11777 SENATE HEALTH,EDUCATION & SOCIAL SERVICES

Chermack, S. T., & Taylor, S. P. (1995). Alcohol and human physical aggression: Pharmacological versus expectancy effects. *Journal of Studies on Alcohol, 56*, 449–456.

Critchlow, B. (1983). Blaming the booze: The attribution of responsibility for drunk behavior. *Personality and Social Psychology Bulletin, 9*, 451–473.

Fals-Stewart, W. (2003). The occurrence of partner physical aggression on days of alcohol consumption: A longitudinal diary study. *Journal of Consulting and Clinical Psychology, 71*, 41–52.

Fals-Stewart, W., Birchler, G. R., & Kelley, M. L. (2003). The timeline followback spousal violence interview to assess physical aggression between intimate partners: Reliability and validity. *Journal of Family Violence, 18*, 131–142.

Fals-Stewart, W., Birchler, G. R., & O'Farrell, T. J. (1999). Drug-abusing patients and their intimate partners: Dyadic adjustment, relationship stability, and substance use. *Journal of Abnormal Psychology, 108*, 11–23.

Fals-Stewart, W., Kashdan, T. B., O'Farrell, T. J., & Birchler, G. R. (2002). Behavioral couples therapy for drug-abusing patients: Effects on partner violence. *Journal of Substance Abuse Treatment, 21*, 1–10.

Fals-Stewart, W., O'Farrell, T. J., Freitas, T. T., McFarlin, S. K., & Rutigliano, P. (2000). The timeline followback reports of psychoactive substance use by drug-abusing patients: Psychometric properties. *Journal of Consulting and Clinical Psychology, 68*, 134–144.

First, M., Spitzer, L., Gibbon, M., & Williams, J. (1995). *Structural clinical interview for Axis I DSM-IV disorders (SCID)*. Washington, DC: American Psychiatric Association.

Gold, M. S. (1997). Cocaine (and crack): Clinical aspects. In J. H. Lowinson, P. Ruiz, R. B. Millman, J. G. Langrod (Eds.), *Substance abuse: A comprehensive textbook* (3rd ed.) (pp. 181–206). Baltimore, MD: Williams & Wilkins.

Goldstein, H., Rasbash, J., Plewis, I., Draper, D., Browne, W., Yang, M., Woodhouse, G., & Healy, M. (1998). *A user's guide to MlwiN*. London: Institute of Education, University of London.

Hotaling, G. T., & Sugarman, D. B. (1986). An analysis of risk markers in husband to wife violence: The current state of knowledge. *Violence and Victims, 2*, 101–124.

Hosmer, D. W., & Lemeshow, S. (1989). *Applied logistic regression*. New York: Wiley.

Landis, J. K., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics, 33*, 159–174.

Leonard, K. E. (1993). Drinking patterns and intoxication in marital violence: Review, critique, and future directions for research. *U.S. Department of Health and Human Services, research monograph 24: Alcohol and interpersonal violence: Fostering multidisciplinary perspective* (pp. 253–280). Rockville, MD: National Institutes of Health, NIH Publication No. 93-3496.

Miczek, K. A., DeBold, J. F., Haney, M., Tidey, J., Vivian, J., & Weerts, E. M. (1994). Alcohol, drugs of abuse, and violence. In A. J. Reiss, & J. A. Roth (Eds.), *Understanding and preventing violence* (pp. 377–570). Washington, DC: National Academy Press.

Mickey, J., & Greenland, S. (1989). A study of the impact of confounder selection criteria on effect estimation. *American Journal of Epidemiology, 129*, 125–137.

Mundt, J. C. (1997). Interactive voice response systems in clinical research. *Psychiatric Services, 48*, 611–612.

Murphy, C. M., & O'Farrell, T. J. (1994). Factors associated with marital aggression in male alcoholics. *Journal of Family Psychology, 8*, 321–335.

Raudenbush, S. W., & Bryk, A. S. (2002). *Hierarchical linear models: Applications and data analysis methods*. Thousand Oaks, CA: Sage.

Schafer, J., Caetano, R., & Clark, C. (1998). Rates of intimate partner violence in the United States. *American Journal of Public Health, 88*, 1702–1704.

Schumacher, J. A., Feldbau-Kohn, S., Slep, A. M. S., & Heyman, R. E. (2001). Risk factors for male-to-female partner physical abuse. *Aggression and Violent Behavior, 6*, 281–352.

Spanier, G. (1976). Measuring dyadic adjustment: New scales for assessing the quality of marriage and similar dyads. *Journal of Marriage and the Family, 38*, 15–30.

Straus, M. A. (1990). The Conflict Tactics Scale and its critics: An evaluation and new data on validity and

reliability. In M. A. Straus, & R. J. Gelles (Eds.), *Physical violence in American families* (pp. 49–73). New Brunswick, NJ: Transaction.

Straus, M. A., & Gelles, R. J. (1990). *Physical violence in American families: Risk factors and adaptations to violence in 8145 families*. New Brunswick, NJ: Transaction Publishers.

Wekerle, C., & Wall, A. -M. (2002). *The violence and addiction equation: Theoretical and clinical issues in substance abuse and relationship violence*. Philadelphia, PA: Brunner/Mazel.

Drug and Alcohol Review (December 2003), 22, 401 – 408

Taylor & Francis
Healthsciences

Substance use and the prediction of young offender recidivism

ALDIS L. PUTNIŅŠ

Department of Human Services, South Australia and School of Psychology, University of South Australia, Australia

Abstract

The problem considered is whether self-reported substance use can be used in the estimation of recidivism risk among youths placed in secure care. The Secure Care Psychosocial Screening (SECAPS) and offending records of 447 youths admitted to detention centres in South Australia were examined. The target outcome was any new offending within 6 months of release. Use of a psychoactive substance at the time of committing the most recent offence was not a significant predictor of subsequent offending, nor was acknowledging having a problem with drug or alcohol use. In relation to the recent use of alcohol, marijuana, hallucinogens, sedatives/hypnotics, narcotics, stimulants and inhalants, only the use of alcohol and inhalants appeared to have significant relationships with recidivism. While the relationships were too small to permit using these items on their own to estimate re-offending risk, recent alcohol and inhalant use could be included as part of a broader recidivism risk assessment. [Putniņš AL. Substance use and the prediction of young offender recidivism. *Drug Alcohol Rev* 2003;22:401 – 408]

Key words: juvenile delinquents, prediction, recidivism, risk, substance use, young offenders.

Introduction

A number of studies have indicated that substance use and juvenile offending are often closely associated activities [1]. This link has been confirmed in research investigations of young offenders in both police detention [2] and in youth detention centres in different Australian states [3 – 14]. The largest Australian study of a detention population to date describes patterns of substance use among 900 incarcerated young offenders in South Australia [12]. The major findings were:

- Incidences of any use of various substances by the young offenders during a recent 1-month period ranged from 10% for narcotics (opioids) to 81% for marijuana.
- The young offenders more often reported having used various psychoactive substances (other than alcohol) during a 1-month period than did a large national sample of high school students [15]. The incidences were 2 – 5 times greater, depending on the particular class of substance.
- While any use of alcohol during a 1-month period was similar between the two groups (namely 73% of young offenders and 68% of students), moderate drinking by young offenders was rare, with more than half (53%) consuming 10 or more drinks on a typical drinking occasion.
- Although a wide range of substances was used by many of the offenders, daily use was largely restricted to alcohol and marijuana (10% and 44% of respondents, respectively). No other substance was used daily by more than 3% of the respondents.
- A majority (60%) of the offenders reported having used substances at the time of committing their last offence.
- Among those who used a substance at the time of their last offence, the substances most often used were alcohol (62%) and marijuana (53%).

The above results are based on data gathered from psychosocial screening assessments that are carried out routinely to inform staff about each youth's needs and risks. Among the risks often considered in assessments of young offenders is that of re-offending. To assess this risk it is important to know what relationships various background factors and behaviours have with later offending. This includes knowing whether predictive relationships exist between substance use and recidi-

Aldis L. Putniņš Dip App Psych, MA, Chief Clinical Psychologist, Family and Youth Services, Department of Human Services, and doctoral candidate in the School of Psychology at the University of South Australia. Correspondence to Aldis L. Putniņš, Family and Youth Services, 2 Norton Summit Rd, Magill SA 5072, Australia. Tel. (08) 8333 1920; Fax. (08) 8364 7041; E-mail: putnins.aldis@saugov.sa.gov.au

Received 22 April 2002; accepted for publication 27 June 2003.

ISSN 0959-5236 print/ISSN 1465-3362 online/03/040401-08 © Australian Professional Society on Alcohol and Other Drugs
DOI: 10.1080/09595230310001613912

vism. The causal relationships between the use of various substances and offending by youths are complex and likely to consist of both bi-directional relationships and common causes [16] and might be different for different substances. This clouds the question of to what degree substance use is predictive of, rather than just concurrently associated with, offending.

The aim of this investigation is to examine the longitudinal relationship between substance use and criminal recidivism. Few such studies with young offenders have been undertaken elsewhere [17] and none previously in Australia.

Method

Subjects and procedure

Subjects were youths who: a) were admitted between 1994 and 1996 to either of South Australia's two secure care centres for young offenders, b) underwent a standardised psychosocial screening assessment while in secure care, and; c) were released from secure care at least 6 months before the end of 1997. Youths whose stays are longer than a few days are routinely assessed in relation to various aspects of psychological and social adjustment, including substance use. Assessed youths include both those on remand as well as those serving detention orders. Reassessments are usually undertaken if a youth is readmitted and more than 12 months have elapsed since they were last assessed.

Measure

The assessment items are part of the Secure Care Psychosocial Screening (SECAPS) [18]. This is a standardized individually administered assessment that includes items about how often various substances were used during the last month before being placed in secure care. The assessments are carried out by youth workers, psychologists or social workers and take about 30 minutes to complete. As given on the questionnaire, substances listed are: alcohol (beer, wine, spirits); marijuana (grass, hash); acid, LSD, datura [also known as Angel's Trumpet—a garden plant with hallucinogenic properties], magic mushrooms or other hallucinogens; sedatives or sleeping tablets such as Mogadon, Serepax, Valium, Rohypnol or similar (without doctor's prescription); heroin, morphine or other narcotics; cocaine, speed, pep pills or other stimulants (without doctor's prescription); sniffed (breathed deeply) from spray cans or sniffed items such as glue, petrol, thinner, butane gas or similar. These are summarized (together with their abbreviations) as: alcohol (Alco); marijuana (Marij); hallucinogens (Hallu); sedatives/hypnotics (Sed/Hyp); narcotics (Narc); stimulants (Stim); inhalants (Inhal). Substances under "Sedatives/Hypnotics" included anti-anxiety agents. Frequency of use was indicated on a five-point scale, namely: never; once or twice a month; once or twice a week; several times a week; daily or almost daily. Other items from the SECAPS assessment considered here include what substances, if any, were used at the time of their last offence, whether the respondent felt they had a problem with substance use and whether any natural parents or siblings had had problems with drugs or alcohol.

As the information about substance use derives from self-reports, questions about its accuracy can be raised. Error due to poor recall is minimized here by restricting questions about frequency to use during the last month in the community. Similarly, the item about substances used when they offended was restricted to the most recent offence. Error can also result from deliberate distortion. Youths in justice settings could be expected to under-report rather than over-report their substance use more often. Studies that have used chemical analyses to check the accuracy of self-reports [2,19–21] indicate that under-reporting is more likely in relation to very recent use (previous 2–3 days) of hard drugs such as cocaine and heroin and that reports of use over the last 4 weeks tend to be more accurate. It is the longer period that is inquired about in the SECAPS. There are other factors that reduce the tendency to not reveal the full extent of substance use. First, the SECAPS assessment is introduced to each youth as a routine assessment to help staff become more aware of needs and issues concerning the youth. It is pointed out that it is not a court-ordered assessment. This helps to reduce the anxiety that some youths might have about the assessment. Secondly, the substance use items in the SECAPS assessment come after a number of more general background items and basic skills tests. This allows the assessor to establish rapport with the youth and for the youth to settle in to the assessment. Thirdly, substance use is so widespread among young offenders that it is normative in this group. In a facility for young offenders, fear of condemnation by admitting to substance use is minimal.

An aid to fuller disclosure in the SECAPS is the use (conditional on each youth's literacy level) of a self-administered pencil-and-paper questionnaire to describe substance use. Studies have found greater disclosure using this format compared to face-to-face interviews [22].

Generally it has been the experience of SECAPS assessors that, when other sources of information have been available, on most occasions there has been a reasonably good concordance between the youth's report and other sources.

The examination of the relationship between self-reported substance use and subsequent re-offending used the full court-adjudicated offending histories of all youths who completed SECAPS assessments during

the first 3 years of the assessment program (namely 1994–96). The outcome was each youth's recidivism status (dichotomous outcome) at 6 months from the time of their next release. This was determined by whether any proven (court-adjudicated) offending had occurred within 6 months of the first release after the SECAPS assessment. As previous predictive studies have often examined violent recidivism as a separate outcome [23], note was also taken of whether any of the new offences were violent offences. The follow-up period extended to the end of 1997. Youths not released within 6 months of the end of the follow-up period were excluded from the study. Offence records were obtained from the Office of Crime Statistics in the South Australian Attorney-General's department. Only records of offences dealt with by South Australian courts were available.

Statistical methods

The bivariate associations between the substance use variables (assessed while the young offenders were in secure care) and later recidivism status are examined using product–moment correlations and χ^2 tests. Some of the youths were re-admitted to secure care and were re-assessed a second time. Thus, two sets of substance use and post-release recidivism data are available. As the number of second assessments is smaller than the number of first assessments, greater weight needs to be placed on the first assessment results. From previous prediction studies [17,24] it is known that most single variables have only weak to modest relationships with recidivism and that results often vary between studies. One way of increasing confidence in results is to raise the alpha value so that only highly significant results are accepted. A disadvantage is that relatively weak yet genuine relationships will be dismissed. Another strategy is to set alpha at a modest level and to look for consistency in the results. The latter approach is adopted here. The second assessment data are retained as a means of checking the consistency of the results from the analysis of the first assessment data. It is expected that any significant relationships will, at best, be modest in strength. If a variable has a weak although positive and significant relationship in the analysis of the first assessment data, confidence in the relationship being genuine will be increased if it also observed to have a positive relationship in the analysis of the second assessment data. If, however, the relationship in the second assessment data is zero or negative, then despite the larger subject numbers in the first assessment data, one would be hesitant to accept the first assessment result as genuine. As the second assessment data are based on smaller subject numbers, they have less statistical power. Therefore the levels of statistical significance of the second assessment results are less important than are consistencies in direction and strength compared to the first assessment results.

Regarding scaling, the predicted outcome (recidivism) is dichotomous. Dichotomous variables can be treated as having interval scale properties [25]. The substance use items of greatest interest in this analysis are reports of the frequencies of substances used during the last month before placement in secure care. These are measured on a five-point ascending scale (none; 1–2/month; 1–2/week; several times/week; daily or almost daily). The scale can be treated in one of two ways. The first is as a physical time scale. If, for simplicity, a month is regarded as being 4 weeks (28 days), then the scale values would be as follows: none = 0; 1–2/month = 1.5; 1–2/week = 6; several times/week = 16; daily or almost daily = 26. The values are the midpoints of the frequency ranges. This is an interval scale. The second is to treat the frequencies as a psychological scale of perceived intensity, similar to a psychophysical scale. This is how the measured scale points were selected and how the scale is analysed here. The scale values are 1 to 5 (none = 1 daily = 5), the scale being treated as approximately interval. The two scales are mathematically related ($y \approx x^2 - 1$, where y = time scale values and x = psychological scale values). In practice the results of analyses of the two types of scales yield very similar results, largely because product–moment correlations suffer little error when different scoring systems are compared to any selected "true" scoring system [26].

Results

Patterns of substance use for the offender group from which this sample was drawn are reported in a separate publication [12] and are summarized in the Introduction section of this paper.

Discarding four initial assessments in which no substance use responses were recorded left 454 youths with relevant assessment data. Due to either death or non-release from secure care 6 months or more before the end of the follow-up period, the number of youths whose assessments were included in the recidivism analyses was 447 (first assessment group). Of these, 140 had a subsequent SECAPS assessment within the same assessment period (i.e. 1994–96). One assessment had no substance use responses recorded. Of the remaining youths with a second assessment, 103 were released from secure care 6 months or more before the end of the follow-up period and were included in the recidivism analysis (second assessment group).

Of the first assessment respondents, 92% were males and 27% described themselves as being Aboriginal. Ages for the total group ranged from 11 to 19 years

(mean age = 15.9 years), with most subjects (83%) falling within the 14–17-year age range. Among the second assessment respondents, 95% were males and 31% described themselves as being Aboriginal. Ages for this group ranged from 12 to 18 years (mean age = 16.3 years), with most subjects (89%) falling within the 15–18-year age range. The mean time from release after the second assessment to the end of the follow-up period was 651 days (21 months). All subjects who were 18 years or older were incarcerated for offences committed before they turned 18.

Results are presented in Table 1 for the relationships between the frequencies of substances used during the month before incarceration and subsequent re-offending. None of the relationships are strong. No obvious pattern of use distinguishes recidivism for violent offences from recidivism for non-violent forms of criminal offending. Violent offences here include various threats of harm and offences that can cause a fear of direct harm, but exclude sexual offences. Assaults account for 70.4% of all the offences in the violent offence category, with robbery with violence and robbery with an offensive weapon accounting for a further 14.8% (further details are available on request from the author).

There had been an expectation that greater use of any substance would be related positively to recidivism. While most of the correlations (although not all) were positive, many were too small to be of either practical or statistical significance. The substances related to recidivism most consistently were alcohol and inhalants: nine of the 12 correlations involving these substances were statistically significant, whereas only two of the other 30 correlations were significant.

An examination of the relationship between substances used at the time of the youths' last offences and subsequent recidivism (see Table 2) did not reveal any consistent trends.

The use of particular substances is often transitory or episodic. This is evident from the correlations between substance use frequencies reported at the first and second SECAPS assessments (see Table 3). The [illegible] are from the larger sample of 900 assessed young offenders from which the offender assessments in the recidivism study were drawn. A minimum of 6 months separation between first and second assessments was specified in order to reduce the chances of some subjects not being released between their first and second assessments. (During the initial implementation of the SECAPS program some youths were reassessed in less than 12 months and, in a few cases, without having been released.) It can be seen that the stability coefficients are all quite low, particularly for the less frequently used substances. This can be expected to reduce the strength of the relationship between the use of particular substances and subsequent re-offending. For example, of 12 reassessed youths who in their first assessment reported recent use of narcotics, only two of them reported recent narcotics use in their second assessment. Of the 25 youths who in their second assessment reported recent use of narcotics, only two had reported any use of narcotics earlier.

The relationship between reported problem use of substances by other immediate natural family members and recidivism was examined. Responses were coded as: no = 1, don't know = 2, yes = 3. No significant relationships were found between recidivism status 6 months after release and responses to this item at either the first ($\chi^2 = 0.699$, df = 2, $n = 446$) or second ($\chi^2 = 0.722$, df = 2, $n = 103$) SECAPS assessments.

The use of some substances might be predictive of offending in some demographic groups but not in others. If, however, general predictive measures are sought then these must demonstrate some degree of predictive consistency across different groups. Results are presented in Table 4 that are relevant to this question. Neither self-reported problems with substance use nor the use of a psychoactive substance at the time of the last offence were consistently related to recidivism.

Table 1. *Correlations between frequency of substances used during the last month prior to incarceration and recidivism status 6 months after release*

	Alco	Marij	Hallu	Sedhyp	Narc	Stim	Inhal
1st assess ($n = 445-447$)							
Any offending	0.08*	0.01	0.02	0.04	−0.02	0.05	0.10*
Violent offending	0.13**	−0.01	0.05	0.09*	0.06	0.08	0.16**
Other offending	0.08	0.00	0.03	0.01	−0.03	0.04	0.08*
2nd assess ($n = 103$)							
Any offending	0.23*	−0.02	0.05	0.11	0.10	−0.04	0.21*
Violent offending	0.09	0.05	0.16*	0.04	−0.05	−0.06	0.14
Other offending	0.21*	−0.04	0.02	0.10	0.10	−0.03	0.22*

*$p \leq 0.05$; **$p \leq 0.01$ (one-tailed).

Table 2. *Correlations between the use of various substances at the time of the last offence and recidivism status 6 months after release from secure care*

	Alco	Marij	Hallu	Sedhyp	Narc	Stim	Inhal
1st assess ($n = 444$)							
Any offending	0.04	− 0.01	0.00	− 0.00	− 0.00	0.05	0.03
Violent offending	0.05	− 0.01	− 0.04	0.03	0.10*	0.00	0.17**
Other offending	0.02	− 0.03	0.02	− 0.01	0.01	0.06	0.03
2nd assess ($n = 103$)							
Any offending	0.06	− 0.07	− 0.08	0.01	0.08	0.04	0.14
Violent offending	− 0.06	0.13	0.25**	− 0.01	− 0.04	− 0.02	0.10
Other offending	− 0.08	− 0.06	0.08	0.02	0.08	0.05	0.14

* $p \leqslant 0.05$; ** $p \leqslant 0.01$ (one-tailed).

Table 3. *Correlations between substance use frequencies reported at the first and second SECAPS assessments† where 6 months or more had elapsed between assessments*

	Alco	Marij	Hallu	Sedhyp	Narc	Stim	Inhal
r	0.33**	0.40**	0.14*	0.13*	0.09	0.09	0.12

†Time between assessments ranged between 184 and 1468 days ($M = 554$). $n = 186$ for all substances except inhalants ($n = 185$).
*$p \leqslant 0.05$; **$p \leqslant 0.01$ (one-tailed).

Table 4. *Correlations between self-reported frequencies of substances used during last month before incarceration, any substance use at the time of last offence, self-reported problem use and recidivism status 6 months after release from secure care*

	1st assessments			2nd assessments†	
	Females (all)	Non-Abor. males	Abor. Males	Non-Abor. (m + f)	Abor. (m + f)
Alcohol	0.26	0.04	0.14	0.13	0.37*
Marijuana	− 0.11	0.02	0.06	− 0.14	0.19
Hallucin0.	0.40**	− 0.01	− 0.10	0.04	0.03
Sed/Hyp	0.47**	− 0.06	0.10	0.07	0.21
Narcotics	0.22	− 0.03	− 0.05	− 0.13	0.32*
Stimulants	0.25	0.04	0.01	− 0.09	0.05
Inhalants	0.31*	0.08	0.05	0.22*	0.19
Use offend	0.27	0.04	− 0.02	0.02	− 0.01
Problem	0.28*	− 0.08	0.01	− 0.02	0.31*
ALCIN	0.38**	0.07	0.16	0.22*	0.37*
Other subs	0.35**	− 0.00	0.02	− 0.08	0.24
n	36 − 38	310 − 311	97 − 98	71	32

Use offend = used a substance at the time of the last offence; Problem = youth considers his/her substance use to be a problem; ALCIN = Alcohol use frequency + Inhalant use frequency; Other subs (use frequencies) = Marij + Hallu + Sed/Hyp + Narc + Stim.
†There were too few females ($n = 5$) among those retested to allow separate analysis by gender. *$p \leqslant 0.05$; **$p \leqslant 0.01$ (one-tailed).

Examining the different groups, the results for females stand out. In this group, with the exception of marijuana use, all the substance use variables are either significant or display positive trends in their relationship with recidivism. Some of the relationships are quite strong, such as the correlation of 0.47 between recidivism and the use of sedatives/hypnotics.

Of the individual substances, only alcohol and inhalants had consistently positive correlations across the different groups. The correlations were weakest in

the first assessment non-Aboriginal male subgroup but increased among those who were reassessed. These positive relationships are generally strengthened when alcohol and inhalant use frequencies are summed to form a measure called ALCIN. This combined measure had highly significant correlations with recidivism status after both first ($r = 0.12$, $p = 0.007$, $n = 446$) and second ($r = 0.28$, $p = 0.002$, $n = 103$) assessments. Examining the various subgroups it is seen that all the correlations between ALCIN and recidivism are positive and that three of the five correlations are statistically significant. By contrast, an index of all the other substances used (Other subs) failed to show any relationship with recidivism in the three largest subgroups and was statistically significant only for the female subgroup (Table 4).

The relationship between the ALCIN index and recidivism can be seen clearly in Table 5. A fairly consistent increase in subsequent recidivism is evident as the ALCIN scores increase.

Discussion

Regarding the prediction of recidivism, as no obvious pattern of recent substance use distinguished general offending, violent offending and non-violent offending, further discussion here considers only general offending. Neither the use of a substance in general (*Use offend*) nor the use of any particular substance at the time of the last offence consistently predicted recidivism—nor did self-reported problems with substance use. Although marijuana was the most widely used substance, there was no support for using it as a marker for increased risk of re-offending. That marijuana is often reported to have been used at the time of last offending is not surprising, given that marijuana is the most frequently used of all the psychoactive substances. The close association between offending and marijuana use might be largely coincidental rather than causal, simply because a large proportion of young offenders at any one time are under the influence of marijuana.

Recent alcohol and inhalant use appear to be the substances that are most consistently predictive of recidivism across different offending types and across different demographic subgroups. Both alcohol and inhalants are central nervous system depressants and both have disinhibiting effects. The use of inhalants might also be a marker for less maturity and poorer self-control particularly as, unlike other substances, it tends to be used by younger detainees [12]. The link between alcohol use and offending has been particularly well documented, including among young people [27]. A combined index of recent alcohol and inhalant use might be a useful addition to a broader recidivism risk assessment instrument.

Although the recent use of other substances failed to show any consistent relationship with recidivism in most of the subject subgroups examined, there was a trend among the females for all substances except marijuana to be related to increased recidivism risk. Due to the relatively small number of females here, replication is needed on a larger sample. If confirmed, however, it could indicate that, for the optimal prediction of offending risk, gender differences in the predictive strength of some variables should be taken into account.

Despite much media attention to the link between crime (particularly acquisitive crimes) and narcotic use

Table 5. *Alcohol–inhalant use index (ALCIN)* scores by recidivism status at 6 months follow-up*

ALCIN scores	6 months post-1st			6 months post-2nd		
	SECAPS			SECAPS		
	% not offend	% re-offend	*n*	% not offend	% re-offend	*n*
1.0	37.0	63.0	92	54.2	45.8	24
1.5	35.2	64.8	122	52.6	47.4	19
2.0	36.8	63.2	114	43.5	56.5	23
2.5	16.7	83.3	48	22.2	77.8	18
3.0	26.4	73.6	53	25.0	75.0	16
3.5	20.0	80.0	10	0.0	100.0	2
4.0	20.0	80.0	5	-	-	0
4.5	0.0	100.0	2	-	-	0
5.0	-	-	0	0.0	100.0	1
Total	32.4	67.7	446	39.8	60.2	103

*ALCIN is the sum of the frequencies of alcohol use and inhalant use during the last month before placement in secure care. A score of 1.0 indicates that neither substance was used. A score of 5.0 indicates daily use of both substances.

(predominantly heroin), among the juvenile male subjects studied here recent use of narcotics had no power to predict recidivism. It could be that this is because few youths use narcotics and even fewer are dependent—most of their narcotics use probably still being experimental or recreational. Many youths have not established consistent or regular patterns of use, suggesting that much of their use, particularly of substances other than marijuana and alcohol, is capricious and might often be transitory.

The statistical weakness of recent substance use as a marker for increased offending risk observed here might be due to a number of factors. While there is a close association between substance use and the act of offending, the stability of substance use among youths over time might be too weak for most substances to be reliable predictors. Perhaps the self-reports of recent substance use by youths in secure care are too distorted to be valid. Although there is little evidence to suggest that there is severe distortion in most of the young offenders' self-reports, the use of physical drug testing is one possible way of verifying the accuracy of such assessment items. Dissimulation might be reduced and the stability of patterns of use increased if, rather than very recent substance use, reports of use over a longer period are used. While lifetime use is likely to be difficult to estimate accurately, perhaps the regular use of various substances during the preceding 6 months might be a more stable measure. This possibility is being explored by introducing such an item into the next revision of the SECAPS assessment.

There is also the problem of homogeneity. While there is good concurrent discrimination between young offenders and non-offenders with regard to their substance use, perhaps those who are placed in secure care long enough to be assessed are not all that different from each other. Most markers, including substance use, at least when taken singly, would have no more than weak relationships with recidivism. The situation might be similar to choosing basketball players. While height might be a predictor of who will get into a team, once a team has been established and there has been some attrition among the players, height will probably be a weak predictor of basketball playing ability among existing players.

Substance use in many previous studies has been found to have only a very modest relationship with recidivism. From an analysis of 60 studies involving almost 55 000 subjects, Gendreau, Coggin & Little [24] reported a weighted mean Pearson's r of 0.10 for substance abuse and adult recidivism. Cottle, Lee & Heilbrun [17], in their analysis of studies that examined recidivism among young offenders, obtained weighted mean effect sizes (Z_r) of 0.15 for substance abuse and recidivism (six studies, 1111 subjects) and 0.01 for substance use and recidivism (two studies, 9366 subjects). Overall, substance abuse was very modestly predictive but substance use was not. In the light of these results it is not surprising that stronger relationships were not observed between substance use and recidivism in the present sample.

Conclusions

Despite the prominence given in the media to the issue of "hard drugs", the substances used most frequently by both young offenders and non-offenders are the so-called "soft drugs", namely alcohol and marijuana. Of these, alcohol appears to have a stronger direct association with acts of offending— due most probably to its strong disinhibiting properties. Ways of restricting adolescents' access to alcohol more effectively need to be considered. The other class of substances that showed a degree of predictive consistency across different groups was inhalants. An index of recent alcohol and inhalant use, although predictively too weak to use on its own, might be a useful addition to a broader recidivism risk assessment.

References

[1] Loeber R, Stouthamer-Loeber M. Prediction. In: Quay HC, ed. Handbook of juvenile delinquency. New York: Wiley, 1987:325–82.

[2] Makkai T, McGregor K. Drug use monitoring in Australia: 2001 annual report on drug use among police detainees. Research and Public Policy Series no. 41. Canberra: Australian Institute of Criminology, 2002.

[3] Copeland J, Howard J. Alcohol and other drug issues amongst NSW juvenile detainees. Paper presented at Australia's 1st Forensic Psychology Conference, Sydney, February, 2001.

[4] Crundall I. Survey of drug use among young people in Victorian youth training and reception centres. Melbourne: Victorian Health Department, 1987.

[5] Fasher AM, Dunbar N, Rothenbury, BA, Bebb DK, Young SJW. The health of a group of young Australians in a New South Wales juvenile justice detention centre: a pilot study. J Paediatric Child Health 1997;33:426–9.

[6] Hando J, Howard J, Zibert E. Risky drug practices and treatment needs of youth detained in New South Wales Juvenile Justice Centres. Drug Alcohol Rev 1997;16:137–45.

[7] Howard J, Zibert E. Curious, bored and wanting to feel good: the drug use of detained young offenders. Drug Alcohol Rev 1990;9:225–31.

[8] Howard J, Copeland J, Nicholas J, Karacanta A. Young offenders, substance use, depression and suicidal behaviour. Paper presented at Australia's 1st Forensic Psychology Conference, Sydney, February, 2001.

[9] Lennings C, Pritchard M. Prevalence of drug use prior to detention among residents of youth detention centres in Queensland. Drug Alcohol Rev 1999;18:145–52.

[10] Putniņš AL. The Adolescent Alcohol Involvement Scale: some findings with young offenders. Drug Alcohol Rev 1992;11:253–8.

[11] Putniņš AL. Recent drug use and suicidal behaviour among young offenders. Drug Alcohol Rev 1995;14:151–8.

[12] Putnins AL. Substance use by South Australian young offenders. Information Bulletin, no. 19, Adelaide: Office of Crime Statistics, Attorney-General's Department, 2001. (downloadable at http://www.ocsar.sa.gov.au/docs/information_bulletins/IB19.pdf)
[13] Putnins AL, Harvey SJR. Alcohol abuse among young offenders. Med J Aust 1992;156:753–5.
[14] Watts P. Youth in custody project: a profile of juvenile offenders' drug use patterns. Perth: Department of Community Services, 1992.
[15] Letcher T, White V. Australian secondary students' use of over-the-counter and illicit substances in 1996. Anti-Cancer Council of Victoria: Melbourne, 1998.
[16] Rutter M, Giller H, Hagell A. Antisocial behavior by young people. Cambridge, UK: Cambridge University Press, 1998.
[17] Cottle CC, Lee RJ, Heilbrun K. The prediction of criminal recidivism in juveniles: a meta-analysis. Criminal Justice Behav 2001;28:367–94.
[18] Putnins AL. Secure care psychosocial screening: manual for SECAPS V.4. Adelaide: Department of Human Services, Family and Youth Services, 1998.
[19] Fendrich M, Xu Y. The validity of drug use reports from juvenile arrestees. Int J Addict 1994; 29:971–85.
[20] Feucht TE, Stephens RC, Walker ML. Drug use among juvenile arrestees: a comparison of self-report, urinalysis and hair assay. J Drug Issues 1994;24:99–116.
[21] Mieczkowski T, Newel R, Wraight B. Using hair analysis, urinalysis, and self-reports to estimate drug use in a sample of detained juveniles. Subst Use Misuse 1998;33:1547–67.
[22] Harrison L. The validity of self-reported drug use in survey research: an overview and critique of research methods. In: Harrison L, Hughes A, eds. The validity of self-reported drug use: improving the accuracy of survey estimates. Rockville, MD: National Institute on Drug Abuse, 1997:17–36.
[23] Gendreau P, Goggin C, Smith P. Is the PCL-R really the "unparalleled" measure of offender risk? A lesson in knowledge cumulatio Criminal Justice Behav 2002;29:397–426.
[24] Gendreau P, Goggin C, Little T. Predicting adult offender recidivism: what works! (User Report no. 1996–07). Ottawa: Department of the Solicitor General of Canada, 1996.
[25] Nei NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH. SPSS: statistical package for the social sciences (2nd edn). New York, NY: McGraw-Hill, 1975.
[26] Labowitz S. The assignment of numbers to rank order categories. Am Soc Rev 1970;35:515–24.
[27] Komro KA, Williams CL, Forster JL, Perry CL, Farbakhsh K, Stigler MH. The relationship between adolescent alcohol use and delinquent and violent behaviors. J Child Adolesc Subst Abuse 1999;9:13–28.

3

Stepping-Stones, Gateways, and the Prevention of Drug Problems

Researchers, theorists, politicians, and parents have all expressed concern about marijuana's potential to lead to the use of drugs with worse negative consequences. Proponents of these stepping-stone and gateway theories suggest that even if the adverse effects of marijuana are minimal, the drug can still cause trouble by ushering users toward the consumption of other illicit substances, including heroin and crack. According to this premise, marijuana should remain a primary concern because these other drugs create so many hardships. Comparable arguments appear against underage drinking and cigarette smoking. The gateway and stepping-stone theories have generated considerable research and debate for many years. The research remains difficult to evaluate without clear definitions of a stepping-stone and gateway. Interpreting this literature requires a good understanding of causality. Many popular reports confuse the causes of drug use with simple precursors. Confusion about the actual causes of drug consumption can impair any effort to prevent substance abuse and related problems. Thus, this chapter defines a stepping-stone and gateway, reviews the requirements for causality, examines the literature relating marijuana consumption to the use of harder drugs, and discusses the prevention of drug problems.

A stepping-stone provides a helpful foothold along a path, which serves as an odd metaphor for drug use. Stepping-stone theories often imply that marijuana produces a biological effect that somehow leads to the uncontrollable consumption of other drugs. This sort of theorizing began over 40 years ago (Nahas, 1990). Descriptions in popular culture create the impression that marijuana intoxication produces an insatiable

urge for more and different drugs, something similar to the way eating salt makes people thirsty. Data do not support these ideas. Marijuana and hard drugs do share some biological effects. For example, THC, the opiates, and cocaine alter the dopamine system in comparable ways (Koob & Le Moal, 1997). The cannabinoids, however, have their own receptor that does not react directly to drugs like heroin and cocaine.

Additional evidence against a biological stepping-stone appears in animal research. If marijuana created physiological changes that increased the desire for other drugs, animals exposed to cannabis would likely ingest other intoxicants when given the opportunity. Yet rodents exposed to THC do not show a sudden willingness to press levers for other drugs. They do not even appear willing to give themselves more THC (Schenk & Partridge, 1999; Wiley 1999). Thus, physiological mechanisms do not explain any link between marijuana and the use of other intoxicants (Institute of Medicine [IOM], 1999; Zimmer & Morgan, 1997).

Gateways and Causality

The lack of evidence for an obvious, biological stepping-stone inspired theorists to formulate ideas about marijuana as a gateway drug. A gateway usually serves as a passage to a region. Proponents of gateway theory show that people who use drugs like heroin and cocaine often used alcohol, tobacco, and marijuana first (Kandel, Yamaguchi, & Chen, 1992; Miller, 1994). Confusion about these theories has led to the idea that gateway drugs cause users to consume other substances. The prominent researchers of gateway theory never state that one drug causes the ingestion of another one. They simply report that cigarette smoking often precedes marijuana consumption, which usually precedes the use of other illicit drugs. Nevertheless, a few other authors misunderstand these data and create the impression that smoking marijuana leads inevitably to the use of other drugs (Nahas, 1990). Avoiding these misunderstandings requires a thorough understanding of causes.

The idea that one drug causes the use of another drug is difficult to prove. Proof of a cause creating an effect requires at least three clear criteria. These criteria were first proposed in the 1700s by the Scottish philosopher David Hume, one of the British empiricists. Hume emphasized that a cause creates an effect only under certain conditions. The conditions are association, temporal antecedence, and isolation. Associ-

ation means that the cause and effect must occur together. Temporal antecedence means that the cause must occur first, before the effect. Isolation means that all alternative causes must be ruled out. That is, the effect could not have occurred because of some other potential cause (Hume, 1739).

The best evidence for causality comes from experiments where the hypothesized cause can be manipulated. The cause should lead to the effect; its absence should create no effect (or a different one). For example, an experiment might reveal that marijuana causes intoxication by comparing people who smoked cannabis to an equivalent group of other people who smoked a placebo. This sort of experiment could provide evidence for all three of Hume's criteria. A large group of people who are not under the influence of any drug might begin by rating their intoxication. Presumably, all would claim their intoxication level was 0. The experimenter would then choose half these people at random to smoke marijuana. The other half would smoke a credible placebo. This random selection of people would help ensure that the two groups are similar. A second assessment of intoxication would likely reveal higher ratings of intoxication for the people who smoked marijuana than those who smoked the placebo.

The data from this experiment would satisfy Hume's criteria. The marijuana group's higher intoxication ratings reveal that the drug and the effect are associated. Because the groups did not differ before smoking, only after, the marijuana apparently preceded the effect. Thus, the temporal antecedence is fulfilled. Finally, all other sources of intoxication are ruled out because the two groups only differ in smoking cannabis or placebo. This condition helps eliminate any alternative explanations or causes. These data support the conclusion that marijuana can be isolated as the cause of this intoxication.

A simple experiment works well for proving cannabis causes intoxication, but researchers cannot use a human experiment to test the gateway theory. Randomly exposing people to marijuana to see who goes on to snort coke, bang junk, or drop acid creates many practical and ethical problems. These problems make isolating marijuana as the cause of other drug use impossible in humans. An obvious alternative approach could employ animal participants. No animal experiments have found that exposure to THC increases the likelihood of using other drugs or of even working for more THC (Schenk & Partridge, 1999; Wiley, 1999). Thus, gateway theory's only support comes from correlational studies.

Correlational investigations often examine users of crack or heroin and ask which drugs they used previously. These studies may establish an association and temporal antecedence, but fail to isolate marijuana as a cause. At first glance, the idea that users of hard drugs used marijuana previously seems compelling evidence for marijuana's contribution to drug problems. Nevertheless, a couple of exaggerated examples may illustrate how treating the data as causal evidence is erroneous.

Errors in Causal Reasoning

Suppose data revealed that the crime rate in a city rises as the number of churches increases. This association might lead some cynic to hypothesize that churches cause crime. Data may suggest that the churches are built prior to the increases in crime, further supporting the theory. Both the association between cause and effect and the precedence of the cause appear. Nevertheless, these two facts alone do not establish that churches cause crime. An alternative explanation remains. As cities grow larger, both crime and the number of churches increase. The size of the population accounts for both of these increases. One need not cause the other. Another example concerns shoe size and vocabulary. Data reveal that people who know more words also have larger shoes. One might hypothesize that memory for words is stored in the feet. Obviously, age can account for this relationship. As children grow older their feet grow and they learn more words.

Although these examples appear absurd, data misinterpreted to support that marijuana causes crack addiction are comparable to those in support of churches causing crime and vocabulary increasing shoe size. In fact, the correlations for these absurd examples are probably larger than those linking marijuana to crack. Most studies cited in support of the gateway theory show that people using heroin or crack used marijuana first. Unfortunately, these data tell little about the magnitude of the association between marijuana and hard drugs. Because only the users of hard drugs serve as participants, the data neglect the many, many people who consumed cannabis but no other illicit drugs. The use of marijuana also does not always precede the use of harder drugs, limiting the support for temporal antecedence. That is, some people try crack or ecstasy before they smoke cannabis. These data also fail to isolate mari-

juana as a cause. The association, temporal antecedence, and isolation criteria are explained in more detail below.

Association

Hume's first criterion for establishing a cause concerns association. Despite popular stereotypes, the association between marijuana and harder drugs is not particularly strong. Many people who abuse hard drugs used marijuana first, but few people who smoke marijuana go on to consume other intoxicants. One study found that 75% of men who used marijuana between 10 and 99 times never used any other illicit drug (Kandel & Davies, 1992). Part of the absence of an association stems from the small number of people who use hard drugs relative to the many who have tried marijuana. Data from the Substance Abuse and Mental Health Services Administration (SAMHSA, 2000) can shed light on this question (see table 3.1).

Most associations are expressed as a correlation coefficient, a number ranging from −1 to +1 that depicts how well two phenomena go together. If everyone who tried marijuana also snorted cocaine, their correlation would be 1.0. Few phenomena go together perfectly, but larger numbers (up to 1.0) mean a stronger relationship. Correlations around .3 frequently receive some attention in the social sciences. For example, Scholastic Aptitude Test math scores correlate approximately .3 with grades in math classes (Gougeon, 1984). Personality measures and alcohol consumption often correlate between .3 and .4 (Earleywine, Finn, & Martin, 1990; Earleywine & Finn, 1991). Correlations smaller than .3 often attract little attention.

Table 3.1. 1999 Drug Use Rates in America (in millions)

	Marijuana	Cocaine	Crack	Heroin
Lifetime	76.4	25.4	5.9	3.0
Past year	19.5	3.7	1.0	0.4
Past month	11.1	1.5	0.4	0.2

Data from Substance Abuse and Mental Health Services Administration (SAMHSA) (2000). Summary of findings from the 1999 National Household Survey on Drug Abuse. Rockville, MD: SAMHSA.

Based on the national survey data, the actual correlation between marijuana and crack cocaine use is .02. This calculation assumes that everyone who used crack cocaine used marijuana first. In addition, this calculation takes into account the many people who use marijuana and never try the harder drug. The correlations between marijuana and heroin are even smaller. (See the final section of this chapter for the computations.) Thus, most correlations that scientists view as important are over 15 times larger than the correlation between marijuana and crack cocaine use.

Standard measures of the correlation between marijuana use and the use of harder drugs are very small. They offer little support for an association between marijuana and other drugs. In an effort to depict the link between marijuana and other drugs in a different way, some authors have turned to another statistical procedure known as conditional probabilities. Conditional probabilities reveal the chances that people will try a harder drug if they have tried marijuana. They are computed by dividing the number of users of the harder drug by the number of marijuana users. If everyone who smoked marijuana tried crack, the conditional probability would be 1.0. If half the marijuana users tried crack, the conditional probability would be .5. If we had 100 people and 30 of them used cannabis, the conditional probability of using cannabis would be .3. If 10 of those 30 went on to try crack, the chance of using cocaine given that cannabis was used first would be one-third.

Again, assume that everyone in the national survey who used a hard drug tried marijuana first. The chance of trying powder cocaine after trying marijuana appears to be relatively high. If all 25 million people who tried cocaine were among the 76 million who had also tried marijuana, 25 million divided by 76 million equals .33. Thus, 1 in 3 people who try marijuana also try cocaine. This number is certainly large enough to warrant concern. Statistics like these motivated drug reformers in the Netherlands to remove criminal penalties for small amounts of marijuana in hope of separating it from the cocaine drug market. They also inspired increased penalties for cannabis in the United States.

The conditional probability linking marijuana to cocaine may be alarming. Nevertheless, the number of marijuana users who continue to use cocaine regularly is markedly smaller. Although 25 million Americans have used cocaine in their lifetimes, fewer than 4 million used it in the past year. Assume that these people use the drug at least once a year, and that they all tried marijuana first. Thus, the chances of marijuana

leading to yearly cocaine use are 4 million (the number of yearly cocaine users) divided by 76 million (the number who tried cannabis), or about .05. This means only 1 in 20 people who try marijuana use cocaine once a year or more. Even fewer people used cocaine in the last month (1.5 million). Comparable computations suggest that the chances of trying marijuana and then using cocaine monthly are .02, or about 1 in 50. Thus, less than 2 in 100 marijuana users go on to use cocaine monthly. The probabilities for using crack cocaine and heroin are even lower (see table 3.2).

Thus, the association between marijuana consumption and problem use of other drugs is very small. In fact, studies of the gateway theory do not assess the problems associated with hard drug use; they simply focus on trying other substances. Some studies consider trying a drug a single time as confirmation of use (e.g., Blaze-Temple & Lo, 1992), an approach that has drawn criticism (Zimmer & Morgan, 1997). Trying marijuana is not sufficient to cause the use of harder drugs. Even if the association were markedly larger, this criterion alone does not establish causality. Causal arguments require temporal antecedence and isolation, too.

Table 3.2. Gateway Associations

Of all Americans who have tried marijuana
Few have used marijuana regularly
approximately 1 in 4 used marijuana in the past year (25.5%)
approximately 1 in 7 used marijuana in the past month (14.7%)
Few people who have tried marijuana have used cocaine
approximately 1 in 3 tried cocaine (33.0%)
approximately 1 in 20 used cocaine in the past year (4.8%)
approximately 1 in 50 used cocaine in the past month (2.0%)
Even fewer people who have tried marijuana have used crack
approximately 1 in 13 tried crack (7.7%)
approximately 1 in 100 used crack in the past year (1.3%)
approximately 1 in 200 used crack in the past month (0.5%)
Even fewer people who have tried marijuana have used heroin
approximately 1 in 26 tried heroin (3.9%)
approximately 1 in 200 used heroin in the past year (0.5%)
approximately 1 in 333 used heroin in the past month (0.3%)

These calculations assume that everyone who ever tried hard drugs used marijuana first. Data from Substance Abuse and Mental Health Services Administration (SAMHSA) (2000). Summary of findings from the 1999 National Household Survey on Drug Abuse, Rockville, MD: SAMHSA.

Temporal Antecedence

If marijuana actually caused the consumption of other intoxicants, cannabis consumption must precede the use of hard drugs. Many studies show that hard drug users smoke marijuana first. The stereotyped progression of the use of drugs probably begins with caffeine, but no data address this drug's potential as a gateway. Most research suggests that adolescents first use alcohol or nicotine. Some authors argue that cigarettes serve as the actual gateway to drug problems (Kandel et al., 1992; Labouvie, Bates, & Pandina, 1997). A subset of the people who drink alcohol and smoke cigarettes subsequently use marijuana. A subset of those who try marijuana then use harder drugs like cocaine, crack, and heroin. Some researchers report that over 90% of people who try hard drugs tried marijuana first, suggesting temporal antecedence. They also ate white sugar, breathed air, and attended grade school. Thus, although hard drug users smoke marijuana before turning to other substances, this fact alone does not prove causality.

In addition, the order of drug use is not always perfectly consistent with the idea that marijuana is the gateway. Many hard drug users do not start with marijuana. Allen Ginsberg, the celebrated "beat" poet, serves as one notable exception. He injected heroin before smoking cannabis (Ginsberg, 1966). Large samples with thousands of respondents show a range of hard drug users who did not try marijuana first. One study found that as few as 1% of people who used hard drugs had not tried marijuana (Donovan & Jessor, 1983). In contrast, 15% of another sample of heavy drug users started with cocaine or intravenous drugs before smoking marijuana (Golub & Johnson, 1994). A study of Australian youth found that 29% of those who had used amphetamine, LSD, cocaine, or heroin had not used marijuana first (Blaze-Temple & Lo, 1992). Other research found 39% of a sample used hard drugs before trying cannabis (Mackesy-Amiti, Fendrich, & Goldstein, 1997). Thus, temporal antecedence applies in some cases of hard drug use, but not all. Even a perfect ordering with marijuana preceding all other drug use does not prove causality without isolation.

Isolation: Independent Processes or Problem Behavior?

Hume's last criterion for causality concerns isolation. If marijuana actually causes the use of hard drugs, other explanations should not account

for any association between them. At least two alternative explanations have some empirical support. One concerns the idea that the initiation of each drug arises from its own individual process. Miller (1994) calls this approach the statistical independence hypothesis. This hypothesis states that using one drug stems from its own availability, expectancies, and motivations that are separate from those related to another drug. Thus, the initiation of caffeine may stem from one process; the initiation of heroin may stem from another. The other idea that may account for stages of drug use focuses on the abuse of any and all substances as part of a cluster of larger problem behaviors. This problem behavior theory views substance abuse, unsafe sex, crime, and delinquency as all part of the same underlying trouble.

The rationale for individual processes follows statistical logic. People who participate in rare events likely engage in popular activities first. For example, more people view television than skydive. Thus, we would expect that most skydivers watched TV before they leaped from a plane. This fact need not mean that television causes skydiving. The two acts probably arise from independent, individual processes. Yet the most common one occurs first simply because it is more common. Comparable logic applies to drug consumption. People who use drugs will likely begin with those that are most common. Thus, individuals may use marijuana before cocaine because marijuana is more prevalent in our culture. Yet this fact need not mean that marijuana caused cocaine consumption. A massive study of four national samples including over 6,000 participants suggests that a large portion of the appearance of stages of drug use can be accounted for by statistically independent processes (Miller, 1994). This model does not account for all the data, but independent processes clearly contribute to the progression of drug use.

An alternative way to test the statistical independence hypothesis might examine neighborhoods where crack cocaine is more available than marijuana. If most drug users in such neighborhoods smoked crack before cannabis, the role of availability might receive some support. Under these circumstances, few could conclude that crack is a gateway leading to cannabis. Instead, people use the most available drug first and less available drugs later or not at all.

Another alternative explanation of drug sequencing concerns problem behavior theory. According to this theory, a small group of adolescents engage in a cluster of actions that all may lead to negative consequences (Jessor & Jessor, 1977). These problem behaviors include drug consump-

tion, poor school performance, unsafe sex, and criminal activities. According to this theory, the association between cannabis use and the consumption of other drugs does not arise because marijuana causes problems with other substances. Instead, both marijuana and the use of other intoxicants arise from the underlying problem orientation in a subset of individuals.

Many studies reveal strong correlations among the use of different drugs (Earleywine & Newcomb, 1997). Several potentially dangerous actions also correlate with drug use (Jessor, 1998). Miller (1994) analyzed data from four national surveys including more than 6,000 participants and found that problem behavior theory may account for the appearance of stage-like progressions in substance use. He found a large subgroup who used many drugs, and another set of people who used no drugs at all. These results are consistent with the idea that an underlying "problem-proneness" may account for links between marijuana and other drugs. Essentially, cannabis does not cause cocaine consumption, but a subset of people who like marijuana also like cocaine.

In addition to statistical independence and problem behavior theory, a third set of findings also supports arguments against marijuana as an isolated cause of hard drug use. Studies that show personality traits correlate with the use of multiple substances may mean that a personality characteristic led to both marijuana use and hard drug use. These data suggest that the same personality traits that can lead to smoking cannabis can also lead to snorting cocaine. Thus, the marijuana may not cause the use of the other drugs; both stem from the same underlying characteristic. Although evidence for an addictive personality is clearly limited (Nathan, 1988), people who report strong desires for thrill, adventures, and sensations often use a greater variety and amount of drugs (Simon, Stacy, Sussman, & Dent, 1994).

These findings support the idea that marijuana cannot be isolated as the cause of the use of hard drugs. Simple exposure to cannabis is not strongly associated with the use of other intoxicants. Hard drug users do not always use marijuana first. Causes other than marijuana also lead to the consumption of heroin or cocaine. Nevertheless, some authors argue that marijuana may still contribute to the use of harder drugs, even if it is not a unitary cause. They assert that even if cannabis does not qualify as the cause of other drug problems, it facilitates the use of more substances, increasing the likelihood of trouble.

Marijuana as Contributor Rather than Cause

Few interesting behaviors, including drug use, arise from a single process. Data do not support the idea that smoking marijuana causes the abuse of other substances. Nevertheless, perhaps marijuana "contributes" to the use of other drugs, even if it does not serve as the sole cause. This contributing process could work in several ways. For example, smoking marijuana may lead people to think of themselves as illicit drug users, making hard drug consumption more likely. Another pathway may arise when purchasing marijuana exposes people to the market for other drugs. In addition, marijuana intoxication may limit a person's ability to refuse harder drugs when they are offered. These factors may interact to contribute to the transition from cannabis consumption to hard drug use. Each of these paths is discussed in the following paragraphs.

One potential path for marijuana's impact on substance abuse concerns an individual's identity as an illicit drug user. Despite rampant consumption of caffeine and nicotine, few of us see ourselves as drug users. Most people who have never consumed an illicit drug would claim that they have no intention of snorting cocaine or smoking crack. Yet people might alter their impressions of themselves after smoking marijuana. With continued consumption of cannabis, people may see themselves as illicit drug users. After establishing this identity, their chances of trying other drugs may increase. Thus, people who may have had no intention of using cocaine or crack before they smoked marijuana may consider consuming these drugs after a period of cannabis use. Researchers have yet to examine this potential path, but studies along these lines may prove fruitful. Longitudinal studies might assess each individual's identity as a drug user over time. Some of those who try marijuana may adopt this identity; others may not. If those who smoke cannabis and subsequently consider themselves illicit drug users make the transition to hard drugs, the data would support this theory.

Another pathway may involve exposure to the illicit drug market. Suppliers of cannabis may also sell harder drugs, exposing marijuana purchasers to cocaine or heroin. A supplier may have a set of marketing strategies for these harder drugs, including strong personal testimony about their quality. Each purchase of marijuana may expose people to sales pitches for more harmful drugs, perhaps increasing the likelihood

of eventually trying them. Few studies address this course, but it has a certain intuitive appeal. This line of reasoning motivated marijuana decriminalization in the Netherlands in an attempt to separate it from the hard drug market. Some studies suggest that this move decreased the consumption of hard drugs (see chapter 10). Other studies might include interviewing hard drug users to see if they first obtained these drugs from the same person who supplied their cannabis.

Finally, perhaps marijuana users would be more likely to try other drugs during intoxication. People often use combinations of different substances (Earleywine & Newcomb, 1997). Yet no data address if people first try cocaine or heroin while experiencing cannabis's effects. The cognitive impairments associated with marijuana intoxication might decrease an individual's ability to resist using other drugs. A couple of experiments might shed light on this phenomenon. Researchers might provide access to cocaine or heroin after injecting THC into rodents to see if they are more likely to consume the new drugs during intoxication. Studies of human reactions might rely on self-reported willingness to try other drugs after smoking marijuana. People may claim to be more likely to ingest a new substance after consuming cannabis than they would after smoking a placebo. These data would support the idea that marijuana intoxication alters the probability of consuming hard drugs.

Preventing Substance Abuse

If marijuana consumption led directly to the abuse of hard drugs, preventing substance abuse would be simpler. In fact, drug problems arise from complex interactions of multiple factors. Preventing addiction and other aspects of substance abuse proves difficult. Many attempts have been relatively unsuccessful, including scare tactics, basic drug education, the Drug Abuse Resistance Education (DARE) program, and enhancing self-esteem. Other programs show more promise, including interactive sessions that teach techniques for combating peer pressure to use drugs. Social influence programs combine multiple strategies and have proven effective at decreasing drug use (Sussman, Dent, Stacy, & Craig, 1998). Unfortunately, no program has consistently eliminated drug abuse in all participants, and the impact of prevention efforts can often dissipate quickly (Shope, Copeland, Kamp, & Lang, 1998). Deterring drug problems remains an ongoing challenge.

standing in front of other participants to declare their intention. These activities have helped alter attitudes about drugs in other interventions. In addition to these activities, social influence programs add unique components to improve outcomes.

The unique components of social influence programs include increasing awareness about the actual number of drug users, developing skills for refusing drugs when offered, and combating indirect social pressures to use drugs. Perceptions of the number of users often lead to a skewed sense of drug consumption. People often hold the faulty idea that drug use is extremely common, and may feel that they should use drugs in order to obey this norm. Drug users often assume that everyone else uses drugs, too. Perceptions of norms for behavior guide actions in many domains. Objective information about drug consumption often surprises participants, who frequently overestimate the incidence of use (Perkins, Meilman, Leichliter, Cashin, & Presley, 1999). Accurate assessments of the number of users may minimize pressures related to the idea that everyone else consumes illicit drugs.

Drug refusal skills focus on resisting peer pressure to consume drugs. These include more than Nancy Reagan's simplistic recommendations to "just say no." Students engage in role plays designed to enhance their ability to decline drugs whenever they are offered. Strategies include keeping their refusals direct, suggesting alternative activities, avoiding situations where drugs are prevalent, and walking away if pressures feel threatening. Increasing awareness of indirect pressures to use drugs often focuses on the inaccuracy of glamorized media portrayals of substance use. Indirect pressures also may arise when social models, including parents, older siblings, and peers use drugs. Increasing awareness about these pressures may help inoculate against drug use (Donaldson et al., 1996). The majority of programs that employ this social influence approach have had positive effects (Hansen, 1992). Implementing them in more settings with detailed follow-up research can help minimize drug problems in ways that simplistic assumptions about marijuana as a gateway cannot.

Conclusions

The idea that marijuana serves as a gateway or stepping-stone to the consumption of harder drugs with worse negative consequences has generated considerable interest. There is no evidence that cannabis creates

physiological changes that increase the desire for drugs. The idea that marijuana causes subsequent drug use also appears unfounded. Causes require association, temporal antecedence, and isolation. Evidence for the association between marijuana and other drugs remains limited. Data do reveal that the majority of cocaine and heroin users consumed cannabis first. Nevertheless, only a minority of marijuana smokers try cocaine, crack, or heroin. Only a few people become regular users of these intoxicants. In addition, marijuana does not precede the use of hard drugs in all cases. Finally, correlations between marijuana smoking, hard drug consumption, and other problem behaviors suggest that one drug may not lead to another so much as all use of illicit substances reflects an underlying deviance or personality characteristic. Thus, prevention of drug problems requires more than staying away from cannabis.

Many programs designed to minimize substance abuse have met with only limited success. The scare tactics of the 1960s and 1970s had little impact on drug use. The DARE program, though popular, does little to prevent the use of illicit substances. The enhancement of self-esteem also does not prevent drug problems. One series of studies suggests that programs designed to minimize the impact of social influences to use drugs holds considerable promise. By providing valid information on the relative infrequency of drug use and valuable coaching on ways to resist pressures to use intoxicants, these programs can help decrease the incidence of abuse and dependence.

Comment on the Computation of Correlations

A few adventurous souls may wish to know the exact correlation between marijuana use and the use of other drugs. A Pearson product-moment correlation can be computed from a 2 × 2 table using the cross products and the marginals, in a manner that is easier done than said. Cross products are computed by multiplying the numbers along the diagonal. Marginals are the totals across each row and column. The correlation (R) equals the difference in the cross products divided by the square root of the product of all four marginals (Rosenthal & Rosnow, 1991). Using the data on drug use from 1999, and an assumption of 221 million adult Americans, we can compute the correlation between marijuana and crack cocaine use. Assume absolutely everyone who tried

The idea that
unfounded. Caus
on. Evidence for the
nains limited. Data do
consumed cannabis
nokers try cocaine,
r users of these intox-
ıse of hard drugs in
king, hard drug con-
ıat one drug may not
es reflects an under-
, prevention of drug
innabis.

buse have met with
s and 1970s had little
popular, does little to
ıt of self-esteem also
s suggests that pro-
ıfluences to use drugs
mation on the rela-
ıng on ways to resist
elp decrease the inci-

xact correlation be-
A Pearson product-
2 table using the cross
sier done than said.
ımbers along the di-
nd column. The cor-
ucts divided by the
osenthal & Rosnow,
an assumption of 221
lation between mar-
everyone who tried

Table 3.3. Millions of Americans Who Have Tried Marijuana, Crack Cocaine, Both, or Neither (1999)

	Tried marijuana		
Tried crack	Yes	No	Total
Yes	6	0	6
No	70	145	215
Total	76	145	

crack tried marijuana previously. The number of people (in millions), who tried marijuana, crack cocaine, both, or neither, appears in table 3.3.

Thus, of 221 million adult Americans, 76 million tried marijuana and 145 million did not. Assume that the 6 million Americans who tried crack cocaine also tried marijuana. Thus, 70 million people who tried marijuana never tried crack. The correlation from this table is computed by starting with the difference in the cross products. Moving from the upper left to lower right on the diagonal, we have 6 million × 145 million = 8.7×10^{13}. The upper right times the lower left is 0 × 70 million = 0. The difference between these two equals 8.7×10^{13}. We divide this number by the square root of the product of the four marginals. SQRT (76 million × 145 million × 6 million × 215 million)= 3.77×10^{15}. We divide 8.7×10^{13} by this number and get a correlation of .02. The numbers for heroin are even smaller because so few people have ever tried it. Thus, the correlation between marijuana consumption and the regular use of these harder drugs is negligible.

Cancer Causes and Control, 1997, 8, pp. 722-728

Marijuana use and cancer incidence (California, United States)

Stephen Sidney, Charles P. Quesenberry, Jr., Gary D. Friedman, and Irene S. Tekawa

(Received 16 December 1996; accepted in revised form 12 May 1997)

The purpose of this retrospective cohort study was to examine the relationship of marijuana use to cancer incidence. The study population consisted of 64,855 examinees in the Kaiser Permanente multiphasic health checkup in San Francisco and Oakland (California, United States), between 1979-85, aged 15 to 49 years, who completed self-administered questionnaires about smoking habits, including marijuana use. Follow-up for cancer incidence was conducted through 1993 (mean length 8.6 years). Compared with nonusers/experimenters (lifetime use of less than seven times), ever- and current use of marijuana were not associated with increased risk of cancer of all sites (relative risk [RR] = 0.9, 95 percent confidence interval [CI] = 0.7-1.2 for ever-use in men; RR = 1.0, CI = 0.8-1.1 in women) in analyses adjusted for sociodemographic factors, cigarette smoking, and alcohol use. Marijuana use also was not associated with tobacco-related cancers or with cancer of the following sites: colorectal, lung, melanoma, prostate, breast, cervix. Among nonsmokers of tobacco cigarettes, ever having used marijuana was associated with increased risk of prostate cancer (RR = 3.1, CI = 1.0-9.5) and nearly significantly increased risk of cervical cancer (RR = 1.4, CI = 1.0-2.1). We conclude that, in this relatively young study cohort, marijuana use and cancer were not associated in overall analyses, but that associations in nonsmokers of tobacco cigarettes suggested that marijuana use might affect certain site-specific cancer risks. *Cancer Causes and Control* 1997, 8, 722-728

Key words: Cancer incidence, marijuana, retrospective cohort study, United States.

Introduction

Marijuana use is by far more prevalent than the use of any other illegal drug in the United States. It has been estimated that nearly one-third of the US population aged 12 and older has used marijuana.[1] The most common route of administration is smoking. Since marijuana smoke contains many of the same chemicals as tobacco smoke, in addition to containing 60 cannabinoid compounds, it is plausible to hypothesize that it may be carcinogenic.[2] However, marijuana has not been established as a risk factor for any cancer, although it may play a role in the development of lung cancer [3-5] and of head and neck cancers.[6,7] The uncertainty in establishing marijuana as a cancer risk factor may be due, in part, to the difficulty in separating the effects of marijuana from the effects of tobacco cigarettes, since in many instances the marijuana smoker also smokes tobacco cigarettes. Even though a marijuana cigarette may be smoked in a more intense manner (greater puff volume and length of inhalation)[10] than a tobacco cigarette, the total quantity of marijuana smoked by a user during his/her lifetime is

Authors are with the Division of Research, Kaiser Permanente Medical Care Program, Oakland, California, USA. Address correspondence to Dr Sidney, Kaiser Permanente Division of Research, 3505 Broadway, Oakland, California 94611-5714, USA. *This research was supported by Grant no. R01 DA06609 from the US National Institute on Drug Abuse. Dr Friedman is supported in part by Grant no. R[illegible]49761 from the US National Cancer Institute. Data collection regarding alcohol use was supported by a grant from The [illegible]ic Beverage Medical Research Foundation (Baltimore, MD, USA).*

generally a tiny fraction of the quantity smoked by a tobacco cigarette user.

We report here the findings of a study of the relationship of marijuana use to cancer incidence in a cohort of nearly 65,000 members of a large, prepaid health plan. This study is important because relatively little research has been reported regarding the relationship of marijuana use to human cancer, and we are unaware of any other cohort studies which have examined this issue. We hypothesized that marijuana use would be associated with increased risk of tobacco-related cancers, but that it might be difficult to detect this risk because of the coexistence of tobacco cigarette smoking in marijuana smokers. In previously published work,[11] we did not find an association between marijuana use and cancer mortality.

Materials and methods

Study population

The study cohort is composed of 64,855 Kaiser Permanente Medical Care Program (KPMCP) members, aged 15 to 49 years (mean 33 years), who voluntarily completed self-administered research questionnaires between mid-1979 through 1985 regarding tobacco, marijuana, and alcohol use in the context of a multiphasic health checkup (MHC) conducted in the San Francisco (until 1980) and Oakland Kaiser Permanente facilities in California (US). Written assurance was provided at the beginning of the questionnaires that all answers would be kept confidential, and that the questionnaire would not become part of the medical record or sent to the member's doctor. Follow-up was carried out from the date of the MHC to date of the earliest of the following: cancer diagnosis (n = 1,421); death (n = 495); diagnosis of HIV positivity or AIDS (n = 384); membership termination (n = 34,887); or 31 December 1993 (n = 27,668). The mean length of follow-up was 8.6 years.

Source of follow-up data

Incident cancers through 1993 were determined from computerized databases of confirmed cancer cases maintained by the Northern California Cancer Center (prior to 1988) and from the Kaiser Permanente Northern California Regional Cancer Registry (1988-93). Cancer cases were categorized according to ICD-9 codes.[12] Cancer sites categorized as smoking-related included upper aerodigestive (including esophagus), lung, pancreas, kidney, and bladder, as specified by the International Agency for Research on Cancer (IARC) in 1984.[13] We did not include cervical cancer and acute leukemia in the grouping of smoking-related cancers, although evidence has accumulated linking smoking to cancer of these sites.[14,15] HIV/AIDS cases were ascertained from the Northern California Kaiser Permanente Medical Care Program HIV/AIDS database. We excluded 92 cancer cases that occurred subsequent to or within one year prior to the date of HIV/AIDS diagnosis, distributed as follows: 68 Kaposi's sarcoma; 16 lymphatic cancers; eight other sites.

Mortality was ascertained through 1993 by computer-matching names and other demographic data with the California death file using the California Automated Mortality Linkage and Information System (CAMLIS).[16] In a test subset of 4,695 MHC examinees, this method was found to produce more false-negative results (11 percent *cf* six percent of known dead classified as alive) and fewer false-positive results (0.07 percent *cf* 1.2 percent of known alive classified as dead) compared with utilization of the National Death Index in ascertainment of mortality.[16] Membership status was determined for each year by examination of computerized files maintained by the Northern California Kaiser Permanente Medical Care Program.

Determination of marijuana use

Current marijuana smoking was defined by respondent's admission to smoking currently and more than six times ever. Former marijuana smoking was defined by denial of current smoking but admission to having smoked more than six times ever. Nonsmoking was defined as never having smoked. Experimenters were defined as those admitting to having ever smoked from one to six times. Ever-smokers included current and former users but excluded experimenters. Duration of use (number of years) and frequency of use ('less than once a month,' 'about once or twice a month,' 'about once or twice a week,' and 'almost every day') were assessed in ever-users. Data were not collected in former users regarding the length of time since they last used marijuana.

Determination of tobacco cigarette use and alcohol use

Persons were classified as current, former, or never-smokers of tobacco cigarettes on the basis of their questionnaire responses.[17] Current and former smokers were also questioned about frequency (number of cigarettes per day) and duration (years) of smoking. Alcohol consumption was categorized as current use, former use, and nonuse. Current drinkers were questioned about how many drinks they usually had during the past year: 'nine or more per day,' 'six to eight per day,' 'three to five per day,' 'one or two per day,' 'less than one per day, but more than one per month,' and 'less than one per month (special occasions only).'

Analytic methods

The analyses were planned to compare the risk of cancer associated with ever-use of marijuana and with current use of marijuana relative to never or experimental use.

Statistical analyses were performed using SAS programs.[11] Age-adjusted incidence rates were calculated by the direct method using 10-year age groupings with the 1990 US population as the standard. Cox proportional hazards models were used to examine the joint effect of sociodemographic characteristics, marijuana use, tobacco, and alcohol use on the risk of cancer, from which estimates of relative risks (RR) and associated 95 percent confidence intervals (CI) were obtained.[11] Two-way interactions between marijuana and tobacco use and between marijuana and alcohol were tested by including cross-product terms in the proportional hazards models. The interaction terms were statistically significant in a few of the models (tobacco and ever-use of marijuana for colorectal cancer in women and melanoma in men; alcohol and both ever- and current use of marijuana for prostate cancer in men; alcohol and both ever- and current use of marijuana for breast cancer in women). However, the RR estimates associated with tobacco or alcohol use within any given level of marijuana use in these models had wide CIs and lack of dose-response, so that only the main effects are reported in this paper. To obtain estimates of the overall risk associated with marijuana use, adjusted for tobacco cigarette use, tobacco cigarette use was categorized as a three-level variable (*i.e.*, nonuser, former user, current user).

Results

Sociodemographic characteristics (Table 1)

Current users, former users, experimenters, and nonusers, respectively, comprised 22 percent, 20 percent, 20 percent, and 38 percent of the cohort. The percentage of ever-users was highest in the 20 to 29 year age group. Ever-use of marijuana was more common in men than in women, and more common in Whites followed by Blacks, Hispanics, and Asians.

Tobacco cigarette use

The distribution of marijuana use (ever *cf* nonusers/experimenter) and tobacco cigarette smoking (ever *cf* nonsmoker) are shown in Table 2. The prevalence of a history of tobacco-cigarette smoking was 54 percent higher in ever-users of marijuana (59.9 percent) than in nonusers/experimenters (38.8 percent). A substantial proportion of marijuana users were nonsmokers of tobacco cigarettes (40.1 percent of ever-users of marijuana [10,710 of 26,733]).

Distribution of cancer sites

Overall, there were 379 incident cancers in men, and 1,042 incident cancers in women. We performed analyses for cancers of all sites, tobacco-related cancers, and individual

Table 1. Sociodemographic characteristics of a cohort of Kaiser Permanente Medical Care Program members ($n = 64,855$) by marijuana use status, June 1979 through December 1985

Status at multiphasic health checkup	Never	Experimenter	Former	Current
Age (yrs)				
15-19	902	558	508	757
20-29	5,489	3,864	4,734	5,970
30-39	8,929	5,320	5,751	5,759
40-49	9,647	3,413	1,707	1,547
Gender				
Male	9,072	5,271	6,075	7,502
Female	15,895	7,884	6,625	6,531
Race				
White	9,894	7,316	8,372	8,743
Black	7,654	3,770	2,839	3,974
Asian	6,085	1,082	649	399
Hispanic	1,525	615	476	528
Other/unknown	809	392	365	389
Education				
≤ High school	5,837	2,420	1,887	2,518
Technical/business school/ some college	8,706	4,760	4,458	5,435
College graduate/ post graduate	9,941	5,703	6,034	5,850
Unknown	483	272	321	230
Total	24,967	13,155	12,700	14,033

sites with 30 or more gender-specific incident cancers (colorectal, lung, and melanoma for both genders; prostate for men, and breast and cervix for women). The counts for all these groupings and sites by marijuana and tobacco-cigarette use status are shown in Tables 3 and 4.

Table 2. Distribution of study population by gender and by ever-use of marijuana and tobacco cigarettes, Kaiser Permanente Medical Care Program

Marijuana/cigarette smoking history		Men	Women
Marijuana use	Tobacco cigarette use	No.	No.
Never	Never	8,170	15,360
Never	Ever	6,173	8,419
Ever	Never	5,593	5,117
Ever	Ever	7,984	8,039
Total		27,920	36,935

Marijuana and tobacco cigarette use in relation to cancer incidence

As expected, tobacco cigarette use, regardless of marijuana use status, was associated with increased risks of tobacco-related cancer ranging from 2.7 to 3.8 relative to nonuse of marijuana and tobacco cigarettes in models adjusted for age, race/ethnicity, education, and alcohol use (Tables 3 and 4); RRs for lung cancer associated with tobacco cigarette use ranged from 9.2 to 11.2. Ever-use of marijuana by nonsmokers of tobacco cigarettes was associated with increased risk in men of prostate cancer (RR = 3.1, CI = 1.0–9.5) and with a nearly significant increased risk in women of cervical cancer (RR = 1.4, CI = 1.0–2.1) relative to nonuse of marijuana and tobacco cigarettes. Additional adjustment for marital status did not appreciably alter the estimates of RRs for cancer of these sites, nor was it statistically significantly associated with these cancer sites. We also examined the association of marijuana use with invasive cervical cancer, comprising 11 percent (33 of 302) of all cervical cancers. Ever-use of marijuana in nonsmokers of tobacco cigarettes was asso-

Table 3. Counts, age-adjusted rates, and relative risks (RR) of all cancers, tobacco-related cancers, and cancers of specific sites by marijuana and cigarette smoking status in men, Kaiser Permanente Medical Care Program

Site of cancer	Marijuana, cigarette smoking history	Number of cases	Rate[a]	RR[b]	(CI)[c]
All sites	Never, never	109	149	1.0	—
	Never, ever	138	169	1.1	(0.9-1.5)
	Ever, never	38	186	0.8	(0.5-1.2)
	Ever, ever	96	165	1.1	(0.8-1.5)
Tobacco-related	Never, never	12	19	1.0	—
	Never, ever	56	68	3.1	(1.6-5.8)
	Ever, never	3	7	0.8	(0.2-2.9)
	Ever, ever	28	39	2.8	(1.4-5.6)
Colorectal	Never, never	16	16	1.0	—
	Never, ever	15	18	0.8	(0.4-1.6)
	Ever, never	4	7	0.7	(0.2-2.1)
	Ever, ever	10	29	0.9	(0.4-1.9)
Lung	Never, never	2	3	1.0	—
	Never, ever	32	39	10.3	(2.4-43.7)
	Ever, never	0	—	—	—
	Ever, ever	14	28	9.2	(2.0-42.0)
Melanoma	Never, never	23	27	1.0	—
	Never, ever	10	9	0.5	(0.2-1.0)
	Ever, never	6	11	0.5	(0.2-1.3)
	Ever, ever	19	25	1.1	(0.6-2.0)
Prostate	Never, never	9	16	1.0	—
	Never, ever	21	28	2.0	(0.9-4.5)
	Ever, never	5	95	3.1	(1.0-9.5)
	Ever, ever	7	31	1.7	(0.6-4.9)

[a] Per 100,000 person-years, age adjusted to 1990 US population.
[b] Adjusted for age, race, education, and alcohol use.
[c] CI = 95% confidence interval.

Table 4. Counts, age-adjusted rates, and relative risks (RR) of all cancers, tobacco-related cancers, and cancers of specific sites by marijuana and cigarette smoking status in women, Kaiser Permanente Medical Care Program

Site of cancer	Marijuana, cigarette smoking history	Number of cases	Rate[a]	RR[b]	(CI)[c]
All sites	Never, never	408	260	1.0	—
	Never, ever	353	393	1.3	(1.1-1.5)
	Ever, never	93	403	1.1	(0.8-1.3)
	Ever, ever	188	408	1.2	(1.0-1.4)
Tobacco-related	Never, never	18	12	1.0	—
	Never, ever	55	54	3.8	(2.1-6.7)
	Ever, never	0	—	—	—
	Ever, ever	10	47	2.7	(1.2-6.3)
Colorectal	Never, never	27	18	1.0	—
	Never, ever	28	26	1.4	(0.8-2.4)
	Ever, never	1	2	0.3	(0.0-2.5)
	Ever, ever	6	8	0.9	(0.4-2.3)
Lung	Never, never	5	4	1.0	—
	Never, ever	36	35	9.8	(3.6-26.5)
	Ever, never	0	—	—	—
	Ever, ever	8	44	11.2	(3.3-37.7)
Melanoma	Never, never	23	11	1.0	—
	Never, ever	18	18	1.1	(0.6-2.0)
	Ever, never	8	71	1.0	(0.4-2.3)
	Ever, ever	16	19	1.2	(0.6-2.5)
Breast	Never, never	171	101	1.0	—
	Never, ever	113	117	0.9	(0.7-1.2)
	Ever, never	22	115	0.8	(0.5-1.3)
	Ever, ever	54	138	1.0	(0.8-1.5)
Cervix	Never, never	92	62	1.0	—
	Never, ever	80	118	1.7	(1.2-2.3)
	Ever, never	48	119	1.4	(1.0-2.1)
	Ever, ever	82	125	1.6	(1.2-2.2)

[a] Per 100,000 person-years, age adjusted to 1990 US population.
[b] Adjusted for age, race, education, and alcohol use.
[c] CI = 95% confidence interval.

ciated with a nonsignificant increased risk of invasive cervical cancer (RR = 2.4, CI = 0.8-6.7). The overall risks of cancer associated with ever-use of marijuana are shown in Table 5, adjusted for tobacco cigarette smoking status (non-, former, current) as well as the other variables noted earlier. Ever use of marijuana was not associated in men or in women with increased or decreased risk of any cancer grouping or individual site. The results of analyses of cancer incidence excluding the 222 (15.6 percent of total cancers) which developed within the first two years of follow-up was similar to the overall results, suggesting that the overall results were not affected by symptoms caused by cancer prior to its diagnosis that affected the decision to use marijuana.

The findings in regard to current marijuana use were similar to those for ever-use of marijuana. Current marijuana use also was associated with increased risk of prostate cancer (RR = 4.7, CI = 1.4-15.5) and a nearly

Table 5. Risk of cancer in ever-users of marijuana relative to nonusers/experimenters adjusted for cigarette smoking status and other variables,[a] Kaiser Permanente Medical Care Program

Site of cancer	Men		Women	
	RR[b]	(CI)[c]	RR[b]	(CI)[c]
All sites	0.9	(0.7-1.2)	1.0	(0.8-1.1)
Tobacco-related	0.9	(0.6-1.4)	0.7	(0.3-1.4)
Colorectal	0.9	(0.5-1.8)	0.6	(0.2-1.3)
Lung	0.9	(0.5-1.7)	1.1	(0.5-2.6)
Melanoma	1.2	(0.7-2.1)	1.1	(0.6-1.9)
Prostate	1.3	(0.6-2.6)	—	—
Breast	—	—	1.0	(0.8-1.3)
Cervix	—	—	1.1	(0.9-1.5)

[a] Adjusted for age, race, education, alcohol use, and tobacco cigarette smoking.
[b] RR = relative risk.
[c] CI = 95% confidence interval.

significant increased risk of cervical cancer (RR = 1.6, CI = 1.0-2.5).

We examined the association of duration (years) and frequency of marijuana use for cancer-site groupings and for individual sites with 10 or more gender-specific incident cancers among ever-users of marijuana. Duration of use was not associated with the risk of any cancer site or grouping of cancers. For prostate cancer, a tendency toward an association of frequency of marijuana use with increased risk was evident, with usage one or more times per week (56.3 percent of male ever-users) associated with a nonsignificant twofold increase in the risk of prostate cancer relative to nonusers/experimenters. However, the addition of frequency of use did not improve the fit of any of the models for prostate cancer or for cancer of any other site or grouping of cancers.

Discussion

The main findings were the lack of association of marijuana use with overall cancer incidence and with the incidence of all tobacco-related cancers combined. Marijuana use was associated with an increased risk of prostate cancer in men who were nonsmokers of tobacco cigarettes, and a nearly significant risk of cervical cancer in women who were current marijuana smokers and nonsmokers of tobacco cigarettes.

The association between marijuana use and prostate cancer found in this study raises the question of potential hormonal mediation. There is a significant body of evidence suggesting that androgens are important in prostate carcinogenesis, including the following: (i) androgens are required for the normal growth and maintenance of the prostate;[30] (ii) testosterone has been shown to induce prostate cancer in laboratory rodents;[31] and (iii) prostate cancer rarely occurs in eunuchs, and antiandrogen therapy or ablation results in regression of prostate cancer.[32] Studies examining the relationship of serum testosterone to prostate cancer in humans have not shown consistent results.[33,34] An early study showing that plasma testosterone levels in men were decreased in chronic marijuana users[35] has not been supported by most of the numerous subsequent studies regarding this issue.[36] Sexual activity measures have been inconsistently associated with prostate cancer,[37] although they are associated with psychoactive drug use.[38] It is notable that the prostate cancer cases represented in this study are relatively young compared with those occurring in the general population, since the maximum age reached by this cohort during follow-up was 63 years.

Cigarette smoking has been shown to be a risk factor for cervical cancer in many studies.[15] The association of marijuana use with cervical cancer in nonsmokers might reflect confounding by sexual activity, since number of sexual partners is a risk factor for cervical cancer[39] and is associated with psychoactive drug use.[38]

The major limitations of this study include reliance on self-report for ascertainment of marijuana-use status; inability to study change in marijuana-use status during follow-up; and lack of lengthy follow-up into the geriatric age range, as noted earlier. We do not believe that underreporting of marijuana use is likely to have been a significant problem because data collection on marijuana use was completed before the acceleration of the 'War on Drugs' in the late 1980s, during a period of time when the environment surrounding marijuana use was more accepting. Additionally, marijuana use rates in the study cohort were similar to those reported during the same time period by the National Household Survey on Drug Abuse, the most authoritative source of statistics on illegal drug use in the US.[39] Lack of longitudinal data regarding an exposure is a common feature of many cohort studies. We believe that it is unlikely that ever-use of marijuana would have increased substantially over time, because a relatively small proportion of the cohort are likely to have initiated marijuana use during follow-up which occurred in a period marked by a significant decline in self-reported marijuana use in the US.[1]

In conclusion, this study did not show any significant associations between marijuana use and cancer in overall analyses, but showed associations suggesting increased risk of prostate and cervical cancer in marijuana users who were nonsmokers of tobacco cigarettes. We do not consider any of the findings to be conclusive, and as with all epidemiologic work, we strongly suggest that marijuana use and cancer be studied in other settings.

Acknowledgements — The authors acknowledge Steve Wilson and Anne Zishka for computer programming, Leo Hurley for consultation regarding the use of the Kaiser Permanente HIV/AIDS database, and Diana Holt for technical assistance in preparing the manuscript.

References

1. Substance Abuse and Mental Health Services Administration. *Preliminary Estimates from the 1994 National Household Survey. Advance Report No. 10.* Rockville, MD (USA): SAMHSA, 1995.
2. Hoffman D, Brunnemann KD, Gori BG, Wynder EL. On the carcinogenicity of marijuana smoke. *Recent Adv Phytochem* 1975; 9: 63-81.
3. Gong H Jr, Fligiel S, Tashkin DP, Barbers RG. Tracheobronchial changes in habitual, heavy smokers of marijuana with and without tobacco. *Am Rev Respir Dis* 1987; 136: 142-9.
4. Taylor FM. Marijuana as a potential respiratory tract carcinogen: A retrospective analysis of a community hospital population. *South Med J* 1988; 81: 1213-6.

5. Tashkin DP. Is frequent marijuana smoking harmful to health [Letter]. *West J Med* 1993; 158: 635-7.
6. Caplan GA, Brigham BA. Marijuana smoking and carcinoma of the tongue. Is there an association? *Cancer* 1989; 66: 1005-6.
7. Donald PJ. Advanced malignancy in the young marijuana smoker. In: Friedman H, Specter S, Klein TW, *et al*, eds. *Drugs of Abuse, Immunity, and Immunodeficiency*. New York, NY (USA): Plenum Press, 1991: 33-46.
8. Donald PJ. Marijuana and upper aerodigestive tract malignancy in young patients. In: Nahas GG, Latour C, eds. *Cannabis Physiopathology, Epidemiology, Detection*. Boca Raton, FL (USA): CRC Press, 1993: 165-83.
9. Nahas G, Latour C. The human toxicity of marijuana. *Med J Aust* 1992; 156: 495-7.
10. Wu TC, Tashkin DP, Djahed B, Rose JE. Pulmonary hazards of smoking marijuana as compared with tobacco. *N Engl J Med* 1988; 318: 347-51.
11. Sidney S, Beck JE, Tekawa IS, Quesenberry CP, Friedman GD. Marijuana use and mortality. *Am J Public Health* 1997; 87: 585-90.
12. World Health Organization. *International Classification of Diseases*, Ninth Revision. Geneva, Switzerland: WHO, 1977.
13. International Agency for Research on Cancer. *Tobacco Smoking*. Lyon, France: IARC, 1986. *IARC Monogr Eval Carcinog Risk Chem Hum*, Vol. 38.
14. Friedman GD. Cigarette smoking, leukemia, and multiple myeloma. *Ann Epidemiol* 1993; 3: 425-8.
15. Winkelstein W Jr. Smoking and cervical cancer – current status: A review. *Am J Epidemiol* 1990; 131: 945-57.
16. Arellano M, Peterson G, Petitti DB, Smith R. The California automated mortality linkage system. *Am J Public Health* 1984; 74: 1324-30.
17. Friedman G, Sidney S, Polen M. Smoking habits among multiphasic examinees, 1979 to 1984. *West J Med* 1986; 145: 651-6.
18. SAS Institute, Inc. *SAS User's Guide*. Cary, NC (USA): SAS Institute, Inc., 1990.
19. Cox DR, Oakes D. *Analysis of Survival Data*. New York, NY (USA): Chapman and Hall, 1984.
20. Henderson BE, Ross RK, Pike MC, Casagrande JT. Endogenous hormones as a major factor in human cancer. *Cancer Res* 1982; 42: 3232-9.
21. Noble RL. The development of prostatic adenocarcinoma in Nb rats following prolonged sex hormone administration. *Cancer Res* 1977; 37: 1929-33.
22. Huggins C, Hodges CV. Studies on prostate cancer: Effect of castration, of estrogen, and of androgen injection on serum phosphatase in metastatic carcinoma of the prostate. *Cancer Res* 1941; 1: 293-7.
23. Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. *JNCI* 1996; 88: 1118-26.
24. Hsing AW. Hormones and prostate cancer: Where do we go from here? [Editorial] *JNCI* 1996; 88: 1093-5.
25. Kolodny RC, Masters WH, Kolodner RM, Toro G. Depression of plasma testosterone levels after chronic intensive marihuana use. *N Engl J Med* 1974; 290: 872-4.
26. Block RI, Farinpour R, Schlechte JA. Effects of chronic marijuana use on testosterone, luteinizing hormone, follicle stimulating hormone, prolactin and cortisol in men and women. *Drug Alcohol Depend* 1991; 28: 121-8.
27. Nomura AMY, Kolonel LN. Prostate cancer: a current perspective. In: Armenian HK, Gordis L, Levine MM, Thacker SB, eds. *Epidemiol Rev* 1991; 13: 200-27.
28. Newcomb MD, Bentler PM. *Consequences of Adolescent Drug Use*. Newbury Park, CA (USA): Sage Publications, 1988: 21.
29. Brinton LA. Epidemiology of cervical cancer – an overview. In: Muñoz N, Bosch FX, Shah KV, Meheus A, eds. *The Epidemiology of Human Papillomavirus and Cervical Cancer*. Lyon, France: International Agency for Research on Cancer, 1992: 119; 4-6.
30. National Institute on Drug Abuse. *National Household Survey on Drug Abuse, Population Estimates 1985*. Rockville, MD (USA): NIDA, 1987; DHHS Pub. No. (ADM)87-1539.

Short-Term Effects of Cannabinoids on Immune Phenotype and Function in HIV-1-Infected Patients

Barry M. Bredt, Dana Higuera-Alhino, Starley B. Shade, Samuel J. Hebert, Joseph M. McCune, and Donald I. Abrams

Cannabinoids, including smoked marijuana and Δ^9-tetrahydrocannabinol (THC) (dronabinol, Marinol), have been used to treat human immunodeficiency virus-1 (HIV)–associated anorexia and weight loss. Concerns have been raised, however, that these compounds might have adverse effects on the immune system of subjects with HIV infection. To determine whether such effects occur, the authors designed a randomized, prospective, controlled trial comparing the use of marijuana cigarettes (3.95% THC), dronabinol (2.5 mg), and oral placebo in HIV-infected adults taking protease inhibitor-containing highly active antiretroviral therapy (HAART). Assays of immune phenotype (including flow cytometric quantitation of T cell subpopulations, B cells, and natural killer [NK] cells) and immune function (including assays for induced cytokine production, NK cell function, and lymphoproliferation) were performed at baseline and weekly thereafter. On the basis of these measurements and during this short 21-day study period, few statistically significant effects were noted on immune system phenotypes or functions in this patient population.

Journal of Clinical Pharmacology, 2002;42:82S-89S

In the era prior to the introduction of highly active antiretroviral therapy (HAART), and largely in the absence of any supporting data, smoked marijuana became increasingly used for the treatment of human immunodeficiency virus-1 (HIV)–associated anorexia and weight loss.[1] Legislation was passed in California in 1996 that enabled physicians to recommend marijuana for a number of medical conditions, including the AIDS wasting syndrome. Access to smoked marijuana was facilitated in the San Francisco Bay Area by the creation of numerous cannabis "buyers clubs."[2] At one time, it was estimated that such establishments were providing marijuana to more than 10,000 clients with HIV infection.

Despite anecdotal reports of weight gain and improvement in mood and quality of life in their patients who smoked marijuana, medical providers caring for patients with HIV infection have raised concerns about the safety of marijuana smoking by patients with immune deficiency. Studies of the effect of marijuana on immunity have been contradictory and, when viewed in aggregate, difficult to interpret. The major psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (THC), has been reported to suppress immune functions such as cell proliferation, antibody production, natural killer (NK) cell activity, and macrophage function; to dysregulate production of proinflammatory cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α); and to confer altered susceptibility in vivo to infection with intracellular organisms such as *Legionella pneumophilia* and to herpes simplex virus type-1 infected cells.[3-10]

Two cannabinoid receptors, CB1 and CB2, have been identified.[11] The CB1 receptor, which is preferentially expressed in the brain, has been identified as the

From the Department of Medicine, General Clinical Research Center at San Francisco General Hospital (B. M. Bredt); Gladstone Institute of Virology and Immunology, San Francisco (D. Higuera-Alhino, S. J. Hebert, J. M. McCune); Department of Medicine, Positive Health Program at San Francisco General Hospital (S. B. Shade, D. I. Abrams); and Departments of Medicine and Microbiology and Immunology, University of California, San Francisco (J. M. McCune). Supported by R01 DA/MH 11607, NIAID 2P30 AI27763, and NCRR MO1 RR00083. J. M. McCune is an Elizabeth Glaser Pediatric AIDS Foundation Scientist and a recipient of the Burroughs Wellcome Fund Clinical Scientist Award in Translational Research. Address for reprints: Donald I. Abrams, MD, Professor of Clinical Medicine, San Francisco General Hospital, Ward 84, 995 Portero Avenue, San Francisco, CA 94110.
DOI: 10.1177/0091270002238798

...ely cause of cannabis-mediated central nervous system effects. In contrast, the CB2 receptor is preferentially expressed in peripheral tissues such as the marginal zone of the spleen and on the surface of B lymphocytes and NK cells.[17] Accordingly, the potential exists for interactions between THC and the immune system.

To date, there have been no controlled investigations of the impact of marijuana on immune function in patients with HIV infection. Either as a stimulant or suppressant of immune function, marijuana could potentially lead to increased viral burden. This potential effect also has never been investigated in a prospective, controlled fashion. Finally, the potential for a drug-drug interaction between protease inhibitors and marijuana is particularly worrisome because both are metabolized by the cytochrome P450 enzyme system, and many HIV-infected patients continue to smoke marijuana as an appetite stimulant or to decrease nausea associated with their antiretroviral therapy.[12-16]

To more closely evaluate the possibility of these adverse effects, we designed a study to determine the safety/toxicity profile of cannabinoids in people with HIV infection on protease inhibitor-containing regimens. The specific goals of this study were to determine the short-term effects of cannabinoids (smoked and oral) on HIV RNA levels, the immune system, and the pharmacokinetics of two widely used protease inhibitors, indinavir and nelfinavir. Viral load was selected as the primary endpoint because it might be affected by an interaction between cannabinoids and the metabolism of the protease inhibitor and/or between cannabinoids and the immune system. Reported here are the immune endpoints of this study of the short-term effects of cannabinoids in patients with HIV infection. Published data are reported separately on the short-term effects of cannabinoids on viral load and on the pharmacokinetics of the protease inhibitors.[17,18]

METHODS

Study Population

Subjects were required to be at least 18 years old, have documented HIV infection, and be on a stable antiretroviral treatment regimen that included either indinavir (Crixivan, Merck) or nelfinavir (Viracept, Agouron) for at least 6 weeks prior to enrollment. Upon admission to the San Francisco General Hospital General Clinical Research Center (GCRC) for the 25-day inpatient trial, subjects who had been taking the more recently recommended dose of nelfinavir (1250 mg twice daily) were switched to a dose of 750 mg three times daily for consistency of our pharmacokinetic evaluations.[19] No additional protease inhibitors were allowed during the duration of the study. Subjects were also required to have a stable viral load, defined as less than a threefold (< 0.5 $\log_{10}$) change in HIV RNA level for the 16 weeks prior to enrollment. All subjects were required to have prior experience smoking marijuana (defined as six or more times) to ensure that they knew how to inhale and what neuropsychiatric effects to expect. The study was approved by the Committee on Human Research of the University of California, San Francisco, and signed informed consent was obtained from each participant before enrollment.

Exclusion criteria included the following: any active opportunistic infection or malignancy requiring acute treatment, unintentional loss of ≥ 10% of body weight during the prior 6 months, current substance dependence, methadone maintenance, use of tobacco or cannabinoids (smoked or oral) within 30 days of enrollment, history of serious pulmonary disease, pregnancy, and Stage II or higher AIDS dementia complex. Laboratory exclusion criteria were as follows: hematocrit < 25% and hepatic transaminase elevations greater than five times the upper limit of normal. Therapeutic exclusions were concurrent use of megestrol acetate, nandrolone, oxandrolone, oxymetholone, human growth hormone, thalidomide, pentoxifylline, prednisone, interleukin-2, chemotherapy, radiotherapy, or other investigational agents known to alter immune system function within the prior 8 weeks.

Study Medications

The National Institute on Drug Abuse (NIDA) provided prerolled marijuana cigarettes, weighing on average 0.9 gm and containing 3.95% THC. These cigarettes were kept in a locked and alarmed freezer until they were dispensed to a locked freezer in the GCRC where the inpatient study was conducted. The marijuana cigarettes required rehydration overnight in a humidifier. Subjects randomized to the smoked marijuana arm were housed in a room with a fan ventilating to the outside. To maximize standardization of inhaled doses, research staff monitored subjects while they followed the Foltin uniform puff procedure.[20] Research staff weighed the marijuana cigarettes immediately before and after they were administered to subjects and returned all leftover material to the pharmacy for ultimate return to NIDA. Subjects smoked up to three com-

plete marijuana cigarettes daily, as tolerated, 1 hour prior to meals. Roxane Laboratories (Columbus, OH) supplied dronabinol and matching placebo capsules.

Research Design and Procedures

Subjects were randomized in a double-blind manner to the oral regimens and received either dronabinol 2.5 mg or placebo on the same schedule as the subjects randomized to smoked marijuana. The randomized, placebo-controlled trial was composed of two inpatient phases. The first phase was a 4-day lead-in period, during which time subjects were admitted to the GCRC for measurement of baseline parameters. A urine sample obtained on the day of admission (day −4) was required to be negative for THC. The second phase was a 21-day intervention period beginning with random assignment of treatments on day 0. The subjects were stratified by protease inhibitor (indinavir or nelfinavir) and then allocated with equal probability in blocks of 12 to the study agents (marijuana, dronabinol, and placebo). Subjects were not permitted to have visitors or to leave the confines of the GCRC unless accompanied by research personnel during the 25-day study. All clinical laboratory tests and study procedures were obtained or performed in the GCRC.

Absolute Lymphocyte Counts

Automated complete blood counts with differential were performed in the San Francisco General Hospital Clinical Laboratory, using an automated hematology analyzer (Bayer Technicon H3 System, Bayer Corp., Tarrytown, NY) according to the manufacturer's directions.

Immunophenotyping

Baseline samples were collected on day 0, and follow-up specimens were drawn on days 7, 14, and 21. Four-color flow cytometric immunophenotyping was performed according to the manufacturer's directions with the following panels of antibodies: CD3-Cy5/CD4-PE/CD8-ECD/CD45-FITC, CD3-ECD/CD19-FITC/CD56-PE/CD45-Cy5, CD4-ECD/CD8-Cy5/CD38-PE/HLA-DR-FITC, CD4-ECD/CD8-Cy5/CD25-FITC/CD69-PE, CD4-ECD/CD8-Cy5/CD45RA-FITC/CD62L-PE (all from Beckman Coulter, Inc., Fullerton, CA). Data acquisition and analysis were performed using a Beckman Coulter EPICS XL flow cytometer, running System II, version 3.0.

Cytokine Flow Cytometry

A cytokine flow cytometry assay was used to measure the percentage of CD4+ T cells that are activated (express CD69) and that also synthesize specific cytokines (TNF-α, IFN-γ, or IL-2) in response to stimulation with the CMV antigen.[21] As a positive control, stimulation was carried out with the superantigen *Staphylococcal* enterotoxin B (SEB), and unstimulated cultures served as negative controls. Briefly, heparinized blood was incubated with antibody to CD28 (L293, BD Biosystems, San Jose, CA) alone (negative control), with SEB (Sigma, St. Louis, MO), or with sucrose density gradient-purified virus preparations from human CMV strain AD169-infected human foreskin fibroblast cultures (Advanced Biotechnologies, Inc., Columbia, MD) for 5 hours. Brefeldin A (Sigma) was added during the last 3 hours, followed by addition of FACS™ lysing solution (BD Biosystems), centrifugation, and resuspension of cells in FACS™ permeabilizing solution (BD Biosystems). Cells were then stained with monoclonal antibodies specific for CD4, CD69, and either TNF-α, IFN-γ, or IL-2 and analyzed by flow cytometry. The frequency of CD4+ T cells staining positive for CD69 and for the intracellular cytokine of interest after CMV stimulation was adjusted by subtracting the frequency in unstimulated samples. In preliminary experiments, control stimulants included a mock-infected cell lysate–negative control preparation (BioWhittaker), tissue culture medium including 10% human AB serum, and no stimulation. No significant difference was noted among these negative controls.

Natural Killer Cell Function

The cytolytic activity of NK cells was assessed using K562 erythroleukemic target cells.[22] K562 cell suspensions were labeled with ^{51}Cr for 2 hours at 37°C and supplemented with RPMI 1640 and 10% human AB serum. After centrifugation, cells were stained with trypan blue and counted. Peripheral blood mononuclear cells (PBMC) were isolated by density-gradient centrifugation, counted, adjusted to 1×10^7 cells/ml, and plated in K562 cells at effector:target (E:T) ratios of 6.3:1, 12.5:1, 25:1, 50:1, and 100:1. Culture plates were centrifuged and incubated at 37°C with 5% CO_2 for 4 hours before being harvested and counted. Both net NK cell

[redacted]city and per NK cell cytotoxicity were mea- [redacted]d expressed as percent lysis of target cells at 1 E:T ratio.

mphoproliferation

phoproliferation was measured using a standard tiated thymidine uptake assay.[23] Briefly, PBMC were ubated in quadruplicate with phytohemagglutinin A, Sigma), tetanus toxin (Connaught Laboratories, fwater, PA), CMV antigen (BioWhittaker), or a pool inactivated alloreactive human PBMC for 3 to 6 days then pulsed with 1 μCi of tritiated thymidine. nts per minute (cpm) for each antigen were averged and the stimulation index (SI) calculated. At st one HIV-uninfected control was run weekly ughout the course of the study. In all cases in ich donor cell responses were found to be negative, sitive responses were detected either for that donor h another antigen or for other donors assayed on same day.

tatistical Analysis

e effects of cannabinoids on absolute lymphocyte unts, immunophenotyping analyses, and immune ponses as measured by cytokine flow cytometry, NK [redacted]ny, and lymphoproliferation assay were ana- [redacted]y comparison of the baseline (day 0) parameters ith those derived after cannabinoid treatment (day . Median values of these variables for each arm at eline are reported, as are median values for each m based on the change in each variable between day nd day 21. Because many of the baseline and change iables were not normally distributed, nonparametric tistical tests were performed. Kruskal-Wallis tests ere used to identify statistically significant differences between the placebo arm and each of the nabinoid arms at baseline. Kruskal-Wallis tests ere also used to identify statistically significant differences between the placebo arm and each of the nabinoid arms based on the change in each variable ween day 0 and day 21.

SULTS

ubject Characteristics

ty-two patients completed the study. Twenty paits were randomized to smoke marijuana, 22 to take onabinol, and 20 to take placebo. Of the patients, 55 re male, 3 were female, and 4 were male-to-female transgendered. Half ($n = 31$) of the patients were white, 12 were African American, 10 were Latino, and 9 were of mixed or other ethnicity. More than half of the patients ($n = 33$) were between the ages of 40 and 49, 18 were younger than 40, and 11 were age 50 or older.

Absolute Lymphocytes and Immunophenotyping

Figure 1 shows absolute lymphocyte counts and immunophenotyping results for percent CD4+ T cells, percent CD8+ T cells, percent naive CD4+ T cells, percent naive CD8+ T cells, percent memory/effector CD4+ T cells, percent memory/effector CD8+ T cells, percent CD3-CD19+ B cells, and percent CD3-CD56+ NK cells for all three arms over the 21 days of the study. There were no statistically significant differences in baseline values across the three arms for any of these variables. When we looked at change in these variables between day 0 and day 21, we found only one statistically significant difference when we compared patients in the cannabinoid arms with those in the placebo arm. Changes in absolute lymphocyte counts among those in the marijuana arm were significantly greater compared with changes in the placebo arm (median change = 300 vs. 0.00 cells/μl; $p = 0.01$).

Baseline values were significantly higher in the dronabinol arm compared with the placebo arm for four other immunophenotyping variables: %CD4+HLA-DR+ cells (median = 11.8 vs. 4.5; $p = 0.03$), %CD4+CD38+HLA-DR+ cells (median = 9.0 vs. 4.5; $p = 0.04$), %CD8+HLA-DR+ cells (median = 20.0 vs. 9.6; $p = 0.01$), and %CD8+CD38+HLA-DR+ cells (median = 13.2 vs. 5.2; $p = 0.01$). Although baseline values were also higher for each of these variables in the marijuana arm compared with the placebo arm, this difference was statistically significant only for %CD8+HLA-DR+ cells (median = 13.0 vs. 9.3; $p = 0.03$).

When we looked at change between day 0 and day 21, we observed significant negative changes in the dronabinol arm compared to the placebo arm for two variables: %CD8+CD38+HLA-DR+ cells (median change = −3.50 vs. 0.05; $p = 0.001$) and %CD8+CD69+ cells (median change = −0.30 vs. 0.05; $p = 0.04$). An additional negative change, which approached statistical significance, was seen in %CD4+CD38+HLA-DR+ cells (median change = −1.20 vs. −0.25; $p = 0.06$). However, two of these three variables, %CD8+CD38+HLA-DR+ and %CD4+CD38+HLA-DR+, were significantly higher in the dronabinol arm compared with the placebo arm at day 0. Therefore, the potential confounding

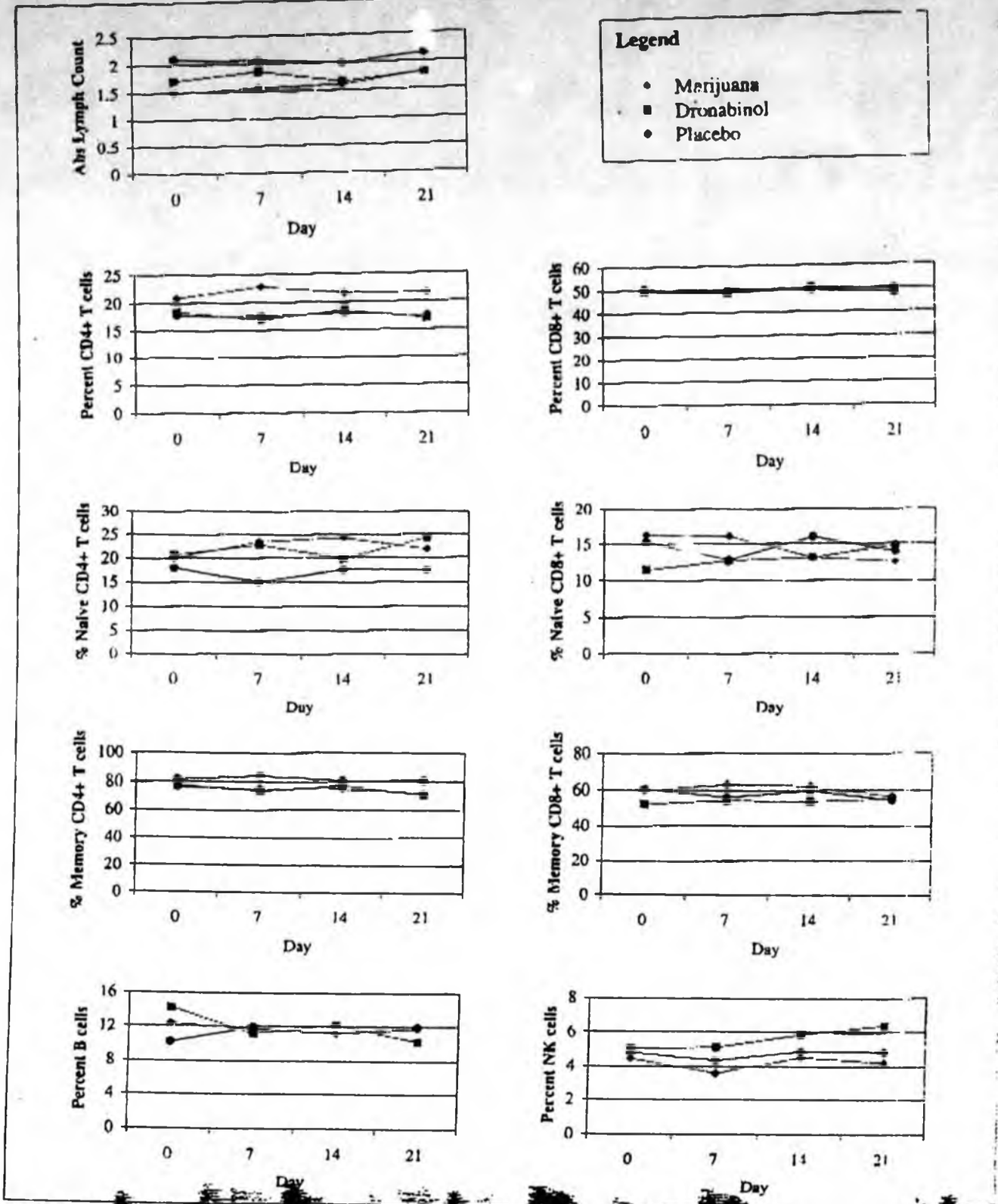


Figure 1. Median absolute and percent lymphocytes for selected variables by arm.

assay) techniques demonstrated decreased interleukin-6 but increased TNF-α levels in a mouse macrophage system.[29]

Many of the reported studies of the immune effects of cannabinoids have been conducted in cell culture systems or animal models. Human studies have evaluated immune function in chronic marijuana smokers. To date, there have been no prospective clinical trials investigating the immune effects of smoked marijuana in patients with HIV infection. Retrospective analyses from the Multicenter AIDS Cohort Study evaluating outcomes in 1662 seropositive users of psychoactive drugs found that none of the drugs used by participants was associated with enhanced clinical or immunologic expression of HIV infection.[30] Of note, use of marijuana in the preceding 2 years was reported by 89% of the seropositive men in the cohort. This was consistent with findings from a previous observation from the San Francisco General Hospital experience.[31] A study of intravenous drug users with HIV infection determined that smoking of drugs such as marijuana was associated with an increased risk of bacterial pneumonia, although there were other confounding associations.[32]

In sum, this study revealed no evidence of detrimental effects of cannabinoids on any of the immune parameters measured. Our conclusions are limited by the short (21-day) duration of this study. In addition, the lack of a blinded control group for the smoked marijuana arm could lead to bias in interpreting some of the results of the main study (e.g., weight changes). However, it is difficult to attribute HIV-1 RNA and lymphocyte subset effects to any such potential bias. We chose not to include a smoked placebo group because we thought it would be impossible to blind marijuana in subjects with prior experience. The disparate results on the effects of THC on the immune system from prior studies may be related to differences in study populations, drug composition, drug concentration, or assay conditions. A key question now will be whether marijuana exerts significant immune effects when administered over longer periods of time.

We are grateful to the research nursing and dietary staff at the SFGH GCRC for the professionalism and compassion with which they conducted the trial. We appreciate the efforts of the SFGH inpatient research pharmacy staff. We are deeply indebted to our committed study participants. Thanks to Roxane Laboratories for the dronabinol and placebo capsules.

REFERENCES

1. Abrams DI: Medical marijuana: tribulations and trials. *J Psychoact Drugs* 1998;30:166-172.

2. Werner CA: Medical marijuana and the AIDS crisis. *J Cannabis Ther* 2001;1(3-4):17-33.

3. Cabral G, Vasquez R: Effects of marijuana on macrophage function. In: Friedman H, Specter S, Klein T (eds.), *Drugs of Abuse, Immunity and Immunodeficiency*. New York: Plenum, 1991.

4. Fischer-Stenger K, Dove Pettit DR, Cabral GA: Delta-9-tetrahydrocannabinol inhibition of tumor necrosis factor-alpha: suppression of post-translational events. *J Pharm Exp Ther* 1993;267:1558-1565.

5. Klein TW, Newton C, Friedman H: Resistance to *Legionella pneumophilia* suppressed by the marijuana component, tetrahydrocannabinol. *J Inf Dis* 1994;169:1177-1179.

6. Klein TW, Newton C, Zhu W, Daaka Y, Friedman H: Delta-9 tetrahydrocannabinol, cytokines and immunity to *Legionella pneumophilin* (43697A). *PSEBM* 1995;209:205-212.

7. Friedman H, Klein TW, Newton C, Daaka Y: Marijuana, receptors and immunomodulation. *Adv Exp Med Biol* 1995;373:103-113.

8. Cabral GA, Pettit D: Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. *Journal of Neuroimmunology* 1998;83:116-123.

9. Friedman H, Klein TW: Marijuana and immunity. *Science and Medicine* 1999;6:12-21.

10. Cabral GA: Immune system, in: Grotenhermen F, Russo E (eds.), *Cannabis and Cannabinoids: Pharmacology, Toxicology and Therapeutic Potential*. New York: Haworth, 2002.

11. Pertwee RG: Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther* 1997;74:129-180.

12. Munro S, Thomas KL, Abu-Shaar M: Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993;365:61-65.

13. Yamamoto I, Watanabe K, Narimatsu S, Yoshimura H: Recent advances in the metabolism of cannabinoids. *Int J Biochem Cell Biol* 1995;27(8):741-746.

14. Acosta EP, Kakuda TN, Brundage RC, Anderson PL, Fletcher CV: Pharmacodynamics of human immunodeficiency virus type-1 protease inhibitors. *Clin Inf Dis* 2000;30(suppl. 2):S151-S159.

15. Child CC, Mitchell TF, Abrams DI: Patterns of therapeutic marijuana use in two community-based cannabis buyers' cooperatives [abstract 60569]. Paper presented at the 12th World AIDS Conference, Geneva, Switzerland, June-July 1998.

16. Braitstein P, Kendall TR, Chan K, Montaner JSG, O'Shaughnessy MV, Hogg RS: Mary-Jane and her patients: sociodemographic and clinical characteristics of HIV+ individuals using medicinal marijuana and antiretrovirals in British Columbia, Canada [abstract ThPeB5055]. *XIIIth International AIDS Conference* 2000;2:326.

17. Abrams DI, Leiser R, Shade S, Aweeka F, Bredt B, Elbeik T, Hilton J, Schambelan M: Short-term safety of cannabinoids in HIV patients [abstract 744]. Paper presented at the 8th Conference on Retroviruses and Opportunistic Infections, Chicago, February 2001.

18. Kosel BW, Aweeka FT, Benowitz NL, Shade SB, Hilton JF, Lizak PS, Abrams DI: The effects of cannabinoids on the pharmacokinetics of indinavir and nelfinavir. *AIDS* 2002;16:543-550.

19. Johnson M, Peterson A, Winslade J, Glendennin N: Comparison of BID and TID dosing of VIRACEPT® (nelfinavir, NFV) in combination with stavudine (d4T) and lamivudine (3TC) [abstract 373]. Paper presented at the 5th Conference on Retroviruses and Opportunistic Infections, Chicago, February 1998.

...Soltin RW, Fischman MW, Byrne MF: Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite* 1988;11:1-14.

...Komanduri KV, Viswanathan MN, Wieder ED, Schmidt DK, Brudt ...Jacobson MA, McCune JM: Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nat Med* 1998;4:953-956.

...Brunner KT, Mauel J, Cerottini JC, Chapius B: Quantitative assay of the lytic interaction of immune lymphoid cells on ^{51}Cr-labeled allogeneic target cells: inhibition by isoantibody and drugs. *Immunology* 1960;4:181.

...Fletcher MA, Morgan R, Klimas NG, Gjerset G: Lymphocyte preparation, In: Rose NR, Conway de Macario E, Fahey JL, Friedman H, Penn GM (eds.). *Manual of Clinical Laboratory Immunology*. 3rd ed. Washington, DC: American Society for Microbiology, 1992,213-219.

...Newton CA, Klein TW, Friedman H: Secondary immunity to *Legionella pneumophilia* are suppressed by Delta-9-tetrahydrocannabinol injection. *Infection and Immunity* 1994;62: 4015-4020.

...Hollister LE: Marijuana and immunity. *J Psychoactive Drugs* 1992;24:159-164.

...Nahas GG, Sutin-Foca N, Armand JP, Morishima A: Inhibition of cellular mediated immunity in marijuana smokers. *Science* 1974: 419-420.

27. White SC, Brin SC, Janicki BW: Mitogen-induced blastogenic responses of lymphocytes from marihuana smokers. *Science* 1975;188: 71-72.

28. Lau RJ, Turbergen DC, Barr M, Domino EF, Benowitz N, Jones RT: Phytohemagglutinin-induced lymphocyte transformation in humans receiving Delta-9-tetrahydrocannabinol. *Science* 1976;192: 805-807.

29. Shivers SC, Newton C, Friedman H, Klein TW: Delta-9-tetrahydrocannabinol (THC) modulates IL-1 bioactivity in human monocyte/macrophage cell lines. *Life Sci* 1994;54: 1281-1289.

30. Kaslow RA, Blackwelder WC, Ostrow DG, et al: No evidence for a role of alcohol or other psychoactive drugs in accelerating immunodeficiency in HIV-1-positive individuals: a report from the Multicenter AIDS Cohort Study. *JAMA* 1989;261:3424-3420.

31. Roland A, Felgal DW, Abrams DI, et al: Recreational drug use does not cause AIDS progression: the UCSF AIDS Registry cohort. Paper presented at the Third International Conference on AIDS, Washington, DC, April 1987.

32. Caiaffe WT, Vlahov D, Graham NM, et al: Drug smoking, *Pneumocystis carinii* pneumonia and immunosuppression increase risk of bacterial pneumonia in human immunodeficiency virus-seropositive injection drug users. *Am J Res Crit Care Med* 1994;150: 1493-1498.

Journal of the International Neuropsychological Society (2003), 9, 679–689.

DOI: 10.1017/S1355617703950016

Non-acute (residual) neurocognitive effects of cannabis use: A meta-analytic study

IGOR GRANT,[1,2,3,5] RAUL GONZALEZ,[4,5] CATHERINE L. CAREY,[4,5] LOKI NATARAJAN,[5,6] AND TANYA WOLFSON[5]

[1]Department of Psychiatry, University of California, San Diego
[2]Center for Medicinal Cannabis Research, University of California, San Diego
[3]Veterans Affairs Healthcare System, San Diego, CA
[4]SDSU/UCSD Joint Doctoral Program in Clinical Psychology
[5]HIV Neurobehavioral Research Center, University of California, San Diego
[6]Department of Family and Preventive Medicine, University of California, San Diego

(Received September 12, 2002; Revised December 4, 2002; Accepted December 23, 2002)

Abstract

The possible medicinal use of cannabinoids for chronic diseases emphasizes the need to understand the long-term effects of these compounds on the central nervous system. We provide a quantitative synthesis of empirical research pertaining to the non-acute (residual) effects of cannabis on the neurocognitive performance of adult human subjects. Out of 1,014 studies retrieved using a thorough search strategy, only 11 studies met essential *a priori* inclusion criteria, providing data for a total of 623 cannabis users and 409 non- or minimal users. Neuropsychological results were grouped into 8 ability domains, and effect sizes were calculated by domain for each study individually, and combined for the full set of studies. Using slightly liberalized criteria, an additional four studies were included in a second analysis, bringing the total number of subjects to 1,188 (i.e., 704 cannabis users and 484 non-users). With the exception of both the learning and forgetting domains, effect size confidence intervals for the remaining 6 domains included zero, suggesting a lack of effect. Few studies on the non-acute neurocognitive effects of cannabis meet current research standards; nevertheless, our results indicate that there might be decrements in the ability to learn and remember new information in chronic users, whereas other cognitive abilities are unaffected. However, from a neurocognitive standpoint, the small magnitude of these effect sizes suggests that if cannabis compounds are found to have therapeutic value, they may have an acceptable margin of safety under the more limited conditions of exposure that would likely obtain in a medical setting. (*JINS*, 2003, *9*, 679–689.)

Keywords: Cannabis, Cannabinoids, Marijuana, THC, Cognitive effects, Neuropsychology, Meta-analysis

INTRODUCTION

Recent developments in the scientific and public sectors have reawakened the possibility that cannabis compounds or their synthetic analogues may be proposed as treatments for several medical conditions. At the research level, the discovery of the first cannabinoid receptor (CB1) in 1986, and a second receptor (CB2) in 1992, paved the way to the identification of endocannabinoid-signaling molecules including anandamide and glyceryl-anandamide (Devane et al., 1992; Herkenham, 1992; Herkenham et al., 19[illegible] Howlett et al., 1990; Matsuda et al., 1990; Munro et [illegible], 1993; Pertwee, 1993, 1997).

The CB receptor system is widely distributed in the body, with CB1 primarily localized in the central nervous system. The highest concentrations are found in deep brain structures and the cerebellum (Childers & Breivogel, 1998; Herkenham et al., 1991a, 1991b). Receptors are also found in other organ systems including the uterus, pancreas, and testes (Pertwee, 1997). The CB2 receptor appears to be primarily localized in the spleen and immune cells (Kaminski et al., 1992). Although the biological functions of the endocannabinoid system remain unclear at this time, it is

Dr. Erin D. Bigler served as action editor during the course of this review.

Reprint requests to: Igor Grant, M.D., University of California, Center for Medicinal Cannabis Research, 150 West Washington Street, Second Floor, San Diego, CA 92103. E-mail: igrant@ucsd.edu

likely that the CB1 receptor system is important in a number of neurobehavioral events, including dampening of neuro-excitability (Samudo-Pena & Walker, 1997), and perhaps in the initiation and maintenance of complex feeding behaviors, including suckling (Fride et al., 2001; Hao et al., 2000).

From the public health standpoint, there have been increasing anecdotal and limited scientific observations to suggest that cannabinoids may have utility in the management of severe pain, especially neuropathic pain (Noyes et al., 1975), muscular spasticity, tremor in conditions such as multiple sclerosis (Baker et al., 2000, 2001), and improved appetite and weight gain in patients with chronic inanition (Gorter et al., 1992; Nelson et al., 1994; Plasse et al., 1991). The possibility that cannabis might have a benefit in conditions such as AIDS or diabetic neuropathy, muscle spasm in multiple sclerosis, and severe weight loss, nausea, and vomiting related to cancer and its treatments, has been raised and reviewed in some detail by recent expert panels, including the NIH Expert Panel Report (U.S. National Institute of Health, 1997) and by the Institute of Medicine (1999).

These developments have converged with an increasing public sense that cannabis might be beneficial to some patients with severe chronic illnesses, and therefore should be made available to them. Evidence of this now comes from the passing of initiative laws seeking to facilitate access to cannabis by medical patients in nine states in the United States. Therefore, it seems reasonable to expect that the convergence of scientific evidence and public pressure may result in increasing use of cannabis products by patients with certain severe chronic illnesses in the future. If this were to happen, it would naturally raise the concern that cannabis may have certain long-term undesirable effects, particularly with respect to the central nervous system. Although the acute neurobehavioral effects of cannabis intoxication have been characterized and reviewed in some detail (e.g., Solowij, 1998), the very long-term effects of cannabis on brain function are not well understood. In order to assess the state of current knowledge on persisting CNS consequences of cannabis use, we have performed a meta-analysis of the existing literature on neuropsychological evaluation of persons who have been exposed to regular, long-term use of cannabis. Previous reviews have provided excellent summaries of investigations on this topic and their findings (Grant & Mohns, 1975; Pope et al., 1995; Solowij, 1998). In order to avoid duplication of previous efforts and provide new information, we have approached this task with a view of arriving at a quantitative estimate of the potential effects of long-term cannabis consumption on various neurocognitive functions. In this way, we have attempted to estimate effect sizes for each of eight neurocognitive domains, as well as a global indicator of overall neurocognitive functioning as it relates to history of cannabis consumption. Thus, the overall objective of this study was to provide a quantitative synthesis of the research investigating the non-acute (residual) effects of cannabis use on the neurocognitive performance of long-term users. Readers interested in more detailed descriptions of studies on this topic are encouraged to examine the aforementioned reviews. In addition, we have recently published a companion paper (Gonzalez et al., 2002) that qualitatively examines the research methodologies of studies included in this meta-analysis.

METHODS

Literature Search and Study Identification Strategy

Two of the investigators (RG and CC) conducted independent literature searches through several online databases, including Medline/HealthSTAR, PsychInfo, BioSys, Current Contents, Dissertation Abstracts International, Article First, Eric, Science Citation Index Expanded, and Social Science Citation Index. The key words used were (marijuana or marihuana or tetra-hydrocannabinol or THC or cannabis) with the Boolean operator "and" connecting (neuro* or cognitive or assessment or ability or effects or processes or impairment or cognition or drug effects). Boolean operators were slightly modified and tailored to each database, in order to comply with specific database guidelines and to ensure a valid search. The search criteria were liberal, purposely so, in order to avoid missing any potentially relevant citations. The results of the two independent searches are presented in Figure 1. The first investigator identified 824 citations (Data Set A) and the second investigator identified 1,006 citations (Data Set B). After a consensus meeting, both databases were combined, and the investigators agreed that there were 1,014 unique citations.

The two investigators then independently rated each citation by title and abstract (if available) and classified these references into one of four relevance categories: CORE, REVIEW, UNKNOWN, and NOT RELEVANT. An article

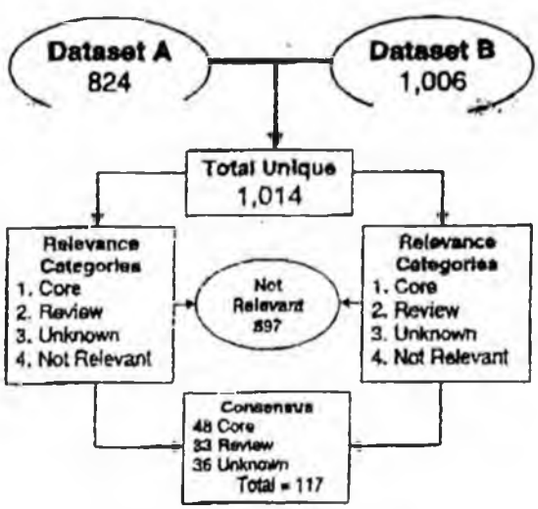


Fig. 1. Literature search process.

was categorized as CORE if, based on title and abstract, the study was highly likely to contain information relevant to the question of persisting, non-acute effects of cannabis exposure on neurocognitive functioning. An article was identified as REVIEW if the title or abstract indicated it was a literature review or an editorial. An UNKNOWN classification was given to an article if there was insufficient information based on an examination of the title or abstract, to confidently place it in one of the other categories. An article was categorized as NOT RELEVANT if it was clear from the title or abstract that it did not address the question of long-term, non-acute effects of cannabis on neurocognitive functioning.

Once these independent classifications were achieved, the two investigators had a second set of meetings to arrive at a consensus classification, resolving the status of the small number of articles upon which their original classification did not agree (seven articles). Figure 1 shows the results of this process. From 1,014 articles, 897 articles were classified as not relevant. Table 1 provides a summary of the major topics represented by articles deemed NOT RELEVANT. The majority of articles were excluded because they either did not deal with human subjects, or they involved studies of children and adolescents who were prenatally exposed or whose exposure to cannabis was not sufficient in duration to explore long-term persisting effects. Another large group of studies concerned psychiatric and other behavioral issues but not neurocognitive functioning *per se*.

After excluding the 33 review articles, the remaining 84 articles originally classified as CORE and UNKNOWN were examined in detail. From these, 38 were ultimately selected for coding as they met the inclusion criteria for this analysis as outlined below. The 38 included 35 of the 48 articles originally classified as CORE. Three additional studies, originally classified as UNKNOWN, were added after being reclassified as CORE upon thorough examination of the complete manuscript.

Before the literature search began, the authors identified a set of criteria that a study should have met in order to adequately answer the principal research question: "Is regular, long-term use of cannabis associated with non-acute (residual) neurocognitive dysfunction, suggestive of brain injury?" The criteria, presented in Table 2, emphasized the necessity of including an appropriate control group (i.e., non-drug using or very lightly drug using), indicating that subjects were drug free (i.e., not acutely intoxicated) at the time of evaluation, addressing potential confounds (e.g., history of heavy use of other substances, presence of other neurological conditions or traumatic brain injury, psychiatric confounds, other neuromedical risks), providing information on cannabis abstinence, and collecting data of sufficient detail to calculate effect sizes for each test administered.

Table 1. Classification of non-relevant studies omitted after initial search (n = 897)

Neurobiology/Pharmacology/Animal	= 314
Psychiatric/Psychological/Prevention	= 231
Prenatal/Infant/Children/Adolescents	= 155
Neurological/Medical/Neuroimaging	= 89
Acute cannabis use or misc. exclusions	= 66
Polydrug/Other drugs	= 24
Completely irrelevant	= 18

Table 2. Original inclusion criteria for studies entering meta-analysis

1. Includes a group of "cannabis only" users
2. Includes an appropriate control group (i.e., non drug-using or very limited cannabis use)
3. Provides sufficient information to calculate effect size
4. Outcome measures include valid neuropsychological tests
5. Cannabis using group is drug-free on day of neuropsychological testing
6. Study addresses other potential substance use in cannabis group
7. Study addresses potential history of neurological or psychiatric problems
8. Study reports length of abstinence from cannabis before testing

We were surprised to discover that only nine studies ultimately met all original inclusion criteria. In a companion review paper (Gonzalez et al., 2002) we provide a more thorough analysis of the methodological limitations that affected many of the studies that we examined. To the nine studies that met all original inclusion criteria, two additional studies were added. One of these studies was brought to the authors' attention during a conference on cannabis organized by the National Institute on Drug Abuse on August 13–14, 2001 in Rockville, Maryland. This article by Pope et al. (2001), in press at the time our analyses were being conducted, met all of the inclusion criteria, and was therefore added to the original meta-analysis of the nine studies. The other manuscript added to our original meta-analysis was authored by Solowij et al. (2002) and was published while this manuscript was under review. Because this study met all of our original inclusion criteria, we felt our analyses would be incomplete without its inclusion. These 11 articles are briefly summarized in Table 3a. The meta-analysis of the 11 studies that met all original inclusion criteria involved data from 1,032 subjects, of whom 623 were regular, moderate, or heavy cannabis users and 409 were either non-users or persons whose exposure to cannabis was extremely limited.

In order to ensure that we were not rejecting potentially informative studies, we reexamined the 38 coded studies with a slightly relaxed set of criteria. We chose to accept a study if it violated no more than one of our *a priori* inclusion criteria (Table 2), as long as neither of two absolutely essential criteria were violated; that is, the study had to have an appropriate non-cannabis using (or extremely limited cannabis using) contrast group, and it had to have enough detail in the presentation of results to permit computation of effect sizes. For example, an often cited investigation by

Table 3a. Descriptions of original 11 studies

Study	Users (n)	Control (n)	Frequency or amount of use	Duration of use (yrs)	Length of abstinence (hr)	Cognitive domains assessed[a]
[1]Block & Ghoneim (1993)	144	72	1 to 7+ times/wk	5.8 (*M*) 4.1 (*SD*)	≥24	AE, L, PM, SRT
[2]Carlin & Trupin (1977)	10	10	NR	5 (*M*) range: 2.5–8	≥24	A, AE, L, M, PM, V
[3]Croft et al. (2001)	18	31	lifetime joints: 5309.8 (*M*) 6517.5 (*SD*)	NR	66.5 (*M*) 42.4 (*SD*)	A, AE, F, L, M, V
[4]Ehrenreich et al. (1999)	99	49	use in last 6 mo: 3.5 dys/wk (*M*) 1.9 (*SD*)	4.2 (*M*) 3.4 (*SD*)	29.8 (*M*) 29.5 (*SD*)	A, SRT
[5]Gouzoulis-Mayfrank et al. (2000)	28	28	20.9 dys/mo (*M*) 10.2 (*SD*)	2.9 (*M*) 2.0 (*SD*)	96 (*M*) 372 (*SD*)	A, AE, F, L, PM, SRT, V
[6]Hanil (1996)	19	19	NR	NR	≥336	F, L
[7]Pope & Yurgelun-Todd . (1996)	65	64	≥22 days of the past 30 days	≥2	≥19	A, AE, F, L, V
[8]Pope et al. (2001)	63	72	≥5000 lifetime episodes ≥7 times/week	≥13 years	~672	A, AE, F, L, PM, V
[9]Rodgers (2000)	15	15	4 dys/wk (*M*)	11 (*M*)	≥720	A, F, L, SRT
[10]Solowij (1995)	60	16	15.3 dys/mo (*M*) 10 (*SD*)	7.8 (*M*) 5.1 (*SD*)	≥1008	A, SRT
[11]Solowij et al. (2002)	102	33	median: 2 joints/day 27.9 dys/mo in past 14 wks	17.1 (*M*) 7.9 (*SD*)	17 (median)	A, AE, F, L, PM, V

Note. Numeric superscripts refer to the data presented in Figures 2A and 2B.
[a]A study may have "assessed" a given domain, but their data may not have been included in our analysis if it was presented in a format that was incompatible with our methods for effect size calculations.
A, Attention; AE, Abstraction/Executive; F, Forgetting/Retrieval; L, Learning; M, Motor; PM, Perceptual Motor; SRT, Simple Reaction Time; V, Verbal/Language; NR, not reported.

Fletcher and colleagues (1996), which examined the cognitive correlates of long-term cannabis use in Costa Rican men, did not meet the latter criterion and was therefore excluded. In addition, a study was also included if criterion items #5 and #8 (see Table 2) were the only two criteria not met. This decision was made based on the interdependence of these two items (i.e., studies that did not quantify length of abstinence from cannabis, also did not confirm if subjects were drug-free at time of testing).

As a result of re-inspecting the 38 studies, an additional four studies met the revised criteria necessary for inclusion. These particular studies were initially excluded due to the lack of abstinence information in all but one instance. Nevertheless, based on the totality of the study design, it appeared unlikely that the authors included acutely intoxicated individuals in their analyses. Thus, this expanded meta-analysis based on 15 studies (the 11 original plus the four added) now included 1,188 subjects (i.e., 484 controls and 704 cannabis users). In terms of the meta-analytic methodology, the analysis of the 15 studies was identical in all respects to that of the original 11 studies described above. A brief description of the four additional studies is provided in Table 3b, along with the original inclusion criteria that were not met.

Statistical Methodology

Using the techniques described by Hedges and Olkin (1985) to combine continuous outcome measures, a standardized mean difference (effect size) d, and its variance were calculated for each neuropsychological test that was administered within each of the 11 studies. In particular, $d = (M_e - M_c)/S$, where M_e and M_c were the mean scores on a neuropsychological test for the cannabis using and control groups respectively and S was the standard deviation for the pooled sample. The expression for variance (v), is $v = (n_e + n_c)/(n_e n_c) + d^2/(2(n_e + n_c))$, with n_e and n_c representing the sample sizes for the cannabis users and controls, respectively. Then, within each of the studies, the individual effect sizes were linearly combined by subsets into one of eight neurocognitive ability domains. Thus, if $d_1, d_2, \ldots d_k$ represented the effect sizes for k tests from a particular study all deemed to measure the same neurocognitive ability, a pooled estimate (d'), $d' = \Sigma(w_i d_i)$, was obtained. The decision to group individual tests into domains was necessary due to the lack of overlap between tests across studies. We acknowledge that individual neuropsychological tests assess multiple cognitive abilities; nevertheless, each test was assigned to the cognitive domain it was determined to

Table 3b. Study characteristics for four additional studies

Study	Users (*n*)	Control (*n*)	Frequency or amount of use	Duration of use (years)	Cognitive domains assessed*	Cognitive impairment concluded?	Criteria not met†
[12]Dulf et al. (1993)	15	10	1.1 gm/dy (*M*) 0.5 (*SD*)	7.5 (*M*) 2.2 (*SD*)	A	No	5, 8
[13]Grant et al. (1973)	29	29	3 times/mo (median)	4 (median)	A, AE, L, PM	No	5, 8
[14]Rochford et al. (1977)	26	25	NR	3.7 (*M*)	L, PM	No	5, 8
[15]Wig & Varma (1977)	11	11	NR	NR	A, AE, F, L, PM	Yes: memory, concentration	6

Note. Numeric superscripts refer to the data presented in Figures 2A and 2B.
*A study may have "assessed" a given domain, but their data may not have been included in our analysis if it was presented in a format that was incompatible with our methods for effect size calculations.
A, Attention; AE, Abstraction/Executive; F, Forgetting/Retrieval; L, Learning; PM, Perceptual Motor; NR, not reported.
†See Table 2.

best assess. These domains were simple reaction time, attention (e.g., WAIS–R Digit Span, Digit Vigilance), verbal/language (e.g., WAIS–R Vocabulary, Verbal Fluency), abstraction/executive functioning (e.g., Wisconsin Card Sorting Test, Raven's Progressive Matrices), perceptual motor (e.g., WAIS–R Block Design, WAIS-R Object Assembly), simple motor (e.g., Grooved Pegboard, Finger Tapping), learning (e.g., California Verbal Learning Test–Learning Trials, Rey Auditory Verbal Learning Test–Learning Trials), and forgetting/retrieval (e.g., California Verbal Learning Test–Delayed Recall, Rey Auditory Verbal Learning Test–Delayed Recall). As different tests measuring the same neurocognitive domain would be expected to be correlated, the method prescribed by Hedges and Olkin (1985; Chapter 10) was adopted, whereby, the vector of weights $(w_1, \ldots w_k)$ above was chosen to be proportional to the inverse of the covariance matrix of the vector $(d_1, d_2, \ldots d_k)$, thus reflecting the dependence between the tests. We assumed a correlation of .7 between any two tests that were purported to measure the same neurocognitive domain. The assumption of this value had to be made because we did not have individual test results for each subject from each study. However, based on extensive experience in the conduct of neuropsychological studies employing comprehensive neuropsychological test batteries with many thousands of subjects over the years (Grant, et al., 1978, 1979, 1982, 1987; Heaton et al., 1981, 1991, 1995), it has been our experience that tests grouped into a particular domain share about 50% of common variance, with a range of 30% to 70%. Using a correlation of .7 ensures a conservative estimate of the variance of the pooled effect size.

A heterogeneity statistic, Q, was calculated in order to assess whether the effect sizes that were pooled within each study could in fact be said to be measuring the same underlying population effect [illegible] cognitive domain. In a few instances, this exploration indicated a large amount of heterogeneity between tests within a given domain, suggesting that a test may have been incorrectly assigned to a particular domain or would better fit classification under a different cognitive domain. In these cases, the tests within a domain were reexamined, and redistributed to another neurocognitive domain if deemed appropriate based on logical grounds and previous experience (e.g., a computer-assisted test which was assigned to the domain of learning *a priori*, better fit the attention domain).

A fixed effects model was used in our computations. Our choice of using fixed effects rather than random effects, was guided by the judgment that in investigating the potential toxic effects of a substance such as cannabis, it was important to be more "permissive" rather than "conservative" in our modeling of the information due to the likelihood of heterogeneity in data across studies. If a subtle signal were present in a heterogeneous data field, it would be more readily detected using a fixed effects model. Thus, the fixed effects approach was expected to produce somewhat smaller confidence intervals around the effect sizes than would a random effects model, thus increasing the likelihood of detecting a cannabis effect.

The above analysis yielded for each study a vector of effect sizes with variances for each of the neurocognitive domains (with the possibility of missing values if the study in question did not measure a particular neuropsychological domain). Effect sizes were again linearly combined (with weights inversely proportional to the variance) across studies to obtain an overall effect size and a variance for each domain. An across study heterogeneity statistic Q was also computed for each domain. Finally, an overall or global neurocognitive effect size and its variance were computed by pooling the effect sizes across domains. The methodology for this final combination mirrored the methods described above for the within study pooling. As it might be expected that scores on tests corresponding to different neuropsychological domains might be (weakly) correlated, the correlation between [illegible] Again, this estimate was based on the authors' experience of the likely correlation of tests from separate domains. Because the overall determination of the association between cannabis use and neuropsychological impairment was based

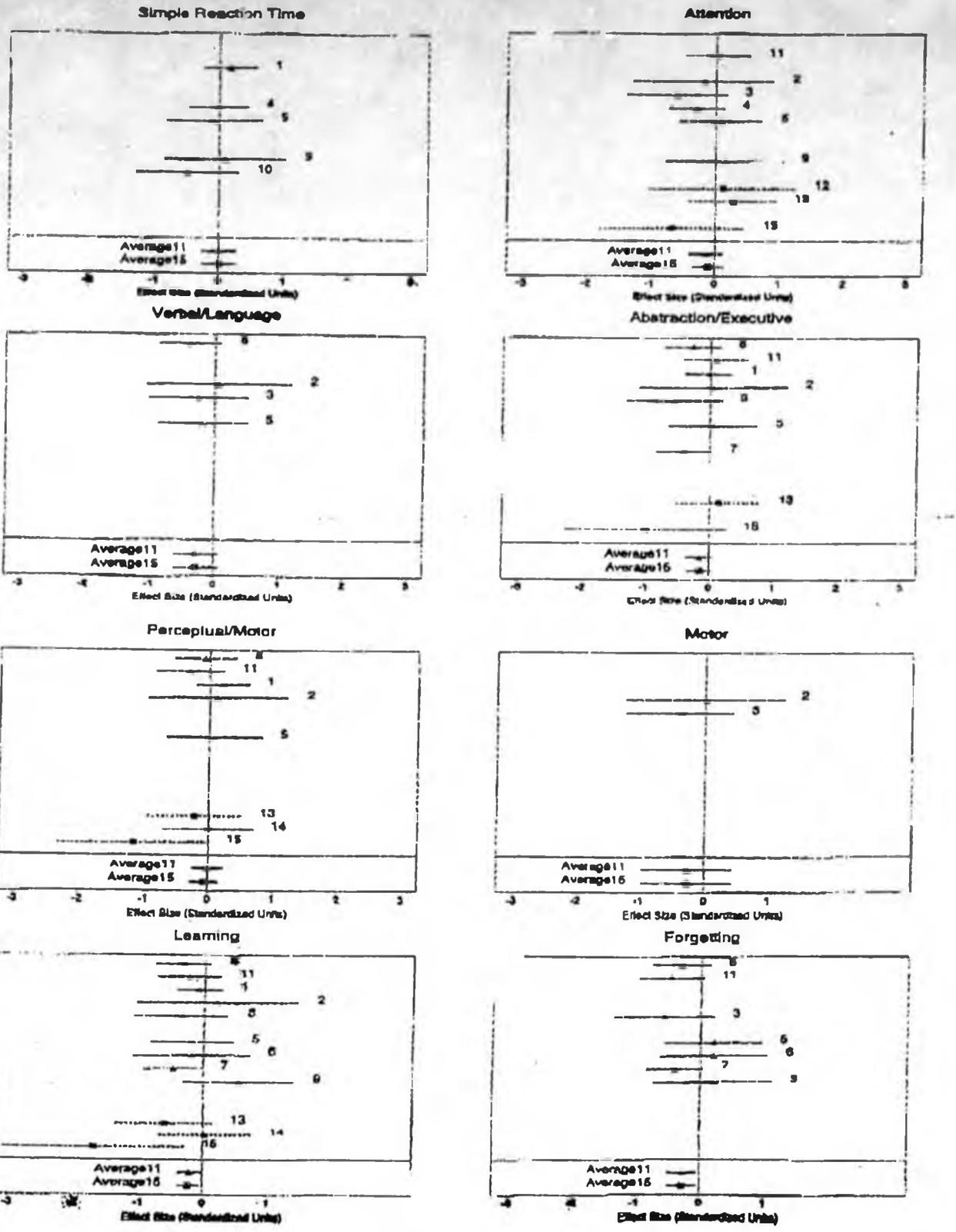


Fig. 2a & 2b.

on a large number of test scores measuring eight different neuropsychological domains, 99% confidence intervals were calculated to allow adjustment for multiple comparisons.

RESULTS

The effect sizes for each neurocognitive domain from each study are presented in Figures 2a and 2b. The effect sizes for each domain across the 11 original studies ranged from +.0086 for the perceptual motor domain, to −.28 for the verbal/language domain. In most instances, the 99% confidence interval surrounding the mean effect size for each domain included zero; therefore, the possibility that the effect size observed was indeed zero could not be discounted. However, in the case of the learning [−.21 99%CI (−.39, −.022)] and forgetting [−.27 99%CI (−.49, −.044)] domains the average effect sizes were found to be significant, albeit of small magnitudes. Comparable results were obtained when all 15 studies were considered.

The global measure of neurocognitive performance was determined through a linear combination of effect sizes across all domains. Data from the 11 studies included under the original criteria indicated an average effect size of −.15 99%CI (−.29, −.019) on the global measure of neurocognitive performance. When all 15 studies were considered, the average global neurocognitive effect size was −.16 99% CI (−.29, −.033). Thus, the results of both sets of analyses suggest a small detrimental cannabis effect on overall neurocognitive performance.

Inspecting the effect sizes for neurocognitive domains by study, we noted that one study examining users in India (class-IV caste) of bhang and charas appeared to be a distant outlier. Due to the fact that this study was based on a population very unlike that of other studies in our pool, we repeated the analysis omitting this study from the pool. Eliminating this study had a negligible influence on effect sizes, but did reduce heterogeneity so that none of the domain-specific Q statistics were statistically significant. Table 4 presents the values of Q for each domain across studies, as well as observed effect sizes, both before and after the removal of the outlier study.

Meta-Regression

To determine whether several study and subject characteristics influenced the observed effect sizes, we performed a set of univariate regressions. These analyses were limited to four of the eight domains (attention, abstraction/executive functioning, perceptual motor, and learning) that contained data from a sufficient number of studies. Variables included whether the cannabis-using and control groups were adequately matched with regard to education or intelligence, as well as the extent to which cannabis users and controls were excluded due to other significant drug use. Furthermore, with respect to cannabis using subjects, variables of interest included whether individuals were ascertained to be abstinent from cannabis for at least 24 hr at time of testing, and their duration of cannabis use. Because of the possibility that sex and years of education might moderate neurocognitive performance in the cannabis group, these variables were also examined. For each of the univariate meta-regressions, years of cannabis use, percent female in the cannabis group, and years of education in the cannabis group, were coded as continuous variables. Dichotomous variables were used to represent whether subjects were known to be abstinent from cannabis for 24 hr, were excluded for other significant drug use, and were matched with the control group on education and/or IQ. The meta-regression results indicated that none of the examined covariates significantly moderated the effect size in any of the regressions.

DISCUSSION

The results of our meta-analytic study failed to reveal a substantial, systematic effect of long-term, regular cannabis consumption on the neurocognitive functioning of users who were not acutely intoxicated. For six of the eight neurocognitive ability areas that were surveyed, the confidence intervals for the average effect sizes across studies overlapped zero in each instance, indicating that the effect size could not be distinguished from zero. The two exceptions were in the domains of learning and forgetting. Here when we averaged across the 11 studies that had the most rigorous inclusion/exclusion criteria and the best designs, the effect size for learning was −.21 99%CI (−.39, −.022) indicating a very small but discernible negative effect. This effect was slightly larger when all 15 studies were included [−.24 99%CI (−.41, −.064)]. Similarly, in the domain of forgetting (failure to recall or recognize) the average effect size was −.27 99%CI (−.49, −.044), again suggesting a very small but measurable decrement.

Fig. 2a & 2b. Effect sizes for each neurocognitive domain. Effect sizes derived from the 11 studies included under the original set of criteria are depicted with solid triangles and long-dashed confidence intervals; data for the four studies added under the relaxed criteria are displayed with solid squares and small-dashed confidence intervals. Each effect size and confidence interval is shown with a number that references the study as presented in Table 3a and 3b. The average effect sizes across studies for each domain, are presented at the base of each of the specific domain related figures. A negative effect size represents poorer performance by the cannabis using groups. "Average 11" refers to the average effect size of the studies included under the original criteria; "Average 15" presents effect sizes for the entire set of studies.

Table 4. Effect sizes and estimates of heterogeneity within domains, across studies

Domain	Effect size (99% CI)	Q-statistic	df for Q	p-value for Q
Attention	−.11 (−.34, .12)	11.26	8	.19
	−.083 (−.32, .15)	9.30	7	.23
Abstraction/Executive	−.15 (−.34, .032)	14.24	8	.08
	−.13 (−.32, .052)	10.73	7	.15
Forgetting/Retrieval*	−.27 (−.49, −.044)	10.81	6	.09
Learning*	−.24 (−.41, −.064)	23.09	11	.02
	−.21 (−.39, −.040)	14.60	10	.15
Motor	−.26 (−.96, .43)	.55	1	.46
Perceptual–Motor	−.065 (−.28, .15)	12.80	7	.15
	−.026 (−.25, .20)	5.57	6	.47
Simple Reaction Time	.0086 (−.25, .26)	4.54	4	.34
Verbal/Language	−.28 (−.62, .060)	1.30	3	.73

Note. * denotes a significant effect size; Rows with two sets of numbers contain the values obtained before and after the removal of an outlier study (i.e., Wig & Verma), in the respective order; *df* = degrees of freedom.

These results can be interpreted in several ways. A statistically reliable negative effect was observed in the domain of learning and forgetting, suggesting that chronic long-term cannabis use results in a selective memory defect. While the results are compatible with this conclusion, the effect size for both domains was of a very small magnitude. The "real life" impact of such a small and selective effect is questionable. In addition, it is important to note that most users across studies had histories of heavy long-term cannabis consumption. Therefore, these findings are not likely to generalize to more limited administration of cannabis compounds, as would be seen in a medical setting.

Some of the studies included in our analyses tested cannabis users with less than 24 hr of abstinence, and others reported no information on abstinence at all. As a result, another factor that may have contributed to the small tendency towards worse performance in the cannabis-using group might be attributable to what Pope et al. have called "residual effects." In a recent study by Pope et al. (2001), three groups of subjects were repeatedly examined over a period of 28 days. They included a group of current heavy cannabis users, a group of persons who had heavy histories of past cannabis use but had not used in the recent past, and a group of controls who had very limited experience with cannabis. The active cannabis users were tested on Days zero, 1, 7, and 28 after ceasing active cannabis use. Abstinence was confirmed through regular urinalysis, which detected declining concentrations of THC in the urines of the active users, and demonstrated that all had undetectable THC levels by 28 days. Pope and colleagues noted subtle impairments on several neurocognitive tests in the active cannabis users who had just become abstinent. However, by 28 days, the active cannabis users who had abstained for almost a month were indistinguishable from former heavy users or non-using controls. Pope et al. suggested that the subtle cognitive impairments observed in the active users during the first week of cessation might represent residual effects (i.e., effects of persisting low levels of THC in the system), abstinence phenomena, or both. The Pope et al. data have direct relevance on the interpretation of results obtained in this study. In nearly all instances, heavier cannabis users were asked to abstain for a period of hours or days before testing. Therefore, many of them could have been at risk for "residual effects" or "abstinence phenomena," which might have contributed to slight decrements in their performance. Given this likelihood, it is even more surprising that our meta-analytic study revealed so few effects.

In interpreting the results of this meta-analytic study, several caveats need to be considered. First, many of the studies examined had significant limitations. For example, several studies had small numbers of subjects, reducing our confidence in the individual study's results and creating concerns about generalizability. Second, many studies had insufficient information about potential confounding factors. These factors included recency of last cannabis exposure, extent of exposure to other drugs of abuse, presence of confounding neuropsychiatric factors (e.g., depression, anxiety, personality disorders, etc.), or other neuromedical risks that can independently affect brain function. As an example, the most recent study by Solowij et al. (2002) focused on patients receiving treatment for cannabis dependence, and the controls were non-patients. This study found negative effects on memory. An unanswered question is whether the cannabis users in that study, being persons who sought or were referred to treatment, might consist of a highly selected group that either were experiencing cannabis related cognitive problems, or who had such difficulties as a function of comorbid psychiatric disorders. No data were presented on mood disorders, which can contribute

both to subtle memory difficulties and account for treatment seeking. In this particular study, the long term using group was, on average, 8 years older than the controls. Although the authors attempted to adjust for age in some of their analyses, there is reason to believe that such covariance adjustments often under-correct, and therefore are not an appropriate substitute for proper age matching, especially when interpreting neuropsychological tests which are influenced by age (Adams et al., 1985).

A general issue affecting most studies was that the premorbid neurocognitive abilities of these subjects were largely unknown, as the studies did not document neurocognitive performance before subjects' onset of regular cannabis use. Although methods are available to estimate premorbid intelligence through consideration of performance on tests that are not likely to be impacted by subtle brain injury (e.g., scores on the Vocabulary subtest of the WAIS series), these techniques are often inadequate. For example, we can never be absolutely certain whether the cannabis users might have been brighter than controls to begin with, and then lost some measure of their cognitive function. Nevertheless, many of the confounds discussed would most likely result in poorer scores in the cannabis group, thus increasing the likelihood and magnitude of observed effects. Only studies that begin with the examination of children and young adolescents before they enter the period of risk to cannabis exposure, can sufficiently reduce the influence of confounds, thus answering this question most effectively. An alternative strategy would be to examine monozygotic twins discordant for cannabis and other substance use. In such studies, one can be more confident of controlling for "native endowment." In the absence of such designs, which can be costly to implement, the approach developed by Pope et al. represents the next best alternative. By examining regular active users who were instructed to abstain, and then repeatedly tested during a lengthy supervised abstinence period, studies such as those designed by Pope et al. bring us closer to understanding the persisting effects of cannabis use, while simultaneously tracking the potential confounds of a "residual effect" and "abstinence phenomena."

In addition to the limitations posed by suboptimal study designs, limiting aspects of the statistical methodology necessarily employed when conducting a meta-analysis should also be considered. It is important to recognize that noise and interpretability, inherent in such analyses, present an additional challenge. To compute the average effect size, as discussed in the statistical methodology section, three types of linear combinations were performed. First, within studies, different tests (from study to study) were combined into the eight neuropsychological domains. Then, across studies, domain effect sizes were linearly combined into eight domain effect size estimates. Finally, the eight effect sizes were linearly combined across domains, yielding a highly processed overall effect size that should be subject to caution in interpretation. In regards to the lack of findings when considering the influence of covariates on observed effect sizes, it is important to note that the meta-regressions were performed on data matrices with as many rows as there were studies testing a particular domain (no more than 10). With less than 10 data points per regression, it is necessarily difficult to make any conclusions about the significance of the model.

Finally, it is important not to generalize these findings to special populations. Many of the studies included in our analyses were conducted with better-educated, younger individuals. We do not know if these mostly negative findings would apply to individuals who have other risk factors for neurocognitive impairment and are then exposed to chronic heavy cannabis use. For example, we cannot be certain if individuals with mild head injuries, attention deficit/hyperactivity disorder, or other neuropsychiatric conditions that may affect cognitive capacity, might be equally resistant to the chronic effects of cannabis. In addition, the fact that cannabinoids appear to be well tolerated by healthy adults does not mean that children and adolescents, who are continuing to undergo neurobiological and cognitive development, will be similarly unaffected. Data from several human studies, as well as animal studies examining the effects on the offspring of cannabis-exposed mothers, suggest that neurodevelopmental difficulties can occur. For example, Fried et al. (2001) have noted executive dysfunction in older children and adolescents of mothers who were substantial cannabis users in the Ottawa cohort. Similar findings were noted in the Pittsburgh longitudinal study (Goldschmidt et al., 2000). Thus, it remains entirely possible that exposure of the developing nervous system to cannabinoids may cause alterations that affect cognitive function in the future.

In conclusion, our meta-analysis of studies that have attempted to address the question of longer term neurocognitive disturbance in moderate and heavy cannabis users has failed to demonstrate a substantial, systematic, and detrimental effect of cannabis use on neuropsychological performance. It was surprising to find such few and small effects given that most of the potential biases inherent in our analyses actually increased the likelihood of finding a cannabis effect. Specifically, our use of a fixed effects model resulted in smaller confidence intervals for the effect sizes we computed, thus facilitating the discovery of statistically significant between-group differences. Moreover, many of the confounds inherent in the studies included in our analyses made it more likely for the cannabis using group to demonstrate poorer performance on neuropsychological tests than controls, irrespective of cannabis consumption. Finally, meta-analytic studies are generally criticized for including only investigations that have been published in peer-reviewed journals, because studies that report statistically significant findings are more likely to be published. This "file-drawer" bias can result in an underrepresentation of studies that did not find statistically significant results, therefore also increasing the likelihood of generating statistically significant effect sizes. Nevertheless, when considering all 15 studies (i.e., those that met both strict and more relaxed criteria) we only noted that regular cannabis users per-

formed worse on memory tests, but that the magnitude of the effect was very small. The small magnitude of effect sizes from observations of chronic users of cannabis suggests that cannabis compounds, if found to have therapeutic value, should have a good margin of safety from a neurocognitive standpoint under the more limited conditions of exposure that would likely obtain in a medical setting.

ACKNOWLEDGMENTS

Supported by the University of California Center for Medicinal Cannabis Research. Special thanks to Michelle Pryberger and Kimberly Jones for assistance in formatting graphs and figures.

REFERENCES

Adams, K.M., Brown, G.G., & Grant, I. (1985). Analysis of covariance as a remedy for demographic mismatch of research subject groups: Some sobering simulations. *Journal of Clinical and Experimental Neuropsychology, 7*, 445–462.

Baker, D., Pryce, G., Croxford, J.L., Brown, P., Pertwee, R.G., Huffman, J.W., & Layward, L. (2000). Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature, 404*, 84–87.

Baker, D., Pryce, G., Croxford, J.L., Brown, P., Pertwee, R.G., Makriyannis, A., Khanolkar, A., Layward, L., Fezza, F., Bisogno, T., & Di Marzo, V. (2001). Endocannabinoids control spasticity in a multiple sclerosis model. *Faseb Journal, 15*, 300–302.

Block, R.I. & Ghoneim, M.M. (1993). Effects of chronic marijuana use on human cognition. *Psychopharmacology, 110*, 219–228.

Carlin, A.S. & Trupin, E.W. (1977). The effect of long-term chronic marijuana use on neuropsychological functioning. *International Journal of the Addictions, 12*, 617–624.

Childers, S.R. & Breivogel, C.S. (1998). Cannabis and endogenous cannabinoid systems. *Drug and Alcohol Dependence, 51*, 173–187.

Croft, R.J., Mackay, A.J., Mills, A.T.D., & Gruzelier, J.G.H. (2001). The relative contributions of ecstasy and cannabis to cognitive impairment. *Psychopharmacology, 153*, 373–379.

Deif, A., El Sheshai, A., & Fawzy, R.K. (1993). Neurological, psychiatric and C.T. evaluation of chronic cannabis smokers. *Journal of the Medical Research Institute, 14*, 151–160.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., & Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science, 258*, 1946–1949.

Ehrenreich, H., Rinn, T., Kunert, H.J., Moeller, M.R., Poser, W., Schilling, L., Gigerenzer, G., & Hoehe, M.R. (1999). Specific attentional dysfunction in adults following early start of cannabis use. *Psychopharmacology, 142*, 295–301.

Fletcher, J.M., Page, J.B., Francis, D.J., Copeland, K., Naus, M.J., Davis, C.M., Morris, R., Krauskopf, D., & Satz, P. (1996). Cognitive correlates of long-term cannabis use in Costa Rican men. *Archives of General Psychiatry, 53*, 1051–1057.

Fride, E., Ginzburg, Y., Breuer, A., Bisogno, T., Di Marzo, V., & Mechoulam, R. (2001). Critical role of the endogenous cannabinoid system in mouse pup suckling and growth. *European Journal of Pharmacology, 419*, 207–214.

Fried, P.A. & Smith, A.M. (2001). A literature review of the consequences of prenatal marihuana exposure. An emerging theme of a deficiency in aspects of executive function. *Neurotoxicology and Teratology, 23*, 1–11.

Goldschmidt, L., Day, N.L., & Richardson, G.A. (2000). Effects of prenatal marijuana exposure on child behavior problems at age 10. *Neurotoxicology and Teratology, 22*, 325–336.

Gonzalez, R., Carey, C., & Grant, I. (2002). Nonacute (residual) neuropsychological effects of cannabis use: A qualitative analysis and systematic review. *Journal of Clinical Pharmacology, 42*, 48S–57S.

Gorter, R., Seefried, M., & Volberding, P. (1992). Dronabinol effects on weight in patients with HIV infection [Letter to the editor]. *Aids, 6*, p. 127.

Gouzoulis-Mayfrank, E., Daumann, J., Tuchtenhagen, F., Pelz, S., Becker, S., Kunert, H.J., Fimm, B., & Sass, H. (2000). Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). *Journal of Neurology, Neurosurgery and Psychiatry, 68*, 719–725.

Grant, I., Adams, K., & Reed, R. (1979). Normal neuropsychological abilities of alcoholic men in their late thirties. *American Journal of Psychiatry, 136*, 1263–1269.

Grant, I., Adams, K.M., Carlin, A.S., Rennick, P., Judd, L.L., & Schooff, K. (1978). The collaborative neuropsychological study of polydrug abusers. *Archives of General Psychiatry, 35*, 1063–1074.

Grant, I., Atkinson, J.H., Hesselink, J.R., Kennedy, C.I., Richman, D.D., Spector, S.A., & McCutchan, J.A. (1987). Evidence for early central nervous system involvement in the acquired immunodeficiency syndrome (AIDS) and other human immunodeficiency virus (HIV) infections. *Annals of Internal Medicine, 107*, 828–836.

Grant, I., Heaton, R.K., McSweeny, A.J., Adams, K.M., & Timms, R.M. (1982). Neuropsychological findings in hypoxemic chronic obstructive pulmonary disease. *Archives of Internal Medicine, 142*, 1470–1476.

Grant, I. & Mohns, L. (1975). Chronic cerebral effects of alcohol and drug abuse. *International Journal of the Addictions, 10*, 883–920.

Grant, I., Rochford, J., Fleming, T., & Stankard, A. (1973). A neuropsychological assessment of the effects of moderate marihuana use. *Journal of Nervous and Mental Disease, 156*, 278–280.

Hamil, W.L. (1996). Auditory learning and memory performance among veterans with a history of stimulant abuse. *Dissertation Abstracts International: Section B: The Sciences and Engineering, 56*, 4906.

Hao, S., Avraham, Y., Mechoulam, R., & Berry, E.M. (2000). Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. *European Journal of Pharmacology, 392*, 147–156.

Heaton, R.K., Grant, I., Anthony, W.Z., & Lehman, R.A. (1981). A comparison of clinical and automated interpretation of the Halstead-Reitan Battery. *Journal of Clinical Neuropsychology, 3*, 121–141.

Heaton, R.K., Grant, I., Butters, N., White, D.A., Kirson, D., Atkinson, J.H., McCutchan, J.A., Taylor, M.J., Kelly, M.D., Ellis, R.J., Wolfson, T., Velin, R., Marcotte, T.D., Hesselink, J.R., Jernigan, T.L., Chandler, J., Wallace, M., Abramson, I., & the HNRC Group. (1995). The HNRC 500–Neuropsychology of HIV infection at different disease stages. *Journal of the International Neuropsychological Society, 1*, 231–251.

Heaton, R.K., Grant, I., & Matthews, C.G. (1991). *Comprehensive norms for an expanded Halstead-Reitan Battery: Demographic corrections, research findings, and clinical applications.* Odessa, FL: Psychological Assessment Resources, Inc.

Hedges, L.V. & Olkin, I. (1985). *Statistical methods for meta-analysis.* Orlando, FL: Academic Press.

Herkenham, M. (1992). Cannabinoid receptor localization in brain: Relationship to motor and reward systems. *Annals of the New York Academy of Sciences, 654,* 19–32.

Herkenham, M., Lynn, A.B., de Costa, B.R., & Richfield, E.K. (1991a). Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Research, 547,* 267–274.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R., & Rice, K.C. (1991b). Characterization and localization of cannabinoid receptors in rat brain: A quantitative in vitro autoradiographic study. *Journal of Neuroscience, 11,* 563–583.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., & Rice, K.C. (1990). Cannabinoid receptor localization in brain. *Proceedings of the National Academy of Sciences of the United States of America, 87,* 1932–1936.

Howlett, A.C., Bidaut-Russell, M., Devane, W.A., Melvin, L.S., Johnson, M.R., & Herkenham, M. (1990). The cannabinoid receptor: Biochemical, anatomical and behavioral characterization. *Trends in Neurosciences, 13,* 420–423.

Institute of Medicine. (1999). *Marijuana and medicine: Assessing the science base.* Washington, DC: Author.

Kaminski, N.E., Abood, M.E., Kessler, F.K., Martin, B.R., & Schatz, A.R. (1992). Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. *Molecular Pharmacology, 42,* 736–742.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., & Bonner, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature, 346,* 561–564.

Munro, S., Thomas, K.L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature, 365,* 61–65.

Nelson, K., Walsh, D., Deeter, P., & Sheehan, F. (1994). A phase II study of delta-9-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. *Journal of Palliative Care, 10,* 14–18.

Noyes, R., Brunk, S.P., Baram, D.A., & Canter, A. (1975). Analgesic effect of delta-9-tetrahydrocannabinol. *Journal of Clinical Pharmacology, 15,* 139–143.

Pertwee, R. (1993). The evidence for the existence of cannabinoid receptors. *General Pharmacology, 24,* 811–824.

Pertwee, R.G. (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology and Therapeutics, 74,* 129–180.

Plasse, T.F., Gorter, R.W., Krasnow, S.H., Lane, M., Shepard, K.V., & Wadleigh, R.G. (1991). Recent clinical experience with dronabinol. *Pharmacology, Biochemistry and Behavior, 40,* 695–700.

Pope, H.G., Gruber, A.J., Hudson, J.I., Huestis, M.A., & Yurgelun-Todd, D. (2001). Neuropsychological performance in long-term cannabis users. *Archives of General Psychiatry, 58,* 909–915.

Pope, H.G., Gruber, A.J., & Yurgelun-Todd, D. (1995). The residual neuropsychological effects of cannabis—The current status of research. *Drug and Alcohol Dependence, 38,* 25–34.

Pope, H.G. & Yurgelun-Todd, D. (1996). The residual cognitive effects of heavy marijuana use in college students. *Journal of the American Medical Association, 275,* 521–527.

Rochford, J., Grant, I., & LaVigne, G. (1977). Medical students and drugs: Further neuropsychological and use pattern considerations. *International Journal of the Addictions, 12,* 1057–1065.

Rodgers, J. (2000). Cognitive performance amongst recreational users of "ecstasy." *Psychopharmacology, 151,* 19–24.

Sanudo-Pena, M.C. & Walker, J.M. (1997). Role of the subthalamic nucleus in cannabinoid actions in the substantia nigra of the rat. *Journal of Neurophysiology, 77,* 1635–1638.

Solowij, N. (1995). Do cognitive impairments recover following cessation of cannabis use? *Life Sciences, 56,* 2119–2126.

Solowij, N. (1998). *Cannabis and cognitive functioning.* New York: Cambridge University Press.

Solowij, N., Stephens, R.S., Roffman, R.A., Babor, T., Kadden, R., Miller, M., Christiansen, K., McRee, B., & Vendetti, J. (2002). Cognitive functioning of long-term heavy cannabis users seeking treatment. *Journal of the American Medical Association, 287,* 1123–1131.

US National Institute of Health. (1997). *Report on the medical uses of marijuana.* Bethesda, MD.

Wig, N.N. & Varma, V.K. (1977). Patterns of long-term heavy cannabis use in North India and its effects on cognitive functions: A preliminary report. *Drug and Alcohol Dependence, 2,* 211–219.

Oncologic, Endocrine & Metabolic

Cannabinoid receptor systems: therapeutic targets for tumour intervention

Sarah Jones & John Howl†
†Molecular Pharmacology Group, Biomedical Sciences Division, School of Applied Sciences, University of Wolverhampton, Wulfruna Street, WV1 1SB, UK

The past decade has witnessed a rapid expansion of our understanding of the biological roles of cannabinoids and their cognate receptors. It is now certain that Δ^9-tetrahydrocannabinol, the principle psychoactive component of the *Cannabis sativa* plant, binds and activates membrane receptors of the 7-transmembrane domain, G-protein-coupled superfamily. Several putative endocannabinoids have since been identified, including anandamide, 2-arachidonyl glycerol and noladin ether. Synthesis of numerous cannabinomimetics has also greatly expanded the repertoire of cannabinoid receptor ligands with the pharmacodynamic properties of agonists, antagonists and inverse agonists. Collectively, these ligands have proven to be powerful tools both for the molecular characterisation of cannabinoid receptors and the delineation of their intrinsic signalling pathways. Much of our understanding of the signalling mechanisms activated by cannabinoids is derived from studies of receptors expressed by tumour cells; hence, this review provides a succinct summary of the molecular pharmacology of cannabinoid receptors and their roles in tumour cell biology. Moreover, there is now a genuine expectation that the manipulation of cannabinoid receptor systems may have therapeutic potential for a diverse range of human diseases. Thus, this review also summarises the demonstrated antitumour actions of cannabinoids and indicates possible avenues for the future development of cannabinoids as antitumour agents.

Keywords: cannabinoid, endocannabinoid, signal transduction, tumour

Expert Opin. Ther. Targets (2003) 7(6):749-758

Expert Opinion

1. Introduction

1.1 The pharmacology of cannabinoid receptors

For many years, the highly lipophilic nature of cannabinoids proved a barrier to the unequivocal demonstration that the biological effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), and other ligands that demonstrate Δ^9-THC-like pharmacological properties, were receptor-mediated. However, the molecular cloning of a brain receptor that bound these lipid moieties [1] finally proved that the biological actions of cannabinoids were mediated by G-protein-coupled receptors (GPCRs), the quantitatively-dominant class of drug target. To date, only two cannabinoid receptor subtypes have been cloned. The central brain cannabinoid receptor (CB_1) [1] and the peripheral cannabinoid receptor (CB_2) [2] share 44% overall sequence homology that predictably rises to 68% within the seven transmembrane-spanning domains. The use of selective agonists and/or antagonists can discriminate between CB_1- and CB_2-mediated responses. However, both receptors predominantly couple to pertussis toxin-sensitive G proteins of the G_i class. Agonist stimulation of either receptor subtype inhibits adenylyl cyclase, reducing intracellular cAMP, and commonly

 ISSN 1472-8222

activates the extracellular signal-regulated kinase (ERK) cascade (see below) [3]. CB_1 receptor activation also inhibits voltage-dependent Ca^{2+} channels and activates inwardly rectifying K^+ channels [4].

1.2 Biological and pathological roles of cannabinoid receptors

The CB_1 receptor is associated with various biological/therapeutic functions, including the inhibition of synaptic transmission, analgesia, reduction in intra-ocular pressure, appetite stimulation, decreased muscle spasms, and various antitumour actions [5]. The known distribution of the CB_1 receptor, expressed by brain and neuronal tissue and particularly enriched in the hippocampus, cerebral cortex, basal ganglia and cerebellum, supports a predominantly central action. In contrast, the peripherally-located CB_2 receptor is predominantly expressed by cells of immune origin, a cellular distribution that supports an immunomodulatory and/or anti-inflammatory role [6-8]. Of particular relevance to this review is an abundance of evidence that the activation of cannabinoid receptor systems can markedly influence cell fate [9,10]. Following brain injury, and in the case of certain neurodegenerative disorders, cannabinoids protect against neuronal insult and promote cell survival [11-14]. Conversely, activation of cannabinoid receptors expressed by tumour cells can inhibit proliferation and induce apoptosis both *in vivo* and *in vitro* [15,16].

1.3 Ligand classification

Cannabinoid receptor ligands may be divided into four distinct groups: i) Classical; ii) non-classical; iii) aminoalkylindoles, and iv) endocannabinoids/eicosanoids. The classical cannabinoids comprise compounds occurring naturally within the *Cannabis sativa* plant, including Δ^9-THC, Δ^8-THC, cannabinol, cannabidiol and their synthetic analogues, including the highly potent HU-210 and desacetyl-L-nantradiol (DALN) [5]. These ABC-tricyclic dibenzopyran derivatives demonstrate equal affinity for CB_1 and CB_2 receptors. Minor modification of these compounds has led to the generation of cannabinoids with either increased affinity (JWH-015 [17]) or selectivity (JWH-133 [18] and HU-308 [19]) for CB_2.

Developed by Pfizer, the non-classical cannabinoids lack the dihydropyran ring of Δ^9-THC [5]. These bicyclic and tricyclic analogues include the highly utilised CP-55940, showing similar affinity for both CB_1 and CB_2 [20].

Aminoalkylindoles with decreased anti-inflammatory properties and derived from analogues of pravadoline [21], were found to possess cannabimimetic activity [22,23]. This group is typified by WIN-55212-2, exhibiting affinity for both CB_1 and CB_2 receptors [5] and the CB_2-selective agonist AM-1241 [24].

In addition to the four main classes of cannabinoids, the diarylether sulfonylester derivative BAY-38-7271 is a new high-affinity CB_1 receptor agonist that is structurally distinct from any cannabinoid receptor ligand known so far [25].

The discovery of putative endogenous ligands, the endocannabinoids, gave further credence to the pharmacological relevance and therapeutic potential of cannabinoids [26,27]. N-arachidonylethanolamine (AEA), more commonly referred to as anandamide, was initially isolated from porcine brain [28], and synthesis of its analogue, arachidonyl-2-chloroethylamide, produced the high-affinity CB_1 agonist ACEA [29]. Shortly afterwards, 2-arachidonyl glycerol (2-AG), isolated from canine gut [30] and rat brain [31], was acknowledged as a potential candidate in the search for further endocannabinoids. Owing to the relatively low affinity displayed by these putative endogenous ligands for cannabinoid receptors, it seems highly probable that further endocannabinoids remain to be isolated. Most recently, a third endocannabinoid candidate, 2-arachidonyl glycerol ether (noladin ether), has gained attention in the quest for further endogenous ligands. Isolated from porcine brain, noladin ether possesses a higher affinity for the central CB_1 receptor than for the peripheral CB_2 receptor, and shows greater metabolic stability than its counterparts, 2-AG and AEA [32]. However, a recent study was unable to detect appreciable amounts of noladin ether in brains from various mammalian species [33].

Synthesis of selective antagonists has greatly assisted our understanding of cannabinoid receptor pharmacology. The most notable CB_1 antagonists include the diarylpyrazole SR-141716 [34], a highly potent CB_1-selective antagonist, and the high-affinity LY-320135 [35]. However, both are reported to demonstrate properties of inverse agonism [36]. CB_2 antagonists comprise the diarylpyrazole, SR-144528 [37], and the aminoalkylindole, AM-630 [38]. Again, both show characteristics of inverse agonism [39], and the latter may exhibit partial agonist activity at the CB_1 receptor [38].

1.4 Biosynthesis and degradation of endocannabinoids

Local production of AEA, and probably other endocannabinoids, is a calcium-dependent process [40]. AEA biosynthesis requires the phospholipase D-mediated hydrolysis of the phospholipid precursor N-arachidonylphosphatidylethanolamine [40,41]. The extracellular signal-regulated activity of phospholipase D could enable many extrinsic factors to regulate AEA biosynthesis. Inactivation of AEA is by a specific membrane transporter [41-43] and subsequent intracellular hydrolysis by the fatty acid amide hydrolase (FAAH) [41,44,45].

2. Antitumour effects of cannabinoids

The antitumour effects of Δ^9-THC and its analogues were first identified in the 1970s. Antineoplastic activities were reported in Lewis lung adenoma cells [46,47], L1210 leukaemia cells [48] and HeLa S3 cells [49]. As detailed below, numerous investigations have since identified antiproliferative, apoptotic, antiangiogenic, and antimetastatic properties of cannabinoids.

2.1 Malignant astrocytomas and gliomas

Attention has recently been focused on the cannabinoid-induced apoptosis of malignant astrocytomas/gliomas both *in vivo* and in cultured cellular systems. Glial cell/astrocyte

malignancies are a relatively rare yet fatal form of brain tumour [10] that can be modelled in rodents by parenchymal inoculation with transformed glioma cell lines. Following inoculation of Wistar rats with C6.9 glioma cells, intratumoural administration of Δ^9-THC and the synthetic cannabinoid WIN-55212-2 reduced tumour growth and promoted regression via apoptotic mechanisms [51]. More recently, an additional mechanism of cannabinoid-mediated glioma/astrocytoma tumour regression has been elucidated by Blazquez and co-workers [52]. Intra-tumoural administration of the non-psychoactive and CB_2-selective ligand, JWH-133, inhibited angiogenesis required for the growth of tumours induced in mice by inoculation with both C6 glioma and human astrocytoma cells.

2.1.1 Signalling

A common second messenger system stimulated by cannabinoid receptor activation is the ERK cascade. The end point of this key signal transduction event is the phosphorylation and activation of ERK1/2, alternatively referred to as p42/44 mitogen-activated protein kinases (MAPKs). Activated ERK1/2, in turn, modulate gene transcription. Although this system is often associated with events leading to cellular proliferation and differentiation, there are paradoxical circumstances during which activation of the ERK cascade leads to cell cycle arrest, apoptosis and necrotic cell death [53,54]. These disparate effects upon cell fate may be a consequence of differences in signal intensity and duration. Cannabinoid receptor stimulation promotes ERK1/2 activation in C6.9 glioma cells, astrocytoma cells and primary astrocytes, and these events are accompanied by the intracellular accumulation of ceramide [55,56]. Ceramide, a sphingosine-based lipid, possesses apoptotic properties. The accumulation of ceramide may be a consequence of the receptor-mediated activation of sphingomyelinase and subsequent hydrolysis of sphingomyelin. More recently, a second pathway leading to ceramide accumulation and apoptosis, that of *de novo* ceramide synthesis [57-59], has also been elucidated. Although the role of cannabinoid-induced sphingomyelin hydrolysis in normal astrocytes is probably the stimulation of glucose metabolism [56,60], evidence strongly indicates that the intracellular accumulation of ceramide contributes to the apoptotic properties of cannabinoids in transformed cells. Moreover, Galve-Roperh and co-workers [51] showed that exposure of C6.9 glioma cells to both Δ^9-THC and synthetic cannabinoid agonists resulted in the generation of two intracellular ceramide peaks that were upstream of ERK activation. The initial peak, a likely response to short-term cannabinoid treatment, probably involved activation of sphingomyelinase by the adaptor protein FAN (factor associated with neutral sphingomyelinase) [61]. This initial rise in ceramide concentration did not play an active role in cannabinoid-mediated apoptosis. A second ceramide peak was observed following 3 days of continued cannabinoid exposure, and this rise was related to C6.9 glioma cell death. Furthermore, following chronic stimulation of cannabinoid receptors on C6.9 gliomas, inhibition of *de novo* ceramide synthesis prevented stimulation of ERK1/2 and also increased the activity of antiapoptotic protein kinase B (PKB) [62] (**Figure 1**).

These observations clearly support a pro-apoptotic role for cannabinoid receptor systems, although there are several caveats to these interpretations. First, other kinase pathways may underlie the effects of cannabinoids on cell fate. Thus, Δ^9-THC-induced cell death, both within C6.9 gliomas and U373MG astrocytoma cells, has also been paralleled to the activation of Jun N-terminal kinase (JNK) [63] and p38 MAPK [51], although selective inhibition would indicate an ERK-mediated apoptotic response [51]. The JNK and p38 MAPK family, in contrast to the ERKs, are considered to be activated by stress signals and contribute to the stress response by altering progression through the cell cycle, DNA repair or apoptosis [64]. Second, the highly potent agonist HU-210 appears to be protective against ceramide-induced apoptosis in the U373MG cell line [65]. CB_1-mediated $\beta\gamma$ subunit dissociation, activation of the Class I_B phosphatidylinositol 3′-kinase isoenzyme, subsequent activation of its target, PKB, and acute ERK activation, appear to constitute pro-survival signals in this cell line [65]. It follows, therefore, that short-term cannabinoid-mediated ERK activation protects against apoptosis, whereas chronic stimulation induces apoptosis. However, as GPCRs, cannabinoid receptors would be expected to desensitise upon stimulation. Thus, it is difficult to understand why cannabinoid receptor-induced apoptosis requires a 3- to 5-day exposure period to cannabinoids.

2.1.2 Functional significance of the CB_2 receptor

CB_2 receptors are largely considered to be peripherally located. However, evidence for CB_2 receptor expression by some gliomas is accumulating and this receptor may also inhibit cell growth. Thus, Δ^9-THC-induced apoptosis of C6.9 glioma cells was only completely inhibited by a combination of both CB_1 and CB_2 antagonists [51]. Moreover, selective activation of the CB_2 receptor induced a regression of glioma cell growth *in vivo* [66].

Of further interest is the finding that CB_2 receptor expression on human astrocytomas appears to be closely correlated with tumour grade and degree of malignancy [66]. Sanchez and co-workers identified both CB_1 and CB_2 receptor expression in 70% of human astrocytomal biopsies [66]. Moreover, Grade IV astrocytomas showed an elevated level of CB_2 receptor expression compared to that of CB_1. The above findings provide a promising therapeutic strategy for cannabinoid treatment of malignant astrocytomas and gliomas, whilst avoiding the unwanted psychotropic affects associated with recruitment of CB_1 receptors.

2.1.3 Cannabinoid-mediated inhibition of angiogenesis and tumour invasiveness

The importance of cannabinoid-based glioma and astrocytoma therapy has increased significantly in the light of recent

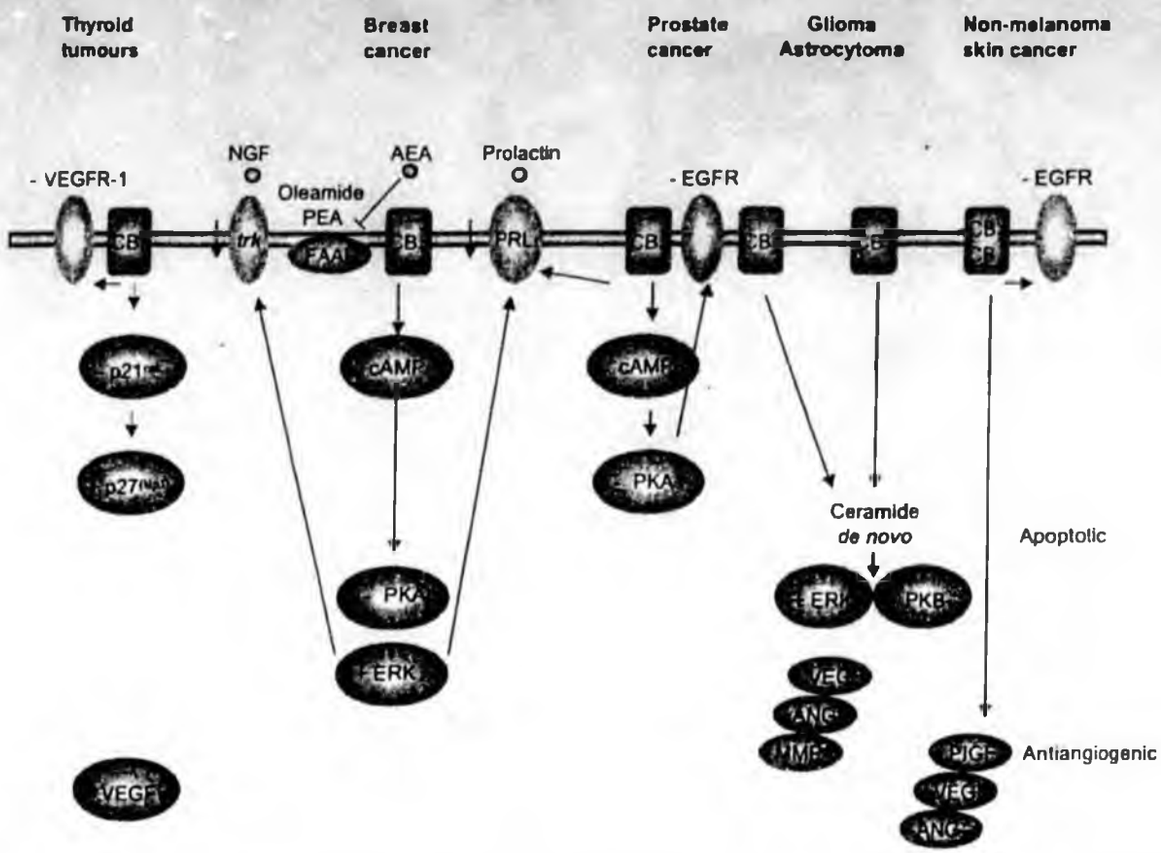


Figure 1. Cannabinoid-mediated antitumour signalling mechanisms. Cannabinoids have been implicated in the tumour suppression of malignant astrocytomas and gliomas, breast and prostate cancer cells, thyroid epithelium, and non-melanoma skin cancer growth. Inhibition of proliferation, induction of apoptosis and impairment of angiogenesis have been attributed to a diversity of intracellular signalling cascades following cannabinoid receptor ligation.

AEA: Anandamide; ANG: Angiopoietin; CB_1: Brain cannabinoid receptor; CB_2: Peripheral cannabinoid receptor; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase; FAAH: Fatty acid amide hydrolase; MMP: Matrix metalloproteinase; NGF: Nerve growth factor; $p21^{ras}$: Monomeric G protein ras; $p27^{kip1}$: Cyclin-dependent kinase inhibitor; PEA: Palmitoylethanolamide; PlGF: Placental growth factor; PK: Protein kinase; PRLr: Long form of the prolactin receptor; *trk*: High-affinity nerve growth factor receptor; VEGF: Vascular endothelial growth factor, VEGFR: Vascular endothelial growth factor receptor.

findings by Blazquez and co-workers [52]. Administration of the CB_2-selective agonist, JWH-133, proved effective in suppressing the release of pro-angiogenic factors, vascular endothelial growth factor (VEGF) and angiopoietin-2 from C6 glioma and human Grade IV astrocytoma-induced tumours in mice. Furthermore, decreased expression of matrix metalloproteinase (MMP)-2 from tumour biopsies was reported (Figure 1). It has therefore been hypothesised that cannabinoids may target and inhibit endothelial cell survival and migration, thereby restricting tumour blood vessel supply [52]. *In vitro* experiments with primary human umbilical vein endothelial cells (HUVECs) and the cell line ECV304 have indicated that such effects are CB_2-mediated. Furthermore, cannabinoid-induced endothelial cell death and inhibition of endothelial cell migration were abrogated by inhibition of MEK (MAPK kinase) 1/2, thereby implicating the involvement of the ERK signalling cascade. However, caution should be applied when interpreting these findings, as ECV304 cells are derived from the human bladder cancer-derived epithelial cell line T24/83 [67].

In summary, cannabinoid receptors and their associated signalling pathways could prove to be a valuable therapeutic target for the treatment of malignant gliomas and astrocytomas. In these situations, cannabinoids could support apoptosis, suppress the release of pro-angiogenic factors and MMPs and, therefore, prevent tumour growth, angiogenesis and cell invasiveness.

2.2 Human breast cancer cell lines

The therapeutic potential of cannabinoids in the treatment of human breast cancer has been well studied by Di Marzo and co-workers, with particular emphasis placed upon the antiproliferative effects of the endocannabinoids in cellular systems. Rather than inducing apoptosis, the endocannabinoid AEA arrested the proliferation of human breast cancer cells by halting progression through the cell cycle at the G1/S transition

45. UEDA N, YAMAMOTO S: Anandamide amidohydrolase (fatty acid amide hydrolase). *Prostaglandins Other Lipid Mediat.* (2000) 61:19-28.

46. MUNSON AE, HARRIS LS, FRIEDMAN MA, DEWEY WL, CARCHMAN RA: Antineoplastic activity of cannabinoids. *J. Natl. Cancer Inst.* (1975) 55:597-602.

47. WHITE AC, MUNSON JA, MUNSON AE, CARCHMAN RA: Effects of Δ^9-tetrahydrocannabinol in Lewis lung adenocarcinoma cells in tissue culture. *J. Natl. Cancer Inst.* (1976) 56:655-658.

48. CARCHMAN RA, HARRIS LS, MUNSON AE: The inhibition of DNA synthesis by cannabinoids. *Cancer Res.* (1976) 31:95-100.

49. MON MJ, JANSING RL, DOGGETT S, STEIN JL, STEIN GS: Influence of Δ^9-tetrahydrocannabinol on cell proliferation and macromolecular biosynthesis in human cells. *Biochem. Pharmacol.* (1978) 27:1759-1765.

50. MAHER EA, FURNARI FB, BACHOO RM *et al.*: Malignant glioma: genetics and biology of a grave matter. *Genes Dev.* (2001) 15:1311-1333.

51. GALVE-ROPERH I, SANCHEZ C, CORTES ML, DEL PULGAR TG, IZQUIERDO M, GUZMAN M: Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nat. Med.* (2000) 16:313-319.

•• A seminal report signifying the antitumour effects of cannabinoids *in vivo*.

52. BLAZQUEZ C, CASANOVA ML, PLANAS A *et al.*: Inhibition of tumor angiogenesis by cannabinoids. *FASEB J.* (2003) 17:529-531.

53. PUNIGLIA KM, DECKER SJ: Cell cycle arrest mediated by the MEK/mitogen-activated protein kinase pathway. *Proc. Natl. Acad. Sci. USA* (1997) 94:448-452.

54. YORK RD, YAO H, DILLON T *et al.*: Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. *Nature* (1998) 392:622-626.

55. SANCHEZ C, GALVE-ROPERH I, CANOVA C, BRACHET P, GUZMAN M: Δ^9-tetrahydrocannabinol induces apoptosis in C6.9 glioma cells. *FEBS Lett.* (1998) 436:6-10.

56. SANCHEZ C, GALVE-ROPERH I, RUEDA D, GUZMAN M: Involvement of sphingomyelin hydrolysis and the mitogen-activated protein kinase cascade in the Δ^9-tetrahydrocannabinol-induced stimulation of glucose metabolism in primary astrocytes. *Mol. Pharmacol.* (1998) 54:834-843.

57. PERRY DK, CARTON J, SHAH AK, MEREDITH F, UHLINGER DJ, HANNUN YA: Serine palmitoyltransferase regulates *de novo* ceramide generation during etoposide-induced apoptosis. *J. Biol. Chem.* (2000) 275:9078-9084.

58. HUWILER A, KOLTER T, PFEILSCHIFTER J, SANDHOFF K: Physiology and pathophysiology of sphingolipid metabolism and signaling. *Biochim. Biophys. Acta* (2000) 1485:63-99.

59. GUZMAN M, GALVE-ROPERH I, SANCHEZ C: Ceramide: a new second messenger of cannabinoid action. *Trends Pharmacol. Sci.* (2001) 22:19-22.

60. BLAZQUEZ C, SANCHEZ C, DAZA A, GALVE-ROPERH I, GUZMAN M: The stimulation of ketogenesis by cannabinoids in cultured astrocytes defines carnitine palmitoyl transferase I as a new ceramide activated enzyme. *J. Neurochem.* (1999) 72:1759-1768.

61. SANCHEZ C, RUEDA D, SEGUI D, GALVE-ROPERH I, LEVADE T, GUZMAN M: The cannabinoid receptor of astrocytes is coupled to sphingomyelin hydrolysis through the adaptor protein FAN. *Mol. Pharmacol.* (2001) 59:955-959.

62. GOMEZ DEL PULGAR T, VELASCO G, GUZMAN M: The CB_1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. *Biochem. J.* (2000) 347:369-373.

63. RUEDA D, GALVE-ROPERH I, HARO A, GUZMAN M: The CB_1 cannabinoid receptor is coupled to the activation of c-Jun-N-terminal kinase. *Mol. Pharmacol.* (2000) 58:814-820.

• These findings prompt an excellent discussion of the diversity of cannabinoid-mediated signal transduction mechanisms in the determination of cell fate.

64. HARPER SJ, LOGRASSO P: Signaling for survival and death in neurons: The role of stress-activated kinases, JNK and p38. *Cell. Signal.* (2001) 13:299-310.

65. GALVE-ROPERH I, RUEDA D, GOMEZ DEL PULGAR T, VELASCO G, GUZMAN M: Mechanism of extracellular signal-regulated kinase activation by the CB_1 cannabinoid receptor. *Mol. Pharmacol.* (2002) 62:1385-1392.

66. SANCHEZ C, DE CEBALLOS ML, GOMEZ DEL PULGAR T *et al.*: Inhibition of glioma growth *in vivo* by selective activation of the CB_2 cannabinoid receptor. *Cancer Res.* (2001) 61:5784-5789.

67. BROWN J, READING SJ, JONES S *et al.*: Critical evaluation of ECV304 as a human endothelial cell model defined by genetic analysis and functional responses: a comparison with the human bladder cancer derived epithelial cell line T24/83. *Lab. Invest.* (2000) 80:37-45.

68. DE PETROCELLIS L, MELCK D, PALMISANO A *et al.*: The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. *Proc. Natl. Acad. Sci. USA* (1998) 95:8375-8380.

69. MELCK D, DE PETROCELLIS L, ORLANDO P *et al.*: Suppression of nerve growth factor *trk* receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. *Endocrinology* (2000) 141:118-126.

70. MELCK D, RUEDA D, GALVE-ROPERH I, DE PETROCELLIS L, GUZMAN M, DI MARZO V: Involvement of the cAMP–protein kinase A pathway and of mitogen-activated protein kinase in the anti-proliferative effects of anandamide in human breast cancer cells. *FEBS Lett.* (1999) 463:235-240.

71. CRAVATT BF, PROSPER-GARCIA O, SIUZDAK G *et al.*: Chemical characterization of a family of brain lipids that induce sleep. *Science* (1995) 268:1506-1509.

72. BISOGNO T, KATAYAMA K, MELCK D *et al.*: Biosynthesis and degradation of bioactive fatty acid amides in human breast cancer and rat pheochromocytoma cells. *Eur. J. Biochem.* (1998) 254:634-642.

73. MECHOULAM R, FRIDE E, HANUS L *et al.*: Anandamide may mediate sleep induction. *Nature* (1997) 389:25-26.

74. DE PETROCELLIS L, MELCK D, BISOGNO T, DI MARZO V: Endocannabinoids and fatty acid amides in cancer, inflammation and related disorders. *Chem. Phys. Lipids* (2000) 108:191-209.

•• A pioneering review exploring novel therapeutic targets of the endocannabinoid system.

75. BISOGNO T, MAURELLI S, MELCK D, DE PETROCELLIS L, DI MARZO V: Biosynthesis, uptake and degradation of anandamide and palmitoylethanolamide in leukocytes. *J. Biol. Chem.* (1997) 272:3315-3323.

76. LAMBERT DM, DI MARZO V: The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic. *Curr. Med. Chem.* (1999) 6:757-773.

77. DI MARZO V, MELCK D, ORLANDO P *et al.*: Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells. *Biochem. J.* (2001) 358:249-255.

78. MIMEAULT M, POMMERY N, WATTEZ N, BAILLY C, HENICHART JP: Anti-proliferative and apoptotic effects of anandamide in human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. *Prostate* (2003) 56:1-12.

79. RUIZ L, MIGUEL A, DIAZ-LAVIADA I: Δ^9-Tetrahydrocannabinol induces apoptosis in human prostate PC-3 cells via a receptor-independent mechanism. *FEBS Lett.* (1999) 458:400-404.

80. KAMIYAMA M, FUKUYA Y, TAKIHANA Y *et al.*: Anandamide and capsaicin inhibit proliferation of human prostate cancer cell via cannabinoid receptor and vanilloid receptor respectively. *Eur. Urol. Suppl.* (2003) 2:36.

81. BIFULCO M, LAEZZA C, PORTELLA G *et al.*: Control by the endogenous cannabinoid system of ras oncogene-dependent tumor growth. *FASEB J.* (2001) 15:2745-2747.

82. PORTELLA G, LAEZZA C, LACCETTI P, DE PETROCELLIS L, DI MARZO M, BIFULCO M: Inhibitory effects of cannabinoid CB_1 receptor stimulation on tumor growth and metastatic spreading: actions on signals involved in angiogenesis and metastasis. *FASEB J.* (2003). Epublished ahead of print.

83. CASANOVA ML, BLAZQUEZ C, MARTINEZ-PALACIO J *et al.*: Inhibition of skin tumor growth and angiogenesis *in vivo* by activation of cannabinoid receptors. *J. Clin. Invest.* (2003) 111:43-50.

84. MCKALLIP RJ, LOMBARD C, FISHER M *et al.*: Targeting CB_2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood* (2002) 100:627-634.

85. DI MARZO V, DE PETROCELLIS L, FEZZA F, LIGRESTI A, BISOGNO T: Anandamide receptors. *Prostaglandins Leukot. Essent. Fatty Acids* (2002) 66:377-391.

86. SMART D, GUNTHORPE MJ, JERMAN JC *et al.*: The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Br. J. Pharmacol.* (2000) 129:227-230.

87. ZYGMUNT PM, PETERSSON J, ANDERSSON DA *et al.*: Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* (1999) 400:452-457.

88. CATERINA MJ, SCHUMACHER MA, TOMINAGA M, ROSEN TA, LEVINE JD, JULIUS D: The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* (1997) 389:816-824.

89. MACCARRONE M, LORENZON T, BARI M, MELINO G, FINAZZI-AGRO A: Anandamide induces apoptosis in human cells via vanilloid receptors. Evidence for a protective role of cannabinoid receptors. *J. Biol. Chem.* (2000) 275:31938-31945.

90. JACOBSSON SO, WALLIN T, FOWLER CJ: Inhibition of rat C6 glioma cell proliferation by endogenous and synthetic cannabinoids. Relative involvement of cannabinoid and vanilloid receptors. *J. Pharmacol. Exp. Ther.* (2001) 299:951-959.

91. SARKER KP, BISWAS KK, TAMAKUCHI M *et al.*: ASK1-p38 MAPK/JNK signaling cascade mediates anandamide-induced PC12 cell death. *J. Neurochem.* (2003) 85:50-61.

92. SARKER KP, OBARA S, NAKATA M, KITAJIMA I, MARUYAMA I: Anandamide induces apoptosis of PC-12 cells: involvement of superoxide and caspase-3. *FEBS Lett.* (2000) 472:39-44.

93. MACCARRONE M, ATTINA M, CARTONI A, BARI M, FINAZZI-AGRO A: Gas chromatography–mass spectrometry analysis of endogenous cannabinoids in healthy and tumoral human brain and human cells in culture. *J. Neurochem.* (2001) 76:594-601.

94. PAGOTTO U, MARSICANO G, FEZZA F *et al.*: Normal human pituitary gland and pituitary adenomas express cannabinoid receptor Type 1 and synthesize endogenous cannabinoids: first evidence for a direct role of cannabinoids on hormone modulation at the human pituitary level. *J. Clin. Endocrinol. Metab.* (2001) 86:2687-2696.

95. LIGRESTI A, BISOGNO T, MATIAS I *et al.*: Possible endocannabinoid control of colorectal cancer growth. *Gastroenterology* (2003) 125:677-687.

96. ZHU LX, SHARMA S, STOLINA M *et al.*: Δ-9-Tetrahydrocannabinol inhibits anti-tumor immunity by a CB_2 receptor-mediated, cytokine-dependent pathway. *J. Immunol.* (2000) 165:373-380.

97. TASHKIN DR, BALDWIN GC, SARAFIAN T, DUBINETT S, ROTH MD: Respiratory and immunologic consequences of marijuana smoking. *J. Clin. Pharmacol.* (2002) 42:S71-S81.

98. JORDA MA, RAYMAN N, VALK P, DE WEE E, DELWEL R: Identification, characterization and function of a novel oncogene: the peripheral cannabinoid receptor CB_2. *Ann. NY Acad. Sci.* (2003) 996:10-16.

99. JOOSTEN M, VALK PJ, JORDA MA *et al.*: Leukemic predisposition of pSca-1/CB_2 transgenic mice. *Exp. Hematol.* (2002) 30:142-149.

100. MELCK D, BISOGNO T, DE PETROCELLIS L *et al.*: Unsaturated long-chain N-acyl-vanillyl-amides (N-AVAMs): vanilloid receptor ligands that inhibit anandamide-facilitated transport and bind to CB_1 receptors. *Biochem. Biophys. Res. Commun.* (1999) 262:275-284.
- Synthesis of novel antitumour agents to target the endocannabinoid system.

101. DI MARZO V, BISOGNO T, MELCK D *et al.*: Interactions between synthetic vanilloids and the endogenous cannabinoid system. *FEBS Lett.* (1998) 436:449-454.

Affiliation

Sarah Jones BSc & John Howl PhD[†]
[†]Author for correspondence
Molecular Pharmacology Group,
Biomedical Sciences Division, School of Applied Sciences, University of Wolverhampton,
Wulfruna Street, WV1 1SB, UK
Tel: +44 (0) 1902 321131;
Fax: +44 (0) 1902 322714;
E-mail: J.Howl@wlv.ac.uk

DOI: 10.1093/brain/awg143 Advanced Access publication April 8, 2003 *Brain* (2003), **126**, 1252–1270

INVITED REVIEW

Cannabis and the brain

Leslie Iversen

Department of Pharmacology, University of Oxford, Oxford, UK

Correspondence to: Leslie Iversen, Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK
E-mail: les.iversen@pharm.ox.ac.uk

Summary

The active compound in herbal cannabis, Δ^9-tetrahydrocannabinol, exerts all of its known central effects through the CB_1 cannabinoid receptor. Research on cannabinoid mechanisms has been facilitated by the availability of selective antagonists acting at CB_1 receptors and the generation of CB_1 receptor knockout mice. Particularly important classes of neurons that express high levels of CB_1 receptors are GABAergic interneurons in hippocampus, amygdala and cerebral cortex, which also contain the neuropeptides cholecystokinin. Activation of CB_1 receptors leads to inhibition of the release of amino acid and monoamine neurotransmitters. The lipid derivatives anandamide and 2-arachidonylglycerol act as endogenous ligands for CB_1 receptors (endocannabinoids). They may act as retrograde synaptic mediators of the phenomena of depolarization-induced suppression of inhibition or excitation in hippocampus and cerebellum. Central effects of cannabinoids include disruption of psychomotor behaviour, short-term memory impairment, intoxication, stimulation of appetite, antinociceptive actions (particularly against pain of neuropathic origin) and anti-emetic effects. Although there are signs of mild cognitive impairment in chronic cannabis users there is little evidence that such impairments are irreversible, or that they are accompanied by drug-induced neuropathology. A proportion of regular users of cannabis develop tolerance and dependence on the drug. Some studies have linked chronic use of cannabis with an increased risk of psychiatric illness, but there is little evidence for any causal link. The potential medical applications of cannabis in the treatment of painful muscle spasms and other symptoms of multiple sclerosis are currently being tested in clinical trials. Medicines based on drugs that enhance the function of endocannabinoids may offer novel therapeutic approaches in the future.

Keywords: cannabinoid CB_1 receptor; Δ^9-tetrahydrocannabinol; rimonabant (SR141716A); anandamide; 2-arachidonylglycerol

Abbreviations: 2-AG = 2-arachidonylglycerol; DSI = depolarization-induced suppression of inhibition; FAAH = fatty acid amide hydrolase; Gi/o = G-proteins negatively linked to adenylate cyclase or to inositol phosphates; LTD = long-term depression; LTP = long-term potentiation; mGlu = metabotropic glutamate; NMDA = *N*-methyl-D-aspartate; THC = Δ^9-tetrahydrocannabinol

Introduction

A large literature exists on the effects of cannabis, with many of the earlier studies conducted in human subjects (Mendelson *et al.*, 1976; Jones, 1978; Hollister, 1986). Unfortunately, much of this research would now be regarded as inadequately controlled and poorly designed. However, research on cannabis has been stimulated in recent years by the recognition that specific receptors exist in the brain that recognize cannabinoids, and by the discovery of a series of endogenous cannabinoids that act as ligands for these receptors. As was the case with opiate research in the 1970s, research on a psychoactive drug of plant origin has revealed a hitherto unknown physiological control mechanism. This review will focus mainly on the more recent literature in this field.

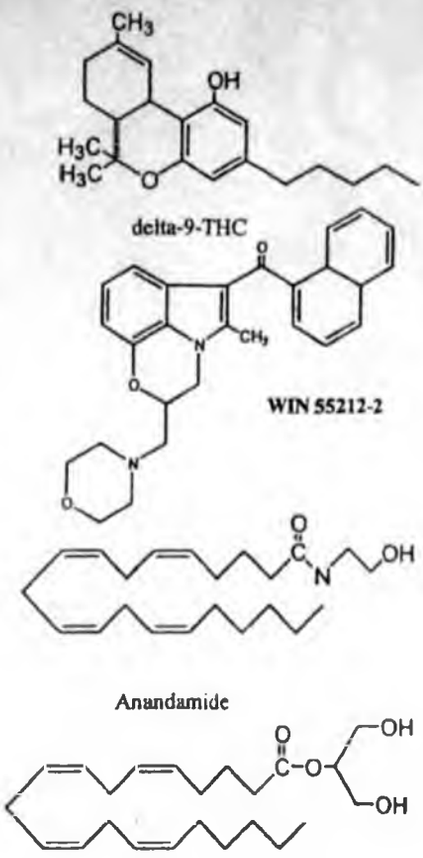
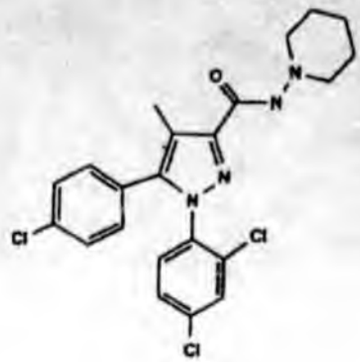


Fig. 1 Chemical structures of THC, the synthetic CB_1 receptor agonist WIN 55,2122 and the endocannabinoids.



SR141716A

Fig. 2 Chemical structure of the CB_1 selective antagonist drug rimonabant (SR141716A).

The cannabinoid system in brain

Exogenous cannabinoids and their receptors

The principal active component in the complex mixture of cannabinoids present in extracts of the plant *Cannabis sativa* is Δ^9-tetrahydrocannabinol (THC) (Mechoulam, 1970) (Fig. 1). THC is a sticky resin that is not soluble in water. Smoking remains the most efficient means of delivering the drug and experienced users can titrate the dose by adjusting the frequency and depth of inhalation (Iversen, 2000). THC or cannabis extracts can also be taken orally in fat-containing foods or dissolved in a suitable pharmaceutical oil, but absorption is delayed and variable (Iversen, 2000). A series of man-made synthetic cannabinoids, some of which are more potent and more water soluble than THC, is also available (Pertwee, 1999) (Fig. 1). All of these compounds act as agonists at the CB_1 cannabinoid receptor (Matsuda *et al.*, 1990), which is the only one known to be expressed in the brain. A second cannabinoid receptor, CB_2, is expressed only in peripheral tissues, principally in the immune system (Munro *et al.*, 1993; Felder and Glass, 1998; Pertwee, 1999). THC and the synthetic cannabinoids also act to some extent as agonists at the CB_2 receptor. Both cannabinoid receptors are members of the G-protein coupled class, and their activation is linked to inhibition of adenylate cyclase activity (Howlett *et al.*, 1988). A series of synthetic drugs is also now available that act as specific antagonists at CB_1 or CB_2 receptors (D'Souza and Kosten, 2001). One of these compounds, rimonabant(SR141716A), which acts selectively to block CB_1 receptors (Rinaldi-Carmona *et al.*, 1994; Compton *et al.*, 1996), has been widely used in studies of the actions of cannabinoids in the CNS (Fig. 2).

Endogenous cannabinoids

Following the discovery of specific cannabinoid receptors, a search was made for naturally occurring ligands of these receptors in mammalian tissues. This led to the discovery of a series of arachidonic acid derivatives with potent actions at cannabinoid receptors. These are: anandamide (*N*-arachidonyl-ethanolamine; Devane *et al.*, 1992), 2-arachidonylglycerol (2-AG; Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995; Stella *et al.*, 1997) and 2-arachidonylglyceryl ether (Hanuš *et al.*, 2001) (Fig. 1). Of these, anandamide is the ligand that has been most extensively studied so far. The endogenous cannabinoids known as 'endocannabinoids' are present only in small amounts in the brain or other tissues. Like other lipid mediators (e.g. prostaglandins) they appear to be synthesized and released locally on demand (see below). Anandamide and the other endogenous cannabinoids are rapidly inactivated by a combination of a transporter mechanism and by the enzyme fatty acid amide hydrolase (FAAH) (Di Marzo *et al.*, 1994; Piomelli *et al.*, 1998; Giuffrida *et al.*, 2001). Genetically engineered mice lacking FAAH displayed elevated levels of anandamide in brain and were supersensitive to the biological actions of anandamide (Cravatt *et al.*, 2001). The discovery of agents that could interfere with the inactivation of endogenous cannabinoids may provide a novel means of pharmaco-

Fig. 3 Distribution of cannabinoid CB_1 receptors in rat brain revealed by an autoradiograph of the binding of radioactively labeled CP-55940 (a high affinity agonist ligand) to a sagittal brain section. The brain regions labelled are: Cb = cerebellum; CbN = deep cerebellar nucleus; cc = corpus callosum; EP = entopeduncular nucleus; fi = fimbria hippocampus; Fr = frontal cortex; FrPaM = frontoparietal cortex motor area; GP = globus pallidus; Hi = hippocampus; IC = inferior colliculus; LP = lateral posterior thalamus; Me = medial amygdaloid nucleus; PO = primary olfactory cortex; PCRt = parvocellular reticular nucleus; SNR = substantia nigra reticulate; Tu = olfactory tubercle; VP = ventroposterior thalamus. Photograph kindly supplied by Dr Miles Herkenham, National Institute of Mental Health, USA.

logically modifying cannabinoid function in the brain (Piomelli *et al.*, 2000).

Neuroanatomical distribution of CB_1 receptors in brain

The distribution of cannabinoid receptors was first mapped in rat brain in autoradiographic studies, using the radioligand [H^3]CP-55,940, which binds with high affinity to CB_1 sites (Herkenham *et al.*, 1991) (Fig. 3). The validity of using this radioligand was confirmed by autoradiographic studies in CB_1 receptor knockout mice, in which no detectable [H^3]CP-55,940 binding sites were observed (Zimmer *et al.*, 1999). More recently, antibodies that target the C- or N-terminal regions of the CB_1 receptor protein have been used for immunohistochemical mapping studies (Egertová *et al.*, 1998; Pettit *et al.*, 1998; Egertová and Elphick, 2000). Immunohistochemistry provides a superior degree of spatial resolution to autoradiography, but the overall pattern of distribution of CB_1 receptors revealed by the two approaches is very similar (Elphick and Egertová, 2001).

The mapping studies in rat brain showed that CB_1 receptors are mainly localized to axons and nerve terminals and are largely absent from the neuronal soma or dendrites. The finding that cannabinoid receptors are predominantly presynaptic rather than postsynaptic is consistent with the postulated role of cannabinoids in modulating neurotransmitter release (see below).

In both animals and man the cerebral cortex, particularly frontal regions, contains high densities of CB_1 receptors. There are also very high densities in the basal ganglia and in the cerebellum (Fig. 3). In the limbic forebrain CB_1 receptors are found particularly in the hypothalamus and in the anterior cingulate cortex. The hippocampus also contains a high density of CB_1 receptors. The relative absence of the cannabinoid receptors from brainstem nuclei may account for the low toxicity of cannabinoids when given in overdose.

The regional distribution of the CB_1 receptor in brain correlates only poorly with the levels of anandamide and other endocannabinoids in different brain regions (Felder *et al.*, 1996; Bisogno *et al.*, 1999). However, measurements of endocannabinoids have yielded variable results, and a strict correlation would not be expected for ligands that are only produced on demand. There is a better correlation between the regional distribution of CB_1 receptors and the enzyme FAAH. FAAH is widely distributed in CNS and other tissues, suggesting that its role is not confined to inactivating endogenous cannabinoids. Nevertheless, particularly high levels of FAAH were found in brain regions that are enriched in CB_1 receptors, and immunohistochemical staining suggested a complementary relationship between FAAH and CB_1 receptors at the synaptic level (Egertová *et al.*, 1998; Elphick and Egertová, 2001). In cerebellum, hippocampus and neocortex FAAH was expressed at high levels in the somato-dendritic regions of neurons that were postsynaptic to CB_1-positive axon terminals. The close and complementary relationship between CB_1 receptors and FAAH led to the hypothesis that FAAH may participate in the inactivation of endogenous cannabinoids released locally at synapses

(Elphick and Egertová, 2001). These authors postulated a retrograde cannabinoid signalling mechanism, whereby endogenous cannabinoids are released in response to synaptic activation, feedback to presynaptic receptors on these axon terminals, and are subsequently inactivated by FAAH after their uptake into the postsynaptic compartment. This hypothesis has been supported independently by neurophysiological findings, as described below.

Effects of cannabinoids on synaptic function

Inhibition of neurotransmitter release

The presynaptic localization of CB_1 receptors suggests a role for cannabinoids in modulating the release of neurotransmitters from axon terminals, and this has been confirmed by a substantial body of experimental data. Early reports (Gill *et al.*, 1970; Roth, 1978) showed that THC inhibited acetylcholine release from electrically stimulated guinea pig ileum. Similar inhibitory effects of THC and other cannabinoids on the release of a variety of neurotransmitters from CNS neurons have been observed in many subsequent studies (Schlicker and Kathmann, 2001). The neurotransmitters involved include L-glutamate, GABA, noradrenaline, dopamine, 5-HT and acetylcholine. The brain regions most often studied *in vitro*, usually in tissue slice preparations, have been cerebellum, hippocampus or neocortex. Neurotransmitter release has been studied directly in superfused preparations, and indirectly by measuring postsynaptic currents. Although most of these studies involved rat or mouse brain, a few studies have shown similar results using human brain tissue (Katona *et al.*, 2000; Schlicker and Kathmann, 2001). Because THC is only poorly water soluble, the more soluble synthetic CB_1 receptor agonists WIN552123, HU210 or CP55-2940 were used in these *in vitro* studies. The specificity of the cannabinoid effects were confirmed by demonstrating that the inhibitory effects of the agonists were completely blocked by the CB_1-selective antagonist rimonabant.

The cellular mechanisms involved in the inhibition of neurotransmitter release by cannabinoids remain unclear. Some have suggested that there is a direct inhibitory effect of CB_1 receptor activation on N-type Ca^{2+} currents (Caulfield and Brown, 1992; MacKie and Hill, 1992). However, the effect appears more likely to involve sites downstream of voltage-dependent Ca^{2+} channels, since a number of studies have shown that cannabinoids reduce the frequencies of miniature excitatory or inhibitory synaptic currents, which are Ca^{2+} independent, rather than altering their amplitude, which is Ca^{2+} sensitive (Schlicker and Kathmann, 2001). Deadwyler *et al.* (1995) suggested that the inhibitory effect of CB_1 receptor activation on adenylate cyclase activity causes a decreased phosphorylation of A-type K^+ channels by the cAMP-dependent enzyme protein kinase A. This, in turn, would activate the A-type K^+ channels and cause a shortening of the duration of presynaptic action potentials as they invade axon terminals.

Biosynthesis of endocannabinoids

Despite their similar chemical structures, the endocannabinoids are produced through distinct biochemical pathways. The formation of anandamide is thought to result from the hydrolysis of the precursor *N*-arachidonoyl phophatidylethanolamine, catalysed by the phosphodiesterase enzyme phospholipase D (Di Marzo *et al.*, 1994; Cadas *et al.*, 1997). 2-AG, on the other hand, is produced by cleavage of an inositol-1,2-diacylglycerol, catalysed by phospholipase C. Although both anandamide and 2-AG can activate CB_1 receptors, it is not clear whether both function as endocannabinoids, and whether their synthesis and release are independently controlled. The levels of 2-AG found in brain (2–10 nmol/g) are 50–1000 times higher than those of anandamide (10–50 pmol/g). There is some evidence for separate control of their biosynthesis. Stimulation of glutamate release from Schaffer collaterals in rat hippocampal slices increased levels of 2-AG, but not anandamide (Stella *et al.*, 1997). On the other hand, another study using *in vivo* microdialysis probes showed that local administration of the dopamine D2 receptor agonist quinpirole caused an increased release of anandamide from rat striatum without affecting levels of 2-AG (Giuffrida *et al.*, 1999). Indeed, despite the much higher tissue levels of 2-AG relative to anandamide and the availability of a very sensitive assay, no 2-AG could be detected at all in the striatal dialysate samples. In cultured rat cortical neurons activation of Ca^{2+} influx by stimulation of glutamate *N*-methyl-D-aspartate (NMDA) receptors caused an increase in 2-AG formation but not anandamide (Stella and Piomelli, 2001). However, if NMDA activation was combined with a cholinergic agonist (carbachol) the formation of both endocannabinoids was increased. In both cases Ca^{2+} influx was required for endocannabinoid synthesis. It is clear that much remains to be learned about the relative roles played by the different endocannabinoids. The biosynthesis of the most recently discovered third endocannabinoid, 2-arachidonylglyceryl ether, remains to be characterized.

Endogenous cannabinoids act as retrograde signal molecules at synapses

Important new insights into the physiological role of cannabinoids has emerged from neurophysiological studies published independently by three different research groups in 2001. A phenomenon known as depolarization-induced suppression of inhibition (DSI) has been known to neurophysiologists for some years (Alger and Pitler, 1995). It is a form of fast retrograde signalling from postsynaptic neurons back to inhibitory cells that innervate them, and is particularly prominent in the hippocampus and cerebellum. Three prop-

erties of DSI suggested to Wilson and Nicoll (2001) that a cannabinoid mechanism might be involved. First DSI, like endocannabinoid synthesis, requires Ca^{2+} influx into the postsynaptic neuron (Lenz *et al.*, 1998). Secondly, DSI is probably presynaptic, since the sensitivity of the postsynaptic cell to GALA is unaffected (Pitler and Alger, 1992). Finally, DSI is blocked by pertussin toxin, which interacts with the Gi-proteins negatively linked adenylate cyclase or to inositol phosphates (Gi/o) protein to which the CB_1 receptor is coupled (Pitler and Alger, 1994). Wilson and Nicoll (2001) used slice preparations of rat hippocampus and induced DSI by brief depolarizing steps in the holding potential of voltage clamped CA1 pyramidal neurons. They found that DSI was completely blocked by the cannabinoid CB_1 receptor antagonists AM251 or rimonabant and could be mimicked by application of the CB_1 receptor agonist WIN55,2122, but the continued presence of the agonist prevented DSI by occlusion. Wilson and Nicoll (2001) were also able to show by recording from pairs of nearby CA1 neurons that depolarizing one of these neurons caused DSI to spread and affect adjacent neurons up to 20 μm away. They suggested that the small, lipid-soluble, freely diffusible endocannabinoids act as retrograde synaptic signals that can affect axon terminals in sphere of influence some 40 μm in diameter.

Ohno-Shosaku *et al.* (2001) came to a similar conclusion using a different experimental paradigm. They recorded from pairs of cultured hippocampal neurons with inhibitory synaptic connections. They found that depolarization of the postsynaptic neurons lead to DSI in approximately two-thirds of the neuron pairs, and showed that this was due to inhibition of GABA release. Those that exhibited DSI, but not the others, proved to be sensitive to the CB_1 receptor agonist WIN55,2122, which mimicked the inhibitory effect of DSI. Both DSI and the cannabinoid effect could be blocked by the CB_1 receptor antagonists AM-281 or rimonabant.

Further support for the conclusion that a cannabinoid-mediated mechanism underlies DSI came from Varma *et al.* (2001), who found that DSI was completely absent in hippocampal slices prepared from CB_1 receptor knockout mice (Ledent *et al.*, 1999). Varma *et al.* (2001) also reported that agonists which stimulate metabotropic glutamate (mGlu) receptors enhanced DSI, whereas the broad-spectrum antagonist of mGlu receptors, LY341495, tended to reduce DSI, suggesting that glutamate may also be involved. Interestingly, Varma *et al.* (2001) found that mGlu agonists failed to have any effect on DSI in the CB_1 knockout animals, suggesting that glutamate acts to enhance the endocannabinoid signal.

Retrograde signalling by endocannabinoids is not restricted to the inhibitory inputs to postsynaptic neurons. Kreitzer and Regehr (2001*a*) showed that depolarization of rat cerebellar Purkinje cells leads to a transient inhibition of excitatory inputs from parallel fibre and climbing fibre inputs, a phenomenon described as depolarization-induced suppression of excitation (DSE). They found that DSE was triggered by Ca^{2+} influx into the Purkinje cells, and could be completely blocked by the CB_1 antagonist AM-251, and mimicked and occluded by the CB_1 receptor agonist WIN55,2122. Kreitzer and Regehr (2001*b*) went on to show that inhibitory inputs to rat cerebellar Purkinje cells from basket cells and stellate cells were subject to DSI, and that this was also blocked by AM-251 and occluded by WIN55,2122. The DSE phenomenon in the cerebellum is also linked to mGlu receptors. Maejima *et al.* (2001) reported that mGlu agonists acting on mouse Purkinje cells mimicked DSE, and the effects could be blocked by CB_1 antagonists.

These findings suggest that endocannabinoids are involved in the rapid modulation of synaptic transmission in CNS by a retrograde signalling system that can influence synapses in a local region of some 40 μm diameter, causing inhibitory effects on both excitatory and inhibitory neurotransmitter release that persist for tens of seconds. This may play an important role in the control of neural circuits, particularly in cerebellum and hippocampus (see below). Exogenously administered Δ^9-THC or other cannabinoids cannot mimic the physiological effects of locally released endocannabinoids. Since they cause long-lasting activation of CB_1 receptors in all brain regions, their overall effect is to cause a persistent inhibition of neurotransmitter release from those nerve terminals that express CB_1 receptors, and as a consequence they temporarily occlude and prevent the phenomena of DSI and DSE.

Effects of cannabinoids on CNS function

Psychomotor control

CB_1 receptors are expressed at particularly high densities in the basal ganglia and cerebellum, so it is not surprising that cannabinoids have complex effects on psychomotor function (reviewed by Rodriguez de Fonseca *et al.*, 1998). One of the earliest reports of the effects of cannabis extracts in experimental animals described the awkward swaying and rolling gait caused by the drug in dogs, with periods of intense activity provoked by tactile or auditory stimuli, and followed eventually by catalepsy and sleep (Dixon, 1899). In rodents cannabinoids tend to have a triphasic effect. Thus in rats low doses of THC (0.2 mg/kg) decreased locomotor activity, while higher doses (1–2 mg/kg) stimulated movements, and catalepsy emerged at doses of 2.5 mg/kg (Sañudo-Peña *et al.*, 2000). Similarly in mice, Adams and Martin (1996) described a 'popcorn effect' in animals treated with THC. Groups of mice are sedated by the drug, but will jump in response to auditory or tactile stimuli, as they fall into other animals these in turn jump, resembling corn popping in a popcorn machine. Interestingly, the CB_1 receptor antagonist rimonabant stimulated locomotor activity in mice, suggesting that there is tonic activity in the endocannabinoid system that contributes to the control of spontaneous levels of activity (Compton *et al.*, 1996).

These effects of cannabinoids may be due, in part, to actions at cerebellar or striatal receptors. Patel and Hillard (2001) used tests of specific cerebellar functions to show that

cannabinoids caused increased gait width and the number of slips on a bar cross test. DeSanty and Dar (2001) observed rotorod impairments in mice after direct injection of synthetic cannabinoids into the cerebellum. These defects were no longer seen in animals pretreated with cerebellar injections of an antisense olgonucleotide directed to a sequence in the CB_1 receptor.

In human subjects it is also possible to demonstrate that cannabis causes impaired performance in test of balance (Greenberg *et al.*, 1994), or in tests that require fine psychomotor control, for example tracking a moving point of light on a screen (Manno *et al.*, 1970). Human cannabis users may also seek isolation and remain immobile for long periods.

A number of authors have attempted to combine what is known of the neuroanatomical distribution of the cannabinoid system and the results of behavioural and electrophysiological studies to speculate on the mechanisms underlying cannabinoid modulation of psychomotor function (Breivogel and Childers, 1998; Sañudo-Peña *et al.*, 1999; Giuffrida *et al.*, 2000; Elphick and Egertová, 2001). The CB_1 receptor is expressed particularly by striatal GABAergic medium-spiny projection neurons, and is abundant in regions containing the axon terminals of these cells (globus pallidus, entopeduncular nucleus and substantia nigra reticulata, and in axon collaterals feeding back to medium-spiny projection neurons in striatum). CB_1 receptors are also abundant on the terminals of glutamatergic projection neurons from the subthalamic nucleus to globus pallidus, entopeduncular nucleus and substantia nigra reticulata. Cannabinoids might thus be expected to inhibit GABA release in striatum and GABA and glutamate release in the other nuclei. Sañudo-Peña *et al.* (1999) suggested that the primary role of the endocannabinoid system may be to inhibit tonic release of glutamate in the substantia nigra, regulating levels of basal motor activity. Exogenous cannabinoids also lead to decreased GABA release in substantia nigra, which could lead to a disinhibition of the inhibitory nigral input to the thalamocortical pathway, resulting in inhibition of movement. To what extent the effects of cannabinoids on motor function are due to actions in the cerebellum remains unclear, although as described above it is likely that effects on posture and balance are mediated in this brain region. As described previously, CB_1 receptors are known to occur abundantly on nearly all of the principal excitatory (glutamatergic) and inhibitory (GABAergic) inputs to cerebellar Purkinje cells.

The results of eliminating the expression of CB_1 receptors in knockout mice have yielded conflicting results. The knockout animals studied by Zimmer *et al.* (1999) displayed reduced levels of basal activity, in support of the hypothesis put forward by Sañudo-Peña *et al.* (1999), suggesting that tonic activation of CB_1 receptors promotes movement. However, the CB_1 knockout animals studied by Ledent *et al.* (1999) showed no change in spontaneous activity, and in some tests they exhibited increased motor activity. This is in line also with the observations of Compton *et al.* (1996) that the CB_1 antagonist SR141716 caused an increase in locomotor activity. The reasons for the discrepant findings in different strains of CB_1 knockout mice are unknown. Clearly, there is as yet only a poor understanding of the actions of cannabinoids in the basal ganglia and cerebellum. Interactions with other chemical signalling systems in the brain are likely to be important. Giuffrida *et al.* (1999) showed, for example, that dopamine D2 receptor agonists caused an increase in anandamide synthesis and release in striatum. Deadwyler *et al.* (1995) described the convergence of multiple presynaptic controls on the terminals of granule cells in cerebellum. In addition to the CB_1 receptor, these terminals also express high densities of kappa opioid, adenosine A_1 and GABA-B receptors, all of which are coupled through a similar Gi/o type G-protein to inhibit adenylate cyclase and are capable of inhibiting glutamate release. Such complexities are likely to prove the norm.

There is anecdotal evidence that cannabis can relieve muscle pain and spasticity in patients suffering from multiple sclerosis (Consroe *et al.*, 1996). Experimental data obtained by Baker *et al.* (2000) in an animal model of multiple sclerosis appears to support such claims. Mice immunized with myelin antigens develop spasticity and tremor. Both symptoms were ameliorated by administration of cannabinoids, and the symptoms were exacerbated by rimonabant, suggesting the involvement of CB_1 receptors and tonic activity in the endocannabinoid system. Controlled clinical trials of cannabis-based medicines for the treatment of multiple sclerosis are currently under way.

Cannabinoid mechanisms in the hippocampus and effects on memory

One of the well established effects of acute intoxication with cannabis in man is an impairment of short-term memory (the extensive literature on human studies is reviewed by Jones, 1978; Miller and Branconnier, 1983; Solowij, 1998; Earleywine, 2002). Many studies have shown significant effects on short-term memory, particularly when tests were used that depend heavily on attention (Abel, 1971; Mendelson *et al.*, 1976). Animal studies have also found that THC, synthetic cannabinoids and anandamide cause deficits in short-term memory in spatial learning tasks (for a review see Hampson and Deadwyler, 1999). These include delayed matching or non-matching tests in rodents (Mallet and Beninger, 1998; Hampson and Deadwyler, 1999), performance in a radial arm maze (Stiglick and Kalant, 1985; Lichtman and Martin, 1996), and a fixed ratio food acquisition task in squirrel monkeys (Nakamura-Palacios *et al.*, 2000). The effects of both cannabinoids (Lichtman and Martin, 1996) and anandamide (Mallet and Beninger, 1998) were reversed by rimonabant, indicating that they are mediated by the CB_1 receptor.

A probable site for these effects is the hippocampus. Hampson and Deadwyler (1999) claimed that the effects of the treatment of rats with cannabinoids on short-term memory in a delayed non-matching to sample test were equivalent to the effects seen after surgical removal of the hippocampus. In each case the animals were unable to segregate information between trials in the task because of disruptions to the processing of sensory information in hippocampal circuits. CB_1 receptors are expressed at high densities in the hippocampus. They are particularly abundant on the terminals of a sub-set of GABAergic basket cell interneurons, which also contain the neuropeptide cholecystokinin (Katona *et al.*, 1999), and this is also the case in human hippocampus (Katona *et al.*, 2000). These are presumably the GABAergic neurons involved in the endocannabinoid-mediated DSI phenomenon described above. The terminals of these cells surround large pyramidal neuron somata in the CA1–CA4 fields. GABAergic neurons in the dentate gyrus also express CB_1 receptors, with terminals concentrated at the boundary of the molecular and granule cell layers (Egertová and Elphick, 2000). In addition CB_1 receptors are expressed, at a lower level, in the glutamatergic pyramidal cells and their terminals. Cannabinoids can thus inhibit both the release of GABA and glutamate in hippocampal circuits.

The mechanisms underlying synaptic plasticity have been studied more intensely in the hippocampus than in any other brain region. In particular, the electrophysiological phenomena of long-term potentiation (LTP) and long-term depression (LTD) are thought to be involved in memory formation at glutamatergic synapses in the hippocampus. A number of studies have shown clearly that cannabinoids inhibit the induction of both LTP and LTD (for review see Elphick and Egertová, 2001). Cannabinoids appear to work by reducing glutamate release below the level needed to activate NMDA receptors, a requirement for LTP and LTD (Shen *et al.*, 1996; Misner and Sullivan, 1999). Although the actions of cannabinoids in reducing GABA release from hippocampal interneurons might have been expected to increase the level of excitability of hippocampal pyramidal cells, it seems that the cannabinoid-induced reduction in glutamate release predominates. The administration of exogenous cannabinoids is, of course, wholly unphysiological and cannot mimic the effects of endocabinnoids that are released in discrete local regions in response to particular patterns of afferent inputs. CB_1 receptors are capable of regulating both inhibitory and excitatory neurotransmitter release in the hippocampus and are thus capable of subtle control of synaptic plasticity. The CB_1-containing GABergic interneurons are thought to control oscillatory electrical activity in the hippocampus in the theta and gamma frequencies, which plays a role in synchronizing pyramidal cell activity (Hoffman and Lupica, 2000). CB_1 agonists decrease the power of such oscillations in hippocampal slices (Hájos *et al.*, 2000) and may thus influence the synchronous activity of pyramidal cells. The physiological importance of cannabinoid-mediated DSI may be to decrease GABAergic inhibition of these cells and thus facilitate learning when hippocampal inputs are active (Wilson and Nicoll, 2001).

One approach to answering the question of what role the tonic release of endocannabinoids may play in hippocampal function has been to examine the effects of CB_1 receptor knockout or of selective CB_1 receptor antagonists. Unfortunately, these studies have so far yielded conflicting results. Bohme *et al.* (2000) reported a significant enhancement of LTP in CB_1 knockout mice, and Reibaud *et al.* (1999) found a significant enhancement of memory in such animals. However, tests with the CB_1 antagonist rimonabant showed no effects on LTP (Terranova *et al.*, 1995) or on learning and memory in a spatial learning task (Mallet and Beninger, 1998), although Terranova *et al.* (1996) reported that rimonabant enhanced memory in a short-term olfactory memory test in rats (social recognition test).

Cannabinoids and the neocortex

Like other intoxicant drugs cannabis causes profound changes in a variety of higher brain functions. The literature on the acute effects of the drug in human subjects is large, and can only be summarized here (for reviews see Jones, 1978; Solowij, 1998; Iversen, 2000; Earleywine, 2002). The distribution of CB_1 receptors in the neocortex has been described in detail (Herkenham *et al.*, 1991; Egertová and Elphick, 2000). As in the hippocampus, the majority of cortical interneurons expressing high levels of CB_1 receptor are GABAergic cells, which also express cholecystokinin (Marsicano and Lutz, 1999). CB_1-positive terminals are concentrated in layers II–III and layers V–VI, with few in layers I or IV. Despite the obvious importance of the abundant CB_1 receptors in the neocortex there have so far been few electrophysiological studies of their effects on neural activity.

The earlier literature, however, contains several reports of the effects of acute and chronic cannabis use on EEG activity, both in man and animals (reviewed by Adams and Martin, 1996; Solowij, 1998). Most studies in man have observed changes consistent with a state of drowsiness, with increases in relative and absolute α power particularly in frontal regions of cortex. In contrast, the CB_1 antagonist rimonabant was shown to induce EEG changes characteristic of arousal in rats, and increased the time spent in wakefulness as opposed to sleep (Santucci *et al.*, 1996). Mechoulam *et al.* (1997) have suggested that anandamide may play a role in the control of the sleep–waking cycle.

Studies of the effects of cannabis on perceptual abilities have yielded a variety of often conflicting results. While users often report a subjective enhancement of visual and auditory perception, sometimes with synesthesia (sounds take on visual colourful qualities), laboratory studies have usually not shown marked changes in visual or auditory perception. One subjective effect that has been confirmed is the sensation that cannabis users experience time as passing more quickly relative to real time. In laboratory tests subjects overestimate

the amount of elapsed time when asked to estimate, or produce shorter than required intervals when asked to signal a period of elapsed time (Hicks *et al.*, 1984; Mathew *et al.*, 1998). This curious effect can also be seen in rats trained to respond for food reward using a fixed interval schedule. When treated with THC or WIN55,2122 the animals shortened their response interval, whereas the antagonist rimonabant lengthened this interval (Han and Robinson, 2001).

There have been many studies of the acute and chronic effects of cannabis on human cognitive function (Jones, 1978; Solowij, 1998; Earleywine, 2002). Performance on a variety of tests of cognitive function is impaired by the drug, but by comparison with alcohol the effects of cannabis are subtle. Whereas even moderate doses of alcohol, for example, impair reaction time, most studies with cannabis have failed to show consistent effects on measures of simple reaction time. Thus the drug's ability to disrupt cognitive function cannot be due to an inability to respond promptly. Among the impairments of cognitive function that have been observed in many, but not all, human studies are: decreased ability to inhibit responses, decreased vigilance, especially for long and boring tasks, decreased ability to perform complex mental arithmetic and impairments in tests of complex reaction times. On the other hand, intoxicated subjects can perform simple arithmetic, learn simple lists of words and recall memories laid down earlier.

Other studies have addressed the question of whether more severe deficits in cognitive function might develop in chronic heavy users of cannabis, or in animals treated for prolonged periods with the drug. The human studies are fraught with difficulties, as described in detail by Earleywine (2002). Among the confounding factors in human studies are that comparisons have to be made between groups of drug users versus non-users, but it is usually impossible to compare the baseline performance of these groups prior to cannabis use to see if they are properly matched. Statistical analysis of such data has often been poor, common errors being the use of so many different tests that the likelihood of finding some significant differences is increased, or the use of inadequate sample sizes. Other drug use can also confound the data. Results have been very variable. Some studies in long-term very heavy users of cannabis (10–20 joints per day for more than 10 years) in Jamaica (Bowman and Pihl, 1973) and Costa Rica (Satz *et al.*, 1976) failed to show any significant difference between users versus non-users using a battery of test assessments of cognitive function, and similar negative results were reported in some studies of US college students (Earleywine, 2002). However, most reports have shown that there are deficits in the performance of complex cognitive tasks in long-term cannabis users, although there is little evidence that these are qualitatively or quantitatively more severe than those seen after acute use of the drug (Earleywine, 2002).

Even more controversial is the question of whether long-term cannabis use can cause irreversible deficits in higher brain function that persists after drug use stops. Many studies have suffered from poor design. It is not sufficient to identify a group of cannabis users and simply to test them after stopping cannabis use. Pope *et al.* (2001), for example, recruited 63 current heavy users, who had smoked cannabis at least 5000 times in their lives, and 72 control subjects. Subjects underwent a 28-day washout from cannabis use, monitored by urine assays. At days 0, 1 and 7 the heavy users scored significantly below control subjects on a battery of neuropsychological tests, particularly in recall of word lists. However, by day 28 there were virtually no differences between the groups on any of the test results, and no significant association between cumulative lifetime cannabis use and test scores. The fact that drug-induced effects on cognitive performance can persist for up to a week after stopping the drug (perhaps because of the persistence of THC in the body, or because of a subtle withdrawal syndrome) means that many earlier studies that did not allow a sufficiently long washout period may be invalid. On the other hand, some well designed studies have shown subtle persistent cognitive deficits in ex-cannabis users. Solowij (1998) recruited a group of people who had used cannabis regularly for at least 5 years but who had stopped on average 2 years before the experiment. The subjects were given a very difficult task. They had to listen to a series of tones, some in the right ear some in the left; the tones were long or short (but differing by only 51 ms) and high or low pitch (but differing very little). Participants had to press a button as fast as possible in response to longer tones of a specified pitch in the correct ear. Previous research using this paradigm showed that current regular cannabis users had difficulty in discriminating between the tones. Measurements of event-related potentials also revealed small but significant abnormalities in the P300 wave (Solowij, 1998). The ex-users continued to make significant errors in the discrimination task, but they showed normal P300 waves. The conclusion of these and many other studies in ex-users seems to be that regular cannabis use can cause small but significant impairments in cognitive function that may persist after drug use stops. Such impairments appear to be associated with long-term heavy use of the drug and are unlikely to affect most recreational users.

Effects of cannabinoids on hypothalamic control of appetite

Many subjective reports suggest that cannabis intoxication is associated with an increased appetite, particularly for sweet foods, even in subjects who were previously satiated. This effect can be confirmed under laboratory conditions (Hollister, 1971; Mattes *et al.*, 1994), although results from studies in human subjects have tended to be variable, perhaps because the increased appetite is focused on certain types of food. Nevertheless, controlled clinical trials showed that THC (dronabinol) had significant beneficial effects in counteracting the loss of appetite and reduction in body weight in patients suffering from the AIDS-related wasting syndrome (Beal *et al.*, 1995), and this is one of the medical indications for which the drug has official approval in the USA.

THC also stimulates food intake in experimental animals, and again the effect is specific for high-fat or sweet high-fat diets, and is not seen in animals offered standard rat chow (Koch, 2001). The endocannabinoid anandamide also stimulates food intake in rats, and the effect is blocked by rimonabant (Williams and Kirkham, 1999). Conversely the CB_1 antagonist rimonabant given on its own suppressed food intake and led to reduced body weight in adult non-obese rats (Colombo *et al.*, 1998). These results suggest that cannabinoids may play a role in the regulation of food intake and body weight (Mechoulam and Fride, 2001). A possible reciprocal link between endocannabinoid mechanisms and the appetite-suppressing hormone leptin was suggested by Di Marzo *et al.* (2001*a*). They found that food-deprived CB_1 receptor knockout mice eat less than their wild-type litter mates, and the CB_1 antagonist rimonabant reduced food intake in the wild-type animals but not in the knockouts. Animals with defective leptin signalling (obese db/db or ob/ob mice and Zucker rats) exhibited elevated hypothalamic levels of anandamide and 2-AG. On the other hand, treatment of normal rats or ob/ob (leptin deficient) mice with leptin caused decreases in hypothalamic levels of the endocannabinoids. These findings suggest that hypothalamic endocannabinoids may play an important role in mediating the appetite-suppressant effects of leptin. At some stages during development these effects of endocannabinoids may be of critical importance. Fride *et al.* (2001) found that administration of the CB_1 antagonist rimonabant to new-born mouse pups had a devastating effect in decreasing milk ingestion and growth, continuing treatment with the antagonist led to death within 4–8 days. The effect of rimonabant could be almost fully reversed by co-administering THC.

Cannabinoids as anti-emetic agents

The ability of THC and the synthetic cannabinoid nabilone to control the nausea and vomiting associated with cancer chemotherapy is one of the few well documented medical applications for these drugs (for reviews of the controlled clinical trials see Vincent *et al.*, 1983; British Medical Association, 1997; Joy *et al.*, 1999; and the meta-analysis reported by Tramèr *et al.*, 2001). THC (dronabinol) and nabilone were approved for medical use in the USA, although neither drug has found much utility. The narrow window between the anti-emetic dose and that causing unwanted psychic effects made these drugs difficult to use. The advent of serotonin 5-HT_3 receptor antagonists as new and more powerful anti-emetic drugs that were free of unwanted psychic effects during the 1980s also made the cannabinoids less attractive.

Studies in experimental animals have confirmed that the anti-emetic effects of cannabinoids are mediated through CB_1 receptors (Darmani, 2002), and in some susceptible species (e.g. the least shrew) the CB_1 antagonist rimonabant is emetic, an effect that can be blocked by THC or WIN55,2122 (Darmani, 2001).

Cannabinoids and pain

Cannabis was widely used in 19th century medicine for pain relief and there is renewed interest in cannabis-based medicines, with pain as one of the key therapeutic targets (British Medical Association, 1997; Joy *et al.*, 1999). Endogenous cannabinoids and cannabinoid receptors exist at various levels in the pain pathways, from peripheral sensory nerve endings to spinal cord and supraspinal centres, in a system that is parallel to but distinct from that involving endorphins and opiate receptors.

Systemically administered THC and synthetic cannabinoids have anti-nociceptive and anti-hyperalgesic effects in a variety of animal models of acute and inflammatory pain (for reviews see Pertwee, 2001; Iversen and Chapman, 2002). Since cannabinoids inhibit motor activity this could prevent animals from exhibiting the normal behavioural reactions in analgesic tests; however, a number of studies have also shown that cannabinoids suppress electrophysiological responses of spinal cord neurons to noxious stimulation, and block spinal c-fos expression in response to such stimulation (Walker *et al.*, 1999; Pertwee, 2001; Iversen and Chapman, 2002). Cannabinoids and anandamide also exert anti-nociceptive effects in animal models of inflammatory pain when injected directly into spinal cord, brain stem or thalamus (Pertwee, 2001). Behavioural studies have shown that cannabinoids reduce thermal and mechanical allodynia in rat models of neuropathic pain (Herzberg *et al.*, 1997; Fox *et al.*, 2001; Iversen and Chapman, 2002). Furthermore, noxious stimulation evoked an increased release of anandamide in the periaqueductal grey region of brainstem, a key site for modulating nociceptive information (Walker *et al.*, 1999). The anti-nociceptive effects of cannabinoids are blocked by the CB_1 antagonist rimonabant, but the antagonist itself does not alter basal pain thresholds, suggesting that these are not controlled by tonic activity in the endocannabinoid system (Compton *et al.*, 1996).

Results obtained with CB_1 receptor knockout mice, however, suggest that not all of the anti-nociceptive effects of THC or anandamide are mediated via CB_1 receptors. Thus, although Di Marzo *et al.* (2000) found that the anti-nociceptive effects of THC were virtually absent in the knockout animals, anandamide continued to show analgesic activity in the hot-plate test. It is possible that the analgesic effects of anandamide are mediated in part through an action at other as yet ill-defined cannabinoid receptors (Breivogel *et al.*, 2001; Hájos *et al.*, 2001). Alternatively, it has been proposed that the effects of anandamide might be mediated through its ability to bind to the vanilloid VR1 receptor, which is present in primary afferent neurons and known to play an important role in nociceptive responses (Di Marzo *et al.*, 2001*b*). To complicate matters further, Zimmer *et al.* (1999), in a different strain of CB_1 receptor knockout mice, found that THC continued to exert some anti-nociceptive actions in hot-plate and formalin tests in the knockout animals. The reasons for the discrepant results obtained

with different strains of CB_1 receptor knockout mice are unknown.

There is evidence for an interaction between cannabinoid and opioid mechanisms. In tests of acute pain (Fuentes *et al.*, 1999) and chronic inflammatory pain (Welch and Stevens, 1992; Smith *et al.*, 1998) THC and morphine acted synergically—one potentiated the anti-nociceptive actions of the other. This potentiation could be blocked by either rimonabant or by naloxone, indicating that both CB_1 and opiate receptors were involved (Fuentes *et al.*, 1999). Meng *et al.* (1998) showed that temporary inactivation of neural activity in the rostral ventromedial medulla (RVM) in rat brainstem prevented the analgesic effects of systemically administered cannabinoids, while leaving their effects on motor activity unaffected. An electrophysiological analysis of the effects of cannabinoids on single cell firing patterns in RVM revealed that the effects of cannabinoids were similar to those elicited by morphine. The authors concluded that cannabinoids may produce analgesia through activation of a brainstem circuit that is also required for opiate analgesia, although the two mechanisms are pharmacologically distinct.

Basic research into the role of cannabinoids and endocannabinoids in pain mechanisms is progressing rapidly. Clinical progress, however, has been slow. A meta-analysis of clinical trials of cannabinoids as analgesics concluded that there was not enough evidence to justify their use in this indication (Campbell *et al.*, 2001). However, this may merely reflect the paucity of data from adequately sized controlled clinical trials, and cannabis-based medicines may yet find genuine medical applications in this field.

Cannabis as an intoxicant and drug of dependence

Cannabis intoxication

Despite being illegal, cannabis is one of the most widely used intoxicants; almost half of all 18 year olds in the USA and in most European countries admit to having tried it at least once, and ~10% of that age group are regular users (Iversen, 2000). There have been many subjective accounts of the cannabis 'high' (see Iversen, 2000; Earleywine, 2002). The experience is highly variable, depending on the dose of drug, the environment and the experience and expectations of the drug user. A typical 'high' is preceded initially by a transient stage of tingling sensations felt in the body and head accompanied by a feeling of dizziness or lightheadedness. The 'high' is a complex experience, characterized by a quickening of mental associations and a sharpened sense of humour, sometimes described as a state of 'fatuous euphoria'. The user feels relaxed and calm, in a dreamlike state disconnected from real world. The intoxicated subject often has difficulty in carrying on a coherent conversation, and may drift into daydreams and fantasies. Drowsiness and sleep may eventually ensue. The feelings of heightened perception, increased appetite and distortion of the sense of time have already been referred to. A survey of 1333 young British cannabis users (Atha and Blanchard, 1997) reported that the most common positive benefits reported were relaxation and relief from stress (25.6%), insight/personal development (8.7%) and euphoria (4.9%); more than half reported some positive benefits. But 21% of the users also attributed some adverse effects to cannabis use, including impaired memory (6.1%), paranoia (5.6%) and amotivation/laziness (4.8%).

As with other intoxicant drugs, little is known about the brain mechanisms that underlie the cannabis 'high'. The intoxicant effects are clearly mediated via CB_1 receptors. Huestis *et al.* (2001) carried out a well controlled study in 63 healthy cannabis users, who received either rimonabant or placebo and smoked either a THC-containing or placebo marijuana cigarette. The CB_1 antagonist blocked the acute psychological effects of the active cigarettes. Interestingly rimonabant itself when given alone (with placebo cigarette) produced no significant psychological effects. Mathew *et al.* (1997) used $H_2{}^{15}O$ and PET to measure changes in regional cerebral blood flow in a double blinded study in 32 volunteers comparing THC with placebo. Self ratings of cannabis intoxication correlated most markedly with increased blood flow in the right frontal region.

Endocannabinoids and CB_1 receptors are present in many regions of the limbic forebrain. For example, Katona *et al.* (2001) reported that CB_1 receptors were expressed in high densities in lateral and basal nuclei in the rat amygdala. As in hippocampus, the CB_1 receptors in these regions were located presynaptically on the terminals of cholecystokinin-containing GABAergic interneurons. Electrophysiological experiments showed that cannabinoids modulated GABAergic synaptic transmission. The authors suggested that such effects might underlie some of the actions of cannabinoids on emotional behaviour. Other experiments have revealed that, in common with other euphoriant drugs, THC selectively activates dopaminergic neurons in the ventral tegmental area. In an electrophysiological study French *et al.* (1997) reported that low doses of THC increased the firing of these cells. Tanda *et al.* (1997) used microdialysis probes to show that low doses of THC (0.15 mg/kg intravenously) caused an increased release of dopamine from the shell region of the nucleus accumbens, an effect that is also seen after administration of heroin, cocaine, d-amphetamine and nicotine. Tanda *et al.* (1997) found that the increased release of dopamine provoked by THC could be blocked by administration of the μ-opiate receptor antagonist naloxonazine, suggesting the involvement of an opioid mechanism.

Tolerance and dependence

Many animal studies have shown that tolerance develops to most of the behavioural and physiological effects of THC (for review see Pertwee, 1991). The earlier clinical literature suggested that tolerance also occurs after repeated administration of THC in man, although many of these studies were poorly controlled (for reviews see Jones, 1978, 1987;

Hollister, 1986). But for many years cannabis was not considered to be a drug of addiction. Withdrawal of the drug did not lead to any obvious physical withdrawal symptoms either in people or in animals, and animals failed to self-administer the drug, a behaviour usually associated with drugs of addiction.

Attitudes have changed markedly in recent years. The DSM-IV (American Psychiatric Association, 1994) defines 'substance dependence' and 'substance abuse' rather than 'addiction'. When the DSM-IV criteria are applied to populations of regular cannabis users surprisingly high proportions appear to be positive by these definitions. Swift *et al.* (2001) undertook a survey of 10 641 Australians aged 18 years and older. They reported that almost one-third of regular cannabis users fell within the definitions of 'substance abuse' (10.7%) or 'substance dependence' (21%). In the USA, Anthony *et al.* (1994) reported the results obtained from a large scale survey which indicated that some 46% of those interviewed had ever used cannabis and 9% of users became dependent. More carefully controlled studies have also shown that a reliable and clinically significant withdrawal syndrome does occur in human cannabis users when the drug is withdrawn. The symptoms include craving for cannabis, decreased appetite, sleep difficulty and weight loss, and may sometimes be accompanied by anger, aggression, increased irritability, restlessness and strange dreams (Budney *et al.*, 2001).

The existence of dependence on cannabinoids in animals is also much more clearly observable because of the availability of CB_1 receptor antagonist drugs that can be used to precipitate withdrawal. Thus, Aceto *et al.* (1996) described a behavioural withdrawal syndrome precipitated by rimonabant in rats treated for only 4 days with doses of THC as low as 0.5–4.0 mg/kg per day. The syndrome included scratching, face rubbing, licking, wet dog shakes, arched back and ptosis—many of the same signs are seen in rats undergoing opiate withdrawal. Similar withdrawal signs could be elicited by rimonabant in rats treated chronically with the synthetic cannabinoids CP-55,940 (Rubino *et al.*, 1998) or WIN55,2122 (Aceto *et al.*, 2001). Rimonabant-induced withdrawal after 2 weeks of treatment of rats with the cannabinoid HU-120 was accompanied by marked elevations of release of the stress-related neuropeptide corticotropin-releasing factor in the amygdala, a result also seen in animals undergoing heroin withdrawal (Rodríguez de Fonseca *et al.*, 1997). An electrophysiological study showed that precipitated withdrawal was also associated with reduced firing of dopamine neurons in the ventral tegmental area of rat brain (Diana *et al.*, 1998). These data indicate clearly that chronic administration of cannabinoids leads to adaptive changes in the brain, some of which are similar to those seen with other drugs of dependence. The ability of THC to cause a selective release of dopamine from the nucleus accumbens (Tanda *et al.*, 1997) also suggests some similarity between THC and other drugs in this category.

Furthermore, although many earlier attempts to obtain reliable self-administration behaviour with THC were unsuccessful (Pertwee, 1991), some success has been achieved recently. Squirrel monkeys were trained to self-administer low doses of THC (2 μg/kg per injection), but only after the animals had first been trained to self-administer cocaine (Tanda *et al.*, 2000). THC is difficult to administer intravenously and these authors succeeded perhaps in part because they succeeded in delivering the drug intravenously in doses comparable to those to which human cannabis users are exposed. The potent synthetic cannabinoids are far more water soluble than THC, which makes intravenous administration easier. Mice could be trained to self-administer intravenous WIN 5,2122, but CB_1 receptor knockout animals failed to exhibit this behaviour (Ledent *et al.*, 1999). Another way of demonstrating the rewarding effects of drugs in animals is the conditioned place preference paradigm, in which an animal learns to approach an environment in which it had previously received a rewarding stimulus. Rats demonstrated a positive THC place preference after doses as low as 1 mg/kg (Lepore *et al.*, 1995).

A number of studies have suggested that there may be links between the development of dependence to cannabinoids and to opiates (Manzanares *et al.*, 1999). Some of the behavioural signs of rimonabant-induced withdrawal in THC treated rats can be mimicked by administration of the opiate antagonist naloxone (Kaymakçalan *et al.*, 1977). Conversely, the withdrawal syndrome precipitated by naloxone in morphine-dependent mice can be partly relieved by administration of THC (Hine *et al.*, 1975) or by endocannabinoids (Yamaguchi *et al.*, 2001). Rats treated chronically with the cannabinoid WIN55,2122 became sensitized to the behavioural effects of heroin (Pontieri *et al.*, 2001). Such interactions can also be demonstrated acutely. A synergy between cannabinoids and opiate analgesics has already been described above. THC also facilitated the anti-nociceptive effects of RB 101, an inhibitor of enkephalin inactivation (Valverde *et al.*, 2001). These authors found that acute administration of THC caused an increased release of Met-enkephalin into microdialysis probes placed into the rat nucleus accumbens.

The availability of receptor knockout animals has also helped to illustrate cannabinoid–opioid interactions. CB_1 receptor knockout mice exhibited greatly reduced morphine self-administration behaviour and less severe naloxone-induced withdrawal signs than in wild-type animals, although the anti-nociceptive actions of morphine were unaffected in the knockout animals (Ledent *et al.*, 1999). The rimonabant-precipitated withdrawal syndrome in THC-treated mice was significantly attenuated in animals with knockout of the pro-enkephalin gene (Valverde *et al.*, 2000). Knockout of the μ-opioid receptor also reduced rimonabant-induced withdrawal signs in THC-treated mice, and there was an attenuated naloxone withdrawal syndrome in morphine dependent CB_1 knockout mice (Lichtman *et al.*, 2001*a*, *b*).

These findings point clearly to interactions between the endogenous cannabinoid and opioid systems in CNS, although the neural circuitry involved remains unknown. Whether this relationship is relevant to the so-called 'gateway' theory is unclear. The US National Household survey of Drug Abuse (US Department of Health and Human Services,

1999) indicated that respondents aged 22 years or older who had started cannabis use before the age of 21 years were 24 times more likely than non-cannabis users to initiate use of hard drugs. But the proportion of cannabis users who progress in this way remains very small (~1% or less), and mathematical modelling using the Monte Carlo method suggested that the association between cannabis use and hard drug use need not be causal but could relate to some common predisposing factor, e.g. 'drug-use propensity' (Morral *et al.*, 2002).

Adverse effects of cannabis on the CNS

Is cannabis neurotoxic?

Although there have been claims that chronic cannabis use may permanently damage the brain, there is little scientific evidence to support these claims (for reviews see Dornbush *et al.*, 1976; Hollister, 1986, 1998; Zimmer and Morgan, 1997). As described above, some studies have revealed a modestly impaired ability to focus attention and filter out irrelevant information in ex-cannabis users (Solowij, 1998), but other studies failed to find any impairments in cognitive function (Pope *et al.*, 2001). There is little evidence that cannabis use impairs work performance or leads to an 'amotivational syndrome' (Dornbush *et al.*, 1976; Hollister, 1986; Abood and Martin, 1992), nor is there any convincing evidence for neuropathological changes in the brains of cannabis users (Hollister, 1986). The earlier studies have been complemented by the application of powerful modern neuroimaging methods. For example, an MRI study compared 18 current, frequent, young adult cannabis users with 13 comparable non-users and found no evidence of cerebral atrophy or regional changes in tissue volumes (Block *et al.*, 2000).

Animal studies have yielded conflicting results. Treatment of rats with high doses of THC given orally for 3 months (Scallet *et al.*, 1987) or subcutaneously for 8 months (Landfield *et al.*, 1988) was reported to lead to neural damage in the hippocampal CA3 zone, with shrunken neurons, reduced synaptic density and loss of cells. However, in another study the potent synthetic cannabinoid WIN55,2122 was administered twice daily (2 mg/kg) to rats and led to an apparent increase in hippocampal granule cell density, and increased dendritic length in the CA3 zone. In perhaps the most severe test of all, rats and mice were treated with THC 5 days each week for 2 years and no histopathological changes were observed in brain, even after 50 mg/kg/day (rats) or 250 mg/kg/day (mice) (Chan *et al.*, 1996). Although claims were made that exposure of a small number of rhesus monkeys to cannabis smoke led to ultrastructural changes in septum and hippocampus (Harper *et al.*, 1977; Heath *et al.*, 1980), subsequent larger scale studies failed to show any cannabis-induced histopathology in monkey brain (Scallet, 1991).

Studies of the effects of cannabinoids on neurons *in vitro* have also yielded inconsistent results. Exposure of rat cortical neurons to THC was reported to decrease their survival, with twice as many cells dead after 2 h exposure to 5 μM THC than in control cultures (Downer *et al.*, 2001). Concentrations of THC as low as 0.1 μM had a significant effect. The effects of THC were accompanied by release of cytochrome *c*, activation of caspase-3 and DNA fragmentation, suggesting an apoptotic mechanism. All of the effects of THC could be blocked by the antagonist AM-251 or by pertussis toxin, suggesting that they were mediated through CB_1 receptors. Toxic effects of THC have also been reported on hippocampal neurons in culture, with 50% cell death after 2 h exposure to 10 μM THC or after 5 days exposure to 1 μM drug (Chan *et al.*, 1998). The antagonist rimonabant blocked these effects, but not pertussis toxin. The authors proposed a toxic mechanism involving arachidonic acid release and formation of free radicals. However, other authors failed to observe any damage in rat cortical neurons exposed for up to 15 days to 1 μM THC, although they found that this concentration of THC killed rat C6 glioma cells, or human astrocytoma U373MG and mouse neuroblastoma N18TG12 cells (Sánchez *et al.*, 1998). In a remarkable study injections of THC into solid tumours of C6 glioma in rodent brain led to increased survival times, and a complete eradication of the tumours was evident in 20–35% of the treated animals (Galve-Roperh *et al.*, 2000). The anti-proliferative effects of cannabinoids has suggested a potential utility for such drugs in cancer treatment (Guzmán *et al.*, 2001).

Some studies have reported neuroprotective actions of cannabinoids. Administration of WIN55,2122 was found to reduce cerebral damage in rat hippocampus or cerebral cortex after global ischaemia or focal ischaemia models *in vivo* (Nagayama *et al.*, 1999). The endocannabinoid 2-AG protected against damage elicited by closed head injury in mouse brain, and the protective effects were blocked by rimonabant (Panikashvili *et al.*, 2001). THC had a similar effect *in vivo* in protecting against damage elicited by ouabain (Van der Stelt *et al.*, 2001). Rat hippocampal neurons in tissue culture were protected against glutamate-mediated damage by low concentrations of WIN55,2122 or CP-55,940 and these effects were mediated through CB_1 receptors (Shen and Thayer, 1998). But not all of these effects seem to require mediation via cannabinoid receptors. Nagayama *et al.* (1999) reported protective effects of WIN55,2122 that did not require either cannabinoid receptor in cortical neurons exposed to hypoxia, and similar findings were reported for the protective actions of anandamide and 2-AG in cortical neuron cultures (Sinor *et al.*, 2000). Both THC and cannabidiol, which is not active on cannabinoid receptors, protected rat cortical neurons against glutamate toxicity (Hampson *et al.*, 1998) and these effects, were also independent of CB_1 receptors. The authors suggested that the protective effects of THC in their studies might be due to the antioxidant properties of these polyphenolic molecules, which have redox potentials higher than those of known antioxidants (e.g. ascorbic acid).

The mixed reports of neurotoxic and neuroprotective effects of cannabinoids are confusing. While it may be possible to demonstrate neurotoxic actions after exposure of neurons to high concentrations of cannabinoids *in vitro*, there is little evidence for any significant neural damage *in vivo* after the administration of pharmacologically relevant doses of these drugs.

Cannabis and psychiatric illness

A temporary form of drug-induced psychosis can occur in some cannabis users. In some of the psychiatric literature this is referred to as 'cannabis psychosis' (or 'marijuana psychosis'). Research psychiatrists, particularly in Britain (Thomas, 1993; Hall and Degenhardt, 2000; Johns, 2001), have studied this condition carefully. It nearly always results from taking large doses of the drug, often in food or drink, and the condition may persist for some time, perhaps as the accumulated body load of THC is washed out. The acute toxic psychosis that is sometimes caused by cannabis can be sufficiently serious to lead to the subject being admitted to hospital, and the initial diagnosis can be confused with schizophrenia, since the patients may display some of the characteristic symptoms of schizophrenic illness. These include delusions of control (being under the control of some outside being or force), grandiose identity, persecution, thought insertion, auditory hallucinations (hearing sounds, usually non-verbal in nature), changed perception and blunting of the emotions. Not all symptoms will be seen in every patient, but there is a considerable similarity to paranoid schizophrenia. This has led some to propose a 'cannabinoid hypothesis of schizophrenia', suggesting that the symptoms of schizophrenic illness might be caused by an abnormal over-activity of endogenous cannabinoid mechanisms in the brain (Emrich *et al.*, 1997).

A number of studies have addressed the more contentious question of whether cannabis use can precipitate long-term psychiatric illness. The strongest evidence seemed to come from a study in Sweden that involved taking detailed medical records and information about the social background and drug-taking habits of 45 570 conscripts on entry to the Swedish army at age 18 years and following up of their subsequent medical history over a 15-year period (Andreasson *et al.*, 1987). A total of 4293 of the conscripts admitted having taken cannabis at least once, but the cannabis users accounted for a disproportionate number of the 246 cases of schizophrenic illness diagnosed in the overall group on follow-up. The relative risk of schizophrenia in those who had used cannabis was 2.4 times greater than in the non-users. In the small number of heavy users (who had taken the drug on more than 50 occasions) the relative risk of schizophrenia increased to 6.0. The authors concluded that cannabis was an independent risk factor for schizophrenia. There have been other similar reports (Mathers and Godse, 1992; Hall and Degenhardt, 2000; Johns, 2001). Hambrecht and Häfner (2000), for example, studied 232 patients in Germany with first-episode schizophrenia. They found that 13% of these had a history of cannabis use, a rate twice that of matched normal controls. At first viewing these findings seem convincing, but they do not prove any cause-and-effect relationship with cannabis. It may simply be that both cannabis use and schizophrenia are related to some common predisposing factor, such as personality. Indeed some psychologists and psychiatrists believe that they can identify psychological traits that are described as 'schizotypy' and which may predict an increased risk of developing clinical psychosis. Some studies in healthy adults have reported that those subjects who used cannabis scored higher on schizotypy scales than non-users (Williams *et al.*, 1996; Skosnik and Spatz, 2001). Half of the cannabis-using subjects in the original Swedish study had used cannabis more than 10 times and subsequently developed schizophrenia had also taken amphetamine, a drug known to be capable of inducing a schizophrenia-like psychosis. The cannabis users also came from deprived social backgrounds, another known risk factor of schizophrenia. More detailed follow-ups of some of the original Swedish cohort, however, claimed to have answered some of these criticisms (Andreasson *et al.*, 1989; Zammit *et al.*, 2002). In addition, further reports from New Zealand (Arseneault *et al.*, 2002; Fergusson *et al.*, 2003), Australia (Patton *et al.*, 2002) and France (Verdoux *et al.*, 2003) add weight to the hypothesis that the development of cannabis dependence in young people is associated with increased rates of psychiatric symptoms, both of psychosis and depression and anxiety (Patton *et al.*, 2002).

Nevertheless, the existence of a causative relationship between cannabis use and long-term psychotic illness remains unproven. If cannabis use did precipitate schizophrenia one might expect to have seen a large increase in the numbers of sufferers from this illness as cannabis use became more common in the West during the past 30 years. However, a detailed review of the epidemiological evidence up to 1990 appeared to show that this has not been the case (Thornicroft, 1990).

On the other hand, it is clear that cannabis can exacerbate the symptoms of existing psychotic illness. While schizophrenic patients seem to use cannabis and other psychoactive drugs as a form of 'self-medication', cannabis can make the key symptoms of delusions and hallucinations worse and it tends to counteract the anti-psychotic effects of the drugs used to treat the illness (Negrete *et al.*, 1986; Linzen *et al.*, 1994). On the other hand, one Swedish study reported that cannabis use made schizophrenic patients less withdrawn and more likely to speak (Peralta and Cuesta, 1992). It would seem prudent, nevertheless, to discourage the use of cannabis in patients with existing psychotic illness.

Conclusion

The discovery of the endocannabinoids and the availability of new pharmacological tools, together with the development of strains of genetically engineered knockout mice that lack

functional cannabinoid receptors, has revitalized the field of cannabis research in the past few years. The effects of administering THC or other cannabinoids can never simulate the highly localized function of the endocannabinoids, which appear to act as a fine control system to regulate neurotransmitter release at the synaptic level. There has been renewed interest in the potential therapeutic applications of cannabis-based medicines (British Medical Association, 1997; Joy *et al.*, 1999; Robson, 2001). THC or other cannabinoid agonists all suffer from the problem of a narrow therapeutic window between the desired clinical benefits and the unwanted psychic side-effects. It is possible that the pharmacological manipulation of the endocannabinoid system, boosting function, for example by drugs that inhibited the inactivation of the endocannabinoids, may offer a safer and more subtle approach to cannabis-based medicines in the future (Piomelli *et al.*, 2000).

References

Abel EL. Marihuana and memory: acquisition or retrieval? Science 1971; 173: 1038–41.

Abood ME, Martin BR. Neurobiology of marijuana abuse. [Review]. Trends Pharmacol Sci 1992; 13: 201–6.

Aceto MD, Scates SM, Lowe JA, et al. Dependence on delta 9-tetrahydrocannabinol: studies on precipitated and abrupt withdrawal. J Pharmacol Exp Ther 1996; 278: 1290–5.

Aceto MD, Scates SM, Martin BR. Spontaneous and precipitated withdrawal with a synthetic cannabinoid, WIN 55212-2. Eur J Pharmacol 2001; 416: 75–81.

Adams IB, Martin BR. Cannabis: pharmacology and toxicology in animals and humans. [Review]. Addiction 1996; 91: 1585–14.

Alger BE, Pitler TA. Retrograde signaling at $GABA_A$-receptor synapses in the mammalian CNS. [Review]. Trends Neurosci 1995; 13: 333–40.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. DSM-IV. 4th ed. Washington (DC): American Psychiatric Association; 1994.

Andreasson S, Allebeck P, Engstrom A, et al. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet 1987; 2: 1483–5.

Andreasson S, Allebeck P, Rydberg U. Schizophrenia in users and nonusers of cannabis. Acta Psychiatr Scand 1989; 79: 505–10.

Anthony JC, Warner LA, Kessler RC. Comparative epidemiology of dependence on tobacco, alcohol, controlled substances and inhalants. Basic findings from the National Comorbidity Survey. Exp Clin Psychopharmacol 1994; 2: 244–68.

Arseneault L, Cannon M, Poulton R, et al. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. BMJ 2002; 325: 1212–3.

Atha MJ, Blanchard S. Regular users. Self-reported drug consumption patterns and attitudes towards drugs among 1333 regular cannabis users. Wigan: Independent Drug Monitoring Unit; 1997.

Baker D, Pryce G, Croxford JL, et al. Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature 2000; 404: 84–7.

Beal JA, Olson R, Laubenstein L, et al. Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. J Pain Symptom Manage 1995; 10: 89–97.

Bisogno T, Berrendero F, Ambrosino G, et al. Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. Biochem Biophys Res Commun 1999; 256: 377–80.

Block RI, O'Leary DS, Ehrhardt JC, et al. Effects of frequent marijuana use on brain tissue volume and composition. Neuroreport 2000; 11: 491–6.

Bohme GA, Laville M, Ledent C, et al. Enhanced long-term potentiation in mice lacking cannabinoid CB_1 receptors. Neuroscience 2000; 95: 5–7.

Bowman M, Pihl RO. Cannabis: psychological effects of chronic heavy use. A controlled study of intellectual functioning in chronic users of high potency cannabis. Psychopharmacologia 1973; 29: 159–70.

Breivogel CS, Childers SR. The functional neuroanatomy of brain cannabinoid receptors. [Review]. Neurobiol Dis 1998; 5: 417–31.

Breivogel CS, Griffin G, Di Marzo V, et al. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol Pharmacol 2001; 60: 155–63.

British Medical Association. Therapeutic uses of cannabis. [Review]. Amsterdam: Harwood Academic; 1997.

Budney AJ, Hughes JR, Moore BA, et al. Marijuana abstinence effects in marijuana smokers maintained in their home environment. Arch Gen Psychiatry 2001; 58: 917–24.

Cadas H, di Tomaso E, Piomelli D. Occurrence and biosynthesis of endogenous cannabinoid precursors, N-arachidonoyl phosphatidylethanolamine, in rat brain. J Neurosci 1997; 17: 1226–42.

Campbell FA, Tramer MR, Carroll D, et al. Are cannabinoids an effective and safe treatment option in the management of pain? A qualitative systematic review. [Review]. BMJ 2001; 323: 13–6.

Caulfield MP, Brown DA. Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. Br J Pharmacol 1992; 106: 231–2.

Chan PC, Sills RC, Braun AG, et al. Toxicity and carcinogenicity of delta 9-tetrahydrocannabinol in Fischer rats and B6C3F1 mice. Fundam Appl Toxicol 1996; 30: 109–17.

Chan GCK, Hinds TR, Impey S, et al. Hippocampal neurotoxicity of delta 9-tetrahydrocannabinol. J Neurosci 1998; 18: 5322–32.

Colombo G, Agabio R, Diaz G, et al. Appetite suppression and weight loss after the cannabinoid antagonist SR141716. Life Sci 1998; 63: PL113–7.

Compton DR, Aceto MD, Lowe J, et al. In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydocannabinol-induced responses and

apparent agonist activity. J Pharmacol Exp Ther 1996; 277: 586–94.

Consroe P, Musty R, Rein J, et al. The perceived effects of smoked cannabis on patients with multiple sclerosis. Eur Neurol 1996; 38: 44–8.

Cravatt BF, Demarest K, Patricelli MP, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci USA 2001; 98: 9371–6.

Darmani NA. Delta 9-tetrahydocannabinol and synthetic cannabinoids prevent emesis produced by the cannabinoid CB_1 receptor antagonist/inverse agonist SR141716A. Neuropsychopharmacology 2001; 24: 198–203.

Darmani NA. The potent emetogenic effects of the endocannabinoid, 2-AG (2-arachidonylglycerol) are blocked by delta 9-tetrahydrocannabinol and other cannabinoids. J Pharmacol Exp Ther 2002; 300: 34–42.

Deadwyler SA, Hampson RE, Childers SR. Functional significance of cannabinoid receptors in brain. In: Pertwee RG, editor. Cannabinoid receptors. London: Academic Press; 1995. p. 206–31.

DeSanty KP, Dar MS. Cannabinoid-induced motor incoordination through the cerebellar CB_1 receptor in mice. Pharmacol Biochem Behav 2001; 69: 251–9.

Devane WA, Hanus L, Breuer A, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992; 258: 1946–9.

Diana M, Melis M, Muntoni AL, et al. Mesolimbic dopaminergic decline after cannabinoid withdrawal. Proc Natl Acad Sci USA 1998; 95: 10269–73.

DiMarzo V, Fontana A, Cadas H, et al. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 1994; 372: 686–91.

DiMarzo V, Breivogel CS, Tao Q, et al. Levels, metabolism, and pharmacological activity of anandamide in CB_1 cannabinoid receptor knockout mice: evidence for non-CB1, non-CB_2 receptor-mediated actions of anandamide in mouse brain. J Neurochem 2000; 75: 2434–44.

DiMarzo V, Goparaju SK, Wang L, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 2001*a*; 410: 822–5.

DiMarzo V, Bisogno T, De Petrocellis L. Anandamide: some like it hot. [Review]. Trends Pharmacol Sci 2001*b*; 22: 346–9.

Dixon WE. The pharmacology of cannabis indica. BMJ 1899; 2: 1354–7.

Dornbush RL, Freedman AM, Fink M, editors. Chronic cannabis use. [Review]. Ann NY Acad Sci 1976; 282: 1–430.

Downer E, Boland B, Fogarty M, et al. Delta 9-tetrahydrocannabinol induces the apoptotic pathway in cultured cortical neurones via activation of the CB_1 receptor. Neuroreport 2001; 12: 3973–8.

D'Souza DC, Kosten TR. Cannabinoid antagonists. [Review]. Arch Gen Psychiatry 2001; 58: 330–1.

Earleywine M. Understanding marijuana. [Review]. Oxford: Oxford University Press; 2002.

Egertová M, Elphick MR. Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB_1. J Comp Neurol 2000; 422: 159–71.

Egertová M, Giang DK, Cravatt BF, et al. A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB_1 receptor in rat brain. Proc R Soc Lond B Biol Sci 1998; 265: 2081–5.

Elphick MR, Egertová M. The neurobiology and evolution of cannabinoid signalling. [Review]. Philos Trans R Soc Lond B Biol Sci 2001; 356: 381–408.

Emrich HM, Leweke FM, Schneider U. Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. [Review]. Pharmacol Biochem Behav 1997; 56: 803–7.

Felder CC, Glass M. Cannabinoid receptors and their endogenous agonists. [Review]. Annu Rev Pharmacol Toxicol 1998; 38: 179–200.

Felder CC, Nielsen A, Briley EM, et al. Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. FEBS Lett 1996; 393: 231–5.

Fergusson DM, Horwood LJ, Swain-Campbell NR. Cannabis dependence and psychotic symptoms in young people. Psychol Med 2003; 33: 15–21.

Fox A, Kesingland A, Gentry C, et al. The role of central and peripheral cannabinoid 1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain 2001; 92: 91–100.

French ED, Dillon K, Wu X. Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. Neuroreport 1997; 8: 649–52.

Fride E, Ginzburg Y, Breuer A, et al. Critical role of the endogenous cannabinoid system in mouse pup suckling and growth. Eur J Pharmacol 2001; 419: 207–14.

Fuentes JA, Ruiz-Gayo M, Manzanares J, et al. Cannabinoids as potential new analgesics. Life Sci 1999; 65: 675–85.

Galve-Roperh I, Sanchez C, Cortés ML, et al. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat Med 2000; 6: 313–9.

Gill EW, Paton WDM, Pertwee RG. Preliminary experiments on the chemistry and pharmacology of cannabis. Nature 1970; 229: 134–6.

Giuffrida A, Piomelli D. The endocannabinoid system: a physiological perspective on its role in psychomotor control. [Review]. Chem Phys Lipids 2000; 108: 151–8.

Giuffrida A, Parsons LH, Kerr TM, et al. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 1999; 2: 358–63.

Giuffrida A, Désarnaud F, Piomelli D. Endogenous cannabinoid signaling and psychomotor disorders. Prostaglandins Other Lipid Mediat 2000; 61: 63–70.

Giuffrida A, Beltramo M, Piomelli D. Mechanisms of endocannabinoid inactivation: biochemistry and pharmacology. [Review]. J Pharmacol Exp Ther 2001; 298: 7–14.

Greenberg HS, Werness SAS, Pugh JE, et al. Short-term effects of smoking marijuana on balance in patients with multiple sclerosis and normal volunteers. Clin Pharmacol Ther 1994; 55: 324–8.

Guzmán M, Sánchez C, Galve-Roperh I. Control of the cell survival/death decision by cannabinoids. [Review]. J Mol Med 2001; 78: 613–25.

Hájos N, Katona I, Naiem SS, et al. Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. Eur J Neurosci 2000; 12: 3239–49.

Hájos N, Ledent C, Freund TF. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. Neuroscience 2001; 106: 1–4.

Hall W, Degenhardt L. Cannabis use and psychosis: a review of clinical and epidemiological evidence. Aust NZ J Psychiatry 2000; 34: 26–34.

Hambrecht M, Hafner H. Cannabis, vulnerability, and the onset of schizophrenia: an epidemiological perspective. Aust NZ J Psychiatry 2000; 34: 468–75.

Hampson RE, Deadwyler SA. Cannabinoids, hippocampal function and memory. [Review]. Life Sci 1999; 65: 715–23.

Hampson RE, Deadwyler SA. Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. J Neurosci 2000; 20: 8932–42.

Hampson AJ, Grimaldi M, Axelrod J, et al. Cannabidiol and (-)delta 9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci USA 1998; 95: 8268–73.

Han CJ, Robinson JK. Cannabinoid modulation of time estimation in the rat. Behav Neurosci 2001; 115: 243–6.

Hanuš L, Abu-Lafi S, Fride E, et al. 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB_1 receptor. Proc Natl Acad Sci USA 2001; 98: 3662–5.

Harper JW, Heath RG, Myers WA. Effects of cannabis sativa on ultrastructure of the synapse in monkey brain. J Neurosci Res 1977; 3: 87–93.

Heath RG, Fitzjarrell AT, Fontana CJ, et al. Cannabis sativa: effects on brain function and ultrastructure in rhesus monkeys. Biol Psychiatry 1980; 15: 657–90.

Herkenham M, Lynn AB, Johnson MR, et al. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 1991; 11: 563–83.

Herzberg U, Eliav E, Bennett GJ, et al. The analgesic effects of R(+)-WIN55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. Neurosci Lett 1997; 221: 157–60.

Hicks RE, Gualtieri CT, Mayo JP Jr, Perez-Reyes M. Cannabis, atropine, and temporal information processing. Neuropsychobiology 1984; 12: 229–37.

Hine B, Friedman E, Torrelio M, et al. Morphine-dependent rats: blockade of precipitated abstinence by tetrahydrocannabinol. Science 1975; 187: 443–5.

Hoffman AF, Lupica CR. Mechanisms of cannabinoid inhibition of $GABA_A$ synaptic transmission in the hippocampus. J Neurosci 2000; 20: 2470–9.

Hollister LE. Hunger and appetite after single doses of marihuana, alcohol, and dextroamphetamine. Clin Pharmacol Ther 1971; 12: 44–9.

Hollister LE. Health aspects of cannabis. [Review]. Pharmacol Rev 1986; 38: 1–20.

Hollister LE. Health aspects of cannabis: revisited. Int J Neuropsychopharmacol 1998; 1: 71–80.

Howlett AC, Johnson MR, Melvin LS, et al. Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model. Mol Pharmacol 1988; 33: 297–302.

Huestis MA, Gorelick DA, Heishman SJ, et al. Blockade of effects of smoked marijuana by the CB_1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry 2001; 58: 322–8.

Iversen LL. The science of marijuana. [Review]. Oxford: Oxford University Press, 2000.

Iversen LL, Chapman V. Cannabinoids: a real prospect for pain relief? [Review]. Curr Opin Pharmacol 2002; 2: 50–5.

Johns A. Psychiatric effects of cannabis. [Review]. Br J Psychiatry 2001; 178: 116–22.

Jones RT. Marihuana: human effects. [Review] In: Iversen LL, Iversen SD, Snyder SH, editors. Handbook of psychopharmacology. Vol. 12. New York: Plenum Press; 1978. p. 373–412.

Jones RT. Drug of abuse profile: cannabis. [Review]. Clin Chem 1987; 33 (11 Suppl): 72B–81B.

Joy JE, Watson SJ, Benson JA, editors. Marijuana and medicine, assessing the science base. Washington (DC): National Academy Press; 1999.

Katona I, Sperlagh B, Sik A, et al. Presynaptically located CB_1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 1999; 19: 4544–58.

Katona I, Sperlagh B, Maglóczky Z, et al. GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. Neuroscience 2000; 100: 797–804.

Katona I, Rancz EA, Acsády L, et al. Distribution of CB_1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci 2001; 21: 9506–18.

Kaymakçalan S, Ayhan IH, Tulunay FC. Naloxone-induced or postwithdrawal abstinence signs in delta 9-tetrahydrocannabinol-tolerant rats. Psychopharmacology (Berl) 1977; 55: 243–9.

Koch JE. Delta 9-THC stimulates food intake in Lewis rats. Effects on chow, high-fat and sweet high-fat diets. Pharmacol Biochem Behav 2001; 68: 539–43.

Kreitzer AC, Regehr WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 2001*a*; 29: 717–27.

Kreitzer AC, Regehr WG. Cerebellar depolarization-induced

suppression of inhibition is mediated by endogenous cannabinoids. J Neurosci 2001*b*; 21: RC174–9.

Landfield PW, Cadwallader LB, Vinsant S. Quantitative changes in hippocampal structure following long-term exposure to delta 9-tetrahydrocannabinol: possible mediation by glucocorticoid systems. Brain Res 1988; 443: 47–62.

Lawston J, Borella A, Robinson JK, et al. Changes in hippocampal morphology following chronic treatment with the synthetic cannabinoid WIN 55,212-2. Brain Res 2000; 877: 407–10.

Ledent C, Valverde O, Cossu G, et al. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. Science 1999; 283: 401–4.

Lenz RA, Wagner JJ, Alger BE. N- and L-type calcium channel involvement in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. J Physiol 1998; 512: 61–73.

Lepore M, Vorel SR, Lowinson J, et al. Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine and food reward. Life Sci 1995; 56: 2073–80.

Lichtman AH, Martin BR. Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology (Berl) 1996; 126: 125–31.

Lichtman AH, Fisher J, Martin BR. Precipitated cannabinoid withdrawal is reversed by delta 9-tetrahydrocannabinol or clonidine. Pharmacol Biochem Behav 2001*a*; 69: 181–8.

Lichtman AH, Sheikh SM, Loh HH, et al. Opioid and cannabinoid modulation of precipitated withdrawal in delta 9-tetrahydrocannabinol and morphine-dependent mice. J Pharmacol Exp Ther 2001*b*; 298: 1007–14.

Linzen DH, Dingemans PM, Lenior ME. Cannabis abuse and the course of recent-onset schizophrenic disorders. Arch Gen Psychiatry 1994; 51: 273–9.

MacKie K, Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. Proc Natl Acad Sci USA 1992; 89: 3825–9.

Maejima T, Hashimoto K, Yoshida T, et al. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. Neuron 2001; 31: 463–75.

Mallet PE, Beninger RJ. The cannabinoid CB_1 receptor antagonist SR141716A attenuates the memory impairment produced by delta 9-tetrahydrocannabinol or anandamide. Psychopharmacology (Berl) 1998; 140: 11–9.

Manno JE, Kiplinger GF, Haine SE, et al. Comparative effects of smoking marihuana or placebo on human motor and mental performance. Clin Pharmacol Ther 1970; 11: 808–15.

Manzanares J, Corchero J, Romero J, et al. Pharmacological and biochemical interactions between opioids and cannabinoids. Trends Pharmacol Sci 1999; 20: 287–94.

Marsicano G, Lutz B. Expression of the cannabinoid receptor CB_1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci 1999; 11: 4213–4225.

Mathers DC, Ghodse AH. Cannabis and psychotic illness. Br J Psychiatry 1992; 161: 648–53.

Mathew RJ, Wilson WH, Coleman RE, et al. Marijuana intoxication and brain activation in marijuana smokers. Life Sci 1997; 60: 2075–89.

Mathew RJ, Wilson WH, Turkington TG, et al. Cerebellar activity and disturbed time sense after THC. Brain Res 1998; 797: 183–9.

Matsuda LA, Lolait SJ, Brownstein MJ, et al. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990; 346: 561–4.

Mattes RD, Engelman K, Shaw LM, et al. Cannabinoids and appetite stimulation. [Review]. Pharmacol Biochem Behav 1994; 49: 187–95.

Mechoulam R. Marihuana chemistry. [Review]. Science 1970; 168: 1159–66.

Mechoulam R, Fride E. Physiology. A hunger for cannabinoids. [Review]. Nature 2001; 410: 763, 765.

Mechoulam R, Ben-Shabat S, Hanuš L, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 1995; 50: 83–90.

Mechoulam R, Fride E, Hanus L, et al. Anandamide may mediate sleep induction. [Review]. Nature 1997; 389: 25–6.

Mendelson JH, Babor TF, Kuehnle JC, et al. Behavioral and biologic aspects of marijuana use. [Review]. Ann NY Acad Sci 1976; 282: 186–210.

Meng ID, Manning BH, Martin WJ, et al. An analgesia circuit activated by cannabinoids. Nature 1998; 395: 381–3.

Miller LL, Branconnier RJ. Cannabis: effects on memory and the cholinergic limbic system. Psychol Bull 1983; 93: 441–56.

Misner DL, Sullivan JM. Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. J Neurosci 1999; 19: 6795–805.

Morral AR, McCaffrey DF, Paddock SM. Reassessing the marijuana gateway effect. Addiction 2002; 97: 1493–504.

Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993; 365: 61–5.

Nagayama T, Sinor AD, Simon RP, et al. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. J Neurosci 1999; 19: 2987–95.

Nakamura-Palacios EM, Winsauer PJ, Moerschbaecher JM. Effects of the cannabinoid ligand SR141716A alone or in combination with delta-9-tetrahydrocannabinol or scopolamine on learning in squirrel monkeys. Behav Pharmacol 2000; 11: 377–86.

Negrete JC, Knapp WP, Douglas DE, et al. Cannabis affects the severity of schizophrenic symptoms: results of a clinical survey. Psychol Med 1986; 16: 515–20.

Ohno-Shosaku T, Maejima T, Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron 2001; 29: 729–38.

Panikashvili D, Simeonidou C, Ben-Shabat S, et al. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature 2001; 413: 527–31.

Patel S, Hillard CJ. Cannabinoid CB_1 receptor agonists produce cerebellar dysfunction in mice. J Pharmacol Exp Ther 2001; 297: 629–37.

Patton GC, Coffey C, Carlin JB, et al. Cannabis use and mental health in young people: cohort study. BMJ 2002; 325: 1195–8.

Peralta V, Cuesta MJ. Influence of cannabis abuse on schizophrenic psychopathology. Acta Psychiatr Scand 1992; 85: 127–30.

Pertwee RG. Tolerance to and dependence on psychotropic cannabinoids. [Review]. In: Pratt J, editor. The biological basis of drug tolerance and dependence. London: Academic Press; 1991. p. 232–65.

Pertwee RG. Pharmacology of cannabinoid receptor ligands. [Review]. Curr Med Chem 1999; 6: 635–64.

Pertwee RG. Cannabinoid receptors and pain. [Review]. Prog Neurobiol 2001; 63: 569–611.

Pettit DAD, Harrison MP, Olson JM, et al. Immunohistochemical localization of the neural cannabinoid receptor in rat brain. J Neurosci Res 1998; 51: 391–402.

Piomelli D, Beltramo M, Giuffrida A, et al. Endogenous cannabinoid signaling. [Review]. Neurobiol Dis 1998; 5: 462–73.

Piomelli D, Giuffrida A, Calignano A, et al. The endocannabinoid system as a target for therapeutic drugs. [Review]. Trends Pharmacol Sci 2000; 21: 218–24.

Pitler TA, Alger BE. Postsynaptic spike firing reduces synaptic $GABA_A$ responses in hippocampal pyramidal cells. J Neurosci 1992; 12: 4122–32.

Pitler TA, Alger BE. Depolarization-induced suppression of GABAergic inhibition in rat hippocampal pyramidal cells: G protein involvement in a presynaptic mechanism. Neuron 1994; 13: 1447–55.

Pontieri FE, Monnazzi P, Scontrini A, et al. Behavioral sensitization to heroin by cannabinoid pretreatment in the rat. Eur J Pharmacol 2001; 421: R1–3.

Pope HG Jr, Gruber AJ, Hudson JI, et al. Neuropsychological performance in long-term cannabis users. Arch Gen Psychiatry 2001; 58: 909–15.

Reibaud M, Obinu MC, Ledent C, et al. Enhancement of memory in cannabinoid CB_1 receptor knock-out mice. Eur J Pharmacol 1999; 379: R1–2.

Rinaldi-Carmona M, Barth F, Heaulme M, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 1994; 350: 240–4.

Robson P. Therapeutic aspects of cannabis and cannabinoids. [Review]. Br J Psychiatry 2001; 178: 107–15.

RodríguezdeFonseca F, Carrera MR, Navarro M, et al. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. Science 1997; 276: 2050–4.

RodríguezdeFonseca F, Del Arco I, Martín-Calderón JL, et al. Role of the endogenous cannabinoid system in the regulation of motor activity. [Review]. Neurobiol Dis 1998; 5: 483–501.

Roth SH. Stereospecific presynaptic inhibitory effect of delta-9-tetrahydrocannabinol on choiinergic transmission in the myenteric plexus of the guinea pig. Can J Physiol Pharmacol 1978; 56: 968–75.

Rubino T, Patrini G, Massi P, et al. Cannabinoid-precipitated withdrawal: a time-course study of the behavioral aspect and its correlation with cannabinoid receptors and G protein expression. J Pharmacol Exp Ther 1998; 285: 813–9.

Sánchez C, Galve-Roperh I, Canova C, et al. Delta 9-tetrahydrocannabinol induces apoptosis in C6 glioma cells. FEBS Lett 1998; 436: 6–10.

Santucci V, Storme JJ, Soubrie P, et al. Arousal-enhancing properties of the CB_1 cannabinoid receptor antagonist SR 141716A in rats as assessed by electroencephalographic spectral and sleep-waking cycle analysis. Life Sci 1996; 58: PL103–10.

Sañudo-Peña MC, Tsou K, Walker JM. Motor actions of cannabinoids in the basal ganglia output nuclei. Life Sci 1999; 65: 703–13.

Sañudo-Peña MC, Romero J, Seale GE, et al. Activational role of cannabinoids on movement. Eur J Pharmacol 2000; 391: 269–74.

Satz P, Fletcher JM, Sutker LS. Neuropsychologic, intellectual, and personality correlates of chronic marijuana use in native Costa Ricans. Ann NY Acad Sci 1976; 282: 266–306.

Scallet AC. Neurotoxicology of cannabis and THC: a review of chronic exposure studies in animals. [Review]. Pharmacol Biochem Behav 1991; 40: 671–6.

Scallet AC, Uemura E, Andrews A, et al. Morphometric studies of the rat hippocampus following chronic delta-9-tetrahydrocannabinol (THC). Brain Res 1987; 436: 193–8.

Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. [Review]. Trends Pharmacol Sci 2001; 22: 565–72.

Shen M, Thayer SA. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. Mol Pharmacol 1998; 54: 459–62.

Shen M, Piser TM, Seybold VS, et al. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 1996; 16: 4322–34.

Sinor AD, Irvin SM, Greenberg DA. Endocannabinoids protect cerebral cortical neurons from in vitro ischemia in rats. Neurosci Lett 2000; 278: 157–60.

Skosnik PD, Spatz-Glenn L, Park S. Cannabis use is associated with schizotypy and attentional disinhibition. Schizophr Res 2001; 48: 83–92.

Smith FL, Cichewicz D, Martin ZL, et al. The enhancement of morphine antinociception in mice by delta-9-tetrahydrocannabinol. Pharmacol Biochem Behav 1998; 60: 559–66.

Solowij N. Cannabis and cognitive functioning. [Review]. Cambridge: Cambridge University Press; 1998.

Stella N, Piomelli D. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. Eur J Pharmacol 2001; 425: 189–96.

Stella N, Schweitzer P, Piomelli D. A second endogenous

cannabinoid that modulates long-term potentiation. Nature 1997; 388: 773–8.

Stiglick A, Kalant H. Residual effects of chronic cannabis treatment on behavior in mature rats. Psychopharmacology (Berl) 1985; 85: 436–9.

Sugiura T, Kondo S, Sukagawa A, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 1995; 215: 89–97.

Swift W, Hall W, Teesson M. Cannabis use and dependence among Australian adults: results from the National Survey of Mental Health and Wellbeing. Addiction 2001; 96: 737–48.

Tanda G, Pontieri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. Science 1997; 276: 2048–50.

Tanda G, Munzar P, Goldberg SR. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat Neurosci 2000; 3: 1073–4.

Terranova JP, Michaud JC, Le Fur G, et al. Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716-A, a selective antagonist of CB_1 cannabinoid receptors. Naunyn Schmiedebergs Arch Pharmacol 1995; 352: 576–9.

Terranova JP, Storme JJ, Lafon N, et al. Improvement of memory in rodents by the selective CB_1 cannabinoid receptor antagonist, SR141716. Psychopharmacology (Berl) 1996; 126: 165–72.

Thomas H. Psychiatric symptoms in cannabis users. [Review]. Br J Psychiatry 1993; 163: 141–9.

Thornicroft G. Cannabis and psychosis. Is there epidemiological evidence for an association? Br J Psychiatry 1990; 157: 25–33.

Tramèr MR, Carroll D, Campbell FA, et al. Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. [Review]. BMJ 2001; 323: 16–21.

US Department of Health and Human Services. US National Household Survey of Drug Abuse. 1999. Washington DC.

Valverde O, Maldonado R, Valjent E, et al. Cannabinoid withdrawal syndrome is reduced in pre-proenkephalin knock-out mice. J Neurosci 2000; 15: 9284–9.

Valverde O, Noble F, Beslot F, et al. Delta 9-tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. Eur J Neurosci 2001; 13: 1816–24.

VanderStelt M, Veldhuis WB, Bär PR, et al. Neuroprotection by delta-9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced in vivo excitotoxicity. J Neurosci 2001; 21: 6475–9.

Varma N, Carlson GC, Ledent C, et al. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. J Neurosci 2001; 21: RC188–93.

Verdoux H, Gindre C, Sorbara F, et al. Effects of cannabis and psychosis vulnerability in daily life: an experience sampling test study. Psychol Med 2003; 33: 23–32.

Vincent BJ, McQuiston DJ, Einhorn LH, et al. Review of cannabinoids and their antiemetic effectiveness. [Review]. Drugs 1983; 25 Suppl 1: 52–62.

Walker JM, Hohmann AG, Martin WJ, et al. The neurobiology of cannabinoid analgesia. [Review]. Life Sci 1999; 65: 665–73.

Welch SP, Stevens DL. Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. J Pharmacol Exp Ther 1992; 262: 10–18.

Williams CM, Kirkham TC. Anandamide induces overeating: mediation by central cannabinoid (CB_1) receptors. Psychopharmacology (Berl) 1999; 143: 315–7.

Williams JH, Wellman NA, Rawlins JNP. Cannabis use correlates with schizotypy in healthy people. Addiction 1996; 91: 869–77.

Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 2001; 410: 588–92.

Yamaguchi T, Hagiwara Y, Tanaka H, et al. Endogenous cannabinoid, 2-arachidonylglycerol, attenuates naloxone-precipitated withdrawal signs in morphine-dependent mice. Brain Res 2001; 909: 121–6.

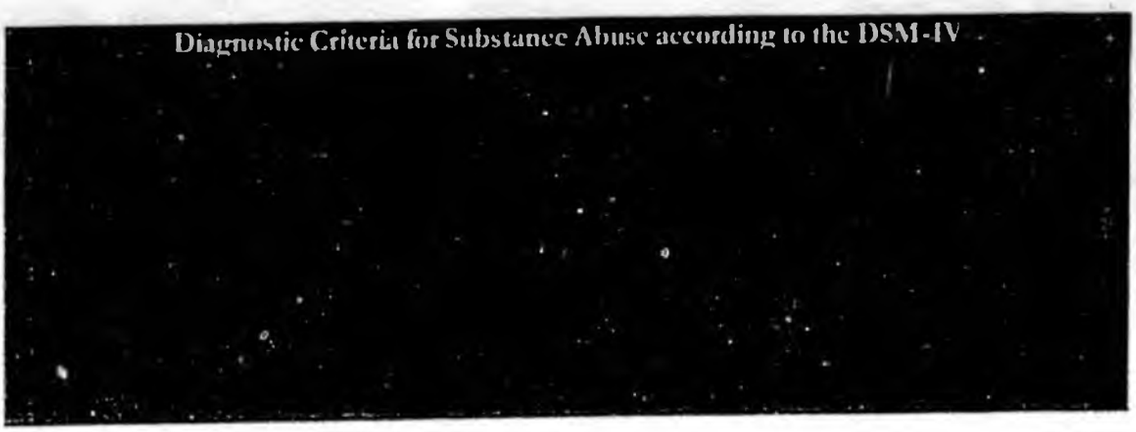
Zammit S, Allebeck P, Andreasson S, et al. Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. BMJ 2002; 325: 1199.

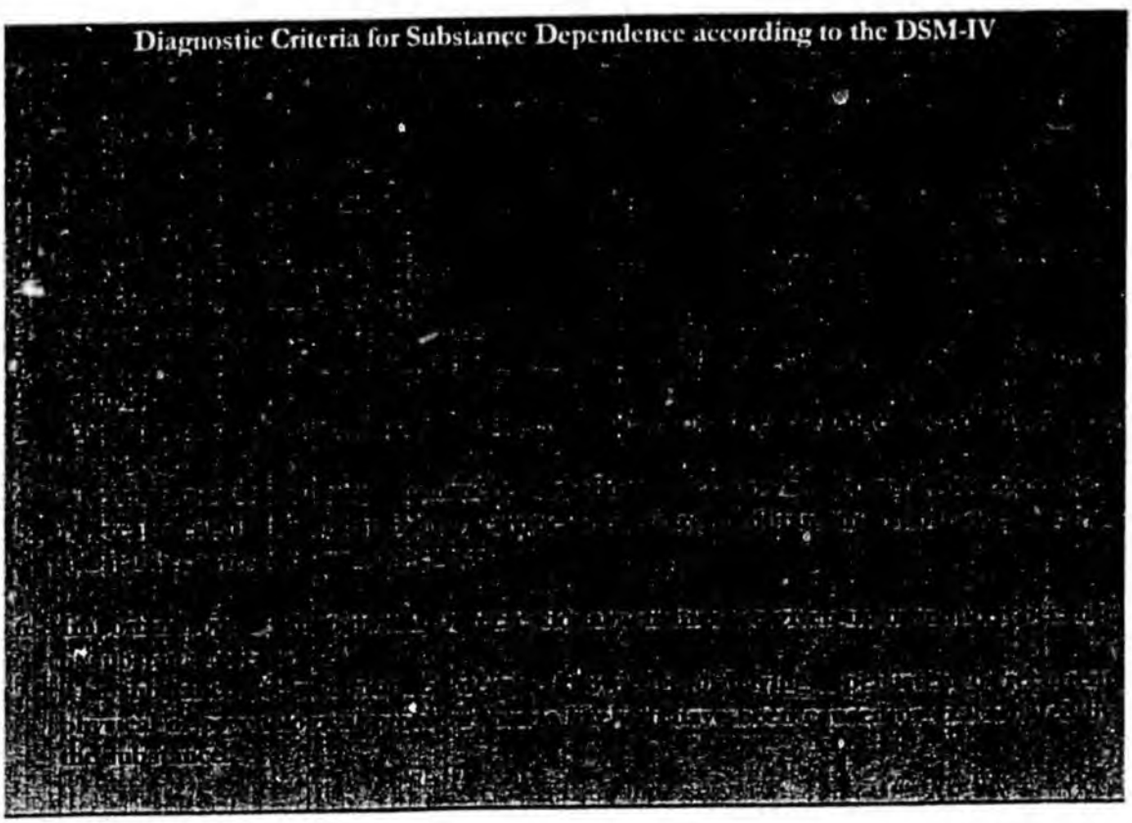
Zimmer L, Morgan JP. Marijuana myths, marijuana facts. [Review]. New York: Lindesmith Center; 1997.

Zimmer A, Zimmer AM, Hohmann AG, et al. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB_1 receptor knockout mice. Proc Natl Acad Sci USA 1999; 96: 5780–5.

Received April 15, 2002. Revised January 13, 2003
Accepted January 15, 2003

N


Diagnostic Criteria for Substance Abuse according to the DSM-IV


Diagnostic Criteria for Substance Dependence according to the DSM-IV

6. Trends in Initiation of Substance Use

Estimates of substance use incidence, or initiation, concern the number of new users of illicit drugs, alcohol, or tobacco during a given year. These estimates supplement prevalence estimates as and measures of the Nation's substance use problem. Where prevalence estimates describe the extent of use of substances over some period of time, incidence data describe emerging patterns of use, particularly among young people. In the past, increases and decreases in incidence usually have been followed by corresponding changes in the prevalence of use, particularly among youths.

The incidence estimates in this report are based on the 2002 National Survey on Drug Use and Health (NSDUH). As the 2002 NSDUH constitutes a new baseline year for the survey, these data should not be compared with previously published data from the National Household Survey on Drug Abuse (NHSDA).

The incidence estimates are based on NSDUH questions on age at first use, year and month of first use for recent initiates, the respondent's date of birth, and the interview date. Using this information along with editing and imputation when necessary, an exact date of first use is determined for each substance used by each respondent. By applying sample weights to incidents of first use, estimates of the number of new users of each substance are developed for each year. These estimates include the number of new users at any age (including those younger than age 12) and also are shown for two specific age groups—persons younger than 18 and adults aged 18 or older. In addition, the average age of new users in each year and age-specific rates of first use are estimated.

Although they are not discussed in this chapter, estimates of age-specific incidence rates also are developed. These rates are defined as the number of new users per 1,000 potential new users because they indicate the rate of new use among persons who have not yet used the substance (i.e., potential new users). More precisely, the rates are actually the number of new users per 1,000 person-years of exposure. This measure is widely used in describing the incidence of disease. The method used for computing these rates is described in Section B.4 in **Appendix B**.

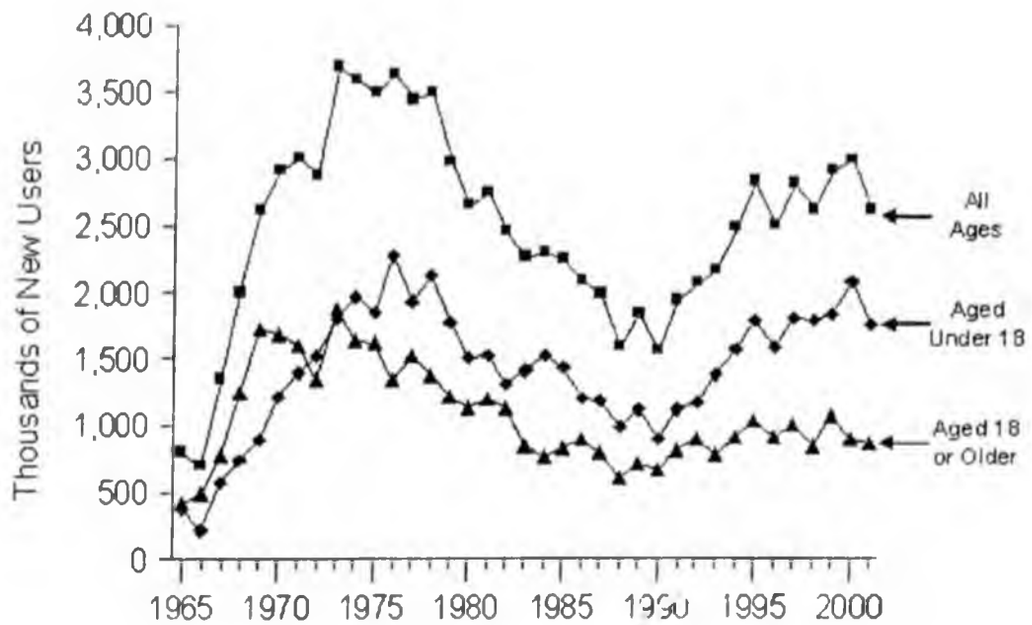
Because the incidence estimates reported herein are based on retrospective reports of age at first substance use by survey respondents interviewed during 2002, they may be subject to several sources of bias. These include bias due to differential mortality of users and nonusers of each substance, bias due to memory errors (recall decay and telescoping), and underreporting bias due to social acceptability and fear of disclosure. See Section B.4 in **Appendix B** for a discussion of these biases. It is possible that some of these biases, particularly telescoping and underreporting because of fear of disclosure, may be affecting estimates for the most recent years more significantly than estimates for earlier years. To account for this bias in the interpretation of the trends, a more stringent standard for determining statistical significance involving estimates from the most recent years (1998 and later) is used in this chapter. Differences are reported to be statistically significant only if they differ at the $\alpha = .01$ level. The usual standard in the rest of the report is the $\alpha = .05$ level. This is an arbitrary standard that provides some protection against incorrect conclusions in the face of potential biases that can fluctuate and even change the direction of estimates from year to year. A more thorough analysis of the problem will be conducted in the future.

Because the incidence estimates are based on retrospective reports of age at first use, the most recent year available for these estimates is 2001, based on the 2002 NSDUH. For two of the measures, first alcohol use and first cigarette use, initiation before age 12 is common. A 2–year lag in reporting for "all ages" estimates is applied for these measures because the NSDUH sample does not cover youths under age 12. The 2–year lag ensures that initiation at ages 10 and 11 is captured in the estimation.

Marijuana

- There were an estimated 2.6 million new marijuana users in 2001. This number is similar to the numbers of new users each year since 1995, but above the number in 1990 (1.6 million).
- In 1965, there were an estimated 0.8 million new users of marijuana. The annual number of marijuana initiates generally increased until about 1973. From 1973 to 1978, the annual number of marijuana initiates remained level at approximately 3.5 million per year. After that, the number of initiates declined to 1.6 million in 1990, then rose to 2.8 million in 1995. From 1995 to 2001, there was no consistent trend, with estimates varying between 2.5 and 3.0 million per year (**Figure 6.1**).

Figure 6.1 Annual Numbers of New Users of Marijuana: 1965–2001



D

- In 2001, about two thirds (67 percent) of new marijuana users were under age 18. This proportion has generally increased since the 1960s, when less than half of initiates were under 18 (**Figure 6.1**). The average age of marijuana initiates was around 19 in the late 1960s and 17.1 in 2001.
- Since 1975, about half of marijuana initiates each year were females (51 percent in 2001). Prior to 1975, females comprised fewer than half of new users, on average. Since 1965, the average age of female initiates has generally been slightly higher than the average age for male initiates.

Cocaine

- Incidence of cocaine use generally rose throughout the 1970s to a peak in 1980 (1.7 million new users) and subsequently declined until 1991 (0.7 million new users). Cocaine initiation steadily increased during the 1990s, reaching 1.2 million in 2001.
- Age-specific incidence rates generally have mirrored the overall incidence trends, with greater initiation among adults than among youths under 18. Approximately 70 percent of cocaine initiates in 2001 were age 18 or older.
- Since 1975, males have generally comprised the majority of cocaine initiates. In 2001, there were 0.7 million new male users and 0.5 million new female users.
- The average age of cocaine initiates rose from 18.6 years in 1968 to 23.8 years in 1990 and subsequently declined to approximately 21 years from 1995 to 2001.

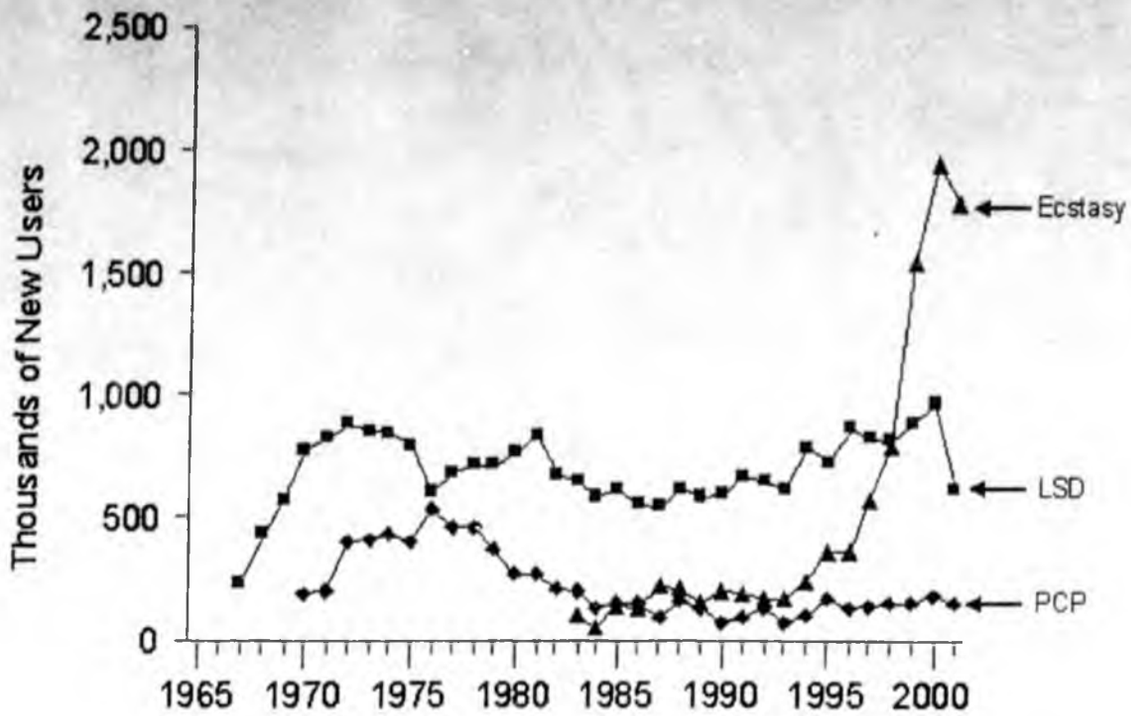
Heroin

- During the latter half of the 1990s, the annual number of heroin initiates rose to a level not reached since the late 1970s. In 1974, there were an estimated 246,000 heroin initiates. Between 1988 and 1994, the annual number of new users ranged from 28,000 to 80,000. Between 1995 and 2001, the number of new heroin users was consistently greater than 100,000.

Hallucinogens

- The incidence of hallucinogen use has exhibited two notable periods of increase. Between 1966 and 1970, the annual number of initiates rose almost sixfold, from 168,000 to 956,000. This increase was driven primarily by use of LSD. The second period of increase in first-time hallucinogen began in 1992 when there were approximately 706,000 new users. By 2000, the number of initiates rose to 1.7 million, which is similar to the number for 2001 (1.6 million). The hallucinogen increase in the 1990s appears to have been driven by increases in use of Ecstasy (i.e., MDMA) (**Figure 6.2**).
- Initiation of Ecstasy use has been rising since 1993, when there were 168,000 new users. There were 1.9 million initiates in 2000 and 1.8 million in 2001 (not a statistically significant decline).

Figure 6.2 Annual Numbers of New Users of Ecstasy, LSD, and PCP: 1965–2001



D

- LSD incidence dropped from 958,000 new users in 2000 to 606,000 in 2001.

Inhalants

- The number of new inhalant users increased from 627,000 new users in 1994 to 1.2 million in 2000. During this period, more males initiated inhalant use than females. The number of new inhalant users in 2001 was similar to the number in 2000 (1.1 million).
- Inhalant initiates in 2001, as well as in prior years, were predominantly under age 18 (71 percent in 2001).

Psychotherapeutics

- This category includes nonmedical use of any prescription-type pain reliever, tranquilizer, stimulant, or sedative; it also includes methamphetamine. This category does not include over-the-counter substances.
- Pain reliever incidence increased from 1990, when there were 628,000 initiates, to 2000, when there were 2.7 million (**Figure 6.3**). In 2001, the number was 2.4 million, not significantly different from 2000. About half (52 percent) of the new users in 2001 were females.

Figure 6.3 Annual Numbers of New Nonmedical Users of Psychotherapeutics: 1965–2001