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# Stimulation Elicits the Chick Crowing with Testosterone in Japanese Quail Chicks

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**ABSTRACT**—Japanese quail chicks produce the *distress calls* at a high rate when socially isolated from other individuals. Under the same conditions, chicks which were given chronical subcutaneous implantation of testosterone (T) produced the *chick crowing* which has similar acoustical structural characteristics to both the chick distress call and the adult crow. Electrical stimulation of dorso-medial areas of chick mesencephalon through a chronically implanted electrode elicited calls in freely moving chicks. The acoustical structure of the elicited call resembled that of the distress call with the harmonic structures and constant frequency before T implantation. At 6 days after T implantation, the chicks produced the chick crowing similar to the adult crow with a trill structure by electrical stimulation. The area in brainstem of chicks that exhibit two different kinds of vocal behavior upon electrical stimulation lies in the medial intercollicular nucleus (ICo). This suggests that the medial ICo of chicks has two different function, production of the distress call and the chick crowing with T implantation. We concluded that continual exposure of the neural substrate in the medial ICo of chick to T induced the functional change from emitting the distress call to producing the chick crowing.

## INTRODUCTION

Japanese quails have a variety of calling patterns depending on sex and age. A chick emits distress calls at high rates when isolated from other individuals. In male and female chicks of domestic fowls treatment with testosterone (T) produced the chick crowing which shows similar acoustical characteristics to adult crow (Marler *et al.*, 1962). The chicks of quail donor - chicken host brain (mesencephalon-diencephalon) chimeras emitted quail-like crowing patterns with T implantation (Balaban *et al.*, 1988). It was reported that electrical stimulation of the mesencephalon elicited some calling patterns (Potash, 1970; Peek and Phillips, 1971). But neither specific sites nor vocal control system in the mesencephalon-diencephalon is understood with respect to calls. Recently we have reported that bilateral lesioning of dorso-medial areas of mesencephalon results in eliminating both the distress call and the chick crowing (Yazaki *et al.*, 1994). In this study we have investigated the site of vocal control system and the function of the vocal system in quail chick brain. We have found that the medial intercollicular nucleus (ICo) of the mesencephalon can produce the distress call, and has the ability to produce the chick crowing with T.

## MATERIALS AND METHODS

Fertilized eggs of Japanese quail were purchased from commercial sources and hatched in an incubator in our laboratory. The chicks were then kept in groups (3–12 chicks) in an air-conditioned chamber (28–33°C) with a LD 14:10 photoperiod. Water and foods were freely available. Only healthy chicks with active locomotion of the age of 0–14 days were used.

Chicks were divided into two experimental groups. In group 1 at 2–7 days post hatch, the chicks (n=12) were anesthetized with the injection of a mixture of xylazine and ketamine and fixed to the stereotaxic apparatus. Head skin and skull were cut and then a stimulation electrode was inserted into the chick brain and fixed to the skull using agar, dental cement and acrylic glue. The implantation of the electrode was performed based on the quail brain atlas (Bayle *et al.*, 1974) after a slight modification. The electrode was made from an insect pin (no. 00) by electrolytically sharpening and insulating with lacquer (bared tip size ranged between 10–30 µm). After the operation chicks recovered from anesthesia, walked around and emitted the distress call normally with the implanted electrode. Electrical stimuli were administered on the day of implantation or the next day.

In group 2 (n=17) the brain of T implanted chick was stimulated using the method of electrical stimulation. In this group a Silastic tube (1 cm) containing T was implanted subcutaneously at 0–3 days post hatch. T was melted with heat and introduced into the tube under negative pressure. Then the tube was cut into a piece of 1 cm. A small incision was made subcutaneous on the right side of a chick, and the tube was inserted, and the incision was sealed with acrylic glue. Before implantation the tube was incubated in saline. Stimulation electrode was implanted in the brain with the same operation as the chicks of group 1 during 0–4 days after implantation of T. The T treated

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chick was subjected to electrical stimulation between 0–6 days after the implantation of the electrode.

A chick with the electrode was placed in a cage furnished with an aluminum board covered with wet paper on the floor. The electrical stimuli were applied by the stimulator with isolator. The aluminum board on the bottom was used as the reference electrode. A microphone was placed in the cage and vocalizations of the chick and the stimuli were monitored on an oscilloscope and recorded with the computer system Sound Master. The electrical stimuli were consisted of 20–100 pulses at 100–250 Hz. The optimal current of the applied stimuli was ranged between 30–300  $\mu$ A.

After the experiment a positive current (5–10  $\mu$ A for 5–10 sec) was passed into the stimulation sites through the electrode for identification of stimulation sites using the Prussian blue reaction. Then the chicks were transcardially perfused with heparinized phosphate buffered saline and then with a fixative (1% paraformaldehyde 1.25% glutaraldehyde in 0.05M phosphate buffer). The brain was dissected out of the skull and placed in a 15% sucrose solution in the same fixative, and embedded in a 15% gelatin. Fifty  $\mu$ m thick frontal sections were cut and immersed in 1% hydrochloric acid containing 2% potassium ferrocyanide for the Prussian blue reaction. The marking spot was plotted using a video micrometer. The timing of the initial vocalizations and their correspondence to the intensity and frequency of the stimulus were analyzed with the Sound Master program. The recorded vocalizations were also analyzed with a Kay DSP Sona-Graph.

## RESULTS

The electrical stimulation of the dorso-medial area of the mesencephalon produced vocal behavior in freely moving chicks. In 8 out of 12 chicks electrical stimulation elicited the calling behavior. The rest of 4 chicks showed no response. These elicited vocalizations were acoustically similar to the normal distress call with harmonic and constant frequency (Fig. 1). The quality of the vocalization was slightly modified with different sets of stimulation with different frequencies and number of pulses of electrical stimuli (Fig. 1 Ba–c). The structures of calls which were evoked by electrical stimulation showed some variations in each chick. But the structures generally had similar harmonic and constant frequency as the distress call. The latency from the first pulse of the stimuli to the call evoked was varied among chicks (50–80 ms), but was the same in the same chick. Some chicks that produced vocal behavior also exhibited behaviors such as wing spreading, inclined head and running. In these case the threshold for calling behavior was higher than that of other behavior and the data was neglected. Thus we chose an optimal current for stimulation of the chick calling behavior,

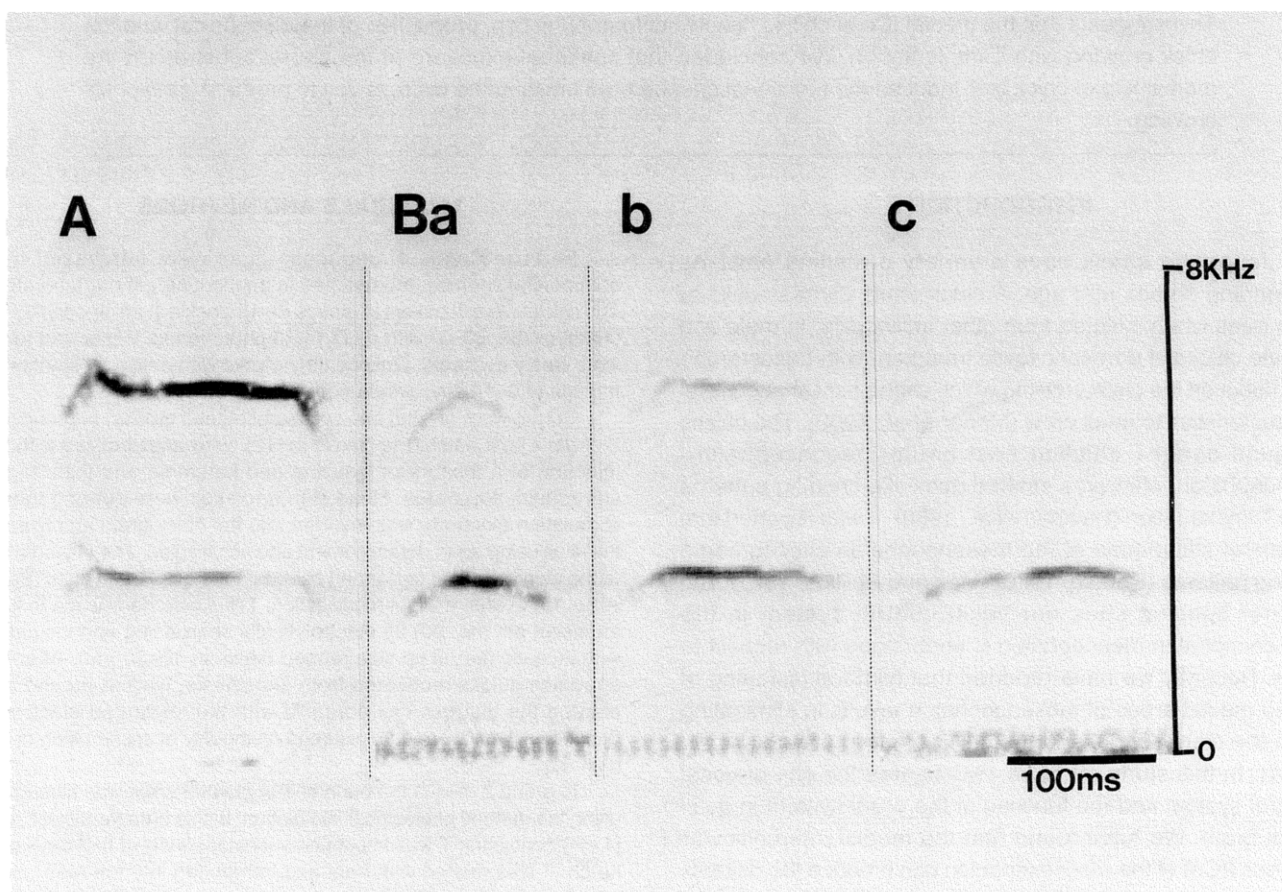


Fig. 1. Features of call in Japanese quail chick. The calling behavior in freely moving chicks was evoked by electrical stimulation of dorso-medial areas of mesencephalon. Each sonographs shows the call of the same chick: (A) normal distress call; (B) examples of the stereotyped call using electrical stimulation. The produced calls were modified depending on frequency and trains: Ba, 200 Hz/sec, 20 pulses; Bb, 200 Hz/sec, 50 pulses; Bc, 100 Hz/sec, 30 pulses. These are consists of harmonic structures and constant frequency as well as spontaneous distress call (A).

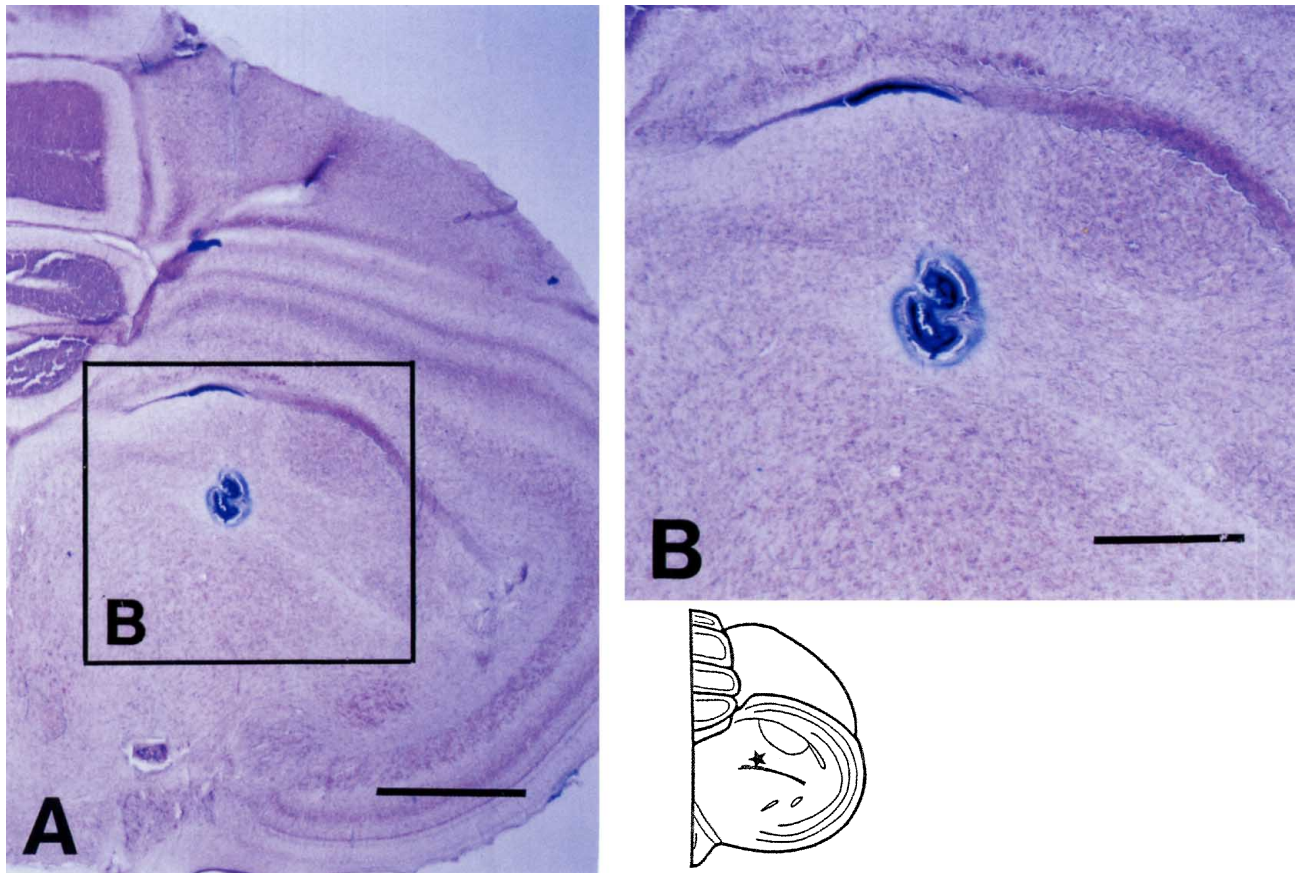


Fig. 2. The blue spot was localized in medial areas of ICo. Fifty  $\mu\text{m}$  frontal sections were cut and stained with Prussian blue reaction. Then the stimulation site was visualized as blue spot, and counter stained with neutral red. Scale bars **A**:1 mm, **B**:500  $\mu\text{m}$

determining the threshold of each behavior. After the calling behavior was evoked by the electrical stimuli, the stimulation site was stained with Prussian blue. The stimulation site as shown in Fig. 2 was histologically verified as a spot in the medial region of ICo. In 8 chicks of group 1 which produced calling behavior using electrical stimulation, and 4 chicks which showed no response stimulation sites were plotted as  $\circ$  and  $\blacktriangle$  in Fig. 3, respectively. The stimulation sites which induced calling behavior were located in the medial ICo as shown in Fig. 3.

The electrical stimulation of the dorso-medial area of the mesencephalon after the treatment with T produced the call with trill elements. The chicks which were implanted with T produced both the chick crowing and the distress call. The rate of the chick crowing gradually increased and that of the distress call decreased. A few days after the T implantation the distress call disappeared in some chicks. In group 2, 11 out of 17 chicks the electrical stimulation produced the calling behavior. In 4 of 11 chicks, at 0~4 days after the T implantation the call with constant frequency, and at 2~7 days after the T implantation the call with trill were produced by the electrical stimulation. The time course until the call with trill was produced after the T implantation by electrical stimulation, were varied in 4 chicks respectively (2~6 days). On the same day the call with trill was elicited depending on pulses of stimuli. For

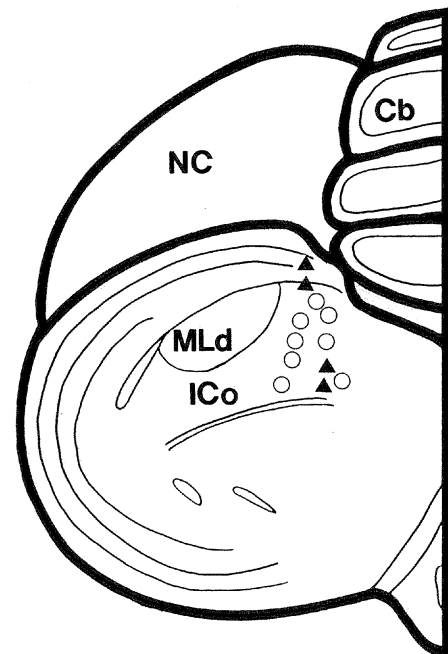


Fig. 3. Distribution of electrical stimulation sites for recorded calling behavior. Histologically verified stimulation sites are plotted as opened circles. Closed triangles showed the stimulation sites with no response.



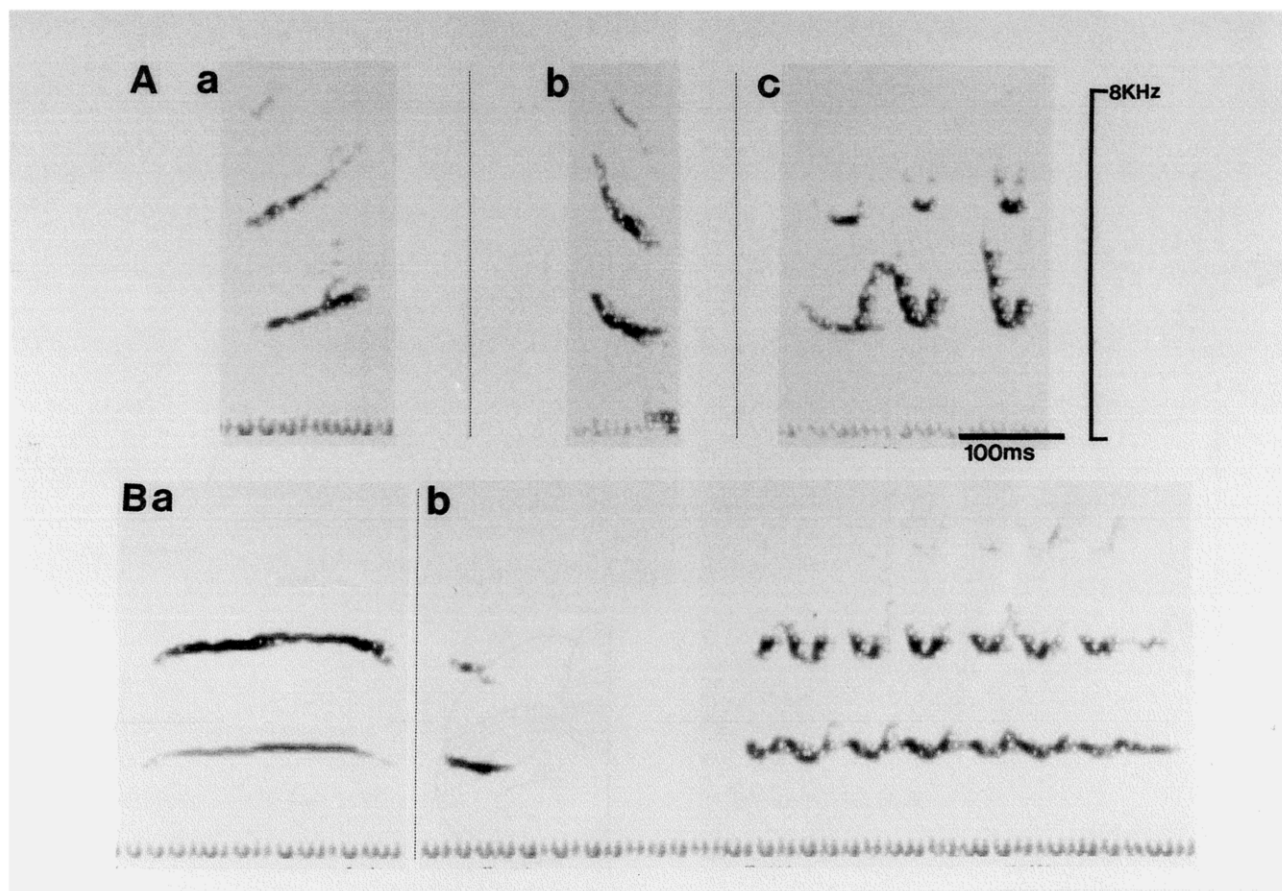


Fig. 4. Examples of the stereotyped calls with trill evoked by means of electrical stimulation after exposure to T. At 4 days after T implantation, the simple call was produced upon the electrical stimulation in mesencephalon (**Aa**). The simple call shows the acoustic features of harmonic structures and constant frequency together with the spontaneously emitted distress call (**Ba**). At 6 days after T implantation, the chick's distress call were almost completely replaced by chick crowing. Electrical stimulation of 200 Hz, 20 pulses produced the simple call (**Ab**). On the same day, electrical stimulation of 200 Hz, 50 pulses produced the call with trill (**Ac**), which resembles chick crowing (**Bb**).

instance (Fig. 4) in one chick at 4 days after T implantation, electrical stimulation produced a call with harmonic and constant frequency (Fig. 4 Aa), while at 6 days after T implantation, electrical stimulation of the same frequency and duration produced a trilled call (Fig. 4 Ac). After T implantation the chick produced the call by electrical stimulation, which has similar harmonic and trill structures to the chick crowing produced without electrical stimulation. The structure of the vocalization varied from short phone (Fig. 4 Ab) to trill structure (Fig. 4 Ac), corresponding to numbers of pulses of stimulus. The other 7 of 11 chicks after the T implantation produced the call with harmonic and constant frequency by electrical stimulation after their spontaneous distress call was totally replaced by the chick crowing. The latency from the first pulse to beginning of call was almost same in the chick, but varied between chicks and days (50~130 ms).

The stimulus sites in the brain of the 11 T implanted chicks which produced the calling behaviors when electrically stimulated were localized in the medial ICo (Fig. 5, ○ ●). But stimulation of other areas outside the ICo did not produce calling behaviors (Fig. 5, ▲). The stimulus sites which

produced calling behaviors were found to occur from rostral to caudal area in the medial ICo. We examined whether or not the response of calling behavior is produced using a concentric electrode (David-Koph) on the medial ICo area, and the results confirmed this localization (data was not shown).

## DISCUSSION

Electrical stimulation of the medial ICo in Japanese quail chick produced calling behaviors resembling the distress call. This suggests that neural activation of this area is sufficient for producing the distress call. The neural substrates for the calling behavior have been demonstrated in medial ICo sites as shown in Fig. 2 by electrical stimulation. But the electrical stimulation not only produced the calling behavior, but also modulated call structures. If neural activation of these sites produced only calling behavior, the call structures might be stereotyped. However some kinds of calling patterns were recorded by different frequencies of electrical stimulation. This suggests that neural system in the medial ICo produces a call

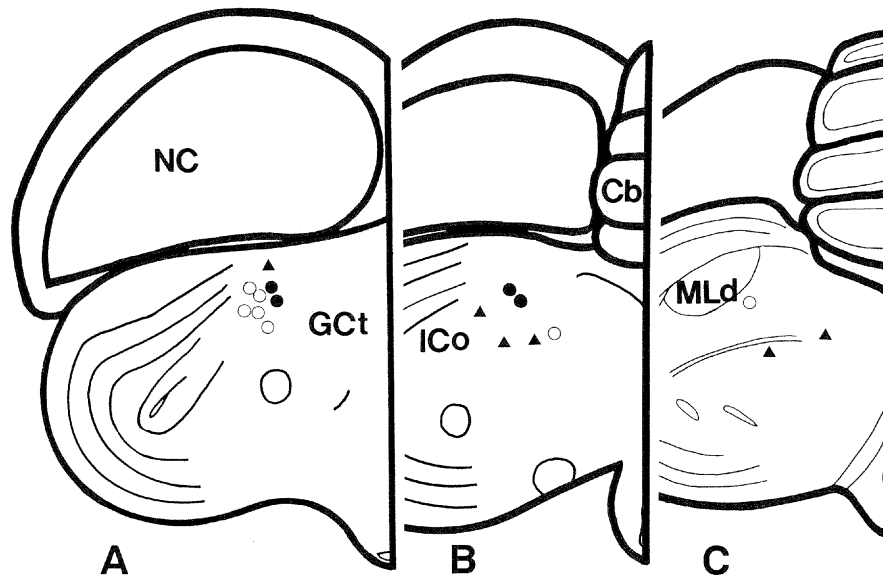


Fig. 5. Distribution of electrical stimulation sites for recorded calls with trill. Histologically verified stimulation sites are plotted as open circles for distress call responses and closed circles for chick crowing responses. The closed triangles show stimulation sites which did not respond to the stimulation. Sections **A** and **B** are 300  $\mu\text{m}$  apart; **B** and **C**, 150  $\mu\text{m}$ .

pattern without command the system of the on/off switch mechanism.

The sites of medial ICo in a quail chick with T implantation produced the chick crowing similar to the adult crow. This suggests the neural substrate in the medial ICo which produce the distress call before T implantation have been modified by T. It has been reported that, in adult quails, the neural substrates in the ICo have androgen receptors (Balthazart *et al.*, 1992), and that steroid modulates the density of transmitter receptor in ICo in quail (Ball and Balthazart, 1990). From these things it was thought that the vocal neural system in medial ICo may be T sensitive. But not all the T implanted chicks produced the chick crowing by means of electrical stimulation. T may modulate the sensitive sites in vocal neural system to change the distress call to the chick crowing. A part of the vocal neural system in the medial ICo which produces the distress call could be modulated to produce the chick crowing by T. T implanted chicks have both the distress call and the chick crowing in a mixed manner about 2–3 days after T implantation. This showed that not all neural system of the distress call change to that of the chick crowing at the same time. Medial ICo can have the ability of producing two different calling patterns, the distress call and the chick crowing. Recently we suggest that T acts on mesencephalic structures to induce the chick crowing. But we have not indicate that T directly acts on the neural substrates in ICo. This study clarified that the neural substrates in the medial ICo relating to the distress call produces the chick crowing with T implantation.

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