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Testosterone Modulates Calling Behavior in Japanese Quail Chicks

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ABSTRACT—Japanese quail chicks have various patterns of calling. The chicks of both sexes emit *distress call* at a high rate when they are socially isolated. Chronical subcutaneous implantation of testosterone (T) induced the *chick crowing* which has similar in acoustical structural characteristics to male adult *crowing*. The chick crowing which was induced by T appeared in a mixed manner with the distress call at high rate when isolated. After T implantation, the frequencies of the chick crowing gradually increased and the distress call disappeared. The chick crowing was induced as the result of androgenic action of T, and the chick crowing disappeared after removal of T. Implantation of a small amount of T directly into mesencephalon, also induced chick crowing after a short latency. The same dose of subcutaneous T implantation failed to induce the chick crowing. This study suggested that T acts on quail chick brain to transform the distress call into the chick crowing.

INTRODUCTION

Japanese quails have a variety of calling patterns depending on sex and age. Chicks of both sexes emit the distress call with intensive frequency when socially isolated. Their distress call disappeared about 3 weeks after hatching. The male quails begin to emit the crow about 5 weeks after hatching. It was reported that the plasma testosterone (T) level was started to increase at 4 weeks and sexually matured at 8 weeks in male quails (Hirano *et al.*, 1978). Castration strongly suppressed crowing and T application restored it (Wada, 1982). T application induced the chick crowing which has acoustically characteristic patterns of the adult crow in domestic cockerels (Marler *et al.*, 1962). In these reports it was suggested that the crow has T dependency. However central nervous action of T nor the relationship between the distress call and the chick crowing was not reported. The chick of quail-donor chicken-host brain (mesencephalon-diencephalon) chimera emitted quail like chick crowing with T (Balaban *et al.*, 1988) suggesting that T acts on chick brain. In this study we confirmed the induction of the chick crowing in quail chicks, and then examined to clarify a hormonal dependency of a change from the distress call to the chick crowing by directly implanting T to the brain.

MATERIALS AND METHODS

General

Fertilized eggs were purchased from commercial sources and incubated until hatching in our laboratory. All chicks were caged in groups of 3~12 individuals and kept in an air-conditioned chamber (28~33°C) with a LD 14:10 photoperiod. Water and foods were freely available. Experiments were performed using chicks between 2~14 days after hatching. Only healthy chicks with active locomotion were used.

For examining calling behavior, each chick was placed in a small experimental cage. The experimental cage was illuminated from above, and settled in a sound attenuating box to avoid possible interactions with other chicks. If not otherwise stated, chick was initially adapted to the cage for a period of 3 min, then spontaneously emitted calls were examined during the following 5 min. The calls were monitored through a microphone in the sound attenuating box. Number of the distress call and the chick crowing was counted during the 5 min.

Silastic tubes (Dow Corning Co., 0.065 inches in I.D.) were filled with steroid hormones and cholesterol as control (T: Sigma, 5 α-DHT, 5 β-DHT: Teikokuzoki Co., estradiol, cholesterol: Wako Chemical Industry Co.). Each steroid hormone and metabolite was melted with heat and filled into the tube under negative pressure. Then the tube was cut into pieces of 1 cm long, and both ends sealed with Silastic glue. Chicks were implanted the tube containing each steroid and cholesterol, respectively at 2~4 days after hatching. For chronic implantation of the tube, a small incision was made in the right side, and then the tube was subcutaneously inserted. The incision was then sealed with acrylic glue. The tube was incubated in saline before implantation. Before and 1, 2, 3, 5 and 7 days after the implantation the distress call and chick crowing were recorded.

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Microimplantation of T into brain

Paraffin pellet was made for implantation of a small amount of T. The pellets were made according to the methods of Hayashi (1974). T and melted paraffin (melting point 50~52°C) were mixed with carbon black under heat, cooled, and punched out into a small pellet (diameter = 0.5~0.8 mm, length = 0.6~1.0 mm) by using a stainless pipe (21 gage).

Surgery was performed under an anesthesia by injection of a mixture of 25 μl/10g body weight xylazine (1 mg/ml saline: Sigma) and ketamine (Ketalar10: Sankyo Ltd.). Chicks were placed sterotaxically, and the operation was performed according to the quail atlas (Bayle *et al*., 1974) after a slight modifications. Head skin and scull were cut and the brain was exposed. Then the pipe containing a T-pellet inside was inserted vertically from dorsal approach into chick brain, and the pellet inside was extruded into the brain tissue by a wire through the pipe. Then the pipe was withdrawn and the exposed brain was covered with wet paper and head skin, and glued with acrylic glue. The implantation was bilateral or unilateral to the brain.

Histology

Before and 1, 2, 3, 5 and 7 days after the operation calling behavior was examined. After examining the calling behavior, chicks were transcardially perfused with heparinized phosphate buffered saline and then with a fixative (1% paraformaldehyde, 1.25% glutaraldehyde in 0.05 M phosphate buffer). Brain was placed in the same fixative for 12 hr ~ 2 days and then remove from head scull and placed in a 15% sucrose solution in the same fixative at 4°C until sink, and embedded in 15% gelatin. Fifty μm thick frontal sections were made, and the sites of pellets were simply drawn to avoid losing sight of pellets. Then the sections were stained with cresyl violet, and the sites of implantation were morphologically identified.

RESULTS

General

Chronic subcutaneous implantation of Silastic tube containing T induced the chick crowing (Fig. 1 Ab) which had acoustical characteristic structures of crow of male adults. Sonagraphic analysis indicated the chick crowing consists of notes (*) and subsequent trill (**). These acoustical structures were similar to those of crow (Fig. 1 B) in a male adult. In addition the chick crowing as well as distress call has harmonic structures.

The chick crowing appeared in a mixed manner with the distress call. The typical one is indicated in Fig. 2. Just before T implantation, the chick emitted the distress call repetitively when socially isolated (pre). One day after the implantation, the chick crowing appeared at the end of the distress call series (day 1). Two days after, the chick crowing was repeated some

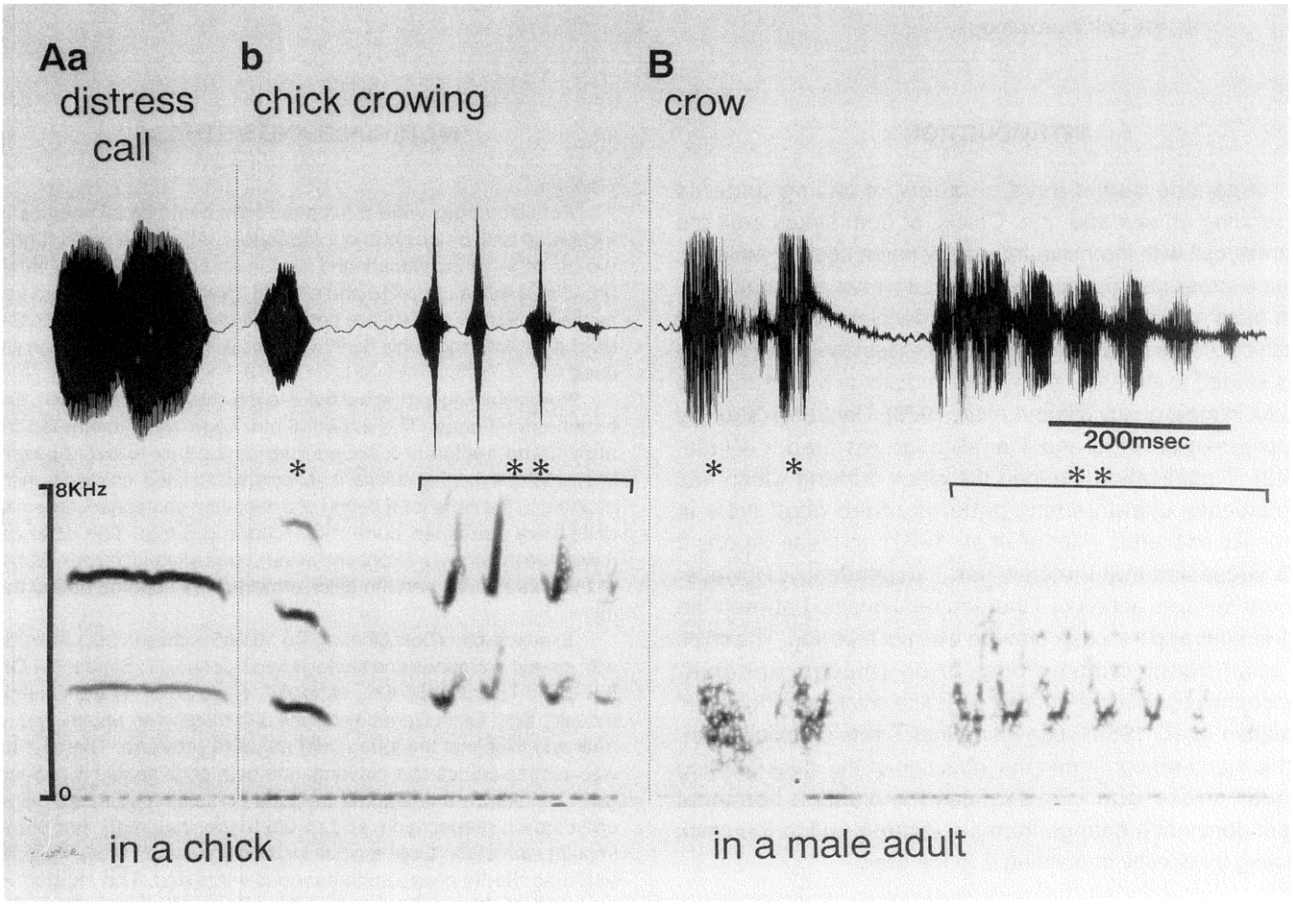

Fig. 1. The sound spectrographs of two kinds of call in chicks and crow of male adults. Chronic and subcutaneous implantation of Silastic tube containing testosterone produced chick crowing, that has characteristic patterns of distress call as well as crowing. Sound spectrograph of distress call (**Aa**) and chick crowing (**Ab**) in a chick and crow (**B**) in a male adult. Chick crowing show acoustically similar harmonic structures to the distress call. Chick crowing resembles crow in the initial short note type (*), and the frequency / amplitude modulation during the late trill (**).

times at the end of the distress call series (day 2). Three days after, series of the chick crowing appeared between the distress call series (day 3). The number of the chick crowing increased gradually after the implantation coincide with decreasing that of the distress call. Then in the following experiments, frequencies of the chick crowing were described as the ratio (%) of the number of the chick crowing shared in total number of calls (distress call + chick crowing).

The chick crowing appeared under the same condition with distress call. Distress call was strongly suppressed by pairing with another chick. It was examined that the chick crowing was affected by another chick. A chick was adapted to the experimental cage for 3 min. The following 10 min the spontaneously emitted vocalization was counted. Next, another chick was introduced to the cage and left for 3 min, and the following 10 min the vocalization was counted. Then the added chick was removed and examined the same way. Before T implantation, chicks emitted the distress call intensively when they were isolated (Fig. 3, pre implantation single1, 2). While when the chicks were paired with another chick, the number of the distress call was very low (paired). Before the implantation the chick crowing was not observed (pre implantation). Five days after the implantation (post day 5), both the distress call and the chick crowing were intensively emitted in isolated condition (single1, 2), and suppressed with another chick (paired).

Androgenic action

T is metabolized into active androgen 5 α-DHT or estradiol (Balthazart and Schumacher, 1983). Which of 5 α-DHT or estradiol is effective to produce the chick crowing was compared. A piece of Silastic tube containing each of T or its metabolite, unaromatizable androgen 5 α-DHT, estradiol (E2), inactive androgen 5 β-DHT, cholesterol (chol) as control was subcutaneously implanted to the chicks (Fig. 4). Unaromatizable androgen 5 α-DHT as well as T induced the chick crowing, while estradiol, 5 β-DHT as well as cholesterol did not induce the chick crowing. There was no significant difference between the ratio of the chick crowing of T implanted chicks and 5 α-DHT implanted chicks.

Reversible action

Steroid hormones have 2 patterns of action, organizational action and activational action (Arnold and Breedlove, 1985). They reported that organizational action is irreversible, and activational action is reversible. We examined that inducement of the chick crowing with T implantation is reversible or irreversible action (Fig. 5). Chicks were implanted with the T

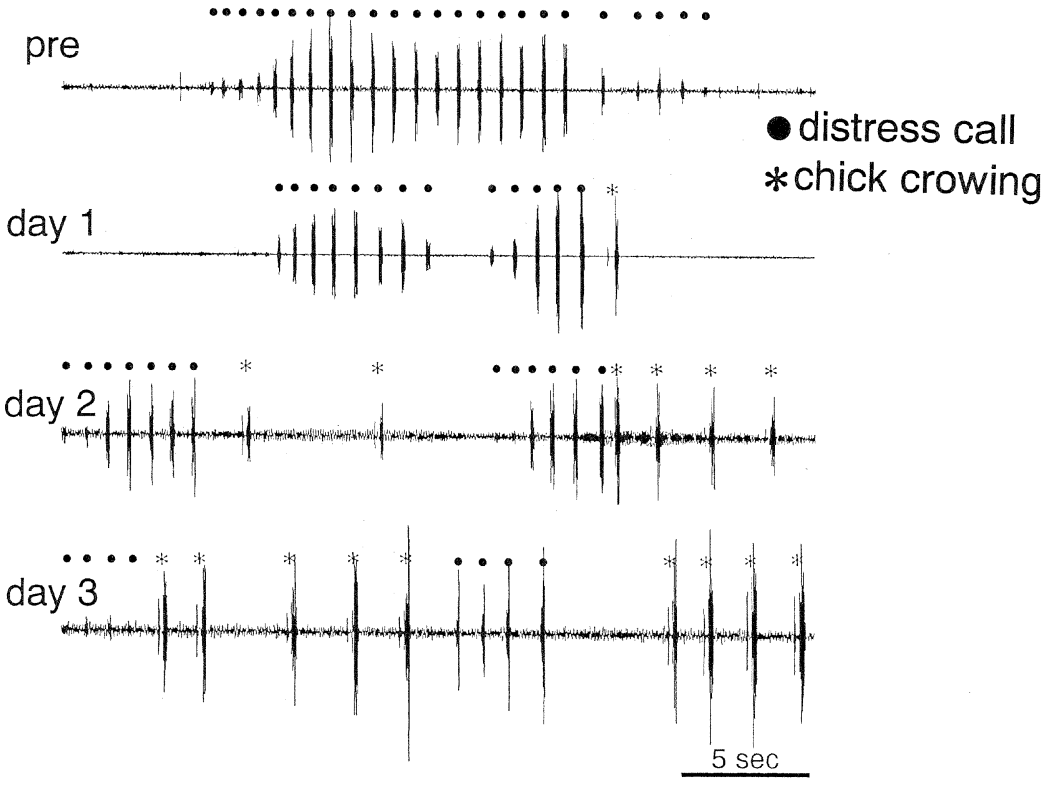

Fig. 2. Distress call and chick crowing appear in a mixed manner. Typical examples of vocalization emitted by an isolated chick before (pre) and after (day 1, day 2, day 3) the chronic implantation of testosterone. Filled circles and asterisks denote distress call and chick crowing, respectively. When socially isolated, the chick emitted distress call repetitively (pre), just before the implantation. One day after the testosterone implantation, chick crowing was emitted at the end of distress call series (day 1). The number of chick crowing gradually increased the following day (day 2, day 3), while that of distress call decreased.

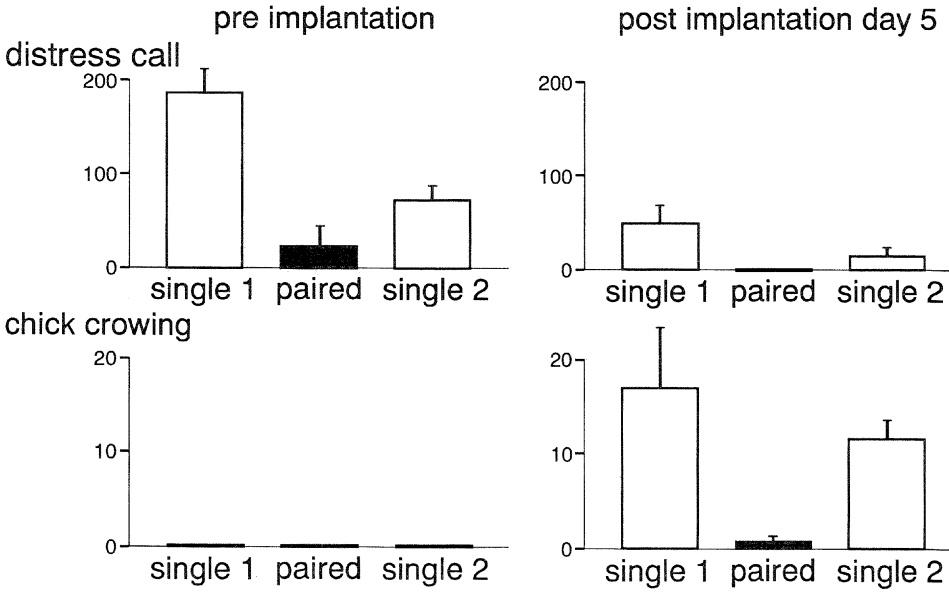

Fig. 3. The distress call and chick crowing intensively induced by social isolation, and suppressed by pairing with other chicks. Before implantation (pre implantation), number of distress call under pairing condition (paired) is much lower than that in isolated (single 1, single 2). Chick crowing was not emitted at that time. Five days after the implantation (post implantation day 5), both distress call and chick crowing were emitted under isolated conditions much higher than that under pairing conditions. The result was expressed as the mean for 7 chicks.

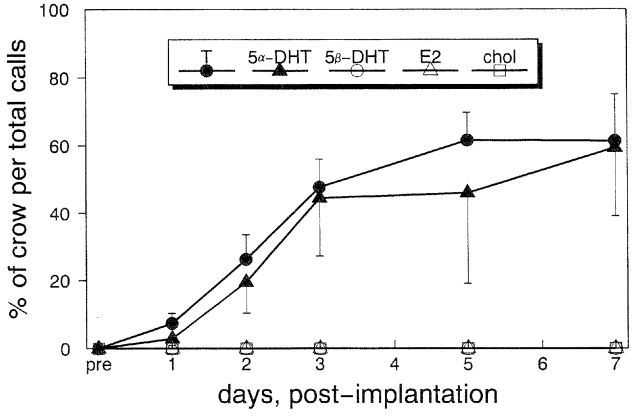

Fig. 4. Unaromatizable androgens, but not estradiol, induce the chick crowing. Effects of testosterone and other steroid derivatives were compared with the ratio (%) of the produced chick crowing divided by the number of total calls (distress call + chick crowing). Symbols and vertical vars denote means and s.e.m., respectively. Both of unaromatizable and aromatizable androgen (testosterone (T) and 5 α-DHT, respectively) were similarly effective. On the other hand, inactive testosterone metabolite, 5 β-DHT, estradiol (E2), and cholesterol (chol) had no effects. T (n=17), 5 α-DHT (n=6), 5 β-DHT (n=5), E2 (n=6), chol (n=6).

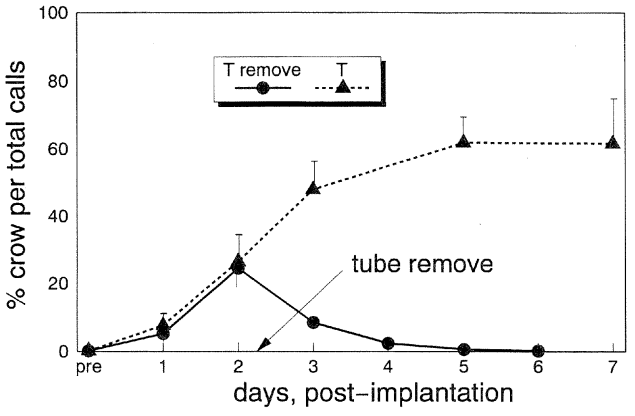

Fig. 5. The reversible effect of testosterone. The testosterone containing in Silastic tube was subcutaneously implanted on the day 0 (pre), and then removed 2 days after the testosterone implantation after examining vocalizations. Three day after the implantation and afterwards, the ratio of chick crowing gradually decreased, and finally chick crowing disappeared on day 6. Dashed line (T) is identical to the data of T in Fig. 3. Similar format as in Fig. 3. T (n=18), T remove (n=8).

tube subcutaneously, and the chick crowing was produced. Two days after the implantation, the tube was removed after examining vocalizations. From 1 day after the removal of T tube, the ratio of the chick crowing began to decrease, and finally disappeared. In another experiment five days after the subcutaneously implantation of T when chicks produced the chick crowing only, the T tube was removed. From 1 day after the removal of T tube the distress call reappeared and the chick crowing decreased, and finally the chick crowing disappeared.

Dose dependency

T, diluted with cholesterol 4 times (1/4 T) or 8 times (1/8 T), also produced the chick crowing (Fig. 6). But the ratio of the chick crowing produced by 1/4 T and 1/8 T were lower than that by full T. Particularly 4 out of chicks of 10 1/8 T

implanted chicks did not emit the chick crowing at all. The other 6 chicks emitted the chick crowing only a few times at 1~3 days after the implantation. So the inducement of the chick crowing had dose dependency.

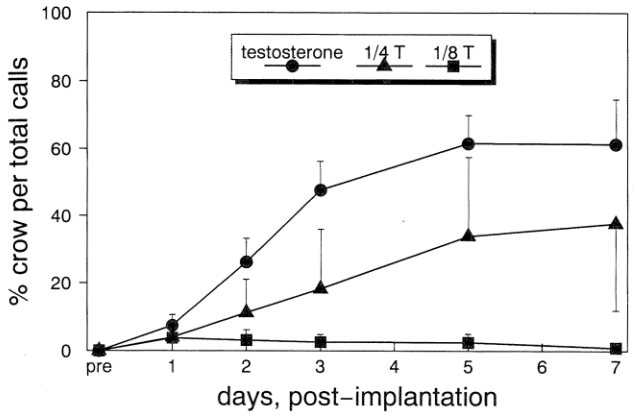

Fig. 6. Testosterone activity with dose dependency. Chicks were implanted testosterone or testosterone diluted with cholesterol 4 times (1/4 T) or 8 times (1/8 T) containing in Silastic tube. The ratio of chick crowing in chicks implanted diluted testosterone (1/4 T, 1/8 T), is lower than that in testosterone implanted chicks. Furthermore 1/8 testosterone implanted chicks emitted chick crowing very low frequency. Eight times diluted testosterone (1/8 T) is the limit dose to induce chick crowing.

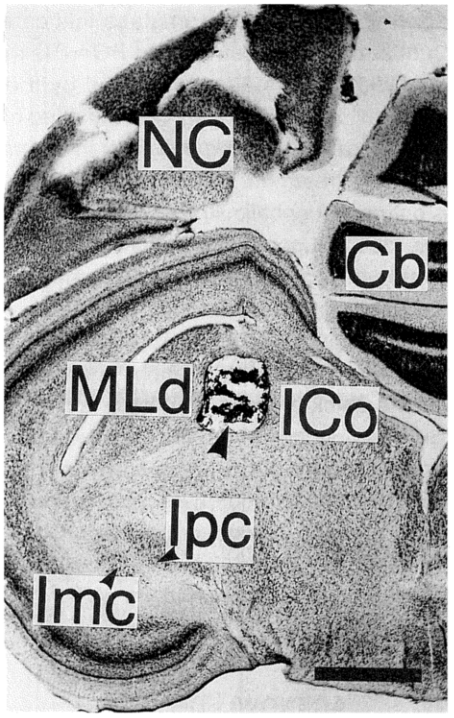

Fig. 7. The photograph of the section of brain after the testosterone micro-implantation. Paraffin pellet containing in testosterone is implanted directly into mesencephalon. The brain sections were stained with cresyl violet. Arrowhead shows implanted pellet. Scale bar : 1 mm.

Microimplantation into brain

T could act on the central nervous system to produce the chick crowing. It was also reported that androgen receptors localize in the lateral and medial area of intercollicular nucleus (ICo) (Balthazart *et al.*, 1992). ICo was also reported of call eliciting area by electrical stimulation in quail and chicken (Potash, 1970; Peek and Phillips, 1971). We hypothesized that T act on the area of ICo to produce the chick crowing. Paraffin pellets containing a small amount of T were directly implanted into mesencephalon aiming at near the area of ICo. The chick which implanted the pellets in mesencephalon emitted the chick crowing (Fig. 7). Implantation of the high dose of T (containing about 40 μg T in each pellet) induced the chick crowing at high frequency and with short latency (Fig. 8A). One chick implanted high dose of T emitted the

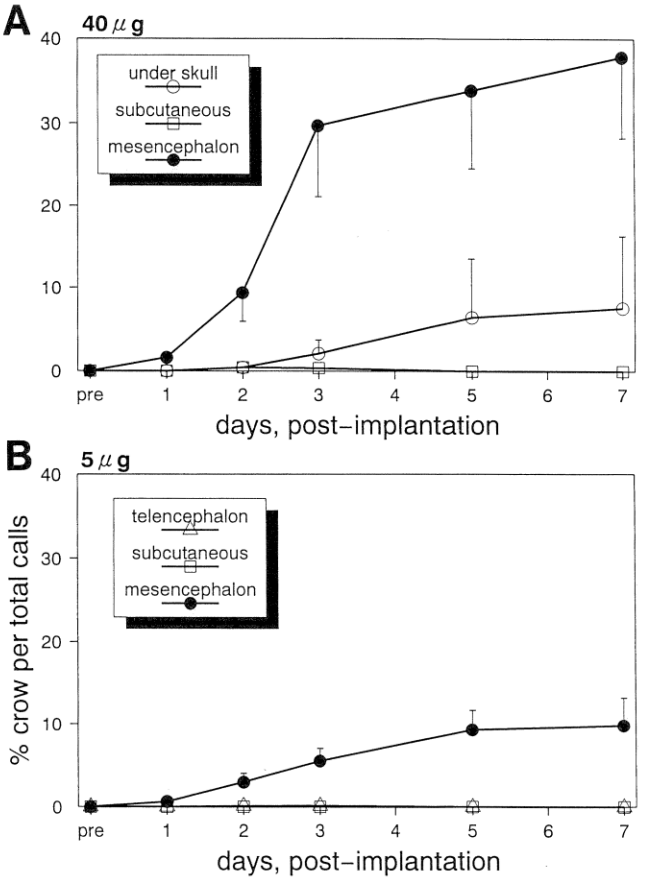

Fig. 8. Testosterone acts on mesencephalic structures to modify the call pattern. Chicks were implanted paraffin pellets containing a small amount of testosterone (**A** : 40 μg, **B** : 5 μg) directly into mesencephalon at 5~7 days after hatching. In other chicks as controls, the same pellets were implanted subcutaneously or under skull (40 μg) or into telencephalon (5 μg). Mesencephalic implantation showed the results of the highest ratio of chick crowing with the shortest latency. Particularly with a lower dose of 5 μg (**B**), only the mesencephalic implantation was effective. The dose-dependency is also evident (● in **A** vs **B**). Similar format as in Fig. 3. 40 μg: mesencephalon (n=17), subcutaneous (n=5), under skull (n=4), 5 μg: mesencephalon (n=17), subcutaneous (n=5), telencephalon (n=5).

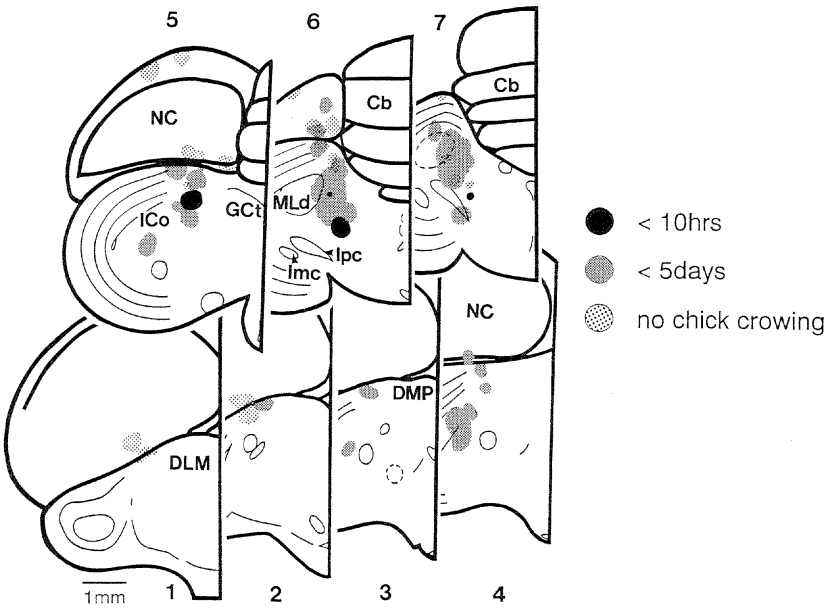

Fig. 9. Distribution of the testosterone pellets in the mesencephalon and telencephalon. The pellets containing 5 μg testosterone were implanted into brain of 17 chicks. The pellets were implanted bilateral or ipsilateral. All pellets were described into one side. The positions of the pellets were described each section where implanted. Filled area is for the chicks that began to emit chick crowing within 10 hr after operation. Striped area the same within 5 days after operation. Dotted area indicated that of chicks that did not emit chick crowing. Each section is 500 μm apart from the next.

chick crowing within 1 hr after the implantation. At low dose of T (5 μg, Fig. 8B), mesencephalic implantation induced the chick crowing, while subcutaneous implantation of the same size of pellets did not produce the chick crowing and telencephalic implantation hardly produced the chick crowing. It was evident that the chick crowing was produced with dose dependency of T. The latency from implant of T to emission of the chick crowing, was varied between each chick of both doses. We examined the latency correspond to the implanted sites of the pellets. Pellets were implanted near the action sites of T, the latency could be short. But there was no correlation between implanted site and the latency (Fig. 9). Each chick implant of the pellet at the same site, emitted chick crowing with different latency. We supposed that T leaks out of the pellets and diffuses all directions. Thus accurate sites of T action are uncertain. But the medial areas of ICo in posterior mesencephalon (Fig. 9, 5~7) were inclined to be effective to produce the chick crowing.

DISCUSSION

In this study we confirmed that quail chick produced the chick crowing by T implantation. Acoustical characteristic patterns of the chick crowing resemble the crow of male adults as well as the distress call. The chick crowing appeared under the same condition as the distress call after T implantation. From these results we hypothesized that the chick crowing is produced in the vocal system of the distress call in the brain by androgenic action of T.

The distress call changed to the chick crowing as the result of the androgenic action of T. This suggests the chick crowing has nothing to do with copulatory behavior and aggressive behavior, because of the report that copulatory behavior requires enzymatic activity of aromatase that catalyzes the conversion of androgen to estrogen in POA (Balthazart and Surlemont, 1990). It was also supported by the fact that aggressive behavior was not observed in pairing experiment (Fig. 3). But failure of restoring the crow in castrated adult males by 5 α-DHT treatment was reported (Wada, 1982).

T acts on mesencephalic structures to produce the chick crowing. This idea is supported by the fact that mesencephalic implantation of a small amount of T produces the chick crowing, while subcutaneous implantation of the same size of pellets did not. In this manner the chick crowing was induced within a short latency, sometimes within 1 hr. It was suggested that induction of the chick crowing may be direct effect of T for neurons. Some evidences of the effects of steroid hormones for neurons were reported (Erylkar and Wetzel, 1987). In this paper effect of steroids for neurons is rapid action, sometimes in a few ms. However it was not proved that ICo is the target of T to produce the chick crowing. The accurate action sites of T in the chick brain and mechanism of T action at a cell level are not clarified yet.

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