Mechanisms of action of antipsychotic drugs: 
Time-course and pattern of AP-1 complex and ATF/CREB immediate 
early gene expression on chronic treatment with antipsychotics. 

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ABSTRACT 

Acute, intermediate, and chronic effects of clozapine, haloperidol, and risperidone on 
AP-1 complex and CREB immediate early gene expression in the medial prefrontal 
cortex and the cingulate/motor cortices were quantified. Rats were subjected to clozapine, 
haloperidol, risperidone and saline as control for three different durations of two weeks, 
four weeks and eight weeks. Neurons immunoreactive to CREB-1 and c-fos were 
oberved and statistical analysis performed to assess the significance of the results. While 
there is a need to increase sample size, there appeared to be a significant up-regulation in 
c-fos expression in rats treated with risperidone, suggesting that risperidone play a role in 
increasing neuronal activity in schizophrenic rats. 

Schizophrenia refers to a group of mental illnesses or related disorders characterized 
by delusions, prominent hallucinations, disorganized speech, grossly disorganized or 
catatonic behaviour and negative symptoms such as affective flattening, alogia, or 
avolition in the affected individual. The current treatment involves the use of 
antipsychotic drugs (APD) for example, haloperidol and clozapine. The latter, an atypical 
antipsychotic drug, has been more popular clinically as it induces fewer extrapyramidal 
side effects (EPS) while alleviating cognitive impairments in the patient. 

The therapeutic effects of all APD can be attributed to their ability to bind to the 
dopamine receptors (DAR) and antagonize the action of dopamine. However, as the APD 
display selectivity in their binding with DAR, the localization of the blocked DAR marks 
a possible reason for the difference in the therapeutic effects in typical and atypical 
APD. 

The central serotonergic system has also been suggested to be implicated in 
schizophrenia. While serotonergic mechanisms appears to remain intact in schizophrenia, 
the receptor density of 5-HT3 in the human brain is extremely low, thus suggesting that 
the deficit could play a role in the pathogenesis of schizophrenia. On the other hand, 5-
HT2 receptor antagonists have been found to be efficient in treating schizophrenia 
symptoms, thus also suggesting the involvement of the 5-HT2 receptors. 

Rats were randomly assigned to four treatment groups (n=12). One group was treated 
with vehicle (acidified water) while the other three groups each treated with a different 
APD (haloperidol, clozapine, and risperidone). Each treatment group was further divided 
into 3 sub-groups (n=4) receiving 2-weeks, 4-weeks, or 8-weeks treatment. At the end of 
the treatment, they were terminally anesthetized and the brains fixed through perfusion 
with formalin. Coronal blocks were made and embedded in paraffin wax before
sectioning into 8 um using a microtome. Sections were mounted on Poly-L-Lysine solution coated slide.

Immunocytochemical methods were used to visualize the presence of both the CREB-1 and c-fos proteins. After removal of the paraffin with xylene, tissues were first incubated with hydrogen peroxide to quench endogenous peroxidase action and then with pepsin to ensure the proper binding of the primary antibody to the antigen of interest. Sections were then incubated with the primary antibody at a 1:50 concentration for 24 hours at 4°C. At the end of the incubation period, sections were again incubated, first with biotinylated secondary antibody followed by avidin and biotin conjugated horse radish peroxidase (ABC-HRP). Diaminobenzidine (DAB) was then added, and a brown precipitate observable under the light microscope on positive immunoreaction. The sections are then mounted with cover slips for viewing under the microscope and digital images are taken to count the number of immunoreactive cells.

An analysis of variance (ANOVA) was first performed in order to ascertain if the results of the overall experimental design shows any significance. In the presence of significance, the effects of the various contributors were then subsequently tested. Within each variable, the Tukey HSD test was used to ascertain if there was any significance between any two treatments. The results were then tabulated in the least-squares means table and plotted in the least-squares chart. Non-parametric methods were used as the sample size was small and therefore, of non-normal distribution.

Significant differences in the different treatments were found for CREB-1 in both the
medial prefrontal cortex (mPFC) \( (F_{11, 35}= 3.48, P< 0.0024) \) and the cingulate/motor cortex (CGC) \( (F_{11, 35}= 4.53, P< 0.0003) \). In the CGC, the effects of the different drug groups \( (F_3, 35= 4.61, P< 0.008) \) and the effects of the different durations \( (F_2, 35= 5.60, P< 0.0077) \) were found to be significant. The interaction between drug groups and durations, however, were insignificant \( (F_6, 35= 2.09, P< 0.08) \). In the mPFC, the effects of the two variables—groups \( (F_3, 35= 7.08, P< 0.0008) \); and the interaction between drug groups and durations \( (F_{11, 35}= 3.78, P< 0.0052) \)—were significant. The effects of the durations of treatment \( (F_2, 35= 2.96, P< 0.06) \) were not significant.

Significant differences were also found in the different treatments for c-fos in both the mPFC \( (F_{11, 34}= 13.72, P< 0.0001) \) and CGC \( (F_{11, 34}= 6.25, P< 0.0001) \). In the CGC, the effects of the three variables were significant—groups \( (F_3, 34= 6.88, P< 0.001) \); duration \( (F_2, 34= 13.31, P< 0.0001) \) and the interaction between groups and duration \( (F_6, 34= 3.52, P< 0.008) \). In the mPFC, the three variables were also found to be significant \( (F_3, 34= 19.35, P< 0.0001) \) for groups; \( (F_2, 34= 22.98, P< 0.0001) \) for weeks; \( (F_6, 34= 7.33, P< 0.0001) \) for the interaction between drug groups and duration.

The functionality of APD is generally attributed to the selective blocking of dopamine (DA) receptors throughout the brain and the body (Kalant, 1998). One feature common to all APD is their ability to bind to the dopamine receptors and antagonize the action of dopamine. The therapeutic sites of APD are the D_2 receptors, which are localized in the caudate and putamen. The atypical APD bind more effectively to D_3 and D_4 receptors, which are primarily expressed in the limbic system and cortex. The localization of the various DA receptors has been suggested to be important in the induction of EPS (Kandel et al., 2000).

The dopaminergic system arises from groups of cells in the midbrain and hypothalamus, and is organized into four major systems—the tuberoinfundibular, nigrostriatal, mesolimbic, and mesocortical systems. The nigrostriatal system consists of neurons ascending from the substantia nigra to the striatum, and being primarily involved in the modulation of motor behaviour, is thought to be responsible for the parkinsonian side effects induced by typical APD. The other prominent midbrain projections arise from the ventral tegmental area project to various limbic regions (mesolimbic pathway) and cortical areas, the latter will include the CGL and PFC (mesocortical pathway). These neurons are involved in cognition, modulation of motivation, reward-linked behaviour, and emotion (Kalant, 1998).

Previous studies have indicated the efficacy of HAL to be related to increasing DA turnover in the mPFC (Hernandez & Hoebel, 1989). In the CGC however, since HAL is unable to bind to D_3/D_4 receptors, it does not enhance DA turnover within the area. The up-regulation of CREB-1 and c-fos in the CGC could correlate to an increased synthesis of DA within the CGC and this would subsequently result in a hyperactivity of the mesolimbic and mesocortical pathway. The role of HAL in activating the two pathways is probably the basis for its inability to ameliorate cognitive impairments.

The central serotonergic system has also been suggested to be implicated in schizophrenia (Owen & Cross, 1992). Three serotonin receptor subtypes have been known to date: 5-HT_1, 5-HT_2, and 5-HT_3. While serotonergic mechanisms appears to remain intact in schizophrenia, the receptor density of 5-HT_3 in the human brain is extremely low. This deficit could play a role in the pathogenesis of schizophrenia. On the other hand, 5-HT_2 receptor antagonists have been found to be efficient in treating
schizophrenia symptoms, as illustrated by the atypical APD risperidone (Tnmble, 1996), thus suggesting the involvement of the 5-HT₂ receptors. Close examination of anatomical connectivity of the serotonergic and dopaminergic systems in the brain reveals several levels at which modulation of dopaminergic function by the serotonergic system could occur. 5-HT₂ receptors are found in high density in the limbic cortical areas, suggesting that axo-axonic modulation can occur (Pazos et al., 1985).

There appeared to be a rapid increase in c-fos synthesis in the PFC for all the drug groups from the 2nd week of treatment to the 4th week. This strongly suggests that all APD induce increased neuronal activation upon administration. However, differences for the mechanism of typical and atypical APD can be justified to the trend in the 8-weeks treated rats. The lowered c-fos protein expression for the HAL-treated rats as compared to CLZ and RIS appears to support in vivo imagining studies which have suggested that a reduction in neuronal activity of the PFC might underlie the symptoms of schizophrenia (Berman et al., 1986). This would therefore explain why HAL is not as therapeutically effective as the atypical APD. Also, the decreased synthesis of c-fos proceeding from the 4-weeks treated to the 8-weeks treated rats appears to support Sebens et al. (1995)’s statement that long-term treatment with APD leads to the development of differential biochemical tolerance, which could contribute to their differing clinical profile.

The results as obtained in this study appear to suggest the potentiality of the risperidone being a therapeutically effective agent. This can only be assessed with the administration of more comparative experiments. At the same time, a larger sample size is necessary for results to be more conclusive.

While the mechanisms of schizophrenia is still largely unknown, the dopaminergic pathway serves to be the most likely pathway involved in the mechanism of antipsychotic drugs. However, more studies are starting to examine the interaction of the serotonergic pathway with the dopaminergic pathway as a likely mechanism for antipsychotic drug action.

REFERENCES

Hernandez L & Hoebel BG. (1989) Haloperidol Given Chronically Decreases Basal Dopamine in the Prefrontal Cortex more than the Striatum or Nucleus Accumbens as Simultaneously measured by microdialysis. Brain Res. Bull 22: 763-769