for analytical purposes that the molecular weight of the substance is indicated.

(1) S. Meyerson and A. W. Weitkamp, *Org. Mass Spectrom.*, **1**, 659(1968).

(2) M. F. Grostic and K. L. Rinehart, *J. Org. Chem.*, **33**, 1740 (1968).

(3) V. I. Zaretskii, N. S. Wulfson, V. G. Zaikin, V. N. Leonov, and I. V. Torgov, *Tetrahedron*, **24**, 2339(1968).

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Audiosensitization: Potential Screening Method for Drugs Affecting the CNS

Keyphrases □ CNS active drugs—screening method □ Stress, sound induced—seizures

Sir:

With the advent of new types of CNS active drugs, new screening tests with predictive association for subtle drug effects are needed. The phenomenon of audioconditioned convulsions (1, 2) affords unique potential as such a screen.

The exaggerated and abnormal responses of psychiatric patients to auditory stimuli (3) prompted the use of audiogenic seizures in genetically susceptible strains of mice as an analogous reaction pattern for CNS drug research (4, 5). The present report suggests the use of audioconditioning and the subsequent susceptibility to sound-induced seizures as a simple and more informative analogy. Both analogies are based on the hypothesis that stress-induced neurosis can be measured by quantal observations of CNS hyperexcitability in response to a specific triggering mechanism. The use of audioconditioning has the advantage of offering the induction of stress susceptibility, as well as the stress-induced crisis for drug modification and study.

Susceptibility to sound-induced seizures can be conditioned in "sound-resistant" strains of mice by a short period (30 sec.) of auditory stimulation at a critical early age (1, 2). In mice, early experiments with the classical conditioning method of physiology showed that pretest exposure to sound elevates or reduces seizure threshold, depending upon the temporal parameters of treatment (6). In such reports, however, the durations of both the conditioning stimulus and the condition–test interval have been short, generally only a few seconds. The audioconditioned convulsions described here are inherently similar, but the condition–test interval is much longer and is measured in days.

Sound-resistant mice [*e.g.*, CAW:CF-1 (SW)][1] display an auricular startle upon initial sound exposure (audioconditioning), but less than 5% convulse (2). The sound source is a 6.3-cm. doorbell which produces approximately 95 db. (relative to 0.0002 dyne/cm.^{2}) within a glass testing chamber, 25 cm. in diameter by 15 cm. deep. If conditioned at the optimally sensitive age (18–20 days for CF-1), virtually all mice will convulse upon the second (test) sound exposure 2–3 days later. The initial conditioning stimulation is absolutely essential for the genesis of convulsions.

The typical seizure in such sensitized animals consists of a sudden burst of wild running, followed by clonic and then tonic convulsions. Less severe seizures terminate after running or clonus. Estimates of seizure severity can be derived from latency and duration times as well as from seizure pattern (2, 7). The following experimental factors affect these parameters (2, 7, 8):

1. The interval between conditioning and testing is critical. Maximal clonic–tonic convulsions characterize seizures after a 2- or 3-day condition–test interval; with a 1- or 5-day interval, only clonus is seen.

2. Repeated auditory stimulation prior to the development of convulsibility makes mice temporarily refractory to seizure, and prolongation of the initial conditioning sound (over 6 hr.) imparts permanent seizure resistance without causing deafness. Once an animal experiences a convulsion, however, seizure susceptibility persists for several weeks. This indicates that audiosensitization and seizure susceptibility are mediated by separate mechanisms.

3. The tonal characteristics of the sound stimulus are equally or more important than the intensity. Although reproducibility is excellent, it is necessary to bioassay each bell periodically. After extensive use, a bell may no longer induce maximal seizures, despite no alteration in intensity.

4. Genetic and environmental factors must be controlled. Noises in the animal quarters, such as the clatter of metal garbage cans, have profound influence. The critical age for sensitization and the optimum condition–test interval vary from strain to strain. CF-1 mice have a high incidence of maximal seizures, a short duration of audiosensitivity, and a low death risk.

When these experimental factors are controlled, seizures of predictable incidence, severity, and latencies are produced (2).

Theoretically, pharmacologic alteration of audioconditioned seizures should be afforded by: (*a*) drugs that impair hearing or otherwise interfere with input of the sound stimulus; (*b*) drugs that block central perception of the stimulus; (*c*) drugs that inhibit or enhance the slow process of sensitivity development; (*d*) drugs that block the effect of intertrial stress; and (*e*) drugs that modify the mechanism of seizure production. The novel interest in audioconditioning as a screen will be for drugs that alter the development of sensitization (*b* and *c* as previously mentioned). For these drugs, this screen is unique because the potential drug need not be present at the time the animals are challenged for a test response. Thus, the prosensitizing

[1] Carworth Farms, New City, N. Y.

or antisensitizing effects of the drug can be isolated from any sedative–anticonvulsant action it may have.

Physical impairment of hearing (*e.g.*, glycerin in the ear) during conditioning reduces the incidence of sensitization (and subsequent seizures); impairment at the time of testing reduces seizure severity. When hearing is impaired on both occasions, the two effects are combined (7). The latter should be the case when ototoxic drugs are tested with this screen. Transient and permanent effects can be partitioned by subjecting the mice to a third sound exposure 3 days after the test exposure.

A drug that blocks central perception of the sound stimulus should be detected by its ability to prevent sensitization when present during conditioning, but not when administered immediately afterward. Prototypes of many pharmacologic classes have been tested, but no drug has been found that effectively blocks audioconditioning at nontoxic doses. Latency changes and a decreased incidence of maximal seizures can be observed. However, because of the reduced metabolic and excretory potential of young mice, it is difficult to determine whether these are due to impairment of audioconditioning or to residual drug effects on seizure response. The latter remains a possibility even after the 2- or 3-day condition–test interval. Phenobarbital and diphenylhydantoin have been shown to be proconvulsant (rather than anticonvulsant) 2 days after their administration (8).

It is interesting to note that ether and pentobarbital anesthesias do not block central perception of the stimulus. Their presence during conditioning does not prevent sensitization but rather appears to enhance its development and to counteract the antisensitization effects of unilateral ear blockade (7).

In contrast to drugs that only inhibit or enhance sensitization, we recently have discovered that high doses of atropine sulfate (25 mg./kg., i.p.) completely block the development of sensitization. While this admittedly is an extremely high dosage, it is the first indication that audioconditioning can be prevented by drugs. Furthermore, since it is an ED_{100} dose, it is likely that a lower dose range can be found.

A drug that alters postconditioning development of sensitization would be detected by its ability to inhibit or enhance sensitization when administered after the conditioning stimulus. Several drugs of this type have been observed in our laboratory (8). Low doses of edrophonium (1–2 mg./kg., i.p.) inhibit sensitization when administered 30 min. after conditioning. When tested 2 days later, seizure incidence and severity are reduced and latencies are prolonged. The effect is similar but less striking when edrophonium is given before conditioning. This perhaps is explained by the fact that the postconditioning duration of drug action is shorter in this case. When edrophonium-treated mice are challenged at a 3-day condition–test interval, the usual seizures are elicited, indicating that this drug slows rather than blocks the development of sensitization.

A multitude of drugs promote or inhibit the seizure response when present at the test exposure to sound. In general, convulsants promote seizures, whereas anticonvulsants, sedatives, and tranquilizers inhibit their onset and severity (8). These drugs produce similar effects on audiogenic seizures in genetically susceptible strains of mice.

The advantages of audioconditioned seizures as a screening method lie in several areas. As a biomodel of stress-induced neurosis, it does not require the use of genetically susceptible strains, special diets, chemicals, or surgical manipulation. The test response is a quick quantal observation which can be easily assessed by a technician. Also, since it involves the use of immature animals, this screen may have special predictive value for drugs to treat neurologic diseases of children.

It is our conclusion that audioconditioning should serve as an important experimental approach to the study of neural hyperexcitability and as a useful screening method for drugs affecting the CNS.

(1) W. B. Iturrian and G. B. Fink, *Fed. Proc.*, **26**, 736(1967).
(2) W. B. Iturrian and G. B. Fink, *Develop. Psychobiol.*, **1**, 230 (1968).
(3) R. B. Malmo, *Trans. N. Y. Acad. Sci.*, **18**, 545(1956).
(4) N. P. Plotnikoff, *Arch. Int. Pharmacodyn. Ther.*, **116**, 130 (1958).
(5) G. B. Fink and E. A. Swinyard, *J. Pharmacol. Exp. Ther.*, **127**, 318(1959).
(6) W. Bevan, *Psychol. Bull.*, **52**, 473(1955).
(7) W. B. Iturrian and H. D. Johnson, *Pharmacologist*, **11**, 248 (1969).
(8) W. B. Iturrian, to be published.

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Binding of Salicylate to Crystalline Bovine Serum Albumin and to Fraction V Bovine Serum Albumin

Keyphrases ☐ Salicylate binding—bovine serum albumin ☐ Bovine serum albumin, crystalline, Fraction V—salicylate binding comparison ☐ Equilibrium dialysis—bovine serum albumin salicylate binding

Sir:

In the course of developing analytical methods for protein-binding studies, we have routinely used Fraction V bovine serum albumin (BSA) rather than the more costly crystalline BSA in preliminary work. For a drug such as sulfaethidole (SETD), there appears to be little difference in binding of the drug to Fraction V BSA and to crystalline BSA; a survey of references cited in a recent review (1) indicates that many workers have