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Molecular mechanisms of cannabinoid addiction

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Cannabis is the world's most widely used illicit substance, with an estimated number of 119–224 million users worldwide. In recent years we assisted to an increased effort aimed to individuate the brain circuits underlying cannabis addiction and dependence. Similarly to other drugs of abuse, repeated exposure to cannabinoids causes brain neuroadaptations that persist long after drug effects, contribute to the negative affective states during withdrawal, and ultimately facilitate relapse. Recently, considerable progress has been made in understanding the cellular and molecular consequences of prolonged cannabis use, among which is the identification of specific set of transcriptional regulations that develop differently after chronic cannabinoids and in the abstinent brain.

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Introduction

According to the last World Drug Report, cannabis still remains the most widely used drug worldwide [1], and is able to induce craving [2], dependence [3], and drug-seeking behavior [4]. A withdrawal syndrome has been described after chronic marijuana smoking, characterized by a time-dependent constellation of symptoms including irritability, anxiety, craving, sleep disturbances, and decreased appetite [5].

Over the last two decades, cannabinoid research has made huge progress. A major breakthrough has been the discovery of a multifaceted endogenous cannabinoid system (ECS), a neuromodulatory system comprised of: firstly, cannabinoid receptors (CBRs), among which the subtype 1 (CB1R) is the most abundant in the brain, secondly, endogenous ligands for CBRs, like anandamide and 2-arachidonylglycerol, thirdly, a putative membrane transporter, and finally, enzymes involved in the synthesis and inactivation of the endogenous ligands [6,7]. The CB1R was initially identified as the neuronal target of Δ(9)-tetrahydrocannabinol (THC), the major psychoactive ingredient of marijuana, and numerous endocannabinoid-like compounds have been identified so far.

Recent developments in cellular and molecular neurobiology have provided new tools for disentangling the mechanisms by which cannabinoids produce long-lived alterations in brain functioning, may facilitate the development of drug dependence and lead to compulsive drug-seeking. Here we highlight latest advances in the understanding of the consequences of prolonged exposure to cannabinoids on several components of the brain neuronal circuits, among which are neurotransmitters receptors, signaling cascades, and gene expression.

Synaptic plasticity

Neuronal circuits and their elements undergo important adaptations in response to external insults, including exposure to addictive drugs. By modulating the number and strength of neuron–neuron connections, synaptic plasticity (i.e. the dynamic adjustment of synaptic efficacy occurring in response to environmental or internal stimuli) is unanimously recognized as a major mechanism for experience-dependent changes in brain neuronal responsiveness.

Endocannabinoids suppress presynaptic glutamate release, leading to a depolarization-induced suppression of excitation (DSE), and inhibit presynaptic GABA release, leading to depolarization-induced suppression of inhibition (DSI), both effects lasting less than one minute. Accordingly, cannabinoid agonists (like THC) not only act via presynaptic CB1Rs to inhibit release of glutamate and GABA in the striatum [8], but also cause long-term forms of synaptic plasticity like long-term potentiation (LTP) and long-term depression (LTD), that is, persistent increase and weakening, respectively, in the strength of synaptic signaling that can last hours or weeks.

At the mouse prefrontal cortex (PFC)-nucleus accumbens (NAc) synapses, endocannabinoid-mediated LTD is abolished after an intraperitoneal injection of a non-aversive dose of THC (3 mg/kg). Yet, LTD can still be observed after repeated (1 week) drug administrations, suggesting that prolonged exposure to THC triggers

compensatory mechanisms allowing the recovery of LTD expression [9]. Notably, the same treatment induces functional tolerance and desensitization in the NAc core CB1Rs [9] (see 'Changes in CB1R density and function' section). Preservation of LTD after 1-week THC described by Mato *et al.* [9] is in contrast with the finding of an abolished endocannabinoid-mediated LTD of excitatory signaling in the rat NAc after 1-week of exposure to a higher dose of THC (10 mg/kg), an effect that authors ascribed to a downregulation of CB1R function at both excitatory and inhibitory synapses [10]. Yet, differences in THC doses (3 mg/kg versus 10 mg/kg) and rodent species (mice versus rats) used in the two studies may account, at least partly, for the reported discrepancies.

Changes in CB1R density and function

CB1R is coupled predominantly to G-proteins of the Gi/o class, which implies that its activation inhibits adenylate cyclase and voltage-gated Ca^{++} channels, activates G-protein-coupled inwardly rectifying K^+ channel, and hinders synaptic transmission. Uncoupling and downregulation of brain CB1Rs after prolonged THC exposure are key players in the development of cannabinoid addiction and tolerance [11]. Repeated THC treatment reduces the coupling efficacy of CB1Rs to Gi/o transduction proteins in the NAc, which could respond for the reported loss of CB1R-mediated synaptic function after chronic THC [10] (see 'Synaptic plasticity' section). The magnitude of desensitization following chronic THC is greater in adolescent female animals than in adults or males [12•], confirming the female sex and adolescence as vulnerability factors to disruption of CB1R-mediated signaling by cannabis abuse [13] (see Figure 1). CB1R adaptations that occur following subchronic THC administration are regulated in a region-dependent manner by beta-arrestin2 [14••], which is one of two arrestin isoforms in the brain which mediates receptor desensitization, internalization, and signaling. Region-dependent neuroadaptations were reported during spontaneous cannabinoid withdrawal in mice [15], after cannabinoid self-administration in rats [16], and in humans (see Table 1). Brains of chronic cannabis smokers display decreased mRNA expression in several limbic area [17] and a reversible and regionally selective downregulation of cortical CB1Rs [18••]. Yet, cannabinoid-induced alterations in the number and function of CB1Rs recover within weeks upon cessation of exposure to the drug [18••], implying additional mechanisms in mediating long-lasting neurobiological changes.

Signaling cascades

The members of the mitogen-activated protein kinase (MAPK) signaling system, such as Raf-1 (an effector of the Ras proteins), MEK1/2 (MAPK kinase), JNK (c-Jun N-terminal kinase) and ERK (extracellular signal-regulated kinases), are major modulators of cell functions, including proliferation, differentiation, and survival. In particular, the MAPK/ERK1/2 cascade plays a key role in neuronal plasticity and is activated by drugs of abuse, including cannabinoids [19]. Activation of this cascade by acute THC exposure in the caudate putamen and cerebellum shows homeostatic adaptation after chronic treatment; yet, it is increased in the PFC and hippocampus after repeated THC exposure, ventilating the hypothesis that different neuronal circuits might be involved in the early and late phases of cannabis addiction [20]. Repeated THC administrations induce neurophysiological adaptive mechanisms at cerebellar parallel fiber (PF)-Purkinje cell (PC) synapses which are dependent on the ERK pathway activation [19]. These alterations deregulate the physiological long-term synaptic plasticity between PF and PC, potentially affecting cerebellar functions. In light of the evidence that cerebellum is a critical area for the chronic effects of THC and for the expression of THC withdrawal [21,22], these forms of pathological synaptic plasticity might play a role in cannabinoid dependence likely contributing to the development of behavioral tolerance.

Chronic THC increases the expression of brain derived neurotrophic factor (BDNF), a neurotrophin involved in the differentiation, survival, and repair of neurons in reward-related brain areas of rats [23]. CB1R knockout mice exhibited decreased BDNF levels in the hippocampus [24], while adolescent cannabinoid exposure induced long-term, sex-dependent effects on hippocampal BDNF levels [25] (see Figure 1). Intriguingly, BDNF levels were found to change over time in both light users of cannabis and healthy control subjects receiving THC intravenously over 20 min at a dose resembling recreational cannabis use [26••]. Yet, serum BDNF levels increased more rapidly in healthy controls than in light users, which in turn had lower basal serum BDNF levels [26••]. The expression of BDNF is partially regulated by the transcription factor cAMP responsive element binding protein (CREB), which represents a central integrator of signaling from a number of extracellular stimuli that influence neuronal plasticity and survival. Chronic THC exposure markedly attenuates phosphorylation of CREB in the rat cerebellum [27] and mouse hippocampus [28], an effect that persists three weeks after withdrawal from THC [27], suggesting that reduced levels of CREB account for the impaired long-term hippocampal synaptic plasticity induced by THC exposure (see 'Synaptic plasticity' section).

Changes in gene expression

A number of gene modifications occur in the brain during drug abstinence, mostly related to three main molecular networks: the glutamate/corticoids and the CREB/ERK networks at the level of hypothalamus, striatum and amygdala [29–31], and a third one involving Nf-κB, a transcription factor regulating addiction-induced