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# Regulation of gene expression in brain areas following chronic exposure to cannabinoids

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The use of marijuana and hashish, which goes back centuries, is widespread in human populations. The abuse of these substances is attributed to the psychotropic effects of the principal active compound, $\Delta^9$-THC, and to the euphoric sensation it imparts to the user. While there was debate about the addictive potency of $\Delta^9$-THC, it is now accepted that cannabinoids are addictive, as animals self-administer these drugs and withdrawal symptoms appear upon the injection of cannabinoid antagonist following chronic cannabinoid exposure.

Previous experiments by us and others have shown that chronic exposure to drugs of abuse (including cannabinoids) induces changes in the expression of several specific gene products. Most of this work has been done by analyzing the effects of the administered ligand on the expression of the mRNA of individual genes. However, recent developments in gene array technology provide a faster and more advanced approach to investigating the changes in gene expression for thousands of genes simultaneously. Our goal is to identify the genes affected by acute and chronic exposure to cannabinoids, as well as following cannabinoid withdrawal, and to subsequently determine the role of these gene products in regulating the rewarding and addictive properties of these drugs. We are focusing on specific rat brain regions, which have been shown to play a role in reward and addiction, *e.g.* the nucleus accumbens (NAc), the ventral tegmental area, and the medial prefrontal cortex. The dorsal striatum, which contains cannabinoid receptors and is in close proximity to the NAc, but is not involved in reward, is examined in parallel as a control brain area. The results for mRNAs of interest are corroborated by other methods (such as quantitative real-time PCR), and their distribution in the brain determined by *in situ* hybridization. Subsequently, we are using various immunological approaches (immunohistochemistry, Western blotting) to follow the changes in expression of these genes at the protein level.

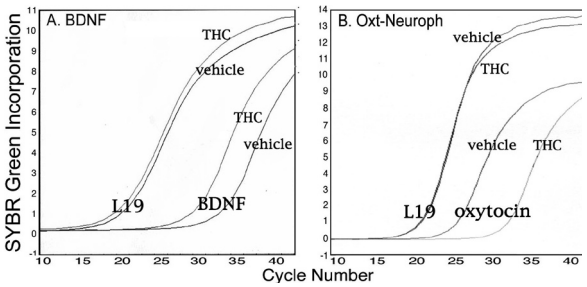


**Fig. 1** *Quantitative real-time PCR analysis for BDNF and oxytocin-neurophysin. Changes in gene expression are shown, compared with L19 (used for calibration), in the NAc of control and $\Delta^9$-THC (1.5 mg/kg/day for 7 days)-treated rats.*

Our preliminary experiments demonstrated that this approach is successful. Utilizing the U34A Affymetrix rat GeneChip system, we found that the expression of 40 genes was upregulated and 6 downregulated (by more than 2 fold) by one week exposure to a physiologically rewarding concentration of $\Delta^9$-THC (1.5 mg/kg/day i.p.). The results for several of these gene products, including the growth factors brain-derived neurotrophic factor (BDNF), platelet-derived growth factor (PDGF), and glial-derived neurotrophic factor (GDNF) (which were upregulated) and for oxytocin-neurophysin (which was downregulated) were validated by real-time quantitative PCR. In addition, we were able to show changes in protein immunostaining for BDNF and oxytocin-associated neurophysin in the NAc and certain other specific brain areas (see examples in Figs. 1 and 2).

Knowledge of the genes whose expression is affected by cannabinoid exposure will allow us, in the more distant future, to study their role in reward and drug abuse. We intend to use mice in

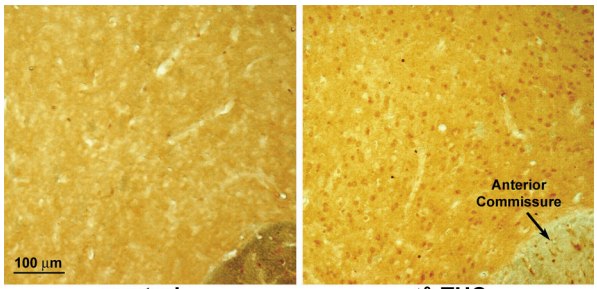


**Fig. 2** *BDNF staining in the NAc. Rats were treated with $\Delta^9$-THC or vehicle (control). Sections were treated with chicken anti-BDNF followed by biotinylated goat anti-chicken antibody, and avidin–biotin–peroxidase.*

which such genes are lacking or modified (knocked out, reduced, overexpressed, etc.), and test the rewarding and addictive effects of cannabinoids using behavioral tests such as conditioned place preference. In this regard, it is important to note that heterozygous BDNF knockout mice (homozygotic BDNF knockouts are not viable) show an altered response to cocaine, and we would like to study their response to cannabinoids. Mice lacking the oxytocin gene are viable, and can thus be used to study the role of this gene in the cannabinoid reward mechanism. Similar experiments with other transgenic mice will become possible when more genes are identified (by us and others) as being affected by the cannabinoid exposure. Another approach is to inject, into the desired brain areas, Lentivirus expressing specific siRNA to the genes of interest.

These studies should advance our understanding of the genes regulated by cannabinoid treatment and their interrelationships, and of the role of these gene products in regulating the rewarding properties of the cannabinoids. These studies will eventually lead to a better understanding of the adaptive changes that take place during chronic exposure to these widely abused drugs, and could eventually allow the identification of gene products and signaling steps that could be targeted, in the future, for pharmaceutical intervention to prevent or reverse drug addiction.

### Selected Publications

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