Conditioned Seizures Susceptibility in the Hamster Induced by Prior Auditory Exposure

The occurrence of sound-induced seizures as a result of conditioning implies a relationship to basic physiologic processes rather than to specific species or strain aberrations. The significance of sound-induced seizures will be greatly enlarged if such seizures can be demonstrated in a broad spectrum of species (perhaps including man.) In our laboratory we have recently elicited audioconditioned seizures in the golden hamster (*Mesocricetus auratus*).

Sound-induced seizures in genetically susceptible strains of mice have been known for many years, and have been designated as audiogenic seizures. Animals of susceptible strains convulse upon both initial and repeated exposures to a suitable sound stimulus (e.g. a 95 db doorbell for 60 sec) during the first few months of life[1].

More recently, similar seizures have been elicited in several audioresistant strains of mice through a process of auditory conditioning[2–4], and are referred to as audioconditioned[3] or audiosensitized seizures[5]. Seizure susceptibility is induced during a 'critical period' of development by a single brief auditory exposure during which no seizure occurs. Seizures occur only when the sound stimulus is repeated after a suitable interval. Unlike the short intervals usually associated with classical conditioning methods, the critical condition-test interval in this case is measured in days.

Audiogenic seizures have been demonstrated in a number of species[1], but implicitly always in the milieu of genetic susceptibility. Audiosensitized seizures have been studied chiefly in mice, and have been reported in rats[6].

Our interest in investigating the hamster for audiosensitized seizure susceptibility was based on several factors. Hamsters are easy to breed for dated pregnancies; their gestation period is short, and postnatal development is rapid. The species has been widely used for embryologic research, and its developmental processes have been studied in detail. Furthermore, hamsters in the normal state are not known to be susceptible to audiogenic seizures, although such seizures have been observed in magnesium deficient animals[7].

Hamsters (LVG: LAK) for our study were obtained from Lakeview Hamster Colony, Newfield, New Jersey. Our sound stimulus consisted of a 95 db (above 0.002 dyne/cm^2) electric doorbell (10 cm diameter) suspended in a glass chamber (28 cm diameter by 25 cm deep). For audiosensitization, the bell was rung for 60 sec. In testing for seizure response, it was rung for 60 sec, or until clonus occurred if this was in less than 60 sec. Treatments were assigned by the split litter technique.

The challenges in demonstrating audiosensitized seizures are in discovering a) the age of susceptibility to sensitization and b) the duration of the condition-test interval. Hamsters from 2 weeks to 5 months of age were exposed to an initial sound stimulus. No seizures resulted from any of these initial exposures. The animals were then exposed to a second (test) stimulus 1 to 25 days later.

Audiosensitized seizures did indeed occur in hamsters, with the greatest seizure incidence (56%) occurring when the animals were sensitized at 28 days of age and tested 14 days later. Typical seizure data are shown in the Table. The seizure in hamsters approximates that in mice with regard to seizure pattern and latencies[4]. A maximal seizure begins with wild running, proceeds to clonus, and then to tonus. As in mice, milder seizures may terminate after running or clonus.

A number of distinctive features were noted in hamsters. The wild running was often prolonged and might continue for several minutes after termination of the test stimulus. During clonus, the animals appeared to be vocalizing although no audible sound was heard. A prolonged catatonic state lasting up to 1 h usually followed seizure in hamsters, even after mild seizures terminating with running or clonus. Prolongation of the sensitizing stimulus (to 300 sec) greatly increased seizure severity upon testing. None of these features are associated with audiosensitized seizures in mice.

On repeated testing at 2-week intervals, cyclic susceptibility to seizures appears to occur in hamsters. Of the group that had convulsed at the first test stimulus, approximately $^1/_3$ responded at each of the repeated tests. The animals involved on each occasion were different, however, but most of the group eventually re-seizured. In several cases of retesting, the hamsters seizured upon being placed in the test environment without the bell being rung. (Spontaneous seizures were not noted in any other circumstance.)

Among nonresponders at initial testing, approximately 15% eventually seizured upon repeated testing. Seizure incidence in hamsters drops off sharply after 5 months of age. As in mice, ether anesthesia did not prevent audiosensitization, nor did any other drug tested.

The demonstration of audiosensitized sound-induced seizures in hamsters supports the hypothesis that audiosensitization is a response common to many species. Furthermore, audiosensitization may be but one facet of a general phenomenon of sensory-conditioned hypersensitivity. Flashing light induces epileptic activity in adolescent baboons[8], and Soviet authors describe conditioned seizures in the dog (see reference[9]). Increased responsiveness of

Profile of seizure susceptibility in hamsters on the second exposure to sound

Age at audiosensitization (days)	N	Sensitization-test interval (days)[a] 0–6	7–9	10–12	13–15	16–18	over 18
21	(52)	0	0	0	0	0	0
28	(155)	0	30	20	43	32	3
31	(120)	0	9	17	35	9	0
35	(104)	0	12	0	11	0	0
42	(50)	0	0	0	0	0	0

[a] Average daily seizure incidence (%).

[1] A. Lechmann and R. G. Busnel, in *Acoustic Behavior of Animals* (Ed. R. G. Busnel; Elsevier Publisher, Amsterdam 1963), p. 244.
[2] K. R. Henry, Science *158*, 938 (1967).
[3] W. B. Iturrian and G. B. Fink, Devl. Psychobiol. *1*, 230 (1968).
[4] G. B. Fink and W. B. Iturrian, in *Physiological Effects of Noise* (Eds. B. L. Welch and A. S. Welch; Plenum Press, New York 1970), p. 211.
[5] J. L. Fuller and R. L. Collins, Devl. Psychobiol. *1*, 185 (1968).
[6] W. B. Iturrian, in *Defining the Laboratory Animal in the Search for Health.* IV int. ILAR-ICLA Symp. (Ed. W. I. Gay; Nat. Acad. Sci., Washington, in press).
[7] R. A. Patton, J. comp. Physiol. Psych. *40*, 283 (1947).
[8] K. F. Killam, E. K. Killam and R. Naquet, Electroenceph. clin. Neurophysiol. *22*, 497 (1967).
[9] L. Chocholova, in *Comparative and Cellular Pathophysiology of Epilepsy* (Ed. Z. Servit; Excerpta Medica, Amsterdam 1966), p. 285.

withdrawal reflexes upon repeated stimulation has been observed in kittens[10] and frogs[11]. Conditioned auditory triggering of epileptic seizures has been reported in humans[12]. Unfortunately the precise role of conditioning is not clear in all of these situations.

The cyclic seizure susceptibility in hamsters upon repeated testing has a striking parallel in the light-induced epilepsy of baboons. KILLAM, KILLAM and NAQUET[8] reported that although some baboons were highly sensitive and always responded to flashing light, many had a cyclic variability in responsiveness when tested at weekly or biweekly intervals. Such variation was thought to be a function either of the duration of the intertest interval or of some general physiologic cycle.

The significance of audiosensitized seizure susceptibility as a generalized physiologic phenomenon is great. Its general parameters – a brief susceptibility period at a critical age, followed by a long condition-test interval measured in weeks – suggest that sensory conditioning may be operative but overlooked in many experimental situations. In clinical situations a conditioning factor may be significant in some apparently spontaneous seizures. The study of audiosensitized seizure susceptibility as a model system may provide instructive insight not only into basic neurologic processes, but into their derangements as well[13].

Zusammenfassung. Nachweis audiosensibilisierter Anfälle beim Hamster *Mesocricetus auratus* nach Konditionierung auf akustischen Reiz.

W. B. ITURRIAN and H. D. JOHNSON

Department of Pharmacology, University of Georgia Athens (Georgia, 30601, USA), 21 April 1971.

[10] A. A. BUERGER and A. M. DAWSON, Expl Neurol. *23*, 457 (1969).
[11] L. FRANZISKET, Anim. Behav. *11*, 318 (1963).
[12] H. GASTAUT, H. REGIS, S. DONGIER and A. ROGER, Rev. Neurol. *94*, 829 (1956).
[13] Supported in part by grant No. EC 00447-01 from the PHS. We thank Mrs. BARBARA BEAGLE for technical assistance.

Potentialisation de l'isoprénaline par l'association 6-hydroxydopamine et réserpine

L'inhibition de l'accumulation intraneuronale présynaptique des catécholamines (uptake-1) se traduit habituellement par une sensibilisation des effets des catécholamines sujettes à cette accumulation. Par contre, pour ce qui est de l'isoprénaline, dont l'inactivation s'effectue surtout par accumulation extraneuronale[1-6] et *O*-méthylation[7], ses effets β-adrénergiques ne sont pas modifiés par l'inhibition de l'uptake-1. Cependant, dans le présent travail, nous avons observé une potentialisation des effets de l'isoprénaline sur l'oreillette et le duodenum isolés de Rat, prétraités par deux substances généralement considérées comme agissant à un niveau présynaptique, la 6-hydroxydopamine (6-OH-D)[8-10] et la réserpine[11-14].

Méthodes. Les coeurs et duodenums de rats albinos Wistar (200 à 300 g) ont été prélevés aussitôt après que les animaux aient été assommés. Les oreillettes sont montées dans une chambre à organe, contenant 30 ml de solution de MORAN[15] à 31 °C où barbote un courant de carbogène. Les duodenums, dont on utilise des fragments de poids identique quel que soit le prétraitement auquel les animaux sont soumis, sont préparés selon la technique de HORTON[16]. On applique des tensions de 1 g aux oreillettes et de 0,5 g aux duodenums. Les contractions et relaxations des préparations sont mesurées dans des conditions isotoniques et amplifiées au moyen d'un microdynamomètre Ugo Basile et enregistrées. Les effets de 3 à 5 concentrations d'isoprénaline puis de noradrénaline (temps d'exposition: 2 min pour les oreillettes et 30 sec pour les duodenums) sont étudiés sur chaque préparation. Les valeurs moyennes des contractions ou des relaxations observées, mesurées en mm, sont calculées et comparées entre elles par le test *t* de Student.

Quatre groupes de 6 animaux chacun ont été étudiés: Groupe 1 (témoin): rats isolés en cages individuelles à 20°C, 14 jours avant l'expérience. Groupe 2 (réserpine): rats isolés comme ci-dessus et recevant 10 mg/kg de réserpine, i.p., 19 h avant l'expérience. Groupe 3 (6-OH-D): rats isolés comme ci-dessus et recevant 14 jours avant l'expérience 2×50 mg/kg de 6-OH-D, i.v. et 7 jours avant l'expérience 2×100 mg/kg de 6-OH-D, i.v.[8]. Groupe 4 (6-OH-D + réserpine): rats traités comme ceux du groupe 3 et recevant en outre 19 h avant l'expérience 10 mg/kg de réserpine, i.p.

Résultats. Ces résultats sont illustrés par les Tableaux I et II. La 6-OH-D seule ou la réserpine seule ne modifient pas significativement les effets de l'isoprénaline mais potentialisent ceux de la noradrénaline sur les deux préparations.

Par contre, le prétraitement associant 6-OH-D et réserpine potentialise considérablement les effets de l'isoprénaline, les valeurs observées dans le Groupe 4 étant significativement supérieures à celles de tous les autres groupes. Il potentialise aussi les effets de la noradrénaline, significativement plus que la réserpine seule, mais pas significativement plus que la 6-OH-D.

Discussion. L'inhibition de l'uptake-1, que réalisent la 6-OH-D[8,9] et la réserpine[12,13] potentialise classiquement les effets de la noradrénaline[10,17]. Nous avons retrouvé ce phénomène dans nos expériences, mais observé aussi que l'association 6-OH-D et réserpine ne potentialisait pas les effets de la noradrénaline davantage que la 6-OH-D seule, bien que des réponses maximales n'aient pas été atteintes.

[1] B. A. CALLINGHAM et A. S. V. BURGEN, Molec. Pharmac. *2*, 37 (1966).
[2] R. W. FOSTER, Br. J. Pharmac. *35*, 418 (1969).
[3] S. L. LIGHTMAN et L. L. IVERSEN, Br. J. Pharmac. *37*, 638 (1969).
[4] W. J. DAVIDSON et I. R. INNES, Br. J. Pharmac. *39*, 175 (1970).
[5] P. R. DRASKOCZY et U. TRENDELENBURG, J. Pharmac. exp. Ther. *174*, 290 (1970).
[6] A. J. KAUMANN, J. Pharmac. exp. Ther. *173*, 383 (1970).
[7] G. HERTTING, Biochem. Pharmac. *13*, 1119 (1964).
[8] H. THOENEN et J. P. TRANZER, Naunyn Schmiedebergs Arch. Pharmak. exp. Path. *261*, 271 (1968).
[9] J. P. TRANZER et H. THOENEN, Experientia *24*, 155 (1968).
[10] G. HAEUSLER, W. HAEFELY et H. THOENEN, J. Pharmac. exp. Ther. *170*, 50 (1969).
[11] G. HERTTING, J. AXELROD et L. G. WHITBY, J. Pharmac. exp. Ther. *134*, 146 (1961).
[12] N. KIRSHNER, J. biol. Chem. *237*, 2311 (1962).
[13] A. CARLSSON, N. A. HILLARP et B. WALDECK, Acta physiol. scand. *59*, suppl. 215, 1 (1963).
[14] L. L. IVERSEN, Adv. Drug Res. *2*, 1 (1965).
[15] N. C. MORAN, Circulation Res. *21*, 727 (1967).
[16] E. W. HORTON, Br. J. Pharmac. *14*, 125 (1959).
[17] J. H. BURN et M. J. RAND, J. Physiol., Lond. *144*, 314 (1958).