Pharmacology Biochemistry and Behavior, Vol. 1, pp. 557–567. ANKHO International Inc., 1973. Printed in the U.S.A.

Sleep-waking Patterns and Brain Biogenic Amine Levels in Cats after Administration of 6-Hydroxydopamine into the Dorsolateral Pontine Tegmentum[1]

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(Received 25 July 1973)

ZOLOVICK, A. J., W. C. STERN, J. E. JALOWIEC, J. PANKSEPP AND P. J. MORGANE. *Sleep-waking patterns and brain biogenic amine levels in cats after administration of 6-hydroxydopamine into the dorsolateral pontine tegmentum.* PHARMAC. BIOCHEM. BEHAV. 1(5) 557–567, 1973.–Results from lesion and pharmacological experiments have implicated brainstem monoaminergic systems in the regulation of sleep-waking behavior. The serotonin-rich neurons of the raphé complex function mainly in the genesis of slow-wave sleep (SWS) while the catecholamine-rich neurons of the dorsolateral pontine tegmentum (locus coeruleus) appear to function in the maintenance of cortical desynchronization of wakefulness and in the expression of various tonic and phasic components of rapid eye movement sleep (REM). 6-Hydroxydopamine, an analogue of dopamine, which rather selectively destroys catecholaminergic neurons, was injected into the catecholamine-rich dorsolateral pontine tegmentum of cats in order to evaluate the role of catecholamines in sleep-waking behavior. Five weeks after injection, the animals were sacrificed and regional brain levels of norepinephrine and serotonin were determined in order to assess the effects of this treatment on monoamine metabolism. The acute affect of the treatment was to increase total waking time at the expense of both SWS and REM sleep indicating an initial release of synaptically active norepinephrine. The chronic effect of the treatment was to increase the occurrence of SWS and to decrease REM sleep, an effect consistent with disruption of an ascending cortical activating mechanism of REM sleep or disinhibition of a SWS mechanism. This was accompanied by a selective and highly reliable reduction in cortical and hippocampal levels of norepinephrine. However, coincident decreases in forebrain levels of serotonin and norepinephrine preclude a definitive conclusion regarding the relative contribution of catecholamine and indoleamine mechanisms in the maintenance of sleep-waking patterns after intracerebral 6-hydroxydopamine.

6-Hydroxydopamine Biogenic amines Sleep-waking behavior Locus coeruleus REM Norepinephrine Serotonin

BASED on extensive pharmacological and lesion studies in cats Jouvet [14–19] has postulated that states of vigilance are regulated largely by activity in monoaminergic systems located in the brainstem. According to Jouvet the serotonergic system, originating mainly in the mid-line raphé complex, appears to regulate the quality and quantity of slow-wave sleep (SWS) and the triggering of rapid-eye-movement sleep (REM), while wakefulness, behavioral arousal and phasic and tonic events of REM sleep appear to be under the control of catecholaminergic systems located in the lateral brainstem and in diffusely organized cholinergic systems.

Lesions confined to the serotonin-containing raphé nuclei, or treatment with parachlorophenylalanine (PCPA), a drug which inhibits the synthesis of serotonin and decreases its levels in the brain, have been shown to reduce SWS, which can be restored by administration of 5-hydroxytryptophan, the precursor of serotonin. Also, three-way correlations between the reduction of SWS, the extent of damage to the serotonin-containing cells of the raphé and the subsequent reduction of serotonin in the basal forebrain add additional support for a role of serotonin in the genesis of SWS. Results from lesion and pharmacological studies indicate that catecholaminergic

[1] Supported by Grants MH 10625 and MH 02211, NIMH; we gratefully acknowledge the assistance of R. Miller for analyses of biogenic amines and C. Westfall, Jr. and K. McKnight for technical assistance.

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mechanisms are primarily involved in the regulation of wakefulness and REM sleep [18]. For example, total bilateral destruction of the dorsolateral pontine tegmentum has been shown to produce a selective and total suppression of REM sleep, while less extensive damage to the nucleus locus coeruleus (LOC) only reduced the amount of REM sleep or suppressed the various phasic or tonic components of this state [18]. Thus, the component nuclei of the dorsolateral pontine tegmentum, LOC, subcoeruleus and parabrachial nuclei, appear to be critical elements in governing the occurrence of the various components of REM sleep.

Regarding the role of the catecholamine-containing neurons of the LOC in REM sleep; destruction of the caudal third of the LOC eliminates the tonic muscular atonia associated with this state while destruction of the middle third of the LOC results in a selective suppression of some phasic components of REM sleep, i.e., the ponto-geniculo-occipital (PGO) activity. The anterior third of the LOC appears more to function in cortical desynchronization of waking rather than in the regulation of REM sleep per se [18].

Pharmacological data regarding the involvement of catecholamine mechanisms in the regulation of wakefulness and REM sleep appear less convincing. Treatment with disulfiram, an inhibitor of norepinephrine (NE) synthesis, leads to a decrease in REM sleep and waking [10], while alpha-methyl-dopa, which leads to synthesis of a false transmitter in NE neurons, suppresses REM sleep [11]. However, unlike PCPA which reduces SWS, administration of alpha-methyl-tyrosine, an inhibitor of catecholamine synthesis, fails to inhibit REM sleep in man [42], cat [20,36] and rat [27]. Thus, pharmacological evidence for the role of catecholamines in the genesis or regulation of REM sleep is confusing despite the rather striking effects on REM suggested by lesion experiments.

Recently, administration of 6-hydroxydopamine (6-OHDA) has been used to induce a relatively specific destruction of neurons in rat brain as evidenced by a long-lasting decrease in brain levels of NE without coincident decreases in serotonin [47]. Furthermore, intraventricularly administered 6-OHDA has recently been shown to produce a long-lasting specific reduction in forebrain NE accompanied by a reduction in REM sleep in cats [22,23]. Since the catecholamine-containing cells of the dorsolateral pontine tegmentum (LOC) constitute the primary source of noradrenergic afferents to cortical structures [40], an attempt was made to establish a relationship between the noradrenergic components of this brain region and sleep-waking mechanisms more precisely by injecting 6-OHDA directly into the dorsolateral pons. This approach avoids much of the indiscriminate destruction of fibers of passage and non-catecholaminergic components of this area as well as a disruption of neuron-glial-vascular relationships produced by electrolytic lesions which could conceivably affect sleep.

METHOD

Six mongrel, female cats weighing 2.5–4.0 kg were anesthethized with pentobarbital sodium (Diabutal – 15 mg/kg), supplemented with α-chloralose (30 mg/kg), and stereotaxically implanted with electrodes for chronic recording of cortical EEG, neck EMG, and dorsal hippocampal, lateral geniculate and eye movement activity. Kopf cannulae were bilaterally implanted into the dorsolateral pontine tegmentum; either at the level of the LOC (Anterior-Posterior = –3.0: Lateral = 2.8: Vertical = +8.5 mm) or the sub-coeruleus area (AP –5.0: L 2.0: V +4.5 mm) according to the stereotaxic atlas of Reinoso-Suárez [30]. Eight-hour continuous polygraphic recordings were obtained from animals placed in an electrically and acoustically shielded, dimly lit and temperature controlled sleep chamber (130 x 75 x 65 cm) with food and water available ad lib. A flexible cable was attached to a counterweighed 15-lead slip-ring system permitting unrestrained movement. Grass model 5 and 7 polygraphs were run at 3 mm/sec and the resulting records scored in 10 sec epochs according to the criteria of Sterman *et al.* [34]. Scoring of sleep-waking activity was according to 3 categories: (1) awake; (2) SWS (characterized either by cortical spindles interspersed with brief periods of desynchronization or by continuous cortical synchronization; and (3) REM sleep (characterized by cortical desynchronization, neck atonia, rapid eye movements, PGO waves recorded from the lateral geniculate nucleus and hippocampal theta activity). Baseline values for the three states of vigilance in untreated cats were averaged from 4–6 recording sessions taken between 08.30 and 17.00 hr and beginning at least 14 days after surgery.

6-Hydroxydopamine hydrobromide (4.0 mg/ml) was prepared in deoxygenated saline (0.9%) containing ascorbic acid (1.0 mg/ml) to reduce oxidation. Animals were placed in a restraining jacket and 20 μl of solution was injected slowly into each cannula (15 μl volume) over a period of 20 min. The cannulae were then flushed with 10 μl of vehicle over a period of 10 min. The animal's sleep-waking patterns were recorded on the day of injection and at various intervals during the next 3–4 week period. Behavioral observations during the recording sessions supplemented polygraphic indicators of the vigilance states.

Five days after the final recording session the subjects were sacrificed and the brains removed for analyses of NE and serotonin. The method of sacrifice and regional brain dissection have been reported previously [35]. Brain regions taken for analyses were: temporal cortex, occipital cortex, anterior pyriform lobe (amygdala), posterior pyriform lobe (hippocampus), basal forebrain area, striatum, lateral and medial hypothalamus, lateral lobe of the cerebellum and the medulla. Tissue levels of NE and serotonin were assayed according to the fluorometric method of Thompson *et al.* [37] derived from Maickel *et al.* [26]. The mesencephalon and pons were dissected out and placed in 10% buffered Formalin (pH = 7.1). Histological identification of the cannular placements and degree of tissue damage were estimated from serially prepared frozen sections (20 μ) stained according to the method of Klüver-Barrera [21].

The effects of 6-OHDA-induced reductions in brain biogenic amines on vigilance states were assessed using paired *t*-tests in which the percent of time spent in the three sleep states during the 8-hr recording sessions were compared to the results from the same time period of the untreated baseline conditions. Regression analyses were determined for the three vigilance states from each cat's accumulated sleep records beginning from the day following the injection. The effect of 6-OHDA on regional brain levels of NE and serotonin were compared to corresponding regional amine levels of 17 untreated cats using Student's *t*-test.

TABLE 1

EFFECTS OF 6-HYDROXYDOPAMINE DIRECTLY INJECTED INTO THE DORSOLATERAL PONTINE TEGMENTUM ON THE VIGILANCE STATES. VALUES EXPRESSED AS THE MEAN (± SE) DIFFERENCES BETWEEN THE PERCENT OF TIME SPENT IN A GIVEN SLEEP STATE AFTER DRUG ADMINISTRATION FROM THAT PERCENT WHICH OCCURRED DURING THE PRE-DRUG BASELINE CONDITION. MEAN ± SE BASELINE VALUES AS PERCENT OF RECORDING TIME: AWAKE = 31.2 (2.8); SWS = 51.3 (3.0); REM = 17.5 (1.2)

Vigilance State	Day of Injection	Days 1–5	Days 6–10	Days 11–15	Days 16–20	Days 21–25
Awake	+38.2 (6.9)‡	+0.3 (4.1)	+3.1 (4.9)	−0.6 (4.3)	−10.2 (5.3)	+0.6 (3.4)
SWS	−20.7 (7.1)‡	+3.2 (2.9)	+4.5 (2.5)	+7.7 (3.1)*	+12.0 (5.9)*	+7.1 (1.7)*
REM	−17.4 (1.1)§	−3.5 (3.2)	−7.6 (3.6)	−7.2 (2.8)*	− 2.9 (0.1)†	−7.9 (2.9)‡

*$p<0.05$ †$p<0.02$ ‡$p<0.01$ §$p<0.001$ correlated t (2-tail)

RESULTS

During the initial 2–3 hr following injection of 6-OHDA into the dorsolateral pontine tegmentum, all animals exhibited restlessness accompanied by increased locomotor activity. For the total initial 8-hr postinjection recording period REM sleep was completely absent, SWS was significantly reduced to half of its baseline value (Table 1). From 1–5 days after injection of 6-OHDA all vigilance states had returned to baseline values except for REM sleep in 2 animals which remained at 45 and 5% of baseline and waking in one animal which was 132% of baseline (Fig. 1). REM sleep progressively decreased in all animals from posttreatment Day 5 throughout the remaining 3 weeks of observation, reaching significantly lower levels from 11–25 days after treatment (Table 1). During the same period SWS progressively increased in 4 cats while SWS in 1 cat initially rose to a peak value at Day 2 after treatment then plateaued at a level greater than baseline for the remaining period of observation (Fig. 1). The mean value for SWS was significantly greater than baseline throughout the 11–25 days posttreatment period. The 6-OHDA treatment produced no consistent effects on percent of waking time throughout the 1–25 day posttreatment period. Two animals exhibited elevated levels of wakefulness while waking time was slightly depressed in the other 3 animals.

Regression analyses of the percent of time spent in each state of vigilance versus posttreatment time revealed a significant downward trend in REM sleep in 3 animals while significant upward trends in SWS were noted in 3 of 5 animals (Table 2). In addition, 2 of the cats exhibited significant downward trends in total waking time throughout the posttreatment period.

Other than slight ataxia which occurred during the initial 2–4 hr after injection of 6-OHDA into the dorsolateral pontine tegmentum, few behavioral effects were noted in the above 5 animals throughout the 3 week period of observation. Polygraphic indices of sleep states revealed little change in bioelectrical activity except for an increase in the number of PGO spikes discharged during the waking state on the day of injection (mean control value = 1.7 spikes/min vs. mean treated value = 23 spikes/min; $p<0.05$; N = 5) and for a loss of the atonicity in the antigravity muscles which normally occurs during REM sleep. The atonicity slowly disappeared beginning 1–2 days after injection in all animals, reaching completion by the 6–8th day and then returning to normal between 16–18 days after injection.

Histological examination of the midbrain-pontine region revealed bilateral destruction of neural elements in the inferior colliculus, LOC, brachium conjunctivum, nucleus parabrachialis, the dorsomedial portion of the nucleus reticularis pontis oralis, the tractus tegmentalis and, occasionally, the ventrolateral segment of the central gray substance (Fig. 2). However, areas of destruction common to all animals were the LOC, the brachium conjunctivum and the nucleus parabrachialis. The rostral extent of the lesion corresponded to the −1.0 mm plane of the Reinoso-Suárez atlas while the caudal extent of the affected area corresponded to the −4.0 mm plane.

Analyses of regional brain levels of NE one week after the final recording session yielded a highly significant reduction in all areas of the brain with the most pronounced decreases occuring in neocortical areas, basal forebrain area, hippocampus and cerebellum (Table 3). Significant reductions in tissue levels of serotonin were noted in all cortical areas, medulla, striatum, pyriform lobe and the hypothalamus, but not in the basal forebrain area and cerebellum.

Except for first day effects, an entirely different spectrum of sleep patterns emerged in one cat given 6-OHDA into the subcoeruleus area and the nucleus reticularis pontis oralis than in the previous 5 animals. During the first week after treatment, SWS increased dramatically, total waking time decreased, and REM sleep remained unchanged from its baseline value (Fig. 3). However, on the tenth day after administration of 6-OHDA, SWS suddenly decreased and then slowly increased again for the remainder of the period of observation. Although, a significant upward trend ($r = +0.86$, $p<0.005$) in SWS was evident during the secondary rise, i.e., from Day 10–32, the overall trend in SWS was not significant nor was the absolute increase when compared to baseline values (Table 2). The daily pattern of wakefulness was essentially a mirror image of the SWS pattern throughout the entire 32 day posttreatment period. The decreasing trend in waking time from 10–32 days was highly signifi-

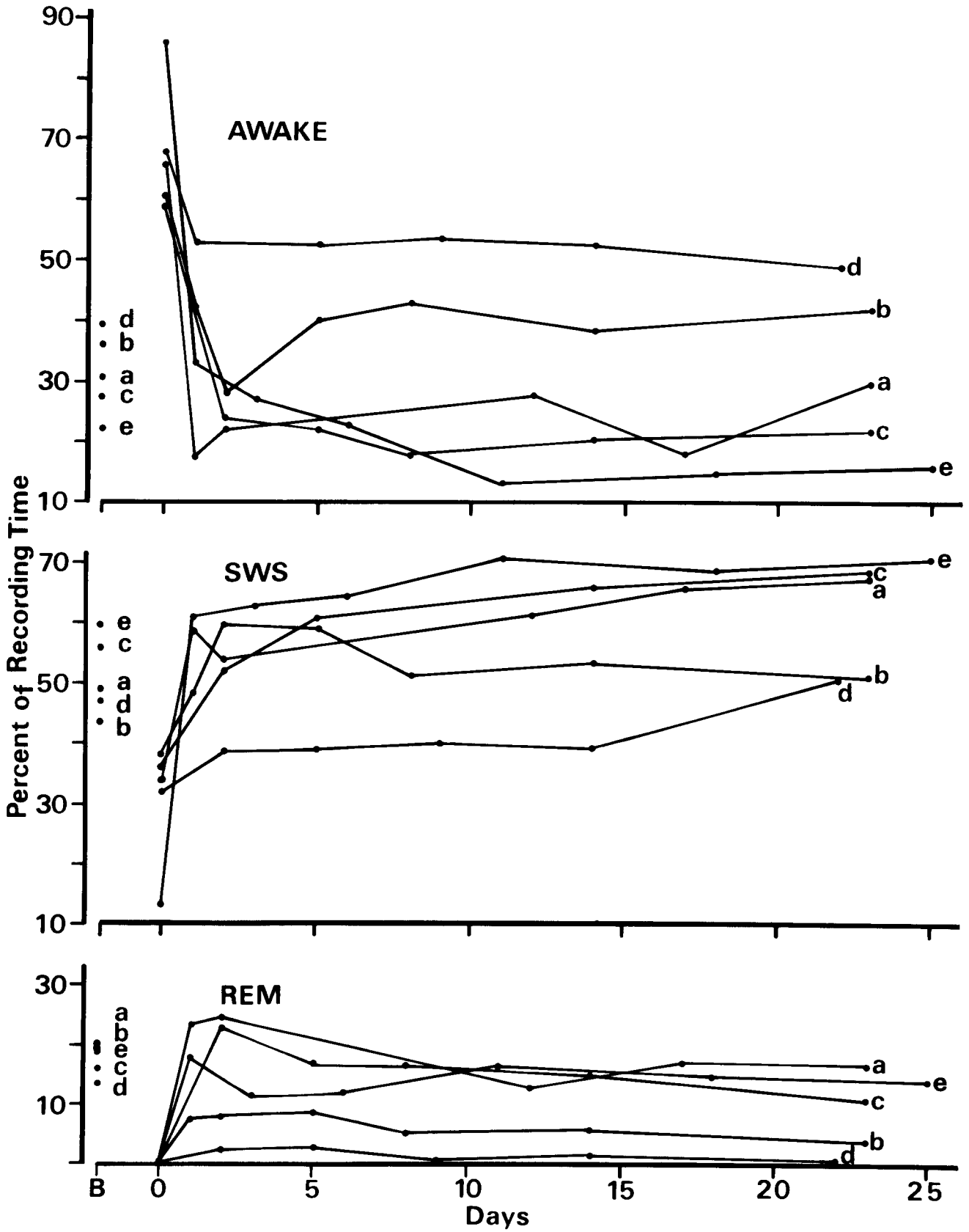

FIG. 1. Sleep-waking patterns of Cats A through E after bilateral injection of 80 μg of 6-hydroxydopamine into the dorsolateral pontine tegmentum. B in lower left corner refers to mean of 3–6 baseline sleep state values for the respective cats. Vigilance state values are expressed as percent of recording time.

TABLE 2

REGRESSION EQUATIONS AND COEFFICIENTS OF VIGILANCE STATES FROM 1–25 DAYS AFTER BILATERAL INJECTION OF 6-HYDROXYDOPAMINE INTO THE DORSOLATERAL PONTINE TEGMENTUM. DATA COMPUTED AS VIGILANCE STATE (PERCENT OF RECORD) VERSUS DAYS AFTER TREATMENT.

Cat	Awake	SWS	REM
A	19.91 +0.43X, r = +0.45	55.54 +0.55X, r = +0.94†	23.74 –0.42X, r = –0.81†
B	39.14 +0.18X, r = +0.25	55.34 –0.14X, r = –0.46	9.26 –0.22X, r = –0.83†
C	22.39 –0.02X, r = –0.48	56.23 +0.59X, r = +0.81†	22.82 –0.50X, r = –0.92†
D	55.22 –0.25X, r = –0.91†	42.68 +0.22X, r = +0.11	2.24 –0.05X, r = –0.42
E	27.99 –0.69X, r = –0.79†	61.87 +0.39X, r = +0.81†	14.84 –0.03X, r = –0.10
F*	31.37 –0.37X, r = –0.51†	58.10 –0.13X, r = –0.14	10.39 +0.57X, r = +0.81‡

*data collected for 32 days †$p<0.05$ ‡$p<0.005$ one-tail t-test

cant (r = –0.91, $p<0.005$) as well as its overall trend. REM sleep percentages remained unchanged throughout the first 10 days after administration of 6-OHDA then progressively increased to a peak value at 32 days, accounting for 30% of the recording at that time. The upward trend in REM sleep from 10–32 days was significant (r = +0.68, $p<0.025$) as well as its overall trend (Table 2, Cat F).

In contrast to the other five animals, the behavior of this cat was dramatically affected after administration of 6-OHDA. In addition, to the behavioral excitement which occured on the day of injection, Cat F exhibited episodes of hallucinogenic-like behavior, poorly directed sham rage and unilateral locomotor activity (circling) beginning on the day of injection and terminating on Day 11 after treatment. The hallucinogenic-like episodes occurred in the awake state shortly after a REM period. Polygraphic examination at the moment preceding the onset of the hallucinogenic-like episodes revealed seizure activity throughout the cerebrum. The duration of the seizure activity was from 50–135 sec and first appeared simultaneously in the hippocampal and LGN leads. Similar to the previous 5 cats, both the release of PGO activity into the awake state on the day of injection of 6-OHDA as well as the onset and duration of the loss of atonicity in postural muscles during REM sleep occurred in this animal.

Histological examination of the pons in Cat F revealed extensive bilateral damage to the subcoeruleus area, nucleus reticularis pontis oralis, and ventral portion of the central gray substance (Fig. 4a). Although a considerable loss of neural elements was evident within the injected area many neurons escaped the cytolytic effects of 6-OHDA (Fig. 4b). Regional levels of NE in Cat F were reduced in all areas of the brain except in the striatum and medulla with the greatest reduction occurring in the basal forebrain area (80%) and in all neocortical areas (6–51%). Levels of NE were reduced by 30 and 32% in the hypothalamus and anterior pyriform lobe, respectively. Regional levels of serotonin were reduced by 70% in the medial hypothalamus and posterior pyriform lobe while serotonin remained unchanged in the basal forebrain area, occipital cortex and striatum. Serotonin levels in the remaining areas of the brain were reduced by 28–41%.

DISCUSSION

Results from the present study show that injection of 6-OHDA into the LOC and adjacent tissue produces a long term reduction in REM sleep and an increase in SWS. These results are consistent with results from previous studies which also show a marked reduction in REM sleep [5, 8, 31, 32] and an increase in SWS [5] after electrolytic destruction of the dorsolateral pontine tegmentum sparing the midline raphé nuclei. However, the location of the pacemaker for genesis of REM sleep in the pons is less clearly defined. Jouvet [15,18] places the REM sleep generator in a diffuse area of the pontine tegmentum containing the middle and caudal region of the LOC along with the dorsocaudal area of the nucleus reticularis pontis oralis (NPO) and the rostral area of the nucleus reticularis pontis caudalis. However, Carli and Zanchetti, favor a mediolateral position in the middle and caudal third of the NPO [7, 8, 43]. The present study suggests an important role for the catecholamine-containing neurons of the LOC and immediately surrounding tissue in the induction of REM sleep rather than the NPO, since extensive damage to the NPO (Cat F) failed to reduce REM episodes and, in fact, caused an increase in REM sleep, while destruction of the LOC reduced REM sleep in all subjects. The results of Carli *et al.* [8,43] can be explained, in part, by an interruption of ascending projections from the catecholamine-containing neurons of the LOC and adjacent area which course diffusely through the dorsal NPO at the level they made their lesions [25,33]. The reduction in forebrain NE (particulary in cortical and hippocampal tissue) in Cat F after placement of 6-OHDA into the dorsorostral portion of the NPO lends support to the above interpretation. Similarly, the increase in SWS might then be accounted for by a disruption of ascending activating influences originating from the LOC [18, 28, 29], i.e., removal of a system which probably exerts an inhibitory effect on a SWS mechanism [3,24].

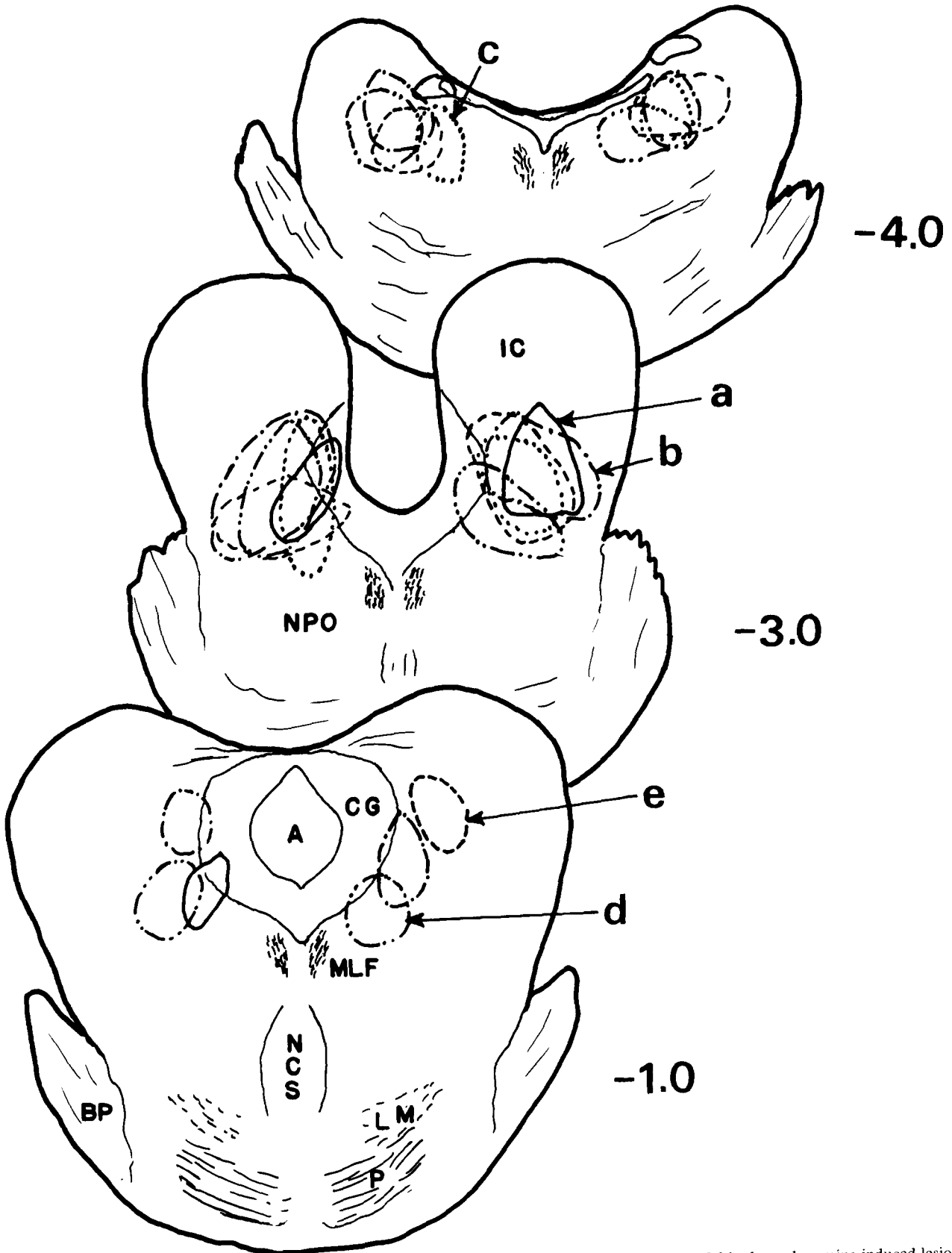

FIG. 2. Diagrammatic reconstruction of mesencephalic-pontine region of cat brain illustrating extent of 6-hydroxydopamine-induced lesions in Cats A thru E. Arrows indicate line-cat code of lesion. Numbers on right of figure refers to corresponding anterior-posterior planes (in mm) from the Reinoso-Suarez cat atlas. A = cerebral aqueduct, BP = brachium pontis, CG = central gray substance, IC = inferior colliculus, LM = lemniscus medialis, MLF = medial longitudal fasciculus, NCS = nucleus centralis superior of Bechterew, NPO = nucleus reticularis pontis oralis, P = pons.

TABLE 3

EFFECTS OF 6-HYDROXYDOPAMINE INJECTED INTO THE DORSOLATERAL PONTINE TEGMENTUM ON REGIONAL BRAIN LEVELS OF NOREPINEPHRINE AND SEROTONIN. TISSUE VALUES EXPRESSED AS ng OF amine/g OF TISSUE (MEAN ± SE). CATS WERE SACRIFICED 5–6 WEEKS AFTER INTRACEREBRAL INJECTION OF 6-HYDROXYDOPAMINE.

Brain Region	Norepinephrine		Serotonin	
	Normal N = 17	Treated N = 5	Normal N = 17	Treated N = 5
Temporal Cortex	298 ± 11	144 ± 30‡	473 ± 15	358 ± 13‡
Anterior Pyriform Lobe (Amygdala)	356 ± 13	220 ± 42‡	1529 ± 62	1097 ± 226†
Posterior Pyriform Lobe (Hippocampus)	237 ± 12	130 ± 16‡	1486 ± 107	795 ± 154*
Basal Forebrain Area	1183 ± 70	449 ± 58‡	1673 ± 71	1674 ± 128
Striatum	300 ± 12	135 ± 13‡	1140 ± 76	810 ± 149*
Occipital Cortex	297 ± 14	82 ± 4‡	412 ± 22	245 ± 26*
Lateral Hypothalamus	906 ± 52	392 ± 47‡	1769 ± 81	1116 ± 118‡
Medial Hypothalamus	1998 ± 90	1085 ± 107‡	1785 ± 92	1140 ± 110*
Cerebellum (Lateral Lobe)	285 ± 10	140 ± 15‡	371 ± 21	300 ± 50
Medulla	320 ± 16	200 ± 22*	1119 ± 58	623 ± 52‡

*$p<0.01$ †$p<0.02$ ‡$p<0.001$ Student's t-test (2-tailed)

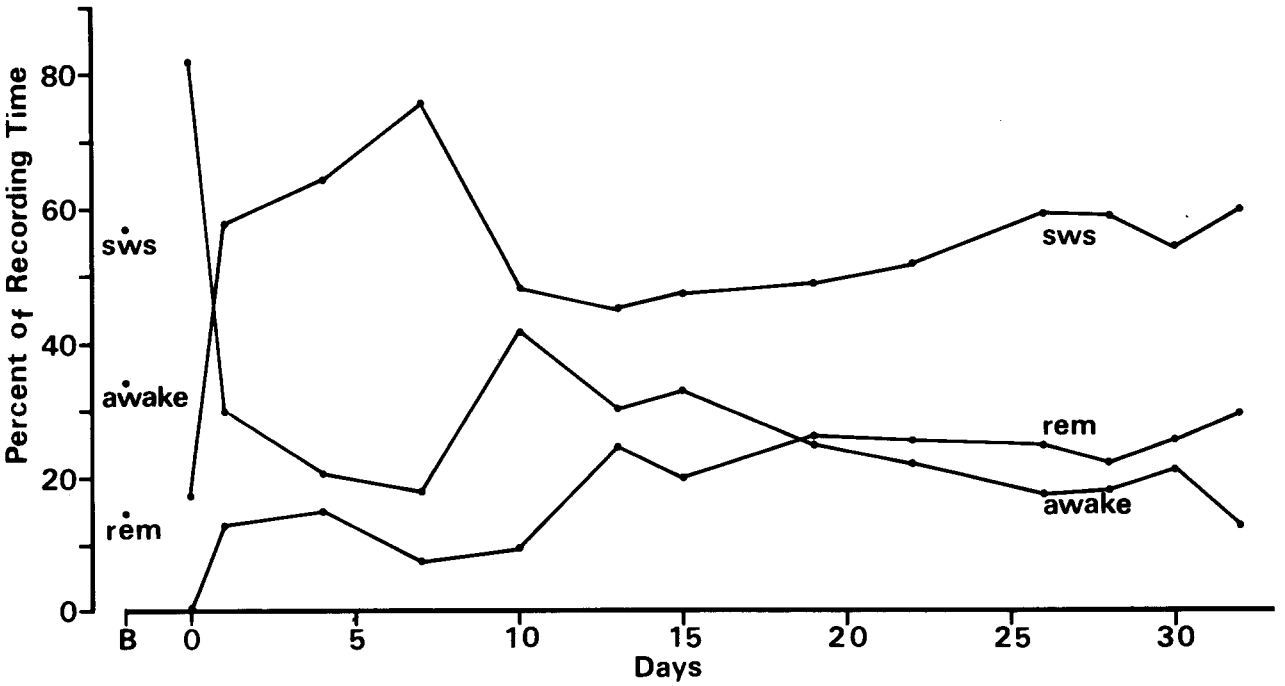

FIG. 3. Sleep-waking patterns of Cat F after bilateral injection of 80 μg of 6-hydroxydopamine into the dorsomedial pontine tegmentum. B in lower left corner refers to mean of 6 baseline values for the respective vigilance state. Vigilance state values expressed as percent of recording time.

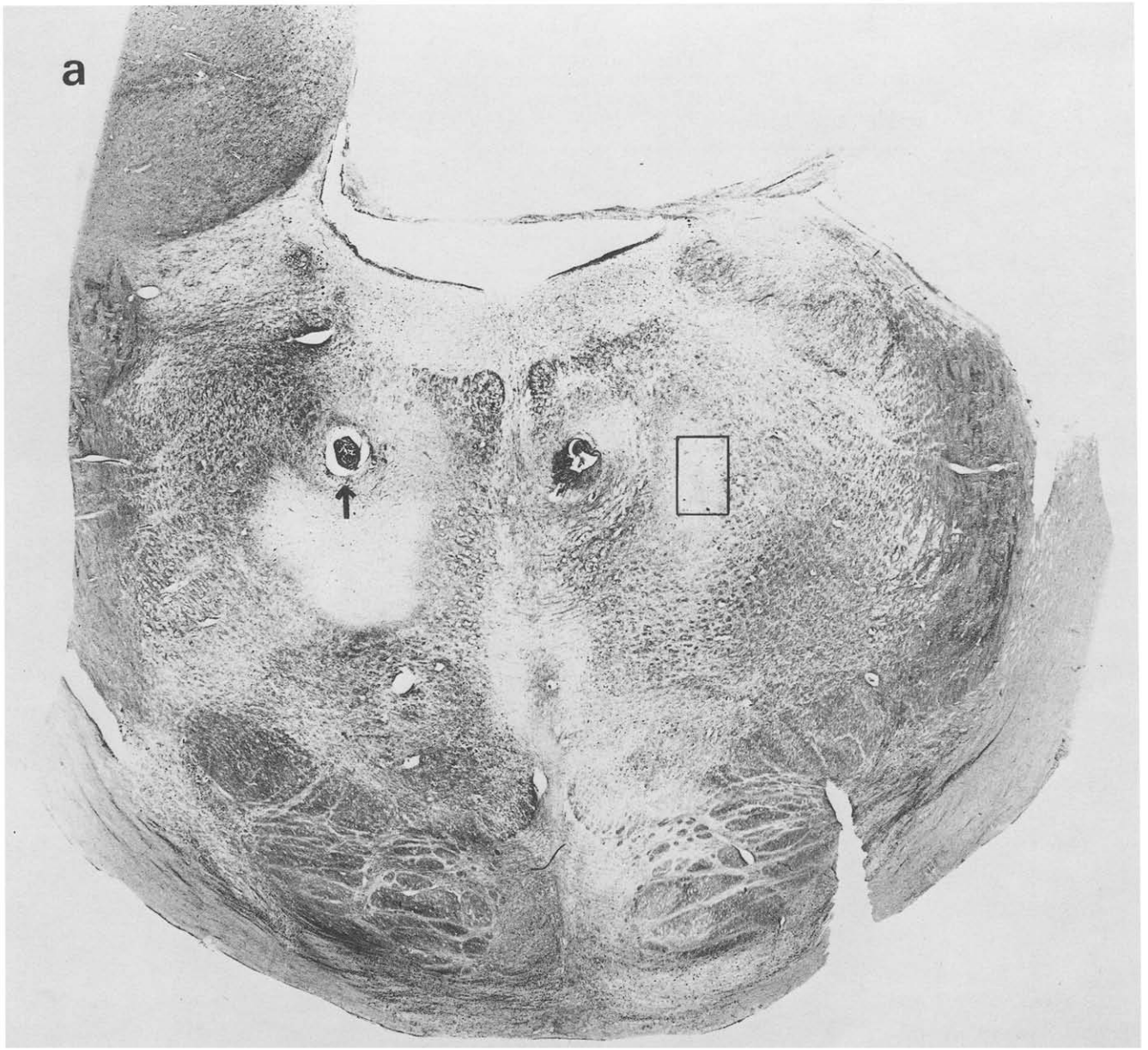

FIG. 4a. Histological section illustrating lesion in pontine tegmentum of cat F sacrificed 35 days after receiving bilateral injections of 80 μg of 6-hydroxydopamine. Arrow indicates point-of-entry of cannyla. Magnification is 5.7X. Kluver-Barrera stain.

The present study also confirms the original observation by Jouvet and co-workers [22,23], that 6-OHDA produces two distinct time-dependent effects on sleep-waking behavior. The initial arousal which occurs immediately after injection of 6-OHDA into the pontine tegmentum or ventricles may be due to an acute liberation of synaptically active NE [2,12], while the chronic sleep effects of the treatment may be due to permanent degeneration of an ascending noradrenergic system resulting in an imbalance in catecholaminergic-serotonergic tone [18]. However, certain differences between these studies can be noted. In our study, the initial behavioral excitation terminated 2–3 hr after administration of 6-OHDA, whereas the behavioral excitation was more pronounced, of a longer duration and accompanied by a severe disruption in autonomic function – often leading to death – when the LOC was destroyed via intraventricular injections of 6-OHDA [9,22]. This latter result suggests a more widespread effect of the drug when given via the intraventricular route. The acute autonomic response appears to result from a liberation of both NE and serotonin, since the excitation can be prevented by prior inhibition of catecholamine synthesis, while the hypothermia and initial release of PGO spikes can be prevented by pretreatment with chlorimipramine, a drug that prevents the nonsepcific uptake of 6-OHDA into serotonin neurons [23]. The initial release of PGO activity that occurred in 5 of 6 cats in this study combined with the long term reduction in brain levels of serotonin suggest a 6-OHDA-induced

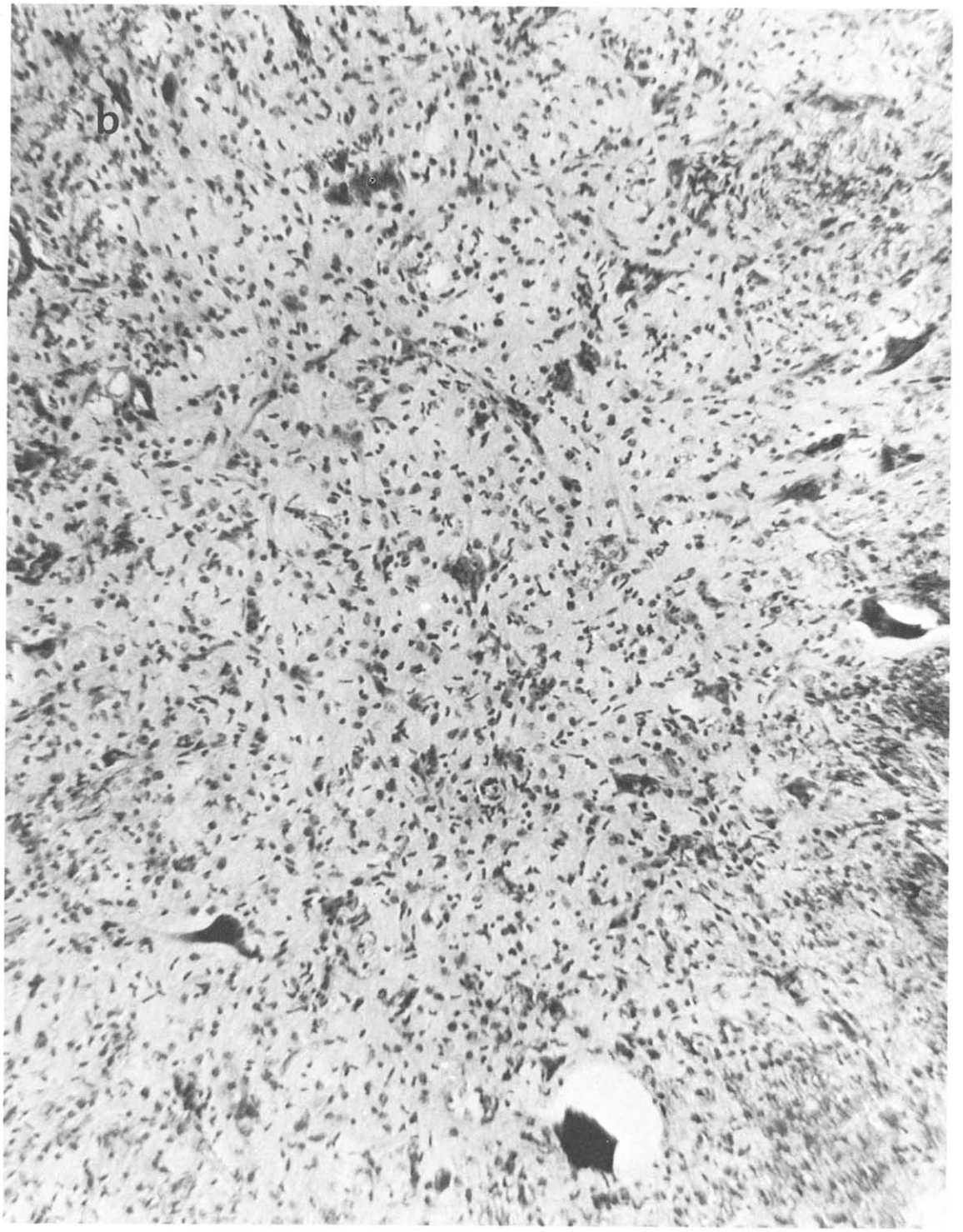

FIG. 4b. High-power photomicrograph of area on right side of lesion (see Fig. 4a) illustrating 6-hydroxydopamine-induced cytological damage. Magnification is 202X.

disruption of serotonergic mechanisms. Secondly, the time course and duration of the abolition of atonicity in anti-gravity muscles during REM sleep after injection of 6-OHDA into the pontine tegmentum is similar to that produced following electrocoagulation of the LOC [18]. However, this effect was not produced after intraventricular administration of 6-OHDA [23]. Since decrements in NE were of a similar magnitude in this study and the latter

study, it can be concluded that either 6-OHDA was unable to diffuse into the critical elements subserving this function via the intraventricular route or the inhibitory influence on antigravity muscles should not be attributed solely to descending catecholaminergic influences as originally envisioned by Jouvet [16]. In the latter regard George *et al.* [13] and Baxter [1] have emphasized the importance of a descending cholinergic pathways originating in the ponto-mesencephalic reticular formation in the induction of some tonic phenomena associated with REM sleep. Since we did not assess damage to the cholinergic system originating in the dorsal pons produced by our 6-OHDA injections, it is possible that the decrease in REM sleep or the effects on components of REM sleep were due to interference with a cholinergic sleep mechanism. Finally, neither injection of 6-OHDA into the coeruleus complex or into the ventricle elicits hallucinogenic-like behavior which has been reported following bilateral coagulation of the LOC [16]. Although, we have been able to confirm the occurrence of this abnormal behavior in one animal which received 6-OHDA primarily into the NPO, it appears doubtful that such behavior can be attributed to disruption of the catecholamine mechanisms of the coeruleus complex.

Excluding the acute effects of administration of 6-OHDA, sleep parameters were not significantly different from baseline conditions until after postinjection Day 10. At that time, SWS increased and REM sleep decresed and remained so for the duration of the experiment. The time lag required to achieve this effect combined with the significant correlation of the sleep parameters with time (Table 2) is consistent with a slow degeneration of, or a progressive dysfunction of sleep mechanisms within the coeruleus complex.

Histological verification of the affected area, biochemical analysis of regional levels of NE and behavioral effects on sleep in Cats A–E (Figs. 1 and 2) indicate extensive but not total or permanent dysfunction of the LOC after injection of 6-OHDA. The recovery of the neck muscle atonia of REM sleep between 16–18 days after the injection combined with only a 38% increase in medullary NE suggest that the descending limb originating from the caudal LOC [18] is either resistant to the chemolytic effects of 6-OHDA [39] or that this tonic event of REM sleep is not solely dependent upon the catecholaminergic components of the LOC. Failure to detect cytological changes in the caudal LOC suggests that this structure was not sufficiently perfused by the injection of 6-OHDA or that a partial reestablishment of functional connections had taken place in the intervening time period. Since complete destruction of the caudal 2/3 of the LOC may be necessary to achieve total suppression of REM sleep [18], failure to completely destroy this structure in any of the cats in the present study precluded a complete loss of REM sleep. However, a permanent 95% reduction in REM sleep was noted in one animal (Cat D) which sustained the greatest degree of damage to the LOC. It would appear from the present data that the ascending projections from the anterior LOC, which may subserve cortical desynchronization during wakefulness, were not sufficiently affected by our treatment as evidenced by a failure to observe a significant decrease in waking time [18]. Histology revealed that the cannular placements were not sufficiently rostral to completely destroy the diffuse rostral and lateral catecholamine-containing neurons which contribute to the ascending cortical system [16].

Regarding the lesion itself, *in situ* injection of 6-OHDA by no means results in the global tissue destruction produced by electrocoagulation. Although a large number of neurons were destroyed (Fig. 4a, b) many neurons appeared to escape the cytolytic effects of 6-OHDA. Thus, it may be assumed that at least a portion of the affected area can remain functionally active. The latter assumption may in fact account for the disparity between results from electrolytic lesion studies and results from the present study.

The significant reduction in NE throughout the forebrain after injection of 6-OHDA into the dorsolateral pontine tegmentum confirms previous reports of the catecholaminolytic effect of the drug in brain tissue [4,39]. However, coincident reductions in brain levels of serotonin following this treatment indicates that the drug lacks the degree of specificity that has been reported for the rat [4, 38, 41]. Since depressed levels of serotonin generally lead to a reduction in SWS [18], it appears unlikely that the lowered levels of serotonin played a significant role in the changes seen in sleep-waking behavior in the present study in view of the reliable increase in SWS that occurred between Days 10–25 after treatment.

REFERENCES

1. Baxter, B. L. Induction of both emotional behavior and a novel form of REM sleep by chemical stimulation applied to cat mesencephalon. *Expl Neurol.* **23:** 220–230, 1969.
2. Bell, L. J., L. L. Iversen and J. J. Uretsky. Time course of the effects of 6-hydroxydopamine on catecholamine containing neurones in rat hypothalamus and striatum. *Br. J. Pharmac.* **40:** 790–799, 1970.
3. Berman, E., W. Stern and P. Morgane. Effects of locus coeruleus stimulation on raphé unit activity. *Soc. Neurosci.* 555, 1973.
4. Breese, C. R. and T. D. Traylor. Effects of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp. Ther.* **147:** 413–427, 1970.
5. Buguet, A., F. Petitjean and M. Jouvet. Suppression des pointes PGO du so sommeil par lésion ou injection *in situ* de 6-hydroxydopamine au niveau du tegmentum pontique. *C.r. Soc. Biol.* (Paris) **164:** 2293–2298, 1970.
6. Camacho-Evangelista, A. and F. Reinoso-Suárez. Activating and synchronizing centers in cat brain: electroencephalograms after lesions. *Science* **146:** 268–270, 1964.
7. Carli, G., V. Armengol and A. Zanchetti. Electroencephalographic desynchronization during deep sleep after destruction of midbrain-limbic pathways in the cat. *Science* **140:** 677–680, 1963.
8. Carli, G. and A. Zanchetti. A study of pontine lesions suppressing deep sleep in the cat. *Archs. ital. Biol.* **103:** 751–788, 1965.
9. Descarries, L. and G. Saucier. Disappearance of the locus coeruleus in the rat after intraventricular 6-hydroxydopamine. *Brain Res.* **37:** 310–316, 1972.
10. Dusan-Peyrethon, D. and J. L. Froment. Effets du disulfiram sur les états de sommeil chez le chat. *C.r. Soc. Biol.* (Paris) **162:** 2141–2145, 1968.

11. Dusan-Peyrethon, D., J. Dusan-Peyrethon and M. Jouvet. Suppression élective du sommeil paradoxal chez le chat par alpha méthyl-dopa. *C.r. Soc. Bio.* (Paris) **162:** 116–118, 1968.
12. Evetts, K. D., J. T. Fitzsimons and P. S. Setler. Eating caused by 6-hydroxydopamine-induced release of noradrenaline in the diencephalon of the rat. *J. Physiol.* **223:** 35–47, 1972.
13. George, R., W. L. Haslett and D. J. Jenden. A cholinergic mechanism in the brainstem reticular formation: induction of paradoxical sleep. *Int. J. Neuropharmac.* **3:** 541–552, 1964.
14. Jones, B., P. Bobillier and M. Jouvet. Effets de la destruction des neurones contenant des catecholamines du mesencephale sur le cycle veille-sommeil du chat. *C.r. Soc. Biol.* (Paris) **163:** 176–180, 1969.
15. Jouvet, M. Reserches sur les structures nerveuses et les méchanisms responsables des différents phases du sommeil physioloqique. *Archs. ital. Biol.* **100:** 125–206, 1962.
16. Jouvet, M. Neurophysiology of the states of sleep. *Physiol. Rev.* **47:** 117–177, 1967.
17. Jouvet, M. Biogenic amines and the states of sleep. *Science* **163:** 32–41, 1969.
18. Jouvet, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergebn. Physiol.* **64:** 166–307, 1972.
19. Jouvet, M. and F. Delorme. Locus coeruleus et sommeil paradoxal. *C.r. Soc. Biol.* (Paris) **159:** 895–899, 1965.
20. King, D. and R. E. Jewett. The effects of alpha methyltyrosine on sleep and brain norepinephrine in cat. *J. Pharmac. exp. Ther.* **177:** 188–194, 1971.
21. Klüver, H., H. Barrera and E. Barrera. A method for the combined staining of cells and fibers in the nervous system. *J. Neurophath. exp. Neurol.* **12:** 401–407, 1953.
22. Laguzzi, R., F. Petitjean, J. F. Pujol and M. Jouvet. Effets de l'injection intraventriculaire de 6-hydroxydopamine sur les états de sommeil et les monoamines cérébrales du chat. *C.r. Soc. Biol.* (Paris) **165:** 7–8, 1971.
23. Laguzzi, R., F. Petitjean, J. F. Pujol and M. Jouvet. Effets de l'injection intraventriculaire de 6-hydroxydopamine. II. Sur le cycle veille-sommeils du chat. *Brain Res.* **48:** 295–310, 1972.
24. Loizou, L. A. Projections of the nucleus locus coeruleus in the albino rat. *Brain Res.* **15:** 563–566, 1969.
25. Maeda, T. and N. Shimizu. Projections acendantes du locus coeruleus et d'autres neurones aminergique pontiques au niveau de prosencéphale du rat. *Brain Res.* **36:** 19–35, 1972.
26. Maickel, R. P., R. H. Cox, J. Saillant and F. R. Miller. A method for determination of serotonin and norepinephrine in discrete areas of rat brain. *Int. J. Neuropharmac.* **7:** 275–281, 1968.
27. Marantz, R. and A. Rectschaffer. Effect of alpha-methyltyrosine on sleep in the rat. *Percept. Mot. Skills* **25:** 805–808, 1967.
28. Moruzzi, G. The sleep-waking cycle. *Ergebn. Physiol.* **64:** 1–165, 1972.
29. Moruzzi, G. and H. W. Magoun. Brain stem reticular formation and activation of the EEG. *Electroenceph. clin. Neurophysiol.* **1:** 455–473, 1949.
30. Reinoso-Suárez, F. *Topgraphischer Hirnatlas der Katze.* Merck: Darmstadt, 1961.
31. Rossi, G. F., K. Minobe and O. Candia. An experimental study of the hypnogenic mechanisms of the brain stem. *Archs ital. Biol.* **101:** 470–492, 1963.
32. Roussel, B., A. Buguet, P. Bobillier and M. Jouvet. Locus coeruleus-sommeil paradoxal et noradrénaline cérébral. *C.r. Soc. Biol.* (Paris) **161:** 2537–2541, 1967.
33. Sladek, J. R. Jr. Differences in the distribution of catecholamine variocosities in cat and rat reticular formation. *Science* **174:** 410–412, 1971.
34. Sterman, M. B., T. Knauss, D. Lehmann and C. D. Clemente. Circadian sleep and waking patterns in the laboratory cat. *Electroenceph. clin. Neurophysiol.* **19:** 509–517, 1965.
35. Stern, W. C. and P. J. Morgane. Effects of reserpine on sleep and brain biogenic amine levels in the cat. *Psychopharmacologia* **28:** 257–286, 1973.
36. Stern, W. C. and P. J. Morgane. Effects of α-methyltyrosine on REM sleep and brain amine levels in the cat. *Biol. Psychiat.* **6:** 301–306, 1973.
37. Thompson, J. H., C. A. Spezia and M. Angulo. Fluorometric detection of serotonin using O-phthaldehyde: an improvement. *Experientia* **15:** 327–329, 1970.
38. Tranzer, J. P. and H. Thoenen. An electronmicroscopic study of selective, acute degeneration of sympathetic nerve terminals after administration of 6-hydroxydopamine. *Experientia* **24:** 155–156, 1968.
39. Ungerstedt, U. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In: *6-Hydroxydopamine and Catecholamine Neurons,* edited by T. Malmfors and H. Thoenen. North Holland, Amsterdam. 1971, pp. 101–127.
40. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand.* **367:** 1–48, 1971.
41. Uretsky, N. J. and L. L. Iversen. Effects of 6-hydroxydopamine on noradrenaline-containing neurons in the rat brain. *Nature* (Lond.) **221:** 557–559.
42. Wyatt, R. J., T. N. Chase, D. J. Kupfer, J. Scott, F. Snyder, A. Sjoerdsma and K. Engleman. Brain catecholamines and human sleep. *Nature* (Lond.) **233:** 63–65, 1971.
43. Zanchetti, A. Brain stem mechanism of sleep. *Anesthesiology* **28:** 81–99, 1967.