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Short communication

Changes in hippocampal morphology following chronic treatment with the synthetic cannabinoid WIN 55,212-2

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Abstract

Learning and memory are often correlated with cellular changes within the hippocampus, and drugs or environmental factors which affect learning and memory will thus often induce observable morphological changes in this structure. Like tetrahydrocannabinol (THC) itself, many synthetic cannabinoids such as the CB-1 receptor agonist WIN 55,212-2 will induce learning and memory changes. In the current study, we investigate whether or not these changes could be related to structural changes within the hippocampus. Adult male Sprague–Dawley rats were injected twice daily (12:00 and 0:00 h) subcutaneously with WIN 55,212-2 (2.0 mg/kg) in DMSO or DMSO for 21 days. On day 22, animals were perfused and stained immunochemically for the dendritic marker MAP-2, or with cresyl violet. Morphometric analysis showed dendritic rearrangement with increased staining of MAP-2 in CA3 and the lower blade of the dentate gyrus. However, a loss of staining was observed in CA1. Counting of cresyl violet stained sections showed an apparent increase in granule cell number in the lower blade of the dentate gyrus. This work shows the potential for cannabinoids to influence hippocampal morphology. The pattern of changes may be similar to that seen after ischemic or toxic damage, but may be opposite to changes seen in stress.

Theme: Neural basis of behavior

Topic: Psychopharmacological agents

Keywords: Marijuana; WIN 55 212-2; Hippocampus; Dendrite; Stress; Ischemia; MAP-2

Delta 9-tetrahydrocannabinol (THC), the major psychoactive ingredient in marijuana, produces many acute effects in humans including, euphoria, analgesia, appetite stimulation and sedation. In addition, marijuana causes alteration in cognitive processes, specifically in learning and short-term memory [3]. Chronic use of marijuana may lead to lasting changes in cognition [25] and decreased functioning in temporal lobes [2].

Cannabinoids also impair learning and memory in laboratory animals [11]. The acquisition and retention in the radial arm maze is impaired by acute THC administration [14,15,23]. Deficits in spatial delayed nonmatching-to-position working memory task have been reported in rats [12,17] and in the comparable delayed nonmatching-to-sample task in monkeys [1]. Additionally, there is evidence that hippocampally-mediated sensory-attentional processes are disrupted by acute THC administration [6,26].

The acute memory effects of cannabinoids are thought to be due to their effects on the hippocampus, which contains a large number of cannabinoid receptors in humans [16], non-human primates [24] and rat [4]. The hippocampus may also be the site of the longer-lasting cognitive deficits seen after chronic use. Learning and memory deficits, whether from toxins, ischemia or stress, often have associated morphological changes in the hippocampus.

The current study, therefore, tests the hypothesis that chronic THC use results in morphological changes in the hippocampus. For this study, we chose the synthetic cannabinoid WIN 55,212-2, because of its high potency and its solubility, compared to THC itself.

Eight male Sprague–Dawley rats (265–285 g) were received into the animal care facilities and maintained

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under a 12 h light/dark cycle with food ad libitum for 1 week prior to injections. Following that time, rats were divided into treatment and control groups. Treated animals were injected twice daily (12:00 and 0:00 h) subcutaneously with WIN 55,212-2 (2.0 mg/kg; Research Biochemical Institute) in DMSO into the nape of the neck. Control animals were injected with an equal volume of DMSO. Injections continued for 21 days. This time was chosen because previous neurochemical and behavioral studies have shown widespread cannabinoid CB1 receptor down-regulation [27] and a suppression of exploratory activity [18] after 2–3 weeks of chronic THC exposure.

On day 22, animals were anesthetized with a rompun/ketaset mixture and transcardially perfused with icec–cold normal saline (approximately 300 ml, or until the effluent ran clear) followed by ice-cold 4% paraformaldehyde in phosphate buffer (pH 7.2). The brains were removed and postfixed for 1 h in 4% paraformaldehyde, then placed in sucrose, before sectioning at 20 microns on a sliding microtome with a freezing stage (American Optical Corporation). Sections were collected into phosphate buffered saline (PBS). Alternate sections were transferred to an eight-chamber Plexiglas staining tray or mounted onto slides. For immunochemistry, the staining tray was used. Each chamber received the sections from one animal, thus all eight animals (control and treated) were stained identically. Sections were incubated for 48 h at 4°C. with a mouse monoclonal MAP-2 antibody (Sigma catalogue number M-4403) at a dilution of 1/500 in PBS containing 3% normal goat serum and 0.3% triton. MAP-2 is a microtubule associated protein found in dendrites and immunochemistry of MAP-2 is a commonly used method for quantification and visualization of dendrites. After three 5 min washes in PBS, sections were incubated for 2 h with mouse IgG Fab-specific biotinylated conjugate and processed with the Vector Elite ABC kit with diaminobenzidine (DAB, 0.05%) as substrate. For the cresyl violet staining, mounted sections were immersed in cresyl violet dye for 15 min then rinsed with de-ionized water. The slides were processed through a series of alcohol baths (70%, 95% (with glacial acidic acid) and 100%) for 2 min each. The final rinse was for 6 min in xylene.

Dendritic length was determined by capturing images (at 600×magnification) with an Olympus photomat system and measuring MAP-2 immunoreactive processes using a program established in our laboratory which is based on intersections with random lines (Bouffon's principle). At least three determinations were made for each region in each section. Cell number was analyzed by three independent raters counting cells at 600× within a one square micron grid in a predetermined area of each region. Means were determined for each region for each subject for each measure. Final results are expressed as the means of all four animals and analyzed with a two-tailed Student's *t*-test.

Immunochemical staining of dendrites with the MAP-2 antibody in control animals is as we have previously seen [20,32]. Briefly, there was intense staining in the stratum radiatum of the CA fields and subiculum where the pyramidal dendrites are located. There was also heavy staining in the molecular layer of the dentate gyrus, where granule cells have their dendrites. In the control animals, the MAP-2 immunoreactive dendrites appear as thin continuous processes.

Compared to control, the WIN 55212-2 animals showed an apparent re-organization of dendrites within the hippocampal subfields in all treated animals. Specifically, MAP-2 staining was increased in the dendritic fields of CA3 and the lower blade of the dentate gyrus. Conversely, overall dendritic length in CA1 was decreased. These results are given in Table 1 and Fig. 1. At higher magnification in the figure, the specific changes in dendritic morphology in CA1 are evident. In treated animals, the dendrites of CA1 appear as disjointed segments rather than the continuous structures seen in control animals.

WIN 55212-2 also had an effect on cell counts. The data, given in Table 2, show an apparent increase in cell number in the lower blade of the granule cells in the dentate gyrus of the treated animals. No other changes were observed.

Chronic treatment with a THC analogue results in morphological changes in the hippocampus. These changes may represent the structural changes underlying the learning and memory changes reported by others. There are several interesting and important interpretations of our observations.

Our results showed a significant change in morphology of the dendrites in CA1 with no associated pyramidal cell loss. This morphological change, wherein the dendrites appear twisted or broken with a beaded aspect, could be associated with dendritic degeneration and/or retraction from the pyramidal cells without actual cell death. CA1 damage of this type is commonly seen after ischemia [19,20,31,32], toxins [13] or traumatic head injury [8]. This type of morphological change is often associated with a decreased capacity for LTP and some types of spatial learning [30]. Interestingly, WIN 55212-2 has been shown to inhibit LTP in CA1 [29].

An increase in MAP-2 staining appears in subiculum, the lower blade of the dentate gyrus and CA3. Interestingly, these three regions are not generally damaged by

Table 1
Estimated total length of MAP-2 immunostained dendrites ($\mu m/mm^2$ + S.E.M.)

Region:	Control	WIN 55,212-2	
Subiculum	348±32	444±52	$P<0.025$
CA1	412±48	324±24	$P<0.025$
CA3	388±28	469±53	$P<0.05$
Dentate gyrus			
Upper blade	423±27	428±37	NS
Lower blade	411±16	520±48	$P<0.025$

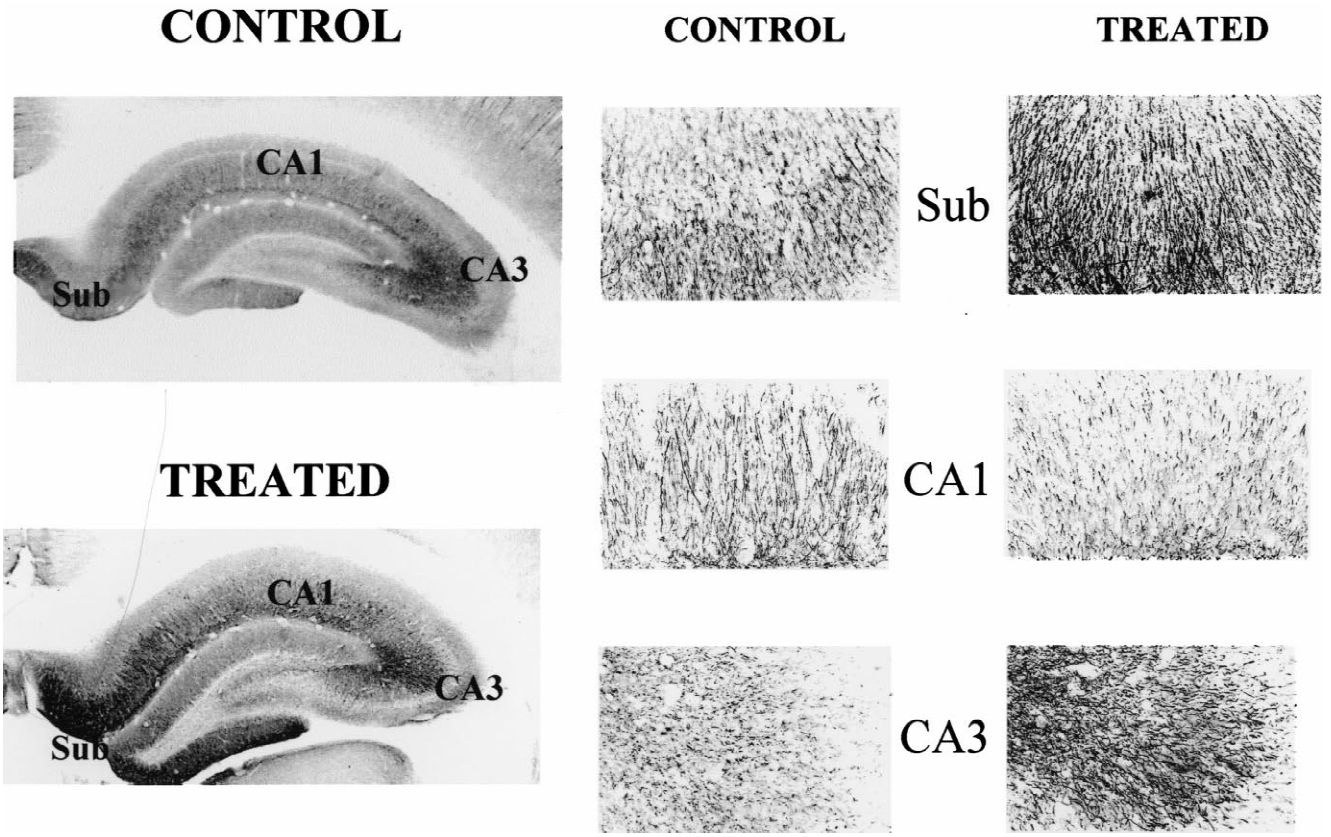

Fig. 1. Representative photomicrographs of MAP-2 immunostained dendrites in rat hippocampus. The lefthand panels are 40×, while the righthand are 400×. In the subiculum (Sub) and CA3 regions of hippocampus, the total dendritic length appears greater in the animal treated with WIN 55,212-2. Conversely, in the CA1 region of treated animals, the dendrites appear sparser and more segmented.

ischemia or head trauma [8], but are severely damaged by stress [9,21,28]. Stress is also known to decrease adult neurogenesis of granule cells in the dentate gyrus [5,10]. Taken together, the findings of increased MAP-2 immunoreactive dendrites in CA3, subiculum and dentate plus the apparent increase in granule cell number, suggests that the WIN 55,212-2-treated animals have experienced less stress than control animals. It is possible that the stress of daily injections was damaging to the control animals, but that the treated animals were protected from the normally damaging effects. This would be in agreement with the known anxiolytic effects of THC in humans.

There are other studies which may appear contradictory to those reported here. For example, other researchers have not seen this pattern of THC-induced toxicity in CA1, and have in fact suggested that THC and its analogues might be useful as neuroprotectants against ischemic damage [22]. However, other researchers have seen a greater degree of toxicity than we have reported, suggesting cell death might occur [7].

Table 2
Estimated granule cell number by cresyl violet counts (cells/mm^2±S.E.M.)

Region	Control	WIN 55,212-2	
Upper	883±86	856±73	NS
Lower	842±72	1137±89	$P<0.02$
Intersection	641±67	608±53	NS

Our findings suggest several important future directions. The capacity for THC analogues to prevent stress-induced damage to the hippocampus should be tested. This may be done simply by repeating our studies, with the inclusion of an uninjected control group, or by using the more standard models of stress used by others [21]. There is also the question of whether or not our morphological results are reversible. In human studies of cognition and marijuana use, there is some indication that the changes are permanent. In this study, animals were perfused and examined 1 day after the final injections so that no period for recovery was observed. Finally, our studies were performed in adult animals. In humans, heaviest marijuana usage is associated with adolescence. Thus, in a future study, we will look at adolescent animals. In this case, the results may be less severe or more reversible, due to the greater adaptability of the immature brain, or the consequences may be more severe, if cannabinoids have the teratological effects seen with many psychoactive drugs.

In summary, the known effects of marijuana and its analogues on learning and memory may have associated

morphological changes in the hippocampus. While these changes are reminiscent of that seen after ischemic or toxic damage, they are in fact opposite to that seen after stress.

References

[1] T. Aigner, Delta-9-tetrahydocannabinol impairs visual recognition memory in rhesus monkeys, Psychopharmacology 95 (1988) 507–511.

[2] D.G. Amen, M.J. Waugh, High resolution brain SPECT imaging of marijuana smokers with AD/HD, Psychoactive Drugs 30 (1998) 209–214.

[3] A. Ameri, The effects of cannabinoids on the brain, Prog. Neurobiol. 58 (1999) 315–348.

[4] F. Berrendero, N. Sepe, J.A. Ramos, V. Di Marzo, J.J. Fernandez-Ruiz, Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid content in the developing rat brain during late gestation and early postnatal period, Synapse 33 (1999) 181–191.

[5] E.S. Brown, A.J. Rush, B.S. McEwen, Hippocampal remodeling and damage by corticosteroids: implications for mood disorders, Neuropsychopharmacology 21 (1999) 474–484.

[6] K.A. Campbell, T.C. Foster, R.E. Hampson, S.A. Deadwyler, Effects of Delta-9-tetrahydrocannabinol on sensory-evoked potentials in the granule cells in the dentate gyrus of behaving rats, J. Pharmacol. Exp. Ther. 239 (1986) 941–945.

[7] G.C. Chan, T.R. Hinds, S. Impey, D.R. Storm, Hippocampal neurotoxicity of Delta 9-tetrahydrocannabinol, J. Neurosci. 18 (1998) 5322–5332.

[8] M.M. Folkerts, R.F. Berman, J.P. Muizelaar, J.A. Rafols, Disruption of MAP-2 immunostaining in rat hippocampus after traumatic brain injury, J. Neurotrauma 15 (1998) 349–363.

[9] L.A. Galea, B.S. McEwen, P. Tanapat, T. Deak, R.L. Spencer, F.S. Dhabhar, Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress, Neuroscience 81 (1997) 689–697.

[10] E. Gould, B.S. McEwen, P. Tanapat, L.A. Galea, E. Fuchs, Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation, J. Neurosci. 17 (1997) 2492–2498.

[11] R.E. Hampson, S.A. Deadwyler, Role of cannabinoid receptors in memory storage, Neurobiol. Dis. 5 (1998) 474–482.

[12] C.J. Heyser, R. E Hampson, S.A. Deadwyler, Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: Alterations of short-term memory associated with changes in task specific firing of hippocampal cells, J. Pharmacol. Exp. Ther. 264 (1993) 294–307.

[13] D. Koczyk, Differential response of microtubule-associated protein 2 (MAP-2) in rat hippocampus after exposure to trimethyltin (TMT): an immunocytochemical study, Acta Neurobiol. Exp. (Warsz) 54 (1994) 55–58.

[14] A.H. Lichtman, K.R. Dimen, B.R. Martin, Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats, Psychopharmacology 119 (1995) 282–290.

[15] A.H. Lichtman, B.R. Martin, Delta-9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism, Psychopharmacology 126 (1996) 125–131.

[16] P. Mailleux, M. Parmentier, J.J. Vanderhaeghen, Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides, Neurosci. Lett. 143 (1992) 200–204.

[17] P.E. Mallet, R.J. Beninger, The endogenous cannabinoid receptor agonist anandamide impairs memory in rats, Behav. Pharmacol. 7 (1996) 276–286.

[18] J. Masur, R.M.W. Martz, E.A. Carlini, Effects of acute and chronic administration of cannabis sativa and (−)delta-9-tetrahydrocannabinol on the behavior of rats in the open field arena, Psychopharmacologia 19 (1971) 388–397.

[19] D.F. Matesic, R.C. Lin, Microtubule-associated protein 2 as an early indicator of ischemia-induced neurodegeneration in the gerbil forebrain, J. Neurochem. 63 (1994) 1012–1020.

[20] C. Mazer, J. Muneyyirci, K. Taheny, N. Raio, A. Borella, P.M. Whitaker-Azmitia, Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: a possible model of neurodevelopmental disorders with cognitive deficits, Brain Res. 760 (1997) 68–73.

[21] B.S. McEwen, Stress and hippocampal plasticity, Annu. Rev. Neurosci. 22 (1999) 105–122.

[22] T. Nagayama, A.D. Sinor, R.P. Simon, J. Chen, S.H. Graham, K. Jin, D.A. Greenberg, Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures, J Neurosci. 19 (1999) 2987–2995.

[23] E.M. Nakamura, E.A. da Silva, G.V. Concilio, D.A. Wilkinson, J. Masur, Reversible effects of acute and long-term administration of delta-9 tetrahydrocannabinol (THC) on memory in the rat, Drug and Alcohol Dependence 28 (1991) 167–175.

[24] W.Y. Ong, K. Mackie, A light and electron microscopic study of the CB1 cannabinoid receptor in primate brain, Neuroscience 92 (1999) 1177–1191.

[25] H.G. Pope Jr, D. Yurgelun-Todd, The residual cognitive effects of heavy marijuana use in college students, J. Am. Med. Assoc. 275 (1996) 521–527.

[26] G. Presburger, J.K. Robinson, Spatial signal detection in rats is differentially disrupted by delta-9-tetrahydrocannabinol, scopolamine, and MK-801, Behav. Brain Res. 99 (1999) 27–34.

[27] J. Romero, F. Berrendero, J. Manzanares, A. Perez, J. Corchero, J.A. Fuentes, J. J Fernandez-Ruiz, J.A. Ramos, Time-course of the cannabinoid receptor down regulation in the adult rat brain caused by repeated exposure to Delta-9-tetrahydrocannabinol, Synapse 30 (1998) 298–308.

[28] Sunanda, M.S. Rao, T.R. Raju, Effect of chronic restraint stress on dendritic spines of hippocampal CA3 pyramidal neurons — a quantitative study, Brain Res. 694 (1995) 312–317.

[29] J.P. Terranova, J.C. Michaud, G. Le Fur, P. Soubrie, Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors, Naunyn-Schmiedeberg's Arch. Pharmacol. 352 (1995) 576–579.

[30] C. Vaillend, J.M. Billard, T. Claudepierre, A. Rendon, P. Dutar, A. Ungerer, Spatial discrimination learning and CA1 hippocampal synaptic plasticity in mdx and mdx3cv mice lacking dystrophin gene products, Neuroscience 86 (1998) 53–66.

[31] Y. Yamasaki, T. Shigeno, Y. Furukawa, S. Furukawa, Reduction in brain-derived neurotrophic factor protein level in the hippocampal CA1 dendritic field precedes the delayed neuronal damage in the rat brain, J. Neurosci. Res. 53 (1998) 318–329.

[32] P.M. Whitaker-Azmitia, A. Borella, N. Raio, Serotonin depletion in the adult rat causes loss of the dendritic marker MAP-2, Neuropsychopharm. 12 (1995) 269–272.