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Actions of dexamphetamine and amphetamine-like amines in chickens with brain transections

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Summary

1. A method for preparing the encéphale isolé preparation in young fowls is described. Certain important differences were found between electrocortical activity of chicken and mammalian encéphale isolé preparations. Electrocortical effects of excitant sympathomimetic amines and their antagonism were readily quantified because of stable electrocortical activity of the chick encéphale isolé preparation.

2. Amphetamine-like excitant amines ((+)- and (−)-amphetamine, α-methyltryptamine, tryptamine, β-phenethylamine, cyclopentamine, β-tetrahydronaphthylamine and tuaminoheptane) evoked electrocortical desynchronization in chick encéphale isolé preparations, confirming the central origin of these effects. Behavioural changes were also observed.

3. The electrocortical response to these amines was antagonized by methysergide, a selective tryptamine antagonist and by a catecholamine, α-methylnoradrenaline. Behavioural changes were also antagonized.

4. Electrocortical desynchronization to dexamphetamine was prevented by an anterior transection of the brain which separated the telencephalon from the diencephalon. More posterior transections reduced the duration of the electrocortical response to dexamphetamine; intensity of response was either increased or decreased.

Introduction

The blood-brain barrier of the chick is incomplete, or absent for the first 3–4 weeks after hatching (Bakay, 1956; Lajtha, 1957) permitting entry of intravenously administered noradrenaline to the brain (Spooner, Winters & Mandell, 1966). During this period, sympathomimetic amines—depending on their chemical structure, produce behavioural excitation or sedation. Thus amphetamine-like amines elicited arousal, whereas catecholamine-like substances induced sleep (Clymer & Seifter, 1947; Zaimis, 1960); these behavioural changes were accompanied by appropriate electrocortical patterns (Key & Marley, 1962).

If the central actions of catecholamines and amphetamine-like amines in chicks and mammals are similar, then the chick would be a useful preparation for studying the central actions of these amines since, in spite of the absence of the blood-brain barrier, the central nervous system is sufficiently mature for the chick to be behaviourally self sufficient and for electrocortical activity to correlate highly with behavioural sleep (Key & Marley, 1962).

In this paper, the actions of amphetamine-like amines were investigated using chick encéphale isolé preparations, to exclude the possibility of arousal being mediated by spinal afferents. The action of dexamphetamine was localized by determining the level at which a transection of the brain prevented its electrocortical effects. This technique has been used successfully for localizing the central action of dexamphetamine in the cat (Bradley & Elkes, 1953; Hiebel, Bonvallet, Huvé & Dell, 1954). The central actions of catecholamines in chickens have already been investigated using intracerebral infusion techniques (Marley & Stephenson, 1968, 1970).

Transection experiments in young and adult chickens with acutely implanted cortical recording electrodes and intravenous cannula described briefly (Marley, 1963; Dewhurst & Marley, 1964), indicated that arousal effects of dexamphetamine and allied amines were determined in the brain stem and that these effects were abolished by transection above the brain stem. In chicks, unilateral thalamo-mesencephalic transection prevented electrocortical arousal to (±)-amphetamine in the ipsilateral, but not the contralateral hemisphere (Spooner & Winters, 1966).

The earlier of the above experiments (Marley, 1963; Dewhurst & Marley, 1964) had two major defects. These were: (1) drugs were tested after recovery from a 60 min period of anaesthesia, and (2) location of transections was determined macroscopically and not microscopically, so vitiating comparison with results obtained in mammals because of the important differences in organization between avian and mammalian brains. Our investigation avoids these defects and considerably extends the earlier findings.

Methods

Animals

Rhode Island Red pullets, usually 12–19 days old, were kept in a thermostatically controlled cage maintained at 33°–34° C for the first week after hatching and for the next 2 weeks at 29°–31° C. In addition, one 5-day and two 8-week chicks were used as well as one adult pigeon.

Operative procedures

Operations were performed under halothane anaesthesia (Marley & Payne, 1964) on 2 successive days. On the first, cortical recording electrodes and an intravenous jugular cannula were implanted (Key & Marley, 1962). In chicks which were later to be used as encéphale isolé preparations, a hole was drilled in the cranium to expose the left side of the cerebellum. For chicks in which an anterior transection was to be made, the cranium was drilled bilaterally in the coronal plane, immediately anterior to the junction of the fronto-parietal bones, a narrow section of skull being removed from the lateral margin almost to the midline.

On the second day, the chick was incubated and halothane readministered via a Palmer Miniature Ideal respiration pump with ventilation at 24/minute. When artificial respiration was satisfactory, the midline incision was reopened and the dura incised. For encéphale isolé preparations, a blunt leucotome was inserted, through the cerebellum, and gentle pressure exerted to transect the brain stem immediately above its junction with the spinal cord. Anaesthesia was stopped, and artificial respiration continued. For anterior transections, the leucotome was inserted

through each of the apertures at the fronto-parietal junction and gentle pressure exerted until the floor of the cranium was felt; lateral movement of the leucotome completed the transection. Anaesthesia was terminated and when spontaneous respiration returned, artificial ventilation was stopped. For both encéphale isolé preparations and anterior transections, the procedures were complete and anaesthesia concluded within 2 minutes. To ensure satisfactory recovery after halothane in encéphale isolé preparations, 30 min elapsed before recording began whereas in many of the anterior transections, the experiment was not begun until 6 h later.

In the case of stereotactic implantation of a brain stem stimulating electrode in encéphale isolé preparations, the recording electrodes and intravenous cannula were implanted 24 h previously. On the second day, the chick was anaesthetized and placed in a Stoelting Stellar stereotactic instrument (Marley & Stephenson, 1970). The transection was then made and a coaxial stimulating electrode positioned stereotactically in the brain stem 2–3 mm anterior to the transection. Anaesthesia was terminated and 30 min allowed for recovery.

In three encéphale isolé preparations, blood pressure (1 mmHg≡1·333 mbar) was recorded on a Devices polygraph from a cannula tied into an ischiadic artery immediately before brain transection.

Positions of the transections and stimulating electrodes were subsequently located histologically, the brain having been fixed in formol saline, then stained with luxol fast blue and cresyl violet after rapid embedding in celloidin (Inman, 1968).

Testing arrangements

The chick after transection was placed on a rubber mat, 2 feet from a 100W reflector lamp to provide heat. The electroencephalograph was situated close to the chick so that electrocortical and behavioural changes could be observed simultaneously. Encéphale isolé preparations were laid on their side. While recording electrocortical activity, the head was covered with a small box to exclude light from the eyes. This was necessary, because retraction of the eyelids caused by the excitant amines would itself lead to electrocortical arousal since passive eye opening in the light, achieved by elevating the upper eyelid with the tip of a soft brush, evoked electrocortical desynchronization. Electrocortical activity was automatically integrated at 1 min intervals by a modification of the method of Dewhurst & Marley (1965a), large amplitude potentials producing high integral counts and low voltage electrocortical patterns giving low integrals.

Drugs

These (with the molecular weights of the salts in parentheses) were the hydrochlorides of tryptamine (196); (±)-α-methyltryptamine (210); (±)-cyclopentamine (178); β-tetrahydronaphthylamine (181); (−)- and (±)-α-methylnoradrenaline (220); β-phenethylamine (158); the sulphates of (+)- and (−)-amphetamine (368) and (±)-tuaminoheptane (328). Also tested were methysergide bimaleate (470) and mebanazine oxalate (226). All injections were given intravenously.

Results

Encéphale isolé preparations

Electrocortical activity and behaviour

The transection was made in the plane indicated in Fig. 1 (transection 3) after which the anaesthetic was withdrawn and artificial ventilation continued. If the section was complete, the chick lay on its side, as placed, with its eyes closed, the only movement being of the nictitating membranes. The electrocorticogram (Fig. 2A) resembled that from the intact sleeping chick with respect to amplitude (100–150 μV) but was of a slower frequency (predominantly 2–4 Hz with some 4–8 Hz). Unlike electrocorticograms from intact sleeping chicks there were no cyclic sequences of low amplitude (10–25 μV) paradoxical sleep activity interrupting the large amplitude slow frequency activity.

Unilateral passive opening of the eyelids (see **Methods**) produced marked desynchronization (Fig. 8B) confined to the contralateral hemisphere in eighteen of twenty-four chickens tested. Desynchronization was 'phasic', that is it did not outlast the stimulus. Stimuli to the body below the level of transection or to the head did not alter the electrocorticogram provided the eyes remained closed.

Central excitant amines

Dexamphetamine. Dexamphetamine, 1·0 μmol/100g, evoked immediate behavioural and electrocortical arousal in twenty-three chickens tested. Control electrocortical activity (Fig. 2A) changed to a low amplitude (15–30 μV), desynchronized pattern with peak frequencies occurring in the 10–12 Hz and 40 Hz bands (Fig. 2B); electrocortical integrals fell most markedly in the 5 min after injection when a 70–95% fall in integrals occurred (Fig. 2E); control activity returned after 90–100

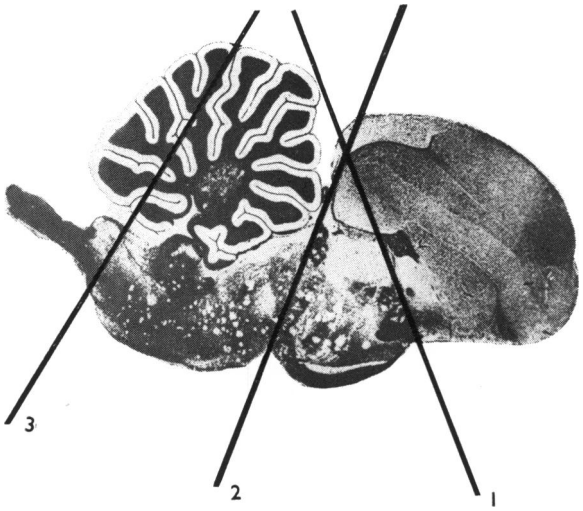


FIG. 1. Microphotograph of a midline sagittal section of the brain of a 16-day chick (85g). 1. Position of a transection anterior to the thalamic nuclei. 2. Position of a transection passing through the posterior commissure in a plane anterior to the nucleus ruber but posterior to the optic chiasma. 3. Approximate position of the encéphale isolé transection.

minutes. Similar effects were obtained with dexamphetamine in one adult pigeon and two adult fowl encéphale isolé preparations.

Electrocortical desynchronization was associated with behavioural and other changes. These were retraction of the upper eyelids, repetitive depression and elevation of the mandible, movements of the wings and reciprocal flexion and extension of the limbs. The sclera, not normally visible above the upper margin of the iris, became visible so suggesting onset of exophthalmos. Retraction of the eyelids developed slowly. Initially there were frequent movements of the nictitating membranes but these gradually ceased with the membranes in the retracted position. The behavioural signs waned in reverse order of appearance. The effects on the wings and limbs were observed in spinal chicks and are presumably due to an action of dexamphetamine on the spinal cord (unpublished results).

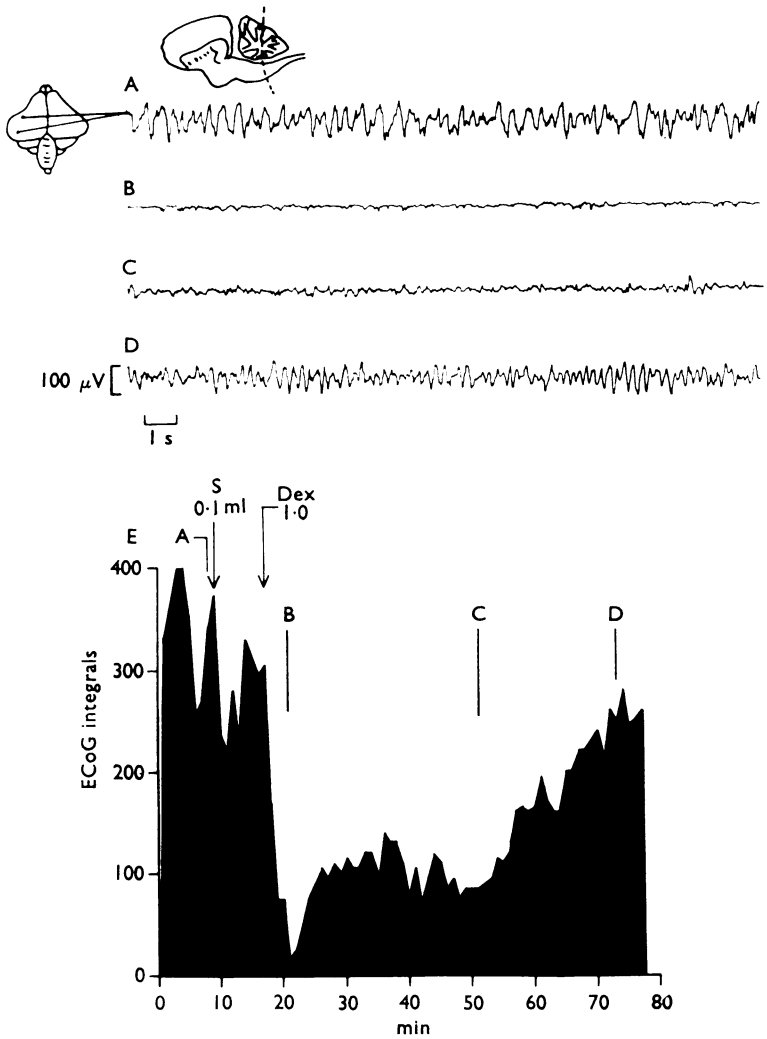


FIG. 2. Records of electrocortical activity (A–D) and histogram of integrated electrocortical activity in a 5-day chick encéphale isolé preparation (40g). S, saline. A, Control record with predominantly 2–4 Hz, 100 μV activity; B, electrocortical desynchronization produced by dexamphetamine, 1·0 μmol/100g; C and D, gradual recovery of electrocortical activity.

Subthreshold doses of dexamphetamine, 0·4 μmol/100g in four equally divided doses, converted 'phasic' desynchronization elicited by passive eye opening into a 'tonic' effect persisting for 45 s after eye closure. Similarly, dexamphetamine, 0·4–0·5 μmol/100g, a dose insufficient to produce electrocortical desynchronization, enhanced that produced by threshold (0·75–1 V) electrical stimulation of the brain stem reticular formation of three chick encéphale isolé preparations. For example, in one of the chicks tested, dexamphetamine, 0·4 μmol/100g increased persistence of electrocortical desynchronization after stimulation from 12 to 30 s; desynchronization was intensified.

(−)-*Amphetamine.* In three chickens (−)-amphetamine, 1·0 μmol/100g reduced mean electrocortical integrals an average of 22%; an additional 2·0 μmol/100g had little effect. Behavioural effects were restricted to occasional movements of the legs.

α-*Methyltryptamine.* In two chickens α-methyltryptamine, 0·5 μmol/100g produced immediate long-lasting behavioural and electrocortical arousal (Fig. 3B). In one, electrocortical integrals were reduced 60% and did not recover over the

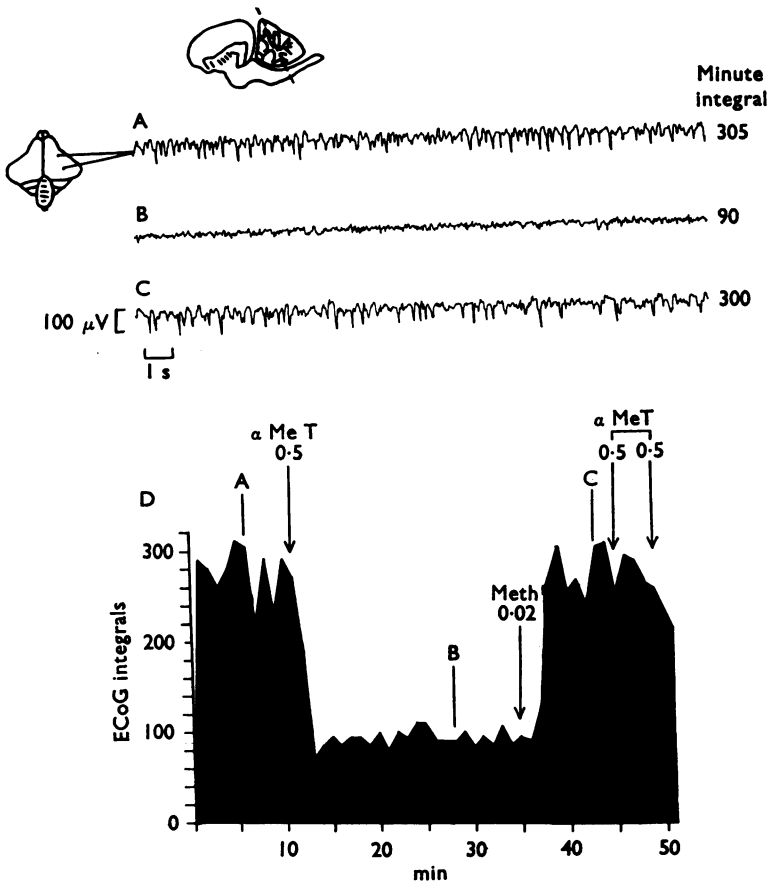


FIG. 3. Records of electrocortical activity (A–C) and histogram of integrated activity (D) in an 8-week chicken encéphale isolé preparation (level of transection shown by dotted line on sagittal section of brain). Wt, 275g. A, Control record with predominantly 4–6 Hz, 100 μV activity; B, electrocortical desynchronization produced by α-methyltryptamine, 0·5 μmol/100g and antagonized (C, D) by methysergide, 0·02 μmol/100g.

following 120 min and in the other, integrals fell 63% and were promptly returned to preinjection values at 25 min by methysergide, 0·02 μmol/100g (Fig. 3C and 3D).

Cyclopentamine. In two chickens cyclopentamine, 2·5 and 10 μmol/100g elicited electrocortical and behavioural arousal persisting for 90 min; integrals fell a maximum of 60% and 50% respectively.

β-Tetrahydronaphthylamine. Behavioural and electrocortical arousal were obtained with β-tetrahydronaphthylamine, 3·0 μmol/100g in three chickens, electrocortical integrals declining a maximum of 50% with recovery in 15 minutes.

Tuaminoheptane. Tuaminoheptane, 10 μmol/100g evoked behavioural and electrocortical arousal in three chickens with maximal reductions in integrals of between 17 and 28%. In one, a second injection of 10 μmol/100g given 10 min later reduced integrals by 16% to 44% of their control value and in another, injection of 20 μmol/100g further reduced integrals by 23% to 48% of their control value.

Potentiation

The degree of monoamine oxidase inhibition depends on dose, duration of pretreatment and recency of last dose. Unless stated otherwise, mebanazine, which did not affect behaviour or electrocortical activity in the doses used, was given in divided doses over the previous 1–3 days followed by a dose 1 h before the experiment.

Dexamphetamine. The behavioural and electrocortical effects of dexamphetamine, 1·0 μmol/100g in two chickens were not affected by pretreatment with mebanazine, 120 μmol/100g.

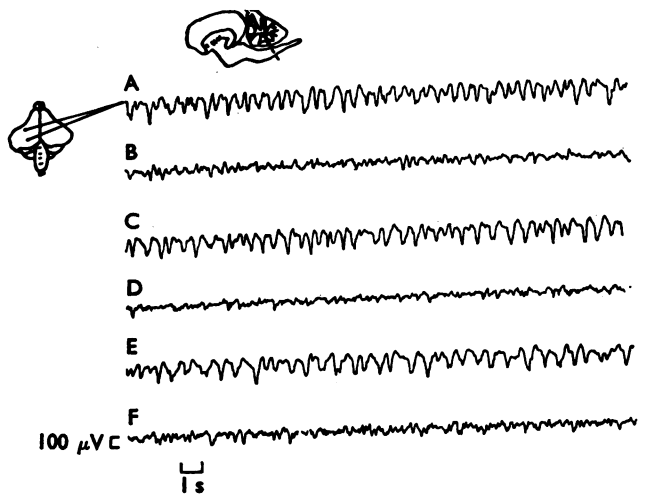
β-Phenethylamine. Experiments were made in five chickens. In a representative experiment in which the chick had been pretreated with mebanazine, 80 μmol/100g, β-phenethylamine, 4.0 μmol/100g, a dose ineffective in non-pretreated chicks, produced behavioural and electrocortical arousal (compare Fig. 4A and 4B), integrals declining to a maximum of 56% (Fig. 4G).

Tryptamine. Since intravenously injected tryptamine often produced immediate death in non-pretreated chicks [a consequence of a fall in blood pressure secondary to pulmonary vasoconstriction (Eble, 1963)] but not in those given monoamine oxidase inhibitors, tryptamine was not given to non-pretreated encéphale isolé preparations. In intact chicks, tryptamine 1·0 μmol/100g produced electrocortical arousal lasting 10 min (Dewhurst & Marley, 1965b).

The effects of tryptamine, 0·5 μmol/100g were tested in two chickens. In the first, pretreated with mebanazine (20 μmol/100g 3 h previously) tryptamine reduced electrocortical integrals a maximum of 40% for at least 30 minutes. More intense desynchronization was evoked in the second chicken given mebanazine (140 μmol/100g in divided doses over 3 days); tryptamine reduced integrals a maximum of 80% and at 28 min when arousal was still as intense, the behavioural and electrocortical effects were antagonized by methysergide, 0·008 μmol/100g.

Antagonism

Two modes of antagonism were studied, antagonism by methysergide, a selective tryptamine antagonist, and antagonism by α-methylnoradrenaline, a central depressant amine.



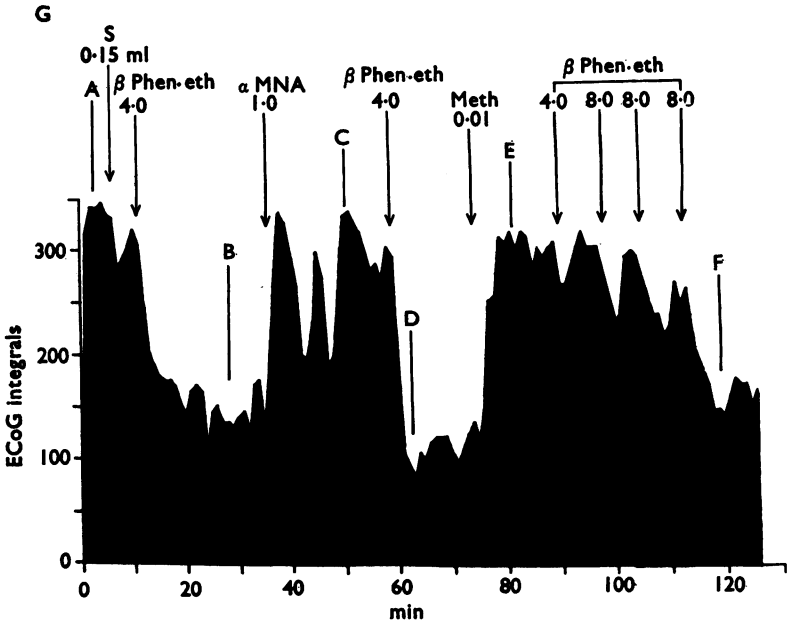


FIG. 4. Records of electrocortical activity (A–F) and histogram of integrated activity (G) in a 12-day chicken encéphale isolé preparation (level of transection shown by dotted line on sagittal section of brain). Wt, 55g. The chicken was pretreated with mebanazine (20 μmol/100 g 24 h previously; 40 μmol/100g, 3 h previously and 20 μmol/100g, 30 min previously). Epochs corresponding to A, B, C, D, E and F are indicated in the histogram. S, saline. A, Control record with predominantly 2–4 Hz, 200 μV activity; B, electrocortical desynchronization produced by β-phenethylamine, 4·0 μmol/100g; C, antagonism produced by (−)-α-methylnoradrenaline, 1·0 μmol/100g and surmounted (D) by β-phenethylamine, 4·0 μmol/100g; E, antagonism produced by methysergide, 0·01 μmol/100g and surmounted (F) by a total of 28·0 μmol/100g β-phenethylamine. In contrast to antagonism with (−)-α-methylnoradrenaline, antagonism with methysergide was only surmounted by increasing the dose of β-phenethylamine.

Methysergide

Dexamphetamine. In a chicken dexamphetamine, 3·0 μmol/100g evoked immediate behavioural and electrocortical arousal reducing integrals 75% from 100 to 25/minute. Methysergide, a total of 0·024 μmol/100g in three doses, restored control electrocortical activity and integrals, behavioural alerting abating at the same time. Antagonism was not due to a central depressant action of methysergide since passive eye opening still produced contralateral electrocortical desynchronization. Antagonism was partially surmounted by dexamphetamine, 3·0 μmol/100g, and completely surmounted by a second similar injection. These results were confirmed in two other chicks; in one, methysergide, 0·001 μmol/100g, completely antagonized electrocortical desynchronization elicited by dexamphetamine, 1·0 μmol/100g.

β-Phenethylamine. In a chicken pretreated with mebanazine, 80 μmol/100g, β-phenethylamine, 40 μmol/100g, produced behavioural arousal and electrocortical desynchronization (Fig. 4D), integrals falling 66% from 300 to 100/minute. Methysergide, 0·01 μmol/100g promptly antagonized behavioural and electrocortical effects (Fig. 4E and 4G). Antagonism was surmounted by β-phenethylamine, 28·0 μmol/100g (Fig. 4F), given in four doses, integrals falling 44%.

Tryptamine. In a chicken pretreated with mebanazine, 140 μmol/100g, tryptamine, 0·5 μmol/100g, produced behavioural arousal and intense electrocortical desynchronization; integrals fell a maximum of 80%. At 28 min, when the response was still as intense, methysergide, 0·008 μmol/100g (in two equal doses), completely antagonized behavioural and electrocortical responses within 10 min; a total of 7·0 μmol/100g tryptamine given in divided doses over 20 min only partially surmounted antagonism, integrals falling 21%.

α-Methyltryptamine. α-Methyltryptamine, 0·5 μmol/100g, produced behavioural arousal and electrocortical desynchronization, integrals falling 63% (Fig. 3B). At 25 min, the effects were just as intense but methysergide, 0·02 μmol/100g immediately restored electrocortical activity to the control level (Fig. 3C, D) and antagonized behavioural arousal. Antagonism was partially surmounted by α-methyltryptamine, 1·0 μmol/100g given in two doses, integrals falling 30%.

α-Methylnoradrenaline

Demonstration of central depressant activity of a drug in the encéphale isolé preparation is not straightforward since the chick exhibits continuous behavioural and electrocortical sleep. It was achieved in three ways: (1) abolition of 'phasic' electrocortical arousal on passive eye-opening; (2) abolition of behavioural and electrocortical arousal on electrical stimulation of the brain stem; (3) abolition of behavioural and electrocortical arousal induced by dexamphetamine or, in chicks pretreated with a monoamine oxidase inhibitor, by β-phenethylamine.

Antagonism of electrocortical desynchronization on eye-opening or on brain stem stimulation. Passive eye opening produced marked electrocortical desynchronization confined to the contralateral hemisphere in 75% of twenty-four chickens tested. In a representative experiment, left eye opening for 15 s evoked electrocortical desynchronization over the right hemisphere which persisted throughout the 15 s and returned to the control pattern within 2 s of eye closure. The response was reproducible. Four minutes after (±)-α-methylnoradrenaline, 1·0 μmol/100g, passive

eye opening for 15 s produced desynchronization which persisted for only 4 s, after which slow waves reappeared in the electrocorticogram; the desynchronization observed for 2 s after control eye closure was abolished. Four minutes after (±)-α-methylnoradrenaline, 2·0 μmol/100g, electrocortical desynchronization was more attenuated, persisting for only 2–3 s after commencing eye opening for 15 seconds.

(−)-α-Methylnoradrenaline (2·0 μmol/100g) prevented electrocortical desynchronization produced by threshold (0·75–1·0 V) electrical stimulation of the brain stem of three chick encéphale isolé preparations. In one such experiment, in which threshold stimulation (0·75 V) produced electrocortical desynchronization throughout

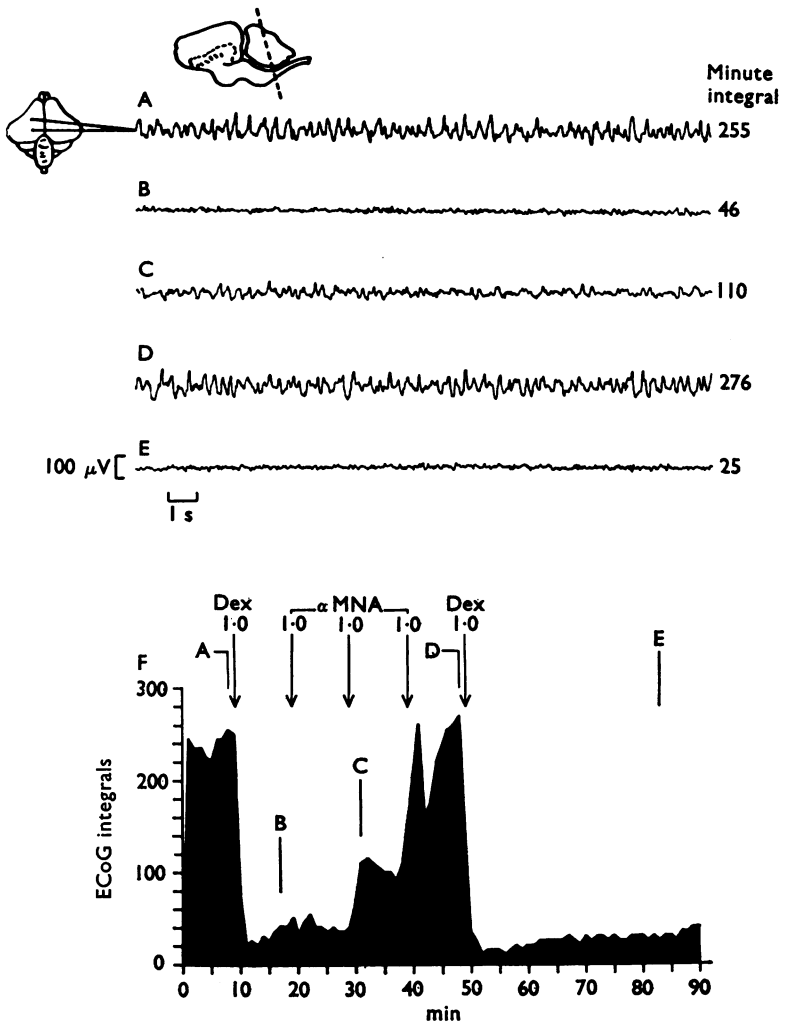


FIG. 5. Records of electrocortical activity (A–E) and histogram of integrated activity (F) in a 12-day chicken encéphale isolé preparation (level of transection shown by dotted line on sagittal section of brain). Epochs corresponding to A, B, C, D and E are indicated in the histogram. Wt, 80g. A, Control record with predominantly 2–4 Hz, 100 μV activity; B, electrocortical desynchronization produced by dexamphetamine, 1·0 μmol/100g; C, partial antagonism produced by (±)-α-methylnoradrenaline, 2·0 μmol/100g. D, Antagonism produced by (±)-α-methylnoradrenaline, 3·0 μmol/100g; E, antagonism surmounted by dexamphetamine, 1·0 μmol/100g.

excitation, and persisting an additional 8–12 s thereafter, stimulation at 0·85V between 2 and 30 min after (−)-α-methylnoradrenaline reduced electrocortical amplitude but did not change the frequency. However, the effects of α-methylnoradrenaline were surmounted by increasing the intensity of excitation; thus stimulation at 2V during the same period elicited electrocortical desynchronization for the duration of excitation.

Antagonism of dexamphetamine. (−)- or (±)-α-Methylnoradrenaline, 1·0 μmol/100g was given at 10 min intervals, 10 min after dexamphetamine, 1·0 μmol/100g. The racemate completely antagonized the behavioural and electrocortical effects of dexamphetamine in three chicks after 3·0 μmol/100g and in one after 4·0 μmol/100g (Fig. 5), that is within 30–40 min of injecting dexamphetamine compared with a normal duration of at least 90 min; 3·0 μmol of the laevo isomer was an effective antagonist in four chicks. In contrast to the racemate, even the first dose of the laevo isomer evoked slow frequency large amplitude electrocortical activity with increase in integrals. Antagonism by α-methylnoradrenaline was readily surmounted by a further dose of dexamphetamine, 1·0 μmol/100g (Fig. 5E, F), its effects continuing undiminished for the remaining 40 min of the experiment.

Antagonism of β-phenethylamine by (−)-α-methylnoradrenaline is shown in Fig. 4.

Preparations with anterior transections of the brain

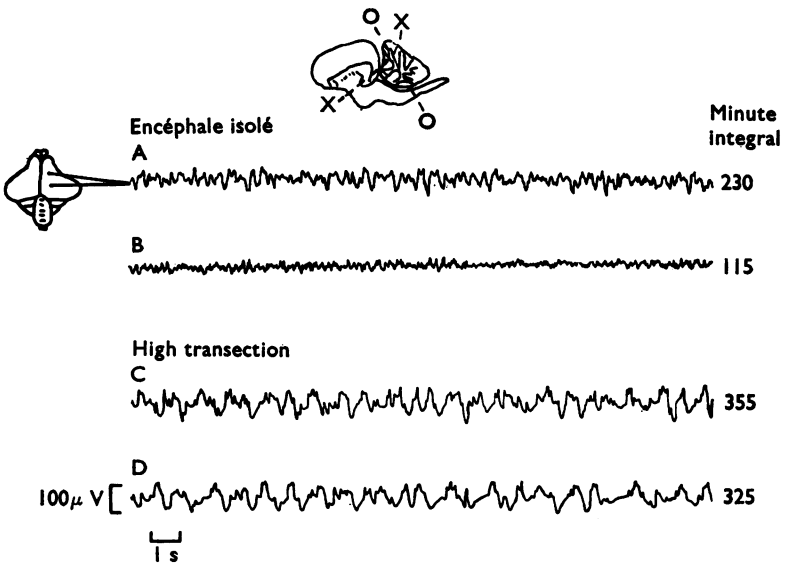
Two types of preparation were used. In the one, only a high transection was made using a bilateral approach through the posterior portion of the cerebral hemispheres immediately anterior to the junction of the frontal and parietal bones. In these preparations, respiration was spontaneous and the ability to stand usually returned 60–90 min after stopping anaesthesia. Should this not have returned, then it was restored by dexamphetamine or α-methyltryptamine which also evoked the postural changes and tachypnoea observed in normal chickens; vocalization did not occur. Unless stated otherwise, experiments were of this type. In the other, an encéphale isolé preparation was made and electrocortical arousal with dexamphetamine established. The preparation was reanaesthetized and a higher transection made via an approach through the cerebellum. This transection abolished electrocortical desynchronization produced by dexamphetamine in the encéphale isolé (Fig. 6C); 30 min after stopping anaesthesia the effects of dexamphetamine were again tested. The chick was unable to right itself because of the lower brain stem transection.

Because of anatomical differences between mammalian and avian brains, the term cerveau isolé is misleading. Instead, levels of transections were confirmed histologically and their position related to the thalamic and mesencephalic nuclei.

Transection anterior to the diencephalon. In this preparation the cerebral hemispheres were separated from the diencephalon by transection 1 shown in Fig. 1. Immediately after transection, the electrocorticogram frequently contained recurrent 'silent periods' of approximately 2–3 s duration. As the after effects of anaesthesia diminished, these 'silent' periods became less frequent and gradually disappeared; if they persisted, the preparation was discarded.

After anterior transection, control electrocortical activity consisted entirely of large amplitude (up to 400 μV) slow frequency (up to 4 Hz) waves (compare Fig. 6C with 6A). In contrast to chicks with more posterior transections, passive eye opening

was without effect on the electrocardiogram. In five chickens, dexamphetamine, 1·0 μmol/100g, a dose that would have elicited arousal in an encéphale isolé preparation (Fig. 2), was without effect on electrocortical activity. In a representative



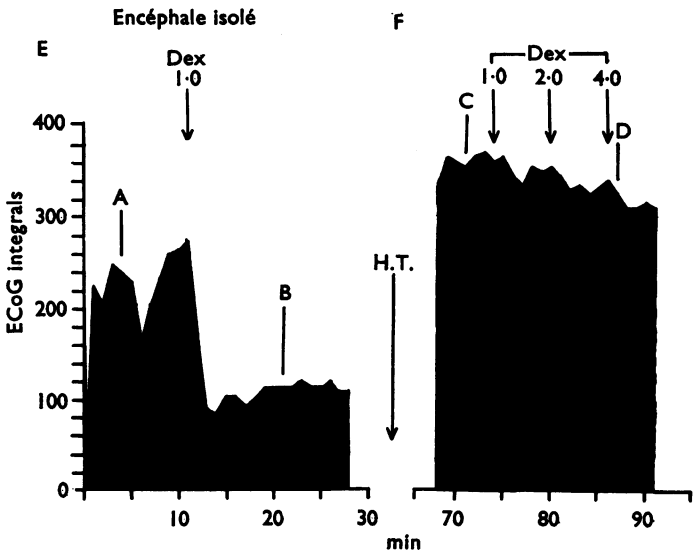


FIG. 6. Records of electrocortical activity (A–B) and histogram of integrated activity (E) in a 12-day chicken encéphale isolé preparation and records of electrocortical activity (C–D) and histogram of integrated activity (F) in the same chicken after an anterior transection, H.T. The level of the encéphale isolé transection is indicated by the dotted line, O.....O on the sagittal section of the brain, the level of the anterior transection is indicated by the dotted line X.....X. Epochs corresponding to A, B, C and D are indicated in the histograms. Wt, 60g. A, Control record with predominantly 4–6 Hz, 100 μV activity; B, electrocortical desynchronization produced by dexamphetamine, 1·0 μmol/100g ; C, control record with predominantly <2 Hz, 100 μV activity; D, lack of effect of dexamphetamine on electrocortical activity after a total dose of 7·0 μmol/100g in three divided doses.

experiment, control electrocortical activity (Fig. 6C) consisted of slow waves with a frequency up to 2 Hz and amplitude 150 μV with superimposed 4–6 Hz waves of lower amplitude; control integrals were approximately 350/minute. Dexamphetamine, 1·0, 2·0 and 4·0 μmol/100g was without significant effect on electrocortical activity (Fig. 6F). Before the high section the brain stem had been transected to make an encéphale isolé preparation in which dexamphetamine, 1·0 μmol/100g had reduced electrocortical integrals from control values between 220 and 280/min to about 100/min (Fig. 6E). Electrocortical desynchronization (Fig. 6B) persisted for 15 min before the chick was reanaesthetized for a high transection.

In addition to abolition of the electrocortical effects of dexamphetamine, two other types of response were encountered. Thus, in four other chicks in which

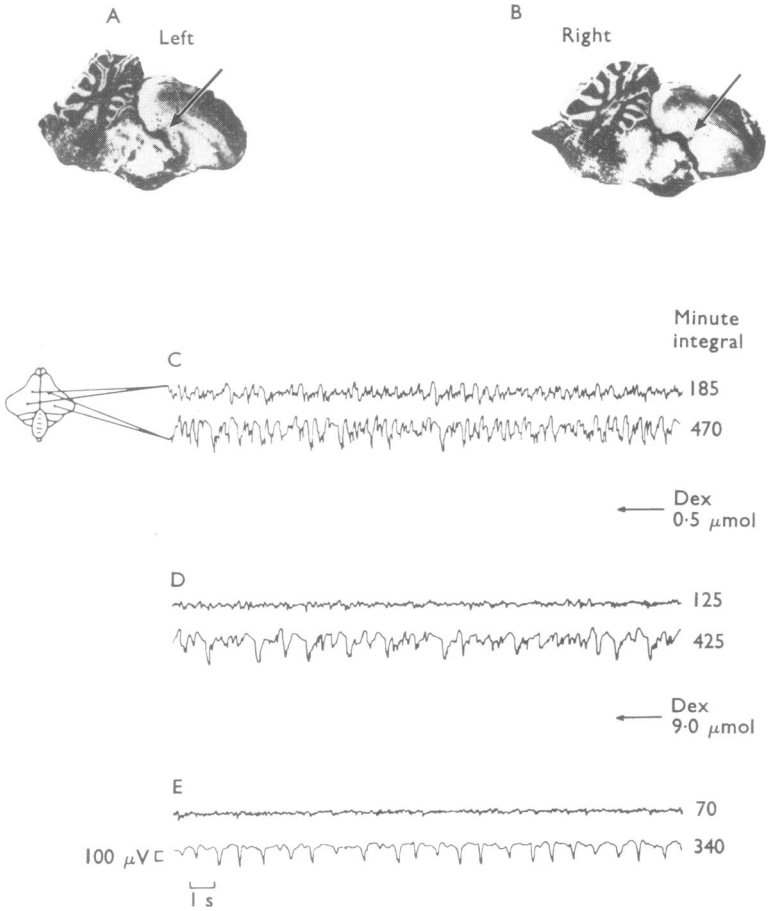


FIG. 7. Records of electrocortical activity (C–E) in a 12-day chicken with an anterior transection of the brain. The transection was incomplete on the left side of the brain (A) but complete on the right side (B). The transection is indicated by the arrow in the two microphotographs. Wt, 65g. C. Control record consisting of predominantly 6–8 Hz, 150 μV activity in the left cerebral hemisphere and predominantly <4 Hz, 300 to 350 μV activity in the right cerebral hemisphere; D, dexamphetamine, 0·5 μmol/100g produced electrocortical desynchronization (10–12 Hz, 50–75 μV activity) in the left cerebral hemisphere but not in the right cerebral hemisphere; E, a total of 9·5 μmol/100g dexamphetamine produced intense electrocortical desynchronization in the left cerebral hemisphere (20–25 Hz, 50 μV activity) and electrocortical slowing in the right cerebral hemisphere (<4Hz, 250 μV activity).

dexamphetamine, 1·0 μmol/100g was without effect on electrocortical activity, further equal doses produced electrocortical 'flattening' lasting 15–20 minutes. There was a rapid reduction in amplitude until the electrocorticogram became isoelectric, followed after a few minutes by a gradual increase in amplitude. On recovery, the electrocorticogram frequently contained 4–6 Hz activity, not present before dexamphetamine. In a chicken in which electrocortical activity was unaffected by dexamphetamine, 1·0 μmol/100g, further equal doses produced progressively larger amplitude reductions (no frequency change) until, after a total of 6·0 μmol/100g, the electrocorticogram became isoelectric; integrals and electrocortical activity returned to the control pattern within 15 min of each injection. Thus tachyphylaxis to this 'flattening' response did not occur.

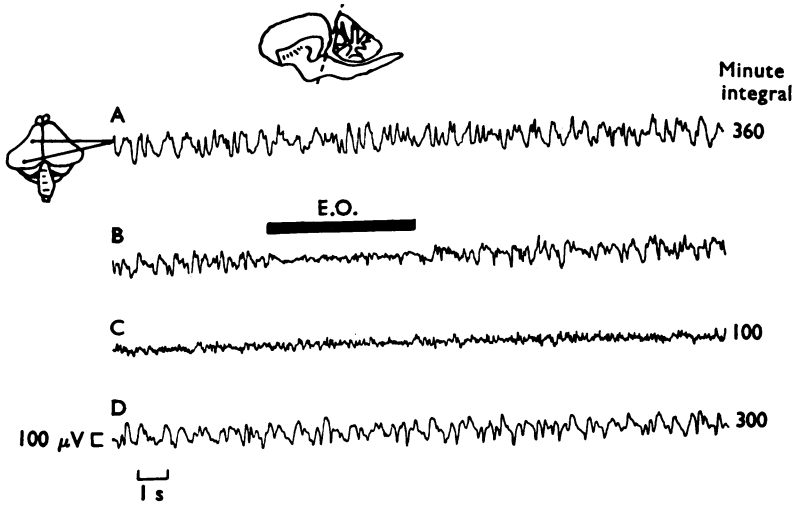
Four chickens, in which section was complete on one side but either incomplete contralaterally or complete in a plane posterior to some thalamic nuclei, provided additional information as to the extent of the lesion required to prevent the electrocortical alerting effects of dexamphetamine. In such an experiment, the section isolated the right hemisphere from the diencephalon (Fig. 7B) but was incomplete on the left so that ascending connexions between certain lateral thalamic nuclei, for example nucleus rotundus and the corresponding hemisphere remained intact (Fig. 7A). Dexamphetamine, 0·5 μmol/100g, evoked electrocortical desynchronization in the left cerebral hemisphere, integrals falling 46% from 185 to 100/minute. In contrast, desynchronization was not obtained in the right hemisphere even after a total of 9·5 μmol/100g given in divided doses over 60 min (Fig. 7E); integrals declined from 470 to 340/min, a reduction of 28%.

Transections through the diencephalon but anterior to nucleus ruber. Seven chickens were prepared. The most caudal transection in this group passed through the posterior commissure in a plane anterior to nucleus ruber but posterior to the optic chiasma (Fig. 1, transection 2). In those preparations in which the transection was posterior to the optic chiasma, ascending retino-tectal pathways were undamaged since passive eye opening produced contralateral electrocortical desynchronization. Two types of response to dexamphetamine were observed, not attributable to different levels of transection; in each, dexamphetamine elicited electrocortical desynchronization and not 'flattening'.

The first type of response, a reduction in duration and intensity of the electrocortical effect of dexamphetamine, was obtained in four chicks. Dexamphetamine, 1·0 μmol/100g, reduced integrals by an average of 15%; a second injection reduced integrals an average of 75% but the effect was of short duration, integrals returning to preinjection values within 30 minutes. Thus, after dexamphetamine, 1·0 μmol/100g, control electrocortical activity which consisted of high amplitude (300 μV) slow frequency (<2 Hz) waves was reduced slightly in amplitude, electrocortical integrals falling 13% from 375 to 325/min (Fig. 8E). A second injection of 1·0 μmol/100g 12 min later produced electrocortical desynchronization (Fig. 8C), integrals falling a further 72% from 325 to 90/min with a return to preinjection values after 2 min (Fig. 8E).

The second type of response to dexamphetamine—an increase in intensity but a decrease in duration—was obtained in three chicks. In two, dexamphetamine, 1·0 μmol/100g, lowered integrals an average of 93% and in one, dexamphetamine 0·25 μmol/100g lowered integrals 80%, effects lasting 20 minutes. The response to

dexamphetamine was more intense than in the encéphale isolé preparation. In such an experiment, dexamphetamine, 1·0 μmol/100g, reduced integrals 93% from 350/min to 25/min; integrals and electrocortical activity returning to preinjection patterns within 15 minutes. A second similar injection produced a slightly greater effect both in reduction of integrals, 97% and duration, 20 minutes.



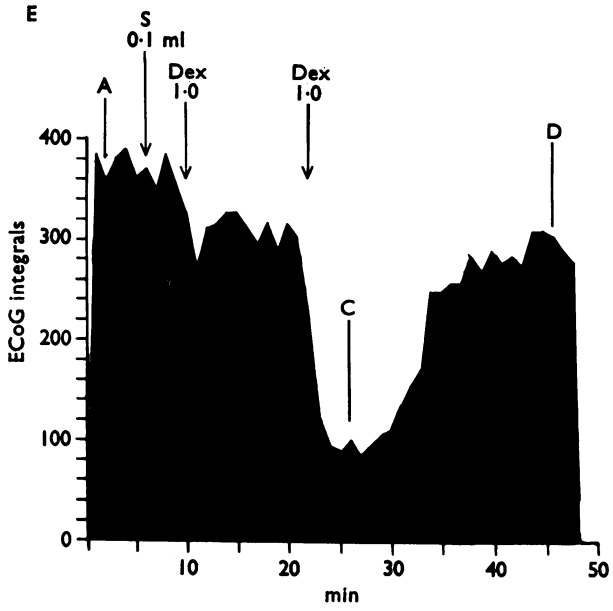


FIG. 8. Records of electrocortical activity (A–D) and histogram of integrated activity (E) in a 12-day chicken with an anterior transection through the diencephalon but anterior to the nucleus ruber (level of transection shown by dotted line on sagittal section of brain). Epochs corresponding to A, C and D are indicated in the histogram. Wt, 40g; S, saline. A, Control record with predominantly 2 Hz, 300 μV activity; B, effect of passive right eye opening (indicated by bar) on control electrocortical activity; C, electrocortical desynchronization obtained after a total of 2·0 μmol/100g dexamphetamine; D, return of electrocorticogram to control record.

Transections anterior to the encéphale isolé transection but posterior to nucleus ruber. In two chickens maintained on artificial ventilation in spite of slight spontaneous respiratory movements, responses to dexamphetamine, 1·0 μmol/100g, were indistinguishable from those in the encéphale isolé. In the first, electrocortical integrals fell 47% from 320 to 170/min and in the second, 64% from 220 to 80/minute. In the second preparation, dexamphetamine produced tachypnoea, respiratory rate increasing to 144/min despite continuing artificial ventilation at 24 strokes/minute.

Discussion

The encéphale isolé preparation enables drug effects on electrical activity of the brain anterior to the transection to be studied, unaffected by actions on sites posterior to the section. Effects on behaviour are limited to movements of mandible, eyelids, eyes, nictitating membranes and in mammals, the ears and vibrissae. A feature of our experiments was the preparation of the encéphale isolé; because anaesthesia tends to modify or even abolish drug effects, implantation of electrodes and other procedures were completed 24 h previously and the chick was anaesthetized only for 1–2 min to make the transection.

Behavioural and electrocortical activities of avian and mammalian (for example cat) encéphale isolé preparations differ markedly. The feline preparation exhibits periods of wakefulness alternating with sleep (Bremer, 1936), whereas chick preparations displayed uninterrupted behavioural and electrocortical sleep (followed up to 4 h after transection), passive eye opening being the only sensory stimulus to evoke electrocortical desynchronization. Because blood pressure in cat encéphale isolé preparations approximated that for cerebral anoxaemia (Rothballer, 1959), a small rise in blood pressure, spontaneous or due to drugs, could restore normal electrocortical function in an otherwise alert cat and the electrocorticogram would change from synchronized to desynchronized activity. It is not always easy, therefore, to decide whether electrocortical alerting is a consequence of the rise in blood pressure or a central action of the drug. This problem did not arise in the chick, since the mean blood pressure of three preparations tested was 133 mbar and central excitants, which elicited behavioural and electrocortical alerting, had little effect on or lowered blood pressure, whereas central depressant amines such as α-methylnoradrenaline raised blood pressure (Dewhurst & Marley, 1965b).

The predominantly contralateral electrocortical desynchronization elicited by unilateral passive eye opening was presumably a reflection of the complete decussation of retinal fibres in the optic chiasma (Cowan, Adamson & Powell, 1961) and the paucity of commissural fibres. Electrocortical desynchronization in the chick encéphale isolé preparation did not outlast eye opening, that is was of the 'phasic' variety ascribed by Sharpless & Jasper (1956) in cats to diencephalic mechanisms in contrast to 'tonic' arousal, which outlasts the eliciting stimulus, observed in mammalian encéphale isolé preparations and attributed to reticular mechanisms.

A dose of dexamphetamine, insufficient by itself to elicit electrocortical arousal, converted 'phasic' desynchronization obtained on contralateral eye opening to 'tonic' arousal and elicited ipsilateral cortical desynchronization; the persistence of electrocortical desynchronization after electrical stimulation of the brain stem was also more marked. In cat encéphale isolé preparations, amphetamine 0·01–1·0

mg/kg intravenously, progressively lowered the threshold for electrocortical desynchronization, elicited by stimulation of the reticular formation, as the total quantity of drug injected increased (Bradley & Key, 1958).

α-Methylnoradrenaline, in contrast to dexamphetamine, raised the threshold at which stimulation of the reticular formation produced electrocortical desynchronization and abolished 'phasic' cortical desynchronization elicited by passive eye opening. Comparison of these results with those from other species is difficult since, so far as it is known, the young chick is the only species in which systemically administered catecholamines have central depressant effects, behavioural and electrocortical arousal being elicited in the young and adult of other species. The findings with α-methylnoradrenaline in the chick encéphale isolé preparation are compatible with its central depressant effects in intact young chicks, for behavioural sleep, lowering of temperature and oxygen consumption were obtained on microinfusing the drug into the hypothalamus (Marley & Stephenson, 1968, 1970).

α-Methylnoradrenaline was a less efficient antagonist of dexamphetamine in the encéphale isolé preparation than in intact chicks suggesting that transection precluded some influence of α-methylnoradrenaline acting on the brain as a consequence of its effect on the spinal cord. Certainly, in spinal chicks, a crossed extensor reflex of the lower limbs was extremely sensitive to the depressant actions of α-methylnoradrenaline, 0·05 μmol/100g intravenously, reducing peak tension by 75% for 2–3 min with recovery over 15 min (Marley & Stephenson, 1971).

Behavioural and electrocortical arousal elicited in chick encéphale isolé preparations with dexamphetamine, (−)-amphetamine, α-methyltryptamine, cyclopentamine, β-tetrahydronaphthylamine and tuaminoheptane were also observed with similar doses in intact chicks (Key & Marley, 1962; Dewhurst & Marley, 1965b, c). Phenethylamine did not produce arousal in encéphale isolé preparations although it does briefly in intact chicks. However, after pretreatment with an amine oxidase inhibitor, mebanazine, long lasting behavioural and electrocortical arousal were elicited in chick encéphale isolé preparations with phenethylamine or tryptamine as noted (Marley, 1968) in intact chicks. Its duration of action was now similar to that of its α-methyl derivative, amphetamine.

That tryptamine had similar excitant effects to dexamphetamine and the other excitant amines tested and that these excitant effects were antagonized by methysergide, a selective tryptamine antagonist, suggests an action on central tryptamine receptors as proposed for amphetamine by Vane (1960), and Gelder & Vane (1962). Similar conclusions were reached with intact chicks (Dewhurst & Marley, 1965c) although observations from operant studies in chicks indicate that an action on central tryptamine receptors cannot explain the entire action of dexamphetamine (Marley & Morse, 1967).

The site of central action of dexamphetamine in chicks was further investigated by studying its effects on behaviour and electrocortical activity after acute mesencephalic and diencephalic transections. In cats, behavioural and electrocortical alerting to amphetamine in encéphale isolé preparations were abolished by a cerveau isolé transection and were not obtained in cats with a cerveau isolé transection (Bradley & Elkes, 1953, 1957); it was necessary for the anterior transection to pass through the posterior third of the thalamus to emerge at the base of the brain, just

anterior to the mammillary bodies (Hiebel *et al.*, 1954). In rabbits, in which the basilar artery was ligated at the midpontine level, the area anterior to ligation was supplied by the carotid arteries whereas that posterior was supplied by the vertebral arteries; intracarotid but not intravertebral injections of dexamphetamine evoked electrocortical arousal indicating an action localized to the anterior midbrain reticular formation (van Meter & Ayala, 1961).

Responses of mammals and birds to acute mesencephalic and diencephalic lesions differ but because of their different neuroanatomical organizations, comparison is difficult. The feline cerveau isolé preparation exhibits behavioural and electrocortical sleep and is unrousable (Bremer, 1935) save for electrocortical desynchronization with olfactory stimuli (Morruzi, 1952). In contrast, cats with a more caudal transection, for example the ' pretrigeminal midpontine ' preparation, exhibit persistent (up to 9 days) low voltage desynchronized activity for 70 to 90% of recording time (20–50% in isolated intact cats), whereas a slightly more rostral transection (pretrigeminal rostropontine transection) produced uninterrupted (up to 7 days) synchronized activity (Batini, Moruzzi, Palestini, Rossi & Zanchetti, 1958). Influence of time upon recovery of chickens with anterior transections of the brain is not known, but in contrast to the absence of righting and other postural activities of cats with acute mesencephalic transection, chickens with equivalent transections usually and spontaneously developed righting and standing within 60–80 min of transection: righting and standing, if not present, were elicited by dexamphetamine. Facilitation of righting by dexamphetamine (Maling & Acheson, 1946; Macht, 1950) or by pipradol (Brown & Werner, 1954) occurs in chronic decerebrate cats.

Chickens with acute transections of the diencephalon or mesencephalon exhibited only slow frequency electrocortical activity, indicating the existence of an autochthonous synchronizing centre in the cerebral hemispheres. This slow wave activity was present even in chicks with righting and standing reflexes and with open eyes. The fast frequencies superimposed on electrocortical slow wave activity noted in encéphale isolé preparations presumably depends on intact connexions with the reticular formation, since with more anterior transections which would sever these connexions, the superimposed fast frequencies were absent. Spontaneous desynchronized activity was not observed after any of the transections.

In chicks with transection anterior to a plane passing dorsally through the posterior commissure and ventrally between the optic chiasma and mammillary bodies and posterior to one passing through the junction of the telencephalon with the diencephalon, the duration of electrocortical arousal after dexamphetamine was reduced compared to that with the encéphale isolé preparation; the intensity of effect varied, being greater or less than that in the encéphale isolé preparation. Decreased sensitivity to amphetamine (viz. less than that in the encéphale isolé preparation) might explain the failure of a subcutaneous injection of (±)-amphetamine (0·5 mg/100g, that is, 2·7 μmol/100g) to elicit electrocortical desynchronization in one cerebral hemisphere of a chicken with an ipsilateral thalamo-mesencephalic hemisection (Spooner & Winters, 1966). Increased intensity of electrocortical arousal, albeit of reduced duration, may be likened to that induced by amphetamine in cats with prebulbar transection (Hiebel *et al.*, 1954). A pontine homologue is present in birds (Brodal, Kristiansen & Jansen, 1950) but lies posterior to the transections under consideration.

Dexamphetamine failed to produce electrocortical desynchronization in chicks with a transection at the diencephalic-telencephalic junction; in chicks with an oblique section, desynchronization was restricted to the hemisphere with intact connexions to the diencephalon also (Spooner & Winters, 1966). However, the isolated cerebral hemisphere is conceivably incapable of desynchronized electrocortical activity, in which case such a supposition would be groundless. We tried to meet this objection by eliciting electrocortical arousal with physostigmine in chicks with a diencephalic-telencephalic transection and which did not respond to amphetamine; however, even extremely small doses of physostigmine were lethal in such preparations.

In the best cat isolated cerebral hemisphere preparations, electrocortical activity consists of 300–1,000 μV, 0·5–4·0 Hz potentials with superimposed 25–70 Hz rhythm (Kellaway, Gol & Proler, 1966). In poorer preparations, this activity was interrupted by brief recurrent isoelectric periods (' flattening '), prolonged and more frequent during hypoxia, and considered an expression of a ' pathological ' state. Nevertheless, in anaesthetized cats or non-anaesthetized encéphale isolé preparations, electrocortical ' flattening ' in response to brain stem excitation was deemed physiological (Ingvar & Söderberg, 1958). Similar isoelectric periods interrupting the large amplitude potentials were observed in chicks immediately after but not before anterior transections.

Electrocortical ' flattening ' frequently occurred after dexamphetamine in chicks with a transection separating the telencephalon from the diencephalon. This was unlikely to be due to reduced cortical circulation, since the vascular effects of repeated doses of dexamphetamine diminished whilst duration of electrocortical ' flattening ' increased. Moreover, transections above the pons do not affect blood pressure (Bard, 1960; Reis & Cuénod, 1964), and in cats, at least, dexamphetamine increases cortical blood flow (Ingvar, 1958). A possible explanation is that if the cortical blood supply is barely adequate, dexamphetamine may so increase metabolic demands as to render the sectioned hemisphere hypoxic, electrocortical ' flattening ' being an expression of this.

The results indicate that integrated electrocortical activity of chick encéphale isolé preparations is sufficiently stable for central effects of excitant amines, and their antagonism, to be quantified. More anterior transections of the brain suggested that dexamphetamine produced electrocortical arousal in the chick by a mechanism similar to that postulated for cats (Bradley & Elkes, 1953 ; Hiebel *et al.*, 1954), that is, a generalized action on the reticular formation. The degree of electrocortical desynchronization was related directly to the amount of brain stem having connexions with the cortex. Not surprisingly therefore, microinfusions of dexamphetamine into discrete areas of the brain stem lacked effect on behaviour and electrocortical activity (Marley & Stephenson, 1968). However, the brain region from which dexamphetamine elicited electrocortical desynchronization differed from that in mammals in that the entire diencephalon was included. Indeed, the area involved extended rostrally to include the posterior portion of the paleostriatum.

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