Psychopharmacologia 7, 115—122 (1965)

From the Department of Psychology, Yale University, New Haven, Connecticut

The Effect of Amobarbital Sodium on Conditioned Fear as Measured by the Potentiated Startle Response in Rats *

By
CARL C. CHI

With 2 Figures in the Text

(Received June 24, 1964)

The purpose of this study was to test the effects of different doses of amobarbital sodium (sodium amytal) on the potentiated startle response and on various control startle responses. BROWN et al. have shown that the rat's startle response to a sudden loud sound is increased when this sound is preceded by a conditioned stimulus (CS) that has previously been paired with shock. These authors have shown in some detail that such potentiation of the startle shows the effects that would be expected if it were produced by a learned fear of the CS.

Amobarbital is known to reduce the height of the avoidance gradiant in the cat (BAILEY and MILLER) and in the rat (MILLER and BARRY) in a conflict situation. DAVIS and MILLER found that rats which received occasional electric shocks pressed a bar which caused them to be injected with amobarbital, while control rats not given shocks pressed much less, if at all. MILLER (1964) has summarized evidence from clinical observations and a variety of experimental studies supporting the hypothesis that one of the effects of amobarbital is to differentially reduce fear. Thus, we would expect this drug to have a greater effect upon the potentiated startle than upon the startle to sound alone. Such a result would constitute an additional test by a different technique of the fear-reducing effects of amobarbital. The value of using a variety of tests to avoid side-effects that may be specific to a single kind of test has already been pointed out by MILLER and BARRY.

In addition to measuring the effects of different doses of amobarbital on startle to a sudden sound as potentiated when preceded by a CS that has been paired with electric shock, the effects of the drug were tested:

a) on the startle to the sound when preceded by the same cue that was used as a CS, but without it having been paired with shock and

* Work on this study was supported by Grant MY 2949 from the National Institute of Mental Health, United States Public Health Service.

The author wishes to thank Dr. N. E. MILLER who was consulted on every phase of the research and Dr. J. D. DAVIS who designed the transducer and suggested the system for recording startle responses.

b) on the startle to an electric shock just strong enough to elicit a startle response of the same size as that of the potentiated startle to sound.

Finally, a special control test was run to show that the CS after pairing with shock did not elicit any startle response of its own when it was not followed by the loud sound.

Method

Subjects. The S's were 120 naive 90 to 120 day old male albino rats of the Sprague-Dawley strain supplied by the Holtzman Company of Madison Wisconsin.

Apparatus. S was confined in a movable cage attached to a mechano-electrical transducer. The startle cage was made of wire mesh with inside dimensions 7″ long, 4″ wide, and 4″ high. There were three $^1/_2$″ diameter tubes as a grid floor. These tubes and the walls of the cage could be electrified. A piece of stiff spring steel was fastened to the center of the top of the cage and used to suspend it so that any movement of the rat caused the cage to move. One end of the cage was attached to a loudspeaker voice coil through an oil coupling in such a manner that movements of the cage caused movements of the voice coil. The minute voltages thus generated were proportional to the rate of change of movement of the cage. The voltage output of the voice coil was coupled through an impedence matching transformer (Stancore A-3332) to a conventional high fidelity amplifier (Eico model HF-12). The amplifier output was coupled through another impedence matching transformer (Stancore A-3876) and a .047 mfd series capacitor to a voltage-to-frequency converter (Dymec model 2210). The output of this converter was fed to a digital counter (Hewlett-Packard model 5212A), and a digital recorder (Hewlett-Packard model 562A) was connected to the output of the counter. The apparatus was programmed so that the counter was opened to accept the output of the voltage-to-frequency converter .15 sec before the delivery of the startle stimulus or the electric shock and then remained open for another .85 sec. The raw data recorded was therefore a number which was a function of the force of the rat's jump to loud sound or electric shock. This system provided an accurate and automatic means of quantifying the relative force of the startle response.

The startle cage was mounted inside a sound and light proof box which also contained three 5″ loudspeakers mounted 4″ from the cage and a 28 volt light bulb mounted 5″ from one side of the cage. This bulb was used to deliver the CS which was 7.5 sec of light flashing at the rate of 4 times a second. The startle stimulus was a .1 sec 3,000 cycle per sec square wave tone delivered to the three loudspeakers. The intensity of

this tone in the startle cage was 122 db above .0002 dynes/cm^2. Two electric shock intensities were used. At the source the weak shock was 30 volts AC and the strong one was 158 volts AC. Both shocks were .2 sec long and were delivered to the rat through a .03 mfd series capacitor, which is believed to result in a better compromise between constant voltage and constant current shocks than does a series resistor of intermediate ohmage (TROTTER). Ventilation was provided by a blower mounted outside the box.

Procedure and experimental design. S's were on *ad libitum* food and water.

All trials in all groups were presented on a variable interval schedule with a $2^1/_2$ min mean, and each S was tested in one session of 32 trials (one hour and twenty minutes).

Habituation: Each S was placed in the startle cage for five minutes and then given ten startle trials with the tone alone.

Groups and Dosages: There were three groups—non-potentiated startle, potentiated startle, and shock. Rats were assigned to groups at random. Each group was divided into four subgroups. One subgroup was injected i.p. with .5 ml/kg of isotonic saline (equal to the quantity of fluid injected into the rats in the 20 mg/kg amobarbital subgroup). The members of the three remaining subgroups were injected with 10 mg/kg, 20 mg/kg, and 40 mg/kg respectively of amobarbital sodium[1]. The injection was given to each S 10 min before testing began.

The non-potentiated startle group was used to test the effect of amobarbital on the non-potentiated startle response and to determine any possible aversiveness of the flashing light to be used as a CS in the potentiated group. Each of the 32 trials consisted of a 7.5 sec presentation of the flashing light which was followed immediately by the loud sound used as a startle stimulus. Since the flashing light had never been paired with shock, it was presumably a neutral CS for this group.

Each animal in the potentiated startle group was given 32 training trials in each of which the CS was followed immediately by a .2 sec 158 volt shock, and then these animals received test trials 18 hours later. An 18 hour interval between training and testing was selected on the basis of preliminary work which showed that the startle was most strongly potentiated by the fear-eliciting CS when a number of hours elapsed after training. The test trials were identical to the training trials except that the startle stimulus was presented at the moment the shock would normally have occurred.

The shock group was designed to test the effects of amobarbital on a startle elicited in a different way. Animals in this group received thirty-

[1] Supplied by Eli Lilly and Company, Indianapolis, Ind.

two shocks of 30 volt intensity. Thirty volts was used because it was found that a .2 sec 30 volt shock elicited a response approximately equal in magnitude to the potentiated startle response under placebo conditions.

In all groups the relative force of S's startle response was automatically recorded as previously described. Altogether there were three groups and four dosages making a 12 cell design. Ten animals were run in each cell. In order to analyze the time course of the action of the drug each block of 32 trials was divided into four quarters each consisting of 8 trials during 20 min. This is a type III LINDQUIST design. Analyses of variance were performed on the logs of the mean startle scores of each sub-block of eight test trials for each S. Mean scores were used because the variance between subjects was much greater than the variance within subjects. Thus four scores corresponding to the four quarters of the test period were obtained for each rat making a total of 480 scores. A log transformation was used to equalize the variances over doses.

In order to test for the possibility that the response of the rats in the potentiated startle group was a direct conditioned response to the CS and not a potentiation of the innate, unconditioned startle to the sudden loud tone, four rats were trained in the same manner as the potentiated startle group and then tested 18 hours later by presenting on each trial only the CS without the tone.

Results

For the group to which the CS was presented without the tone on each trial, no movement at all was recorded at the time the tone would have normally been presented. This result confirms the interpretation by BROWN et al. that the potentiation of the startle response is a dynamogenic effect of fear.

Observation of the rats during the experiment was impossible, but informal observation during exploratory work indicated that the startle response, a quick phasic jump, was changed only in magnitude by the drug, not in topography.

Fig. 1 shows the mean log of the startle score averaged for all S's in each group as a function of drug dosage. Note that the data for the quarters are combined. No drug effect was observed in the non-potentiated startle group, but the mean log of the startle score which is proportional to the force of the startle response decreased with increasing dosages of amobarbital in both the potentiated startle and shock groups. These drug effects were highly significant (Table 2), and in addition the difference between the potentiated startle and shock groups was reliable at the .01 level. At each dose level amobarbital had a greater depressing effect upon the reaction to shock than upon the potentiated startle

response. Even a dose as small as 10 mg/kg had a depressing effect on the shock group, but the dose response curve for this group approached its asymptote at 20 mg/kg. A dose of 40 mg/kg had a hypnotic effect,

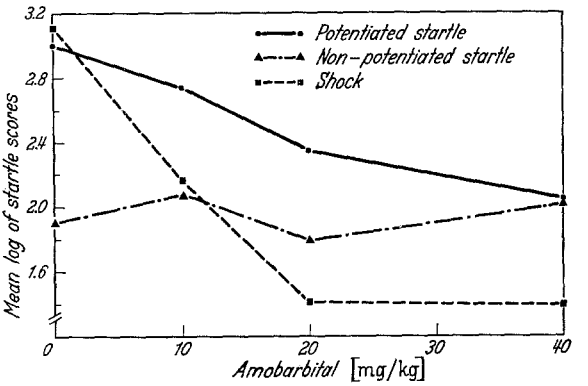

Fig. 1. Effect of i.p. injections of amobarbital on potentiated startle, non-potentiated startle, and startle response to electric shock

but although it eliminated the righting reflex it did not appreciably reduce the non-potentiated startle to loud sound.

The overall analysis of variance for group, dosage and quarter yielded the results summarized in Table 1:

Table 1. *F-values and levels of significance for the analysis of variance by group, dosage, and quarter*

Effect	F-Value	DF	P-Value
Group	11.6	2,108	<.001
Dosage	13.9	3,108	<.001
Quarter	5.4	3,324	<.005
Group X Dosage	5.0	6,108	<.001
Group X Quarter	5.1	6,324	<.001
Dosage X Quarter	1.9	9,324	<.05
Group X Dosage X Quarter	2.4	18,324	<.005

Notice that all the effects and interactions are significant.

Fig. 2 shows the mean log of startle score as a function of quarter of the test run. For each group two lines are plotted, one representing animals who got only saline injections and the other representing animals who got 10, 20, or 40 mg/kg of amobarbital. The three drug doses are combined in this graph because there were no significant dosage by quarter interactions when the saline animals were eliminated from the analysis of variance (see below). The largest quarters effect was shown by the shock group rats who received amobarbital. In this group the drug produced its maximal effect (depression of response to shock) about

one hour after i.p. injection. Although the quarters effects in the other groups were significant due to a very small within subject variance, the absolute magnitudes of these effects are very small and do not give any

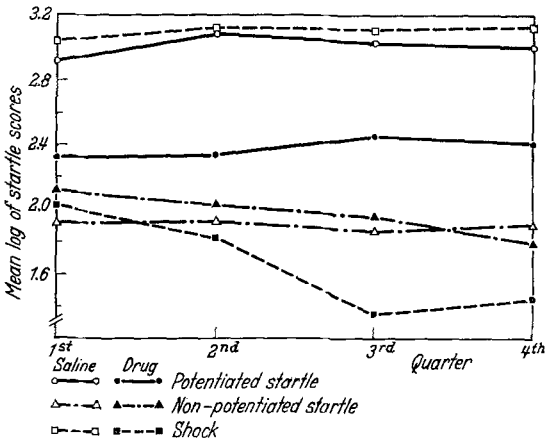

Fig. 2. Mean log of startle scores for saline and amobarbital animals as a function of quarter of test run (see text)

indication that the drug wore off during the 90 min period after i.p. injection during which data was collected. It should be noted in this connection that the 40 mg/kg animals were still in a hypnotic state when removed from the startle cage at the end of testing.

The analysis of variance for dosage and quarter in each group is shown in Table 2:

Table 2. *F-values and levels of significance for the analysis of variance by dosage and quarter for each group*

Group	Effect	F-Value	DF	P-Value
Non-potentiated startle	Dosage	0.4	3,36	NS
	Quarter	8.5	3,108	<.001
	Dosage X Quarter	0.9	9,108	NS
Potentiated startle	Dosage	4.1	3,36	<.025
	Quarter	7.6	3,108	<.001
	Dosage X Quarter	9.9	9,108	<.001
Shock	Dosage	31.6	3,36	<.001
	Quarter	6.6	3,108	<.001
	Dosage X Quarter	2.2	9,108	<.05

Only the dosage effect and the dosage by quarter interaction in the non-potentiated startle group are insignificant. The analysis of variance in each group was done a second time eliminating all animals who had

saline injections. This procedure reduced the dosage by quarter interactions to insignificant levels although there was still a significant quarters effect in all three groups and a significant dosage effect in the shock and potentiated startle groups.

Discussion

The greater effect of amobarbital on the potentiated than on the non-potentiated startle is what was predicted by the hypothesis that this drug reduces fear. Taken together with the results of other experiments using operantly conditioned responses, this experiment using the potentiation of an innate, unconditioned response lends further support to the hypothesis. It should be noted, however, that other interpretations are not ruled out. For example, amobarbital may interfere with the rat's ability to discriminate the CS and in this way "reduce" the fear elicited by it. The evidence on this point is conflicting (MILLER 1961, 1964), and the possibility that the effect of the drug on the potentiated startle response can be explained in this way must be kept in mind.

In any event, the fact that the non-potentiated startle was relatively unaffected by the drug rules out any interpretation in terms of inactivation of the final common motor pathways.

The finding that the drug had its greatest effect on the group receiving weak shock had not been expected. It can be explained post hoc if one assumes, as MILLER (1951) has done, that fear is a component, and perhaps the major motivational one, of the innate reaction to pain. In this case, the reduction in the startle to shock would be explained by the amobarbital's reduction in the fear component of the reaction to pain. This explanation is consonant with the observation that in man the barbiturates reduce the aversiveness of pain, but do not dull its perception, producing an effect similar to prefrontal lobotomy. According to the foregoing interpretation the amobarbital reduces the fear potentiated component of the startle, but after it has removed this component the innate startle to sound remains relatively unaffected, so that the net result is less than it was in the case of the response to shock.

Again, other interpretations are possible, but one cannot explain the effects on the potentiated startle solely by assuming that the drug reduces the aversiveness of electric shock because the original training with strong shock for the potentiated startle group was given before the drug was administered.

Whatever the correct final interpretation may turn out to be, this technique has shown that amobarbital produces a differential effect on startle responses elicited in different ways. An investigation of such differential effects may prove useful in studying other drugs.

Summary

The startle response in rats to a sudden sound was increased when the startle was elicited in the presence of a flashing light (CS) which had previously been paired with electric shock. The magnitude of this potentiated startle was used as a measure of the conditioned fear elicited by the CS.

The effects of different doses of amobarbital sodium on the potentiated startle, the startle in the presence of a flashing light which had not been paired with shock, and the startle to electric shock were tested. It was found that the drug reduced the magnitude of the potentiated startle response and reduced even more the startle to electric shock, but apparently had little, if any, effect on the startle to loud sound in the presence of a neutral CS. The effects lasted for at least 90 min after i.p. injections of the drug.

The action of amobarbital was interpreted in terms of a selective reduction in the strength of the fear drive, but other interpretations were not ruled out.

The fact that this drug produced differential effects on startle responses elicited in different ways, suggests that such responses may be useful measures of differential psychopharmacological effects.

References

BAILEY, C. J., and N. E. MILLER: The effect of sodium amytal on an approach-avoidance conflict in cats. J. comp. physiol. Psychol. **45**, 205—208 (1952).

BROWN, J. S., H. I. KALISH, and I. E. FARBER: Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. J. exp. Psychol. **41**, 317—328 (1951).

DAVIS, J. D., and N. E. MILLER: Fear and pain: their effect on self-injection of amobarbital sodium by rats. Science **141**, 1286—1287 (1963).

LINDQUIST, E. F.: Design and analysis of experiments in psychology and education. Boston: Houghton Mifflin 1953.

MILLER, N. E.: Learnable drives and rewards. In: S. S. STEVENS (Ed.): Handbook of experimental psychology, p. 435—472. New York: Wiley 1951.

— Some recent studies of conflict behavior and drugs. Amer. Psychologist **16**, 12—24 (1961).

— The analysis of motivational effects illustrated by experiments on amylobarbitone sodium. In: STEINBERG, H., A. V. S. DE REUCK, and J. KNIGHT (Editors): Animal behavior and drug action, p. 1—18. London: Churchill 1964.

—, and H. BARRY, III: Motivational effects of drugs: methods which illustrate some general problems in psychopharmacology. Psychopharmacologia (Berl.) **1**, 169—199 (1960).

TROTTER, JOHN: Personal communication.

Dr. CARL C. CHI,
Department of Psychology, Yale University, 333 Cedar Street, New Haven, Connecticut (U.S.A.)