AMPHETAMINE AND THE CNS: ELECTROCORTICAL, BEHAVIORAL AND PHARMACOLOGICAL CONSIDERATIONS IN THE LIGHT OF CENTRAL 6 - HYDROXY-DOPAMINE INDUCED ALTERATIONS

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to Claude

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ABSTRACT

Amphetamines are generally thought to produce their effects by the indirect action of excitatory catecholamines. During the process of obtaining behavioral, electrocortical and biochemical data, evidence was acquired which casts some doubt on the importance of this currently accepted concept. Stereotypy and locomotor behavior, changes in electroencephalographic records and in auditory evoked potentials as well as ∝-methyl-para-tyrosine (tyrosine hydroxylase inhibitor) induced toxicities have been observed in rats trained in positive and negative discrimination tasks and possessing either normal or extensively damaged (via 6-hydroxy-dopamine) central dopaminergic/noradrenergic pathways. It was concluded that both direct and indirect central actions affecting various systems, such as those mediated by acetylcholine for example, would be relatively important in producing amphetamine effects. Evidence of an inhibitory catecholaminergic central effect was also obtained.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF FIGURES

LIST OF FIGURES CONTINUED

LIST OF TABLES

I. INTRODUCTION AND STATEMENT OF THE PROBLEM

Amphetamine effects have for some time been correlated with their actions on catecholamines. These properties of amphetamines are presently recognized as their predominant modes of action and explain the physiological as well as behavioral manifestations of the drug.

A. Amphetamine Action

1. Chemistry

Since amphetamine continued to exert its effects after reserpinization, whereas cocaine did not, amphetamine was believed to directly excite post-synaptic catecholamine receptors (1, 2, 3).

These early theories have since been completely overshadowed by the advent of hypotheses favouring an indirect mode of action in the CNS which finds its strongest supporting evidence in the inhibition of the catecholamine biosynthetic pathway. Behavioral manifestations of amphetamines can seemingly be abolished with α-methyl-p-tyrosine (αMpT) (4, 5, 6) an inhibitor of tyrosine-hydroxylase, the rate limiting enzyme of catecholamine synthesis. Readily available newly synthesized catecholamines have thus recently emerged as primary causative factors (7), amphetamine competitively inhibiting norepinephrine (NE) reuptake at presynaptic endings for example, inhibiting their granular binding and thereby inducing their premature release (8). An important role for stored NE in excitation has long been postulated by Stein (9) and further supported by the fact that its total drain concentrations may severely be depleted in the course of amphetamine intoxication (8). In the course of exhaustive research several other actions of amphetamines have been unveiled. They include the release of stored NE, monoamine oxidase inhibition and the production of false transmitters (8, 10). In view of the synthesis inhibiting experiments these are considered minor.

2. Behavior

The very specific behavioral manifestations of amphetamine intoxication have been linked to catecholamine alterations. Dopamine (DA)

release has been related to the advent of stereotypy whereas increased alertness, orientation or locomotion have been related to NE release. In these studies its loss after lesioning the striatum (location of greatest DA concentration in CNS), its restoration with Dopa after tyrosine hydroxylase inhibition and the lack of its disappearance following DA-β -oxidase inhibition, among others, was taken as an indication of the necessary involvement of DA in stereotyped behavior. The loss of amphetamine induced locomotor hyperactivity with tyrosine hydroxylase inhibition or DA-β -oxidase inhibition was taken to indicate the necessary involvement of NE (see 11 for cit.). Of interest, however, is the fact that these effects, stereotypy for example, can be reproduced by other drugs which are not so easily related to catecholamines (12, 13, 14).

B. Catecholamines as Depressants

Other researchers, totally disregarded in amphetamine studies, have found that with equally large changes in catecholamine concentrations within the CNS sedative effects can be obtained.

Systemic injections of large doses of catecholamines which do cross the blood-brain-barrier in appreciable amounts (15) produce behavioral inhibition and EEG deactivation (16, 17, 18). In young chickens with underdeveloped blood-brain-barriers the same is obtained (19). Systemic administration of catecholamine precursors DA (3,4-dihydroxyphenylethylamine) (20) and DOPS (3,4-dihydroxyphenylserine) (21, 22) have resulted in essentially identical findings.

Intralumbar (23, 24), intracisternal (23) and intraventricular injections (25 - 31) of catecholamines again produce similar results on behavior and EEG, the latter action only being altered effectively by adrenergic receptor blockers (31).

With microinjections of NE into brain tissue, behavioral depression has been reported (32, 33). Predominantly inhibitory effects of microiontophoretic applications of NE and DA upon spontaneous and evoked spike activity at different levels of the CNS are cited (34). These effects are more marked for the solutions nearest physiological pH's (35).

C. Miscellaneous Amphetamine Actions

The behavioral and electrocortical signs of depression listed in the above reports are a far cry from the amphetamine behavioral and electrocortical forms of excitation (36 - 40 and 41 for cit.). This suggests that other actions may exist.

Pronounced nicotinic effects have been observed peripherally (ganglia) (42) and centrally (43) and proposed as possible central modes of action. Lending support to this idea is the sympathomimetic presynaptic facilitation of acetylcholine (ACh) release at ganglia (44) and at the neuromuscular junction (45). (Anticurare actions have also been reported before and antigallamine actions observed personally). Arousal, tremorigenisis and/or catatonia are observed with the use of amphetamine and ACh (46, 47) as well as with nicotine (47, 48, 49). Hypothalamic cholinergically mediated phenomena such as hypophagia (50) and hyperthermia (51) are commonly produced by amphetamine. A non-competitive inhibition of ACh uptake into cortex slices by amphetamine has also been reported (52) which may explain some reports that agents which decrease cholinergic function can lead to amphetamine potentiation (53, 54). All these effects of amphetamine are rather interesting in the light of electrocortical and behavioral changes obtainable with cholinergics and anticholinergics (55).

Amphetamine (as does ACh) can induce intestinal contractions and such properties have been ascribed to serotoninergic (5HT) receptors (56, 57) and amphetamine/5HT involvement generalized to the CNS (58, 59). Interestingly enough, a direct stimulatory effect on cells of the raphe nuclei (5HT) by amphetamine has been reported (60).

D. Proposal and Logic of Experiments

It is beyond the scope of this dissertation to discuss fully those actions of amphetamines which are not mediated by catecholamines; however, it is the author's impression that the views relating amphetamine effects to catecholamine action are oversimplifications and in need of revision.

Ideally, if a drug existed which could selectively destroy, either partly or fully, central catecholaminergic pathways, the catecholamine mediation theory would be subjected to the severest test and detrimental, damage-related alterations in amphetamine responses should be observed.

Such a drug, capable of disappearing from an animal's CNS within hours, without affecting the periphery (61), does exist. Recent biochemical (61 - 65), histochemical (66, 67, 68) and ultrastructural (62, 69, 70) investigations have established that intracerebral administration of the drug 6-hydroxydopamine (6-OH-DA), leads to selective degeneration of central DA and NE terminals. Within 2 hours for NE and 2 days for DA of central injection of the drug, exudation of these neurotransmitters has reached its peak and brain levels their minimum (71). Monoamine oxidase (MAO) inhibition can potentiate DA depletion and of great importance is the tremendous tyrosine hydroxylase inhibition that results (61,62,72). Destruction of tissue and reduction of catecholamine concentrations are greatest in areas with nerve terminals and least in those which contain biogenic amine cell bodies (62,69); hence, complete brain depletion of both catecholamines is not a necessity. Brain reduction of these amines is not the best indicator of pathway damage and cell body retention of these amines is not a manifestation of functional systems.

This drug is therefore more capable of altering central catecholaminergic systems than any drug previously used in the study of amphetamine pharmacodynamics. It has the combined action of αMpT, popular DA-β-oxidase inhibitors such as FLA63, reserpine and others. Therefore, if amphetamine effects are in fact mediated predominantly by catecholamines, as widely held, subsequent to an adequate convalescence period these should be at least decreased or even completely abolished in the case of 100% destruction.

The purpose of the present experiments was therefore, by means of 6-OH-DA, to shed new light on amphetamine modes of action in the CNS, by observing fairly well understood electrocortical phenomena as well as spontaneous and conditioned behaviors and in so doing to put catecholamines, particularly NE, in their proper perspective, that of central inhibitory agents.

II. METHODS

A. Subjects and Surgery

Male Sprague-Dawley rats, weighing approximately 200g, were implanted (under pentobarbital anesthesia) with 0.016 inch diameter platinum wire electrodes and permanently fitted with female connectors secured in inert acrylic onto their skulls. All electrodes were supradural, one reference electrode in the nasal bone, two pairs of electrodes over both sensory motor areas and one electrode in each primary auditory area (Fig. 1). Local anesthesia was used when needed and followed up with local and general antibiotics.

B. Destruction of Catecholaminergic Pathways

After a recovery period of no less than one month, catecholaminergic pathway destruction was accomplished under light ether anesthesia by means of one 20µl intracisternal injection of 6-hydroxydopamine (6-OH-DA). The 6-OH-DA was dissolved (10 mg/ml base) in Elliott's "A" solution (73) which contained 1mg/ml of ascorbic acid to prevent its oxidation. In an attempt to decrease deamination of 6-OH-DA and thereby potentiate its destructive effect, some animals (used in behavioral studies only) received 100 mg/kg of Nialamide i.p. 60 minutes before 6-OH-DA.

C. Drugs

During and subsequent to surgery, a majority of the following drugs were used: sodium pentobarbital (Nembutal, Abott), lidocaine (Xylocaine, Astra), bacitracin (Baciguent, Upjohn), sodium pennicillin G (Ayerst), tetracycline (Tetracyn, Pfizer) and chlortetracycline (Aureomycin, Cyanamide).

The key drugs used in this study were d-amphetamine sulphate (Dexedrine, Smith, Kline and French), nialamide (Nialamide, Pfizer), 6-hydroxydopamine hydrochloride (H88/32, AB Biotec) and dl- α -methyltyrosine-methylester HCl (H44/68, AB Biotec). These latter drugs, with the exception of nialamide and αMpT, were injected according to free base calculations. Amphetamine, nialamide and αMpT were dissolved in saline and injected i.p. in concentrations of 1 mg/ml, 40 mg/ml, 50 mg/ml respectively.

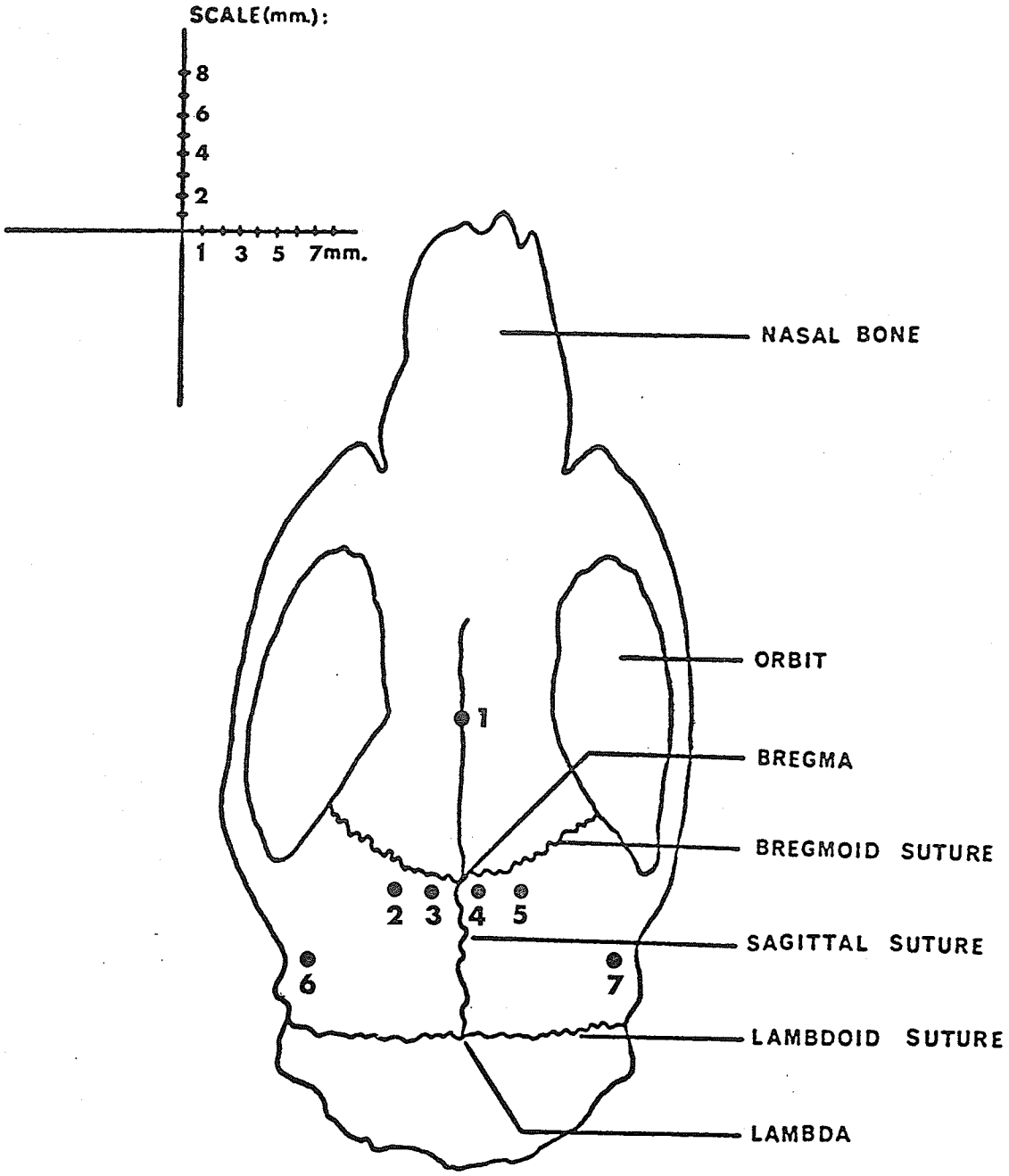

Fig. 1. Dorsal surface of rat skull and electrode placements. No. 1, reference; no's 2, 3, 4 and 5, sensory-motor; no's 6 and 7, auditory.

D. Animal Upkeep and Conditioning

Rats were fed Purina Laboratory Chow and watered once daily in the late afternoon.

Conditioning of the partly trained rats was completed within roughly three weeks subsequent to a short postoperative convalescence period. The rats were trained to criterion of 80% correct response on positive (Rf^{+}) and negative (Rf^{-}) reinforcement schedules. Haphazardly presented ten second trains of clicks (3 Hz, 5 ms, 60 db, 3000 Hz pitch) served as the conditioned stimuli (CS) during which the animals either had to depress a lever to obtain one drop of water on the "drinking side" of the modified Skinner-box or await, without escape attempts, an unavoidable shock (10 Hz, 1 ms, 0.1 - 0.5 ma net current through rat) which was delivered to the "shock side" of the cage's grid-bottom subsequent to the CS.

E. Electrocortical Recordings

One recording session for an animal lasted roughly 15 - 20 minutes and consisted of recording EEG and averaged auditory evoked potentials (EP) while the animal performed in sequence the positively and negatively motivated tasks. One such control session was always recorded within 1 - 2 hours of drug treatment as well as 45 minutes following saline or d-amphetamine.

Continual bipolar EEG recordings were taken unilaterally from paired electrodes located in one sensory motor cortex. Unipolar recordings were simultaneously obtained from an electrode at one auditory cortex which was referred to the electrically inactive nasal electrode. EEG corresponding to the animal's "tonic" reactions to both environments were evaluated.

At least 3 auditory EP, each averaged from 100 clicks, were obtained in both environments.

F. Equipment

Clicks were delivered through a 2 1/2 inch speaker placed 32 inches over the floor of the modified Skinner-box. The box, effectively sheilded from from electrical interference by a surrounding copper wire cage, was located in a room relatively free of extraneous sounds.

EEG was amplified by Grass 7P511 amplifiers, graphically displayed by a Model 7 Grass Polygraph and fed to a Series 3960 Hewlett Packard 4 channel FM analogue tape recorder and stored on Ampex magnetic tape. Filtering provided a band pass of 1 - 25 Hz. For safe measure, all EEG at recorder inputs and outputs were monitored on a Tektronix oscilloscope.

Amplified and filtered unipolar EEG signals were fed to the Didac 800 digital computer which directly averaged and displayed 100 auditory evoked potentials. Such averaged potentials were photographed for permanent record and subsequent evaluation.

G. Statistical Evaluations of Electrocortical Data

1. Raw data

EEG stored on magnetic tape was fed to a CDC 1700 Computer which via fast Fourier analysis evaluated in 0.1 sec steps amplitudes of 10 sec epochs for a spectrum of 7 frequency ranges (D (delta) 1.5 - 3.5 Hz, T_1 (theta) 3.5 - 5.5 Hz, T_2 (theta) 5.5 - 7.5 Hz, A_1 (alpha) 7.5 - 9.5 Hz, A_2 (alpha) 9.5 - 12.5 Hz, B_1 (beta) 12.5 - 17.5 Hz, B_2 (beta) 17.5 - 25.0 Hz). In the process, EEG was also integrated over the range 1 - 25 Hz and frequency dominance indicated.

Ten second epochs of EEG, free of artifacts, were then chosen and categorized as either CS ("phasic" reaction to clicks) or pre CS ("tonic" reaction to environment) for both unipolar and bipolar leads, in both drinking and shock situations and for each session pair (Control + Drug) for individual rats.

Within each animal/experiment, by means of an IBM 360 Computer, all EEG data was averaged and statistically evaluated by means of one way analyses of variance and multiple range tests.

Evoked potentials, on the other hand, for session pairs were categorized as either Rf^+ or Rf^-. Seven amplitude and four latency measurements were taken of each averaged evoked potential (Fig. 2) and statistically evaluated as above.

Through these procedures the "raw data" was acquired for one experiment on one rat.

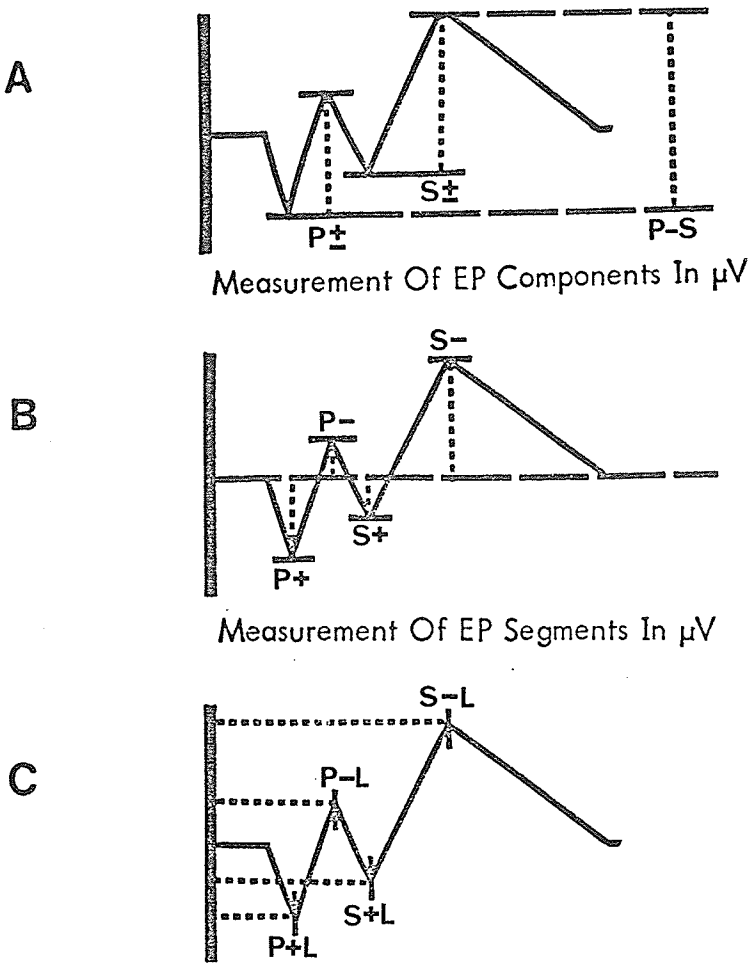

Fig. 2. The measurement of averaged auditory evoked potentials (EP). Negativity is up and positivity down. Primary and secondary portions of EP are P and S respectively.

2. Final data

"Raw" EEG and EP data as such or as percentages of control readings for individual rats, were averaged for a maximum of 26 experiments and the statistical significance of trends of electrocortical changes within the populations were evaluated by the appropriate tests. (Analyses of variance, paired t - tests and unpaired t - tests (for either equal or unequal variances of 2 compared populations)).

H. Evaluation of Behavior

1. Conditioned pressing response

Correct pressing response was expressed as a percentage of total CS presentations (12 - 16) within a session. Response changes in control/drug session combinations for individual rats were evaluated and averaged for all rats.

2. Amphetamine induced behavior

Progressive intoxication by amphetamine (i.e. d-amphetamine) was categorized into 6 stages according to various correlates of behavior as specified in Table 1.

3. Active-dull rat classifications

Classification of rats into active or dull categories was accomplished according to the general concept of Lat (74) who used rat rearings as activity indicators. Modifications were made as follows to render the test more sensitive. On five subsequent days, the behaviors of the trained animals in five new quiet environments (small rat cages of various forms) were observed for 10 minutes. Within a 3 sec accuracy times spent rearing, sniffing, walking and generally orienting (all indicative of active behavior) as well as cleaning, sitting, lying and sleeping (all indicative of inhibition) were recorded. The former were reflections of the more active and peppy "boss" rats whereas the latter revealed the greater inhibition of duller rats. The ratio active/dull behavior was taken as the final index of rat non-specific excitability levels.

Table 1. Stages of d-amphetamine intoxication as defined by behavior in the rat

normal	0	- asleep
	1	- alert, little locomotion, grooming, eating
abnormal	2	- intermittent compulsive sniffing and periods of locomotor activity
	3	- increasingly rapid locomotion, much rearing and active sniffing often directed toward upper half of cage - horizontal displacement maximal and highly stereotyped - eating and drinking absent or decreased but short grooming attempts may be seen
	4	- horizontal displacement minimal or absent - stereotypy consists mostly of compulsive sniffing (often with side to side head movements) in restricted area of bottom half of cage
	5	- licking or gnawing wires in one area of cage floor
	6	- seizures, coma, death

III. RESULTS

A. Electrocortical Readings

1. Control responses of normal rats to positive and negative reinforcement environments

The amplitudes of all frequency bands, including the integrated EEG for the spectrum, are significantly different in Rf^+ and Rf^-. All, with the exception of B_2, were lowered in Rf^- (Fig. 3).

All EP measurements in both situations were also significantly different... ..those of the shock situations, excluding S^-, being of a greater amplitude. These results are depicted in Fig. 4 and 5 for actual averaged amplitude measurements or composite EP respectively.

Conditioned behavior during these recordings was of course at or above criterion.

2. Control responses of 6-OH-DA treated rats to positive and negative reinforcement environments

i) Earlier reactions

Immediately after the injection of 6-OH-DA, rats became increasingly sedated and remained very lethargic for a minimum of some 24 hours. Conditioned behavior was abolished during that period. Rectal temperature dropped maximally at about 4 hours approaching ambient temperature and recovered fully within 24 hours. Electrocortical signs of sleep such as EEG synchronization and spindling were common. Tremors, seizures and hyperactivity to physical stimuli were observed in the following days along with partial or complete recovery of conditioned behaviors. As may be noted in Fig. 6 concomitant with NE accumulation one hour after Nialamide (MAO inhibition) a slight EP synchronization in Rf^+ occurred. Accompanied by a possibly larger accumulation of catecholamines at receptor sites subsequent to the injection of 6-OH-DA, EP and behavior were

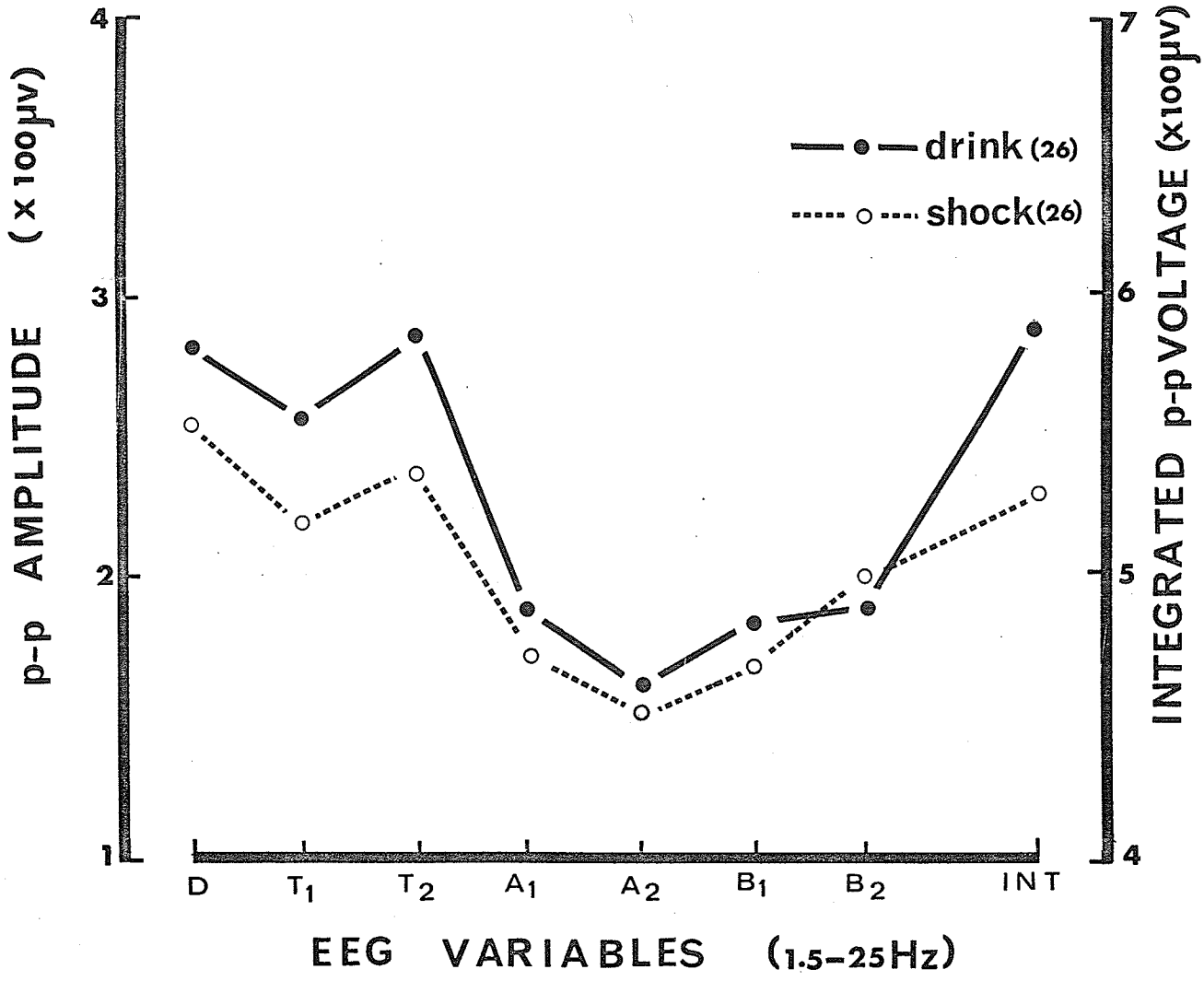

Fig. 3. Integrated EEG and amplitudes of various frequency bands in positive (Rf^+) and negative (Rf^-) reinforcement situations for normal rats. Bracketed figures in this and succeeding graphs represent the number of experiments and/or rats. Abbreviations used hold for following graphs. Difference of two treatments: $p \lesssim 0.005$, unpaired t-test, two tailed.

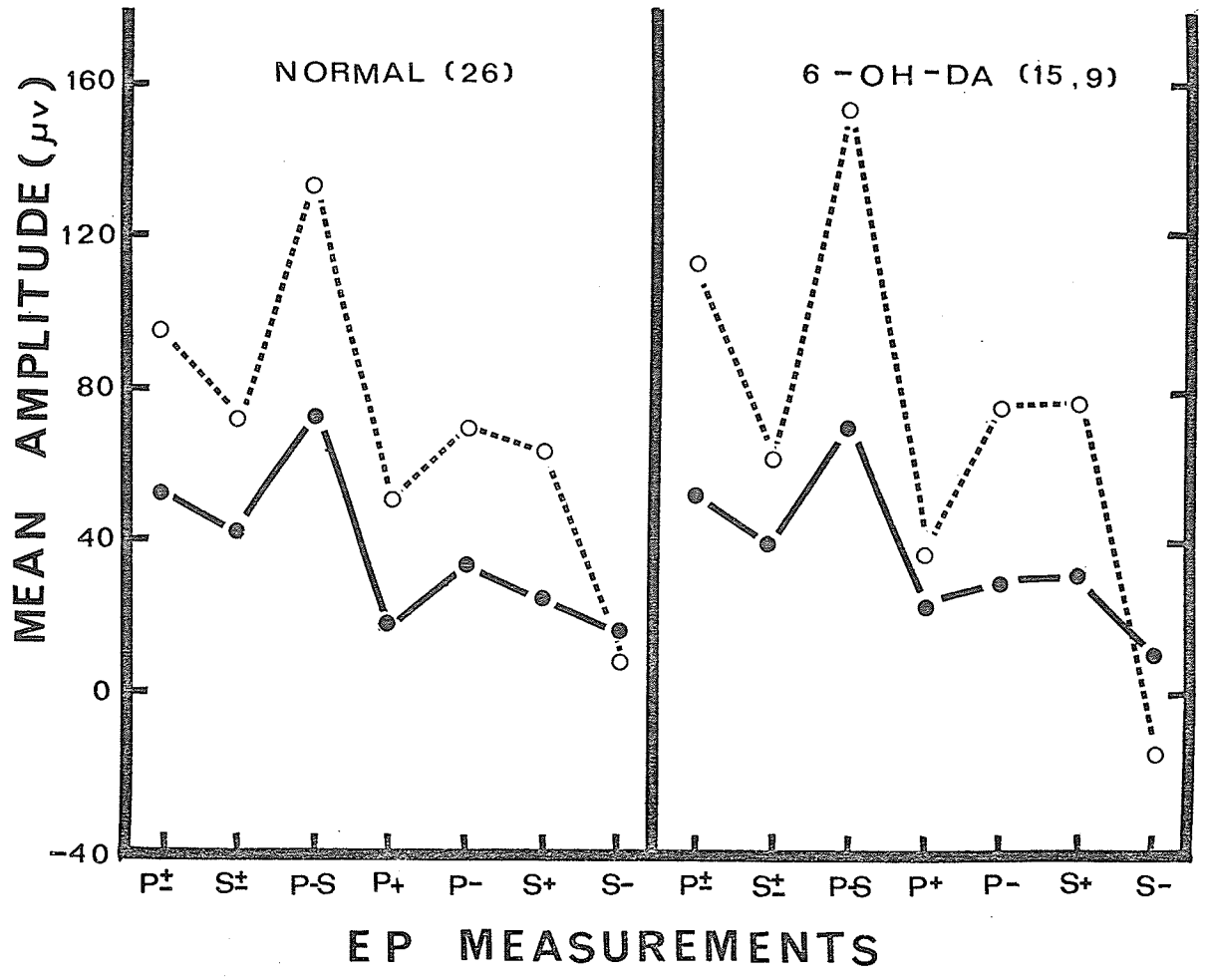

Fig. 4. Mean EP amplitude measurements in Rf^+ and Rf^- for normal and 6-OH-DA treated rats (6HD-rats). Legend as in Fig. 3. Readings ($9Rf^+$, 15 Rf^-) were taken 9-30 days following 6-OH-DA injections (not supplemented with nialamide). Latency changes showed no obvious trends. Difference between Rf^+ and Rf^- within either sample: $p < 0.05$, t-test, one tailed.

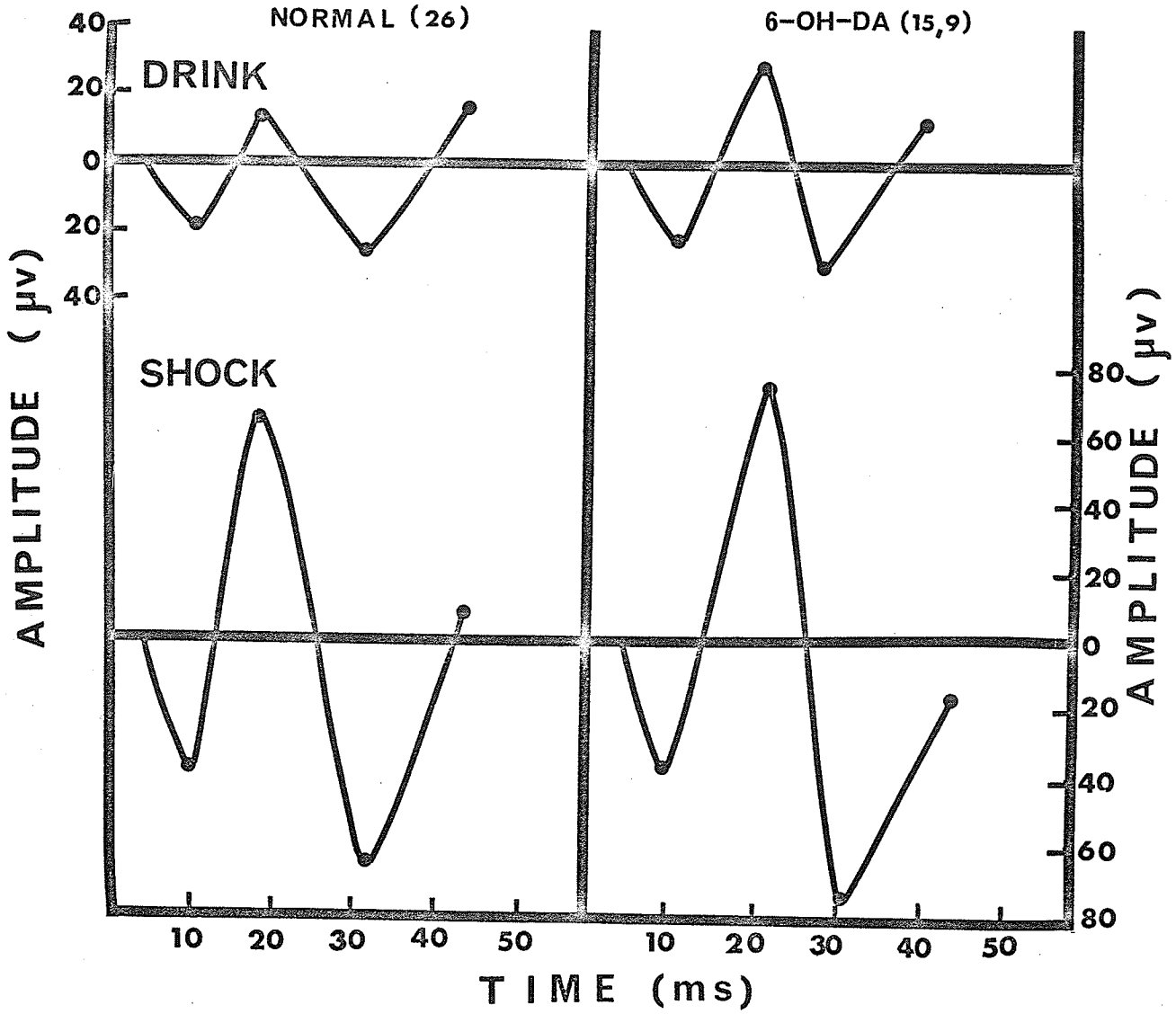

Fig. 5. Composite EP obtained in Rf^+ and Rf^- for normal and 6 HD-rats. Data taken from Fig. 4. Composite EP are means of a minimum of 5500 evoked potentials for normal and 3800 for 6 HD-rats. Differences in Rf^+ and Rf^- for normals: $p < 0.01$, ($p < 0.05$ for S^-). Similarly, $p < 0.05$ for 6 HD-rats for S^- in Rf^-: $p < 0.05$. All tests are one tailed t-tests.

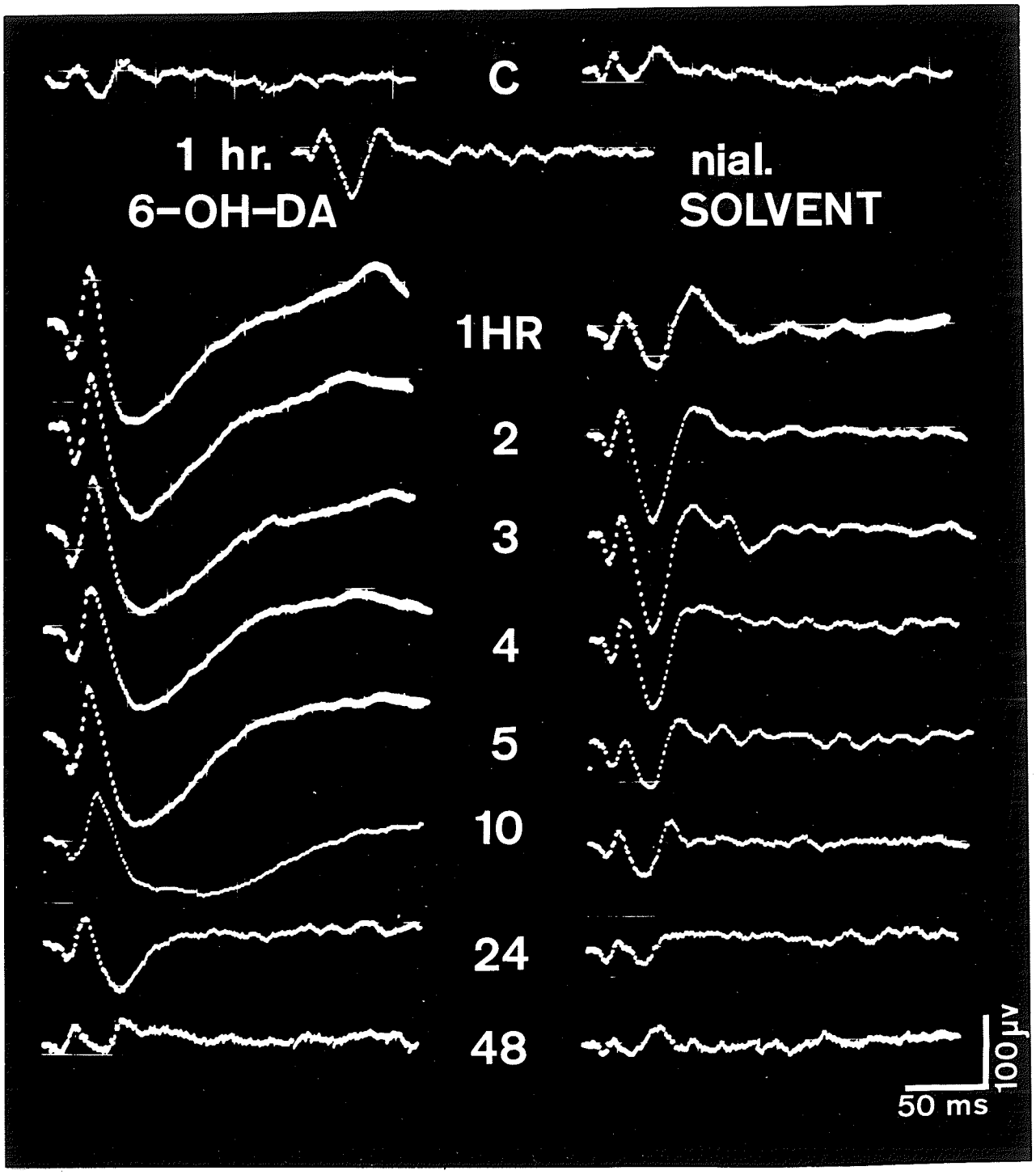

Fig. 6. EP changes in Rf^{+} for one rat injected with nialamide (100 mg/kg) and either solvent or 6-OH-DA (200 μg, intracisternally). Each tracing is the average of 100 click-evoked potentials. Readings were taken prior to injections (C) and one hour after nialamide. Further recordings were taken at various intervals subsequent to intracisternal injections which immediately followed.

very much more sleep-like. Previously, in a sham operation performed on the same rat, EP synchronization and behavioral depression had been comparatively slight (Fig. 6).

ii) Later reactions

Nine to thirty days following the injection of 6-OH-DA (without Nialamide), the EEG responses of trained rats in Rf^+ and Rf^- were apparently back to normal, with the exception of a significant fall in the B_2 ratio Rf^-/Rf^+ (Table 2).

EP measurements and composite pictures (Fig. 4, 5) in 6-OH-DA pretreated rats (hereinafter referred to as 6 HD-rats) were as different in Rf^+ and Rf^- as were those of normal rats. One obvious difference between normal and 6 HD-rats was the latter's significantly more positive orientation of the secondary negativity (S^-) in Rf^-.

3. Reactions of normal and 6-OH-DA treated rats to saline and d-amphetamine (2 mg/kg)

D-amphetamine induced a significant lowering of the EEG amplitudes of normal rats in Rf^+ (Fig. 7) but a predominant increasing of amplitudes in Rf^- (Fig. 8).

For 6 HD-rats a general fall in EEG amplitudes was observed in Rf^- (Fig. 8) with the exceptions of an increase in T_2 which is probably an indication of greater arousal and hippocampal firing. In the latter environment, interestingly enough, amphetamine does not seem to induce significant EEG changes in the 6 HD-rats (Fig. 8), as it probably could not release as much NE (synchronizing factor) to postsynaptic receptor sites.

If, in lieu of comparing the mean of drug induced changes for the individual rats, in order to obtain the mean change for a sample (Fig. 7, 8), changes of sample means, as formulated, are compared instead, the results obtained from rats in which absolute changes are largest and most obvious become biased through

Table 2. The maintanence of consistant electrocortical relationships in positive and negative environments after 6-OH-DA.

EEG Variable	Value $((Rf^-/Rf^+) \times 100)$ for Normal rats (26)	6 HD-rats (9)
D	90.0 ± 4	83.5 ± 6
T_1	85.5 ± 4	103.3 ± 12
T_2	82.0 ± 3	83.7 ± 6
A_1	91.5 ± 3	99.5 ± 4
A_2	97.2 ± 4	97.2 ± 3
B_1	92.9 ± 2	93.3 ± 2
B_2	106.0 ± 2	100.4 ± 2
Integrated	89.9 ± 3	93.5 ± 6

Each value is a Rf^- variable expressed as a percentage of the Rf^+ variable for individual rats and averaged for the sample.

B_2 is significantly higher in normal rats: $p < 0.05$, one tailed t-test. All other differences N.S.

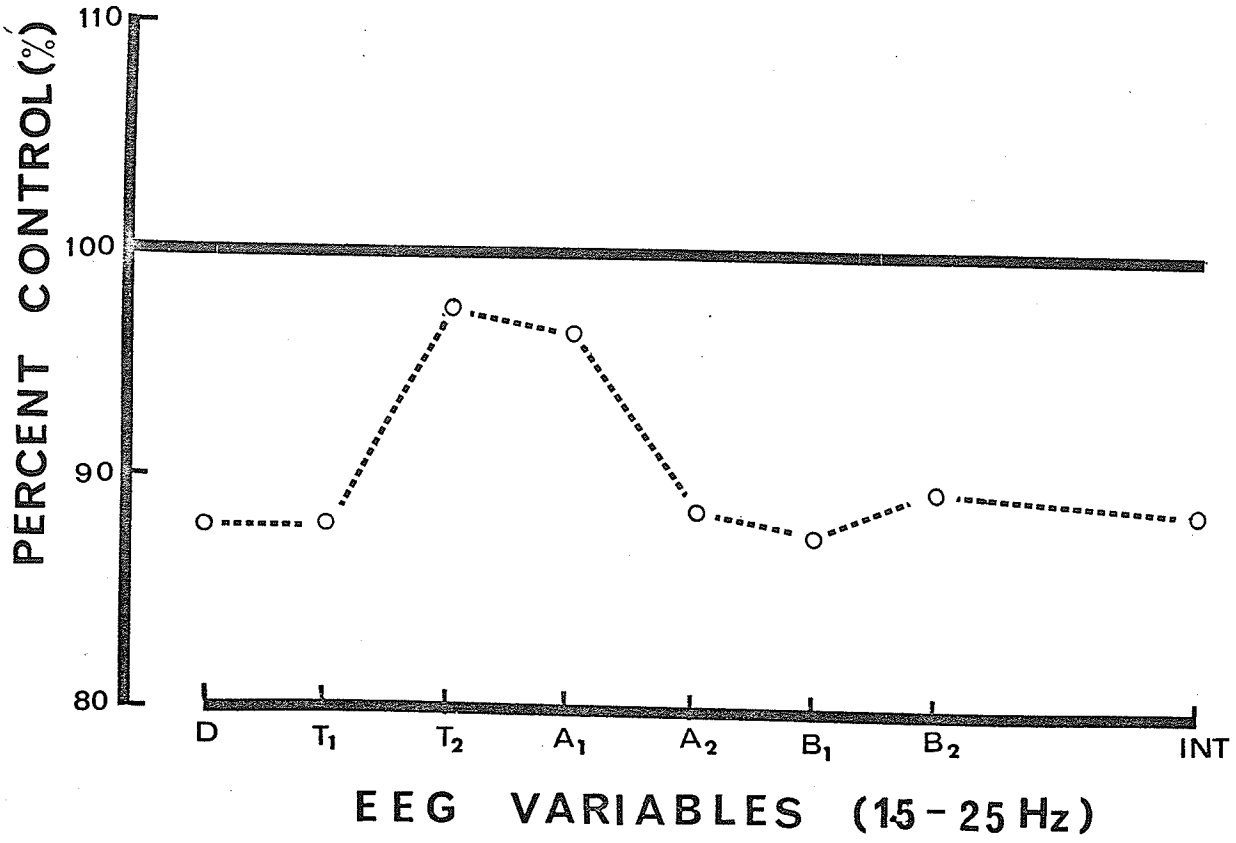

Fig. 7. EEG changes in Rf^{+} induced by d-amphetamine in normal rats. D-amphetamine (2mg/kg, i.p.) was injected into 9 normal rats. Formula used in calculations was $(X_{1A}/X_{1C} \cdots\cdots X_{nA}/X_{nC})/n$ where C, A and n are control, drug and total number of readings.

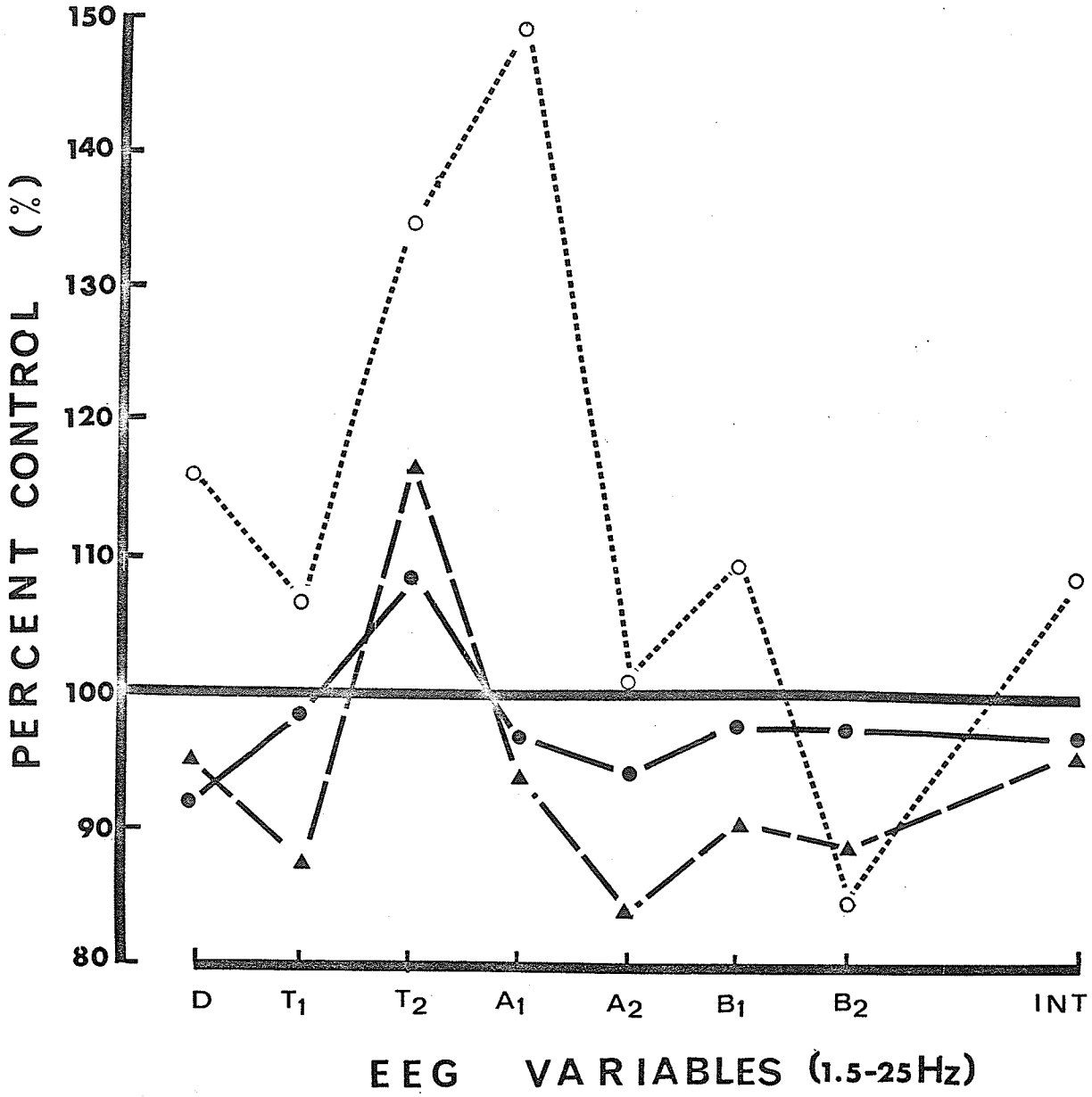

Fig. 8. EEG changes in Rf^{-} induced by saline and d-amphetamine in 6 HD-rats and by d-amphetamine in normal rats. Saline was injected into 4 6 HD-rats at 50 days (——●——). D-amphetamine (2mg/kg) was injected into 9 normal rats (·····○·····) and into 6 6 HD-rats at 17 days (— — ▲ — —). Differences relative to saline: amphetamine in 6 HD-rats N.S., except A_2 where $p < 0.05$, one tailed t-test; amphetamine in normal rats all significant, except T_2 ($p < 0.05$, one tailed t-test).

mathematical manipulation (Fig.9,10). For normal rats a large flattening of EEG in Rf^+ (Fig. 9) as well as an increase in Rf^- (Fig. 10) is again seen; however, an apparent reversal of normal effects in the latter condition is detected for 6 HD-rats, perhaps unveiling in reference to the electrocorticogram, non-catecholaminergic activating properties of amphetamine.

Although not graphically exemplified here, in Rf^+, from general observations in our laboratory, saline produces only inconsistent EP changes in normal and 6 HD-rats (Fig. 11). As readily detectable in Fig. 12 corresponding changes in the three major EP components were not altered significantly by amphetamine except for $S^{\pm}$ which would not be expected to differ from saline controls.

In Rf^- (Fig. 13), again saline produced no real significant changes in 6-OH-DA treated animals whereas amphetamine had the property of very significantly decreasing all measurements, with the exception of S^- which was increased. Basically, similar but less significant results were obtained with normal rats. If the three major EP components are considered (Fig. 14), saline pretreatment does not differ significantly from amphetamine pretreatment in 6 HD-rats; however, in comparison to the former these same measurements are significantly lower. The differences observed in the above three treatments should be expected if the proposed synchronizing or deactivating factor (e.g. NE) is absent or decreased in 6 HD-rats.

EP latencies on the average were not dramatically altered in any of the above cases (Table 3).

4. Summary of Electrocortical Data

Figure 15, although not a perfect representation of the data averaged for the total population, depicts the EP and EEG tracings obtained for one normal rat. While EEG amplitudes are smaller in control Rf^- than control Rf^+, EP are larger. In the former, the slow waves which predominate the EEG record are believed to be artifacts and were not evaluated by fast Fourier analysis.

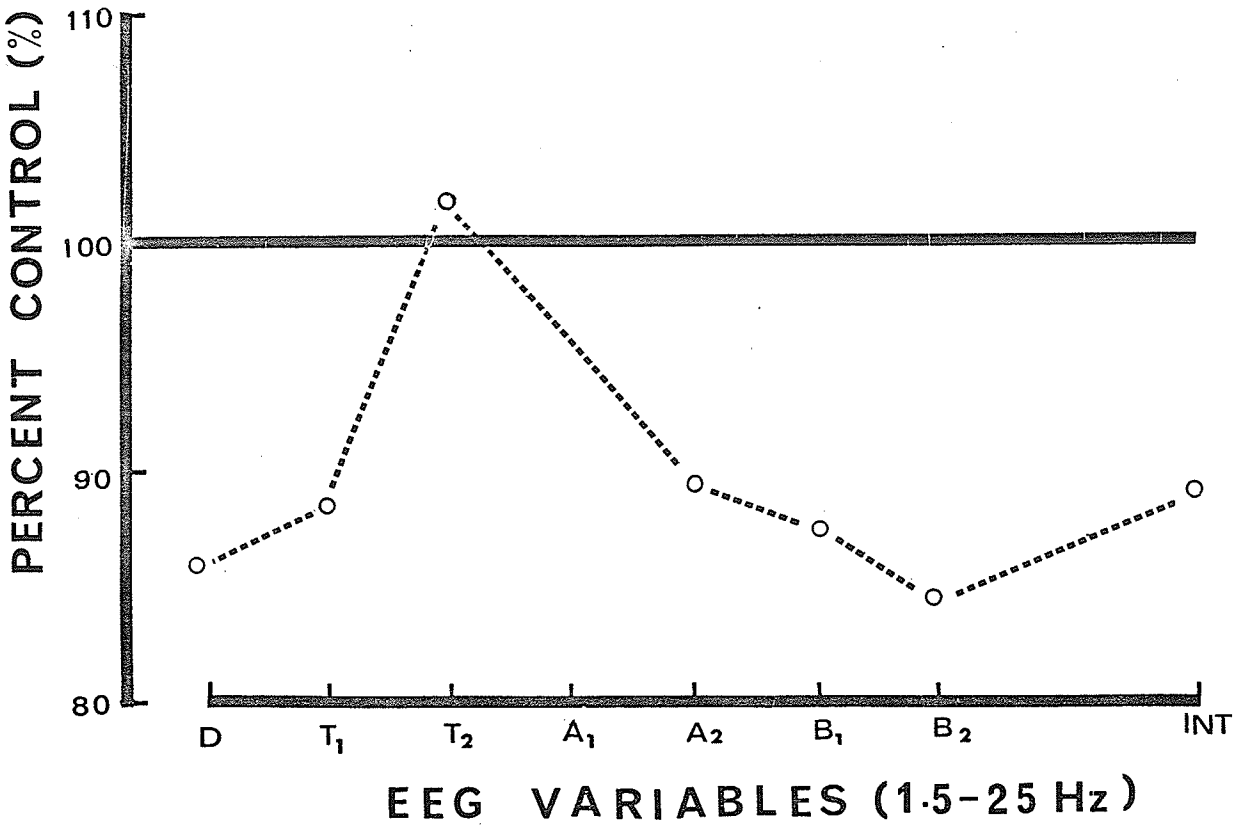

Fig. 9. EEG changes induced by d-amphetamine in normal rats. Data and all other conditions identical to those of Fig. 7, except that the following formula was used in computations (X_{nA}/X_{nC}).

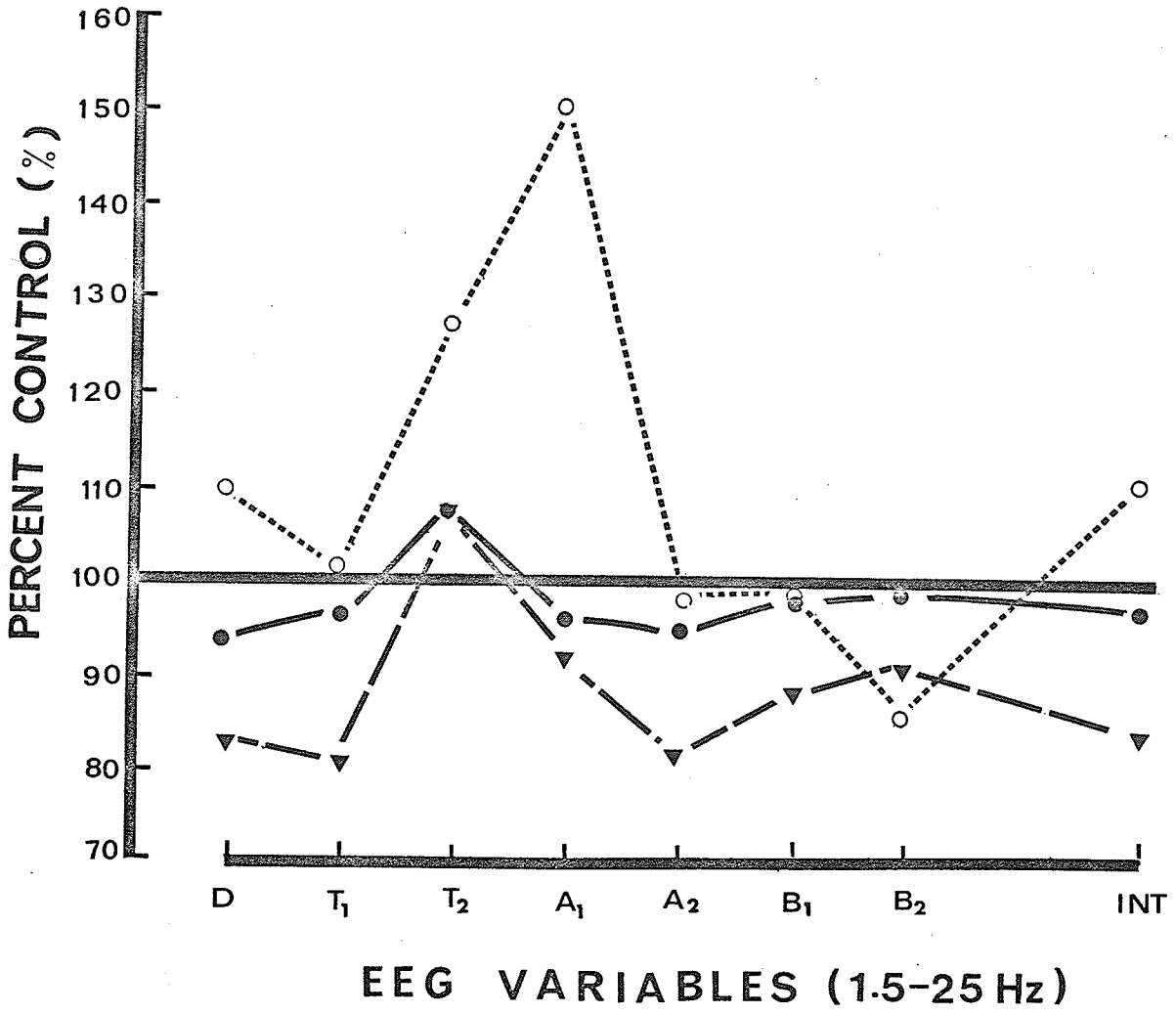

Fig. 10. EEG changes in Rf^{-} induced by saline and d-amphetamine in 6 HD-rats and by d-amphetamine in normal rats. Legend, data and all other conditions identical to those of Fig. 8. Calculation of variables as in Fig. 9.

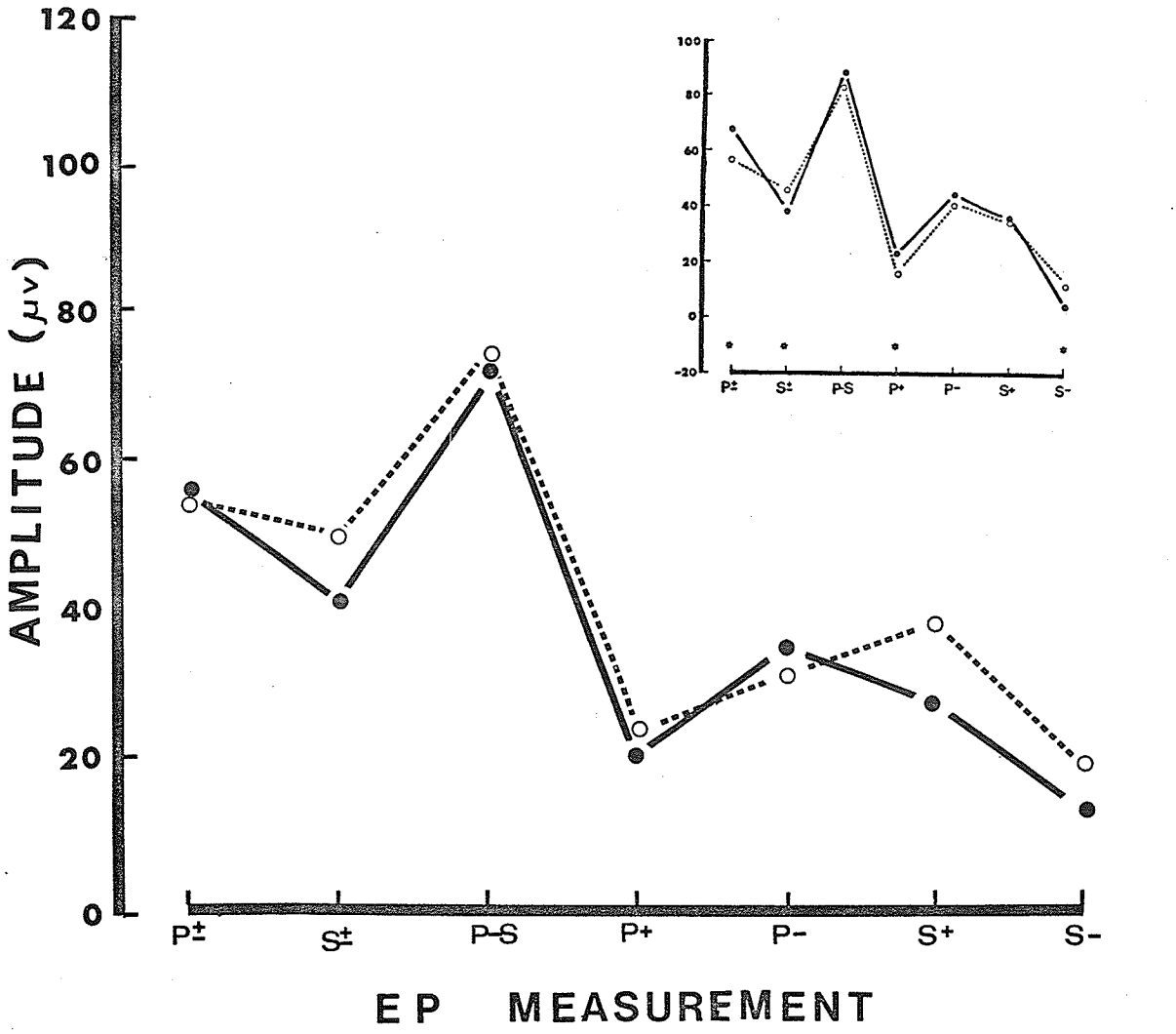

Fig. 11. EP changes in Rf^{+} induced by saline in 6 HD-rats and by d-amphetamine in normal rats. Solid lines are pre-drug and dashed lines post-drug. Bottom curves are reactions of normal rats to d-amphetamine (2 mg/kg) while insert are those of 6 HD-rats to saline. Difference of curve pairs: N.S., except * $p < 0.05$, one tailed t-test. Experiments, animals and all other conditions correspond to those of Fig. 9.

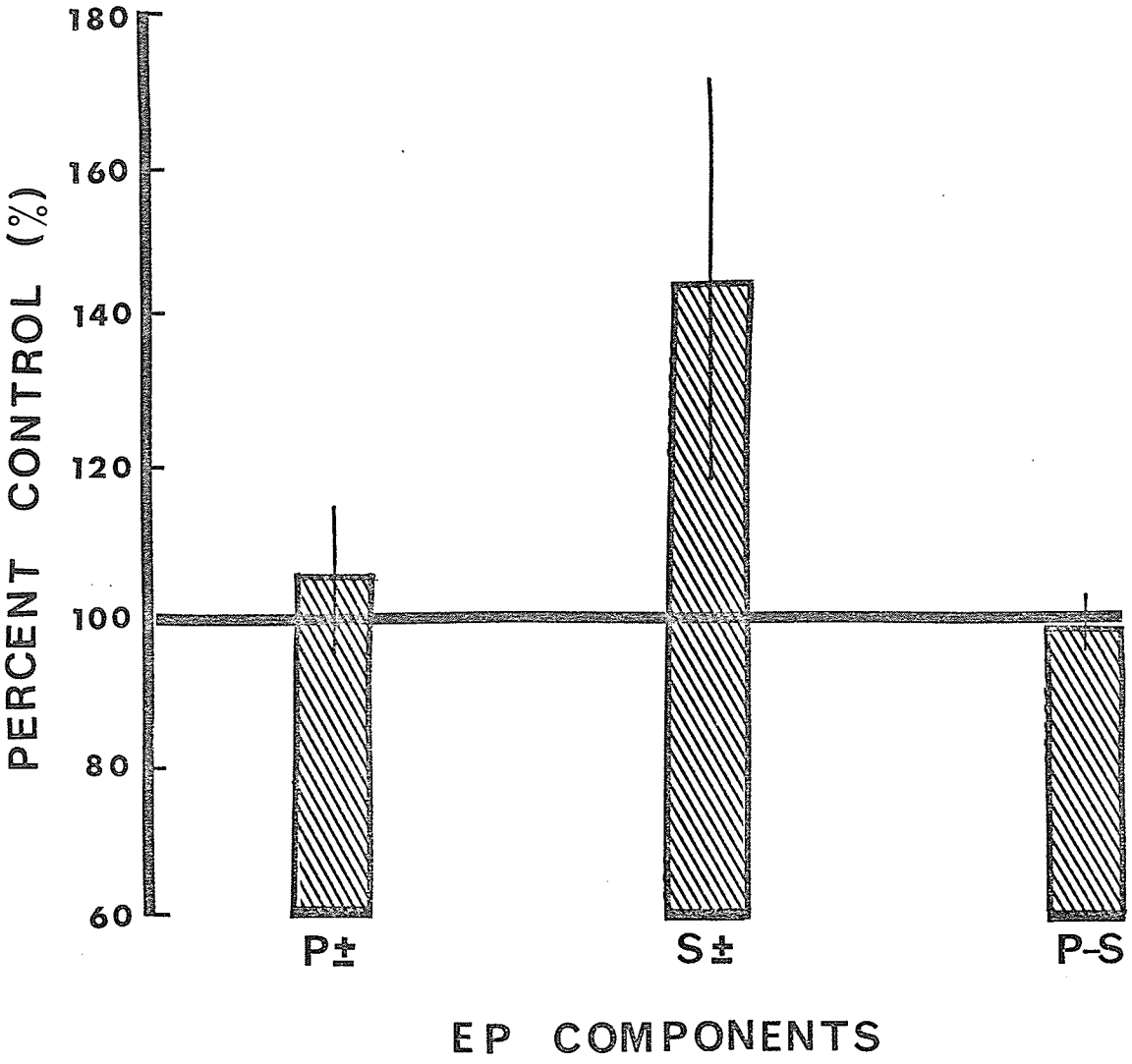

Fig. 12. EP component changes in Rf^{+} induced by d-amphetamine in normal rats. Experiments as in Fig. 7. Percent control values were calculated for individual rats and averaged. Values represent mean ± SE.

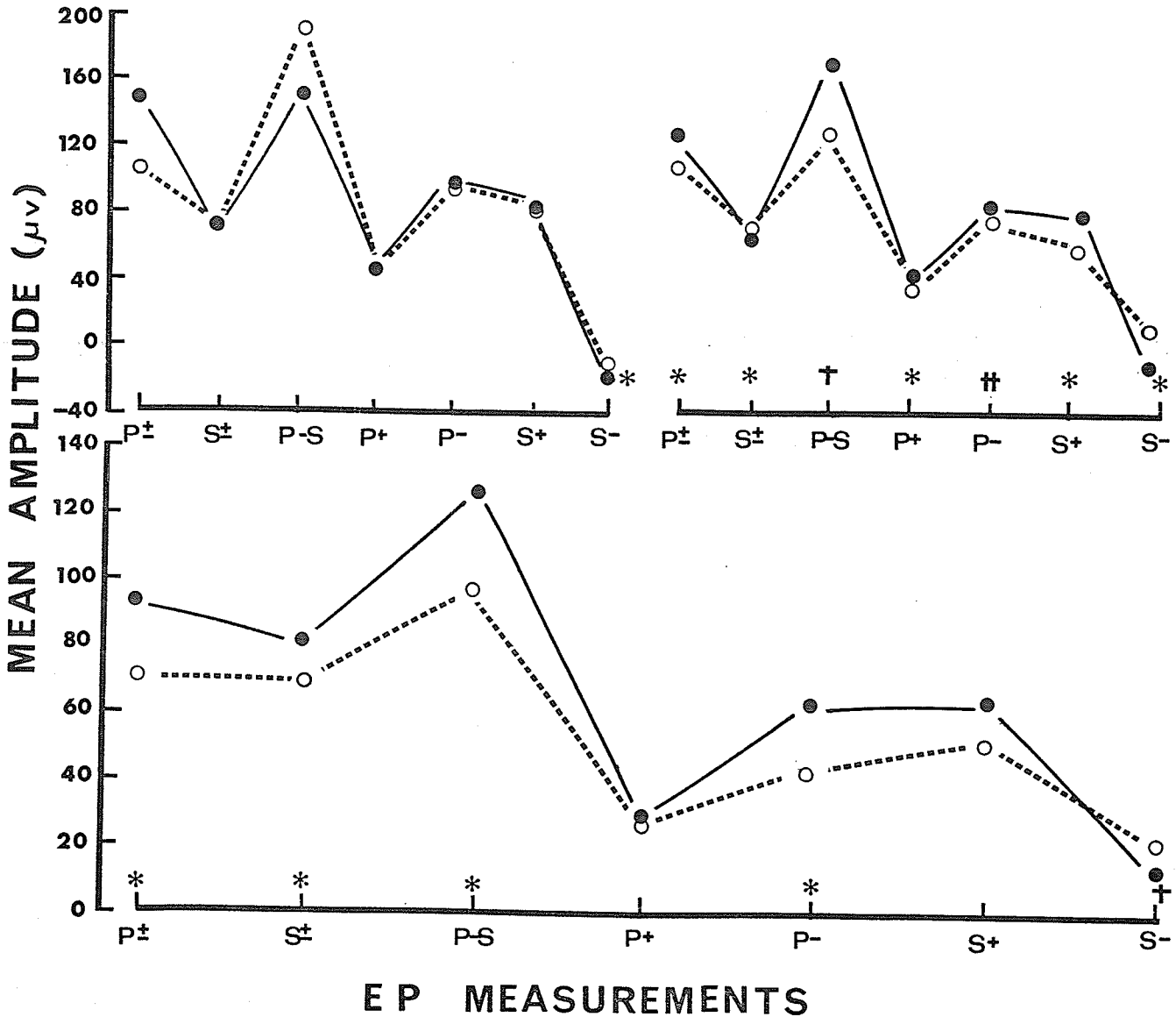

Fig. 13. EP measurement changes in Rf^{-} induced by saline and d-amphetamine in 6 HD-rats and by d-amphetamine in normal rats. Experiments, rats and all other conditions same as in Fig. 10. Solid and broken lines are pre- and post-drug readings, respectively. Top left: effect of saline on 6 HD-rats. Top right: effects of d-amphetamine on 6 HD-rats. Bottom: effect of amphetamine on normal rats. Differences relative to control: * $p < 0.05$, † $p < 0.06$, ‡ $p < 0.08$, one tailed t-tests.

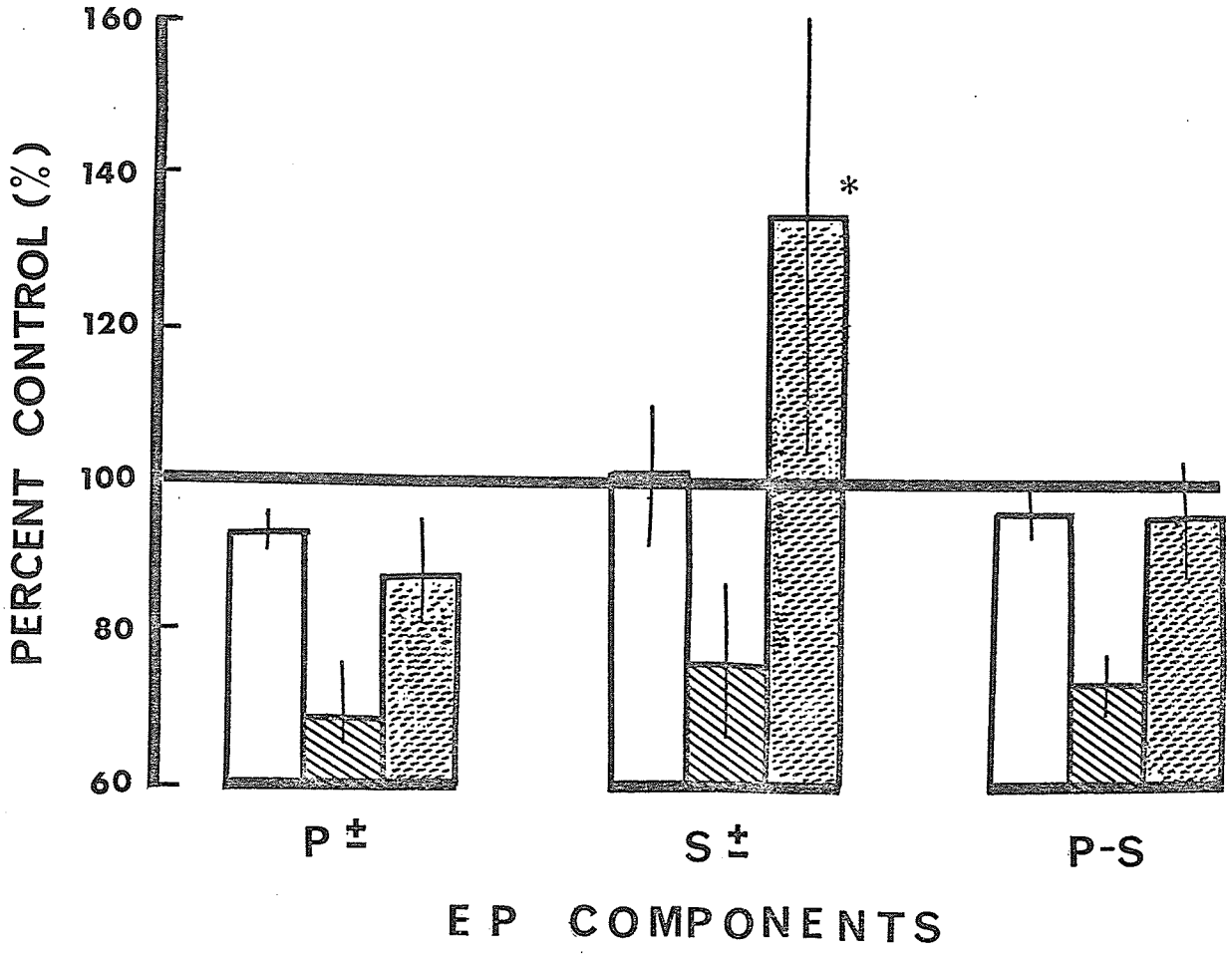

Fig. 14. EP component changes in Rf^{-} induced by saline and d-amphetamine in 6 HD-rats and by d-amphetamine in normal rats. Data is identical to that of Fig. 13. Open columns: saline treated 6 HD-rats. Hatched columns: amphetamine treated normal rats. Stipled columns: amphetamine treated 6 HD-rats. All variables in the amphetamine injection of normal rats are significantly lower than other treatments ($p < 0.05$ one tailed t-test). * value in one rat increased by 265%. Values are means ± SE.

Table 3. Mean latencies of EP in Rf^+ and Rf^- for saline and/or amphetamine treatments of normal and 6-OH-DA pretreated rats.

Injection	Measurement	Drinking		Shock	
		Control	Drug	Control	Drug
Saline (6-OH-DA)	P+L	10.1	10.1	10.1	10.1
	P-L	17.8	16.3	16.7	17.0
	S+L	29.8	28.4	31.6	31.4
	S-L	41.9	42.9*	45.0	43.3
Amphetamine (6-OH-DA)	P+L	-	-	9.34	9.6
	P-L	-	-	17.0	17.2
	S+L	-	-	31.1	29.9*
	S-L	-	-	43.4	43.3
Amphetamine (normal)	P+L	10.67	10.6	10.57	10.65
	P-L	18.7	19.2	18.60	19.17
	S+L	32.2	29.4	32.23	29.44
	S-L	40.96	41.02	40.95	41.04

* $P < 0.05$, paired t-test, one tailed.
Brackets specify if rat was pretreated with 6-OH-DA or not.

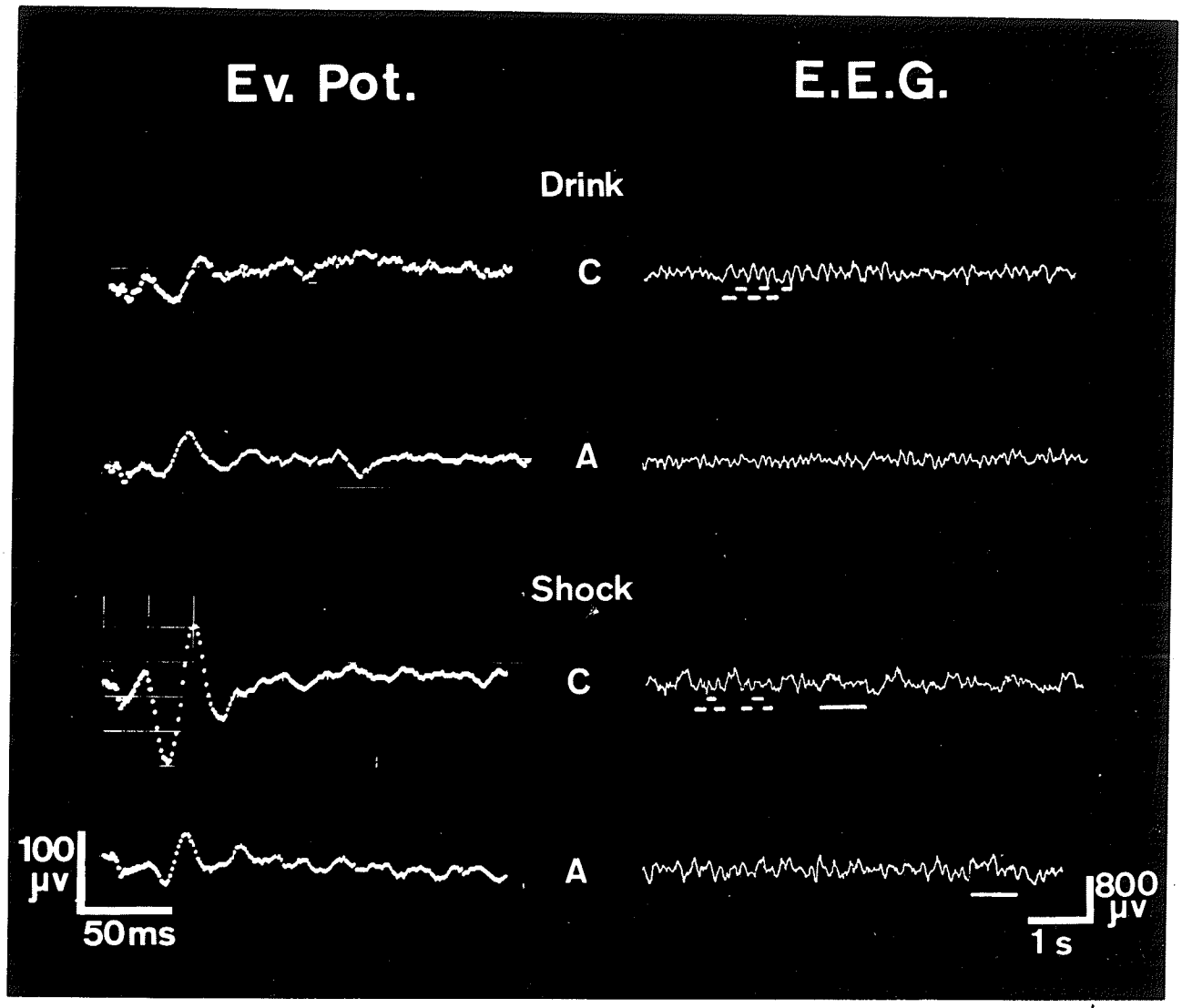

Fig. 15. The effects of amphetamine on EP and EEG tracings of a normal rat in Rf^+ or Rf^-. Pre-drug control sessions and post-amphetamine (2 mg/kg) sessions are identified as C and A respectively. Underscoring of EEG waves permits their comparison.

After amphetamine injection (2 mg/kg) EEG was flattened in Rf^+ whereas in Rf^- frequency bands were predominantly increased in amplitude. Significant EP changes consist of a flattening in Rf^- only.

On the other hand 6 HD-rats, although their electrocortical behavior was similar in many ways to that of normal rats, did not exhibit in Rf^- the amphetamine induced rise in EEG amplitudes, which tends to suggest the lack of an amplitude increasing factor at the cortex or elsewhere.

B. Behavioral Observations

1. Reactions of normal and 6-OH-DA treated rats to saline and d-amphetamine (0.5, 2.0 mg/kg)

Conditioned pressing behavior (Fig. 16) in normal rats (as well as those treated with 6-OH-DA without nialamide) was, if anything, increased in a repeat performance following saline injection.

A low dose of amphetamine (0.5 mg/kg) tended to slightly decrease pressing success in normal rats, whereas a higher dose (2.0 mg/kg) depressed averaged correct performance much more extensively. The degree of performance inhibition was potentiated at 6 and 7 days after 6-OH-DA/nialamide pretreatment by both doses of amphetamine. In sham operations (not graphed) such potentiations were not observed. At 15 days after a solitary injection of 6-OH-DA, the higher dose of amphetamine still seemed to completely depress conditioned behavior, whereas at 50 days, in the same animals, same indication of possible CNS recovery from 6-OH-DA induced alteration of catecholaminergic systems, was seen in the normalized response to the lower dose of amphetamine.

Such inhibitions of behavior were always paralleled with similar distortions in Rf^-, although these were less quantifiable.

2. Stages of amphetamine intoxication reached before and after 6-OH-DA treatment

The extent of intoxication induced by amphetamine in the above mentioned conditioned behavior experiments parallels changes induced in home cage behavioral manifestations as previously described and defined by stages (Fig. 17).

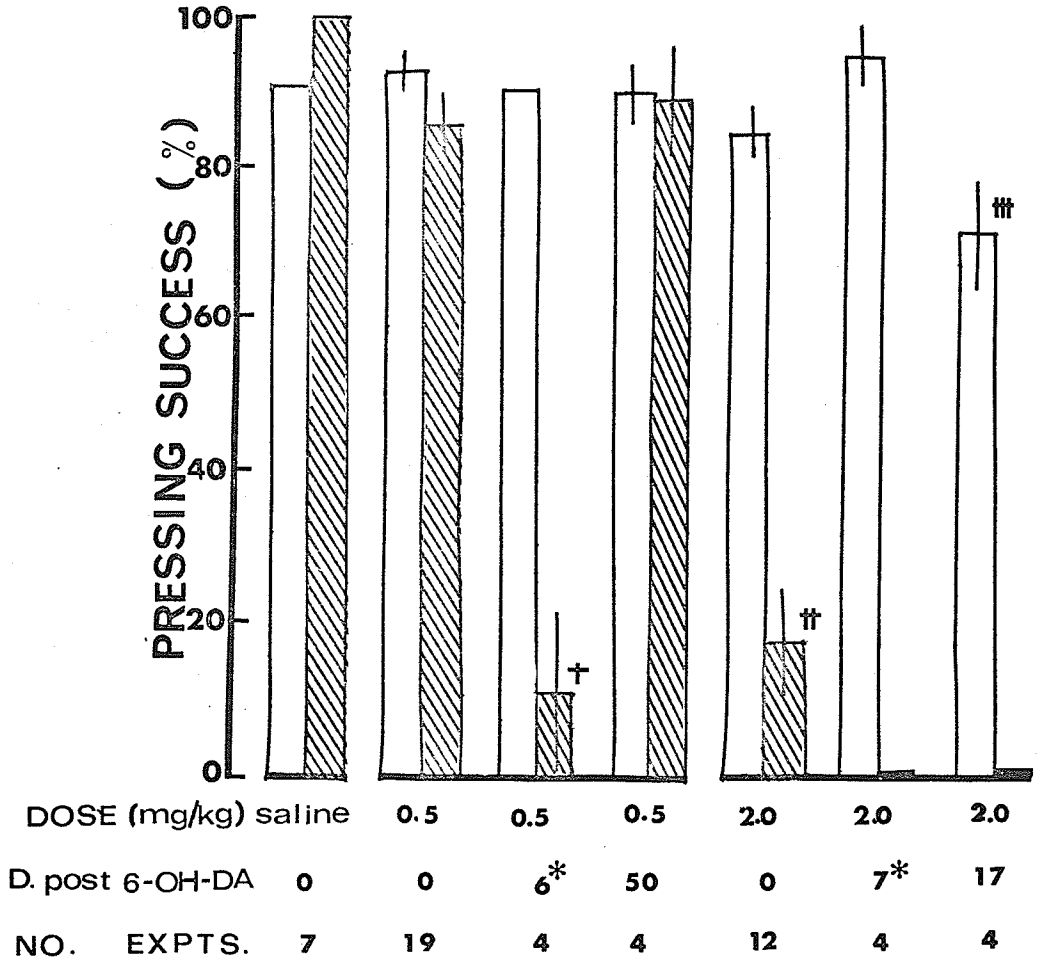

Fig. 16. Amphetamine induced conditioned pressing response (Rf^{+}) changes in normal and 6 HD-rats. Open column: before 6-OH-DA. Hatched column: after 6-OH-DA. * 6-OH-DA (200 µg) supplemented with nialamide 100 mg/kg †: one rat pressed. ††:3 of 12 rats pressed. †††: one rat pressed poorly. Values represent mean ± SE.

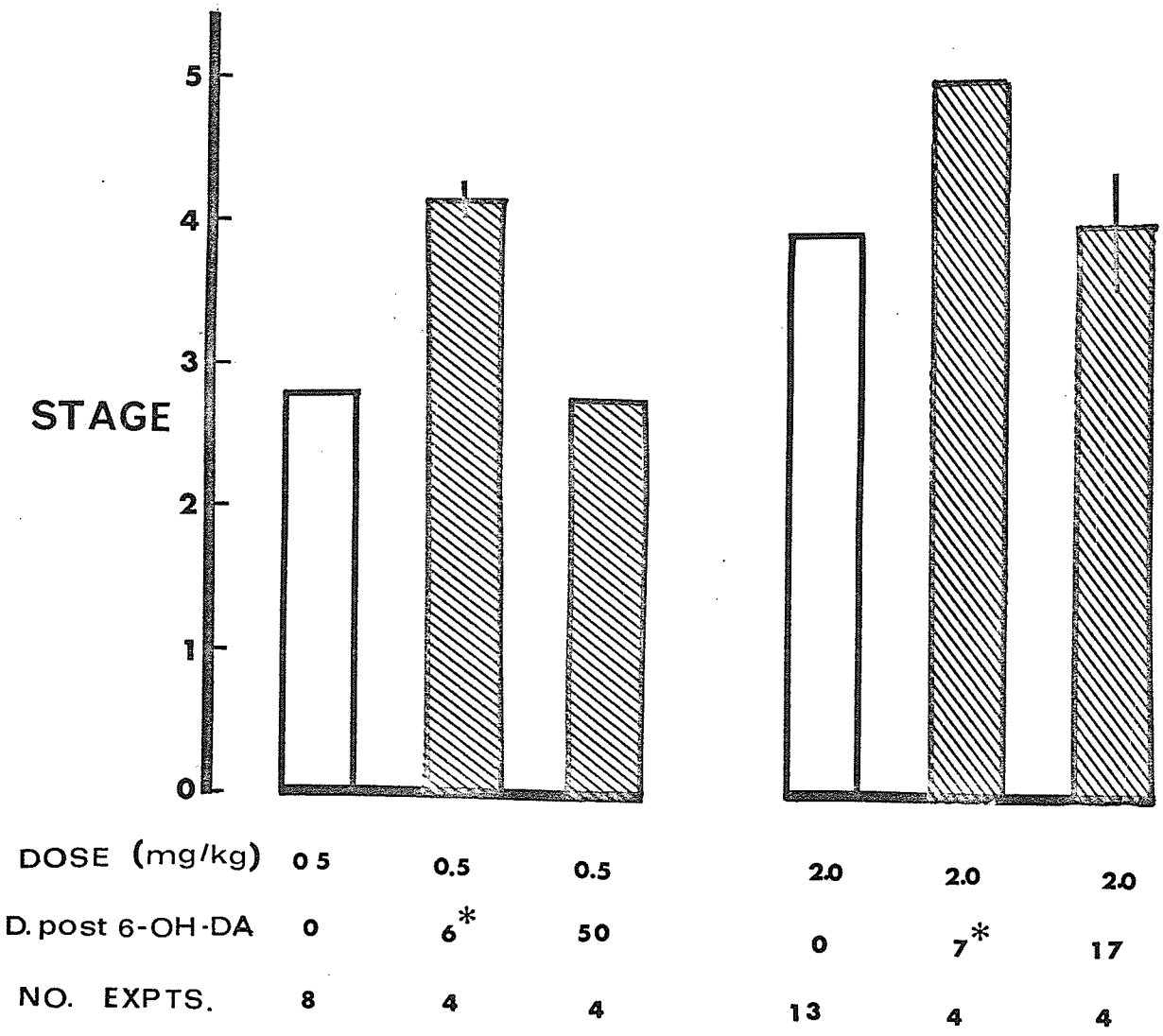

Fig. 17. Stages of amphetamine intoxication reached before and after 6-OH-DA treatment. Open column: before 6-OH-DA. Hatched column: after 6-OH-DA. * : 6-OH-DA supplemented with nialamide. Values represent mean ± SE.

In normal rats, increasing the dose of amphetamine brings the animal to a higher stage. Massive irreversible destruction of catecholaminergic pathways on the other hand, permits the same doses of amphetamine to exact greater effects, whereas such demonstrations of amphetamine toxicity are back to control levels even for the higher dose of amphetamine at 17 days.

3. Amphetamine effects in active and dull rats

In seven select rats, categorized as either inhibited or uninhibited, amphetamine (2 mg/kg) induced behavior was not obviously different (Fig. 18).

Although control pressing responses in both dull and active categories were similar, this behavior was completely inhibited in the former and only partially altered in the latter.

4. Untoward effects of ∝-methyl-p-tyrosine (∝MpT)

In the course of what must still be considered pilot experiments on a total of 12 rats either injected 2 - 6 months beforehand with 6-OH-DA or its solvent, interesting observations on the effects of 2 doses of ∝MpT (150, 250 mg/kg, salt) were made.

6-HD-rats on the average seemed to reach a later stage of intoxication than did normal rats following amphetamine injections (2 - 5 mg/kg) at 2 or 3 hours after the above doses of ∝MpT. Eating and orienting in a new environment was here noted in two 6 HD-rats (with and without ∝MpT) but not in two normal rats (with ∝MpT). Catalepsy was indueed in 2 rats not injected with amphetamine and tremendously potentiated by 5 - 14 mg/kg doses of d-amphetamine in 4 other animals. Although this was not the case for all rats injected with 150 mg/kg ∝MpT, catalepsy resulted in all rats given 250 mg/kg ∝MpT plus amphetamine. Temperature falls were observed soon after ∝ MpT or 48 hours after ∝MpT/amphetamine (maximum, 4C°). Amphetamine potentiation of catalepsy seemed to occur with greatest ease (a greater effect at lower doses in 4 rats) in 6-OH-DA treated animals. Hungry and thirsty animals would not eat or drink 2 hours subsequent to an injection of ∝MpT, nor did amphetamine induce urination (a usual peripheral effect) in any of the animals, nor would diuresis follow water loading (10 ml saline i.p.), in 6 animals tested.

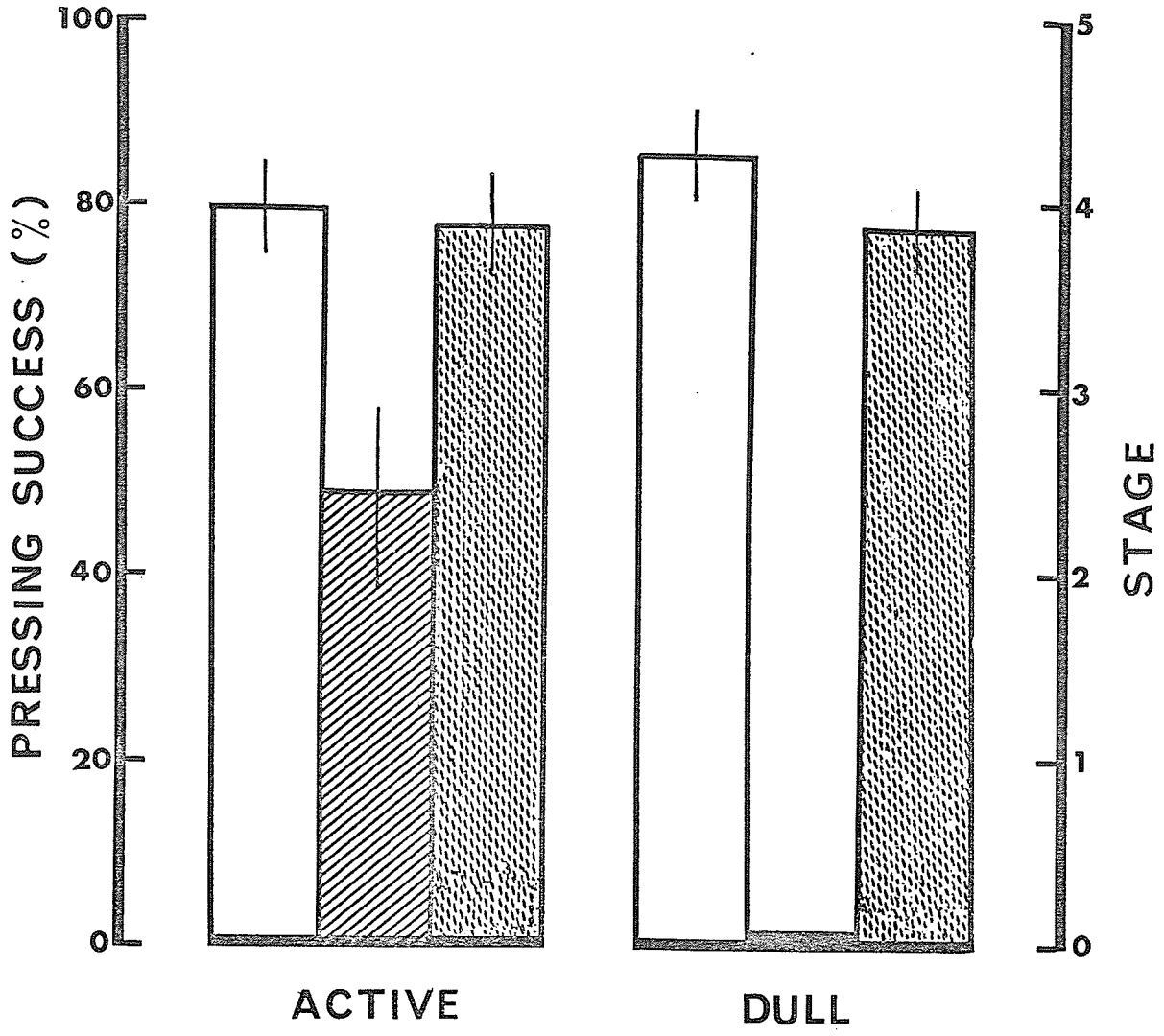

Fig. 18. Effect of amphetamine on the spontaneous and conditioned behavior (Rf^{+}) of active and dull rats. Open column: control pressing. Hatched column: pressing after amphetamine. Stippled column: stages. Differences for the two groups (5 active and 3 dull rats) are seen for pressing behavior under amphetamine (2 mg/kg) only. ($p < 0.05$, one tailed t-test).

None of the animals survived beyond 48 hours with the exception of two rats which were water loaded subsequent to ∝MpT/amphetamine experiments (over 15 ml saline i.p. and a diuretic).

IV. DISCUSSION

A. Electrocortical Recordings and Behavior

1. Control responses of normal rats to positive and negative reinforcement environments

That the integrated EEG and amplitudes (and consequently the power) at all frequency bands examined were lower in Rf^- than Rf^+, with the exception of B_2, is interesting in relation to the EP amplitudes which basically subserved reciprocal relationship to the former in both environments. Such a relationship (75), as well as discrepancies between EEG and EP (76), have been noted by others. As will be discussed in later sections , the probability of multiple systems controlling EEG amplitudes and frequencies, as well as EP amplitudes, renders difficult the comparison of these two "cortical" phenomena; therefore, it may be stated not that EP and EEG have different origins but rather that the mechanisms controlling these two amplitudes differ.

2. Control responses of 6-OH-DA treated rats to positive and negative reinforcement environments

Rats whose central catecholaminergic pathways were probably only partially destroyed with 6-OH-DA (NE concentrations in cortex, cerebellum, lower brainstem and hypothalamus/upper brainstem were 73%, 0%, 78% and 58% of control levels respectively, while striatal DA was 66%) displayed EEG and EP which were very close to normal. This again stresses the fact that mediation of EEG and EP changes are highly complex processes which are modulated also by other than catecholamine neurotransmitters. This is very much the case considering the extensively documented electrocortical changes induced by 5HT, ACh and their assorted mimetics and antagonists. Of major importance is also the probability that subsequent to the large fall in catacholamine inhibitory processes, a central homeostatic balance is preserved by an equilibration of antagonistic systems.

This latter concept appears most enticing when considering the fact, as also reported by others following 6-OH-DA (77, 78, 79, 80) or reserpine, (81, 82, 83, 84) that NE and DA releases are accompanied by sedation, which is followed

by a period of hyperirritability and excitability and an eventual return to various behavioral parameters of normalcy. The early period of sedation also correlates well with electrocortical signs of inhibition reported herein and elsewhere (38, 79, 85). Also, the period of irritability, as observed in the present experiments and in some of those already cited (among others 86) correlates well with reports of increased desynchronized sleep (87) indicating a greater need for rest, best obtained in deeper sleep. Jouvet's hypothesis that NE triggers and maintains desynchronized, low voltage, fastwave sleep (88, 89), is not supported by the former observations, again indicating the probability of multiplicity in the controls of sleep mechanisms.

That a multitude of cholinergic influences reproduce so many of the behavioral and electrocortical observations made during such periods of hyper-excitabiltiy (79) (among others, arousal, 41, 90, 91, tremors and seizures, 41, 92, 93, 94) and that furthermore ACh biosynthesis in the early days only subsequent to 6-OH-DA injection be decreased (95, 96), is very much an indication of possible excessive central cholinergic tone following abrupt and massive alterations of possibly central inhibitory catacholaminergic systems.

In nialamide/6-OH-DA injected rats, all these behavioral effects were potentiated and it appears from experiments now in progress that as a possible result of too extensive a destruction of the proposed inhibitory pathways, central equilibration of systems may have been rendered impossible, rats maintaining higher levels of excitability and aggressiveness (NE concentrations in cortex, cerebellum, lower brainstem and hypothalamus/upper brainstem were 34%, 0%, 48% and 23% of control levels respectively, while striatal DA was 19%).

3. Amphetamine induced electrocortical changes in normal and 6-OH-DA treated rats

It is consistently reported in the literature that typical amphetamine induced EEG changes consist of a flattening and shift to higher frequencies, saline changes being similar but relatively minor (39, 40). This popular concept of electrocortical amphetamine induced "arousal" may need slight revision considering the

present findings that this seems to barely hold true in a positive reinforcement environment (Fig. 7, 8). In a negative reinforcement situation, as could possibly be the case in experiments performed on restricted animals, an increase in the amplitudes of most frequencies occurred (Fig. 8, 10).

It is of interest to note again, that the predominant increase in amplitude of the EEG patterns, as visually detectable in the polygraph recordings (Fig. 15) or as better demonstrated by the integration of the EEG spectrum (obtained through the use of the fast Fourier analysis) maintains an inverse relation to EP amplitudes. In Rf^{+} there is also an apparent tendency of this kind, but, significance could probably only be reached with a larger sample of animals.

Considering, the central and peripheral release of NE in stressful situations (97, 98, 99), the general increase in central turnover (100, 101, 102) which accompanies the release of this presumably inhibitory agent, the potentiation of its release by amphetamine generating greater extraneuronal accumulations of the neurotransmitter, and the possibility of amphetamine, intoxication being in itself highly stressful, NE and/or perhaps catecholamines and their products appears to be good candidates in the nomination of "deactivating factors".

Also of great interest is the fact that for 6 HD-rats such electrocortical changes were no longer observed. That EEG amplitudes were no longer increased by amphetamine in Rf^{-} should be expected if the normally induced increases of NE at receptor sites are largely prevented. The fact that an actual reversal of normal effects on EEG amplitude may possibly have been obtained, is indicative of the likelihood that non-catecholaminergic activating, desynchronizing, flattening properties of amphetamine may also be evidenced through other systems, as previously discussed. Also, it has been proposed by some that the visually observed amphetamine induced cortical arousals as well as some of the behavioral effects are reticular or midbrain in origin (36, 37, 41, 103); however, the non-catecholaminergic predominance of amphetamine action is strongly endorsed by the fact that cholinergic mediation of such phenomena has been established at those levels (104). That reticular stimulation can incite arousal, that amphetamine can facilitate reticular unit firing activity (31, 41, 103, 105) but that NE depress it (31) is again an indication that such catecholamines cannot be responsible for amphetamine action in

producing arousal. Were the EEG changes seen in Rf^- perhaps an indication of electrocortical and behavioral deactivation?

The maintenance and greater significances of amphetamine induced EP reductions of most measurements obtained after 6-OH-DA treatment again indicated the possible loss of some factors that inhibit or deactivate and the predominance of others remaining that activate. The insignificance of EP component changes in Rf^- seen for 6 HD-rats when compared to the highly significant desynchronization in normal rats also favors this argument. The discrepancy in EP and EEG amplitude changes observed as before could be the result of multiplicity in control/modulation mechanisms. Being that cholinergically mediated olivo-chochlear-bundle (OCB) inhibition of auditory evoked potentials is known (106, 107), as is amphetamine facilitation of ACh release, it is the author's belief that cholinergic actions of amphetamine may well lead to attenuated auditory nerve potentials via interactions at hair cells or via middle-ear muscle contractions, thereby differentially affecting EEG and EP. That sympathomimetics such as amphetamine (the l isomer is more potent than the d isomer) can if peripherally administered induce hypertension (18, 40, 41, 108, 109) which may lead to EEG arousal (41, 108, 109) has been reported; however, this is generally considered not to be a serious factor in EEG studies (38, 41). Also of great interest to this dissertation, is the corroboration of ACh-activation/NE-deactivation evidences of central antagonism by chronic amphetamine over stimulation of the CNS which is purported to induce an increase in catecholaminergic tone (increased biosynthesis) and simultaneously a decrease in cholinergic tone (increased mediator breakdown) (110).

4. Behavioral effects of amphetamine in normal and 6-OH-DA treated rats

The inhibition of conditioned pressing behavior particularly at the higher doses of amphetamine and the potentiated effect at least within the first week or so after 6-OH-DA, are also in line with the concept that amphetamine effects on behavior are not necessarily directly related to cerebral NE and DA. The potentiation of extraneuronal NE relase in Rf^- by amphetamine in normal rats corroborates our expectation that NE may perhaps be an inhibiting, deactivating drug at the cortex and/or reticular formation (Fig. 8, 10).

That amphetamine induction of stereotypy and locomotor behavior was NOT DECREASED as a result of massive central destruction and biosynthetic inhibition in noradrenergic and dopaminergic pathways, which very definitely occurred at proposed alerting sites, but was on the contrary FACILITATED, is strongly suggestive of the relative unimportance of these pathways in accentuating stereotypy and locomotion. Indeed an inhibitory quality on amphetamine induced behavior MUST be hypothesized.

The hereto stressed and restressed possibilities of NE inhibitory powers and the lack of dependance of amphetamine on catecholamines could be easily refuted if catecholamine depletions led to supersensitivity in adrenoreceptors; however, this is not the case. Reserpine induced potentiation of amphetamine toxicity, for example, has often been reported (111) but serious considerations of this fact have consistently led to the conclusion that this was not a supersensitivity phenomenon (112, 113, 114, 115). Hypophagia and weight loss have been suggested as the actual cause in the case of reserpine (115); however, in our experiments with 6-OH-DA and those reported by others (71, 116), food and water intake were very soon adequate and mature rats maintained normal growth rates. In fact, in the latter, it even appears that postsynaptic receptors are damaged (117). It has been reported that in cases of reserpine induced supersensitivity the effects of indirect acting sympathomimetics were all in fact decreased, whereas those of direct acting ones were increased (118). Hence, if supersensitivity was indeed the case in the experiments performed for this thesis, amphetamine effects would still have to be considered the result of direct post-synaptic action and not that of indirect NE/DA mediation.

In the light of previous reports could amphetamine not directly stimulate ACh release presynaptically or perhaps even be a general stimulant? This possibility as well as others are considered in Fig. 19. The predominance of behavioral and/or electrocortical effects would of course depend on the predominant tone in a system involving activating and deactivating central pathways. The actual extent of feed-back (more or less inhibition, for example) to or within a particular system responsible for a specific phenomenon (e.g. behavioral) would be of importance. The most "potent" or effective overall or predominating action of amphetamine at various levels in the

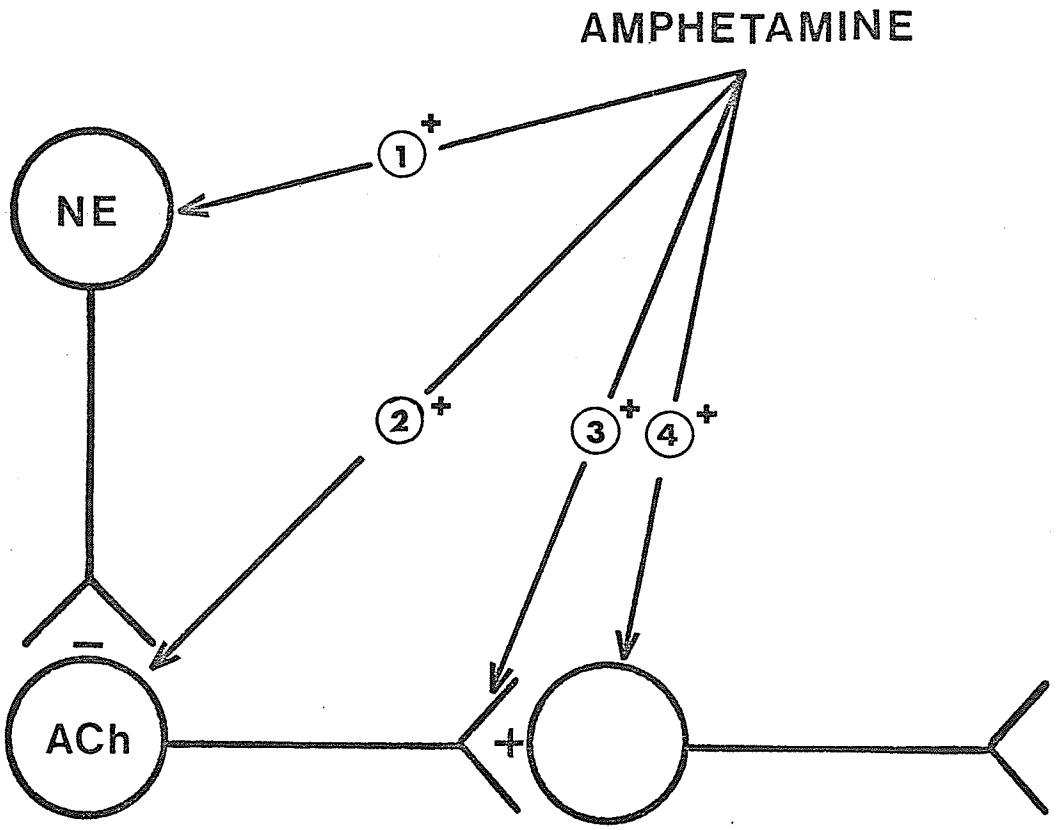

Fig. 19. A proposal for the multiphasic action of amphetamine on, among others, two antagonistic activating (ACh) and deactivating (NE) pathways. Amphetamine may mimic the effects of neurotransmitters by direct postsynaptic stimulant action, 1, 2 and 4 or by presynaptic potentiation of cholinergic transmission, 3.

CNS, be they direct stimulating or indirect releasing capabilities, could also be control factors explaining some of the discrepancies within conditioned and spontaneous behaviors and electrocortical phenomena. There are innumerable allusions in the literature NE/ACh as well as to other combinations of neurotransmitter actions which lead to the above hypothesis. These, however, cannot be dealt with in detail within the scope of this dissertation.

To add to the problems of amphetamine studies it seems that on close examination of highly quoted papers (116) some data therein are simply poorly interpreted, in the obvious reluctance of disfavoring current concepts of catecholaminergic involvement in amphetamine action. In the report in question, the decreased exploratory activities of amphetamine in rats pretreated with higher doses of 6-OH-DA should have been taken to indicate that the animals approached the later stages of amphetamine intoxication, which are ultimately coma and death. The literature is burdened with many such misinterpretations.

Problems may also arise from other sources. Conditioned pressing behavior in the duller more inhibited rats (which are known to have greater stores but lower turnovers of centrol NE (119, 120, 121) or other catecholamines (122) than do active rats) was completely inhibited, unlike that in active rats. This again implies the inhibitory quality of the probably more extensively released NE or possibly the greater tone of inhibitory pathways in dull rats or still alternately, the lower protection against overstimulation in active rats. Outstanding differences for both types of rats, not having been observed, in gross homecage behavior, as well as stage-defined, is perhaps again an indication of the relative lack of importance of catecholamine stores and biosynthetic rate in those specific behaviors for the seven rats studied. The importance of not relying solely on spontaneous behavior to interpret amphetamine action is quite obvious and the concomitant observation of several types of effects is a distinct advantage. The popular use of jiggle cages, or the like, to interpret activity can lead to errors, as pentobarbital and amphetamine in appropriate doses can both produce periods of hyper- and hypo-activity and surely the predominant effect of both is not the same.

In using small populations of animals in the study of amphetamine pharmacodynamics, the importance of classifying animals by general activity, as was done

for this series of experiments, is again stressed by the differential effects to similar doses of the drug, that can be observed in animals of dissimilar baseline activities (110, 123, 124). These contrasting effects of amphetamine can be summarized in Figure 15. Pronounced spontaneous behavioral and electrocortical changes were indicative of amphetamine intoxication in this rat, however, a normal pressing response in Rf^{+} was maintained.

5. Inhibition of catecholamine synthesis

The serious drawbacks observed in the present experiments, with the use of ∝MpT at doses of 150 and 250 mg/kg i.p., which are comparable or considerably smaller than those used by many reputable laboratories, seriously question the specificity of this drug...even at lower doses, if extrapolation is possible. The great toxicity (catalepsy, uremia etc.) of ∝MpT has been observed by others (125, 126, 127), and an LD 50 of 160 mg/kg and ID 50 (dose producing 50% inhibiton of tyrosine hydroxylase activity) of 29 mg/kg i.p. in rats, has been approximated for similar compounds (5, 128).

That ∝MpT brain concentrations and extent of tyrosine hydroxylase inhibition could not be directly related to the degree of locomotor activity, and that although previously disclaimed (131), ∝MpT may itself produce substantial amounts of false transmitters, such as ∝-methyl-DA and ∝-methyl-NE (129) which can displace NE and DA (hence sedation and hypothermia?) (133) and possibly result in reported falls in motility for normal control animals (132), leads the author to conclude that its effects are very unspecific <u>in vivo</u> and conclusions on amphetamine action drawn from its extensive use, very speculative. That another potent tyrosine hydroxylase inhibitor (H 59/64), although quite lethal, produced for depletions of catecholamines comparable to those of ∝MpT, only modest alterations of a conditioned avoidance response (129), supports the former allegations, as does the fact that threshold doses of the latter do not change this response (128).

The drug ∝MmT although a potent inhibitor of Dopa decarboxylase (134) and reported not to alter spontaneous orientational activity (5, 135), is completely unacceptable in amphetamine behavioral studies. Considering its high rate of degradation into metaraminol, a by-product reported to produce NE displacement

concomitant with sedative effects, as also often reported interestingly enough, activity measurements, as taken 7 hours following injection for example (135), are absolutely meaningless; however, as an aside, tetrabenazine induced falls in spontaneous activity (135), as well as the tetrabenazine reserpine and ∝MmT potentiations of ∝-methyl-tyrosine antagonism of amphetamine hyperactivity (5) are most interesting and revealing of these studies.

Ignorance of the full scope of drug effects, ironically, has led to the eventual recycling within the literature of the theory of motor activity dependance on stored catecholamines (127, 130). Experiments that did nevertheless manage to correlate the inhibition of tyrosine hydroxylase by ∝MpT (in mice and rats, 127, 136) to locomotor behavior, did so at a time when catalepsy is reported maximal (8 - 9 hours) (126) and at which time brainstem concentrations of the drug (for about 1/4 dose in guinea pig) are only 25% of the maximum reached in this species (131).

The deactivation of EEG obtained subsequent to ∝MpT injection and its potentiation by reserpenization (137), and for that matter the similar observation after reserpine, NE, 6-OH-DA, induced deactivations, are all in line with the concept that catecholamines are inhibitory. On observing stereotypy following ∝MpT/amphetamine/reserpine treatment, some preach the merits of amphetamine's direct stimulating properties (126), whereas others, exemplifying the confused state of affairs, have adopted both theories of direct and indirect catacholamine mediation (38).

That, in the above study (126), close mimicking of 6-OH-DA effects by means of reserpine plus tyrosine hydroxylase inhibition, still permits amphetamine induced activity (112) is very meaningful, as is the fact that tyrosine hydroxylase inhibition cannot completely inhibit the effect of higher doses of amphetamine (128, 138). That amphetamine further potentiate ∝MpT toxicity (126) renders the latter's use in amphetamine studies practically worthless.

B. Concluding Remarks

Reports based on data obtained from inconclusive and contradictory catecholaminergic receptor blockade, on the extrapolations of basically biochemical

experiments involving huge doses of amphetamine and from the flagrant use of synthesis inhibitors, cannot be accepted at par, if at all, in formulating non-speculative opinions on amphetamine's central mediation of electrocortical and behavioral effects.

The central mechanism of amphetamine action is an extremely complex process which encompasses many chemical events mediated by various central neurotransmitters. It is the author's belief that the experiments presented in this thesis, those cited herein, as well as a multitude of others which cannot be discussed within the scope of this dissertation, correlate complexly the predominantly excitatory cholinergic but inhibitory catecholaminergic and serotoninergic mediations of highly interdependant central pathways and systems. These led the author to conclude and theorize as to the multiphasic direct and indirect effects of amphetamine on the CNS.

Biosynthetic rate and newly synthetized freely available catecholamines are probably of no great importance in the mediation of central amphetamine effects; hence, NE and striatal DA are probably not crucial in the production of stereotypy and the enhancement of locomotor behavior. On the other hand, the depleting of catecholamines (in the active sense) appears to be centrally inhibiting whereas, depletion (in the past sense) appears to be exciting; hence, a central catecholaminergic inhibitory system cannot be expected to mediate amphetamine excitation.

V. SUMMARY OF FINDINGS

1) With respect to Rf^+, in Rf^- most EEG frequency bands decreased in amplitude, whereas EP amplitudes increased in normal rats.

2) Evidence of the preservation of a reciprocal relationship of EEG to EP amplitudes in various situtations (Rf^+ and Rf^-) and following various treatments (saline, amphetamine (2 mg/kg) and 6-OH-DA (200 µg, intracisternal)) was presented.

3) Following the injection of 6-OH-DA (200 µg only without nialamide) and the resultant release of central catecholamines, hypothermia and lethargy were observed. A period of irritability and excitability followed with the eventual return of fairly normal behavioral and electrocortical parameters. The maintainance of hyperexcitability in 6-OH-DA/nialamide treated rats was discussed in terms of the lack of preservation of homeostatis by cental antagonistic systems.

4) The arousing capacity of amphetamine, typically pictured in normal animals as EEG flattening was with some reservations found to hold true in Rf^+ only. On the contrary, as indicated by the integrated EEG, a predominant increase in EEG amplitudes was found in Rf^- for normal rats.

5) The EEG amplitude increasing capacity of amphetamine in Rf^- was not observed in 6 HD-rats whose catecholaminergic systems were greatly damaged and a suggestion of a reversal of effects was taken as an indication of a non-catecholaminergic arousing capacity of amphetamine. The normal flattening of EP in such conditions was taken as an indication of the differential control/modulation mechanisms on EP and EEG.

6) The differential effects of amphetamines on active and dull categorized rats, in respect to conditioned and spontaneous behaviors was suggestive of the disadvantage of relying solely on one type of test in determining the effects of amphetamine. The advantages of classifying small rat samples into such categories was also discussed.

7) The severe toxic effects of αMpT, at the usual doses of 150 and 250 mg/kg i.p., which included antidiuresis, hypothermia, catalepsy and death, as well as potentiation of this toxicity by amphetamine, were observed and interpreted to indicate the inadequacies of this drug in amphetamine studies.

8) A dose dependant depression of conditioned behavior (Rf^+ and Rf^-) by amphetamine was observed in normal rats. This was highly potentiated at 6 and 7 days after 6-OH-DA/nialamide treatment and as determined by spontaneous behavior the animals also reached later stages of amphetamine intoxication.

9) That amphetamine effects were actually accentuated rather than depressed by massive destruction of brain catecholaminergic systems, was taken as evidence of the probability that catecholamines such as NE and DA were not involved in the mediation of amphetamine central effects.

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