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THEORETICAL REVIEW

Use of the Immobility Reflex ("Animal Hypnosis") in Neuropharmacological Studies[1]

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KLEMM, W. R. *Use of the immobility reflex ("animal hypnosis") in neuropharmacological studies.* PHARMAC. BIOCHEM. BEHAV. 4(1) 85–94, 1976. – The immobility reflex (IR), a reversible, involuntary, immobility response in certain species is advocated as a uniquely useful assay system for testing of psychoactive drugs. One of the two potential areas of application is that measures of IR duration or arousal threshold serve to screen drugs to help establish drug classification, relative potency, and degree of extrapyramidal side effects. Drugs can also be tested for their neural target sites and modes of action by recording electrographic responses in various brain areas during IR. Electrographic activity (EEG, averaged evoked responses, multiple-unit activity) is relatively stable, artifact-free, and less influenced by behavioral feedback and other variables that are problems with alternative experimental preparations. The reversibility of the IR offers the advantages of chronic studies (evaluation of long-term effects, replication of results, and dose-response testing in which each animal can serve as his own control). Results from both areas of application would ultimately need cross-checking by other methods to rule out interactions of IR and the independent variables being tested. Further possible interactions in long-term studies include potential interactions between the degree of tolerance developed to repeated IR trials and to repeated drug administration.

Immobility reflex Neuropharmacology Psychoactive drugs

THE immobility reflex (IR) ("animal hypnosis") is a unique experimental preparation for psychoactive drug testing. It has two potential areas of application for drug research and development: (1) screening of psychoactive compounds to help establish class, potency, and motor side effects, and (2) testing of drugs for their neural sites and modes of action. The main limitation is that only certain species are highly susceptible (arthropods, amphibians, reptiles, birds, and among mammals, guinea pigs and rabbits). A similar state can be induced in many insects and crustaceans, but the neural mechanisms may not be identical.

THE PHENOMENON

Characteristics of the IR in vertebrates have been reviewed [7, 21, 36]. The state is usually induced by manual restraint, with the animal struggling initially until after a few seconds of continued restraint immobility suddenly ensues and further restraint becomes unnecessary.

The phenomenon can be illustrated most vividly by a brief account of the historical development of terminology. Some of the early names for the state are seldom used, because they are anthropomorphic: "animal hyponosis, death feint, playing possum, mesmerism" and others [7]. Contemporary terminology seems to focus on one of two descriptors: "tonic immobility" or "immobility reflex". Both focus appropriately on the immobility, which is the cardinal sign of the state (Fig. 1). "Tonic immobility" has the advantage of being a seemingly strict and unambiguous behavioristic description; however, since immobility only lasts for a few minutes, one could argue with equal logic that a more appropriate descriptor would be "phasic immobility". In fact, the term "paroxysmal inhibition" has been used [39]. "Immobility reflex" has the advantage of describing the state in a way that fosters thinking along lines of the neurophysiological bases. The state does exhibit most of the characteristics of a reflex, as classically defined: it is a reversible, involuntary, unconditioned, and stereotypical response to specific kinds of sensory stimulation (commonly manual restraint, especially with inversion).

Terminological controversy has arisen over the breadth of the operational definition of reflex. Many would argue that reflexes have to involve movement, not absence of movement. On the other hand, reflex action, as viewed neurophysiologically, very often includes postsynaptic inhibition (examples: ipsilateral Renshaw cell inhibition of alpha motoneurons; inhibition of contralateral flexor

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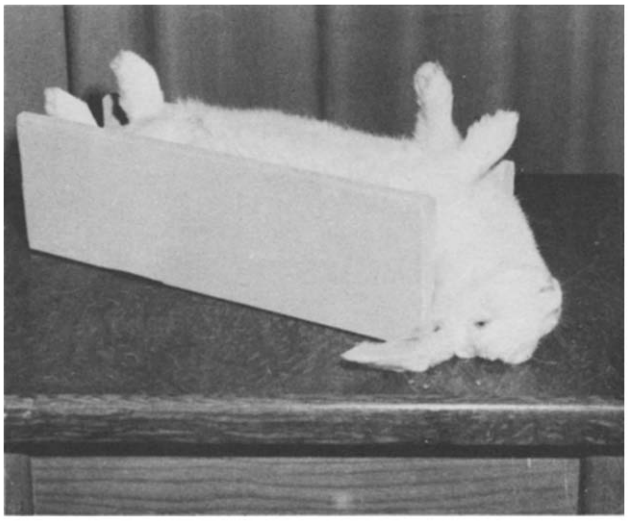

FIG. 1. IR in the rabbit. The chute is used to prolong IR, but it is not essential for inducing the state (from Klemm [17], reprinted with permission).

motoneurons in the crossed extensor spinal reflex). Others would argue that reflexes do not habituate, at least not permanently. Aside from the fact that only arbitrary definitions preclude reflexes from being habituatable, no very rigorous tests have been done to indicate that the habituation of IR (which is not prominent in all species) is in fact absolute or permanent; in some species at least, IR might recover, given sufficient time and absence of the unconditioned stimuli. Finally, it could be argued that IR behavior is neurophysiologically too complex to be called a reflex. However, the IR has not been claimed to be "simple" [21,22] and many other generally accepted reflexes may be equally as complicated (examples: spinal walking/swimming reflexes, suckling reflexes of newborn). Finally, there does seem to be well-established terminological precedent for identifying as reflexes states that do not necessarily involve movement, that can habituate, and that are relatively complex (examples: conditioned reflex, orienting reflex.)

Voluntary somatic motor activity is abolished during IR, by definition; certain spinal reflexes are suppressed, but not abolished [3,4]. In all species, mild sensory stimulation can evoke reflex withdrawal responses without disruption of the IR, but strong stimulation disrupts the state. Animals respond to most kinds of stimuli during IR, but the threshold for overt response is elevated; a slight degree of analgesia is present [10,35].

Muscle tone varies with species as well as with induction procedures. Animals such as frogs and guinea pigs may exhibit pronounced catalepsy (waxy flexibility). In rabbits the limbs may be extended initially or a fine tremor may occur spontaneously or be induced by stimulation such as tapping the patellar tendon or abdomen. The eyes in rabbits are invariably open during IR and do not move; pupils often constrict suddenly after induction. The corneal reflex is quite active. Biphasic responses of heart and respiratory rates have been noted in rabbits; initially rates may be unchanged or even accelerated, but later the rates tend to decrease [16]. Chickens may close their eyes occasionally, may defecate, and may vocalize intermittently toward the end of an episode [7].

Mechanisms of the IR are not thoroughly understood, but neurons which cause and sustain the state are clearly located in the brain stem and/or spinal cord [21,22]. Transections at the midbrain level, with or without accompanying decerebellation, do not prevent IR [3, 4, 22, 31]. The profound motor inhibition may arise reflexly from cutaneous and proprioceptive stimuli that activate a relatively small population of brain stem reticular formation neurons that diffusely inhibit flexor and extensor spinal motoneurons [21,22] (Fig. 2), without a corresponding change in sensory or integrative functions (see below).

SCREENING OF PSYCHOACTIVE COMPOUNDS

A wide variety of drugs can be screened for ability to potentiate or interfere with IR. Such screening could have several practical applications: (1) establishing drug class, (2) estimation of relative potencies, and (3) detection of excitatory or disinhibitory extrapyramidal side effects.

Testing IR Duration

The IR appears to be a useful addition to the battery of tests which are used to screen potential psychoactive drugs. A summary of some studies that measured drug effect on spontaneous duration is presented in Table 1. Most of these studies have examined only one or a few drugs in limited dose ranges. All studies have disclosed large IR duration variances in both control and experimental groups, and it is common practice to transform data before statistical analysis. In general, the collective results indicate that durations are prolonged by tranquilizers and sedative-hypnotics and are shortened by stimulants. There are exceptions, most notably with the tranquilizers metoserpate [8] and high doses of chlorpromazine [7] which decrease IR durations in chickens.

Testing IR Arousal Threshold

Drug action on arousal threshold has been evaluated in two studies in which the IR-disrupting electrical stimulus was delivered to the external ear of rabbits. The first study disclosed that the tranquilizers meprobamate and chlorpromazine elevated arousal thresholds [15]. The other study demonstrated the value of this approach for industrial drug development [43]. In general, arousal threshold data paralleled that from IR duration studies; i.e., thresholds were elevated by tranquilizers and sedative-hypnotics and decreased by stimulants (Fig. 3). The degree of threshold elevating activity was in the following ascending order: diphenhydramine, nialamide, ectylurea, chloral hydrate, pentobarbital, meprobamate, chlordiazepoxide, phenobarbital, haloperiodol, reserpine, morphine, and chlorpromazine. All but nialamide have been used clinically as tranquilizers or sedatives. Only the stimulant amphetamine lowered arousal thresholds.

Conceivably, tranquilizers and sedatives could lower arousal thresholds if they have disinhibitory or extrapyramidal side effects. Time of testing could govern whether or not such effects were observed; for example, pentobarbital was reported to lower threshold at 15 min postinjection [15] but to elevate it at 30 min [43]. Since extrapyramidal side effects are common with tranquilizers and sedatives, the IR could be useful in screening new drugs for such adverse reactions.

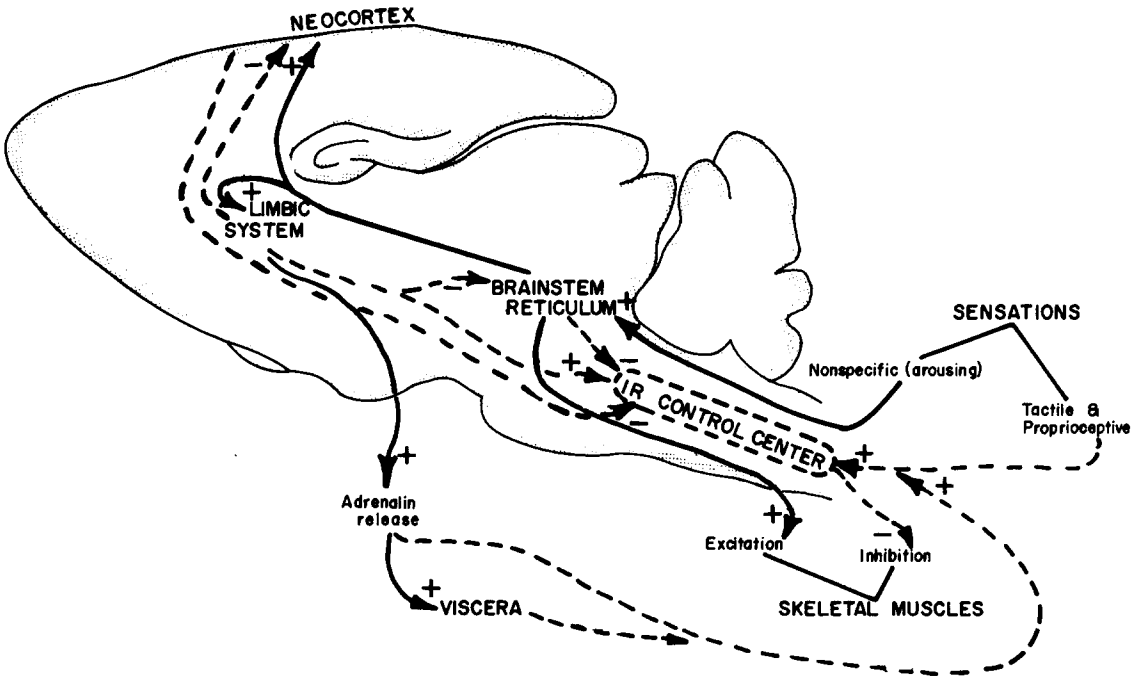

FIG. 2. Diagramatic representation of a unified theory to explain the sensory and motor mechanisms of the IR. All dashed lines refer exclusively to IR-related influences. Plus signs refer to an excitatory action, minus signs, an inhibitory action. The IR control system (which is much smaller than indicated in drawing) is a specific group of neurons in the brain stem reticular formation which is presumed to inhibit skeletal muscle reflexly when the neurons are activated by a certain pattern of tactile and proprioceptive input. Among the rostral brain structures that modulate activity in the control system is the limbic system, which under fear-producing conditions, potentiates the IR; the mechanism is not known, but could include a direct inhibition of neocortex or certain brainstem neurons, a direct excitation of the IR center, and a release of epinephrine that excites the IR center directly or via visceral efferents to the center. Inhibition of the IR control center appears to come from the neocortex, as well as ascending arousal portions of the brain stem reticulum when they are activated by nonspecific, arousing somaesthetic sensations to produce a generalized activation of the neocortex and skeletal muscle. (from Klemm [22], reprinted with permission).

The major drawback of arousal threshold testing is that the electrical stimulation used, if sufficiently intense, can confound the interpretation of drug action, because stressful stimuli are known to potentiate the IR [7]. There is evidence that the potentiation of stress may be mediated via endogenous release of epinephrine, although it is not known whether epinephrine is acting centrally or peripherally [2,22].

Control of Experimental Variables

It is imperative to control for variables other than drug effect that can affect IR duration and arousal threshold. Most data that are relevant to this question are based on IR duration, but parallel phenomena may occur with arousal threshold.

The most studied confounding variables are number of repeated trials and the inter-trial interval. In some species, particularly chickens, habituation develops with repeated trials (i.e. durations get shorter); these effects have been attributed to the disfacilitating effects of fear reduction as the birds become accustomed to human handling [7]. Durations are also affected, in frogs at least, by the interval between successively repeated trials, an effect which could be explained by stress-released epinephrine at induction and the rate of its catabolism [31]. Another explanation for intense massed trials is that induction is aversive, and the animal is being instrumentally conditioned by that punishment to avoid the righting response that terminates IR [7]. Subjecting animals to aversive stimuli, such as electric shock, prior to IR testing increases susceptibility and prolongs the response [7].

The time required to induce IR also affects susceptibility and duration. This factor is almost impossible to study adequately because of confounding variables associated with the manual restraint technique, but one might expect prolonged restraint to promote susceptibility and duration because of heightened affective and hormonal influences.

Genetic factors within a species clearly govern the degree of susceptibility. Strain differences have been documented for both rats and chickens [7].

No sex differences have been reported.

Interpretation of Results

While screening of drugs for their effect on IR duration or threshold seems to hold great promise for empirical purposes, such studies probably do not have much utility in explaining mechanism of drug action. Unfortunately, it is too easy to draw misleading conclusions from drug studies of IR, and this has contributed unnecessarily to certain controversies over IR mechanisms.

TABLE 1

DRUG EFFECTS ON IR DURATION

Drug	Species	Reference
Drugs Prolonging Duration		
carisoprodol	rabbit	5
chloral hydrate	rats	40
chlorpromazine	rabbit	5, 15, 37
	guinea pig	25
	chicken	30
Deanol	rabbit	5
epinephrine	frog	22
	lizard	14
	chicken	2
ergotamine	lizard	14
ethylmethyl-aminoethanol	rabbit	5
iproniazid	chicken	29
d-LSD	chicken	29
meprobamate	rabbit	15
morphine	rabbit	5
pargyline	chicken	29
pentobarbital	rabbit	5, 15
physostigmine	chicken	42
reserpine	guinea pig	27
Drugs Decreasing Duration		
amphetamine	rabbit	5
caffeine	rabbit	38
chlorpromazine*	chicken	30
imipramine	chicken	44
iproniazid	guinea pig	26
levallorphan	rabbit	5
LSD	guinea pig	26
meprobamate†	rabbit	5
metoserpate	chicken	8
scopolamine	chicken	42
serotonin	chicken	29

*high doses only †low dose (10 mg/kg)

For the sake of illustration, let us accept the view that the IR is produced by a population of supraspinal neurons that actively causes a massive inhibition of spinal motoneurons. Clearly, such a population would be influenced by many other brain areas and also by peripheral inputs. A drug that affects IR could therefore act in many indirect ways. IR could be enhanced by a drug action that suppresses activity in a population of neurons that normally inhibits the IR-causing neurons; i.e., the IR neurons would be disinhibited. Drugs could also enhance IR by inhibiting other, IR-independent motor systems, that normally would promote general movements. Conversely, drugs could interfere with IR by depressing neural systems which normally supply excitatory drive to IR-producing neurons, i.e. the IR neurons would be disfacilitated. Or, drugs could suppress IR by exciting other, IR-independent systems, that promote general body movements.

The seriousness of such ambiguities is magnified in studies which attempt to identify the neurochemical transmitter mechanisms of IR by such gross procedures as measuring IR duration in response to systemically administered agonist and antagonist drugs [29,42].

Such interpretative problems have been particularly manifest in the recent research aimed at pharmacological tests of the so-called fear hypothesis. Some investigators hold that any drug which heightens fear should prolong IR and any drug which suppresses fear would shorten IR [7, 8, 11, 29].

However, it is difficult to test such ideas because a given fear-influencing drug may have other independent, IR-relevant properties. As an example, chlorpromazine, in doses that protentiate IR in mammals [5, 15, 25, 37], has marked depressant properties [12,13], and the interruption of ascending arousal influences, which are patently antagonistic to the IR [22,41], would certainly tend to enhance IR independently of any fear effect.

A recent study of a wide dose range of chlorpromazine in chickens disclosed that the highest doses (18 mg/kg and above) had opposite effects from low doses, interfering with IR [30]. The doses below 18 mg/kg in this and all previously reported studies were considered as low doses, apparently too low to produce the full degree of tranquilization required to interfere with IR. However, IR disruption by higher doses could reflect toxic action, which in mammals causes a paradoxical activation of the brain stem reticular formation [12] and promotes electrographic seizures in the amygdala which spread into other limbic structures and into motor cortex [13], causing convulsions at around 45 mg/kg.

It would seem that there is no totally satisfactory way to resolve such problems. The closest obvious approach is to test a variety of drugs, all of which are known to alleviate fear, but which have known, diverse motor effects. Testing in species other than the ones commonly used (rabbits and chickens) is also advisable.

TESTING NEURAL SITE AND MODE OF ACTION OF DRUGS

Need for a New Experimental Preparation

The 3 experimental preparations in common use for investigating drug effect and mechanisms of action involve testing of (1) freely behaving animals, (2) animals that are immobilized with muscle relaxant and artificially respired, and (3) surgically deafferented preparations (cerveau or encéphale isolé). All of these have the same limitation in a

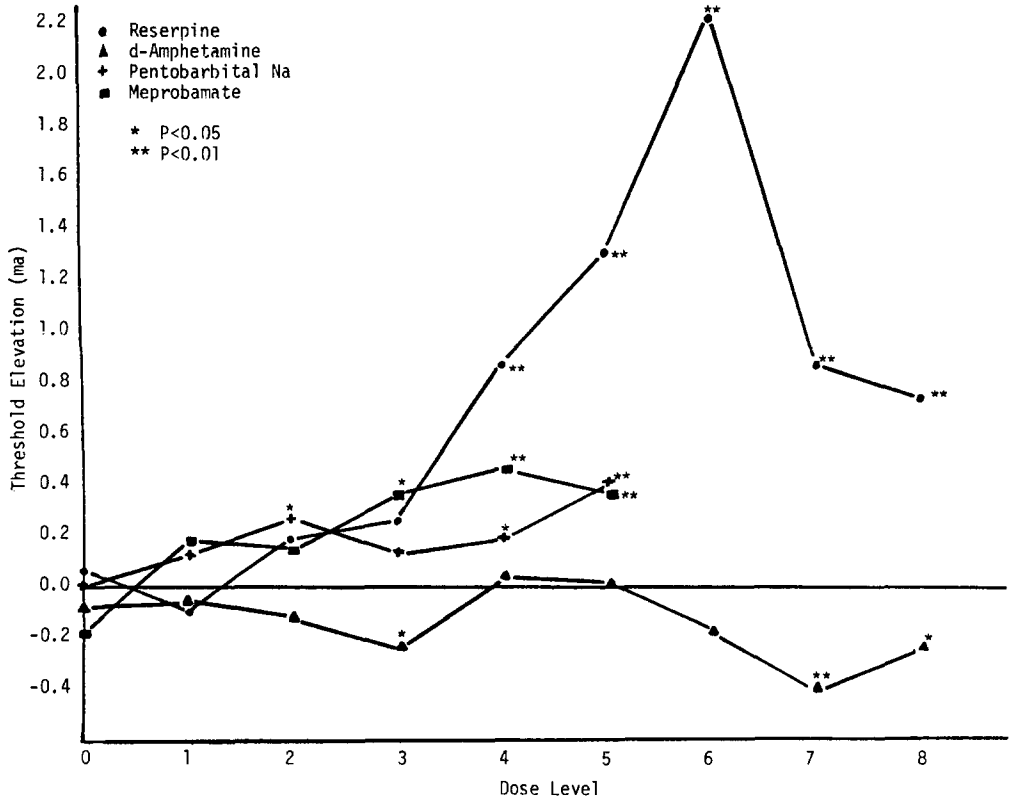

FIG. 3. Drug-induced changes in IR-disruption threshold. Electrical stimulation of the ear of rabbits served as the disrupting stimulus. Data are expressed as change from corresponding pre-treatment current levels. The highest dose level was 0.2 log units below the minimum lethal dose; subsequent doses decreased successively by 0.2 log units. In general, sedatives potentiate the IR (i.e., increase threshold), whereas stimulants interfere with IR (from Tompkins [43], reprinted with permission).

varying degree, namely, an inability to maintain a constant background state of electrophysiological activity upon which to assess drug effect.

Only the freely behaving animals can be conveniently used for chronic evaluation of long-term effects, but these animals have the most variable brain electrical activity due to numerous variables associated with the consequences of behavior.

Significant electrographic variability can be seen in paralyzed preparations, for different reasons. An animal may fall asleep due to lack of sensory input or may become hyperaroused due to variable stimuli from artificial respiration, metabolism of local anesthetics around wound margins, affective reactions to the imposed unnatural conditions, etc.

Surgically deafferented preparations also are quite unstable, especially the encéphale isolé preparation, which may undergo alternating arousals and sleep periods because of variables in sensory input through the cranial nerves. Cerveau isolé preparations tend to sleep constantly, hindering study of deactivating drugs. Both kinds of surgical deafferentation are undesirable in that the drug is being studied in an animal with a massive upset in neural homeostasis.

Advantages of the IR

IR's reproducibility allows chronic testing in the same animal, under conditions which do not impose the shock and massive trauma of surgical procedures. All stimuli still have access to the brain and some, such as autonomic and somatic reflex inputs, can cause reflex responses without necessarily terminating the state.

The key remaining concern is then the relative stability of electrographic activity and its underlying biochemical bases during IR. The available data are thus summarized:

Brain electrographic characteristics during IR. The EEG is mildly activated shortly after induction, with relatively low voltage, fast activity in cortex and theta activity in certain sub-cortical regions and sometimes in cortex [16]. The activated phase is not rapid-eye movement (REM) sleep (no REM, nuchal atonia, or limb muscle twitching). Within a few seconds, the EEG becomes more deactivated and is also associated with a corresponding decrease in muscle tone and heart and respiratory rates. Arousing stimuli evoke typical EEG arousal responses even in the absence of overt behavioral response. When IR is terminated, either spontaneously or by stimulation from the investigator, EEG and the monitored somatic functions become activated again.

Occurrence of an activated EEG during IR is an EEG-behavioral dissociation and this dissociation was the first ever reported in animals [9], although that fact is not generally recognized. This type of dissociation belongs to a seemingly growing list of diverse circumstances in which such dissociations can occur (Table 2). The dissociation can be abolished, for example, by a tranquilizer [15] or can be

TABLE 2

EEG – BEHAVIORAL DISSOCIATIONS (ACTIVATED EEG, SEDATED BEHAVIOR)

Condition	Reference
eserine + chlorpromazine	1
reserpine	34
immobility reflex	9
hypnosis (human)	28
dream sleep	6
anesthesia + certain encephalopathies (dogs)	18
coma & certain encephalopathies (human)	33
eserine + alcohol	23

magnified by seizure-producing drugs [16] in doses that cause low level muscular spasms that are abolished by IR although electrographic seizures persist.

Stability of electrographic activity during IR. The IR does produce relatively superior electrographic stability, as will be documented for the electroencephalogram (EEG), average evoked response (AER), and multiple-unit activity (MUA).

EEG. Many external and internal influences alter the EEG independently of drug action and can readily confound attempts to identify drug actions and target sites. Target sites would be those areas where electrographic activity changed in response to low drug doses at a short postinjection latency. Such a paradigm requires a stable baseline of EEG within each given animal.

The most convenient way to quantify shifts in arousal state is to calculate the incidence of hippocampal theta rhythm in the EEG. Data from 5 rats that were paralyzed with muscle relaxant and artificially respired were randomly selected as a basis for comparison with IR; for each successive minute, the percentage of time for theta was counted. Rats differed widely from each other (Fig. 4a), with some showing almost continuous theta and others having almost none. Worse yet, the EEG of 4 of the 5 rats changed drastically and unpredictably at one or more periods during the recording session.

Quite different results occurred when the same analysis was applied to 5 randomly selected rabbits during the IR (Fig. 4b). All were apparently in a relatively drowsy state, as indicated by the low incidence of theta. Records were not scored for the first minute after induction, because the higher initial theta incidence then was decreasing. The greatest variation for a given rabbit was on the order of only 20 percent.

Even when the rabbits spontaneously disrupted the IR and required reinduction, there was very little change in EEG stability, as long as one did not score for 1 sec before disruption and during the up to 8 sec delay required for reinduction. This was true even for a very poor subject such as the rabbit represented by closed circles which had to be reinduced 6 times (indicated by circled data points). Should spontaneous termination present a problem in a given experiment, the problem can be minimized in two ways: (1) preselect subjects which sustain the IR for long durations, although that introduces a genetic bias, or (2) tie feet and block head in such a way as to stretch the neck [10]. With the latter procedure, however, the experimenter cannot know if the animal is really in the IR state.

The stability during IR may be due in part to a basement effect in that the brain may be near the maximum deactivation possible in a normal, undrugged state. However, sedative drugs do exaggerate the deactivation during the IR, but arousal effects during the IR would be more conspicuous.

AER. AER variability is a common and serious problem that is especially increased by a wide range of changes of internal state, especially by shifts along an arousal-sleep continuum.

Two AER studies have been completed on untreated rabbits in the IR [19,20]. Both involved paired stimulation of various structures within the brain. All stimulated sites produced clear primary and secondary responses; the primary responses were unusually reproducible, at least for the waveforms during the first 125 msec of the response (Fig. 5). Moreover, these primary responses were practically the same during IR as during the non-IR state, even with paired stimulation of progressively decreasing intervals. Measurement of amplitudes of primary responses disclosed no significant change in either the first or second responses during IR, even under those conditions in which second responses were attenuated in the IR and non-IR states. Secondary or long-latency responses did, however, vary within a given animal.

These results, and the other data already cited, suggest that the IR is characterized by a profound inhibition of skeletal movement without corresponding decrease in primary excitability and discriminative capacity, although many secondary neural processing reactions may be affected.

MUA. In a study on alcohol effect in paralyzed and artificially respired rats [24], the MUA was much more stable than the EEG, even though both were recorded from identical electrodes. The MUA was still sensitive to drug effect, and clear topographically differential actions were evident, even though no such distinctions were evident among EEG traces. However, MUA did change sometimes during testing with saline-injected controls.

In order to minimize this baseline instability, ethanol was tested in rabbits during the IR. (A full report of this is now being prepared for separate publication.) Changes in these patterns seldom occurred in saline injected controls, but frequent and long-lasting changes occurred in certain brain areas at each dose level of 300, 600, 900 and 1200 mg/kg. There were also significant differences in the temporal sequence in which MUA patterns changed in the various brain areas (Fig. 6).

Procedure for electrographic studies during IR. Rabbits were implanted in the usual way with chronic electrodes and head-mounted cable connectors. After the rabbit fully recovered, he was subjected to repeated test sessions at several-day intervals. During each session he received, in random order, saline control injections or one of the doses of drug under study. Thus, each rabbit served as his own control for each test condition. Habituation effects could

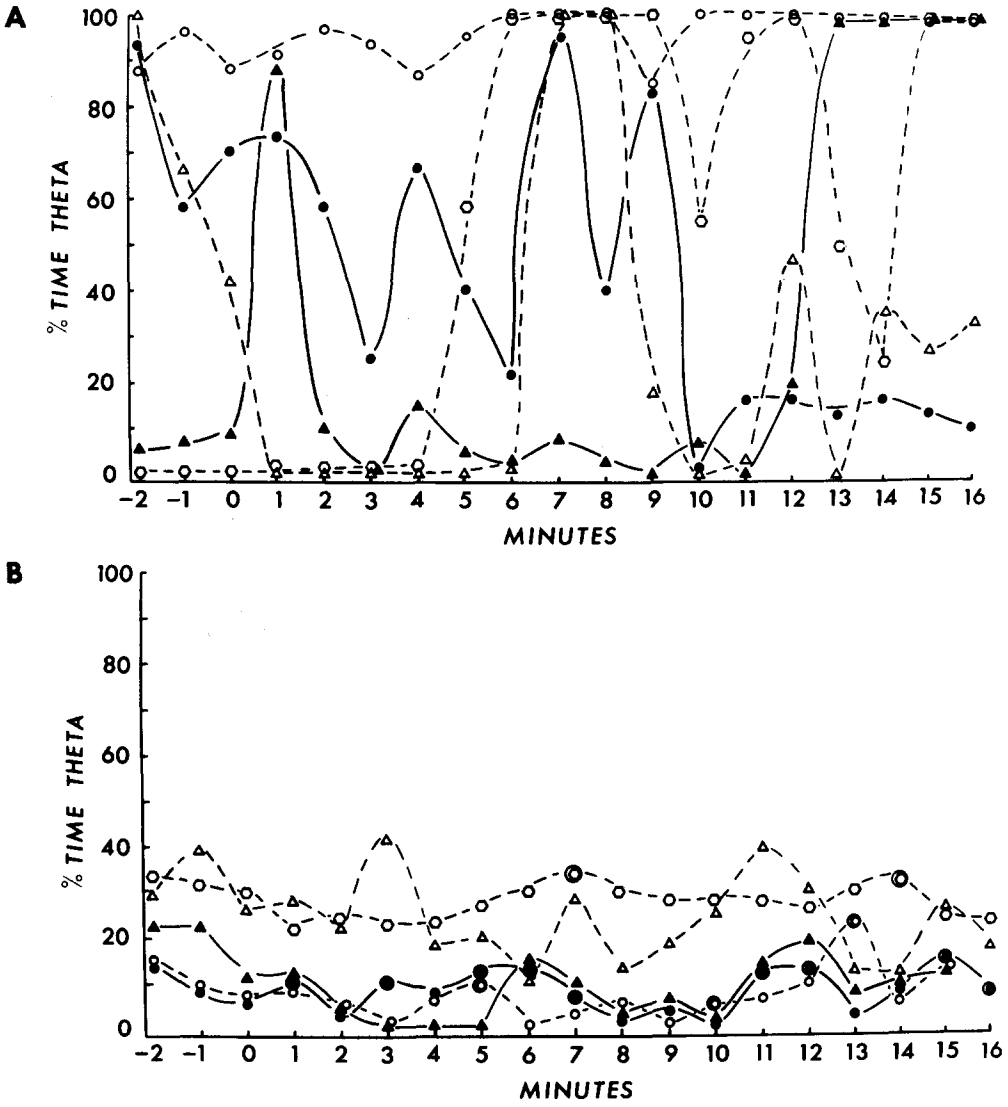

FIG. 4. EEG instability in 5 randomly selected drug paralyzed and artificially respired rats. At time zero, a saline control solution was injected intraperitoneally. The gross fluctuations in theta incidence reflect underlying shifts in alertness (much theta) and drowsiness (little theta). (B) Stability in the EEG of 5 randomly selected rabbits during the IR. Saline injection given at time zero. Circled data points represent periods when IR was spontaneously terminated and was reinduced.

occur if IR were repeated often at short intervals; however, this does not seem to be a problem in rabbits in which re-test intervals are spaced at least 4 days apart, as is desirable to reduce sequential drug effects.

The IR was produced by rapid inversion in a snug-fitting wooden chute. The head was held with one hand while pressing down firmly on the lower abdomen with the other hand, which also was held against the upper hindlimbs to restrict their movements and to avoid scratching. The head was positioned vertically and ears were pulled to lie on the floor of the chute. After about 5 sec of such restraint, the rabbit sustained the immobility without further restraint. Hands were withdrawn gently and slowly. Next a sterilized hypodermic needle with attached tubing was inserted into the abdomen at the midline; an attendant placed his hands on the rabbit in the induction positions during the time for needle insertion to prevent disruption of the IR.

Then after 1 min, recording of EEG and MUA began. After 2 min of control recording, saline or drug was injected remotely through the previously inserted needle, while recording continued for 15 more min Often there was a transient EEG activation for a few seconds after injection, even though neither rabbit nor needle and tubing were touched or moved.

Research applications. The IR preparation should be quite suitable for recording any kind of electrographic activity: ultra slow, EEG, MUA, or single unit. Presumably the electrographic stability reflects a stability of the various underlying physico-chemical events; thus, the IR also appears to be admirably suited for the assay of drug effects

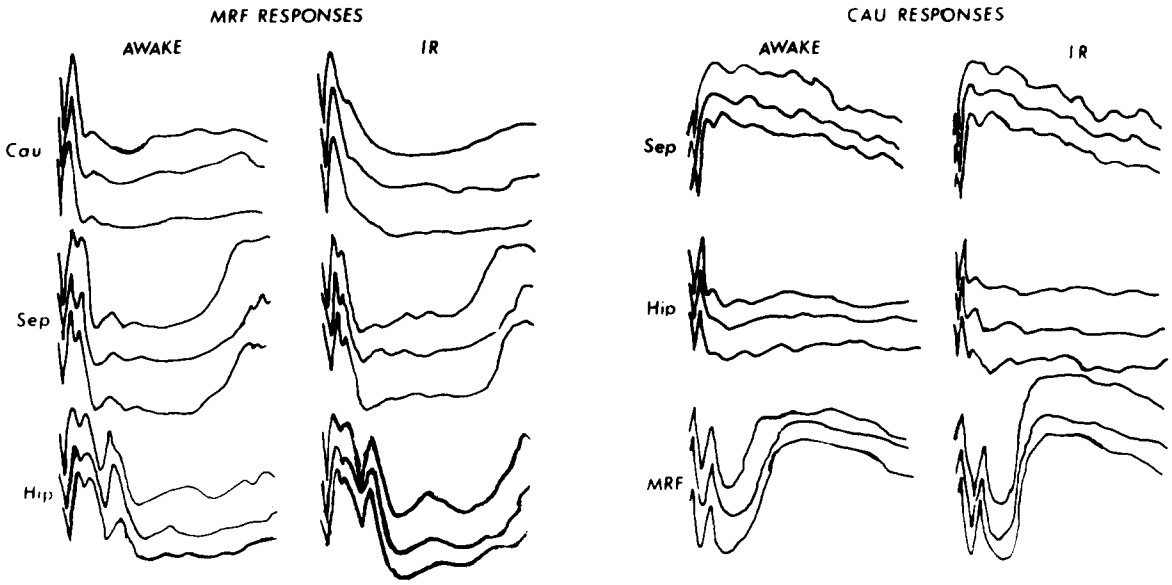

FIG. 5. Illustration of stability of AERs during Awake and IR states with short analysis periods (total time span = 125 msec). Shown are medullary reticular formation (MRF) responses to stimulation of the caudate (CAU), septum (Sep), and Hippocampus (Hip), as well as caudate responses to stimulation of the Sep, Hip, and MRF. The results of 3 consecutive trials are also presented to indicate the degree of reproducibility. No distinct changes in early, primary components are evident during the IR (from Klemm [20], reprinted with permission).

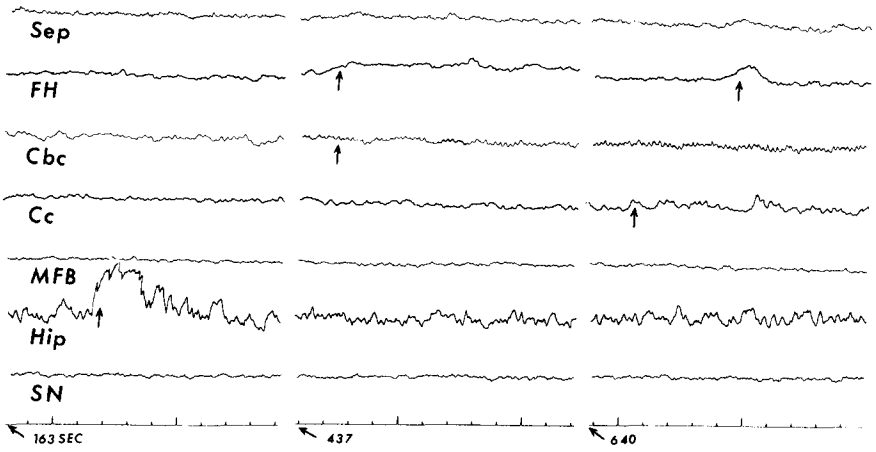

FIG. 6. Illustration of differential MUA responses to alcohol in the rabbit during IR. Traces reflect the instantaneous integral of MUA (envelop of the MUA). Before and up to 163 sec after injection of alcohol (600 mg/kg), MUA patterns were stable. The first response, in the hippocampus (Hip), was a massive increase (arrow) which subsided after about 5 sec (large time marks 5 sec apart); scattered phasic increases occurred occasionally in this lead thereafter (not shown). Beginning around 240 secs an oscillating pattern began to develop in the cerebellar cortex (Cbc) (arrow) which progressed and remained throughout the 15 min recording period. Soon after 437 sec, there was a tonic increase in the fimbria of the hippocampus (FH) (arrow) which subsided in about 10 sec, followed by a few phasic increases thereafter (second arrow). Another clear response was in the cerebral cortex (Cc), in which the pattern converted from tonic to phasic, about 1 min before the tracing of the last panel (arrow). Among the other areas, the septum (Sep) was the third to show a response (not illustrated) at about 300 sec which consisted of increased amplitude and duration of some of the integration waveforms. The medial forebrain bundle (MFB) exhibited a few phasic increases and decreases (not shown) after about 500 sec and the substantia nigra (SN) never indicated a response.

on those physico-chemical systems. The preparation is especially valuable for unit activity signals, because these are more easily contaminated by a wide variety of artifacts, the sources of which are generally eliminated or at least reduced during the IR. The special advantages include decreased feedback variables, reduced artifact, electrographic stability, and the ability to test the same sites repeatedly with vehicle or different doses.

Clearly, the approach is applicable for testing any systemically administered fast-acting drug. Depending on the drug used, the IR state could be shifted either in the arousal direction (termination of IR, seizures) or in the drowsy direction (sleep, anesthesia). Drugs with a long latency of onset may require a different protocol, perhaps with pre- and post-drug recording sessions interrupted by a long period of non-IR.

The IR also permits chronic testing of electrographic responses to topographically applied chemicals, either to populations of neurons via cannula or to single neurons via microiontophoresis. The IR preparation could also be used with certain nonelectrographic data collection methods, such as push-pull perfusion techniques [32] for collecting released transmitter.

ACKNOWLEDGEMENT

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