The effect of a heroin diastereomer on memory formation and retention through GABA level analysis from short-term memory testing on mice

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Introduction:

The process by which memories are formed is a question which has intrigued scientists for many years. Said process, along with multiple other processes in the brain, is believed to be one of the more complex chemical reactions in the human body, and one which is not completely understood even today. However, with current technologies we are able to learn more about the neurophysiological processes that govern how the brain functions, and why certain compounds affect these processes. Research up to this point has also shown that drugs, used for a variety of purposes, have an affect on the memories of those who use them. Illegal recreational drugs, in particular, tend to have adverse effects on the cognitive functions and memories of their users. Heroin, also known as 6-monoacetylmorphine, is a drug that, when metabolized by the human body, floods the brain with dopamine and causes impairment of both short- and long-term memory formation for various reasons. However, stereochemical studies performed on cocaine, another dopamine-flooding drug, have shown that a diastereomer of cocaine (Benzatropine) can actually be used as a dopamine-impulse control treatment for Parkinson disease due to how differently it alters the dopamine transporter (Reith, 2001). This lends support to the idea that stereoisomers of known illegal drugs can have a different effect than their known compounds.

We know from previous chemical studies how the brain seems to react to external stimuli to produce thoughts and form memories. This process involves the storage of information in the nervous system and can be divided into two separate mechanisms: short-term memory and long-term memory (Tsukada, 1988). Short-term memory has been summarized as a result of the reversible chemical modification of the synapses in the brain via neurotransmission, while long-term memory is a result of reorganization of synapses in the brain, as well as protein synthesis within those synapses. In other words, neurotransmitters affect short-term memory while protein synthesis affects long-term.

Neurotransmission is the process by which signaling molecules called neurotransmitters transfer electric impulses from one neuron into certain receptors in another neuron, which either excite or inhibit a physiological response. An example of a neurotransmitter involved in short-term memory formation is Gamma-aminobutyric acid, also known as GABA. GABA is an inhibitory neurotransmitter, which means that its function is to slow or limit the production of another neurotransmitter, in this case: dopamine. Heroin is a known inhibitor of GABA, and by slowing the release of GABA, it allows dopamine to flood the many regions of the brain (Clear Detox Center, 2017). This excess of dopamine has many adverse side effects, one of them being memory loss due to the chemical imbalances in the synapses of the brain. Recent studies have shown that more GABA in certain regions of the brain (the dorsolateral prefrontal cortex (DLPFC) specifically) is linked to better memory (Goldman, 2016). When heroin is introduced into the brain, it binds to the opiate receptors, which ultimately results in GABA inhibition -- note that heroin is not a GABA agonist or antagonist, rather it inhibits GABA production from a receptor that is not involved with GABA (National Institute of Drug Abuse, 2007). In a study titled "The effect of heroin on verbal memory," it was experimentally proven that "heroin abuse, lasting longer than one year, is connected with impairment of short-term and delayed verbal memory." This shows that heroins inhibitory effect on GABA results in some sort of memory impairment; in other words confirming that high levels of GABA in certain parts of the brain (the DLPFC) correlate with better memory.

Now that the role that neurotransmitters such as GABA play in the process of short-term memory formation has been established, the role of protein synthesis must also be taken into account. Previous studies had been performed which suggested a link between protein synthesis in the brain and learning. Hyden and Egyhazi noticed that when rats were subjected to training for tightrope walking, there was an increase in RNA and a change in the composition of proteins in the sensory-motor cortex of the rat brain (Hyden and Egyhazi, 1962). This finding suggested that memory consolidation, or long-term memory formation involved in learning, requires the synthesis of particular proteins in the cerebrum. Agranoff furthered this research by studying the effect of protein synthesis inhibitors such as puromycin on learning and memory on fish being conditioned to avoid electric shock. In this study, fish that were administered puromycin while being conditioned failed to remember the shock avoidance behavior the next day, while fish that were not administered puromycin were able to remember the shock avoidance behavior for almost a month after being conditioned (Agranoff, 1967). These findings support the conclusion that protein synthesis in the brain plays an important role in long-term memory formation, and that certain chemicals can affect this process in a positive or negative manner. GABA is also involved in protein synthesis, as evidenced by a fairly recent study in which an increase in ribosomal kinase SK61 (involved in translation) was observed upon administration of GABA in rats (Thanapreedawat, 2013). Since protein synthesis has been found to be linked with memory consolidation, inhibition of GABA by heroin would not only lead to impairment of short-term memory through flooding of dopamine, but long-term memory formation as well. This strengthens the case for the use of GABA as an indicator of memory formation in our experiment. Any effects on GABA-levels in regions of the brain could potentially indicate an effect on memory formation.

Researching a heroin diastereomer's effect through GABA levels could allow for further insight into heroin and its diastereomer's effect on memory formation and retention. Studies have shown that heroin proves to have a negative effect on memory formation, but would its diastereomer also have an effect?

Goals Statement:

The goal of this research experiment is to determine whether or not a diastereomer of the opioid heroin has an effect on memory formation and retention by observing memory changes in mice and analyzing GABA levels in the dorsolateral prefrontal cortex after the memory tests. If successful, the proposed experiments will result in a more detailed understanding of how stereochemistry affects the process by which heroin interacts with the brain.

Proposed Research:

Animal testing:

The initial part of the experiment would be initiated on animals specifically on mice/rats. A series of tests would be conducted where the half of the animal test subjects would receive normal heroin and the other half of the animal test subjects would receive the diastereomer of heroin.

Experiment 1: Barnes Maze Test

A standard Barnes Maze Test (Rosenfeld, 2014). In this test the speed of the mice or rat will be measured to see how fast they can get to the escape cage at the end of the maze. The test will be given numerous times and the times will be recorded. The test is supposed to check the short-term memory of the mice. The faster they move through the maze would show the creation of a short-term memory. The mice and rats would then be given heroin or the diastereomer of heroin and they would be put in the maze again. The times would be recorded again and would later be compared to their initial times. All side effects will all also be recorded.

Experiment 2: Novel Object Recognition Test

A second animal test would be a 2-Object Novel Object Recognition Test (Stanford Medicine). The second test would measure a mouse or rat short-term memory by being able to identify the unfamiliar new object. The test will be timed and observation on the animal test subject's ability to recognize the new object will be observed. The animals will then be given heroin or the diastereomer and the test will be given again. The times and object identification will be observed and compared to the preliminary test without heroin or its diastereomer.

Experiment 3: fMRI Testing

fMRI testing would be conducted on the mice to see the normal functions of the brain. The mise or rat would then be given heroin and the fMRI test would be administered. The fMRI test would be mainly focused on the GABA receptors specifically in the dorsolateral prefrontal cortex as well as other parts of the brain. The mice or rat would at a later time be given the diastereomer of heroin and the MRI test would be administered again. The results from all three tests would be recorded and compared to each other.

Human Testing:

If animal testing is found to be successful, test will then be tested on volunteer human test subjects. The human tests subjects will be subjected to various preliminary testing before being given heroin or its diastereomer.

Experiment 1: Psychiatric Testing/ fMRI

The first test would be a psychiatric test to ensure that the test subject is of sound mind to endure the rest of the testing. The second test would be an initial fMRI scan to ensure there is no existing brain damage or discrepancies. The test subjects will then be put back in the fMRI machine and would given cards and they would need to find the pair and match them up. The MRI scan will be focused on the GABA receptors as well as the brain as a whole to see what part of the brain is lighting up during memory formation. The test will be given multiple times and the results will be compared to each other. The test subjects will then be given heroin first and then they will be given the diastereomer of heroin. The test subjects will be put back into the fMRI and will given the exact same test. The effects will heroin and the diastereomer will be observed. The effects on the GABA receptors will be observed as well as the other parts or the brain. The main focus will be on the dorsolateral prefrontal cortex and the GABA receptors. The test will be administered at least twice, and the results will then be compared to each other.

Experiment 2: Flash Card recognition

A second test will be administered which would be a simple flash card recognition test. During this test subjects will be given flash card with word pairs. The test subjects will need to be able to recognize word pairs. The test will be timed to see the speed of short-term memory recognition. The test subject will then be given heroin and then the diastereomer and will be given the cards again. The speed will be recorded and compared to the initial test. The test will show how fast short-term memory recognition is.

Experiment 3: Health Test

As a final test the test subjects will be separated into groups and the groups will be given either a placebo, heroin or its diastereomer. This test is to determine if there is other side effects in other parts of the body that could possibly make it unusable. Blood test will be taken as well as ultrasounds of the liver and kidneys.

Materials:

Material needed for this experiment are readily available. Heroin and its diastereomers can be purchased from any big drug distributor. The mice for the purpose of research are easily obtained. Mice can be obtained from the website titled: “Find & Order Mice.” Access to a an organic laboratory would also be necessary but can be accommodated by the University of San Diego. An MRI and an UltraSound Machine would also be required. Access to these machines will be more complex to obtain. These machines could be possibly accessed at the university if available if not permission would be needed to get access to machines at another University or even possible a local hospital.

Possible outcomes:

This particular experiment could result in a number of outcomes including: the diastereomer treated group showed either a improvement, impairment, or no change in memory when compared to the heroin treated group. These results were further verified through GABA level analysis derived from the MRI scans of the dorsolateral prefrontal cortex. The subjects treated

with the diastereomer may also have had no change in memory, but some sort of adverse effect; this effect could either be fatal or potentially serendipitous. Side note: because GABA also has an effect on memory, it can be conclude that the increase or decrease of GABA levels observed indicates an improvement or impairment of both short-term and long-term memory. This will not be discussed in depth because the focus of this project is short-term memory formation and retention.

If the diastereomer treated group resulted in an improved or impaired memory as opposed to the heroin treated group, and its GABA levels were either increased or decreased respectively, it could be concluded that the diastereomer had an effect on memory. If no change were to occur between the two groups, then the heroin diastereomer had no effect on memory. Both these results would ascertain whether or not the heroin diastereomer is involved in memory formation and retention.

Another possible outcome is that the diastereomer of heroin at the dosage we used actually ends up having no effect on the altered GABA levels. In this case measurements would have to be reevaluated and re-administered. Finally, the diastereomer may result with a serendipitous effect: say it turned out to be a agonist for norepinephrine; a drug could be created from this diastereomer that would give people momentary energy and extreme speed. On a more serious note, the diastereomer may actually be a dangerous toxin for humans (no toxicity in the animal which was originally tested), attacking the body or brain and leading to serious pathologic outcomes including nervous system failure, loss of muscle function, and even death.
Side effects must also be considered when conducting this type of research: Augmented GABA levels in the brain have been known to lead hyperalgesia (pain amplification, a key feature of FMS), anxiety, restlessness, and ADHD-like symptoms, such as inability to focus. These confounding variables by themselves could affect learning capability, which would be detrimental to the experiment. Similarly, reduced levels could potentially generate panic, anxiety, depression, alcoholism, and bipolar disorders which also provide as confounding variables.

Because of the likelihood of so many different things going wrong in this experiment, or the development of a myriad of unfavorable side-effects, it would be insufficient to simply conduct this one experiment in hopes to ascertain the effect of a heroin diastereomer on memory. In order for this to actually occur, many more experiments will have to be conducted so as to avoid any of the many side effects that may result.

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