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Telencephalic Lesions and Behavior in the Teleost, *Macropodus opercularis*: Further Analysis of Reproductive and Operant Behavior in the Male

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Ablation of the telencephalic hemispheres blocked reproductive behavior and decreased the rate of operant responding for conspecific visual reinforcement. Repeated administration of operant conditioning sessions for 4 weeks following the ablation resulted in a partial recovery of the operant rate but not of reproductive behavior. Social isolation, which increases reactivity in *Macropodus,* had no significant effect on the operant rate in sham-operated or lesioned males. Lesioning resulted in a significant reduction of testes weight and the gonadosomatic index in 8 weeks.

INTRODUCTION

Investigations of the functions of the teleost telencephalon show that lesions or complete removal of both hemispheres interfere with various aspects of social behavior, including schooling (Aronson and Kaplan, 1968), fighting (Hale, 1956), and reproductive behavior (Noble and Borne, 1941; Segaar and Nieuwenhuys, 1963; Ribbink, 1972; Overmier and Gross, 1974). Most reports indicate that response frequency is decreased, response latency is increased, and behavior is less efficient but that no class of behavior is eliminated. In a previous experiment in our laboratory, bilateral ablation of the telencephalon anterior and dorsal to the preoptic nucleus eliminated spawning and nest-building behavior in male *Macropodus opercularis* (Davis, Kassel, and Schwagmeyer, 1976). Thus, the telecephalon may play a critical role in activating reproductive behavior in this species. The present investiga-

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tion examined the possibility that the lesion results in aversive responses to strong social and nonsocial stimulation which are incompatible with reproductive behavior.

The previous investigation in *Macropodus* revealed that in addition to blocking reproductive behavior, the telencephalic ablation greatly reduced operant responding for conspecific visual reinforcement. The fish were administered several 1-hr conditioning sessions prior to the spawning trial and a 1-hr and a final 24-hr session 3 weeks postoperatively. Some recovery of the operant rate was seen in the 24-hr session. This suggested that the earlier 1-hr sessions were too short to accommodate the slower-adapting lesioned males. Longer sessions might result in increased responding and, thereby, increased conspecific stimulation. Operant responding might further be increased by socially isolating the fish between sessions of operant conditioning. Social isolation increases the reactivity to external stimuli in *Macropodus* (Davis, 1975). Recovery of the operant rate, evoked by any behavioral manipulation, would imply that the lesioned male had neither a perceptual nor motor disability and that any initial, postoperative aversion to strong stimulation, including conspecific stimuli, was decreased. If extended recovery time and operant conditioning also result in increased reproductive behavior, it would suggest that the telencephalon is not exclusively responsible for the organization and integration of mating and nest building in *Macropodus.*

METHOD

Subjects

Adult male paradise fish, *Macropodus opercularis* (L.), $\bar{x}$ = 4.9 cm body length and $\bar{x}$ = 3.3 g body weight, were obtained from domesticated stocks in Florida. The daily cycle of diffuse natural light in the laboratory was augmented by a 14:10-hr L:D cycle of "daylight" flourescent light. The fish received Tetramin staple conditioning food and frozen brine shrimp in one or two daily feedings. The fish were placed in individual 9-liter isolation tanks (Davis *et al.*, 1976) for 1 to 2 days to screen subjects for readiness to respond to social reinforcement. Temperatures were 24 to 26°C.

Operant Tank

The apparatus was identical to that described in Davis *et al.* (1976), except that an opaque plastic partition decreased the length of the tank to 22 cm. The stimulus fish was kept in a compartment behind an opaque screen containing a vertical sliding door. Raising the door revealed a window through which the subject could view the stimulus compartment. The swim-through manipulandum consisted of an infrared beam across a 2.5-cm ring. When the fish interrupted the beam, the stimulus compartment door was opened for 30 sec. The photodetector was interfaced to a PDP-8F digital computer which registered response frequency, the duration which the fish remained in the beam, presented the visual reinforcements, and compiled the data. Responses made during a 30-sec reinforcement were not reinforced and were tallied separately from reinforced responses.

PROCEDURE

Preoperative Operant Conditioning

The subjects were screened for performance of the swim-through response when reinforced with a 30-sec view of the stimulus male. During this period an opaque partition between the isolation tanks prevented the subject from seeing other fish. An 18-hr overnight session of continuous reinforcement (CRF) in continuous light was then presented, and the mean hourly rate of responding was used as the preoperative baseline. Several hours after the session, 10 males were telencephalonectomized (tele-x) and eight received a sham operation as previously described (Davis *et al.* 1976).

Postoperative Reinforcement Sessions

When the males were returned to the isolation tanks following surgery, the partitions between half the tanks were removed, allowing the subject to view an adjacent tank which contained an intact male of approximately equal size. Each male was administered eight, 18-hr CRF sessions at 3- to 4-day intervals, beginning on Day 3 following surgery. The session was started by delivering a 30-sec free reinforcement. During the free reinforcement, the observor noted the occurrence of lateral and frontal displays and attacks (Davis and Kassel, 1975) directed toward the stimulus fish.

Extinction Sessions

Following the reinforcement sessions, four 18-hr extinction sessions were given. In the first three, the stimulus compartment was empty but the door opened on each swim-through response. The sessions started with a 30-sec presentation of the empty stimulus compartment. In the final sessions, swim-through responses were registered but the door was not opened.

Spawning Trial

Four days after the last extinction session, approximately 7 weeks following surgery, the male was placed in a 38-liter tank with a female until they spawned, or for a maximum of 10 days. The frequencies of approach, lateral and frontal displays, quivers, and attacks by the male were recorded during the first 10 min of the trial. The pair was observed briefly three times daily to determine the time of spawning.

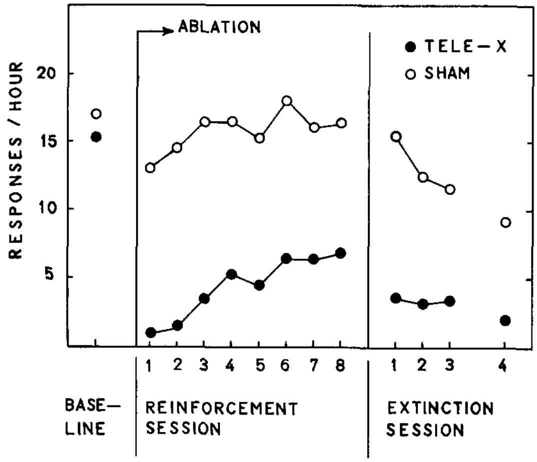

Fig. 1. The effects of the telencephalic ablation and the sham operation on the mean rate of swim-through responding. Following the operations, the fish received eight biweekly 18-hr CRF sessions followed by four extinction sessions. In the first three extinction sessions, the stimulus fish was removed but the stimulus compartment was opened for 30 sec following each response. In the final session the stimulus compartment remained closed.

Histology

Following the spawning trial, the male was killed in ice water, weighed, measured, and the brain was prepared for histology. The entire head was fixed in neutral formalin for 48 hr, decalcified, embedded in paraffin, and cut in 8-μm parasaggital or coronal sections. The sections were stained with hematoxylin and eosin. The extent of the ablations was determined by inspection of serial brain sections. The testes were removed, fixed in Bouins solution, and weighed. The gonadosomatic index was calculated for each subject (GSI = (testes weight/body weight) X 100).

RESULTS

Operant Responding

Socially isolated and nonisolated fish did not differ significantly in the mean rate of responding. Accordingly, the data were combined to obtain single tele-x and sham groups (Fig. 1). Two of the sham males were eliminated from the experiment after session 3 when they developed bacterial skin lesions.

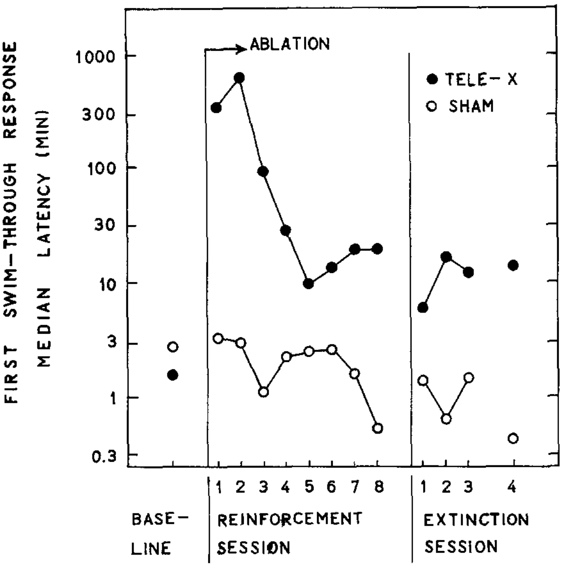

Fig. 2. The effects of the telencephalic ablation and the sham operation on the latency of the first swim-through response following the free reinforcement at the start of the 18-hr session. In the eight postoperative CRF sessions, the free reinforcement consisted of a noncontingent, 30-sec visual presentation of the stimulus fish. In the first three extinction sessions, it consisted of a 30-sec presentation of the empty stimulus compartment. In the final session the compartment remained closed.

The tele-x and sham males showed equivalent levels of responding prior to surgery. Following surgery, the tele-x males initially responded at a low rate. The response rate increased to a stable level in six to eight sessions. In reinforcement session 1, three of the ten males responded consistently at a mean rate of 3 per hour, three made only a few responses, and the remaining four did not respond. By session 8, one tele-x male had not responded and the other nine had operant rates of 4 to 14 responses per hour. The sham-operated males performed at a significantly higher rate than the tele-x males in each of the eight reinforcement sessions ($P < 0.003$, Student's t test).

Extinction resulted in decreased operant rates in both groups but the shams continued to respond more frequently than the tele-x males ($P < 0.02$). It is difficult to evaluate the possible difference in the rate of extinction between the groups owing to the differences in reinforcement history and response rates prior to extinction (Fig. 1).

In most of the 18-hr sessions the tele-x males showed a longer latency to perform the first swim-through response than the shams (Fig. 2). The median latency was significantly different for the two groups ($P < 0.02$, Mann-Whitney U test) except in sessions 6 and 7. The tele-x males also showed a longer median latency in the four extinction sessions ($P < 0.05$).

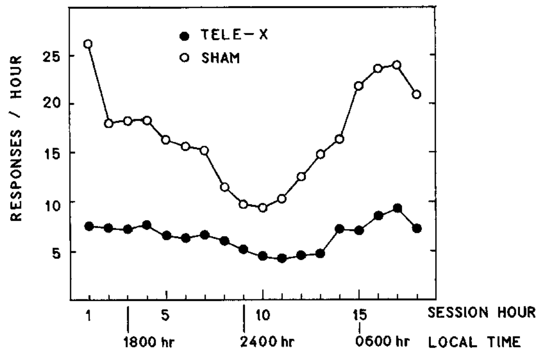

Fig. 3. The variation in the mean rate of swim-through responding by lesioned and sham-operated males during the 18-hr CRF session. The mean values were obtained by combining data for sessions 6, 7, and 8. The sessions started at 1500 hr and ended at 0900 hr the following day, in continuous illumination. Between sessions, the fish were exposed to a daily cycle of illumination with the dark, or dim, period from approximately 2000 to 0600 hr.

The shams showed a pronounced variation in operant rate during the 18-hr reinforcement session, while the tele-x males responded relatively uniformly. To illustrate the difference, the mean hourly rate was determined for reinforcement sessions 6 to 8 (Fig. 3). For the shams, the response rate was highest during the first and last several hours of the session and lowest during the hours corresponding to the middle of the night. In contrast, the lesioned males responded at a low rate throughout most of the session, showing a slight increase during the last few hours. The cycle of responding exhibited by the shams corresponded, approximately, to the day-night changes in illumination in the laboratory in which the fish were kept between operant sessions.

Spawning Trial

None of the tele-x males spawned during the 10-day trial, and none blew a foamnest. Four of six sham males spawned and all six built nests. During the first 10 min of the trial, the tele-x males showed fewer lateral and frontal displays and fewer quivers (Table 1) than the shams. However, the two groups showed equivalent frequencies of approach, and they both exhibited the brisk body movements and bright coloration of reproductively active males.

Histology and Gonadosomatic Index

Examination of serial sections of the whole cranium revealed that both telencephalic lobes and olfactory bulbs were ablated as in the previous

TABLE 1

Frequency of Behaviors during the First 10-Min of the Spawning Trial, and Biometrics

		Behavior				Biometrics			
	N	Approach	Lateral display	Frontal display	Quiver	Body length (cm)	Body weight (g)	Testes weight (mg)	GSI
Sham	6	4.3	49.2	15.0	11.3	4.8	3.2	4.9	0.15
Tele-x	10	4.1	20.1	6.8	3.9	4.9	3.3	3.5	0.11
Student's t test		ns	$P < 0.002$	$P < 0.05$	$P < 0.03$	ns	ns	$P < 0.007$	$P < 0.04$

experiment (Davis *et al.*, 1976). The subpallium rostral to the anterior commissure, and the entire pallium, were removed. The sham-operated males showed no brain damage when the dorsal surface of the brain was inspected with a dissecting microscope.

The mean testes weight and the GSI were significantly decreased in the tele-x group (Table 1). The values varied more for the tele-x males than for the shams; the GSI of one tele-x male was in the range shown by the shams but otherwise the GSI values of the two groups did not overlap. Histological cross sections of testes, which were embedded in paraffin and stained with hematoxylin and eosin, showed similar numbers of cells in various stages of gametogenesis and well developed seminiferous tubules in both sham and tele-x groups.

DISCUSSION

The postoperative recovery of the operant rate is noteworthy in two respects. First, the recovery was incomplete after eight sessions and it appeared to reach asymptote in the sixth. Bilateral telencephalic ablation thus appears to produce a lasting decrease in operant responding. Second, although the recovery was only partial it was sufficient to show that the conspecific visual stimulation, and possibly other stimuli associated with the door operation, was positively reinforcing to the lesioned males. The decrease in rate during the extinction session confirmed that the operant was maintained by the 30-sec reinforcements. The tele-x males did not subsequently mate or build nests in the spawning trial. However, in contrast to the males in the previous experiment (Davis *et al.*, 1976), which received a shorter postoperative recovery period and less operant conditioning experience, these males were brightly colored and active, and they interacted continuously with the female. The behavior of the lesioned males was qualitatively similar to the premating behavior of the shams during the entire 10-day trial. Thus, the additional conditioning and longer recovery period resulted in the lesioned male being less submissive to the female, as postulated, but reproductive behavior was not facilitated. On the basis of these results, the elimination of mating and nest building can not readily be attributed to incompatible social responses or to perceptual or motor deficits. Rather, we conclude that the extensive lesion disrupts brain mechanisms which are specifically responsible for activating reproductive behavior.

The decrease in testes weight and the GSI in the lesioned males implies that blockage of reproduction is correlated with a decrease in the level of gonadotropic hormone. Noble (1939) briefly reported that: "Complete removal of the forebrain of fish has a detrimental effect upon the pituitary with the result that the gonads degenerate. If small rudiments of the forebrain are left, these fish may be brought to spawning by pituitary replacement therapy." Unfortunately, the details of the research have apparently not been described. It is conceivable that the lesions in our experiment destroyed hypothalamic connections which regulate hypophyseal secretions. The afferent and efferent projections of the *Macropodus* telencephalon have not yet been elucidated. In two other teleosts, *Eugerres* and *Holocentrus*, a medial and lateral forebrain bundle and a telencephalo-lobar bundle project to hypothalamic targets (Vanegas and Ebbesson, 1975). Preoptic area lesions decrease reproductive activity in some teleosts but not in others (Peter, 1970). Gonadotropic hormone(s) has been implicated in the control of reproductive behavior in other teleosts (Liley, 1969). Decreased testicular androgen is probably not responsible for the blockage of reproduction, as surgically castrated *Macropodus* males readily spawn, blow foamnests, and care for the eggs (Villars and Davis, in preparation). Machemer (1971) found that foamnest building in *Macropodus* was facilitated by exogenous testosterone and that prolactin increased oral mucous secretion and hence the foaminess of the nest. It should be noted that the testes of the tele-x male were only slightly atrophied, suggesting that gonadtropic secretion was not completely blocked.

The increased latency to the first swim-through response following administration of the lesion is consistent with proposals that removal of the telencephalon results in hyperreactivity (Regestein, 1968; Davis *et al.*, 1976). The decrease in the median response latency over the first five postoperative sessions (Fig. 2) indicated that the males partially adapted to the experimental procedure, such as the handling and the free reinforcement at the start of the session. The decrease in latency closely corresponded with the increased operant rate (Figs. 1 and 2). The increased latency could also reflect an impairment of brain mechanisms of arousal, which has been postulated to occur following removal of the telecephalon (Aronson and Kaplan, 1968). The behavioral effects of increased reactivity and decreased arousal may be difficult to distinquish (Davis *et al.*, 1976).

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