Commentary

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# New Insights Into the Neural Consequences of Synthetic Cannabinoids During Adolescence: The Critical Role of Reelin at Prefrontal Synapses

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The recreational use of synthetic cannabinoids (SCs) has increased markedly in recent years, with a particularly high prevalence among young people (1). Originally designed to mimic the effects of $\Delta^9$-tetrahydrocannabinol (THC), the main psychotomimetic compound in cannabis, SCs have proliferated in the illicit drug market under the generic term "new psychoactive substances" (2). This heterogeneous group of substances often exhibits a higher binding affinity for the $CB_1$ cannabinoid receptor (CB1R) than THC, which acts as a partial agonist at CB1Rs. Additionally, SCs interact with other components of the endocannabinoid system, potentially resulting in distinct pharmacological effects and diverse toxicity profiles. These characteristics make SCs unsuitable for medical use because they have a high potential for abuse and addiction, which poses significant challenges for human dosing. Reports of acute intoxications, hospitalizations, and, in some cases, fatalities, particularly coinciding with the general trend toward the appearance of increasingly potent SCs (3), underscore the global public health concern posed by these substances.

Considering the acknowledged risks associated with SC use during adolescence and the substantial prevalence of experimentation during this critical developmental period (4,5), it is crucial to address the limited understanding of the neurobiological effects of early SC initiation and the long-lasting consequences of initial exposure to these substances. This is particularly relevant in the prefrontal cortex (PFC), which is the latest brain region to fully mature in both humans and rodents and particularly vulnerable to cannabinoid exposure during adolescence.

The glycoprotein reelin regulates the developmental trajectory of pyramidal PFC neurons and associated behaviors, making it a strong candidate in the etiology of several psychiatric disorders that are accompanied by cognitive and executive function deficits associated with PFC dysfunctions, including schizophrenia, major depression, bipolar disorders, and autism spectrum disorder. During adolescence, reelin regulates long-term synaptic plasticity and spinogenesis in the PFC, as well as fear memory processing through glutamatergic signaling, suggesting that reelin is essential for the proper structural, functional, and behavioral development of juvenile prefrontal circuits (6). Furthermore, reelin is necessary for the correct maturation and refinement of GABAergic (gamma-aminobutyric acidergic) synaptic circuits and for the physiological excitatory-inhibitory balance in the maturing PFC (7). Considering the vulnerability of reelin to environmental insults (including nutritional stress, exposure to drugs of abuse), understanding whether reelin serves as a link between initiation of SC use during adolescence and prefrontal dysfunctions remains crucial.

A recent study by Silva-Hurtado *et al.* (8) in *Biological Psychiatry: Global Open Science* identified and thoroughly characterized a previously undisclosed mechanism that underlies the cellular and synaptic consequences of a single exposure to the synthetic cannabinoid WIN 55,212-2 (which shows higher potency than THC for CB1Rs) in adolescent male mice, highlighting a functional interaction between CB1R and the reelin signaling pathway in the PFC.

The authors first showed that a single intraperitoneal injection of WIN 55,212-2 impacted the cellular density of reelin 24 hours after treatment via CB1Rs in specific PFC layers. Specifically, acute administration of WIN 55,212-2 significantly decreased reelin-positive cell density in PFC layers 2/3 and 5/6 (but not layer 1) of WIN-treated mice, and co-administration of the CB1R antagonist SR141716A normalized these effects. These results suggested a layer-specific decrease in reelin levels induced by WIN 55,212-2 acting on CB1Rs.

To evaluate the coexpression of CB1R and reelin-positive neurons in layers 2 to 6 of the adolescent mouse PFC, Silva-Hurtado *et al.* (8) analyzed the expression profile of reelin in the PFC of naïve male mice. They found that reelin-positive neurons expressed GABAergic or glutamatergic markers across superficial (layers 2/3) and deep (layers 5/6) PFC layers, with reelin-positive cells being primarily positive for GABAergic markers. Reelin was also found to be coexpressed with the neuropeptide cholecystokinin, with a similar distribution across PFC layers. Additionally, CB1Rs were expressed across layers 2 to 6, and reelin showed a high level of coexpression with CB1R in both superficial and deep PFC layers. These findings represent the first evidence of an enrichment of CB1Rs in postnatal prefrontal reelin-expressing neurons beyond their previously recognized coexpression during the embryonic stage.

These intriguing observations were also supported by pharmacological and genetic experiments, which revealed that the reduction of reelin levels induced by WIN 55,212-2 was 1) blocked by the CB1R antagonist SR141716A and 2) abolished in CB1R knockout mice.

Based on the results indicating that most neurons that coexpressed reelin and CB1R in the PFC were GABAergic, the authors used conditional knockout mice to specify the cell types of CB1R-expressing neurons responsible for the reduction in reelin expression. Notably, CB1R deletion in GABAergic neurons (but not in glutamatergic neurons) abolished the reduction in prefrontal reelin expression 24 hours after SC exposure, highlighting an obligatory role of CB1Rs on GABAergic neurons in regulating reelin expression.

Interestingly, a single SC exposure also modulated the proteolytic fragmentation of reelin; for example, 24 hours after

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exposure to WIN 55,212-2, the amount of full-length reelin protein decreased together with the amount of one of the cleaved reelin products containing the N-terminal domain (i.e., N-R6 fragment). No changes in reelin messenger RNA levels were observed, excluding any alteration in transcription. This reduction supports the notion of decreased secretion combined with increased N-terminal processing of reelin in the presence of SCs.

The alterations in reelin expression triggered by SC exposure also affected cortical theta burst stimulation–induced long-term potentiation (TBS-LTP) at deep PFC excitatory synapses, consistent with previous evidence from the same group that showed that normal levels of reelin were necessary for the expression of NMDA receptor–dependent LTP in the PFC (6,7). Specifically, the magnitude of TBS-LTP was significantly reduced in WIN-treated mice 24 hours postinjection and remained diminished for several days after drug clearance. Notably, this LTP reduction was absent when the SC was coadministered with a CB1R antagonist. To explore the underlying mechanisms, the authors investigated whether the reduction in TBS-LTP was due to altered transcription of NMDA receptor subunits. However, no significant differences were found in the messenger RNA levels of NMDA receptor subunits in the PFC of WIN-treated mice compared with controls.

Overall, these results raise an intriguing question about whether specific N-terminal reelin fragments are required for the adequate expression of synaptic plasticity at prefrontal glutamatergic synapses. The authors hypothesized that in the absence of SC exposure, the regulation of N-terminal cleavage may be important for controlling the diffusion of the N-R6 fragment from the site of reelin secretion, allowing it to reach and activate signaling at distant glutamatergic synapses and facilitate TBS-LTP.

Finally, the authors established a functional relationship between CB1R-induced reelin reduction and LTP impairment using two approaches. First, they demonstrated that the LTP impairment following WIN 55,212-2 exposure closely resembled the impairment that has been observed in a mutant mouse model that is haploinsufficient for reelin, highlighting that a genetic reduction in endogenous reelin mimics the detrimental effects of SC on PFC plasticity. Second, they showed that bilateral injection of recombinant reelin into the PFC of WIN-treated mice completely prevented the WIN-induced decrease in TBS-LTP, thereby restoring LTP magnitude. This further supports the hypothesis that reelin downregulation functionally contributes to the synaptic impairments induced by SC exposure.

Overall, the work by Silva-Hurtado *et al.* (8) highlights the detrimental effects of SC initiation and establishes a causal link between CB1R activation and deficits in prefrontal reelin signaling for the first time. One compelling hypothesis is that CB1R activation by WIN 55,212-2 reduces reelin secretion in the PFC, thereby impairing its paracrine function on surrounding excitatory neurons. As noted by the authors, the molecular mechanisms by which CB1R activation disrupts reelin proteolysis require further investigation, as do potential sex- and age-specific effects (9,10).

Although SCs have become popular among adolescents for recreational purposes, the long-term effects of early exposure to these substances remain largely underexplored. The study by Silva-Hurtado *et al.* (8) represents a significant step forward in understanding how adolescent exposure to CB1R agonists can disrupt synaptic plasticity mechanisms and provides the first evidence of a functional connection between reelin and CB1Rs in GABAergic neurons during developmental stages. This knowledge is critical for translational applications, including informing public policies and educational strategies aimed at addressing the potential risks associated with SCs.

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## Article Information

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