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Review Article

Drug Effects on Animal Performance and the Stress Syndrome

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INTRODUCTION

STRESS is the response of an organism to a variety of challenging and threatening events which inevitably occur and recur throughout life. Each individual's health and survival depend on effective physiological and behavioral responses to stressors on repeated occasions. Many compounds, including those classified as tranquilizers, sedatives, and antidepressants, are used for the purpose of aiding an adaptive response to stressors. The actions of these drugs, administered under stress, may depend partly on their interactions with endocrine and other systems which are stimulated by stressors.

Stressors and Stress Responses.—The environmental stimulus which constitutes the stressor is generally distinguished from the physiological reactions which have been described as the stress syndrome or general adaptation syndrome (1–3). The existence of a stressor is generally inferred from the strength of the noxious stimulus and confirmed by observation of the stress reaction. All environmental events and changes are stimuli which threaten the organism's state of biological equilibrium or homeostasis (4). Those stimuli which merely require slight and well-established adjustments are not considered to be stressors, but any stimulus, if sufficiently intense, may evoke the stress syndrome. Many types of stressors have been classified as biological drives, including hunger, thirst, pain, and excessive heat or cold. The physiological reaction of fatigue, during or after exertion, and the mental state of fear or anxiety, aroused by

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realistic or unrealistic anticipation of a threatened stressor, likewise are stressors. Deprivation of environmental stimulation for several hours may also be stressful (5). Different types of stressors require different behavioral responses and physiological changes to maintain or restore homeostasis. However, all stressors, if sufficiently intense and prolonged, evoke the general adaptation syndrome, with the successive stages of alarm reaction, adaptation, and exhaustion.

The various physiological and endocrinological changes in different stages of the general adaptation syndrome are accompanied by behavioral responses which the stressed animal learns and performs. The alarm reaction generally includes vigorous muscular movements of attack or flight to destroy or escape the stressor. If the stress situation cannot be terminated by the initial violent reaction, the animal eventually resumes a more normal response, thus conserving energy and prolonging resistance to exhaustion by means of behavioral as well as physiological adaptation. The initial agitated behavior tends to improve the animal's ability to destroy or escape the stressor but curtails its survival in a situation of severe, inescapable stress. The choice of a therapeutic compound may depend on whether it is needed to enhance the initial alarm reaction or to prolong resistance to exhaustion.

Purposes of This Review.—The present paper attempts to review and evaluate the experimental techniques which have been used to test effects of compounds on behavioral responses to pain, threat of pain, and other stressors. The most frequently used test situations may be classified as measures of avoidance, escape, or approach-avoidance conflict. The physiological and endocrinological reactions involved in the stress syndrome are also summarized and related to the effects of drugs on behavioral performance during stress. The principal purpose of this review is to identify the features of the test situations which influence drug effects on behavioral performance. In order to enable such comparisons, emphasis is placed on techniques which have been used most frequently, such as the conditioned avoidance response, and on those compounds, notably chlorpromazine, whose effects have most commonly been tested with these techniques.

A series of excellent reviews of behavioral effects of drugs (6–10) have included various stressful test situations. Their coverage of the literature was necessarily selective and abbreviated. A comprehensive review of drug effects on the conditioned avoidance response (11) was limited to this one major technique and was primarily concerned with identifying the effects of a wide variety of drugs rather than comparing the various techniques with respect to their measurement of drug effects. Other articles (12–14) have described and evaluated certain selected techniques for testing drug effects on behavioral performance but without any attempt at a comprehensive coverage. Many of the studies described or cited in these prior articles are included in the present review, which aims to provide a new summary and interpretation of accumulated findings rather than merely bringing the literature up to date. Two collections of abstracts, available from the U. S. Public Health Service, have provided a number of useful references: "Psychopharmacology Handbook," vols. 1–3, for publications in 1954–1961, and "Psychopharmacology Abstracts," vols. 1–4, for publications in 1961–1964.

In spite of the fact that the preponderance of studies on behavioral drug effects have been published since 1953, the high and rapidly accelerating rate of output since that time has accumulated several thousand articles, only a small proportion of which can be cited in the present paper. The main criterion for including an article in this review was the description and use of an important behavioral technique for measuring drug effects in a stressful situation. Preference is given to large-scale studies, testing a number of doses of several compounds on a sizable number of animals. Most of the studies have tested drug effects on performance of a previously established avoidance, escape, or conflict response, but some have tested drug effects on acquisition of the response or on persistence of the avoidance during extinction, when removal of the threatened stressor has made it unnecessary for the animal to continue responding to the warning signal. Painful electric shock has been used as the stressor in most studies; objective records of performance have been ensured by automatic recording in almost all of the studies cited, and automatic programming of the experimental events was also used in many of them. The majority of the experiments were performed on rats, but many other species have been used, including mice, dogs, cats, and monkeys in a substantial number of the studies. This review excludes the few pertinent studies on humans. The use of infrahuman animals permits much greater control of the stress conditions and also has the advantage that the data are free from variations due to verbal learning and cultural expectations of the

subjects. The behavioral tests on animals were usually designed to measure general features of motivation and performance which are common to all species, including humans. Certain types of stressful situations have been discussed in the Review Article in the October 1966 issue of *J. Pharm. Sci.* (15) and are not included here. These include the relatively mild stress of exposure to a novel environment, measured by defecation and locomotor activity in the open field test, and test methods which involve manually pinching or otherwise stimulating the animal to elicit a reaction which is observed and rated rather than being automatically recorded. Measurements of forced locomotor activity, also included in this prior review (15), are stressful situations but have generally been used as tests of muscular coordination.

The present review is expected to be particularly useful for those who conduct, direct, or evaluate experiments on animal behavior. The comparisons among commonly used techniques are intended to help in the choice of experimental methods and in the interpretations of the findings, whether the purpose is basic scientific knowledge or screening for clinically useful compounds. However, the authors hope that this review will also be read with appreciation by those with a purely clinical interest in the drugs and by those with a scientific desire for further understanding of the interrelations between drugs and behavior in stressful situations.

MANIPULATIVE RESPONSE

A frequently used test situation is a chamber (Skinner box) equipped to deliver painful electric shocks to the grid floor; the animal is trained to escape or avoid the stressful shocks by means of an "operant" manipulative response, usually pressing a lever or rotating a wheel attached to one of the walls. This situation is generally used for testing drug effects on performance in test situations which last several hours, after the avoidance or escape response has been thoroughly established in a number of prior sessions. Most animals readily learn to press a lever to escape the shock, but many fail to perform the same response consistently in order to avoid the shock. The manipulative avoidance response must compete with an immobile, crouching response to the threat of shock. The test session comprises an inescapable, chronic stress situation in which crouching is a strong behavior tendency; thus, the animal's normal performance and the drug effects show the outcome of a conflict between opposing response tendencies rather than measuring the strength of a simple avoidance response.

Continuous Avoidance.—The procedure of continuous avoidance without a warning signal is one of the most recent of the commonly used behavioral tests. It was devised by Sidman (16) in 1953, at approximately the same time as the beginning of the recent upsurge in rate of publications on behavioral drug effects. The animal receives a brief electric shock at a fixed interval of once every few seconds. Each lever press postpones the next shock by a fixed interval, so that the animal can avoid the shock indefinitely by pressing this lever before the expiration of the fixed interval between shocks. Several different studies on rats (17–20) showed that a low dose of chlorpromazine (CPZ) substantially decreased the rate of avoidance lever presses, thus increasing the number of shocks received. The number of shocks is a more valid measure of the drug effect than the avoidance rate; one of these studies (18) showed an increase in shocks and an increase in the rate of lever presses with a low CPZ dose, because of a tendency for the animal to make a rapid burst of lever presses after each shock. Low doses of reserpine or tetrabenazine likewise markedly impair avoidance, thus increasing the number of shocks received by rats (21, 22) and by monkeys (23). The doses at which these compounds prevent avoidance are too small to cause any marked analgesia or ataxia; however, it is possible that the drugs intensify the immobile, crouching response which competes with the lever-pressing avoidance. Relatively high doses of chlordiazepoxide (17) and alcohol (24) caused only a moderate decrement in lever pressing by rats, and half the anesthetic dose of pentobarbital was required to impair avoidance in monkeys (23).

The avoidance performance of rats improved after injection of adrenocorticotropic hormone (ACTH) or dexamethasone (25). Lysergic acid diethylamide (LSD) improved performance at a low dose and impaired it at higher doses (26). Administration of amphetamine or one of its isomers, at low or moderate doses, increases the rate of lever pressing under a variety of continuous avoidance conditions (19–21, 27–30). A toxic depression of responding is produced by doses only slightly above those inducing maximal stimulation of lever pressing. Even at low doses, there is generally little or no decrease in the number of shocks received; an analysis of lever-pressing inter-response times (21) showed that amphetamine increased the incidence of responses in rapid succession while decreasing the incidence

of the more effective avoidance responses at times shortly before the next shock was scheduled. Two alternative schedules, requiring rats to lick a water tube at a high or low rate for shock avoidance, were used to demonstrate that low doses of amphetamine improved avoidance performance on either schedule, whereas higher doses caused the animals to lick at a faster rate on either schedule (31). Amphetamine greatly increased the rate of lever pressing during nonshock time-out periods which were designated by a visual signal (29). In a procedure with omission of shocks for 0.5-hr. periods without any signal (19), response rates decreased during the nonshock period in nondrug tests but not under dextroamphetamine. Three anticholinergic compounds (scopolamine, atropine, and benactyzine) generally produced an elevation in lever-pressing rate with no consistent effect on the number of shocks received (19, 20); thus, as in the case of amphetamine, efficiency of performance was impaired. Likewise, monkeys responded to scopolamine and atropine with a marked increase in avoidance lever presses and in unnecessary responses during a signaled nonshock period. Much larger doses of methyl scopolamine and methyl atropine were required for equivalent activity, suggesting that the behavioral effects were mediated by central rather than peripheral anticholinergic action (32).

The fact that the continuous avoidance schedule does not require any signals for experimental events facilitates the use of this procedure as one component of a multiple schedule, in which different experimental events are associated with different signals, at different times during the same session. This has the advantage of permitting a comparison of shock avoidance with food-rewarded or other types of performance, in the same animal and session. Drug effects on a continuous avoidance component of a multiple schedule appear to agree well with drug effects reported in other studies on a simple avoidance schedule. CPZ impaired avoidance in rats (33) and in dogs (34) at doses which produced no decrement in other components of the multiple schedule. Doses of amphetamine which greatly increased lever presses during the food-reinforced and time-out stages of the multiple schedule had no consistent effect on the number of shocks received during continuous avoidance (33). A high dose of pentobarbital was required to increase the number of shocks received by rats (33) and by rhesus monkeys (35). Low doses of scopolamine increased the rate of avoidance lever presses, and high doses depressed food-reinforced much more than shock-avoidance lever pressing, in rats (36) and in monkeys (35).

A method for differentiating avoidance from escape performance is through the use of a two-lever system whereby depression of the escape lever terminates the shock, while the separate avoidance lever is ineffective during this shock period. In tests with a variety of compounds (37), low doses of CPZ consistently increased the number of shocks received, indicating impairment of avoidance. Other compounds reliably increasing the occurrence of shocks at low doses include morphine and chlordiazepoxide, whereas high doses were required for detrimental effects of barbiturates, alcohol, and meprobamate. The animals always escaped the shock in nondrug sessions, and much higher doses of CPZ, morphine, and chlordiazepoxide were required to cause escape failures than to impair avoidance, whereas the compounds which required a high dose to impair avoidance were found to cause escape failures at slightly higher doses. An increase in rate of avoidance responses was produced by low doses of dextroamphetamine, cocaine, and anticholinergic compounds. In general, the drug effects in this study (37) agree well with the findings obtained with other continuous avoidance procedures. Detailed analysis of inter-response times, shock escape latencies, and other measures of performance on this two-lever avoidance schedule is feasible with a recently reported system for punched paper tape records and computer analysis (38). With the use of this system, the probability of avoidance responses shortly before shock is scheduled was greatly decreased by CPZ at doses which had little effect on the bursts of response in rapid succession (39).

Warning Signal for Avoidance.—If the continuous avoidance schedule is modified by presentation of a signal several seconds before the shock, rats (40, 41) and monkeys (42) generally do not perform the response until the signal appears. In most studies, the warning signal is presented at fixed or varied intervals, according to a schedule determined by the experimenter rather than by the animal, and lever presses during the intertrial interval have no effect. Generally, the same response which terminates the signal and avoids the shock also terminates the shock if the animal fails to avoid, so that it is possible to compare the drug dosage which impairs avoidance with the higher dose which impairs escape. A crouching tendency interferes with the lever-pressing avoidance response, so that typically only a minority of animals acquire consistent

avoidance performance (43), but the warning signal apparently has a stimulating effect which results in greater resistance to disruption by drugs than is found in continuous avoidance. Six depressant compounds (tetrabenazine, CPZ, chlordiazepoxide, diazepam, meprobamate, and pentobarbital) all impaired continuous avoidance at a lower dose than that which impaired the avoidance response to a warning signal (44, 45). The differential sensitivity of these testing methods was apparently greatest for chlordiazepoxide and smallest for meprobamate and pentobarbital (45). The fact that CPZ and thiopropazate, another phenothiazine, had similar magnitudes of effect on continuous avoidance and on avoidance with a warning signal (46) might be explained by the fact that the continuous avoidance was measured throughout 90 min. after drug administration, whereas avoidance with a warning signal was measured only for the 30 min. of maximum drug effect. Doses of scopolamine and atropine causing a large increase in shocks received by monkeys in a continuous avoidance schedule had less effect on avoidance by monkeys which were required to perform or inhibit an avoidance response by discriminating between two warning signals (32). The drugs produced a much greater detrimental effect with warning signals which were more difficult to discriminate.

Differential drug effects on avoidance with a warning signal in general appear to agree well with findings in continuous avoidance situations. A wheel-turning avoidance response was impaired at much lower doses of CPZ than secobarbital (47) or pentobarbital (48), and the dose required to prevent shock-escape was much greater than the avoidance-blocking dose for CPZ but not for the barbiturates. Morphine appeared to be intermediate in these respects (49). The ratio between escape-blocking and avoidance-blocking doses was reported to be highest for CPZ, intermediate for chlordiazepoxide, diazepam, and meprobamate, and lowest for pentobarbital (45). Avoidance in response to a warning signal was decreased by low doses of anticholinesterase drugs (50, 43, 51) and was increased by dextroamphetamine (52) at a dose which generally caused a toxic decrement of responding in the continuous-avoidance situation. A "trace" avoidance procedure consists of following the 5-sec. warning noise by 5 sec. of silence before the shock is delivered. A dose of chlordiazepoxide, diazepam, and meprobamate, which prevents avoidance during the signal, permits avoidance in the postsignal period, indicating that these drugs tend to delay rather than block the response to the warning signal, whereas in CPZ, pentobarbital, and nondrug tests, the animal generally responds either during the warning signal or not at all (45).

A lever-pressing avoidance response to a warning signal has been used as one component of a multiple schedule, compared with a milk-reinforced approach response in the same sessions. Low doses of CPZ, which greatly decreased avoidance in rats, had little effect on the approach response. Similar differential effects, requiring rather high doses, were induced by meprobamate and reserpine, whereas a high dose of pentobarbital had almost equal effects on avoidance and approach (53). In contrast, LSD, mescaline, serotonin, dextroamphetamine, and iproniazid impaired approach with a much smaller detrimental effect on avoidance (54). In a similar schedule, reserpine had a much greater inhibitory effect on lever pressing by cats for shock avoidance than for milk reward (55). These findings with a multiple schedule agree well with each other and with drug effects on avoidance in other situations.

LOCOMOTOR RESPONSE

Animals may avoid or escape shocks by the more naturally occurring response of running or jumping. Such a procedure has the advantage of enabling quicker and easier training of the avoidance response, with few animals being discarded due to insufficient performance. Drug effects on various locomotor responses have been investigated in many studies.

Avoidance by Running.—A test apparatus which has been widely used, for many years prior to its recent extensive application to drug research, is the two-compartment shuttle box. The animal avoids or escapes the shock by running into the other compartment, usually through a door or across a hurdle; successive trials can be programmed automatically, shifting the shock from one compartment to the other. In a shuttle box for continuous avoidance by mice, low doses of CPZ were found to decrease performance, with one of three strains tested being much more resistant to the drug effect than were the other two (56). A number of investigators have tested drug effects on shuttle-box avoidance by rats or other species in response to a warning signal. Low or moderate doses of CPZ decreased avoidance in rats (57–59), mice (60), and monkeys (61), with much higher doses being required to affect escape. The same doses of CPZ had a greater inhibitory effect on the escape response when shocks were delivered on the same schedule

without the warning signal (indicating a function of the signal in arousing the animal and thus facilitating the escape response) at doses which prevented the avoidance response (57). Reserpine likewise produced a great decrement in shuttle-box avoidance of mice (62, 63), rats (62, 64), cats (65–68), and monkeys (69). A very high, ataxia-inducing dose is required for ethyl alcohol (70) or pentobarbital (61) to impair avoidance. Amphetamine or its congeners effectively improved shuttle-box avoidance of rats (71–73) and cats (68), apparently by decreasing the crouching tendency (72). Performance was also improved by a low dose of benactyzine or LSD (71) and by a high dose of benactyzine, which reduced various rated measures of tension (74), but not by scopolamine, which likewise reduced tension (75). A general excitatory or disinhibitory effect of some compounds which improve avoidance is indicated by the finding that amphetamine and several anticholinergic compounds increased the frequency of incorrect, shuttling responses to a second warning signal which indicated punishment if the animal crossed to the other compartment, whereas it would not be shocked if it remained in the same one (76). Another procedure required animals to remain motionless on the grid floor in order to avoid shock; this response was readily learned in the nondrug condition, and activity was increased by CPZ, imipramine, and methylphenidate in test sessions when shocks were omitted (77). Nearly all other experiments have used some form of active behavior as the avoidance response, so that it is difficult to distinguish the specific drug effect on avoidance performance from a general stimulant or depressant effect.

In general, the shuttle-box avoidance seems to be more readily improved by stimulants and less easily impaired by depressants than is a lever-pressing avoidance response. A higher dose of CPZ was required to block a shuttle-box than lever-press avoidance response in rats (78). Contrary to this finding, the same doses of CPZ, secobarbital, and morphine appeared to produce a greater decrement in avoidance and escape for a shuttle-box than for a wheel-turning response, perhaps because the wheel-turning response was extensively trained to a high level of performance (79). Avoidance performance in the shuttle-box may be impaired by the fact that the animals on each trial are required to return to the compartment in which they previously received shock (13). A four-compartment box (80), permitting the animals to progress in a clockwise or counterclockwise direction, has been shown to improve performance. A still more effective method for increasing the attractiveness of the escape or avoidance response might be a safety compartment where the animals are never shocked, from which they are manually removed before being placed into the starting compartment at the start of each trial.

Rats (81) which were manually placed in the same compartment of a two-compartment shuttle-box, thus never receiving shock in the other compartment, required rather high doses of CPZ and reserpine for inhibition of avoidance. The same conclusion appears to be valid for other studies on effects of CPZ and reserpine in rats (82) and mice (83). Barbiturates, anticholinergics, and meprobamate were even less active in this situation. Drug-induced inhibition of avoidance may have been enhanced in one of these studies (82) by the use of a long, 60-sec. interval before shock, without any warning signal other than placement in the starting compartment of the test box. Rats which have learned to avoid shock by running to a safe compartment at the end of an alley are highly resistant to inhibiting effects of CPZ (84, 85) and other compounds (85). Different groups were trained to run down an alley for food reward, shock escape, or shock avoidance, using a higher shock intensity for the avoidance than escape group in order to equalize nondrug running speed. Most of the compounds had similar effects on the three groups, but amobarbital caused the greatest decrease in avoidance and the smallest decrease in approach speed (85). CPZ does appear to inhibit avoidance performance at relatively low doses in locomotor response situations involving a multiple schedule or a discriminative choice. CPZ greatly impaired avoidance with very little effect on approach, whereas reserpine impaired approach with very little effect on avoidance, in rats trained to avoid shock or approach food during different trials in the same runway (86). Effects of compounds have been studied in a situation with a visual stimulus identifying the correct exit for avoidance or escape from shock (87–91). CPZ decreased avoidance at a low dose, with a higher dose being required to decrease the percentage of correct choices during escape. Benzquinamide, chlordiazepoxide, meprobamate, and hydroxyzine likewise had greater effects on avoidance than on discrimination. In contrast, reserpine and pentobarbital affected both measures of performance almost equally, and alcohol had a greater detrimental effect on discrimination than on avoidance. All of the

compounds required higher doses to suppress escape from the shock than to inhibit avoidance.

Avoidance by Jumping.—When shock is delivered to a grid floor, a response of jumping up to a safe area may provide quicker escape than running across the electrified grid. A rather high dose of CPZ but a fairly low dose of meprobamate was required to inhibit an avoidance response of rats trained to jump onto a stand (92). A relatively high dose of CPZ was required to inhibit a similar avoidance response in rats (93). In mice, an avoidance response of jumping onto a net was not affected by amphetamine and required high doses of CPZ to impair this response (94). Other studies on mice indicated that a jumping avoidance response was more resistant to inhibition by meprobamate and barbiturates than by CPZ, reserpine, and chlordiazepoxide (95–97).

Many investigators have tested drug effects on an avoidance response of jumping onto a vertical pole, which is usually constructed of wood with a rough surface, so that the animal can cling to it and must be removed manually. A number of investigators have shown that rather high doses of CPZ were required to inhibit the pole-jumping avoidance response of rats (98–103). The dose which prevented escape from shock was generally much higher than the avoidance-inhibiting dose. A comparison of pole-jumping with shuttle-box avoidance has been reported in *Peromyscus maniculatus gracilis*, an arboreal species of mice (104). Animals trained in the pole-jumping apparatus acquired a higher percentage of avoidances and were more resistant to suppression of avoidance by CPZ and pentobarbital than animals trained in the shuttle-box. A similar conclusion may be drawn from the report (105) that a much higher dose of a cholinesterase inhibitor was required to suppress avoidance by rats in a pole-jumping than lever-pressing situation.

Pole-jumping avoidance was inhibited in rats by cholinergic compounds (106) and by benzoquinolizine derivatives, monoamine oxidase inhibitors, and catecholamines (107), generally at much lower doses than those required to prevent the escape response. Morphine has also been reported to inhibit the pole-jumping avoidance at a much lower dose than escape (98, 99, 103), whereas pentobarbital and meprobamate impair both avoidance and escape at very high doses, which usually also induce ataxia (98, 99). In general, pole-jumping avoidance was highly resistant to the effects of the above compounds, reserpine (99), and other drugs (103). However, the "secondary conditioned response" of jumping onto the pole as soon as the animal was placed into the chamber, prior to the warning signal, was inhibited by much lower doses, especially by CPZ, meprobamate, and morphine (99). The drug effect on this unnecessarily early avoidance response may be primarily an index of general central nervous system depression.

Avoidance trials have been programmed automatically at regular intervals, using a metal (108) or plastic (109) pole which causes the animals to slide down to the grid floor. With this procedure, avoidance is inhibited by CPZ at lower doses, reserpine at approximately the same doses, and pentobarbital only at higher doses in comparison with brief pole-jumping sessions (108, 109). A finding that CPZ at low doses inhibited pole-jumping avoidance, with very brief intertrial intervals (110), suggests that rapidly repeated trials, in addition to a continuous session, may enhance the inhibitory effect of CPZ. However, even under these conditions a jumping avoidance response seems to be more resistant to inhibition by CPZ than is a lever-pressing avoidance response.

Flinching and Fighting.—A leg-flexion avoidance response by dogs requires only a slight movement, is performed very reliably, and is highly resistant to inhibition by CPZ, meprobamate, phenobarbital, and morphine (111). The dose necessary to prevent shock-escape is much higher than the avoidance-inhibiting dose for CPZ but not for meprobamate and phenobarbital, indicating differential effectiveness of these drugs on performance despite the high dose of each compound necessary to inhibit avoidance. An increase in heart rate during the warning signal (found in nondrug tests) was blocked by the doses of chlorpromazine, phenobarbital, and meprobamate which suppressed avoidance. Morphine, which failed to suppress avoidance, also failed to alter the heart rate response to the warning signal (112). A similar leg-flexion avoidance response in cats (113) was inhibited by cholinergic drugs, and this technique was described as showing an all-or-none effect in comparison with a shuttle-box avoidance response in a different group of cats. When an i.v. injection of *l*-epinephrine was used as the warning signal 30 sec. before shock (114), the leg-flexion avoidance response in dogs was inhibited by a low dose of chlorpromazine which did not block the usual physiological effects of the epinephrine. The conditioned avoidance response may have been weakly established, with the use of a drug as warning signal and the unusually long interval until shock.

Pain threshold is generally measured by gradually increasing the intensity of a painful stimulus until the animal performs an escape or other response. It is difficult to specify whether a drug alters the motivation, the intensity of the stimulus, or the capability for performance, in this as in other behavioral tests. A number of compounds require higher doses to inhibit the initial flinch response to electric shock on a grid floor (115, 116) or foot-licking response on a hot plate (117) than to inhibit the subsequent response of jumping. The effects of several doses of the same compounds have been compared in a test of pain threshold (response to electric stimulation of the tail root) and pole-jumping avoidance in rats (118, 119). Much higher doses of CPZ were required to increase the pain threshold than to decrease the probability of avoiding. A similar differential effect was found with pentobarbital, bulbocapnine, and reserpine. Morphine, and to some degree dextroamphetamine, showed opposite differential effects, with higher doses being required to decrease the probability of avoiding than to increase the pain threshold. Several cholinergic compounds had a similar magnitude of effect on both measures. In mice, a much higher dose of CPZ than morphine was required to inhibit the response of squeaking when electric shock was applied to the tail (120) and to inhibit the reaction when heat was applied on a hot plate (100) or to the tail (121). Contrary to these findings of analgesic effects of morphine, higher doses of morphine were required to inhibit the reaction to these two types of heat stimulation in rats than to inhibit the pole-jump avoidance response (122). Rats which had been trained to terminate a progressively increasing tail shock by turning their head in one direction required a higher dose of morphine than CPZ to cause an elevated shock intensity threshold at which this response occurred (123). In general, tests of pain threshold appear to be rather insensitive to drug effects, requiring high doses of morphine and even higher doses of most other compounds to produce reliable changes. However, analgesic effects with low doses of morphine have been reported using grid shock (124) and ultrasonic pain stimulation (125) in rats and with the jaw-jerk response to electrical stimulation of the tooth pulp in dogs and cats (126). Sympathomimetic compounds have shown analgesic effects, with ACTH and cortisone causing an elevated pain threshold, measured by thermal stimulation of mice on a hot plate (127), and with amphetamine and norepinephrine (NE) likewise causing an elevated pain threshold, measured by the inflamed-foot method in rats (128) and by electric shock to the tooth pulp in guinea pigs (129). Most tests of analgesia have been based on a motor response of flinching, withdrawal, or vocalization. However, a well-trained lever-pressing response has been used successfully in several experiments. With progressively increasing shock on a grid floor, the threshold for a lever-pressing escape response by rats (130) or for an active motor reaction by mice (124) was increased by moderate doses of morphine, sodium salicylate, and acetylsalicylic acid, whereas a sizable dose of pentobarbital had no effect (130). A similar procedure likewise showed analgesic effects with low doses of morphine in monkeys (131).

The startle response to a loud noise associated with painful shocks may be a sensitive measure of anxiety or fear, but there have been few tests of drug effects on this response because of the technical difficulties in constructing and using an appropriate measuring device. Alcohol (13) and amobarbital (132) reduced the motor response to a loud sound when it occurred during a visual warning signal for shock, at doses which had little effect on the startle response to the sound alone. Amobarbital was even more effective in reducing the startle response to shock, without loud noise or warning signal; however, fear rather than pain may have been the principal reaction to the mild shock used (132). A variety of severe stressors have been used for the measurement of drug effects on escape. The speed with which rats escaped from electric shock in a runway was slightly increased by dextroamphetamine and slightly decreased by pentobarbital and chlordiazepoxide (133, 134). Swimming has been used as an escape response, and drug effects may be influenced greatly by variations in the procedures. Barbital (135) and amphetamine (136) greatly slowed swimming of rats to an escape ramp when they were required to pull a weight, at doses with little effect on swimming time under normal conditions. CPZ and meprobamate also decreased swimming speed at fairly low doses, especially when the animals were required to pull a weight, with generally smaller effects on a shuttle-box avoidance response tested in the same rats during the same session (137). Escape of rats from audiogenic stimulation was inhibited by fairly low doses of CPZ but not by high doses of phenobarbital and meprobamate (138, 139). Rats housed and tested in isolation generally fail to escape audiogenic stressors, and amphetamine or other stimulant drugs enabled a substantial proportion of them to escape (140). A lever-pressing escape response has also been used in tests of drug

effects. Amphetamine increased the rate of lever pressing by rats to terminate loud noise (141) and moderate doses of amphetamine increased, CPZ decreased, and pentobarbital briefly decreased rate of lever pressing by rats to turn on a heat lamp in a cold environment (142).

Painful electric shocks on the grid floor may induce pairs of animals to attack each other, indicating that a stressor is likely to elicit aggression if the test situation permits this response. Fighting in rats was inhibited by high doses of CPZ and benactyzine; however, high doses of meprobamate and reserpine had no effect (143, 144). Fighting of mice in response to grid-floor shocks is suppressed by meprobamate and to a lesser degree by CPZ, barbiturates, and chlordiazepoxide (145–147). Another method for inducing aggressive behavior in mice is to house the animal in isolation for several weeks prior to testing with another mouse. Suppression of attack behavior was found at approximately the same dose of CPZ for both techniques, but much higher doses of phenobarbital and meprobamate were necessary to inhibit isolation-induced than shock-induced fighting (148). Isolation-induced aggression of mice was also suppressed by a moderate dose of benactyzine and a very high dose of reserpine (149). A comparison of aggression with analgesia and other behavioral measures showed that isolation-induced fighting was suppressed at a lower dose by CPZ, other phenothiazines, and morphine, but not by barbiturates and meprobamate (150). In a comparison of aggression with shuttle-box avoidance, isolation-induced attack was suppressed at a lower dose by chlordiazepoxide, at the same dose by CPZ, and at a higher dose by pentobarbital and meprobamate (97). The doses of pentobarbital and meprobamate required to suppress aggression also prevented escape from shock in the shuttle-box. Isolation-induced attack has been reported to be inhibited by high doses of LSD, psilocybin, and mescaline (151, 152). The aggressive response to stressors generally appears to be highly resistant to effects of most compounds, even in one study (97) in which it was characterized as being weak and unstable in nondrug tests. A different type of attack behavior, found in a minority of rats, is to kill a mouse placed into the rat's cage. This has been described as aggression but may be related to predatory or feeding behavior, and is highly resistant to drugs. A variety of depressant compounds inhibited this behavior only at severely ataxic doses; hydroxyzine was the only drug tested which abolished the mouse-killing response at a moderate dose (153, 154). However, low doses of several antidepressant compounds effectively inhibit the mouse-killing response (155).

APPROACH–AVOIDANCE CONFLICT

A conflict may readily be established by punishing the animal for responses which procure a desired goal. The stress of the punishment is augmented by the thwarted need for food or other reward formerly obtained, and the conflict itself may be an additional stressor (13). The strength of the opposing approach and avoidance tendencies can be measured only in relation to each other, but this disadvantage is offset by the advantage that the opposing tendencies are likely to be affected equivalently by any drug-produced changes in activity or freezing. This equivalence is not complete, because the approach response is generally acquired first, is more strongly established, and requires more active behavior. Whereas the active lever-pressing or locomotor response is motivated by fear of shock in the usual avoidance test situation, in the conflict test it is the suppression of an active response which is motivated by fear. Therefore, the conflict situation may indicate whether CPZ and other compounds suppress performance of an avoidance response during a warning signal because they intensify an incompatible freezing tendency or because they decrease the fear-producing effect of the signal.

Manipulative Response.—One technique for measuring conflict is to present a signal, terminated by inescapable electric shock, while an animal is pressing a lever for food reward. The "conditioned emotional response" to this stimulus includes "conditioned suppression" of the lever-pressing response. Reserpine, in doses which decreased normal lever-pressing rate, increased the number of responses during the aversive stimulus in rats and monkeys (156, 157) but not in guinea pigs (158). Procedures which caused almost complete suppression of responding by rats during the signal (159–161) prevented any substantial recovery under reserpine. On the other hand, when rats were trained to press a lever in response to a signal that food was available, instead of on the usual free-operant schedule, reserpine greatly increased the rate of suppressed lever pressing during a concurrent signal for inescapable shock (162). Lauener (163) trained rats on a fixed-interval schedule, with water reward obtained by the first lever press 5 sec. or more after the last reward, instead of using the more customary variable-interval schedule. The high, stable performance rate generated is very resistant to disruption by drugs and thus is advantageous for

testing drug effects on conditioned suppression. The responding during the signal was greatly increased by chlordiazepoxide, several barbiturates, and to a lesser degree by meprobamate, but not by CPZ, morphine, ethanol, and amphetamine. Morphine has been reported to increase suppressed responding (164), but most of Lauener's findings are supported by other studies, which show that suppressed responding was greatly increased by amobarbital (165, 166), increased by meprobamate in one study (167), but not in another (162), not increased by CPZ (160, 162), and decreased by amphetamine (156, 157).

A more direct conflict procedure is to punish the animal only when it presses the lever, so that the aversive shock is associated specifically with the food-rewarded lever-pressing response. Comparisons between these procedures have given evidence that reserpine increases responding during a signal for inescapable shock but not when the animal is punished for pressing the lever (156, 157); whereas, meprobamate increases responding during a signal for punishment but not inescapable shock (168). Effects of several compounds have been tested in a conflict situation where lever presses by rats are rewarded on the average of once every 2 min. on an unpredictable, variable-interval schedule, and at periodic intervals a tone is presented for 3 min., during which every lever press is punished by shock and rewarded by food (169–172). The number of lever presses during the conflict signal was increased greatly by meprobamate, substantially by barbiturates and chlordiazepoxide, slightly by reserpine, and decreased by CPZ and morphine, at doses which had little effect on the lever-pressing rate during the unpunished portion of the schedule. Another conflict procedure (173), for rats trained to press a lever for milk in response to a signal, is to accompany this reward signal with an additional stimulus indicating that each of the next four lever presses will be rewarded and also punished. The number of lever presses during the conflict signal was increased by meprobamate and pentobarbital and not by CPZ and reserpine. Punished responding was likewise increased by a barbiturate (amobarbital) but not by CPZ in pigeons pecking a key for food reward on a variable-interval schedule and punished by shock for every response during the conflict signal (174).

When every response is punished during the conflict period, as in the above studies, drug effects might be due to changes in the aversiveness of the shocks, based on the immediately preceding experience with shocks in the same session, rather than being due to changes in fear or avoidance of the threatened shocks. A procedure for measuring fear rather than pain, by omitting shocks during the conflict signal in some test sessions, showed a large increase in lever pressing during the conflict signal under amobarbital but not under the other compounds tested (175). Contrary to the failure of CPZ to increase suppressed responding, in several of the above studies, CPZ produced a slight but reliable increase in lever pressing during the conflict signal in this situation with shocks omitted (175). CPZ produced a large increase in lever pressing during the conflict signal in a study using similar procedures (176) but with a more prolonged duration of the conflict signal.

Drug effects on conflict have been tested in situations without a signal for punishment. Alcohol and amphetamine decreased rate of rewarded and punished lever pressing by rats (177), in agreement with the effects of these drugs during the conflict signal in another study (175). Morphine greatly increased the number of punished water-drinking responses by rats during a prolonged conflict session (178); a similar effect of CPZ during a prolonged conflict signal (176) suggests that the duration of the conflict period may be a factor in the drug effect. In two other studies on rats (179, 180), the frequency of punished drinking responses was increased by meprobamate, amobarbital, and methylpentynol, decreased by CPZ, and not significantly changed by benactyzine. A measure of agitated approach-withdrawal responses showed a decrease under benactyzine (179).

Locomotor Response.—Conditioned suppression of a running response was tested in rats previously trained to obtain water reward by shuttling back and forth in a two-compartment box (181). In this situation, the signal for an inescapable shock caused slightly less suppression of the running response in animals injected with CPZ than in a control group. However, conflict has generally been induced by direct punishment of the locomotor approach response. The use of a long runway permits measures of speed and distance of approach, and provides a test of drug effects on fear of punishment as well as on the immediate effects of punishment. Conger (182) showed that alcohol restored the approach response in rats which had been shocked at the food cup. Measures of strength of pull in the same apparatus showed that alcohol greatly reduced the vigor of running in a shock-avoidance group but not a food-approach group. A further demonstration of the avoidance-reducing effect of alcohol was with a method of omitting shocks after alcohol injection for one group and after

placebo for another group; approach in the nonshock condition was more rapidly learned by the group for which shocks were omitted under alcohol rather than placebo. Barry and Miller (183) devised a "telescope alley" in which progressive changes in runway length signaled increases in shock intensity delivered at the food or water cup, during several trials of the same day. This technique measured drug effects on normal approach speed and on the intensity of punishment or fear of punishment required to prevent the approach response, with repeated tests of the same animals under different drugs on successive test days. Amobarbital and alcohol generally decreased approach speed in the initial safe trial of the day but consistently increased speed and probability of approach in the series of trials with increasing shocks, whether the shocks were delivered or omitted (183–185). Other compounds tested (CPZ, morphine, cocaine, methamphetamine) tended to decrease approach speed, generally with a greater effect in the safe trial than in the conflict test (183). One study gave evidence that CPZ increased approach during trials with shock but decreased approach during trials when shocks were omitted (184).

Drug effects on conflict in a runway or other locomotor situation have also been studied in other species. Amobarbital effectively restored the approach response of cats in a runway (186). In a more complex situation, designed to induce neurotic behavior (187), alcohol restored food-approach responses of cats and had other beneficial effects in the conflict test. A series of studies on cats and monkeys in the same situation (188) showed even greater beneficial effects of barbiturates but little or no effect of reserpine, CPZ, and mephenesin. A similar procedure (189) was used to test effects of several compounds (190) on conflict behavior of cats after food deprivation of only a few hours instead of 24 hr. Approach responses were increased by benactyzine, by related anticholinergic agents, and by alcohol, with no beneficial effect of CPZ and scopolamine. Cats which were punished by electric shock when they seized a mouse resumed the punished response under the influence of a low dose of meprobamate, but high doses of benactyzine and CPZ were required to elicit the response (191). Dogs resumed a punished food-approach response under the influence of barbiturates, alcohol, and meprobamate but not benactyzine (192).

The most consistent finding in the locomotor conflict studies is that barbiturates and alcohol increased approach performance of all species tested, in all of the situations which included tests of these compounds. The failure of alcohol to increase approach in a lever-pressing conflict (175, 177) may be due to the greater detrimental effect of alcohol-induced ataxia on this type of response. CPZ fails to increase approach in both lever-pressing and locomotor conflicts, with a few exceptions (175, 176, 184, 191). Other tranquilizing agents (meprobamate, benactyzine) apparently increase approach responding in some situations but not in others.

Other Conflict Tests.—An approach-avoidance conflict occurs during "experimental extinction," when the cessation of rewards for a learned approach response results in a conflicting avoidance response motivated by the aversive experience of frustration (193, 194). In this situation, the inhibited approach response is increased by amobarbital (193, 195) and by alcohol (193); this drug effect has been attributed to a reduction in frustration-motivated avoidance (193, 194). Likewise, amobarbital gives evidence of counteracting inhibition due to a frustrating schedule in which many of the responses are not rewarded (194, 196, 197) or due to a portion of a schedule associated with nonreward (198). These findings have been reported for locomotor responses of rats (193–197) and for lever pressing by pigeons (196, 198). Responding of rats inhibited by a signal for nonreward was increased by amobarbital in a runway but not in a lever-pressing situation (166), indicating a stronger drug effect for the locomotor response. A temporary increase in lever pressing by rats during the first few minutes of nonreward, attributed to frustration-produced emotionality, was enhanced by CPZ (199). A comparison of this compound with phenobarbital (200) showed that the barbiturate elicited a larger number of unrewarded responses, following a smaller initial increase in lever pressing at the beginning of extinction. Scopolamine and other anticholinergic compounds have been shown to increase preservation and to retard the inhibition caused by nonreward under a wide variety of conditions (201–203). However, scopolamine gave no evidence of diminishing the aversive effects of punishment (201), and the effects of anticholinergic drugs were attributed to a specific antagonism of the inhibitory effects of nonreward (203).

Drugs may help or hinder performance in a conflict situation by affecting the specific motor actions which are required. An example is found in the requirement that a pigeon hold its head for a specified duration in a restricted spot, intersecting two photocell beams, in order to receive food reinforcement (204). The birds were observed to be very excited and agitated in this situation, and the time they were able to remain sufficiently immobile was increased by CPZ and decreased by

pentobarbital. This conflict situation, in which the obstacle to the necessary response is the animal's own motor activity, is one of the few instances in which CPZ has been found to improve the performance of animals.

Drug effects on performance of rats in a complex conflict situation have been reported in a series of studies (205–209). The Lashley jumping apparatus is used to induce a maladaptive, perseverative-choice response during a long series of test sessions. The hungry animal jumps from a platform to one of two windows in a situation where the chosen window has an equal probability of opening to give access to food reward or of punishing the choice by failing to open so that the animal falls into a net below. An electric shock, delivered to the platform after 30 sec., forces the animal to make a choice and adds a further stressor to the situation. These procedures are highly stressful as shown by frequent urination and defecation on the platform and by the fact that the hungry animal usually does not eat on the trials when the door opens to make the food available (205, 206). The maladaptive, perseverative-choice response developed in this situation is highly resistant to therapeutic modification by drugs, but chlordiazepoxide (205, 206) and diazepam (206) gave evidence of reducing emotionality and improved the performance of some animals after a number of days of drug treatment. Under certain conditions, however, the reduced motivation under the influence of chlordiazepoxide prevented animals from acquiring an adaptive choice response (207). The other compounds tested did not have any therapeutic effect in this situation; high doses of CPZ, reserpine, and meprobamate but not phenobarbital gave evidence of reducing avoidance of the shock on the platform by causing the animals to delay jumping to one of the windows under some conditions until the shock was administered (205, 208). The therapeutic effectiveness of a guidance method for breaking a maladaptive, perseverative choice was apparently enhanced by amphetamine and retarded by CPZ and meprobamate (209). The maladaptive, perseverative-choice behavior seems to resemble certain types of neurotic behavior in humans, but this test situation has the disadvantage of being extremely complex, and the behavior was apparently resistant to the rather high drug doses used in these studies.

PERSISTENT BEHAVIOR ALTERATION

Most of the studies reviewed thus far tested the acute effect of a single drug administration on performance which had previously been well established and stabilized. Drug effects on acquisition, extinction, and relearning of responses are also of interest, especially for potential applications to therapy in humans.

Drug Effects on Acquisition.—CPZ (82, 210–214) and reserpine (82, 212) impair performance during acquisition of various types of avoidance responses in rats, but the magnitude of these drug effects does not appear to exceed their inhibitory effects on a well-established avoidance response. The magnitude of the drug effects may depend partly on the test situation; for example, CPZ caused slight decrement in acquisition of runway avoidance, but when the test was made more difficult by requiring the animal to select the lighted one of two adjacent compartments, this compound produced a much greater decrement (213). Likewise, CPZ but not pentobarbital reduced the percentage of rats learning to make the correct choice in a swimming escape situation which was made more stressful by forced immersion for 30 sec. prior to the start of each trial (215).

A variety of depressant drugs have been found to facilitate acquisition of an avoidance response in rats. These include reserpine at low doses (216), amobarbital (212, 217), meprobamate (218), alcohol (219, 220), chlordiazepoxide (221), and benactyzine (222). Some of the drugs which improved shuttle-box avoidance during the warning signal also were shown to increase the frequency of intertrial crossings from one compartment to the other (217–219) indicating that these ordinarily depressant compounds apparently decreased the tendency for immobile, freezing behavior in this stressful situation. These drug effects are influenced by certain characteristics of the test situation. Acquisition of a pole-jumping avoidance response was impaired by amobarbital (223); another pole-jumping situation where amobarbital facilitated acquisition (212) differed in several procedural conditions, including a longer interval between onset of the warning signal and the shock, and a longer intertrial interval. The same dose of benactyzine which improved acquisition of a shuttle-box avoidance response (222) impaired acquisition of a lever-pressing continuous avoidance response (224). On the other hand, a dose of scopolamine which improved acquisition of a lever-pressing continuous avoidance response (225) impaired acquisition of a pole-jump avoidance response (226). Various stimulant compounds have been shown to facilitate acquisition of avoidance, including amphetamine (212

223), pipradol (224), epinephrine (227), and ACTH (228–230).

Acquisition of an approach-avoidance conflict response was studied with the use of a signal that a lever press would deliver water reward to the thirsty rat, with a painful shock being delivered 15 sec. after onset of the signal (231). Inability to control the shock duration apparently enhanced its stressful effect, as indicated in a comparison of rats which escaped the shock by pressing a lever with matched animals which received the shock for the same length of time; after several days of training the latency of drinking was much shorter for the escapable-shock animals than for their paired inescapable-shock controls. A phenothiazine (thioridazine), administered chronically throughout training, substantially decreased the latency of responding, with a greater effect on the inescapable-shock than on the escapable-shock animals. This technique thus gives evidence for a tranquilizing effect of a phenothiazine not usually found with the more commonly used tests of conditioned suppression.

Drug Effects on Extinction.—An animal which always makes the avoidance response when the warning signal is presented will continue to respond unnecessarily even if failure to avoid is no longer punished by shock. Therefore, occasional failures to avoid serve an adaptive purpose, and when the warning signal is repeatedly presented without shocks, in a test of extinction of the avoidance response, excessive persistence of the learned response is maladaptive. However, if animals acquire the avoidance response in a nondrug condition followed by extinction trials under a drug, their performance may be influenced not only by the drug itself but also by the novelty of their drugged condition. It is necessary to have separate drugged and nondrugged groups in acquisition, so that the effects of the drug and of a change in condition can be equalized by changing half the animals of each group to the other condition at the start of extinction (13). The change in condition may have an important effect, as indicated by an experiment in which rats, following punishment for a lever-pressing food-rewarded response, resumed pressing and obtaining food without punishments under the influence of amobarbital but failed to continue pressing the lever in a subsequent placebo test (232). A decrease in avoidance response, due to a change from drugged to placebo or from placebo to drugged condition, has been shown in rats with amobarbital (185), phenobarbital (233), chlordiazepoxide (221), and CPZ (214).

A dose of CPZ which produced a moderate decrement in acquisition of avoidance response also moderately decreased the number of extinction trials before the animals stopped responding to the warning signal (210). CPZ decreased the probability of an avoidance response to the warning signal during extinction (234); all of the animals were in the nondrug condition throughout acquisition, so that the drug effect was associated with a change in condition, but phenobarbital had no effect on extinction performance after nondrug acquisition. CPZ had little effect on extinction of a runway avoidance response (213), but the requirement of a choice response almost completely eliminated avoidance responses during extinction under CPZ. A dose of phenobarbital which had no effect on speed of shock escape in a runway decreased persistence of the response during extinction, when shock was omitted (233). Other compounds have the opposite effect of increasing persistence of avoiding after shocks are omitted. Extinction of shuttle-box avoidance was greatly retarded by a dose of ACTH which had very little effect on acquisition (228). A dose of demeton,[1] which greatly reduced brain cholinesterase also retarded extinction of a platform-jumping avoidance response, with little effect on acquisition (235). Anticholinergic drugs have likewise been shown to increase persistence of avoidance responses in a variety of situations (202, 203).

A passive instead of active avoidance response may be tested by placing a rat in a box which it has previously explored without shock and measuring the amount of time spent in an adjoining, smaller compartment where it previously received painful shock (236–238). Drug effects have been reported with a similar procedure adapted for mice (239). This technique has generally been used as a measure of impairment in the passive avoidance response, presumably due to loss of memory, after administration of anticholinergic drugs. However, prolonged or repeated test sessions would provide a measure of extinction of avoidance.

There have been some studies of drug effects on extinction of avoidance in conflict situations. Rats trained to press a lever for water and punished for this response by shocks normally resumed pressing the lever in subsequent nonshock tests but not if ACTH was administered during punishment and subsequent test sessions (240). A higher level of performance found in animals punished under ACTH and tested without drug than in the placebo group might be due to the

[1] Marketed as Systox by the Chemagro Corp., New York, N. Y.

change in condition (13). Jumping or running was measured as the response to a signal for inescapable shock in mice (241), shocked under CPZ or placebo and all given nonshock extinction trials under placebo. Fewer extinction trials were required to abolish this active response to the signal in the animals which had been given acquisition under CPZ. This might indicate a tendency for CPZ to cause the acquisition of a freezing rather than active response to the signal for inescapable shock. Drug effects on learning to reverse a choice response were tested in rats which were trained to turn their head in one direction to turn off a gradually increasing shock, followed by trials in which only the opposite direction of head-turn escaped the shock (123). The reversal learning was greatly impaired by phenobarbital (123) and by meprobamate (242).

Prolonged Drug Effects.—Most of the studies have investigated the acute effects of a single dose of a compound. The chronic effects of repeated administrations may result in decreased drug effect on behavior, indicating tolerance, or else increased effect, indicating sensitization. Behavioral tolerance to the effect of a high dose of CPZ is shown in the finding (243) that there was progressively less suppression of a locomotor avoidance response on successive test days under the drug. A progressive development of tolerance is indicated by the finding that CPZ produced less decrement in a lever-pressing continuous avoidance response (244) if progressively higher doses were given, starting with a very low dose, than if the doses were given in a descending sequence. A more acute instance of behavioral tolerance is shown by the finding (245) that CPZ caused less decrement in a shuttle-box avoidance response if the test session began immediately after injection, providing a gradual onset of drug effect, rather than at the time of peak drug action. These and other factors influencing behavioral tolerance or sensitization to drug effects may alter the results of experiments, especially those which use repeated administrations of a drug. Furthermore, test compounds themselves are stressors if given in high doses or if they impair the performance of avoidance or escape in a stressful situation. Thus, the development of behavioral tolerance to the drugs may represent the stage of resistance to a chronic stressor.

A different type of response, suitable for measurement of the chronic effects of a prolonged stressor, is an increase or decrease in voluntary consumption of certain drugs. A number of the behavioral tests are designed to assess drug effects in alleviating or intensifying anxiety in a stressful situation. If animals could be trained to consume a drug in order to relieve their anxiety, this might confirm the tranquilizing drug effect and also provide a method for measuring the stress response to various experimental situations. A tendency for an increase in choice of an alcohol solution, during or after stress, has been found in cats (187), rats (246, 247), and monkeys (248). This behavioral response generally seems to be slight, with no resemblance to the human alcoholic's craving for liquor, but it apparently does occur in several species of animals in spite of the obstacles of the delayed pharmacological effect after drinking, the unpleasant effects of excessive quantities, the unpalatability of alcohol solutions except at low concentrations, and the difficulty of inducing consummatory behavior in stressful situations. A technique for self-injection, which may overcome most or all of these obstacles, was used in a study with rats, showing that inescapable electric shocks caused an increase in rate of lever presses which injected amobarbital into their jugular vein (249). Diminution of this response after several 1-hr. sessions indicates the possibility of habituation to the drug or aversive physiological effects of the injected substance. Tests in rats showed that stress failed to increase the choice of a solution containing reserpine (246) and actually decreased the choice of a solution containing chlordiazepoxide (250).

THE STRESS SYNDROME

Selye (1–3) has postulated that organisms subjected to alarming stimuli will respond in a given manner, which he termed "the general adaptation syndrome" or "stress syndrome." Briefly, the general adaptation syndrome (GAS) can be divided into three distinct stages. The first is the alarm reaction, associated with the discharge of adrenocorticotropic hormone (ACTH), cortical steroids, and catecholamines, plus various other physiological changes. The second is the stage of resistance, in which adaptation to the stressor results in a diminished reaction and thus increased resistance. The third is the stage of exhaustion, during which adaptation can no longer be maintained because of prolonged overexposure to the stressor. Different homeostatic adjustments may be aroused by various types of stressors, such as the contrasting stimuli of excessive heat or excessive cold. However, in the case of severe stressors the universal, nonspecific stress syndrome is generally the most prominent response.

Acute Exposure to Stress.—The following physiological responses to stress have been suggested by Selye (3). The stressor (stimulus) acts on the target (the body or some part of it) directly and by way of the pituitary and adrenals. An immediate discharge of ACTH stimulates the release of corticoids from the adrenal cortex. If the stress is extremely severe, the adrenal cortex shows morphologic changes characteristic of hyperactivity. Simultaneously, the animal's corticoid requirement markedly increases and there is an increase in the blood concentration and urinary excretion of corticoids and their metabolites. There is a general stimulation of the sympathetic division of the autonomic nervous system and the splanchnics induce the adrenal medulla to discharge epinephrine (E) and norepinephrine (NE), thus increasing the discharge of NE at various peripheral receptor sites and causing the cardiovascular responses of vasoconstriction and hypertension. Other marked physiological changes include alterations in water and electrolyte metabolism, gluconeogenesis and increased blood sugar levels, alteration in both red and white blood cell counts, and increased renin production by the kidney.

Some of these components of the stress syndrome have been measured in animals which were subjected to painful electric shocks in behavioral test situations. Elevated plasma 17-hydroxycorticosteroid (251) and NE levels (252) have been found in monkeys after sessions of pressing a lever on a continuous shock-avoidance schedule. There was also an increase in plasma steroid and NE levels after sessions of pressing a lever for food rewards in which no shocks were delivered but a conditioned emotional response was aroused by presentation of a clicking noise previously associated with shocks. Aceto *et al.* (109) reported that rats subjected to the pole-climbing avoidance test developed hypertension within 4 weeks. A recent study (253) showed that corticosterone concentration is elevated in rats at the end of a session of inescapable shocks, and an adaptive behavioral function of this physiological response is suggested by the further finding that the animals successfully acquired a shuttle-box avoidance response if they were trained immediately following their exposure to the warning signal paired with inescapable shocks; the animals did not acquire this response if the avoidance training began 1 to 4 hr. afterward, at which time the corticosterone had dropped to a normal level. Inescapable shocks delivered to the grid floor at regular intervals, for 1.5 hr., caused a 38% reduction in brain NE of guinea pigs (254). Another measure of sympathetic activation is the skin resistance of the paws, which was lowered in rats by a severe, single 5-sec. shock to the grid floor (255).

Compounds Altering Acute Stress Response.—There is potential clinical value as well as basic scientific information to be gained by identifying compounds which alleviate or aggravate the components of the stress syndrome. Several compounds have shown evidence of protective effects, with somewhat conflicting findings for CPZ. The large decline in brain-stem NE in rats, resulting from the stressor of inescapable electric shocks on the grid floor, was partly reversed by large doses of CPZ and pentobarbital but not by morphine (256). In another study, the decrease in adrenal ascorbic acid in rats due to the stressor of excessive heat or cold was partly counteracted by a moderate dose of CPZ and by a low dose of reserpine (257). A large CPZ dose had a similar effect on rats subjected to restraint at room temperature (258). The increase in blood glucose after rotation stress was partly counteracted by methylpentynol but not by CPZ (259). Sedative doses of CPZ and other phenothiazines have been reported to stimulate secretion of ACTH, mimicking the effect of exposure to cold temperature (260).

Stress increases the urinary excretion of catecholamines, especially E (261–263). Although E is the main catecholamine excreted in the urine during stress, a more important component of the stress response might be the general stimulation of the sympathetic nervous system and the increased discharge of NE at the peripheral receptor sites. Since the uptake of NE at the peripheral receptor site is very rapid, a more prolonged physiological change may be preferable as a measure of the stress response. Maickel *et al.* (264) reported that adipose tissue lipase was stimulated and plasma free fatty acid (FFA) elevated in rats by catecholamines, ganglionic stimulants, and exposure to cold, but plasma FFA was unaffected by cold exposure in the absence of a functional sympathetic nervous system. Gilgen *et al.* (265) found that an intact sympathetic nervous system was essential for increasing the output of FFA and glucose on exposure to cold and concluded that NE at peripheral nerve endings was essential for this reaction. Plasma FFA levels in rats are significantly increased by inescapable electric shocks delivered to the grid floor, and the degree of increase in plasma FFA is proportional to the duration of the stress. The effect of the stressor (electric shock) on plasma FFA was either blocked

or markedly attenuated by CPZ and meprobamate (266). The elevation of plasma FFA in rats by a similar schedule of inescapable electric shocks was effectively blocked by several tranquilizers (reserpine, benzquinamide, CPZ, meprobamate, hydroxyzine, and chlordiazepoxide). Two sedative compounds (pentobarbital and ethanol) were only partially effective; however, rather small doses were given. Two stimulants (dextroamphetamine and caffeine) elevated plasma FFA, in both shocked and nonshocked rats (267).

Since the stress syndrome may have an adaptive function in preparing the animal to resist a stressor, compounds which diminish the physiological reactions do not necessarily have beneficial effects. A more valid criterion for a protective effect of a compound might be a prolonged survival time during exposure to an acute stressor which is severe enough to cause rapid death. CPZ prolonged survival of rats subjected to combined heat and vibration stress (268) and of pigs subjected to combined heat and restraint stress (269). Survival of mice subjected to rapid mechanical vibrations was prolonged after pretreatment with large doses of chlordiazepoxide, reserpine, pentobarbital, and phenobarbital, and curtailed after large doses of iproniazid, dextroamphetamine, and morphine (270). Swimming time of mice in agitated cold water was prolonged by meprobamate (271) and by morphine (272), with no beneficial effect of hexobarbital or of several stimulants (271).

The generalized increase in sympathetic outflow, occurring in the alarm reaction to acute stressors, rapidly elicits a reciprocal stimulation of the parasympathetic nervous system. This reciprocal activation is not usually included in descriptions of the stress syndrome, but it adds a high level of internal stimulation to the effects of the original stressor and in some situations may be the immediate cause of the sudden lethal effect sometimes observed in cases of intense, acute stress. Richter (273) described this type of reaction, which is almost invariably lethal when wild rats are forced to swim in a vertical position with their whiskers clipped. In this situation, there is a marked slowing of heart rate, accompanied by decreased respiration and hypothermia, and at the time of death the heart is stopped in diastole, indicating a massive overstimulation of the parasympathetic nervous system. The lethal effect is aggravated by cholinergic drugs and retarded by atropine. This stress reaction may be influenced by the animal's experience with the situation, producing variations in the arousal of hopelessness or helplessness as a component of the perceived situation. Animals are much more resistant to the lethal effects if they are allowed to escape from the situation a few times instead of being maintained in the situation continuously.

Another consequence of excessive parasympathetic stimulation during stress may be the development of gastric ulcers. Reserpine has been shown to increase the incidence of ulcers in rats subjected to physical restraint for a number of hours (274–277). In one study (277), pretreatment with reserpine for several days prior to the restraint lowered the incidence of ulcers to the level of the nondrugged controls; this effect of more extensive premedication was attributed to the tranquilizing action of the drug. The incidence of ulcers after restraint stress has been found to be decreased by imipramine (275, 276, 278), thalidomide (279), cortisone (280), and a variety of other compounds, including anticholinergics, barbiturates, and CPZ (276, 281). A method for inducing ulcers without physical restraint is to immobilize rats for 24 hr. by punishing every motion with electric shock; ulcers were prevented by an extremely low dose (5 mg./Kg.) of meprobamate, but higher doses (10 and 20 mg./Kg.) were less effective, perhaps because they reduced the animal's ability to remain sufficiently immobile to avoid the shocks (282).

Chronic Exposure to Stress.—The alarm reaction cannot be maintained for long, and the process of adaptation or habituation enables most of the physiological reactions to return to their normal homeostatic level of functioning, even if the stressor continues unabated. This is identified as the stage of resistance, which continues until the stressor ceases or until exhaustion overcomes the adaptation. Mice forced to swim in cold, agitated water (272) were described as showing within the first 6 min. the agitated reactions of alarm reaction followed by the slower, energy-conserving behavior of the resistance stage and finally exhaustion when they sank beneath the surface. However, a much longer time span is generally required as a criterion for the stage of resistance.

Not all of the components of the stress response appear to return to normal levels of functioning during this stage. Aldosterone production increases, whereas corticosterone production is normal (283, 284), giving evidence that the renin–angiotensin II–aldosterone complex is involved in the response of the organism to chronic stress. Investigators (285–287) have

shown that the kidney is the source of an aldosterone-stimulating hormone and that the renin–angiotensin II system is involved in the stimulation of aldosterone production by the zona glomerulosa of the adrenal cortex. Miller (288) has shown that the glomerular zone increases in weight and hypertrophies by stress in hypophysectomized rats. Feldberg and Lewis (289) have reported that angiotensin is one of the most potent compounds inducing a release of catecholamines from the adrenal medulla, and other investigators (290–294) have provided evidence that there is an interrelationship between the activity of angiotensin II and the sympathetic division of the autonomic nervous system.

In a chronic stress situation, gastric ulcers have developed in monkeys performing a continuous shock-avoidance lever-pressing response for 6-hr. sessions, alternating with 6-hr. rest periods (295). The ulcerogenic effect was apparently a consequence of the chronic rather than the acute stress situation, because the gastric acid secretion was suppressed during the test sessions and greatly elevated in the rest periods. In rats, a chronic approach-avoidance conflict situation lasting 18 days gave rise to gastric ulcers which were greatly increased in animals given reserpine (296).

Gastric ulcers are not the only consequences of chronic stress. Friedman and Ader (297, 298) delivered inescapable electric shocks to the grid floors of the home cages of mice for 15 hr. per day, during a span of 7 days. The most stressful experimental condition, as indicated by the greatest loss in body weight (297) and the greatest susceptibility to the effects of injected coxsackie virus (298), was the presentation of the 2-sec. shock once every 15 min., at regular instead of irregular intervals, and with a stimulus light being presented for 15 sec. immediately before each shock instead of at different times. These conditions are similar to the typical schedule for a conditioned avoidance response. In another study (299), reduced weight gain and enhanced susceptibility to a toxic virus (herpes simplex) were found in mice after 28 days of 6-hr. sessions in a shuttle-box conditioned avoidance response. Animals tested for 1 or 14 days did not differ significantly from nonstressed controls. Measurements of blood pressure in rats tested for 42 weeks in a pole-climbing conditioned avoidance response (109) showed a rapid hypertensive response within the first few weeks on this schedule, persisting at approximately the same elevated level thereafter.

Rats subjected to a chronic variable stress program consisting of visual, auditory, and mechanical stimulation (flashing bright light, noxious intermittent sounds, and 120 oscillations per minute) for 4 hr. per day developed hypertension and had a high incidence of mortality within 20 weeks (300). Rosecrans *et al.* (301), using a similar stress protocol, also induced experimental hypertension in rats and found significant increases in both urinary NE and E following a single stress exposure, with a return to normal range by the eighth week of chronic stress. After the initial increase in secretion of adrenal E, adaptation occurred within 8 weeks. In contrast to acclimation of the sympathetic nervous system, the pituitary-adrenal axis appeared to continue to function maximally throughout the study, as indicated by high plasma steroid levels. The authors suggested that adrenal medullary activity appeared to be more important in acute stress situations, whereas the pituitary-adrenal axis appeared to play a more important role in adaptation during the long-sustained phase of chronic stress.

In spite of the great clinical importance of identifying protective or harmful drug effects in chronic stress, little research has been reported on drug effects in prolonged stress situations. Moderate doses of reserpine and CPZ failed to counteract the hypertension induced by chronic stressors but, on the contrary, potentiated the lethal effects of the stressors, apparently by the action of these compounds on the anterior pituitary-adrenocortical system (302). When reserpine treatment was begun after the seventh week of stress, blood pressures dropped to the control level but there was some indication of a higher mortality rate among the reserpinized than nondrugged animals (303). Acetylsalicylic acid failed to reduce blood pressures and greatly increased mortality of rats subjected to this chronic stress program; the deaths were apparently due to perforated gastric ulcers (303). The stress of physical restraint for 3 hr. per day caused 50% mortality within 32 days in rats pretreated with a large dosage of reserpine compared to successful adaptation and no mortality in nondrugged animals (304).

Drug Effects Modified by Stress.—In view of the physiological alterations involved in the stress response, the effects of some exogenous compounds may be expected to differ, depending on whether they are administered to a stressed or tranquil animal. Such differential effects have been found in a behavioral test situation in which rats turn a wheel to terminate a progressively increasing electric shock delivered to the grid

floor. There was an exaggerated stimulant effect of methamphetamine and caffeine and an exaggerated depressant effect of CPZ on escape performance of a group of animals previously given severe, inescapable shocks in the same apparatus. In contrast, alcohol had less depressant effect on the stressed animals than on the control group (305, 306). Another study has also provided evidence that the stress reaction potentiates the effects of stimulant drugs and of CPZ. With the use of a method of rating various measures of fright in rats introduced to a novel situation, animals whose fear was aroused by loud noises and by a bright, flashing light showed a greater increase in fright under the influence of E and a greater decrease under the influence of CPZ than did the low-fear controls (307). The finding that stress counteracted the depressant effect of alcohol (306) is convincingly supported by a report that rats under the influence of alcohol were better able to cling to a tilted plane after the stress of forced swimming or, to a lesser degree, after exposure to inescapable electric shocks or loud noise compared to nonstressed control animals. Amphetamine and E also improved performance under the influence of alcohol. Forced swimming likewise improved the performance of hypophysectomized rats, indicating that the depressant effect of alcohol was counteracted by a general arousing mechanism rather than by adrenocortical secretion activated in the stress reaction (308).

Reports have shown that stress may markedly alter the activity and toxicity of compounds. The survival time of guinea pigs administered emetine hydrochloride (a cardiotoxic agent) was reduced in animals which had been trained in a shuttle-box conditioned avoidance response and was further reduced in animals which, after training, had been subjected to conflict by being punished with shock when they made the conditioned avoidance response (309). The LD_{50} for amphetamine is less than one-tenth the dosage for mice or rats after receiving a brief severe inescapable shock every 8 or 10 sec. for 3 hr. than for nonshocked control animals (310). Rats being trained in a lever-pressing avoidance may be killed by normally sublethal doses of dextroamphetamine (19). Aggregation in a confined space may be a stressor, and there have been many replications of the original report (311) that aggregation greatly increases toxicity of amphetamine in mice. However, this effect depends partly on genetic factors, with some strains of mice showing little or no difference in amphetamine toxicity between the aggregated and isolated conditions (312). The toxicity of amphetamine in aggregated mice is greatly reduced if the animals have had 40 hr. of previous habituation to the same group of three in which they are placed after amphetamine administration (313). A study of the effects of several variables showed that amphetamine toxicity was increased by the stressors of elevated environmental temperature and forced activity as well as aggregation. Aggregation failed to increase toxicity under conditions in which motor activity was not stimulated (314).

A variety of other stressors have also been found to potentiate toxicity and pharmacological effects of compounds. Amphetamine toxicity was greater in mice after 4 weeks of chronic isolation stress, whether they were isolated or placed in a group after amphetamine injection (315). A similar result was reported after only 13 days of isolation, beginning at weaning (316). Isoproterenol toxicity was likewise found to be greater in rats after 13 weeks of isolation (317). The pentylenetetrazol seizure threshold in mice was lowered by restraint for a very brief (15-sec.) period immediately prior to the test (318). A subsequent study in the same laboratory showed similar effects on seizure threshold after more prolonged body immobilization (for 7.5 to 60 min.) and also after 20 presentations of inescapable electric shocks, at 1-min. intervals (319). In both studies, there was evidence for adaptation to the effects of more prolonged restraint. A study of the effects of three environmental temperatures on acute toxicity of a number of compounds showed the greatest toxicity at the hottest temperature (37°) for amphetamine and most of the other compounds tested, with the least degree of toxicity at 28° for the tranquilizers and at the coldest temperature (18°) for the stimulants (320). The lethal effects of scorpion and rattlesnake venom were potentiated by either cold (2°) or heat (35–38°) stress, with the greatest resistance being found at normal room temperature (321).

Contrary to these reports on the potentiation of drug effects in stressed animals, rats subjected to unilateral hindleg ligation showed shorter sleeping time after injection of hexobarbital, meprobamate, or zoxazolamine (322). This stressor was shown to lower the blood levels of hexobarbital, pentobarbital, and meprobamate (323). However, phenobarbital produced no significant difference in sleeping time (322) and blood levels (323) between the stressed and nonstressed animals. Shorter sleeping time was reported after injection of pentobarbital or a com-

TABLE I.—SUMMARY OF THE EFFECTS OF SEVERAL COMPOUNDS ON THE SPECIFIED BEHAVIORAL RESPONSE IN SEVERAL TYPES OF SITUATIONS

		Avoidance Response[b]		Avoidance Component of Conflict[b]		Stress Syndrome[b]	
	mg./Kg.[a]	Performance	Acquisition	Unavoidable Shock	Avoidable Shock	Alarm Reaction	Ulcers
CPZ	2	− −	− −	0	0	−	−
Reserpine	0.5	− −	−	− −	0	−	+
Chlordiazepoxide	10	−	+	− −	−	−	
Morphine	5	−	+	−	0	+	
Meprobamate	50	0	+	− −	− −	− −	−
Pentobarbital	5	0	+ +	− −	− −	−	−
Alcohol	1000	0	+	0	−	−	
Benactyzine	10	+	+	0	− −		
Scopolamine	0.5	+	−				− −
Dextroamphetamine	1	+	+ +	0	0	+ +	

[a] i.p. in rats. [b] +, increase; −, decrease; 0, unchanged; no entry, insufficient information.

bination of pentobarbital and CPZ, in rats which had been isolated for 4 weeks previously (315). The writhing response of mice to benzoquinone may be inhibited by electric shocks prior to the drug injection (324).

These studies, showing various ways in which a stressful situation influences the action of compounds, indicate that the physiological and endocrinological components of the stress syndrome interact with the administered compound. It would be useful to determine for each important drug whether its effects are potentiated, counteracted, or unaffected by stress, as an aid in determining appropriate doses during stress and normal conditions and also as an addition to scientific knowledge about the drug's mechanisms of action. The drug effects might also be influenced differentially by different intensities or types of stressor or at different stages of the stress syndrome. In contrast to the finding that severe stress potentiates the depressant effect of CPZ on behavior of rats (305–307), evidence has been reported (325) that the mild stress of exposure to a novel environment counteracts the depressant effect of CPZ on spontaneous motor activity of mice.

DISCUSSION

During stress, the physiological and behavioral alterations occur in an attempt to maintain or restore homeostasis. The marked increase in endocrine secretions and activation of the autonomic nervous system during the alarm reaction prepare the animal for violent fighting or flight. The energetic behavior may succeed in terminating the stressful situation and also discharges the excess energy potential, thus helping to restore the organism to its normal, homeostatic state. If the stressor continues, as in an inescapable situation, the best chance for survival is to conserve strength, with most of the physiological and behavioral responses returning to normal during the stage of resistance. The freezing, crouching reaction, often seen during prolonged stress, may also occur during the alarm reaction if the initial attempt to destroy or escape the stressor is unsuccessful. Violent, agitated behavior very quickly leads to exhaustion; the rigid, tense posture of crouching conserves energy and also keeps the animal alert to the environment and in a good posture for springing forward as soon as there is an opportunity for escape or attack. Another adaptive value of freezing in small animals is that a moving object is more likely to be seen by an enemy; furthermore, a predator is less likely to attack an animal that is immobile (326).

Summary of Drug Effects.—The findings reviewed in this paper may be classified as showing either a decrease (−), an increase (+), or no change (0) in the behavioral and physiological responses to stressors. Table I shows a classification of the effects of the compounds most commonly included in these studies for several of the most frequently used measures of behavior. The number of plus or minus symbols (one or two) indicates the degree of consistency with which the effect has been reported in the various studies. All species and routes of administration are included in the compilation of Table I, although the sample doses are specified as i.p. in rats. The absence of a symbol (+, −, or 0) indicates that the information is lacking. The behavioral tests of pain threshold and pain-induced aggression are not included here because almost all of the compounds have effects only at higher doses than those cited here. The data forming the basis for this table are rather meager and often inconsistent for most of these compounds; CPZ is the only one of these which has been

tested by more than one investigator for each of the six measures shown.

In spite of deficiencies in the available information, some meaningful patterns are apparent. The performance of an avoidance response is inhibited by several tranquilizing and general depressant drugs, unaffected by therapeutic doses of a muscle relaxant and hypnotic, and is increased by anticholinergic and adrenergic agents. Drug effects on acquisition of avoidance show a less consistent pattern, perhaps because drug effects on learning of a new response are more complex than drug effects on performance of a well-established response. Drug-produced decrements in avoidance performance cannot reasonably be attributed to a specific reduction of avoidance motivation, because the compound which most consistently inhibits avoidance (CPZ) has no effect in the approach-avoidance conflict situation, whereas the compounds which reduce avoidance of the shock in conflict tests (meprobamate, pentobarbital, and alcohol) have no effect on performance of avoidance and actually improve acquisition of an avoidance response. The two additional compounds which generally increase an animal's willingness to accept avoidable shocks, in a conflict situation where the food-rewarded responses are punished by shock (chlordiazepoxide and benactyzine), likewise improve acquisition of an avoidance response. Three compounds which reduce performance of avoidance (CPZ, reserpine, chlordiazepoxide) reduce physiological components of the alarm reaction, and dextroamphetamine increases both the behavioral and physiological responses, but the other compounds do not show much correspondence between behavioral and physiological effects.

These drug effects cannot be adequately explained in terms of general stimulation or depression. The first seven compounds listed in Table I might all be classified as depressants, but they show very different patterns of effects. A distinction between sympathetic and parasympathetic dominance may explain why the effects of reserpine, which depletes catecholamines, and of CPZ, a centrally acting α-adrenergic blocker, are generally opposite to the effects of the sympathomimetic agent, dextroamphetamine. Scopolamine and benactyzine, which reduce cholinergic stimulation, tend to resemble dextroamphetamine and differ from CPZ and reserpine in certain respects. The few studies on effects of cholinergic drugs, such as physostigmine, have shown profound decrements in avoidance. Five of the compounds listed in Table I (chlordiazepoxide, morphine, meprobamate, pentobarbital, and alcohol) do not have a marked preponderance of adrenergic or cholinergic effects. However, the balance or imbalance of the two divisions of the autonomic nervous system is a critical factor in the stress syndrome, as shown by the adrenergic stimulation characterizing the alarm reaction, followed by reciprocal parasympathetic stimulation which may lead to sudden death (273) or gastric ulceration. Some behavioral drug effects have been convincingly attributed to central sympathetic or parasympathetic stimulation. Avoidance is greatly enhanced by the combination of an adrenergic and anticholinergic compound (28); many other behavioral effects of drugs have been attributed to their central adrenergic or cholinergic activating or blocking effects.

The choice of drugs for protective or therapeutic effects may be expected to depend on certain features of the stress situation. Adrenergic or anticholinergic compounds may enhance and prolong the alarm reaction, helping the animal to destroy or escape the stressor. Termination of the stress situation is the purpose of the vigorous alarm reaction, and if successful this eliminates the need for adaptation to the stressor. However, in most cases the organism's own mechanisms provide sufficient adrenergic stimulation. Administration of certain compounds is likely to be disruptive, as indicated by the lethal effect of moderate doses of amphetamine in acute stress situations (19, 310). The most conspicuous behavioral effect of adrenergic or anticholinergic compounds is the persistence of unnecessary avoidance responses (203). Anticholinergics can be beneficial in counteracting the parasympathetic overstimulation which may cause sudden death (273) or gastric ulcers. However, therapeutic effects are found more often with tranquilizing drugs which prolong the stage of resistance. This might be due to the fact that in most experimental test situations the stressor is inescapable, so that survival is prolonged by physiological and behavioral adaptation rather than by increasing efforts to escape. In nature both escapable and inescapable stress situations occur, and the adrenergic and cholinergic systems apparently provide a mechanism for an appropriate response in either type of situation. Drugs influence simultaneously the behavioral and physiological responses to stress, but our knowledge of these effects is severely limited by the fact that drug effects on behavior have usually been tested in animals previously subjected to the stress situation repeatedly, for

TABLE II.—EFFECTIVE DOSE[a] OF CPZ AND MEPROBAMATE FOR INHIBITING AVOIDANCE PERFORMANCE OF RATS

	Lever Pressing		
	Continuous	Warning Signal	Pole-Jump
CPZ	1.1	3.0	3.5
Meprobamate	103.0	135.0	72.0
Ref.	(37, 45)	(45)	(92)

[a] mg./Kg. i.p.

many days or even weeks, whereas drug effects on physiological stress responses have usually been measured by restraint or some other acute stress procedure during a single session of several hours.

Variations in Test Procedures.—Each compound has a wide variety of effects in addition to the stimulant, depressant, adrenergic, or cholinergic action by which it is often classified. Differential effects may be analyzed by comparing drug effects in situations which differ in a particular specified feature. An important methodological aid for such comparisons is to measure effects of several doses in order to estimate the effective dose, usually defined as the dose which causes a definite change in the behavior of half of the animals (ED_{50}). This measure of the response to a drug is concise and may be standardized and used for comparing data obtained in different laboratories. Unfortunately, a wide range of variations in ED_{50} values has been reported by different investigators, even when using apparently the same procedures for the same compound, administered by the same route in the same species. Similar wide variation is found also in measurements of lethal dose (LD_{50}). Since the variations among laboratories which influence the toxicity or effectiveness of a compound would generally be expected to influence other compounds in the same way, the relationship among compounds in ED_{50} or LD_{50} might be expected to show more consistent results than the levels found for each compound singly. Table II compares the effective doses of CPZ with meprobamate for inhibiting avoidance in three different situations. A CPZ effect was found at a much lower dose in the chronic, continuous avoidance situation than when the extra stimulation of a warning signal was provided, and the pole-jumping response, which was least compatible with a crouching response, was least readily affected by this compound. Meprobamate affected avoidance only at doses which caused marked skeletal muscle relaxation; with this compound the warning signal had very little stimulating effect, and the pole-jumping response was most readily impaired, presumably because it required the highest degree of muscular coordination. These differential situations indicate some specific drug actions which may be identified; a great deal more could be learned from large-scale studies in which a number of different compounds are tested in several different situations, using the same species and route of administration. Unfortunately, such information is apparently very scarce at the present time.

The experimental findings reviewed in this paper permit some additional conclusions about interactions between drug effects and behavioral test situations. Generally, a lever-pressing avoidance response was most susceptible to drug effects, whereas avoidance by jumping on a pole or platform was most resistant to drug effects, with a running response being intermediate in this respect. The lever-pressing response was generally the most difficult to train, whereas the jumping response was usually the quickest way to escape the electrified grid and was readily learned. In the lever-pressing situation, it is probable that the usual procedure of training the animals to a stable level of avoiding over a long period of time somewhat counteracted the tendency to be more readily affected by the compounds. CPZ appeared to block avoidance at lower doses in rodents than in cats; this species difference might be interpreted as showing that the crouching reaction, which is potentiated by this compound, is a stronger response tendency in rodents. However, reserpine showed no such species difference, suggesting a different mechanism for the action of this compound in decreasing avoidance. A much greater decrement in a locomotor avoidance was caused by CPZ when the animal was required to select the correct one of two exits rather than being able to avoid by either route (213); perhaps the process of choice or decision potentiates the inhibitory crouching tendency. In an approach-avoidance conflict, alcohol has been shown to decrease the avoidance response in a runway (183, 184) but not in a lever-pressing test (175, 177), perhaps because the drug causes greater muscular interference with the manipulative than with the locomotor response. Some substantial differences in drug effects have resulted from seemingly minor procedural variations. Studies which isolate and experimentally manipulate the situational features influencing drug effects may contribute valuable information about drugs and behavior through the measurement of their interactions.

Recommendations for Experimenters.—Effective research in behavioral pharmacology requires testing several doses of each drug, to

obtain an ED_{50}. In order for this measure to be reliable it should be determined over a wide time range after administration, on a sizable number of animals. The value of an experiment is greatly increased by the use of several drugs in the same test situation and by a comparison of several measures of performance and several related procedures. These requirements can only be fulfilled by large-scale studies. It is often possible to use the same animals in testing different time intervals, doses, and compounds. This use of each animal as its own control reduces the number of animals needed, increases the sensitivity of the statistical comparisons, and also saves time in preliminary training. However, these advantages can only be obtained if drug effects are tested on performance of an already learned response, rather than on the process of acquisition or extinction. Also, the experimenter must be alert to the possibility that the nondrug performance may change during repeated tests, and that a test under a particular drug condition may influence performance in the following test session. In addition, the possibility of cumulative drug effects and development of tolerance should be taken into consideration.

Experimenters are encouraged to select test techniques which have already been used in a number of previous drug studies, such as the lever-pressing, shuttle-box, and pole-jumping avoidance. Most of the meaningful conclusions in this review have been based on results reported with the most frequently used techniques. If novel procedures are used, the data are greatly increased in value when the investigator also obtains comparable data from a related, commonly used technique. Some of the most novel techniques, such as immobility as an avoidance response (77) and lever-pressing escape from cold temperature (142), are potentially valuable methods which deserve and need a great many more studies to establish a pattern of drug effects under these experimental conditions. The characteristics of the test situation to be used may depend on the purposes. Performance that is difficult to acquire, such as a lever-pressing avoidance response, may have the advantage of being a measure of learned rather than innate behavior that is readily altered by drugs. On the other hand, a pole-jumping avoidance response has the advantage of being easier to train, and its similarity to natural behavior tendencies may be advantageous for certain clinical applications.

It is to be hoped that the findings on drug effects in animals will lead to useful clinical applications. This is a necessary and challenging task, with many complex factors to be taken into account in applying results to a different species under varied conditions. However, the experimenter should select techniques which are simple and yield readily understandable results. The complex, multiple-conflict situation tested in a series of studies with a jumping-stand discrimination (205–209) does not permit isolation and identification of the determinants of behavior. Even though the drugs may be used to alleviate multiple conflicts and complex neuroses, the preclinical tests should measure simple, prototype components of the naturalistic situation. A preliminary step is to obtain more data and gain better understanding of the drug effects in animal test situations. Some glib assumptions, such as the belief that CPZ decreases avoidance performance because of reducing fear of the shock, have been based on an inadequate amount of data. At present we still do not fully understand the crouching response pattern and the situations and drugs which influence it. The collection of further data will greatly increase the validity and usefulness of theories about drug effects on animal behavior and thus provide a firm basis for clinical applications. It would be easy to deplore the shortcomings of behavioral pharmacology research to date, but it is more constructive to emphasize its recency, with nearly all of the studies having been published since 1953. It is inevitable that such a new field of scientific knowledge is largely characterized by diverse methods, conflicting findings, and small-scale studies. Already the research seems to show improvement in scope and methods as well as a rapid increase in number of published studies. We can expect very rapid advance in the next few years.

The stress syndrome includes both behavioral and physiological components, which should be measured concurrently, such as by testing effects of drugs on behavior and also on brain amines, blood pressure, and other physiological measures in the same situation. Most of the behavioral tests currently being used to investigate centrally acting compounds involve a stress reaction to the animal. Cardiovascular, endocrine, and a variety of biochemical changes most likely occur; however, the quantitative changes will vary from animal to animal and will also depend upon the intensity of the stressor involved. Effects of the psychotropic compounds on animal behavior may in reality be effects on stress-induced alteration in one or more of the biological systems within the organism. The behavioral and physiological components of the stress syndrome interact with each other and with experimentally administered

compounds. The inclusion of both behavioral and physiological measures adds a new element of difficulty to pharmacological studies, but the value of the additional information may be expected to outweigh the disadvantage of the extra work required.

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