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DEVELOPMENT OF EEG SEIZURE ACTIVITY DURING AND AFTER CHRONIC KETAMINE ADMINISTRATION IN THE RAT

S. MANOHAR,* D. MAXWELL and W. D. WINTERS†
Departments of Anatomy and Pharmacology and the Brain Research Institute,
UCLA School of Medicine, Los Angeles, California 90024

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Summary—Daily assessment of gross behavior and periodic EEG brain wave activity was performed in rats receiving daily ketamine administration for 1, 2 and 3 months. Three groups were used for these studies; the first group received 80 mg/kg ketamine intraperitoneally (i.p.), a dose which produces a cataleptoid-like behavior and characteristic EEG hypersynchronous patterns; the second group received 30 mg/kg ketamine i.p. which induces behavioral excitation followed by ataxia and desynchronized EEG; the third group received saline control with no change in EEG or behavior noted. Abnormal spike and hypersynchronous bursting activity appeared in both the amygdala and dorsal hippocampus during the course of chronic administration of both the low and high dose of ketamine. The incidence of abnormal brain wave spiking was approximately 50% with 3 out of 5 animals demonstrating abnormal brain wave activity at the high dose and 1 out of 3 at the lower dose. During the course of the three months' administration of both the low and high dose of ketamine there was no evidence of tolerance or potentiation of the drug effect based on EEG or behavioral criteria. Withdrawal of the drug for 5 days following three months of treatment resulted in the appearance of a progressive increase in epileptiform activity without gross behavioral manifestations. The abnormal spike and slow wave EEG activity was not correlated with an observable abnormality in behavior.

PHENCYCLIDINE was removed from clinical trial as an anaesthetic because it induced a high incidence of profound post-operative emergence delirium and acute psychotic reactions. Phencyclidine has found its way into the illicit drug market and is one of the more popular hallucinogens. A derivative of phencyclidine, ketamine (Ketalar®) is presently used clinically as an anaesthetic agent. In a prior study (WINTERS, FERRAR-ALLADO, GUZMAN-FLORES and ALCARAZ, 1972) varying degrees of neuropharmacological similarities between ketamine, phencyclidine and other hallucinogens such as LSD-25 and mescaline were demonstrated. In addition, the similarity between ketamine and other anaesthetic agents such as nitrous oxide, alpha-chloralose, trichlorethylene and gamma-hydroxybutyrate were examined. The conclusion drawn from these studies indicated that these agents induced varying degrees of CNS excitation resulting in an anaesthetic state unlike the CNS depression of halothane or the barbiturates, but instead characterized by a preconvulsant state of catalepsy. Both the cataleptoid agents and CNS depressant agents induce loss of responsiveness and amnesia, the basic requirement for clinical anaesthesia (WINTERS, MORI, SPOONER and BAUER, 1967; WINTERS *et al.*, 1972).

*Present Address: Department of Anatomy, Faculty of Medicine, University of Ottawa, Ottawa 2, Canada.
†Present Address: Department of Pharmacology, UCD School of Medicine, Davis, California 95616.

The demonstration of the hallucinoid and cataleptoid properties of ketamine (WINTERS *et al.*, 1972) raised the possibility that this drug has a high abuse liability; unfortunately, this has already begun to be apparent (REIER, 1971).

Some diagnostic and therapeutic procedures require thirty or more exposures to ketamine. Since ketamine induces an excitatory pre-convulsant state (WINTERS *et al.*, 1972), it is critical to assess the effect of chronic administration of the agent on the CNS. The present study in the rat was instituted to examine the action of repeated ketamine administration for one, two or three months. Daily assessment of gross behavior and periodic brain wave activity was made. In addition, the brain tissue is being examined under light and electron microscopy for changes in cellular and subcellular brain structure (MANOHAR and MAXWELL, unpublished observations).

METHODS

Eleven male Sprague–Dawley rats weighing between 200 and 260 g were used in these experiments. During sodium pentobarbital (50 mg/kg i.p.) anaesthesia, stainless steel bipolar electrodes, insulated with two coats of baked Epoxylite, with 1 mm bared tips were implanted in the right amygdala (A 4·2; L 4·7; H −3·7) and the right dorsal hippocampus (A 1·8; L 3·0; H 2·0) utilizing the stereotaxic coordinate system of DE GROOT (1967). Cortical screws were placed extradurally in the parietal cortex, 4 mm posterior to the coronal suture and 4 mm to the right of the sagittal suture. A reference electrode was implanted in the nasal bone. All recording leads were soldered to a plug which was mounted on the skull with methylacrylic cement.

After surgery the rats were allowed to recover for 7–10 days. EEG recordings were then obtained using an 8 channel Grass polygraph. The animals were divided into three groups. One group of 5 received a daily injection of 80 mg/kg i.p. ketamine, the dose which induced catalepsy in all rats. A second group of 3 animals received a daily injection of 30 mg/kg i.p. ketamine which induced ataxia that lasted for 40–50 min. A control group of 3 rats received 0·9% sodium chloride i.p. daily in a volume identical to the volume of drug administered to the other groups. All the injections were given between 11 a.m. and 1 p.m. daily. Behavioral manifestations, the latencies for the development of ataxia or catalepsy and the duration of such effects were noted. In each animal, EEG recordings were made once every 3–5 days, prior to and after the drug or saline administration. Animals from each of the above three groups were sacrificed at intervals of 1, 2 and 3 months. Electrode placements were confirmed histologically.

RESULTS

Control

Control animals received daily intraperitoneal injections of saline and were sacrificed after 1, 2 or 3 months. In all cases, the EEG and gross behavior remained normal during the period of study. In addition, intermittent administration of saline to animals receiving daily doses of ketamine demonstrated that saline did not induce changes in behavior or EEG (Fig. 1B).

Ketamine 80 mg/kg *i.p.*

Initial administration. The initial dose of 80 mg/kg i.p. of ketamine induced excitation and ataxia within 1–2 min. Three to 6 min after the injection, the animal was cataleptoid in that it was nonresponsive to noxious stimuli, lost the righting reflex, and lay in a crouched

position with the eyes open. This cataleptoid state lasted for 20–30 min. On emergence the animal was again ataxic, then excited for approximately 1 hr.

The EEG changes induced by this initial dose of ketamine are depicted in Fig. 1A. Within 1–2 min following ketamine the EEG pattern changed from the control level of desynchronization to higher frequency, slightly higher amplitude, desynchronization with the appearance of occasional high amplitude slow waves, especially in the neocortex. Within 5 min, these high amplitude slow wave bursts in the neocortex became more apparent. The frequency was 2–3 Hz, occurring intermittently between the high frequency waves (intermittent hypersynchrony). Slowing of the intermittent hypersynchronous activity to 1·5 Hz in the neocortex was always correlated with the onset of the loss of the righting reflex. The hypersynchronous slow wave activity in the neocortex persisted throughout the duration of the cataleptoid state and disappeared when the animal regained the righting reflex 30 min after ketamine administration. During the period of the catalepsy, the dorsal hippocampus and the amygdala demonstrated occasional high frequency, high amplitude spindle waves, and spiking activity. Sixty minutes after administration of ketamine the animal was ataxic, moved around the cage continuously and the EEG was still grossly different from the pattern noted during the control period (Fig. 1A).

Daily administration. Administration of the same 80 mg/kg dose of ketamine to the same animal described above (Fig. 1) on Day 20 and Day 30 was grossly similar to Day 1 during the control pre-drug injection periods, and 10 min following ketamine. A second rat, receiving the same dose of ketamine demonstrated a slightly different EEG pattern. Two minutes after receiving ketamine on Day 4 (Fig. 2), the initial period of excitation was correlated with an increased frequency EEG in the neocortex and slow, high amplitude wave activity in the

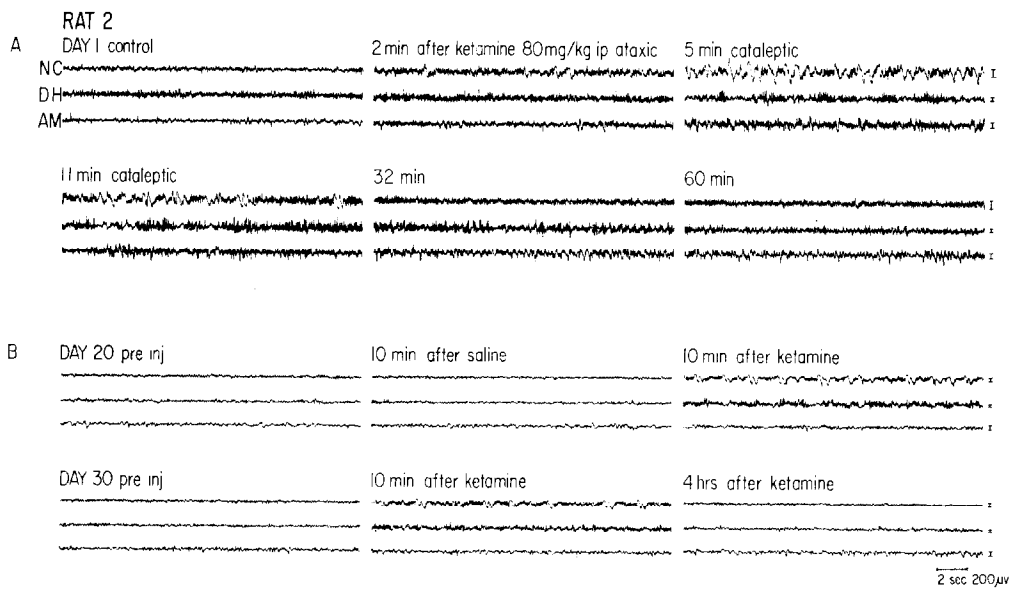

FIG. 1. EEG during control and following administration of ketamine 80 mg/kg i.p. on (A) Day 1 and (B) Day 20 and Day 30. Abbreviations: NC, neocortex; DH, dorsal hippocampus; AM, amygdala. During the cataleptic state (A, 5 and 11 min; B, 10 min), 2–3 Hz waves occurred in NC and high frequency, high amplitude spindle activity and spiking in the DH and AM. On emergence these changes appeared in DH (A, 32 min).

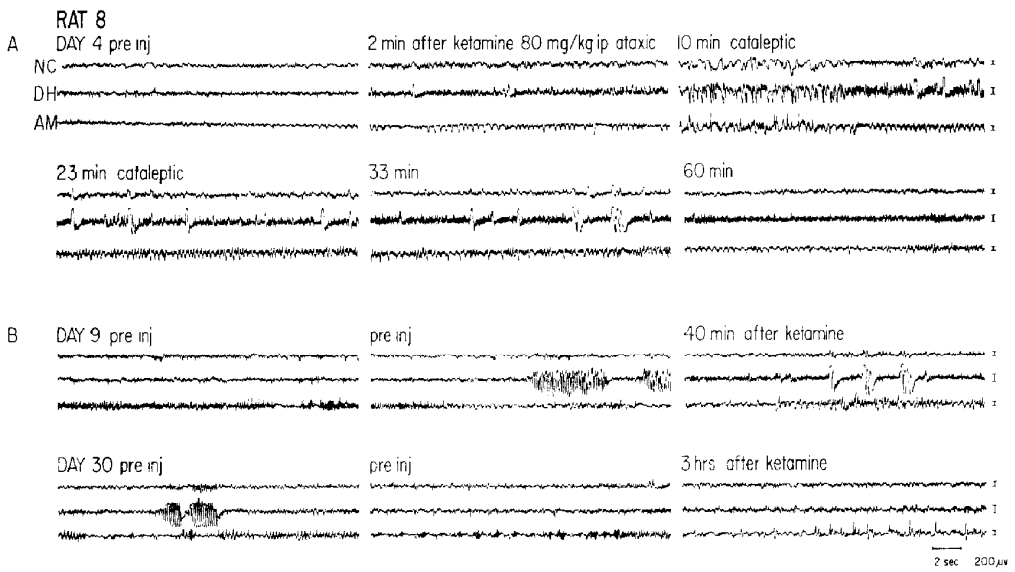

FIG. 2. EEG effects of ketamine 80 mg/kg i.p. (abbreviations see Fig. 1). Two minutes after ketamine (A) on Day 4, the period of excitation was associated with increased frequency in NC, and slow wave, high amplitude activity in the DH and AM. During the cataleptic state (10 min) there were 2–3 Hz waves in NC. Spike-like activity in DH and AM disappeared when the animal emerged from the cataleptic state. On Day 9 and Day 30 (B) spindle like bursts occurred in DH and some spiking activity in AM, during the pre-drug phase. On Day 30 spiking in AM persisted 3 hr after ketamine.

dorsal hippocampus and amygdala. During the cataleptic phase, the hypersynchrony was again seen in the neocortex and there was high amplitude spike-like activity in both the dorsal hippocampus and amygdala, which disappeared when the animal emerged from the cataleptic state. The EEG was relatively normal 60 min after administration of the drug. On Day 9, prior to the administration of ketamine, there were high amplitude, low frequency spindle-like bursts in the dorsal hippocampus and some spiking activity in the amygdala during the pre-drug phase. Forty minutes after ketamine these high amplitude, slow wave bursts persisted in the dorsal hippocampus and amygdala. By Day 30, these bursts were more apparent not only in the dorsal hippocampus but also in the neocortex and spiking and slow wave activity became more apparent in the amygdala during the pre-drug phase. In addition, 3 hr after ketamine administration, profound spiking activity in the amygdala persisted. In two rats receiving 80 mg/kg daily for three months, the pre-injection EEG was not grossly abnormal compared to that noted on Day 1 control, and 2–3 hr post-drug the EEG appeared to be similar to the pre-injection pattern (Fig. 3). However, 5 days after the last injection all leads demonstrated abnormal slow wave and spiking activity without gross behavioral manifestations of seizure activity.

Repeated drug administration of ketamine, 80 mg/kg, did not demonstrate any change in latency to the onset of the cataleptoid state, nor was there any evidence of a change in the duration of the state during chronic drug administration. Of the 5 rats which received 80 mg/kg of ketamine, three animals had abnormal EEG patterns develop in the pre-injection EEG recording. The time of development of the abnormal control pattern was variable;

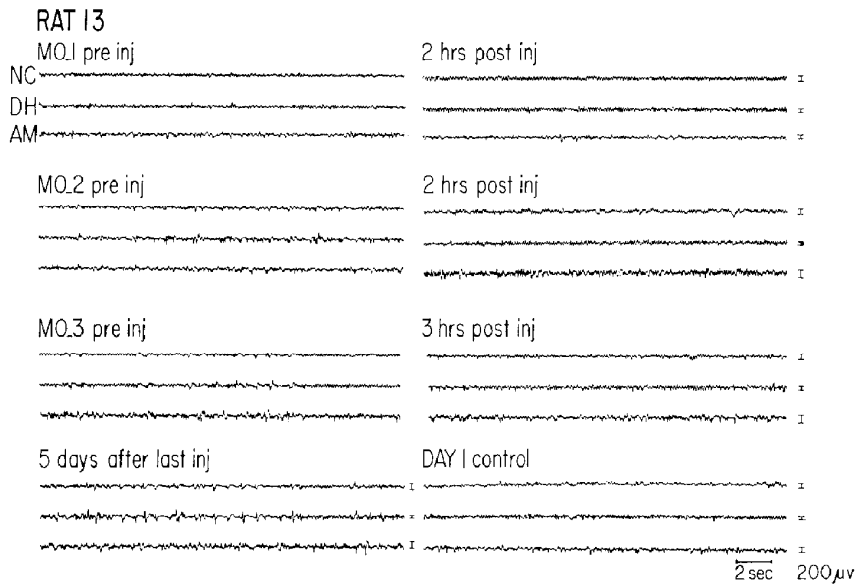

FIG. 3. EEG progression following daily administration of ketamine 80 mg/kg i.p. (abbreviations see Fig. 1). Spike bursts appeared in DH 5 days after the last injection. MO-1, month 1; MO-2, month 2; and MO-3, month 3.

one rat demonstrating the activity after 9 days, the second rat after 30 days and the third rat after 75 days.

Ketamine 30 mg/kg *i.p.*

Initial administration. This lower dose of ketamine induced an initial excitation with ataxia within 2 min. The ataxia usually lasted approximately 20 min and the animal appeared behaviorally normal within 50–60 min. The EEG changes (Fig. 4A) following the initial dose of ketamine, was not different from that observed during the initial non-cataleptic phase of the 80 mg/kg dose (Fig. 1). During the ataxia there was an increase in amplitude in the neocortical EEG and minimal low voltage slow wave activity in the dorsal hippocampus and amygdala. There was no high amplitude slow wave activity (hypersynchrony) in these animals and the EEG pattern returned to baseline appearance within 1 hr.

Daily administration. With the appearance of the ataxia there was high amplitude slow wave activity in the amygdala and higher frequency rhythmic activity in the hippocampus by Day 15, this pattern was quite apparent on Day 30 (Fig. 4B). A semblance of this activity persisted for $1\frac{1}{2}$ hr after drug by Day 15 and the pre-injection ongoing EEG activity was similar to the pre-injection record of Day 1. However, a second rat demonstrated abnormal spiking activity in the dorsal hippocampus in the pre-injection period 2 months after the beginning of chronic ketamine administration which became more prevalent during the pre-injection period following $2\frac{1}{2}$ months of chronic ketamine administration (Fig. 5). This abnormal spiking and slow wave activity was noted primarily in the dorsal hippocampus during the initial period of ketamine action and persisted for 2–20 hr post injection.

After three months of daily administration of 30 mg/kg of ketamine, the drug was discontinued and 5 days after the last injection abnormal spike and polyphasic burst activity was noted in both amygdala and dorsal hippocampus. As was the case for the higher dose of

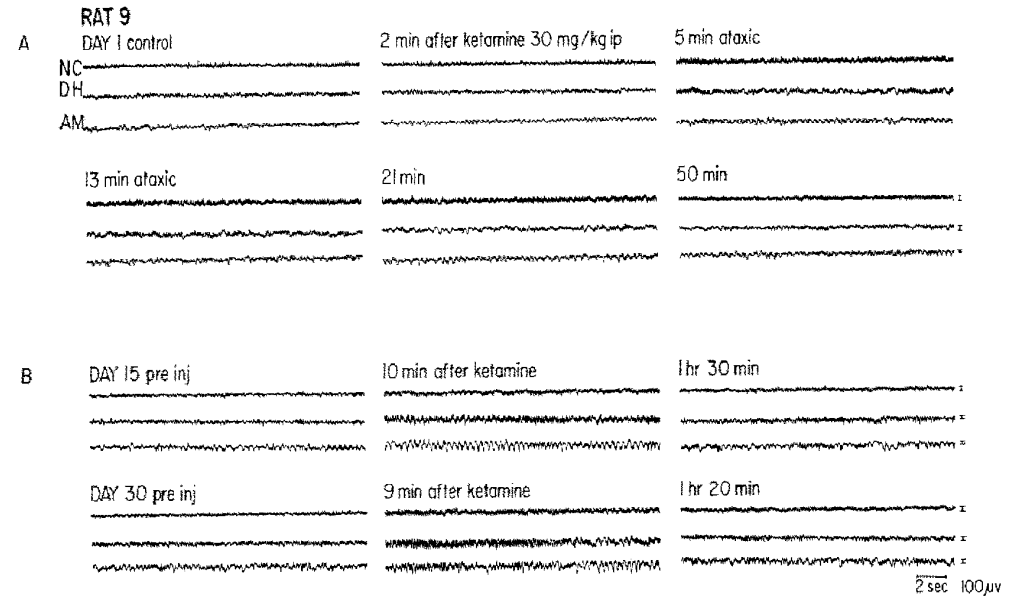

FIG. 4. EEG effects of ketamine 30 mg/kg i.p. on (A) Day 1 and (B) Day 15 and Day 30. (abbreviations see Fig. 1). There was an increase in amplitude in the NC during the ataxic period. There was slow wave activity in the AM and high frequency rhythmic activity in DH (B, 10 and 9 min after ketamine).

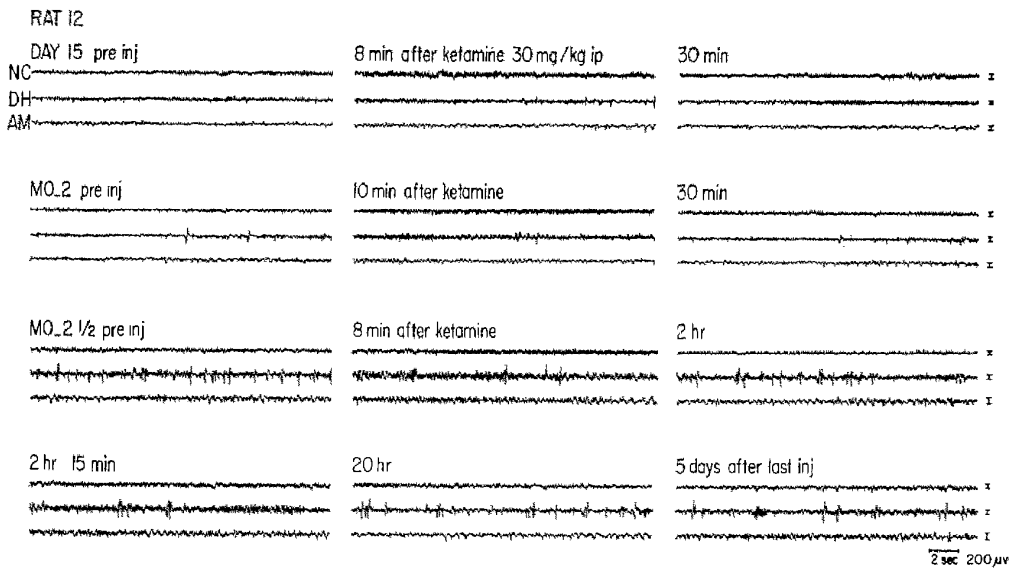

FIG. 5. EEG progression following daily administration of ketamine 30 mg/kg i.p. (abbreviations see Fig. 1). Spiking in DH in the pre-injection control period, 2 and 20 hr after ketamine. Abnormal spiking was also seen 5 days after the last injection.

ketamine, these abnormal brain waves were not correlated with any grossly observable behavioral change in the animals.

There was no change in the latency to onset of drug effect with chronic administration nor was there any significant change in the total duration of drug action during the 3 months of ketamine administration.

DISCUSSION

Prior studies by WINTERS *et al.* (1972) demonstrated bursts of hippocampal seizure activity without overt behavioral manifestation in cats receiving single cataleptic doses of ketamine. In addition, MIYASAKA and DOMINO (1968) and WINTERS *et al.* (1972) demonstrated a progression of EEG changes in both the mesodiencephalon and cortex which were characterized by initial desynchronization, intermittent hypersynchrony (low frequency, high amplitude waves) followed by continuous hypersynchrony. The behavior and EEG pattern induced by a single dose of ketamine appears similar to that demonstrated by other known hallucinogenic agents such as LSD, phencyclidine, and mescaline.

Abnormal spike and hypersynchronous bursting activity was demonstrated during the course of chronic ketamine administration in some rats receiving either the low or the high doses. The incidence of abnormal brain wave spiking in ketamine-treated rats was approximately 50% of the animals tested, i.e. 3 of 5 at the high dose and 1 of 3 at the lower dose. The group receiving daily saline injections did not show any abnormal spike or epileptiform EEG activity. The mere presence of the recording electrodes in the hippocampal or mesodiencephalic regions of the brain did not produce focal lesions with epileptiform discharges since none were noted in the saline group. However, it cannot be ruled out that the presence of electrodes in various brain areas may have induced subclinical epileptic foci in some of the rats. Thus ketamine could serve as a trigger of these foci, whereas saline is not capable of triggering an epileptic focus. Histological examination of electrode tracks indicated no differences between the saline and ketamine groups. Those animals which received ketamine could not be differentiated by gross histological examination into those with epileptiform discharges as compared with those which did not have epileptiform discharges. Perhaps the electron microscopic studies will be able to demonstrate a subcellular basis for these data (MANOHAR and MAXWELL, unpublished observations).

GODDARD, MCINTYRE and LEECH (1969) have shown that brief subconvulsant bursts of repeated electrical stimulation of limbic structures in rats induced localized seizure discharges, behavioral automatisms and at a later date convulsions. Their control observations revealed that the effect was due to electrical activation of the tissue and not due to damage caused by electrodes, the type of metal or insulation used in constructing the electrodes, or gliosis.

Aside from the development of epileptiform EEG activity in 50% of the animals receiving chronic ketamine administration there did not seem to be any significant development of tolerance or potentiation of the drug. The duration of action of each of the various stages of EEG and behavioral activity did not significantly differ from day to day during 1, 2 or 3 months of treatment.

Withdrawal of the drug for 5 days following three months of treatment at both dose levels resulted in the appearance of a progressive increase in epileptiform activity with no gross behavioral manifestations. This type of release phenomenon is curious since the drug did not grossly induce tolerance.

The appearance of epileptiform EEG patterns without behavioral manifestations

following chronic administration of ketamine indicates that one must be very careful in assessing the safety of this agent clinically without the use of the EEG. The ability to demonstrate these epileptiform patterns in mature, fully developed rats can be extrapolated to imply that even greater abnormal brain wave activity might be induced in the developing CNS. There is little data demonstrating the safety of prolonged ketamine administration on the development of the CNS. Since many of the procedures in which pediatric patients receive ketamine anesthesia are related to neurosurgical or neurological diagnosis of suspected brain disease, it should be kept in mind that prolonged or multiple administration of ketamine for diagnostic procedures may induce some degree of iatrogenic damage to the CNS. A further point is, that even single doses of ketamine induce changes in electrical activity which are identical with epileptiform brain patterns. This should be kept in mind when using ketamine for diagnostic procedures, so that a misdiagnosis of clinical epilepsy is not made when in fact the epilepsy is a transient drug-induced pattern.

The significance of the present findings with respect to the chronic use of hallucinogenic or cataleptic agents is interesting. While the hazards of chronic ketamine administration appear quite clear, it will be interesting to determine whether abnormal brain waves will likewise appear after chronic administration of other hallucinogenic or cataleptic drugs of abuse.

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