Chemical transfer of learned fear: Failure to replicate Ungar

L. D. HUTT[1] *and LUKE ELLIOTT, Memphis State University, Memphis, Tenn. 38111*

Thirty naive male Sprague-Dawley rats were used as donors and were randomly divided into three groups of 10. One group was shocked only in the black box, according to Ungar's paradigm. The second group received shock equally in the white and black boxes, to control for the possible activating effects of shock, regardless of where administered. The third group was a control group that received handling only, with no shock. The prepared brain extracts were injected into recipient mice, prescreened to eliminate those showing no dark preference. There was no evidence of a reliable differential avoidance of the dark box by any treatment group. Activity measures also failed to differentiate the three groups. Further, the difficulty of obtaining mice with a dark preference was discussed.

In recent years, one of the more vigorously researched and contested topics has been transfer of training via a brain extract or homogenate. Results of chemical-transfer research have been far from unequivocal. Some researchers offer evidence of a chemical-transfer phenomenon (Babich et al, 1965; Fjerdingstad et al, 1965; Jacobsen et al, 1965); other investigators have been unable to find a chemical-transfer effect (Byrne, 1966; Carron & Nutter, 1966; Gross & Carey, 1965; Hoffman et al, 1967; Luttges, 1966), and still others have offered alternative explanations (e.g., activation and/or sensitization) of chemical-transfer effects (Dyal & Golub, 1968; Harty et al, 1964; Walker, 1966; Walker & Milton, 1966).

Proponents of the chemical-transfer hypothesis have argued that conflicting results are produced by the wide variety of situations in which chemical transfer has been investigated and also have been prone to point an accusing finger at the experimental techniques of those reporting negative results. For these reasons, the paper by Ungar et al (1968) created much excitement in both camps of chemical-transfer investigators. Ungar stated that "the experiments reported here are easily and rapidly reproducible and yield unequivocal results which clearly demonstrate the possibility of a purely chemical transfer of some type of acquired information [p. 1259]." Thus, Ungar promised to resolve the conflicting findings by providing a "pure" situation in which chemical transfer might be studied.

The purpose of the present experiment was twofold: (1) to test the validity of Ungar's rather strong statement by carrying out a direct replication of his experiment, and (2) to include groups of animals that would allow us to assess sensitization and/or activation effects vs specific transfer effects of injections from trained donors. The second question was stimulated by a detailed analysis of Ungar's data and data presented by Gay and Raphelson (1967). Gay and Raphelson trained rats to avoid the dark box by administering shock therein. Trained extract was then injected into an experimental group of mice recipients, and untrained extract was injected into a control group of mice. In a test period of 180 sec, the control animals spent 0.0 sec in the white box and 120 sec in the dark box; experimental animals spent an average of 22 sec in the white box and an average of 16 sec in the dark box. The reduction in time in the dark box in the experimental group is as would be expected if the learned dark avoidance had been transferred chemically in the injection. A question arises, however, when one considers time *unaccounted* for in each group. In the control group, only 60 sec (33% of the total time) is unaccounted for, whereas in the experimental group, 142 sec (79% of the total time) remains unaccounted for. Obviously, if the animal is in neither the black box nor in the white box, it must be in the center start box, perhaps en route from black to white or vice versa. This suggests that the experimental animals were considerably more active than were control animals and implies that the apparent avoidance of the dark box is perhaps a little more than an artifact of increased activity produced by the "trained" extract. A quite similar trend may be seen in data supplied to us by Ungar (1968) based on 12 animals. Experimental animals spent an average of 50 sec in the dark box and an average of 75.5 sec in the white box, leaving 54.5 sec (30% of the total time) unaccounted for. For control animals, the dark-box average was 137 sec, and the white-box average was 14.3 sec, leaving only 28 sec (15.7% of the total time) unaccounted for.

To assess the effects of increased activity, a group of animals was included which received shock equally in the black box and in the white box. It was reasoned that, if activity is increased by the "trained" injection, the important variable is shock, regardless of where administered. Thus, the animals shocked in both boxes should show a dark-avoidance artifact similar to that of the animals shocked in the dark box only. A group of animals, which received handling only without shock, was also included. This group controlled for possible activating effects of handling.

METHOD

With the exception of the additional groups, an effort was made to duplicate Ungar's apparatus and procedure as closely as possible. Donor animals were 30 naive, male Sprague-Dawley rats (200-250 g) and naive random-bred mice recipients. The apparatus consisted of start, white, and

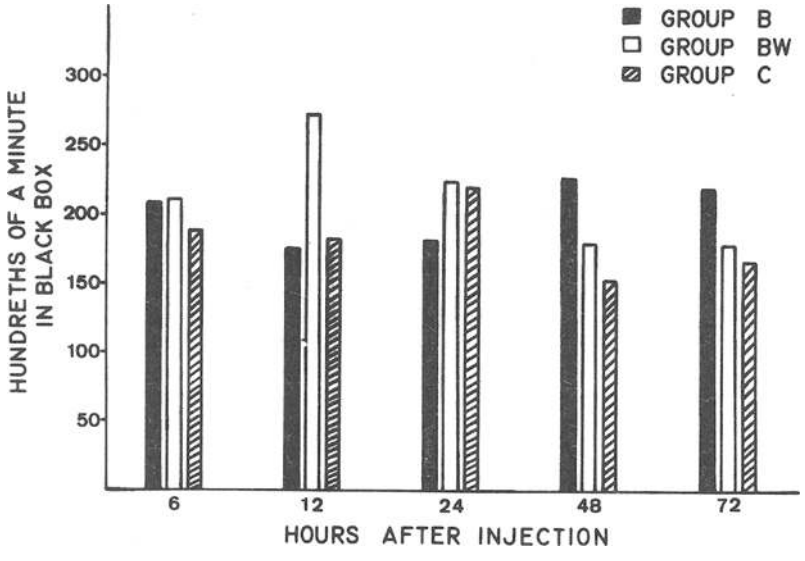

Fig. 1. Time in black box.

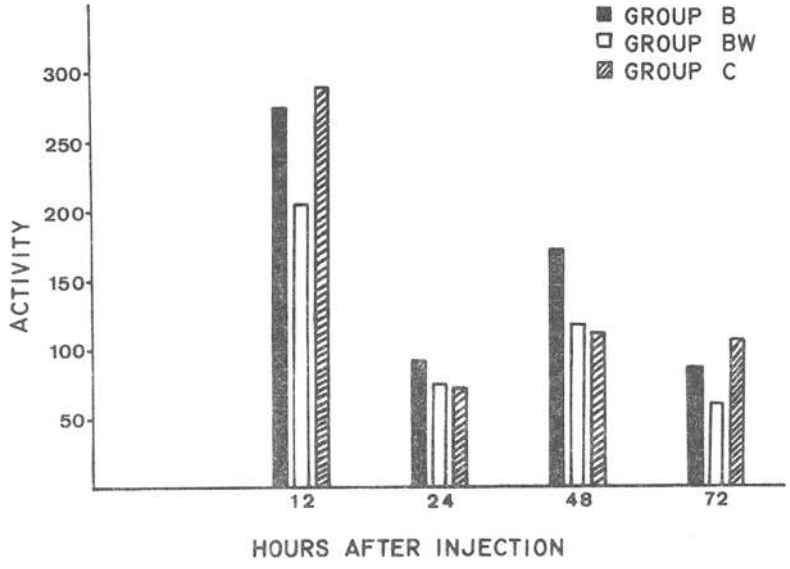

Fig. 2. Activity measures.

dark boxes built and joined to Ungar et al's (1968) specifications. Donors were divided randomly into three groups of 10 each. Group B animals were shocked in the black box; Group BW received an equal number of shock trials in the black box and in the white box, with total number of shock trials equal to those of Group B; Group C was handled as Groups B and BW were but received no shock.

Training trials (four/day for 8 consecutive days) consisted of placing the animal in the start box for a few seconds, then lifting the animal out and placing him through the top of the appropriate black or white box. Animals were administered a 2-mA shock for a 5-sec duration. On cessation of shock, the guillotine door to the start box was raised, and the animal was allowed to enter the start box. If the start box was not entered, the animal was placed in it, again through the top, by the E. For Group C, this sequence and timing of events was maintained, but shock was not administered.

Within 2 h of the final training session, the rats were decapitated, and the brains, excluding the olfactory bulb, were removed and stored on dry ice (within 2 min following decapitation) at −20 deg C until used. Brains from each group were pooled, weighed, and then homogenized in a pestle. The homogenate was preparatorily centrifuged for 10 min at 30,000 g, and the supernatant was drawn off and centrifuged for 60 min at 80,000 g. The volume of the final supernatant was adjusted with distilled water so that 1.0 ml was equivalent to 1 g of brain.

Recipient mice were screened prior to injection to eliminate those not showing a definite dark preference. Those not spending at least 150 hundredths of a minute out of 300 hundredths of a minute were eliminated. This criterion was applied for 3 consecutive days.

Animals reaching criterion received 1 ml of brain extract intraperitoneally and were tested at 6, 12, 24, 48, and 72 h after injection. Testing consisted of a 1-min activity count on a jiggle platform, followed by 3 min in the boxes with all doors raised. Time spent in each box was recorded. The E had no knowledge of the treatment received by the animals.

RESULTS

Figure 1 presents data on time in the dark box of the three groups, assessed at the various intervals following injection. As can be seen, there was no evidence of a reliable differential avoidance of the dark box by a particular treatment group. Neither was there evidence of decay of dark avoidance over time as Ungar reported. Although not presented in Fig. 1, there was also no evidence of a tendency in any group to spend less time in the dark box after injection as compared to preinjection levels. Actually, all groups spent slightly *more* time in the dark box, though not reliably so, following injection.

Activity data taken at various postinjection intervals are presented in Fig. 2. It can be seen that there are no reliable differences in activity levels of the groups. There was a significant decrease in activity level over time, but this probably reflects diminution of novelty of the apparatus.

DISCUSSION

Under the conditions of this experiment, no evidence was found of chemical transfer of specific information from trained to naive animals via injection. There was also no evidence of an increased activation level or increased reactivity to external stimuli produced by injection of brain extracts from trained animals. In short, Ungar's findings were unreplicated by us.

There are, of course, two possible explanations for the failure to replicate. The first is that our procedure deviated in some significant ways from Ungar's. One deviation occurred during training in that animals were lifted from the start to the shock box rather than being forced through the passageway. Another deviation was that all animals received total shock ranging from 30 sec to 300 sec. These deviations do not, however, appear sufficient to account for the failure to replicate. The handling during training is more in line with usual training procedure than is Ungar's, and as to total shock, Ungar reported transfer effects with 150 sec total shock.

In conclusion, this experiment is one other bit of evidence failing to support a chemical-transfer phenomenon. It also indicates that Ungar's hope of resolving contradictions in the chemical-transfer literature by providing a situation in which transfer effects are "easily and rapidly reproducible and yield unequivocal results" was somewhat premature and overly optimistic. The shortcomings of the paradigm become even more apparent when one looks at initial dark-preference data. In this study, 81% of the animals screened were rejected because they did not show a definite preference for the dark box. Thus, the "well-known preference shown by rats and mice for dark rather than lighted enclosures [Ungar et al, 1968, p. 1259]" did not materalize in our Ss, perhaps suggesting that animals included as Ss were atypical.

REFERENCES

BABICH, F. R., JACOBSON, A. L., BUBASH, S., & JACOBSON, A. Transfer of a response to naive rats by injection of ribonucleic acid extracted from trained rats. Science, 1965, 149, 656.

BYRNE, W. L., et al. Memory transfer. Science, 1966, 153, 658.

CARRAN, A. B., & NUTTER, C. P. Heredity-environment interaction in brain extract transfer in highly inbred mice. Psychonomic Science, 1966, 5, 3-4.

DYAL, J. A., & GOLUB, A. M. Further positive transfer effects obtained through injections of brain homogenates. Psychonomic Science, 1968, 11, 13-14.

FJERDINGSTAD, E. J., NISSEN, T. H., & RØIGAARD-PETERSEN, H. H. Effects of RNA extracted from the brain of trained animals on learning in rats. Scandinavian Journal of Psychology, 1965, 6, 1-6.

GAY, R., & RAPHELSON, A. "Transfer of learning" by injection of brain RNA: A replication. Psychonomic Science, 1967, 8, 369-370.

GROSS, C. G., & CAREY, F. M. Transfer of learned response by RNA injection: Failure of

attempt to replicate. Science, 1965, 150, 1749.
HARTY, A. L., KEITH-LEE, P., & MORTON, W. D. Planaria: Memory transfer through cannabalism re-examined. Science, 1964, 146, 274-275.
HOFFMANN, R. F., STEWART, C. N., & BHAGORAN, H. M. Failure to transfer a learned response in rats using a brain extract containing RNA. Psychonomic Science, 1967, 9, 151-152.
JACOBSON, A. L., BABICH, F. R., BUBASH, S., & JACOBSON, A. Differential approach tendencies produced by injection of RNA from trained rats. Science, 1965, 150, 636.
LUTTGES, M., et al. An examination of "transfer of learning" by nucleic acid. Science, 1966, 151, 834-837.
UNGAR, G., GELSON, L., & CLARK, R. H. Chemical transfer of learned fear. Nature, 1968, 217, 1259-1261.
WALKER, D. R. Memory transfer in planarians: An artifact of the experimental variables. Psychonomic Science, 1966, 5, 357-358.
WALKER, D. R., & MILTON, G. A. Memory transfer vs. sensitization in cannibal planarians. Psychonomic Science, 1966, 5, 293-294.

NOTES

1. The authors wish to express their appreciation for the invaluable assistance of Mrs. June Blackwell in the surgical and brain-extract procedures.

2. Ungar, G. Personal communication, 1968.

Cerebral lesions and the excretory alkali metal response (EAMR) in reptile[1]

IRWIN M. SPIGEL,[2] ALEX RAMSAY, and JO-AN SEGGIE, University of Toronto, Toronto 5, Canada

Unlike the climbing-response decrement observed in cerebrally lesioned turtles, and which was independent of contingent shock, lesioned and sham-operated Ss alike produced elevated urinary Na^+ and K^+ levels when under periodic shock. Some consistency with earlier behavioral observations was evident in the significantly lower cation concentrations of shocked lesioned Ss for the first 2 days of the experiment.

Cerebral lesions in the painted turtle, *Chrysemys picta marginata*, have been observed to impair the ubiquitous climbing response of confined Ss (Spigel & Ellis, 1966). The climbing decrement was evident whether or not such behavior resulted in a contingent electric shock. A virtually identical climbing deficit, independent of consequent shock, was also shown to follow intraperitoneal injection of d-amphetamine (Spigel & Ellis, 1967). The production of a similar deficit following either cerebral insult or the administration of a sympathomimetic agent presumed to act on lower brain structures suggested that the reptilian cerebrum and lower centers may be somewhat antagonistic in nature. The data were also viewed as supporting the speculation of Goldby & Gamble (1957) that parts of the reptilian hemispheres may be of significance in the orientation of this species to its environment and in the participation of this tissue in emotionally integrative behavior patterns.

More recently, Spigel & Ramsay (1969) have shown that total alkali metal (combined sodium and potassium) excretion in the turtle is a consistent index of stress. This excretory alkali metal response (EAMR) was not only greater in turtles administered periodic electric shock, but persisted after shock termination when Ss were retained in the surround associated with the antecedent noxious stimulation.

Consideration of these observations led to the question of whether cerebral lesions might not affect the EAMR in a manner consistent with the decremental climbing behavior that was shown to follow such treatment.

SUBJECTS

Forty male central painted turtles *(Chrysemys picta marginata)*, 4 to 5 years of age as estimated from carapace length, were employed. All had been maintained in a colonial Wahmann tank for at least 3 weeks prior to the experiment.

APPARATUS

An IL flame photometer was used for Na^+ and K^+ determinations. Four white plastic food-preserving boxes (8 x 4 x 4 in.) with tightly fitted lids, perforated with four small holes to permit air to enter, housed Ss during the experimental treatment period. A timer, connected to a variable-shock generator, was set to deliver a 1.5-V shock of 3-sec duration every 57 sec for 12 h each day to Ss in groups so designated. The current was administered to Ss by means of insulated alligator clips inserted through holes in the boxes and connected to the tail and right hindleg. The timer was automatically reset by a gear-activated switch.

PROCEDURE

The Ss were assigned randomly to one of four conditions: cerebrally lesioned and shocked, sham-operated and shocked, cerebrally lesioned and nonshocked, and sham-operated and nonshocked. One S in each of the lesioned-shocked and sham-operated, nonshocked groups died, and the data were discarded. Twenty-four hours after surgery, Ss were placed individually in the plastic containers with 25 ml of distilled water, and the electrodes were attached. Although all Ss were fitted with the clips, only those in one of the lesioned and one of the sham-operated groups received the periodic shock. Habitat water was analyzed for Na^+ and K^+ on Days 2, 4, and 6 of the 6-day period of the experiment, and the water was replaced on Days 2 and 4. On Day 6, Ss were sacrificed by decapitation, and the heads were stored in 10% formalin for histological verification of the lesion.

SURGERY

Heads of Ss were extended and taped in position. Anaesthesia was accomplished by retaining Ss in crushed ice for 2 h. Lesions were placed medially in the cerebral hemispheres. Holes were bored bilaterally through the skull with a dental drill and the tip of a hyfrecator needle inserted through the apertures to a depth of 2 mm. During a 10-sec current application, the needle was rotated constantly at an angle of about 60 deg from the ventricle. Ss were returned to the home tank immediately. Sham lesions followed the same procedure up to, but not including, placement of the needle.

RESULTS

Total excretory alkali metal remained significantly higher for all shocked Ss over the entire 6-day experimental period. It was only in the analysis of Day 2 residual fluid that shocked cerebrally lesioned turtles revealed a significantly reduced excretion of Na^+ and K^+ as compared with

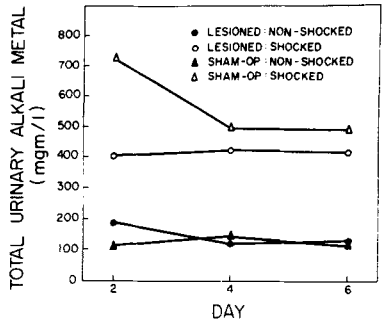

Fig. 1. Concentrations of total excretory alkali metal in the habitat water for the four treatment groups on Days 2, 4, and 6. Values represent means for combined Na^+ and K^+ in milligrams per liter.