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Acute Δ^9-Tetrahydrocannabinol-Induced Deficits in Reversal Learning: Neural Correlates of Affective Inflexibility

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Despite concerns surrounding the possible adverse effects of marijuana on complex cognitive function, the processes contributing to the observed cognitive deficits are unclear, as are the causal relationships between these impairments and marijuana exposure. In particular, marijuana-related deficits in cognitive flexibility may affect the social functioning of the individual and may contribute to continued marijuana use. We therefore examined the ability of rats to perform affective and attentional shifts following acute administration of Δ^9-tetrahydrocannabinol (THC), the primary psychoactive marijuana constituent. Administration of 1 mg/kg THC produced marked impairments in the ability to reverse previously relevant associations between stimulus features and reward presentation, while the ability to transfer attentional set between dimensional stimulus properties was unaffected. Concurrent *in situ* hybridization analysis of regional c-*fos* and *ngfi-b* expression highlighted areas of the prefrontal cortex and striatum that were recruited in response to both THC administration and task performance. Furthermore, the alterations in mRNA expression in the orbitofrontal cortex and striatum were associated with the ability to perform the reversal discriminations. These findings suggest that marijuana use may produce inelasticity in updating affective associations between stimuli and reinforcement value, and that this effect may arise through dysregulation of orbitofrontal and striatal circuitry.

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INTRODUCTION

Marijuana use is widely assumed to compromise cognitive ability, but controlled scientific investigations of these impairments have yielded mixed conclusions (Chait and Perry, 1994; Fant *et al*, 1998; Hart *et al*, 2001; Pickworth *et al*, 1997). Possible deleterious effects of marijuana use on cognitive flexibility, a cardinal feature of the primate prefrontal cortex (PFC) (Dias *et al*, 1996a; Owen *et al*, 1991), may be of particular importance; inflexibility in attentional and affective control may be deleterious to intellectual and social functioning (Pope and Yurgelun-Todd, 1996), and may underlie perseveration to continued drug administration (Bolla *et al*, 2002; Jentsch and Taylor, 1999; Volkow and Fowler, 2000). While some studies have indicated that impairments in mental flexibility persist after approximately 1 day (Pope and Yurgelun-Todd, 1996) and 28 days of abstinence from marijuana (Bolla *et al*, 2002), other studies performed at similar time points have yielded negative or minimal results (Fletcher *et al*, 1996; Pope *et al*, 2001). Some of the discrepancies between these human studies may be due to inherently variable factors such as the frequency and duration of marijuana use, polydrug abuse, or premorbid cognitive ability, confounds that have formed the topic of a lively ongoing debate surrounding the effects of marijuana on complex cognitive function (Block, 1996; Pope, 2002; Scheier and Botvin, 1996; Solowij *et al*, 2002).

Mental flexibility allows mammals to adjust behavioral output according to changing environmental demands or conditions; when conditions change, animals must often learn a new strategy while inhibiting previously appropriate responses. Impairments in cognitive flexibility may therefore manifest in perseveration towards responses or behaviors that are inappropriate in current contexts, resulting from a failure to respond to alterations in task contingencies or outcome valences. Mental flexibility may involve two dissociable types of cognitive control, extra-dimensional set shifting and reversal learning. While extradimensional (attentional) set shifting ability refers to the capacity to shift attentional bias between different perceptual features of complex stimuli, reversal learning relates to capacity to update associations between exteroceptive stimuli and reinforcement presentation when the contingencies between stimuli and reward presentation are

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reversed. These processes are also anatomically dissociable; lesions of the monkey lateral PFC (Dias *et al*, 1996a, b, 1997) and the equivalent prelimbic and infralimbic regions of the rat medial frontal cortex (Birrell and Brown, 2000) markedly disrupt extradimensional attentional set shifting ability, while lesions of the orbitofrontal cortex (OFC) selectively impair reversal learning in both species (Butter, 1969; Dias *et al*, 1996a, 1997; Ferry *et al*, 2000; Iversen and Mishkin, 1970; Jones and Mishkin, 1972; McAlonan and Brown, 2003; Schoenbaum *et al*, 2002). Recently, studies have also emphasized a role for the ventral striatum in transforming reversed stimulus reward contingencies into altered behavioral responses (Cools *et al*, 2002, 2004; Crofts *et al*, 2001; Divac *et al*, 1967; Monchi *et al*, 2001; Rogers *et al*, 2000; Stern and Passingham, 1995).

We therefore investigated the ability of rats to perform affective and attentional shifts following acute administration of Δ^9-tetrahydrocannabinol (THC). In order to investigate the neural substrates mediating THC-induced alterations in task performance, we also characterized regional alterations in the expression levels of mRNA encoding two immediate-early genes (IEGs), c-*fos* and *ngfi-b*, as a consequence of both THC administration and discrimination performance. IEG expression provides a marker of alterations in regional neural activation occurring in response to several stimuli (Morgan and Curran, 1989). *C-fos* and *ngfi-b* were selected as markers for use in the present study as they belong to complementary transcription factor families (Persico and Uhl, 1996) and because initial studies showed that these IEGs were sensitive to THC administration.

MATERIALS AND METHODS

Animals

A total of 36 male hooded Long-Evans rats (Harlan Olac, UK) were housed individually in standard conditions (a temperature-regulated room with a 12 h dark/light cycle (lights on at 0600)) and were maintained on a diet of 18–22 g food per day for a minimum of 2 weeks prior to commencement of behavioral testing. Under this schedule, all animals gained weight and no animals showed a weight of less than 85% *ad libitum* body weight. Water was always available in the home cage. All testing was conducted in the light phase of a 12 h dark/light cycle. The experiment was carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986, and associated guidelines.

Drug Administration

In order to distinguish between the effects of THC administration and behavioral testing on IEG expression, and any interaction between the two, animals were initially subdivided into behavior-positive and behavior-negative groups that did and did not undergo behavioral testing, respectively. Each cohort was then further subdivided into three treatment groups receiving vehicle (1% Tween 80 in saline), 0.01 mg/kg THC, or 1.0 mg/kg THC (Sigma, UK). THC was prepared according to a previously published method (Pertwee *et al*, 1992). To permit counterbalancing in the behavioral task, an $n = 12$ per treatment group was employed for behavior-positive animals, but, to reduce animal use, an $n = 8$ per treatment group was employed in the behavior-negative animals, which were paired as far as group numbers permitted with behavior-positive animals receiving the same drug treatment. Animals received drug administration i.p. 30 min prior to the start of the behavioral test. In cases where behavior-positive and behavior-negative animals were paired, one animal from each group received identical drug administrations at the same time.

Behavioral Apparatus

Small ceramic pots (diameter 7 cm, depth 4 cm) were used as digging bowls, which could contain the food reward of one-half of a Honey Nut Loop ($\frac{1}{2}$ HNL) (Kellogg, Manchester, UK). The bowls were filled with different digging media that could be scented. The test apparatus consisted of an adapted plastic home-cage ($40 \times 70 \times 18$ cm), with sawdust covering the base. One-third of the box was divided into two sections by Plexiglas panels, into which the bowls were placed. A removable divider separated these sections from the rest of the box, so the rat could be given access to the bowls by lifting the divider. In addition, another removable divider was used to block one of the two compartments when an error was recorded (see below). Matched animals in behavior-negative groups were placed in the test room but remained in their home-cages. During the session, they were fed the same amount of food reward as eaten by the behavior-positive cohort during the test.

Habituation Phase

The habituation and testing procedures were originally adapted from Birrell and Brown (2000) and have since been detailed elsewhere (Barense *et al*, 2002; Fox *et al*, 2003; McAlonan and Brown, 2003; Tunbridge *et al*, 2004). Up to 48 h before testing, animals in the behavior-positive groups were habituated to the behavioral task. Animals were initially given access to two digging bowls filled with cork pieces, each containing the $\frac{1}{2}$ HNL food reward. The bowls were re-baited six times so that the rat was reliably digging for the food reward. Rats then performed two sequential simple discriminations (SDs), in which they were presented with two bowls of different stimulus properties, one of which contained the reward. Rats were initially trained on a discrimination based on the texture of the digging medium, where the reward was paired with tealeaves but not tea granules. Next followed odor discrimination training, where the reward was paired with basil but not rosemary in sand. In each case, rats were trained to criterion performance levels of six consecutive correct digs and these stimuli were not used again during the experiment.

Behavioral Testing Paradigm

During a single session, all rats performed the series of discriminations in the order outlined in Table 1. The combinations of stimulus exemplars that were employed are given in Table 2. At each discrimination stage, trials began by raising the divider to allow the animal to explore two bowls, one of which contained the positive stimulus

Table 1 Order of Discriminations Performed

	Dimensions		Exemplar combinations	
	Relevant	**Irrelevant**	**S+**	**S−**
SD	Odor	Medium	**O1**	O2
CD	Odor	Medium	**O1**/M1	O2/M2
			O1/M2	O2/M1
Rev1	Odor	Medium	**O2**/M1	O1/M2
			O2/M2	O1/M1
IDS	Odor	Medium	**O3**/M3	O4/M4
			O3/M4	O4/M3
Rev2	Odor	Medium	**O4**/M3	O3/M4
			O4/M4	O3/M3
EDS	Medium	Odor	**M5**/O5	M6/O6
			M5/O6	M6/O5
Rev3	Medium	Odor	**M6**/O5	M5/O6
			M6/O6	M5/O5

The table illustrates examples of combinations of exemplars into stimulus pairs for a rat shifting set from odor to digging medium at the EDS stage. An equal number of rats in each treatment group shifted from odor to medium and from medium to odor. On every trial except the SD, the pair of stimuli presented differed along both the relevant and irrelevant dimensions. The correct exemplar is shown in bold, and was paired with either exemplar from the irrelevant dimension. The combination of exemplars into positive (S+) and negative (S−) stimuli and their left–right position of presentation in the cage was a pseudorandom series (adapted from Birrell and Brown, 2000).

Table 2 Exemplar Combinations Employed

Odor pairs	**Medium pairs**
Cloves *vs* nutmeg	Fine *vs* coarse sawdust
Thyme *vs* paprika	Small *vs* large pebbles
Oregano *vs* mint	Confetti *vs* polystyrene

The exemplars within a dimension were presented in pairs and varied so that an equal number of animals in each treatment group received each exemplar combination at each stage of the test.

associated with the food reward. A correct response was recorded if the first dig occurred in the correct bowl. The first four trials were always discovery trials in that the animal was allowed to explore both bowls, but in subsequent trials the divider prevented access to the correct bowl if the first dig occurred in the non-rewarded bowl. Once the animal had reached criterion levels of six correct consecutive digs, testing progressed to the next discrimination.

Initially, rats were trained on an SD where stimuli differed only along one dimension (medium or odor). At the compound discrimination (CD) acquisition stage, an additional dimension was introduced but the relevant dimension and stimuli remained the same. Two further acquisition discriminations were employed in the test, the intradimensional shift (IDS) and extradimensional shift (EDS) stages. At both the IDS and EDS stages, the animals were presented with completely new sets of stimuli. However, whereas at the IDS stage the relevant dimension remained the same as in previous discriminations, at the EDS stage the relevant and irrelevant dimensions were reversed; so, for example, an animal initially trained with odor as the relevant dimension would shift to medium at the EDS stage. Each acquisition stage was followed by a reversal discrimination (Rev1, Rev2, Rev3), during which the stimuli remained the same as in the preceding acquisition discrimination, but positive and negative stimuli were reversed. An equal number of rats were trained with odor as the initial relevant dimension, shifting to medium at the ED stage, and *vice versa*. The order of presentation of stimulus pairs was also counterbalanced within treatment groups, and the sequential order and left/right presentation of stimuli were pseudorandomly determined.

Brain Section Collection and Preparation

Following the completion of the behavioral task, animals were killed by cervical dislocation and brains were removed and frozen. Coronal sections (20 μm) were taken at levels 3.2 mm (medial frontal cortex) and 1.6 mm (striatum) anterior to bregma according to Paxinos and Watson (1998), and collected onto poly-L-lysine-coated slides. Once dry, sections were fixed in ice-cold, 4% (wt/vol) paraformaldehyde in phosphate-buffered saline (PBS) for 5 min. After rinsing in PBS for 5 min, the sections were dehydrated by 5 min consecutive immersions in 70, 95, and 100% ethanol.

In Situ Hybridization

Oligonucleotide (45-mer) probes of sequence complementary to c-*fos* (Curran *et al*, 1987) and *ngfi-b* (Berke *et al*, 1998) mRNA (Cruachem Ltd, UK) were 3′ end-labeled with 5-α-^{35}S-dATP (specific activity 1250 Ci/mmol, NEN Life Science Products, UK Ltd) using terminal deoxyribonucleotidyl transferase enzyme (Amersham Pharmacia, UK) and incubated at 37°C for 90 min. A volume of 40 μl of diethylpyrocarbonate (DEPC)-treated water was added to terminate the reaction and the labeled probes were purified using QIAquick nucleotide removal kits (Qiagen Ltd, UK). The extent of probe labeling was determined using β-scintillation counting; probes labeled from 100 000 to 300 000 d.p.m/μl were used for *in situ* hybridization. Radiolabeled probes were hybridized onto coronal brain sections overnight at 42°C in 200 μl of a hybridization buffer (50% deionized formamide, 20% 20 × standard saline citrate (20 × SSC: 3 M sodium chloride; 0.3 M sodium citrate, pH 7), 5% 0.5 M sodium phosphate (pH 7), 1% 0.1 M sodium pyrophosphate, 2% 5 mg/ml polyadenylic acid, 10% dextran sulfate, and 1 M dithiothreitol) containing 0.05 ng/μl labeled probe. Following overnight hybridization, the sections were washed for 30 min in 1 × SSC at 60°C. The sections were then washed in 1 × SSC and 0.1 × SSC, and dehydrated through emersion in a graded series of ethanol solutions (70–100%). Once dry, the sections were exposed to autoradiographic film (Kodak Biomax MR1), and the resulting autoradiograms were developed according to the manufacturer's instructions. Levels of regional mRNA expression were quantified using the MCID densitometry system. Bilateral relative optical density (ROD) measurements were taken from duplicate sections from each animal from the following prefrontal and striatal regions as anatomically defined by Paxinos and Watson (1998): prelimbic cortex, infralimbic cortex, ventral and lateral orbital cortices, dorsolateral striatum, and the core and shell subdivisions of the nucleus accumbens.

Analysis of Behavioral Data

For each of the discriminations, the number of trials to criterion was recorded for each rat. Data were analyzed using repeated measures ANOVA with three factors, one within subjects (*discrimination*: SD, CD, Rev1, IDS, Rev2, EDS, Rev3) and two between subjects (*group*: vehicle, 0.01 mg/kg THC, or 1.0 mg/kg THC; *initial relevant dimension*: odor or medium) with simple main effects *post hoc* tests (Bonferroni method).

As THC administration may also stimulate or inhibit motor output (Sañudo-Peña *et al*, 2000) and increase sucrose palatability (Higgs *et al*, 2003), it is possible that THC administration may also affect the rate of responding on the behavioral task. To investigate this possibility, the time taken by the animals to complete each of the discriminations was recorded. To obtain an approximate value, the average time to dig after stimulus presentation was calculated by dividing the total time to complete the discrimination by the number of trials required to complete that discrimination. Data were analyzed using repeated measures ANOVA with discrimination as the within-subjects factor (SD, CD, Rev1, IDS, Rev2, EDS, Rev3) and drug treatment group as the between-subjects factor (*group*: vehicle, 0.01 mg/kg THC, or 1.0 mg/kg THC). In addition, as the length of the time period between drug administration and euthanasia may affect mRNA expression levels, the total time required to complete the entire task was recorded to confirm that there were no between-group differences in this variable. These data were analyzed using one-way ANOVA with treatment group as the between-subjects factor.

Analysis of mRNA Expression Data

The effects of THC administration and completion of the behavioral task on regional mRNA expression were examined using two-way ANOVA. Where appropriate, subsequent *post hoc* analysis was performed using Tukey's HSD procedure. Where behavioral testing significantly altered regional mRNA gene expression, the contribution of the individual discrimination types to the observed effect was further examined by calculation of the partial correlation coefficients between mRNA expression levels and performance at each discrimination stage, while correcting for treatment group. All analysis was performed using SPSS software for Windows (SPSS Inc. Version 11) and the threshold for statistical significance was defined as $p<0.05$.

RESULTS

THC Administration and Discrimination Performance

During task habituation, and therefore prior to drug administration, all rats learned to dig in bowls to retrieve the food reward and perform the SDs.

Figure 1 illustrates the number of trials required to reach criterion performance levels on the series of discriminations presented during the test session. Two rats in the highest dose THC group (1 mg/kg) failed to complete the task, as they stopped responding for over 2 h during either the first or second reversal stage. These rats were therefore excluded from further analysis.

Overall, the discriminations tested were not of equal difficulty, as ANOVA revealed a significant main effect of task stage on the trials required to reach criterion performance ($F_{(6,168)}=10.34$; $p<0.001$). Although drug treatment affected overall task performance, as indicated by significant main effects of treatment group ($F_{(2,28)}=19.12$; $p<0.001$), THC administration did not affect task performance to an equivalent degree at each discrimination stage, as realized in the significant treatment group × task stage interaction ($F_{(12,168)}=1.914$; $p=0.036$). Subsequent ANOVA analysis of individual task stages confirmed results indicated in Figure 1; while there were no significant effects of treatment group on performance at the SD, CD, and ED stages ($F_{(2,28)}=2.147$–2.365; NS), drug treatment did affect performance on the ID shift ($F_{(2,28)}=5.495$; $p=0.010$) and first ($F_{(2,28)}=4.464$; $p=0.021$), second ($F_{(2,28)}=7.847$;

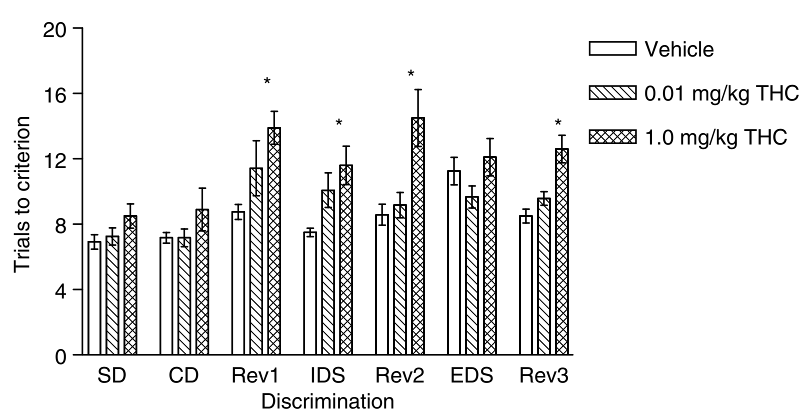

Figure 1 Effect of acute THC administration on task performance. At 30 min before the start of the task, rats were administered vehicle ($n=12$), 0.01 mg/kg THC ($n=12$), or 1.0 mg/kg THC ($n=10$) and the number of trials to reach criterion performance was recorded for a series of discriminations (SD: simple discrimination; CD: compound discrimination; Rev1,2,3: first, second, and third reversal stages; IDS: intradimensional shift; EDS: extradimensional shift). Animals in the 1 mg/kg THC treatment group exhibited marked deficits in performance at each of the reversal stages and during the IDS, *$p<0.05$ *vs* vehicle-treated control.

$p = 0.002$), and third ($F_{(2,28)} = 14.076$; $p < 0.001$) reversal stages. *Post hoc* analysis of these results confirmed that animals receiving 1.0 mg/kg THC treatment group required more trials to reach criterion than vehicle-treated control animals at both the ID shift ($p = 0.009$), and first ($p = 0.018$), second ($p = 0.003$), and third ($p = 0.002$) reversal stages. No significant behavioral effects were detected at the lower dose of 0.01 mg/kg THC.

Although there was no significant main effect of the dimension on which the animals were initially trained on overall performance during the test ($F_{(1,28)} = 1.75$; NS), there was a significant interaction between the initial relevant dimension and discrimination performance ($F_{(6,168)} = 2.783$; $p = 0.013$). Further analysis showed that the effect of relevant dimension was significant at the CD ($F_{(1,28)} = 8.367$; $p = 0.007$) and ED ($F_{(1,28)} = 8.402$; $p = 0.007$) stages, with fewer trials being required to reach criterion when the relevant dimension was the digging medium. These results suggest that acquisition of the discrimination rule was easier when medium was the relevant stimulus and highlight the importance of counterbalancing shift directions across treatment groups. Importantly, no significant two-way interaction was detected between initial relevant dimension and treatment group ($F_{(2,28)} = 1.471$; NS), or three-way interaction between initial relevant dimension, treatment group, and task stage ($F_{(12,168)} = 0.773$; NS) suggesting that, although the relevant dimension may have contributed to discrimination performance, this was not influenced by THC administration.

There were no significant main effects of treatment group ($F_{(2,31)} = 0.618$; NS), discrimination stage ($F_{(6,186)} = 1.009$; NS), or significant treatment group × discrimination stage interactions ($F_{(12,186)} = 1.180$; NS) on the average time to dig on each trial, suggesting that the potential motoric or appetitive effects of THC did not affect task performance (data not shown). In addition, after the exclusion of the two rats in the 1 mg/kg THC group that failed to complete the test, there was no significant effect of treatment group for the time required by the remaining rats to perform the series of presented discriminations ($F_{(2,33)} = 0.072$; NS). Any

Table 3 Effect of THC Administration and Behavioral Testing on Regional *c-fos* Expression

	Behavior negative			Behavior positive		
	Vehicle	**0.01 mg/kg THC**	**1.0 mg/kg THC**	**Vehicle**	**0.01 mg/kg THC**	**1.0 mg/kg THC**
PrL	0.070±0.010	0.067±0.008	0.047±0.001*	0.085±0.008	0.067±0.006	0.059±0.004*
Il	0.088±0.015	0.091±0.007	0.052±0.001*	0.093±0.007	0.074±0.006	0.071±0.005*
VO	0.109±0.011	0.095±0.012*	0.066±0.003*	0.104±0.009	0.082±0.005*	0.076±0.004*
LO	0.077±0.008	0.077±0.007	0.048±0.003*	0.094±0.006#	0.079±0.006#	0.077±0.006*,#
dlStr	0.024±0.002	0.017±0.003	0.021±0.004	0.021±0.004#	0.025±0.002#	0.030±0.003#
NAcC	0.025±0.003	0.021±0.003	0.020±0.004	0.028±0.004#	0.031±0.003#	0.032±0.005#
NAcS	0.023±0.003	0.016±0.003	0.018±0.004	0.027±0.003#	0.026±0.003#	0.025±0.003#

c-fos expression is shown as mean ± SEM ROD in the prelimbic cortex (PrL), infralimbic cortex (Il), ventral orbital cortex (VO), lateral orbital cortex ((LO), dorsolateral striatum (dlStr), nucleus accumbens core (NAcC), and nucleus accumbens shell (NAcS) of animals that either did (behavior-positive) or did not (behavior-negative) perform the attentional set shifting task. Administration of THC significantly decreased *c-fos* expression in several cortical regions (*$p < 0.05$ *vs* vehicle-treated control). Significant main effects of behavioral experience on *c-fos* expression were also detected (#$p < 0.05$ *vs* nonbehaviorally tested animals). No significant THC × behavioral testing interactions were apparent.

Table 4 Effect of THC Administration and Behavioral Testing on Regional *ngfi-b* Expression

	Behavior-negative			Behavior-positive		
	Vehicle	**0.01 mg/kg THC**	**1.0 mg/kg THC**	**Vehicle**	**0.01 mg/kg THC**	**1.0 mg/kg THC**
PrL	0.093±0.011	0.091±0.006	0.108±0.014	0.136±0.014#	0.123±0.010#	0.118±0.012#
Il	0.084±0.012	0.085±0.007	0.100±0.010	0.104±0.009#	0.106±0.012#	0.115±0.012#
VO	0.143±0.014	0.140±0.013	0.156±0.014	0.166±0.021	0.151±0.010	0.151±0.014
LO	0.093±0.009	0.086±0.008	0.088±0.008	0.125±0.012#	0.131±0.007#	0.124±0.012#
dlStr	0.078±0.009	0.079±0.005	0.113±0.009*	0.112±0.005	0.102±0.006	0.128±0.007*
NAcC	0.053±0.007	0.055±0.006	0.062±0.006	0.081±0.008#	0.081±0.008#	0.083±0.007#
NAcS	0.048±0.006	0.046±0.007	0.056±0.008	0.064±0.008#	0.067±0.006#	0.070±0.005#

ngfi-B expression is shown as mean ± SEM ROD in the prelimbic cortex (PrL), infralimbic cortex (Il), ventral orbital cortex (VO), lateral orbital cortex ((LO), dorsolateral striatum (dlStr), nucleus accumbens core (NAcC), and nucleus accumbens shell (NAcS) of animals that either did (behavior-positive) or did not (behavior-negative) perform the attentional set shifting task. Administration of THC significantly increased *ngfi-b* expression in the dorsolateral striatum (*$p < 0.05$ *vs* vehicle-treated control). Significant main effects of behavioral experience on *ngfi-b* expression were also detected (#$p < 0.05$ *vs* nonbehaviorally tested animals). No significant THC × behavioral testing interactions were apparent.

THC-induced alterations in regional mRNA expression were therefore unlikely to be a consequence of group differences in the length of the time period between drug administration and euthanasia.

THC Administration and Regional c-*fos* and *ngfi-b* mRNA Expression

The regional expression levels of c-*fos* and *ngfi-b* following both THC administration and behavioral testing are given in Tables 3 and 4 respectively. THC administration altered c-*fos* expression in several prefrontal cortical regions (Figure 2). Specifically, ANOVA revealed significant overall effects of THC administration on c-*fos* expression in prelimbic ($F_{(2,46)} = 4.756$; $p = 0.014$), infralimbic ($F_{(2,46)} = 5.503$; $p = 0.008$), ventral orbital ($F_{(2,46)} = 8.164$; $p = 0.001$), and lateral orbital ($F_{(2,46)} = 5.668$; $p = 0.007$) cortices. *Post hoc* analysis revealed that administration of 1.0 mg/kg THC produced highly significant decreases in c-*fos* expression from vehicle-treated control levels ($p = 0.001$–0.009) in these regions. In addition, significant decreases in c-*fos* expression in the ventral orbital cortex were observed at the lower dose of 0.01 mg/kg THC ($p = 0.035$). No THC-induced alterations in c-*fos* expression were detected in the striatal areas ($F_{(2,51)} = 0.023$–1.462; NS).

As illustrated in Figure 3, although THC administration did not significantly alter *ngfi-b* expression in any of the cortical regions examined ($F_{(2,52)} = 0.046$–0.879; NS), alterations were detected in *ngfi-b* expression in the dorsolateral striatum ($F_{(2,52)} = 10.349$; $p < 0.001$), with *post hoc* analysis revealing significant increases at 1.0 mg/kg THC compared to control levels ($p = 0.006$).

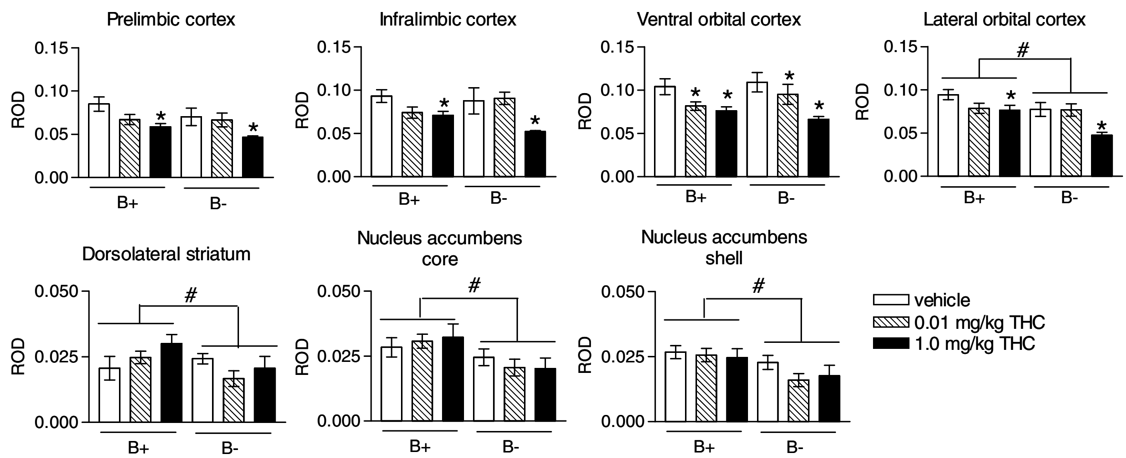

Figure 2 Effect of THC administration and behavioral testing on regional c-*fos* expression. c-*fos* expression is shown as mean ± SEM ROD in the prelimbic cortex, infralimbic cortex, ventral and lateral orbital cortices, dorsolateral striatum, nucleus accumbens core, and nucleus accumbens shell of animals that either did (behavior-positive, B+) or did not (behavior-negative, B−) perform the attentional set shifting task. Administration of THC significantly decreased c-*fos* expression in several cortical regions (**p* < 0.05 *vs* vehicle-treated control). Significant main effects of behavioral experience on c-*fos* expression were also detected (#*p* < 0.05 *vs* nonbehaviorally tested animals). No significant THC × behavioral testing interactions were apparent.

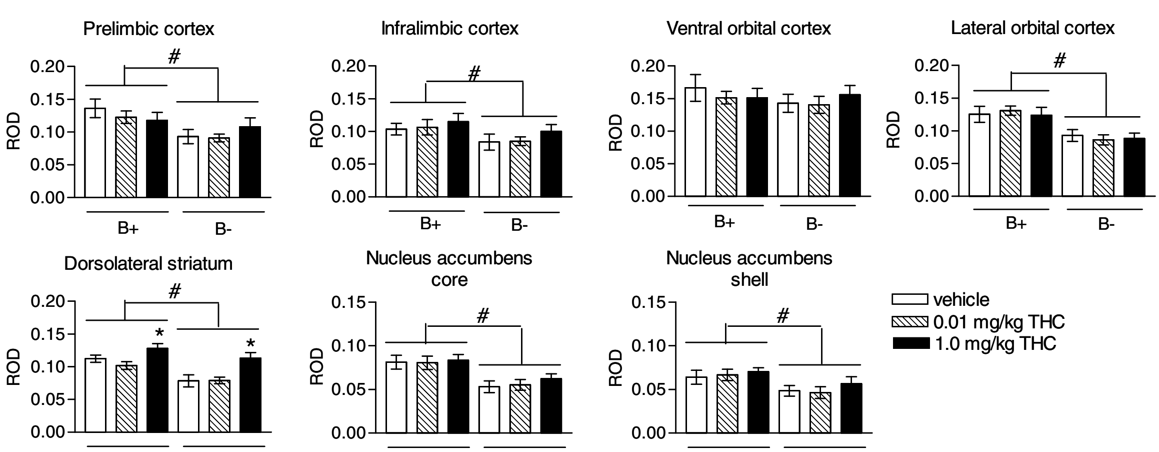

Figure 3 Effect of THC administration and behavioral testing on regional *ngfi-b* expression. *ngfi-b* expression is shown as mean ± SEM ROD in the prelimbic cortex, infralimbic cortex, ventral and lateral orbital cortices, dorsolateral striatum, nucleus accumbens core, and nucleus accumbens shell of animals that either did (behavior-positive, B+) or did not (behavior-negative, B−) perform the attentional set shifting task. Administration of THC significantly increased *ngfi-b* expression in the dorsolateral striatum (**p* < 0.05 *vs* vehicle-treated control). Significant main effects of behavioral experience on *ngfi-b* expression were also detected (#*p* < 0.05 *vs* nonbehaviorally tested animals). No significant THC × behavioral testing interactions were apparent.

Task Performance and Regional c-*fos* and *ngfi-b* mRNA Expression

As illustrated in Figure 2, behavioral testing increased c-*fos* expression in the lateral orbital cortex ($F_{(1,46)} = 7.276$; $p = 0.010$), dorsolateral striatum ($F_{(1,51)} = 6.692$; $p = 0.013$), nucleus accumbens core ($F_{(1,51)} = 7.320$; $p = 0.009$), and nucleus accumbens shell ($F_{(1,51)} = 7.652$; $p = 0.008$). Significant main effects of behavioral testing on *ngfi-b* expression were detected in the prelimbic ($F_{(1,52)} = 8.177$; $p = 0.006$), infralimbic ($F_{(1,52)} = 4.211$; $p = 0.046$), and lateral orbital cortices ($F_{(1,52)} = 18.889$; $p < 0.001$), dorsolateral striatum ($F_{(1,52)} = 17.263$; $p < 0.001$), nucleus accumbens core ($F_{(1,52)} = 17.070$; $p < 0.001$), and nucleus accumbens shell ($F_{(1,52)} = 8.437$; $p = 0.006$).

No significant drug treatment × behavioral testing interactions were detected in any of the regions examined.

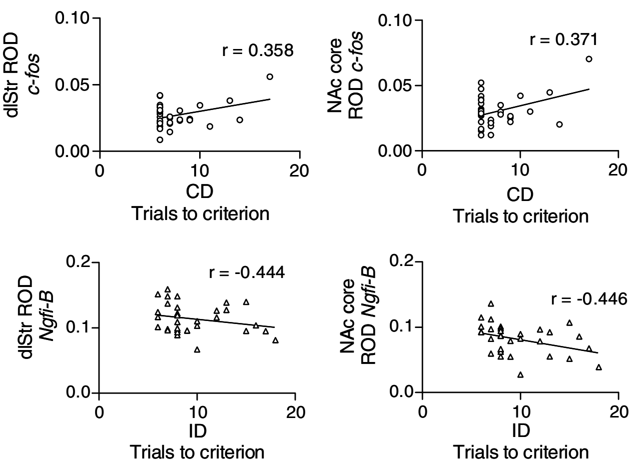

Figure 4 Correlations between regional c-*fos* and *ngfi-b* expression and acquisition performance. Accounting for effects of THC administration, significant correlations were detected between c-*fos* expression (o) in the dorsolateral striatum (dlStr) and nucleus accumbens core (NAc core) and performance of the CD, while *ngfi-b* expression (Δ) in these regions correlated with performance of the IDS. mRNA expression is given as ROD and behavioral performance is illustrated as the number of trial to reach criterion performance levels.

Relationships between Alterations in mRNA Expression and Task Performance

In brain areas where behavioral testing was found to alter mRNA expression levels, the relationships between regional activation and behavioral performance were further explored by correlational analysis. As shown in Figure 4, performance at the initial stage of task acquisition (CD) was associated with c-*fos* expression in both the dorsolateral striatum ($r = 0.358$; $p = 0.048$) and nucleus accumbens core ($r = 0.371$; $p = 0.040$). Interestingly, activation of these areas was also associated with performance at the IDS acquisition stage, but, in contrast to the CD stage, this association was signaled by alterations in *ngfi-b* expression. Thus, significant correlations were detected between IDS performance and *ngfi-b* expression in the dorsolateral striatum ($r = -0.444$; $p = 0.012$) and nucleus accumbens core ($r = -0.446$; $p = 0.012$).

As illustrated in Figure 5, analysis also revealed brain areas that may be associated with reversal learning performance in the rat. Specifically, performance on the first reversal stage was associated with c-*fos* expression in the dorsolateral striatum ($r = 0.381$; $p = 0.035$), nucleus accumbens shell ($r = 0.477$; $p = 0.007$), and lateral orbital cortex ($r = 0.351$; $p = 0.049$). Although no significant correlations were detected for performance at the second reversal stage, performance at the third reversal correlated with *ngfi-b* expression in the dorsolateral striatum ($r = 0.505$; $p = 0.004$), nucleus accumbens core ($r = 0.375$; $p = 0.038$), and prelimbic cortex ($r = 0.412$; $p = 0.021$).

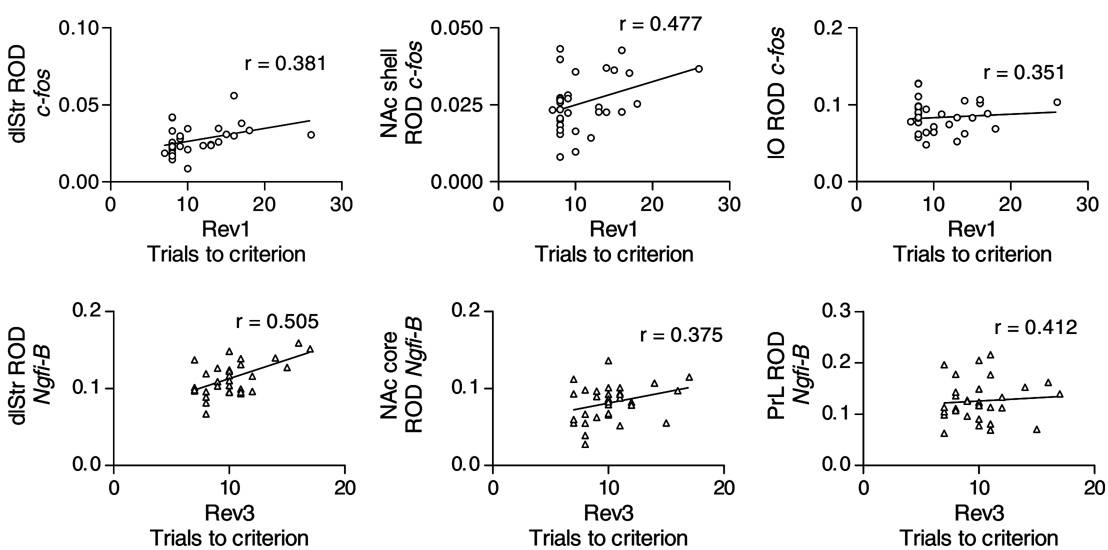

Figure 5 Correlations between regional c-*fos* and *ngfi-b* expression and reversal learning performance. Accounting for effects of THC administration, significant correlations were detected between c-*fos* expression (o) in the dorsolateral striatum (dlStr), nucleus accumbens shell (NAc core) and lateral orbital cortex (lO) and performance of the first reversal (Rev1), while *ngfi-b* expression (Δ) in the caudate putamen, nucleus accumbens core (NAc core), and prelimbic cortex (PrL) correlated with performance of the third reversal (Rev3). mRNA expression is given as ROD and behavioral performance is illustrated as the number of trial to reach criterion performance levels.

DISCUSSION

Acute administration of THC impaired performance on an attentional set shifting task when rats were required to reverse stimulus reward associations (Rev) or shift cognitive set between stimuli belonging to the same perceptual dimension (IDS). In contrast, the ability to shift attentional set between perceptual dimensions (EDS) was unaffected by THC administration. The observed deficits in reversal learning, together with the preservation of ability to shift strategy, suggest that acute THC administration selectively increases rigidity in the processes required to update responses based on affective associations between stimuli and reward presentation, but does not affect ability for higher order attentional flexibility. These effects occurred at doses of THC relevant to human use; applying dose-scaling factors from humans to rodents (Mordenti and Chappell, 1989), we estimate that the dose of 1 mg/kg employed in the present study would equate to moderate levels of cannabis intake in humans (Atha, 2003).

As stated in the introduction, deficits in mental flexibility have been observed in marijuana users (Bolla *et al*, 2002; Pope and Yurgelun-Todd, 1996) although other studies have reported no differences compared to nonusers (Fletcher *et al*, 1996; Hart *et al*, 2001). However, the cognitive tasks employed in these studies involve several different cognitive components, and inflexible responding may therefore arise as a consequence of an impairment at one of several levels of cognitive processing (Rogers *et al*, 2000). The componential analysis provided in the present study suggests that acute administration of THC results in impairments in affective flexibility, rather than in the ability to shift strategy or set *per se*. It is possible that a similar analysis of cognitive flexibility in human marijuana users may parallel these findings, or, alternatively, future studies employing repeated THC administration regimes in rodents may demonstrate that additional deficits in the ability to shift attentional set at the dimensional level arise on recurrent exposure to the drug. Nonetheless, the present results do suggest a causal association between marijuana intake and impairments in aspects of cognitive control in humans.

Deficits in reversal learning may be of particular relevance to continued self-administration of marijuana that occurs in human populations. Inflexibility in stimulus–reward associations may contribute to continued propensity to self-administer drugs, as the reward value of the drug or associated stimuli may not be updated in response to devaluation by the emergence of tolerance or adverse social consequences (Bolla *et al*, 2002; Jentsch and Taylor, 1999; Volkow and Fowler, 2000). Deficits in reversal learning may reflect either a failure in learning new associations between stimuli or deficits in the ability to inhibit previously learned stimulus–reward contingencies. Although we were unable to distinguish these possibilities in the present task, disinhibition has also been reported following marijuana intake in humans (Liraud and Verdoux, 2000; Spinella, 2003).

THC-treated rats also exhibited impaired performance at the IDS stage of the task, where the dimensional discrimination rule must be transferred to novel stimuli. This impairment may indicate THC-induced deficiencies in the ability to either maintain attentional set toward a particular dimension (which may also impact on reversal learning), or in ability to generalize previously learned strategies to novel situations. The difference in the difficulty in performing EDS and IDS stages may be used as evidence that subjects have effectively formed an attentional set toward a dimension (Eimas, 1966). In the current study, although this difference was present in vehicle-treated control animals, indicating that the task was working well, the extension of the number of trials to criterion required to complete the IDS in THC-treated animals resulted in the loss of difference in difficulty between the IDS and EDS transfers. Interestingly, in the rat OFC lesion study performed by McAlonan and Brown (2003), a similar but nonsignificant increase in IDS ability in the OFC lesion group resulted in the loss of a difference in ability at IDS/EDS discriminations.

Analysis of alterations in c-*fos* and *ngfi-b* expression in response to THC administration revealed that THC decreased c-*fos* expression in frontal cortical regions and increased *ngfi-b* expression in the dorsolateral striatum. This regional profile of effects is largely in accordance with the distribution of CB1 cannabinoid receptors at which THC acts (Devane *et al*, 1988; Mechoulam *et al*, 1970; Glass *et al*, 1997; Herkenham *et al*, 1990, 1991a, b), and THC-induced alterations in activity in these areas have also been demonstrated in previous IEG and metabolic mapping studies performed in rodents (Bloom *et al*, 1997; Erdtmann-Vourliotis *et al*, 1999; Mailleux *et al*, 1994; Margulies and Hammer, 1991; McGregor *et al*, 1998; Whitlow *et al*, 2002). In addition, human imaging studies have consistently demonstrated marked alterations in activity in frontal brain regions following acute marijuana/THC intake or chronic marijuana use (Lundqvist *et al*, 2001; Mathew and Wilson, 1993; Mathew *et al*, 1997, 2002; O'Leary *et al*, 2000, 2002; Volkow *et al*, 1996).

Expression of IEG mRNA was also altered in several cortical and striatal regions as a composite result of test experience, but, although not assessed in the present investigation, it is likely that other regions, such as the parietal cortex (Fox *et al*, 2003), amygdala (Schoenbaum *et al*, 2000), and mediodorsal thalamic nucleus (Chudasama *et al*, 2001), additionally contributed to task performance.

While the above findings represent the regional alterations in activity occurring in response to performance of the series of discriminations, we also sought to outline associations between the relative activation of discrete brain loci and ability at different task stages. As correlation analysis was performed between performance levels at all behavioral task stages and mRNA expression in several regions, it is likely that some associations may be statistically significant by chance and these results should be viewed with caution prior to further investigation. However, several of the findings appear meaningful within the context of previous literature and therefore warrant some discussion.

With respect to acquisition stages, potential associations were detected between performance on both the CD and ID discriminations and alterations in IEG mRNA expression in the dorsolateral striatum and core portion of the nucleus accumbens, areas that are strongly implicated in stimulus-reinforcement learning (Berridge and Robinson, 1998;

Cardinal *et al*, 2002; Schoenbaum and Setlow, 2003). Interestingly, while this association was signaled by c-*fos* expression at the initial CD stage, performance–region associations at the later ID acquisition stage were signaled by alterations in *ngfi-b* expression.

Of particular interest are the potential associations that were observed between regional IEG mRNA expression and performance on the reversal learning stages, at which THC produced marked disruptions in ability. As with the acquisition stages, reversal learning was also associated with alterations in mRNA expression in the dorsolateral striatum and nucleus accumbens. These results are largely in agreement with those of human imaging studies that have revealed associations between striatal activation and reversal learning (Rogers *et al*, 2000). Interestingly, in the present study, some dissociation between acquisition and reversal stages was apparent with respect to accumbal subdivisions; while the core portion was implicated in discrimination acquisition performance, activity in the shell portion was associated with reversal learning ability. Furthermore, consistent with the role of the OFC in encoding and updating associations between stimuli and reward values (Cardinal *et al*, 2002; Rolls, 1996, 2000, 2004; Tremblay and Schultz, 1999; Winstanley *et al*, 2004), and the lesion studies that have shown the dependence of effective reversal learning upon the integrity of this region (Butter, 1969; Dias *et al*, 1996a, 1997; Ferry *et al*, 2000; Iversen and Mishkin, 1970; Jones and Mishkin, 1972; McAlonan and Brown, 2003; Schoenbaum *et al*, 2002), performance of the first reversal was associated with alterations in activity in the OFC. Although further investigation is required, these results therefore appear to lie in close accordance with those of several previous studies strongly implicating orbitofrontal striatal circuitry in the processes required to update affective associations and alter behavioral output accordingly when stimulus–reward associations change.

Finally, performance of the third reversal stage was associated with alterations in *ngfi-b* expression in the prelimbic area of the medial frontal cortex. This result is perhaps surprising given the proposed role of the rat prelimbic cortex in control of extradimensional but not reversal shifts (Birrell and Brown, 2000). However, the third reversal stage is performed subsequent to the EDS, and effective performance still requires an ability to attend to stimulus attributes that were not relevant in the pre-EDS task stages. Performance of the third reversal will therefore also relate to the extent of set transfer on the preceding EDS stage, and may possibly explain the association between performance at the third EDS stage and recruitment of the prelimbic cortex.

In summary, in similarity to the profile of effects observed following orbitofrontal lesions (McAlonan and Brown, 2003), acute administration of THC produced marked deficits in the ability to update affective associations between stimuli and reward presentation in the rat, while attentional set shifting ability was unaffected. Furthermore, the concurrent investigation of regional IEG mRNA expression suggested that reversal-learning ability was associated with alterations in neural activity in orbitofrontal and striatal regions. Together, these results suggest that, at least on acute intake, marijuana may not disrupt mental processes requiring 'higher order' cognitive flexibility of abstract concepts, but may affect the ability to modify reward-driven behavior when the consequences of those actions become unfavorable. This inflexibility in the ability to update affective associations may be attributable to disruption of orbitofrontal striatal circuitry as has been suggested to be of importance in tendency toward the continued self-administration of other psychoactive drugs (Volkow and Fowler, 2000).

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