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40. We thank Dr. J. Alderson, Dr. L. E. Zimmerman, Dr. J. M. Henry, Dr. K. M. Earle, and Dr. C. J. Davis of the Armed Forces Institute of Pathology (AFIP), Dr. H. L. Stewart, Dr. J. M. Rice, Dr. Clyde Dawe, and Dr. W. G. Flamm of NIH, and Dr. B. F. Fetter and Dr. D. Hackel of the Duke University Medical Center for comments and suggestions; L. Miller of AFIP and C. Lewis of Duke University Medical Center for gross photography; R. Nye, W. Sewald, and J. Romine of NIH, J. Edwards and L. Duckett of AFIP, and B. Boyarsky and C. M. Bishop of Duke University Medical Center for photomicroscopy; T. L. Johnson of NIH and the Division of Laboratory Animal Resources at Duke University for animal care; and R. L. Dunn and H. Snells of NIH for technical assistance.

11 December 1975; revised 11 May 1976

Cholinergic Changes During Conditioned Suppression in Rats

Abstract. *Levels of acetylcholine were significantly elevated in the telencephalon and diencephalon + mesencephalon of rats killed by near-freezing during conditioned suppression of food-reinforced lever pressing, whereas levels of serotonin, dopamine, and norepinephrine were not altered. These neurochemical changes were not seen in rats serving as controls for conditioning experience, activity levels, or stimulus presentation.*

In a series of studies on drug-induced atypical behavior in rats, a special type of behavioral excitation was found to be temporally related to decreased total levels of acetylcholine (ACh) in the telencephalon (*1*), whereas depressed responding was accompanied by increased levels of this putative transmitter in the diencephalon + mesencephalon (*1, 2*). In both cases, the behavioral states were attributed, at least in part, to changes in release of ACh at cholinergic synapses (*3*).

One disadvantage in using drugs to induce abnormal behavior is the confounding variable of complex interactions among drug, behavior, and neurochemical changes. In addition, drug administration may result in large changes in neurotransmitter levels when relatively small differences may be responsible for the observed atypical behavior. Therefore, to provide more precise correlations between behavior and neurotransmitter levels, it is also necessary to use nondrug methods for altering behavioral states. The conditioned emotional response (CER) procedure has been used extensively to produce conditioned suppression without the use of drugs (*4, 5*), and provides one method for investigating neurochemical correlates of substantial decreases in rates of responding. We report here that levels of ACh are elevated in certain areas of the brain during conditioned suppression of food-reinforced behavior in the rat.

Four groups of adult, male albino rats (Wistar strain) were trained to press a lever for condensed milk in an operant conditioning chamber that contained a lever, a dipper for presentation of liquid reinforcement, a grid floor, and a speaker for presentation of auditory stimuli. The rats were maintained at 85 percent of their free-feeding weights. Once the lever-pressing response was established, the rats were given daily sessions on a variable interval 1 (VI 1) schedule of reinforcement in which 0.15 ml of milk was presented to the responding animal on the average of once per minute. During each session 30 reinforcements were delivered over a period of about 30 minutes.

After levels of stable responding were reached (mean response rate, 42 per minute), the rats continued to receive daily VI 1 sessions. However, at another time of day, at least 2 hours after a VI session, three groups of rats received CER training, whereas a fourth group received only auditory stimulus (S) training. The CER or S training sessions were given in a grid floor apparatus which contained no lever or dipper. The CER training procedure was a modification of the method of Hunt and Brady (*4*). A CER session consisted of a 15-minute period in which six electric grid shocks (2.0 ma, 0.5-second duration) were presented, with each shock preceded by an average of 2 minutes of white noise. The presentations of white noise and shock were interspersed with periods of silence. Defecations or urinations (or both) occurred during 98 percent of the CER sessions. The S training followed the same procedure as the CER training except that no shocks were ever given. In only 5 percent of these latter sessions were defecations or urinations noted. Both CER and S training sessions were given once per day for 7 days, with the final session in a lever-pressing apparatus (again at least 2 hours after a VI session).

On the day after the final CER or S training session, two groups of CER trained rats were placed in the lever-pressing apparatus as usual and allowed to work on the VI schedule. After 5 minutes of responding, the first group was presented with 15 minutes of continuous white noise (which was never followed by shock). The rats were then quickly removed from the chambers and killed by the near-freezing method (*6*). The brains were removed and dissected (*6*) and the parts assayed (*7*) for ACh, serotonin (5-HT), dopamine (DA), and norepinephrine (NE). The second group of CER rats was killed after 20 minutes of working on the VI schedule, never having received white noise during the VI session. The final CER group did not receive any VI experience on the day they were killed, but were killed at comparable times after removal from their home cages following the ingestion of an amount of condensed milk equivalent to that consumed by the first CER group. The S trained rats were presented with the white noise after 5 minutes of working on the VI schedule

Table 1. Levels of four putative neurotransmitters in the telencephalon and diencephalon + mesencephalon of rats killed during conditioned suppression or three control conditions. Rats in the conditioned emotional response (CER) trained suppression group were killed after 15 minutes of white noise during a period of complete behavioral suppression. Rats in one CER trained control group received no white noise during the variable interval (VI) session before being killed. Rats in the activity control group were given CER training but received no VI session on the day of killing and were killed after removal from the home cage. Rats in the auditory stimulus (S) trained control group received no CER training but were killed after presentation of 15 minutes of white noise during a VI session; S.E.M., standard error of the mean.

Group	Number	Content (nanomoles per gram of wet tissue)			
		ACh (mean ± S.E.M.)	5-HT (mean ± S.E.M.)	DA (mean ± S.E.M.)	NE (mean ± S.E.M.)
		Telencephalon			
CER trained suppression	8	21.30 ± 0.53*	3.22 ± 0.19	7.06 ± 0.41	2.42 ± 0.13
CER trained control	13	19.58 ± 0.51	3.38 ± 0.12	6.90 ± 0.33	2.70 ± 0.21
Activity control	6	20.62 ± 1.10	3.60 ± 0.06	6.11 ± 0.38	2.18 ± 0.11
S trained control	14	20.77 ± 0.44	3.21 ± 0.14	7.15 ± 0.28	2.62 ± 0.15
		Diencephalon + mesencephalon			
CER trained suppression	8	34.40 ± 1.11*	5.44 ± 0.29	0.67 ± 0.18	3.82 ± 0.24
CER trained control	13	31.30 ± 0.82	5.97 ± 0.34	0.99 ± 0.17	4.08 ± 0.24
Activity control	6	28.20 ± 1.18*	5.57 ± 0.47	1.06 ± 0.20	3.72 ± 0.21
S trained control	14	30.48 ± 0.71	5.64 ± 0.26	0.74 ± 0.37	3.68 ± 0.17

*$P < .05$; two-tailed t-test. Comparisons made were as follows: CER trained suppression group versus CER trained control group; CER trained control group versus S trained control group; and CER trained control group versus activity control group.

and then killed after 15 minutes of continuous white noise.

The behavioral states of the various groups immediately before being killed were as follows. The CER trained rats that were presented with white noise for 15 minutes before being killed (CER trained suppression group) displayed conditioned behavioral suppression in that their response rates were zero during this period. The suppression period was also accompanied by defecations and urinations. Some rats in this group, however, were suppressed for only a few minutes and had resumed normal responding by the end of the 15-minute period of white noise. These rats were also killed, but were not included in the CER trained suppression group in Table 1, which contains only those rats that remained totally suppressed for the entire 15-minute period. The CER rats that were presented with no white noise (CER trained control group), as well as the rats that received only S training (S trained control group), were responding as usual on the VI schedule when they were killed. The CER rats that received no VI session before they were killed (activity control group) were removed from their home cages where they were relatively inactive, but awake.

Significantly elevated levels of ACh (Table 1) were found in both the telencephalon (9 percent increase) and the diencephalon + mesencephalon (10 percent increase) of the brains of rats displaying conditioned behavioral suppression. Since the CER trained controls appeared no different neurochemically than the S trained controls, the higher ACh levels during suppressed responding cannot be attributed simply to the experience of the CER training and grid shock itself. In the case of the telencephalon, the general activity level of the rats does not appear to be a likely factor, either, since the inactive rats had about the same levels of ACh as the rats working on the VI schedule. Moreover, in another study, no changes in content of brain ACh were found in rats with three different rates of food-reinforced lever pressing (0, 13, and 24 responses per minute) (*8*). There were apparent differences due to activity in the case of the diencephalon + mesencephalon, however, but in a direction opposite to that observed during suppression. Although conditioning and shock experience and dramatic changes in activity levels cannot be completely ruled out as playing a contributory role, the emotional component of the conditioned suppression (as evidenced by the high frequency of defecations and urinations) appears to be an important factor associated with the observed changes in the cholinergic system.

Adding further weight to the apparent relationship between behavioral suppression and elevated ACh levels is the fact that in the group of CER rats that failed to display a full 15 minutes of conditioned suppression (and were killed during normal responding), the levels of ACh in both the telencephalon (19.98 ± 0.52 nmole/g; $N = 6$) and the diencephalon + mesencephalon (32.24 ± 0.84 nmole/g; $N = 5$) were normal when compared to their appropriate controls (CER trained control group in Table 1). No significant changes in the other three neurotransmitters measured (5-HT, DA, and NE) were noted in any of the groups.

These data are consistent with the findings from the earlier psychopharmacological and neurochemical studies of our group in which elevated ACh levels were found in the diencephalon + mesencephalon during drug-induced depressed responding on shock-avoidance schedules after administration of tetrabenazine (2 mg/kg) (*1, 2*). Opposite effects were demonstrated after excitation induced by combined administration of iproniazid (50 mg/kg) and tetrabenazine (2 mg/kg); that is, lower levels of ACh accompanied the period of elevated responding (*1*).

The predominant biochemical theories of the affective psychoses have stressed possible roles for the catecholaminergic or serotonergic systems (or both) (*9*). Although early suggestions of cholinergic involvement were generally ignored (*10*), recent clinical evidence has revived interest in the consideration of ACh as an important factor in these psychotic disorders (*11*). The latter studies demonstrated that physostigmine, an inhibitor of acetylcholinesterase, can reduce manic symptoms and increase depression in manic, depressive, or schizoaffective patients.

The changes in levels of ACh during conditioned suppression (as in the present study) and drug-induced depression or excitation reported previously (*1–3*) may have no direct relevance to the role of the cholinergic system in affective disorders. On the other hand, the hypothesis derived from the recent clinical drug studies (*11*), associating higher ACh levels with depression and lower ACh levels with mania, is supported by the data from the present and previous animal studies (*1–3*). Further investigations of the neurochemical correlates of the de-

velopment, maintenance, and recovery from conditioned suppression could provide additional clues to possible neurotransmitter-behavior interactions in both normal and abnormal states.

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12. Supported in part by NIMH grants MH-03225-17, MH-10695-10, and MH-05986-20.

17 March 1976

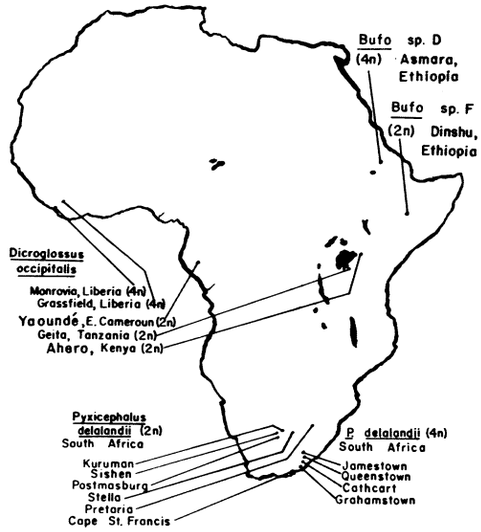
Polyploid Amphibians: Three More Diploid-Tetraploid Cryptic Species of Frogs

Abstract. *The nominal African species* Pyxicephalus delalandii *and* Dicroglossus occipitalis *have diploid and tetraploid populations. There are also cryptic tetraploid and diploid species similar to* Bufo kerinyagae. *These represent the first bisexual polyploid "species" so far encountered in the major frog families Ranidae and Bufonidae. The contention that polyploidy is a widespread and important evolutionary phenomenon in anuran amphibians is supported.*

The prior assumption that bisexual polyploid animal species would not be able to overcome sexual imbalances in gametogenesis as proposed by Muller (*1*) is no longer tenable because of the recent recognition of several bisexual, naturally occurring, populations of polyploid animals. Unlike fishes, where polyploidy has been involved in past evolutionary dichotomies (*2*), frogs appear to be the only unique group of vertebrates that have diploid and polyploid populations included in the same bisexual species or in closely related "cryptic species" (*3–6*). It is expected that future investigations will reveal these diploid and polyploid populations to be distinct and separable. This tenet has been discussed (*3, 6*).

During an extensive survey of the chromosomes of African anurans, two "species" demonstrated diploid and tetraploid populations. Two cryptic species, one diploid and one tetraploid, were also found. Chromosomes were obtained from epithelial squashes of adult cornea or tadpole tail tips by means of standard techniques (*7*). *Bufo* sp. D from Asmara, Ethiopia, are tetraploid ($2n \rightarrow 4n = 40$), but *Bufo* sp. F from Dinshu, Ethiopia, are diploid. South African *Pyxicephalus delalandii* populations from Kuruman, Sishen, Postmasburg, Stella, Pretoria, and Cape Saint Francis are diploid ($2n = 26$) but this species is tetraploid ($2n \rightarrow 4n = 52$) at Jamestown, Queenstown, Cathcart, and Grahamstown. At Ahero in Kenya, Geita in Tanzania, and Yaoundé in East Cameroun *Dicroglossus occipitalis* are diploid ($2n = 26$) but populations from Monrovia and Grassfield, Liberia, are tetraploid ($2n \rightarrow 4n = 52$). The distribution of the sampled populations is provided in Fig. 1, and representative karyotypes are presented in Fig. 2.

Polyploid frogs are known to occur in the families Leptodactylidae (*3, 5, 9*), Hylidae (*3, 4, 6, 10*), and even the "primitive" family Pipidae (*11*). The present recognition of polyploids in the Ranidae (*Dicroglossus occipitalis*, *Pyxicephalus delalandii*) and the Bufonidae (*Bufo* sp. D) helps to establish the fact that polyploidy is a general phenomenon in frogs and may appear in any genus. The rela-



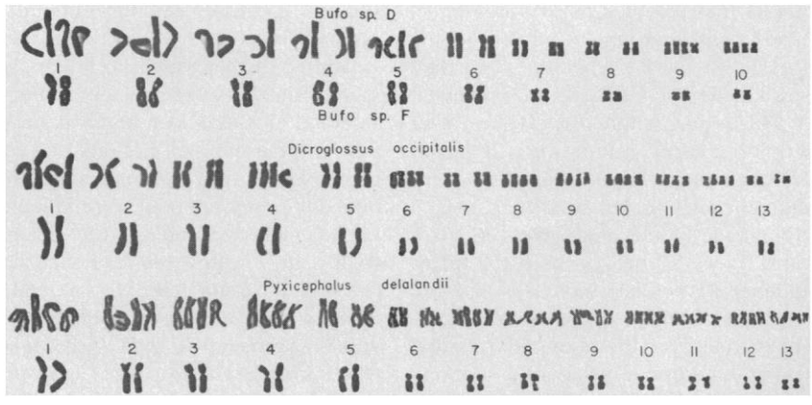


Fig. 1 (left). Map of Africa showing the localities of the diploid ($2n$) and tetraploid ($4n$) populations. Fig. 2 (right). Representative karyotypes of the diploid and tetraploid "cryptic" species. The chromosome sets are numbered from longest to shortest for each respective population.