The Neurobiological Effects of Methamphetamine Abuse

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Widespread abuse of central nervous system stimulants, such as methamphetamine, is increasing astronomically, most notably here in the Central Valley of California. Users of these drugs find them to be affordable and easily attainable, hence the increasing popularity and usage. It transiently increases the levels of dopamine in the brain, which is a highly desirable side effect of using the drug. However, the neurobiological effects of this chemical are severely detrimental, including changes to dopaminergic terminals and transporters, and impairment of mental functions. It has also been found to increase levels of oxidative stress when used over a long period of time.¹

Methamphetamine is a potent synthetic central nervous system stimulant. It is a member of the phenethylamine family which include many other illicit drugs, such as hallucinogens. Furthermore, methamphetamine is a derivative of amphetamine which is typically used in small doses to treat attention deficit hyperactivity disorder (ADHD) and the like. The molecular formula is C₁₀H₁₅N and this compound can be precipitated into an off-white crystalline powder. The typical therapeutic dosing of methamphetamine is 25 mg orally, and it is a substance which becomes rapidly absorbed into the blood plasma. This psychoactive, widely-abused drug has been shown to affect the functioning of the central nervous, cardiovascular, and respiratory systems. Users also exhibit psychologically detrimental effects with long-term use of the drug.

One example of the toxicity of this drug can be attributed to the scientific contributions of researchers at Qazvin’s University of Medical Science in Qazvin, Iran. They performed an
assessment of a population of 96 prior methamphetamine abusers to demonstrate the production of free radicals in users of methamphetamine abuse\(^1\). Oxidative stress in cells generally indicate a problem with the ability to detoxify reactive intermediates, such as superoxide or hydroxyl radicals\(^1\). Furthermore, lipid peroxidation occurs when free radicals take electrons from membranous lipids\(^1\). These lipids are required for proper functioning of the cell, such as signaling. Hence, both oxidative stress and lipid peroxidation in methamphetamine users were the primary focus of this research cohort.

In this study, researchers gathered blood samples from the sample population of methamphetamine abusers (versus a control group of individuals whom had never used methamphetamine), and they performed a ferric reducing ability of plasma assay (FRAP), in which total antioxidant power is assessed\(^6\). With this assay, ferrous ion (Fe\(^{2+}\)) is created from ferric ion (Fe\(^{3+}\)) at a low pH, which causes a colored compound to form, called ferrous-tripyridyltriazine \(^6\). Additionally, serum levels of malondialdehyde (a biomarker for oxidative stress) were measured to assess the initial ability of the body to peroxidize lipids, as well as antioxidant power as a whole\(^8\). Malondialdehyde is a toxic compound which is the product of polyunsaturated fatty acid peroxidation. It has the ability to mutate DNA and denature proteins, and is used in scientific research to identify the occurrence of lipid peroxidation. Lipid peroxidation is generally a mechanism which occurs in the body by way of free radicals, resulting in damaged tissues and decreased cell viability\(^8\). The conclusions of this experiment provided evidence that methamphetamine abusers, over a prolonged period of time, exhibit high levels of oxidative stress and increased peroxidation of lipids\(^1\). They believe that the cognitive effects of using this drug can be treated by including antioxidants in the regimen of drug users, however this idea requires further experimentation\(^1\).
Considering this information, scientists have contemplated the effect of methamphetamine abuse on further neurobiological mechanisms. A group of researchers at the Department of Pharmacology, Physiology, and Therapeutics at the University of North Dakota School of Medicine and Health Sciences published a study of the effects of methamphetamine administration on the nematode Caenorhabditis elegans. The reason this organism was chosen for the study was because C. elegans is known to display amphetamine-induced behavioral modifications\textsuperscript{2}. The scientists had previously known that amphetamine targets dopamine transporters in the brain, however they wished to discover other mechanisms previously unknown. Notably, they wishes to focus on the ligand-gated chloride channel LGC-55 which is a high-affinity tyramine receptor\textsuperscript{2}. Activation of these channels by various amines, according to previously studies, can modify the behavior of C. elegans\textsuperscript{2}.

The nematodes were collected from the C. elegans Genetics Center at the University of Minnesota, Minneapolis. A SWIP assay was performed in which the adult worms were placed in water with (experimental group) or without (control group) amphetamine in a Pyrex spot plate\textsuperscript{2}. Subsequently, the number of paralyzed worms were recorded every minute with inverted microscopy. Additionally, a head immobilization assessment was performed on 24 hour post-larvae worms in which they were placed in an agar plate and given doses of methamphetamine with glacial acetic acid, which is essentially the undiluted, more acidic form of the compound; acetic acid without methamphetamine was used as a control. If the nematodes were unable to swing their heads laterally after this process, they were defined as “immobile”\textsuperscript{2}.

Conclusively, the study showed evidence that LGC-55 did indeed have an effect on the behavioral modifications of C. elegans worms intoxicated with methamphetamine\textsuperscript{2}. When compared with control organisms with no LGC-55 gene activated, amphetamines caused 100%
head immobilization in the wild types, whereas in the mutants it only caused immobilization in 25%\textsuperscript{2}. This is suggestive of a few important key ideas. First, it suggests that the amphetamine apparently has an effect on the LGC-55 channel. Second, it suggests that amphetamines may mimic physiological tyramine. Previous research has shown that tyramine can cause head immobilization via the process of activating LGC-55 channels because tyramine is the main ligand of the LGC-55. Thus the mimicking of tyramine that amphetamines are capable of can contribute to head immobilization in the worms; tyramine binds to the LGC-55 channels and causes hyperpolarization of neurons, leading to the head movements being suppressed. Researchers suggested that further studies be done, particularly with mammalian subjects, to see if these results are duplicated.

As previously discussed, methamphetamine is known to have a negative impact on the dopamine transporters in the brain. The Department of Pharmacology and Toxicity at the University of Utah wanted to further their knowledge of this, so they decided to perform an *in vivo* assessment of amphetamine and methamphetamine administration on the dopamine transporter function in the brain. The dopamine transporter’s role in the brain is to relocate extracellular dopamine into presynaptic terminals after they are released from neurons as neurotransmitters\textsuperscript{3}. Methamphetamine and amphetamines have been hypothesized to reduce these aforementioned transporter functions. In this experiment, male Sprague-Dawley rats were used as test subjects\textsuperscript{3}. They were kept initially at a warm environment, and were administered doses of either 1) a control solution of sterile 0.9% saline solution (1 ml/kg), 2) methamphetamine (15 mg/kg) dissolved in 1 ml/kg of 0.9% saline solution, or 3) amphetamine (15 mg/kg) dissolved in 1 ml/kg of 0.9% saline solution\textsuperscript{3}. There were both single-treatment (one injection) and multiple treatment (many injections) paradigms being tested\textsuperscript{3}.
After the injections were administered, the rats were decapitated and their striatal synaptosomes were isolated using sucrose buffer and a Dounce homogenizer to create a homogenous solution\(^3\). After centrifuging the mixture, the resultant synaptosomal pellet was used to separate the synaptosomal vesicles using vesicle fractionation\(^3\). Additionally, the remaining striatal synaptosomes were analyzed to see if dopamine uptake had occurred; this was done by using an assay buffer and 10 minutes of incubation, followed by adding dopamine for three minutes\(^3\). The protein concentrations all the samples were then assessed with SDS-PAGE and Western Blotting.

Results show that the amphetamine and methamphetamine acutely decreases dopamine transporter function in the synapse of the striata\(^3\). It was also concluded that dopamine transporters were not transported out of the terminals, nor were they degraded\(^3\). It is unknown if the dopamine transporters change their location during the analyzed points of interest, and this is a question that should be further pursued in other studies. This study concluded that the drugs had detrimental effects on the dopamine transporters of neuronal tissues\(^3\). This leads to the long-term decreased transport and uptake of dopamine in the brain, leading to many psychological and neurological disorders.

A group of researchers from The Department of Biochemistry and Molecular Biology at Miller School of Medicine in Miami, Florida wished to analyze the blood-brain barrier (BBB) with respect to the use of methamphetamine. Prior to this publication, knowledge of the specific effect on the actin cytoskeleton and BBB integrity after methamphetamine abuse had been limited. The researchers wanted to focus on the actin cytoskeletal mechanisms in the brain, particularly regarding endothelial cells associated with the BBB, and to support the hypothesis that methamphetamine intoxication leads to rearrangement of actin by activating a complex
called actin-related protein 2/3 (Arp). Human brain endothelial cells were cultured, and an endocytosis assay was done to measure the amount of occludin, a tight junction protein, moving in the brain during methamphetamine exposure. Furthermore, a sucrose gradient analysis was done to measure which compartments the endocytosed occludin are transferred to. Immunofluorescence, immunoblotting, and immunoprecipitation were done to stain, isolate, and examine the occluding proteins as well as Rab7 and Rab11 layers of the endothelial cells. Coronin-1b silencing with siRNA was then used to inhibit the coronin-1b factor which can block activation of actin rearrangement in the brain. Monocyte transmigration assays were also used; they measure the levels of monocyte (a type of white blood cell) movement, which was hypothesized to increase during methamphetamine exposure. Isolation of brain microvessels, occludin assessment in brain microvessels, and BBB permeability assays were finally performed on an in vivo mouse model; methamphetamine was injected into the mice and the brain microvessels were isolated and analyzed to see the effects, primarily on the hippocampus.

The researchers concluded that methamphetamine administration disrupts the blood-brain barrier by resulting in methamphetamine-induced inflammation. Methamphetamine intoxication stimulates polymerization of actin by activating the Arp 2/3 complex and phosphorylating coronin-1b. This causes increased movement of inflammatory cells and disrupts the endothelial barrier in the brain, leading to neuroinflammation, and disrupting the BBB integrity. This is significant because the BBB is vital in the protection of the brain. It is highly selective in the substances that are allowed to permeate through it. Disruption of the BBB by methamphetamine can result in the passage of undesirable toxins.

Incidentally, the immune system is also affected by the use of this illicit drug. The Oregon Health and Science University’s Department of Psychiatry performed a cross-species,
translation report on the dysregulation of the immune system caused by methamphetamine use. Mice and human samples were used in this study for comparison. The researchers subcutaneously injected thirty-two male C57BL/6J mice with 1 mg/kg of methamphetamine, or saline (0.09%) as a control, over a period of seven days. They were then euthanized after the last drug dose, and blood/brain samples were obtained, the plasma were isolated by centrifugation, and they were stored at low temperatures until they could be assayed.

Additionally, 40 human candidates were selected to participate; there were methamphetamine-dependent and non-dependent (control) groups. Blood samples were also collected from the human subjects to assess the amount of cytokines, chemokines, and cellular adhesion molecule levels. To determine immune factor levels in the mice and human plasma, bead-based suspension protein arrays and the Bio-Plex 200 Bead Reader System were used. bead array assays consist of beads of fluorescence and wavelengths that will detect multiple molecules from a single sample. The beads are read by the Bead Reader System which is able to detect the fluorescent dyes. The same assay was used for the brain tissue from the mice.

It was concluded that humans and mice alike who were previously exposed to methamphetamine showed changes in cytokine and chemokine levels, as well as altering the production of T-helper cells in the brain. Cytokines and chemokines are signaling molecules produced by a cell to elicit an immune response. These immune responses (or lack thereof) can alter the production of T-helper cells, which are essential in the adaptive immune system. T-helper cells activate the B cell antibodies as well as produce cytotoxic T cells for further protection. In summary, the fact that the use of methamphetamine decreases the production of the aforementioned chemicals and cells provides more support for the hypothesis that methamphetamine alters the health of its users in a negative way.
Given these points, it is evident that the abuse of this drug poses an enormous threat to the health of users. As previously stated, immunity, blood-brain barrier, dopamine transporter, and ion channel mechanisms were altered during these studies. Additionally, increased levels of oxidative stress and lipid peroxidation were noted in the studies. Methamphetamine is a highly addictive substance that is readily available to people of all walks of life. It is single-handedly destroying the health and mental functioning of millions of people, and little can be done to stop this. However, research in the area is very limited and should be pursued further if change is to happen. It is hopeful that as more information becomes available about this drug, more people will be aware of the harmful effects and avoid it.

**Literature Cited**


