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Antagonistic Effects of Vasotocin on Parental Behavior in Convict Cichlids

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I. Introduction

The peptide hormones vasopressin and oxytocin perform peripheral functions such as osmotic regulation and lactation, respectively. Recent studies have demonstrated that these hormones also perform central functions such as regulation of various social behaviors in mammals. In particular, studies on monogamous and biparental prairie vole (*Microtus ochrogaster*) have shown that both vasopressin and oxytocin play important roles in pair-bond formation (Young & Wang, 2004) and regulation of parental behavior (Bales et al., 2004). Non-mammalian vertebrates possess homologous peptides of vasopressin and oxytocin, namely, vasotocin and isotocin (fishes) or mesotocin (amphibians, lizards, and birds). Comparative studies have revealed that some social behaviors such as vocalization and aggression are commonly regulated by peptide hormones in vertebrates (Goodson & Bass, 2001). However, it is not known whether parental behavior is also regulated by peptide hormones in non-mammalian vertebrates.

Cichlidae is a highly diverse family of teleost fishes, comprising more than 2,000 species. Cichlids exhibit diverse forms of mating, including monogamy, polygyny, polygynandry, and polyandry (Barlow, 2000). Convict cichlid (*Amatitlania nigrofasciata*) is a member of the family

Cichlidae, and it originally inhabited Central America (Schmitter-Soto, 2007). This species shows monogamy and biparental care. Parental care by convict cichlids includes retrieving fry and fin-digging. Pair individuals frequently take their fry away from a group by carrying them in their mouth. At dusk, the parents retrieve fry into the nest (Reebs, 1994). Fin-digging involves settling onto the substratum and stirring up loose material by a short bout of vigorous and rapid beating of the pectoral fins. The fry immediately converge to the dug site to feed on the suspended or newly exposed food material. Thus, this behavior assists in feeding the young (Wisenden et al., 1995). These behaviors in cichlids might be comparable to the parental behavior in mammals. The present study investigated the antagonistic effects of vasotocin on parental behavior in convict cichlids.

II. Methods

1. Subjects

Eight pairs of convict cichlids were used. They were kept in a stock tank, and both sexes were separated from each other. Water temperature was maintained at 25 °C, and the fishes were exposed to a 12-h photoperiod (0800 h–2000 h) followed by 12-h darkness (2000 h–0800 h). The cichlids were fed commercially available pellets (Tetra) once a day.

2. Apparatus

Six experimental tanks (60 [width] × 30 [depth] × 36 [height] cm) were used, with sand-gravel forming the substratum. Each tank was equipped with a heater and an air pipe. A clay flower pot was placed in each tank, which served as the nest for a pair. A styrofoam board was placed between the tanks such that the pairs were visually isolated from each other. A video camera (HDR-HC1, Sony) was paced in front of each tank during the experiment.

3. Procedure

3.1 Preparation

The cichlids were kept in a stock tank for at least 2 weeks before the experiment. First, 2 females were introduced into an experimental tank. After 24 h–48 h, 2 males were introduced into the tank. The criterion of pair-bond formation was sharing of a territory and its protection together by a male and a female. After a pair was formed, the other individuals were returned into the stock tank. After spawning, the day on which a fry began swimming was defined as day 1. The experiments were conducted on days 4 and 7 for males (or females) and on days 12 and 15 for females (or males), in randomized order.

3.2 Recording of behavior

On the experimental day, a pair's behavior was video-recorded for 60 min. The subject was then gently captured using a hand net, and the drug/saline administration experiment was performed. Immediately after the administration, the pair's behavior was again video-recorded for 60 min. All the recordings were made from 1500 h to 1800 h.

3.3 Drug administration

The subject was administered a vasopressin V1a receptor antagonist (Manning compound, Sigma) or saline for fish (0.81% NaCl). Manning compound was dissolved in saline. A total volume of 10 μ l/g of body weight of the drug was administered, with each dose of 2.5 μ g/g of body weight. Identical volume of saline was administered in the saline condition. Either the drug or saline was administered on the first experimental day and the other on the second experimental day. The order was balanced. Both the drug and saline were administered by intraperitoneal (i.p.) injection, using a 1-ml syringe and 30-gauge 1/2-inch needle.

4. Data analysis

Five categories of behavior were observed: retrieving, fin-digging, touching, nest, and foraging (Table 1). The former 2 behaviors were parental behaviors. Frequency of each behavior in the post-injection phase minus that in the pre-injection phase was used as an index of behavioral change, because baseline frequency might change with the age of the fry. Thus, a value of above 0 indicated increased frequency of the post-injection behavior compared with the pre-injection behavior. In addition, the first and last 30 min of the 60-min recording were analyzed separately.

Table 1. Definition of behaviors.

behavior	definition
retrieving	carrying offspring with mouth
fin-digging	scattering substrate with body
touching	touching mate with mouth
nest	entering the nest
foraging	biting substrate or water surface

III. Results

In the first 30-min of injection, the retrieving behavior significantly differed between the sexes (Figure 1). The females increased retrieving of fry after the injection, regardless of the administration of the drug or saline. No significant change was observed in the other behaviors.

In the last 30 min of injection, no change was observed in the retrieving behavior. On the other hand, fin-digging was significantly affected by drug injection. Both the sexes increased fin-digging after the administration of Manning compound than after saline administration. A similar tendency was observed in the foraging behavior. Manning compound administration significantly increased foraging frequency in both the sexes as compared with saline administration (Figure 2). No significant change was observed in the other behaviors.

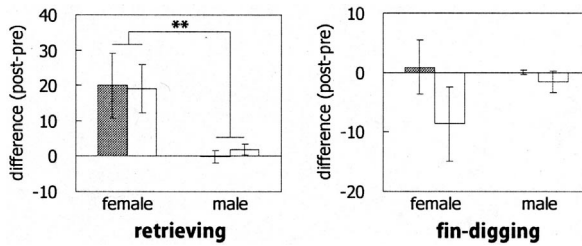


Figure 1. Mean change of frequency of retrieving (left) and fin-digging (right) in the first 30-min recordings. Filled bar and open bar indicate Manning compound and saline condition, respectively. Error bar indicates \pm S. E. M. ($n=8$ each). ** $p<.01$

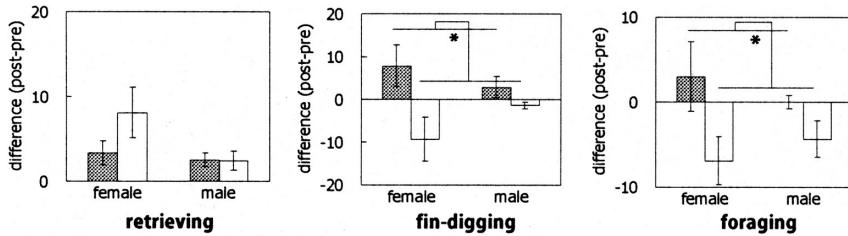


Figure 2. Mean change of frequency of retrieving (left), fin-digging (middle), and foraging (right) in the last 30-min recordings. Filled bar and open bar indicate Manning compound and saline condition, respectively. Error bar indicates \pm S. E. M. ($n=8$ each). * $p<.05$

IV. Discussion

Our experiment demonstrated that (1) the females and not the males increased retrieving of fry immediately after the injection and (2) Manning compound injection increased fin-digging and feeding in both the sexes.

Two categories of parental behavior showed changes after drug administration. Retrieving of fry was increased only in females immediately after the injection, regardless of the administration of the drug or saline. This result suggested that the injection itself altered the behavior of the females. On the other hand, fin-digging and feeding were increased only after the injection of Manning compound. These results suggest that parental behavior is regulated not only by the vasotocinergic system but also by other mechanisms.

Retrieving and fin-digging might be affected by sex hormones. In the pre-injection recording, the females showed higher frequency of parental

behaviors than the males (data not shown). In addition, retrieving increased only in the females immediately after drug administration. These sex-based differences suggest a role of sex hormones in regulating parental behavior.

Retrieving behavior also seems to be affected by stress hormone(s). In the post-injection recording, retrieving increased only in the females regardless of the administration of the drug or saline. In the present study, the cichlids were captured using a hand net and then injected the drug or saline. Although direct evidence could not be obtained from the present experiment, this procedure seemed to be highly stressful for the fishes. Such stressor might have affected the behaviors by the mediation of stress hormone(s). A previous study on prairie vole, which demonstrated positive correlation between corticosterone and frequency of retrieving (Bales et al., 2006), supports this suggestion.

On the other hand, vasotocin might mediate fin-digging and foraging. It should be noted that both the drug and saline were administered by i.p. injection in the present experiment. Thus, the peripheral antagonistic effects of vasotocin could not be ruled out. However, it is known that teleost fishes have vasotocin receptors in the brain (Moons et al., 1989). Moreover, central administration of vasotocin affects vocalization in plainfin midshipman fish (*Porichthys notatus*) (Goodson & Bass, 2000), courtship behavior in white perch (Salek et al., 2002), and social approach in goldfish (*Carassius auratus*) (Thompson & Walton, 2004). These findings suggest the central effects of vasotocin on parental behavior.

In summary, the present study demonstrated that one of the parental behaviors investigated in the study was mediated by vasotocin. In addition, it suggested that parental behavior may also be affected by sex and stress hormones. Further study is necessary to investigate such mechanisms and illustrate a system for adaptive regulation of parental behavior in convict cichlids.

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