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Metrazol Potentiated After-Discharges: Dose-Response Relationships and Effects of Selective Lesions

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BIGLER, E. D., D. E. FLEMING AND D. E. SHEARER. *Metrazol potentiated after-discharges: dose-response relationships and effects of selective lesions.* PHARMAC. BIOCHEM. BEHAV. 5(4) 423–429, 1976. – In Experiment 1 the dose-response effects of pentylenetetrazol (Metrazol) on photically evoked after-discharge (PhAD) parameters were examined. Metrazol potentiated PhAD activity by affecting all measured parameters – PhAD frequency, amplitude, burst duration and spindle composition – in particular PhAD burst duration and spindle composition. A minimum effective dose of 10 mg/Kg of Metrazol was required for some statistically reliable potentiation. Metrazol dosage levels of 20 and 25 mg/Kg induced lengthy bouts of EEG spindling and spiking as well as near maximized PhAD component augmentation. In Experiment 2 stereotaxically oriented knife-cuts isolated the thalamus from cortical and/or midbrain and brainstem input. Such lesions did not block the capacity of Metrazol to potentiate PhADs, although the lesions altered evoked activity. These findings are discussed in terms of the current thought of Metrazol action and thalamic mechanisms in the control of after-discharge activity.

Metrazol Photically evoked after-discharge Dose-response effects Lesions Thalamus

A REPEATED observation in our laboratories [2–4, 11, 20], as well as others [16,25] has been that pentylenetetrazol (Metrazol) and its derivatives reliably augment the photically evoked after-discharge (PhAD) in the rat. These findings have established the PhAD response as an effective model in the study of convulsant and anticonvulsant action. For the present experiments, in terms of the Metrazol potentiated PhAD, we wanted to clarify two points: (a) Experiment 1 – the dose-response effects of Metrazol on specific PhAD components, an aspect not systematically examined in previous research, and (b) Experiment 2 – the effects of selective lesions on the capacity of Metrazol to potentiate PhAD bursting.

EXPERIMENT 1

Method

Animals and surgery. Twelve male Holtzman albino rats between the ages of 90–120 days at the start of the investigation were anesthetized with pentobarbital sodium (45 mg/kg) and surgically prepared with indwelling extradural stainless steel electrodes implanted over the right and left visual cortices at a point 7 mm posterior to the bregma and 3 mm lateral to the midline. Electrodes were also placed in the calvarium over the cerebellum and frontal sinus for reference and grounding, respectively. Seven days of recovery were allowed prior to initiation of the drug

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treatment sessions. All experimental procedures were carried out on awake animals with mydriatic pupils (1% opthalmic atropine sulfate).

Apparatus. A Grass Model PS2C photostimulator was used to present 10 μsec light pulses to a reflecting hemicylinder. The hemicylinder was placed in front of a hammock in which the animal was held under light restraint. With the photostimulator lamp placed 70 cm behind and slightly above the hemicylinder, the illumination of the reflecting surface was approximately 5 ft-c. Brain responses were amplified with Grass 7P5A preamplifiers and Model 7 Polygraph amplifiers (bandwidth, 0.3 Hz-3KHz; time constant, 0.24) and ink written records were obtained.

Procedure. Stabilized PhADs in terms of reliable elicitation rates and consistent waveforms develop when the awake non-anesthetized animal has been well acclimated to being restrained and the presentation of iterative photic stimuli [5]. Accordingly, each animal was taken through several short-term habituation sessions to insure that AD activity was occurring at a reliable and stable rate. There were 6 separate drug conditions which consisted of a normal saline control and five dosage levels of Metrazol (5, 10, 15, 20 and 25 mg/kg). Only one drug treatment condition was given on a single day and at least 5 days separated subsequent drug sessions. The drugs were injected IP in equal volume amounts according to an individualized random schedule. The experimental procedures were carried out in the following manner: a rat was placed in the restraining hammock and allowed to dark adapt for 15 min after which time photic stimulation was initiated at a rate of 1/7 sec for a period of 5 min at which time the next 25 visually evoked responses (VERs) were recorded for purposes of data analysis. PhAD activity at this point has been repeatedly demonstrated to possess uniform and stabilized patterns and hence provides the best measure for comparative purposes. When recording of this block of 25 responses had been completed, photic stimulation was interrupted and a drug treatment given. Photic stimulation was then resumed and at a period of 8 min postinjection another block of 25 VERs was recorded for analysis purposes. EEG was continuously recorded for the duration of the experiment.

Data analysis. Although VERs were recorded from both hemispheres only those in the right visual cortex were utilized for data analysis. The modulation of PhAD waveform was determined by 4 representative measures: (a) the percentage occurrence rate of PhAD activity for each block of 25 responses recorded, with a scoreable PhAD consisting of a minimum of two distinct sinusoidal waves developing in time after the late negative component of the VER (see [2,5]), (b) the number of spindle-like waves per the largest PhAD burst, (c) the peak-to-peak amplitude (in μV) of the largest wave component of PhAD activity and (d) the longest burst duration (in msec) of PhAD activity. Cortical EEG was also examined in terms of the following: (a) incidence of spontaneous spindles, (b) frequency and amplitude of spontaneous spindles, (c) changes in the amplitude and rhythmicity of cortical EEG activity, and (d) the incidence of cortical spiking. The subjects were also observed for overt signs of seizure activity.

The data were analyzed by several statistical techniques. Pre- and postdrug comparisons for each separate and individual treatment were carried out with *t*-test analysis. For comparisons across treatments, pre- and postdrug conditions were subjected separately to analysis of variance with repeated measures tests (see [26]). Where the resulting F-score indicated statistical significance, Newman-Keuls tests (see [26]) were incorporated for treatment mean pair comparisons.

Results and Discussion

The results are summarized in Figs. 1, 2 and 3. While Metrazol increased the frequency of PhAD occurrence during the various postMetrazol conditions, the degree of enhancement as compared to postsaline control values did not reach a significant level, $F(5,55) = 2.00$, $p>0.01$. Individual analysis between separate pre- and postdrug measures can be made by inspection of Fig. 1.

Results of Newman-Keuls tests, based on a reliable analysis of variance, $F(5,55) = 7.81$, $p<0.01$, indicated that the mean number of spindles per largest PhAD during postdrug conditions was significantly increased at dosage levels of 15, 20 and 25 mg/kg as compared to saline and 5 mg/kg levels. Individual analysis of the separate drug levels (see Fig. 1) indicated that postMetrazol mean spindles per largest PhAD could be reliably differentiated from predrug measures during the 15 mg/kg ($t = 2.09$, $df = 22$, $p<0.01$), 20 mg/kg ($t = 3.12$, $df = 22$, $p<0.01$) and 25 mg/kg ($t = 2.06$, $df = 22$, $p<0.01$) treatment levles. The effect of Metrazol on spindle composition was to increment spindle frequency not in a dose-response fashion, but rather to enhance frequency by a mean constant factor of approximately four waves above predrug baseline measures (see Fig. 1 and Fig. 2A).

In terms of postMetrazol effects on mean peak-to-peak amplitude of the largest spindle component, Newman-Keuls tests, based on a reliable analysis of variance score, $F(5,55) = 8.78$, $p<0.01$, indicated that the dose levels of 15, 20 and 25 mg/kg could be statistically separated from the dosage levels of 5 mg/kg and saline control. Individual analysis of the separate drug levels can be made by inspection of Fig. 1. Metrazol affected peak-to-peak amplitude in an incremental dose-response fashion irrespective of the baseline control values.

With respect to postMetrazol effects on mean PhAD duration per largest burst, Newman-Keuls tests, based on a reliable analysis of variance score, $F(5,55) = 9.79$, $p<0.01$, revealed that the 10, 15, 20 and 25 mg/kg levels could be statistically differentiated from post-saline values. In addition, the 15, 20 and 25 mg/kg levels could be reliably distinguished from the 5 and 10 mg/kg levels. Individual analysis (see Fig. 1) of the separate drug levels indicated that the 10 mg/kg ($t = 2.31$, $df = 22$, $p<0.05$), 15 mg/kg ($t = 2.66$, $df = 22$, $p<0.01$), 20 mg/kg, ($t = 4.29$, $df = 22$, $p<0.01$) and 25 mg/kg ($t = 3.69$, $df = 22$, $p<0.01$) could be reliably separated from their respective pre-Metrazol baseline controls. Metrazol affected burst duration in a manner similar to that observed with the spindle component – burst duration was enhanced by a mean constant factor (approximately 800 msec) with respect to preMetrazol measures.

In terms of cortical EEG activity, Metrazol did not systematically alter cortical activity at the 5 and 10 mg/kg levels. At 15 mg/kg and above (see Fig. 3) Metrazol induced a time-locked effect of the development of a low amplitude synchronous rhythm which preceded the development of large amplitude spindling. At 20 and 25 mg/kg levels, this large amplitude spindling was followed in most subjects by

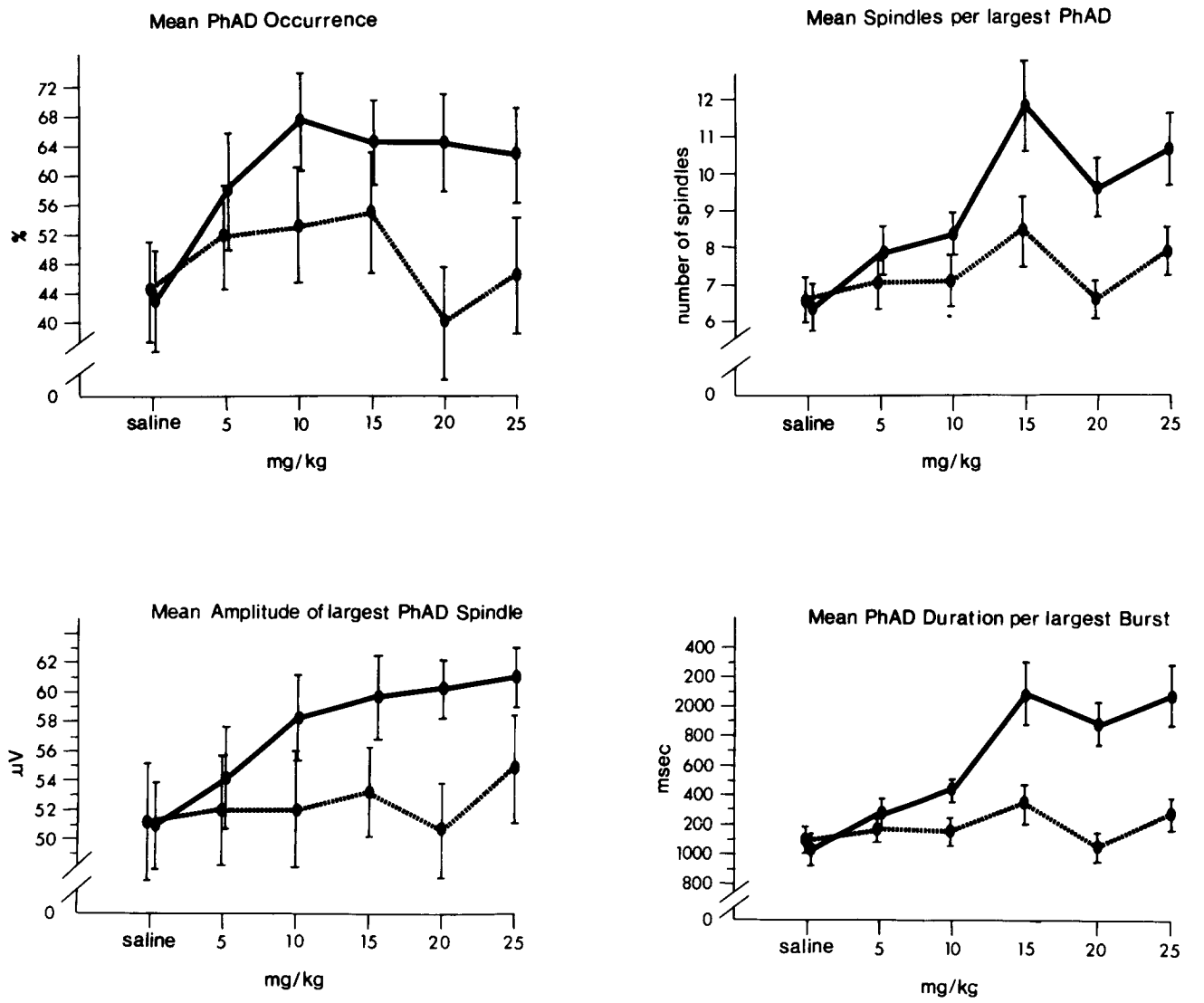

FIG. 1. Effects of Metrazol on PhAD parameters. Mean and (±) standard error of the mean values are presented. Broken lines = predrug; solid lines = postdrug.

the development of spiking activity at about 3 min postinjection (see Fig. 3). In Fig. 2B it is seen that Metrazol induced spindling or spiking does not preclude PhAD elaboration. Point in fact, Metrazol induced spindling has a similar amplitude (at 25 mg/kg, Metrazol induced spindles have a mean amplitude of 64.0 μV, PhAD spindle amplitude at this dosage level was 61.0 μV) and frequency 5–7 Hz) as that of PhAD bursting. The low amplitude synchronous pattern induced by Metrazol which preceded Metrazol induced spindling was found to be at variance with PhAD elicitation (see Fig. 2B). This type of cortical pattern has been considered to be a by-product of limbic-diencephalic arousal which blocks PhAD elaboration [10].

EXPERIMENT 2

The visual cortical PhAD is a product of discharge synchronization in dorsal lateral geniculate neurons (dLGN) which form the geniculo-striate projections [2]. While Metrazol induces a hypersynchronization in these geniculate neurons [2,4], we have not determined whether this effect is strictly a thalamic event or dependent upon existing reciprocal connections between thalamus and cortex or thalamus and midbrain or brainstem. Several reports have demonstrated the role of nonthalamic structures in Metrazol induced seizures [1, 23, 27]. In the following experiments we selectively eliminated input to the thalamus while attempting PhAD augmentation by Metrazol.

Method

Experiments were performed on both hooded and albino rats anesthetized with urethane (2 g/kg). There are strain determined differences in PhAD patterns between albino and hooded rats but in both strains PhADs are readily elicited and augmented by Metrazol [12]. In the following experiments we were primarily concerned with quantitative changes in the ability of Metrazol to augment PhAD

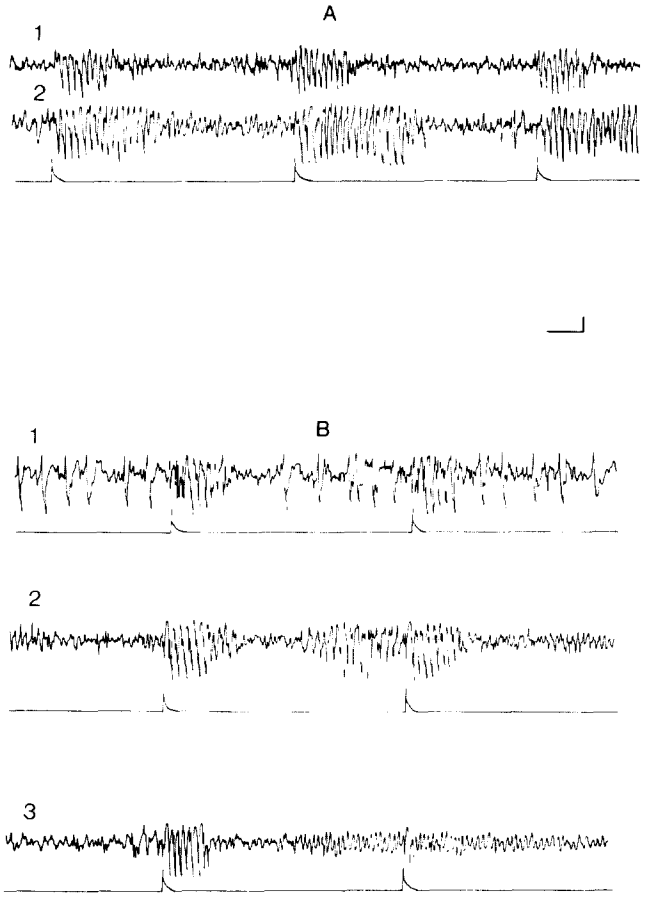

FIG. 2. (A) Comparison of (1) pre-Metrazol PhAD activity with (2) post-Metrazol (20 mg/kg) potentiated PhADs. An upward deflection in the line beneath the EEG tracings, and in all subsequent tracings, represents the presentation of the flash stimulus. (B) (1) PhAD activity during Metrazol induced (25 mg/kg) EEG spiking. (Note the similar amplitudes between PhAD spindles and EEG spiking), (2) PhAD activity with spontaneous spindling preceding the second PhAD burst. Note the similarities between spontaneous spindling and PhAD activity, (3) suppression of PhAD activity during low amplitude electrocortical rhythmical activity (calibrations, 50 μV; 1 sec, in this and following in Figures unless noted).

activity following selective brain lesions and not with existing species-specific differences in PhAD patterns. Animals were secured in a stereotaxic frame with the head being positioned to conform to the atlas of Skinner [21]. VERs were recorded from the right visual cortex via silver ball electrodes placed on the overlying dura (7 mm posterior to the bregma, 3 mm lateral to the midline). Photic pulse stimulation was produced by a Strobotac unit (Model No. 1531, set at maximum intensity) and guided by a fiber optic bundle, the radiating end of which was positioned within 5 mm of the left eye. Photic pulse stimulation was initiated and after 5 min of iterative stimulation at a frequency of 1/7 sec a block of 25 VERs was averaged online over a 750 msec epoch with a Princeton Applied Waveform Eductor (model TDH-9). The thalamus was then isolated by stereotaxically oriented transections (knife-cuts) of the frontal cortex (1–3 mm anterior to bregma) or at midbrain and brainstem levels (5–12 mm posterior to bregma). Care was taken to avoid damage to retino-geniculate pathways and/or visual cortical areas. Once lesioned, photic stimulation was resumed and after 5 min another block of 25 VERs was averaged. Photic stimulation was then discontinued and the animals injected with 10–40 mg/kg of Metrazol. Photic stimulation was then resumed and after 10 min a third block of 25 VERs was averaged. Subsequent to these procedures the animal was sacrificed by an intracardial perfusion of KCl and the brain extracted for histological purposes. The data were compared in terms of Metrazol induced changes in averaged VERs with reference to PhAD activity.

For cortical ablations of the striate area, since the nature of the lesion precludes cortical recording of PhADs, unit and multiple unit recording of dLGN was utilized. Using aspiration techniques ipsilateral cortical ablations were performed which included all of area 17 and most or all of areas 18 and 18a. Following ablation a glass microelectrode filled with 3 Molar KCl (DC resistance range in saline of 0.5–2.0 meg Ω) was lowered to the right dLGN, by a remotely controlled microdrive, until a unit could be isolated. All units examined were lateral geniculate principal (P) cells which pace the cortical PhAD response [2,4]. These cells were identified by their response pattern to antidromic stimulation (see [3, 4, 6]). Since in these preparations a prelesion control could not be taken, control consisted of a preMetrazol postablation period with photic stimulation parameters identical to those specified above. Following the control record, 10–40 mg/kg of Metrazol was injected, photic stimulation was resumed and after 10 min evoked P cell activity was examined for afterdischarge potentiation by Metrazol. Data were also recorded on magnetic tape for delayed data analysis (see [2–4]). At the end of experimentation the animals were terminated as described above and the brains extracted for histological examination.

Results and Discussion

The results are summarized in Fig. 3 and demonstrate that the various brain transections altered VER components, but cortical PhADs could still be elicited and augmented in the postlesion+Metrazol conditions. In terms of cortical ablations, it was demonstrated that cortico-geniculate connections were not essential for the augmentation of P cell after-discharges by Metrazol. These results indicate that Metrazol augmented cortical PhADs are a local product of thalamic mechanisms. While frontal, cortical and mesencephalic regions all unequivocally modulate visual system activity [6, 7, 13, 16], these regions are apparently not essential for the Metrazol potentiation of PhAD bursting. However, Metrazol does affect these areas (see [19,23]) and such effects are likely additive to the intrathalamic ones in terms of PhAD augmentation.

GENERAL DISCUSSION

Metrazol potentiated PhAD activity by affecting a variety of interrelated PhAD parameters, in particular, burst duration and spindle wave composition of the burst. Metrazol had similar effects on visual cortical EEG spindles. The following dose related effects could be documented: (a) a dose of 10 mg/kg was the required minimum for the induction of reliable augmentation in some parameters of PhAD activity, (b) a dose of 20 mg/kg produced maximum potentiation of all PhAD parameters along with inducing

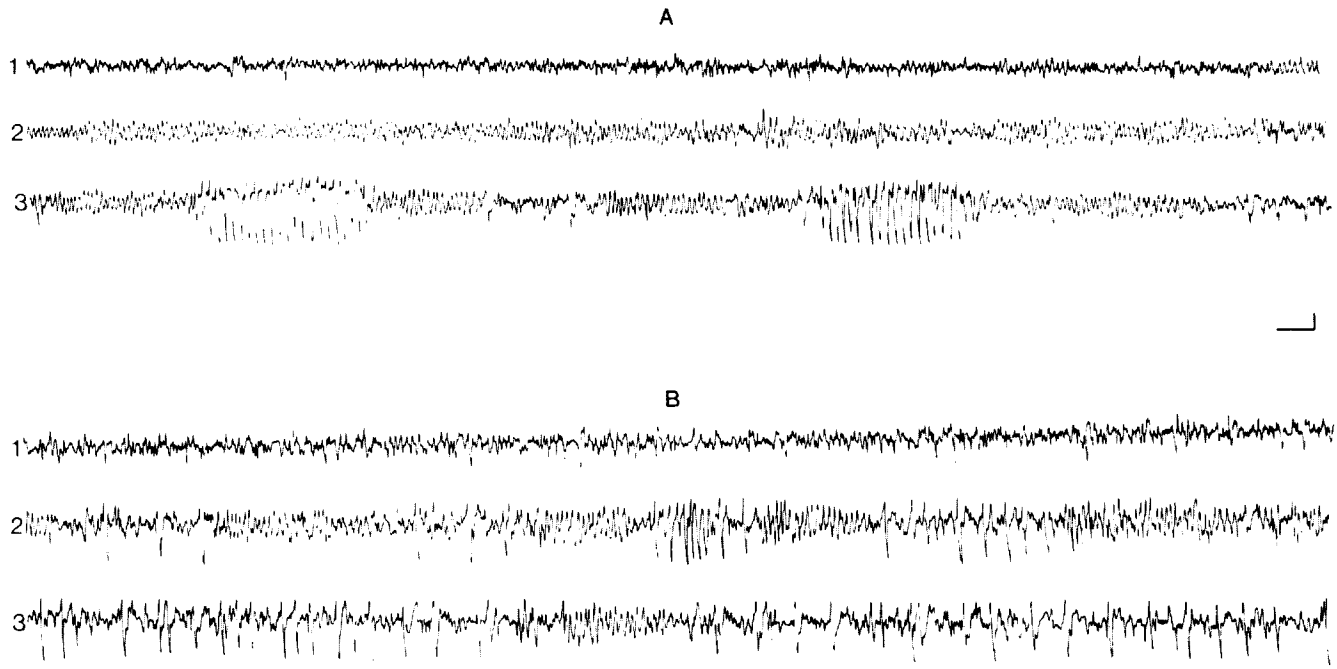

FIG. 3. Segments of visual cortical EEG activity during the first 3 min following a 20 mg/kg (A) and 25 mg/kg (B) dose of Metrazol. The numbers on the left border indicate the number of minutes postinjection during which recording took place. Two different animals are represented in A and B, respectively.

lengthy spindle activity and some seizure spiking, and (c) a dose of 25 mg/kg was required for maximum PhAD augmentation with extensive overt seizure activity, both behaviorally and electrophysiologically. With respect to PhAD bursting, these Metrazol effects were localized at the thalamic level.

Although much of the activity in the visual cortex is a direct product of lateral geniculate P cell input, it appears that the mechanism subserving Metrazol potentiation of cortical PhAD bursting has a locus in addition to the lateral geniculate (see [4]). Accordingly, the thalamic reticular nucleus (nR) has been shown to be the source of inhibitory input required for the development of evoked synchronous and repetitive discharges in lateral geniculate P cells [22]. Such evoked discharges in lateral geniculate P cells drive the cortical PhAD response [2]. The nR has long been suspected as a major control center in thalamic and cortical synchrony (see [15,24]. Similarly, rostral thalamic areas including nR have been implicated as central to Metrazol induced spindling and seizures [9]. Taken together, this evidence strongly suggests the crucial role of nR in PhAD bursting and its augmentation by Metrazol. It is also very likely that nR plays the central role in Metrazol induced EEG spindling and spiking in the visual cortex and that this would represent the communality between visual cortical PhAD activity and visual cortical EEG spindling and spiking.

Although the initial burst in P cell activity is strictly a result of retinal input [6], the subsequent repetitive bursting, as alluded to above, is a result of rebound excitation within a synergistic recurrent inhibitory system [2–4, 6]. Metrazol has been demonstrated to facilitate rebound excitation [8]. Thus, we submit that Metrazol potentiates PhAD bursting via a facilitatory predisposition of rebound excitation, this being localized at the diencephalic level. Similar actions likely control the Metrazol induced visual cortical spindling and spiking. The present results of thalamic control over visual cortical PhADs are in agreement with other experimental epilepsy models demonstrating similar diencephalic mechanisms in control of cortical after-discharge activity [14, 18, 19].

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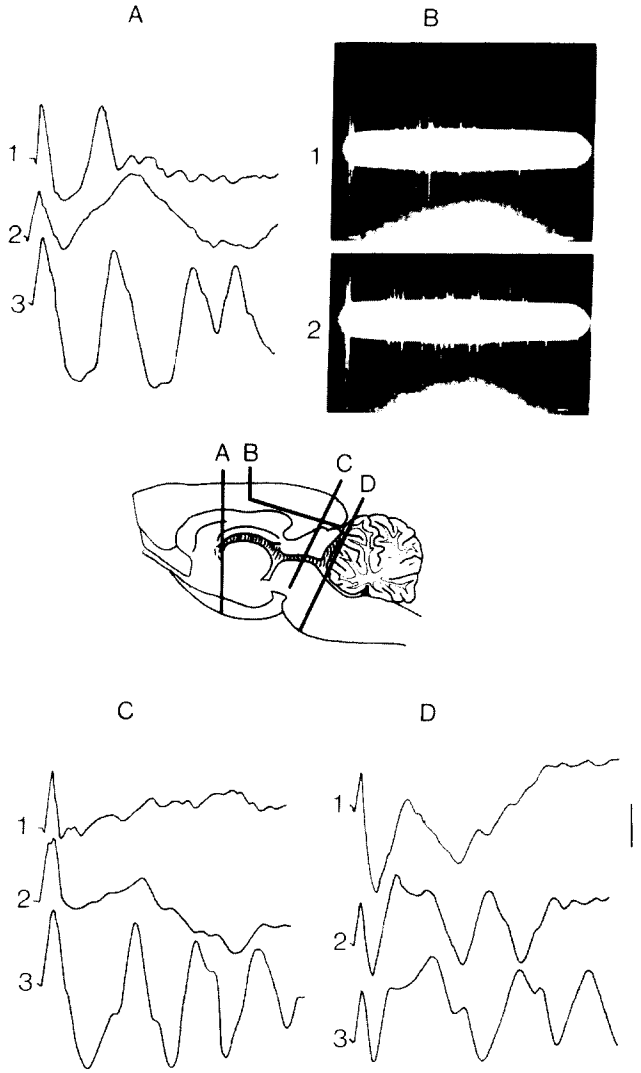

FIG. 4. Representative effects of brain transections on Metrazol capacity to potentiate PhADs. A, C, and D represent averaged VERs (750 msec duration, calibration: 20 μV) during the following conditions: (1) prelesion control, (2) postlesion, preMetrazol and (3) postlesion and Metrazol. B is a photograph (5 sweeps, oscilloscope tracings) of lateral geniculate P cell activity during (1) postvisual cortical ablation, preMetrazol and (2) postvisual cortical ablation, postMetrazol. The details of the surgical techniques are as follows: A – Frontal transections were performed by first opening the skull in a 1 mm strip bilaterally, 1–3 mm anterior to bregma extending 1 mm from midline to the outer skull crest. A scalpel-mounted No. 11 surgical blade was attached to a standard stereotaxic carrier, with the angle of the carrier arm being positioned at 20°. The blade was then lowered approximately 6.5 mm from brain surface at a point 1 mm lateral to the midline. The blade was then moved to the lateral extent of the skull opening, at which point the angle was returned to the 0° position and the blade moved laterally back across to the medial extent of the skull opening. The above procedures were repeated on the contralateral side. B – The skull overlying the right posterior neocortex was removed and the overlying visual cortex aspirated. The area directly over the LGN was filled with agar with the remaining exposed tissue being bathed in mineral oil and covered with a saturated gauge strip to prevent tissue drying. C and D – At the left lateral aspect of the skull a large opening was made which exposed the posterior neocortex and anterior aspect of the cerebellum. Knife cuts above, between or below the colliculi were done with either a stereotaxically mounted blade (as described above) or by hand-held scalpel. In both cases the orientation of the blade was done by visual inspection of the landmarks (i.e. colliculi) following the gentle lifting of the left occipital pole by a laboratory spatula. Some difficulty was encountered with excessive bleeding in these preparations. Such experiments were eliminated from data analysis.

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