Rhythmicity in the early swimming of anuran larvae

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INTRODUCTION

Previous observations of the development of somatic movements in amphibians concentrated their attention on 'reflex' mechanisms (for review, see Hooker, 1952). Extensive study has been made of the types of movement evoked by tactile stimulation at different stages in early development and on the anatomical basis for such responses. Emphasis was thus placed upon the formation of direct pathways through the central nervous system from receptor to effector. For the swimming movements, this approach was able to account for (a) the cranio-caudal wave of somite contractions, in response to sensory input from any part of the body surface, and (b) the existence of a phase difference between the waves on the two sides of the body. A quantitative characterization of behavior at successive stages has also been made, in terms of the distances travelled per response (cf. Detwiler, 1948; Sladećek, 1960). Early spontaneous movements have also been reported, but there is uncertainty as to whether these are myogenic or neurogenic. Little attention appears to have been paid to spontaneous activity after the time when characteristic swimming movements have become established.

Recent observations of neuromuscular activity in vitro in embryonic tissues of the two anurans, *Xenopus laevis* and *Rana pipiens* (Corner, 1964, Corner & Crain, 1964) called attention to spontaneous muscular activity which, moreover, revealed a pronounced rhythmicity. Bursts of twitching showed a tendency to fall at intervals of usually 4–5 sec. and multiples thereof, for periods of several minutes at a time. They were similar in all preparations and were often strikingly consistent in a given preparation with respect to the frequency, duration and intensity of the twitches, and even in certain irregularities in timing. Furthermore, they were apparently identical to bursts evoked by gentle direct manipulation of the nerve tissue or by stimulation of included epidermal tissue. Such a burst was, in fact, with very rare exceptions, the minimal response which could be evoked and was usually consistent and characteristic for the individual

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preparation. When attention was then turned to the early swimming stages of normal and operated larvae, these same phenomena were found.

**OBSERVATIONS AND EXPERIMENTS**

*Normal swimming*

Spontaneous swimming was first seen in *Rana* at stage 20–21 (Shumway), at which time many embryos still showed only occasional single or double ‘coil’ movements. The swimming bursts were seldom longer than $\frac{1}{4}$–1 sec. and occurred at intervals of seldom less than 10 sec. Each burst consisted of several rapid twitches, slightly irregular in their timing. Such a burst was also the reaction to stroking the skin or to poking it several times in quick succession. There were decided slow fluctuations in sensitivity to stimulation, the relatively unresponsive periods coinciding with periods of little or no spontaneous activity.

In *Xenopus*, the first stages were essentially the same as in *Rana* and were then followed by a stage in which little or no spontaneous movements occurred (all the larvae hanging motionless from a branch or from the air–water interface). There subsequently appeared in succession (a) occasional short bursts of swimming, (b) swimming for periods of $\frac{3}{4}$–1 min. (rapid ‘quivering’ of the somites, punctuated every few seconds by a short burst of strong twitches), alternating with a few minutes of inactivity, and (c) continual swimming, as described for the previous stage. Touching the skin or giving it an electric shock always provoked a burst of movement, beginning with one or more large contractions and followed by weak rapid twitches. The duration varied with the stage and the sensitivity of the animal and often also with the stimulus strength. An inhibitory reflex was found, elicited only from the anterior part of the head, whereby a single touch immediately stopped the contractions. This reaction could occur even immediately after contractions had started. Gill depression always accompanied somite contractions, and also often occurred independently.

*Operations on Rana*

In some embryos sections were made through different levels of the cord shortly before the earliest swimming stage. In addition, the donors for the *in vitro* experiments (Corner & Crain, 1964) were studied; these consisted of a spinal cord, lacking the most anterior segments, separated from the brain. The first group showed clearly that the burst pattern was being generated independently in the hind-brain, the cervical spinal cord and the thoracic cord. The bursts were not necessarily identical in duration at the different levels of a given preparation, but all were within the limits of $\frac{1}{3}$–3 sec. The frequency with which they occurred varied widely from one individual to the next, but in all cases there was a progressive decrease in frequency at successively more posterior levels. Thus, while hind-brain discharges could occur as often as every 5–10 sec., contractions
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did not occur at the thoracic level of the same animal at intervals shorter than \( \frac{1}{2} \) min. Even the frequent discharges, however, occurred only during periods of a few minutes at a time, separated by one or more intervals of half to several minutes during which at most an occasional twitch would be observed. Reflex bursts could be elicited separately in the three levels.

The second group, i.e. that with the upper cord segments excised during neurulation, also showed independent spontaneous activity in both hind-brain and spinal cord. In addition, there were a few cases where a relatively long piece of neural tissue was removed, leaving only 'tail-level' cord. The trunk and tail musculature of these preparations was never seen to twitch spontaneously, further suggesting the existence of an antero-posterior gradient of endogenous 'central excitation'. The typical burst of activity, both in intact larvae and in those with isolated cords, was two or three strong twitches (causing large tail movements) followed by a burst of very rapid, weak contractions of all the axial somites. These two components proved to be dissociable, for in some of the operated animals either the strong twitches or the burst would occasionally occur by itself. Also of interest is the fact that at some times in some preparations the bursts of twitching involved only the most anterior muscle fibers.

If any spinal cord was left attached to the brain, stimulation of the skin anterior to the gap evoked bursts of twitching only in the somites at the level of the head (together with gill depression) while stimulation posterior to the gap evoked only trunk contractions. This was also the situation in a number of preparations where the hind-brain could be seen not to include any length of cord. Other animals without any cord connected to the brain, on the other hand, showed no muscle activity at all anterior to the gap, while stimulation of any part of the head evoked typical trunk and tail movements. Finally, some cases were found where head stimulation evoked gill depression but never somite contractions, or only rarely so. These results probably indicate that the functional primitive sensory system extends from the spinal cord for only a short distance into the hind-brain. They furthermore confirm the impression, gained from stimulating intact larvae and from observing spontaneous activity, that the threshold for reflex activation of the gill muscles is considerably lower than that for the somites.

Operations on Xenopus

In these experiments attention was directed to the rôle of different levels of the brain stem, and the excisions were controlled histologically. The operated larvae were tested during the stage of no spontaneous movements but the picture of the reflex responses is quite similar to that for Rana. In no case where the mid-brain and/or the forebrain was isolated from the rest of the nervous system did the pattern of swimming response differ from the normal. That is, a light touch to any part of the body evoked two or three strong somite contractions, followed by a few seconds of very rapid, weak twitching which then stopped
abruptly. This was also true in half of the twenty cases where a part of the hind-brain was isolated from the rest, including a few cases where only a small length of hind-brain was left attached to the spinal cord. There were also many cases where the hind-brain was continuous but morphologically severely distorted in a variety of ways (see Corner, 1963), in none of which was the reaction pattern abnormal. The seven preparations which were found to have abnormal swimming responses all had considerably less than half of the hind-brain intact, suggesting that in most cases where only a small amount of hind-brain is available for control of somite contractions, abnormalities will occur. Functional regulation can, however, sometimes occur (N.B. these operations were performed early in the ‘neurulation’ process). The nature of these abnormal swimming responses was of two kinds: four cases responded with the initial strong contractions only, while the other three continued to swim steadily for as long as 30 sec. Six cases of completely isolated spinal cord were also prepared, and in all of them continual spontaneous ‘quivering’ developed in the somites, causing a very slow, steady forward progression. Stimulation of the skin caused a brief burst of stronger contractions. There were two other larvae which were spontaneously active at this stage. Both of them had a gap through the brain which left the greater part of the hind-brain attached to the cord. In these animals, brief bursts of increased speed were superimposed upon the ‘quivering’ movement at intervals of usually 5–10 sec., and the movements also stopped occasionally for a few seconds.

Taken as a whole, the observations suggest that the hind-brain of *Xenopus* at early stages is the source both of a rhythmic excitation and of an inhibition of spontaneous spinal cord activity. A gradual weakening of this inhibition in later stages would account for the development of spontaneous sustained swimming with periodic bursts of increased speed. The various abnormalities in spontaneous and reflex swimming patterns seem to reflect varying degrees of interference with the inhibitory system. The presence of both endogenous and reflex excitation and inhibition in a system with normally only a single, simple output pattern should make the *Xenopus* larva useful for investigating the origin and development of patterned nervous activity in general.

**DISCUSSION AND CONCLUSIONS**

Utilizing both the *in vivo* and the *in vitro* observations referred to previously, and assuming that these two forms are representative, we can draw the following conclusions about early behavior in the anurans.

(a) There is no myogenic phase of development; the earliest movements are neurogenic and result from an occasional brief output pulse. (This conclusion is also in accord with the observations made by Hooker (1911) on nerveless frog embryos.)

(b) Spontaneous neural activity continues throughout development and is a
fundamental feature of the behavior at all stages. It has a rhythmic basis, with at least two cycles of oscillation.

(c) Reflex swimming responses are stereotyped and essentially the same as those occurring spontaneously, although supra-threshold stimulation can sometimes progressively prolong the response. There are fluctuations in the excitability which coincide with the cycles of spontaneous activity and inactivity.

(d) The forebrain and the mid-brain play no rôle in determining the basic swimming pattern in *Xenopus*. (This had already been established for one species of *Rana* (Wang & Lu, 1941).)

(e) Rhythmic spontaneous activity and stereotyped responses to tactile stimulation are produced both by the hind-brain and by the spinal cord. There is a cranio-caudal gradient in the frequency of endogenous discharges.

(f) With development the intervals between bursts of activity become more consistent and the modal values become somewhat shorter.

(g) The durations of the bursts of twitching fall within the approximate limits of $1-3$ sec. at early swimming stages; they often have a characteristic value for each individual, especially as the larvae mature.

It would appear, then, that reflex activity in these animals cannot be understood primarily in terms of direct pathways for nerve impulses between receptors and effectors. One must consider a structural plan within the central nervous system whereby a basic pattern of pulses can be consistently released. Moreover, there must exist a background of endogenous rhythmic activity which at intervals reaches an excitation level adequate to trigger off this output pattern. Sensory input would then constitute simply an addition to the endogenous excitation in the neural network and would not play a rôle in determining the pattern of the efferent pulses. Even when supra-minimal responses are evoked by relatively strong stimulation, the output pattern appears to be unchanged, but only to persist longer. One is reminded of a clock, where the ‘ticking’ is determined solely by the characteristics of the mechanism while its duration varies with the amount of winding of the mainspring. This nervous ‘clockwork’, however, permits both some irregularity in the successive pulses, and rhythmic fluctuations in their mean frequency and amplitude.

Neural rhythms underlying somatic movements have also been described recently in the chick embryo (Hamburger & Balaban, 1963), in which form too the reflex responses have the nature of a triggered pattern which is essentially identical to that produced endogenously (cf. Orr & Windle, 1934; Tuge, 1937; and own observations). It was even suggested some time ago by Weiss (cf. 1941a, 1941b) that both endogenous rhythmicity and determination of coordinated efferent patterns may be intrinsic properties of neural networks in general. The same idea has more recently also emerged in the discussions of Bullock (cf. 1957). It will be instructive now to explore in detail the developing nervous systems of various vertebrates for the generators of rhythmic ‘central excitation’ and the networks involved in stereotyped efferent activity. The rôle
which the embryonic patterns of nervous functioning then play in the later behavior of animals of different evolutionary 'position' and ecological 'niche' should also be a fruitful subject for investigation.

SUMMARY

Observations were made upon two species of anurans, *Xenopus laevis* and *Rana pipiens*, of the early body movements and their modification after making sections through various levels of the central nervous system. Bursts of muscle contractions, effecting swimming, occur spontaneously and are identical to those evoked by tactile stimulation. Bursts are usually consistent in duration and occur at fairly regular intervals. In *Rana* they are generated by the spinal cord as well as by the hind-brain, but with a decreasing frequency at successively posterior levels. Early behavior in *Xenopus* is unchanged by removal of the forebrain and mid-brain, or by removal of the most anterior portion of the hind-brain. More extensive hind-brain removal alters the swimming pattern in most cases so that the bursts become either prolonged or abortive. Complete removal results in continual rather than periodic muscular activity. The early amphibian central nervous system is concluded to consist functionally of several excitatory and inhibitory rhythms acting upon an efferent mechanism which is capable of generating pulses at a constant mean frequency. Sensory input is held to alter briefly the net level of central excitation resulting from these endogenous rhythms.

RÉSUMÉ

*Rythmicité de la nage des larves d’Anoures à ses débuts*

On a fait des observations sur les premiers mouvements du corps chez deux espèces d’Anoures, *Xenopus laevis* et *Rana pipiens*, et sur leurs modifications après sections pratiquées transversalement à divers niveaux du système nerveux central. Des poussées de contractions musculaires, produisant la nage, se produisent spontanément et sont identiques à celles que provoquent des stimulations tactiles. Elles sont habituellement de durée consistante et se produisent à intervalles assez réguliers. Chez *Rana*, elles sont engendrées par la moelle épinière aussi bien que par le cerveau postérieur, mais avec une fréquence décroissante aux niveaux postérieurs successifs. Le comportement initial chez *Xenopus* n’est pas modifié par ablation du cerveau antérieur et du cerveau moyen, ou par ablation de la partie la plus antérieure du cerveau postérieur. Des exérèses plus étendues du cerveau postérieur altèrent la nage dans la plupart des cas, de sorte que les poussées d’activité ou bien sont prolongées, ou bien avortent. L’exérèse complète produit une activité musculaire continue plutôt que périodique. On conclut que le système nerveux central du jeune Amphibien consiste fonctionnellement en plusieurs rythmes excitateurs et inhibiteurs agissant sur un mécanisme efferent capable d’engendrer des pulsations à une fréquence moyenne
donnée. Les influx sensoriels doivent altérer brièvement le niveau d’excitation centrale résultant de ces rythmes endogènes.

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REFERENCES


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