FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

TRABALHO FINAL DO 6º ANO MÉDICO COM VISTA À ATRIBUIÇÃO DO GRAU DE MESTRE NO ÂMBITO DO CICLO DE ESTUDOS DE MESTRADO INTEGRADO EM MEDICINA

PATRÍCIA FILIPA AFONSO PAIS PACHECO MENDES

ABUSE OF METHAMPHETAMINE AND CONSEQUENT ABNORMALITIES IN HUMAN GLIAL CELLS

REVIEW ARTICLE

ÁREA CIENTÍFICA DE FARMACOLOGIA

TRABALHO REALIZADO SOB A ORIENTAÇÃO DE:
FREDERICO GUILHERME SOUSA COSTA PEREIRA

ABRIL/2011

INDEX

RESUMO 3

ABSTRACT 4

INTRODUCTION 6

METHODS 10

REVIEW DESCRIPTION 12

1. Theoretical anatomical and histological fundamentals 13
2. Methamphetamine: mechanism of action 17
3. Gliosis and neurotoxicity 19
4. Gliosis induced by methamphetamine 21

4.1. Pre-clinical studies: animal models of methamphetamine use 21

4.2. Human studies: abstinent methamphetamine addicts 27

4.3. Human studies: methamphetamine addicts - Post-mortem analysis 35

CONCLUSIONS 37

REFERENCES 41

RESUMO

O abuso de metanfetamina tornou-se uma epidemia à escala global, sendo um importante problema de saúde pública. Assim, é imperativo caracterizar melhor o perfil neurotoxicológico da metanfetamina de forma a propor novas estratégias para o tratamento da dependência desta droga. **Objectivos:** Neste artigo de revisão propõe-se uma abordagem da gliose que se pensa estar na base das alterações metabólicas e estruturais no cérebro de consumidores adultos de metanfetamina e ex-consumidores. Estudos pré-clínicos que olharam especificamente para as alterações gliais induzidas pela metanfetamina foram também revistos. **Descrição da revisão:** Os estudos efectuados por imagiologia cerebral (Ressonância Magnética) em cérebros de humanos adultos em abstinência de metanfetamina mostraram volumes estriatais aumentados bem como alterações na matéria branca, no córtex parietal, hipocampo e tálamo compatíveis com gliose. Consistentemente, estudos efectuados com recurso a Tomografia por Emissão de Positrões demonstraram alterações no metabolismo cerebral da glicose em consumidores em abstinência de metanfetamina no córtex parietal e frontal, neocórtex, tálamo e estriado. Este facto é sugestivo de reactividade das células da glia. No entanto, autópsias de consumidores de metanfetamina mostraram resultados inconsistentes com os anteriores, já que demonstraram que a reactividade das células gliais era rara. Estudos pré-clínicos em roedores mostraram consistentemente alterações no cérebro exposto de forma aguda e crónica a metanfetamina, com particular relevância para a reactividade das células gliais devida a toxicidade provocada por esta droga. **Conclusões:** Os resultados obtidos em estudos em modelos pré-clínicos sobre a toxicidade da metanfetamina suscitaram estudos sobre alterações idênticas no cérebro humano. Estudos de imagiologia cerebral mostraram alterações no cérebro de consumidores adultos de metanfetamina, particularmente no estriado, na substância branca, córtex parietal e hipocampo que foram interpretadas maioritariamente como sendo devidas a gliose reactiva. Contudo, um estudo post-mortem não estava em

consonância com os resultados anteriores. Como ainda permanecem muitas questões e os estudos clínicos tinham algumas limitações (amostra pequena, heterogeneidade entre grupos, consumo de outras drogas, entre outros), mais estudos in-vivo e post-mortem em consumidores de metanfetamina afiguram-se necessários. É também fundamental avaliar o impacto das estratégias terapêuticas na dependência à metanfetamina nas células gliais.

ABSTRACT

Methamphetamine abuse has become a global epidemic and is an important public health issue. For that matter, it is imperative to discover ways to prevent methamphetamine-induced toxicity in order to propose new strategies for the treatment of this drug dependence. **Aims:** In this review we propose an approach of gliosis thought to be the basis of structural and metabolic changes in the brain of adult methamphetamine abusers and former abusers. Preclinical studies that looked specifically for glial changes induced by methamphetamine have also been reviewed. **Discussion:** Studies performed by brain imaging (Magnetic Resonance Imaging) in adult former methamphetamine abusers brain showed increased striatal volumes as well as changes in white matter, parietal cortex, hippocampus and thalamus, consistent with gliosis. Consistently, studies using Positron Emission Tomography showed changes in glucose brain metabolism in parietal and frontal cortex, neocortex, thalamus and striatum of abstinent methamphetamine abusers. This fact suggests glial cells reactivity. However, when comparing the previous studies with those of methamphetamine abusers autopsies, the results were inconsistent since they showed rare glial reactivity. Preclinical studies in rodents have consistently shown changes in the brain exposed to acute and chronically to methamphetamine, with particular relevance to the reactivity of glial cells due to toxicity caused by this drug. **Conclusions:** Preclinical studies results of methamphetamine toxicity evoked new studies about similar alterations in the human brain.

Cerebral neuroimaging studies showed brain modifications in methamphetamine abusers, particularly in the striatum, white matter, parietal cortex and hippocampus which were interpreted mostly as being due to reactive gliosis. However, a post mortem study was not in consonance with former results. Because many questions still remain and the trials had a few limitations (small size of sample, sex-heterogeneity in groups, exposure to other drugs, among others), more in vivo and post mortem studies of methamphetamine abusers are indispensable. It is also of particular interest to evaluate the impact of therapeutic strategies on glial cells on methamphetamine dependence.

Keywords: Methamphetamine, Astrocytes, Microglia, Oligodendrocytes, Gliosis, Striatum, Cortex, Hippocampus, Thalamus, Human

INTRODUCTION

INTRODUCTION

Drugs of abuse have been becoming a growing epidemic worldwide which raises economic, social and public health concerns to our society. According to United Nations Office of Drug and Crime (2010), the Amphetamine-like substances stand in a prominent rank on account of its large abuse – between 13.7 and 52.9 million people aged 15 to 64 worldwide used amphetamines at least once in 2008, including methamphetamine (METH). Nowadays, the amphetamine-group substances presently rank as the second most commonly used illicit drug. It is likewise noteworthy to highlight that METH abuse is becoming progressively more widespread in many developed countries because of its somewhat simple production and low price when compared with other drugs such as heroin and cocaine (Marwick, 2000). East Asia, South-East Asia as well as North America and Oceania are the world regions most affected by problems caused by METH over the last decade (United Nations Office of Drug and Crime, 2010).

Methamphetamine is a synthetic psychostimulant drug which fits in a larger group: the Amphetamine-type stimulants (ATS). The Amphetamine-group substances were firstly synthetized in the late nineteenth century, more specifically in 1887, with the primary purpose of being used as a nasal decongestant (UNDCP, 1996). Six years later, the Japanese Nagayoshi Nagai synthetized by the first time the drug that would be named methamphetamine. Its use became generalized during The Second World War by the soldiers and civilian workers (Weisheit and White, 2009).

This drug is available in many forms like powder, tablet, paste or crystalline, varying in purity (United Nations Office of Drug and Crime, 2010), and can be taken by four routes: (1) **Intravenous,** taking less than 15 minutes to peak effect; (2) **Smoking,** taking circa 20 minutes to peak effect; (3) **Oral,** the route with the longest time to peak effect (180 minutes)

and (4) **Intranasal**, an easy available route with similar times of peak effect to those of intravenous route (Reviewed by Cruickshank and Dyer, 2009).

METH acts in the sympathomimetic system and generates its effects through numerous mechanisms following the increase of synaptic levels of biogenic amines: norepinephrine, dopamine and serotonin. The release of dopamine from vesicular storage pools into the cytoplasm, permits this amine to be oxidized to produce neurotoxic quinones and additional reactive oxygen species like hydroxyl free radicals, superoxide and hydrogen peroxide (Graham, 1978) that are probably involved in methamphetamine-induced neurite degeneration (Larsen et al., 2002; Pubill et al., 2003). On the other hand, methamphetamine abuse constitutes a major health risk and has been linked to persistent neurotoxicity like psychiatric disorders (Yoshimoto et al., 2001), such as schizophrenia, psychosis or persistent paranoia, even years after abstinence, as well as memory, cognitive and psychomotor impairment (Kalechstein et al., 2003; Nordahl et al., 2003; Scott et al., 2007). Impulsivity and aggressive behavior were also reported. The acute effects of METH consist of alertness, euphoria, decreased appetite, increased locomotor activity and hyperthermia. At the same time, these individuals present alterations in general metabolic activity as well as in dopaminergic and serotoninergic neurotransmitter systems within basal ganglia, frontal cortex and hippocampus in line with the pre-clinical studies (Seiden et al., 1987; Melega et al., 1997; Harvey et al, 2001; Chang et al., 2007). In addition, recent evidence suggests that structural brain modifications occur in a methamphetamine misuse setting, specifically, glial adaptative and reactive alterations in clinical models.

Glial cells include astrocytes and oligodendrocytes as macroglial cells, and microglia which are specialized macrophages from the Central Nervous System. It is already well known that glial cells provide structural, metabolic and trophic support to neurons (homeostatic functions) and also produce myelin in the Central Nervous System. For

example, the loss or dysfunction of astrocyte connexins and gap junctions leads to dysmelination, exerting a negative impact on the development of white matter (Lutz et al., 2009). Moreover, glia is suspected of being involved in a wide range of Central Nervous System pathologies, in which drug addiction is included. Although reactive gliosis has been observed both *in vitro* and in animal models of methamphetamine neurotoxicity, there is scarce information on the status of glial activation in human methamphetamine abusers.

METHODS

METHODS

The review of scientific literature was performed using Pubmed and Science Direct databases offered by both "Biblioteca Central dos Serviços de Documentação dos Hospitais da Universidade de Coimbra" and "Biblioteca do Polo de Ciências Médicas de Coimbra". The following keywords were used: Methamphetamine, glia, gliosis, human. All available years were surveyed and only articles written in English were included.

In this review we also cite the following books: "Basic and Clinical Pharmacology", "Clinical neuroanatomy", "The pharmacological basis of therapeutics" and "Methamphetamine: its history, pharmacology and treatment". Also the publications by the following organizations were considered: *United Nations Office on Drugs and Crime* (UNODC) and *United Nations International Drug Control Program* (UNDCP).

In the present review, preclinical and clinical studies on neurotoxicity of methamphetamine abuse, focusing mainly on glial alterations within white matter, striatum, cortex and hippocampus were used as inclusion criteria. Using methamphetamine and (glia or gliosis) as keywords, the search revealed 115 papers. Papers which were not written in English, which were about methamphetamine abusers with Human Immunodeficiency Virus (HIV) or other viruses and papers where gliosis was either an incidental finding or was not analyzed were excluded.

Overall, our objective was to review the new reports on abnormalities found in human glial cells consequent to methamphetamine abuse. The inclusion of a description of recent in vitro and in vivo animal methamphetamine studies serves the purpose of better elucidating this matter. Because there have not been numerous post-mortem studies published in methamphetamine abusers that could directly assess glia, we will focus on imaging studies and demonstrate that these findings can be inferred as glial cell alterations.

REVIEW DESCRIPTION

REVIEW DESCRIPTION

1. Theoretical anatomical and histological fundamentals

As addressed in the introduction, METH neurotoxicity afflicts mostly the basal ganglia (Figure 1). These are a collection of masses of gray matter located within each cerebral hemisphere. We will direct our attention to the corpus striatum which is the largest part of the basal ganglia and is enriched with dopaminergic terminals.

It is called striatum because of its striated appearance caused by the strands of gray matter passing through the internal capsule, a band of nerve fibers that divide it. This structure is constituted by three nuclei buried deep in the white matter of the cerebral hemisphere: (1) the **caudate nucleus** is a large C-shaped mass of gray matter that is located closely to the lateral ventricle and lies lateral to the thalamus. Its lateral surface is related to the internal capsule, which separates this nucleus from the lentiform nucleus. The lentiform nucleus – related laterally to the external capsule and medially to the internal capsule - is divided in two by a vertical plate of white matter into (2) the **putamen**, the dark lateral portion and (3) the **globus pallidus**, an inner lighter portion. (Clinical Neuroanatomy, 2010).

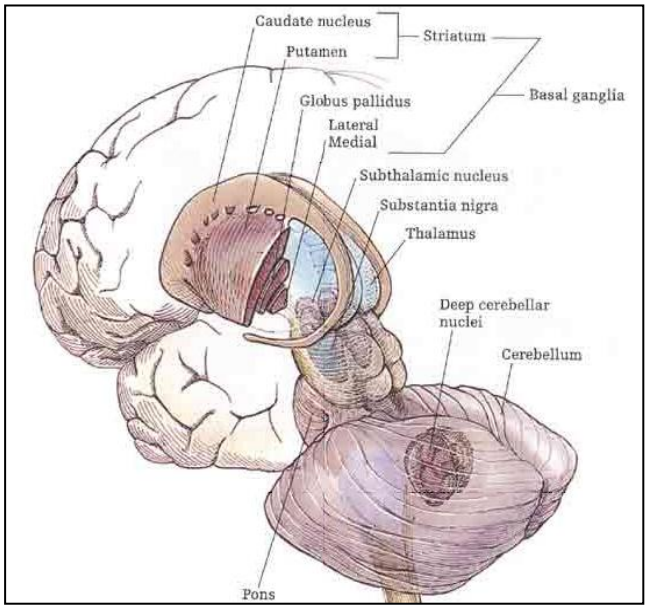

Figure 1- Neuroanatomy of the basal ganglia (Adapted from Rhawn Joseph, 2000)

Both grey and white matter, the two components of the Central Nervous System (CNS), and more specifically the neurons of the CNS, are supported by varied nonexcitable

cells – the neuroglia (Figure 2). These cells exceed in number by five to ten times the neurons. There are four different types of glial cells, which main characteristics we will briefly address to next, supporting on the information given by the book Clinical Neuroanatomy (2010), so that some interpretations of the studies' results analyzed next are more easily understood:

(1) The astrocytes are small cells with branched processes found both in gray and white matter. They are divided in two types according to their location: **fibrous astrocytes,** found mainly in the white matter, with long but not much branched processes; **protoplasmatic astrocytes**, found mostly in the gray matter, which shorter but branched processes which pass between the nerve cell bodies. The astrocytic processes end in expansions on brain blood vessels, where they are named perivascular feet, and are also found around the initial segment of most axons and at the nodes of Ranvier (zone of axons without myelin). Astrocytes main functions are molding a supporting framework for the nerve cells and fibers, serving as electric insulators by their location in between neuron fibers, releasing neurotransmitters and neurotrophic factors, contribute to the metabolism of neurotransmitters and regulate extracellular pH and K+ levels by removing excess K^+ ions from the extracellular space (Vesce et al., 2001; Bohn, 2004; Fellin and Carmignoto, 2004), storing glycogen within their cytoplasm, serving also as phagocytes by removing degenerating synaptic axon terminals and supporting blood vessels. They are also essential for the formation and function of developing synapses (Ullian et al., 2001; Christopherson et al., 2005; Barres, 2008). Another important role of these glial cells is that they can proliferate and occupy the gap left by neurons, a process called replacement gliosis.

(2) Microglia, the smallest of the neuroglial cells. These cells have a different embryonic origin than the others since they are derived from macrophages outside the nervous

system. One can verify its increase in number in the presence of damaged nervous tissue and of diseases such as Alzheimer disease, AIDS, multiple sclerosis and Parkinson disease. In the normal brain these cells appear to be inactive (resting microglia) though when there is brain injury they migrate to the impaired location where they can become antigen presenting cells or phagocytes.

(3) Ependyma, the ciliar cells which line the brain cavities (ventricles) and the central canal of the spinal cord and that can be divided into ependymocytes – cells that circulate and absorb cerebral spinal fluid (CSF), tanycytes – cells that transport substances from the CSF to the hypophyseal-portal system - and choroidal epithelial cells that produce and secrete the CSF.

(4) The oligodendrocytes, other small cells which are usually found in rows along myelinated nerve fibers and surround nerve cell bodies. These cells are responsible for the formation of the central nervous system axons' myelin sheath. They are also thought to influence the biochemical environment of neurons because some of these cells surround nerve cell bodies.

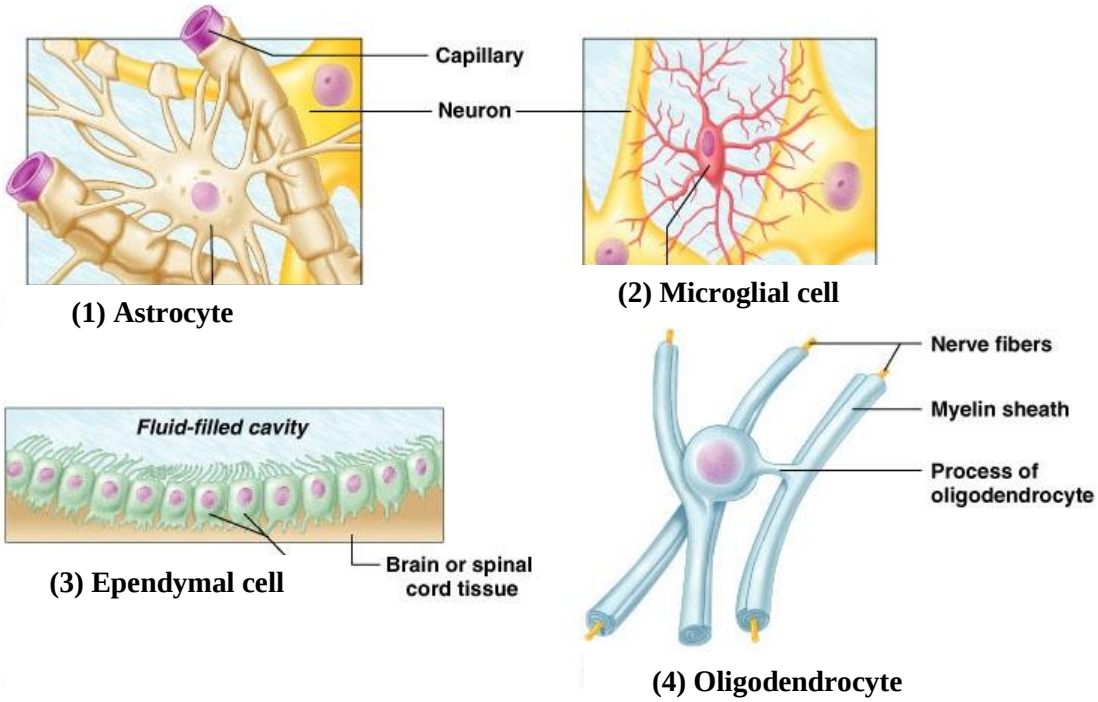

Figure 2- The four different types of glial cells. Adapted from Pearson Education Inc., publishing as Benjamin Cummings

2. Methamphetamine: mechanism of action

To understand the chronic modifications induced by METH, we must identify its initial molecular and cellular targets.

Methamphetamine (*N*-methylamphetamine), a synthetic drug related with amphetamine, is a potent sympathomimetic that induces extensive release of dopamine in the brain evoking a state of euphoria and amplifying confidence and alertness in its users (Chang et al., 2004).

It exercises its effects by reversing the action of biogenic amine transporters at the plasma membrane of synaptic terminal of dopaminergic neurons (Figure 3).

In more detail, due to structural similarity, METH substitutes for monoamines and is taken up into the cell by dopamine transporters (DAT) competitively inhibiting dopamine transport. Once in the cell, METH bounds to vesicular monoamine transporter-2 (VMAT2) blocking the filling of synaptic vesicles leading to an increase of cytoplasmatic dopamine. Consequently, there is a reversal of DAT direction, with a high increase of non-vesicular release (abnormal pathway) of dopamine and augmentation of this biogenic amine's extracellular concentration in the synapse leading to oxidative damage of terminal components. The primary function of VMAT is to gather neurotransmitters in vesicles, and therefore determines the receptor sensitivity, quantal size and synaptic plasticity, but it can also translocate toxicants and dopamine away from cytosolic sites of action before it can be oxidized playing an important role in neuroprotection. When VMAT2 expression or function is diminished, dopaminergic neurons are more susceptible to damage (Takahashi et al., 1997; Gainetdinov et al., 1998; Fumagalli et al., 1999; Caudle et al., 2007; Vergo et al., 2007; Guillot and Miller, 2009).

Not only does this drug exert its effects on VMAT2 and DAT but also in serotonin transporter (SERT) and norepinephrine transporter (NET). (Basic and Clinical Pharmacology, 2009; The pharmacological basis of therapeutics, 2010)

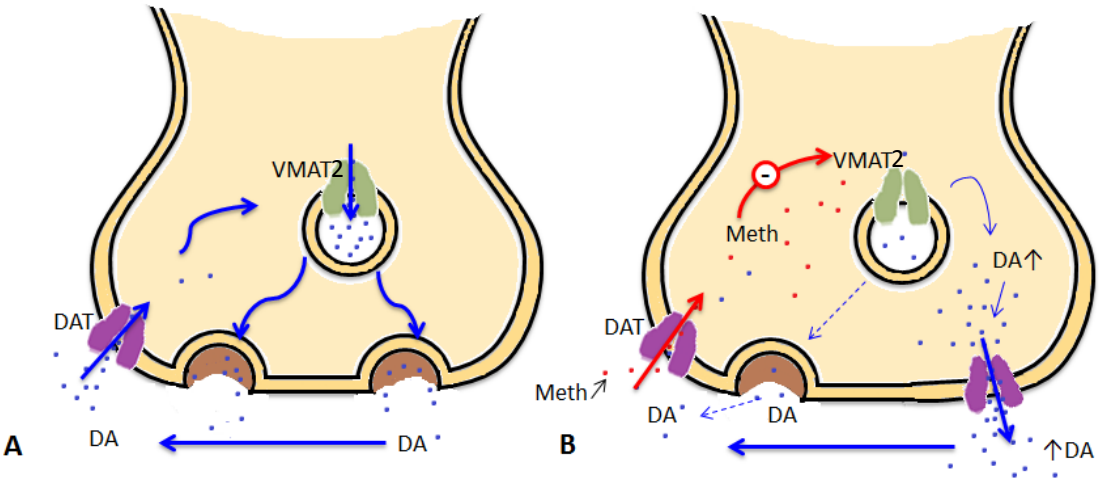

Figure 3- Mechanism of action of Methamphetamine (Meth) on synaptic terminal of Dopamine (DA). A- Usual pathway of DA liberation into the extracellular space by synaptic vesicles. **B-** Mechanism of action of Meth: by competing with DA in the dopamine transporter (DAT), Meth enters in the neuron inhibiting vesicular monoamine transporter-2 (VMAT2) and impeding the filling of

3. Gliosis and neurotoxicity

When the brain is submitted to an insult, glia is considered to be essential in determining the extent of neuronal damage. Gliosis is the name given to the reaction of neuroglial cells to injury and is mainly characterized by the proliferation and hypertrophy of the astrocytes and microglia although it can also occur with the oligodendrocytes (Figure 4).

In more detail, the proliferation and hypertrophy of astrocytes, named astrogliosis or astrocytosis, are accompanied by functional and morphological alterations, including augmentation of glial fibrillary acidic protein (GFAP) (Ullian et al., 2004; Abrous et al., 2005). On the one hand reactive astrocytes are responsible for rescuing neurons in the injured area and also for extracting excessive glutamate at the synapse but on the other hand they may also aggravate the injury (Bemarroch, 2005). This gliosis is much greater if there is residual damaged neuronal tissue than when someone is submitted to a clear surgical excision. Histologically, when the brain is exposed to injury the cytoplasm of these cells, which contains large numbers of fibrils and glycogen granules, enlarges and the extense network of astrocytic processes produces the so-called gliotic scar.

As to oligodendrocytes, the response to injury induces vacuolation and expansion of their cytoplasm. If the injury is severe, the consequence will be demyelination.

Not only exists activation of astrocytes and oligodendrocytes but also of microglia. Microglial cells respond retracting their processes and migrating to the areas of injury. At the sites of lesion, these cells proliferate and become phagocytes, also releasing inflammatory molecules. (Clinical Neuroanatomy, 2010)

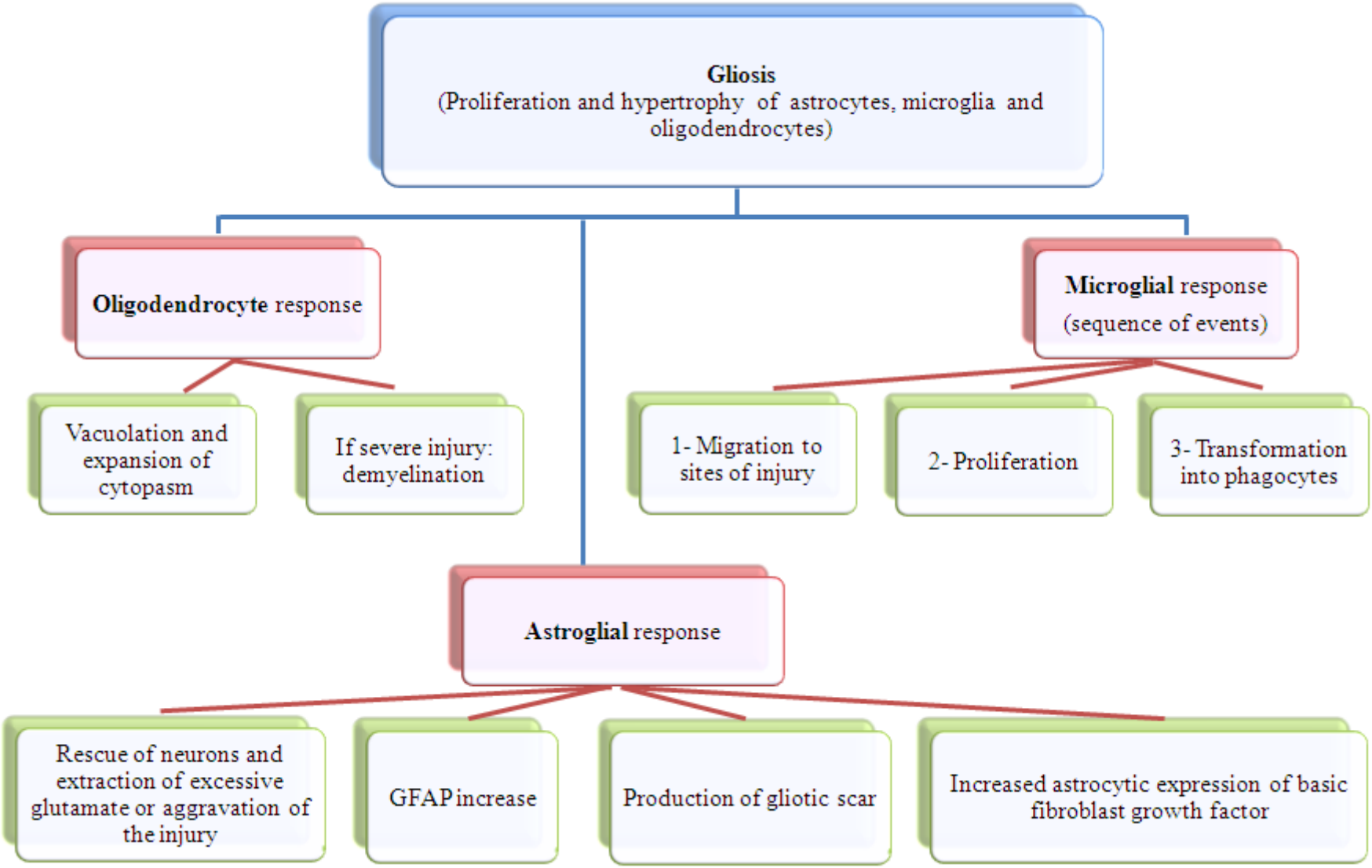

Figure 4- Gliosis. Summary of glial cells reactive responses to injury. GFAP- glial fibrillary acidic protein.

4. Gliosis induced by methamphetamine

4.1.Pre-clinical studies: animal models of methamphetamine use

Before proceeding to studies on the impact of METH on human glial cells we will review in vitro as well as in vivo pre-clinical data that were instrumental to human studies.

It was recently shown that high METH concentrations caused disruption of dopamine homeostasis and consequent oxidative stress, activation of apoptotic cascades and inflammatory mediators and finally activation of microglia and astrocytes (Thomas et al., 2004; Quinton and Yamamoto, 2006). Although there is evidence that both astroglia and microglia are activated after the administration of methamphetamine, the exact mechanism of glial perturbation remains uncertain (Hess et al., 1990; O'Callaghan and Miller, 1994; Sheng et al., 1994; Escubedo et al., 1998; LaVoie et al., 2004). There are two strains of theories: gliosis is consequent to neuronal damage and gliosis is somewhat responsible for the neuronal damage.

Regarding astrocytic cells, a study conducted by Narita et al., 2006, showed that the activation of astrocytes in the mouse Nucleus Accumbens and cingulate cortex provides a powerful signal for dopamine-associated behaviors, habits and addiction by METH. These authors further suggested that astrocytes-related soluble factors could amplify the development of the rewarding effect evoked by METH. Finally they reported a major activation of astrocytes in the mouse purified cortical astrocytes challenged with METH. Suzuki et al. (2007) showed that treatment of mouse limbic neuron/ glia co-cultures with either METH or methylphenidate for 3 days caused a robust activation of astrocytes (astrogliosis). While investigating the maintenance of the astrocyte activation, their results showed that after 2 days with METH the induced astrocytic activation remained whether with methylphenidate the induced astrocytic activation was reversed. This long-lasting astrocytic activation induced by METH may contribute to the development of synaptic plasticity

underlying the sensitization induced by this stimulant. Flores et al. (2000) showed that bFGF (basic fibroblast growth factor) expressed by astrocytes is necessary for the development of sensitization to amphetamine.

Although the mechanism of astrocytic activation remains uncertain, the activation of cortical astrocytes induced by METH exposure appears to have its origins in Glutamate (GLU) release and protein kinase C activation and is inhibited by GLU receptor antagonists. (Reviewed by Yamamoto et al., 2010)

Microglia, CNS macrophageal cells, activation by neuronal injury has also been proved to occur after administration of METH and other neurotoxic amphetamines (Guilarte et al., 2003).

Thomas et al. (2004a) focused on investigating the microglial activation time-course in the striatum as part of the methamphetamine neurotoxic cascade by using isolectin B_4 (IB_4, a specific marker for staining microglia in brain). These authors showed a striatal microglial activation 24 to 48 hours after methamphetamine injection (4 x 5 mg/Kg i.p., 2 h apart). However, this response was transitory and returned to levels similar to control within 7 days suggesting only an acute microgliosis. This microglial response is somewhat coincident with the initiation and amplification of the process of damage to dopamine nerve terminals suggesting that these cells are contributing to dopamine nerve terminal damage. Although microglial cells activation towards METH use arose throughout the striatum, it was highest in its lateral aspects, the same pattern seen for dopamine depletion. Microgliosis was greater with higher doses of METH and with lesser striatal dopamine levels. Thomas et al. (2004b) reported that METH induced simultaneously GFAP over expression as well as microgliosis. These authors further suggested that microglial activation evoked by METH could be a prompter of astrogliosis. LaVoie et al. (2004) also studied the temporal correlation of reactive microgliosis with neuropathological modifications of striatal dopaminergic axons subsequent

to exposure to METH. The rats exposed to METH (total of 4 injections – 15 mg/kg, s.c. - each separated by 2 hours) presented the following results: microglia activation was absent in the striatum or any other brain area after twelve hours exposure to METH. However, 1 day after METH administration, activated microglia was found throughout the striatum, with more intensity in the ventrolateral region. 2 days after it generalized through the whole striatum, maintaining the regional differences though. This activation faded progressively after those 2 days but never returned to control levels. There was also focal microglial reaction in the parietal and piriform cortices and in the ventromedial column of the periaqueductal gray, with no attenuation after 6 days unlike the striatal microgliosis. The authors also suggested that the reactive microgliosis preceded the axonal pathology observed in the striatum, and the effect of hypothermia on METH-induced reactive microgliosis with results that support previous studies which hypothesized the need of hyperthermia to METH-induced tocixity. This study was in agreement with those two discussed previously suggesting that microgliosis may be a contributor to METH-induced neuropathogenesis rather than being a secondary consequence of neuropathology. Yamamoto et al. (2010) also emphasized that activated microglia can initiate, intensify, and perpetuate METH neurotoxicity.

Besides astrocytes playing a role on METH-induced synaptic plasticity, these glial cells support the brain vessels and it should not be excluded the role of these cells in the disruption of the brain blood barrier (BBB) possibly due to astrocytes reactive impairment. Bowyer et al. (2006, 2008) showed that a single injection of 40 mg/kg of METH induced minor changes in BBB at time-points subsequent to terminal damage. Moreover the time-course of microglia activation suggests that these cells are responding to dopaminergic terminal dysfunction. These findings are in contradiction with Thomas et al. (2004a, 2004b) and LaVoie et al. (2004). The CPu areas with neurodegeneration also evidenced myelin damage, expressed by changes in the morphology of the myelinated fibers. These

modifications of myelin may be due to neuron damage or death or even impairment of the oligodendrocytes. Guillot et al. (2008) also supported the hypothesis that injury to the dopaminergic neurons comes before gliosis. In fact, these authors showed an elevation of the GFAP expression as well as microgliosis in the striatum after METH administration in mutant mice with decreased VMAT2 levels. Since VMAT2 is an intraneuronal transporter, these results suggest that METH must access the terminals and cause neuronal damage before occurring gliosis. Another study supports this theory: Fumagalli et al. (1998) showed that genetic deletion of DAT, which denies terminal access of METH and MPTP, prevented astrogliosis. Further studies should be taken so that the mechanism of damage of myelin in this brain region can be understood. In addition it was showed that doses of METH that cause BBB instability also induce neuronal damage, myelin degeneration, and astrogliosis in the parietal and occipital cortices (Bowyer et al., 2008; Krasnova and Cadet, 2009a).

Pubill et al. (2003), Coutinho et al. (2008), and Kuhn et al. (2006) also showed METH-induced microglial activation in rat and mouse striatum, rat cortex (including frontal and somatosensory cortices) and hippocampus. However, these alterations were not found in areas where dopamine levels were unaltered by METH, such as substantia nigra. It was further suggested that METH is a substantial inducer of microglial response in the areas of the brain that show neuronal degeneration. Brain modifications were also shown in non-human primates. (Krasnova and Cadet, 2009b).

Most studies previously analyzed about METH -induced toxicity share the protocol of single-day-single-dose (20-100 mg/Kg) or single-day-multiple-dose (5-10 mg/Kg, given four times at 2 hours intervals). The problem with this type of procedure is that it only mimics the human overdose scenario, as it is an acute administration of METH, maintaining an enormous gap on information of the process of chronic use of this drug.

For that matter Simões et al., 2007 performed a study where they demonstrated that both acute (30 mg/kg, s.c) and sub-chronic METH (10, 15, 15, 20, 20, 25, 30 mg/kg/day) administration induced changes on hippocampal N-methyl-D-aspartate (NMDA) and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) ionotropic Glutamate (Glu) receptor subunit levels. These glutamatergic changes could underlie METH -induced neurotoxicity. Although neither models exhibited signs of neurodegeneration as shown by the absence of Fluoro-Jade C staining (reveals degenerating neurons, dendrites, axons and terminals), the acute METH model evoked astrogliosis in the hippocampus 24h following METH injection.

Using a rat model of METH self-administration, which can mimic some aspects of human drug-taking behaviors, Krasnova et al. (2010) showed that rats allowed to intravenously self-administer METH (0.1 mg/kg/injection, i.v.) 15 hours per day for 8 days presented significant dose-dependent increases in GFAP in both striatum and cortex, but with a greater magnitude in the striatum. This data is consistent with the usual modifications present in astrogliosis. On the other hand, Mandyam et al., 2007 showed that three methamphetamine (dose of 0.05 mg/kg per infusion) self-exposure rat models (intermittent 1 h short access– I-ShA; daily 1 h short access – ShA; daily 6 h long access – LgA) exhibited different glial behaviour. In fact, intermittent short access (I-ShA) to methamphetamine increased medial prefrontal cortex (known for its gliogenic precursors) proliferation, survival (oligodendrocytes) and cell death. This glial cell proliferation supports the reactivity glial cells display towards METH intake not only in acute administration but also in a sub-acute model. Contrasting to intermittent access, daily short and extended access to METH (ShA; LgA) decreased medial prefrontal cortex proliferation and survival (neurogenesis and gliogenesis) and increased cell death which the authors interpret as probable mechanisms underlying the functional neurotoxic effects caused by METH amphetamine dependence. The

precedent results evidence that even limited exposure to METH leads to early modifications in medial prefrontal cortex cell proliferation, survival and cell death.

Glial cells are also being investigated as potential targets for neuroprotective strategies under METH neurotoxic settings.

For example, the endogenous nociceptin/orphanin FQ (N/OFQ) peptide modulates astrogliosis and microgliosis (Buzas et al.., 1998, 1999, 2002; Takayama and Ueda, 2005; Fu et al.., 2007), possibly via NOP receptors expressed on those glial cells (Zhao et al.., 2002). Sakoori and Murphy (2010) showed that METH induced toxicity to striatal dopaminergic terminals, as reflected by the decrease in tyrosine hydroxylase (TH) - a marker of dopaminergic terminals. However this event was less marked in the NOP receptor knock-out group. Additionally, the NOP receptor knock-out group showed an exaggerated astrocytic reactive response (astrogliosis), as reflected by GFAP immunoreactivity, when compared with the control group. These results indicate that endogenous nociceptin/orphanin FQ exacerbates the neurotoxic effects of methamphetamine on striatal dopamine neurons, and suggests this is due in part to an astrocyte-mediated event. Additionally, the endogenously occurring opioid peptide N/OFQ may be involved in METH addiction (Zhao et al., 2003).

Finally, there are evidences that there is significant release of inflammation mediators such as cytokines, interleukins, prostaglandins, neurotrophic factors and reactive oxygen species. Glial-derived neurotrophic factor (GDNF), which appears to play a role in neuroprotective mechanisms, is present in neurons, astrocytes and microglial cells, although it seems to be principally produced by astrocytes. This neurotrophic factor exerts its effects by influencing the survival and differentiation of dopaminergic cells and protecting those cells against METH -induced neurotoxicity as shown in wild-type mice and in heterozygous mice with a partial deletion of the GDNF gene (Miguel-Hidalgo, 2009). It was further investigated whether the pre-treatment with anti-inflammatory agents would attenuate the

methamphetamine-associated microglial activation and depletion of dopamine and DAT in the striatum. The anti-inflammatory agents indomethacin and ketoprofen as well as dextromethorphan, n-acetyl-l-cysteine (an antioxidant agent) and a NMDA receptor antagonists dizocilpine (MK-801), attenuated microglial response to methamphetamine-induced toxicity (Reviewed by Chang et al., 2007 and Krasnova and Cadet, 2009a). Tamoxifen can also cause anti-inflammatory reactions in microglial cells and protect glial cells against glutamate toxicity (Krasnova and Cadet, 2009a).

Therefore, further investigations in humans should be taken so that the efficacy of these glutamatergic antagonists, anti-inflammatory agents and antioxidants could be evaluated and compared the results with those from the pre-clinical studies.

4.2.Human studies: abstinent methamphetamine addicts

Several human studies have been conducted, mostly in former chronic METH abusers, with the aim of investigating structural and metabolic brain changes reflecting glial alterations using either Magnetic Resonance Imaging (MRI) or Positron Emission Tomography (PET) imagiology.

As seen in pre-clinical trials, METH acts preferably by modifying dopamine pathways in humans. In fact it was shown that there is a decline in the density of dopamine transporters (DAT) in the striatum and, to a lesser magnitude, in the frontal cortex of abstinent users after chronic METH abuse (Volkow et al., 2001: Sekine et al., 2003; McCann et al., 2008) by using PET. Regarding the serotonin transporters (SERT) it is verified that its number is decreased in several brain regions in abstinent METH addicts (Sekine et al., 2006).

Numerous magnetic resonance and PET imaging studies discovered abnormal structural modifications in cortical and subcortical brain regions in abstinent METH users.

Thompson et al. (2004) conducted a study with 22 human subjects and 21 controls in which they evaluated structural abnormalities with MRI imaging in individuals who had consumed METH and were abstinent for less than 4 months. When mapping cortical gray matter (cingulate gyrus, subgeminal cortex and paralimbic belts- ring of cortex encircling the corpus callosum), the authors reported that the most significant impairment arose in cingulate regions with volumes 11.3% below control average. Also in the METH abusers, medial frontal cortices exhibited only a tendency for loss when compared to the limbic cortices that they surrounded and the right medial wall displayed severe deficits. These MRI-based maps were highly consistent with formerly stated maps of group differences in glucose metabolism in a partially overlapping sample. In what concerns to hippocampal volumes, they concluded that both left and right hippocampi were atrophied in METH abusers. These volume deficits were correlated with minor memory performance. These authors also evaluated whole brain gray and white matter, demonstrating a significant hypertrophy of the white-matter in the METH abuser group. In fact, the white-matter was 7.0% greater in this group, compared to the control group verified a 6.6% excess in the left hemisphere and a 7.5% excess in the right hemisphere. Moreover, the MRI scans showed a hypertrophy of temporal and occipital white-matter, adjacent to some regions in which hippocampal and cortical gray-matter changes were detected, suggesting a reactive glial event with neuronal damage. There were also alterations usually found in neurodegenerative and psychotic disorders such as the right cingulate gray-matter loss was accompanied of substantial frontal horn enlargement in right lateral ventricles. The early enlargement of the brain structures indicated above suggests inflammation or reactive gliosis induced by METH.

Chang et al. (2002) studied 20 abstinent (for at least 2 weeks) METH abusers and 20 controls using perfusion MRI imaging These authors showed decreased regional cerebral blood flow (relative rCBF) bilaterally in putamen/insular cortices and the right lateral parietal brain region, contrasting with increased relative rCBF bilaterally in the left temporoparietal white matter, the left occipital brain region and the right posterior parietal region. Gender differences were observed in the right occipital cortex and a mid-saggital brain region as female METH users showed increased relative rCBF whereas the male METH users had decreased relative rCBF. The authors interpreted the increases in rCBF in parietal regions as glial response since previous studies of this brain region showed increased metabolism and glial cells have a significantly higher metabolic rate than neurons. They hypothesized that hypoperfused regions had more neuronal injury/ loss and less glial activity while regions with hyperperfusion are richer in glial activity in response to brain injury due to METH.

Because induction of aromatase, the enzyme that produces estrogen de novo in astrocytes, seems to be part of the glial repair response to brain injury (Garcia-Segura et al., 1999), the authors thought that it could be the explanation for the gender-related differences: female METH users might have a stronger glial response (more regional hyperperfusion). These structural changes were accompanied by slower reaction times on computerized measures of cognitive functions.

Chang et al. (2005) evaluated 50 METH abusers and 50 non-consumers by both PET and MRI imaging techniques. The 50 abstinent METH-dependent subjects had used METH 6.3 ± 1.3 days/week at 1.6 ± 1.6 g/day for 110 ± 68 months, and had an average lifetime METH use of 4,519 ± 5,730 g. They were abstinent for 4.0 ± 6.2 months. The 43 METH subjects who also completed the neuropsychological tests had similar METH usage as a group: METH 6.2 ± 1.4 days/week at 1.5 ± 1.7 g/day for 118 ± 68 months, and a lifetime METH use of mean 4,870 ± 6,101 g; average duration of METH abstinence was 4.0 ± 6.5

months). The volumetric analysis showed that the METH group had a larger averaged globus pallidus and putamen volumes with no significant differences between genders. In addition, feminine individuals with METH abuse history presented a larger posterior middle portion of corpus callosum when comparing to non-drug using women. This striatal structures enlargement occurred in METH users conceivably as a compensatory mechanism to repeated METH -induced striatal injury during early phases of drug dependence. However, a continuous and cumulative METH usage implies a decrease in striatal volumes and a poorer cognitive performance, as seen in some of the studied METH -abusers. As to gender differences, the authors propose a stronger glial response (hence more inflammatory changes) by the female METH users possibly due to the neuroprotective effect of estrogen in specific brain regions. When they correlated brain volumes with cognitive performance and drug usage, those with a smaller putamen volume in the METH group had poorer performances on verbal fluency and Grooved Pegboard (a manipulative dexterity test consisting of 25 holes with randomly positioned slots. Pegs with a key on one side must be rotated to match the hole before being inserted). In conclusion, cumulative METH abuse was inversely correlated with putamen and globus pallidus volumes. Consistently with what other researches suggested, possible mechanisms for the striatal enlargement include glial activation.

Proton magnetic resonance spectroscopy (1H-MRS) was performed in abstinent methamphetamine abusers (the median period of time since last methamphetamine use was 4.25 months) and 24 healthy subjects without a history of drug abuse to study metabolic profile from frontal cortex, frontal white matter, and basal ganglia. The methamphetamine users showed significantly reduced total creatine (Cr, cellular energy metabolism) in the basal ganglia (-8%), and increased choline-containing compounds ([CHO], +13%) and myo-inositol ([MI], +11%) in the frontal grey matter. CHO is a marker for membrane turnover. In addition, the concentrations of CHO and Cr are three times more elevated in glia than in neurons and

MI is only found in glial cells making these good markers of glial content (Brand A et al., 1993). These results show that these METH abusers brains showed evidences of reactive gliosis. (Ernst et al., 2000)

Also taking advantage of this neuroimaging technique, Sung et al. (2007) compared 30 abstinent METH abusers with 20 healthy subjects. Moreover, the authors divided METH users in two sets depending on abstinence duration (greater or less than 6 months) or the total cumulative MA dose (greater or less than 100 g lifetime). They showed significantly increased MI concentration in left frontal white matter of METH abusers when compared to the healthy subjects, increase that may indicate a glial cell proliferation in response to the neuronal toxicity produced by METH since MI is a specific marker for activated glia. However, this MI concentration did not correlate with abstinence duration, the cumulative METH dose or the duration of METH use since there were no differences between the different METH user subgroups for MI.

In another study, Sekine et al. (2008) investigated the microglial activation in the brain of METH abusers. A group of 12 individuals who consumed METH for 6 years and were abstinent for 2 was compared to a control group also with 12 individuals. Both groups underwent positron emission tomography (PET) scans using radiotracer for activated microglia ([^{11}C](R)-(1-[2-chlorophenyl]-N-methyl-N-[1-methylpropyl]-3-isoquinoline carboxamide - [^{11}C](R)-PK1195). When comparing the [^{11}C](R)-PK1195 binding between METH abusers and control subjects, the METH group presented a higher binding potential than the control group in the midbrain, striatum, thalamus, orbitofrontal cortex and insular cortex. (Figure 5)

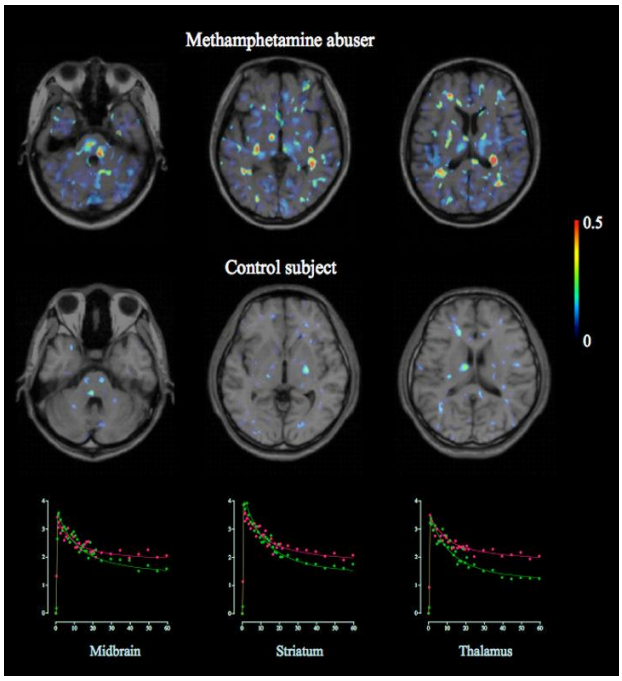

Figure 5- MRI-PET fusion parametric images of [11C](*R*)-PK11195 binding potential in a control subject and a methamphetamine abuser.
There is a marked increase in [11C](*R*)-PK11195 binding in widespread areas of the brain of the Meth abuser. The color bar indicates a level of binding potential. Scatter grams show the time activity curves of [11C](*R*)-PK11195 for each region in the methamphetamine abuser (in red) and healthy control (in green). Adapted from Sekine et al. (2008)

There was no significant correlation between the duration interval of METH usage and the [^{11}C](R)-PK1195 binding potential in any of the brain regions. However, the length of METH abstinence revealed to have a negative correlation with [^{11}C](R)-PK1195 binding points in three brain areas: midbrain, thalamus and striatum. This is consistent with the levels of microglial activation inversely correlating with duration of METH abstinence. Since there was no history of other illicit drug consumption or psychiatric illness, the authors concluded that the reactive microgliosis observed could mirror the effect of METH on the human brain. They further added that METH -induced activated microglia might play a key role in mediating the toxic effects of this drug on monoaminergic terminals in METH users. The authors also emphasized that reactive microgliosis can remain for at least 2 years of abstinence suggesting that METH -induced neurodegeneration might be an ongoing process. These results show the importance of early therapeutic intervention with anti-microglial action, including anti-inflammatory approach.

By quantifying regional cerebral glucose metabolism in brain using PET ([18F]fluorodeoxyglucose), Berman et al. (2008) showed large increases of glucose metabolism (more than 20%) in parietal regions, lesser increases in the neocortex and no

changes in sub-cortical regions during the initial month of abstinence of former methamphetamine-dependent subjects. However, during the initial week of abstinence from METH, these individuals showed no difference in parietal glucose metabolism compared to healthy individuals. On the contrary there was a higher striatal activity during this time-frame.

Even though METH abuse induces most of its toxic effects in the dopamine-rich basal ganglia, it is also known that METH consumption increases proliferation of cortical astrocytes and microglia too (LaVoie et al., 2004). Hence, Berman et al. (2008) suggested that the increased cerebral glucose metabolic rate was caused by an increase in number of astrocytes and microglia. This is consistent with Volkow's (2001) suggestion that higher parietal activity in METH abusers could be a reflection of reactive gliosis after excitotoxic damage to parietal neurons.

Wang et al. (2004) also evaluated brain glucose metabolism by using PET ([18F]fluorodeoxyglucose) in five methamphetamine abusers evaluated after both short (less than 6 months) and prolonged (12–17 months) abstinence interval, eight methamphetamine abusers evaluated only after prolonged abstinence and 11 healthy comparison subjects without any history of drug abuse. They showed significantly greater metabolism in the thalamus but not in the striatum following prolonged abstinence when compared with the metabolism assessed after a short period of abstinence. When compared to the control subjects, the methamphetamine abusers tested after prolonged abstinence had lower metabolism in the striatum (most accentuated in the caudate and nucleus accumbens) but not in the thalamus. The recovery of thalamic metabolism could represent adaptative response to compensate for the dopamine deficits in striatum. The authors suggested that while prolonged abstinence may reverse some of the methamphetamine-induced alterations in brain function, other deficits persist. These striatal findings are in contradiction with those obtained by Berman´s study.

In 2010, in order to investigate whether prefrontal white-matter gliosis, axonal or myelin damage was present in the first weeks of METH abstinence, Tobias et al. (2010) used diffusion tensor imaging (DTI) to measure fractional anisotropy (FA) – as a possible index of gliosis - in prefrontal white matter. FA in the corpus callosum at the midline and at eight bilateral, fiber-tract sites in other regions implicated in effects of methamphetamine were also sampled.

White matter has high directional diffusion and consequently high FA and, in contrast, gliosis is nondirectionally oriented and therefore could decrease FA of local tissue (Jones et al. 1999). The results revealed decreases in FA in the right prefrontal white matter, bilateral midcaudal superior corona radiate, genu corpus callosum and right perforant path which signifies low tissue integrity and/or organization suggesting that the abnormal microstructure may be a neuropathological effect on early abstinent METH abusers however it is difficult to predict whether it is because of myelin, axonal damage or gliosis.

4.3.Human studies: methamphetamine addicts - Post-mortem analysis

Post-mortem studies in METH addicts have found reduced levels of dopamine terminal markers in the striatum which are possibly related to an acute dopamine depletion caused by METH overdose (Wilson, 1996). Despite that, it should not be excluded the possibility of recovery (except for DAT) by the time of the tissue analysis (Schwendt et al., 2009). However little is known on glial cells from METH users that died from overdose. Unfortunately this area remains unexplored, possibly due to legal issues, as only one study has been performed concerning post-mortem modifications in former METH abusers.

Kitamura et al. (2010) examined glial - microglia and astrocytic - reactions by immunohistochemistry in the striatum of chronic METH abusers who were never abstinent and perished of overdose, using human glucose transporter 5 (hGLUT5) for resting and reactive microglia, CR3.43 (a marker for activated human microglia) for reactive microglia, GFAP and S100B (S100 calcium binding protein B) for astrocytes.

Despite most microglial cells were in the resting state, statistical analysis shown an important increase in hGLUT5-positive cells in the putamen, nucleus accumbens and caudate. However staining with CR3.43 detected almost none reactive microglia and these cells proliferation was not apparent even in the striatum of METH former abusers and the control group. Moreover, GFAP immunostaining showed that almost all astrocytes appeared to have normal morphology in both groups although the METH group presented a non-significant increase in the density of positive-marked cells in each subdivision of the striatum, results superimposable with S100B staining. Astrocytes consistent with morphological changes due to reactive activation were rarely detected.

These findings on glial reactivity were inconsistent with previous studies on animal models of METH treatment with high doses as we reviewed earlier possibly due to some limitations of the current study such as lack of detailed clinical information of the individuals,

duration and daily pattern of the METH abuse. Moreover, this study is about individuals who died from METH toxicity unlike those addressed earlier which concerned abstinent METH users. Nevertheless, these observations suggest that chronic METH use by itself does not lead to glial reactive responses in humans and further studies on glial response to METH should be developed for a better understanding of the mechanisms of gliosis in humans.

CONCLUSIONS

CONCLUSIONS

Methamphetamine abuse has turned progressively more prevalent in many developed countries and, therefore, becoming a major public health and social problem. For that matter, more investigation is needed so that the major disabilities that these individuals acquire by chronic abuse or the toxicity of high doses acute consumption can be attenuated or even prevented. From the information reviewed above, we can infer that many of the METH harmful effects could be due to glial activation.

Regarding gliosis, studies undertaken in rodents showed consistent evidences of brain gliosis, with particular incidence in striatal structures. Several investigations showed microgliosis in the striatum with acute exposure to METH which is concurrent with the initiation of dopamine nerve terminals damage and astrogliosis, suggested by increased GFAP levels in this structure (LaVoie et al., 2004; Thomas et al., 2004 a, b; Guillot et al., 2008). Although microglial striatal activation seemed only present with acute METH exposure and for a short period, in the parietal and piriform cortices and ventromedial column of the periaqueductal gray there was no attenuation after 6 days (LaVoie et al., 2004). More importantly, these glial activations seem to be attenuated by the administration of anti-inflammatory agents, glutamatergic antagonists and antioxidants in animals (Reviewed by Chang et al., 2004). This should be faced as an important stimulator for further studies in METH human abusers with the aim of investigating if the same results can be obtained. If so, it could signify a gigantic advance in METH-induced toxicity prevention.

As for human METH abusers investigation, PET scans of abstinent METH abusers' brain were highly suggestive of reactive microgliosis (Sekine et al., 2008). In addition, Volkow et al. (2001), and Berman et al. (2008), interpreted the increased glucose metabolism in the brain as a reflection of reactive gliosis. However, Wang et al. (2004) had inconsistent results showing decreased glucose metabolism in the striatum in both short and prolonged

periods of METH abstinence. The small size sample and the different studied time-points could justify the differences found. For that reason, we suggest more studies using similar methodologies including larger sample sizes and chosen abstinence length so that the inconsistencies can be clarified.

MRI imaging studies show an initial enlargement of parietal cortices, white matter and basal ganglia in early abstinence that is not verified in individuals abstinent for a longer period (Thompson et al, 2004; Chang et al., 2005). This suggests that methamphetamine-induced inflammation or reactive gliosis may normalize with longer abstinence of METH abuse. The decreased volumes of globus pallidus and putamen in contrast suggest an eventual loss of basal ganglia volumes (Chang et al., 2005). So, in order to confirm this hypothesis and clarify these contradictions, there should be an effort to perform longitudinal studies.

Diffusion tensor imaging in abstinent METH users also resulted in observations suggestive of oligodendrocytes impairment and/or reactive gliosis, although neuronal damage cannot be excluded as a prompter or the abnormal microstructures found (Tobias et al., 2010).

All the studies previously enunciated were about abstinent METH abusers. This only clarified the implication of glia during METH-induced neurotoxicity recovery. The role of glia during active METH consumption remains an unexplored issue. We therefore suggest that longitudinal neuroimaging studies should be performed on active METH abusers so that we can be able to compare brain and glial modifications between active and delayed METH-induced toxicity.

Post-mortem studies, which can precisely evaluate glial modifications by histologically analysis of the brain, are scarce. Kitamura et al. (2010) findings were inconsistent with previous studies on animal models and imaging studies of METH abusers since glial reactive activation was rarely detected. Immunohistochemical studies in both abstinent and active METH users could clarify the extent of METH-induced glial activation.

However the need of detailed clinical information as well as having controls for co-morbilities and ethical issues bring these studies into difficult endeavors.

Finally, the limitations or the possible reasons for inconsistency may be due to small sample size, groups with sex-heterogeneity, differences in the duration of abstinence, consumption of other substances such as nicotine, differences on the severity of methamphetamine abuse and the interpretation and correlation of results from trials that used different methodologies of studying brain modifications.

In order to give a better care of these individuals, regarding the prevention of many pathologies caused by the METH abuse and consequent induced toxicity, further studies should be conducted so that the role of gliosis in METH addiction should be better elucidated.

REFERENCES

REFERENCES

Abrous DN, Koehl M, Le Moal M (2005) Adult neurogenesis from precursors to network and physiology. *Physiol Rev* 85:523-569

Barres BA (2008) The mystery and magic of glia: a perspective on their roles in health and disease. *Neuron* 60:430–440

Benarroch EE (2005) Neuron-astrocyte interactions: partnership for normal function and disease in the central nervous system. *Mayo Clin Proc* 80:1326-1338

Berman SM, Voytek B, Mandelkern MA, Hassid BD, Isaacson A, Monterosso J, Mioto K, Ling W, London ED (2008) Changes in cerebral glucose metabolism during early abstinence from chronic methamphetamine abuse. *Mol Psychiatry* 13(9):897-908

Bohn MC (2004). Motoneurons crave glial cell line-derivated neurotrophic factor. *Exp Neurol* 190:263-275

Bowyer JF, Ali S (2006) High doses of methamphetamine that cause disruption of the blood-brain barrier in limbic regions produce extensive neuronal degeneration in mouse hippocampus. *Sinapse* 60:521-532

Bowyer JF, Robinson B, Ali S, Schmued LC (2008) Neurotoxic-related changes in tyrosine hydroxylase, microglia, myelin, and blood-brain barrier in the caudate-putamen from acute methamphetamine exposure. *Sinapse* 62:193-204

Brand A, Richter-Landsberg C, Leibfritz D (1993) Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Deveplopmental Neurosciences* 15:289-298

Brunton LL, Lazo JS, Parker KL (2010) The pharmacological basis of therapeutics, 12th edition. Chapter 23. United States: McGraw-Hill Medical

Buzas R, Rosenberg J, Cox BM, (1998) Activity and cyclic AMP-dependent regulation of nociceptin/orphanin FQ gene expression in primary neuronal and astrocyte cultures. *J Neurochem* 71:556-563

Buzas R, Symes AJ, Cox BM (1999) Regulation of nociceptin/orphanin FQ gene expression by neuropoietic factors in neurons and astrocytes. *J Neurochem* 72:1882-1889

Buzas R, Rosenberg J, Kim KW, Cox BM (2002) Inflammatory mediators increase the expression of nociceptin/orphanin FQ in rat astrocytes in culture. *Glia* 39:237-246

Caudle WM, Richardson RJ, Wang MZ, Taylor TN, Guillot TS, McCormamack AL et al (2007) Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *J Neurosci* 27:8138-8148

Chang L, Ernst T, Speck O, Patel H, DeSilva M, Leonido-Yee M, Miller EN (2002) Perfusion MRI and computerized cognitive test abnormalities in abstinent methamphetamine users. *Psychiatry Research Neuroimaging* 114:65–79

Chang L, Cloak C, Patterson K, Grob C, Miller EN, Ernst T (2005) Enlarged striatum in abstinent methamphetamine abusers: a possible compensatory response. *J Biopsych* 57:967-974

Chang L, Alicata D, Ernest T, Volkow N (2007) Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. *Addiction* 102(Suppl.1):16-32

Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA (2005) Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* 120:421–433

Coutinho A, et al. (2008) Chronic methamphetamine induces structural changes in frontal cortex neurons and upregulates type I interferons. *J Neuroimmune Pharmacol* 3:241–245.

Cruickshank CC, Dyer KR (2009) A review of the clinical pharmacology of methamphetamine. *Addition* 104(7):1085-1099

Ernst T, Chang L, Leonido-Yee M, Speck O (2000) Evidence for long-term neurotoxicity associated with methamphetamine abuse: A 1H MRS study. *Neurology* 54:1344–1349.

Escubedo E, Guilart L, Sureda FX, Jimenez A, Pubill D, Pallas M, Camins A, Camarasa J (1998) Microgliosis and down-regulation of adenosine transportr induced by methamphetamine in rats. *Brain Res* 814:120-126

Fellin T, Carmignoto G (2004) Neurone-to-astrocyte signaling in the brain represents a distinct multifunctional unit. *J Psysiol* 559:3-15

Flores C, Samaha AN, Stewart J (2000) Requirement of endogenous basic fibroblast growth factor for sensitization to amphetamine. *J Neurosci* 20:RC55

Fu X, Zhu ZH, Wang YQ, Wu GC (2007) Regulation of proinflammatory cytokines gene expression by nociceptin/orphanin FQ in the spinal cord and the cultured astrocytes. *Neuroscience* 144:275-285

Fumagalli F, Gainetdinov RR, Valenzano KJ, Caron MG (1998) Role of dopamine transporter in the methamphetamine-induced neurotoxicity: evidence from mice lacking the transporter. *J Neurosci* 18:4861-4869

Fumagalli F, Gainetdinov RR, Wang YM, Valenzano KJ, Miller GW, Caron MG (1999) Increased methamphetamine neurotoxicity in heterozygous vesicular monoamine transporter2 knock-out mice. *J Neurosci* 19:2424-2431

Garcia-Segura LM, Naftolin F, Hutchison JB, Azcoitia I, Chowen JA, (1999) Role of astroglia in estrogen regulation of synaptic plasticity and brain repair. *Journal of Neurobiology* 40:574–584.

Graham DG (1978) Oxidative pathways for catecholamines in genesis of neuromelanin and cytotoxic quinines. *Mol Pharmacol* 14:633-643

Guilarte TR, Nihei MK, McGlothan JL, Howard AS (2003) Metahmphetamine-induced deficits of brain monoaminergic neuronal markers: distal axotomy or neuronal plasticity. *Neuroscience* 122:499-513

Guillot TS, Shepherd KR, Richardson JR, Wang MZ, Li Y, Emson PC, Miller GW (2008) Reduced vesicular storage of dopamine exacerbates methamphetamine-induced neurodegeneration and astrogliosis. *J Neurochem* 106:2205-2217

Guillot TS, Miller GW (2009) Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons. *Mol Neurobiol* 39(2):149-70

Harvey D., Lacan G., Tanious S., Melega W. (2000) Recovery from methamphetamine induced long-term nigrostriatal dopaminergic deficits without substancia nigra cell loss. *Brain Research* 2000; 871: 259-70

Hess A, Desiderio C, McAuliffe WG (1990) Acute neuropathological changes in the caudate nucleus caused by MPTP and methamphetamine: immunohistochemical studies. *J Neurocytol* 19:338-342

Jones DK, Lythgoe D, Horsfield MA, Simmons A, Williams SCR, Markus HS (1999) Characterization of white matter damage in ischemic leukoaraiosis with diffusion tensor MRI. *Stroke* 30:393-397

Kalechstein AD, Newton TF, Green M (2003) Methamphetamine dependence is associated with neurocognitive impairment in the initial phases of abstinence. *J Neuropsychiatry Clin Neurosci* 15:215-220

Katzung BG, Masters SB, Trevor AJ (2009) Basic and Clinical Pharmacology, 11th edition. Chapter 32. United States: McGraw-Hill Medical

Kitamura O, Takeichi T, Wang EL, Tokunaga I, Ishigami A, Kubo S (2010) Microglial and astrocytic changes in the striatum of methamphetamine abusers. *Legal Medicine* 12:57-62

Krasnova IN and Cadet JL (2009a) Molecular Bases of Methamphetamine-Induced Neurodegeneration. International Review of Neurobiology 88:101-119

Krasnova IN and Cadet JL (2009b) Methamphetamine toxicity and messengers of death. *Brain Res Rev*. 60(2):379–407

Krasnova IN, Justinova Z, Ladenheim B, Jayanthi S, McCoy MT, Barnes C, Warner JE, Goldberg SR, Cadet JL (2010) Methamphetamine self-administration is associated with persistent biochemical alterations in striatal and cortical dopaminergic terminals in the rat. *PLoS One* 5(1):e8790.

Kuhn, Francescutti-Verbeem, Thomas (2006) Dopamine quinones activate microglia and induce a neurotoxic gene expression profile: relationship to methamphetamine-induced nerve ending damage. *Ann. N. Y. Acad. Sci.* 1074:31–41

Larsen KE, Fon EA, Hastings TG, Edwards RH, Sulzer D (2002) Methamphetamine-induced degeneration of dopaminergic neurons involves autophagy and upregulation of dopamine synthesis. *J Neurosci* 22:8951-8960

LaVoie MJ, Card JP, Hastings TG (2004) Microglial activation precedes dopamine terminal pathology in methamphetamine induced neurotoxicity. *Exp Neurol* 187:47-57

Lutz SE, Zhao Y, Gulinello M, Lee SC, Raine CS, Brosnan CF (2009) Deletion of astrocyte connexins 43 and 30 leads to a dysmyelinating phenotype and hippocampal CA1 vacuolation. *J Neurosci* 29:7743–7752

Mandyam CD, Wee S, Eisch AJ, Richardson HN, Koob GF (2007) Methamphetamine self-administration and voluntary exercise have opposing effects on medial prefrontal cortex gliogenesis. *J Neurosci* 27(42):11442-11450

McCann UD, Kuwabara H, Kumar A, Palermo M, Abbey R, Brasic J, Ye W, Alexander M, Dannals RF, Wong DF, Ricaurte GA (2008) Persistent cognitive and dopamine transporter deficits in abstinent methamphetamine users. *Synapse* 62:91-100

Melega W., Raleigh M., Stout D., Lacan G., Huang S., Phelps M. (1997) Recovery of striatal dopamine function after acute amphetamine- and methamphetamine-induced neurotoxicity in the vervet monkey. *Brain Research*; 766:113-20

Miguel-Hidalgo JJ (2009) The role of glial cells in drug abuse. *Curr Drug Abuse Rev*. 2(1):76–82.

Narita M, Miyatake M, Narita M, Shibasaki M, Shindo K, Nakamura A, Kuzumaki N, Nagumo Y, Suzuki T (2006) Direct evidence of astrocytic modulation in the development of rewarding effects induced by drugs of abuse. *Neuropsychopharmacol* 36:2476-2488

Nordahl TE, Salo R, Leamon M (2003) Neuropsychological effects of chronic methamphetamine use on neurotransmitters and cognition: a review. *J Neuropsychiatry Clin Neurosci* ; 15:317-325

O'Callaghan JP and Miller DB (1994) Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. *J Pharmacol Esp Therapeut* 270:741-751

Pubill D, Canudas AM, Pallas M, Camins A, Camarasa J, Escubedo E (2003) Different glial response to methamphetamine and methylenedioxymethamphetamine-induced neurotoxicity. *Naunyn Schmiedebergs Arch Pharmacol* 367:490-499

Quinton MS and Yamamoto BK (2006) Causes and consequences of methamphetamine and MDMA toxicity. *Aaps J* 8:E337-347

Rhawn Joseph (2000) Neuropsychiatry, Neuropsychology, Clinical Neuroscience 3rd Edition. New York: Academic Press. Chapter 6

Sakoori K, Murphy NP (2010) Reduced degeneration of dopaminergic terminals and accentuated astrocytes activation by high dose of methamphetamine administration in nociceptin receptor knock-out mice. *Neuroscience Letters* 469:309-313

Schwendt M, Rocha A, See RE, Pacchioni AM, McGinty JF, Kalivas PW (2009) Extended methamphetamine self-administration in rats results in a selective reduction of dopamine transporter levels in the prefrontal cortex and dorsal striatum not accompanied by marked monoaminergic depletion. *J Pharmacol Exp Ther* 331(2):555-562

Scott JC, Woods SP, Matt GE, Meyer RA, Heaton RK, Atkinson JH, Grant I (2007) Neurocognitive effects of methamphetamine: a critical review and meta-analysis. *Neuropsychol Rev;* 17:275-297

Seiden L. S., Ricaurte G. (1987) Neurotoxicity of methamphetamine and related drugs. In: Meltzer H. Y. editor. *Psychopharmacology: The Third Generator of Progress.* New York: Raven Press. p.359-65.

Sekine Y, Minabe Y, Ouchi Y, Takei N, Iyo M, Nakamura K, Suzuki K, Tsukada H, Okada H, Yoshikawa E, Futatsubashi M, Mori N (2003) Association of dopamine transporter loss in the orbitofrontal and dorsolateral prefrontal cortices with methamphetamine related psychiatric symptoms. *Am J Psychiatry* 160:1699-1701.

Sekine Y, Ouchi Y, Takei N, Yoshikawa E, Nakamura K, Futatsubashi M, Okada H, Minabe Y, Suzuki K, Iwata Y, Tsuchiya KJ, Tsukada H, Iyo M, Mori N (2006) Brain serotonin transporter density and aggression in abstinent methamphetamine abusers. *Arch Gen Psychiatry* 63:90-100.

Sekine Y, Ouchi Y, Sugihara G, Takei N, Yoshikawa E, Nakamura K, Iwata Y, Tsuchiya KJ, Suda S, Suzuki K, Kawai M, Takebayashi K, Yamamoto S, Matsuzaki H, Ueki T, Mori N,

Gold MS, Cadet JL (2008) Methamphetamine causes microglial activation in the brains of human abusers. *J Neurosci* 28:5756–5761

Sheng P, Cerruti C, Cadet JL (1994) Methamphetamine (METH) causes reactive gliosis in vitro: attenuation by the ADP-ribosylation (ADPR) inhibitor, benzamide. *Life Sci* 55:PL51-PL54

Simões PF, Silva AP, Pereira FC, Marques E, Grade S, Milhazes N, Borges F, Ribeiro CF, Macedo TR (2007) Methamphetamine induces alterations on hippocampal NMDA and AMPA receptor subunit levels and impairs spatial working memory. *Neuroscience* 150:433-441

Snell RS (2010) Clinical neuroanatomy, 7th edition. Wolters Kluwer Health – Lippincott Williams & Wilkins pp 53-63 and pp 316-327

Sung YH, Cho SC, Hwang J, Kim SJ, Kim H, Bae S, Kim N, Chang KH, Daniels M, Renshaw PF, Lyoo IK (2007) Relationship between N-acetyl-aspartate in gray and white matter of abstinent methamphetamine abusers and their history of drug abuse: a proton magnetic resonance spectroscopy study. *Drug Alcohol Depend* 88(1):28-35

Suzuki T, Shindo K, Miyatake M, Kurokawa K, Higashiyama K, Suzuki M, Narita M (2007) Lack of development of behavioral sensitization to methylphenidate in mice. Correlation with reversible astrocytic activation. *J Pharm* 574:39-48

Takahashi N, Miner LL, Sora L, Ujike H, Revay RS, Kostic V, Jackson-Lewis V, Przedborski S, Uhl GR (1997) VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward , enhanced amphetamine locomotion, and enhanced MPTP toxicity. *Proc Natl Acad Sci USA* 94:9938-9943

Takayama N, Ueda H (2005) Morphine-induced overexpression of prepronociceptin/orphanin FQ in cultured astrocytes. *Peptides* 26:2513-2517

Thomas DM, Walker PD, Benjamins JA, Geddes TJ, Kuhn DM (2004a) Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. *J Pharmacol Exp Ther* 311:1-7

Thomas DM, Dowgiert J, Geddes TJ, Francescutti-Verbeem D, Liu X, Kuhn DM (2004b) Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. *Neurosci. Lett* 367:349-354

Thompson PM, Hayashi KM, Simon SL, Geaga JA, Hong MS, Sui Y, Lee JY, Toga AW, Ling W, London ED (2004) Structural abnormalities in the brains of human subjects who use methamphetamine. *The Journal of Neuroscience* 24(26):6028-6036

Tobias MC, O'Neill J, Hudkins M, Bartzokis G, Dean AC, London ED (2010) White-matter abnormalities in brain during early abstinence from methamphetamine abuse. *Psicopharmacology* 209: 13-24

Ullian EM, Sapperstein SK, Christopherson KS, Barres BA (2001) Control of synapse number by glia. *Science* 291:657–661

Ullian EM, Christopherson KS, Barres BA (2004) Role for glia in synaptogenesis. *Glia* 47: 209-216

UNDCP Technical Series Number 3 (1996). Amphetamine-type stimulants: a global review

United Nations Office of Drug and Crime, *World Drug Report* 2010

Vergo S, Johansen JL, Leist M, Lotharius J (2007) Vesicular monoamine transporter 2 regulates the sensitivity of rat dopaminergic neurons to disturbed cytosolic dopamine levels. *Brain Res* 1185:18-32

Vesce S, Bezzi P, Volterra A (2001) Synaptic transmission with the glia. *News Physiol Sci* 16:178-184

Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler M, Gatley SJ, Miller E, Hitzemann R, Ding YS, Logan J (2001) Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *J Neurosci* 21:9414-9418

Wang GJ, Volkow ND, Chang L, Miller E, Sedler M, Hitzemann R, Zhu W, Logan J, Ma Y, Fowler JS (2004) Partial recovery of brain metabolism in methamphetamine abusers after protracted abstinence. *Am J Psychiatry* 161(2):242-8.

Wilson JM, Kalasinsky KS, Levey AI, Bergeron C, Reiber G, Anthony RM, Schmunk GA, Shannak K, Haycock JW and Kish SJ (1996) Striatal dopamine nerve terminal markers in human, chronic methamphetamine users. *Nat Med* 2:699-703.

Weisheit R and White W (2009) Methamphetamine: its history, pharmacology and treatment. Hazelden Publishing. pp26-27

Yamamoto BK, Moszczynska A, Gudelsky GA (2010) Amphetamine toxicities: Classical and emerging mechanisms. *Ann. N.Y. Acad. Sci.* 1187:101–121

Zhao H, Huang HW, Wu GC, Cao XD (2002) Effect of orphanin FQ on interleukin1 beta mRNA transcripts in the rat CNS. *Neuroscience* 114:1019-1031

Zhao RJ, Woo RS, Jeong MS, Shin BS, Kim DG, Kim KW (2003) Orphanin FQ/nociceptin blocks methamphetamine place preference in rats. *Neuroreport* 14:2383-2385