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Authors

Wada, SI
Starr, A

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Experimental Section

GENERATION OF AUDITORY BRAIN STEM RESPONSES (ABRs). I. EFFECTS OF INJECTION OF A LOCAL ANESTHETIC (PROCAINE HCl) INTO THE TRAPEZOID BODY OF GUINEA PIGS AND CAT¹

SHIN-ICHI WADA² and ARNOLD STARR³

Department of Neurology, University of California at Irvine, Irvine, Calif. 92717 (U.S.A.)

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The short-latency auditory evoked potentials, commonly referred as auditory brain stem responses (ABRs), consist of 7 vertex-positive peaks within the first 10 msec after stimulus onset (Jewett and Williston 1971; Lev and Sohmer 1972). The short latencies of the ABR peaks implicate origins in peripheral and brain stem portions of the auditory system. The neural origin of wave I seems well established since it mimics the characteristics of the cochlear nerve action potential recorded from the human middle ear (Picton et al. 1971). Correspondence between other components of the ABR and the function of particular auditory nuclei and tracts derive from animal studies showing high amplitude evoked potentials and time-locked single unit discharges in different brain stem regions at latencies corresponding to particular ABR components (Jewett 1970; Lev and Sohmer 1972; Huang and Buchwald 1977) as well as changes in the ABR accompanying experimental brain stem lesions in animals (Buchwald and Huang 1975; Goldenberg and Derbyshire 1975; Achor and Starr 1980b). Furthermore, there has been clinical correlation between abnormalities of ABRs recorded in patients with brain stem disorders and the site of brain stem lesions as defined by CT scan or autopsy (Sohmer et al. 1974; Starr and Achor 1975; Starr and Hamilton 1976; Thornton and Hawkes 1976; Gilroy et al. 1977; Rosenhamer 1977; Stock-

ard and Rossiter 1977; Gilroy and Lynn 1978; Uziel and Benezech 1978).

However, the precise generator of each component is still uncertain. Initially, several investigators (Jewett 1970; Lev and Sohmer 1972) suggested that each peak of the ABR in the cat has a single main generator. Consequently, Jewett (1970) and Buchwald and Huang (1975) proposed a generator scheme for each component, i.e., P1, VIIIth nerve; P2, near the cochlear nucleus; P3, near the superior olive; P4, lateral lemniscus or preolivary region; and P5, inferior colliculus. In contrast, Achor and Starr (1980a) concluded from a study correlating potentials measured from within the brain stem with the surface ABR that most of the components of the ABR in the cat may have major contributions from several brain stem sites. Moreover, the results from their lesion study in cat (Achor and Starr 1980b) also were interpreted as indicating that a single auditory pathway site contributes to more than one component of the ABR.

In clinical testing situations in man, a useful rule has been that wave I originates from the VIIIth nerve, wave III from the pons (trapezoid body, superior olive), and wave V from the mid-brain (lateral lemniscus, inferior colliculus), while the generators of waves II, IV, VI and VII are still uncertain (Starr and Hamilton 1976).

The trapezoid body has widespread effects on the generation of the ABR (Buchwald and Huang 1975; Achor and Starr 1980b; Britt and Rossi 1980). It is unclear whether the trapezoid body is one of the generators itself or only a pathway interconnecting the generators of the ABR compo-

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² Present address: Department of Neurosurgery, Kumamoto University Medical School, 1-1-1 Honjo, Kumamoto 860, Japan.

³ To whom direct all correspondence should be addressed.

nents. The experimental techniques for investigating the generation of the ABR such as destructive lesions of the brain stem, or defining the correspondence between surface potentials and brain stem potentials, have certain limitations. For example, transection of the neuroaxis may have profound effects on structures remote from the lesion, while the definition of a large amplitude voltage field in a brain stem site does not insure that this field is reflected in the surface recordings.

The purpose of the present study was to clarify the relationship of the trapezoid body to each component on the ABR using a reversible lesion method, that of placing a topical anesthetic in the trapezoid body from the ventral approach, thereby avoiding trauma to the remainder of the brain stem. This method was then compared with a surgical section of the trapezoid body and other brain stem structures to be presented in companion papers (Wada and Starr 1983a, b).

Methods

Subjects

Sixteen guinea pigs and one cat were studied. The guinea pigs were 0.6–1.0 kg in weight and the cat was 3.2 kg.

Surgery

The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A small screw was fixed at the point of the skull 3 mm posterior to the bregma and served as the recording electrode. A needle electrode was placed in the neck and served as a reference electrode. The animals were placed in the supine position and their head held securely by a mouth clamp and hollow ear bars. The skin was incised from the upper neck to the upper level of the chest just to the left of the midline. A tracheotomy was performed in the guinea pigs, while an endotracheal tube was placed in the cat. The subcutaneous tissue and the muscle were divided carefully and retracted. All subsequent procedures were performed using an operating microscope. A few of the superficial veins or small muscle fibers in the neck were coagulated but all of main arteries and

veins were maintained. The clivus was exposed and removed carefully in the midline with a dental drill. The base of the brain stem was then easily visualized and the dura incised and retracted to obtain access to the trapezoid body.

Stimulus generation

Monaural or binaural 'click' stimuli were produced by activating Beyer transducers with a 100 μ sec square wave pulse at a rate of 25.6/sec. The earphones were coupled to hollow ear bars by a 3.5 cm length polyethylene tubing, containing fine steel wool for acoustic damping. The intensity of click was 94 dB SPL peak equivalent or 65 dB above threshold for a jury of 3 normal hearing human subjects. The acoustic wave form has been previously defined (Achor and Starr 1980a).

Recordings

The ABR was recorded between a screw electrode in the skull, 3 mm posterior to the bregma, and a reference needle electrode at the midline of the base of the neck. In 7 guinea pigs, the ABR was recorded also from needle electrodes at the pinnae referenced to the neck electrode.

Battery-operated amplifiers located inside the sound attenuating room amplified the brain potentials 100,000 times with a bandpass of 100–3000 Hz (–6 dB points, 12 dB/octave). The amplified signals were led to a computer and monitored on an oscilloscope. Positivity at the vertex or pinna was displayed in upward direction. The evoked activity was sampled at a rate of 40 kHz (25 μ sec bin width) and 150 trials were averaged. The analysis epoch of 12.8 msec (512 points) consisted of a 3.0 msec prestimulus period and a 9.8 msec post-stimulus period. The digitized data were stored on disk for subsequent analysis.

Procaine solution (1%), prepared with pure powder procaine and isotonic saline, was injected with a number 30 needle and a 1 ml volume syringe fixed in a micromanipulator. The tip of the needle was lowered 1.5 mm below the surface of the trapezoid body in the midline under visual control using the operating microscope. Thirty μ l of the procaine solution was injected in each guinea pig and 50 μ l in a cat. In many cases this entire volume was not retained in the brain stem as some

of the injected procaine solution flowed out into the cerebrospinal fluid space from the trapezoid body. The procaine solution injected in 5 of the guinea pigs contained a few drops of methylene blue or Pontamine sky blue for subsequent histological observation as to the spread of the injected solution in the brain stem. In 6 of the guinea pigs 30 μ l of only the isotonic saline solution was injected into the trapezoid body as a control procedure.

The ABR was recorded in 3 epochs: prior to exposing the brain stem; after the brain stem was exposed but prior to injecting the solution; and after the injection. There were no significant changes in the ABR between the first two epochs. After the injection significant changes appeared and recordings of the ABR were continued for 4–18 h. During the recordings the rectal temperature was monitored and maintained at 36–38°C by a circulating water pad. At the end of the experiment the animals were perfused through the heart with normal saline followed by 10% buffered formalin. The entire brain was removed, the brain stem portion containing the trapezoid body blocked and stored in 10% buffered formalin for 1 week prior to processing. Serial transverse 60 μ m frozen sections were made, except in the 5 guinea pigs in which the dye solution was injected. In these latter animals thick sections (200 μ m) of the brain stem were made and left unstained so that the distribution of the blue dye could be followed. In all other animals the sections were stained with cresyl violet and examined for evidence of hemorrhage or necrosis at the needle's entry into the brain stem.

Quantification

ABR amplitudes were defined between pre-stimulus baseline and the peak (P1–P5) and trough (N1–N5) for each component as well as combining the peak to following trough values for waves I (P1–N1), II (P2–N2), etc., for 3 pre- and post-injection tracings. All amplitudes were then converted to a percentage of the pre-injection values of the largest component, P3 (or wave III when comparing peak-to-trough measures), to control for variations in absolute amplitude measures between animals. Base-to-peak measurements were gener-

ally more useful than peak-to-trough measurements in determining whether a change in a given portion of the evoked potential was due to an effect on a peak or the following trough (or vice versa), while the peak-to-trough measure avoided the problem of a component's changing polarity due to baseline shifts.

The effects of the injection on amplitude were expressed

$$\frac{\text{control amplitude} - \text{post-injection amplitude}}{\text{amplitude of control P3 (or wave III)}} \times 100 = \% \text{ of P3 (or wave III)}.$$

Increment of amplitude was expressed by a plus (+) and decrement by a minus (–).

Latency was measured at the peak of each component and any change was expressed in absolute terms. Latency changes were not considered to be due to experimental procedures if they were associated with changes in body temperature (Williston and Jewett 1977).

The ABR was very stable in both amplitude and latency during control recording. After the injection, the ABR stabilized within several minutes and it is from these stable ABRs that measures of the initial post-injection results were obtained.

Results

Guinea pig

(I) *Normal ABR.* Auditory brain stem responses of guinea pigs (Fig. 1A), recorded between a 'vertex' electrode in the midline of the skull near the bregma referenced to a non-cephalic site, consists of up to 5 positive and 4 negative waves in the first 10 msec after stimulation (see Dum et al. 1981). The components are designated by their polarity at the vertex (P for positivity and N for negativity) and their approximate latency in msec to high intensity clicks (94 dB SPL). Fig. 1A contains the ABRs from one of the guinea pigs tested over a 50 dB intensity range. Graphs relating the latency and amplitude of the components as a function of signal intensity are Fig. 2. The latency/intensity function for the 4 components are parallel (Fig. 2A) whereas the amplitude/la-

Fig. 1. The ABR from normal guinea pigs. In this animal and in all subsequent figures the recordings are derived from the vertex to a neck reference. A: ABRs to clicks of decreasing intensity; dB refers to peak equivalent SPL. The components are labeled by P or N (referring to their vertex polarity, positive or negative) and their approximate latency in msec; vertical lines descend from the largest components, P3 and P4. B: ABRs to clicks of differing rate; the numbers to the left refer to the interstimulus interval. C: ABRs at the vertex and the pinnae ipsilateral and contralateral to the stimulated ear, all referenced to a non-cephalic site.

tency function for component P3 differs from that of the other components (Fig. 2B). For a 40 dB increase in signal intensity P3 grows more than 4-fold whereas the other components only double

in size. At threshold both P3 and P4 are the only components remaining. The ABRs recorded at the lateral surface of the head (the pinnae) differ in some detail from the ABR recorded at the vertex (Fig. 1C, Tables I and II) with some of the components being clearly lateralized: waves P1 and N1 were not detected at the pinna contralateral to the stimulated ear in 3 of the 14 animals, while waves P2 and N2 were not detected at the ipsilateral pinnae in 7 of the 14 animals. Furthermore, wave P5 was not detected at the pinnae in 4 of the 14 animals and at the vertex 2 of the 14 animals. All the other components were always detected at the 3 sites. Table I contains the mean and S.D. of the latency and the amplitude of each component at the 3 sites. Amplitudes have been normalized so that the amplitude of the components at Cz in

Fig. 2. Graphs of the mean and standard deviation of amplitude and latency of ABR components measured at the vertex as a function of stimulus variables. A: latency as a function of stimulus intensity. B: amplitude as a function of stimulus intensity. C: latency as a function of stimulus rate. D: amplitude as a function of stimulus rate.

TABLE I

Component	Ipsilateral pinna			Vertex			Contralateral pinna		
	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.
<i>(A) Latency (msec) of guinea pig ABR components</i>									
P1	14	2.06	0.07	14	2.01	0.08	11	2.01	0.01
N1	14	2.50	0.12	14	2.39	0.08	11	2.32	0.09
P2	7	2.79	0.11	14	2.65	0.07	14	2.59	0.09
N2	7	2.95	0.07	14	2.91	0.08	14	2.94	0.10
P3	14	3.30	0.08	14	3.31	0.08	14	3.29	0.08
N3	14	3.76	0.08	14	3.82	0.12	14	3.79	0.12
P4	14	4.24	0.15	14	4.31	0.14	14	4.30	0.14
N4	14	5.05	0.18	14	5.02	0.15	14	4.91	0.17
P5	10	5.51	0.23	12	5.58	0.30	10	5.65	0.19
<i>(B) Amplitude of guinea pig ABR components</i>									
I	14	108.8	43.0	14	100	11	30.0	24.3	
P1	14	74.4	35.2	14	100	11	44.6	25.8	
N1	14	280.0	181.0	14	100	11	"		
II	7	18.9	11.9	14	100	14	147.2	67.9	
P2	7	24.9	37.5	14	100	14	101.0	43.6	
N2	7	"		14	"	14	"		
III	14	38.6	11.5	14	100	14	56.6	12.3	
P3	14	43.3	14.9	14	100	14	42.7	14.7	
N3	14	20.0	19.4	14	100	14	76.5	27.1	
IV	14	39.8	12.0	14	100	14	40.2	14.4	
P4	14	45.2	23.7	14	100	14	28.7	19.8	
N4	14	34.6	9.3	14	100	14	44.5	27.5	
V	10	83.0	71.0	12	100	10	71.6	69.3	
P5	10	"		12	"	10	"		
N5	10	39.7	26.3	12	100	10	36.2	23.7	

Measured as % of vertex amplitude.

" Component of opposite polarity.

each animal is considered as a 100%. Wave I is significantly ($P < 0.05$) larger at the pinna ipsilateral than contralateral to the ear stimulated whereas the reverse applies for wave II. Note that P1 is positive at the base of the pinna ipsilateral to the stimulated ear whereas it is negative at the mastoid (Dum et al. 1981). The negative components of waves I (N1) and II (N2) are positive in polarity at the ipsilateral mastoid. All succeeding components were largest at the vertex with the only lateralization occurring for wave III with its component N3 being larger at the pinna contralateral rather than ipsilateral to the stimulated ear. No significant differences in latency as a function of recording site were noted except for N1 at the ipsilateral pinna which was delayed

compared to the contralateral pinna ($P < 0.05$).

The effects of repetition rate on the ABR are shown in Fig. 1B and measures of the latency and amplitude of components P1 through P4 at the vertex are plotted in Fig. 2C and D. When stimulus repetition rate increased from 10/sec to 100/sec (equivalent to a change in the interstimulus interval from 100 msec to 10 msec (Fig. 1B)), there was an increase in the latency of the components that became progressively larger with each successive component (P1, 100 μ sec; P2, 125 μ sec; P3, 200 μ sec; P4, 425 μ sec). An increase in the stimulus repetition rate was also associated with a decrease in the amplitude of the components which was profound for P4, modest for P3, and minimal for P1 and P2. These parametric studies provide

quantitative distinctions between the components of the ABR in their behavior to stimulus variables which will be used to help identify the ABR components following trapezoid body lesions.

Binaural interaction was examined in 16 normal guinea pigs. Binaural interaction represents a non-linear processing of binaurally evoked ABRs when compared to the sum of the ABRs evoked by separate monaural stimulation. In the guinea pig the interaction takes the form of a lower amplitude of the binaurally evoked ABR compared to the sum of the separately evoked monaural ABRs (Fig. 3). This amplitude disparity occurs in the time domain of P4 and N4 and amounts to a 50–60% reduction in the amplitude of the ABR. The effects of trapezoid body lesions on this form of binaural interaction were examined.

(II) *Procaine injection into the trapezoid body.* Shortly after the injection of the procaine solution into the region of the trapezoid body the ABR to monaural stimulation underwent dramatic changes (see Fig. 4 for an example and Tables II and III for quantitative measures). Component P2 broadened in duration (average 335 μ sec) and its peak

Fig. 3. Binaural interaction in the guinea pig ABR. Stimulation of the right ear (R, top trace), left ear (L, second trace), binaural stimulation (solid line, third trace) and algebraic sum of the monaural stimulations (R+L, dotted line, third trace), and binaural interaction representing the sum of the monaural stimulations minus the binaural stimulation (R+L-Binaural, fourth trace).

Fig. 4. ABRs following procaine injection into the trapezoid body (dotted dark traces) compared with control ABRs (solid light traces) in a guinea pig. Monaural stimulation (upper two traces), binaural stimulation (third trace) and binaural interaction (fourth trace) are shown. The label Px refers to a component occurring in the ABR following procaine injection.

was delayed 0.1–0.2 msec in approximately 50% the instances: N2 was uniformly delayed and peaked at the time that P3 normally occurred. P3 and N3 were almost always lost and if present their amplitudes were reduced. P4 broadened in duration and its peak occurred earlier (20 of 25 instances) without any consistent change in amplitude. N4 was attenuated without any regular latency change. Component P5 when present occurred earlier in 7 of the 18 instances. Components P1 and N1 were unaffected. In response to binaural stimulation (Fig. 4) P2 and P4 increased in amplitude when compared to those components in the control period. Moreover, all components of the ABR to binaural stimulation now equalled the sum of the monaural responses with a loss of binaural interaction in the region of P4 and N4.

The identity of the components following the procaine injection is uncertain because of the change in morphology of the entire response. The issue is particularly relevant for the component occurring around the time of P4. Following the injection this component appeared at an earlier latency, was broader in duration, and showed no evidence of binaural interaction compared to the

TABLE II
Effects of procaine injection into trapezoid body on ABR in guinea pig.

Guinea pig	Ear	Thresh- old (dB SPL)	Injected material	% change in amplitude as an (f) of control P3 or wave III											Recov- ery		
				I	P1	N1	II	P2	N2	III	P3	N3	IV	P4		N4	P5
GPO	R	44	+						+10	*	*	*		+18	-21		4(+)
	L	44							+10	*	*	*		+10	-49		4(+)
GPQ	R	54	+				+16	+28		*	*	*		+23	-27		4(+)
	L	54					+56	+68		*	*	*					4(+)
GPX	R	44	+					+10		*	*	*		+17	-36	+35	2(+)
	L	34						+12		*	*	*			-43		2(+)
GPY	R	44	+				+26	+20	+14	*	*	*		+19	-36	+20	3(+)
	L	44					+23	+18	+18	*	*	*		+10	-43	+32	4(+)
GPZ	R	74	+				-10	-13		*	*	*		+21	-25	**	
	L	74								*	*	*		+23	-41	**	
GA	R	54	+							*	*	*		-18	-30		4(+)
	L	54								*	*	*		+26	-43	+29	4(+)
GD	R	54	+							*	*	*				**	3(+)
	L	64						+15	-12	-88	-86	-28			-28	**	2(+)
GPU	R	44	+				-13	-20		*	*	*			-31	+29	2(+)
	L	44								-96	-85	-44"			-53	+19	2(+)
GPV	R	34	+				+15	+25		*	*	*		-19	-73		3(+)
	L	44					+11	+15		*	*	*		-25	-50	**	3(+)
GPW	R	34	+					+12		-98	-84	-61"		-64	-32	+32	2(+)
	L	34					+13		+18"	*	*	*		-16	-58	+19	2(+)
GPS	R	34	+				-15	-37		*	*	*		-46	-60	+27	1(+)
	L	44								*	*	*		-12	-67	+58	1(+)
GPT	R	34	+						+14	*	*	*		+33	-38	**	1(+)
	L	34					-15		-22	-97	-100"	-42		-52	-70	-23	2(+)
GPR	R	64	+				+52	+69		*	*	*			-44	**	4(+)
	L	44					Footnote (1)										4(+)
GE	R	44					+14		+23	-78	-64	-61		-19	-34		1(+)
	L	44					+33		+50	-64	-63	-30		-33	-12	-10	1(+)
GF	R	44															
	L	44															
GG	R	54															
	L	44															

Recovery: 4(+), 75%; 3(+), 50-75%; 2(+), 25-50%; 1(+), 25%. The blank spaces under '% change in amplitude' represent amplitude shifts of <10%.

* Component lost.

** Component not identified prior to injection.

" Polarity change. + = increase in amplitude as a % of control P3 (or III) amplitude. - = decrease in amplitude as a % of control P3 (or III) amplitude. P = procaine, D = dye, S = saline.

TABLE III
Latency shifts (msec) of ABR components following procaine injection into trapezoid body of guinea pig.

Guinea pig	Ear	P1	N1	P2	N2	P3	N3	P4	N4	P5
GPO	R				+0.25	*	*	-0.4		
	L				+0.3	*	*	-0.6		-0.2
GPQ	R			+0.15	+0.5	*	*	-0.35	+0.4	
	L				+0.52	*	*	+0.12	+0.25	+0.3
GPX	R			+0.07	+0.2	*	*	-0.2		
	L			+0.15	+0.3	*	*	-0.15	-0.1	
GPY	R				+0.37	*	*	-0.4	-0.15	
	L			+0.15	+0.72	*	*	-0.25	-0.15	-0.42
GPZ	R			+0.27	+0.4	*	*	-0.67	-0.6	**
	L			+0.3	+0.5	*	*	-0.6	-0.52	**
GA	R			+0.2	+0.3	*	*	-0.12	+0.22	
	L				+0.37	*	*		+0.12	
GD	R			+0.17	+0.17	+0.1				**
	L				+0.15			-0.15		**
GPU	R				+0.4			-0.15		
	L				+0.17		-0.15	-0.1		-0.1
GPV	R				+0.27	*	*	-0.25		
	L				+0.4	*	*	-0.1		**
GPW	R				+0.15		-0.3	-0.15	-0.2	-0.17
	L			+0.1	+0.32	*	*	-0.22		-0.17
GPS	R			+0.2	+0.35	*	*	-0.15		
	L			+0.17	+0.37	*	*			-0.12
GPT	R			+0.15	+0.27	*	*	-0.1	+0.12	**
	L			+0.15	+0.27		-0.17	-0.17	+0.35	-0.32
GPR	R			+0.3	+0.7	*	*	+0.35	+0.83	**

* No component.

** Component not present in control period.

+ = increase in latency.

- = decrease in latency

control P4. The component in question (designated Px) might be a delayed P3, a fused P3 and P4, a P4 shifted in latency and changed in form or an entirely new component. To evaluate among these alternatives the effects of click intensity (Fig. 5A) and click rate (Fig. 5B) on Px was tested in 3 animals while its scalp distribution (Fig. 5C) was defined in 7 animals.

The latency/intensity function of Px was the same as P4. The amplitude/intensity function also resembled that of P4 showing slightly more than a 2-fold increase over a 40 dB intensity range. The effects of stimulus rate on both latency and amplitude of component Px also strongly resembled those effects on component P4 rather than on P3. When stimulus rate changed between 10/sec and 100/sec there was an increase in latency of Px of

400 μ sec similar to the 425 μ sec defined for P4 rather than 200 μ sec defined for P3. Moreover the amplitude of Px diminished precipitously at the fast stimulus rates similar to P4. Thus, Px in its behavior to stimulus rate and intensity, resembled component P4 rather than component P3. Finally, the scalp distribution of Px was similar to that of P4 being of larger amplitude at the pinna ipsilateral than contralateral to the stimulated ear while P3 was of equal amplitude at these two sites (Tables I and IV).

In 6 animals a control injection of an equivalent quantity of isotonic saline was made into the trapezoid body. In 5 of the animals there was little (within 10%) or no effect noted on the latency or amplitude of the ABR components. In one animal (guinea pig GE) there were changes in amplitude

Fig. 5. ABRs following procaine injection as a function of (A) signal intensity, (B) stimulus rate, (C) scalp distribution.

and latency noted that were of smaller magnitude but similar in direction to that observed following procaine injection. Histological examination of this animal's brain stem showed a moderately sized hemorrhage in the trapezoid body at the injection site. Thus, placement of a needle in the trapezoid body without causing a hemorrhage and injection of a small volume of saline does not affect the ABR.

In 5 animals the distribution of the injected procaine solution in the brain stem was assessed by mixing in several drops of methylene blue or Pontamine sky blue. The immediate effects on the

Fig. 6. The reconstruction of the histology in a guinea pig in which the procaine, mixed with dye solution, spread along the decussating fibers in the trapezoid body laterally, rostrad, and caudad but remained localized to the trapezoid body. LSO, lateral superior olive; MSO, medial superior olive; TB, trapezoid body; VAS, ventral acoustic stria; DCN, dorsal cochlear nucleus; VCN, ventral cochlear nucleus; LL, lateral lemniscus.

ABR were similar to those observed following the injection of procaine alone. However, the recovery of the ABR differed in these two groups of animals as will be discussed below. On thick sections of the brain stem the blue stain was observed to spread from the injection site through the trapezoid body in both a rostral and caudal direction as well as laterally (Fig. 6). Thus, assuming that the procaine solution's distribution resembled that of the dye's distribution, the anesthetic agent was localized in the brain stem to the auditory pathway spreading beyond the point of injection to involve the trapezoid body widely.

(III) *Recovery of ABR.* Following the injection of procaine, the ABR was studied for periods up to 12 h in all but one animal (guinea pig GPZ).

TABLE IV

Component	Ipsilateral pinna			Contralateral pinna			Vertex		
	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.
<i>(A) Latency (msec) of ABR components in guinea pig following procaine injection into trapezoid body</i>									
P1	14	2.06	0.12	10	1.95	0.19			
N1	14	2.55	0.16	10	2.30	0.09			
P2	9	2.85	0.17	14	2.58	0.12			
N2	9	3.20	0.33	14	3.20	0.16			
P3	7	3.36	0.06	4	3.70	0.28			
N3	7	3.59	0.13	4	3.84	0.26			
P4	14	4.10	0.16	14	4.06	0.16			
N4	14	4.97	0.26	14	4.89	0.30			
P5	10	5.27	0.12	12	5.29	0.22			
<i>(B) Amplitude of ABR components in guinea pig following procaine injection into trapezoid body</i>									
I	14	115.8	41.0	10	32.3	14.1	14	88.7	29.1
P1	14	59.5	27.6	10	43.8	25.6	14	86.1	33.3
N1	14	376.0	225.1	10	"	"	14	127.0	136.0
II	9	37.7	30.6	14	167.1	70.6	14	123.4	70.8
P2	9	"	"	14	112.0	51.0	14	112.0	51.6
N2	9	"	"	14	"	"	14	"	"
III	7	3.8	2.4	4	9.0	7.7	3	2.6	0.5
P3	7	9.9	7.1	4	9.6	9.5	3	8.0	9.3
N3	7	"	"	4	4.8	7.8	3	"	"
IV	14	26.2	7.4	14	20.9	5.4	14	53.6	12.3
P4	14	42.7	8.2	14	22.9	13.5	14	95.5	20.4
N4	14	11.0	12.4	14	19.0	8.2	14	14.5	13.6
V	10	94.1	42.5	12	117.6	54.2	12	210.3	89.3
P5	10	"	"	12	"	"	12	"	"
N5	10	40.1	20.2	12	55.3	22.0	12	81.6	27.4

Measured as % of vertex amplitude prior to procaine injection.

" Component of opposite polarity.

In this animal, the trapezoid body was sectioned 20 min after the injection as part of a different experimental study. The degree of recovery was defined by the extent of amplitude restoration of component P3; 4+ (> 75% recovery), 3+ (50–75% recovery), 2+ (25–50% recovery) and 1+ (< 25% recovery). The pattern of recovery of the ABR that was seen in 10 of the 12 animals is depicted in Fig. 7A. P3, which had been lost immediately after the injection, reappeared 25 min later at a reduced amplitude but at the latency of P3 in the control period. With the appearance of P3, P2 decreased in duration and P4 shifted later closer to the latency for P4 and decreased in duration. Thereafter P3 continued to grow in amplitude achieving approximately 80% recovery by 7 h following the

injection. In two animals, P3 reappeared as a deflection on the ascending portion of P4 at a delayed latency (Fig. 7B, 1 h). Thereafter P3 both grew in amplitude and shifted earlier to its appropriate control latency value. In the example shown recovery of P3 was complete by 3 h.

Of the 7 animals injected with procaine solution P3 amplitude from at least one of the stimulated ears recovered to more than 75% of the control in 5 animals, to between 50% and 75% in 1 animal, and to between 25% and 50% in 1 animal. In contrast, of the 5 animals injected with both procaine and dye solution none of the ABRs recovered to 75% of the control value, one recovered to between 50% and 75%, three recovered 25–50%, and one animal only recovered less than 25%. It is

Fig. 7. Recovery of ABR following procaine injection into the trapezoid body. The time in minutes (min) and hours following injection are to the left of the traces. Note that in A, waves P3 and N3 abruptly appear at 25 min, and thereafter stay at the same latency and grow in amplitude. In B, P3 appears as a notch on the ascent of P4 at 15 min, and thereafter both grow in amplitude and shorten in duration.

likely that the dye produces long-lasting changes in neural activity as manifest by the diminished recovery of the ABR in animals receiving this material.

Fig. 8. Same as Fig. 4 except substitute cat for guinea pig.

Cat

In one cat procaine injection was made into the trapezoid body in the midline from a ventral approach (Fig. 8). In distinction to the results from the guinea pig, P2 was attenuated in the cat by 50%. However, similar to the findings in guinea pigs, N2, P3 and N3 were lost. In the cat, P4 was broadened in duration and shifted to a longer latency and N4 was attenuated and delayed even further in latency, whereas these components shifted to a shorter latency in the guinea pig. Binaural interaction in the cat was also lost.

Discussion

The results of these experiments in guinea pigs and cat show that injection of a local anesthetic agent (procaine) into the trapezoid body affects many of the components of the scalp-derived ABR: N2 was delayed making P2 broader in duration, P3 and N3 were lost, P4 was shortened in latency, broadened in duration but unaffected in amplitude, and N4 was considerably attenuated. Only P1 and N1 were unaffected by the procaine injection. These changes were temporary and recovery

of the components proceeded as the effects of the procaine were off.

Previous studies utilizing complete midline surgical section of the brain stem (Buchwald and Huang 1975; Britt and Rossi 1980) or electrolytic lesions (Achor and Starr 1980b) of the trapezoid body are in agreement that P3 is lost following an extensive lesion. However, in these latter experiments P4 was also considerably attenuated whereas in the present experiment this component's amplitude was unaffected. We too have utilized surgical section but limited only to the trapezoid body (Wada and Starr 1983a) and the changes in P4 (latency shifts but no amplitude decrement) are similar to the results obtained using procaine injection. It may be that a total midline surgical section of the brain stem or electrolytic lesions extending beyond the trapezoid body affects other auditory brain stem pathways not traveling in the trapezoid body (i.e., dorsal and middle acoustic striae for example) or produces significant generalized brain stem dysfunction to affect P4 amplitude.

An advantage of the use of a local anesthetic agent is that the effects on the ABR are reversible. A limitation is that the procaine spreads laterally along the trapezoid body fibers making it difficult to ascribe the ABR changes to an effect on any limited portion of the trapezoid body. Bipolar recordings from several different portions of the surface of the trapezoid body in 5 animals showed the amplitude of electrical activity evoked by monaural stimulation to be reduced more than 75% following the injection of procaine. Moreover, the spread of anesthetic may have been sufficient to affect the function of adjacent auditory pontine nuclear groups such as the nucleus of the trapezoid body or the superior olivary nuclear complex. Control injections with saline solution were without effect on the ABR indicating that the changes accompanying procaine injection were not due to the trauma of the needle's penetration into the brain stem nor to the effect of a fluid volume into the fiber pathway.

Following the procaine injection, the morphology of the ABR changed significantly raising questions as to the identity of the components. Attention to those stimulus and recording varia-

bles that have specific effects on different components of the ABR assisted in the definition of the changed components. This was particularly apparent for component P4 which shifted to an earlier latency following the procaine injection. This component was initially called Px because it could have been a delayed P3, a P3 fused with P4, or shortened P4. The behavior of Px in terms of its latency/intensity function, amplitude/intensity function, latency/stimulus rate function, and amplitude/stimulus rate function were all consistent with its identification as P4. These same variables can also be used along with scalp distribution in clinical application of ABR testing to aid in the identification of altered ABR components. It is intriguing in this experimental study that a brain stem lesion was accompanied by a shortening of both absolute (P4) and intercomponent latencies (P1-P4) whereas in clinical situations this phenomenon has only been noted with lesions of the cochlea (Coats and Martin 1977) and in children with Trisomy 21 syndrome (Squires et al. 1980). Destructive lesions of the central auditory pathway in human have been associated with a prolongation of absolute and intercomponent conduction times (Starr 1977; Stockard and Rossiter 1977).

Homologies between ABR components in animals and humans merits comment. Components P1 and N1, P2 and N2, and P3 and N3 in animals appear to be homologous to waves I, II and III respectively in humans based on comparable relative latencies and scalp distributions of these events (Picton et al. 1974; Williston and Jewett 1977; Allen and Starr 1978). Uncertainty exists as to the animal homologue of the IV-V complex in humans. P4 and N4 in monkey and cat (but not guinea pig and rat) are both the largest components of the ABR and the easiest to detect at low signal intensities, findings that are similar to wave V in humans. Moreover, binaural interaction in the ABR is restricted to components P4 and N4 in monkey, cat and guinea pig and to wave V in humans. Components P5 and N5 in animals are of low amplitude, variable in occurrence, and are not detected at low signal intensities comparable to wave VI in humans.

The results from this study also bear on the phenomenon of binaural interaction in the ABR.

The non-linear interaction of simultaneous monaural stimuli was first reported by Dobie and Berlin (1979) in guinea pigs and our results confirm their observations. Subsequently binaural interaction in the ABR has been described in humans (Levine 1981; Werge and Starr 1981) but questions have been raised as whether the interaction is due to an artifact of acoustic crossover (Ainslie and Boston 1980; Levine 1981). This possibility is rendered invalid by the results of the present experiment in which binaural interaction in the ABR was lost following procaine injection into the trapezoid body even though component P4 was preserved. In fact, P4 amplitudes to binaural stimulation were almost twice as large following the injection into the trapezoid body even though component P4 was preserved. Thus, binaural interaction is dependent upon the integrity of function of fibers of the trapezoid body and is not an artifact of acoustic crossover.

We conclude that components P3 and N3 are completely dependent on the function of the trapezoid body since they cease to occur following procaine injection into that structure; N4 depends, in part, on the integrity of this structure since its amplitude was reduced as a consequence of the injection whereas both P4 and P2 are relatively independent of the functioning of the trapezoid body. However, to binaural stimulation P4 was significantly altered following the trapezoid body lesion. The delay in latency of N2 following procaine injection indicates the dependence of this component as well on normal trapezoid body function. Only P1, N1 and P2 are independent of trapezoid body function.

The integrity of the trapezoid body is critical for the generation of the normal ABR pattern. Temporary cessation of its function by a local anesthetic is associated with profound changes in the components that are unlikely to be due to the trapezoid body being the sole generator of the components. Rather the trapezoid body serves as a pathway to other auditory brain stem structures that may be responsible for generating certain of the ABR components. We will examine the effects of other types of brain stem lesions on the ABR in companion papers to help clarify these alternatives.

Summary

Auditory brain stem potentials were recorded between the skull and a non-cephalic reference electrode in anesthetized guinea pigs before and after the injection of a local anesthetic agent (procaine HCl) into the trapezoid body from a ventral approach. All components except P1, N1 and P2 were affected; N2 was delayed; P3 and N3 were lost; P4 was both broadened in duration and shortened in latency; N4 was attenuated in amplitude. All of these changes were temporary and recovery of the components occurred. Identification of the altered components was aided by their latency and amplitude changes as a function of both stimulus intensity and rate. This study implicates the trapezoid body as contributing to the generation of auditory brain stem components beginning with N2.

Résumé

Genèse des réponses auditives du tronc cérébral. I. Effets d'une injection d'un anesthésique local (chlorhydrate de procaine) dans le corps trapézoïde de cobaye et de chat

Des potentiels auditifs du tronc cérébral ont été enregistrés entre le crâne et une électrode de référence non céphalique chez des cobayes anesthésiés, avant et après injection d'un agent anesthésique local (procaine HCl) à l'intérieur du corps trapézoïde, ceci par approche ventrale. Tous les composants à l'exception de P1, N1 et P2 ont été affectés; N2 a été retardé; P3 et N3 ont disparu; P4 a présenté à la fois un allongement de sa durée et une diminution de sa latence, l'amplitude de N4 a été réduite. Toutes ces modifications étaient temporaires et une restauration des composants s'est produite. L'identification des composants touchés a été facilitée par la modification de leur latence et de leur amplitude en fonction à la fois de l'intensité du stimulus et de sa cadence. Cette étude suggère une contribution du corps trapézoïde dans la genèse des composants du potentiel auditif du tronc cérébral à partir de N2.

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