Pharmacology Biochemistry & Behavior, Vol. 40, pp. 97–101. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Effects of Single and Repeated Marijuana Smoke Exposure on Fetal EEG[1]

HAZEL H. SZETO,[2,3] DUN-LI WU, JOHN A. DECENA AND YI CHENG

Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021

Received 16 April 1991

SZETO, H. H., D.-L. WU, J. A. DECENA AND Y. CHENG. *Effects of single and repeated marijuana smoke exposure on fetal EEG.* PHARMACOL BIOCHEM BEHAV **40**(1) 97–101, 1991.—The effect of single and repeated marijuana smoke exposure on fetal EEG was investigated in the chronic fetal lamb model using power spectral analysis. Maternal inhalation of marijuana smoke (n = 9) resulted in a significant reduction in total power and power distribution in the delta (1–4 Hz) band, and an increase in power distribution in the faster frequencies in the first h after smoke inhalation. These EEG changes were not observed following maternal inhalation of placebo smoke (n = 5), nor in animals with 3–5 prior exposures to marijuana smoke (n = 5). These results suggest that the effects of marijuana smoke exposure on fetal EEG is short-lived and tolerance develops rapidly with repeated exposure.

Marijuana Cannabis EEG Fetus Tolerance

A large number of studies have reported adverse effects of marijuana use during pregnancy on birth weight and gestational length. Our understanding of the effects of marijuana use on neurobehavioral development is much more limited. Increased tremors and startles, and poorer habituation to visual stimuli, have been reported in marijuana-exposed neonates and infants up to one month of age (13,14). Disturbances in sleep cycling and motility were also reported in marijuana-exposed infants at 24 to 36 h of age (24). It is not clear whether these sleep disturbances are part of a mild withdrawal syndrome, or whether they are consequences of direct action of marijuana on the development of the nervous system.

Acute exposure to marijuana smoke has been reported to affect both waking and sleep EEG in adult humans and animals (12). Most studies which report EEG changes have noted that tolerance develops with repeated drug exposure; and long-term use of marijuana has not been shown to produce persistent changes in EEG in either moderate (11,23) or heavy users (16). There is currently no information on the effects of single or repeated marijuana exposure on fetal EEG in either humans or animal models. Whether tolerance develops to the effect on fetal EEG may have significant impact on the long-term consequences of marijuana use on fetal brain development.

The investigation of the effects of marijuana smoke in an animal model has been greatly hampered by the lack of a convenient method to introduce marijuana smoke to an alert animal. Furthermore, the large number of cannabinoids in marijuana smoke, many of which are pharmacologically active and may interact with each other, has made it difficult to justify using only a single active ingredient [such as Δ^9-tetrahydrocannabinol (THC)] in these studies. The recent development of a simple smoking machine has made it possible to introduce marijuana smoke to conscious pregnant ewes (1). The objective of this study was to study the effect of single and repeated maternal marijuana smoke inhalation on fetal EEG in the sheep.

METHOD

Animal Preparation

Fourteen fetal lambs were surgically instrumented for chronic intrauterine recording of EEG between 110–118 days of gestation (term = 145 days), in accordance with guidelines approved by the Institution for the Care and Use of Animals. Details of the surgical procedure have been described previously (1,25). Briefly, four stainless steel screws (size 0-80) were implanted over the parietal cortex for recording EEG activity. A polyvinyl catheter was placed in the distal aorta to permit collection of arterial blood for blood gas determinations. The EEG leads and catheter were tunneled SC to the maternal flank and stored in a pouch. Intraoperatively, 2 g of ampicillin was placed in the amniotic cavity and 1 g in the peritoneal cavity of the ewe. In addition, a silicone rubber T-tube was implanted in the maternal trachea to facilitate inhalational exposure to marijuana smoke.

Study Design

Ewes were allowed at least 5 days for recovery after surgery before they were randomized to either the placebo or marijuana treatment group. Only fetuses with arterial pH > 7.3, $pCO_2 < 50$ mmHg and $pO_2 > 16$ mmHg were included in the study. Fetal EEG recordings were obtained with the ewe standing or lying

[1]This work was supported, in part, by the National Institute on Drug Abuse (DA-03722).

[2]Dr. Hazel H. Szeto is a recipient of a Research Scientist Development Award (DA-00100) from the National Institute on Drug Abuse, ADAMHA.

[3]Requests for reprints should be addressed to Hazel H. Szeto, M.D., Ph.D., Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

quietly in a cart. The ewe had free access to food and water throughout the recording period. Control EEG was recorded for 3 h prior to smoke exposure. Over a 10-min period, the ewe "smoked" either a marijuana (n = 9) or placebo (n = 5) cigarette supplied by the National Institute on Drug Abuse containing either 1.84% or 0% THC, respectively. The cigarettes were prehumidified and burned in a small Plexiglas cylinder that was hung around the ewe's neck (1). During inhalation the cigarette smoke is mixed with residual and inspired air, and delivered to the lower trachea and bronchial systems. Fetal EEG recording continued for 2 h after smoke inhalation. In 5 fetuses, exposure to marijuana smoke was repeated once every third day. The effect of repeated exposure to marijuana smoke was studied again after 3–5 exposures.

Power Spectral Analysis

Details of the EEG acquisition method have been published previously (25). Briefly, the EEG signal was amplified and filtered (1–100 Hz), and digitized at a rate of 256 Hz. Fast fourier transform (FFT) was performed on consecutive series of 1024 data points (4 s). Trend removal was included to reduce the effect of movement artifacts on the transform. A Hamming (raised cosine) window was applied to control side-lobe leakage due to finite record length. Details of the method can be found in earlier publications (11,12). The resolution of the FFT was 0.25 Hz. The maximum detectable frequency was designated to be 64 Hz, although data for frequencies >32 Hz were discarded. Five power spectra were then averaged to produce a single mean power spectrum every 20 s. The transformed data were then further compressed into 4 frequency bands (delta, 1–4 Hz; theta, 5–7 Hz; alpha, 8–13 Hz; beta, 15–32 Hz) and relative power distribution was calculated for each band (24). The 90% spectral edge frequency was also calculated as the frequency below which 90% of the power resides (25).

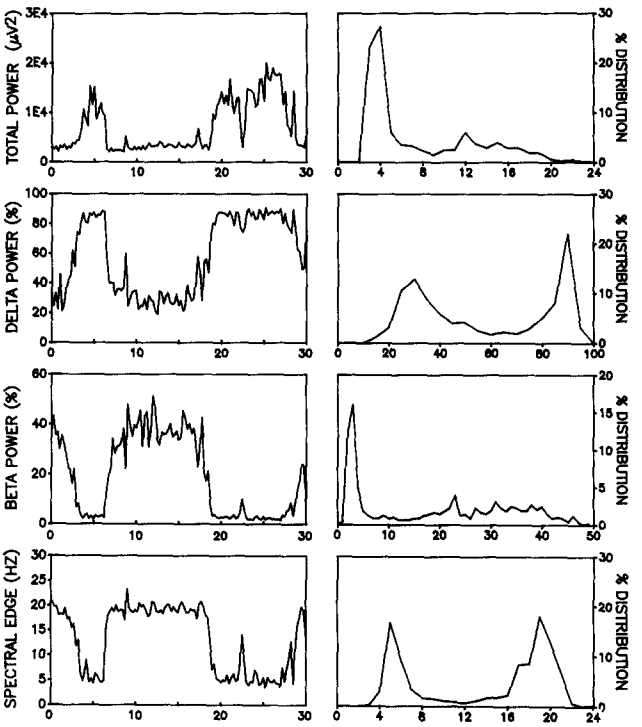

FIG. 1. Left panel: Time series plot of total power, relative power distribution in the delta (1–4 Hz) and beta (15–32 Hz) band, and the 90% spectral edge frequency. Representative data obtained from a 131-day fetal lamb during control recording. Total time represented is 30 min. Right panel: Corresponding normalized distribution of total power, relative power in the delta (1–4 Hz) band and beta (15–32 Hz) band, and the 90% spectral edge for data shown in the left panel. Distribution was determined from the entire data set of 180 min.

Automated EEG State Scoring

Two distinct EEG patterns were clearly distinguishable by visual analysis in the control presmoke recordings, a synchronized high voltage slow activity (HVSA) pattern and a desynchronized low voltage fast activity (LVFA) pattern. A transitional (TRANS) pattern of intermediate amplitude and frequency could also be identified. The time series plot of total power, power distribution in the different bandwidths and the spectral edge provided a guide to the range of spectral parameters to be found in stable episodes of HVSA and LVFA (Fig. 1, left panel). The HVSA is readily identified by high total power, large distribution of power in the delta band and very low distribution of power in the beta band. In contrast, the LVFA state can be identified by low total power, a large distribution of power in the beta band, and a high spectral edge frequency. The numerical criteria used in the computerized scoring of HVSA and LVFA were obtained from the distribution histograms of total power, spectral edge frequency, and power distribution in the delta and beta bands (Fig. 1, right panel). All remaining unassigned runs were designated as TRANS. Due to large variation in spectral estimates as a function of gestational age of the fetus, these scoring criteria were established for each individual fetus on a daily basis. The same criteria were then used for the postsmoking data on the same day. Details of the method have been published previously (26).

TABLE 1

SPECTRAL PARAMETERS OF THE THREE EEG STATES IN A 125-DAY FETAL LAMB

State	Total Power	Percent of Total Power in 1–4 Hz	5–7 Hz	8–13 Hz	15–32 Hz	Spectral Edge (Hz)
LVFA (n = 183)	3939 ± 27	54.6 ± 0.3	12.5 ± 0.4	15.5 ± 0.1	15.0 ± 0.1	16.4 ± 0.1
TRANS (n = 103)	6073 ± 47	69.0 ± 0.2	10.7 ± 0.1	11.6 ± 0.1	7.5 ± 0.1	12.0 ± 0.1
HVSA (n = 241)	13402 ± 4	84.0 ± 0.0	6.4 ± 0.1	5.6 ± 0.1	3.4 ± 0.0	6.5 ± 0.1

TABLE 2

EFFECT OF SMOKE ADMINISTRATION ON INCIDENCE OF EEG STATES

	Single Exposure				Repeated Exposure	
	Marijuana (n=9)		Placebo (n=5)		Marijuana (n=5)	
EEG State	Before	After	Before	After	Before	After
LVFA	44.7 ± 2.7	55.0 ± 5.3*	44.1 ± 5.2	48.4 ± 5.3	35.0 ± 3.6	33.6 ± 2.8
TRANS	10.5 ± 1.8	14.0 ± 3.4	14.8 ± 3.5	11.5 ± 2.8	13.2 ± 1.8	13.8 ± 3.3
HVSA	44.8 ± 1.6	31.2 ± 2.3†	42.3 ± 4.5	40.0 ± 4.5	51.5 ± 3.0	52.6 ± 2.6

Data are mean ± S.E.
*$p<0.05$; †$p<0.001$ (compared to values before smoke exposure; paired t-test).

Statistical Analysis

All data are presented as mean ± S.E. Due to the relatively large interindividual variation in spectral characteristics as a function of gestational age of the fetus, the effects of placebo and marijuana smoke inhalation were compared to control presmoke values with each animal serving as its own control. The paired t-test was used to determine statistical significance.

RESULTS

Under control conditions, spectral parameters showed cyclic fluctuations over a 3-h period that strongly suggested the presence of relatively stable EEG states (Fig. 1). The distribution of the various spectral parameters supported the presence of three distinct EEG patterns with significantly different spectral characteristics (Table 1). The incidence of HVSA, LVFA and TRANS in the placebo and marijuana treatment groups prior to smoke inhalation are shown in Table 2. There was no difference in the incidence of the 3 states between the two groups.

Marijuana smoke exposure resulted in a significant disruption of the normal EEG cyclic pattern. Figure 2A and B show the EEG cycling pattern in a 125-day fetal lamb prior to, and after marijuana smoke inhalation, respectively. There was a decrease in HVSA which was compensated by much longer LVFA episodes. The changes in the incidence of the 3 EEG states are summarized in Table 2 for all the animals. There was a significant decrease in the incidence of HVSA ($p<0.001$) and a compensatory increase in LVFA ($p<0.05$). In contrast, placebo smoke administration did not affect the incidence of the 3 EEG states (Table 2).

Power spectral analysis revealed a significant reduction in mean total power ($p<0.05$) and an increase in 90% spectral edge frequency ($p<0.005$) in the first hour after marijuana smoke inhalation, but not in the second hour (Table 3). There was also a redistribution of the power in the different wavebands, with a significant decrease of relative power in the delta band ($p<0.005$), and an increase in all the faster frequencies ($p<0.05$) (Fig. 3). Placebo smoke inhalation did not affect any of the spectral characteristics.

The effects of marijuana on fetal EEG were completely abolished by repeated drug exposure. Following 3–5 exposures given once every three days, marijuana exposure was no longer associated with any change in the incidence of the 3 EEG states (Table 2) or the spectral characteristics of the EEG (Table 3, Fig. 4).

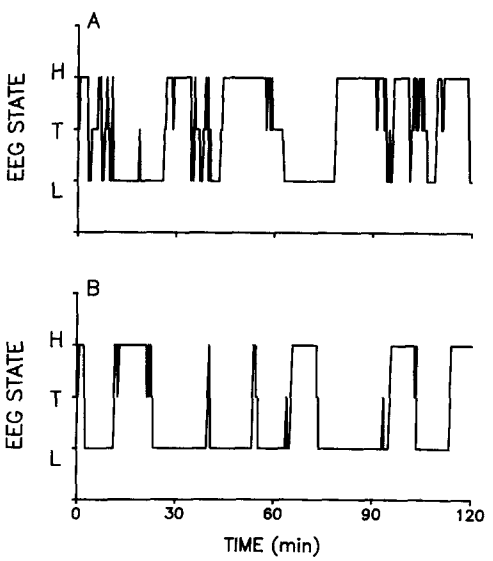

FIG. 2. EEG state transitions before (A) and after (B) marijuana smoke inhalation. [L, low voltage fast activity (LVFA); T, transitional; H, high voltage slow activity (HVSA).]

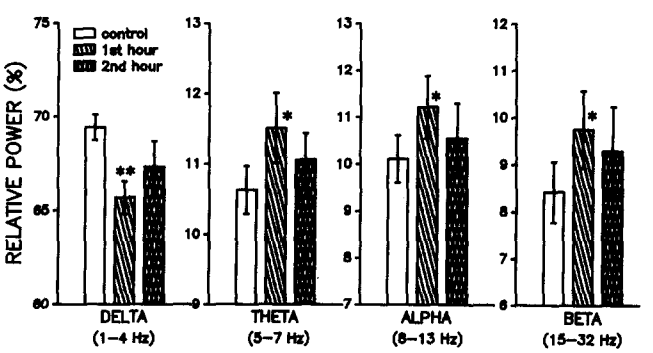

FIG. 3. Effects of single marijuana smoke inhalation on relative power distribution in the different wavebands. Data are mean ± S.E. from 9 animals who have never been exposed to marijuana smoke. Data obtained during the 1st and 2nd hour postsmoke were compared to control values (3 h) with each animal serving as its own control. *$p<0.05$; †$p<0.005$.

TABLE 3

EFFECT OF SMOKE ADMINISTRATION ON EEG SPECTRAL CHARACTERISTICS

Treatment	Total Power ($\times 10^3$ units)			Spectral Edge Frequency (Hz)		
	Control	1st Hour	2nd Hour	Control	1st Hour	2nd Hour
Single Exposure:						
Marijuana	26.2 ± 3.0	17.4 ± 2.1*	20.3 ± 3.6	11.3 ± 0.3	12.4 ± 0.4†	11.8 ± 0.6
Placebo	20.4 ± 3.5	16.5 ± 4.2	16.2 ± 3.5	11.6 ± 0.7	12.1 ± 0.9	12.3 ± 0.9
Multiple Exposures:						
Marijuana	30.3 ± 7.9	37.6 ± 4.9	42.1 ± 8.8	11.8 ± 0.8	11.3 ± 0.6	11.5 ± 0.8

*$p<0.05$, compared to control.
†$p<0.005$, compared to control.

DISCUSSION

The recent development of a simple system for smoke delivery to conscious animals has made possible the assessment of the effects of maternal marijuana smoking on various maternal and fetal physiologic parameters. This smoke production and delivery system has been carefully characterized in both in vitro and animal studies (1,2). The system is capable of delivering large amounts of marijuana smoke to undisturbed ewes efficiently and with minimal inconvenience over a time period (10 min) similar to that typically used in human studies. The plasma THC profile achieved in maternal plasma following inhalation of marijuana smoke generated by this system is very similar to that found in nonpregnant human subjects (2,21). Peak plasma THC levels occurred at the end of the smoking period, and were found to be comparable to those reported in human volunteers (21). THC was detectable in fetal plasma by 10–15 min after onset of smoke inhalation, but peak levels were not reached until 1.5–2 h (2). Thereafter, fetal plasma THC levels declined slowly and remained at approximately 60% of maternal levels over 24 h.

The results of the present study show that this level of marijuana exposure can significantly decrease the incidence of HVSA and result in activation of the fetal EEG, as shown by the decrease in total power and acquisition of faster frequencies. This appears to be a direct drug effect and not due to the stress of smoke inhalation as the changes were not observed after inhalation of placebo smoke. EEG changes have been reported with marijuana exposure in the adult human. A number of studies reported an increase in alpha activity and decreased beta activity with marijuana smoking (11,23). Others reported a reduction in EEG voltage and increase in faster frequencies (15,27). A similar desynchronizing action of marijuana has also been reported in adult rats (5,22). Use of higher doses or direct administration of THC seem to result in EEG synchrony or epileptiform activity (19). Our results in the fetus appear to be consistent with the low-dose findings in the adult.

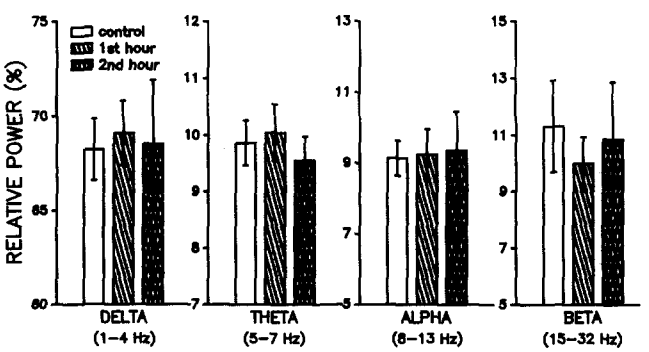

FIG. 4. Effects of repeated marijuana smoke inhalation on relative power distribution in the different wavebands. Data are mean± S.E. from 5 animals who have previously received 3–5 marijuana smoke exposures. Data obtained during the 1st and 2nd hour postsmoke were compared to control values (3 h) with each animal serving as its own control.

A very interesting finding in this study was the rapid adaptation of the fetus to the effect of marijuana. The effect on EEG was completely abolished with as few as three prior exposures given once every three days. Tolerance has been observed to develop to many, but not all, of the effects of marijuana and THC in adult animals (18). The development of tolerance to the EEG effects of marijuana is consistent with the general lack of EEG changes in chronic marijuana users (11, 16, 23). In rats, daily administration of THC resulted in a loss of the EEG desynchronizing effect after 5–12 doses (22). In dogs, tolerance to the behavioral effects of THC could be observed when the cannabinoid was injected only once every eight days (10). The short duration of action of marijuana smoke on fetal EEG, despite fairly constant plasma THC levels, suggest that acute tolerance may be developing with the first exposure. On the other hand, other effects of marijuana have been shown to be rather long-lasting. Barrett and Adams (3) examined the effects on sleep-wakefulness cycles after marijuana was administered for 180 days to cats, and found that the decrease in slow-wave sleep and increase in drowsy-light sleep continued through the 40-day postdrug period. However, the changes in both REM sleep and awake time did not persist throughout the drug exposure period. In humans, slow-wave sleep was found to increase during the first 4 days following marijuana smoking and then progressively decreased until it was significantly below baseline levels by the 8th day (4). Thus whether tolerance develops to repeated marijuana use appears to depend to some extent on the duration and frequency of exposure as well as the specific effect examined.

In addition to the effects on fetal EEG, previous studies using this smoking system in pregnant sheep have reported a number of other cannabinoid effects in the mother and fetus (7, 8, 20). With cigarettes containing 1.84% THC, the same batch as used in this study, Clapp et al. (7) reported a 55–70% reduction in maternal respiratory rate, and a significant fall in maternal heart rate and mean arterial pressure. Despite the reduction in mean arterial pressure, there was no change in uterine blood flow. In the fetus, this level of exposure resulted in a significant reduction in arterial pO_2 (to 17 mmHg) with no change in pCO_2 or pH (7,8). Although this reduction in fetal pO_2 may partly be due to the reduction in maternal pO_2, the longer time course in

the fetus suggests that other mechanisms may be involved (7,8). The effect on fetal EEG does not appear to be secondary to the reduction in fetal pO_2 since mild to moderate hypoxia has been reported to either result in a decrease (6,9) or no change (17) in the incidence of LVFA, whereas an increase in LVFA was found in this study.

In summary, we have found that maternal marijuana smoke exposure can result in significant changes in the fetal EEG which are similar to those reported in adult humans. The effects were short-lived, and the fetus appears to adapt very quickly so that no significant effects were observed upon repeated exposure. This study, however, did not address the issue of chronic marijuana smoke exposure on the development of fetal EEG. These studies are now ongoing in our laboratory.

REFERENCES

1. Abrams, R. M.; Davis, K. H.; Jaeger, M. J.; Szeto, H. H. Marijuana smoke production and delivery system. In: Marijuana '84. Oxford: IRL Press Limited; 1985:205–211.
2. Abrams, R. M.; Cook, C. E.; Davis, K. H.; Niederreither, K.; Jaeger, M. J.; Szeto, H. H. Plasma 9-tetrahydrocannabinol in pregnant sheep and fetus after smoking a marijuana cigarette. Alcohol Drug Res. 6:361–369; 1985.
3. Barrett, E. S.; Adams, P. M. Chronic marijuana usage and sleep-wakefulness cycles in cats. Biol. Psychiatry 6:207–214; 1973.
4. Barrett, E. S.; Beaver, W.; White, R. The effects of marijuana on human sleep patterns. Biol. Psychiatry 8:47–54; 1974.
5. Buonamici, M.; Young, G. A.; Khazan, N. Effects of acute Δ9-THC administration on EEG and EEG power spectra in the rat. Neuropharmacology 21:825–829; 1982.
6. Boddy, K.; Dawes, G. S.; Fisher, R.; Pinter, S.; Robinson, J. S. Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxia and hypercapnia in sheep. J. Physiol. (Lond.) 243:599–618; 1982.
7. Clapp, J. F.; Keve, T. M.; Vial, C.; McLaughlin, M. K. Ovine placental perfusion balance: effect of marijuana smoke. Am. J. Obstet. Gynecol. 159:1430–1434; 1988.
8. Clapp, J. F.; Wesley, M.; Cooke, R.; Pekala, R.; Holstein, C. The effects of marijuana smoke on gas exchange in ovine pregnancy. Alcohol. Drug Res. 7:85–92; 1986.
9. Clewlow, F.; Dawes, G. S.; Johnston, B. M.; Walker, D. W. Changes in breathing, electrocortical and muscle activity in unanesthesized fetal lambs with age. J. Physiol. (Lond.) 341:463–476; 1983.
10. Dewey, W. L.; Jenkins, J.; O'Rourke, T.; Harris, L. S. The effects of chronic administration if trans-Δ^9-tetrahydrocannabinol on behavior and the cardiovascular system of dogs. Arch. Int. Pharmacol. Ther. 198:118–131; 1972.
11. Fink, M. Effects of acute and chronic inhalation of hashish, marijuana, and delta-9-tetrahydrocannabinol on brain electrical activity in man: Evidence for tissue tolerance. Ann. NY Acad. Sci. 282: 387–398; 1976.
12. Fried, P. A. Behavioral and electroencephalographic correlates of the chronic use of marijuana—A review. Behav. Biol. 21:163–196; 1977.
13. Fried, P. A.; Makin, J. E. Neonatal behavioral correlates of prenatal exposure to tobacco, alcohol, cannabis and caffeine on birth size and subsequent growth. Neurotoxicol. Teratol. 9:1–7; 1987.
14. Fried, P. A. Cigarettes and marijuana: are there measurable long-term neurobehavioral teratogenic effects? Neurotoxicology 10:577–584; 1989.
15. Jones, R. T.; Stone, G. C. Psychological studies of marijuana and alcohol in man. Psychopharmacologia 18:108–117; 1970.
16. Karacan, I.; Fernandez-Salas, A.; Coggins, W. J.; Carter, W. E.; Williams, R. L.; Thornby, J. I.; Salis, P.J.; Okawa, M.; Villaume, J. P. Sleep electroencephalographic-electrooculographic characteristics of chronic marijuana users. Ann. NY Acad. Sci. 282:348–374; 1976.
17. Koos, B. J.; Sameshima, H.; Power, G. G. Fetal breathing, sleep state, and cardiovascular responses to graded hypoxia in sheep. J. Appl. Physiol. 62:1033–1039; 1987.
18. McMillan, D. E.; Dewey, W. L.; Harris, L. S. Characteristics of tetrahydro-cannabinol tolerance. Ann. NY Acad. Sci. 191:83–99; 1971.
19. Martinez, J. L.; Stadnicki, S. W.; Schaeppi, U. H. Δ^9-tetrahydrocannabinol: effects on EEG and behavior of rhesus monkeys. Life Sci. 11:643–651; 1972.
20. Niederreither, K.; Jaeger, M. J.; Abrams, R. M. Cardiopulmonary effects of marijuana and delta-9-tetrahydrocannabinol in sheep. Res. Commun. Subst. Abuse 6:87–98; 1985.
21. Perez-Reyes, M. ; Owens, S. M.; Diguiseppi, S. The clinical pharmacology and dynamics of marijuana cigarette smoking. J. Clin. Pharmacol. 21:201S–207S; 1981.
22. Pirch, J. H.; Cohn, R. A.; Barnes, P. R.; Barratt, E. S. Effects of acute and chronic administration of marijuana extract on the rat electrocorticogram. Neuropharmacology 11:231–240; 1972.
23. Rodin, E. A.; Domino, E. F.; Porzak, J. P. The marihuana-induced "social high." Neurological and electroencephalographic concomitants. JAMA 213:1300–1302; 1970.
24. Scher, M. S.; Richardson, G. A.; Coble, P. A.; Day, N. L.; Stoffer, D. L. The effects of prenatal alcohol and marijuana exposure: Disturbance in neonatal sleep cycling and arousal. Pediatr. Res. 24: 101–105; 1988.
25. Szeto, H. H. Spectral edge frequency as a simple quantitative measure of the maturation of electrocortical activity. Pediatr. Res. 27: 289–292; 1990.
26. Szeto, H. H. Morphine-induced activation of fetal EEG is mediated via central muscarinic pathways. Am. J. Physiol. 260:R509–R517; 1991.
27. Williams, E.; Himmelsbach, C. K.; Wikler, A.; Ruble, D. C. Studies on marihuana and parahexyl compounds. Publ. Health Rep. 61: 1059–1083; 1946.