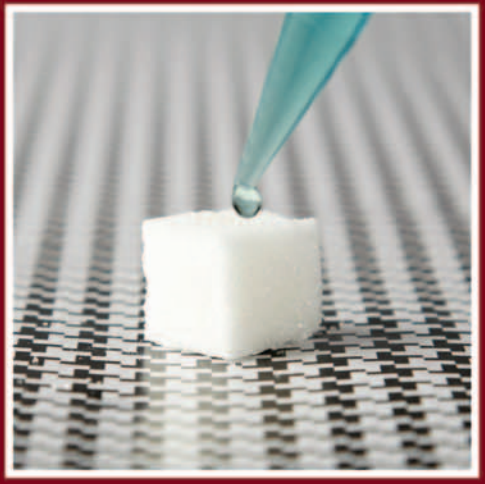
*Neuropathology of*

# DRUG ADDICTIONS AND SUBSTANCE MISUSE

## Stimulants, Club and Dissociative Drugs, Hallucinogens, Steroids, Inhalants and International Aspects



EDITED BY VICTOR R. PREEDY

VOLUME 2

# Neuropathology of Drug Addictions and Substance Misuse

Volume 2: Stimulants, Club and Dissociative Drugs, Hallucinogens, Steroids, Inhalants, and International Aspects

# Neuropathology of Drug Addictions and Substance Misuse, Volume 2

*Victor R. Preedy, Editor*

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# Neuropathology of Drug Addictions and Substance Misuse

## Volume 2: Stimulants, Club and Dissociative Drugs, Hallucinogens, Steroids, Inhalants, and International Aspects

*Edited by*

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# Preface

The well-being of the individual is highly dependent on maintaining neurophysiological processes in a functional state but also on the ability to adapt to changes in the internal and external environments. However, adaptive changes may be pathological in some circumstances, with devastating consequences for the individual. Triggers for these neurological abnormalities are varied and may be due to life stages (e.g., aging), nutrition (e.g., nutrient deficiency or excess such as iodine and iron, respectively), trauma (e.g., metabolic or physical trauma, such as that due to hypoglycemia or blunt instruments), or drugs of addiction and substance misuse (e.g., nicotine, alcohol, caffeine, inhalants, and myriad others). The latter are common and preventable to some extent. For example, in the United States alone, there are an estimated 22 million illegal drug users. Of these, 60 million use tobacco, and 50 million misuse alcohol. Millions of individuals are also addicted to, or misuse, caffeine and prescription or over-the-counter medications.

As a consequence of addictions and substance misuse, adverse changes occur in affected tissues. These range from molecular and cellular perturbations to structural and functional abnormalities. It is possible that some of the science behind these changes may be applicable to other modes of neurophysiological imbalance. That is, lessons and features in one form of addiction and substance misuse may be transferable to another. Indeed, there are other forms of nonsubstance addictions such as gambling, gaming, and workaholism that may share common features, mechanisms, or outcomes. Understanding commonality provides a platform for studying specific addictions in more depth and allows one to speculate about new modes of understanding, causation, prevention, and treatment.

There is some difficulty in describing changes in human tissues, as this sort of information is rather limited in scope and analytical depth. Preclinical or nonclinical studies have advanced the detailed understanding of addictions and substance misuse considerably. These range from isolated structures, cells, and perfusions to invertebrates, rodents, and primates. It is thus essential to have both clinical and preclinical information within the same authoritative textual platform to advance our understanding of addictions and substance misuse.

Understanding neuropathology by itself can be somewhat problematic, especially in terms of addictions. This information needs to be placed within its wider context—from procurement of drugs, to altered behavior and psychosocial conditions. For some substances, there is very little molecular information, whereas for other drugs there is an abundance. The information on behavioral and psychosocial aspects is similarly divergent among the different addictions. Thus, any textual information on addictions and substance misuse/use requires a scientific continuum of information; with neurological features as a central core.

However, marshalling all the aforementioned information is somewhat difficult due to the wide array of material. To address this, the Editor has compiled *The Neuropathology of Drug Addictions and Substance Misuse*. It has three separate volumes:

**Volume 1:** Foundations of Understanding, Tobacco, Alcohol, Cannabinoids, and Opioids
**Volume 2:** Stimulants, Club and Dissociative Drugs, Hallucinogens, Steroids, Inhalants, and International Aspects
**Volume 3:** General Processes and Mechanisms, Prescription Medications, Caffeine and Areca, Polydrug Misuse, Emerging Addictions, and Nondrug Addictions

In compiling these volumes, we interspersed chapters to aid the holistic understanding of addictions and substance misuse. We present material not only on specific substances but also in major sections on the following:

**Foundations for Understanding Substance Misuse and Their Effects**
**Emerging Addictions and Drugs of Abuse**
**International Aspects**
**Principles of Addictions, Overviews, Detailed Processes, and Mechanisms**
**Dual and Polydrug Abuse**
**Nondrug Addictions as Comparative Neuropathology**

**For Volume 1, the main parts are:**

1—[1] Setting the Scene: Foundations for Understanding Substance Misuse and Their Effects
1—[2] Tobacco

1—[3] Alcohol
1—[4] Cannabinoids
1—[5] Opioids

**For Volume 2, the main parts are:**

2—[1] Stimulants
2—[2] Club Drugs
2—[3] Dissociative Drugs
2—[4] Hallucinogens
2—[5] Anabolic Steroids, Inhalants, and Solvents
2—[6] International Aspects

**For Volume 3, the main Parts are:**

3—[1] General Aspects: Principles of Addictions, Overviews, Detailed Processes, and Mechanisms
3—[2] Prescription Medications
3—[3] Caffeine and Areca (Betal Nut)
3—[4] Dual and Polydrug Abuse
3—[5] Emerging Addictions and Drugs of Abuse
3—[6] Nondrug Addictions as Comparative Neuropathology

Each part is split into different subsections:

**General Aspects**
**Molecular and Cellular Aspects**
**Structural and Functional Aspects**
**Methods**

It is tempting to focus exclusively on detection, prevention, and treatment. However, this would far extend the remit of the book. For example, the analysis of markers in alcoholism itself would merit a single book, as would public health prevention or treatment regimens. Instead, the book is focused on neuropathology with upstream and downstream causative scenarios, effects, and consequences. In the section **General Aspect**s, basic information is provided to place the substance in context or to set the scientific scene. The section **Molecular and Cellular Aspects** provides greater detail. The section **Structural and Functional** aspects is more broad-based and includes imaging, psychosocial, and behavioral aspects and other wider information. The section **Methods** contains selective techniques for screening and/or analysis. Of course, these are generalized divisions, and this is recognized by the Editor. Some articles in one section may also be well suited to many other sections. Indeed, in a few cases we have located chapters within sections to complement other chapters; to impart a broader example of ideas, coverage, or concepts; to provide a more in-depth discourse that may be relevant to other drugs and their interactions; or to provide a greater understanding of substance and polysubstance misuse in general. However, the well-structured and professional index, provided by Elsevier, addresses issues in locating information, and so relevant material can be quickly found.

Each chapter has the following subheadings:

**Applications to Other Addictions and Substance Misuse**
**Definition of Terms**
**Key Facts**
**Summary Points**

These subheadings encompass unique features in the book that bridge the intellectual divide, so experts in one area of addiction may become more knowledgeable in another. These features will be very useful for the novice, student, or newly qualified health care professional. Others who wish to gain a broader understanding of addictions and substance misuse will also find these features of benefit.

The subheading **Application to Other Addictions and Substance Misuse** is intended to provide practical, speculative, or broader information. This is particularly useful when applied to those addictions in which there is a paucity of scientific material. For example, detailed molecular or functional information gathered from studying one addiction may be applicable to another.

Contributors are either international or national experts, leaders in the field, or trendsetters, and from respected institutions. Emerging fields of addictions and substance misuse are also incorporated in *Neuropathology of Drug Addictions and Substance Misuse*. This book is essential reading for addiction scientists, health care professionals, research scientists, molecular and cellular biochemists, and medical professionals including physicians and other practitioners, as well as those interested in health in general. It is also designed for professsors, teachers, and lecturers; undergraduates, graduates, postgraduates, and libraries.

**The Editor**

Chapter 39

# MDMA (Ecstasy) and Gene Expression in the Brain: An Overview of Microarray and Candidate Gene Studies Assessing Transcriptional Changes in Rodents

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## Abbreviations

**BCL** B cell leukemia/lymphoma
**BDNF** Brain-derived neurotrophic factor
**CREB** cAMP response element-binding protein
**ERK** Extracellular signal-regulated kinase
***Fos*** FBJ osteosarcoma oncogene
**GABA** Gamma-aminobutyric acid
**IEGs** Induced early genes
**KO** Knockout
**LTD** Long-term depression
**LTP** Long-term potentiation
**MAPK** Mitogen-activated protein kinase
**MDMA** 3,4-Methylenedioxymethamphetamine (ecstasy)
**NF-κB** Nuclear factor kappa–light-chain-enhancer of activated B cells
**NMDA** *N*-Methyl-D-aspartate
**SERT** Serotonin transporter

## INTRODUCTION

MDMA or 3,4-methylenedioxymethamphetamine (ecstasy) is a recreational drug of abuse that is widely used among adolescents and young adults. MDMA is a psychostimulant that induces euphoria, self-confidence, friendliness, empathy, and happiness in humans. MDMA also induces hyperthermia, which can eventually lead to toxicity and death. Chronic exposure to MDMA is related to hallucinations and to verbal, visual, and memory impairment, as well as psychiatric disorders such as psychosis and depression in humans (Baylen & Rosenberg, 2006). Much evidence supports the neurotoxic effects of MDMA in serotonergic neurons and the degeneration of neuronal fibers (dopaminergic neurons in mice) (Green, Mechan, Elliott, O'Shea, & Colado, 2003). MDMA has a high affinity for the serotonin transporter (SERT) and it increases serotonin release to the synaptic cleft. MDMA self-administration and its stimulant locomotor effect were abolished in mice lacking *Sert* (–/–), showing the importance of SERT and the serotonergic system for the behavior and reward effects of MDMA (Trigo et al., 2007).

Compared to other drugs of abuse, such as cocaine, few studies have assessed the changes induced by MDMA in gene expression in brain. Most of them focus on specific candidate genes, selected on the basis of their function or their relation to MDMA targets or effects. This approach leads to bias and complicates the elaboration of hypotheses about the effect of the drug on gene expression and the possible mechanisms involved. In contrast, high-throughput approaches such as microarray studies allow examination of a wide range of genes without prior assumptions.

Here we review the main studies that have assessed changes in gene expression induced by MDMA intake by using high-throughput approaches, as well as some work that focuses on specific candidate genes. The studies analyze different brain regions involved in the behavioral and rewarding effects of MDMA (see Table 1 for details): (1) the brainstem (including raphe nuclei), which contains the serotonergic cell bodies that send axons to the cortex, limbic areas, and spinal cord; (2) the ventral striatum and the nucleus accumbens, which are the main areas involved in reward and are mostly mediated by dopaminergic neurons; (3) the amygdala, which is involved in mood; (4) the hippocampus, which is involved in memory; and (5) the frontal cortex, which regulates cognition, memory, and perception, exerts inhibitory control, and is involved in decision making.

Since acute effects of MDMA differ from those of chronic exposure, which causes serotonin or dopamine depletion in rodents, here we discuss separately the results obtained after passive acute and chronic administration, self-administration, and prenatal exposure to MDMA. Most of the studies reported here considered acute administration of the drug; a few examined chronic administration; and only one assessed self-administration or prenatal exposure (Table 1). We conclude our review by considering the outputs of all the studies to draw some hypotheses

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**TABLE 1** Microarray Studies of the Effects of MDMA on Gene Expression

| Animal Model | Brain Region | MDMA Dose | Treatment | Time after Last Administration (Animals Killed) | Array Platform | Noteworthy Changes in Gene Expression | References |
|---|---|---|---|---|---|---|---|
| Rat | Frontal cortex | 20 mg/kg intraperitoneally | Acute | 0.5 h, 1 h, 2 h, 4 h, 8 h, 16 h, 1 day, 3 days, 7 days | Clontech Ratox12 microarray. 1176 genes | Cytokines, cytoskeleton, Egr, serotonin receptor 3 | Thiriet et al. (2002) |
| Mouse | Substantia nigra (dopamine neurons) | 47 mg/kg subcutaneously | Acute | 8 h | 15K Mouse Developmental cDNA Microarray. 15,264 genes | Metallothioneines | Xie et al. (2004) |
| Mouse | Dorsal striatum | 9 mg/kg intraperitoneally | Acute | 2 h | Affymetrix Mouse GeneChips, MGU74A. v2 and MG-U74B. v2. 24,000 genes | ERK signaling; transcription factors Fos and Egr; heat shock protein | Salzmann et al. (2006) |
| Rat | Frontal cortex, hippocampus, raphe | 15 mg/kg intraperitoneally | Acute | 3 weeks | Illumina RatRef-12 v1 beadarray expression chip. 15,983 genes | LTP, calcium, and ephrin signaling and neurotransmission | Petschner et al. (2013) |
| Mouse | Cerebral cortex, pons, cerebellum, midbrain, and hippocampus | 1.25, 5, and 20 mg/kg orally | Chronic. Adolescent mice. Daily injection during 4 weeks. | 11 days | AB Mouse Genome survey microarray. 32,381 genes | Cerebral cortex (20 mg/kg) MAPK, Wnt signaling, LTP, LTD | Eun et al. (2009) |
| Mouse | Frontal cortex, hippocampus, ventral striatum, dorsal raphe | 0.125 mg/kg intravenously Total cumulative 19.7 mg/kg avg | Chronic. Active and passive (yoked self-administration). 3 h sessions during 11 days. | 8 h | Affymetrix GeneChip Mouse Expression Set 430 array. 34,000 genes | Inflammatory and immune response LTP and MAPK signaling in active administration only in hippocampus and raphe | Fernàndez-Castillo et al. (2012) |
| Rat | Hippocampus | 10 mg/kg intraperitoneally | Binge administration. 4 injections, each every 2 h. | 18 h | Affymetrix GeneChip Rat Gene 1.0 ST array. 27,342 genes | Heat shock proteins and chaperones. Neuropeptide signaling. When previously chronically stressed: neuronal ensheathment | Weber et al. (2014) |
| Mouse | Cerebral cortex | 20 mg/kg orally (mother) | Prenatal exposure (indirect). Daily for 4 weeks, from gestation day 6 to 3 weeks after birth. | 8 weeks | AB Mouse Genome survey microarray. 32,381 genes | MAPK and Wnt signaling, axon guidance, cytoskeleton | Eun et al. (2010) |

This table includes all microarray studies that have assessed MDMA-induced changes in gene expression, along with the main details of the experimental conditions, such as the animal used as a model, brain regions assessed, drug dose, and treatment. The main genes showing altered expression are highlighted.

regarding the mechanisms that underlie the response to MDMA exposure, from the first few hours to several days or weeks after administration.

## ACUTE MDMA ADMINISTRATION

The main changes in gene expression observed after acute MDMA administration can be classified according to the functions that are affected (Table 2).

The first large-scale study that assessed the effect of MDMA on gene expression was performed in rats by Thiriet, Ladenheim, McCoy, and Cadet (2002); it assessed changes in gene expression in the frontal cortex of rats that had received a single injection of MDMA. A wide range of time points were considered after MDMA treatment. A total of 28 genes, divided into nine functional groups, showed differential expression over time: cytokines (*Mip1α* and *Mip3*), cell surface antigens (*Cd28* and *Iap*), BCL2 family proteins (*Bok*), cytoskeleton and matrix proteins (*Fib*, *Lamα3*, *Nglyc*, and *Tubα1*), G-proteins (*Gγ9* and *Rab12*), intracellular kinase and the phosphatase network (*Cakβ*, *Mos*, *Ptp*, *Plcβ3* and *Rptpα*), metabolism (*Rps29*, *Gpx1*, *Hmox2*, *Hprt*, *Gapdh*, *Ldh-b*, and *Pub*), receptors (*5-ht3* and *Pgdr2*), and transcription (*Hox1.3, Egr-1* or *Ngfi-a* and *Ngfi-b*).

Those authors highlight changes in the expression of the gene for the serotonin receptor, *5-ht3*, which displays a 50% upregulation 4 h after MDMA administration, then returns to its normal range and increases again after 3–7 days. The serotonergic system is the main MDMA target and is responsible for its behavioral effects. Other serotonin-related genes have also been assessed in different studies. Transcription of the serotonin receptor genes *5-ht1a* and *5-ht2c* was found to be diminished in the hippocampus after acute MDMA administration (Yau, Noble, & Seckl, 1997). A study performed by García-Osta, Del Rio, and Frechilla (2004) identified enhanced expression of *Tph*, encoding tryptophan hydroxylase, in the frontal cortex, and decreased expression in the hippocampus in rats after 2 days of acute MDMA administration. Also, expression of *Sert* increased in the raphe *pallidus* and *obscurus* 7 days after a single MDMA administration (Kovacs et al., 2007).

Peng and Simantov (2003) assessed gene expression changes in the frontal cortex and midbrain of mice 2 h after acute MDMA treatment. Using Droplet Digital PCR (DD-PCR) they cloned 11 cDNA sequences that showed differential expression. Four of them corresponded to the genes coding for synaptotagmin 4 (*Syt4*), dystrophin (Dmd), septin (Nedd5), and GABA transporter (*Gat1*). The authors then focused on GABA transporters (GAT) and identified changes in gene expression in *Gat1* and *Gat4*, but not in *Gat2*. Both *Gat1* and *Gat4* displayed increased expression in the frontal cortex and midbrain, and expression of *Gat1* was sustained for 7 days after treatment. Furthermore, when *Gat1* expression was assessed in *Sert* knockout mice (–/–), which do not respond to MDMA, no significant induction of this GABA transporter was seen. They also studied the possible role of GAT in the toxic effects of MDMA and observed that after treatment with GAT inhibitors, a lethal MDMA dose decreased its toxicity significantly. The expression of the genes for the synaptic vesicle proteins SYT4 and SYT1 were further assessed by the group (Peng et al., 2002). It was observed that *Syt4* expression decreased, at RNAm and protein levels, in the midbrain and frontal cortex, whereas expression of *Syt1* increased in the midbrain, and that these changes in gene expression did not occur in the *Sert* KO mice.

Xie et al. (2004) performed a microarray study to identify genes involved in murine MDMA-induced toxicity in dopaminergic neurons. Mice were treated with a high dose of MDMA that produced significant dopaminergic depletion 1 week later. Substantia nigra was isolated 8 h after treatment to compare transcriptomic profiles, and 10 genes showing differential expression were identified: *Mt1* and *Mt2* (metallothioneins), *Ef1* and *Ef4* (translation factors), *Sgk*, *Cst3*, *Nd1*, *Mapk14*, *Hat1* and *Macf7*. *Mt1* and *Mt2* showed an upregulation peak 4 h after MDMA administration and may protect dopaminergic neurons against MDMA-induced toxicity, since Mt-KO mice (*Mt1 (-/-) + Mt2 (-/-)*) showed larger dopamine deficits after repeated MDMA administration.

Salzmann, Marie-Claire, Le Guen, Roques, and Noble (2003) demonstrated that extracellular signal-regulated kinase (ERK) signaling plays an important role in MDMA-induced reward and behavioral responses in mice. Based on this, a subsequent study detected ERK activation by MDMA in dorsal striatum (Salzmann, Canestrelli, Noble, & Marie-Claire, 2006). Microarray technology was then used to study MDMA-induced changes in gene expression that were dependent on or independent of the ERK pathway. To that end, dorsal striatum profiles were analyzed in mice treated with an acute injection of MDMA with or without the ERK inhibitor SL327. Twenty-seven genes were identified, most upregulated, with differences in expression after acute MDMA administration; 16 of them were partially or totally inhibited by SL327 pretreatment. Nine of the ERK-dependent genes were validated (*Fos*, *Fosl2*, *Fosb*, *Egr1*, *Egr2*, *Rhoe*, *Dnajb5*, *Nts* and *Ttr*).

Among the genes altered by MDMA treatment that are affected by ERK inhibition, *Fos* and *Egr*-related transcripts (*Fos*, *Fosl2*, *Fosb*, *Egr1* and *Egr2*) deserve to be highlighted. Consistently, in the previous study, Salzmann et al. (2003) observed that 1 h after acute MDMA administration, *c-fos* expression was greatly induced in the nucleus accumbens, caudate putamen, and hippocampus, and the expression of the *Egr1* and *Egr3* genes was increased in the caudate putamen. Other studies also identified *c-fos* induction in several brain regions after acute MDMA administration in mice and rats (Dragunow, Logan, & Laverty, 1991; Erdtmann-Vourliotis, Mayer, Riechert, & Hollt, 1999; Hashimoto, Tomitaka, Narita, Minabe, & Iyo, 1997; Stephenson, Hunt, Topple, & McGregor, 1999). Increased expression of *Egr1* after acute MDMA exposure was also observed in the prefrontal cortex, striatum, and hippocampus of mice and also in the rat frontal cortex (Shirayama et al., 2000; Thiriet et al., 2002). *Fos* and *Egr* encode transcription factors and are MDMA-induced early genes (IEGs) that control late-response gene expression and may play an important role in the transition from short-term neuronal stimulation to long-lasting changes in neuronal function (O'Donovan, Tourtellotte, Millbrandt, & Baraban, 1999).

Another interesting result, which was explored further in another study by the same group (Marie-Claire, Benturquia, Lundqvist, Courtin, & Noble, 2008), is the increased expression of genes coding for several phosphatases in striatum following acute MDMA treatment. Upregulation of *Dusp14* depends on ERK, whereas *Dusp1* and *Dusp5* upregulation is ERK-independent. These three phosphatase-encoding genes are involved in the negative regulation of MAPK signaling; and regulation of protein

**TABLE 2** Gene Expression Changes in Response to Acute MDMA Administration

| Molecular Function | Representative Genes | Gene Symbol | References |
|---|---|---|---|
| Neurotransmission | Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 | *Slc6a3* (*Sert*) | Garcia-Osta, Del Rio, and Frechilla (2004), Kovacs et al. (2007), Marie-Claire, Palminteri, et al. (2008), Nawata et al. (2010), Peng et al. (2002), Peng and Simantov (2003), Petschner et al. (2013), Thiriet et al. (2002), and Yau et al. (1997) |
| | 5-Hydroxytryptamine (serotonin) receptor 3A | *5-ht3* | |
| | 5-Hydroxytryptamine (serotonin) receptor 1A | *5-ht1a* | |
| | 5-Hydroxytryptamine (serotonin) receptor 2C | *5-ht2c* | |
| | Tryptophan hydroxylase 1 | *Tph1* | |
| | Tryptophan hydroxylase 2 | *Tph2* | |
| | Solute carrier family 6 (neurotransmitter transporter, GABA), member 1 | *Slc6a1* (*Gat1*) | |
| | Solute carrier family 6 (neurotransmitter transporter, GABA), member 11 | *Slc6a11* (*Gat2*) | |
| | Gamma-aminobutyric acid (GABA) A receptor, subunit epsilon | *Gabre* | |
| | Glutamate receptor, ionotropic, AMPA 3 | *Gria3* | |
| | Glutamate receptor, ionotropic, *N*-methyl D-aspartate 1 | *Grin1* | |
| | Glutamate receptor, ionotropic, *N*-methyl D-aspartate 2a | *Grin2a* | |
| | Glutamate receptor, ionotropic, *N*-methyl D-aspartate 2b | *Grin2b* | |
| | Solute carrier family 1 (glial high affinity glutamate transporter), member 3 | *Slc1a3* | |
| | Solute carrier family 1 (glial high affinity glutamate transporter), member 2 | *Slc1a2* | |
| | Cannabinoid receptor 1 (brain) | *Cnr1* (*Cb1*) | |
| | Synaptotagmin IV | *Syt4* | |
| | Neurotensin | *Nts* | |
| Inflammatory and immune response and apoptosis | Chemokine (C–C motif) ligand 3 | *Ccl3* (*Mip-1a*) | Thiriet et al. (2002) and Torres et al. (2010) |
| | Chemokine (C–C motif) ligand 20 | *Ccl20* (*Mip-3*) | |
| | Cd28 antigen | *Cd28* | |
| | Cd47 molecule | *Cd47* (*Iap*) | |
| | BCL2-related ovarian killer protein | *Bok* | |
| | Cannabinoid receptor 2 (macrophage) | *Cnr2* (*Cb2*) | |
| Protection from toxicity and hyperthermia | Metallothionein 1a | *Mt1* | Adori et al. (2006), Escobedo et al. (2007), Stetler et al. (2010), Thiriet et al. (2002), Torres et al. (2010), and Xie et al. (2004) |
| | Metallothionein 2 | *Mt2* | |
| | DnaJ (Hsp40) homolog, subfamily B, member 5 | *Dnajb5* (*Hsc40*) | |
| | Heat shock protein 1b | *Hspa1b* (*Hsp70*) | |
| | Heat shock protein 2 | *Hspb2* (*Hsp27*) | |
| | Heat shock protein 90, alpha (cytosolic), class A member 1 | *Hsp90aa1* (*Hspca*) | |

**TABLE 2** Gene Expression Changes in Response to Acute MDMA Administration—cont'd

| Molecular Function | Representative Genes | Gene Symbol | References |
|---|---|---|---|
| | Heat shock factor 2 | *Hsf2* | |
| | Glial fibrilliary acidic protein | *Gfap* | |
| Neurotrophic factors | Brain derived neurotrophic factor | *Bdnf* | Adori et al. (2010) and Martinez-Turrillas et al. (2006) |
| Signal transduction | Eph receptor A4 | *Epha4* | Marie-Claire, Benturquia, et al. (2008) and Petschner et al. (2013) |
| | Eph receptor A5 | *Epha5* | |
| | Eph receptor A6 | *Epha6* | |
| | Calcium/calmodulin-dependent protein kinase II inhibitor 1 | *Camk2n1* | |
| | Calcium/calmodulin-dependent protein kinase II inhibitor 2 | *Camk2n2* | |
| | Calcium/calmodulin-dependent protein kinase II gamma | *Camk2g* | |
| | Calcium/calmodulin-dependent protein kinase II beta | *Camk2b* | |
| | Dual specificity phosphatase 1 | *Dusp1* | |
| | Dual specificity phosphatase 5 | *Dusp5* | |
| | Dual specificity phosphatase 14 | *Dusp14* | |
| Transcription factors | FBJ osteosarcoma oncogene | *Fos* (*c-fos*) | Dragunow et al. (1991), Erdtmann-Vourliotis et al. (1999), Hashimoto et al. (1997), Rodriguez-Alarcon, Canales, and Salvador (2007), Salzmann et al. (2003), Shirayama et al. (2000), Stephenson et al. (1999), and Thiriet et al. (2002) |
| | FBJ osteosarcoma oncogene B | *Fosb* | |
| | Fos-like antigen 2 | *Fosl2* | |
| | Early growth response 1 | *Egr1* (*Ngfia*) | |
| | Early growth response 2 | *Egr2* | |
| | Early growth response 1 | *Egr3* | |
| | Nuclear receptor subfamily 4, group A, member1 | *Nr4a1* (*Ngfib*) | |
| Cytoskeleton | Rho family GTPase 3 | *Rnd3* | Beveridge et al. (2004), Marie-Claire et al. (2007), and Thiriet et al. (2002) |
| | Rad and gem related GTP binding protein 2 | *Rem2* | |
| | Tubulin, alpha 1A | *Tuba1a* | |
| | Activity regulated cytoskeletal-associated protein | *Arc* | |

This table shows the main genes showing differences in gene expression after an acute MDMA administration. Genes are classified according to their molecular function, and references are specified for each functional category.

phosphorylation by phosphatase activity seems to be crucial for synaptic plasticity (Gurd, 1997).

Neurotensin (*Nts*), which modulates dopaminergic neurotransmission and is involved in several behavioral functions (reward, stress, and locomotion), was found to be upregulated in the microarray experiment (Salzmann, Canestrelli, Noble, & Marie-Claire, 2006) showing an overexpression peak 6 h after acute MDMA treatment. Moreover, increased *Nts* expression was observed after chronic treatment, and treatment with a neurotensin receptor antagonist modulated MDMA-conditioned place preference (CPP) and hyperlocomotor activity (Marie-Claire, Palminteri, et al., 2008). In rats, neurotensin also showed increased expression in the striatum 3 h after acute treatment, as did two other neuropeptide genes: *Ppd* (preprodynorphin) and *Ppt* (preprotachykinin) (Adams, Hanson, & Keefe, 2005). *Ppd* was also found to be upregulated in rats in the prefrontal cortex, brainstem, and caudate, and downregulated in the ventral tegmental area, 2 h after acute MDMA

treatment (Di Benedetto, Bastias Candia Sdel, et al., 2011; Di Benedetto, D'Addario, Candeletti, & Romualdi, 2006).

The gene for the Rho GTPase involved in regulating actin cytoskeleton (*Rnd3*) showed overexpression both in the microarray and in a follow-up study in the hippocampus, striatum, and prefrontal cortex of mice treated acutely with MDMA (Marie-Claire, Salzmann, et al., 2007; Salzmann, Canestrelli, Noble, & Marie-Claire, 2006). Another gene involved in cytoskeleton reorganization (*Rem2*) also showed MDMA-induced expression in the microarray experiment.

The *Dnajb5* gene, encoding the heat shock protein HSC40, was upregulated, and another heat shock protein gene, *Hspa1b*, coding for HSP70, also showed increased expression in the frontal cortex 3 h and 7 days after acute MDMA administration in rat; this increase was dependent on the hyperthermic response (Escobedo, Peraile, Orio, Colado, & O'Shea, 2007). Elevated HSP27 was also identified in rat frontal cortex and hippocampus in astrocytes, as was GFAP in hippocampal astrocytes (Adori, Ando, Kovacs, & Bagdy, 2006). Heat-shock proteins can protect against damage caused by hyperthermia, free radicals, and ischemia (Stetler et al., 2010). Another study showed that MDMA induced significant hyperthermia, together with serotonin depletion and increased expression of the *Arc* gene in cortical regions, and the caudate putamen and hippocampus (Beveridge et al., 2004).

The last microarray study that evaluated acute MDMA effects on gene expression was performed by Petschner et al. (2013). Rats were treated with a single dose of MDMA and gene expression profiles of the hippocampus, frontal cortex, and dorsal raphe were assessed 3 weeks afterward. The authors identified a total of 615 genes differentially expressed in the MDMA-treated group: 481 of them in the hippocampus, 155 in the frontal cortex, and 14 in the dorsal raphe.

In the hippocampus, enrichment analysis identified clusters of genes involved in protein phosphorylation, dendrite and synapse development, synaptic plasticity, and transmembrane transport. Several genes encoding neurotransmitter receptors showed altered expression, such as the glutamate receptor genes *Gria3* and *Grin2a*, which were upregulated after acute MDMA administration, or the gene coding for the GABA-A receptor, epsilon subunit (*Gabre*), which was downregulated. The genes for several ephrin receptors (*Epha4*, *Epha5*, and *Epha6*), which modulate synapse formation and long-term potentiation (LTP) of glutamate, were found to be upregulated. Also, genes for members of the calcium signaling pathway (*Camk2n1*, *Camk2n2*, *Camk2g,* and *Camk2b*) and for calcium transporting ATPases (*Atp2b1* and *Atp2b3*) showed altered expression, as did genes encoding voltage-gated potassium transporters (*Kcn2* and *Kcnd2*). The cannabinoid receptor 1 gene (*Cnr1* or *Cb1*) was upregulated in this study and was also found to be increased in mouse hippocampus 7 days after repeated MDMA administration, whereas a CB1 receptor antagonist attenuated the cognitive deficits induced by MDMA (Nawata, Hiranita, & Yamamoto, 2010). Another study revealed increased expression of the CB2 receptor in the frontal cortex and hypothalamus in microglia after acute MDMA administration in rats, and showed that CB2 activation reduces neuroinflammatory response following MDMA administration (Torres et al., 2010).

In the frontal cortex, gene sets were related to protein synthesis and localization, transmembrane and nucleocytoplasmic transport, cell growth, chromatin maintenance, dendrite and synapse development, and oxidoreductase activity. In this brain region, expression changes were also identified in genes related to calcium signaling (*Camk2g* and *Camk1g*), as well as an NMDA glutamate receptor (*Grin2b*) and a glutamate transporter (*Slc1a3*). A study performed in cortical cells in vitro identified an increase in the NMDA glutamate receptor NR1 (*Grin1*) and a decrease in the glutamate transporter EAAT2-1 (*Slc1a2* or *Glt1*) (Kindlundh-Hogberg et al., 2010).

Also, the genes coding for the heat shock protein HSPCA and the heat shock factor HSF2 were upregulated. In agreement with the results obtained by Thiriet et al. (2002), several growth factor gene sets showed upregulation, and others related to cytoskeletal transport showed downregulation.

In the dorsal raphe, only a few genes showed altered expression, among them the one encoding the glycine neurotransmitter transporter (*Slc6a5*).

If we consider all the above studies, which were performed following acute MDMA administration, it is possible to group the observed gene expression changes according to the distinct biological processes that they affect, which helps to elucidate the underlying molecular mechanisms (Table 2). The early events that occur after MDMA administration appear to be related to ERK activation and signal transmission (both ERK dependent and independent), which involve several kinases, phosphatases, and transcription factors (*Fos*- and *Egr*-related transcripts) and are an early response to MDMA. Afterward, some events involve changes in the regulation of neurotransmission: the serotonergic, glutamatergic, GABAergic, and cannabinoid systems. Also, MDMA-induced toxicity and hyperthermia activate inflammatory and immune responses, since some cytokines and cell surface antigens were found to be upregulated, as were some genes encoding proteins that protect against toxicity, such as heat shock proteins and metallothioneins. The later response to MDMA seems to involve synaptic plasticity, possibly mediated through calcium and ephrin signaling, and changes in the cytoskeleton and matrix proteins involved in neuroadaptation.

## REPEATED AND CHRONIC MDMA ADMINISTRATION

The main gene expression changes that occur after repeated and chronic administration of MDMA are listed in Table 3.

Several studies that focus on repeated and chronic MDMA administration have assessed serotonergic, dopaminergic, and glutamatergic candidate genes. The serotonergic system is affected by long-term exposure to MDMA, which causes neurotoxicity due to serotonin depletion, leading to neurotransmitter dysregulation. After four binge administrations to rats (one per week), Kindlundh-Hogberg, Svenningsson, and Schioth (2006) observed increased expression of the *5-ht1b* gene in several brain regions (cortex, caudate putamen, and hypothalamus). In the same study, the *5-ht2a* and *5-ht2c* genes were also upregulated in the cortex; *5-ht2c* and *5-ht3* were upregulated in the hypothalamus; and *5-ht6* showed increased expression in the forebrain cortex and the amygdala. In another study, MDMA intake was found to diminish *5-ht1a* mRNA in the hippocampus and brainstem and to increase its expression in the frontal cortex (Aguirre, Frechilla, Garcia-Osta, Lasheras, & Del Rio, 1997). An in vitro study of rat cortical cells exposed to MDMA for 5 days identified a significant

**TABLE 3** Gene Expression Changes in Response to Repeated and Chronic MDMA Administration

| Molecular Function | Representative Genes | Gene Symbol | References |
|---|---|---|---|
| Neurotransmission | 5-Hydroxytryptamine (serotonin) receptor 1A | *5-ht1a* | Aguirre et al. (1997), Biezonski and Meyer (2010), Bonkale and Austin (2008), Cuyas et al. (2014), Eun et al. (2009), Kindlundh-Hogberg et al. (2008), Kindlundh-Hogberg et al. (2010), and Kindlundh-Hogberg et al. (2006) |
| | 5-Hydroxytryptamine (serotonin) receptor 1B | *5-ht1b* | |
| | 5-Hydroxytryptamine (serotonin) receptor 2A | *5-ht2a* | |
| | 5-Hydroxytryptamine (serotonin) receptor 2C | *5-ht2c* | |
| | 5-Hydroxytryptamine (serotonin) receptor 3 | *5-ht3* | |
| | 5-hydroxytryptamine (serotonin) receptor 6 | *5-ht6* | |
| | Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 | *Slc6a4* (*Sert*) | |
| | Tryptophan hydroxylase 2 | *Tph2* | |
| | Tyrosine hydroxilase | *Th* | |
| | Monoamine oxidase b | *Maob* | |
| | Solute carrier family 18 (vesicular monoamine), member 2 | *Slc18a2* (*Vmat2*) | |
| | Glutamate receptor ionotropic, AMPA1 (alpha1) | *Gria1* | |
| | Glutamate receptor ionotropic, AMPA2 (alpha2) | *Gria2* | |
| | Glutamate receptor metabotropic 1 | *Grm1* | |
| | Glutamate receptor metabotropic 3 | *Grm3* | |
| | Glutamate receptor metabotropic 5 | *Grm5* | |
| | Glutamate receptor ionotropic, NMDA1 (zeta1) | *Grin1* | |
| | Glutamate receptor, ionotropic, NMDA2A (epsilon 1) | *Grin2a* | |
| | Glutamate receptor, ionotropic, NMDA2B (epsilon 2) | *Grin2b* | |
| | Solute carrier family 1 (glial high affinity glutamate transporter), member 3 | *Slc1a3* (*Eaat1*) | |
| | Solute carrier family 1 (glial high affinity glutamate transporter), member 2 | *Slc1a2* (*Eaat2-2*) | |
| | Cholinergic receptor, muscarinic 3, cardiac | *Chrm3* | |
| | Glycine receptor beta | *Glrb* | |
| | Neuropeptide Y | *Npy* | |
| | Solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7 | *Slc17a7* | |
| Inflammatory and immune response and apoptosis | Lipocalin 2 | *Lcn2* | Fernandez-Castillo et al. (2012), Soleimani Asl et al. (2012), and Stumm et al. (1999) |
| | Cytotoxic T lymphocyte-associated protein 2 alpha | *Ctla2a* | |
| | Guanylate binding protein 2 | *Gbp2* | |
| | Interferon gamma induced GTPase | *Igtp* | |
| | Interferon inducible GTPase 1 | *Iigp1* | |
| | Interferon inducible GTPase 2 | *Iigp2* | |
| | T cell specific GTPase 1 | *Tgtp* | |
| | B cell leukemia/lymphoma 2 | *Bcl-2* | |
| | BCL2-associated X protein | *Bax* | |
| | BCL2-like 1 | *Bcl2l1* (*Bcl-x*) | |

*Continued*

**TABLE 3** Gene Expression Changes in Response to Repeated and Chronic MDMA Administration—cont'd

| Molecular Function | Representative Genes | Gene Symbol | References |
|---|---|---|---|
| Protection from toxicity and hyperthermia | Heat shock protein 8 | *Hspa8* | Weber et al. (2014) |
| | DnaJ (Hsp40) homolog, subfamily B, member 1 | *Dnajb1* (*Hsp40*) | |
| | Heat shock protein 1 (chaperonin) | *Hspd1* | |
| | Heat shock protein 90 alpha (cytosolic), class B member 1 | *Hsp90ab1* | |
| | DnaJ (Hsp40) homolog, subfamily A, member 4 | *Dnaja4* | |
| | DnaJ (Hsp40) homolog, subfamily B, member 11 | *Dnajb11* | |
| | DnaJ (Hsp40) homolog, subfamily B, member 4 | *Dnajb4* | |
| | DnaJ (Hsp40) homolog, subfamily A, member 2 | *Dnaja2* | |
| | Serine (or cysteine) peptidase inhibitor, clade H, member 1 | *SerpinH1* (*Hsp47*) | |
| | Chaperonin containing Tcp1, subunit 5 (epsilon) | *Cct5* | |
| | Chaperonin containing Tcp1, subunit 6a (zeta) | *Cct6a* | |
| Neurotrophic factors | Brain derived neurotrophic factor | *Bdnf* | Eun et al. (2009) and Hatami et al. (2010) |
| | Neurotrophin 4 | *Nt-4* | |
| Signal transduction | Purinergic receptor P2X, ligand-gated ion channel, 7 | *P2rx7* | Eun et al. (2009) |
| | Vasoactive intestinal peptide receptor 1 | *Vipr1* | |
| | Glycogen synthase kinase 3 beta | *Gsk3b* | |
| | Seven in absentia 1A | *Siah1* | |
| | Mitogen activated protein kinase kinase 7 | *Map2k7* | |
| | Mitogen activated protein kinase kinase 2 | *Map2k2* | |
| | Protein phosphatase 3, regulatory subunit B, alpha | *Ppp3r1* | |
| | Protein kinase C, beta | *Prkcb1* | |
| | DNA damage inducible transcript 3 | *Ddit3* | |
| | TAO kinase 1 | *Taok1* | |

This table includes the main genes showing differences in gene expression after repeated or chronic MDMA administration. Genes are classified according to their molecular function, and references are specified for each functional category.

decrease in *5-ht3* expression and an increase in *5-ht1a* (Kindlundh-Hogberg et al., 2010). Seven days after a binge administration to rats, increased expression of the genes for tryptophan hydroxylase 2 (*Tph2*) and monoamine oxidase B (*Maob*), enzymes involved in serotonin synthesis and degradation, respectively, was observed in the hippocampus. The gene for tyrosine hydroxilase (*Th*), involved in dopamine synthesis, was downregulated in the striatum, and the serotonin transporter gene *Sert* and the vesicular monoamine transporter gene *Vmat2* showed diminished expression in the brainstem (Cuyas et al., 2014). Also, both in rat dorsal and medial raphe, *Tph2* expression was increased and *Sert* decreased 2 weeks after chronic and binge MDMA exposure, respectively (Biezonski & Meyer, 2010; Bonkale & Austin, 2008).

In rats, MDMA induces pronounced overexpression of the glutamate receptor and transporter genes *Gria2*, *Grm1*, *Grm5*, *Grin1*, *Grin2a, Grin2b*, *Eaat1*, and *Eaat2* in the cortex. The receptor genes *Gria2*, *Grin2a*, and *Grin2b* were increased in the caudate putamen, and *Gria1*, *Gria3*, *Grm1*, and *Grm3* were upregulated in the hypothalamus, whereas *Gria1* was downregulated in the hippocampus (Kindlundh-Hogberg, Blomqvist, Malki, & Schioth, 2008). The expression of dopamine receptor genes was altered only in the hypothalamus (Kindlundh-Hogberg et al., 2006).

The first high-throughput study of gene expression changes was performed in adolescent mice several days after chronic MDMA exposure (Eun et al., 2009). The drug was administered daily at different doses for 4 weeks, and the highest dose showed the largest number of gene expression changes. The main changes were observed in the cerebral cortex, involving a total of 1028 genes, approximately half upregulated and half downregulated. These genes were involved mainly in signal transduction, transcription, protein modification, cell proliferation and differentiation, cell communication, transport, immunity, defense, apoptosis, and neurogenesis. The signaling pathways that were most altered were those of MAPK, Wnt, long-term potentiation (LTP),

long-term depression (LTD), and the neuroactive ligand–receptor interaction pathway. The set of differentially expressed genes included the following: *Npy*, *Chrm3*, *Grm1*, *P2rx7*, *Glrb,* and *Vipr1* (neuroactive ligand receptor interaction pathway); *Gsk3b* and *Siah1* (Wnt pathway); and *Bdnf*, *Map2k7*, *Map2k2*, *Ppp3r1*, *Prkcb1*, *Ddit3,* and *Taok1* (MAPK pathway). The MAPK signaling pathway is activated by growth factors, inflammation, stress, and cytokines and modulates several processes such as apoptosis, inflammation, differentiation, and cell growth (Bonni et al., 1999; Kaminska, 2005; Karin, 1998). This microarray experiment also showed upregulation of *Bdnf*, encoding a neurotrophic factor involved in the regulation of synaptic plasticity, survival, and the functioning of serotonergic neurons, together with many other processes. *Bdnf* is upregulated in response to brain damage and neuronal injury as a compensatory effect (Hicks, Martin, Zhang, & Seroogy, 1999). In the parietal cortex of MDMA-treated rats, BDNF protein levels showed a robust peak increase 8 weeks after an acute administration (Adori et al., 2010). Another study (Martinez-Turrillas, Moyano, Del Rio, & Frechilla, 2006) focused on *Bdnf* expression and its relation with serotonin after MDMA administration in rats, since BDNF induces serotonin synthesis by enhancing *Tph* expression (Siuciak, Clark, Rind, Whittemore, & Russo, 1998). The study identified increased *Bdnf* expression in the frontal cortex 24–48 h after acute MDMA administration, and decreased expression in the hippocampus 2–7 days after drug intake. The investigators also studied the effect of MDMA on serotonin levels, and observed that upregulation of *Bdnf* in the frontal cortex seemed to play a role in the recovery of the levels of this neurotransmitter in this brain region, in contrast to the hippocampus, which showed no recovery after 7 days and which correlated with no increased expression of this gene. Augmented *Bdnf* expression correlated with higher levels of active CREB, enhanced expression of the *Tph* gene, and recovery of serotonin levels. *Tph* expression was increased after acute, repeated, and chronic MDMA administration in rats, as discussed above (Bonkale & Austin, 2008; Cuyas et al., 2014). Another neurotrophin gene, *Nt-4*, which encodes a molecule that acts through the same receptor as BDNF (TRKB), also showed increased expression in the brainstem, cerebellum, and cerebral hemisphere of rats after chronic MDMA administration for 5 days (Hatami, Hossainpour-Faizi, Azarfarin, & Azarfam, 2010). Neurotrophins might be involved both in protecting against MDMA-induced brain damage and in the neuronal remodeling that occurs after chronic drug use.

Another microarray study, performed by Fernandez-Castillo et al. (2012), evaluated changes in gene expression in the frontal cortex, ventral striatum, hippocampus, and dorsal raphe nucleus of mice after chronic MDMA administration (both passive and active) using a yoked control operant intravenous self-administration paradigm for 11 days. Gene expression was evaluated 8 h after the last administration. In the experiment, contingent mice were trained to self-administer MDMA. Each contingent mouse was connected to two other mice, one passively receiving an identical dose of MDMA and another receiving saline solution. This set-up allowed the identification of gene expression changes in active and passive administration. In what remains of this section, we focus on the effects of chronic MDMA administration on gene expression (shared between active and passive administration), and we leave for the next section the discussion on specific changes due to active self-administration. Changes in gene expression due to direct effects of chronic MDMA, and displaying the same direction in both active and passive administration compared to the effects observed with saline, were identified in the ventral striatum (101 genes), frontal cortex (129), hippocampus (183), and dorsal raphe nucleus (16); most genes were upregulated in all brain regions. Similar enriched functions were observed in all brain regions, most of them involving immune and inflammatory responses, response to wounding, and stress. Similar enriched pathways were also identified, such as natural killer-mediated cytotoxicity, complement and coagulation cascades, and B cell receptor signaling. These functions and pathways emphasize the immunological and inflammatory nature of the response to chronic MDMA administration. The NF-κB complex was a central node of gene networks for all four regions, and NF-κB and RELA transcription factors (NF-κB complex) were predicted to be responsible for the gene upregulation in all brain structures. Inflammatory and immunological responses might be modulated by NF-κB, considering these results and previous studies showing that MDMA may induce NF-κB activation (Montiel-Duarte, Ansorena, Lopez-Zabalza, Cenarruzabeitia, & Iraburu, 2004; Orio et al., 2010; Tiangco et al., 2005).

Some of the genes showing differential expression have immunological functions (*Lcn2*, *Ctla2a*, *Gbp2*, *Igtp*, *Iigp1*, *Iigp2,* and *Tgtp*), whereas others are involved in neurological processes (*Sgk1*, *Sgk3,* and *Slc17a7*). Most of the cited genes involved in immunological functions code for GTPases inducible by interferon-γ (INF-γ) and mediate interferon control of inflammatory and immunological responses. The lipocalin 2 gene (*Lcn2*), which is strongly overexpressed in all four brain regions, is induced after chronic and thermal stress and mediates astrocytosis under inflammatory conditions (Krishnan et al., 2007; Lee et al., 2009; Roudkenar et al., 2009).

Other studies showed that chronic MDMA exposure in rats induced neurotoxicity and apoptosis and altered the expression of some apoptotic genes. Long-term exposure to MDMA decreased cell viability in cultured rat cortical cells in a dose-dependent manner, and cell death was accompanied by differential expression of anti- and pro-apoptotic *Bcl-x* splice variants (Stumm et al., 1999). In the hippocampus of rats treated chronically, MDMA increased the expression of the pro-apoptotic gene *Bax* and decreased the expression of the anti-apoptotic gene *Bcl-2*, also in a dose-dependent manner (Soleimani Asl et al., 2012). As mentioned above, another gene of the *Bcl-2* family, *Bok*, was also altered after acute MDMA administration (Thiriet et al., 2002).

Another microarray study, by Weber, Johnson, Yamamoto, and Gudelsky (2014), assessed MDMA transcriptional changes in the hippocampus of rats exposed to chronic stress, which has been shown to increase MDMA-induced serotonergic toxicity. Rats under chronic stress were exposed to binge, repeated MDMA administration and killed 18 h after the last injection. The authors observed that MDMA alone (in nonstressed rats) induced changes in 1225 genes in the hippocampus, approximately half upregulated and half downregulated, involving functions such as calmodulin activity, protein kinase activity, protein folding, and neuropeptide signaling pathways. Regarding protein folding, a large number of genes for heat shock proteins and chaperones showed MDMA-induced overexpression (e.g., *Hspa8*, *Hsp40, Hspd1*, *Hsp90ab1*, *Cct5*, *Dnajb1*, *Dnaja4*, *Dnajb11*, *Dnajb4*, *Cct6a*, *SerpinH1,* or *Dnaja2*), in agreement with the results of other studies mentioned above (Adori et al., 2006; Escobedo et al., 2007; Salzmann et al., 2003).

MDMA combined with chronic stress induced altered expression of genes involved in responses to brain damage, especially neuronal ensheathment, categories in which changes were not observed when MDMA was administered to nonstressed rats. Also, in a context of chronic stress, MDMA altered the expression of genes involved in neurotransmission and sensory perception. Chronic stress seems to enhance neuronal damage caused by MDMA.

Taking into account all these studies of repeated and chronic administration, we can highlight some of the most notable gene expression changes induced by the drug, most of them related to its neurotoxic effects (Table 3). A few hours after the last administration, we observe pronounced immune and inflammatory responses that are probably induced by axonal serotonergic or dopaminergic depletion. These responses may be mediated by NF-κB activation induced by MDMA. There is also increased neuronal death, accompanied by alterations in the expression of apoptotic genes. Brain damage can increase even more if the drug is consumed under stress conditions, altering neuronal ensheathment. Also, some heat shock proteins and chaperones are induced to provide protection against MDMA-induced toxicity. Neurotransmission-related genes, such as those encoding proteins related to serotonin and glutamate, are altered both as an adaptation to repeated exposure to the drug and also due to the depletion of the neurotransmitter. After a few days, several processes aid recovery from the damage, involving neuroadaptations and plasticity. These changes involve functions or molecules such as MAPK signaling and neurotrophins, Wnt signaling, LTP, LTD, and neuroactive ligand receptor signaling.

## MDMA SELF-ADMINISTRATION

The only study of changes after MDMA self-administration that more closely mimics human MDMA intake was performed by Fernàndez-Castillo et al. (2012). As mentioned above, both chronic active self-administration (contingent) and passive administration induced changes in the expression genes involved in immune and inflammatory responses. That study also examined those genes involved in active self-administration learning processes compared to passive administration and saline solution. Positive genes were identified only in the hippocampus (645) and dorsal raphe nucleus (61), most of them downregulated and upregulated, respectively, in contingent mice. No significant differences in gene expression were identified in the frontal cortex or ventral striatum. Genes differentially expressed in the hippocampus were involved in neurological functions such as neurotransmission, regulation of synaptic plasticity, axonogenesis, learning, and memory. Also, several pathways were found to be altered both in the hippocampus and dorsal raphe nucleus, such as long-term potentiation (LTP), the MAPK signaling pathway, and the Wnt signaling pathway, in which most genes were downregulated in the hippocampus of contingent mice. All these pathways were found to be altered in adolescent mice after chronic MDMA administration (see above, Eun et al., 2009). Altered LTP genes included glutamate receptors (*Grin1*, *Grin2a,* and *Grin2b*) and phosphatases (*Ppp1cb* and *Ppp3ca*). Genes altered in the MAPK signaling pathway included *Ntrk2* (encoding a BDNF receptor), *Akt1,* and *Jund*.

Some of the genes involved in neurological processes that show differences in gene expression in the hippocampus were *Clpx2*, *Vamp2*, *Nrxn1*, *Nrx2*, *Amigo1*, *Bzrap1*, *Gprin1*, *Mapk8ip1*, *Nlgn2*, *Vgf*, *Madd,* and *Axin2*; and in the dorsal raphe nucleus they were *Camk2a*, *Kalrn*, *Ddn,* and *Egr3*. These results suggest that both the hippocampus and dorsal raphe nucleus are involved in the motivation and learning processes associated with active MDMA seeking behavior, and that those processes are mediated through LTP, MAPK, and Wnt signaling pathways.

## PRENATAL EXPOSURE TO MDMA

The effect of prenatal exposure to MDMA on gene expression has been investigated only by Eun et al. (2010). Unborn mice (male and female) were exposed indirectly to MDMA from gestation day 6 until 21 days after birth (during pregnancy and lactation), and cerebral cortex expression profiles were assessed 11 weeks later, using microarray technology. Prenatal exposure to MDMA induced differences in gene expression in 1784 genes in the female group and in 804 genes in the male group. Of these, 54 upregulated genes and 36 downregulated genes were common to both males and females. Enriched pathways shared by the male and female gene sets were the MAPK signaling pathway, Wnt signaling pathway, neuroactive ligand–receptor interaction pathway, calcium signaling pathway, and axon guidance and focal adhesion. This suggests that, although the genes showing differential expression are in general not the same between males and females, the processes involved in transcriptomic changes in both sexes are similar. Some of the genes differentially expressed under prenatal MDMA exposure were *Akt1*, *Atp1a2*, *H2afy*, *Ifit1*, *Rnase1,* and *Dctn1* in females and *Egr2*, *Arc*, *Rps2*, *Ppp3rl*, *Prkcb1,* and *Bcds3* in males. *Atp1a2*, *Dctn1,* and *Akt1* were highly upregulated (six- to sevenfold) in females.

Changes in gene expression that are present several days after prenatal MDMA exposure would involve neuroadaptive events, such as remodeling and synaptic plasticity. These events would help the brain to adapt to the effects caused by the drug during brain development.

## MOLECULAR AND CELLULAR EVENTS TRIGGERED BY EXPOSURE TO MDMA

In the previous sections, we have seen how acute or chronic exposure to MDMA alters gene expression in the brain. This has been assessed using different experimental procedures and timings. Although MDMA-induced toxicity is much more pronounced after repeated and chronic administration, we have observed that common pathways are altered under both acute and chronic administration. The results obtained in each individual study provide only a static picture of the alterations in gene expression for a given time and experimental situation. By considering the data produced by all the above studies together, some conclusions can be drawn concerning the molecular events that take place upon MDMA administration, from the very first hours after exposure to the drug to several days or weeks afterward.

### Early Response to MDMA Administration (Up to 2 Hours)

After MDMA administration, in which neurotransmission alterations such as increased serotonergic activity occur, the main downstream mechanism that is activated seems to be signal transmission

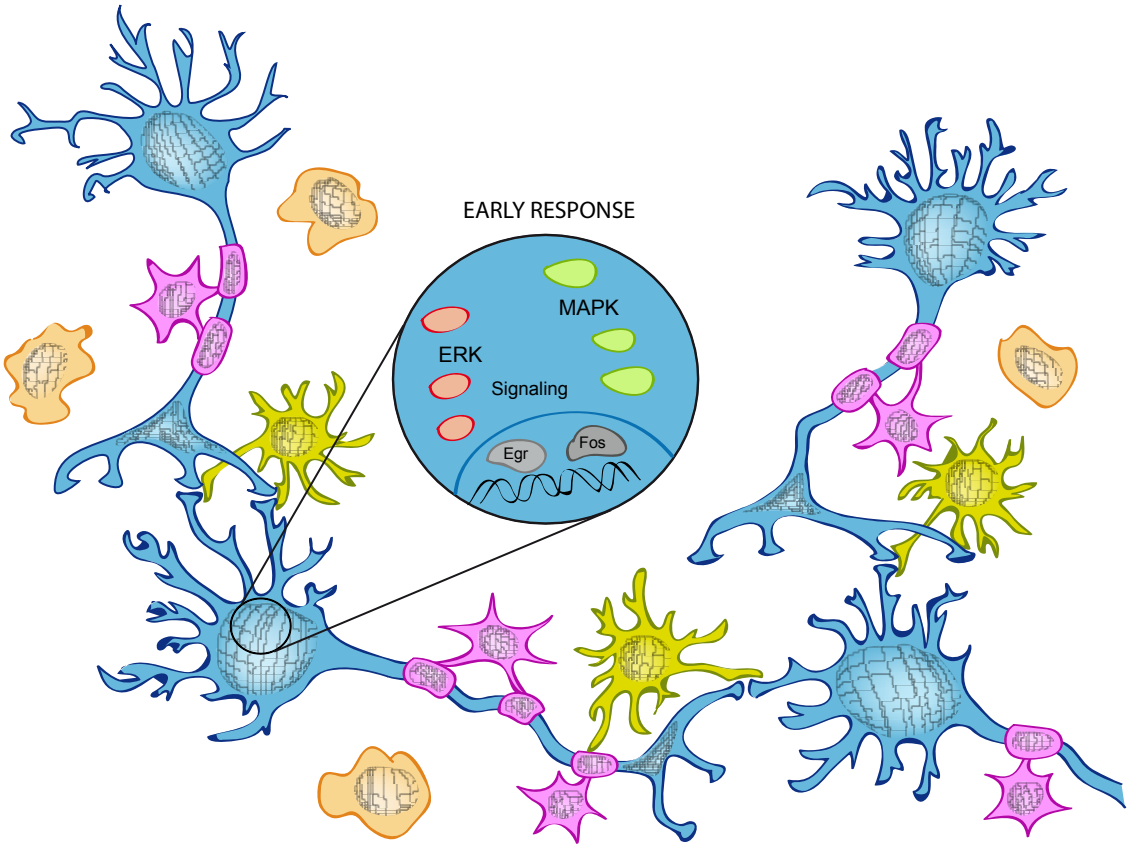


**FIGURE 1** **Early response to MDMA administration.** Changes in gene expression up to 2 hours after drug administration affect mainly signal transmission (ERK and MAPK pathways) and *Fos*- and *Egrs*-related transcription factors, which produce the early response to MDMA. Neurons are indicated in blue, astrocytes in green, oligodendrocytes in purple, and microglia in orange.

(either through ERK activation or independently). MDMA induces changes in gene expression all along this signaling pathway, from kinases and phosphatases to transcription factors, such as FOS and EGR-related transcripts, forming the early response to the drug. Figure 1 shows some neuronal and glial cells, and the signaling pathway and transcription factors that are altered in the postsynaptic neuron.

## Later Response to MDMA Administration (More than 8 Hours)

After MDMA administration, several events take place. We observe expression changes in neurotransmission-related molecules, including receptors, transporters, neurotransmitter enzymes, and neuropeptides (Figure 2). These changes involve most neurotransmission systems, including the serotonergic, glutamatergic, GABAergic, dopaminergic, and cannabinoid systems, and they may be involved in compensating MDMA stimulation. Also, several signaling pathways are altered, such as MAPK, Wnt, and LTP, that are associated with learning and memory processes in active administration (such as memories related to the drug consumption involved in cue-induced craving). Finally, neurotoxicity-related processes occur, which are more intense at higher doses and with repeated administration. MDMA causes axonal depletion, and this induces an inflammatory and immune response that tries to control brain damage. Glial cells probably help to remove cell debris and cellular content and act as a barrier to avoid toxicity in neighboring cells. Also, there is a cellular response that protects against MDMA-induced hyperthermia, which causes cell death.

## Long-Term Response to MDMA Administration (Several Days to Weeks)

After brain damage, some neurotrophins help to recover synaptic function. Also, other synapses are potentiated (Figure 3). Several signaling pathways are required for neuroadaptations and synaptic plasticity, including the MAPK, Wnt, LTP, and LTD pathways, as well as cytoskeleton and matrix proteins.

Overall, only a few studies have assessed the effect of MDMA administration on gene expression. Microarray studies have some limitations, and better technologies are currently available, such as RNA-seq, which allows gene expression to be followed both quantitatively and qualitatively (i.e., by providing data about alternative splicing events). Future studies should consider different time points after the last drug administration; self-administration paradigms in rodents need to be explored further, as they mimic human MDMA use more closely. Also, studies of post mortem human brains should be performed.

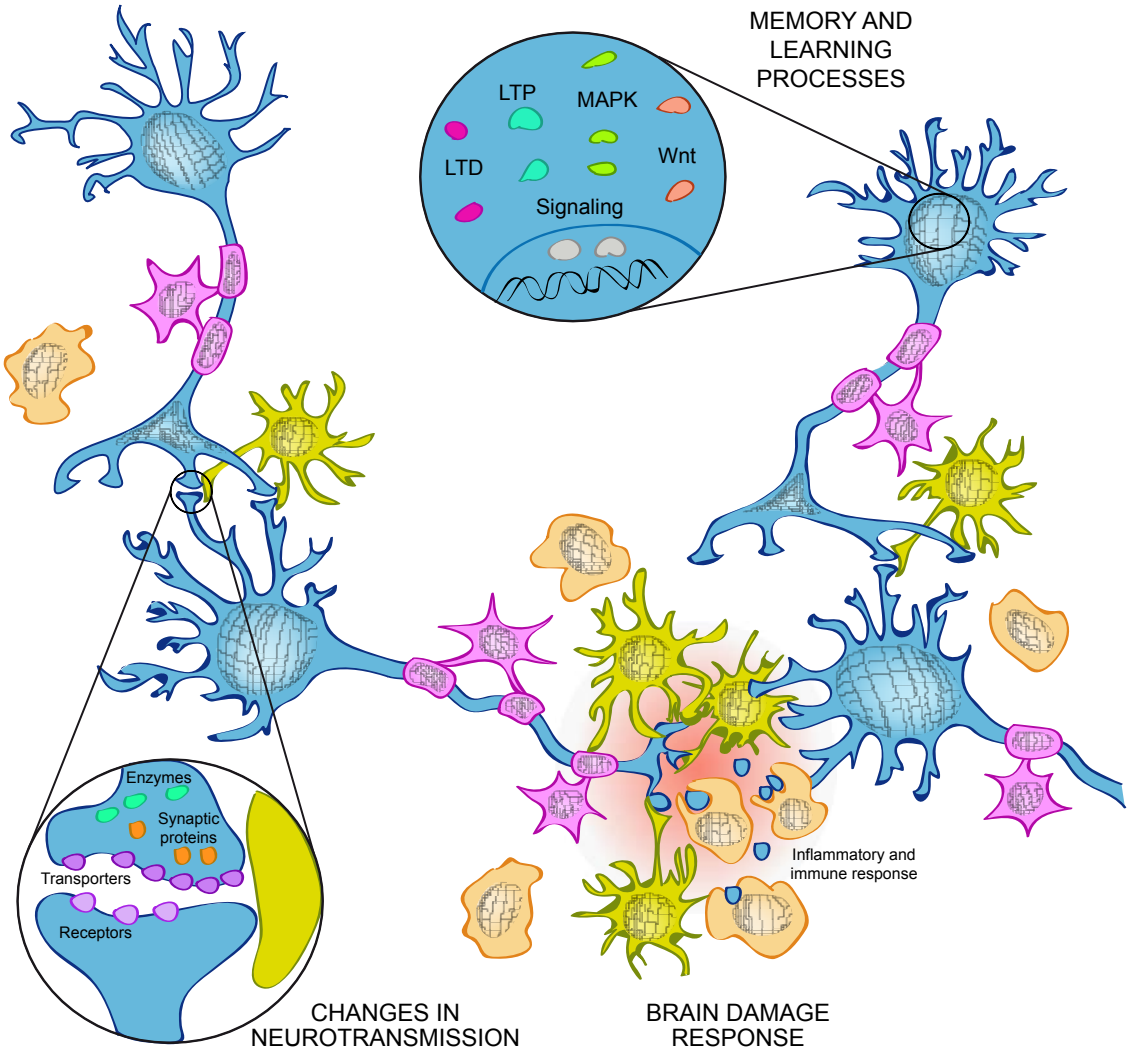


**FIGURE 2** **Late response to MDMA administration.** Changes in gene expression more than 8 hours after drug administration affect different mechanisms. As a compensatory mechanism, several changes in genes related to neurotransmission occur, including those that encode synaptic proteins and neurotransmitter transporters, receptors, and enzymes. Gene expression changes in signaling pathways such as LTP, LTD, MAPK, and Wnt are involved in memory and learning processes in active self-administration. Also, due to toxicity and axonal depletion caused by MDMA, there are expression changes in genes involved in inflammatory and immune responses, in which astrocytes and microglia play an important role.

In summary, MDMA causes numerous alterations in gene expression, the most notable ones indicating induced toxicity in the brain and neuroadaptive processes that are activated to promote recovery from brain damage. These molecular and cellular alterations may have an impact on brain function, as several studies report cognitive impairments in ecstasy users.

## APPLICATION TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

MDMA is an amphetamine derivate. Amphetamines and methamphetamines are, like MDMA, directly neurotoxic; and expression changes caused by these drugs of abuse seem to involve the same mechanisms as those involved in the effects of MDMA on brain (Yuferov, Nielsen, Butelman, & Kreek, 2005). Amphetamines alter the expression of some of the same genes altered after MDMA administration, such as *Ngfi-a*, *Ngfib*, *Arc,* and *Sgk*. Methamphetamine-induced gene expression changes detected a few hours after drug intake involve transcription factors such as *Fos* and *Jun*, whereas genes related to apoptosis, inflammation, and neuroprotection become important only after several hours; similar to the expression changes induced by MDMA. Methamphetamine also activates microglia and increases *Bdnf* expression.

## DEFINITION OF TERMS

**Binge administration** Administration of multiple doses within 24 h or less.

**Expression microarray** Matrix containing DNA fragments used as targets to hybridize a sample that allows the expression of thousands of genes to be tested.

**Gene expression** Process in which the information encoded in a gene is used to synthesize a gene product, which in most cases is, ultimately, a protein. When a gene is upregulated, its expression is

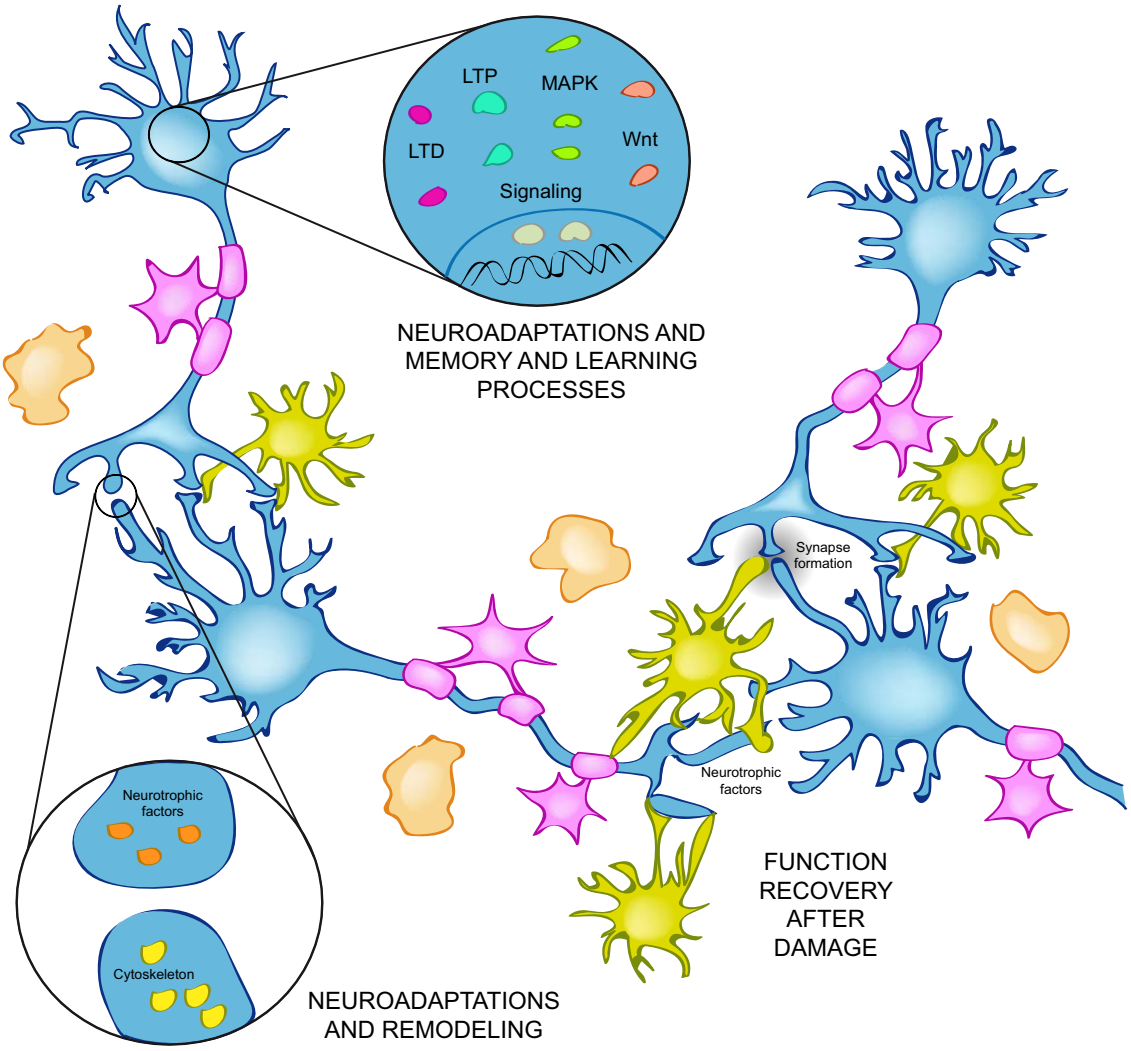


**FIGURE 3** **Long-term response to MDMA administration.** Changes in gene expression days to weeks after drug administration affect mainly synapse formation, neuroadaptations, remodeling, and function recovery after damage. The underlying genes encode neurotrophic factors and proteins related to signaling pathways (LTP, LTD, MAPK, and Wnt) or the cytoskeleton.

increased, whereas when it is downregulated, its expression is decreased.

**Long-term potentiation or depression** Long-lasting potentiation or reduction, respectively, of the efficacy of the synapses between two neurons. They are considered the main mechanisms underlying learning and memory.

**Neurotransmission** Process of communication between neurons in which neurotransmitters are released by a presynaptic neuron and bind receptor molecules in one or more postsynaptic neurons.

**Neurotrophic factor** Growth factors involved in survival, growth, differentiation, and maintenance of neurons.

**Signal transduction or signaling pathway** Biochemical chain of events inside the cell triggered by an extracellular molecule that activates a receptor. The activation results in a response such as alteration of gene expression, cell shape, cell cycle, or metabolism.

**Synaptic plasticity** Changes in synapses resulting from increases or decreases in their activity: for instance, changes in receptor or transporter densities or in the amount of neurotransmitter released.

## KEY FACTS

### Key Facts about Drugs of Abuse

- All drugs of abuse converge on the activation of the reward system of the brain, causing pleasure, reward, and reinforcement.
- Drugs of abuse induce neuronal adaptations in the brain that can become stable over time.

### Key Facts about Neurotransmission

- Neurons connect to each other through the release and capture of chemical compounds called neurotransmitters.
- Drugs of abuse alter the way that neurons communicate.

### Key Facts about Gene Expression

- Drugs of abuse induce changes in gene expression in the brain.
- Expression of all genes in the genome can be easily monitored using microarrays.

## SUMMARY POINTS

- This chapter focuses on the effects of MDMA on gene expression in the brain.
- No human studies are available yet, but animal models using different paradigms and conditions help to elucidate the molecular and cellular events that occur upon MDMA administration.
- Acute MDMA effects on gene expression differ from those caused by chronic exposure, in which neuronal depletion and cytotoxic effects are enhanced.
- During the first few hours after MDMA intake, the changes in gene expression involve signal transmission, which activates transcription factors that trigger an early response.
- Several hours after these initial molecular events, many neurotransmission-related genes show altered expression.
- At this point, genes related to the inflammation and immune responses are upregulated due to the cytotoxic effect of MDMA.
- Changes in the expression of specific genes occur as a response to MDMA-induced hyperthermia.
- Days after MDMA intake, the expression of genes involved in neuroadaptation and synaptic plasticity is altered to promote recovery from brain damage and adaptation of neuronal circuits.
- Therefore, MDMA causes brain damage and toxicity that can lead to cognitive impairments.

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VOLUME 2

# *Neuropathology of* DRUG ADDICTIONS AND SUBSTANCE MISUSE

## Stimulants, Club and Dissociative Drugs, Hallucinogens, Steroids, Inhalants and International Aspects

**Edited by Victor R. Preedy**, BSc, PhD, DSc, FSB, FRSH, FRIPH, FRSPH, FRCPath, FRSC

Research shows that the neuropathological features of one addiction are often applicable to those of others, and understanding these commonalties provides a platform for studying specific addictions in more depth and may ultimately lead researchers toward new modes of understanding, causation, prevention, and treatment. However, marshaling data on the complex relationships between addictions is difficult owing to the myriad of materials and substances. The three volumes that make up **Neuropathology of Drug Addictions and Substance Misuse** address this challenge, offering comprehensive coverage on the adverse consequences of the most common drugs of abuse. Each volume serves to update the reader's knowledge on the broader field of addiction as well as to deepen understanding of specific addictive substances. Volume 2 addresses stimulants, club and dissociative drugs, hallucinogens, inhalants, and international aspects, with each section providing data on the general, molecular/cellular and structural/functional neurological aspects of a given substance, with a focus on the adverse consequences of addictions.

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To his credit, Professor Preedy has published over 600 articles, which include peer-reviewed manuscripts based on original research, abstracts and symposium presentations, reviews and numerous books and volumes.

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