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Effect of Pharmacologically-Induced Arousal on the Evoked Potential in the Unanesthetized Rat

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FLEMING, D. E., D. E. SHEARER AND D. J. CREEL. *Effect of pharmacologically-induced arousal on the evoked potential in the unanesthetized rat.* PHARMAC. BIOCHEM. BEHAV. 2(2) 187–192, 1974. – Three positive-negative wave complexes of the visually evoked response (VER) in the unanesthetized rat were examined following iterative photic stimulation. EEG activation was induced by physostigmine, amphetamine, or pilocarpine in order to compare the peak latency and amplitude values of the VER components during arousal. It was observed that the peak latency of only one component, a late negative wave occurring at approximately 160 msec was affected by the drug treatments. Except for one negative-positive amplitude measure occurring between 70 and 90 msec, the effect of drug-induced activation was to suppress the amplitude excursion of the remaining wave components. These observations agree with other VER studies in which arousal level was modified by type of behavioral task. It is suggested that the late negative wave peaking at 160 msec comprised the first wave of a photically evoked after-discharge burst.

Visually evoked potentials Pharmacologically-induced arousal Wave components

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IN PREVIOUS reports it was observed by Fleming *et al.* [9,10] that certain drugs which produce behavioral and/or pharmacological arousal profoundly suppressed the elicitation of photically evoked after-discharge (AD) bursts in the albino rat. Along these lines, Rhodes and Fleming [20] had shown that increased arousal levels, produced behaviorally, not only resulted in suppressed AD bursting, but in addition, resulted in amplitude changes in components of the visually evoked response (VER). In particular, the amplitude values of the early VER components, peak latencies between 21 and 33 msec, were reliably suppressed in the experimental animals perhaps due to a cumulative effect of sensory restriction. On the other hand, the late components, peak latencies between 82 and 115 msec, were not different between the control and experimental animals at the initiation of the recording procedure. However, with short-term habituation, the amplitude of the late components of the control animals diminished at a greater rate than did the late component amplitude of the experimental animals.

Because latency and amplitude parameters of the VER late components have been shown to be more variable than those of the early waves [19], it has been suggested that modulations in the early components reflect changes in stimulus value while changes in the late waves reflect shifts in behavioral state [19]. The late waves seem to be particularly susceptible to changes in arousal level, although conflicting evidence has been reported with both human and animal subjects [19]. There is little or no evidence relative to the effects of pharmacologically increased arousal on the components of the rat VER (cf. [29]). One may question whether the early and late components could be differentiated in the rat by modifying level of arousal. Moreover, as photically evoked AD bursting is markedly suppressed by pharmacologically-induced arousal increases [9], it also may be questioned whether there would be a parallel suppression of VER components, especially the late waves. These questions bear directly on the degree of communality

of function between the neural mechanisms responsible for the production of the various waves of the VER and of AD bursting. Accordingly, the present study was undertaken to examine the rat VER following the injection of amphetamine, physostigmine, and pilocarpine. Specific attention was directed to the components of the VER which just precede in time behaviorally labile AD bursting. Each of the drugs used has been shown to increase level of arousal [4, 15, 30], although amphetamine and physostigmine have been shown to have differential subcortical effects [29,30].

METHOD

Animals

Eleven albino rats of the Holtzman strain, 90–120 days of age at the start of the investigation, were anesthetized with pentobarbital sodium (50 mg/kg) and were surgically prepared with indwelling stainless steel screw electrodes implanted over the right and left visual cortices at points 7 mm posterior to the bregma and 3 mm lateral to the midline. Electrodes were also placed in the clavarium overlying the cerebellum and frontal sinus for reference and for grounding, respectively. At least 7 days of recovery were allowed before any treatment sessions were initiated. All experiments were carried out with waking animals with mydriatic pupils (1% atropine sulfate.)

Apparatus

A Grass Model PS2C photostimulator was used to deliver 10 μsec light pulses to a reflecting hemicylinder. The hemicylinder was placed in front of a hammock in which an animal was held under light restraint. With the photostimulator lamp placed 70 cm behind and slightly above the hemicylinder, and with the stimulator intensity setting at 4 on a 1–16 scale, the illuminance of the reflecting surface was approximately 5 ft-C.

Brain responses were amplified with Grass 7P5A preamplifiers and Model 7 Polygraph driver amplifiers (bandwidth, 0.3 Hz-3 kHz; coupling time constant 0.24 sec) and recorded on magnetic tape. VERs were summed with a Model 400B Computer of Average Transients (CAT) over a 1 sec epoch and were plotted on 25 × 38 cm graph paper.

Procedures

Each rat was administered the following drugs: amphetamine sulfate (2.5 mg/kg), physostigmine (0.4 mg/kg), pilocarpine hydrochloride (10 mg/kg), atropine sulfate (3.0 mg/kg), atropine methyl nitrate or bromide (3.0 mg/kg), and 0.9% saline. The drugs were injected in equal volume amounts subcutaneously according to an individualized random schedule. A minimum of 4 days elapsed between drug treatments. The experiment was carried out in this manner: The rats were acclimated daily to the hammock for 15 min. Single photic pulses were then presented at a rate of 1/7 sec. Following 5 min of iterative stimulation a block of 25 consecutive responses was summed by the CAT. Following recording, the photic stimulation was interrupted, a drug injected, and a 15 min period elapsed before the iterative stimulation was resumed. A second block of 25 consecutive responses was recorded 5 min following resumption of the iterative stimulation.

Measurement and Statistical Analysis

VER from the right visual cortex were plotted for each set of 25 photic pulses. These responses typically included 3 positive-negative component complexes labelled P_1-N_1, P_2-N_2, and P_3-N_3. The peak latency was determined for each positive or negative component and the peak-to-peak amplitude was determined for each component (e.g. P_1-N_1, N_1-P_2, P_2-N_2, etc.). The data were treated with analysis of variance techniques.

RESULTS

Following the injection of amphetamine, physostigmine, or pilocarpine, the cortical EEG pattern shifted from one of mixed synchronized and desynchronized rhythms to a pattern dominated by fast low-voltage waves indicative of electrocortical activation. Atropine injection resulted in a predominantly synchronized pattern. Following methyl atropine and saline injections the EEG rhythms did not appear to change with respect to control observations. The recording procedure precluded a direct observation of the animal's behavioral arousal level. Observations made following the recording sessions did not reveal any apparent differences among animals in terms of exploratory behavior.

A representation of a characteristic rat VER is displayed in Fig. 1. The mean VER peak delay values for the various drug treatments are presented in Table 1. By inspection of the Table it can be observed that with the exception of the third negative wave in the VER complex (N_3) the peak latency measures for the components were generally unaffected by the drug treatments. Newman-Keuls tests based on a reliable N_3 drug-peak latency analysis of variance (cf. Table 1) indicated that physostigmine significantly reduced N_3 peak latency. This was the only reliable drug-induced modification of the temporal features of the VER.

The mean VER peak-to-peak amplitude values for the various drug treatments are presented in Table 2. The amplitude of the N_2P_3 wave complex was unaffected by any of the drug treatments. Each of the other wave complexes was reliably affected by one or more of the chemical compounds. The results of Newman-Keuls tests based upon reliable analysis of variance (cf. Table 2) are presented in Table 3. Of the drugs used in this investigation, amphetamine and physostigmine were most effective in modifying VER component amplitude. With the exception of the N_2P_2 and N_2P_3 wave complexes amphetamine amplitude values were significantly lower than the corresponding saline control values for the remaining wave complexes. Physostigmine amplitude measures were reliably lower than the saline measures for the N_1P_2 and P_3N_3 when compared with the saline amplitude value. Atropine and methyl atropine values did not differ significantly from the saline values for any of the wave complexes although with the P_2N_2 and P_3N_3 complexes atropine slightly enhanced the wave amplitudes. With the P_2N_2 complex the amphetamine and physostigmine values differed reliably from the atropine measures but did not differ from the saline values.

Of particular interest is the observation that while amphetamine had a more profound effect on P_1N_1 amplitude than did physostigmine, physostigmine had a significantly greater effect on P_3N_3 amplitude whereas the effects of this compound on the other wave components had not been statistically significant.

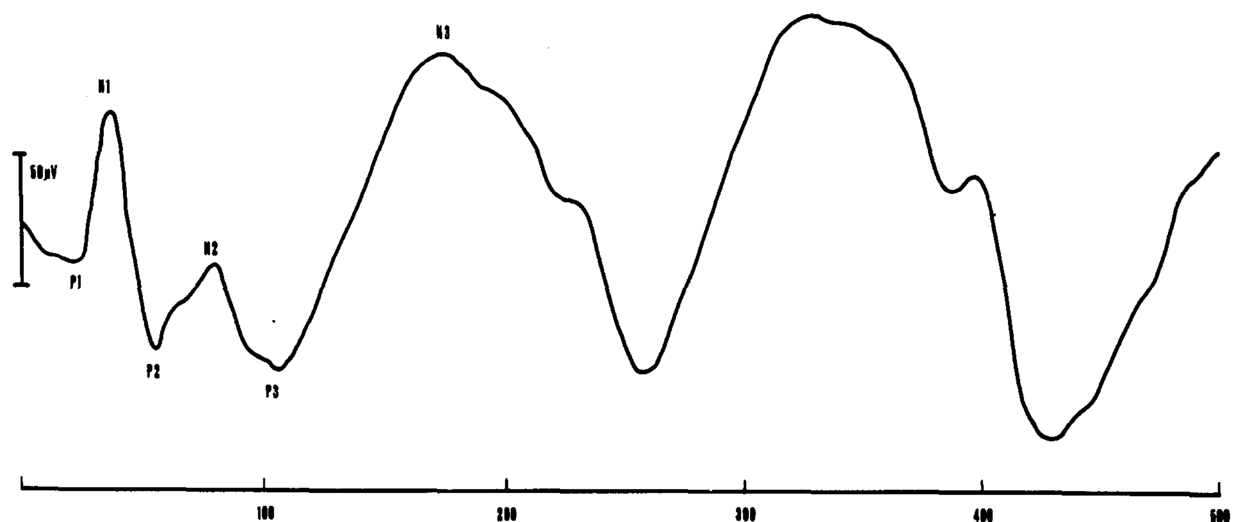

FIG. 1. Example of rat visually evoked potential. The 6 components examined are indicated. Negativity at the recording electrode is indicated by an upward deflection of the tracing. Amplitude and latency calibrations are indicated respectively by vertical and horizontal lines.

TABLE 1

MEAN PEAK LATENCY VALUES IN MILLISECONDS (± STANDARD ERROR OF THE MEAN) FOR THE VARIOUS VER COMPONENTS ACROSS EACH DRUG TREATMENT

VER Component	Saline	Atropine	Methyl Atropine	Pilocarpine	Amphetamine	Physostigmine	F ratio $df = 5/50$	P*
P_1	16.39 ± 0.37	15.68 ± 0.46	15.67 ± 0.66	16.27 ± 0.72	16.86 ± 0.44	15.68 ± 0.62	1.06	NS
N_1	24.86 ± 1.19	25.11 ± 1.53	24.70 ± 1.29	24.74 ± 1.50	24.76 ± 1.67	24.25 ± 1.46	0.23	NS
P_2	44.33 ± 1.23	44.70 ± 1.29	44.94 ± 1.59	45.19 ± 1.97	44.70 ± 1.62	45.94 ± 1.99	0.39	NS
N_2	73.41 ± 2.05	71.24 ± 2.05	70.90 ± 1.78	74.34 ± 2.71	72.09 ± 2.50	73.87 ± 2.69	1.44	NS
P_3	94.80 ± 3.58	91.09 ± 2.91	86.31 ± 2.38	89.06 ± 2.40	88.95 ± 1.22	91.09 ± 3.05	1.80	NS
N_3	159.71 ± 3.46	156.36 ± 3.33	163.06 ± 4.14	159.23 ± 3.26	162.14 ± 3.63	141.76 ± 5.31	5.95	0.001

*Significance level

TABLE 2

MEAN PEAK-TO-PEAK AMPLITUDE VALUES IN MICROVOLTS (± STANDARD ERROR OF THE MEAN) FOR THE VARIOUS VER COMPONENTS ACROSS DRUG TREATMENT

VER Component	Saline	Atropine	Methyl Atropine	Pilocarpine	Amphetamine	Physostigmine	F ratio $df = 5/50$	P
P_1N_1	74.73 ± 7.09	72.27 ± 7.43	68.90 ± 6.32	70.27 ± 6.45	53.73 ± 5.53	59.91 ± 4.29	5.04	0.001
N_1P_2	93.72 ± 12.27	93.91 ± 11.79	84.72 ± 12.73	88.63 ± 12.14	64.45 ± 10.39	65.36 ± 6.42	4.17	0.001
P_2N_2	73.40 ± 9.07	84.64 ± 9.09	66.64 ± 8.25	63.09 ± 7.81	53.82 ± 7.60	59.54 ± 5.60	4.87	0.001
N_2P_3	30.00 ± 5.39	26.45 ± 2.92	33.00 ± 5.57	23.27 ± 4.01	29.73 ± 5.87	23.00 ± 3.91	1.56	NS
P_3N_3	86.73 ± 8.58	94.73 ± 10.71	89.73 ± 10.28	73.91 ± 11.87	46.00 ± 4.80	21.00 ± 3.95	46.15	0.001

DISCUSSION

There is ample evidence to indicate that a number of pharmacological agents modify the parameters of rat VERs [3, 5, 11, 17, 18, 28]. However, with respect to the drugs used in this investigation, there appears to be a paucity of studies using rat preparations with which to compare the present results. On the other hand, the neuropharmacological properties of amphetamine, physostigmine, and pilocarpine are well-known. For example, it has been reported that unanesthetized rabbits and cats typically display an electrocortical arousal pattern following the injection of either amphetamine, pilocarpine, or physostigmine [2, 4, 15]. In addition, Bradley [4] reported that amphetamine and physostigmine produce LVF EEG patterns in encéphale isolé cat preparations. Behavioral excitement was shown to be associated with amphetamine but not with physostigmine injections. Cortical EEG patterns observed following the injection of amphetamine, physostigmine, or pilocarpine in the present study were consistent with the foregoing observations. However, there is ample evidence to indicate that amphetamine and physostigmine, while producing similar electrocortical indices of arousal, have different modes of action on the arousal mechanism. With cerveau isolé cat preparations, on the one hand, the injection of physostigmine produced a fast low-voltage EEG activation pattern while on the other hand, amphetamine was ineffective in modifying ongoing electrocortical slow wave patterns [4]. It also has been shown that midbrain reticular formation evoked potentials are differentially affected by amphetamine and physostigmine treatments [29,30]. Since arousal processes are presumably mediated by cholinergic neurons at several levels of the nervous system [7, 14, 23, 26], physostigmine and pilocarpine may have several sites of action. Ostensibly the site of interaction between amphetamine and the arousal mechanism resides in the brain stem [4].

In the present investigation, the actions of amphetamine and physostigmine were generally parallel for both peak latency and peak-to-peak amplitude VER measures. Nevertheless, these two drugs could be differentiated in terms of effect on specific waves of the VER complex. The analysis of the latency values of the various VER peaks revealed that amphetamine values were nearly identical with the values associated with the control substances. On the other hand, the peak latency of the N_3 component was significantly reduced by the injection of physostigmine. This was the only peak latency affected by any of the drug treatments. With respect to peak-to-peak amplitude values, amphetamine and physostigmine did not significantly differ from one another in terms of the early VER components. These drugs did have a differential effect on the P_3N_3 late wave complex, however. Physostigmine profoundly suppressed the amplitude of the P_3N_3 complex. Amphetamine also suppressed the amplitude of this component although to a degree that was significantly less than that of physostigmine. Pilocarpine reliably suppressed P_3N_3 amplitude but not to the same degree as either physostigmine or amphetamine. This was the only statistically reliable effect pilocarpine exerted on any latency or amplitude measure.

It had been expected that the effects of physostigmine and pilocarpine would be quite similar. Perhaps insufficient time had been allowed for the peak effects of pilocarpine to develop before recording. Little information is available regarding the central uptake and activity of pilocarpine [16] although the time-course of physostigmine activity has been documented [21].

The P_2N_2 and N_2P_3 complexes were not reliably suppressed by the drug treatments. This observation is somewhat at variance with the report of Fleming [8] in which it was noted that during trace conditioning in the cat, intercorrelations between individual components are high, i.e., VER components display a good degree of communality in amplitude potentiation or suppression during behavioral tasks. Hall [12] has demonstrated, however, that components of the rat VER show differential modulation during conditioning procedures. If, as suggested by Torres and Warner [27] that the various components are mediated by different neural systems, then it would not be surprising that some components of the VER may not be responsive to certain classes of drugs while others may be.

It is of particular interest to note that the N_3 component displayed a differential response to physostigmine,

TABLE 3

PAIR-COMPARISON MATRICES OF MEAN DIFFERENCES BETWEEN DRUGS FOR COMPONENT AMPLITUDES SHOWN TO BE SIGNIFICANT BY ANALYSES OF VARIANCE

P_1N_1

	Saline	Atropine	Pilocarpine	Methyl Atropine	Physostogmine
Amphetamine	21.00*	18.54*	16.54	15.17	6.18
Physostigmine	14.82	12.36	10.36	8.99	
Methyl Atropine	5.83	3.37	1.37		
Pilocarpine	4.46	2.00			
Atropine	2.46				

N_1P_2

	Atropine	Saline	Pilocarpine	Methyl Atropine	Physostigmine
Amphetamine	29.46*	29.27*	24.18*	20.27†	0.91
Physostigmine	28.55*	28.36*	23.27*	19.36*	
Methyl Atropine	9.19	9.00	3.91		
Pilocarpine	5.28	5.09			
Saline	0.19				

P_2N_2

	Atropine	Saline	Methyl Atropine	Pilocarpine	Physostigmine
Amphetamine	30.82*	19.58	12.82	9.27	5.72
Physostigmine	25.10*	13.86	7.10	3.55	
Pilocarpine	21.55	10.31	3.55		
Methyl Atropine	18.00	6.76			
Saline	11.24				

P_3N_3

	Atropine	Methyl Atropine	Saline	Pilocarpine	Amphetamine
Physostigmine	73.73*	68.73*	65.73*	52.91*	25.00*
Amphetamine	48.73*	43.73*	40.73*	27.91*	
Pilocarpine	20.82*	15.82*	12.82*		
Saline	8.00	3.00			
Methyl Atropine	5.00				

*Significant mean difference (0.01 Level) as determined by Newman-Keuls Test.
†Mean difference approached but did not reach significance.

amphetamine, and pilocarpine. That pilocarpine was ineffective in modulating either latency or amplitude of any of the other VER components may suggest that the mechanisms for the production of this wave is markedly sensitive to cholinergic stimulation. In a previous investigation [9] it was shown that photically evoked after-discharge bursting was suppressed in a manner similar to the suppression of the P_3N_3 complex in this study. Physostigmine markedly suppressed AD bursting, amphetamine had an intermediate effect, and pilocarpine had the least, albeit a statistically significant, effect. With the cat preparation, the N_3 component has been considered to be the first wave of the AD burst [6,24]. The results of this investigation coupled with the observations reported in related studies [9,10] suggest this to be the case with the rat also. It has been determined with the rat that AD bursts are initiated in the rat lateral geniculate nucleus presumably by a recurrent inhibitory mechanism [13,25]. Andersen and Andersson [1] suggest

that it is an intrinsic property of thalamic nuclei to generate spindle discharges. Such discharges are then projected to specific cortical areas rather than widespread cortical loci. In intact preparations, the initiation of spindles can be suppressed by activation of the thalamic nuclei by discharges from brain stem pathways. Furthermore, it has been demonstrated that thalamocortical synchronized activity can be desynchronized by the local application of acetylcholine to cells within thalamic nuclei [1]. Therefore, with respect to the present results, it may be suggested that the N_3 component of the rat VER is initiated by a mechanism of thalamic origin, i.e., within the lateral geniculate nucleus. Furthermore, it seems reasonable to assume that the N_3 component can be modulated along two dimensions, direct cholinergic activation, or by input from brain stem adrenergic structures activated by amphetamine. Direct cholinergic stimulation has the more profound effect on the elicitation of the wave, reducing peak latency and markedly attenuating amplitude excursion. The other components investigated did not display a differential response to adrenergic and cholinergic agents and it may be speculated that these waves of the VER complex do not rely on lateral geniculate recurrent inhibitory processes but may depend on the state of cortical excitability as a change in cortical excitability is a common feature of physostigmine and amphetamine treatments.

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