REVIEW ARTICLE

BIOLOGICAL ASSESSMENT OF TRANQUILLISERS. PART II*

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2. BEHAVIOURAL METHODS (not involving conditioning)

The methods of measuring sedation described under 1H and 1I might also have been described under behavioural methods, and form a link with this new section. We have grouped under behavioural methods those involving careful rather than superficial observation of responses of animals to their environment. These responses, though they may involve non-experimental conditioning, are spontaneous in the sense that experimental conditioning is excluded.

J. *Observational Methods*

Observational methods are often used as preliminary screening tests. Chen[203] discriminates between anaesthetic, hypnotic and sedative effects by the posture of rats and their reaction to tail-pinching, and Rubin and Burke[204] assay reserpine by grading the eye closure of mice. Similar methods combined with activity recording were used by Lim and co-workers[205]. Such techniques commonly detect sedative rather than tranquillising activity, and the effects of the sedative tranquillisers are the most impressive: thus reserpine, characteristically produces hunched posture and eye closure in laboratory animals[206]. The effects of tranquillisers have also been studied in fascinating experiments on the web-spinning behaviour of spiders[207], or on the fighting response of the Siamese fighting fish, *Betta splendens*[208,209], or its behaviour under stress[210], but it seems unwise to predict actions in man from results in these primitive creatures.

A more advanced technique for investigating the effects of tranquillisers on behaviour has been devised by Norton and co-workers using mainly the cat[211,212]: hamsters and monkeys are also suitable[213]. Briefly the method consists of observing the behaviour of untreated animals, selecting general patterns of behaviour, e.g., sociability, contentment, excitement, and aggressive or defensive hostility, and finding behaviour components that as far as possible are specific to one of the behaviour patterns. Five such components are chosen for each pattern, and for scoring purposes they are weighted, the least frequently occurring component being given the highest score. Each type of drug is said to have its own profile of action. Chlorpromazine predominantly reduces defensive hostility. Rauwolfia reduces sociability and, slightly, contentment. Meprobamate reduces hostility and sociability. Azacyclonol resembles rauwolfia in its effects but also increases excitement slightly[211]. Methamphetamine and LSD are found to have much in common; their effects being in general

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the converse of those produced by chlorpromazine[213]. There was not a perfect correlation of effects of drugs in different species, perhaps because the criteria chosen for each behavioural pattern were not the same in different species. This method, though involving a praiseworthy attempt to introduce subtlety of observation into a field where subtlety of action is frequently claimed but rarely demonstrated, appears extremely complex and time-consuming. Its value is not yet fully determined, but appears doubtful because of the rather similar profiles of action that have been recorded for some drugs the clinical effects of which are dissimilar (e.g., rauwolfia, azacyclonol).

The use of naturally aggressive animals should enable calming effects to be distinguished more readily from purely sedative effects. Rhesus monkeys are wild and aggressive and so make good subjects for this test. Chlorpromazine[214] and other phenothiazines[215,216], reserpine[217,218], meprobamate[148] and hydroxyzine[13] are all said to tame monkeys though their effects are not identical. Hendley and co-workers[219] claimed that chlorpromazine and reserpine insulate the animals from their environment most effectively by almost completely depressing reactivity to stimulation, but that meprobamate tames them more reliably while not affecting alertness. Hydroxyzine has similar effects to those of chlorpromazine[13]. Hosko and co-workers[215,216] also concluded that taming is not necessarily a function of sedation or resistance to arousal. Lysergic acid diethylamide and bufotenine also tame, probably due to sensory block as blindness, ataxia and analgesia accompany the tameness[220]. Benactyzine does not tame[132].

Chlorpromazine does not always tame; one calm monkey was made aggressive by a dose that calmed aggressive monkeys[214], and chronic administration of large doses of chlorpromazine caused major convulsions in four monkeys and produced apparent hallucinations in three of the four[221].

We have used these methods in Rhesus monkeys: our opinion is that the great variation in response of different monkeys, and of the same monkey on different occasions, and the difficulty of handling the large number that such variation requires makes these methods less attractive than they superficially appear to be. Also we are less convinced of the difference between tameness and sedation than some workers are, but we must admit that our experience is rather small.

K. *Direct Intracerebral Injection*

Drugs may be injected directly into the brains of conscious animals by using permanently implanted cannulae. The technique for implanting cannulae into the lateral ventricles of cats was described by Feldberg and Sherwood[222,223] and Haley and Dickinson[224] described a modification of this method for use in dogs. After recovery from the anaesthetic, the animals behave normally and show no ill-effects, appearing to be undisturbed by the presence of the cannula. Drugs can then be injected quickly and painlessly into the brain and their effects studied. Most drugs have pronounced autonomic effects, but more specific effects are

often observed as well. The actions of some psychotomimetics and tranquillisers have been investigated in cats. Mescaline produces bouts of violent scratching[225] and lysergic acid diethylamide (LSD) produces an initial restless state followed by drowsiness accompanied by slow waves in the EEG[225,226]. In the dog LSD has been said to produce a reversion from an adult to a puppy behaviour pattern[227]. In both animals a "fear complex" has been described[228]. Serotonin produces muscular weakness[225,227,229,230] and reserpine in suitable doses produces marked sedation[228,230,231] differing from that of serotonin especially in its time course[231]. The effects of the two drugs do not summate and the effects of reserpine cannot be duplicated by its metabolites[231]. Chlorpromazine in dogs also produces depression[232,233].

Haley observed similar results when the drugs were injected directly into the brains of mice[234,235]. Mescaline again produces scratching and, like LSD[235,236], causes aggressiveness at low doses and depression at higher doses[236]. Chlorpromazine produces alternating bouts of sedation and hyperexcitability in many animals[236].

Although these techniques were designed to eliminate peripheral drug effects, they seem to lack specificity and results have been disappointing. Many of the observed effects may be due to vascular changes.

L. *Sham Rage*

Normally friendly animals can be made aggressive by surgical interference with the brain, and the rage reaction found in such animals is usually called sham rage because it is elicited by slight stimulation and is inappropriate and purposeless. Chlorpromazine suppresses the symptoms of sham rage in decorticate and diencephalic cats[237] and rabbits[238], at doses that have no effect on the behaviour of normal animals. Reserpine also protects against the appearance of rage in similarly operated cats[239,240]. Rats made savage by septal lesions are tamed by meprobamate but not by chlorpromazine[241].

Rage reactions are also produced by electrical and chemical stimulation of the amygdala and surrounding areas of the brain[242]. Naquet, cited by Gloor[243], states that the behavioural effects of amygdaloid stimulation are only apparent during seizure-like discharge of the amygdala. However, chlorpromazine produces a marked increase in the spontaneous electrical activity of the amygdaloid complex amounting to seizure activity at high doses[244] and reserpine prolongs the duration of evoked seizures[245].

It has been reported that rage reactions in cats appear after lesions of the amygdala[246] but later work has shown that such lesions produce relative docility[247], and as similar lesions in rats[248,249] and monkeys[250] also produce docility, the balance of the evidence is in favour of taming by amygdaloid lesions. Weiskrantz and Wilson[251] pointed out the similarity between the effects produced by reserpine and those produced by lesions of the amygdala and adjacent structures in monkeys. However, by testing the effects of reserpine in amygdaloidectomised animals, these workers concluded that the amygdala is not a critical site of action of reserpine, and further observations have confirmed them in this view[252]. The exact

importance of the electrical changes induced by drugs at this site remains to be determined.

3. Neurophysiological Methods

The experiments last mentioned are part of a wider and still expanding branch of tranquilliser research summarised here as neurophysiological. The neurophysiological and biochemical approaches should eventually provide us with information first on the parts of the brain concerned with emotionality and other properties of mind, second on the chemical transmitters important at these sites. A more rational design of tranquillising drugs will then become possible. Currently we think that these extremely important researches have much investigative and doubtful predictive value. We therefore consider them only briefly.

M. *Arousal and Recruitment*

Understanding of consciousness and attention was greatly increased by the discovery of Moruzzi and Magoun[253] that electrical stimulation of the brain stem reticular formation produced changes in the EEG consisting of abolition of slow synchronous discharge and its replacement by low voltage fast activity. This response is now called the arousal reaction. The authors suggested that the cortical arousal reaction to natural stimuli is mediated by collaterals of afferent pathways passing to the brain stem reticular formation and thence through the ascending reticular activating system to the cortex. Other workers[254] had noted that sciatic stimulation produced a secondary diffuse cortical response as well as the primary response localised to the somatic sensory areas. Later work on afferent conduction showed that potentials in the classical lateral sensory pathways showed projection to specific areas of the cortex, segregation of modality, and rapid conduction, whereas medially conducted potentials displayed slower conduction, no segregation of modality and distribution in wide areas of the cortex by way of the diffuse thalamic system[255]. The response via the medial system is thought to provide a background of alertness upon which the sensory discrimination mediated by the lateral pathway can act effectively.

Confirmation of the importance of the reticular activating system in maintaining consciousness was provided by investigations of the effect of chronic brain stem lesions in cats[256] and monkeys[257]. Lesions interrupting this system were followed by chronic somnolence and EEG synchrony. The effects appeared to be more severe in the monkey. Temporary interference with the activity of the reticular activating system had early been postulated as the possible mechanism by which anaesthesia is induced, and indeed it was found that this system is extremely susceptible to the action of anaesthetics[258,259]. It was suggested that this susceptibility is due to the complex multisynaptic nature of the reticular formation[258]. However, some spinal interneuronal depressants have little or no effect on the reticular activating system[260,261] and therefore it was suggested that the pathway for cortical arousal may not depend on interneuronal connections but that there may be an extra-thalamic pathway involving the

direct passage of impulses from the reticular arousal system into the internal capsule[261]. It is, of course, difficult to visualise any central pathway that does not involve numerous relays: a particular type of interneuronal network may, however, display drug specificity.

In close functional connection with the midbrain reticular formation is the diffuse thalamic projection system[262,263]. Electrical stimulation of this system produces high voltage, slow wave recruiting responses over large areas of the cortex, the waves coming in "bursts" or "spindles"[264] and this response can be blocked by activating influences from the midbrain reticular formation[265].

Many drugs have different action on the "arousal" and "recruitment" systems; thus barbiturates block arousal but enhance recruitment, mephenesin and other interneuronal depressants depress recruitment without affecting arousal, whereas ether depresses both systems[260,261]. The action of barbiturates on cortical arousal is characteristically biphasic as an excitatory phase occurs before the depression; alcohol has a similar effect though the excitatory phase is less pronounced[266]. Since anaesthetics modify activity so profoundly, techniques have been evolved for chronically implanting electrodes in the brain of animals so that electrical recordings may be taken during consciousness[267–270]. Evoked potentials may be recorded if stimulating electrodes are also implanted[271].

Chlorpromazine induces EEG synchrony similar to that seen in sleep[272–280] and this has been said to be due to depression of the midbrain reticular system[272,277,280–285]. However, although the behavioural arousal response to auditory or sciatic nerve stimulation is depressed considerably[105,274,275,277,278], the threshold for electrical arousal by reticular stimulation is only slightly raised[286–288]. Chlorpromazine appears to enhance recruiting activity[285].

Like chlorpromazine, meprobamate[105,234,289,290], hydroxyzine[236], benactyzine[236,291] and methyprylone[105,289], all induce EEG synchrony. These drugs also block or depress the behavioural alerting response to auditory stimulation[236,289,291].

Reserpine is not generally reported to produce an electroencephalographic picture of sleep[217,292], though there are some reports of an initial "sleep" pattern[231,232,234] followed by an arousal pattern, but without behavioural arousal. There is also a report of EEG activation followed by depression[293]. The threshold for stimulation of the midbrain reticular formation is unaltered[232,287,288] or, more probably, lowered[294].

Although reserpine does not depress the midbrain reticular activating system, it produces behavioural non-reactivity[295]. The dissociation of behavioural and electrographic arousal shows that the two cannot be simply equated. The suggestion has been made[296] that in the normal animal, attention is selective, but reserpine, by making the activating system more sensitive, destroys the selective suppression of sensory input so that the mechanisms of attention no longer function. It is perhaps doubtful whether the effects of reserpine on the activating system are sufficiently striking to justify this view.

Other examples of dissociation are known. Physostigmine produces EEG arousal without a corresponding change in behaviour[297–303] and atropine produces a synchronised EEG without impairing behavioural alertness[298–303]. Such actions are responsible for the suggestion that cholinergic neurones are concerned in the function of the activating system[304,305], but adrenaline and related drugs also have powerful effects[306]. Substances such as adrenaline[297], ephedrine[307], amphetamine[297–299,308] and methyl phenidate[307] produce behavioural as well as EEG arousal, and so do the psychotomimetic drugs mescaline[309] and LSD[298,309]. Both these classes of drug also produce cerebral synaptic inhibition in the transcollosal preparation of Marrazzi and Hart[310] as does serotonin[311]. It is suggested that the naturally occurring inhibitory synaptic transmitters are adrenaline, noradrenaline and serotonin, of which the last is the most powerful[312,313]. The psychotomimetic substances mescaline, adrenochrome, adrenolutin, LSD and bufotenine resemble these transmitters in structure and in their inhibitory synaptic action[311,312]. Purpura[314] has suggested that the inhibitory action of LSD is due to an activation of inhibitory synapses. This property could be an important one in the production of "psychotic" states, especially as it has also been shown that the tranquillisers chlorpromazine, promazine, reserpine and azacyclonol prevent or reduce this inhibitory action in doses at which they have no effect alone on synaptic transmission[313,315].

These stimulating researches provide a variety of ideas for the synthesis of potential tranquillisers of novel type: for the present it would probably be wisest to test such drugs by methods described elsewhere in this review.

4. Antagonism to Psychotomimetic Drugs

Certain drugs induce behavioural syndromes which have been presumed by some authors to imitate neurotic and psychotic states, and the actions of tranquillisers on these syndromes have been investigated.

Morphine produces a characteristic state in cats[316], consisting of a variety of autonomic effects such as mydriasis, motor effects such as tremor, ataxia and hyperactivity, and "psychic" effects manifested as anxiety, negativism and apparent hallucinations. The state is sometimes termed mania but is sharply distinguished from human mania, particularly by negativism. The amount of hyperactivity and restlessness varies with the degree of restraint such as the size of the cage in which the cat is confined. Chlorpromazine reduces morphine excitement[317–319] but does not alter the autonomic effects[318,319]. Reserpine is also an antagonist when given after morphine[319] though when it is given before morphine sometimes it is reported to antagonise[320] and sometimes to enhance excitement[319]. It is confirmed that low doses of reserpine enhance morphine excitement[317]. Azacyclonol is variously reported as being an effective antagonist[130], having only a weak effect[320], and having no effect[319]. Meprobamate and benactyzine diminish the signs[320]. The difference in results may be due to the different ways of assessing the morphine effects but even where the

results are consistent they are of doubtful clinical significance because the actions of morphine in man are so different from those in the cat.

Mescaline, however, is a drug that produces "psychotic" changes in man. These properties have been known for a long time and the drug has been used as a ritual poison. Intravenous mescaline in dogs produces characteristic behavioural changes such as apparent anxiety, negativism and "catatonia" with autonomic symptoms such as mydriasis and salivation[321,322]. Premedication with azacyclonol prevents the catatonia[322]. Similar effects are observed in the cat when mescaline is administered intraperitoneally[322]. The "mescaline response" of cats given mescaline intraventricularly has been used as a basis for testing tranquillisers[322]. Predosing with reserpine prevented the symptoms from progressing to catatonia. Chlorpromazine suppressed the mescaline response but large doses deepened the catatonia. Azacyclonol changed the catatonic state to excitement[322]. These workers did not find that intraventricular mescaline caused violent bouts of scratching, though it has been reported by others[225]. In mice subcutaneous mescaline produces a marked psychomotor stimulation which is antagonised by chlorpromazine, reserpine, meprobamate, azacyclonol and serotonin, but enhanced by benactyzine and phenobarbitone[63,64]. Mescaline given daily to rats has a non-specific stressing effect[323]. In conditioned rats, mescaline interferes with the conditioned response so that it becomes disorientated and though the stimulus still keeps its significance it is said to produce a hallucinatory crisis[324], inhibited by chlorpromazine. Unfortunately a full account of this crisis has not been given.

Stoll in 1947[325] was the first to report the psychotic effects of lysergic acid diethylamide (LSD) in man, and since then this compound has been studied extensively, many of the experiments being done on human volunteers[326 329]. LSD, besides having subjective psychotomimetic actions in man, is said to produce a change in urinary phosphate excretion which is like that seen in schizophrenics. The phosphate excretion in schizophrenics and in volunteers after LSD administration is much lower than in normal controls, and in a stressful situation or following adrenocorticoid administration the phosphate excretion of both groups shows a marked increase, an effect not seen in the controls[330,331].

LSD reactions have been studied in various animals. The Siamese fighting fish (*Betta splendens*) has been used to study LSD since it gives characteristic responses to this drug[332], responses that are suppressed by crude beef brain extract[333] but not by a variety of amino acids[333] or by reserpine[334]. Similar responses are observed with methyl lysergic acid diethylamide[335,336] but not with some other closely related ergot drugs[337]. The effects of LSD on a stress response of these fish have also been investigated[338]. LSD produces a characteristic state in the guppy (*Lebistes reticulatus*) which can be antagonised by reserpine but not by chlorpromazine[339]. The behavioural changes can be prolonged indefinitely by exposing the treated fish to indole or tryptamine[339].

In cats LSD produces a state of co-ordinated aggression in response to visual and tactile stimuli[340], but of the tranquillisers chlorpromazine,

promazine, meprobamate, azacyclonol and reserpine, only chlorpromazine was consistently effective in antagonising the LSD response, and reserpine intensified the reaction[340]. In rats trained to climb a rope, LSD produces confusion and prolongs climbing time[341] and though meprobamate, benactyzine and serotonin have antagonistic actions, chlorpromazine and reserpine enhance the effects[342]. LSD produces excitation in mice[343] and causes them to walk backwards showing behaviour similar to that of normal mice facing down a slippery inclined plane[344,345]. Another behaviour pattern that appears in LSD-treated mice is a rapid head-shaking response or "twitch" to a light touch at the back of the head[346]. A similar response appears in a proportion of mice when they are kept in solitary confinement for three weeks. If it appears within two days of isolation, it remains permanently when the mice are returned to groups after the three weeks period. If the response only appears after a week of isolation, it is lost gradually when the mice are returned to groups[346]. The effect of reserpine on permanent "twitchers" is that the response is lost only during the period of drug administration, but in the less-permanent "twitchers" the lost response does not reappear when the drug is withdraw[346]. We have confirmed these results on the "twitch" that develops after solitary confinement, and consider that the technique is a promising one for the assessment of tranquillisers. The techniques involving antagonism of psychotomimetic drugs are in our opinion less useful than one would expect them to be. The results of attempted antagonism by tranquillisers are often feeble and variable and no single technique can be selected and confidently proposed as a particularly useful method of assessing tranquillising drugs. Further research on this type of method and on new psychotomimetic drugs is needed.

5. Conditioning Methods

The terminology of conditioning was devised by Pavlov though his rigid methods are very little used to-day except in Russia, and the conditioning techniques now commonly used elsewhere bear very little relationship to those originally put forward by the Pavlovian school.

Conditioning methods are often classified into classical and instrumental methods, and instrumental methods can be further classified into respondent and free-operant types. A classical conditioned response fails to secure reinforcement from the environment; an instrumental response secures reinforcement. A respondent conditioned response is elicited by an environmental stimulus; free-operant behaviour is emitted "spontaneously".

O. *Avoidance Conditioning*

In the search for a convenient conditioning technique, Warner[347], in 1932, described a method of conditioned avoidance that has been widely used. Rats are put individually into a box that has a grid floor and is divided into two compartments by a low fence. The unconditioned stimulus is an electric shock applied through the grid, and by terminating

the shock as soon as the rat scrambles over the fence, the crossing from one compartment to the other is soon established as a consistent response to the shock. Then a buzzer is introduced at a set interval before the shock, and provided this interval is not too long, crossing the barrier becomes established as a conditioned response to the buzzer and shock is avoided. This is not conditioning by Pavlovian standards because the response to the shock is not a naturally-occurring response and also because, as Warner pointed out, the responses to shock and buzzer, although in end-result the same, differ considerably, that to the shock being scrambling and inefficient whereas that to the buzzer is smooth and fast.

Warner's avoidance conditioning technique has been used by very many workers with very little modification[348 352]. Usually the experiments have been done with rats but a similar technique has also been used with monkeys[353,354]. The effect on conditioning of such variables as number of trials given per day and their spacing has been studied[355,356].

In the monkey, reserpine produces a decrement in performance[353,354] as do two other rauwolfia alkaloids, desmethoxyreserpine and rescinnamine[354]. Serotonin, like reserpine, produces a decrement in the performance of rats[357], and so does pentobarbitone: its action has been attributed to ataxia[354]. Azacyclonol has no effect[354]. Benactyzine, under the specialised conditions described by Jacobsen[349], improves the avoidance response. Chlorpromazine retards the acquisition of the conditioned avoidance response in the rat[352] and in trained rats it decreases resistance to extinction of the response[352,358,359]. Control experiments have shown that the effect of chlorpromazine on extinction is not due to a motor deficiency or to accumulation of the drug[359], but to relearning during the chlorpromazine treatment[359]. The effect of chlorpromazine on extinction of avoidance conditioning is so reliable with regard to dosage that it can be used as a bioassay technique[358].

A slightly different method of avoidance conditioning has been used by Courvoisier[324,360,361] and Cook[128,362]. In this method the rat clings to a vertical pole to avoid the shock. Chlorpromazine is said to produce a specific block of the conditioned response (C.R.), that is, it blocks the response to the warning signal but not to the shock[128,324,361,362]. Reserpine also has this effect[361,362]. Meprobamate, however, does not block the C.R. except in ataxic doses[362]. Other substances that do produce a specific block of the C.R. are morphine, serotonin and LSD[362]. Low doses of LSD antagonise the blocking effects of serotonin, reserpine, chlorpromazine and the non-specific action of meprobamate but have no effect on morphine action[362]. Barbitone and methylpentynol produce a non-specific block but pentobarbitone has a more specific effect though only at neurotoxic doses[362]. Pfeiffer and Jenney[363,364] tested the effects of various muscarinic drugs on the avoidance response while protecting the animal from unwanted peripheral effects by using methylatropine nitrate which does not pass the blood-brain barrier. Arecoline, pilocarpine and eserine inhibit the C.R. under these conditions, so the mixture of arecoline and methylatropine was tried clinically and was found to produce a striking lucid interval in schizophrenics.

Another type of avoidance conditioning has been tried in cats and suggested as a possible basis for testing tranquillisers[365]. Cats with a natural tendency to attack mice are conditioned by being given an electric shock every time they attempt to pick up a mouse. This is a very rapid conditioning procedure and an average of only three trials is required to produce mouse avoidance behaviour that persists for several weeks without reinforcement. Under the influence of chlorpromazine, benactyzine, and to a lesser extent meprobamate, cats so trained will continue to pick up the mouse again and again though dropping it when shocked[365]. This procedure is claimed to demonstrate the same relative order of activity of drugs as is obtained by more elaborate conditioning methods, and therefore appears promising.

P. *Free Operant Conditioning*

Avoidance conditioning is not the only type that can be used for testing the effects of centrally-acting drugs. Another that has been adopted successfully to the study of drug effects is the free operant response for a food or water reward. This technique is due to Skinner who conditioned hungry or thirsty rats to obtain pellets of food or drops of water by lever-pressing. The animals will work for a very small reward so that the experiment can be quite long. One of the reasons why this technique has become so important is that the rate of lever-pressing provides a quantitative objective measure of the animal's behaviour, which, under these conditions, is emitted quite spontaneously. In Skinner's early studies on discrimination the correct response was always rewarded, but this meant that the eating of the food pellet interfered with the rate of lever-pressing. Dinsmoor[366] showed that periodic reinforcement is a suitable procedure and that the rate of lever-pressing is a satisfactory index of learning. If the periodic reinforcement is at a fixed interval a temporal discrimination tends to be set up so that the reinforcement schedules must be carefully selected.

It has been shown that the rates of response are markedly affected by the schedule of reward[367]. Dews, using the pecking response of pigeons for a food reward, showed that a fixed ratio of reinforcement, for example, a reward for every fiftieth peck, produced a high and constant rate of pecking, whereas a fixed interval schedule produced a low rate of pecking which increased in rate throughout the interval[368]. The effect of drugs depends very much on the schedule of reinforcement used. For example, pentobarbitone on a fixed interval schedule causes a markedly reduced rate of pecking, but has no effect or even increases the pecks on a fixed ratio schedule[368]. In using this technique for screening purposes Dews[369] suggests that the ratio performance should be used as an indication of the physical capabilities of the animal, and that the effects to be noted on interval schedules are the total number of pecks made and the amount of pausing of more than 10 seconds that occurs. Reserpine has a characteristic effect of producing bursts of high rates of response interspersed with long pauses, and this is interpreted as the release of the pigeon from normally powerful stimulus control and an analogy is drawn between this

effect and the reduction of obsessive compulsive drives[369]. Not only the schedule but also its parameters alter the effects of drugs[370].

Pigeons form especially suitable subjects for studying the effects of drugs on discrimination because their vision is so good. Instead of having a single key to peck the pigeons are faced with two keys that can be illuminated separately and they have to learn to peck the lighted key when an intervening bar is dark and the dark key when the bar is lighted[371]. This provides a sufficiently fine discrimination task, and both correct and incorrect responses can be recorded. Drugs can be even more accurately differentiated by this method. Blough[372] showed that LSD elevates the visual threshold at doses which cause no disturbance in motor performance or discrimination and that although LSD and chlorpromazine both reduce the rate of pecking LSD improves discrimination whereas chlorpromazine impairs it. Pentobarbitone and alcohol, like chlorpromazine, also reduce accuracy but, unlike chlorpromazine, they increase the response rate. This technique probably provides one of the best means available for investigating subtle differences in central actions of drugs, but is, of course, elaborate and time consuming.

The mechanical set-up used in free-operant conditioning can be adapted to include study of avoidance conditioning. For example, lever-pressing behaviour can be maintained by having a high frequency auditory stimulus continuously present unless the lever is pressed to give a silent period[373]. More generally, however, an electric shock is employed as the aversive stimulus. This technique has been used with rats[374] and monkeys[251]. Shocks are given at regular intervals unless the lever is pressed, when the next shock is delayed. Sometimes a warning signal is given before the shock and the shock can then be avoided by responding during the warning period, or escaped by responding during the shock[375]. Reserpine depresses avoidance behaviour under these conditions, but pentobarbitone affects it only slightly or not at all[251].

On theoretical grounds it would seem that the screening of tranquillisers should be based on avoidance rather than on positive-reinforcement conditioning. However, an even better method might be to investigate drug effects on the emotional disturbance or "anxiety" caused by expectation of the unpleasant stimulus. This is a classical, not an instrumental response.

Q. *Conditioned Emotional Responses*

Jacobsen and co-workers[349] have studied the stress, partly an unconditioned and partly a conditioned emotional response, shown by rats subjected to repetitive buzzer-shock sequences. This stress is scored quantitatively by allotting positive marks for muscular tension, immobility, arched back, pilo-erection, etc., and negative marks for relaxed posture, and movements. At the same time conditioned avoidance responses may be studied though the circumstances are not ideal for this purpose. In our experience the scoring system may be much simplified, the score for "tense-immobile" alone showing a satisfactory slope against log-dose of benactyzine. Benactyzine, a known parasympathocolytic drug, was

selected by Jacobsen as the best of a series of compounds tested for their ability to reduce the stress score. It was also shown to increase the number of avoidance responses. This drug was one of the earliest, if not the earliest, to be selected by a planned programme of behavioural research based on an analysis of the type of action on conditioned responses to be expected of a clinically useful tranquilliser, and Jacobsen's contribution to methodology in this field has been outstanding. It is unfortunate that benactyzine, whose actions on conditioned responses, both emotional and avoidance, are more impressive than those of any other drug we have handled, should have made so little progress clinically. This fact suggests that a wider evaluation of benactyzine, particularly perhaps under free-operant conditions, is desirable so that correlation between its laboratory and clinical properties may be reassessed.

On Jacobsen's test a few other compounds such as hyoscine and high doses of reserpine can also "normalise" behaviour[376,377] though reserpine does not increase the number of avoidance responses. Meprobamate has an effect similar to that of benactyzine but at higher doses, and it does not make the animals more lively as benactyzine does[377]. We find meprobamate only feebly active at doses that just fail to cause ataxia. Chlorpromazine, alcohol, the barbiturates and other compounds tested do not improve performance or "normalise" behaviour[376–378].

R. *Conditioned Emotional Responses During Free-operant Behaviour*

A previously neutral stimulus such as a "clicker" signal can acquire an emotional significance by being paired with an electric shock. Usually the "clicker" is sounded for two or three minutes and is followed by a shock. If the conditioned emotional response to this signal is superimposed on a lever-pressing habit, lever-pressing is inhibited during the signal, and by comparing the rates of response before and during the signal a quantitative measure of the conditioned emotional response can be obtained[379,380]. Usually a variable interval schedule is used because this gives a high, constant rate of response. It is convenient to record the response cumulatively on a slowly-moving kymograph; the control period can then be seen as a steeply sloping line and the conditioned emotional period as a nearly horizontal one. The effects of drugs can then be noted both on the slope of the control part of the graph and on the difference in slope between emotional and non-emotional responses. As many drugs cause a change in overall rate of responding[381], Brady[382] expresses his results in terms of an "inflection ratio". The inflection ratio is the ratio of the difference between response rates during and before the stimulus to the rate before the stimulus. Complete cessation of lever-pressing during the conditioned emotional periods gives a ratio of -1 and a constant rate of responding before and during these periods shows as a ratio of 0.

Reserpine is the drug that has been most widely tested by this technique. Brady reported that reserpine reduces the overall rate of lever-pressing by rats and monkeys but abolishes the distinction between "clicker" and "non-clicker" periods[381,382]. Weiskrantz and Wilson[383], supplementing

the objective measurement with observational ratings, and testing monkeys for acquisition, extinction and retention of the conditioned emotional response also concluded that the reserpine-treated animals showed considerably less disturbance during the emotional stimulus than did saline controls. However, Stein[384], using slightly different techniques, found that rats drugged with reserpine almost to the point of inactivity, still acquired an intense conditioned emotional response. The discrepancy between the results is unexplained. Brady[382] has studied the point of action of reserpine on his conditioned emotional response by introducing a behavioural control. Rats are trained on a schedule identical to that described for emotional conditioning except that the electric shock is only given if the lever is pressed during the "clicker" signal. Under these conditions reserpine does not restore lever-pressing during the signal. Its action can therefore not involve failure to hear the signal or indifference to the shock, and may reasonably be supposed to involve reduction of anxiety associated with an expectation of unavoidable shock. Chlorpromazine does not block the conditioned emotional response but weakens it so that the behaviour of treated animals is like that of animals conditioned with weaker shocks[385]. Chlorpromazine blocks extinction of the response[385]. Meprobamate does not block the conditioned emotional response though observationally the rats are calmer and more relaxed[385]. Morphine has been shown to restore bar-pressing inhibited by anticipation of shock in in rats, and the effect is proportional to the dose until doses causing confusion are reached, so the conditioned emotional response superimposed on bar-pressing in response to positive reinforcement has been proposed as a suitable test for analgesics[386–388].

The conditioned emotional methods described in this section are among the most impressive yet proposed and further work with them must be eagerly awaited.

S. *Conflict Neurosis*

Another study has developed from Pavlov's work on conditioning, that of "experimental neurosis". In Pavlov's experiments the neurotic state was brought about by forcing the animal to make increasingly fine discriminations until discrimination became impossible. A technique has been described for inducing this type of neurosis in the rat by training it to discriminate between light signals securing on the one hand positive and on the other negative reinforcement in the Skinner box[389–391]. It appears not to have been used for drug evaluation. The essential element in production of neurotic behaviour by such methods is not the increasing difficulty of discrimination *per se*, but the associated conflict. Masserman[392] produced approach-avoidance conflict in cats by training them to secure food at a bell-signal by switch-pressing, and then intermittently superimposing an aversive air-blast stimulus. This approach-avoidance conflict gave rise to a form of behaviour designated as "neurotic". The cat showed signs of fear and tension, feeding behaviour was disrupted, displacement activities appeared, and some of the behavioural abnormalities persisted out of the experimental situation. Masserman[392]

studied means of making the neurosis more severe and long-lasting, such as forcing the animal to the food box by reducing the size of the cage, and he also studied possible methods of curing the neurosis; methods such as rest and the display of a normal cat. The effect of drugs on this type of conflict behaviour in cats has been studied by Jacobsen and co-workers[377,393,394]. Of the drugs tested, benactyzine gave the best results[377,393,394] though meprobamate also had a considerable normalising effect but only at ataxic doses[377]. Alcohol also produced some improvement in behaviour but at ataxic doses[377,393,394]. Chlorpromazine[377,393,394] and reserpine[377] were ineffective and it was suggested that this is possibly because of the stupor they caused which masked any return of feeding to normal. This is an interesting method but it requires further study: the activity of tranquillisers does not correlate well with their usefulness in clinical psychoneurosis.

T. *Motivation*

Any conditioning procedure involves the use of appropriate motivation. Avoidance conditioning makes use of aversion to noxious stimuli such as electric shock; free-operant conditioning makes use of appetitive drives; and the conditioned emotional techniques of Brady and Hunt and approach-avoidance conflict methods use both types of motivation. In interpreting the effects of drugs on these tests, therefore, it is important to consider the effects of the drugs on motivation. As Miller has pointed out[395] various methods have been described and used for measuring "fear" or "emotional" responses without there being much work on the relationship between the measures obtained by such different techniques. There has been some work on the differences in drug effects when different motivations are used during free-operant conditioning[396,397]. In tranquilliser work it is particularly important to determine drug effects on appetitive as well as on fear drives. For example, tetraethylammonium was reported to reduce fear in rats but experiments by Brady[398] showed that this drug depressed hunger- or thirst-motivated behaviour as well as that due to fear.

But the effects of psychotropic drugs on motivation are important in their own right. In a situation of conflict, either experimentally produced or as found clinically, reduction in intensity of one of the conflicting drives may well be a satisfactory means of resolving the conflict. Miller[395,399] has emphasised the need for using a number of diverse techniques for evaluating the effects of drugs on motivation. He[399] cites the work of Conger[400] on the effect of alcohol on approach-avoidance conflict in rats. Hungry rats are discouraged from running down an alley for food by means of electric shocks, the intensity of which is increased until the rat refuses to go to the food box. After alcohol administration the animal resumes eating at the box. This effect was shown to be due to a reduction in the fear of shock rather than to an increase in hunger by testing the strength of each motivation separately. However, it was also found that altering the width of illumination of the alley produced the same changes as injection of alcohol, so that the effect of the alcohol might simply be

due to change in perception of the situation. Carefully devised experiments permit analysis of such complex effects, which exemplify the need for caution in interpreting the actions of tranquillising drugs in any single experimental situation.

U. *Neurophysiology and Conditioning*

Neurophysiological evidence indicates that there are two stages in conditioning, the first involving more general and diffuse effects in the brain, and the second more specific and localised effects[401,402]. An intermediate stage has also been described in which the visual cortical rhythm tends to follow the frequency of the intermittent photic stimulation used as the unconditioned stimulus, but during the presentation of the conditioned stimulus[402]. Recordings from the reticular formation suggest that it is of great importance in the elaboration of the conditioning processes[402,403] and therefore that drugs which affect the activity of this structure must also affect the expression and acquisition of conditioned responses[403]. The work on and the evidence for the action of tranquillisers on the midbrain reticular formation is reviewed in the section on neurophysiology (M). An investigation on the differential action of chlorpromazine on reflexes conditioned to central and peripheral stimulation[404] showed that this drug produced a greater depression of the peripherally-induced conditioned response and it was suggested that this differential action might be due to the action of chlorpromazine on the arousal system.

As tranquillisers affect conditioning, it is interesting to note the effects on conditioning of various procedures used clinically in the treatment of mental disorder. Gellhorn[348,405] found that leptazol, insulin hypoglycaemia and electrically-induced convulsions cause recovery of an inhibited conditioned avoidance response in rats. A similar effect is produced by benactyzine[406]. Hunt and Brady have shown that a series of electro-convulsive shocks will diminish or eliminate a conditioned emotional response[380,407,408] in the Skinner box and that if the convulsions are prevented by giving the electroshock under ether anaesthesia the effect of the electroshock is not nearly so marked[409]. Later experiments showed that audiogenic convulsions also eliminate the conditioned emotional response whereas auditory stimulation of animals that are not susceptible to the convulsions has no effect[410]. Neither auditory stimulation nor audiogenic seizures destroy memory as indicated by the number of errors made in running a maze[411]. Therefore it is possible that convulsions affect emotional behaviour selectively, though it is probable that care must be taken in applying results on the so-called emotional conditioning of laboratory animals to the treatment of emotional disorders clinically.

V. *Self-stimulation of the Brain*

Delgado observed that animals showed anxiety in a situation which had been previously associated with electrical stimulation of parts of the brain[412,413] and this anxiety could be used to motivate avoidance and operant conditioning[414]. Olds and Milner[415,416] found that stimulation of other parts of the brain acted as a positive reinforcement or "reward" effective in operant conditioning and in maze running. There is some

suggestion that increase in amperage of the stimulating current increases the reward value of the stimulation[417]. The reinforcing value of the stimulation and effects of drugs can be assessed by the rate of bar-pressing in a Skinner-box[418]. Drug effects vary with different electrode sites. Chlorpromazine and reserpine depress response rates for hypothalamic stimulation but have less effect when the electrodes are implanted in the septal area[418]. Azacyclonol depresses hypothalamic and septal stimulation rates but its effects are only of short duration[419]. Pentobarbitone has little effect[419]. Amphetamine increases rates of responding but pipradrol only does so with laterally placed electrodes[419] and LSD is a non-selective depressant[420].

Some electrode positions elicit a curious alternation of behaviour: when rats are trained to make one response to turn the stimulation on and another to turn it off, they alternate repeatedly between the two responses[399]. It was found[399] that methamphetamine and chlorpromazine both depress the response rates but whereas the main effect of methamphetamine is to delay turning the stimulation off, chlorpromazine causes a large increase in the time before the stimulation is turned on.

These interesting and ingenious techniques should provide information about both the motivational effects of drugs and their sites of action.

Discussion

We have already commented briefly on most of the methods that we have listed and classified, and now propose to discuss only their selection for screening purposes, and the directions in which improvement seems necessary or likely.

It is probable that at present no single method can confidently be used for the evaluation of novel drugs, unless prediction of their properties is unusually precise, and most workers in this field use a battery of tests. Tripod's interesting paper[63] provides an excellent illustration of the way in which such a battery can be used to discover the spectrum of activity of tranquillisers, sedatives, hypnotics and anticonvulsants. Obviously more reliance must be placed on some tests than on others and, if random screening is being used, economy will usually require that some sequential design be adopted. More informed searches for new drugs will probably require test patterns that differ according to the types of drug being studied: for example, meprobamate and chlorpromazine have very different activity spectra. Nevertheless, most useful test patterns will probably include methods from all, or nearly all, of the five classes we have reviewed. They should certainly include some of the more specific methods, and, if possible, some of the methods that are known, or believed, to correlate best with clinical usefulness.

It is this last respect in which improvement is most necessary. All of us should like to have methods of inducing in animals recognisable mental disorders simulating the main clinical disorders. We do not think that such experimental neuroses as have been described are completely satisfactory, and the problem of setting up model psychoses is almost wholly unsolved.

It is, of course, debatable whether the non-human brain, even of a primate, is sufficiently advanced to display that complexity of irrational behaviour which characterises psychosis. Rodents and carnivores are probably still less promising experimental subjects. Nevertheless there is much evidence that the neurophysiological events underlying the most complex human behaviour, rational or irrational, are unlikely to differ in kind from those of more primitive creatures. We think that advance is most likely to result from increased knowledge of the links between behaviour and such neurophysiological events. It is probable that much of this knowledge will be obtained from studies of tranquillisers in unified programmes of behavioural and neurophysiological research, and that such knowledge will allow the design of still better tranquillisers, and their recognition by improved methods.

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