



Effects of methamphetamine on rat brain

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Introduction

During the winter break of 07-08 I was involved in a study at National Institute on Drug Abuse, part of National Institute of Health (NIDA/NIH). In that study, we wanted to know the effects of methamphetamine on a particular part of the rat brain called the Ventral Tegmental Area (Figure 1). This area is involved in the pleasure (or reward) system and when activities that produce pleasure is done this area also activates. Since the purpose of NIDA is to ultimately cure drug addiction, studying this part of the brain would give some insight into the nature of drug addiction.

Hypothesis

Methamphetamine may increase the function of glutamatergic neurons in the Ventral Tegmental Area or VTA, because methamphetamine is a psychostimulant, which mimics the effect of norepinephrine and epinephrine. In addition to this, methamphetamine is also involved in releasing dopamine and serotonin, all of which are involved in sympathetic nervous system.

Methods

In this study four groups of rats were used of which two of them were used as control and the other two as experimental. The rats were either injected with methamphetamine or saline for a week. After two weeks the rats were then given another treatment of either saline or methamphetamine. For the negative control group, which gives negative results, the rats were injected with saline both times while the positive control group, which gives positive results, was injected with methamphetamine the first time and then with saline the second time. For the experimental group they were injected with either methamphetamine or saline but in the third week they were both injected with methamphetamine. The brains of those rats were extracted and frozen to cut the brains into 20 micrometer sections. The sections then were mounted on the slides to look for markers.

Before looking at the sections in the microscope, *in situ* hybridization and immunohistochemistry were done on the sections. *In situ* hybridization, single stranded RNA strands were treated with radioactive phosphorus (³³P) and sulfur (³⁵S) to make a cRNA probe. When looking for the radioactive signal, silver has to cover the slide because the radioactivity itself will not show up on a microscope. When the slide is covered with the silver, the radioactivity from phosphorus and sulfur will expose the silver, which then it will be visible on fluorescence microscope. In immunohistochemistry antibodies are tagged with markers and attached to a specific protein. To look at an antibody-antigen interaction, an antibody is conjugated to an enzyme, in this case peroxidase, so that it results in a color-producing reaction.

The sections are then seen through dark field and fluorescent microscope to look for VGluT-2 and c-Fos markers respectively (Figure 2). The HOT VGluT-2 marker's signal shows up with fluorescence as green dots (Figure 6) while the marker for c-Fos marker's signal shows up as brown (Figure 5). From a microscope with two different light sources, a high resolution camera takes pictures in small pieces so one has to put the pictures back together piece by piece. After reassembling the picture one can tell a few things. From the *in situ* hybridization, the radioactive RNA strand attaches to the VGluT-2 mRNA, if it is present, and if it shows up on fluorescence microscope, it means that particular cell is an excitatory neuron. C-Fos marker shows up when the antibodies tagged with fluorescent signal attaches to a specific protein, c-Fos in this case. When the c-Fos marker shows up it means that on that particular neuron, it is firing action potentials. Co-localization or overlapping of the two signals is also looked for in the sample as an indicator for an actively firing neuron. After finding both markers as well as the co-localization, they have to be counted so the number of markers between the 4 rat groups can be compared. In this case the mean, or the average, of the cells in the four groups is used to compare the numbers. Standard deviation is also included with the means because tells how widely spread the numbers are.

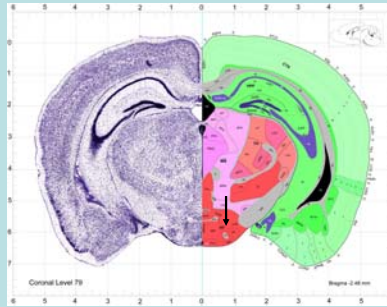


Figure 1. VTA below the arrow. (courtesy of www.caseyfaber.com/html/graphics.html)

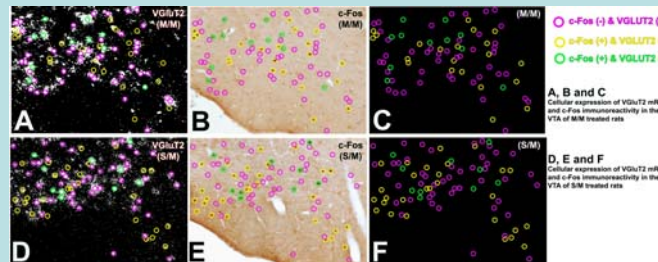


Figure 2. Cells expressing VGluT-2 and c-Fos.

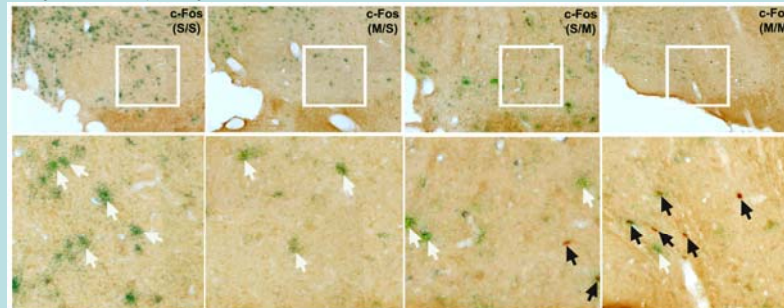


Figure 3. White arrows on 1st (S/S) and 2nd (M/S) panels show vglut-2 signal only while black arrows on 3rd (S/M) and 4th panel (M/M) shows c-Fos specific signal as well as vglut-2 signal.

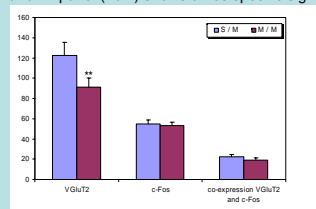


Figure 4. Cell count in a graph



Figure 5. picture of c-Fos expression on a brain section



Figure 6. Picture of VGluT-2 marker showing up as fluorescence

Results

After detecting the signals they were counted. After counting the cells it was found that VGluT-2 signals were more numerous on group that was give Saline first then Methamphetamine (S/M) than on the group that was give Methamphetamine both times (M/M) with the means of 122.76 (sample size n=6015) and 91.05 (sample size n=3890) respectively (Table 1). The expression of c-Fos was found to be about the same level for both S/M and M/M with means of 54.85 (sample size n=2688) and 53.31 (sample size n=2239) respectively (Table 1). Co-expression of VGluT-2 and c-Fos were also found to be similar for both S/M and M/M with the means of 22.37 (sample size n=1096) and 19.17 (sample size n=805) (Table 1).

Discussion

Injecting meth into the rat brain shows that the level of VGluT-2 cells goes down between the two experimental groups as shown above. This means that depending on whether the rat has been is given methamphetamine for only the third week or for both first and third week, the number of excitatory neurons change as the S/M rats had larger amount of VGluT-2 mRNA expression, with 122.76 cells, than the M/M group, with 91.05 cells. However the number of active neurons as well as the co-localization is similar within both of the experimental groups with 53.31 and 22.37 cells for M/M respectively and 54.85 and 22.37 cells for S/M respectively. So it can be concluded that meth decreases the total number of excitatory neurons with each injection of methamphetamine while the number of excitatory neurons that are active stays about the same. Because the control groups show no c-Fos markers, we concluded that methamphetamine does indeed increase the function of glutamatergic neurons.

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LOWER EXPRESSION OF VGluT2 mRNA IN THE VTA OF M/M RATS COMPARED TO THE S/M RATS

Treatment	VGluT2 labeled cells (mean ± sem)	c-Fos labeled cells (mean ± sem)	c-Fos labeled cells with VGluT2 (mean ± sem)
M / M	91.05 ± 8.96 (n=6, count cells=3890)	53.31 ± 3.14 (n=6, count cells=2239)	19.17 ± 2.14 (n=6, count cells=805)
S / M	122.76 ± 12.68 (n=6, count cells=6015)	54.85 ± 3.84 (n=6, count cells=2688)	22.37 ± 2.45 (n=6, count cells=1096)

Table 1. Cell count in numbers