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POSTNATAL HIPPOCAMPAL GRANULE CELL AGENESIS IN THE RAT: EFFECTS ON TWO TYPES OF RHYTHMICAL SLOW ACTIVITY (RSA) IN TWO HIPPOCAMPAL GENERATORS

IAN Q. WHISHAW, BRIAN H. BLAND and SHIRLEY A. BAYER

Psychology Department, The University of Lethbridge, Lethbridge, Alberta TlK 3M4, Psychology Department, University of Calgary, Calgary, Alberta (Canada), and Biology Department, Purdue University, West Lafayette, Ind. (U.S.A.)

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SUMMARY

Hippocampal slow wave activity was studied in rats which were normal or had been subjected to dentate gyrus granule cell agenesis by focal X-irradiation starting at birth (0-day group) or two days (2-day group) of age. X-irradiation reduced adult brain weight, abolished most (2-day) or all (0-day) granule cells in the lower (endal) blade of the dentate gyrus, and reduced granule cell density by up to 70% in the upper (ectal) blade of the dentate gyrus. X-irradiation did not affect pyramidal cells of the hippocampus proper. Tracking with microelectrodes in urethane anesthetized rats given eserine, sensory, or brain stimulation showed two foci of hippocampal rhythmical slow activity (theta or RSA), one in stratum oriens of CA1 and one in stratum moleculare of the dentate gyrus. These were opposite in phase by approximately 180° and separated by a null zone and phase reversal point occurring at stratum radiatum. There were no significant differences in the amplitude, frequency, null, or phase reversal points in the normal or X-irradiated groups. However, the width of the RSA amplitude peak in the ectal blade was reduced, correlated with the reduction in the length of the ectal blade, and the RSA amplitude peak in the lower blade was absent, correlated with the absence of the lower blade. The fast activity recorded in the hilus of normal rats was absent in the X-irradiated groups. RSA recorded during spontaneous movement (walking) had identical amplitude, frequency, anatomical foci, and phase in both the hippocampus proper and dentate gyrus in normal and X-irradiated rats. Antimuscarinic, but not antinicotinic, agents abolished anesthesia-related RSA, but not movement-related RSA, in all groups of rats. The results are discussed in terms of their relevance to the two-generator hypothesis of RSA.

INTRODUCTION

Several recent studies have provided support for the view that there are two anatomically separate generators of hippocampal rhythmical slow activity (RSA or 'theta' rhythm) in the rat[19,37,40,41], rabbit[17,42] and cat[18]. One (dorsal) generator is located in the stratum oriens of the CA1 region of the hippocampus and the other (ventral) in the stratum moleculare of the dentate gyrus, both in the upper (ectal) blade and the lower (endal) blade. Additional support for the 'two-generator' hypothesis comes from demonstration of a tight coupling of the extracellular discharges of CA1 pyramidal cells and dentate granule cells to hippocampal RSA[8,12,35] and demonstration of synchrony between the membrane potentials of dentate granule cells and dentate gyrus RSA[10].

Attempts to test the two-generator hypothesis have included selective interference with one generator. Surgical interference with the CA1 generator abolishes CA1 RSA leaving dentate RSA virtually unaltered[17,19]. Unfortunately, the converse of this experiment has been precluded to date since interference with the dentate region also interferes with the CA1 region blood supply[17]. However, one way to do this is to utilize X-irradiation procedures in neonatal rats. The multiplying cells of the nervous system are extremely radiosensitive[6,7,14] allowing the selective elimination of postnatally forming granule cells. Since most of the granule cells of the dentate gyrus of the hippocampus originate postnatally in altricial species such as the rat[1,4,5,11,23], focal X-irradiation of the hippocampus during infancy results in granule cell agenesis without direct harm to the prenatally formed pyramidal cells of the hippocampus proper[15,24].

In the present study we compared the generator profiles and topography of hippocampal RSA in normal anesthetized and freely moving rats which had received focal X-irradiation of the hippocampus starting on the day of birth (0-day) or second postnatal day (2-day). It was expected that changes in the foci of RSA in the generator might be correlated with changes in the size of the dentate gyrus or the reduced number of granule cells. In addition, it seemed possible to determine, by comparing amplitude profiles[19] in normal and neonatally X-irradiated rats, whether there is a relation between the number or density of granule cells and RSA amplitude.

Since some recent experimental work[25,26,31,34] has suggested that there are two types of RSA, a movement-related anticholinergic-resistant form, and a immobility-related anticholinergic-sensitive form, a further object of the experiments was to see if both forms of RSA were still present at both generator sites in normal and X-irradiated groups. Both the anticholinergic-sensitive and anticholinergic -resistant forms of RSA have been shown to be present at both generator sites in normal rats paralyzed by neuromuscular blockade[37]. In the present paper we extend this work to include normal freely moving animals and freely moving animals previously subjected to radiation-induced granule cell agenesis.

METHODS

Animals. Sixty-five Purdue–Wistar male albino rats, cross-fostered and raised

6 to a litter were used in the experiments. Thirty-eight rats (11 control, 13 2-day, and 14 0-day) were used in the acute experiments and 27 rats (15 control, 6 2-day, and 6 0-day) were used in the chronic experiments.

X-irradiation. The details of the X-irradiation technique[2,16] and the morphological effects of the X-irradiation exposures[14] have been provided in previous papers. Before irradiation the experimental pups were immobilized in holders and placed under two protective lead sheets separated by a narrow slit so only that portion of the head containing the hippocampus was exposed. The location of the hippocampus from 2 to 18 days of age had been previously determined in sagittal slices of the head by measuring the length from the snout to the anterior and posterior borders of the dorsal hippocampus. The X-ray source was a Maxitron 300 kV unit and exposure rate was 50 R/min with the beam filtered through 1.5 mm copper. The 2-day X-irradiated group received: 200 R on postnatal days 2 and 3 followed by 150 R on days 5, 7, 9, 11, 13 and 15. The 0-day X-irradiated group received: 200 R immediately after birth and on postnatal day 1, followed by 150 R on days 3, 5, 7, 9, 11 and 13. The control animals were wrapped and handled but did not receive X-ray exposure. Electrophysiological analysis was made when the animals weighed between 350-450 g.

Acute experiments

Surgical procedure. In the acute experiments animals were anesthetized with ethyl ether, Xylocaine hydrochloride (4%) was topically applied to exposed tissue, the trachea cannulated to facilitate breathing, and the external jugular vein cannulated for infusions. Urethane (1 g/ml) was administered intravenously to 30 rats to maintain anesthesia. Each animal was placed in a stereotaxic instrument and appropriate connections made for monitoring heart rate and body temperature. Rectal temperature was maintained at 38 °C by means of a servo system. Narrow slits or holes were drilled into the skull to allow access to the brain.

Eight rats were studied under curare, the only difference in procedure being that instead of urethane they received intramuscular injections of D-tubocurarine chloride (0.8 mg/kg). All pressure points and surgical incisions were treated with Xylocaine hydrochloride (4%) at regular intervals and breathing was maintained with a small animal respirator (tidal volume 10–12 ml, rate 60–70 strokes/min.).

Recording. Recording of unit potentials and gross electrical activity was made with either glass micropipettes (2 M NaCl, 1–5 MΩ) or 127 μm electrolytically sharpened and kynar insulated tungsten microelectrodes (1 μm tip, 5–10 MΩ). Some glass microelectrodes were also prepared for dye marking by saturating the sodium chloride with Fast Green FCF. Neocortical activity was monitored with two 250 μm diameter stainless steel electrodes insulated except for the cross-sectional area of the tip. For bipolar recording one electrode was cut 0.5 mm longer than the other[19]. For brain stimulation a similar bipolar electrode with tips cut at the same length was used. The biological potentials were recorded on a Grass Model 7C polygraph or stored on tape for subsequent analysis.

RSA was elicited by sensory stimulation (stroking the back), intravenous eserine (0.1–1 mg/kg) or electrical stimulation of the posterior hypothalamus of the rat.

Square wave stimulation parameters were: current 50–400 μA; duration 0.1 msec; frequency 100 Hz; pulse train duration 2–30 sec.

Hippocampal electrical activity was routinely monitored by an exploratory and reference electrode, amplified, and displayed in the conventional manner. The reference electrode was located at an appropriate depth in either the stratum oriens of CA1 or stratum moleculare of the dentate gyrus (see refs. 17 and 19) of the hippocampus ipsilateral or contralateral to the exploratory electrode. Profiles[19] of hippocampal electrical activity were made at 0.25–1.0 mm steps through the extent of the hippocampus in its sagittal dimension. In each profile EEG was sampled during stimulation at 25–100 μm steps to a depth of 6–8 mm below the dura. Profiles were made at distances of 2.5 and 4.0 mm lateral to the midsagittal fissure.

In experiments using glass micropipettes, localization of the tips in nervous tissue was achieved by ejecting a small quantity of dye from the electrodes saturated with Fast Green FCF[30]. In experiments utilizing tungsten electrodes, tip locations were marked by passing a DC current through the electrodes in order to make a small lesion (5–10 μA for 1 sec, electrode negative).

Analysis. Measurements of frequency and amplitude of hippocampal activity were made directly from the analogue signals. Phase changes between the reference electrode and the roving electrode were analyzed using 4 different techniques[19]. (1) An x/y display was used, the x-axis being the reference electrode and the y-axis being the roving electrode after amplitude normalization. When the phase angle was 0° the Lissajous pattern was an oval 45° to the x-axis. When the phase angle was 180° the Lissajous pattern was an oval 45° to the x-axis but in the opposite direction to the 0° phase angle. (2) By superimposing the baselines of the oscilloscope records of the reference and roving electrode 0° and 180° phase angles were directly visualized along with null zones and zones of fast activity. (3) One channel of the oscillograph was used to record between the reference and roving electrode (i.e. a bipolar recording). With such a difference recording, out of phase waves were summated additively and in phase waves were subtracted. (4) Detailed experiments were carried out on one control, one 2-day X-irradiated rat, and one 0-day X-irradiated rat, analyzing phase changes first under a curare condition and then repeating the profile with urethane added. For these experiments a second polygraph was used with the chart speed set at 100 mm/sec. Measurements were made directly from the chart in the following manner: On the reference and roving electrodes the DC baseline was taken as a zero crossline, negativity down and positivity up. The reference electrode was located in stratum moleculare of the fascia dentata in all tests. A minimum of 5 consecutive waves were analyzed at each step, the steps varying from 25 to 100 μm. On the reference record the distance in mm was measured for each wave from the 0 cross point for positivity to the 0 cross point for negativity and then averaged. This value gave the distance for 180° phase shift of the reference record. A vertical line was then drawn from the 0 cross point for positivity (leading edge of the wave) down through the matching wave on the roving record for each of the 5 waves. Each of the 5 waves of the roving record were measured from this vertical line to the 0 cross point for positivity (leading edge) of the wave and the mean value calculated. This value over the mm distance for 180°

times 180 gave the mean phase relation between the reference and roving electrode.

Histological analysis. At the completion of the experiments animals were deeply anesthetized with sodium pentobarbital and perfused intracardially with 10% neutral formalin. The brains were carefully removed, weighed, placed in Bouin's fixative for 24 h, further fixed in 10% neutral formalin, and embedded in paraffin. Sagittal sections or coronal sections of the hippocampal region were cut at 6 μm. Sections were mounted and stained with hemotoxylin and eosin, or thionin. Granule cells in the ectal limb (facing the cerebral hemispheres) and the endal limb (facing the thalamus) of the dentate molecular layer (excluding endothelial cells) were counted in coronal sections at a level 3.0 mm posterior to bregma. In addition, granule cells in the dorsal part of the hippocampal region were counted in the sagittal plane at a point 2.5 mm lateral from the midline. Finally, the length of the granule cell extension in the dentate gyrus was measured in the sagittal plane 2.5 mm from the midline.

Chronic experiments

Electrodes and surgery. Under pentobarbital anesthesia, tungsten microelectrodes were implanted stereotaxically in the rats in the chronic series. Subminiature connectors with a length of number 26-gauge hypodermic tubing soldered to the bottom were friction-fitted on the external ends of tungsten microelectrodes. Eight microelectrodes were implanted in each animal, 4 on each side. For a given animal the array was a straight line running either sagittally or coronally through the hippocampal formation, the interelectrode distance being 1 mm. Typically, the array on one side was aimed at regio superior and the array on the other side at regio inferior. The electrode assembly was fixed to the skull with stainless steel screws and dental cement. One of the screws with an attached male connector served as the ground connection.

Drugs. The drugs used and their intraperitoneal doses were: atropine sulfate, 50 mg/kg; scopolamine, 10 mg/kg; mecamylamine hydrochloride, 10 and 20 mg/kg; and nicotine hydrobromide, 5 mg/kg. The dosages for mecamylamine and nicotine were arrived at by determining the LD_{50} in naive rats.

Procedure. Behavioral testing consisted of observing spontaneous behaviors in an open-field situation. The open-field was a 46.5 cm square Faraday cage constructed of wood and copper mesh. The animals were connected directly to cathode follower inputs with shielded phono-pickup cable. Typical behaviors observed were walking, rearing, turning, face-washing, postural shifts, head movements, and alert immobility. The behaviors were indicated on the polygraph chart with the use of signal markers by the experimenter observing the animal and by written notations by a second experimenter operating the polygraph. Once baseline testing was completed the animals were randomly assigned to various drug groups and testing was repeated.

A total of 15 rats were tested during spontaneous or tail-pinch-induced walking and the data were analysed for quality, frequency, and amplitude of RSA in dorsal and ventral generator sites. All rats were tested with atropine sulfate and scopolamine on separate days, the sequence for a given rat determined in a random fashion. At least two days elapsed between tests. The rats were then placed into 3 additional drug testing groups in a random fashion, 5 rats per group. The three groups were: (a) low

TABLE I

Perfused brain weights (g) of control rats and X-irradiated rats

Controls	*2-Day X-irradiated*	*0-Day X-irradiated*
2.175	1.970	1.786
2.055	1.810	1.738
2.035	1.954	1.790
2.170	1.913	1.782
2.089	1.899	1.805
2.135	1.915	1.882
2.109	1.910*	1.797**,***

* Different from control t = 5.99, $P < 0.001$
** Different from control t = 10.10, $P < 0.001$.
*** Different from 2-day t = 3.78, $P < 0.01$.

dose (10 mg/kg) mecamylamine followed 10 min later by scopolamine (10 mg/kg); (b) high dose (20 mg/kg) mecamylamine followed 10 min later by scopolamine (10 mg/kg); (c) nicotine (5 mg/kg) followed by scopolamine (10 mg/kg). All above mentioned drug combinations were administered to the same animals under urethane anesthesia (1 mg/kg). Hippocampal RSA after urethane injection either occurred spontaneously, was elicited by tail pinches or injections of eserine chloride (1.5 mg/kg i.p.). At the completion of the experiments electrode placements were verified histologically.

RESULTS

Acute experiments

Brain weights. Table I summarizes the data for brain weights of 6 rats in each of the 3 groups. The X-irradiation treatment had a significant effect on brain weight, $F(2,15) = 5.17$, $P < 0.05$. The brains of the X-irradiated rats weighed significantly less than the brains of the control rats, control versus 0-day $t(10) = 10.10$, $P < 0.001$, control versus 2-day, $t\ (10) = 5.99$, $P < 0.001$. The brains of the 0-day X-irradiated rats also weighed significantly less than the 2-day X-irradiated rats, $t(10) = 3.78$, $P < 0.01$.

Cell counts. The X-irradiation treatment had a significant effect on the length of the granule cell layer in the hippocampus, $F(2,9) = 14.1$, $P < 0.001$. Measures from sagittal sections taken 2.5 mm lateral to the midline showed that the mean overall length of the granule cell layer was: control, 5.26 mm; 2-day, 2.16 mm; 0-day, 1.71 mm. Examples of the extent of the granule cell layer can be seen in Fig. 1. As can be seen in Fig. 1 the greatest reduction in extent was in the endal blade which consisted of only a small 'hook' in the 2-day rats and was usually absent altogether in the 0-day X-irradiated rats. The mean length of the ectal blade was: control, 3.1 mm; 2-day, 1.9 mm, 0-day, 1.7 mm, which at the extremes represents a reduction of 55% in length between the 0-day as compared with control rats.

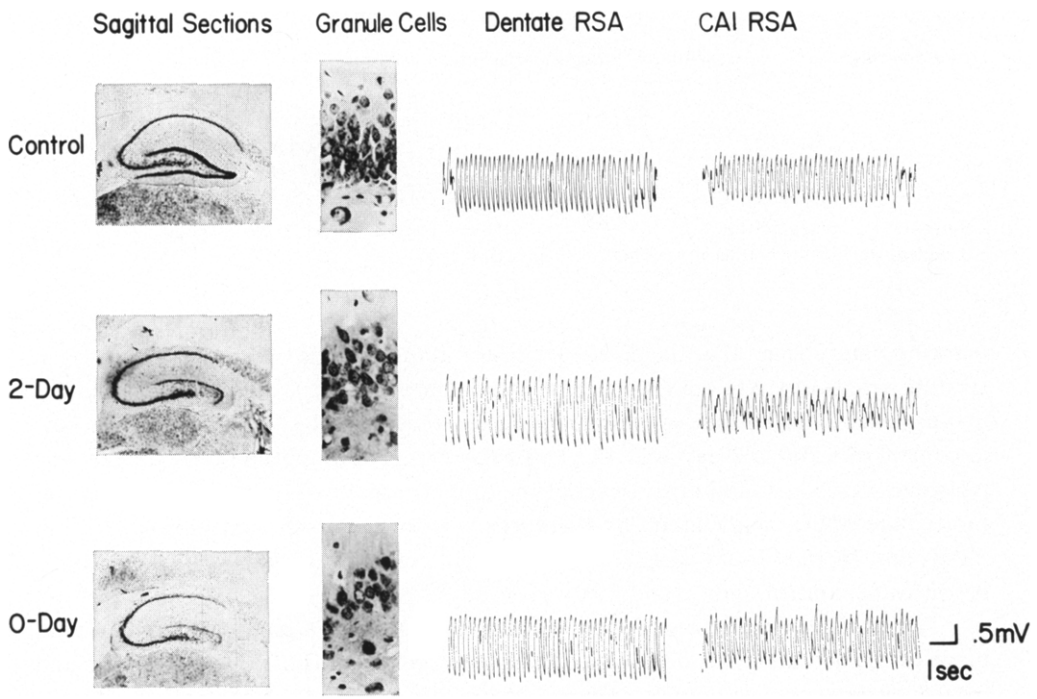

Fig. 1. Histological appearance of the hippocampus of control, 2-day X-irradiated, and 0-day X-irradiated rats and corresponding RSA recorded from the CA1 and dentate generators. Sagittal sections stained with thionin from rats from which depth profiles were obtained. Granule cells located in the ectal limb of the dentate granular layer at 30 days of age. Hematoxylin and eosin, × 640. The 2-day X-irradiated rats received 8 exposures to irradiation between days 2 and 15. The 0-day X-irradiated rats received 8 exposures to irradiation between days 0 and 13.

Total cell counts made in the coronal plane and in the sagittal plane are given in Table II. The effects of X-irradiation treatment on cell number was significant, $F(2,9) = 1{,}575$, $P < 0.001$. The granule cell count for control rats differed significantly from the 2-day group ($t = 52.16$, $P < 0.001$) and the 0-day group ($t = 51.98$, $P < 0.001$). The 2-day group also differed significantly from the 0-day group ($t = 2.33$, $P < 0.05$). The smallest number of cells counted in a sagittal section in a 0-day rat was 159 and the smallest number in a control rat was 2131. Thus, in the extreme, the reduction was up to 92% in cell number between control and X-irradiated rats. The mean granule cell density per mm length in the ectal blade was calculated to be approximately 420 cells/mm in the control rats, 140 cell/mm in the 2-day X-irradiated rats, and 119 cells/mm in the 0-day X-irradiated rats. Fig. 1 gives photomicrographs showing examples of this relative difference in cell density in the ectal blade of the different groups of rats.

Depth distribution of hippocampal RSA in CA1 dentate profile. All microelectrode tracks were successfully located in the histological material as were tip locations marked by the dye or electrolytic lesion technique. The majority of tracking was done in the sagittal plane with the distance between tracks varying from 0.25 to 1.0 mm. The

TABLE II

Dentate granule cell counts (mean and standard error of six sections)

	Controls (n = 4)	*2-Day X-irradiated (n = 4)*	*0-Day X-irradiated (n = 4)*
2.5 mm lateral (parasagittal)	2193 ± 29	304 ± 28	207 ± 30
3.0 mm posterior (coronal) to bregma	2048 ± 57	350 ± 27	224 ± 11

marking data verified that the dorsal generator amplitude maximum was localized in stratum oriens of CA1: 6/6 lesions or dye spots in control rats, 7/7 in 2-day, and 9/9 in 0-day rats. The null zone was located in stratum radiatum by 8/8 lesions or dye spots in control rats, 6/6 in 2-day, and 11/11 in 0-day rats. The amplitude peak for the ventral generator was found at the level of the hippocampal fissure proximal to stratum moleculare of the facia dentata by 19/19 lesions or dye spots in control rats, 14/14 in 2-day, and 16/16 in 0-day rats. There were no differences with respect to these points between the control, 0-day, and 2-day groups.

Despite the dramatic reduction of granule cells the RSA recorded from the ectal blade of the dentate gyrus of the 2-day and 0-day groups was of equal amplitude and frequency compared to the RSA recorded from this region of the control rats. As can be seen from Table III, which gives the mean amplitude and standard error from 39 profiles in normal rats, 29 profiles in 2-day rats, and 45 profiles in 0-day rats, there were no significant differences in RSA amplitude of the different groups in the ventral maxima at a sagittal plane 2.5 mm lateral to the midline, $F(2,167) = 0.61$, $P > 0.05$, or at a sagittal plane 4.0 mm from the midline, $F(2,64) = 1.1$, $P > 0.05$. The RSA amplitude in stratum oriens was significantly smaller in all of the groups than the RSA amplitude recorded in stratum moleculare: $F(2,167) = 37.05$, $P < 0.001$ at 2.5 mm lateral; $F(1,64) = 152.3$, $P < 0.001$ at 4.0 mm lateral to the midline.

Fig. 2 shows the mean amplitude and phase of RSA in profiles taken through

TABLE III

Amplitude (μV) of RSA in the CA1 stratum oriens and ectal blade of dentate molecular layer (mean and standard error)

	Controls	*2-Day X-irradiated*	*0-Day X-irradiated*
Stratum oriens			
2.5 mm Lateral	1080 ± 38 (n = 30)	1070 ± 55 (n = 14)	1000 ± 37 (n = 32)
4 mm Lateral	1065 ± 80 (n = 7)	1030 ± 80 (n = 13)	1185 ± 60 (n = 13)
Stratum moleculare — ectal blade*			
2.5 mm Lateral	1535 ± 20 (n = 30)	1575 ± 23 (n = 14)	1460 ± 23 (n = 32)
4 mm Lateral	1600 ± 45 (n = 9)	1625 ± 25 (n = 15)	1610 ± 39 (n = 13)

* Amplitude in stratum moleculare significantly different from stratum oriens 2.5 mm lateral to the midline, $F(2,167) = 37.05$, $P < 0.001$ and 4 mm lateral to the midline, $F(1,64) = 152.3$, $P < 0.001$.

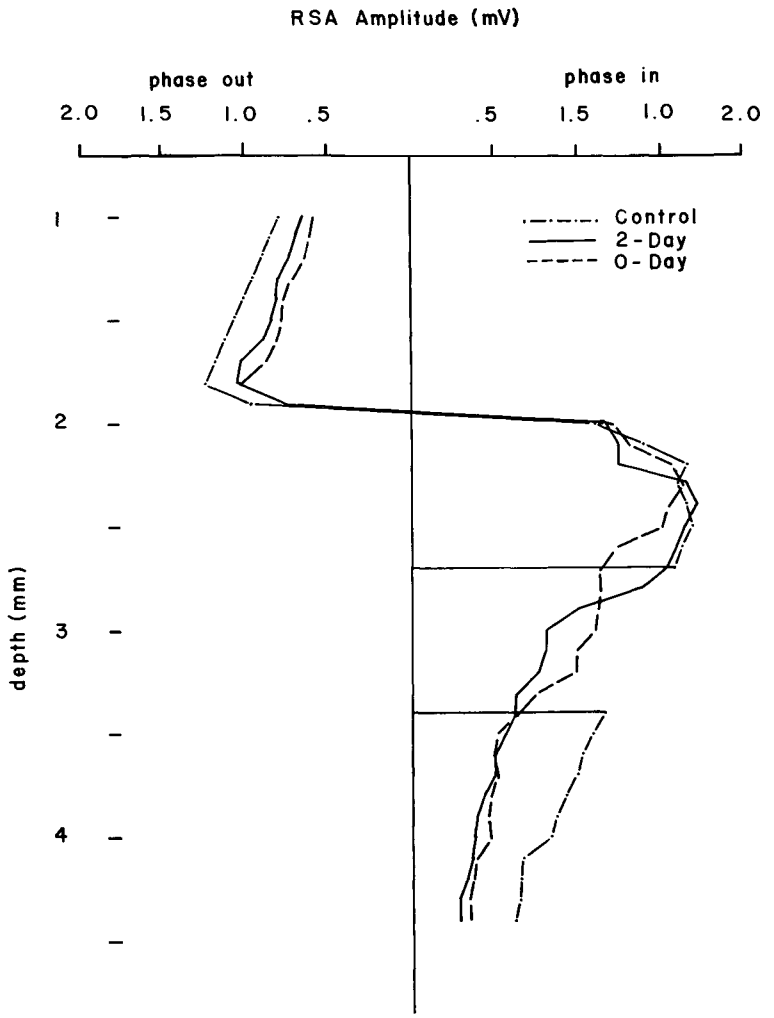

Fig. 2. Depth distribution and phase changes of hippocampal RSA in a CA1–dentate profile in control, 2-day X-irradiated, and 0-day X-irradiated rats. The graph indicates the mean amplitude (n = 4 rats per group) of RSA (mV) as recorded from the roving microelectrode and the phase compared to the fixed electrode in stratum moleculare. Parallel lines on the right side of the central axis represent the extent of the hilus of the fascia dentata. Only fast activity was recorded from control rats in this zone. Stratum oriens is located at a depth of 1.7 mm, the hippocampal fissure at a depth of 2.3 mm.

both generators from 4 rats in each of the treatment groups. The profiles were located between 1 and 1.6 mm from the blade of the dentate gyrus in each case as is indicated diagrammatically in Fig. 3. From Fig. 2 the similarity in amplitude in the two maxima as well as the similarity in the phase reversal point between the three treatment groups can be seen.

From Fig. 2 it may also be seen that hippocampal RSA of gradually decreasing amplitude was recorded through the hilus of the X-irradiated rats. In control rats predominantly large amplitude fast activity (30–50 Hz) was recorded from the hilus[19].

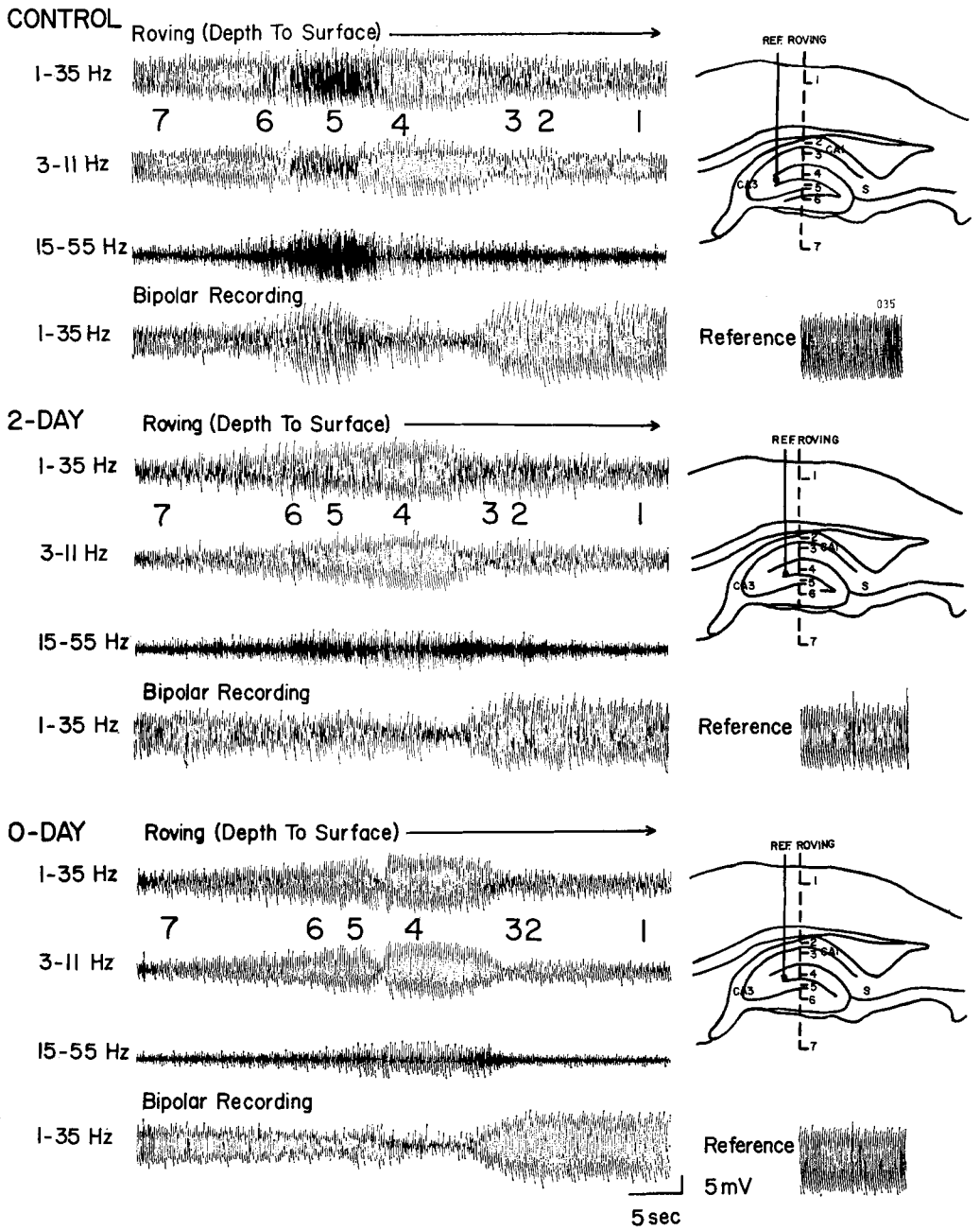

Fig. 3. Depth to surface recordings of hippocampal RSA in a CA1–dentate profile in control, 2-day X-irradiated, and 0-day X-irradiated rats. First 3 recordings of each panel were taken from the roving electrode while it was being withdrawn with the activity recorded at filter settings of 1 and 35 Hz, 3 and 11 Hz, and 15 and 55 Hz respectively. The last recording in each panel represents a bipolar recording between the roving and reference microelectrodes made as the roving microelectrode was withdrawn from the thalamus to brain surface at a constant rate. The numbers 1 through 7 correspond to depths indicated by these numbers on the diagrammatic representation of a sagittal section of the hippocampal formation. The bottom recording in each panel was taken from the reference electrode located in stratum moleculare of the fascia dentata as shown on the diagrammatic insert.

Fig. 2 shows that as the microelectrode leaves the hilus and continues through the endal blade to the thalamus of X-irradiated rats the hippocampal RSA continues to decline in amplitude. At the thalamus, RSA is lower compared to that of control rats. This difference appears to be related to the fact that the peak of RSA associated with stratum moleculare[19] in normal rats is absent in the X-irradiated rats as is this portion of the endal blade.

Examples of the recordings taken during the CA1-dentate profiles are presented in Fig. 3. The roving electrode was being withdrawn at a constant speed from depth to the neocortical surface by a hydraulic microdrive. The bottom tracing of each panel represents a bipolar recording between the roving electrode and the fixed electrode in stratum moleculare. As we have described previously[19], recording in such a manner resulted in the activity being 'subtracted' when the signals at both electrodes were isomorphic and 'summated' when out of phase. Fig. 3 illustrates the absence of fast activity in the hilus region of the 2-day and 0-day X-irradiated rats (at no. 5) and the decline in RSA amplitude from the ectal blade through to the thalamus.

One further difference between control and X-irradiated rats was that the profiles showing a ventral amplitude maxima, such as those shown in Fig. 3, were located within a narrower area in the X-irradiated rats. Such profiles were only obtained with electrode tracking that penetrated close to granule cells. This zone was reduced by approximately 50% in the X-irradiated groups. The configuration of the profiles obtained outside of this area were similar to those described in our previous work[19,37].

Analysis of phase in CA1–dentate profiles. Detailed phase analysis showed that the phase differences between the CA1 and dentate generators and the phase values relative to depth were not significantly different for each of the 3 groups. Furthermore, the phase relations were identical for the curare and curare plus urethane conditions. In 18 CA1–dentate profiles measured the range for the phase difference between the CA1 generator and the dentate generator was 142–180°. The mean phase difference for control rats (N = 9) was 160 ± 9.4°, for 2-day X-irradiated rats (N = 5) 166 ± 10.3°, and for 0-day X-irradiated rats (N = 4) 162 ± 4.7°.

TABLE IV

Phase values (degrees) relative to the stratum radiatum compared to a reference electrode located in stratum moleculare of the ectal granular layer

Depth (mm)	*Controls (n = 9)*	*2-Day X-irradiated (n = 5)*	*0-Day X-irradiated (n = 4)*
1925	170.00	177.26	166.00
1950	167.36	180.00	166.00
1975	163.96	176.86	160.00
2000	156.66	145.27	141.76
2025	21.36	32.00	38.00
2050	13.24	7.87	15.58
2075	4.39	2.22	6.80
2100	0.00	0.00	0.00
2125	0.00	0.00	0.00

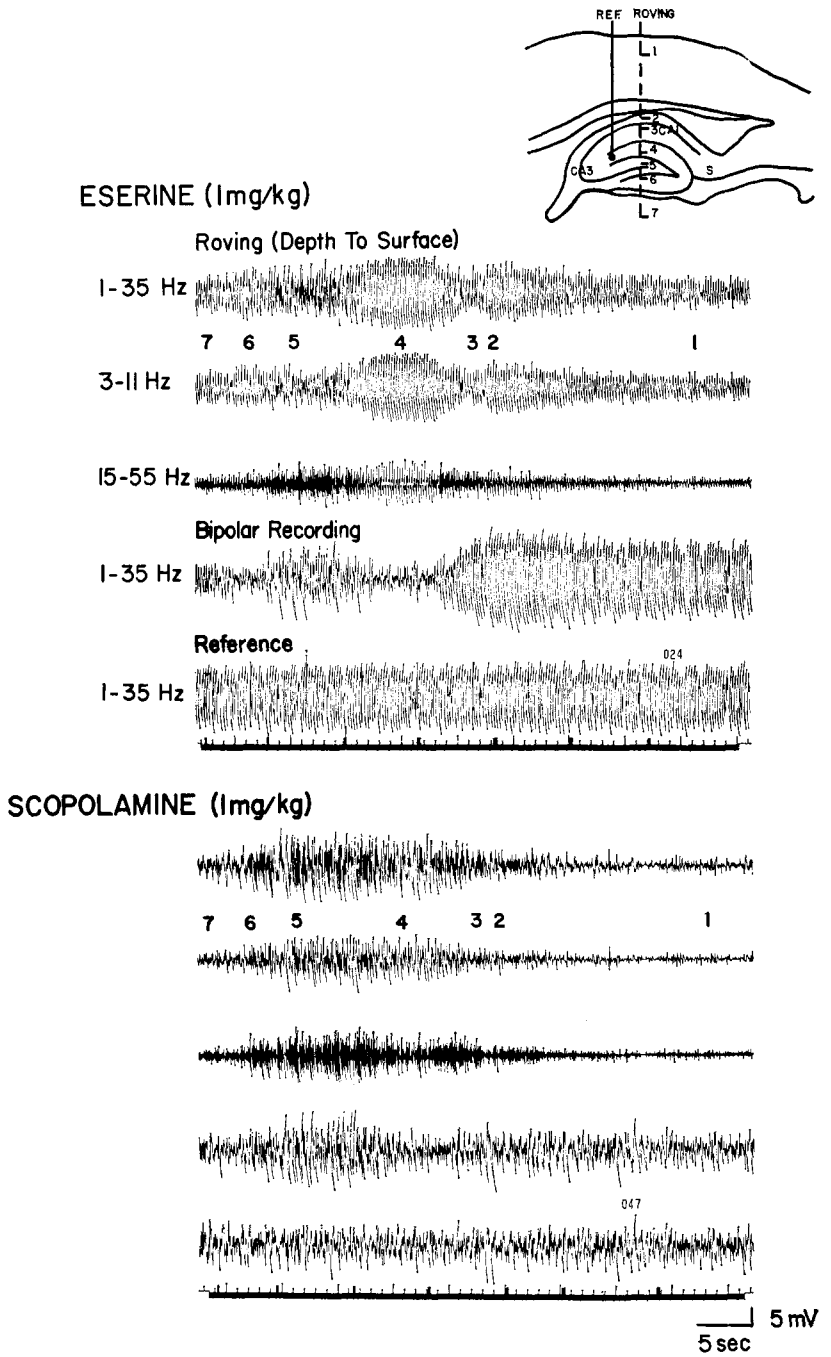

Fig. 4. Depth to surface recordings of hippocampal RSA in a CA1–dentate profile made in a control rat treated first with eserine (upper 5 tracings) and then with scopolamine added (lower 5 tracings). The first 3 recordings of each panel were taken from the roving electrode while it was being withdrawn and the activity was recorded at filter settings 1 and 35 Hz, 3 and 11 Hz, and 15 and 55 Hz respectively. The fourth recording in each panel represents a bipolar recording between the roving and reference microelectrodes made as the roving microelectrode was withdrawn from the thalamus to brain surface at a constant rate. The numbers 1 through 7 correspond to depths indicated by these numbers on the diagrammatic representation of a sagittal section of the hippocampal formation.

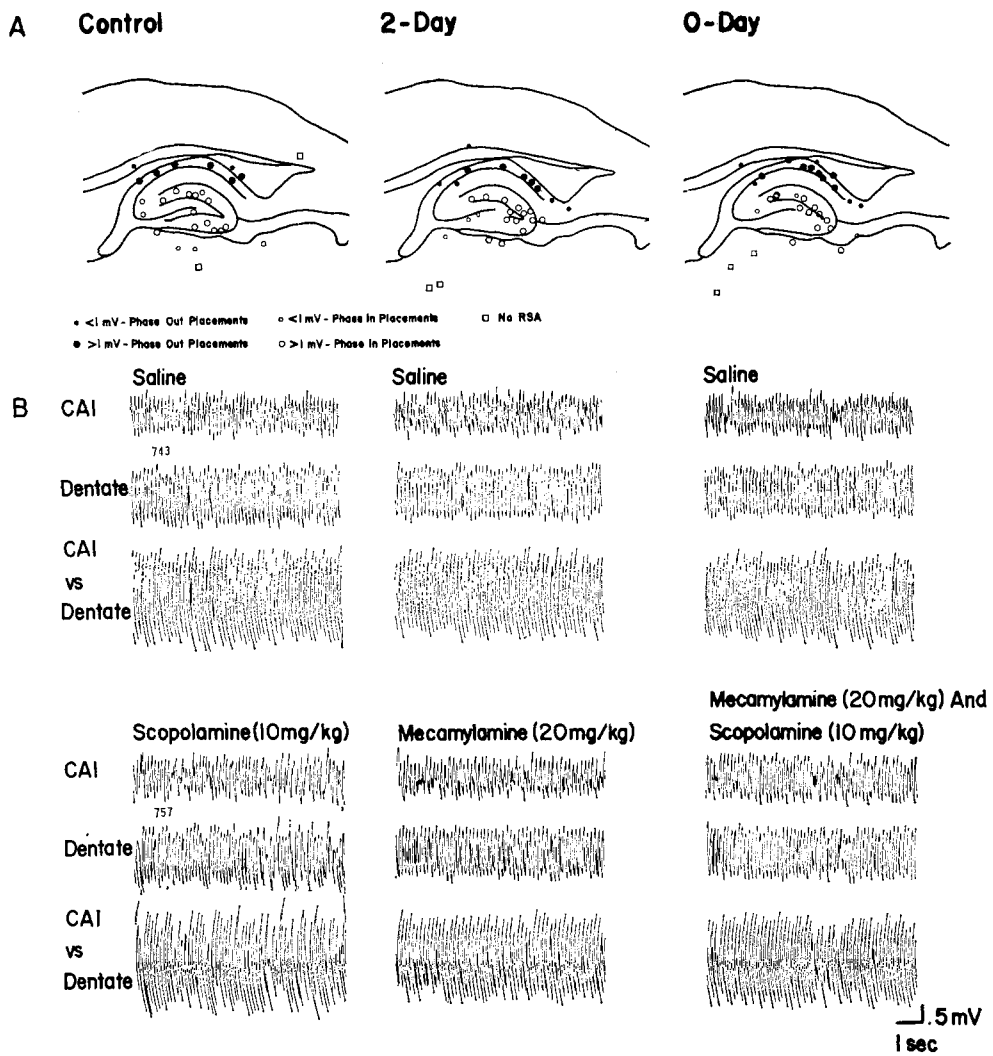

Fig. 5. Electrode tip locations in regio superior and regio inferior of the hippocampal formation of control, 2-day X-irradiated, and 0-day X-irradiated rats studied during spontaneous behavior with recordings made during walking for saline and drug conditions. A: filled circles represent placements yielding RSA either greater than or less than 1 mV located in regio superior, open circles represent placements yielding RSA either greater than or less than 1 mV located in regio inferior and open squares represent placements yielding no RSA. B: recordings shown in the first line of each panel were taken from regio superior (CA1) placements, the second line from regio inferior (dentate) placements, and the third line represents a 'bipolar' recording between the CA1 and dentate placements, indicating that the recordings were out of phase (the out of phase recordings summate). The recordings were made while the animals were walking following treatment with saline, scolopamine, mecamylamine, or mecamylamine plus scopolamine.

Table IV summarizes the data for the rate of phase reversal at representative depths for the 3 groups of rats. Below the CA1 pyramidal layer the signal reverses to an approximately 21–38° phase lag compared to the reference electrode in stratum moleculare and takes 75 μm before becoming isomorphic. Thus, within 50 μm phase reverses sharply but still has a significant lag before the signals become isomorphic. Analysis of variance carried out on these data revealed no significant differences for the phase values of the three groups of rats at equivalent depths, $F(1,17) = 1.75$, $P > 0.05$.

Effect of scopolamine. Fig. 4 illustrates a CA1–dentate profile recorded while withdrawing from the thalamus to the neocortex at a constant rate in a urethanized rat using eserine to produce the RSA. Profiles taken under such conditions were identical to profiles recorded in urethanized rats using hypothalamic stimulation or sensory stimulation to elicit RSA. The bottom panel of Fig. 5 shows that with the addition of scopolamine (1 mg/kg, i.v.) all RSA was abolished. Scopolamine abolished RSA in the profiles of 4/4 control, 4/4 2-day and 4/4 0-day rats. Thus, RSA recorded under urethane in all of the groups was of the anticholinergic sensitive type.

Chronic experiments

Histology. Diagrammatic reconstructions of the electrode placements for the control, 2-day X-irradiated, and 0-day X-irradiated rats are presented in Fig. 5A.

TABLE V

Mean amplitude (mV) and frequency (Hz) with standard errors of CA1 and dentate RSA measured during walking for undrugged and drugged (muscarinic blockers) conditions (n = number of electrode placements)

	Controls	*2-Day X-irradiated*	*0-Day X-irradiated*
Amplitude — saline			
CA1	920 ± 41 (n = 8)	905 ± 63 (n = 10)	990 ± 57 (n = 13)
Dentate	1085 ± 53 (n = 17)	1174 ± 45 (n = 17)	1065 ± 51 (n = 16)
Amplitude — atropine sulfate			
CA1	1025 ± 59 (n = 8)	1005 ± 70 (n = 10)	1060 ± 51 (n = 13)
Dentate	1155 ± 67 (n = 17)	1240 ± 37 (n = 17)	1145 ± 46 (n = 16)
Amplitude — scopolamine			
CA1	975 ± 23 (n = 8)	1005 ± 57 (n = 10)	1035 ± 58 (n = 13)
Dentate	1045 ± 65 (n = 17)	1215 ± 37 (n = 17)	1090 ± 50 (n = 16)
Frequency — saline			
CA1	7.15 ± 0.07 (n = 8)	7.13 ± 0.13 (n = 10)	7.28 ± 0.10 (n = 13)
Dentate	7.20 ± 0.07 (n = 17)	7.21 ± 0.09 (n = 17)	7.30 ± 0.12 (n = 16)
Frequency — atropine sulfate			
CA1	7.75 ± 0.09 (n = 8)	7.68 ± 0.08 (n = 10)	7.81 ± 0.16 (n = 13)
Dentate	7.77 ± 0.08 (n = 17)	7.58 ± 0.06 (n = 17)	7.93 ± 0.11 (n = 16)
Frequency — scopolamine			
CA1	7.75 ± 0.13 (n = 8)	7.45 ± 0.09 (n = 10)	7.96 ± 0.17 (n = 13)
Dentate	7.71 ± 0.10 (n = 17)	7.64 ± 0.08 (n = 17)	7.72 ± 0.17 (n = 16)

Filled circles represent locations of CA1 generator placements, open circles represent locations of dentate generator placements, and the open triangles represent placements where no RSA was recorded. A total of 28 placements were analyzed in the control group, 29 placements in the 2-day X-irradiated group, and 31 placements in the 0-day X-irradiated group. There were no significant differences in CA1 or dentate gyrus RSA amplitude and frequency from control or X-irradiated rats.

Amplitude and frequency analysis — muscarinic antagonists. Table V summarizes the data for amplitude and frequency of hippocampal RSA measured during walking following injections of saline, atropine sulfate, and scopolamine for the 3 groups of rats. Analysis of variance showed there was no significant effect of X-irradiation induced granule cell agenesis on RSA amplitude, $F(5,61) = 0.76$, $P > 0.05$, or frequency, $F(5,61) = 0.56$, $P > 0.05$, for the three groups of rats. Analysis of variance carried out on the amplitude data revealed a significant difference between the two generator sites, $F(1,75) = 12.89$, $P < 0.01$, and a significant drug effect $F(2,150) = 17.99$, $P < 0.01$. The mean amplitude in regio superior placements was lower than amplitude in regio inferior placements and both atropine and scopolamine slightly increased the amplitude of RSA at all placements[27]. Analysis of variance carried out on the frequency data showed that there was no significant placement effect but that the frequency was slightly but significantly increased for all placements following atropine or scopolamine treatment, $F(2,150) = 72.9$, $P < 0.01$. Samples of recordings taken during walking from representative CA1 and dentate placements in each of the three groups are presented in Fig. 5B. Fig. 5B also shows a sample of recordings from the two generators in a control rat treated with scopolamine.

Amplitude and frequency analysis — nicotinic antagonists. Analysis of variance indicated no significant effect of granule cell agenesis on amplitude and frequency, $F(5,61) = 0.69$, $P > 0.05$. Analysis of variance indicated a significant generator effect, $F(1,37) = 11.69$, $P < 0.01$. As described above the CA1 amplitude was smaller than the dentate gyrus amplitude. There were no significant drug, nicotine or mecamylamine, effects on the RSA amplitude or frequency recorded during walking.

Amplitude and frequency analysis — combinations of muscarinic and nicotinic antagonists. Mecamylamine tested in combination with scopolamine did not produce significant changes in the control or X-irradiated groups, RSA amplitude or frequency. Nicotine in combination with scopolamine did not produce changes in RSA frequency but amplitude was slightly but significantly reduced by 40–100 μV in both generators, $F(2,58) = 7.11$, $P < 0.01$. The combination of nicotine and scopolamine also made the rat's movements extremely sluggish. A sample of recordings from the CA1 and dentate generators taken during walking in a rat drugged with mecamylamine and scopolamine is given in Fig. 5B.

Amplitude and frequency analysis — effects of urethane and urethane plus muscarinic or nicotinic antagonists. The amplitude difference between the CA1 generator and dentate generator in the undrugged rats was present in the urethane anesthetized rats, $F(1,37) = 27.64$, $P < 0.01$. In addition, urethane produced a mean amplitude reduction of 200 μV in 'CA1' sites, $F(1,37) = 25.49$, $P < 0.01$. Histological analysis revealed that this effect was due to a reduction of amplitude at dorsal placements

located close to the corpus callosum. Urethane significantly reduced RSA frequency of undrugged rats by a mean of 2.0 Hz at all placements, $F(1,36) = 474.8$, $P < 0.01$. The addition of mecamylamine or nicotine to the urethane-drugged rat had no effect on the amplitude or frequency of RSA. The addition of atropine or scopolamine to the urethane-drugged rat abolished RSA from all sites.

DISCUSSION

The results of the present study confirm recent observations on the cytogenesis of the hippocampal formation[3] as well as observations on the influence of postnatally applied low level focal radiation[3,13–16]. The length of the dentate granular layer was reduced as were the number of granule cells. The reduction of cells was more severe in the subgranular (endal) layer and, in animals in which X-irradiation treatment was begun immediately after birth, this layer was absent. Pyramidal neurons of the hippocampus proper were not affected by X-irradiation. Our results also suggest that the afferent pathways to the CA1 pyramidal cells and the dentate granule cells subserving RSA were still intact[9].

The findings for the distribution of rhythmical slow wave activity in the hippocampal formation of the control rats confirmed in all aspects our previous studies[19,37]. The profiles were similar to Winson's type 1 profiles for curarized rats[41]. The profiles of RSA down to the depth of the ectal blade of the dentate gyrus in the X-irradiated rats did not differ significantly from control rats despite the great reduction in the number of granule cells. In all animals there was a CA1 RSA amplitude maxima in stratum oriens, a null zone and phase reversal point at stratum radiatum, and a ventral RSA amplitude maxima at stratum moleculare. At these points there were no amplitude differences in the different groups of rats.

There were some differences in the distribution of RSA in the normal and X-irradiated rats which support the idea that there are two generators of RSA and that dentate granule cells play a role in the generation of RSA in the ventral generator. Specifically, there was a reduction in the length of the ectal blade and a corresponding reduction in the extent of the hippocampus in which the two amplitude maxima were seen. In addition, a large portion of the endal blade in 2-day X-irradiated rats and almost the total extent in 0-day X-irradiated rats was abolished. Correspondingly, there was a much reduced RSA amplitude in this region of the X-irradiated rats compared to control rats. However, due to shrinkage and volume conduction it was not possible to demonstrate a total absence of RSA in this area. Despite these points, the idea that the CA1 area is the real source generator with the dentate simply acting as a sink cannot be definitely ruled out. From the present results it would appear that unequivocal demonstration that there are two independent generators which can produce RSA requires a preparation in which dentate granule cells are completely absent.

Several hypotheses may be presented as possible explanations for the failure to see a reduction in the amplitude of the extracellular RSA wave despite the reduction in granule cell density. The assumptions could be made that RSA in the dentate is generated by the extracellular current associated with postsynaptic potentials in gra-

nule cells and that each granule cell generates a voltage, then, provided that synaptic input is adequate and that the major change is the decrease in number of granule cells, there would be no change in RSA amplitude because voltages in parallel do not add. A number of other hypotheses could also be entertained. (1) X-irradiation somehow forces more granule cells to participate in the generation of the rhythmic waveform than is the case in the normal animal. Thus, 'overworked' neurons compensate for the cell loss. (2) Glial or other cells are responsible for the generation of the waveform. (3) The CA1 is the real source generator with the dentate simply acting as a sink. (4) Shrinkage[3] and glial proliferation[13], with glial cells possibly forming 'tight junctions', results in a reduction of extracellular space and an increase in extracellular resistance thereby increasing the extracellular current density and compensating for the loss of cells. Our present level of knowledge does not allow us to eliminate any of these possibilities.

An interesting finding was that, unlike control rats, the X-irradiated rats did not show the characteristic 30–50 Hz fast activity localized to the hilus of the fascia dentata. At present we do not know what is responsible for this activity. There does appear to be a species difference in that rats have this activity[19,39] and rabbits do not[17,21].

The analysis of phase changes presented in the present paper confirm and extend our previous observations[19] and are in essential agreement with Winson's[41] results. However, Winson[41] reports that after a null zone extending approximately 90 μm he observed high-amplitude RSA that was isomorphic with the ventral reference electrode. We observed that by 90–100 μm below the null zone the signals were isomorphic, but before this, clear RSA was present that was phase-reversed with 21–38° lag. The lag gradually decreased within 75 μm and the signals were isomorphic by 90–100 μm.

Analysis of behavior in freely moving rats implanted with microelectrodes in the CA1 and dentate generators confirmed a preliminary report that RSA recorded from these areas was systematically related to certain types of motor behavior[19]. The results also confirmed the observations made of amplitude and phase of RSA in the acute series. The RSA recorded from the CA1 area was lower in amplitude and approximately 180° out of phase compared to that recorded from ectal granule blade placements for both control rats and X-irradiated rats.

There has been argument about whether there are differences in the pharmacology of RSA in the dorsal and ventral generators[29,32]. In a previous report we demonstrated that the RSA in both generators of the anesthetized rat was abolished by atropine[37] and in the present study we extended this observation to scopolamine[33]. We have also previously shown that both the anticholinergic-sensitive and the anticholinergic-resistant forms of RSA can be recorded from both generators in rats paralyzed by neuromuscular blockade[37]. By using freely moving rats in the present study we have confirmed that both generators produce the anticholinergic resistant form of RSA. These findings supplement the results of previous studies[31,34,36–38] and support the idea that there are two pharmacologically distinguishable types of RSA.

Mecamylamine and nicotine administered to urethane-anesthetized or freely moving rats did not abolish RSA. Nicotine initially has a stimulant effect, mimicking the nicotinic action of ACh, and then subsequently produces a blockade of such action

(see ref. 22). During the stimulant phase the rats evidenced respiratory difficulties and convulsive-like behavior. Low frequency (5–7 Hz) RSA was recorded throughout this stage[28]. Rats administered with a combination of mecamylamine and scopolamine did not show any changes in RSA amplitude or frequency recorded during walking. Nicotine in combination with scopolamine reduced the amplitude slightly but had no effect on the frequency of RSA recorded during walking. This change was probably related to effects of the drugs on motor behavior. These results suggest that neither the anticholinergic-sensitive nor the anticholinergic-resistant forms of RSA are mediated by nicotinic-type synapses.

Although the present experiments studied the dorsal hippocampal formation we have also examined the extracellular activity in the ventral hippocampus of normal and X-irradiated rats[20]. The relation between bioelectrical activity and anatomical locus in the ventral hippocampus appears similar in all major respects to that reported here for the dorsal hippocampus.

In summary, the results of the present study have demonstrated that a dramatic reduction in the density of dentate granule cells produced by focal X-irradiation of newborn rat pups does not affect the amplitude, frequency, or pharmacology of extracellulary-recorded slow wave activity. The amplitude finding suggests that there is not a linear relationship between the amplitude or frequency of EEG activity and the number of cells subserving this activity. The findings related to the loss of amplitude or RSA in the lower (endal) blade, where cell loss was nearly complete, support the conclusion that granule cells are responsible for the ventral maxima of RSA. However, this cannot be viewed as conclusive due to volume conduction. The pharmacological findings presented in this report support the view that immobility-related RSA is cholinergic and mediated by muscarinic-type synapses and not nicotinic-type synapses. The pharmacological studies also suggest that granule cell agenesis produces no obvious changes in the contribution of cholinergic mechanisms to generation of hippocampal RSA. Furthermore, movement-related RSA is unlikely to be activated by a cholinergically mediated system.

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REFERENCES

1 Altman, J., Autoradiographic and histological studies of postnatal neurogenesis. II. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in infant rats, with special reference to postnatal neurogenesis in some brain regions, *J. comp. Neurol.*, 128 (1966) 431–474.

2 Altman, J., Anderson, W. J. and Strop, M., Retardation of cerebellar and motor development by focal X-irradiation during infancy, *Physiol. Behav.*, 7 (1971) 143–150.
3 Altman, J. and Bayer, S. A., Postnatal development of the hippocampal dentate gyrus under normal and experimental conditions. In R. L. Isaacson and K. H. Pribram (Eds.), *The Hippocampus, Vol. I. Structure and Development*, Plenum Press, New York, 1975.
4 Altman, J. and Das, G. D., Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats, *J. comp. Neurol.*, 124 (1965) 319–335.
5 Altman, J. and Das, G. D., Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonatal rats, with special reference to postnatal neurogenesis in some brain regions, *J. comp. Neurol.*, 126 (1966) 337–390.
6 Altman, J., Anderson, W. J. and Wright, K. A., Differential radiosensitivity of stationary and migratory primitive cell in the brains of infant rats, *Exp. Neurol.*, 22 (1968) 52–74.
7 Altman, J. and Nicholson, J. L., Cell pyknosis in the cerebellar cortex of infant rats following low-level X-irradiation, *Radiat. Res.*, 46 (1971) 476–489.
8 Andersen, P., Bland, B. H. and Ganes, T., Activity of identified hippocampal and dentate granule cells during theta activity, unpublished manuscript (1977).
9 Andersen P., Bland, B. H., Myhrer, T. and Schwartzkroin, P. A., Septo-hippocampal pathway necessary for dentate theta production, unpublished manuscript (1977).
10 Andersen, P. and Schwartzkroin, P. A., Mechanisms of dentate theta generation in rabbits, in preparation (1977).
11 Angevine, J. B., Time of neuron origin in the hippocampal region: an autoradiographic study in the mouse, *Exp. Neurol.*, 2, Suppl. (1965).
12 Artemenko, D. P., Role of hippocampal neurons in theta-wave generation, *Nierofiziologiya*, 4 (1972) 531–539.
13 Bayer, S. A. and Altman, J., Hippocampal development in the rat. Cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation, *J. comp. Neurol.*, 158 (1974) 55–80.
14 Bayer, S. A. and Altman, J., Radiation-induced interference with postnatal hippocampal cytogenesis in rats and its long-term effects on the acquisition of neurons and glia, *J. comp. Neurol.*, 163 (1975) 1–20.
15 Bayer, S. A., Brunner, R. L., Hine, R. and Altman, J., Behavioral effects of interference with the postnatal acquisition of hippocampal granule cells, *Nature New Biol.*, 242 (1973) 222–224.
16 Bayer, S. A. and Peters, P. J., A method for X-irradiating selected brain regions in infant rats, *Brain Res. Bull.*, 2 (1977) 153–156.
17 Bland, B. H., Andersen, P. and Ganes, T., Two generators of hippocampal theta activity in rabbits, *Brain Research*, 94 (1975) 199–218.
18 Bland, B. H., Sainsbury, R. S. and Creery, B., Electrical activity of the feline hippocampus: anatomical and behavioral correlations, in preparation (1977).
19 Bland, B. H. and Whishaw, I. Q., Generators and topography of hippocampal theta (RSA) in anesthetized and freely moving rat, *Brain Research*, 118 (1976) 259–280.
20 Bland, B. H., Whishaw, I. Q. and Bayer, S. A., The topography and generators of hippocampal slow wave activity (RSA) in the ventral hippocampus of normal rats and rats subjected to neonatal X-irradiation, unpublished manuscript.
21 Creery, B. and Bland, B. H., Post-natal development of hippocampal electrical activity and motor behavior, unpublished manuscript (1977).
22 Goodman, L. S. and Gilman, A., *The Pharmacological Basis of Therapeutics*. Macmillan, Toronto, 1970.
23 Haggbloom, S. J., Brunner, R. L. and Bayer, S. A. Effects of hippocampal granule cell agenesis on acquisition of escape from fear and one-way active-avoidance responses, *J. comp. physiol. Psychol.*, 86 (1974) 447–457.
24 Hicks, S. P. and D'Amato, C. J., Effects of ionizing radiation on mammalian development. In D. H. M. Wollam (Ed.), *Advances in Teratology*, Logos Press, London, 1966.
25 Kolb, B. and Whishaw, I. Q., Effects of brain lesions and atropine on hippocampal and neocortical electroencephalograms in the rat, *Exp. Neurol.*, 56 (1977) 1–22.
26 Kramis, R., Vanderwolf, C. H. and Bland, B. H., Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethylether, urethane and pentobarbital, *Exp. Neurol.*, 49 (1975) 58–85.
27 Robinson, T. E., Kramis, R. C. and Vanderwolf, C. H., Two types of cerebral activation during active sleep: relations to behavior, *Brain Research*, 124 (1977) 544–549.

28 Stumpf, C., Drug action on the electrical activity of the hippocampus, *Int. Rev. Neurobiol.*, 8 (1965) 77–138.
29 Teitelbaum, H., Hippocampal activity and scopolamine, *Science*, 192 (1975) 192–193.
30 Thomas, R. C. and Wilson, V. J., Precise localization of Renshaw cells with a new marking technique, *Nature (Lond.)*, 206 (1965) 211–213.
31 Vanderwolf, C. H., Neocortical and hippocampal activation in relation to behavior: effects of atropine, eserine, phenothiazines and amphetamine, *J. comp. physiol. Psychol.*, 88 (1975) 300–323.
32 Vanderwolf, C. H., Hippocampal activity and scopolamine, *Science*, 192 (1975) 192–193.
33 Vanderwolf, C. H., Bland, B. H. and Whishaw, I. Q., Diencephalic, hippocampal, and neocortical mechanisms in voluntary movement. In J. Maser (Ed.), *Efferent Organization and Integration of Behavior*, Academic Press, New York, 1973.
34 Vanderwolf, C. H., Kramis, R., Gillespie, L. and Bland, B. H., Hippocampal rhythmical slow activity and neocortical low voltage fast activity: relations to behavior. In R. L. Isaacson and K. H. Pribram (Eds.), *The Hippocampus, Vol. 2, Neurophysiology and Behavior*, Plenum, New York, 1975, pp. 101–128.
35 Vinogradova, O. S., Functional organization of the limbic system in the process of registration of information: facts and hypotheses. In R. L. Isaacson and K. H. Pribram (Eds.), *The Hippocampus, Vol. 2, Neurophysiology and Behavior*, Plenum Press, New York, 1975.
36 Whishaw, I. Q., The effects of alcohol and atropine on EEG and behavior in the rabbit, *Psychopharmacologia (Berl.)*, 48 (1976) 83–90.
37 Whishaw, I. Q., Bland, B. H., Robinson, T. E. and Vanderwolf, C. H., Two types of rhythmical slow activity (RSA) in hippocampus and dentate gyrus during neuromuscular blockade, *Brain Res. Bull.*, 1 (1976) 573–581.
38 Whishaw, I. Q., Robinson, T. E. and Schallert, T., Intraventricular anti-cholinergics do not block cholinergic hippocampal RSA or neocortical desynchronization in the rabbit or rat, *Pharmacol. Biochem. Behav.*, 5 (1976) 275–283.
39 Whishaw, I. Q. and Vanderwolf, C. H., Hippocampal EEG and behavior: changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement patterns in rats and cats, *Behav. Biol.*, 8 (1973) 461–484.
40 Winson, J., Patterns of hippocampal theta rhythm in the freely moving rat, *Electroenceph. clin. Neurophysiol.*, 36 (1974) 291–301.
41 Winson, J., Hippocampal theta rhythm. I. Depth profiles in the curarized rat, *Brain Research*, 103 (1976) 57–70.
42 Winson, J., Hippocampal theta rhythm. II. Depth profiles in the freely moving rabbit, *Brain Research*, 103 (1976) 71–79.