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Prolonged Electrophysiological and Behavioral Alterations Following a Single Injection of Methadone in the Cat

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SNYDER, E. W., D. E. SHEARER, C. SCHLEHUBER, R. E. DUSTMAN AND E. C. BECK. *Prolonged electrophysiological and behavioral alterations following a single injection of methadone in the cat.* PHARMAC. BIOCHEM. BEHAV. **12**(6) 893–898, 1980.—Visual evoked potentials (VEPs), EEG, plasma methadone concentrations, blood gas values, and behavioral ratings were recorded periodically for 4 days following a single injection (4 mg/kg IP) of methadone in cats. Most measurements indicated a prolonged drug effect which lasted into the 4th day. Independent alterations of VEP component amplitudes suggested site-specific variations in the time-course of the drug effects. An early component, reflecting activity of classical ascending pathways, was quite resistant to the drug effects immediately following injection. Only after 31 hours was this component significantly attenuated. The amplitude approached predrug values at the time of final measurement. A late VEP component was quickly suppressed following methadone and returned to predrug values after 55 hours. Decreases in EEG frequency, on the other hand, evidenced no time-dependency following drug administration. All animals were behaviorally normal after 55 hours with no further evidence of mania. The results confirm the prolonged effects of methadone in a species known for its unusual and complex response to opiates. Sites within the visual system are apparently highly sensitive to the drug and are differentially altered over time following methadone injection. These alterations are, for the most part, correlated with plasma methadone concentration.

Visual evoked potential Behavior Methadone Prolonged effects Cats EEG

CATS respond to opiates with central nervous system excitation and depression which vary among structures within the brain and depend upon drug-administration parameters [5]. Despite the intricate nature of the feline response, the prolonged effects (e.g., many days) of a single dose of an opiate have not been evaluated. Such investigations are warranted since measureable quantities of methadone were found to persist in various CNS structures of the dog for up to three weeks following a single injection [11]. The pharmacological significance of such extended exposure to the drug or its metabolites has not been established but we have observed that cats were electrophysiologically responsive to methadone at 2, 3 and 4 mg/kg and were behaviorally responsive to the 4 mg/kg dose for at least 12 hours following the injection [20]. There was suggestive evidence of continued response in some animals up to 48 hours past injection. Therefore, the present study was designed to determine the course and nature of extended EEG, evoked potential and behavioral alterations following a high dose of methadone. Electrophysiological changes were compared to alterations in plasma methadone concentration. Furthermore, since evoked potentials are sensitive to alterations in blood oxygen concentration [16], PO_2, PCO_2, and pH were assessed.

METHOD

Methodological details are provided elsewhere [19,20].

Animals

Briefly, 15 healthy cats (8 males, 7 females) were studied. Body weights ranged from 3.5 to 5.4 kg; ages ranged from 2 to 5 years. Electrodes were implanted in 3 male and 3 female cats. Nine additional animals were treated with methadone and used for blood gas determinations (N=6) and radioimmunoassay (N=3). The animals were maintained on a standard laboratory diet and a 12-hour light-dark cycle. Prior to all recording sessions pupils were maximally dilated with 1% Mydriacyl.

Surgical Procedure

Six cats were anesthetized, positioned in a stereotaxic instrument and stainless steel screws were threaded through the calvarium to contact dura overlying posterior marginal (striate cortex) and posterior suprasylvian gyri. The frontal sinus served as a reference. Referential techniques, commonly used in similar protocols [3], are sensitive to altera-

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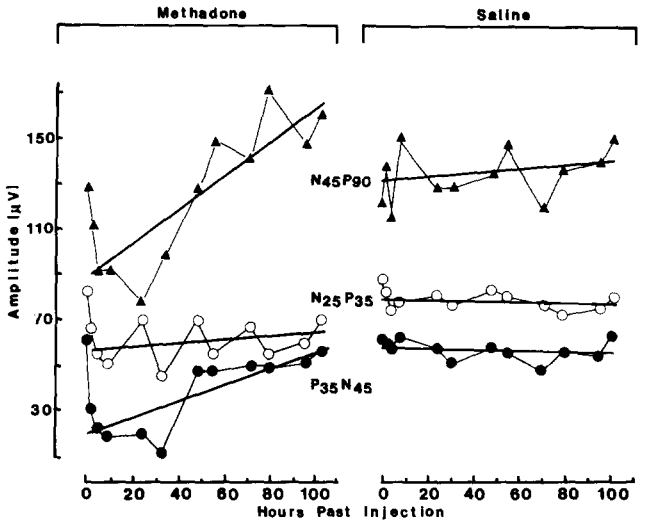

FIG. 1. Amplitudes of three VEP components recorded from striate cortex of six cats following methadone (4 mg/kg, IP) and saline injections. Least squares calculations excluded the predrug (0 hr) value. Each component under the methadone condition was significantly attenuated ($p<0.05$) as described in the text. Saline had no significant ($p>0.10$) effects.

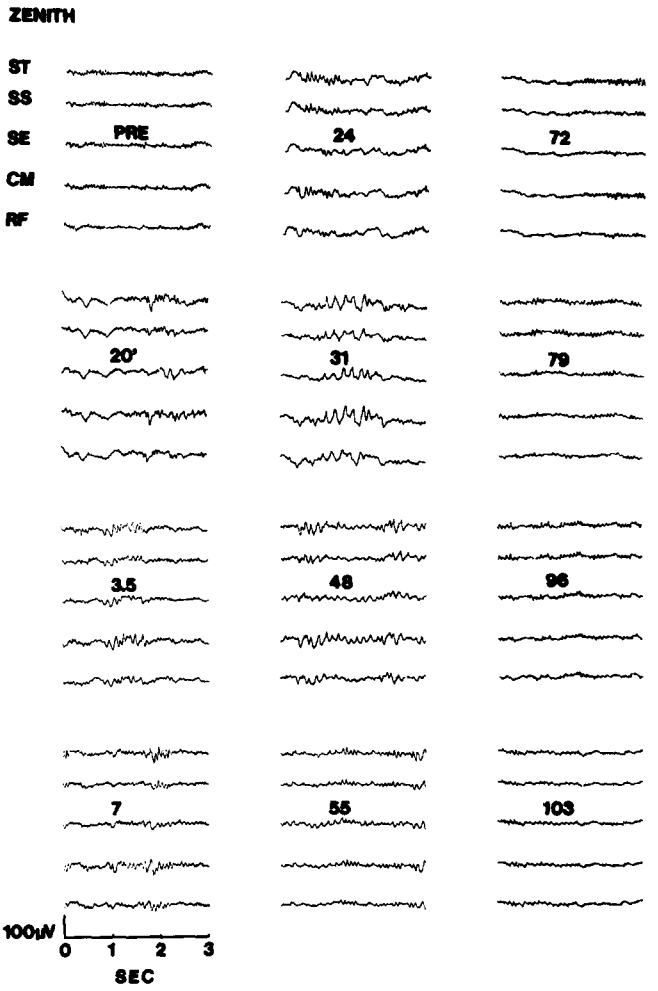

FIG. 2. EEG recorded from striate cortex (ST), suprasylvian gyrus (SS), septum (SE), centre median (CM), and midbrain reticular formation (RF) at various times preceding and following methadone in one cat (Zenith).

tions in "distant" sources of evoked potential components as compared to bipolar techniques which are acutely sensitive to "local" potential differences. Subcortical electrodes were directed towards the hippocampus, centre median, septum, and reticular formation. Electrode positions were histologically verified. Although all cortical electrodes were properly positioned, there were an insufficient number of verified placements in each subcortical area to permit statistical analyses of VEPs recorded from these areas.

Apparatus

All experimental procedures were conducted in a semidarkened and electrically shielded recording room. A photostimulator was used to present light pulses reflected from a white hemicylinder in front of which the animals were held under light restraint. EEG and accompanying trigger pulses were conventionally amplified and recorded on magnetic tape. Single photic pulses were presented at 2–3 sec intervals during periods of artifact-free EEG. Single evoked potentials to 50 flashes were averaged to produce each average evoked potential (VEP).

Procedure

Following postoperative recovery each cat was thoroughly habituated to the restraining hammock and recording techniques. All animals quickly learned to lie passively in the hammock; the resultant EEG records were largely artifact free. Prior to recording sessions each cat was allowed to readapt to restraint for approximately 5 minutes. VEPs and EEG were obtained 5 min preceding the administration (IP) of methadone HCl (4 mg/kg) or saline and at the following times postinjection: 20 min, 3.5, 7, 24, 31, 48, 55, 72, 79, 96, and 103 hours. Methadone and saline were administered in random order to each cat with at least 2 weeks between treatment. Animals were released from restraint between recording sessions. Blood was drawn from six nonimplanted cats prior to methadone or saline injection and at 20 min, 7, 24, and 96 hours past injection for analysis of PO_2, PCO_2, and pH levels using standard techniques. Venous blood for radioimmunoassay (RIA) of methadone content [18] was drawn from the implanted cats and 3 additional nonimplanted cats at various times following methadone.

Behavioral observations were conducted following each recording session when the animals were released from restraint or at comparable times following drug or saline administration in nonimplanted cats. Animals were observed throughout each recording session and subsequently for at least one hour. Four behaviors were rated for their presence or absence in each cat by an evaluator who was unaware of the experimental condition. Salivation was scored when a cat evidenced rapid licking motion and/or when frothy saliva was visible around the mouth. Loss of righting reflex was scored when the animal did not rotate properly to strike the floor with legs extended. Rear leg paralysis was recorded when an animal moved by dragging its splayed hind legs. An excited animal paced back and forth, ran in circles or vigorously attempted to escape. The behaviors were not mutually exclusive.

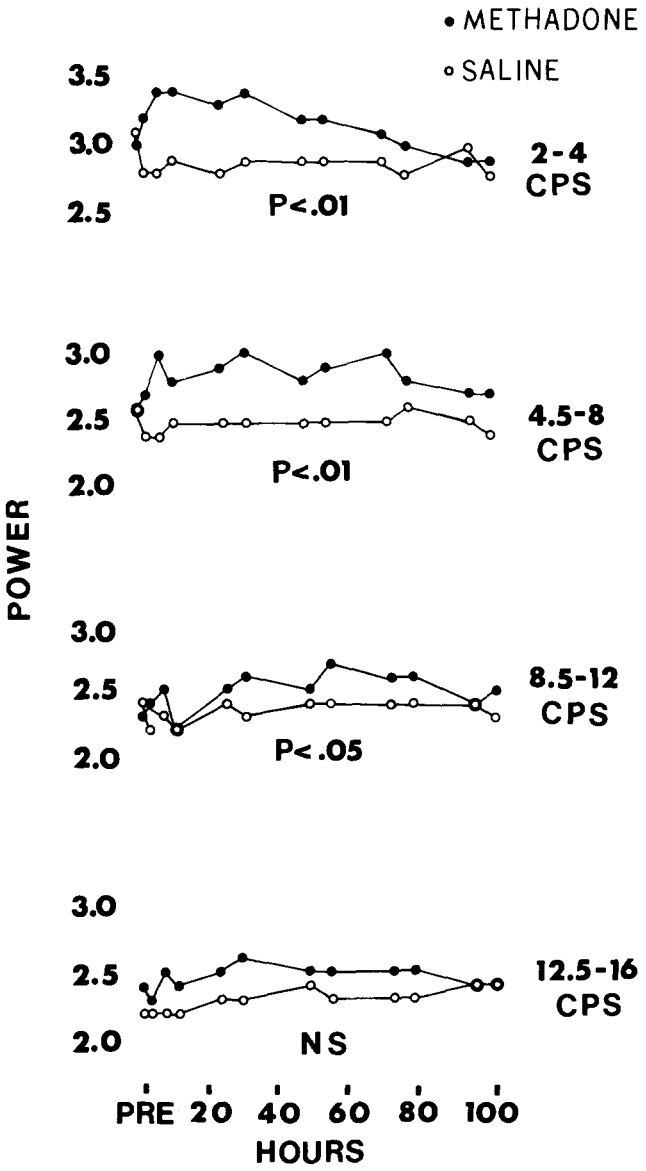

FIG. 3. Power spectra density (means across animals) at each recording session following methadone (4 mg/kg; IP) and saline. Values were based upon EEG derived from striate cortex of 4 animals. NS=nonsignificant main effect. Power is expressed as $\log_{10}(\mu V^2)$.

Statistical Analysis

The latency to consistent peaks of each VEP components and peak-to-peak amplitudes were compared across time periods by analyses of variance with repeated measures [22]. Significant individual sources of variation were determined with the use of Duncan's Multiple Range Test [6]. pH, PO_2, PCO_2 blood levels following methadone were analyzed with the *t*-test.

Power spectra density of the EEG was computed using the fast fourier transform on data sampled at 256 points per second. A total of 20 contiguous periods of 2 sec each were analyzed and averaged under each condition. The power values were scaled in μV^2. Since the distribution of power in a given population is not normal, the power values were transformed to a $\log_{10}$ scale for analyses of variance.

RESULTS

VEPs

The typical wave form of the feline VEP, as recorded from occipital cortex, consists of a series of positive (P) and negative (N) components within the first 300 msec [19,20]. The amplitude of the earliest negative component (P15-N35; numerical values refer to peak latency in msec) was not significantly different from the predrug value at any time following methadone. Amplitudes of subsequent waves were significantly reduced following the drug. Furthermore, the time course of these effects varied significantly ($p<0.01$) among waves as determined by two factor analysis of variance with repeated measures. These differential effects following methadone are evident in Fig. 1 where the best fit lines are clearly divergent.

Single factor analyses of variance based upon each VEP component revealed the following: The early positive component peaking at approximately 35 msec (N25-P35) was not significantly attenuated until 31 hours past injection ($p<0.01$). The component was significantly ($p<0.05$) suppressed at subsequent determinations and in the course of the experiment never achieved predrug values. The next wave (P35-N45) was significantly ($p<0.01$) suppressed at 20 min past injection and remained so for 31 hours when the mean amplitude approached but did not equal ($p<0.05$) predrug values. The third component (N45-P90), significantly ($p<0.05$) suppressed at 20 min, was at its lowest amplitude ($p<0.01$) at 24 hours postinjection. The mean amplitude then gradually increased to equal the predrug value at 55 hours. The time course of the last component (P90-N220) closely paralleled that of the preceding wave but the changes were not statistically significant ($p>0.10$).

No significant differences ($p>0.10$) over time were observed in VEPs following saline injection. Amplitudes fluctuated minimally about the predrug values without evidencing a systematic increase or decrease. Interactions between time and VEP component amplitudes were not significant ($p>0.10$) as exemplified by the near-parallel lines under the saline condition (Fig. 1).

EEG

Samples of EEG from a representative cat ("Zenith") are provided in Fig. 2. The increase in slow frequency EEG after 20 min is apparent. The record appears completely normal by 72 hours postinjection.

Power spectra density was computed on the EEG recorded from the striate cortex of four cats following saline and methadone treatment. The $\log_{10}$ of the power at lower frequencies (2–12 cps) was significantly increased following methadone as compared to saline treatment (Fig. 3). However, no significant ($p>0.10$) variation occurred *over time* in any of the bands. At the lowest frequency band (2–4 cps) power values for the two treatments evidenced distinct separation in the early hours followed by convergence in the final hours of study.

Three animals with histologically verified subcortical electrodes evidenced obvious spiking in the hippocampus following methadone. This activity began at 3.5 hours and continued through the 55 hour recording session. EEGs recorded from other subcortical sites showed no such activity.

Behavior

While in restraint all cats remained alert with no signs of

TABLE 1

NUMBER OF ANIMALS (N=15) EXHIBITING VARIOUS BEHAVIORS AT TIMES BEFORE (PRE) AND FOLLOWING METHADONE (4 mg/kg IP)

Time	Salivation	Excitation	Rear leg paralysis	Loss of (R) reflex
Pre	0	0	0	0
20 min	15	15	7	10
3.5 hr	15	15	7	10
7 hr	5	15	5	8
24 hr	2	12	2	5
31 hr	0	8	0	2
48 hr	0	5	0	2
55–103 hr	0	0	0	0

Data from nonimplanted cats included. None of the above behaviors occurred following injections of saline. (R)=Righting.

TABLE 2

PLASMA METHADONE CONCENTRATION (ng/ml) AS DETERMINED BY RADIOIMMUNOASSAY PRECEDING (PRE) AND AT VARIOUS TIMES FOLLOWING METHADONE (4 mg/kg IP)

Time	Mean	SEM	N
Pre	0	0	9
20 min	188	32	9
3.5 hr	265	27	9
7 hr	211	32	9
24 hr	218	40	8
48 hr	148	40	9
72 hr	81	34	8
96 hr	27	11	6

SEM is standard error of the mean. N (number of animals) decreased as repeated extractions made samples more difficult to obtain. The RIA has been previously described [18].

TABLE 3

PEARSON PRODUCT-MOMENT CORRELATIONS ($\bar{r}$) BETWEEN PLASMA METHADONE CONCENTRATION AND VEP VALUES (LATENCIES AND AMPLITUDES)

	Latencies					
	P15	N25	P35	N45	P90	N220
$\bar{r}$	+.10	−.45	+.65	−.19	−.52	−.61
p	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10

	Amplitudes				
	P15-N25	N25-P35	P35-N45	N45-P90	P90-N220
$\bar{r}$	−.80	−.10	−.92	−.97	−.95
p	<0.01	>0.10	<0.01	<0.01	<0.01

Coefficients, calculated across times as listed in Table 2, represent means across 6 animals. Means were derived from Z scores and reconverted to $\bar{r}$.

struggle. Consequently, EEG records remained largely free of muscle artifact. Although salivation was not formally scored while the cats were restrained, many animals did salivate during recording sessions.

Upon release from restraint the animals' behavior included the classical signs of feline mania (Table 1). When left completely undisturbed in their cages, the animals often assumed an elongated, prone posture with rapid breathing and quick, jerky head movements. No animal was observed to sleep during the period of study. After 55 hours all cats appeared behaviorally normal. All cats survived the present experiment. However, previous work [20] suggested that 4 mg/kg is lethal to some cats (roughly 10%).

Blood Values

Changes in plasma methadone concentrations following drug administration are shown in Table 2. A marked elevation of plasma methadone was seen at 20 minutes past injection; maximum concentration occurred at 3.5 hours following the injection. Measureable quantities of the drug were still evident at 96 hours.

In most cases component amplitudes of the VEP were strongly correlated with plasma methadone concentration while latencies were not (Table 3). The negative correlations indicate an inverse relationship over time between concentration of the drug in plasma and the amplitude of the VEP.

Blood-gas values are presented in Table 4. A significant

TABLE 4

MEAN, RANGE AND STANDARD DEVIATION (SD) OF pH AND BLOOD GAS VALUES FOR SIX CATS TREATED WITH METHADONE (4 mg/kg; IP)

	pH			PO_2			PCO_2		
Time	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
Pre	7.33	7.25–7.39	0.05	36.0	24–52	9.4	37.8	19–51	10.5
20 min	7.23*	7.11–7.30	0.07	28.3	21–34	4.8	48.5†	39–56	6.4
7 hr	7.16†	7.07–7.22	0.05	20.8†	18–28	3.7	54.0*	47–66	7.0
24 hr	7.16†	7.11–7.22	0.04	24.3*	17–31	5.0	41.0	30–46	6.1
96 hr	7.35	7.29–7.39	0.04	34.0	27–44	6.1	34.2	31–36	2.1

*$p<0.05$; †$p<0.01$; no significant differences were found following saline treatment.

decrease occurred in pH and PO_2 at 20 min, 7 and 24 hours postinjection. By 96 hours postmethadone, all values had returned to predrug levels.

DISCUSSION

The results demonstrate behavioral disturbance lasting into the second day following a single 4 mg/kg dose of methadone. VEP alterations lasted for up to 4 days. However, a direct comparison between behavioral and electrographic correlates of the drug effects can not be drawn. During restraint, when VEPs were recorded, the animals appeared consistently alert but calm with no signs of struggle. Only upon release were behavioral indices of mania (Table 1) evident. Under restraint the EEG power of slow frequencies increased reliably (Figs. 2 and 3) which is inconsistent with hyperexcitable behavior. These observations suggest a strong environmental contribution (see also [2]) to feline opioid mania which appears to be characteristic only of the free ranging animal. Restraint does not physically preclude manic behavior since cats which are naive to restraint exhibit vigorous and obvious struggle.

For the most part the VEP alterations were significantly correlated with plasma methadone concentration which peaked at 3.5 hours and gradually decreased thereafter to a minimum at the time of final measurement (96 hours). The significant negative correlation between VEP amplitude and plasma methadone concentration supports the contention that the drug rapidly distributes from blood to active sites in the central nervous system [11].

Examination of the individual components of the VEP support the evidence [11] that peak concentrations of the drug in plasma and at various sites in the CNS are achieved differentially. Peak plasma concentrations were reached at 3.5 hours (Table 2) while an early component of the VEP (N25-P35) was not significantly attenuated until 31 hours postinjection (Fig. 1). Unlike other VEP waves (P35-N45 and N45-P90), the time-dependent alterations of N25-P35 were not correlated with plasma methadone concentration. Since various components of the VEP have been shown to reflect the activity of separable neural processes [1,15] our data support the implication [11,12] that methadone gains access to and is eliminated from various structures within the CNS at different rates. One can not, however, discount the possibility that various loci respond at different rates to available methadone or its metabolites.

A late component of the VEP (N45-P90) reached its lowest amplitude at the 24 hour recording session (Fig. 1) and thereafter returned monotonically to predrug values. Earlier components, however, remained significantly suppressed for at least 96 hours past injection. The differential time-course of the effects is best illustrated by the divergence of the best fit lines (Fig. 1). As was the case in the dog [10, 11, 14] some structures within the cat brain would appear to bind methadone for extended periods of time. Based on the present data some hypotheses can be drawn regarding the nature of these structures.

Early components (<50 msec) of the evoked potential recorded from cortex have been associated with excitation along classical pathways ascending through specific thalamic nuclei (lateral geniculate) to primary receiving cortex [1, 15, 16, 17]. Two early components (P15-N25; N25-P35) of the feline VEP have been found to be insensitive to a wide range of doses of methadone at 20 minutes after injection [20]. In the present study 31 hours elapsed before one of these components (N25-P35) was significantly attenuated. These results suggest that structures along the primary ascending pathways accumulate methadone (or its metabolites) at a relatively slow rate and thereafter remain influenced by the drug for days.

The neurogenesis of late VEP components has been vigorously debated. However, most workers agree that the midbrain reticular formation and midline thalamus affect these waves [1, 4, 15]. In the present study, and in previous work conducted in this laboratory [20], VEP components peaking at 45 msec or beyond were significantly attenuated at 20 min following methadone administration suggesting a relatively rapid accumulation of the drug in secondary pathways. Likewise, other workers have proposed opiate-induced alteration of mechanisms that modulate thalamocortical activity [4, 9, 13, 17]. However, we have not found VEPs recorded directly from reticular formation and thalamus to be influenced by methadone [20], at least at 20 min past injection. Taken together the findings suggest a cortical or at least supra-thalamic mechanism of late VEP component attenuation. The increased power at lower frequencies (Figs. 2 and 3) is consistent with a methadone-induced depression at cortex [7].

The hippocampal spiking we observed is consistent with the well established subcortical effects of opiates in cats [2, 8, 21]. Our results suggest persistence of this activity for approximately 55 hrs following a high dose; a term coincident with the alteration of the late VEP wave (N45-P90). However, the delayed onset of spiking (3.5 hr) is noteworthy since electrographically active blood levels [19] were achieved at 20 min postinjection. This delay would suggest that blood levels approaching 265 ng/ml (Table 2) are necessary to precipitate spiking activity.

During the course of the procedure, animals treated with methadone evidenced hypoxia which peaked by the 7th hour and had abated by the 96th hour (Table 3). We doubt that the observed VEP alterations were secondary to the decreased oxygenation of the blood. However, the specific contribution of such disturbance to the complexity and duration of the feline response to methadone is not known. The effects of graded hypoxia in monkeys have been shown to include increased latencies and decreased amplitudes of early components and an "upward (positive) shift of late components" [16]. These effects were only evident with a 66% reduction in PO_2 and were quite unlike what we have observed in the present study. Our animals' PO_2 decreased, at most, by 50%. Furthermore, while the cats were hypoxic by the time of the first VEP recording session (20 min), one component (N25-P35) was not significantly attenuated until the 31st hour. Without implicating a delayed effect of minimal hypoxia, one must look elsewhere for an explanation of the drug effects on the VEP.

In summary, the findings suggest a prolonged duration of action of methadone in the feline CNS. The time-course of the drug's effect appears to differ among structures within the visual system which remain sensitive long after gross behavioral disturbances (e.g., mania) have waned.

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