1·0

2·8   2·5

3·15   2·2

3·5

1·1   4·0   2·0

4·5

1·8

1·25   1·4   1·6

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MICROCOPY RESOLUTION TEST CHART

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REPORT NUMBER 8

A NEUROPSYCHOLOGICAL BASIS FOR DRUG SUBSTITUTION

ANNUAL PROGRESS REPORT

HAROLD C. NIELSON PH 801 581-8889

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The effects of librium, meprobamate, chloral hydrate, pentobarbital, paraldehyde and ethanol upon brain thresholds is detailed. In addition, the sequence of changes of the reticular formation and the caudate nucleus during the acquisition of physical dependence to pentobarbital, and also during withdrawal from pentobarbital is described. It was hypothesized that the development of drug dependence is characterized by a double dissociation, the peripheral nervous system from the central nervous system and the motor system from the arousal system.

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FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide of Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences-National Research Council.

TABLE OF CONTENTS

SUMMARY

Experiment I: Effects of drugs on Brain Thresholds.

Cats, under general anesthesia, have been fitted with chronic electrodes, that have been stereotaxically placed in the medial geniculate body, mesencephalic reticular formation, and the caudate nucleus. Following recovery from this surgery, they were trained to avoid a right foreleg footshock by making a conditioned response (CR was a right foreleg flexion) to the conditioned stimulus (CS) which was direct electrical stimulation of one of the implanted neural sites. When the cats were trained, conditioning thresholds were determined by repeatedly lowering the intensity of the CS until no CRs were obtained. The CS intensity that maintained 50% CRs was defined as the conditioning threshold. After stable conditioning thresholds were determined the effects of various drugs in various dosage levels and combinations, upon the conditioning thresholds was determined. The drugs, tested singly and in combination, at various dosage levels are pentobarbital, paraldehyde, librium, chloral hydrage, meprobamate, and ethyl alcohol.

Experiment II: Drug Substitution.

Cats have been fitted with chronically implanted electrodes, and received avoidance training. The surgical and training procedures are identical to those of the first experiment with one exception. Whereas the cats in the first experiment were trained while they were in the normal, non-drugged state, these cats were trained while they were intoxicated, following an ip injection of pentobarbital (12.5 - 15.0 mg/kg), so that their responses would be state dependent and give CRs when they are drugged but not while they are sober. Thus, when the animal is given a substitute drug it will give CRs if the substitute drug produces the same effects as pentobarbital. In this report we show that paraldehyde, librium, chloral hydrate, meprobamate, in single doses, and in drug combinations that abolish CRs in experiment I effectively substitute for 15 mg/kg pentobarbital. In addition, we show that the effect of drugs upon brain thresholds, as measured by changes in conditioning thresholds, is a function of the interaction of the drugged state and learning, and not strictly upon the pharmacological effects of the drug alone. The conditioning thresholds of cats initially trained under pentobarbital being progressively elevated as the dosage level of pentobarbital was progressivly decreased. This is in direct contrast to the normally trained cats where progressive increases in pentobarbital produced progressive increases in conditioning thresholds.

Experiment III. Effects of barbiturate addiction upon brain thresholds.

The method and procedure is like that of experiment I in that the cats are surgically fitted with brain electrodes, conditioned, and conditioning thresholds taken. In addition, forced movements were elicited, and kindling seizures established and the threshold for both the forced movement and the seizures were determined. The cats were then made physically dependent upon, and withdrawn from pentobarbital. The course of the addiction and withdrawal upon the conditioning, seizure, and forced movement thresholds was followed. In this report we show that there is no supersensitivity of the central nervous system during withdrawal seizures. There were only slight increases

in the seizure and forced movement thresholds but not decreases in thresholds as demanded by a supersensitivity theory. In this report we summarize and discuss the method of producing physical dependence upon pentobarbital in cats; we detail the dissociation of pentobarbital effects upon the elevation in conditioning thresholds of caudate nucleus and the mesencephalic reticular formation, and show the relatively small increases in seizure and forced movement thresholds.

Experiment IV. A comparison of drug effects upon conditioned responses established to peripheral stimulation or to direct electrical stimulation of the brain.

The method and procedure was like that of experiment I except that these cats were conditioned to a 1000 hz tone as well as to direct electrical stimulation of brain tissue. The results show that in every instance, and in the same cats, the CRs established to the tone were abolished before those established to direct brain stimulation. Furthermore, attempts to establish CRs to either a tone, or a light CS while the cat was drugged with 12.5 mg/kg pentobarbital were unsuccessful despite extensive training while CRs to electrical stimulation of the brain were successful.

BODY

The ultimate goal of this research goal is to determine which drugs, or drug combinations can effectively substitute for pentobarbital and block withdrawal seizures in cats that have been made dependent upon pentobarbital. A second goal is to determine what brain mechanisms may be operating during the addictive process. To achieve these goals, a series of four related experiments have been, and still are being conducted.

The function of the first experiment is to provide a screening of drugs, thought to have some effects in common with pentobarbital. The screening of these drugs is to determine whether they have similar effects upon brain excitability levels, determined by conditioning thresholds, as does pentobarbital and to provide a means of equating dosage levels of the various drugs to produce equivalent effects. The function of the second experiment is to determine whether the cat equates the drugs the same way that the experimenter does and what drugs and at what dosage level they can substitute for pentobarbital. The purpose of the third experiment is to follow the course of the development of physical dependence upon barbiturates, the time course of withdrawal symptoms, and the changes in the excitability levels that accompany withdrawal seizures and symptoms. The fourth experiment was designed to determine whether the drugs used in the first three experiments differentially alter or affect conditioned responses elicited by peripheral (tone) stimulation or by direct electrical stimulation of the brain.

In all of the experiments the method for recording changes in brain excitability levels is the conditioning procedure described by Doty, Rutledge, and Larsen (1956) and modified by Nielson, Knight, and Porter (1962). Cats are trained to give a right foreleg flexion conditioned response (CR) with direct electrical stimulation of neural tissue as the conditioned stimulus (CS). When the CR is well established, the intensity of the CS is lowered until no responses are elicited, and raised again until the animal is again responding. Threshold intensities, the measure of brain excitability used here, is defined as the intensity of the CS that produces CRs 50% of the time. Such thresholds are stable over long periods of time in the normal animal (Nielson and Davis, 1966), yet are sensitive to changes in neural excitability levels produced by electroconvulsive shock (Nielson, 1968), brain lesions remote from the site of CS stimulation (Nielson and Davis, 1966), anticonvulsant drugs (Nielson, Justesen, and Porter, 1968) and the drugs reported here. Furthermore, the acquisition and maintenance of these CRs can be state dependent, (Pusakulich and Nielson, 1972).

Experiment I: Experiment I is designed to determine which drugs, and at what dosages, produce changes in brain thresholds comparable to those produced by an intraperitoneal injection of 15 mg/kg sodium pentobarbital. This was accomplished by stereotaxically placing bipolar electrodes in a variety of brain areas of cats, but especially in the medial geniculate body, mesencephalic reticular formation and the caudate nucleus. When the cats had recovered from this surgery they were habituated to a conditioning apparatus, which allowed movement of the head and limbs but limited gross locomotion until they became tolerant of this restraint and remained quiet. Then, avoidance

conditioning training was started. The conditioned stimulus (CS) was a train of electrical square wave pulses delivered to a particular brain site, through the chronically implanted electrodes, for 2 sec. The unconditioned stimulus (US) was also a train of electrical square wave pulses, .2 sec. in duration and overlapping the CS by 50 msec., that was delivered to the cat's right foreleg through a leg cuff and a grid upon which its leg is placed. The conditioned response (CR) was a flexion of the right foreleg which broke the US circuit and allow the animal to avoid the US. When the animal had learned to give a high number of CRs, the same training procedure was carried out with electrical stimulation of another brain site as the CS. Each cat was trained to give CRs to electrical stimulation of at least two different brain sites.

When avoidance training was complete the threshold of each brain site was determined. This was done by lowering the intensity of the CS in blocks of five trials until no CRs were obtained. The intensity of the CS was then gradually increased until a high level of performance was again obtained. This process was repeated several times a day for several days until stable thresholds, defined as the CS intensity that gives 50% CRs, has been determined. After stable thresholds had been determined, the experimental animal was given a low drug dose and thresholds were again determined. If there were no identifiable threshold changes produced by the drug, the animal was returned to its home cage. The following day a larger drug dosage was given and thresholds again taken. The drug dosage was progressively increased until threshold changes did occur or until the animal was sufficiently intoxicated that it could not perform. When a given dosage of a drug results in a shift in brain thresholds, the animal was tested two, four and eight hours later until the drug wore off and the thresholds returned to normal. After thus determining the drug effect and its time course upon the brain thresholds, another drug dosage level was given and the duration of effect determined. This continued until a range of dosage levels had been given so that the smallest dosage had no effect upon the threshold while the largest dose completely abolished the CRs. When a dose response curve was thus determined in one cat it was verified in other cats that had never received that drug before. The same procedure was used when different drug combinations were investigated.

The effects of the various drugs and drug dosages are summarized in figures 1-12. Each of the curves in figures is based upon the data obtained from at least three cats with that electrode placement and that drug, and each figure is based upon at least five cats. The effects of various drug dosages upon the conditioning threshold of the caudate nucleus is summarized for paraldehyde in figure 1, for librium in figure 2, for chloral hydrate in figure 3, for meprobamate in figure 4, and for ethyl alcohol in figure 5. Similar dose response curves were obtained for these drugs upon the conditioning thresholds of the medial geniculate body and the mesencephalic reticular formation. The only differences in the drug effects upon the medial geniculate body and the caudate nucleus were found with librium and meprobomate. The effects of these two drugs upon the excitability level of the medial geniculate body is shown in figure 6 for librium, and figure 7 for meprobomate. The differences in the drug effects upon the caudate nucleus and the medial geniculate body are directly compared in figure 8 for librium and figure 9 for meprobamate. Inspection of these figures shows that there were differences in CR thresholds of these two brain structures only with the intermediate dosage levels, and these differences showed the caudate nucleus to be slightly more sensitive

to the drugs than was the medial geniculate body. There were no other dif-
ferences in the drug effect between the caudate nucleus and the medial
geniculate body. The drug effects upon the mesencephalic reticular formation
were identical to those upon the caudate nucleus and are not reproduced here.

The different drug dosage combinations were next determined. Because
of the similarity of the drug effects upon the three brain structures the
drug combination effects are detailed only for the caudate nucleus. Figures
10 and 11 show the effects of different combinations of librium and meprobamate
upon the conditioning thresholds of the caudate nucleus. Figure 10 compares
the low dosage levels and shows that the combination of 5 mg/kg librium,
which singly produced only a transient effect upon brain excitability, and 25
mg/kg of meprobamate, which singly had no effect upon brain excitability,
produced a small, but very long lasting depression of brain excitability.
Figure 11 shows the effect of larger dosages of the librium-meprobamate
combination. A single dose of meprobamate of 50 mg/kg decreased the ex-
citability of the caudate nucleus for eight hours when given alone. However,
when this dose of meprobamate was combined with librium of 10 mg/kg, which
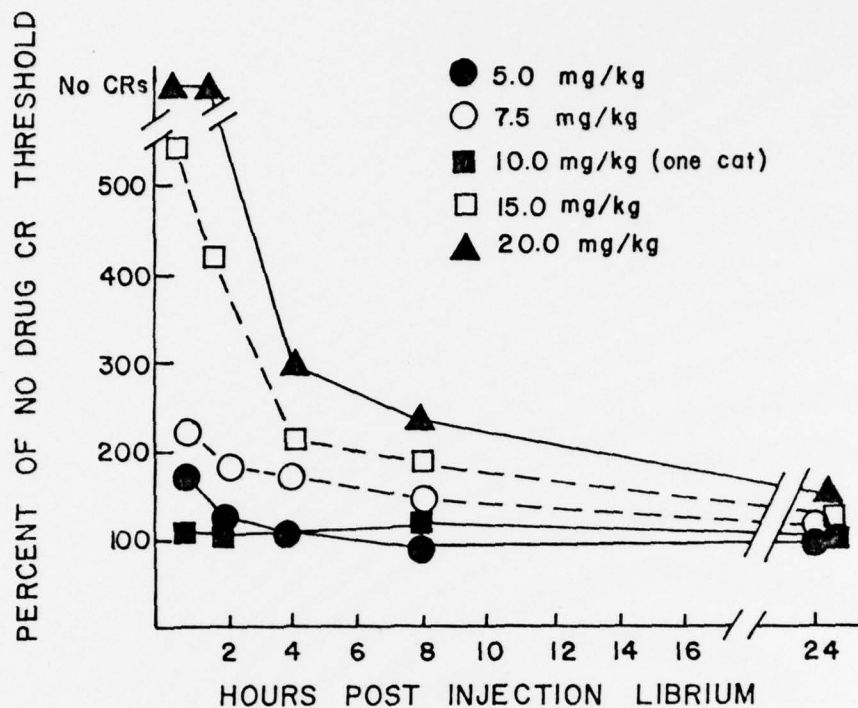singly had no effect upon brain excitability levels, produced a profound
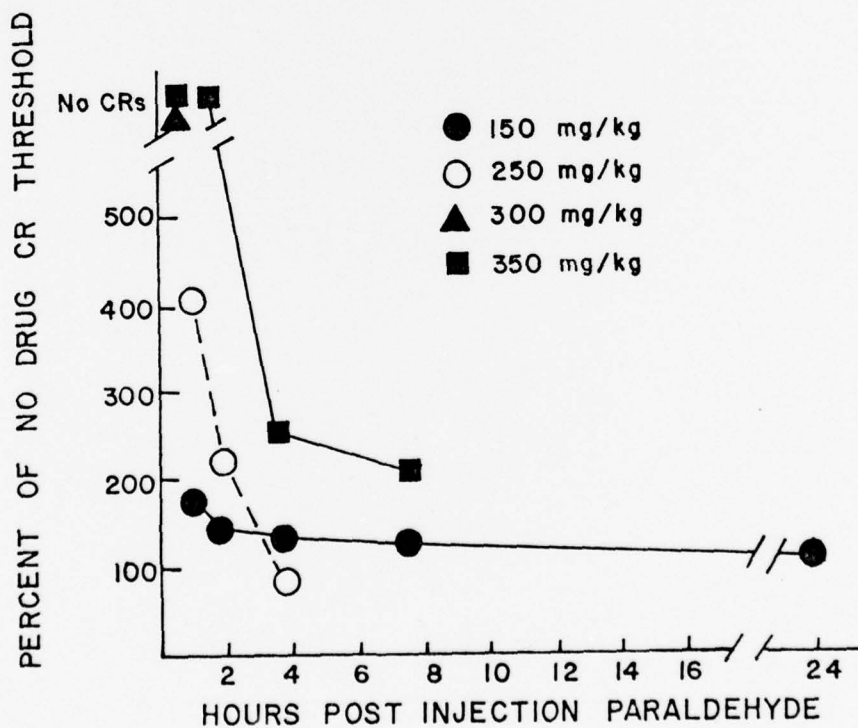
Figure 2. Changes in conditioned response thresholds of the caudate nucleus as a function of dosage level and time since administration.
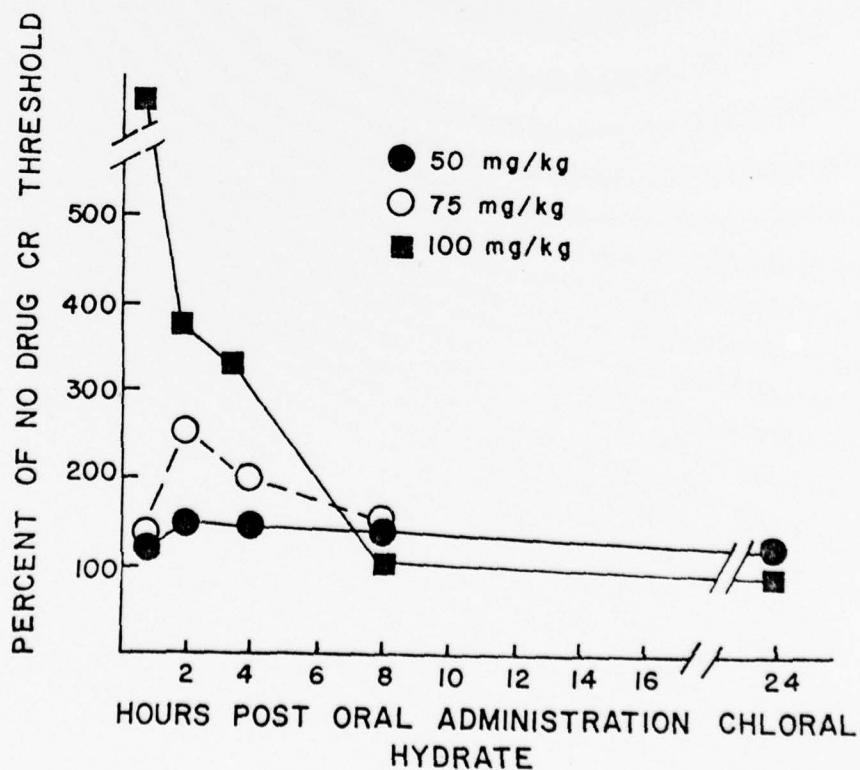

Figure 3. Changes in conditioned response thresholds of the caudate nucleus as a function of dosage level and time since administration.
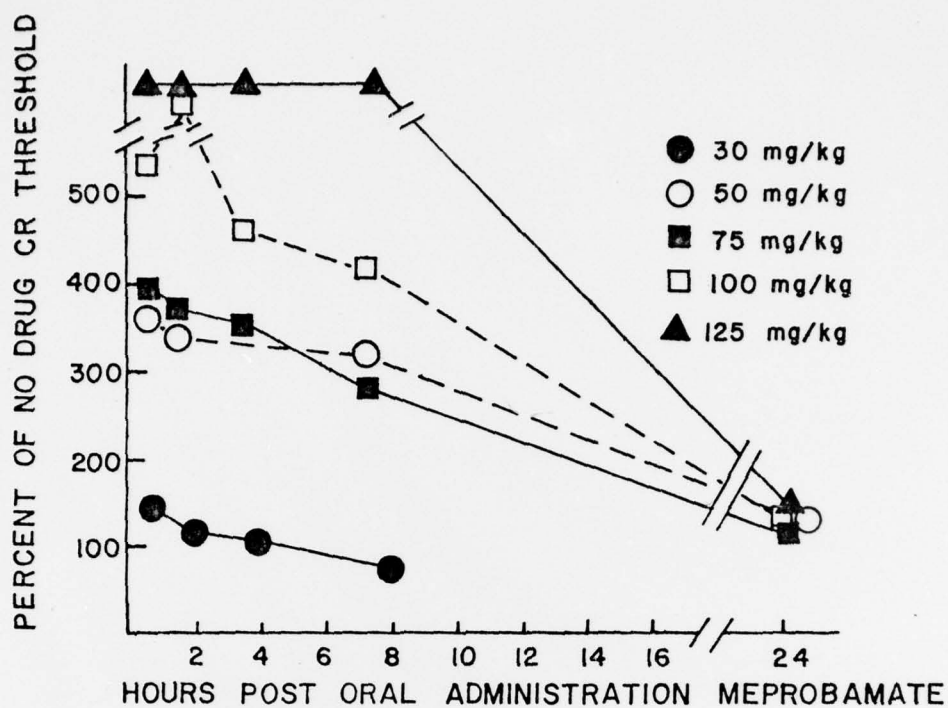
Figure 4. Changes in conditioned response thresholds of the caudate nucleus as a function of dosage level and time since administration.
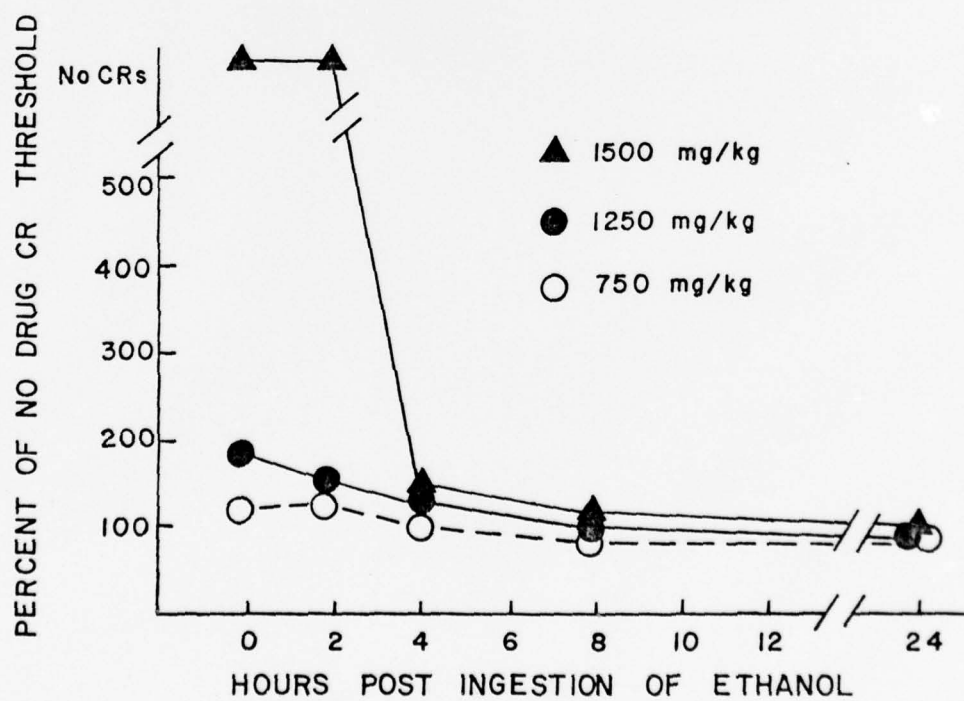

Figure 5. Changes in conditioned response thresholds of the caudate nucleus as a function of dosage level and time since administration.
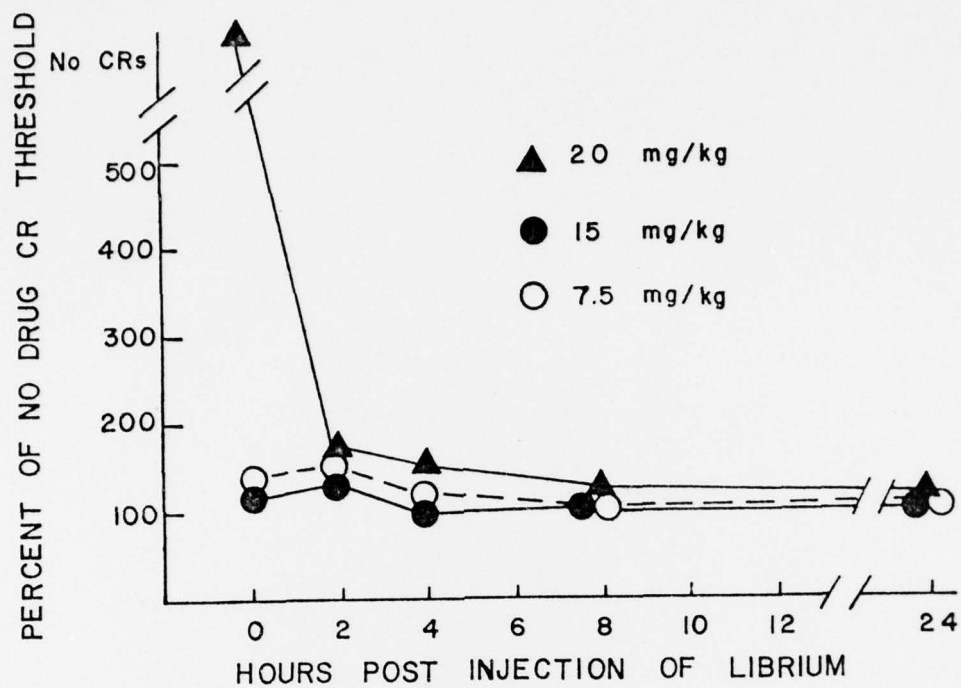
Figure 6. Changes in conditioned response thresholds of the medial geniculate as a function of dosage level and time since administration.
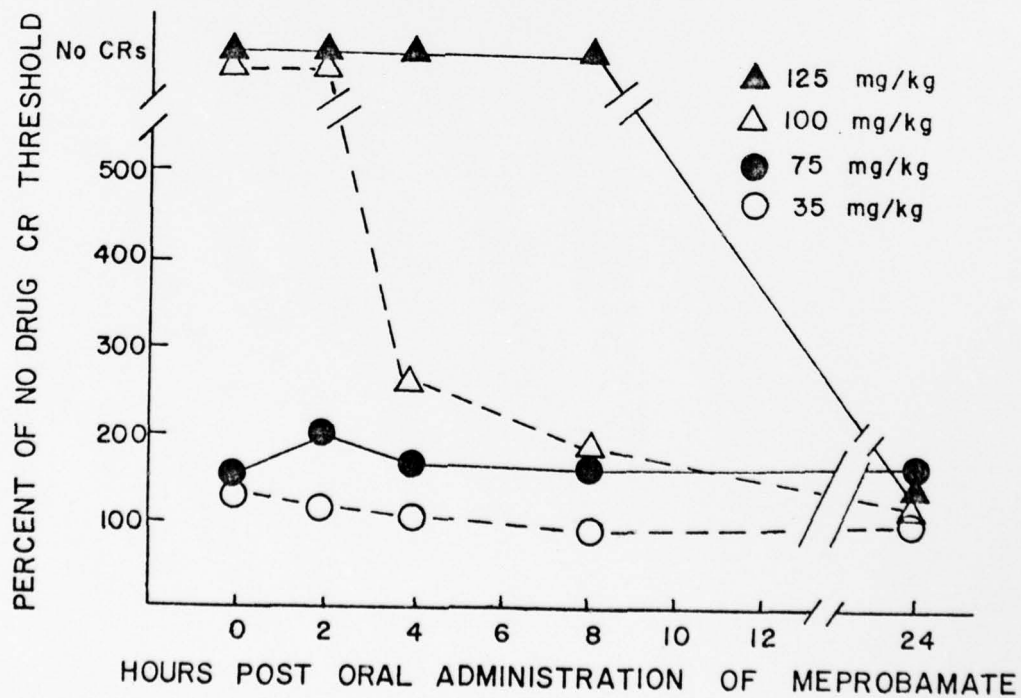

Figure 7. Changes in conditioned response threshold of the medial geniculate as a function of dosage level and time since administration.
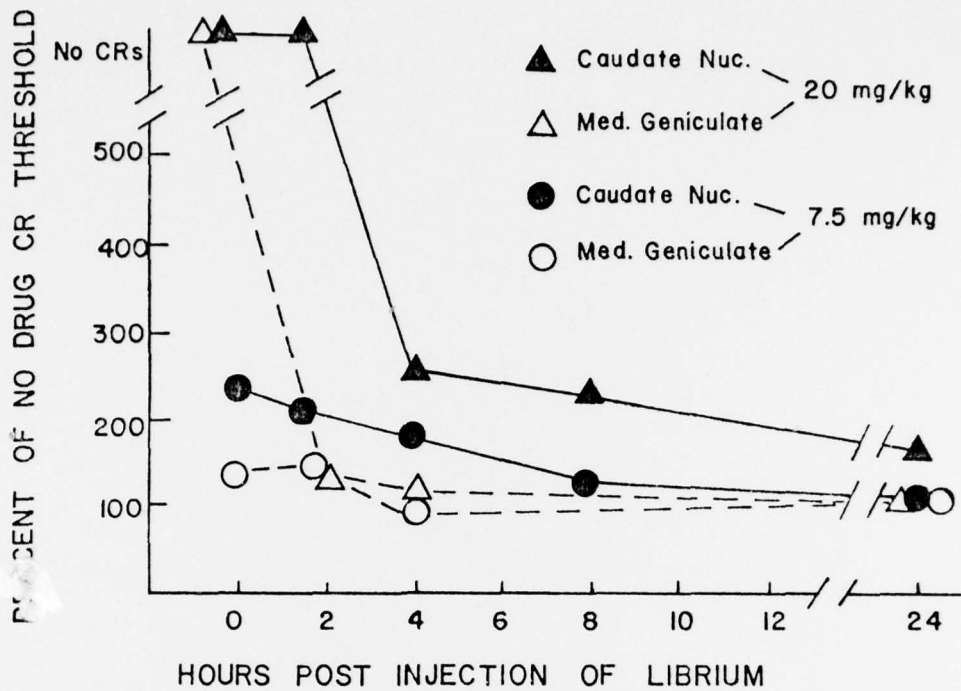
Figure 8. Comparison of the effects of Librium (20 mg/kg and 7.5 mg/kg) upon the conditioned response thresholds of the caudate nucleus and the medial geniculate.
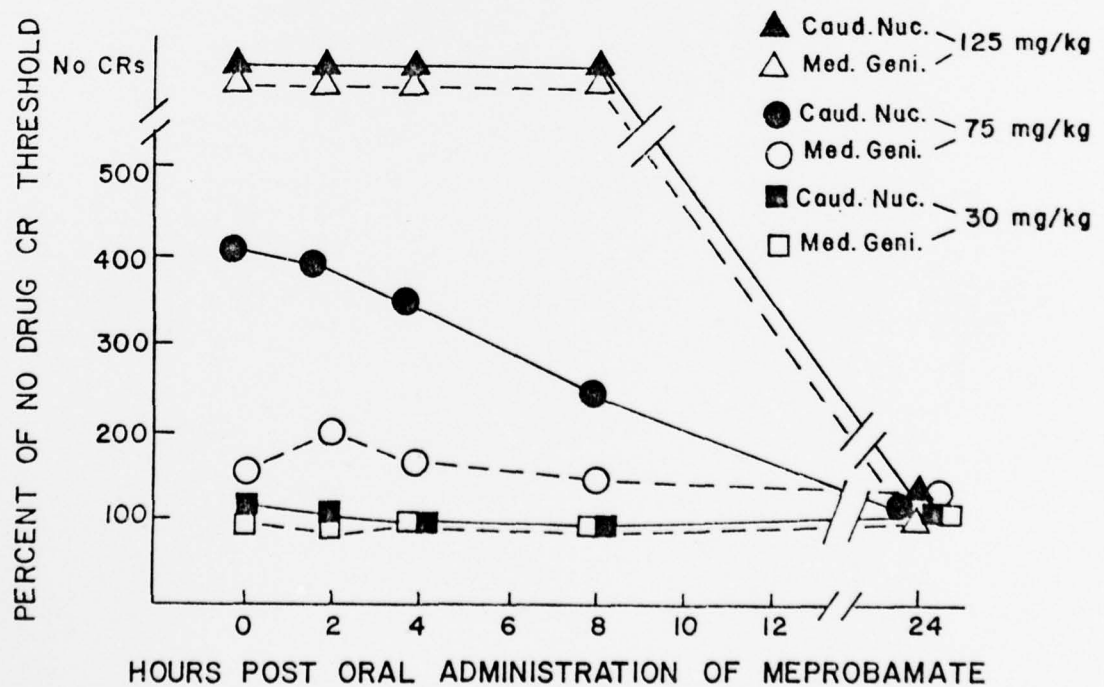

Figure 9. Comparison of the changes in CR threshold for the caudate nucleus and medial geniculate following oral administration of meprobamate.

depression of the excitability level of the caudate nucleus such that the conditioned responses were lost for over eight hours. We now believe that any combinations of paraldehyde, librium, chloral hydrate, meprobamate, and ethyl alcohol, where the combination is one half of the dosage level that abolished the CRs for any drug is combined with one half of the dosage level of any other drug that abolished the conditioned response the two drugs will combine and abolish the CR for long periods of time. The potentiation of drug effects is great when its depression upon the conditioning thresholds is measured, but the greatest effect of the drug potentiation is upon prolonging the depression of brain excitability. Thus, the combination of these drugs produces a depression of CRs for four to six times as long as does a single dose of a single drug given in twice the amount of any of the drug given in a drug combination.

To emphasize the nearly identical effects of the drugs effects upon the caudate nucleus and the mesencephalic reticular formation the responses of these two structures to the meprobamate-librium drug combinations is shown in figure 12. The data for the figures were taken from cats from which CR thresholds were taken from both the caudate nucleus and the mesencephalic reticular formation, so that it represents data within cats. Similar
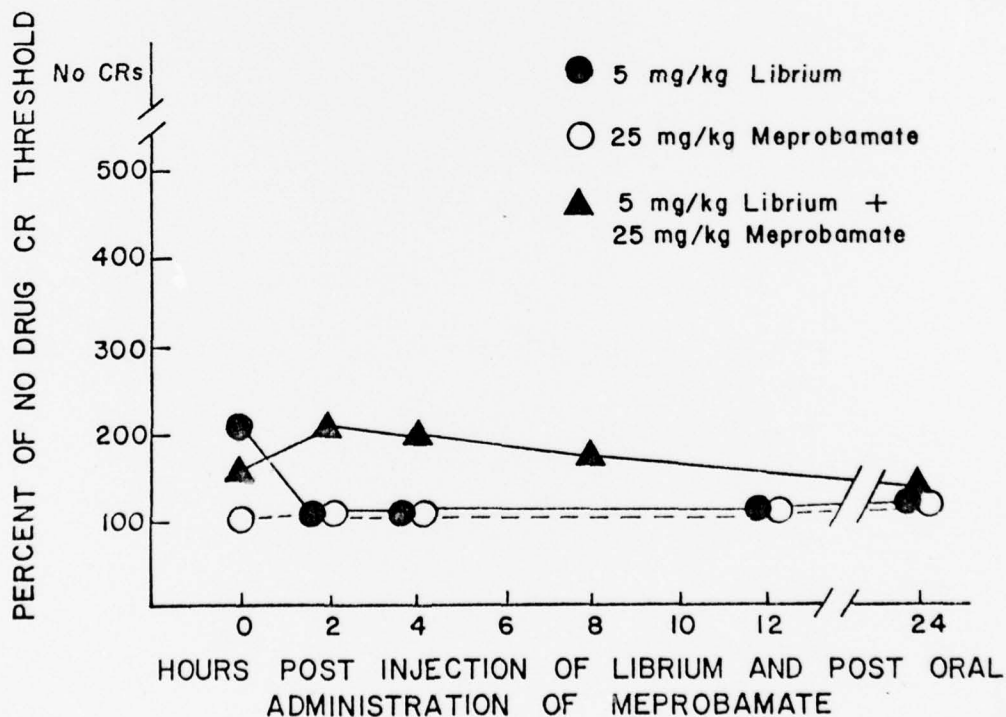

Figure 10. Effects of Librium (5 mg/kg) and Meprobamate (25 mg/kg) administered singly or in combination upon the conditioned response threshold of the caudate nucleus.
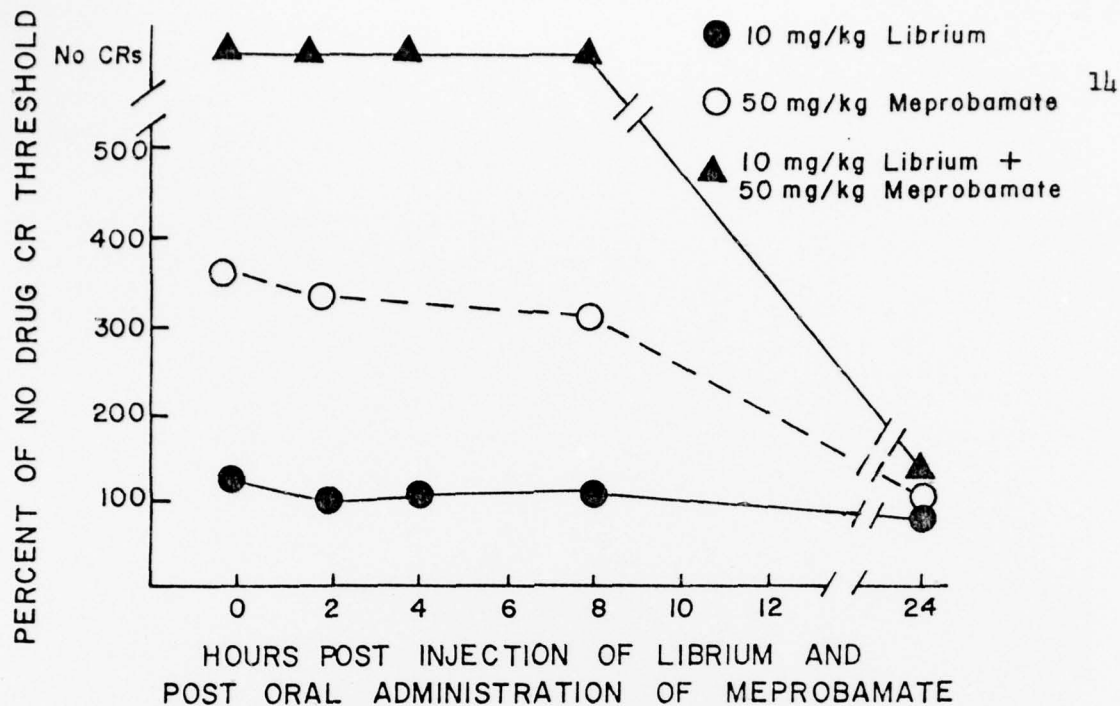
Figure 11. Effect of Librium (10 mg/kg) and Meprobamate (50 mg/kg) administered singly or in combination upon the conditioned response thresholds of the caudate nucleus.
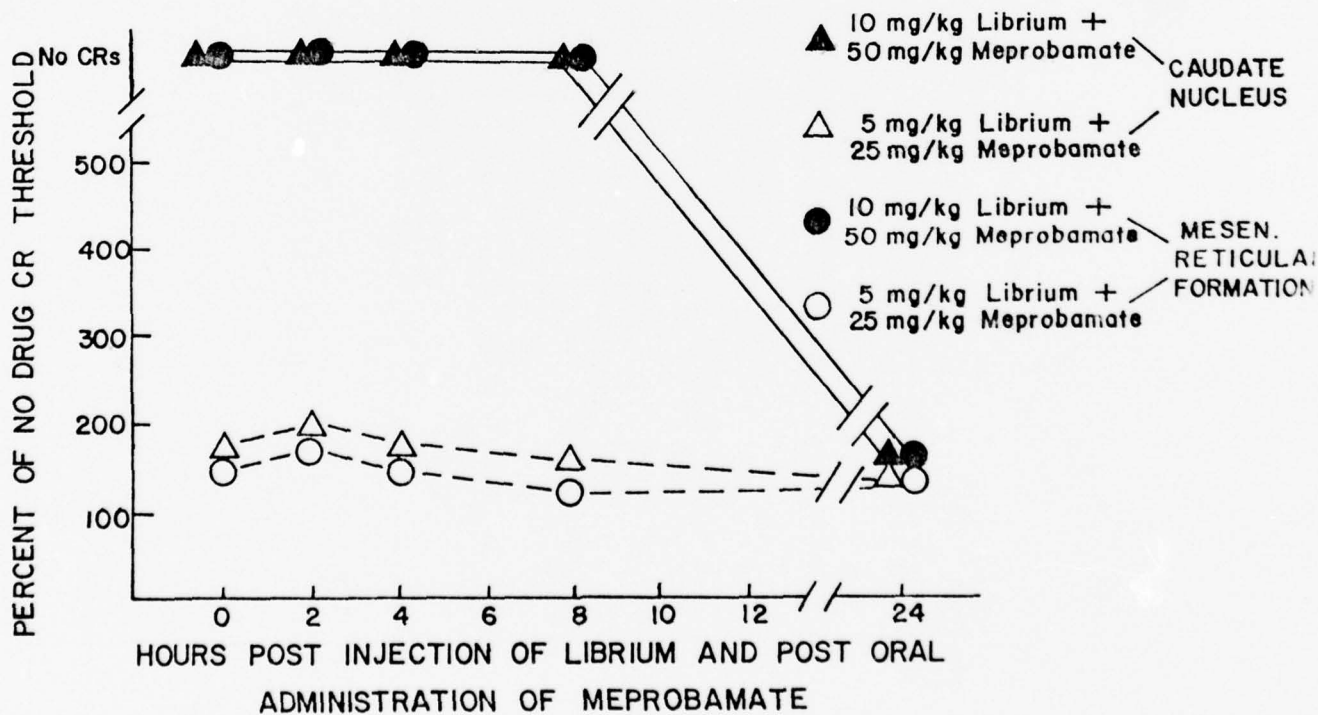

Figure 12. Comparison of the effects of combinations of Librium and Meprobamate upon the conditioned response thresholds of the caudate nucleus and the Mesencephalic Reticular Formation.

comparisons between cats that had CR thresholds from only one or the other cites showed the same thing. The drugs all effected the caudate nucleus and the mesencephalic reticular formation in the same way. The identical nature of the drug effects upon these two structures is emphasized here because we find a dissociation of the drug effects on these two structures as the cat becomes addicted to pentobarbital.

Experiment II. Drug substitution. In this experiment we determine the extent to which a cat views a variety of dosage levels given singly and in combinations, of paraldehyde, librium, chloral hydrate, and meprobamate as producing the same drugged state as that produced by an ip injection of 15 mg/kg of pentobarbital. We can do this because these drugs all produce state dependent learning. This is a phenomenon in which a response acquired by an animal under the influence of a drug is lost when the animal is no longer under the influence of that drug, but re-appears when the animal is again in that drugged state. Conversely, responses acquired while the animal is in the normal non-drugged state are lost when the animal is in a drugged state but re-appear as the drug wears off. Thus the maintenance of the response is dependent upon the maintenance of the drugged or non-drugged state that existed at the time of response acquisition. If both these conditions are met, the response is said to be state dependent.

While not all drugs produce state dependent learning, the action of those that do is principally upon the central nervous system. These drugs can also serve as discriminative stimuli, may be used clinically to alleviate fear and anxiety, and are frequently abused and produce drug dependence. These drugs may produce similar effects, they develop cross tolerance, produce somewhat similar intoxications and are frequently substituted for each other. This last fact is particularly important in the initiation of abstinence (Vallient, 1969) in that, since the time of Himmelsbach and Andrews (1943) drug substitution has played a major role in drug therapies for addiction. The state dependent learning paradigm offers a particularly good method for determinining the extent to which one drug can substitute for another because the substitute drug must maintain a behavior. In this experiment it is a conditioned response maintained in a drugged state produced by an ip injection of 15 mg/kg pentobarbital.

The specific training procedure is identical to that of the first experiment. The general training procedure differs from the first experiment only in that these animals begin each training session after receiving an ip injection of 12.5 to 15.0 mg/kg pentobarbital. Because all training is conducted while the cat is in the drugged state the CRs are state dependent.

To put the results of the drug substitution tests in perspective the effects of various dosage levels of pentobarbital upon the training thresholds and percent CRs of drugged trained and non-drugged (normal) trained cats is shown in Figures 13 and 14. These figures show that as the dosage level of pentobarbital changed away from the training state there was a progressive loss of CRs and an increase in CR threshold. Thus giving normally trained animals (those in experiments I and 4) increasing doses of pentobarbital progressively elevates the CR threshold and decreases the percentage of CRs. Nearly the reverse was true for those cats trained in the drugged state

produced by 12.5 mg/kg of pentobarbital. For these cats the thresholds in-
creased as the dosage level of pentobarbital was decreased. Similarly, the
percentage of CRs decreased as dosage level of pentobarbital decreased until
CRs were lost. Thus we can conclude that CR thresholds are a function of the
animals training state, and deviations from the training state produce eleva-
tions in CR thresholds, a reduction and finally a loss of CRs. We can con-
clude that the CR thresholds are a function experience in a state and not
the pharmacological action of the drug alone.

The dissociative dose of pentobarbital for the normally trained cats
was 12.5 mg/kg which was the training dose for the drugged trained cats.
The results from our drug substitution tests show that in each instance, the
dissociative dose of the substitute drug, the dosage level that abolished the
CRs in experiment I, was an adequate substitute for 15 mg/kg penobarbital.
Paraldehyde at 300 and 350 mg/kg, chloral hydrate at 100 mg/kg, librium at
20 mg/kg and meprobamate at 100 mg/kg all substituted for 12.5 mg/kg of
pentobarbital and maintained state dependent responding. Ethyl alcohol was
not tested because these cats were not fitted with tubes so that alcohol
could be administered without the cats vomiting it up. Smaller drug dosage
levels of these drugs that were not dissociative and did not abolish CRs in
experiment I, did not substitute for pentobarbital and produced very limited
responding. Thus the adequate substitute dose in this experiment was the
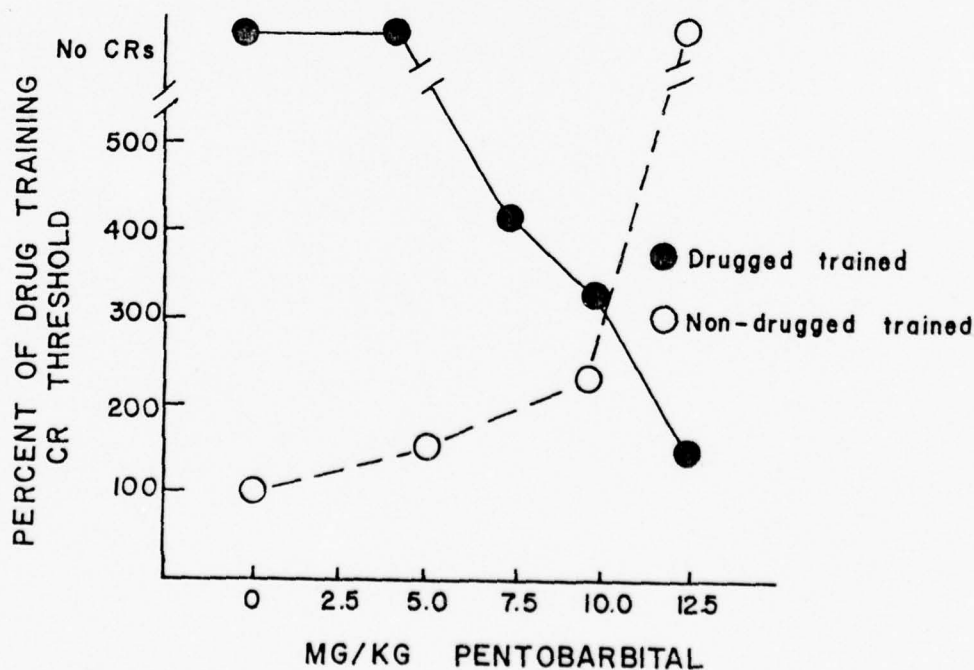dissociative dose found in experiment I.

Figure 13. Comparison of the effects of variation in the dosage levels
of pentobarbital upon the training threshold of drugged and nondrugged trained
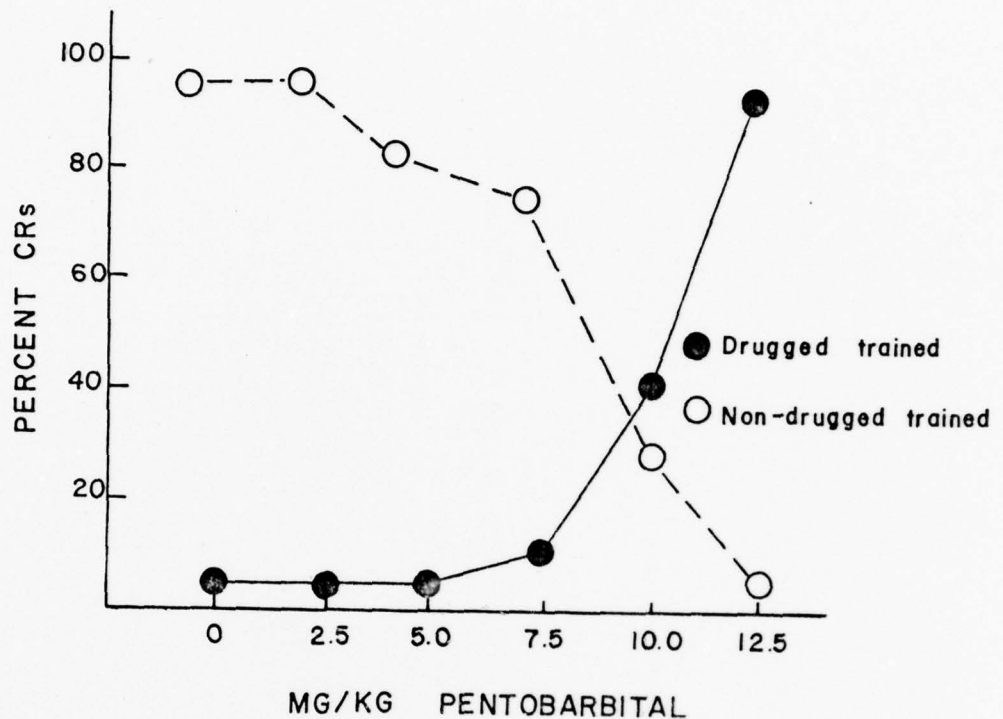cats.

Figure 14. Comparison of the effects of various dosage levels of pentobarbital upon the percent CRs of cats trained in the drugged (12.5 mg/kg) state and the nondrugged (normal) state.

The different substitute drugs had somewhat different side effects upon the cats. Paraldehyde (300 mg/kg) produced a cat that early in the training session seemed much more sedated than with 12.5 mg/kg pentobarbital, but as testing continued these cats, unlike those in the first experiment that were normally trained and received the same dosage level, seemed to experience a general distress. However, they continued to give good CRs throughout the session. When the cats received chloral hydrate (100 mg/kg) they were calm and quiet throughout the sessions. Their CRs were good but they were not as good as they were with pentobarbital. Librium (20 mg/kg) produced cats that were jittery and agitated and seemed very nervous and restless. Their CRs were good and remain so. Meprobamate (100 mg/kg) produced a cat that was calm and seemed to be less sedated than with pentobarbital. The CRs were slow but full. The drug combination of librium (10 mg/kg) and meprobamate (50 mg/kg) which also produced dissociation of the CRs in experiment I also substituted for the pentobarbital. This drug combination produced cats that were very difficult to handle. They seemed more jittery and agitated than when they received librium alone. Their CRs were good, however. A combination of chloral hydrate and meprobamate, made up of half the dissociative dose of each (chloral hydrate 50 mg/kg and meprobamate 50 mg/kg) was also tested. These drugs separately had produced cats that were calm and well

behaved. This drug combination substituted for pentobarbital in that the cats gave better than 50 percent CRs, although the CRs were poor in quality. The cats themselves were very calm, easy to handle and seemed alert. Thus a drug combination, mixed 1/2 and 1/2 of the dissociative doses of the respective drugs can substitute for 15 mg/kg pentobarbital.

Drug	Dose mg/kg	% Pentobarbital Threshold
Paraldehyde	300	90
Chloral hydrate	100	90
Librium	20	140
Meprobamate	100	50
Librium and meprobamate	10 and 50	120
Chloral hydrate and meprobamate	50 and 50	25

Table 1.   The CR threshold differences of substitute drugs and drug combinations given in percentage of pentobarbital state (12.5 mg/kg) CR thresholds.

Threshold determinations taken for each of the drugs and drug combinations is shown in Table 1. The thresholds are presented as percentage of pentobarbital state (12.5 mg/kg). CR thresholds: Most of the thresholds were fairly close to the CR thresholds obtained in the pentobarbital state. However, the chloral hydrate-meprobamate combination produced thresholds that were considerably lower than the pentobarbital state threshold. It should also be pointed out that this deviation in threshold was of the greatest magnitude and also produced the poorest CRs. It is different from the expected changes in that all previous deviations from the training state have produced increased in CR thresholds. Except for librium, all the other substitute drugs have produced decreases in CR thresholds. This was not particularly expected. Nevertheless, we have found in experiment III that there is an increase in CR thresholds of the caudate nucleus as the cat becomes addicted, and have hypothesized that this increase may represent a partial, but functional loss of reflex inhibition.

Experiment III: Drug induced changes of barbiturate withdrawal symptoms.
    Phenobarbital has been selected as the drug of choice for treatment of withdrawal from barbiturate dependence (Smith and Wesson, 1971) even though synergistic action of a variety of other drugs with the barbiturates has been reported (Wahlstrom, 1970; Gibbins, Kalant, LeBlanc, & Clark, 1971; Davis, Kind, & Babbini, 1971; Smith & Wesson, 1971). The extent to which those drugs that have a synergistic action with the barbiturates will alter pentobarbital

withdrawal symptoms is the ultimate goal of this experiment. A further goal is to determine the extent to which the withdrawal of pentobarbital from animals that are physically dependent upon pentobarbital, is reflected in changes in brain excitability levels, and whether the blocking of withdrawal symptoms by phenobarbital and other drugs represents a restoration of neural thresholds to pre-withdrawal or pre-addiction levels. Furthermore, the drug used to block or alter the withdrawal symptoms will actually be a combination of drugs, identified in the first experiment by their ability to reproduce the threshold shifts produced by pentobarbital, and identified in the second experiment by their ability to substitute for pentobarbital and maintain state dependent CRs.

Method and Procedure:

Cats have been surgically implanted with bipolar electrodes in a variety of subcortical areas, with emphasis upon the caudate nucleus and the mesencephalic reticular formation. When the cats recovered from this surgery, they were habituated to a hammock and conditioned responses have been established to electrical stimulation of subcortical areas and then to a tone CS. The conditioning procedure and apparatus are identical to those described for experiment I except for two variations. In addition to establishing CRs and then determining the CR thresholds, kindling seizures were established in some of the cats and the seizure thresholds have been determined. In addition, the thresholds for any forced movements were determined and followed throughout the experiment. After the cats were conditioned, CR, forced movement, and seizure thresholds determined, they were made physically dependent upon pentobarbital. The addiction procedure is one that we have developed. Each cat is given a single ip injection of 30 mg/kg pentobarbital for 10 days. A withdrawal probe was then given where pentobarbital was withheld and CR, forced movement and seizure thresholds were determined for the next three days. At the end of the three days withdrawal probe the cats were given 45 mg/kg pentobarbital in two ip injections of pentobarbital for 10 days. The first ip injection is 30 mg/kg followed 7-10 hours later with a second ip injection of 15 mg/kg. At the end of this 10 day period another three day withdrawal probe was given and CR, forced movement, and seizure thresholds was taken. A third 10 day addiction period followed the second withdrawal probe during which the cats received 60 mg/kg pentobarbital in two ip injections of 40 mg/kg followed 7-10 hours later by a 20 mg/kg injection. At the end of this 10 day addiction period there was another withdrawal probe of three days followed by a fourth addiction period where the cats received 70 mg/kg day for 10 days with the first ip injection of 40 mg/kg followed in 7-10 hours by a second ip injection of 30 mg/kg. This addiction period was repeated and followed by withdrawal of all barbiturates. During the withdrawal periods behaviors such as righting responses, being handled by the experimenter and eating, were filmed.

We have not, to date, tried any drugs to alter the course of withdrawal seizures. We have followed the changes in conditioning thresholds for the caudate nucleus and the mesencephalic reticular formation. Figure 15 details the chances in CR threshold for the caudate nucleus and the mesencephalic reticular formation for a single cat that had placements in both these structures. The respective curves are very similar to those obtained from cats with only one or the other placements. We have illustrated this data within a cat

Figure 15. Sequence of conditioning threshold changes for the caudate nucleus and the mesencephalic reticular formation during the development of physical dependence and withdrawal from pentobarbital. Baseline is in days of treatment, daily treatment dosage levels of pentobarbital, and withdrawal probes and periods.

because it is representative of the data we have obtained between cats as well.
The first withdrawal probe, following daily injections of 30 mg/kg pentobarbital
shows that the conditioning thresholds of the caudate nucleus and the mesencephalic
reticular formation are somewhat elevated on the first day of the withdrawal
but return to near normal by the end of the withdrawal period. The drug
produced nearly identical threshold shifts with short term drug exposure as
seen in Figure 12. The second withdrawal probe that followed 10 daily doses
of 45 mg/kg pentobarbital shows a beginning of a dissociation of the drugs
effect on the caudate nucleus and the mesencephalic reticular formation. Now,
when the drug is withdrawn the threshold for the reticular formation is low and
increases as the withdrawal period continues, while the conditioning threshold
of the caudate nucleus is elevated and falls during the withdrawal period.
This elevation in the reticular threshold, as the withdrawal period continues,
is similar to the effect seen in Figure 13 and described by Pusakulick and
Nielson (1972) for the reticular formation, where animals that were trained
in the drugged state showed an elevation of threshold as the drug dosage de-
creased. In the next withdrawal probes we clearly see that there is progres-
sive di-sociation of the conditioning thresholds of the caudate nucleus and
the mesencephalic reticular formation. After the last withdrawal period, and
with no further administration of the drug we see that the threshold of the
reticular formation recovers as it returns to its normal, pre-addiction levels.
We can also see that the conditioning thresholds of the caudate nucleus remain
elevated and do not recover even sixty days after the last injections of
pentobarbital.

Another important feature of Figure 15 is indicated by the circles. This
cat had withdrawal seizures two hours before and two hours after those thresholds
were taken. The conditioning thresholds are elevated, and the kindling seizure
thresholds are normal or above, but they are lower than normal. These data
make it extremely difficult to maintain that withdrawal seizures are the re-
sult of a central nervous system hyperexcitability. It would seem that if the
central nervous system is hyperexcitable it would show up when the reticular
formation (the arousal system) is stimulated, or when a kindling seizure is
evoked, if not when part to the motor system itself, the caudate nucleus, is
stimulated. If there is hyperexcitability of the CNS it should show up when
it is directly stimulated. We have tentatively concluded that the withdrawal
seizures are due to a loss of inhibition, exercised by the caudate nucleus,
on either the alpha or gamma motor neuron. We think it unlikely that the
reticular system is directly involved in the withdrawal seizures since its
thresholds were near normal when the withdrawal seizures occurred. These con-
clusions are subject to modification as further data is collected. We have
previously reported that the conditioning threshold of the amygdala was
elevated during the second withdrawal probe. Unfortunately, the two cats with
those placements have died (choked on their own food while intoxicated) and no
conclusions can be drawn. They may have returned to near normal, as the
reticular thresholds did, or they may have increased as did the caudate
thresholds. Only further data will clear up this detail. A final observation
is that placements in the medial geniculate body, like those in the amygdala,
have not been through the complete sequence and hence, do not provide us with
enough information to determine what the sequence of excitability shifts, if
any, are associated with the establishment of dependence and withdrawal
seizures.

Figure 16   (Nielson, et al., 1962) Thresholds of electrical stimulation
             provoking avoidance conditioned responses. (Symbols indicate
             stimulus frequencies in pulses per second: Triangles, 300 p/s;
             crosses, 150 p/s; circles, 30 p/s; squares, 3 p/s). Note--
             VA, N. ventralis anterior; R+Cd, N. caudatus, right head;
             RET MES, substantia reticularis mesencephalica; Mm, Corpus mam-
             millare; R, N. reticularis; LM, lemniscus medialis; L+Cd,
             N. caudatus, left head; Lim, N. limitans; CM, N. centrum
             medianum; Put, Putamen; H_1-Spf, Forel's field H_1 and N. sub-
             parafascicularis; VPM-VL, N. ventralis posteromedialis and
             N. ventralis lateralis; CI, capsula interna; NCM-MD, N. cen-
             tralis medialis and N. medialis dorsalis; NCP, N. commissurae
             posterioris; LP, N. lateralis posterior; Ped, pedunculus cere-
             bralis; CS, colliculus superior; Hippo, hippocampus.

Another aspect of this experiment is the unknown relationship between food intake and the ip injections of pentobarbital in these cats. We have had several deaths in these cats and had stopped the addiction sequence until we were sure that the schedule of drug administration was not responsible for their deaths. Within the last couple of months we have noticed that these cats were not eating much and had lost weight. To combat this we changed their diet from dry cat chow to their particular favorite canned cat food. We now feed these cats the canned cat food of their choice. This is because we noticed that these cats tended to not eat their food until they received their injection of pentobarbital. This gives them a limited time to eat, the time between their ip injection of pentobarbital and sleep. Consequently we fed them something we hoped they would gulp down in a hurry. We have not, until now, kept accurate records of the relationship between their eating behavior and their pentobarbital injections so we do not know whether this is an example of state dependent control of food motivation or not. Nevertheless, we have been able to maintain their weight only by feeding them highly preferred foods following their ip injections of pentobarbital withdrawal. The consequence of feeding them the highly preferred food, that they will gulp down has been unfortunate. The cats, while they are still very drunk try to eat it. We have had several of them choke to death on their food while they have been heavily intoxicated and one of them has apparently drowned in its water bowl. We now allow them access to food and water several times a day but only while someone can watch them.

Experiment IV. A comparison of drug effects upon conditioned responses established to peripheral stimulation or to direct electrical stimulation of the brain.

This experiment was conducted to compare the effect of pentobarbital upon CRs established to peripheral stimulation, i.e., to a tone CS, and to a CS applied directly to the brain. We wanted to know whether drugs, in this case pentobarbital, differentially influence central or peripheral neural processes.

The same general procedure was used in this experiment as in the first experiment. However, one difference was that these cats were trained to give CRs to a 1000 Hz tone in addition to having CRs established to electrical stimulation of the medial geniculate body as the CS. One other difference is that some of these cats were trained while drugged following an ip injection of 12.5 mg/kg pentobarbital, as they were in experiment II, to electrical stimulation of the medial geniculate body. We also attempted to train them to give CRs to the tone CS while they were drugged but the training was unsuccessful. When the CRs were established conditioning thresholds were established of the electrical stimulation delivered to the medial geniculate body. Similarly, the intensity of the tone CS was varied from 60 to 105 decibels, measured at the cats ear, to determine whether the intensity of the tone CS was a factor in maintaining the CR when the cats were in a drugged state.

The results from the cats first trained in the normal state, summarized in Figure 17, show that without exception the CRs established to the tone CS were abolished at a lower drug dosage than were the CRs elicited by electrical stimulation of the medial geniculate body. The CRs established to the tone
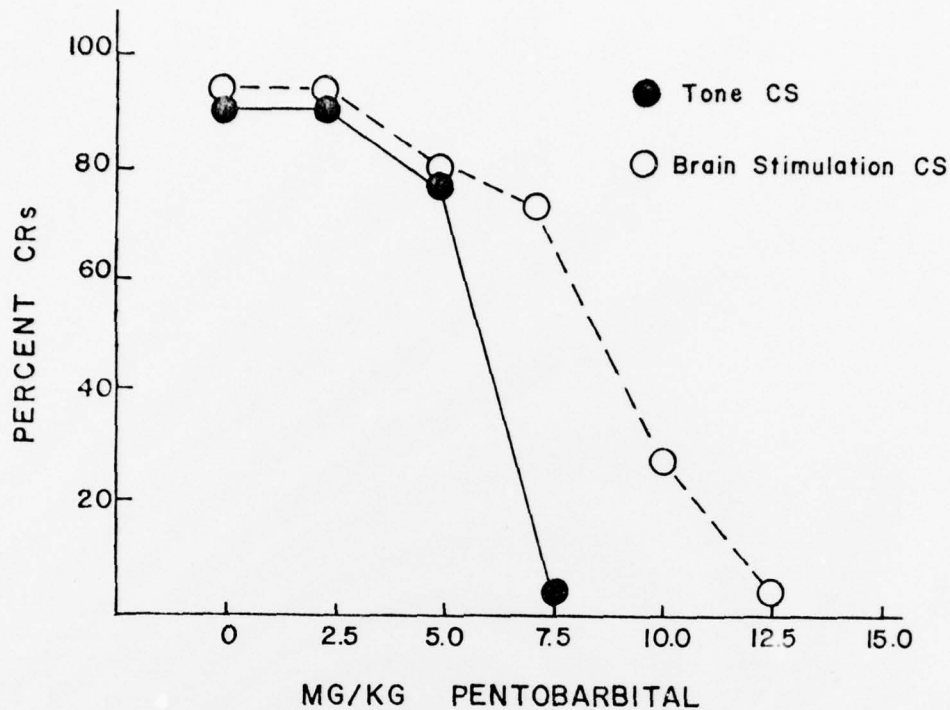
Fig. 17. Comparison of effect of Pentobarbital upon conditioned responses elicited by either a tone CS or by direct electrical stimulation of the central nervous system with animals trained in the non-drugged state.
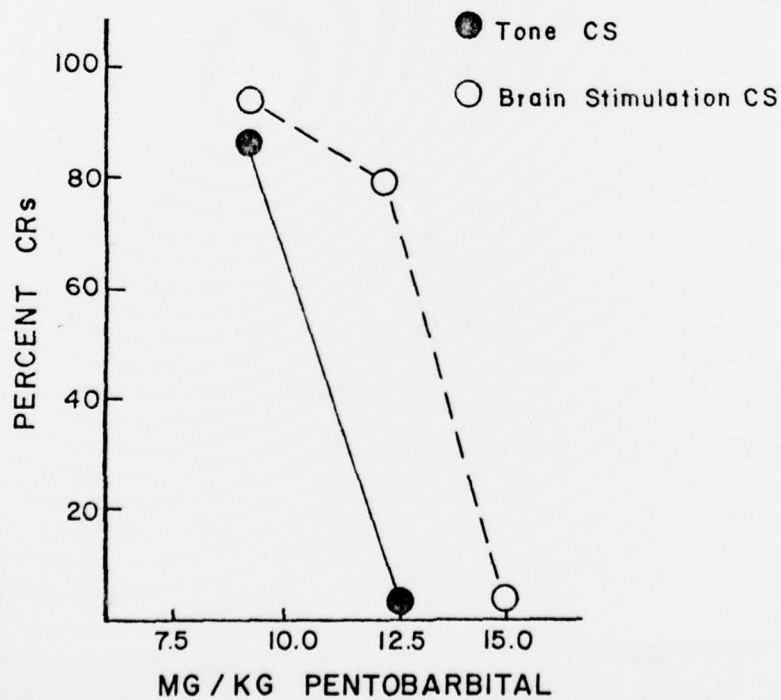

Fig.18. Comparison of effect of pentobarbital upon conditioned responses elicited by either a tone CS or by direct electrical stimulation of the central nervous system with the animals trained in the drugged state.
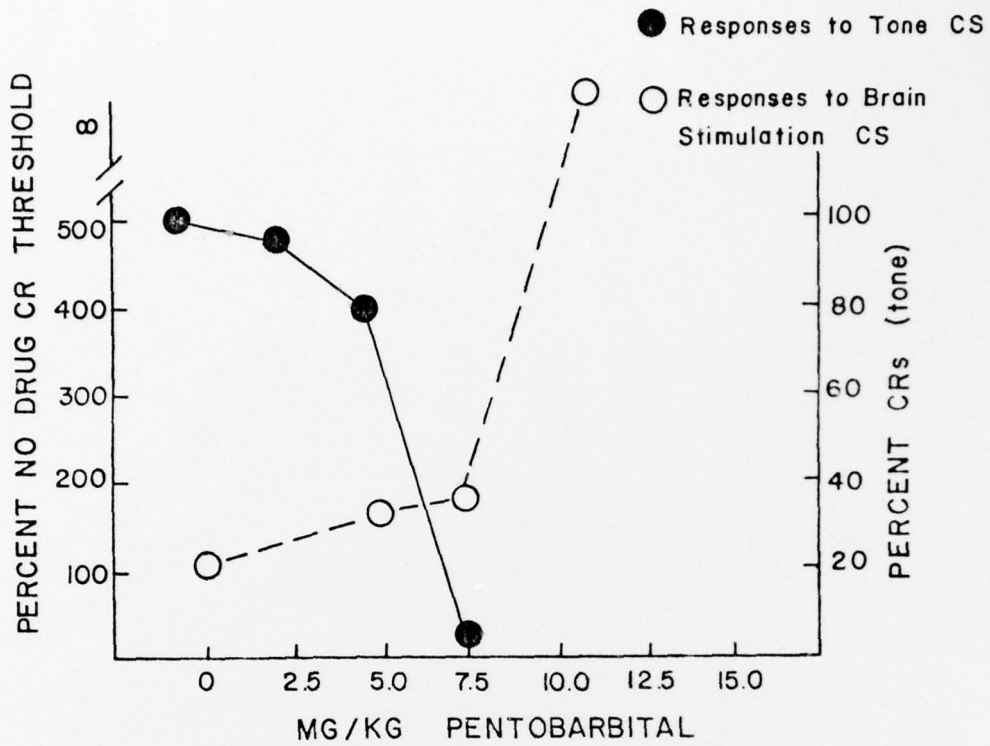
Figure 19. Comparison of the effects of Pentobarbital upon the conditioned responses established in the normal state to a tone or to brain stimulation as the CS.
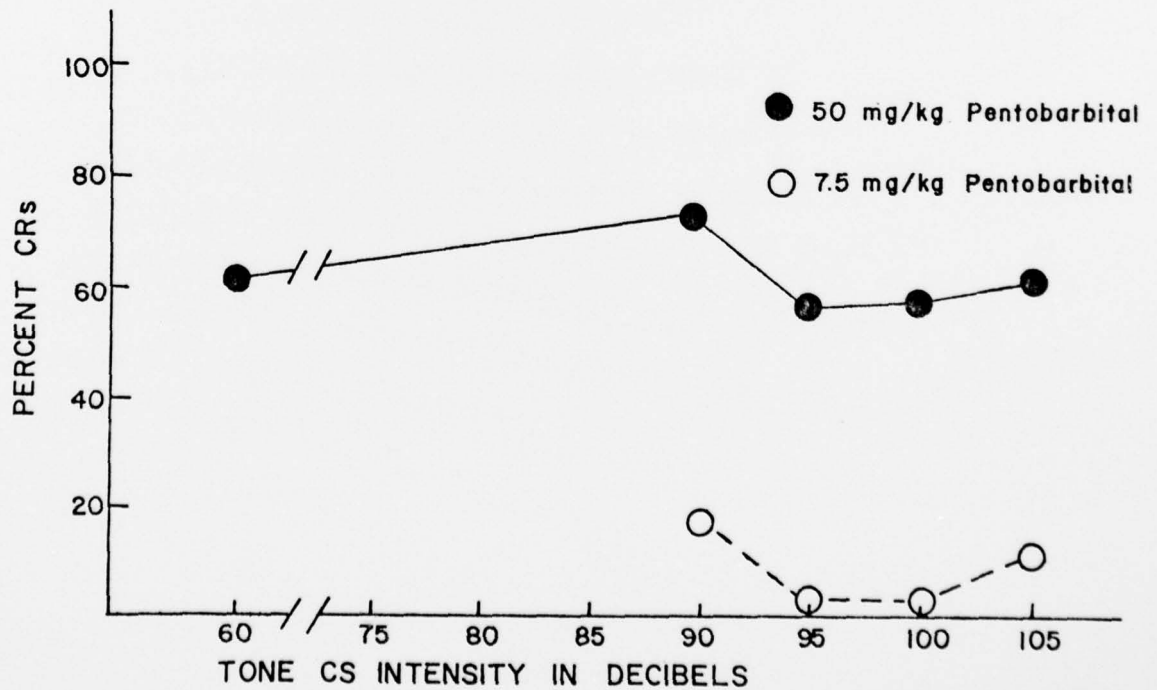

Figure 20. Percent CRs as a function of the intensity of the tone CS. The intensity of the CS was measured at the cats ears.

CS are abolished at much lower dosages than are those established to stimula-
tion of the medial geniculate body. It is pointed out in Figures 18 and 19
that the failure to respond to the tone cannot be due to any motor impairment
since the same animals were still responding to medial geniculate stimulation
with only slight increases in the CR thresholds. Four cats were given exten-
sive training to the tone CS while drugged, with some receiving over three
thousand trials. All failed to learn. We thought, that maybe we could train
cats to a tone CS if they had already learned a CR while in the drugged state.
Consequently, cats were first trained to give CRs to electrical stimulation of
the medial geniculate body while they were drugged with an ip injection of
12.5 mg/kg pentobarbital. When they had acquired this response, they were
then trained to give CRs to the Tone CS while drugged with an ip injection of
10 mg/kg pentobarbital. The results, graphed in Figures 18 and 19 again show
that the CRs established to the tone are abolished by smaller dosages of the
drug than are the CRs elicited by the central stimulation. Furthermore, the
fact that we could not train a cat to respond to the tone in the drugged state
unless it had first been conditioned to medial geniculate stimulation, sup-
ports the position that pentobarbital has a greater impact on peripheral
stimuli than they do on central stimuli. Another aspect of this impairment
of CRs established to the tone is that it is clear that the sensory process-
ing of stimuli that have great importance for the animal is not altered. These
cats, at all dosages that abolished the tone CRs, would still catch or at-
tempt to catch a mouse and attempt to flee from a dog. Thus the sensory
systems still process biologically important stimuli. To determine whether
a higher intensity tone would produce more responses at a given drug level,
i.e., does the tone show elevation in thresholds with increasing drug dosages,
we plotted the intensity of the tone in decibels measured at the cat's ear,
against the percentage of CRs. This is shown in Figure 14. There was no
relationship between the intensity of the tone CS and the number of CRs that
were elicited. There was no evidence that the sensory systems had changed
threshold.

Discussion and Conclusions

I wish to comment about the range of stimulus intensities that we use in
these experiments. When a cat has been implanted and before it is trained to
give CRs to electrical stimulation of the brain it is stimulated at all
electrode placements with a wide range of stimulus intensities. This pre-
liminary screening of the animal is so that we will know what kinds of move-
ments and at what intensities movements are elicited. This is a routine
screening of animals so that we do not select a conditioning site which gives
us any forced movement that could interfere with the cats learning the flexion
CR, that could masquerade as a conditioned response, or that is obtained with
low intensity stimulation. We do try to find forced movements that won't
interfere with the cats learning and that are obtained with moderate (.9 to
1.2 milliamperes) stimulation. We then follow the effects of the drugs and
drug treatments upon these forced movement thresholds. In fact, Girden and
Culler, when they first described "dissociation of learning" measured
elevation in forced movement thresholds and described the dissociation of
learning as a functional decortication. The reason we do not routinely de-
scribe in our method section how we obtain forced movement thresholds, and
the fact that we follow them is because we cannot always count on obtaining

them. It is something we follow when we can.

The thresholds for seizures were determined and we have filmed the entire seizure sequence with the seizure elicited by several different stimulus intensities. The topography of the seizures are reliable and the same to different intensities. The duration of the components is related to the intensity of the eliciting stimulus. However, as the cats have been receiving the addicting drug sequence, their seizure thresholds have increased. The topography of the seizures when they are in a drugged condition is not related to the stimulus intensity but rather to the current that the stimulating intensity is above the threshold. This is true for the CR thresholds also. Intensities of CS stimulation that the animal would not and could not tolerate in the normal state are frequently below threshold when the animal is drugged. Thus we use a wide range of stimulus intensities. it is just that often the threshold has been elevated and the cat does not respond. Thus the stimulus intensity is related to the animal's drugged state, or threshold, and has meaning only in that context.

Furthermore, when we initially train an animal we always try to use a stimulus intensity that we are reasonably sure the animal can perceive, i.e., one that is well above threshold. We have no desire to spend time training a cat with a stimulus that the cat might not be able to respond to. Consequently, we select a fairly high CS intensity. A scan of the training protocols show that the intensity of our training stimuli range from 2 to 4 times their threshold. We have no way of knowing before the animal is conditioned where we are in relationship to the threshold. The cat can only tell us whether he can detect the stimulus or not by making a conditioned response.

There is one final point and that is the range of CR thresholds in the normal state. The range of the CR thresholds for the mesencephalic reticular formation is from .03 to .5 milliamperes with a mode at .05 milliamperes; the caudate nucleus from .2 to .7 milliamperes with a mode of .5 milliamperes: and the medial geniculate body from .1 to .2 milliamperes with a mode of .14 milliamperes. In the drugged state the thresholds for the mesencephalic reticular formation may reach .35 mA before the CRs are lost, while those of the caudate have reached 3.5 milliamperes and the medial geniculate thresholds have reached 1.45 before the CRs are lost. This brings us to another point about our range of CS intensities. When a cat has been drugged with a dose of pentobarbital that abolishes CRs, we use stimulating intensities up to 10 times those found in the normal state and frequently five times the CS intensities that last produced CRs. Thus we commonly use a range of CS intensities that range from normal state CR threshold intensities up to 10 times the threshold intensities.

To compare the drug effects we have obtained with different thresholds, even within the same structure, we have made the comparison on the basis of percentage CR threshold change. We could have graphed the results in terms of threshold intensity (milliamperes) and shown that there were threshold differences across structures. This information is available, however, and we chose to present the data in terms of relative effect, or percentages, because the drugs do seem to have the same relative effect. This we believed to be more important than to restate the findings that areas of the brain vary

in sensitivity. I have taken the liberty of enclosing Figure 20, which is previous work which shows the CR thresholds of various brain areas as a function of frequency of the CS.

Experiments I and II were conducted so that the time course of action for each of the drugs could be determined, and the equivalence of the different drugs at different dosages could be established. This has been done. The equivalence of any drug at any dosage to any other drug upon CR thresholds can be determined. This is done by reference to the figures showing the effects of those two drugs. The equivalent drug dosages are those that produce equivalent shifts in CR threshold. That these drugs could be so equated was established by the fact that half of the dissociative dose of one drug added with half of the dissociative dose of another drug produced dissociation (meprobamate and librium, meprobamate and chloral hydrate). Further support for conclusion that these drug combinations, or drug dosages were equivalent came from experiment II where the dissociative dosages of drugs given singly or in combination in dissociative dosages maintained drugged state learning and responding. Smaller drug dosages did not produce dissociation and did not maintain drugged state learning and responding. We can conclude, then, that the equivalence of different drugs dosages and the time course of their actions can be determined by their effect upon Conditioning thresholds. These results represent the first systematic attempt to equate drug dosages and their time of action on the central nervous system as they influence behavior. No other studies have equated dosages as they influence both the central nervous system and behavior.

A second conclusion that we can reach from experiment II is that the effect of a particular drug, in this experiment pentobarbital, will have upon brain excitability levels is a function of the animals experience in the drugged state and not simply due to the pharmacological action of the drug alone. This was seen in experiment II, and graphed in figures 13 and 14 where brain excitability levels, as determined by CR thresholds, decreased when pentobarbital dosage was reduced in the drugged trained animals, and was also decreased as the pentobarbital dosage was increased in the nondrugged trained animals. Thus the effect of pentobarbital upon brain excitability was a function of the animals training states (see also Pusakulich and Nielson, 1972).

While experiments I and II were designed to determine the duration of drug action and establish and equivalence of the drug dosages so that the dose and duration parameters that might block withdrawal seizures could be inferred, Experiments III and IV had as one of their goals a description of the addictive process itself. The findings of these experiments is that during the addictive process there is an alteration in the normal relationship between the caudate nucleus and the reticular formation. When the cats were fully dependent, the thresholds of the reticular formation had nearly returned to their pre-addictive levels while the threshold of the caudate nucleus were greatly elevated and remained so after withdrawal. Thus, the major change that in the central nervous system, a change that occurred during addiction but and after remained after withdrawal was in the caudate nucleus. This is especially interesting since the caudate nucleus is particularly high in dopamine, and changes in dopamine levels have been associated with motor disturbances, particularly parkinsonism. These results suggest that the neurotransmitter substance that is probably the most acutely influenced during

the development of physical dependence is dopamine.

We have come to the following conclusions from Experiment IV: 1) it is extremely difficult, if not impossible, to train cats to give state dependent responses to a tone CS without first learning to give CRs to direct electrical stimulation of the central nervous system. 2) The sensory systems are still functional when an animal is under a sufficient amount of drug to produce state dependent learning since they will attempt to catch mice and run from dogs, yet they do not respond to stimuli that represent shock. This, we believe, suggests that instinctual type behavior can still be guided by sensory stimulation, but softer type of behavior that is heavily dependent upon learning is disrupted by drug dosages necessary to produce state dependent learning, and furthermore, new learning requiring sensory guidance cannot occur.

With the addition of the results presented here, there is a considerable amount of evidence that pentobarbital and other pharmacologically related drugs differentially affect the utilization of peripheral and central stimulation. Pusakulich and Nielson (DADA-17-73-C-3058; report #4) found that it was virtually impossible to train drugged rats to utilize distal cues in the solution of a water maze escape problem. The same animals, however, acquired response solutions with relatively little difficulty. Bliss (1974) found similar effects of pentobarbital on discrimination responses in monkeys. Visual discriminations proved to be more affected by the drug than did response (pressing a left versus pressing a right door) discriminations. Results of two earlier studies by Weiskrantz (Weiskrantz and Baltzer, 1965; Gross and Weiskrantz, 1961) are consistant with the results of both of these studies and with the results presented here but indicate that there may not be a clear distinction between drug effects on central and peripheral stimuli. The experimenters gave monkeys training in a visual or an auditory discrimination task and in a delayed response task and then tested for retention of responding while the animals were drugged with meprobamate. Of the three tasks, the drug disrupted performance of the auditory task the most severely. Performance on the visual discrimination task was also disrupted by the drug but performance on the delayed response task was the same or better than normal. Apparently related to these same effects, Weiskrantz has also noted that meprobamate ameliorates difficulties that monkeys with frontal cortex lesions have in the solution of delayed response problems. The animals are typically described as being highly distractible and meprobamate presumably modulates the ability of the animals to attend to or otherwise utilize background stimuli.

The exact nature of the changes which might underly drug produced sensory restriction is of course uncertain. It seems unlikely, however, that they are simple reception deficits. Drugged animals behave quite appropriately in a variety of situations and may exhibit behaviors which presumably require the processing of a great deal of sensory stimuli. It is a common observation, for instance, that if food is available, that animals drugged with pentobarbital will eat until they are nearly unconscious. Similarly, though they are quite clumsy, drugged animals will attempt to elude capture if placed in an open area. Drugged cats and drugged rats with histories of killing mice while nondrugged will pursue and kill mice while under doses of barbiturates which completely disrupt conditioned responses. This was most impressively

demonstrated with one of the cats in the experiment described above. The particular animal did not respond to tone or to brain stimulation while under even the smallest test dose of pentobarbital (5 mg/kg) but pursued and killed a mouse while under 7.5 mg/kg. Under 10.0 mg/kg, the animal was in-effectual in its attempts but nevertheless, pursued a mouse for the 3 minute duration of the test. It is of course a truism that an animal cannot be conditioned to respond to a stimulus or configuration of stimuli unless the animal can sense the stimulation. There are reports of successful drug con-ditioning with visual, auditory and position conditioned stimuli. It seems, therefore, that the drugged animal is at once both sensitive and insensitive to sensory stimulation. An explanation for the apparent paradoxical situa-tion is that depressants interfere with cross modality sensory integration. Pusakulich and Nielson, for instance, have noted that maze learning in normal rats involves at the very least, the integration of visual and body position cues. Their findings were that drugged animals cannot learn to utilize distal cues but can learn a sequence of responses to escape a water maze. Learning under a drug may thus be both simpler and more restricted than that in the normal state. This is, in fact perfectly consistent with observations that drugged rats learn some simple approach or avoidance responses more readily than do normal animals but have great difficulty with discrimination tasks and responses requiring delayed responses (Sachs, 1965).

We have tentatively concluded, from our results to date, that the addictive process is characterized by a double dissociation. Initially it is characterized by a dissociation of the peripheral from the central nervous system, and the central nervous system becomes "free running". As drugged conditions continue, there is a further dissociation, between the arousal system and the motor system. This is reflected in the shifts in CR thresholds of the caudate nucleus but not the reticular formation. Furthermore, this dissociation appears to be permanent, and is probably related to shifts in dopamine functioning. It is possible that a fruitful approach to the problem of controlling withdrawal seizures and also returning the animal to its pre-drug dependent state will involve restoring caudate nucleus, and probably dopamine, to its predrug dependent level of functioning.

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