Received: 1 August 2017 | Revised: 6 November 2017 | Accepted: 23 November 2017

DOI: 10.1002/syn.22020

RESEARCH ARTICLE

WILEY SYNAPSE

THC alters alters morphology of neurons in medial prefrontal cortex, orbital prefrontal cortex, and nucleus accumbens and alters the ability of later experience to promote structural plasticity

Bryan Kolb[1,2] | Yilin Li[1] | Terry Robinson[3] | Linda A. Parker[4]

[1]Department of Neuroscience, University of Lethbridge, Alberta, Canada

[2]Child Brain Development Program, Canadian Institute for Advanced Research, Toronto, Ontario, Canada

[3]Department of Psychology, University of Michigan, Ann Arbor, Michigan

[4]Department of Psychology, University of Guelph, Ontario, Canada

Correspondence
Bryan Kolb, Department of Neuroscience, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada.
Email: kolb@uleth.ca

Funding information
Natural Sciences and Engineering Research Council of Canada; The Canadian Institutes for Health Research; The Canadian Institute for Advanced Research; National Institute on Drug Abuse (USA)

Abstract

Psychoactive drugs have the ability to alter the morphology of neuronal dendrites and spines and to influence later experience-dependent structural plasticity. If rats are given repeated injections of psychomotor stimulants (amphetamine, cocaine, nicotine) prior to being placed in complex environments, the drug experience interferes with the ability of the environment to increase dendritic arborization and spine density. Repeated exposure to Delta 9-Tetrahydrocannabinol (THC) changes the morphology of dendrites in medial prefrontal cortex (mPFC) and nucleus accumbens (NAcc). To determine if drugs other than psychomotor stimulants will also interfere with later experience-dependent structural plasticity we gave Long-Evans rats THC (0.5 mg/kg) or saline for 11 days before placing them in complex environments or standard laboratory caging for 90 days. Brains were subsequently processed for Golgi-Cox staining and analysis of dendritic morphology and spine density mPFC, orbital frontal cortex (OFC), and NAcc. THC altered both dendritic arborization and spine density in all three regions, and, like psychomotor stimulants, THC influenced the effect of later experience in complex environments to shape the structure of neurons in these three regions. We conclude that THC may therefore contribute to persistent behavioral and cognitive deficits associated with prolonged use of the drug.

KEYWORDS

D9-THC, golgi-cox, neuroplasticity, enriched environment, dendrite, spines

1 | INTRODUCTION

The evolving views on legalization of marijuana for recreational use in the USA, Canada, and various European countries has led to a growing interest in the long-term effects of cannabis use on the integrity of human brain structures, especially in regions rich in cannabionoid CB1 receptors, including prefrontal cortex, hippocampus, amygdala, striatum, and cerebellum. Although there are now many studies using structural MRIs to evaluate the long-term effects of cannabis use, the results have been inconsistent with early studies showing significant loss of gray and/or white matter (e.g., Battistella et al., 2014; Churchwell, Lopez-Larson, & Yurgelun-Todd, 2010) whereas more recent studies have failed to confirm this, possibly because of various comorbid factors (e.g., Weiland et al. 2015), although one study of a large sample of users showed a correlation between cannabis use and white matter integrity and subcortical, but not cortical, morphometry (Orr, Paschall, & Banich, 2016).

One consistent finding in studies of laboratory animals is that most, if not all, psychoactive drugs alter the structure of neurons in the prefrontal cortex (both medial and orbital areas) and nucleus accumbens (NAcc), although the nature of the changes vary by class of drug (for a review see Robinson & Kolb, 2004). Studies of neuronal morphology following exposure to Delta 9-Tetrahydrocannabinol (THC) injections in laboratory animals have been consistent showing alterations in dendritic arborization in prefrontal cortex, striatum (Kolb, Gorny, G., Limebeer, & Parker, 2006), hippocampus (Lawston, Borella, Robinson, & Whitaker-Azmitia, 2000; Scallet et al., 1987), and reductions in a range of measures of cell size in the ventral tegmental dopamine neurons (Behan et al., 2012; Spiga, Lintas, Migliore, & Diana, 2010).

In view of the evidence that THC modifies the structure of cerebral neurons the question arises as to whether this might influence subsequent experience-dependent plasticity, such as that associated with learning. A learning experience that is known to produce the largest effects on neuron structure results from housing animals in complex environments (e.g., Sirevaag & Greenough, 1987; van Praag, Kempermann, & Gage, 2000). Furthermore, the administration of psychomotor stimulant drugs to rats (amphetamine, cocaine, and nicotine) alters the ability of later experience in a complex environment to change dendritic structure, sometimes occluding such effects (Hamilton & Kolb, 2005; Kolb, Gorny, Li, Samaha, & Robinson, 2003a). We therefore asked whether THC might also alter the brain's subsequent response to complex housing. Given that we had previously shown that the effect of THC appeared to be largely limited to medial prefrontal cortex (mPFC), orbitofrontal cortex, and NAcc we focused on these structures in the current study. In addition, because we had only examined dendritic branching and length in our earlier study we also wanted to examine the effects of THC on spine density because of the large changes in spine density produced by psychomotor stimulants (e.g., Robinson & Kolb, 2004). In view of our previous experience both with psychomotor stimulants and complex housing we predicted that the spine changes would be more likely to be found than the changes in dendritic branching and length, both in response to complex housing and THC. We also predicted that like psychomotor stimulants, THC would alter the morphological changes related to complex housing. Both predictions were confirmed.

2 | MATERIALS AND METHODS

2.1 | Animals

Twenty-four male Long-Evans rats that were born in house from stock originally obtained from Quebec Charles-Rivers Laboratory and weighing between 250 and 300 g at the beginning of the experiment. After weaning the animals lived in pairs in Plexiglas shoebox cages (39 × 57 × 21 cm with corn-cob bedding covering the floor) having ad libitum access to food and water for the duration of the experiment. The colony room was maintained at 21 °C on a 12/12 light dark schedule, with lights on at 7:30 a.m. All experimental procedures were approved by the University of Lethbridge animal welfare committee.

2.2 | Drugs

D9-THC (provided by NIDA) was prepared in a suspension of ethanol:cremophor (Sigma):saline (1:1:18) and prepared as a solution of 0.5 mg/mL. The rats received either THC (0.5 mg/kg) or 0.9% saline i.p. daily for 11 consecutive days. The dose of THC was selected because it is within the estimated range than human marijuana users would obtain in recreational use (Gardner & Lowinson, 1991) and this dose produced structural changes in our previous work (Kolb et al., 2006).

2.3 | Procedure

When the experiment began, the animals were divided into four groups: Saline ($n = 6$), THC ($n = 6$), Saline-complex ($n = 6$) and THC-complex ($n = 6$). All of the animals remained in pairs in the shoebox cages during the drug injection phase of the study. Each day the rats were transported from their housing to a testing room and prior to drug administration they were placed individually in an activity apparatus for 30 min prior to drug exposure. Following drug injection, the activity was monitored for 60 min and the rats were returned to their home cages.

The activity system was an AccuScan Instruments Inc., Versamax animal activity monitoring system© (open field). The activity apparatus consisted of a clear plexi-glass cage measuring 42 × 42 × 30 cm^3 with a removable plexi-glass lid. Each cage was equipped with horizontal and vertical sensors (infra-red beams) that monitored activity in selected intervals for a total of 60 min. Recorded measures of horizontal activity (number of beam breaks on the lower bar in each sample period), and total distance (path traveled in centimeters) were retrieved and used for analysis. Once the testing period was complete, the VersaDat function scanned the files for errors to verify the integrity of data collected. The files were then converted for import to an Excel spread sheet.

On the day following the last injection, the complex-housed animals were transferred for 90 days to complex environments that consisted of a large (61 × 122 × 183 cm) structure. The sides and front of the condo were made of heavy wire mesh and the back vertical wall was galvanized steel. Three platforms were attached to the back wall at various heights with wooden ramps that provided access to each level. The floor was covered with corn-cob bedding. Objects, which included plastic "toys," paper, cardboard boxes, and PVC pipe, were strewn over the floor and the platforms to encourage exploration. The complex environments were cleaned weekly and the objects replaced each time. As with the lab-caged rats, the complex-housed rats were given ad libitum food and water. The complex environments were in the same animal room as the shoebox cages.

Following the complex housing, the rats were administered an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline. The brains were removed and placed in 20 mL Golgi-Cox solution and stored in the dark for 14 days. The brains were then placed in a 30% sucrose solution for at least 3 days prior to coronal sectioning (200 μm), mounted on glass slides, and stained using a procedures described by Gibb and Kolb (1998).

The basilar dendrites from layer V pyramidal neurons were drawn from mPFC (Zilles area Cg3), layer III orbital frontal cortex (OFC, Zilles area AID), and medium spiny neurons from the shell of NAcc. The brain regions were first identified at low power (100×) and five cells from each

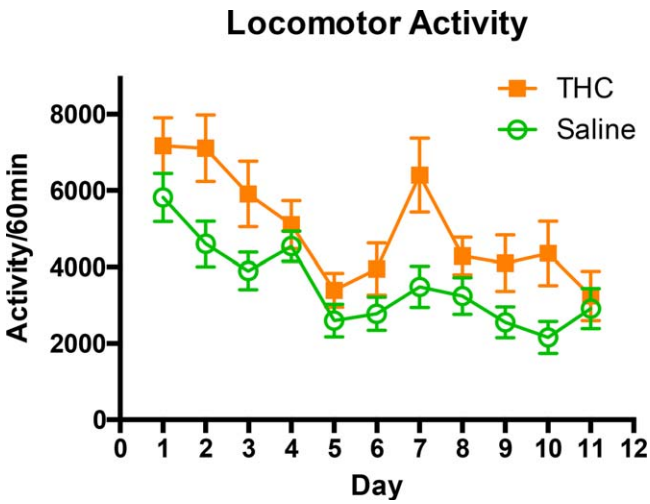

FIGURE 1 Locomotor activity related to THC administration. The THC increased activity but there was no sensitization

hemisphere of each region using camera lucida (250×). To be included in the analysis the dendritic trees needed to be intact and not obscured by other neurons, glia, or blood vessels. The dendritic arbor was quantified by counting the number of dendritic branches (indicated by bifrications) using the method of Coleman and Reisen (1968). Dendritic length was estimated using the Sholl (1956) procedure in which an overlay of concentric circles beginning at the cell body and spaced 20 μm apart. The number of intersections of the dendrites and the rings was counted and the length estimated by multiplying by 20 μm. Spine density was calculated by tracting a length of dendrite (at least 30 μm long) at ×1000. The exact length of the dendrite was calculated, and the number of spines along the entire length was counted. For cortical pyramidal cells, spines were counted on one third-order terminal tip per neuron. For medium spiny NAcc neurons, spines were counted on one terminal tip per neuron. No attempt was made to account for the fact that spines on the back side of the dendrites were obscured from view so the spine estimate necessarily underestimates the total spine number. The values for cells in each hemisphere of each rat were averaged, and hemisphere was used as the unit of analysis. The anatomical analyses were performed by someone (Y.L.) blind to the experimental conditions.

2.3.1 | Statistical analyses

Statistical analyses were performed using SPSS 21 for Mac. For the behavioral measure, two-way ANOVAs with drug (THC, saline) and test day as variables were performed. The anatomical data for each of the five brain areas were analyzed using three-way ANOVA's with treatment (THC and control), housing, and hemisphere as factors. The data points were the mean of 5 cells per area per hemisphere. However, as hemisphere failed to show significant main effects or interactions with other factors the data were collapsed across this variable and the ANOVA reported as a two-way (Drug and Housing). Posthoc tests were conducted using Tukey's ($p < .05$).

3 | RESULTS

3.1 | Locomotor behavior

The THC significantly increased activity across the first ten days of drug treatment (see Figure 1). A two-way ANOVA (THC, Test Day) showed a main effect of drug [$F(11, 264) = 22.49$, $p < .0001$] and test day [$F(11, 264) = 18.05$, $p < 0.001$] but not the interaction [$F(11, 264) = 0.9392$, $p = 0.5031$].

3.2 | Anatomical analysis

There are three different effects to consider, namely the effect of complex housing, the effect of THC, and the interaction. We consider each region separately. We note that the Golgi staining was good and similar to that seen and illustrated in our previous studies (e.g., Kolb et al., 2003a, 2006).

3.2.1 | Cg3

As in our previous studies, dendritic branching, but not length, were affected by complex housing and there was an increase in spine density in the complex-housed rats. THC increased dendritic length in both cage and complex reared animals but branching was increased only in the complex-housed group and the THC increased spine density in the complex-housed group but decreased it in the cage-housed group (See Figure 2, Table 1).

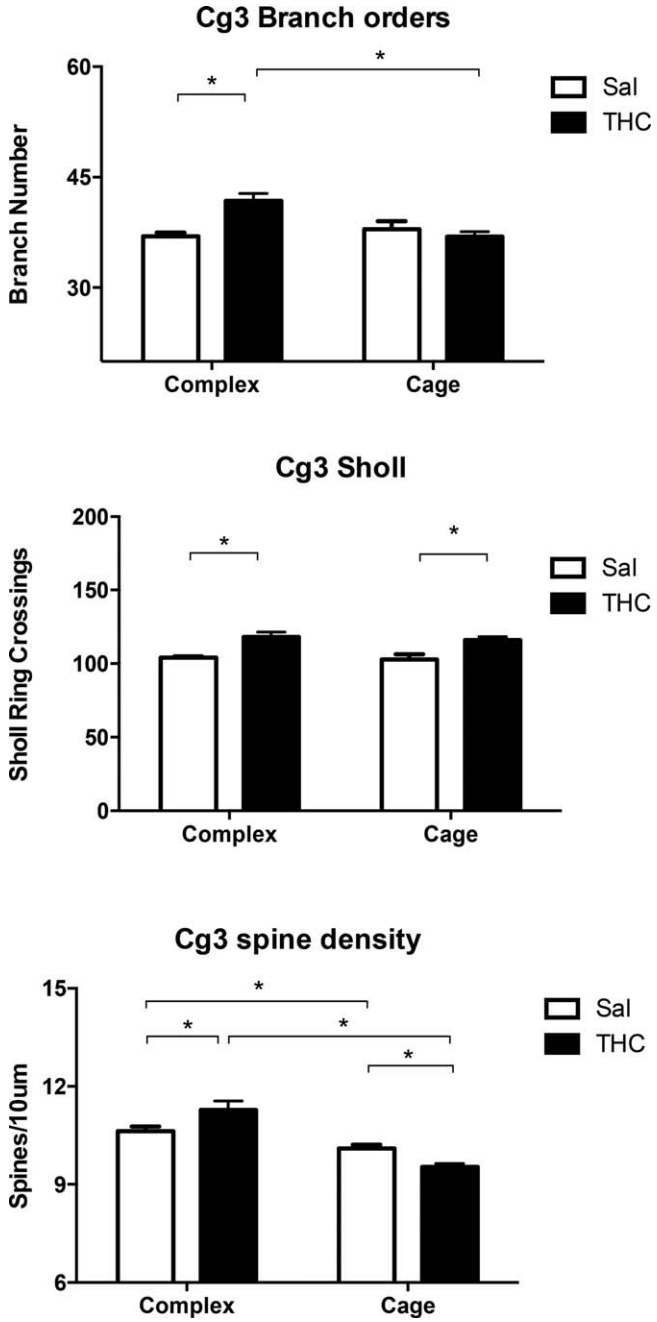

FIGURE 2 Summary of measures of Cg3 neurons. There was a significant effect of complex housing on dendritic braching and spine density but not for dendritic length. THC increased dendritic length but THC had housing-specific effects on dendritic branching and spine density (p's $< .05$ or better)

The statistical analysis for branching showed a significant effect of housing [$F(1,44) = 5.29$, $p = .026$], THC [$F(1,44) = 5.02$, $p = .03$] and the interaction [$F(1,44) = 12.04$, $p = .001$]. For length (Sholl line intersections) there was only an effect of THC [$F(1,44) = 36.41$, $p < .0001$]. Spine density showed a significant effect of housing [$F(1,44) = 43.55$, $p < .0001$] and the interaction [$F(1,44) = 12.63$, $p = .0009$]. Thus, exposure to THC changed the effect of complex housing.

3.2.2 | AID

Complex housing decreased spine density and branching but had no effect on dendritic length. In contrast, THC increased all measures and there were no interactions (see Figure 3, Table1). Analysis of variance on branching showed a significant effect of housing [$F(1,44) = 17.00$, $p = .002$] and THC [$F(1,44) = 6.42$, $p = .04$], but no interaction. The only statistical effect on length was THC [$F(1,44) = 8.48$, $p = .006$]. Spine density showed a significant effect of housing [$F(1,44) = 6.78$, $p = .01$] and THC [$F(1,44) = 5.31$, $p = .03$], but no interaction.

TABLE 1 Summary of dendritic effects

Parameter	Housing	THC	Housing × THC
Cg3 branches	S	S	S
Cg3 length	N	S	N
Cg3 spines	S	N	S
AID branches	S*	S	N
AID length	N	S	N
AID spines	S*	S	N
NAcc branches	S	N	S
NAcc length	N	S	N
NAcc spines	S*	S	S

Abbreviations [S = significant increase ($p < .05$ or better); S* = significant decrease ($p < .05$ or better); N = no effect].

3.2.3 | Nacc

Complex housing increased branching and decreased spine density but did not affect dendritic length. In contrast, THC increased dendritic length and spine density but the branching was only increased in the cage-housed animals (see Figure 4, Table 1). Analysis of variance on branching showed a significant effect of housing [$F(1,44) = 17.05$, $p = .0002$] and THC [$F(1,44) = 14.56$, $p = .0004$], but no interaction. The only effect on length was THC [$F(1,44) = 36.41$, $p = .0001$]. Analysis of spine density showed a significant effect of THC [$F(1,44) = 44.78$, $p < .0001$] and the interaction [$F(1,44) = 6.51$, $p = .01$].

4 | DISCUSSION

The major results of this study support the conclusions that (1) THC alters both dendritic arborization and spine density in prefrontal cortex and NAcc; (2) Like psychomotor stimulants, THC interacts with the effects of complex housing on neuronal morphology in prefrontal cortex and NAcc; and (3) the effects of complex housing vary with cerebral region. We consider each conclusion in turn.

4.1 | Effects of THC on neuronal morphology

The Kolb et al. (2006) study examined the effects of THC on dendritic branching and length in mPFC (Cg3), OFC (AID) parietal cortex (Par1), occipital cortex (Occ1), hippocampus (CA1), striatum, and NAcc. Significant effects were only found in Cg3 and the shell of NAcc. In this study, we replicated our earlier finding of increased branching in Cg3 and NAcc and, in addition, showed significant increases in spine density in NAcc but not Cg3. One different result was that here we found increased branching, length, and spine density in AID. There are three differences between this study and the Kolb et al. (2006) study: (1) the earlier study used Sprague-Dawley rats whereas the current study used Long-Evans; (2) the rats in this study were born and raised in the University of Lethbridge vivarium whereas in the earlier study the rats were purchased from a breeder and housed at the vivarium at Wilfrid Laurier University; and, (3) in the Kolb et al. (2006), there was a withdrawal period of 30 days but in the current study it was 90 days. Therefore, a strain and/or rearing difference may account for the different effects on AID. The 90 day withdrawal period in the current study suggests that the THC effects on neuronal morphology are persistent, much like the effects of psychomotor stimulants (e.g., Kolb et al., 2003a).

Overall, both in this study and in studies of other drug effects on neuronal morphology it appears that the largest changes are seen in spines. This may reflect the fact that spines are the major location of excitatory synapses. The molecular mechanism driving these synaptic changes is uncertain but sensitization to both amphetamine and nicotine is associated with changes in gene expression (Mychasiuk, Muhammad, Ilnytsky, & Kolb, 2013; Robison & Nestler, 2011).

It is also worth noting that as in our earlier study we found no evidence of behavioral sensitization, which is consistent with other studies reporting that low doses of cannabinoids do not produce behavioral sensitization of locomotor activity (Arnold, Topple, Hunt, & McGregor, 1998; Ellgren, Hurd, & Franck, 2004; Muschamp & Siviy, 2002). Thus, it appears that THC can produce persistent changes in synaptic plasticity, even in the absence of behavioral sensitization. As well, the elevated locomotor activity produced by low doses of THC is consistent with reports that at low doses THC enhances locomotor activity but at high doses it suppresses locomotor activity (e.g., Sañudo-Peña, Romero, Seale, Fernandez-Ruiz, & Walker, 2000).

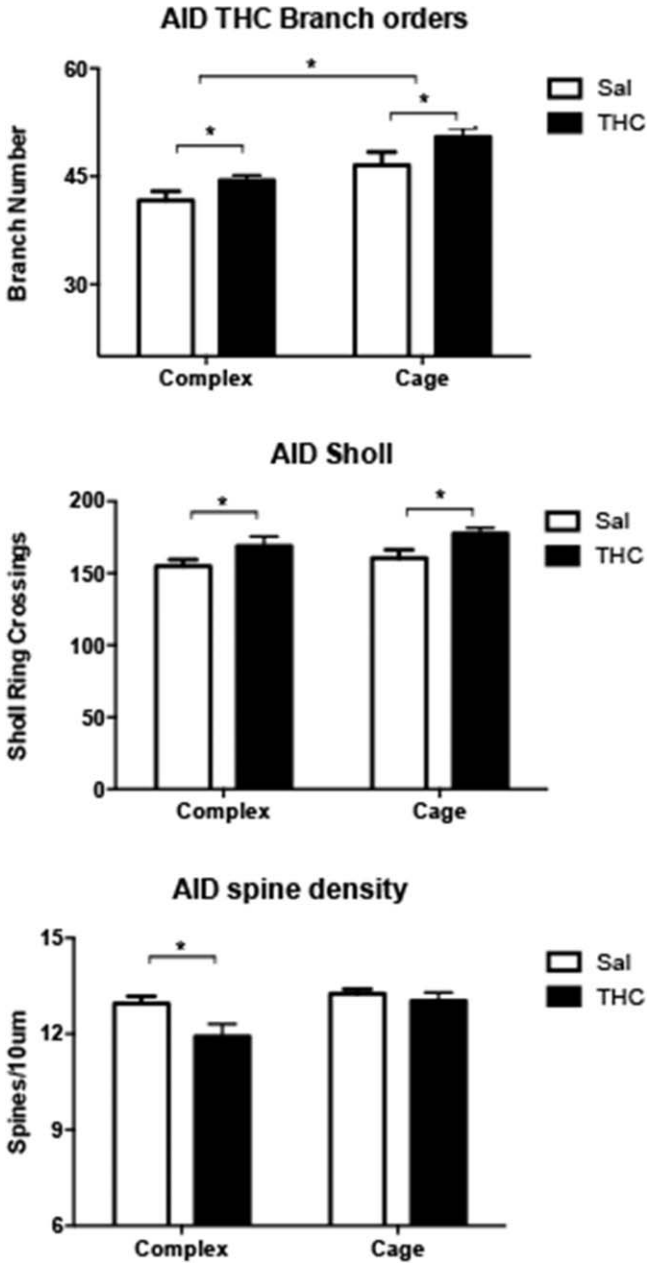

FIGURE 3 Summary of measures of AID neurons. There was a significant effect of housing and THC on branching, and an effect of THC on dendritic length and on spine density, although only in the complex housed group (p's $< .05$ or better)

4.2 | THC interacts with the effect of complex housing

Exposure to THC influences the effect of later experience in complex environments to shape the structure of neurons in prefrontal cortex and NAcc, just as psychomotor stimulants do (amphetamine, cocaine, and nicotine). In fact, it has also been shown that exposure to low doses of nicotine in utero also modifies the effect of complex housing in adulthood (Mychasiuk, Muhammad, & Kolb, 2014). Taken together, these results suggest that psychoactive drugs can limit or alter the effects of later experiences in modifying neuronal circuits. It is unknown whether similar drug effects would be seen with other types of plastic changes in the brain such as those seen in task learning, long-term potentiation, gonadal hormone manipulations, or cerebral injury. This is an important question because the results of this study, as well as our earlier ones with psychomotor stimulants, suggest that one long-term consequence of psychoactive drug exposure may be an alteration in behavior and psychological function.

An obvious question that arises is how the drugs interact with plastic changes related to other experiences. There are multiple mechanisms whereby drugs alter the transcriptional potential of genes (for a review see Robison & Nestler, 2011) and complex housing is associated with changed expression of many genes related to neuronal structure and plasticity (Rampon et al., 2000), increased expression of neurotrophic factors (van Praag et al., 2000), and FGF-2 reactive astrocytes (Kolb, Forgie, Gibb, Gorny, & Rowntree, 1998). It thus seems likely that molecular interactions

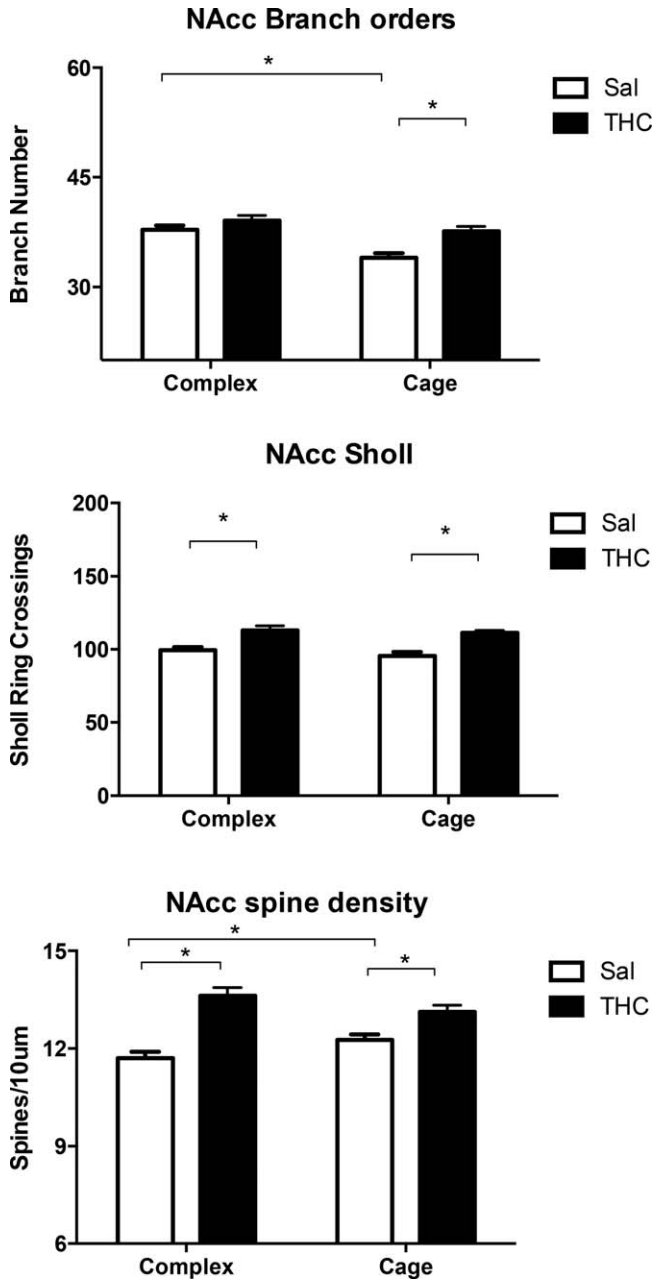

FIGURE 4 Summary of measures of NAcc neurons. THC significantly increased all measures of NAcc neurons. Housing only affected branching and spine density, but the effect on spine density only occurred in THC-treated animals

from both types of experience (i.e., drugs and complex housing) could lead to altered synaptic organization, although the details are currently unknown.

Dong and Nestler (2014) suggested that exposure to psychoactive drugs induces mechanisms of plasticity that normally occur during in reward circuitry during brain development. These re-awakened developmental mechanisms act to form unusually strong and long-lasting maladaptive changes in the brain. Following this logic, we suggest that one of the long-term effects may be an occlusion or modification of other forms of experience-dependent changes, such as what is normally seen in response to complex housing.

4.3 | Site-dependent changes in the effects of complex housing

Historically, most studies of the effects of complex housing have been focused on changes in neocortex and hippocampus (e.g., Bennett, Diamond, Krech, & Rosenzweig, 1964; Sirevaag & Greenough, 1987, 1988; Van Praag et al., 2000). In general, although the effects of complex housing appeared larger in posterior than anterior cortex, it has generally been assumed that complex housing induces synaptogenesis across the brain. In the course of studying the effects of experience on prefrontal regions and NAcc we showed that the effects of complex housing are more subtle

than elsewhere in the neocortex and, as seen in the current study, the changes in the AID were actually in the opposite direction, namely a pruning of dendritic material and synapses (see also Kolb, Gorny, Sonderpalm, & Robinson, 2003b). Thus, whereas sensory and motor regions typically show increases in dendritic branching, dendritic length, and spine density changes in mPFC are seen only in an increase in spine density and in OFC, there is a reduction in branching and spine density. This opposite effect in the two prefrontal regions has precedence as it is seen in response to both psychomotor stimulants and morphine (Robinson & Kolb, 2004). We should note that another study (Mychasiuk et al., 2014) placed rats in complex environments for 35 days beginning on postnatal Day 60 and although the effects on Cg3 neurons was similar to the current study, the effects on AID were different with no change in branching or spine density. This inter-study difference appears to reflect the fact that in contrast to mPFC, in which neuronal morphology appears adult by postnatal Day 60, neurons in the OFC continue to prune until about postnatal Day 90 (Himmler et al., submitted). We might therefore predict that the effects of complex housing will vary by age in OFC (see Kolb, Gibb, & Gorny, 2003c for a parallel effect in parietal cortex).

Finally, there was a reduction in spine density related to complex housing in NAcc in this study, an effect also seen in Mychasiuk et al. (2014). In contrast, two studies found the opposite result, namely an increase (Kolb et al., 2003a,b). The key difference is that the latter studies were conducted with Sprague-Dawley rats and the studies showing a reduction in spine density used Long-Evans rats. This difference suggests that there could be strain differences in the effects of complex housing, at least in some cerebral regions.

ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada, The Canadian Institutes for Health Research, The Canadian Institute for Advanced Research, and the National Institute on Drug Abuse (USA).

ORCID

Bryan Kolb http://orcid.org/0000-0002-2576-0642

REFERENCES

Arnold, J. C., Topple, A. N., Hunt, G. E., & McGregor, I. S. (1998). Effects of preexposure and co-administration of the cannabinoid receptor agonist CP 55,940 on behavioral sensitization to cocaine. *European Journal of Pharmacology*, *354*, 9–16.

Battistella, G., Fornari, E., Annoni, J.-M., Chitoui, H., Dao, K., Fabritius, M., … Giroud, C. (2014). Long-term effects of cannabis on brain structure. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *39*, 2041–2048.

Behan, A. T., Hryniewiecka, M., O'tuathaigh, C. M., Kinsella, A., Cannon, M., Karayiorgou, M., … Cotter, D. R. (2012). Chronic adolescent exposure to delta-9-tetrahydrocannabinol in COMT mutant mice: impact on indices of dopaminergic, endocannabinoid and GABAergic pathways. *Neuropsychopharmacol*, *37*, 1773–1783.

Bennett, E. L., Diamond, M. C., Krech, D., & Rosenzweig, M. R. (1964). Chemical and anatomical plasticity of the brain. *Science (New York, N.Y.)*, *146*, 610–619.

Churchwell, J. C., Lopez-Larson, M., & Yurgelun-Todd, D. A. (2010). Altered frontal cortical volume and decision making in adolescent cannabis users. *Frontiers in Psychology*, *1*, 225.

Dong, Y., & Nestler, E. J. (2014). The neural rejuvenation hypothesis of cocaine addiction. *Trends in Pharmacological Sciences*, *35*, 374–383.

Ellgren, M., Hurd, Y. L., & Franck, J. (2004). Amphetamine effects on dopamine levels and behavior following cannabinoid exposure during adolescence. *European Journal of Pharmacology*, *497*, 205–213.

Gardner, E. L., & Lowinson, J. H. (1991). Marijuana's interaction with brain reward systems: Update. *Pharmacology, Biochemistry, and Behavior*, *40*, 571–580.

Gibb, R., & Kolb, B. (1998). A method for vibratome sectioning of Golgi-Cox stained whole rat brain. *Journal of Neuroscience Methods*, *79*, 1–4.

Hamilton, D., & Kolb, B. (2005). Nicotine, experience, and brain plasticity. *Behavioral Neuroscience*, *119*, 355–365.

Himmler, B. T., Mychasiuk, R., Nakahashi, A., Himmler, S. M., Pellis, S. M., & Kolb, B. Juvenile social experience and differential age-related changes in the dendritic morphologies of subareas of the prefrontal cortex in rats. In submission.

Kolb, B., Forgie, M., Gibb, R., Gorny, G., & Rowntree, S. (1998). Age, experience, and the changing brain. *Neuroscience and Biobehavioral Reviews*, *22*, 143–159.

Kolb, B., Gibb, R., & Gorny, G. (2003c). Experience-dependent changes in dendritic arbor and spine density in neocortex vary with age and sex. *Neurobiology of Learning and Memory*, *791*, 1–10.

Kolb, B., Gorny, G., Li, Y., Samaha, A. N., & Robinson, T. E. (2003a). Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 10523–10528.

Kolb, B., Gorny, G., Limebeer, C. L., & Parker, L. A. (2006). Chronic treatment with Delta-9-tetrahydrocannabinol alters the structure of neurons in the nucleus accumbens shell and medial prefrontal cortex of rats. *Synapse (New York, N.Y.)*, *60*, 429–436.

Kolb, B., Gorny, G., Sonderpalm, A., & Robinson, T. E. (2003b). Environmental complexity has different effects on the structure of neurons in the prefrontal cortex versus the parietal cortex or nucleus accumbens. *Synapse (New York, N.Y.)*, *48*, 149–153.

Lawston, J., Borella, A., Robinson, J. K., & Whitaker-Azmitia, P. M. (2000). Changes in hippocampal morphology following chronic treatment with the synthetic cannabinoid WIN 55, 212-2. *Brain Research*, *877*, 407–410.

Muschamp, J. W., & Siviy, S. M. (2002). Behavioral sensitization to amphetamine follows chronic administration of the CB1 agonist WIN 55, 212-2 in Lewis rats. *Pharmacology, Biochemistry and Behavior*, *73*, 835–842.

Mychasiuk, R., Muhammad, A., Ilnytsky, S., & Kolb, B. (2013). Persistent gene expression changes in NAc, mPFC, and OFC associated with previous nicotine or amphetamine exposure. *Behavioural Brain Research*, *256*, 655–651.

Mychasiuk, R., Muhammad, A., & Kolb, B. (2014). Environmental enrichment alters structural plasticity of the adolescent brain but does not remediate the effects of prenatal nicotine exposure. *Synapse*, *68*, 293–305.

Orr, J. M., Paschall, C. J., & Banich, M. T. (2016). Recreational marijuana use impacts white matter integrity and subcortical (but not cortical) morphometry. *NeuroImage. Clinical*, *12*, 47–56.

Rampon, C., Jiang, C. H., Dong, H., Tang, Y.-P., Lockhart, D. J., Schultz, P. G., … Hu, Y. (2000). Effects of environmental enrichment on gene expression in the brain. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 12880–12884.

Robinson, T. E., & Kolb, B. (2004). Structural plasticity associated with drugs of abuse. *Neuropharmacology*, *47* Suppl 1, 33–46.

Robison, A. J., & Nestler, E. J. (2011). Transciptional and epigenetic mechanisms of addiction. *Nature Review Neuroscience*, *12*, 623–637

Sañudo-Peña, M. C., Romero, J., Seale, G. E., Fernandez-Ruiz, J. J., & Walker, J. M. (2000). Activational role of cannabinoids on movement. *European Journal of Pharmacology*, *391*, 269–274.

Scallet, A. C., Uemura, E., Andrews, A., Ali, S. F., McMillan, D. E., Paule, M. G., … Slikker, W. Jr. (1987). Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydrocannabinol (THC). *Brain Research*, *436*, 193–198.

Sirevaag, A. M., & Greenough, W. T. (1987). Differntial rearing effects on rat visual cortex synapses. III. Neuronal and glial nuclei, boutons, dendrites, and capillaries. *Brain Research*, *424*, 320–332.

Sirevaag, A. M., & Greenough, W. T. (1988). A multivariate statistical summary of synaptic plasticity measures in rats exposed to complex, social and individual environments. *Brain Research*, *441*, 386–392.

Spiga, S., Lintas, A., Migliore, M., & Diana, M. (2010). Altered architecture and functional consequences of the mesolimbic dopamine system in cannabis dependence. *Addiction Biology*, *15*, 266–276.

Van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews. Neuroscience*, *1*, 191–196.

Weiland, B. J., Thayer, R. E., Depue, B. E., Sabbineni, A., Bryan, A. D., & Hutchison, K. E. (2015). Daily marijuana use is not associated with brain morphometric measures in adolescents or adults. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *35*, 1505–1512.

How to cite this article: Kolb B, Li Y, Robinson T, Parker LA. THC alters alters morphology of neurons in medial prefrontal cortex, orbital prefrontal cortex, and nucleus accumbens and alters the ability of later experience to promote structural plasticity. *Synapse*. 2018;72:e22020. https://doi.org/10.1002/syn.22020