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# Suppression Behavior Increased by Telencephalic Lesions in the Teleost, *Macropodus opercularis*

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Previous experiments in several teleost species suggested that defensive reactions are increased following extensive bilateral ablation of the telencephalon. We investigated the effects of varied telencephalic lesions on the reaction of fish to handling and placement in an unfamiliar tank. When intact or sham-operated males were handled, they showed increased rates of surfacing for air. Males which were previously administered bilateral (TLX) or unilateral (UTLX) telencephalon ablation suppressed activity following handling. TLX lesions resulted in the greatest suppression reactions; in addition, the TLX males showed slightly decreased rates of activity prior to handling. Extensive, bilateral lesions of the dorsal telencephalon did not induce the suppression reaction in most males. Based on the results, we propose that one of the functions of the teleost telencephalon is to modulate defensive behavior. The structures which influence defensive behavior appear to be located in the ventral and possibly the rostrodorsal area of the telencephalon.

In teleost fishes, extensive bilateral removal of the telencephalon results in decreased frequencies of behavior, but no behavior pattern is completely eliminated (Aronson and Kaplan, 1968; Flood *et al.*, 1976; Kassel and Davis, 1977). For example, male sexual and parental behaviors are decreased following telencephalon ablation (Segaar, 1965; Overmier and Gross, 1974; Davis *et al.*, 1976; Kassel *et al.*, 1976; Schwagmeyer *et al.*, 1977; Kassel and Davis, 1977). Shock avoidance frequency is decreased and the latency to avoidance is increased in telencephalon-ablated fish (Aronson and Kaplan, 1968; Flood *et al.*, 1976). The rate of operant responding for food (Savage, 1969) or for conspecific visual reinforcement (Davis *et al.*, 1976) is also decreased. These results suggest that one of the functions of the telencephalon in teleosts is to facilitate arousal, or responsivity to external stimulation (Aronson and Kaplan, 1968).

TLX fish have also been reported to exhibit exaggerated startle or defensive reactions which indicate that they are hyperreactive to some forms of external stimulation. When laboratory fish are startled, they



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variously show increased activity, such as rapid withdrawal or escape, or decreased activity, such as hovering, hiding, and lying motionless on the substrate. Incidents of unusually violent escape behavior have been observed in TLX fish during handling (Steiner, 1886; Hosch, 1936), optomotor behavior trials (Janzen, 1933; Shaw and Sherman, 1971), and in social behavior trials (Atema, 1969; Ribbink, 1972). A previous investigation in the paradise fish, *Macropodus,* showed that freezing or suppression behavior following presentation of a visuoacoustical startle stimulus is increased in TLX males (Davis *et al.*, 1976). The telencephalon may thus play a role in regulating defensive reactions to novel or intense stimulation.

If TLX males are hyperdefensive, exaggerated flight or suppression reactions could interfere with investigations of other behaviors which entail presentation of strong stimulation, such as sociosexual behavior and learning. Regestein (1968) pointed out the possibility that the increased shock avoidance latency of TLX fish could represent suppression, in response to the punishing stimulation, rather than depression, or low arousal. Telencephalon ablation could conceivably also result in hypoactivity, or a decreased level of body motion and locomotion, in addition to increased defensive reactions. Further investigations are needed to clarify the relationship between defensive behavior and other behavioral correlates of telencephalon removal.

The present experiments in *Macropodus* were to investigate telencephalic lesions and suppression behavior following handling. Experiment 1 was designed to determine whether TLX males are less active following handling than sham-ablated and intact males, and whether recovery occurs within several hours. Preliminary observations showed that handling of the fish by the investigator evokes profound suppression of swimming and air breathing in a TLX male lasting several hours. Intact males typically are only briefly disturbed by handling. The reactions of males to handling following partial telencephalon ablations were investigated in Expts 2 and 3. The mapping of telencephalon structures in which lesions result in increased suppression behavior is the principal goal of this research.

## EXPERIMENT 1: THE EFFECT OF TLX LESIONS ON ACTIVITY

Direct visual observation of TLX males in home tanks showed that suppression of activity which is produced by brief handling was most profound during the first hour following handling and that recovery occurred within several hours. A conspicuous feature of the suppression reaction was that the fish remained immobile on or near the bottom except to rise to the surface to breathe, and that the rate of surfacing for air was decreased. *Macropodus* is an obligate air breather. In daylight, males kept at 24 to 26°C rise to the surface to breathe air 10 to 50 times per hour, and the rate varies with the amount of swimming activity. Movements of the opercula, or gill covers, are nil, suggesting that water ventilation of the gills is decreased in air-breathing individuals. After handling, a TLX male may surface once or not at all during the first hour and rhythmic opercular movements are increased.

We used the rate of surfacing as an index of swimming activity. Surfacing is a distinct unit of behavior which is easy to identify and to register electronically with a photodetector beam. The handling procedure consisted of removal of the fish from the home tank and placement into an unfamiliar experimental tank. In the home tank, the fish were kept so that they could see other fish in nearby tanks and movements in the surrounding laboratory. This procedure reduces freezing behavior in *Macropodus* (Davis, 1975). In the experimental tank, fish were visually and physically isolated from other fish to eliminate disturbances which could affect recovery of activity during the handling trial.

The present experiment examined the rates of surfacing activity of TLX, sham-ablated, and intact males in weekly, 3-hr handling trials. We wished to determine whether telencephalon ablation results in decreased surfacing activity following handling, and whether recovery occurs during the weekly trial and between successive trials.

*Method*

*Fish.* Twenty-four *Macropodus opercularis* (L.) ($\bar{x}$ = 4.7 cm standard length, or body length) were obtained from domesticated stock in Hong Kong. The fish were kept in bisexual groups in large tanks prior to the experiment and in individual 9.5-liter home tanks in an open laboratory room during the experiment. The daily cycle of diffuse natural light in the laboratory was augmented by a 12:12-hr L:D cycle of "daylight" fluorescent light. The home tank walls were clear glass. Tetramin staple food was provided daily, and water temperatures were kept between 24 and 26°C.

*Experimental tank.* The 57-liter tank described by Davis *et al.* (1975; Fig. 1) was used. A surfacing activity compartment of clear Plexiglas, 9 × 12 × 17 cm tall, was placed into the center against one wall. Fresh water flowed through the tank at 400 ml/min. The water entered through the bottom of the surfacing activity compartment and circulated upward and then through a screen at the bottom into the surrounding tank toward the drain. The top of the compartment was covered except for a 2.5-cm diameter opening which was level with the water surface. To reach the surface to breathe, the fish had to place its head into the opening. An infrared beam (Davis *et al.*, 1975) detected the presence of the fish's head in the opening. The photodetector was connected to a PDP-8F digital computer which registered responses and compiled hourly totals. To register a response, a fish had to remain out of the beam for 10 sec or longer. Opaque, nonmirroring wall coverings prevented fish in neighboring tanks from seeing each other.

*Procedure*

Experimentally naive males were placed into individual home tanks the evening before the experiment. On Day 1, eight of the males received bilateral telencephalon removal (TLX), eight received sham ablations, and eight received no surgery. Each male was subsequently administered a 3-hr handling trial in the experimental tank on Days 8, 15, 22, and 29. At the start of the trial, the male was carried from the home tank in a small container and poured into the surfacing compartment. The compartment was covered, and the experimental room door was closed prior to starting the 3-hr period of response registration. Three experimental tanks were operated simultaneously. At the end of the trial, the male was returned to the home tank.

*Surgical lesions and histology.* Telencephalon ablation and the sham ablation were carried out as previously described (Davis *et al.*, 1976). After the experiment, males were sacrificed by brief immersion in ice water. The brain was fixed in formalin or alcohol–formalin–acetic acid, imbedded in paraffin and cut in 10-$\mu$m vertical, longitudinal sections. The sections were stained with cresyl violet acetate. The lesions were reconstructed by projecting parasagittal sections onto corresponding sections of a reference brain (Kassel and Davis, 1977).

*Results*

The TLX males showed less activity during the handling trial than the sham and intact males (Table 1). A trivariate analysis of variance (ANOVA) on the three groups, the four trials, and the 3 hr of the trial revealed a significant Group ($P < 0.001$) and Hour effect ($P < 0.001$) but a nonsignificant Trial effect ($F < 1$). There was a significant Group $\times$ Hour interaction ($P < 0.01$), indicating that the change in the rate of surfacing activity during the 3-hr trial varied between the groups (Table 2). A univariate ANOVA showed that the mean hourly rate of surfacing did not

TABLE 1
The Mean Hourly Rate of Surfacing in the Weekly Handling Trials in Expt. 1

| | | Postoperative week | | | |
|---|---|---|---|---|---|
| Group | $N$ | 1 | 2 | 3 | 4 |
| Intact | 8 | 45 | 45 | 39 | 40 |
| Sham | 8 | 36 | 38 | 40 | 39 |
| TLX | 8 | 2 | 6 | 9 | 9 |

TABLE 2
Variation of the Mean Hourly Rate of Surfacing During the Four 3-hr Handling Trials in Expt 1

| Group | $N$ | Hour of the trial | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| Intact | 8 | 48 | 41 | 37 |
| Sham | 8 | 47 | 36 | 31 |
| TLX | 8 | 7 | 6 | 6 |

vary significantly for the TLX males. However, the intact and the sham males showed a distinct decrease in activity during the trial ($P < 0.01$). In the final trial, three of the TLX males were moderately active though still less active than the control males (Table 3). One male surfaced not at all in the entire 3-hr period of the final trial and only three times during the entire experiment. All the males appeared to be in excellent physical condition.

*Histological examination.* Parasaggital sections of the TLX brains showed that both hemispheres had been removed at the level of the rostral preoptic area. The variation in the amount of damage to the preoptic nucleus area was small and similar to that described for males in a previous investigation (Kassel and Davis, 1977; see Fig. 1). The extent of preoptic tissue removal was seemingly unrelated to the rate of surfacing activity in the experimental tank. For example, the least active and most active males (Table 3) had similar lesions.

### *Discussion*

The data show that TLX lesions result in low rates of surfacing following handling with no significant recovery within 3 hr. The rate of surfacing

TABLE 3
The Mean Hourly Rate of Surfacing Activity for Individual Males in the Experimental Tank During the Final 3-hr Handling Trial of Expt 1

| Group | | |
|---|---|---|
| Intact | Sham | TLX |
| 24 | 28 | 0 |
| 35 | 29 | 4 |
| 38 | 34 | 6 |
| 39 | 36 | 8 |
| 39 | 42 | 9 |
| 45 | 42 | 15 |
| 47 | 48 | 16 |
| 53 | 55 | 17 |

increased slightly between the weekly trials for some of the TLX males. Direct observation of fish in home tanks indicated that undisturbed TLX and control males had similar rates of surfacing which varied from 15 to 30 per hour. Unfortunately, owing to visual obstructions in the tanks, fish could evade continuous observation and accurate measurements of surfacing were not possible. The method of observation was improved in subsequent experiments so that the rate of activity in the home tank could be compared to the rate following handling. Whereas the TLX males appeared to suppress activity following handling, the control males seemed to increase the rate of surfacing at the start of the trial, as though they were excited by the stimulation. This impression was supported by later experiments. Part of the excitement could have been elicited by the flow of water in the activity compartment. Standing water was used in later experiments, and the rates of surfacing by control males decreased correspondingly.

The TLX males reacted more violently than the control males to being captured and transported to the experimental tank. At the end of the trial, when the compartment was opened to remove the fish, the TLX male was typically seen to be rigid on the bottom, tilted laterally or lying prone on one side with its body curled into the shape of a "potato chip." When approached by the capture net, the TLX male reacted with explosive swimming movements. In contrast, the control males typically were seen to be hovering or moving when the compartment was opened, and they did not struggle violently when captured.

Isolation of the male in the surfacing compartment may add to the stress produced by the handling procedure and delay recovery. Stimulus deprivation can increase reactivity to conspecific visual stimuli in *Macropodus* males within a matter of hours (Davis, 1975). Recovery following disturbances in the home tank may be facilitated by the familiarity of the environment and the availability of reinforcing social stimulation (Davis *et al.*, 1976).

## EXPERIMENT 2: HANDLING REACTION FOLLOWING UNILATERAL TELENCEPHALON LESION (UTLX)

The present experiment was designed to compare the effects of TLX and UTLX lesions on the reaction of males to handling. Several changes in procedure were made, based on our findings from Expt 1 and in unreported experiments. The reaction to handling was measured before and after lesion administration. We had found that while most intact fish show increased activity when handled some slightly suppress activity or show no change. Males that suppressed activity in the preoperative handling trial were not used in the experiment. The rate of surfacing prior to handling was measured by visual observation of the male in the home tank. The posthandling rate of surfacing was measured by the photodetector method for 1 hr immediately after the fish was placed into the experimental tank. The pre- and posthandling rates for each male were used to calculate the percentage change in the rate of surfacing as a measure of the handling reaction.

*Method*

*Fish.* Twenty-seven males ($\bar{x}$ = 4.6 cm body length) were used. The fish were kept in an individual home tank as described for Expt 1.

*Surfacing activity.* To measure the rate of surfacing prior to handling, a removable, surfacing activity compartment similar to the one used in the experimental tank was placed in the home tank. The compartment facilitated direct visual observation of the fish and provided the fish with experience in breathing through a small opening at the surface prior to being placed into the experimental tank. Surfacing for air in the experimental tank was registered electronically as described in Expt 1. Standing, aged water was used instead of flowing, charcoal-filtered tapwater. The tapwater was found to have a high pH (9.5). Aged water was used to avoid possible deleterious physiological effects of changes in pH. The water was buffered to pH 7.3 with a phosphate buffer and continuously filtered, and water temperatures were kept at 24 to 26°C. The bottom was covered with 3 cm of gravel. The surfacing compartment was raised slightly above the gravel so that water in the tank and the compartment could slowly mix through the wire mesh floor of the compartment.

*Procedure*

*Preoperative.* On Day 1, the male was placed into a surfacing activity compartment in an individual home tank. The male could see other fish in adjoining tanks and movements in the laboratory. On Day 2, between 1000 and 1200 hr, an observer recorded the frequency of surfacing for a 10-min period and calculated the hourly rate. The procedure was administered for four fish simultaneously.

A 1-hr handling trial was administered at approximately 1200 hr on Day 2. The male was placed into an individual experimental tank and surfacing responses were registered for 1 hr. After the trial, the fish was returned to the home tank, from which the surfacing activity compartment had been removed. A second 1-hr handling trial was administered at 1200 hr on Day 5, to obtain an additional measurement of the individual's rate of activity. The percentage change in the rate of surfacing was calculated {100 × [1 minus (posthandling hourly rate/prehandling hourly rate)]} for each male. The difference between the mean pre- and posthandling rates of surfacing was evaluated using a $t$ test for correlated means.

The results of the two preoperative trials were similar and only the second trial data are presented. Three males suppressed activity in the preoperative trial and they were eliminated from the experiment. The remaining 24 males were evenly distributed among three experimental groups, a sham lesion, UTLX lesion, and a TLX lesion group, and the groups were balanced to have similar mean rates of surfacing in the experimental tank.

*Lesions.* The TLX and sham lesions were carried out as in Expt 1. The UTLX lesion was done by cutting the commissure of the dorsal telencephalon to separate the two hemispheres and aspirating one hemisphere and the attached olfactory bulb.

*Postoperative.* The male was returned to the home tank and kept undisturbed, except for feeding and routine removal of debris from the tank, for 4 weeks. On Day 33, the male was gently placed into a surfacing activity compartment in the home tank and allowed to adapt overnight. On Day 34, between 1000 and 1200 hr, the male was observed for 10 min to measure the rate of surfacing. At approximately 1200 hr the male received a 1-hr handling trial in the experimental tank. The procedure was administered for four fish at a time. The male was subsequently sacrificed to examine the brain.

*Results*

Handling resulted in a significant increase in activity in the preoperative trial (Table 4). In the postoperative trial, the sham males showed increased activity but the UTLX and TLX males suppressed activity. A bivariate ANOVA of the mean handling reaction on the three groups and the two trials revealed a significant Group effect ($P < 0.001$) and Trial effect ($P < 0.001$), and Group X Trial interaction ($P < 0.001$), which is evident in Table 4. The difference in the handling reaction between the pre- and postoperative trials was significant for the UTLX (paired $t$ test; $P < 0.01$) and TLX ($P < 0.001$) males, but not for the shams. As can be seen in Table 5, the UTLX lesion males showed less suppression than the TLX lesion males.

The rate of surfacing in the home tank varied significantly between the groups (bivariate ANOVA; $P < 0.05$), but the differences between the

TABLE 4
The Mean Rate of Surfacing Activity in the Home Tank and the Percentage Change in the Rate Following Handling in Expt 2

| | | Preoperative | | | Postoperative | | |
|---|---|---|---|---|---|---|---|
| Group | $N$ | Activity (hour$^{-1}$)[a] | Change (%) | $t$ test (P) | Activity (hour$^{-1}$) | Change (%) | $t$ test (P) |
| Sham | 6 | 17 | +78 | <0.01 | 23 | +82 | <0.01 |
| UTLX | 6 | 16 | +32 | <0.05 | 19 | −54 | <0.02 |
| TLX | 6 | 15 | +86 | <0.01 | 12 | −97 | <0.01 |

[a] Hour$^{-1}$ = per hour.

TABLE 5
Postoperative Rate of Surfacing Activity in the Home Tank and the Percentage Change in the Rate in the Handling Trial for Individual Males in Expt 2

| Group | | | | | |
|---|---|---|---|---|---|
| Sham | | UTLX | | TLX | |
| Activity ($hour^{-1}$) | Change (%) | Activity ($hour^{-1}$) | Change (%) | Activity ($hour^{-1}$) | Change (%) |
| 36 | +22 | 24 | −8 | 6 | −92 |
| 30 | +33 | 18 | −44 | 12 | −92 |
| 18 | +67 | 30 | −63 | 12 | −100 |
| 24 | +67 | 18 | −55 | 12 | −100 |
| 18 | +122 | 12 | −75 | 12 | −100 |
| 12 | +183 | 12 | −83 | 18 | −100 |

pre- and postoperative trials were not significant. TLX males were less active in the home tank than the shams while the level of activity of the UTLX males was intermediate (Table 4).

*Lesions*. Examination of the brain of the TLX males with a dissecting microscope showed that both hemispheres were removed at the level of the rostral preoptic area. The lesions resembled those which were described for the TLX males in Expt 1. In the eight UTLX males, one telencephalic hemisphere was seen to be removed at the level of the rostral preoptic area, while the remaining hemisphere and olfactory bulb remained intact.

*Discussion*

This experiment confirmed that handling and placement into an unfamiliar tank stimulates activity in intact males and suppresses activity in telencephalon-ablated males. We infer that the lesions result in lowering the threshhold of freezing, or suppression of behavior. Whether sham lesion males briefly suppress activity at the start of a trial and then increase activity for the remainder of the trial has not been determined. However, we have shown that a visuoacoustic startle stimulus elicits suppression in sham lesion males and much greater suppression in TLX males (Davis *et al.*, 1976). The increased rate of surfacing by the shams may reflect increased exploration and escape behavior.

Activity in the home tank appeared to be decreased by the TLX lesions but not UTLX lesions. This observation suggests that, in addition to increased freezing behavior, TLX lesions also result in decreased rates of activity independent of the effects of handling. The procedure of placing the male into the surfacing compartment in the home tank might have resulted in a partial suppression of activity during the 10-min observation period the next day. The possibility that TLX fish require longer than 24 hr to fully recover or to adapt to the relatively unfamiliar activity compartment needs to be investigated. It is our impression, however, after observing many TLX lesion males that they are slightly less active, less vigorous than control males under some conditions. Telencephalon ablation may result in a decreased metabolic rate. Other investigators have reported that TLX fish are less active than control fish (Aronson and Kaplan, 1968). However, it is difficult to determine from those reports whether the fish were adapted at the time that they were observed and not suppressing activity in reaction to some environmental disturbance. Some experiments revealed no differences in activity between control and TLX fish (eg., Flood and Overmier, 1971).

TLX males which are given extensive operant conditioning experience, in which a simple position response is rewarded by a brief presentation of conspecific visual stimulation, persistently perform the response at a lower rate than sham lesion males (Kassel *et al.*, 1976). The decreased rate might reflect suppression due to the handling and to the excitement of the reinforcement. In addition, brain mechanisms of reinforcement may be impaired by TLX lesions (Flood *et al.*, 1976). However, the possibility that the fish merely move more slowly cannot be excluded. Intact and telencephalon-ablated males perform nonsexual social behaviors at similar rates (Aronson, 1948; Kassel and Davis, 1977), so some forms of activity are not decreased by telencephalon ablation.

UTLX lesions result in suppression behavior but they are less effective than TLX lesions. The regulation of defensive reactions may depend upon the combined function of both hemispheres of the telencephalon. This is in contrast to the influence of the telencephalon on male reproductive behavior. Sexual responses to intact females, including mating, and the performance of parental behaviors, such as nest building and egg care, are decreased by TLX but not UTLX lesions (Kassel and Davis, 1977). The decreased reproductive behavior and increased defensive reaction may thus be separate effects of telencephalon ablation.

## EXPERIMENT 3: HANDLING REACTION FOLLOWING PALLIAL LESIONS

The increased freezing and violent escape behavior of TLX *Macropodus* resemble behavioral effects of telencephalon lesions that have been obtained in rats. Emotional reactivity and acoustical startle reactions are increased in laboratory rats following bilateral, surgical lesions of the septal area (Brady and Nauta, 1953, 1955) and the cingulate cortex (Thomas *et al.*, 1968). Telencephalic organization in the actinopterygian, or rayfinned, fishes is very different from that of land vertebrates and other sarcopterygians, particularly the organization of the dorsal area of the telencephalon, or the pallium (Nieuwenhuys, 1963). Homologies of pallial structures between actinopterygians and land vertebrates have been variously interpreted (e.g., Holmgren, 1922; Kallen, 1951; Droogleever-Fortuyn, 1961; Nieuwenhuys, 1963, 1966; Northcutt and Braford, 1978) but most neuroanatomists homologize ventral, or subpallial cell groups, to those of land vertebrates (Aronson and Kaplan, 1968). Several cell groups in the anterior, ventromedial telencephalon of actinopterygians have been identified as homologs of septal nuclei in land vertebrates (Northcutt and Braford, 1978).

The pallium and the subpallium, which could contain homologs of, respectively, the neocortical cingulate gyrus and the septal nuclei, are both removed by the TLX lesion procedure in our experiments. If the removal of pallial nuclei is responsible for the increased suppression response of TLX males, then administration of pallial lesions which spare the subpallium should also induce the response. This possibility was investigated in the following experiment using methods similar to those described in Expt 2. If extensive pallial lesions do not increase suppression behavior, subpallial structures would be implicated.

*Method*

Twenty males ($\bar{x}$ = 4.7 cm body length) were used. They were kept in individual home tanks as described for Expt 1. The previously described surfacing activity compartments and experimental tanks were used without modification.

*Preoperative.* The male was placed into a surfacing activity compartment in an individual home tank on Day 1. The frequency of surfacing was recorded for 10 min on Days 2, 3, and 4, between 1000 and 1200 hr. The average of the three daily measurements was used to calculate the hourly rate of prehandling activity. The procedure was administered for four fish at a time.

A 1-hr handling trial was administered on Day 4 at approximately 1200 hr. The male was placed into the experimental tank and surfacing activity was recorded for 1 hr. The percentage change in the rate of surfacing during the trial was determined as in Expt 2. After the trial the male was returned to the home tank. Four of the males showed suppression during the trial and were eliminated from the experiment. On Days 4 or 5, eight males were administered pallial lesions (PLX) and eight received sham lesions.

*Lesions.* The PLX lesions were made with a Ziegler knife through a large opening in the cranium. A bone flap was cut in the roof of the cranium between the eyes and reflected caudally to expose the telencephalon. The flap was formed by taking three incisions in the cranium with the point of a scalpel: A 3-mm transverse incision centered between the orbital ridges, and two 3- to 4-mm longitudinal incisions extending caudally from the ends of the transverse cut. The flap was raised so that it remained attached caudally. The loose adipose tissue and primary menix above the brain were aspirated to visualize the telencephalon. A portion of the dorsal and lateral pallium of each hemisphere was removed by cutting with the knife from the caudal to the rostral pole of the telencephalon. The lesioned tissue was removed by aspiration prior to closing the bone flap. The sham lesion procedure consisted of opening the bone flap, aspirating the superficial tissues without touching the brain, and then closing the flap. The males were returned to the home tank following surgery.

*Postoperative.* The rate of surfacing in the home tank was measured for 10 min by direct observation on Days 15, 16, and 17 to calculate the hourly rate of activity. A 1-hr handling trial was given shortly following the home tank observation on Day 17. The PLX males were subsequently sacrificed to prepare the brain for histological examination. To reconstruct the PLX lesions, 10 thick transverse sections of the telencephalon were stained with cresyl violet. A section from the rostral, central, and the caudal areas of the telencephalon was projected onto a corresponding section of an intact reference brain (Fig. 1). Telencephalic cell groups and the outline of the lesion were drawn with a Bausch and Lomb microprojector. Sham-operated brains were examined in the cranial cavity using a dissecting microscope.

### *Results*

A bivariate ANOVA revealed that the differences in home tank activity between the sham lesion and PLX lesion males prior to and following administration of the lesions were not significant (Table 6). Thus, the PLX lesions did not noticeably affect the rate of surfacing in the home tank environment 10 to 12 days following surgery.

Both groups increased activity in the preoperative handling trial, while in the postoperative trial the sham lesion males showed an increase but the PLX males did not (Table 6). The handling reaction (percentage change) did not vary significantly between the sham and PLX males in either trial, but a bivariate ANOVA revealed a significant Group X Trial interaction ($P < 0.01$). The origin of the interaction was evident in the range of variation in the handling reactions of individual males (Table 7). Two PLX males clearly suppressed activity, and two showed little or no

TABLE 6
The Rate of Surfacing Activity in the Home Tank and the Change in the Rate in the Handling Trial in Expt 3

| | | Preoperative | | | Postoperative | | |
|---|---|---|---|---|---|---|---|
| Group | *N* | Activity ($hour^{-1}$) | Change (%) | *t* test (P) | Activity ($hour^{-1}$) | Change (%) | *t* test (P) |
| Sham | 8 | 21 | +34 | <0.02 | 20 | +48 | <0.05 |
| PLX | 8 | 21 | +52 | <0.01 | 21 | +3 | NS[a] |

[a] NS, not significant.

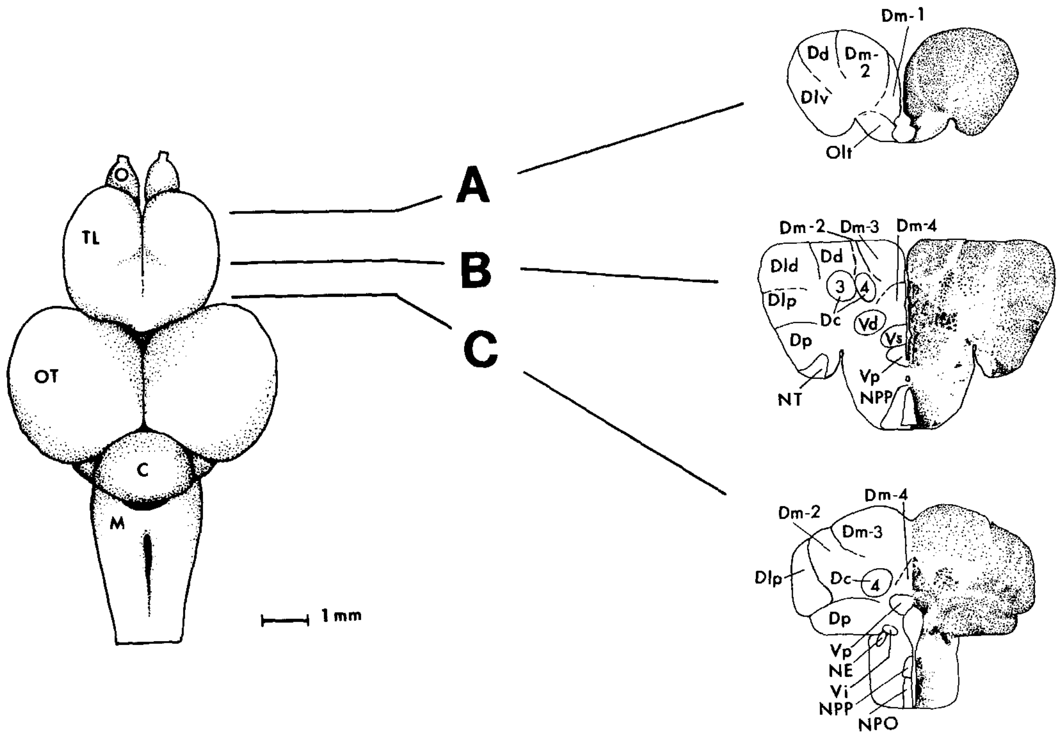


FIG. 1. Dorsal view of the brain of *Macropodus opercularis*. Lines A, B and C denote the levels of the transverse sections that are presented in Fig. 2. Abbreviations (Nieuwenhuys, 1963; Segaar, 1965; Peter and Gill, 1975; Northcutt and Braford, 1978) used: C, cerebellum; D, area dorsalis telencephali; Dc, central part of D; Dc-1,2,3,4, parts of Dc; Dd, dorsal part of D; Dl, lateral part of D; Dld,p,v, dorsal, posterior and ventral parts of Dl; Dm, medial part of D; Dm-1,2,3,4, parts of Dm; Dp, posterior part of D; M, medulla, Ne, nucleus entopeduncularis; NPO, nucleus preopticus; NPP, nucleus preopticus periventricularis; NT, nucleus taenia; O, olfactory bulb; OT, optic tectum; Olt, olfactory tract; TL, telencephalon; V, area ventralis telencephali; Vd, dorsal nucleus of V; Vi, intermedial nucleus of V; Vp, postcommissural nucleus of V; and Vs, supracommissural nucleus of V.

TABLE 7
Postoperative Rate of Surfacing Activity in the Home Tank and the Change Rate During the Handling Trial for Individual Males in Expt 3

| | Group | | | | |
|---|---|---|---|---|---|
| | Sham | | | PLX | |
| Male No. | Activity ($hour^{-1}$) | Change (%) | Male No. | Activity ($hour^{-1}$) | Change (%) |
| S1 | 28 | −11 | P2 | 22 | −86 |
| S2 | 20 | +15 | P5 | 20 | −25 |
| S3 | 30 | +17 | P6 | 12 | −8 |
| S4 | 16 | +36 | P12 | 12 | 0 |
| S5 | 14 | +57 | P17 | 22 | +19 |
| S6 | 20 | +80 | P18 | 28 | +25 |
| S7 | 18 | +94 | P19 | 32 | +49 |
| S8 | 16 | +100 | P20 | 18 | +50 |

change, while the remaining four males increased activity. Among the sham lesion males, one suppressed activity slightly while the others increased activity.

*Lesions*. The PLX lesions varied greatly in size (Fig. 2). In the pallium, cell groups of the Dl series and groups Dd, Dm-2, and Dm-3 were damaged by the lesions in every male (Table 8). Area Dm-4 was damaged in males P2, P5, P6, and P20 and intact in the remaining males. Cell group NT was damaged bilaterally or unilaterally in all the males except P12 and P17. Area Dp was variously damaged in males P2, P5, P6, P12, and P20; these males and males P18 and P19 also had Dc lesions. The subpallium ventral and medial to Dl and ventral to Dm-4 was intact in seven of the

TABLE 8
Telencephalon Cell Groups That Were Extensively or Fully Ablated (a) or Partially Ablated (b) in the PLX Lesion Males

| | Cell group | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Male No. | Dl[a] | Dd | Dm-2 | Dm-3 | Dc | NT | Dp | Dm-4 | Dm-1 | V[b] |
| P2 | a | a | a | a | b | b | b | b | b | |
| P5 | a | a | a | a | a | a | a | a | b | b |
| P6 | a | a | a | a | b | b | b | b | | |
| P12 | a | a | b | a | b | | b | | | |
| P17 | a | a | b | a | b | | | | | |
| P18 | a | b | b | b | | b | | | | |
| P19 | a | b | b | b | | b | | | | |
| P20 | a | a | a | a | b | b | b | b | | |

[a] Includes Dlv, Dlp, and Dld.
[b] Includes Vd, Vs, and Vp.

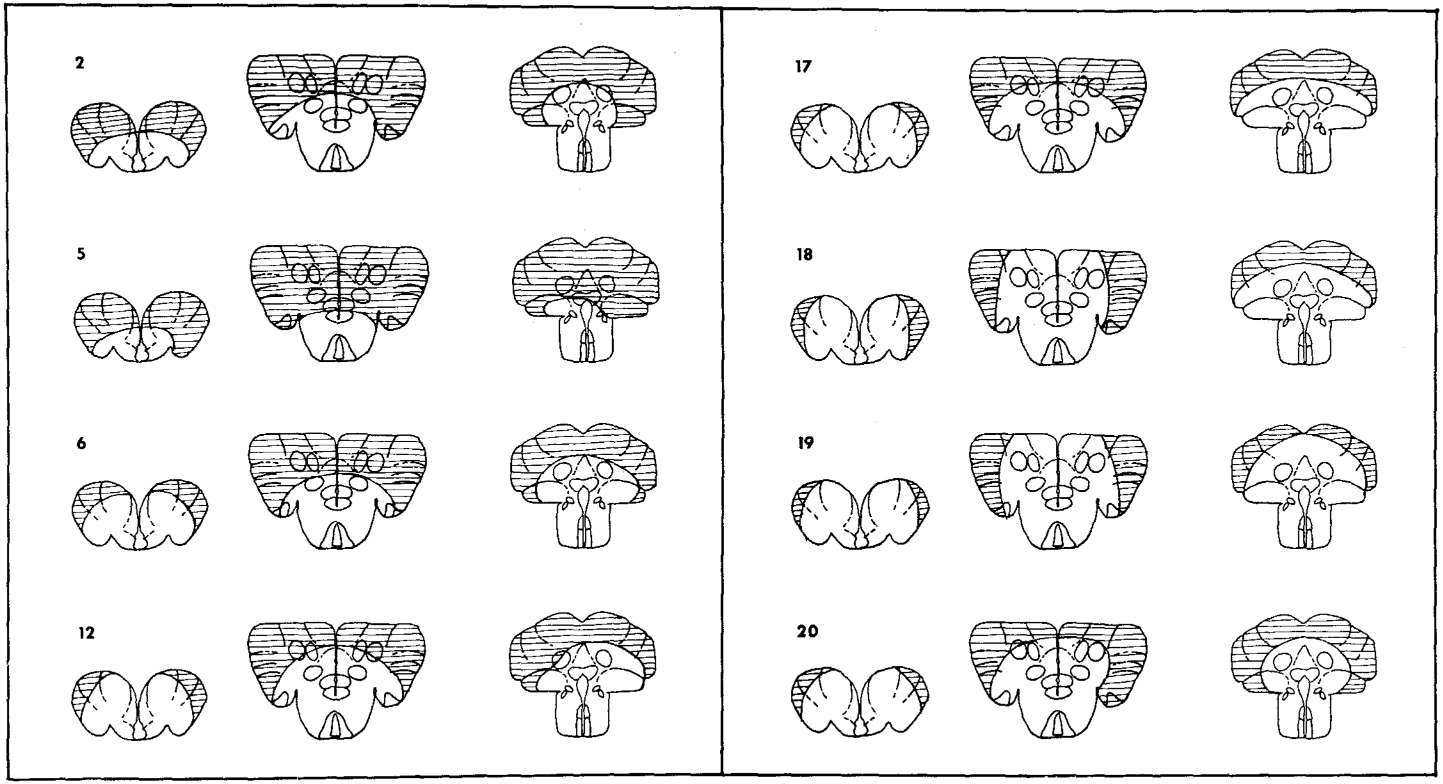


FIG. 2. The extent of the telencephalon lesions sustained by the eight PLX lesion males in Expt 3. The transverse brain sections are from the rostral (A), central (B), and caudal (C) levels of the telencephalon, as shown in Fig. 1. The cross-hatched region of each section shows the area that was removed as a result of the lesion.

eight PLX males. In the eighth, male P5, virtually all telencephalic tissue dorsal to the anterior commissure was ablated, including portions of Vd, Vs, and Vp in Fig. 1.

*Discussion*

The results of Expt 3 show that extensive portions of the pallium can be ablated without increasing the suppression response to handling. Lesions of area Dl, Dd, Dm-2, Dm-3, Dc, and NT occurred in all or most of the males. These areas are thus probably not primarily involved in the suppression response. Areas Dm-1, Dm-4, Dp, Vd, and Vs were damaged mainly in males showing suppression. Male P20 is an exception: the telencephalon was severely damaged with little pallial tissue left intact, yet the fish showed a normal handling reaction. Of the two males that showed a distinct suppression reaction, P2 and P5, only P5 sustained lesions in the V series nuclei which Nieuwenhuys (1967) classified as subpallial. However, the pallial–subpallial boundary has not been clearly established for actinopterygians. Northcutt and Braford (1978) propose that, based on histochemical investigations, parts of areas Dm-3, Dm-4, and Dc are homologous to subpallial structures of land vertebrates. It is interesting to note that the rostral telencephalon, including area Dm-1, received the greatest damage in the males, P2 and P5, which showed the strongest suppression. Our tentative conclusion from these results is that the telencephalic structures that modulate defensive behavior in male *Macropodus* are located in the ventral and possibly the rostrodorsal area of the telencephalon.

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