morbin, M., Ferrari, S., Cavallaro, T., Sparaco, M., Boso, G., Gaetti, L., 1997. Delayed spongiform leukoencephalopathy after heroin abuse. *Acta Neuropathol.* 94 (1), 87–90. <https://doi.org/10.1007/s004010050676>.
- Rudd, R.A., Seth, P., David, F., Scholl, L., 2016. Increases in drug and opioid-involved overdose deaths - United States, 2010–2015. *MMWR Morb. Mortal. Wkly. Rep.* 65 (50–51), 1445–1452. <https://doi.org/10.15585/mmwr.mm650501e1>.
- Rusin, K.I., Giovannucci, D.R., Stuenkel, E.L., Moises, H.C., 1997. Kappa-opioid receptor activation modulates Ca<sup>2+</sup> currents and secretion in isolated neuroendocrine nerve terminals. *J. Neurosci.* 17 (17), 6565–6574. Retrieved from: <https://www.ncbi.nlm.nih.gov/pubmed/9254669>.
- Salazar, R., Dubow, J., 2012. Delayed posthypoxic leukoencephalopathy following a morphine overdose. *J. Clin. Neurosci.* 19 (7), 1060–1062. <https://doi.org/10.1016/j.jocn.2012.01.001>.
- SAMHSA, 2019. Key Substance Use and Mental Health Indicators in the United States: Results from the 2019 National Survey on Drug Use and Health *Substance Abuse and Mental Health Services Administration* iii, pg. 40. Retrieved from: <https://www.samhsa.gov/data/sites/default/files/reports/rpt29393/2019NSDUHFRPDFWHTM12019NSDUHFR1PDFW090120.pdf>.
- Schifano, F., Chiappini, S., Corkery, J.M., Guirguis, A., 2019. Assessing the 2004–2018 fentanyl missing issues reported to an international range of adverse reporting systems. *Front. Pharmacol.* 10, 46. <https://doi.org/10.3389/fphar.2019.00046>.
- Schiller, E.Y., Goyal, A., Mechanic, O.J., 2021. Opioid overdose. *StatPearls. Treasure Island (FL)*.
- Schonfeld, T., Heinemann, A., Schulz, M., Grädl, G., Andresen-Streichert, H., Müller, A., Iwersen-Bergmann, S., 2019. Post-mortem analysis of prescription opioids-A follow-up examination by LC-MS/MS with focus on fentanyl. *Forensic Sci. Int.* 305, 109970. <https://doi.org/10.1016/j.forsciint.2019.109970>.
- Schuckit, M.A., 2016. Treatment of opioid-use disorders. *N. Engl. J. Med.* 375 (16), 1596–1597. <https://doi.org/10.1056/NEJMc1610830>.
- Seifert, C.L., Magon, S., Sprenger, T., Lang, U.E., Huber, C.G., Denier, N., Walter, M., 2015. Reduced volume of the nucleus accumbens in heroin addiction. *Eur. Arch. Psychiatr. Clin. Neurosci.* 265 (8), 637–645. <https://doi.org/10.1007/s00406-014-0564-y>.
- Shichinohe, S., Ozawa, H., Hashimoto, E., Tatschner, T., Riederer, P., Saito, T., 2001. Changes in the cAMP-related signal transduction mechanism in postmortem human brains of heroin addicts. *J. Neural. Transm.* 108 (3), 335–347. <https://doi.org/10.1007/s007020170079>.
- Shichinohe, S., Ozawa, H., Saito, T., Hashimoto, E., Lang, C., Riederer, P., Takahata, N., 1998. Differential alteration of adenylyl cyclase subtypes I, II, and V/VI in postmortem human brains of heroin addicts. *Alcohol Clin. Exp. Res.* 22 (S3 Pt 1), 84S–87S. <https://doi.org/10.1111/acer.1998.22.s3.part1.84s>.
- Sillivan, S.E., Whittard, J.D., Jacobs, M.M., Ren, Y., Mazloom, A.R., Caputi, F.F., Hurd, Y. L., 2013. ELK1 transcription factor linked to dysregulated striatal mu opioid receptor signaling network and OPRM1 polymorphism in human heroin abusers. *Biol. Psychiatr.* 74 (7), 511–519. <https://doi.org/10.1016/j.biopsych.2013.04.012>.
- Strang, J., Volkow, N.D., Degenhardt, L., Hickman, M., Johnson, K., Koob, G.F., Walsh, S. L., 2020. Opioid use disorder. *Nat Rev Dis Primers* 6 (1), 3. <https://doi.org/10.1038/s41572-019-0137-5>.
- Switzer, A.R., Beland, B., Sarna, J.R., Walzak, A., Pfeffer, G., 2020. Fentanyl overdose causing hippocampal ischaemia followed by delayed leukoencephalopathy. *Can. J. Neurol. Sci.* 47 (3), 398–399. <https://doi.org/10.1017/cjn.2020.33>.
- Tabatabai, M., Kitahata, L.M., Collins, J.G., 1989. Disruption of the rhythmic activity of the medullary inspiratory neurons and phrenic nerve by fentanyl and reversal with nalbuphine. *Anesthesiology* 70 (3), 489–495. <https://doi.org/10.1097/0000542-198903000-00020>.
- Tataranno, M.L., Gui, L., Hellstrom-Westas, L., Toet, M., Groenendaal, F., Claessens, N.H. P., Benders, M., 2020. Morphine affects brain activity and volumes in preterms: an observational multi-center study. *Early Hum. Dev.* 144, 104970. <https://doi.org/10.1016/j.earlhumdev.2020.104970>.

- Tateishi, T., Krivoruk, Y., Ueng, Y.F., Wood, A.J., Guengerich, F.P., Wood, M., 1996. Identification of human liver cytochrome P-450 3A4 as the enzyme responsible for fentanyl and sufentanil N-dealkylation. *Anesth. Analg.* 82 (1), 167–172. <https://doi.org/10.1097/0000539-199601000-00031>.
- Thompson, C.M., Wojno, H., Greiner, E., May, E.L., Rice, K.C., Selley, D.E., 2004. Activation of G-proteins by morphine and codeine congeners: insights to the relevance of O- and N-demethylated metabolites at mu- and delta-opioid receptors. *J. Pharmacol. Exp. Therapeut.* 308 (2), 547–554. <https://doi.org/10.1124/jpet.103.058602>.
- Tiong, S.C., Chiang, J.S.L., Khoo, H.W., Ng, C.H., 2019. Methadone-induced toxic encephalopathy in pediatric patients: two case reports. *J. Radiol. Case Rep.* 13 (5), 1–9. <https://doi.org/10.3941/jrcr.v13i5.3658>.
- Tolomeo, S., Matthews, K., Steele, D., Baldacchino, A., 2018. Compulsivity in opioid dependence. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 81, 333–339. <https://doi.org/10.1016/j.pnpbp.2017.09.007>.
- Torrecilla, M., Marker, C.L., Cintora, S.C., Stoffel, M., Williams, J.T., Wickman, K., 2002. G-protein-gated potassium channels containing Kir3.2 and Kir3.3 subunits mediate the acute inhibitory effects of opioids on locus ceruleus neurons. *J. Neurosci.* 22 (11), 4328–4334.
- UNODC, 2019. World Drug Report. United Nations Office on Drugs and Crime. [https://wdr.unodc.org/wdr2019/prelaunch/WDR19\\_Booklet\\_3\\_DEPRESSANTS.pdf](https://wdr.unodc.org/wdr2019/prelaunch/WDR19_Booklet_3_DEPRESSANTS.pdf).
- Upadhyay, J., Maleki, N., Potter, J., Elman, I., Rudrauf, D., Knudsen, J., Borsook, D., 2010. Alterations in brain structure and functional connectivity in prescription opioid-dependent patients. *Brain* 133 (Pt 7), 2098–2114. <https://doi.org/10.1093/brain/awq138>.
- Van Bever, W.F., Niemegeers, C.J., Schellekens, K.H., Janssen, P.A., 1976. N-4-Substituted 1-(2-arylethyl)-4-piperidyl-N-phenylpropanamides, a novel series of extremely potent analgesics with unusually high safety margin. *Arzneimittelforschung* 26 (8), 1548–1551. Retrieved from. <https://www.ncbi.nlm.nih.gov/pubmed/12771>.
- Vila, N., Chamorro, A., 1997. Ballistic movements due to ischemic infarcts after intravenous heroin overdose: report of two cases. *Clin. Neurol. Neurosurg.* 99 (4), 259–262. [https://doi.org/10.1016/s0303-8467\(97\)00088-7](https://doi.org/10.1016/s0303-8467(97)00088-7).
- Wang, F., Xu, H., Zhao, H., Gelernter, J., Zhang, H., 2016. DNA co-methylation modules in postmortem prefrontal cortex tissues of European Australians with alcohol use disorders. *Sci. Rep.* 6, 19430. <https://doi.org/10.1038/srep19430>.
- Wang, W., Wang, Y.R., Qin, W., Yuan, K., Tian, J., Li, Q., Guo, Y.M., 2010. Changes in functional connectivity of ventral anterior cingulate cortex in heroin abusers. *Chin. Med. J.* 123 (12), 1582–1588. Retrieved from. <https://www.ncbi.nlm.nih.gov/pubmed/20819516>.
- Wang, X., Li, B., Zhou, X., Liao, Y., Tang, J., Liu, T., Hao, W., 2012. Changes in brain gray matter in abstinent heroin addicts. *Drug Alcohol Depend.* 126 (3), 304–308. <https://doi.org/10.1016/j.drugalcdep.2012.05.030>.
- Weerts, E.M., Kim, Y.K., Wand, G.S., Dannals, R.F., Lee, J.S., Frost, J.J., McCaul, M.E., 2008. Differences in delta- and mu-opioid receptor blockade measured by positron emission tomography in naltrexone-treated recently abstinent alcohol-dependent subjects. *Neuropsychopharmacology* 33 (3), 653–665. <https://doi.org/10.1038/sj.npp.1301440>.
- Wheaton, T., Toll, B.J., Breznak, K., Da-Silva, S., Melvin, J., Misra, A., Hwang, S.W., 2019. Opioid-induced toxic leukoencephalopathy: a case report and review of the literature. *Heliyon* 5 (12), e03005. <https://doi.org/10.1016/j.heliyon.2019.e03005>.
- Wilson, N., Kariisa, M., Seth, P., Smith, H.T., Davis, N.L., 2020. Drug and opioid-involved overdose deaths - United States, 2017–2018. *MMWR Morb. Mortal. Wkly. Rep.* 69 (11), 290–297. <https://doi.org/10.15585/mmwr.mm6911a4>.
- Winkhofer, S., Surer, E., Ampanozi, G., Ruder, T., Stolzmann, P., Elliott, M., Schweitzer, W., 2014. Post-mortem whole body computed tomography of opioid (heroin and methadone) fatalities: frequent findings and comparison to autopsy. *Eur. Radiol.* 24 (6), 1276–1282. <https://doi.org/10.1007/s00330-014-3128-7>.
- Wolters, E.C., van Wijngaarden, G.K., Stam, F.C., Rengelink, H., Lousberg, R.J., Schipper, M.E., Verbeeten, B., 1982. ["Heroin" leuko-encephalopathy: spongiform leukomyeloencephalopathy following inhalation of the pyrolysate of impure heroin]. *Ned. Tijdschr. Geneesk.* 126 (12), 508–514. Retrieved from. <https://www.ncbi.nlm.nih.gov/pubmed/7070545>.
- Wu, C.H., Henry, J.A., 1990. Deaths of heroin addicts starting on methadone maintenance. *Lancet* 335 (8686), 424. [https://doi.org/10.1016/0140-6736\(90\)90269-b](https://doi.org/10.1016/0140-6736(90)90269-b).
- Xie, C., Shao, Y., Ma, L., Zhai, T., Ye, E., Fu, L., Li, S.J., 2014. Imbalanced functional link between valuation networks in abstinent heroin-dependent subjects. *Mol. Psychiatr.* 19 (1), 10–12. <https://doi.org/10.1038/mp.2012.169>.
- Xu, J., Wang, T., Su, Z., Zhou, X., Xiang, Y., He, L., Zhao, X., 2018. Opioid exposure is associated with aberrant DNA methylation of OPRM1 promoter region in a Chinese han population. *Biochem. Genet.* 56 (5), 451–458. <https://doi.org/10.1007/s10528-018-9852-y>.
- Yoshimura, H., Ida, S., Oguri, K., Tsukamoto, H., 1973. Biochemical basis for analgesic activity of morphine-6-glucuronide. I. Penetration of morphine-6-glucuronide in the brain of rats. *Biochem. Pharmacol.* 22 (12), 1423–1430. [https://doi.org/10.1016/0006-2952\(73\)90320-1](https://doi.org/10.1016/0006-2952(73)90320-1).
- Yuge, O., Kitahata, L.M., Collins, J.G., Matsumoto, M., Tabatabai, M., Suzukawa, M., Tanaka, A., 1985. Fentanyl and alfentanil suppress brainstem pain transmission. *Anesth. Analg.* 64 (6), 597–600. Retrieved from. <https://www.ncbi.nlm.nih.gov/pubmed/3923868>.
- Zou, F., Wu, X., Zhai, T., Lei, Y., Shao, Y., Jin, X., Yang, Z., 2015. Abnormal resting-state functional connectivity of the nucleus accumbens in multi-year abstinent heroin addicts. *J. Neurosci. Res.* 93 (11), 1693–1702. <https://doi.org/10.1002/jnr.23608>.
- Blackwood, C.A., Hoerle, R., Leary, M., Schroeder, J., Job, M.O., McCoy, M.T., Ladenheim, B., Jayanthi, S., Cadet, J.L., 2019. Molecular adaptations in the rat dorsal striatum and hippocampus following abstinence-induced incubation of drug seeking after escalated oxycodone self-administration. *Mol. Neurobiol.* 56 (5), 3603–3615. <https://doi.org/10.1007/s12035-018-1318-z>.