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Cannabinoid-induced motor dysfunction *via* autophagy inhibition

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ABSTRACT

The recreational and medical use of cannabis is largely increasing worldwide. Cannabis use, however, is associated to several undesired and possibly dangerous side effects, so it is crucial that innovative studies aimed to understand and potentially reduce cannabis-evoked harms are conducted. Previous research conducted on cultured neural cells had supported that cannabinoid CB_1 receptor (CB_1R), the main molecular target of cannabis, can alter autophagy. However, it was not known whether CB_1R controls autophagy in the brain *in vivo*, and, eventually, what the functional consequences of a potential CB_1R/autophagy connection could be. We have now found that Δ^9-tetrahydrocannabinol (THC), the major intoxicating constituent of cannabis, impairs autophagy in the mouse striatum. Administration of autophagy activators (specifically, the rapalog temsirolimus and the sugar trehalose) rescues THC-induced autophagy inhibition and motor dyscoordination. The combination of various genetic strategies *in vivo* supports that CB_1R molecules located on neurons belonging to the direct (striatonigral) pathway are required for the autophagy- and motor-impairing activity of THC. By identifying autophagy as a mechanistic link between THC and motor performance, our findings may open a new conceptual view on how cannabis acts in the brain.

KEY WORDS

Cannabinoid; autophagy; striatum; motor behavior; mTOR; drug abuse

Cannabis is the third most popular drug of abuse in the world following alcohol and tobacco, and its use is rapidly increasing. To date, four countries in the world and a dozen territories in the USA have legalized the recreational use of cannabis. In addition, medicinal-cannabis dispensation programs have been implemented in about thirty countries globally, as well as in a similar number of states in the USA, and several cannabinoid-based medicines have been approved by foremost regulatory agencies (*e.g.*, FDA, EMA and Health Canada) as anti-emetic, anti-cachexic, analgesic and anti-spastic adjuvants. However, cannabis use is associated to several undesired and possibly dangerous side effects, so it is crucial that innovative procedures aimed to understand and potentially reduce cannabis-evoked harms are explored. Δ^9-Tetrahydrocannabinol (THC), the key intoxicating constituent of cannabis, exerts its biological effects mainly by activating cannabinoid CB_1 receptor (CB_1R), one of the most abundant metabotropic receptors in the mammalian central nervous system. This receptor is particularly expressed in discrete brain areas involved in the control of learning and memory (cortex, hippocampus), motor behavior (striatum, cerebellum), emotions (amygdala) and autonomic and endocrine functions (hypothalamus, pons, medulla), therefore participating in the modulation of a wide plethora of biological processes. Recent evidence had suggested that CB_1R controls autophagy in cultured neural cells. Strikingly, however, in some cellular settings cannabinoids *via* CB_1R enhance autophagy, while in others they inhibit autophagy. Moreover, it was not known whether CB_1R controls autophagy in the brain *in vivo*, and, eventually, what the functional consequences of a potential CB_1R/autophagy connection could be.

In a recent study [1] we have explored the effect of THC on autophagy in the mouse brain. A single injection of the drug (at 10 mg/kg body weight) concertedly increased LC3-II and p62 levels in the striatum but not in other representative brain

regions as the cortex, the hippocampus and the cerebellum. THC also increased LC3-II and p62 levels in primary cultures of mouse striatal neurons. However, when an accumulation of LC3-II and p62 was achieved by incubating the cells with lysosomal inhibitors (either hydroxychloroquine or an E64d/pepstatin A cocktail), THC was unable to heighten those autophagy protein levels further. Hence, THC seems to inhibit autophagy in striatal neurons both *in vivo* and *in vitro*.

Next, we studied the biological impact of this THC-evoked inhibition of striatal autophagy. For this purpose, mice were subjected to tests of motor behavior, an archetypical process that is controlled by the striatum and impacted by cannabis. As expected, THC impaired motor coordination, as determined by the RotaRod test, and motor activity, as determined by the open-field test. Strikingly, boosting striatal autophagy either by pharmacological manipulation (with the rapalog temsirolimus) or dietary intervention (with the disaccharide trehalose) rescued THC-induced motor dyscoordination but not THC-induced motor inactivity, thus indicating that autophagy selectively modulates the coordination component of overall motor behavior.

What may be the neuroanatomical substrate of the observed THC effects? First, we proved the participation of CB_1R by systemically administering the CB_1R-selective antagonist SR141716 (rimonabant) to wild-type mice. Second, we found that the THC-evoked inhibition of striatal autophagy and motor coordination was not evident in mice in which the gene encoding CB_1R had been knocked-out in striatal neurons belonging to the direct (striatonigral) pathway. Third, in contrast, we observed that mice in which the gene encoding CB_1R had been knocked-out in corticostriatal projections (which are usually considered a key determinant of striatal activity) were fully responsive to the THC-evoked inhibition of striatal autophagy and motor coordination. And fourth, an array of experiments conducted on mice selectively expressing (*i*) reporter genes for

direct-pathway and indirect-pathway (striatopallidal) neurons, (*ii*) dominant-negative Raptor in direct-pathway neurons, or (*iii*) p62 in direct-pathway neurons, provided further support to the selective participation of CB_1R molecules located on neurons belonging to the striatal direct pathway in the autophagy-inhibiting and motor-dyscoordinating activity of THC.

Taken together, these findings suggest that impairment of autophagy may be an unprecedented mechanism involved in at least some cannabinoid-induced motor alterations. On molecular grounds, our data would favor a “two-hit” model by which engagement of CB_1R may impair autophagy. First, CB_1R activation, through its well known coupling to the phosphatidylinositol-3-kinase/Akt/mTORC1 pathway, could lead to ULK1 phosphorylation, which, subsequently, would inhibit autophagosome formation/autophagy initiation. Second, CB_1R activation, by a hitherto undefined mechanism that may conceivably involve an impact on lysosomal function, would inhibit autophagosome clearance/autophagy completion (Figure 1).

We are aware, however, that our work has several shortcomings that could limit the generalization of its conclusions to other experimental conditions (*e.g.*, other types and doses of cannabinoids, times of cannabinoid treatment, and behavioral traits). We also note that our work does not unveil the precise cellular and molecular mechanisms by which the CB_1R-evoked inhibition of autophagy in striatal direct-pathway neurons affects brain functionality to impact on motor coordination. Nonetheless, neuronal communication is known to be sensitive to the status of both the mTORC1 pathway and proteostatic processes as autophagy, which, for example, may clear dysfunctional proteins and fine-tune the trafficking/recycling of membrane neurotransmitter receptors. Hence, it is conceivable that the control of neurotransmission exerted by CB_1R on the striatal direct pathway might be mechanistically connected to the THC-evoked effects

123 on mTORC1/autophagy shown in our study. Further research will be necessary to
124 deepen into the mechanistic and biological details of cannabinoid anti-autophagic
125 action. These issues notwithstanding, our findings might be applicable not only to motor
126 behavior but also to other neurobiological processes that are known to be controlled by
127 the striatum and impacted by cannabis (*e.g.*, cognition, affection and reward). Moreover,
128 from a translational standpoint, our data add to previous reports suggesting that
129 targeting the mTORC1 pathway (and now, as shown in our study, autophagy) might
130 provide a rationale for designing strategies aimed to manage some particular behavioral
131 alterations induced by cannabis consumption.
132

DISCLOSURE STATEMENT

We declare no competing financial interests.

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FIGURE LEGEND

Figure 1

Scheme depicting the impact of THC on striatal autophagy and motor coordination

THC, the main intoxicating ingredient of cannabis, would bind to CB_1R molecules located on neurons belonging to the striatal direct (striatonigral) pathway. Upon engagement, CB_1R, by (*i*) activating the mTORC1 pathway and (*ii*) blocking autophagolysosomal function, would inhibit autophagy. This, in turn, would impair motor coordination by a hitherto unknown mechanism.

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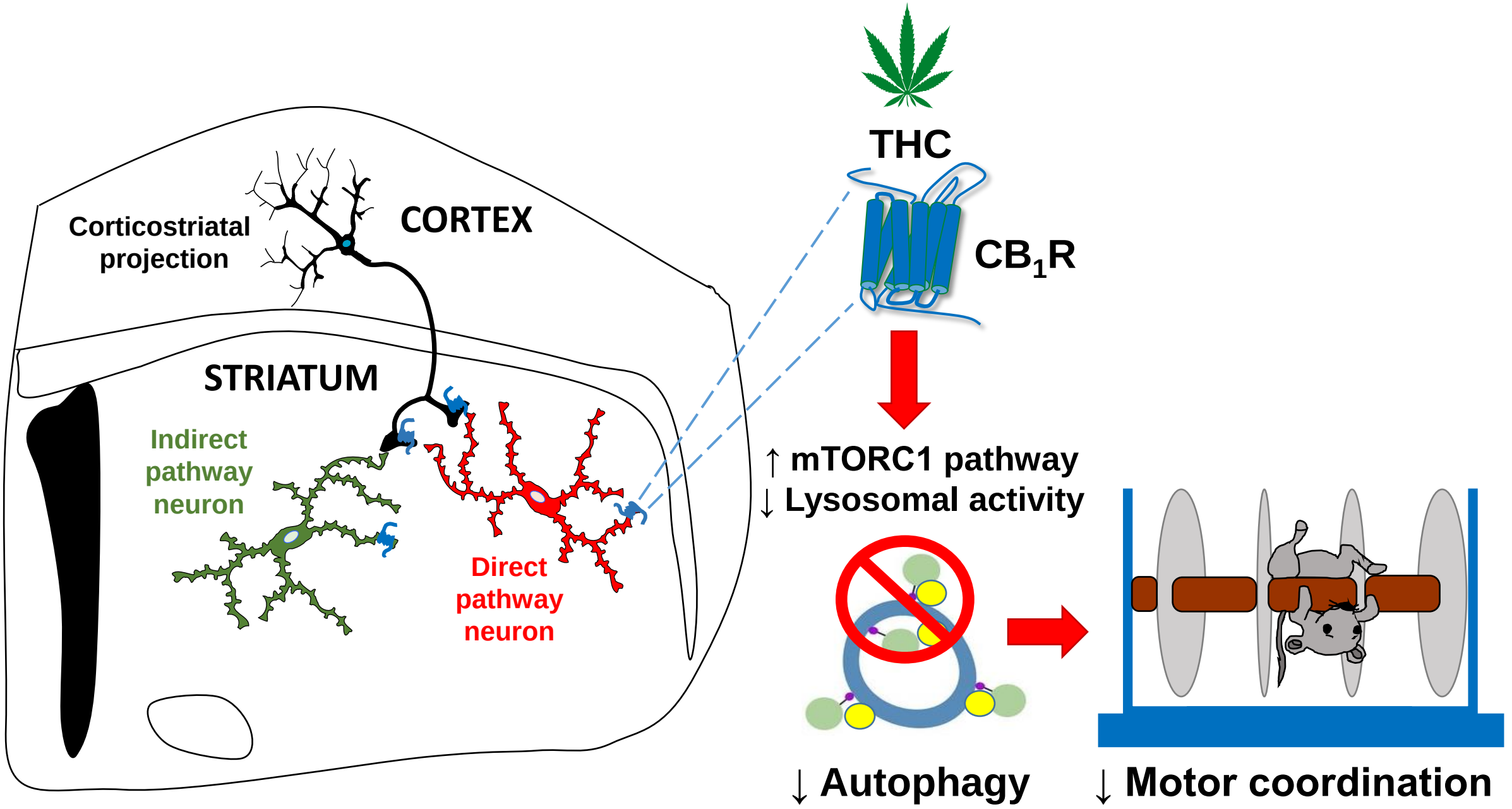
Corticostriatal projection
CORTEX
STRIATUM
Indirect pathway neuron
Direct pathway neuron
THC
CB1R
↑ mTORC1 pathway
↓ Lysosomal activity
↓ Autophagy
↓ Motor coordination