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► **To cite this version:**

Cristina Blázquez, Andrea Ruiz-Calvo, Raquel Bajo-Grañeras, Jérôme Baufreton, Eva Resel, et al.. Cannabinoid-induced motor dysfunction via autophagy inhibition. *Autophagy*, 2020, 145, pp.1-3. 10.1080/15548627.2020.1827560 . hal-02988861

HAL Id: hal-02988861

<https://hal.science/hal-02988861v1>

Submitted on 13 Oct 2021

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Cannabinoid-induced motor dysfunction *via* autophagy inhibition

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27 **ABSTRACT**

28 The recreational and medical use of cannabis is largely increasing worldwide. Cannabis
29 use, however, is associated to several undesired and possibly dangerous side effects, so
30 it is crucial that innovative studies aimed to understand and potentially reduce cannabis-
31 evoked harms are conducted. Previous research conducted on cultured neural cells had
32 supported that cannabinoid CB₁ receptor (CB₁R), the main molecular target of cannabis,
33 can alter autophagy. However, it was not known whether CB₁R controls autophagy in the
34 brain *in vivo*, and, eventually, what the functional consequences of a potential
35 CB₁R/autophagy connection could be. We have now found that Δ⁹-tetrahydrocannabinol
36 (THC), the major intoxicating constituent of cannabis, impairs autophagy in the mouse
37 striatum. Administration of autophagy activators (specifically, the rapalog temsirolimus
38 and the sugar trehalose) rescues THC-induced autophagy inhibition and motor
39 dyscoordination. The combination of various genetic strategies *in vivo* supports that CB₁R
40 molecules located on neurons belonging to the direct (striatonigral) pathway are required
41 for the autophagy- and motor-impairing activity of THC. By identifying autophagy as a
42 mechanistic link between THC and motor performance, our findings may open a new
43 conceptual view on how cannabis acts in the brain.

44

45 **KEY WORDS**

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

47

48 Cannabis is the third most popular drug of abuse in the world following alcohol and
49 tobacco, and its use is rapidly increasing. To date, four countries in the world and a
50 dozen territories in the USA have legalized the recreational use of cannabis. In addition,
51 medicinal-cannabis dispensation programs have been implemented in about thirty
52 countries globally, as well as in a similar number of states in the USA, and several
53 cannabinoid-based medicines have been approved by foremost regulatory agencies
54 (e.g., FDA, EMA and Health Canada) as anti-emetic, anti-cachexic, analgesic and anti-
55 spastic adjuvants. However, cannabis use is associated to several undesired and
56 possibly dangerous side effects, so it is crucial that innovative procedures aimed to
57 understand and potentially reduce cannabis-evoked harms are explored. Δ^9 -
58 Tetrahydrocannabinol (THC), the key intoxicating constituent of cannabis, exerts its
59 biological effects mainly by activating cannabinoid CB₁ receptor (CB₁R), one of the most
60 abundant metabotropic receptors in the mammalian central nervous system. This
61 receptor is particularly expressed in discrete brain areas involved in the control of
62 learning and memory (cortex, hippocampus), motor behavior (striatum, cerebellum),
63 emotions (amygdala) and autonomic and endocrine functions (hypothalamus, pons,
64 medulla), therefore participating in the modulation of a wide plethora of biological
65 processes. Recent evidence had suggested that CB₁R controls autophagy in cultured
66 neural cells. Strikingly, however, in some cellular settings cannabinoids *via* CB₁R
67 enhance autophagy, while in others they inhibit autophagy. Moreover, it was not known
68 whether CB₁R controls autophagy in the brain *in vivo*, and, eventually, what the
69 functional consequences of a potential CB₁R/autophagy connection could be.

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

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

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

88 What may be the neuroanatomical substrate of the observed THC effects? First,
89 we proved the participation of CB₁R by systemically administering the CB₁R-selective
90 antagonist SR141716 (rimonabant) to wild-type mice. Second, we found that the THC-
91 evoked inhibition of striatal autophagy and motor coordination was not evident in mice in
92 which the gene encoding CB₁R had been knocked-out in striatal neurons belonging to
93 the direct (striatonigral) pathway. Third, in contrast, we observed that mice in which the
94 gene encoding CB₁R had been knocked-out in corticostriatal projections (which are
95 usually considered a key determinant of striatal activity) were fully responsive to the
96 THC-evoked inhibition of striatal autophagy and motor coordination. And fourth, an
97 array of experiments conducted on mice selectively expressing (*i*) reporter genes for

98 direct-pathway and indirect-pathway (striatopallidal) neurons, (ii) dominant-negative
99 Raptor in direct-pathway neurons, or (iii) p62 in direct-pathway neurons, provided
100 further support to the selective participation of CB₁R molecules located on neurons
101 belonging to the striatal direct pathway in the autophagy-inhibiting and motor-
102 dyscoordinating activity of THC.

103 Taken together, these findings suggest that impairment of autophagy may be an
104 unprecedented mechanism involved in at least some cannabinoid-induced motor
105 alterations. On molecular grounds, our data would favor a “two-hit” model by which
106 engagement of CB₁R may impair autophagy. First, CB₁R activation, through its well
107 known coupling to the phosphatidylinositol-3-kinase/Akt/mTORC1 pathway, could lead
108 to ULK1 phosphorylation, which, subsequently, would inhibit autophagosome
109 formation/autophagy initiation. Second, CB₁R activation, by a hitherto undefined
110 mechanism that may conceivably involve an impact on lysosomal function, would inhibit
111 autophagosome clearance/autophagy completion (Figure 1).

112 We are aware, however, that our work has several shortcomings that could limit
113 the generalization of its conclusions to other experimental conditions (*e.g.*, other types
114 and doses of cannabinoids, times of cannabinoid treatment, and behavioral traits). We
115 also note that our work does not unveil the precise cellular and molecular mechanisms
116 by which the CB₁R-evoked inhibition of autophagy in striatal direct-pathway neurons
117 affects brain functionality to impact on motor coordination. Nonetheless, neuronal
118 communication is known to be sensitive to the status of both the mTORC1 pathway and
119 proteostatic processes as autophagy, which, for example, may clear dysfunctional
120 proteins and fine-tune the trafficking/recycling of membrane neurotransmitter receptors.
121 Hence, it is conceivable that the control of neurotransmission exerted by CB₁R on the
122 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

133 **DISCLOSURE STATEMENT**

134 We declare no competing financial interests.

135 **ACKNOWLEDGEMENTS**

136 Our work was supported by the Spanish *Ministerio de Ciencia, Innovación y*
137 *Universidades* (MCIU/FEDER; grants SAF2015-64945-R and RTI2018-095311-B-I00 to
138 MG, and SAF2016-78666-R to JAR-N). AR-C was supported by a contract from the
139 Spanish *Ministerio de Ciencia, Innovación y Universidades* (MCIU/FEDER; *Formación*
140 *de Personal Investigador* Program). RB-G was supported by a contract from the
141 Spanish *Ministerio de Ciencia, Innovación y Universidades* (MCIU/FEDER; *Juan de la*
142 *Cierva* Program). GM and LB were supported by INSERM. GM was also supported by
143 *Fondation pour la Recherche Medicale* (grants DRM20101220445 and
144 DPP20151033974), Human Frontiers Science Program (grant RGP0036/2014),
145 European Research Council (Endofood grant ERC–2010–StG–260515, CannaPreg
146 grant ERC-2014-PoC-640923, and MiCaBra grant ERC-2018-AdG-786467), *Region*
147 *Nouvelle Aquitaine*, and *Agence Nationale de la Recherche* (ANR Blanc MitObesity
148 grant ANR-18-CE14-0029-01, ANR LabEx BRAIN grant ANR-10-LABX-0043, ANR
149 Blanc ORUPS grant ANR-16-CE37-0010-01, and ANR Blanc CaCoVi grant ANR 18-
150 CE16-0001-02). L.B. was also supported by *Fondation pour la Recherche Medicale*
151 (grant ARF20140129235) and *Agence Nationale de la Recherche* (ANR Blanc mitoCB1-
152 fat grant ANR-19-CE14). We are indebted to E. García-Taboada, L. Rivera, A.
153 Gaudioso, P. García-Rozas, and M.J. Asensio for expert laboratory assistance; D.
154 Gonzales, N. Aubailly, and all the personnel from the animal facilities of the
155 NeuroCentre Magendie; M. Biguerie from the technical service of the NeuroCentre
156 Magendie; all the personnel from the Bordeaux Imaging Center; and V. Morales for
157 continuous help.

158

159 **FIGURE LEGEND**

160 **Figure 1**

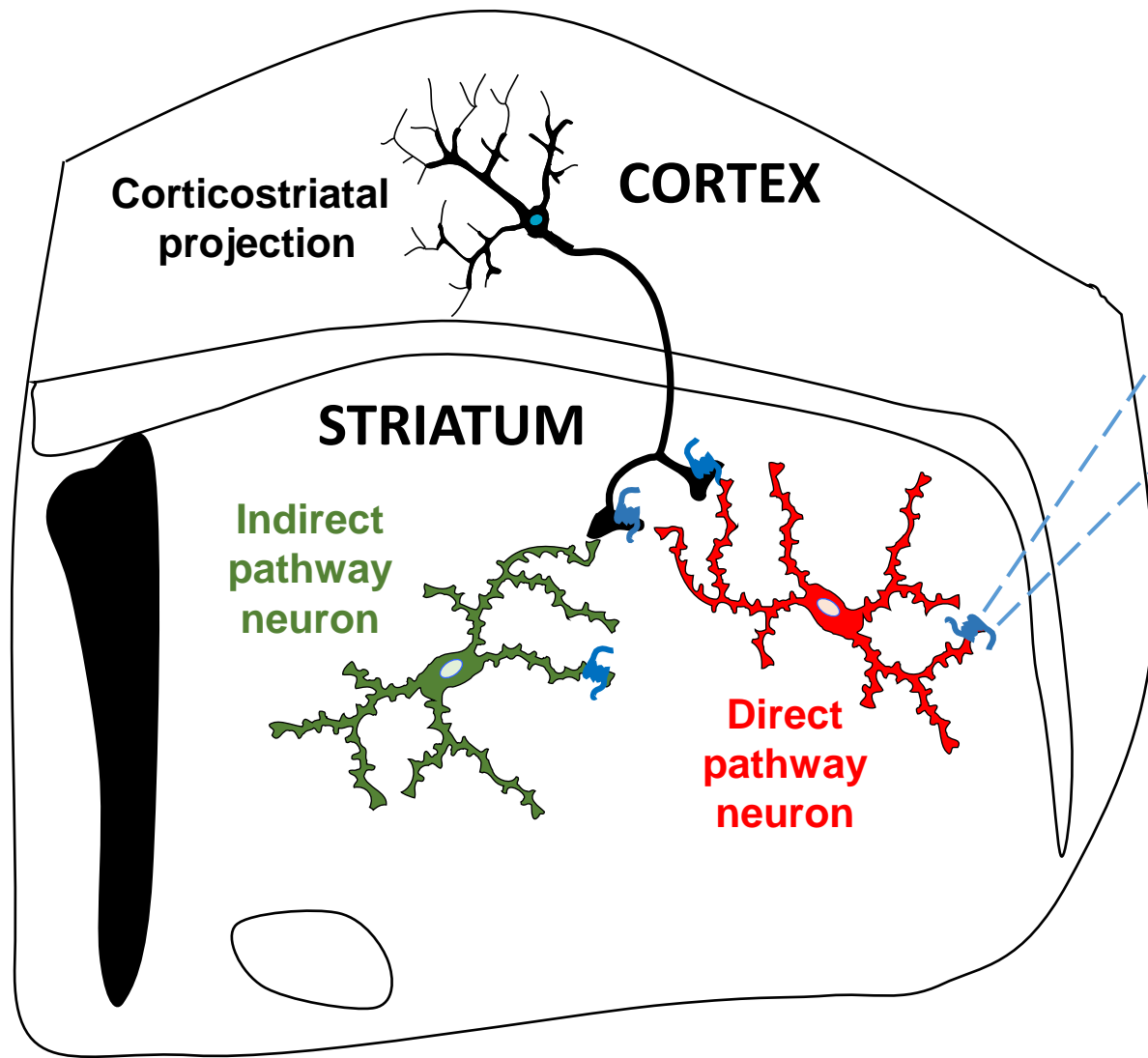
161 **Scheme depicting the impact of THC on striatal autophagy and motor coordination**

162 THC, the main intoxicating ingredient of cannabis, would bind to CB₁R molecules
163 located on neurons belonging to the striatal direct (striatonigral) pathway. Upon
164 engagement, CB₁R, by (i) activating the mTORC1 pathway and (ii) blocking
165 autophagolysosomal function, would inhibit autophagy. This, in turn, would impair motor
166 coordination by a hitherto unknown mechanism.

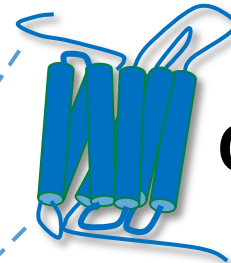
167

168 **REFERENCE**

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172 cannabinoid intoxication and impairment of motor coordination. *Elife*. 2020;9:e56811.



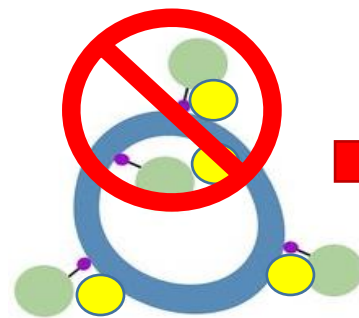
THC



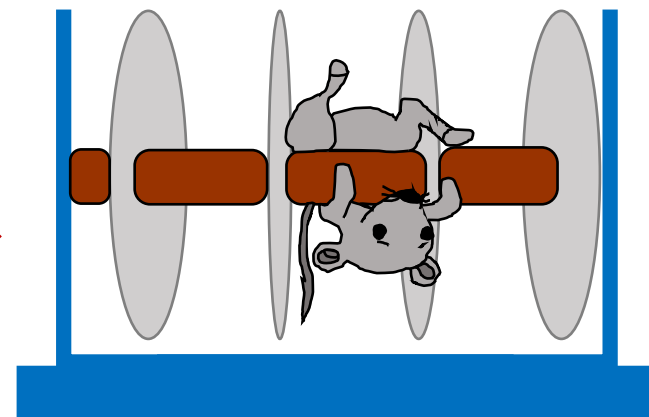
CB₁R



↑ **mTORC1 pathway**
↓ **Lysosomal activity**



↓ **Autophagy**



↓ **Motor coordination**