Title

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Publication Date

1973-05-01

Submitted to
Psychopharmacologia

LBL-1797
Preprint c./

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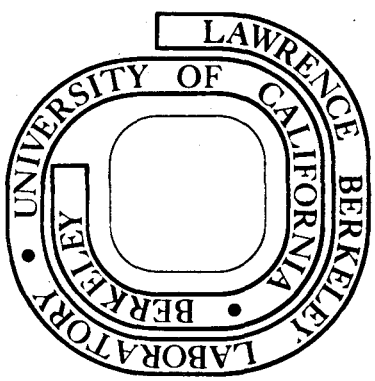
May 1973

Prepared for the U. S. Atomic Energy Commission
under Contract W-7405-ENG-48



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DISCLAIMER

Excitant and Depressant Drugs Modulate Effects of Environment on Brain Weight and Cholinesterases*

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Running Title: Excitants and depressants modulate environmental effects on brain

Footnotes to title:

*This research was supported by Grant No. 0-9-140398-4512(057) from the Office of Education, Department of Health, Education and Welfare; it also received aid from the United States Atomic Energy Commission.

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Excitant and Depressant Drugs Modulate Effects of Environment on Brain Weight and Cholinesterases

ABSTRACT

Certain excitant drugs can enhance the effects of enriched experience on weights of brain sections and on the activities of acetylcholinesterase and cholinesterase in the brain, and certain depressants can lessen these effects. Most experiments were performed with prepubertal male rats. Some rats were exposed in groups of 12 to an enriched environmental condition (EC), usually for 2 h per day and over a 30-day period; other rats remained in their individual home cages (HC) throughout. Some rats received a drug injection and others received a saline injection before the daily period of EC; HC controls received similar injections. The drug injections had no significant effects on brain values of HC rats, but they altered effects of EC, probably by influencing the animals' reactions to the environment. Methamphetamine and d-amphetamine enhanced the EC effects; metrazol had small positive effects, and strychnine was without effect. Phenobarbital depressed the brain weigh' effects, but paradoxically it increased the enzymatic effects. Use of methamphetamine made it possible to find EC effects with short daily periods (30 min) or with a shortened experimental duration (15 days). In two experiments with adult rats, methamphetamine did not clearly modulate the brain weight effects. The results of this study may bear on the use of stimulants to promote recovery from brain damage.

Key words: Methamphetamine d-Amphetamine Phenobarbital Acetylcholinesterase Brain weight Enriched environment Brain injury

The present study demonstrates that certain excitant drugs can enhance the effects of enriched experience on brain measures and that certain depressant drugs can lessen these effects. Effects of enriched versus impoverished experience on chemistry and anatomy of rodent brain have been reported by several investigators and are by now well established (e.g., Bennett, Diamond, Krech and Rosenzweig, 1964; Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech and Bennett, 1966; Ferchmin, Eterovic and Caputto, 1970; Geller, Yuwiler and Zolman, 1965; Henderson, 1970; Huntley and Newton, 1972; Krech, Rosenzweig and Bennett, 1960; La Torre, 1968; Levitan, Mushynski and Ramirez, 1972a and b; Møllgaard, Diamond, Bennett, Rosenzweig and Lindner, 1971; Riege and Morimoto, 1970; Rosenzweig and Bennett, 1969; Rosenzweig, Bennett and Diamond, 1972; Rosenzweig, Krech, Bennett and Diamond, 1962; Bolkmar and Greenough, 1972; Walsh, Budtz-Olsen, Penny and Cummins, 1969; West and Greenough, 1972). One incentive to perform this study came from some still earlier findings--that certain stimulant drugs aid behavioral recovery from the effects of brain lesions whereas certain depressant drugs slow recovery and result in a lower eventual level of performance (Ward and Kennard, 1942; Watson and Kennard, 1945). Then we discovered that 2 h per day of exposure to an enriched environment over a 30-day period sufficed to produce significant changes in brain weights and brain chemistry (Rosenzweig, Love and Bennett, 1968). This suggested that a short daily period of combined drug and environmental treatment might produce relatively large cerebral effects, and that such results might aid in understanding the effects of drugs on recovery from brain injury.

METHODS

The basic experimental design was as follows: All animals were housed in individual home cages. Certain animals were placed for 2 h per day in a group of 10-12 in a large enriched condition (EC) cage. Shortly before being placed in EC, these animals received an injection of a drug or of physiological saline solution. The animals that remained throughout in the home cage (HC) condition also received injections of drug or saline. At the end of the 30-day experimental period, the animals were decapitated, weights of brain sections were taken, and analyses of brain enzyme activities were made later in certain experiments.

Experimental conditions

Except in the first of 20 experiments, all rats were housed in individual colony cages (32 x 20 x 20 cm) in the same room. (In the first experiment, the impoverished-experience animals were housed in individual cages in a separate isolation room and they did not receive injections. A subsequent control experiment showed that individually housed rats develop identical brain values whether or not they are housed in the isolation room and whether or not they receive daily saline injections.) Home cage (HC) rats received an injection each day but were not handled otherwise except for weighing every third day.

In the EC condition, the rats of a group were injected in the room in which their home cages were placed. In the early experiments, upon injection, the rats of a group were put in a holding cage, and then a few minutes after the first rat was injected, the group was taken through an open doorway and placed in one of the EC cages in the

adjoining room. Later we found it simpler to put each rat into EC as soon as it was injected. With practice, the time required to inject a group of 12 rats dropped from about 10 minutes to less than 5 minutes. Our standard EC cages were used (70 x 70 x 46 cm). Each EC cage had a different arrangement of about 6 stimulus objects, most of them from our standard pool of 25 objects (Rosenzweig & Bennett, 1969), and a few other objects were also used. Each group had a different arrangement of stimulus objects each day.

In a few experiments, as will be noted below, the daily EC period lasted only 30 or 60 minutes instead of the usual 2 h. In one case the experimental period was 15 days instead of the usual 30 days. The groups included in the various experiments are listed in Table 1.

Table 1 About Here

Behavioral Observations

Formal observations of behavior during EC sessions were made during several of the experiments. A checklist of 16 behavioral categories was employed. The first observation of a session was made 5 min after the animals were placed in EC and successive observations were made at 10 min intervals. About a min before each observation was to be made, the experimenter stationed himself in front of the cage, attempting not to distract the rats. At the moment when the second hand of the room clock reached zero, the activities of all rats in the group were recorded as quickly as possible. With practice, this could be done accurately, and several experimenters agreed well in their

observations. When several cages were to be observed, as was the case in most experiments, the groups were removed from their holding cages and put into EC cages at 1 min intervals, so that the observation of successive cages were made at 1 min intervals. In experiment 18, behavior was recorded on television tape during some experimental periods; results of these detailed observations will be the subject of a separate report.

Drugs and Injections

Each rat received an intraperitoneal injection of 0.003 ml per gm body weight each day. The injection was either physiological saline or a drug dissolved in saline. The drugs employed were the following: dextro desoxyephedrine hydrochloride (methamphetamine), dextroamphetamine, pentylenetetrazol (Metrazol), strychnine sulphate, pentobarbital sodium, and phenobarbital sodium.

Subjects

The subjects were male rats of the Berkeley S_1 and S_3 lines, bred in the Department of Psychology colony, and of the inbred Fischer strain, obtained from Simonsen Laboratory, Gilroy, California. In the case of the S_1 and S_3 rats, a littermate design was employed. Litters were used that included as many males as the number of experimental groups to be run; the maximum body weight range allowed among littermates was 15%. Littermates were assigned semi-randomly among the number of experimental groups to be included in a particular experiment, the only restriction being that the distributions of body weights among the groups be closely similar; these groups were then assigned at random among the experimental

conditions. In the case of the Fischer rats, animals were matched in terms of body weights and then were assigned at random among the conditions of an experiment. Animals were usually assigned to these experiments about a week after weaning, which was done at about 25 days of age.

Removal and Weighing of Brain Tissue

At the end of the experiment, the animals were put in a multiple-unit cart bearing code numbers that did not reveal the experimental condition of any rat. The animal was decapitated, and the brain was dissected following our standard procedures (Rosenzweig et al., 1962). Using a calibrated plastic T square, we removed standard samples of occipital and somesthetic cortex. The other brain sections were the following: remaining dorsal cortex; ventral cortex, including the hippocampus and corpus callosum; cerebellum and medulla; remaining subcortical brain, including the olfactory bulbs. Measures from all of the cortical sections could be combined to give total cortex; measures from the two remaining sections could be combined to give rest of brain (or subcortex).

As soon as each sample was removed, it was weighed to the nearest tenth of a milligram on an automatic balance. The samples were then frozen on dry ice and stored at -30° C. for subsequent chemical analysis.

Chemical Analysis

The quantitative method of Ellman, Courtney, Andres, and Featherstone (1961) has been adapted for the differential assay of acetylcholinesterase (AChE) and cholinesterase (ChE). Our procedure has

been described in more detail in Rosenzweig and Bennett (1972); a complete description can be obtained from the authors upon request.

Analyses for both AChE and ChE are routinely made in duplicate; two AChE values usually agree within 2%, and two ChE values within 3%.

Statistical Tests

Results of individual experiments were evaluated by two-way analyses of variance (litters vs. treatments). Overall results combining several experiments utilized the same design with replication. Comparisons between different experimental groups were done by Duncan's multiple-range test.

RESULTS

We will take up first results with excitants and then results with depressants.

Excitants

Effects of Methamphetamine in 30-Day EC-IC Experiments

(a) Brain weight effects. Nine experiments investigated effects of methamphetamine when young animals were exposed to the enriched environment for 2 h per day over a 30-day period. All nine of these experiments included both a Methamphetamine-EC (Meth-EC) and a Saline-home cage (Saline-HC) group; eight of these also included the Saline-EC condition and two included Meth-HC. The results for brain weights are shown in Table 2 for the three strains separately and then overall. (The table includes dosages of 1 mg/k for experiments 1 and 2 and 2 mg/k for the other 7 experiments.) It is clear that the Meth-EC animals developed

the largest brain weights of all the groups. Furthermore, Meth-EC was

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Table 2 About Here

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significantly greater on many brain weight measures than was Saline-EC. We can ask whether the Meth-EC effects were more than a simple addition of the separate EC and drug effects. Animals that received a saline injection before being put into EC for 2 h daily (the Saline-EC group) developed clear brain differences from the Saline-HC animals; in the case of the occipital cortex where EC-HC differences tend to be largest, this was 8.0% ($P < 0.001$). But when the drug was given to animals that remained in HC (the Meth-HC group in Table 2), this appeared to be without any consistent or significant effect on the brain weights. A series of 4 experiments with S_1 rats included Meth-HC and Saline-HC rats as controls for animals exposed singly to EC cages; these experiments showed that with sufficiently large numbers, some of the differences between Meth-HC and Saline-HC can reach statistical significance, but the effects remain small (Occipital Cortex, 0.6%, NS; Total Cortex 1.9%, $P < 0.05$; Cortex/Rest, 1.1%, $P < 0.01$) (Rosenzweig and Bennett, 1972). From these results or from the overall Meth-EC results of Table 2, it can be seen that the Meth-HC effect by itself added to the Saline-EC effect is not enough to have yielded the Meth-EC effect in most cortical weights or in the cortical/subcortical weight ratio; this is clearest in regard to the occipital cortex where the Meth-HC versus Saline-HC difference is only 0.6%, the Saline-EC versus Saline-HC difference is 8.0%, but the Meth-EC versus Saline-HC difference reaches 12.1%. Thus the drug and the enriched environment appear to interact as factors in producing the cerebral

effects. (In the case of single rats in EC cages, such interaction between drug and environment is clear only for the occipital cortex and not for weights of other brain regions.)

It should be noted that the Berkeley S_1 and S_3 lines yielded somewhat larger effects, especially in occipital cortex, than did the inbred Fischer strain. Although they are inbred, the Fischers have shown somewhat inconsistent results, so that we have used them less in recent work.

(b) Brain Enzyme Effects. Our usual findings have been that EC rats, as compared to IC littermates, show decreased AChE activity per unit of weight in the cortex and increased ChE activity throughout the brain; the purely chemical ChE/AChE ratio is increased in the cortex and in the cortical/subcortical ratio (Rosenzweig et al., 1972). Results

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Table 3 About Here

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conforming to this pattern were found in the present experiments with S_1 rats, as will be seen in Table 3. The Meth-EC animals were found to deviate further and in some cases more significantly from HC than did the Saline-EC animals. In no case, however, was the difference between Meth-EC and Saline-EC statistically significant. Similarly, when S_1 rats were exposed individually to EC (Rosenzweig and Bennett, 1972) methamphetamine usually produced larger enzymatic differences from HC than were found between the saline EC and HC groups, but the differences were not significant. Thus, the use of the excitant drug had only a questionable effect on the brain enzymes, although it had a clear effect on brain weight measures.

Although we ran almost as many Fischer rats (N = 26 per group) as S_1 rats, we did not find significant chemical effects for either Meth-EC or Saline-EC versus Saline-HC. Perhaps 2 h per day is not sufficient enrichment of experience to develop clear enzymatic effects in this strain, although it produced clear effects in brain weights. The single experiment with S_3 rats showed typical EC-IC enzymatic differences but little indication of a drug versus saline effect.

Effects of Methamphetamine with Shorter Exposure to EC

The enhancement of brain weight effects is also seen when the length of experience is reduced, either by shortening the daily EC period or by reducing the duration of the experiment. Table 4 presents results of two experiments with 30-min daily EC for 30 days; Table 5 presents results

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Table 4 About Here

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for Experiment 8 with 2 h daily EC for 15 days. In Table 4 note that the Meth-EC effects are larger than the Saline-EC effects for every brain region and that several of these differences are significant. Thus, even 30 min per day in the enriched condition brings about cerebral effects, and this is seen more strongly and clearly when the rats are in EC under the influence of methamphetamine.

The use of methamphetamine was important to bring out significant brain weight effects in the 15-day experiment (Table 5, Fischer rats). Here Saline-EC did not differ from the control Saline-HC group for any brain weight measure. (Subsequent 15-day experiments with S_1 rats in EC for

24 h per day have shown significant brain weight effects, but the difference in strain or in daily duration of experience may be important here.) Although Saline-EC was not effective for any brain weight measure,

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Table 5 About Here

Meth-EC differed significantly from Saline-HC in weight of total cortex and in the cortical/subcortical weight ratio.

The pattern of enzymatic results was unusual in this single 15-day experiment. In the case of AChE/weight, the EC groups show higher values than the HC group, whereas in the 30-day experiments, EC was lower in AChE/weight (see Table 3). In the case of ChE/AChE, Saline-EC exceeds Meth-EC in Table 5, whereas in 30-day experiments the reverse is true. More experiments with short EC-IC durations will be required before we can generalize confidently about these effects.

Pattern of Cerebral Effects

Throughout this section and in Tables 2-4, it should be noted that the use of methamphetamine accentuates the EC weight and enzymatic effects but does not substantially alter the pattern of effects. That is, in Meth as in saline groups, the largest difference from Saline-HC occurs in occipital cortex, and the effects throughout the cortex are greater than in the rest of the brain. This, and the fact that methamphetamine has little or no effect in the HC condition, indicates that Meth-EC does not produce "drug" effects but only augments the environmental effects on brain weights.

Dosage Effects with Methamphetamine

As noted in Table 1, all of the Meth experiments with young rats, except 1 and 2, employed a dosage of 2 mg/k. Experiment 1 used 1 mg/k and Experiment 2 had one group at 1 mg/k and another at 3 mg/k. With the dose of 3 mg/k no special effects were noticed during the first two weeks, but then we found that some of these rats began to avoid being removed from the EC cage; they shunned the experimenter's hand and sometimes even jumped out of the cage to the floor. When the experimenter did pick up such a rat, it did not resist and was not aggressive; the next day it would let itself be injected as usual. Because of this striking effect of long continued injections of 3 mg/k, we settled on 2 mg/k as our standard dosage with young rats and never noticed any ill effects of these injections. The cerebral effects of all three dose levels could not be distinguished from each other.

Effects of Methamphetamine on Activity

Records of activity during the 2-h EC period were taken during Experiment 3 with S_1 rats. Littermates given saline, methamphetamine and phenobarbital were observed simultaneously over 10 sessions. A large number of categories of behavior were recorded, but for purposes of summary presentation these were reclassified into three headings-- "active" includes all types of locomotor movement, head movement, sniffing, clawing, etc.; "awake but inactive" was defined as lying down with eyes open; "asleep" was defined as lying down with eyes closed. As can be seen in Figure 1, animals in the saline condition are active at the start but

Figure 1 About Here

become progressively less active, with a quarter of the group asleep by the end of the 2-h session. Under methamphetamine, the animals remain active, ceaselessly moving, exploring, sniffing; only one rat appeared to be asleep during all 10 observation sessions. Under phenobarbital, the animals are active in the early observations, and during the first half hour, it is almost impossible to distinguish the phenobarbital group from the saline group. But the activity of the phenobarbital animals drops off rapidly, and typically three-quarters are asleep by the end of the session.

Effects of Methamphetamine with Older Rats

In order to test whether the enhancement of EC-HC differences would also be found with older rats, we ran Experiments 19 and 20. These followed the procedures of the previous experiments except that at the start of injections the rats in Experiment 19 were about 240 days of age, and those in Experiment 20 were about 210 days of age. In 19, the dosage was 2 mg/k at the start and was later reduced because of behavioral observations. In 20, it was 1 mg/k throughout.

In Experiment 19, after about one week we observed that the Meth-EC rats were extremely sensitive to any noise or sudden movement in their vicinity and would jump if a rat or an experimenter made such a stimulus. Furthermore, a number of rats frequently assumed sparring positions, rearing up on their hind legs and facing each other as if ready to fight. This posture is also seen among young Meth-EC rats, but they do not hold this

pose for as long periods as the older rats do. We therefore cut the dosage of the drug for the adults to 1 mg/k, and when the tense and defensive behavior still persisted, we cut the dosage to 0.5 mg/k for the latter half of the experiment, but the sparring posture remained frequent. In Experiment 20, the dosage was 1 mg/k throughout. Some tenseness and frozen sparring postures occurred almost from the start, but it was not as extreme as in the previous experiment. Records showed that the saline-EC rats moved about freely in the early part of each daily period and then became inactive or slept toward the end. In contrast, the Meth-EC rats showed less exploration of objects; they often huddled together early in the period, although some sparred or engaged in brief attacks. The Meth-EC rats remained awake throughout the 2 h period and were tense when picked up for removal, whereas the saline-EC rats were relaxed.

The results of both experiments showed very similar brain weight values for the Meth-EC and saline-EC groups; see Table 6 for combined values for the two experiments. In Experiment 19 neither EC group differed significantly from saline-HC except on an occasional measure. For the cortical/subcortical ratio, Meth-EC and saline-EC differed from saline-HC

Table 6 Around Here

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by 3.0 and 2.1% respectively; neither difference was significant. In Experiment 20, on the contrary, both EC groups differed significantly from the baseline saline-HC group on several weight measures (occipital cortex, remaining dorsal cortex, total cortex, and the cortical/subcortical ratio). For the cortical/subcortical ratio, Meth-EC and saline-EC differed

from saline-HC by 3.8% ($P < .001$) and 3.4% ($P < .01$) respectively. In neither experiment, nor in the two experiments combined, did Meth-EC differ significantly from saline-EC.

Thus the effects of methamphetamine on both behavior and cerebral effects of differential experience appear to differ for older and younger rats. The results obtained with younger rats in all of the other experiments of this paper cannot safely be extrapolated to older rats.

Effects of Strychnine in 30-Day Experiments

In order to test whether other drugs might yield effects similar to methamphetamine in the EC situation, we then used strychnine in two experiments with Fischer rats (11 and 12). Dosages of 0.125, 0.50 and 1.0 mg/k were employed. The brain weight results for the strychnine groups did not differ significantly among themselves nor from the Saline-EC group. That is, the strychnine-EC groups differed from the saline-HC baseline, but no more than did the saline-EC group, so the strychnine had no apparent effect on the brain values even though it made the rats noticeably more tense. The second of these experiments also included a Meth-EC group, and this group had clearly the largest cortical/subcortical weight ratio. Thus, in direct comparison with methamphetamine, strychnine showed itself to be ineffective to interact with the enriched environment to alter brain weights.

Effects of d-Amphetamine in a 30-Day Experiment

We next tested d-amphetamine sulphate (d-Amphet) in Experiment 13. Eleven sets of three males per S_1 litter were used for 3 groups placed in EC for 2 h per day over a 30-day period: (a) 0.5 mg/k d-Amphet, (b) 2.0 mg/k d-Amphet, and (c) saline. It should be noted that all three groups

were given enriched experience, and the question was whether the d-amphetamine groups would differ from the saline group in the EC direction.

The low dosage d-Amphet group, group a, did not differ significantly from the saline-EC group in brain weights. The dose of 2.0 mg/k caused differences from saline-EC littermates in weight of occipital cortex (7.0%, $P < 0.05$) and in the cortical/subcortical weight ratio (2.0%, $P < 0.10$). It therefore appears that d-Amphet has similar effects to Meth on rats in EC. There were no clear differences among the three EC groups on brain enzyme measures.

Effects of Metrazol in 30-Day Experiments

We next investigated effects of Metrazol-EC versus Saline-EC in three successive experiments (14-16) in which S_1 rats were placed in EC for 2 h per day over 30 days. As in the d-amphetamine experiment described above, the Metrazol-EC groups were compared with Saline-EC groups to see whether Metrazol-EC produced effects that differed significantly from those of Saline-EC. All three experiments included a group with the dosage of 15 mg/k; in addition, the first of these experiments had a group with 7.5 mg/k, and the second experiment included a group that received 30 mg/k at the outset. The higher dosage, 30 mg/k, was found to produce convulsions in some rats after several days of injection, so the dosage for

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Table 7 About Here

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this group was reduced to 15 mg/k during the latter half of the experiment. Occasional rats convulsed even at the dosage of 15 mg/k.

Effects of Metrazol-EC versus Saline-EC on brain weights are presented in Table 7; in all three experiments, results shown are for the dosage of 15 mg/k. It will be seen that the use of Metrazol in EC did produce somewhat greater cortical weights and greater cortical/subcortical ratios than were found with Saline-EC. Few of these effects were statistically significant within a single experiment, but for the three experiments combined there were significant effects in remaining dorsal cortex, total cortex, and the cortical/subcortical ratio. It should be noted that whereas both methamphetamine and d-amphetamine interacted with EC to produce especially large effects in the occipital cortex, Metrazol had no significant effect in this region. It appears that Metrazol is not highly effective in enhancing EC versus HC differences, and that the dosage required to obtain effects is close to the convulsive dose.

Depressants

If excitants enhance the brain's response to an enriched environment, then depressants would be predicted to diminish the cerebral effects of enriched experience. To test this hypothesis, we included the use of depressants in the earlier experiments of this series and in one recent one.

Pentobarbital Sodium (Pento)

Pentobarbital sodium was employed in Experiments 1 and 2. Both experiments included groups given 5 mg/k, and this did not alter the magnitude of EC-IC brain weight differences from what is usually seen with saline injection. The second experiment also included a group run under 10 mg/k, and this appeared to reduce the EC-IC effects. Compared with Saline-EC, Pento-10-EC showed significantly lower weight of occipital cortex (5.5%,

$P < 0.05$) and somewhat lower weights of total cortex (2.4%, NS), rest of brain (2.7%, $P < 0.10$) and total brain (2.6%, $P < 0.10$). It may be that Pento-10 does counter the effects of EC, but rather than attempt to establish this definitively we decided to test another depressant, phenobarbital.

Phenobarbital Sodium (Pheno)

(1) Brain Weight Effects. Phenobarbital sodium, 30 mg/k, was used in four experiments with 2-h EC sessions over 30-day periods--Experiments 3, 5, 9 and 17—as well as three other experiments with shorter daily EC sessions. Brain weight results are shown in Table 8. In experiments 3, 9 and 17, which included concurrent Saline-EC groups, Pheno-30-EC yielded

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Table 8 About Here

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smaller effects than did Saline-EC on every brain weight measure. Combining results of these three experiments, phenobarbital reduced the EC effect at occipital cortex by 4.7% ($P < 0.01$), at total cortex by 2.6% ($P < 0.01$), and it reduced the cortical/subcortical ratio by 1.4% ($P < 0.01$).

Two experiments, 5 and 17, included a Pheno-HC group. Results for the two experiments combined showed no significant differences from Saline-HC except for ventral cortex. On the basis of this control comparison, it appears that overall phenobarbital does not significantly affect brain values in the home cage condition although, as we have just seen, it does reduce the EC effects.

(2) Brain Enzyme Effects. The enzyme analyses were done on the four Pheno experiments. Results for the three experiments run with the S_1 strain are shown in Table 9, and they were surprising. Although

phenobarbital reduced the brain weight effects of enriched experience, it enhanced the enzymatic effects. In the case of acetylcholinesterase

Table 9 About Here

activity per unit of weight, it will be recalled that the usual finding is that EC rats have lower AChE/wt than IC littermates in the cortex and in the cortical/subcortical ratio. Table 9 shows that such reductions were larger in the case of Pheno-EC than in the case of Saline-EC; furthermore, in several brain regions the difference between Pheno-EC and Saline-EC was statistically significant. Cholinesterase activity per unit of weight is usually greater in EC than in IC; this was observed in the present experiments, but in most cases the increase was greater for Pheno-EC than for Saline-EC, although these differences between the EC groups were not significant. Finally, the ChE/AChE ratio is typically greater in EC than in IC. On this measure too, the Pheno-EC condition was more effective than Saline-EC, and for several brain regions the difference between Pheno-EC and Saline-EC was significant.

Pheno in Short-Session Experiments. Pheno-30 was used with 30-min daily EC in Experiments 6 and 10. In the first of these, the Pheno brain weight results scarcely differed from Saline-EC; in the second there were small but nonsignificant differences from the control values. It thus appears that 30 min per day is insufficient for effects of Pheno to manifest themselves. We have seen above that methamphetamine was effective even in 30-min daily EC experiments. The difference in effectiveness of

these two agents in brief daily exposure to EC is understandable in terms of their effects on activity in the EC cage (Fig. 1); the Pheno rats, it will be recalled, were about as active as the Saline rats during the first half-hour of the 2-h period. Limiting EC to 30 min thus did not permit Pheno to produce a differential effect. These results are further evidence that the drug does not produce a cerebral effect by itself but only by altering the behavior of the animals with regard to the EC environment.

DISCUSSION

Both methamphetamine and phenobarbital have been shown above to interact with the enriched laboratory environment in determining brain weight measures. Figure 2 helps to compare and contrast the effects of the two drugs. Each bar in the figure represents the percentage difference between the experimental condition and the baseline group that received control injections of saline solution and that remained in their individual home

Figure 2 About Here

cages throughout the experiment. The left-hand column of bars shows that giving phenobarbital to rats that remained in their home cages had no significant effects on any of the brain measures; the right-hand column shows a similar lack of effect in the methamphetamine-home cage group. The two center columns show the effect of 2 h per day of enriched experience in animals that received saline injections; EC with no drug had highly significant effects on all three brain measures. It should be noted that the left-hand saline column consists of saline-EC groups run in experi-

ments which also included phenobarbital-EC groups; thus this column provides the correct comparison for the Pheno-EC groups. Similarly, the right-hand Saline-EC column provides the comparisons for the Meth-EC groups. Actually, all the experiments that included both Pheno-EC and Saline-EC also included Meth-EC, so the 28 Saline-EC rats in the left-hand Saline-EC column are also included in the 80 Saline-EC rats in the right-hand column. Both Saline-EC columns have quite similar values, indicating the replicability of this condition and the fact that those experiments that included Pheno-EC do not represent any special selection from the overall Saline-EC condition.

Phenobarbitol given to rats in EC is seen clearly to reduce the EC effects; that is, the bars representing Pheno-EC versus Saline-HC are significantly lower than the bars representing Saline-EC versus Saline-HC. Nevertheless, phenobarbital did not completely abolish the EC effects, since these are still significant for occipital cortex and for the cortical/subcortical ratio. Giving methamphetamine to rats in EC clearly increases the EC effect, as is seen when the fifth column of bars (Meth-EC) is compared with the fourth column (Saline-EC). Thus there is a regular progression of effects, from Pheno-EC to Saline-EC to Meth-EC.

Comparisons Among Excitants and Among Depressants

Although the research reported here demonstrates clear interactive effects between methamphetamine and experience and between phenobarbital and experience, it is too early to generalize these effects to excitant drugs as a class or to depressant drugs. We have only begun to test other agents. d-amphetamine, in the one experiment in this series in which it was employed, did appear to act similarly to methamphetamine. Metrazol also

increased the EC effects somewhat, but only at a dose close to the convulsive level, and it did not yield typical effects in the occipital cortex where we usually find the largest effects. Strychnine, in doses that increased muscular tonus and jerkiness of behavior, did not produce effects on the brain measures tested. One might attribute this lack of effect to the fact that strychnine wore off before the end of the daily 2 h session, whereas the other excitants kept up heightened activity throughout the session. Against this reasoning is the fact that methamphetamine increased in EC weight effects when animals were placed in EC for only 30 min daily (Table 4). A more probable reason for the failure of strychnine to alter EC-IC effects is that the EC-IC effects are most pronounced in the cortex, whereas strychnine, unlike certain other excitants, is active chiefly below the level of the telencephalon and diencephalon. Thus in electrical recording, strychnine was reported to produce ". . . a continuous discharge of synchronous waves at 30 c/sec at the level of the spinal cord, the cerebellum and the midbrain, with very little involvement of the thalamic and cortical EEG" (Florio and Longo, 1972, p. 285). Other types of excitant agents will have to be tested before it is clear what pharmacological properties will interact significantly with the enriched environment, and we have some studies of this sort under way.

Age as a Factor in the Methamphetamine Effects

Experiments 19 and 20, done with rats over 200 days in age, did not yield the effects of methamphetamine typical of the young rats in the other experiments. This is not surprising, since the amphetamines have been

demonstrated to produce different results on aspects of behavior of older and younger subjects, human as well as infrahuman. Nevertheless, the factor of age is not always given explicit attention in research with these agents. Thus Kumar (1969) and Robbins and Iversen (1973) both concluded that d-amphetamine increases the locomotor activity of individual rats but reduces their exploratory behavior. Kumar's subjects were female hooded rats, over 100 days old at the start of the experiments. Robbins and Iversen's subjects were male albino Wistar rats; age was not specified, but they were 210 ± 20 g at the start of habituation for testing, so presumably they were at least 50 days old and thus were postpubertal. A week of habituation preceeded the testing. Neither Kumar nor Robbins and Iversen asked whether results similar to theirs might also be found with prepubertal rats, so it would be interesting to apply their techniques to animals of different ages, with and without amphetamines.

Can Excitants Promote Recovery from Brain Damage?

We noted that one incentive to perform these studies was the work of Kennard and collaborators suggesting that stimulant drugs could aid behavioral recovery from effects of brain lesions. Pharmacological and environmental determinants of recovery from cerebral trauma have been investigated by a number of workers (see the review by Rosner, 1970), but the possibility of interaction between drugs and environmental stimulation seems scarcely to have been envisaged. There does not seem to be any consensus among American neurologists as to whether stimulant drugs should be employed to promote recovery. A recent bulletin on Neurochemistry in the Soviet Union (Tower, 1969) reports some relevant material from studies of recovery

of animals from closed head trauma conducted by M. Sh. Promyslov of Moscow. Promyslov is reported to recommend, on the basis of biochemical studies of the brains of animal subjects, that human patients suffering from cranio-cerebral trauma should never be narcotized but should be treated by stimulants. Further research on this topic might be done by adding drug conditions to the design employed by Schwartz (1964). Schwartz subjected rat pups to either a bilateral posterior cortical lesion or to a sham operation, and then animals of each sort were raised in either an enriched or a colony environment. When tested as young adults, both brain damage and an impoverished environment caused larger error scores on the Hebb-Williams maze. Furthermore, the lesioned group raised in the enriched environment was superior in maze scores to the non-lesioned group from the impoverished environment. The environment clearly aided recovery from the brain lesion. We have now found certain excitant drugs to promote brain growth. It remains to be tested whether the conjunction of enriched environment and an excitant drug may be even more favorable for recovery from brain damage than is either treatment alone.

Acknowledgments

The authors are happy to thank Marie Hebert for dissection of the brains, Hiromi Morimoto for the enzymatic analyses, Arun Prakash for the statistical computations, Donald Dryden and Walter Kisack for the behavioral procedures, and Jessie Langford for the secretarial work.

Figure Captions

Figure 1. Activity of S_1 rats during 2 h periods in the enriched condition. Values are means of 10 sessions in Experiment 3.

Figure 2. Differences between brain weights of rats run under various conditions of experience and injection and the Home Cage-Saline group. Pheno = phenobarbital, Meth = methamphetamine, HC = Home Cage, EC = Enriched Condition. Asterisks indicate levels of significance of differences from the baseline of the Home Cage-Saline group: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The left-hand shaded bars show values of the EC-Saline rats that were controls for the EC-Pheno rats; the right-hand shaded bars show values of the EC-Saline controls for EC-Meth rats.

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Table 1

Conditions, Drugs and Dosages in Experiments on Drugs and Environment[a,b]

Experimental Conditions

Exper. No.	Strain	Date of Sacrifice	Home Cage	Saline-EC	Excitant-EC	Depressant-EC	Comments
1.	S_3	8/1/67	No inj.	x	Meth-1	Pento-5	HC in separate room
2.	S_1	11/28/67	No inj.	x	Meth-1	Pento-5	
					Meth-3	Pento-10	
3.	S_1	2/9/68	Saline	x	Meth-2	Pheno-15	
						Pheno-30	
4.	S_1	3/11/68	Saline		Meth-2		
			Meth-2				
5.	S_1	3/11/68	Saline			Pheno-30	
			Pheno-30				
6.	S_3	4/29/68	Saline	x	Meth-2	Pheno-30	30 min daily EC
7.	Fischer	7/17/68	Saline	x	Meth-2		15-day duration
			Meth-2				
8.	Fischer	8/5/68	Saline	x	Meth-2		
			Meth-2				
9.	Fischer	10/15/68	Saline	x	Meth-2	Pheno-30	
10.	Fischer	3/13/69	Saline	x	Meth-2	Pheno-30	30 min daily EC
11.	Fischer	8/25/69	Saline	x	Strychnine-0.125		
					Strychnine-0.50		
					Strychnine-1.0		

Table 1 continued

12.	Fischer	11/17/69	Saline	x	Meth-2		
					Strychnine-0.125		
					Strychnine-0.50		
					Strychnine-1.0		
13.	S_1	2/17/71		x	d-Amphet-0.5		
					d-Amphet-2.0		
14.	S_1	2/17/71		x	Metra-7.5		
					Metra-15		
15.	S_1	4/15/71		x	Metra-15		
					Metra-30		Higher dose of metra reduced in last half of experiment.
16.	S_1	5/19/71		x	Metra-15		
17.	S_1	10/21/71	Saline	x	Meth-2	Pheno-30	
			Pheno-30				
18.	S_1	12/12/72	Saline	x	Meth-2		
19.	S_1 adult		Saline	x	Meth-2		Dosage reduced during experiment.
20.	S_1 adult	3/15/73	Saline	x	Meth-1		

[a] Drug identification: Meth=Methamphetamine, Pento=Pentobarbital sodium, Pheno=Phenobarbital, d-Amphet=d-Amphetamine, Metra=Metrazol.

[b] Doses are mg of drug per kilo of body weight; thus Meth-1 means 1 mg/k of methamphetamine.

Table 2

Effects of Environment and Methamphetamine on Brain Weights and Body Weights

Strain	Brain Measure	% differences of brain and body wts from Saline-HC group: Meth-HC	Saline-EC	Meth-EC	between: Meth-EC and Saline-EC
S_1	Cortex	N=10	N=43	N=48	N=43
(Experiments 2, 3, 4, 17, 18)	Occipital	2.5	9.7***	13.3***	4.3**
	Total	1.3	4.6***	6.4***	1.9*
	Rest of brain	2.2*	1.4	1.9*	0.4
	Cortex/Rest	-0.9	3.3***	4.4***	1.4**
Fischer	Cortex	N=9	N=26	N=26	N=26
(Experiments 8, 9, 12)	Occipital	-1.4	4.1*	8.0***	3.7
	Total	1.2	4.9***	6.5***	1.5
	Rest of brain	-1.2	2.5*	1.7	-0.9
	Cortex/Rest	2.4*	2.2***	4.8***	2.5***
S_3	Cortex	-	N=11	N=11	N=11
(Experiment 1)	Occipital	-	11.8***	17.5***	5.1*
	Total	-	4.3*	6.7***	2.3
	Rest of brain	-	-0.9	0.6	1.5
	Cortex/Rest	-	5.1***	6.2***	1.0
Overall	Cortex	N=19	N=80	N=86	N=80
	Occipital	0.6	8.0***	12.1***	4.2***
	Somesthetic	0.5	4.7***	6.5***	2.0*
	Rem. dorsal	2.9*	5.7***	6.8***	1.1
	Ventral	-0.1	2.4**	4.4***	2.0*
	Total	1.2	4.5***	6.3***	1.8**
	Rest of brain	0.6	1.4*	1.6*	0.2
	Total brain	0.8	2.8***	3.7***	0.9
	Cortex/Rest	0.7	3.1***	4.6***	1.7***
	Terminal body weight	-3.2	0.2	0.9	0.4

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4

Effects of 30-Min Daily EC for 30 Days
on Brain Weights[a]
(Weights given as % differences from Saline-HC)

N=21 (litters)

Cortex	Saline-EC	Meth-EC	Meth-EC vs. Saline-EC
Occipital	6.7*	11.4***	4.4*
Somesthetic	3.1*	6.5**	3.3**
Rem. Dorsal	3.0*	7.3***	4.1*
Ventral	1.6	2.6	1.0
Total	2.8*	5.7***	2.8*
Rest of Brain	0.9	1.7	0.8
Total Brain	1.7	3.5**	1.7
Cortex/Rest	1.8*	3.9***	2.0*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[a] Values are combined for Experiment 6 (S_3) and Experiment 10 (Fischer)

Table 5

Effects of Two-Hour Daily EC for 15 Days
on Brain Weights and Brain Enzymes
(% differences from Saline-HC)[a]

	Meth HC	Saline EC	Meth EC	Meth-EC vs. Saline-EC
A. Brain Weight				
Occip. Cortex	4.0	0.7	6.0	5.3
Total Cortex	-0.5	0.1	3.5*	3.4*
Rest of Brain	-0.8	-1.2	1.0	2.3
Cortex/Rest	0.4	1.4	2.4*	1.0
B. Acetylcholinesterase/wt				
Occip. Cortex	0.1	0.4	3.0*	2.6*
Total Cortex	-0.8	0.1	1.9*	1.7*
Rest of Brain	2.6***	2.6***	1.6*	-1.0
Cortex/Rest	-3.3**	-2.4*	0.3	2.7*
C. Cholinesterase/wt				
Occip. Cortex	-1.1	2.6	1.2	-1.4
Total Cortex	1.6	4.0*	1.0	-2.9
Rest of Brain	0.3	1.5	0.2	-1.3
Cortex/Rest	1.3	2.5	0.8	-1.6
D. ChE/AChE				
Occip. Cortex	-1.2	2.3	-1.6	-3.9
Total Cortex	2.3	3.8*	-0.9	-4.6**
Rest of Brain	-2.3	-1.1	-1.4	-0.3
Cortex/Rest	4.7*	4.9**	0.5	-4.2*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[a] N=11 for all groups.

Table 6

Effects of Environment and Methamphetamine on Brain Weights and Body Weights among Adult S_1 Rats

Measure	% differences of brain and body weights from Saline-HC group: Saline-EC	Meth-EC	between Meth-EC and Saline-EC
Cortex			
Occipital	6.6**	6.7**	0.0
Total	3.7**	2.5*	-1.1
Rest of Brain	1.0	-1.1	-2.1
Cortex/Rest	2.6**	3.6***	1.0
Terminal Body Weight	4.2	0.5	-3.5

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Based on Experiments 19 and 20; N = 23 per condition.

Table 7

Effects of Metrazol on Brain Weights of Enriched-Environment Rats

(% differences between Metrazol-EC and Saline-EC)

Cortex	Experiment: / N(pairs):	14 / 12	15 / 12	16 / 11	Overall / 35
Occipital		1.4	2.3	1.2	1.6
Somesthetic		1.6	-1.5	2.7	0.8
Rem. dorsal		0.0	1.4	5.9**	2.3*
Ventral		0.1	2.4	5.1	2.5
Total		0.3	1.6	4.8**	2.2**
Rest of Brain		-0.2	-0.3	2.5	0.6
Total Brain		0.0	0.5	3.5*	1.3
Cortex/Rest		0.5	2.0*	2.3	1.6*

* $P < 0.05$, ** $P < 0.01$

Table 8

Effects of Phenobarbital on Brain Weights in Home Cage and in Enriched Condition

Exper. No.	Strain	N (litters)	Brain Measure	% differences from Saline-HC: Pheno-HC	Saline-EC	Pheno-EC	% diff. Pheno-EC vs. Saline-EC
3	S_1	9	Occip. Cortex	-	11.5*	4.3	-6.4*
			Total Cortex	-	2.5	-1.1	-3.5*
			Rest of Brain	-	-0.4	-2.9	-2.5
			Cortex/Rest	-	3.0*	2.0	-1.0
5	S_1	8	Occip. Cortex	-0.5	-	5.0	-
			Total Cortex	-0.7	-	0.6	-
			Rest of Brain	-2.9	-	-2.1	-
			Cortex/Rest	2.2	-	2.7	-
9	Fischer	7	Occip. Cortex	-	7.6	3.2	-4.0
			Total Cortex	-	4.1*	0.8	-3.2
			Rest of Brain	-	1.7	-1.0	-2.7
			Cortex/Rest	-	2.3*	1.8	-0.5
17	S_1	12	Occip. Cortex	-4.1*	6.1**	2.0	-3.9
			Total Cortex	-2.0	2.9	1.4	-1.4
			Rest of Brain	-1.8	-1.5	-0.6	0.9
			Cortex/Rest	-0.2	4.4***	2.0*	-2.3*
Overall			Cortex	N=20	N=28	N=36	N=28
			Occipital	-2.6	8.2***	3.5*	-4.7**
			Somesthetic	-1.8	5.5***	4.6**	-0.4
			Rem.dorsal	1.0	3.9*	1.3	-2.5*
			Ventral	-3.6**	0.6	-1.8	-2.5
			Total	-1.5	3.1**	0.5	-2.6**
			Rest of Brain	-2.3	-0.3	-1.6	-1.2
			Total Brain	-1.9	1.2	-0.7	-1.8*
			Cortex/Rest	0.7	3.4***	2.1***	-1.4**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 9

Effects of Phenobarbital on Brain Enzymes in Home Cage and in Enriched Condition (S_1 strain)

Brain Measures	% difference from Saline-HC: Pheno-HC N=20	Saline EC N=21	Pheno-EC N=29	% diff. Pheno-EC vs. Saline-EC N=21
A. Acetylcholinesterase/wt				
Occipital Cortex	0.6	-1.4	-2.0	-2.0*
Total Cortex	0.7	-1.0	-2.8***	-3.0***
Rest of Brain	0.3	1.3	0.0	-1.6*
Cortex/Rest	0.3	-2.2	-2.7**	-1.3
B. Cholinesterase/wt				
Occipital Cortex	0.4	2.4*	2.5	0.3
Total Cortex	-0.3	2.0**	2.6**	0.9
Rest of Brain	0.7	1.1	1.2	0.2
Cortex/Rest	-0.9	0.8	1.4*	0.8
C. ChE/AChE				
Occipital Cortex	-0.4	3.6**	4.3**	2.3
Total Cortex	-1.1	2.9*	5.5***	4.2**
Rest of Brain	0.2	-0.2	1.2	1.8*
Cortex/Rest	-1.3	3.0	4.3***	2.4

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

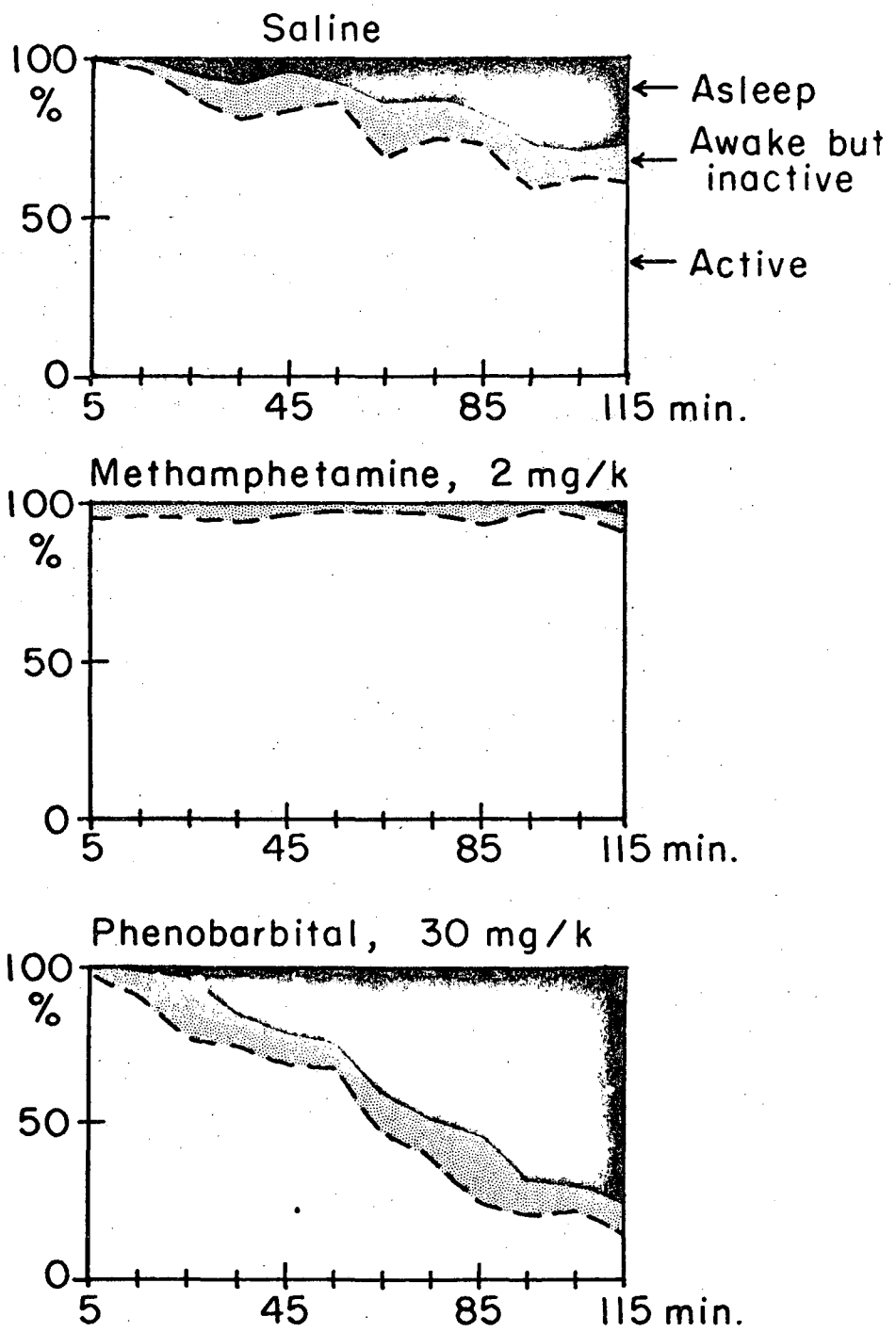

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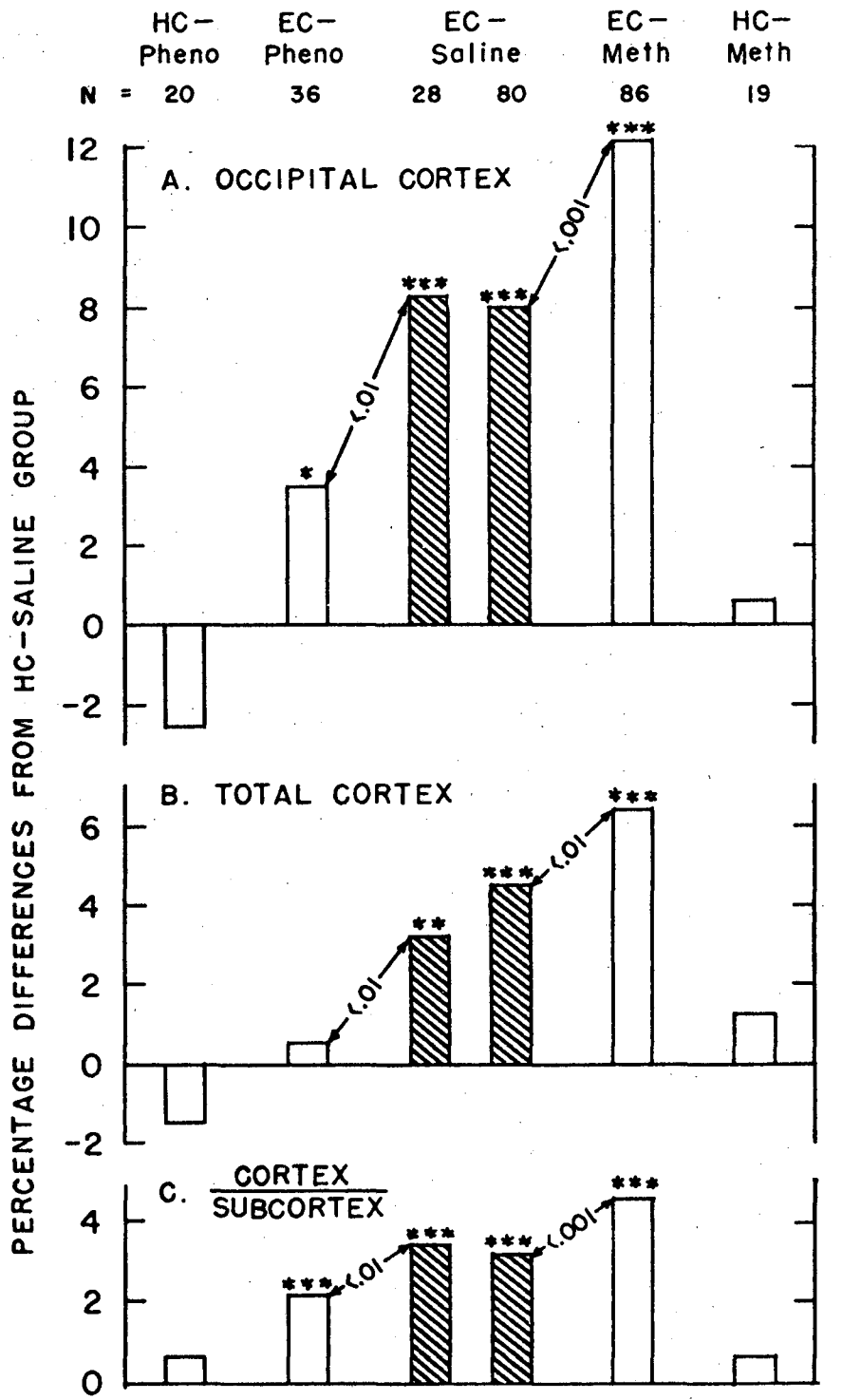
Fig. 1


HC– Pheno
EC– Pheno
EC– Saline
EC– Meth
HC– Meth
N = 20 36 28 80 86 19
A. OCCIPITAL CORTEX
B. TOTAL CORTEX
C. CORTEX / SUBCORTEX
PERCENTAGE DIFFERENCES FROM HC–SALINE GROUP
12
10
8
6
4
2
0
-2
*
**
***
<.01
<.001

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