Brain extracts modify recipient avoidance behavior as a function of performance level of donor goldfish

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Separate RNA-protein extracts were prepared from brains of goldfish that had shown high levels of performance, had shown low levels of performance, or had not performed at all (control), during 7 days of shuttlebox active avoidance acquisition. The resulting three extracts were injected intracranially into three groups of naive recipient goldfish that were given 3 days of nonreinforced testing in the shuttlebox. Performance levels of recipient animals were significantly related to performance levels of donor animals. The data are discussed in terms of their bearing on the behavioral specificity issue in the area of "biochemical transfer."

The "behavioral bioassay" phenomenon refers to the paradigm in which the behavior of recipient animals is modified via injections of brain extracts from donor animals with particular learning histories. To date, the phenomenon has been reported by at least 32 independent laboratories. The biochemical substrates of the phenomenon have been studied most extensively by Ungar and his associates (Ungar, Desiderio, & Parr, 1972; Ungar, Galvan, & Chapouthier, 1972; Guttman, Matwyshyn, & Warriner, 1972).

Workers in our laboratory have been studying the behavioral bioassay phenomenon from the viewpoint of its behavioral specificity. We have shown that both response incremental (e.g., avoidance acquisition) and response decremental (e.g., experimental extinction and habituation) effects may be made to occur in recipients of trained donor brain extracts (Braud, 1970a), that the antagonistic effects just mentioned "transfer" independently but do not "cross-transfer" (Braud & Hoffman¹), that the effects can be stimulus specific (Braud & Braud, 1972; Braud & Hoffman¹), and that the occurrence of the indicant response in donor animals is a necessary condition for a successful instrumental transfer (Braud & Hoffman¹). These findings, together with those of other investigators, strongly suggest that the behavioral bioassay is characterized by considerable behavioral specificity.

In a study recently completed, brain extracts from donor goldfish that had acquired a particular active avoidance response (crossing a hurdle in an aquatic shuttlebox) facilitated that same response in naive recipients of these extracts. However, brain extracts from donors that were exposed to the same stimuli but did not learn to make

the hurdle-crossing response did not have such a facilitating effect (Braud, 1971). Our interest in the present study was to explore further this response-specificity problem. Having observed the "all or none" effect just mentioned, it was interesting to ask whether the response-specificity effect would occur in a graded fashion, i.e., whether the degree to which a response occurred in donor animals determined the extent to which that same response might be facilitated in naive recipient animals.

SUBJECTS AND APPARATUS

The Ss were 60 common goldfish, 7.6-10.2 cm in body length, obtained from Ozark Fisheries, Stoutland, Missouri. The fish were maintained in

the laboratory for 7 days before the experiment began; maintenance conditions were identical to those described by Braud (1970a). Goldfish were chosen as experimental animals because of their unique advantages in behavioral and biochemical research (Braud, 1970b). The training/testing apparatus was a clear plastic aquatic shuttlebox, equipped with completely automated stimulus-presentation and response-recording devices; see Braud (1970a) for a detailed description of the apparatus. The box was programmed so that a fish might terminate or avoid a pulsed 9-V dc electric shock by swimming from the light to the dark compartment.

PROCEDURES

Donor Training

Twelve fish remained in their home tanks and served as naive control donors. Twenty-four fish were given 7 days of avoidance acquisition training in the shuttlebox, at a rate of 20 trials/day. A trial consisted of a 12½-sec presentation of light in the compartment occupied by the fish, then a 12½-sec simultaneous presentation of light plus pulsed shock, then a 35-sec period of darkness and no shock. Light and shock offsets were response contingent. Although environmental variables were constant, there were considerable individual differences among these 24 trained experimental donors, and these differences were exploited in order to constitute two separate donor groups: low-level performers (Group L) and high-level

Fig. 1. Mean percent avoidance responses during acquisition for low-level (Group L) and high-level (Group H) donor goldfish.

Fig. 2. Mean percent avoidance responses during nonreinforced test trials (20 trials per day) for recipients of low-level (Group L'), high-level (Group H'), and naive control (Group C') extracts, 48, 72, and 96 h after injection.

performers (Group H). The 24 trained donors were rank-ordered in terms of median number of avoidance responses (crossing the shuttlebox hurdle during the 12½-sec light-only period) for the last 3 training days; the top half of this distribution became Group H, the bottom half, Group L.

Biochemical Procedures

Ss were sacrificed 20 h following the last training session. Whole brains were removed within 60 sec and frozen in powdered dry ice. Brains were sealed in glass vials and stored in a freezer at -20°C until ready to be processed. The extraction procedure was a cold phenol "RNA" extraction method identical to that described in detail by Braud (1970a). The resultant of this procedure might be more appropriately called "RNA protein," since it does not consist of pure RNA but contains considerable protein; evidence (Ungar & Fjordingstad, 1971) strongly suggests that protein, not RNA, is the active substance. The lyophilized extracts were concentrated with 10⁻³ M NaCl solution so that single 40-microliter injections would each contain 1.5 brain equivalents of material.

Recipient Injection and Testing

Twenty-four naive goldfish were divided randomly into three groups of eight recipient fish. Fish were injected intracranially with 40 microliters (1.5 brain equivalents) of extract from one of the three donor groups (see Braud, 1970a, for injection details). Fish were

returned to their home tanks and were left undisturbed until the first behavioral test 48 h after injection. Additional tests were given 72 and 96 h after injection. Each fish received 20 light-only test trials in the shuttle apparatus each day. Avoidance responses (those during the first 12½ sec of light; no shock was given during testing) were automatically recorded. Both injection and testing of recipients were done blind.

RESULTS

Acquisition performance of the low- and high-level donor groups is presented in Fig. 1. Recall that these two groups were constituted by splitting the distribution of median number of avoidances on the last 3 days into equal upper and lower halves. Terminal acquisition levels for the low- and high-level groups are 70.5% and 91.1% mean avoidance responses, respectively. A Mann-Whitney U test on Day 7 data yielded a U of 4, which has an associated probability of < .001 (one-tailed).

Figure 2 presents curves for number of avoidance responses (mean percent) of the three recipient groups over the 3 test days. Recipients of high-level extract (Group H') are superior to low-level extract recipients (Group L') on all test days, and this difference is statistically significant on Days 2 ($U = 13$, $p = .05$, two-tailed) and 3 ($U = 9$, $p = .014$). Group L', in turn, is superior to Group C' (recipients of naive

control extract) on all 3 test days, with this difference being significant on Days 3 ($U = 12$, $p = .038$) and 4 ($U = 13$, $p = .05$, two-tailed) by a Mann-Whitney test. There is a suggestion that the maximal effect (maximal curve separation) occurs faster in Group H' than in Group L'; whether this kinetic effect is reliable or not must be determined by further research.

DISCUSSION

Injection of brain extracts from trained donors facilitated avoidance behavior in naive recipient fish, while injection of similar extracts from naive control donors did not have a facilitating effect. More importantly, the degree of facilitation of recipient behavior varied systematically with degree of donor performance—i.e., extracts from good donor performers produced a strong modification of recipient behavior, while extracts from poorer performers produced a weaker effect. The performance of control recipients was low, relatively static, and corresponded closely to the kind of performance generated by other sorts of control groups that have been run in this laboratory (viz, groups given light-only trials, light plus unpaired shock, shock-only trials, etc.). The effects are graphically great and statistically significant.

The apparent differential time courses of the effects in the "bright" and "dull" extract recipients is interesting, especially in view of Guttman's observations of interesting kinetic patterns in mice and goldfish injected with both natural and synthetic brain materials (Guttman & Gronke, 1971; Guttman, Matwyshyn, & Warriner, 1972). While it is clear that different recipients show "transfer" effects at different times following injection, the mechanism of this kinetic effect is unclear. In fact, it is not clear whether the effect is time dependent as opposed to experience dependent, because the two factors (time and trials) are confounded in all tests involving repeated reinforced or nonreinforced testing of individual animals. A true time-dependent process might be masked by active (i.e., behavioral) acquisition or extinction processes distinct from the passive (i.e., biochemically induced) effects produced by the brain extracts. An adequate "time study" is sorely needed—one that could disentangle time effects from experience-dependent effects. Such a study would involve injection of a large number of animals with extract and testing of these animals once and only once at various times following injection; confounding experiential factors (especially experimental extinction) would thus be removed.

In summary, the present results provide additional empirical support for the conclusion that the behavioral bioassay phenomenon is "response specific."

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NOTE

1. Braud, W. G., & Hoffman, R. B. Process-, response-, and stimulus-specificity in behavioral bioassays in goldfish. Submitted for publication.