*Pharmacology Biochemistry & Behavior*, Vol. 6, pp. 603–605. 

# BRIEF COMMUNICATION

# Audiogenic Seizures and Brain Extracts: Enhancement by Extracts from C57BL/6J Mice Subjected to Audiogenic Priming[1]

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(Received 17 January 1977)

SCHREIBER, R. A. AND N. N. SANTOS. *Audiogenic seizures and brain extracts: enhancement by extracts from C57BL/6J mice subjected to audiogenic priming.* PHARMAC. BIOCHEM. BEHAV. 6(5) 603–605, 1977. – Donor C57BL/6J mice were given audiogenic priming (AP) by exposure to noise at 16 days of age ($T_O$) and sacrificed at intervals from 1 hr to 18 days thereafter. Brains from AP and littermate non-AP mice were extracted in 1 M acetic acid, and passed through a filter with a nominal mol. wt. cutoff of 10,000 daltons. The filtrates were lyophilized and resuspended in $H_2O$ (0.1 ml/brain). Recipient C57BL/6J mice were also exposed to 30 sec of 127 ± 2 dBA at 16 days of age, and injected IP with one brain-equivalent of extracts from either AP or non-AP donors immediately thereafter. All recipients were tested for convulsability at 18 days of age. There were no differences in audiogenic convulsion rates between groups given AP or non-AP extracts from donors sacrificed 1 to 4 hr after $T_O$. Recipient convulsion rates were higher in AP relative to non-AP extract-injected mice when AP donors were sacrificed 1 to 18 days after $T_O$. The extract taken from donors 1 day after $T_O$ appears more potent than extract taken either before or later.

Audiogenic priming  C57BL/6J mice  Brain extracts  Audiogenic seizures

THERE HAVE been four previously published attempts to isolate and purify brain compounds which confer altered sensitivity to intense noise. Two major categories of compounds have been investigated: substances which should result in lowered recipient reactivity (donor habituated), and substances which should result in heightened recipient reactivity (donor acoustically sensitized).

Experiments in the first category include those of Ungar and Ocequera-Navarro [12] and Daliers and Riguax-Motquin [3]. Both groups habituated their rats to an acoustic stimulus. The former group prepared an acetone-extracted dialysate brain fraction, and the latter group a phenol-extracted fraction. In both studies, habituation to the same acoustic stimulus was significantly enhanced in recipients of habituated extract as compared to recipients of nonhabituated extract.

There are also two published efforts to confer heightened recipient reactivity. Both Fjerdingstad [5] and Corwin and Stanford [2] subjected C57BL/6J mice to audiogenic priming (AP), defined as an initial exposure to noise of sufficient quality to induce susceptibility to audiogenic seizures on a subsequent exposure to a sufficient acoustic stimulus. Donor mice in both experiments were sacrificed at varying intervals after AP; Fjerdingstad extracted the brain with Ringer's solution, and Corwin and Stanford dialyzed against $H_2O$. Recipient mice were tested with a number of various injection-test time combinations; neither of the two experiments generated significant data on an initial recipient test for enhanced convulsability. Corwin and Stanford retested some of their recipients yet again, and in some instances a few more mice convulsed after injection with donor primed extracts as compared with not primed extract.

C57BL/6J mice appear an ideal test system, since first, the later behavioral response to audiogenic priming (induced convulsability) is not unduly complicated by the

[1] A portion of these data were presented at the 67th meeting of the American Society for Biological Chemists, San Francisco, June 6–10, 1976.

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need for repeated trails by either the donor or the recipient, secondly, use of inbred subjects results in virtual elimination of genetically based sources of behavioral variability, thirdly, the response has a number of easily delineated levels, and data are not necessarily complicated by difficulties in attempting to score the behavior of each subject, or the necessity to assign some arbitrary criterion of performance, and fourthly, the time courses of the later effects of given stimuli of particular duration and intensity during AP on C57BL/6J mice of a particular age are now known [9,10]. We report below our first efforts to alter convulsability in recipients by donor substance(s) extracted from selected donors.

On the basis of the experiments mentioned above, there is no reason to expect that the substance of interest may not be a peptide. Maxson, Towle and Sze [8] have supplied additional evidence for a peptide hypothesis by showing that cycloheximide and puromycin blocked the induction of convulsability in C57BL/6J mice when injected prior to AP, but not when injected afterward. Therefore, preliminary efforts to isolate an active fraction of brain have been directed toward techniques of extraction of brain peptides.

## METHOD

### *Animals*

A total of 144 C57BL/6J mice *(Mus musculus)* were used in these experiments (the origins and inbreeding of these mice have been described [7]). Breeding stocks were obtained from the Jackson Laboratory, Bar Harbor, ME. Mice were maintained on ad lib Wayne Mouse Breeder chow and tap water. An automatic switch turned fluorescent lights in the colony room on at 0700 hr and off at 1900 hr. The temperature was maintained at approximately 23° C.

### *Apparatus and Procedures*

Mice were placed in a battery jar, 30 cm (outside diameter) × 46 cm high, enclosed in a sound-attenuating box with an observation window [11]. Each mouse was allowed 15 sec to explore during which time the lids to the jar and the box were put in place. An Edwards No. 340 bell was attached to the jar lid, which generated a broad band output of 127 ± 2 dBA [9]. The bell was then turned on for 30 sec for all mice subjected to AP.

## EXPERIMENTAL

One-half of each donor litter were subjected to AP at 16 days of age (day of birth = Day 0), tail-marked, and placed back with their mother until sacrifice. If any mice responded with a wild run or a more severe seizure during the priming period, the entire litter was discarded.

Donor mice (AP or non-AP) were sacrificed by decapitation at varying intervals after priming. Brains between litters within conditions were pooled, placed on dry ice, assigned a code by R.A.S., and lyophilized. The freeze-dried brains were powdered in a mortar with dry ice, placed in a freezer until the dry ice had sublimed, and extracted with cold 1.0 M acetic acid for 2 hr.

The supernatant was centrifuged for 1 hr at 5° at 6,000 rpm, and lyophilized. The powder was taken up in 0.05 ml $H_2O$ per mg of brain powder, centrifuged as above, and passed through a UM10 Amicon filter, with a mol. wt. cutoff of 10,000 daltons. The filtrate was lyophilized, and resuspended in 0.10 ml $H_2O$ per brain. The two samples (AP or non-AP donors) were then coded again (by N.N.S.)

Litters of recipient mice were subjected to the bell noise for 30 sec at 16 days of age, injected IP with one brain-equivalent of one of the donor extracts in 0.10 ml $H_2O$ immediately thereafter, and tail marked. Recipients were tested for susceptibility to audiogenic convulsions at 18 days of age, a time of an average group convulsion rate and also a time of rapid change from zero to maximal responsiveness [9,10]. All AP and all recipient testing took place between 1100 and 1400 hr to control for circadian rhythms [11].

The codes were broken after all of a particular pair of extracts (AP or non-AP) had been used and the recipient mice tested.

## RESULTS

A mouse must wild run prior to entering into a clonic seizure, and clonic before tonic, etc. Table 1 is constructed to reflect this: If an animal had a tonic seizure, that fact was reflected in the percentage of mice having a wild run and also in the percentage of clonic seizures. This table shows the data pooled across litters for each of the donor-sacrifice intervals examined. There were no significant effects of extracts taken from mice sacrificed 1 to 4 hr after AP. The greatest effect was observed using extract from AP donors sacrificed 1 day later. Slight enhancements in convulsability were seen in the mice injected with AP substance taken 2 to 18 days later, though given the small N and purposefully built-in variability in recipient convulsability at 18 days, these differences were not statistically significant.

Each litter of recipient mice constitutes a separate experiment, the comparison being whether the mice injected with substance from AP mice showed more severe convulsions than those littermates injected with substance from non-AP mice. Mice from nine separate litters were included in the 1-day data. Mice receiving AP extract had a higher mean seizure level in 7 of the 9 comparisons ($\chi^2 = 5.07, p<0.05$).

## DISCUSSION

It should be reemphasized that this first experiment was designed to investigate the possibility that differing donor-prime donor-sacrifice times might affect the quality of the extract. The donor extract used in this experiment was injected into standard recipients which were given AP at 16 days of age and which were then tested 2 days later. Eighteen days of age is one of the intermediate group susceptibility and also high group variability, so that effects in either direction could be detected [10]. Other variables, including the recipient prime-injection interval, the recipient prime-test interval, other routes of injection, and dose-responses remain to be tested. Once the most effective paradigm resulting in the greatest behavioral differences between groups is known, then one could further fractionate the donor brain extract, and continue the search for the active fraction(s) which alter(s) a convulsability behavioral bioassay.

Susceptibility to sound-induced convulsions has been considered an experimental model for the laboratory investigation of sensory epilepsy [1, 4, 6]. The experiment reported here shows that as a result of AP, a substance can

TABLE 1

THE PERCENTAGE OF AUDIOGENIC CONVULSIONS IN RECIPIENT C57BL/6J MICE (DATA POOLED ACROSS LITTERS FOR EACH OF THE DONOR-SACRIFICE INTERVALS EXAMINED)

| Prime to Donor Sacrifice Time | Extract From Primed Mice | | | | | | Extract From Nonprimed Mice | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N= | WR | C | C+ | T | D | N= | WR | C | C+ | T | D |
| 1 hour | 10 | 70 | 50 | 50 | 20 | 0 | 10 | 60 | 30 | 20 | 20 | 0 |
| 2 hours | 5 | 40 | 40 | 0 | 0 | 0 | 5 | 40 | 20 | 20 | 20 | 20 |
| 4 hours | 6 | 66 | 33 | 33 | 17 | 0 | 5 | 80 | 40 | 20 | 20 | 0 |
| $\bar{X}_{1-4}$ hours | 21 | 62 | 43 | 33 | 14 | 0 | 20 | 60 | 30 | 20 | 20 | 5 |
| 1 day | 14 | 86 | 71 | 50 | 29 | 7 | 16 | 75 | 31 | 13 | 6 | 0 |
| 2 days | 17 | 71 | 52 | 35 | 29 | 12 | 17 | 71 | 47 | 24 | 24 | 12 |
| 5 days | 11 | 81 | 81 | 63 | 54 | 27 | 11 | 81 | 54 | 54 | 45 | 9 |
| 10 days | 13 | 62 | 54 | 46 | 31 | 15 | 13 | 77 | 39 | 15 | 15 | 8 |
| 18 days | 17 | 94 | 53 | 53 | 41 | 12 | 15 | 80 | 60 | 40 | 20 | 7 |
| $\bar{X}_{1-18}$ days | 72 | 79 | 61 | 49 | 36 | 14 | 72 | 76 | 46 | 28 | 21 | 7 |

WR = wild run; C = clonic flexion convulsion; C+ = clonic extension convulsion; T = tonic convulsion; D = lethal convulsion.

*Donors: One half of each C57BL/6J litter was subjected to 30 sec of 127 dBA noise Day 16. Mice were then sacrificed at intervals thereafter indicated. Brains within conditions were then pooled, extracted as described, and injected IP.

*Recipients: All recipients were subjected to 30 sec of 127 dBA noise at 16 days of age, and were then injected IP with extract (one brain-equivalent per mouse in 0.1 ml $H_2O$) immediately thereafter. All recipients were then tested for convulsability at 18 days of age.

Statistically significant $\chi^2$ values ($df = 1, p<0.05$) are underlined in the table.

be found for a brief time which enhances seizure levels in recipients. This experiment is the first in a series designed to elucidate the molecule(s) involved in altered reactivity levels in these mice. If this effort proves fruitful, then a step in determining one of many possible mechanisms of induced convulsability will have been taken.

ACKNOWLEDGEMENTS

This research was supported in part by National Institute of Health General Research Support Grant RR 5423 to the College of Medicine, University of Tennessee Center for the Health Sciences. The aid of Ms. Linda Craddock in mouse testing is acknowledged, and we also thank Ms. Debbie Martin for her excellent typing skills.

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