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Carbon Monoxide and Flash Evoked Potentials from Rat Cortex and Superior Colliculus[1]

ROBERT S. DYER AND ZOLTAN ANNAU

Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, MD 21205

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DYER, R. S. AND Z. ANNAU. *Carbon monoxide and flash evoked potentials from rat cortex and superior colliculus.* PHARMAC. BIOCHEM. BEHAV. 6(4) 461–465, 1977. – In view of the conflict between qualitative reports that flash evoked potentials from superior colliculus (SC) and visual cortex (VC) of rats are uniquely sensitive to low levels of carbon monoxide (CO), and a more quantitative report that the visual cortex evoked potential is not sensitive to low levels of CO, the present report documents the effects of different concentrations of CO upon flash evoked potentials from these areas. The amplitude of the P3-N4 component from the SC evoked potential demonstrated the best dose/effect relationship, increasing up to levels of 38% carboxyhemoglobin (COHb). Latencies were affected only when COHb levels reached 55%. The use of the visual system to determine effects of toxic agents upon the central nervous system is discussed.

Carbon monoxide Vision Unanesthetized Evoked potentials

EXPOSURE to carbon monoxide (CO) is known to produce hypoxia, and thus in high concentrations to produce damage to the central nervous system. Although several reports have described the consequences of severe exposure upon CNS function [9,11,15], few studies have described the consequences of exposure to low levels of CO. Among those studies which have included low level exposure, there seems to be considerable disagreement regarding the effect.

According to Petajan *et al.* [15] no change in latency of the flash evoked potential (VEP) from the rat cortex could be observed until Carboxyhemoglobin (COHb) saturations became greater than 65%. Amplitude changes were not quantitatively characterized, but were said to increase initially and then to become depressed. Exposure to 1500 ppm CO was necessary in order to reach COHb levels of 65%. These results contrast markedly with those of Xintaras *et al.* [21], who described changes in both latency and amplitude of VEP's from the rat superior colliculus (SC) at exposures to only 50 ppm CO. Unfortunately both the latency and amplitude changes reported by Xintaras *et al.* [21] were based upon a qualitative analysis of the data. Attempts to demonstrate electrophysiological changes in humans exposed to low levels of CO have been somewhat more quantitative but no more conclusive [13]. The level at which measurable changes in function occur is of more than academic interest since some have suggested that threshold exposure levels set for industrial environments might be in part determined by reference to electrophysiological experiments [2].

In an earlier paper [6], we have characterized the normal VEP from the rat SC, and suggested that without appropriate controls and quantitative evaluations of data it would be difficult to assess the effects of any agent. In the present paper appropriate controls were used to study the effects of mild exposures to CO upon VEP's from both SC and visual cortex (VC) in rats.

Interpretation of experiments with CO requires knowledge of carboxyhemoglobin (COHb) saturations. Since it takes some time for COHb concentrations to reach a plateau and stabilize at a constant concentration of inspired CO, two types of strategies appear available. One can either expose the subject to a constant high level of CO, sampling the COHb concentration and evoked potential parameters at different times during the experiment as the plateau is reached, or alternatively expose animals to various lower concentrations of CO and wait for the plateau to be reached before sampling evoked potential parameters. The first method has one significant advantage: data can be rapidly acquired at various exposure levels during one recording session. The disadvantages of the first method are at least threefold: (a) there is a limited amount of blood one can draw from a rat without blood loss becoming an experimental variable; (b) there would be no control for any changes in evoked potential parameters which occurred over time that were unrelated to COHb; (c) the COHb level would be constantly changing, and since at least several minutes are required to average enough evoked potentials to produce a stable baseline, the COHb level determined could at best be only the average of that experienced by the

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animal during the recording. For these reasons the present experiment used the second method.

METHOD AND MATERIAL

Fifteen adult male hooded rats obtained from Blue Spruce Farms were chronically implanted with recording electrodes under Equithesin anesthesia. Four 0.80 × 1/16 in. stainless steel anchor screws were placed in the skull. One of these screws, placed over the frontal sinus, was used to ground the animal. A second screw placed 1 mm posterior to bregma and 2 mm lateral served as a reference, and a third screw, placed 7 mm posterior to bregma and 3 mm lateral, was used to record the cortical evoked potential. Bipolar 0.25 mm twisted nichrome wires, insulated up to the cut tips, were lowered into SC under stereotaxic guidance according to the atlas of Skinner (1970). The tips of the electrodes were separated from each other in the vertical plane by about 1 mm, and the electrodes were placed at 5.5 posterior to bregma, 1.5 lateral and about 3.8 mm below the cortical surface. All electrodes were cemented in place with dental acrylic and connected to an Amphenol receptacle. The animals were then given IM injections of 0.3 cc Bicillin and allowed at least one week for recovery.

Recording methods have been described in more detail elsewhere [6]. Briefly, the recordings were accomplished as follows.

After pupils were dilated (Cyclogyl) and the animals were connected to the recording apparatus, they were placed in a chamber which had mirrors on 3 walls, ceiling and floor. The fourth clear Plexiglas wall had the lamp from a Grass PS-2 photostimulator mounted flush against it. Either compressed air or a predetermined mixture of CO and air was blown into the chamber at 6 ℓ/min through a 0.6 cm hole located 5 cm from the floor. Recordings were made by connecting the animal to conventional preamplifiers with high and low frequency cutoffs set at 10 kHz and 0.2 Hz respectively. Amplified signals were led to an oscilloscope for monitoring, and to a PDP-8 computer for averaging. The poststimulus analysis epoch was 240 msec, each 1 ms representing one bin of a 240 point plot display.

A signal from the computer triggered the photostimulator which was always set at its greatest value (No. 16), a 10 μsec flash of about 1.5×10^6 cp. Flashes were presented at 0.4 Hz.

Averaged responses were displayed on an oscilloscope, and a cursor controlled by the teletype and one analog channel printed the latency to the nearest ms and amplitude to the nearest 1.0 μV of any bin requested.

In each test session the animal was allowed 10 min to habituate to the recording box, during which time the light was flashed at 0.4 Hz. Following this, the VEP to 500 flashes was obtained, after which the compressed air blowing into the chamber was replaced with the desired concentration of CO throughout the remainder of the session. After 110 min, the light began to flash again at 0.4 Hz, and 10 min later a second series of 500 flashes was begun. Amplitudes and latencies of peaks obtained from this second series of flashes were then taken as a percentage of the preexposure values.

Exposures to 0, 75, 150, 250, 500, and 1000 ppm CO were accomplished, and whenever possible each animal was run at each concentration twice, once to record the SC EP and once to record the VC EP. At least one week intervened between exposures, and they were presented in random order.

The desired carbon monoxide concentrations were produced by mixing compressed air and 100% CO in a 100 gal drum. The mixture was continuously monitored by a Beckman Model 215 infrared gas analyzer, which controlled a solenoid valve attached to the CO input and thus maintained the concentration of CO in the drum at the desired level. The mixture was pumped from the drum to the recording chamber, where occasional samples were drawn and fed back into the analyzer to insure that the concentration in the chamber was the same as that in the drum. It was found that 6 lpm of the flow produced sufficient positive pressure to maintain the desired concentration of gas in the chamber in spite of the recording cable hole.

At the end of the experiment the animals were perfused with normal saline followed by 10% Formalin, and the brains were frozen, sectioned at 90 μ and stained with cresyl violet for verification of the SC placements.

Carboxyhemoglobin saturations at the different exposure levels were determined in a separate series of animals with chronically implanted venous catheters according to the method described by Weinstein and Annau [20]. COHb determinations were made according to the method described by Small *et al.* [17].

RESULTS

The COHb saturations resulting from 120 min exposures to 75, 150, 250, 500 and 1000 ppm CO were 6, 13, 22, 38, and 55% respectively. In the rat, 120 min was sufficient time for the COHb levels to reach equilibrium.

Evaluation of the stained brain section revealed that most animals had electrodes implanted within the superior colliculus. The data from those animals with electrodes not correctly placed were discarded. In addition, due to the length of time required to complete the entire experiment, only a few animals were actually exposed to all conditions. For the SC recordings the distribution of animals across exposure conditions was: 1000 ppm, n = 11; 500 ppm, n = 10; 250 ppm, n = 11; 150 ppm, n = 9; 75 ppm, n = 8; 0 ppm, n = 13. For the VC recordings the distribution was: 1000 ppm, n = 9; 500 ppm, n = 10; 250 ppm, n = 9; 150 ppm, n = 5; 75 ppm, n = 6; 0 ppm, n = 14.

For SC recordings the only amplitudes evaluated were those of the N1-P1, P3-N4, N4-P4 and N5-P5 components. Previous work had shown that only these components are sufficiently stable under normal conditions for a long enough period to provide a meaningful baseline [6]. Latencies of all peaks were evaluated.

The amplitudes of SC components N1-P1, P3-N4, and N4-P4 tended to increase with exposure concentrations up to 500 ppm. Amplitudes of N5-P5 and latencies of all SC peaks appeared unaffected to this point. At 1000 ppm all amplitudes appeared depressed and all latencies except P5 appeared increased. These results are summarized in Figs. 1, 2, and 3. Parametric statistical evaluation of the data supported these descriptive statements. The amplitudes of N1-P1, P3-N4, and N4-P4 as shown in Figs. 1 and 2 were significantly greater than the 0 ppm values at 500 ppm, and P3-N4, N4-P4 and N5-P5 were significantly less than the 0 ppm values at 1000 ppm. All latencies except P5 were significantly increased at 1000 ppm.

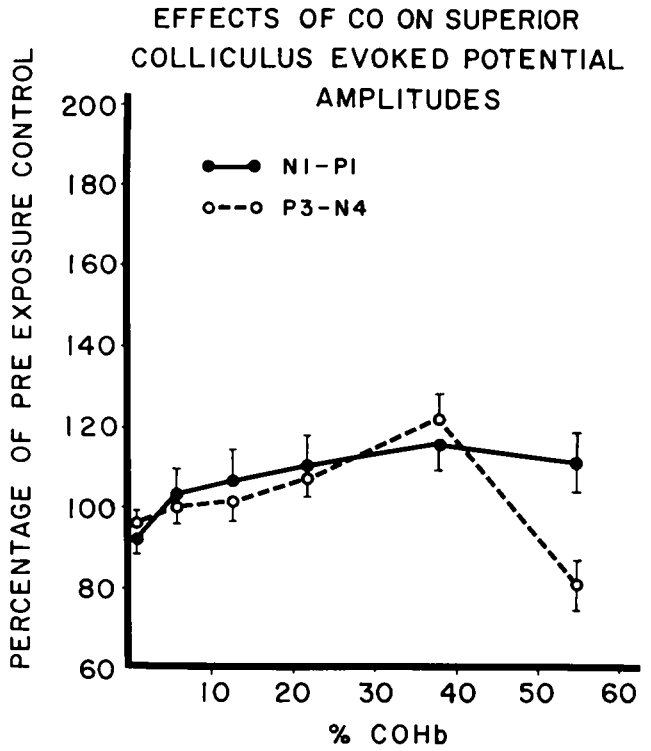

FIG. 1. Effects of different concentrations of inspired CO upon amplitudes of N1-P1 and P3-N4 components of the superior colliculus evoked potential. Since values are expressed as a percentage of pre exposure control, the 0 ppm points reflect variability and change with time.

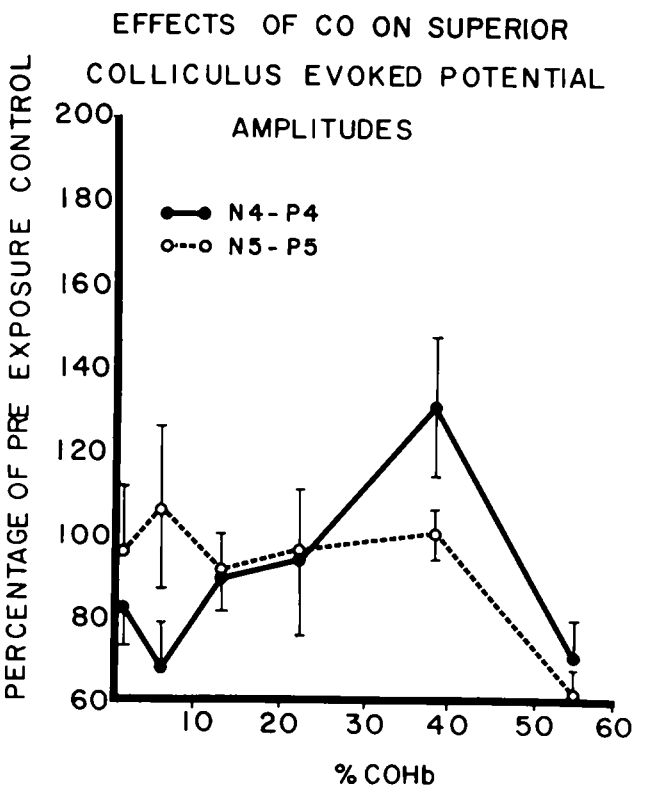

FIG. 2. Effects of different concentrations of inspired CO upon amplitudes of N4-P4 and N5-P5 components of the superior colliculus evoked potential.

For VC recordings the amplitudes evaluated were P1-N1, N1-P2, P2-N2, N2-P3, and P3-N3. Again latencies of all peaks were evaluated. The amplitudes of most peaks appeared increased at most concentrations of CO. These results are summarized in Figs. 4 and 5. Latencies appeared generally unaffected until exposures of 1000 ppm were reached. Statistical evaluation of the data revealed that latencies of N1 and P2 were significantly increased at 1000 ppm CO ($p<0.05$), but that P1-N1 amplitudes were the only ones significantly greater than control at 250 and 500 ppm CO. It should be noted that in most of the figures the 0 ppm points are not at 100% control. The value at the 0 ppm point represents the change which occurred as a function of time.

DISCUSSION

The results show clearly that in both the VC and SC no changes in evoked potential latency occur until the COHb saturation reaches 55%. However, at this exposure level the changes which occur in VC seem somewhat less than those which occur in SC. At the 1000 ppm exposure level the latency of N1 in VC was 112% of control, but at the same exposure level the latency of P1 in SC was 123% of control. A t-test showed these differences to be significant ($p<0.05$). Although exposures below this level had no significant effect upon latency of either VC or SC evoked potentials, they did significantly increase the amplitude of several components. Again these changes were more clearly seen in the SC than in the VC recordings. Indeed, reference to Fig. 1 suggests that changes in amplitude of the P3-N4

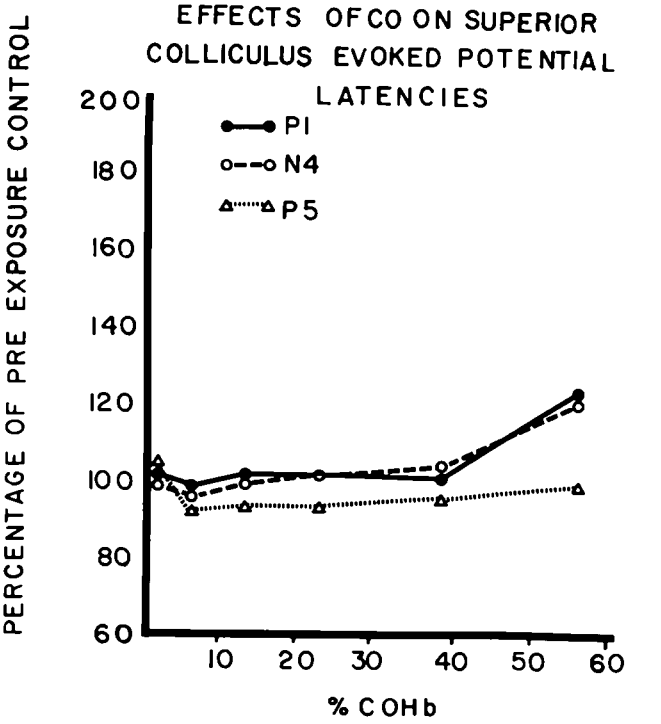

FIG. 3. Effects of different concentrations of CO upon latencies of P1, N4 and P5 components of the superior colliculus evoked potential. Peaks P1 and N4 increased significantly in latency at 1000 ppm CO (55% COHb).

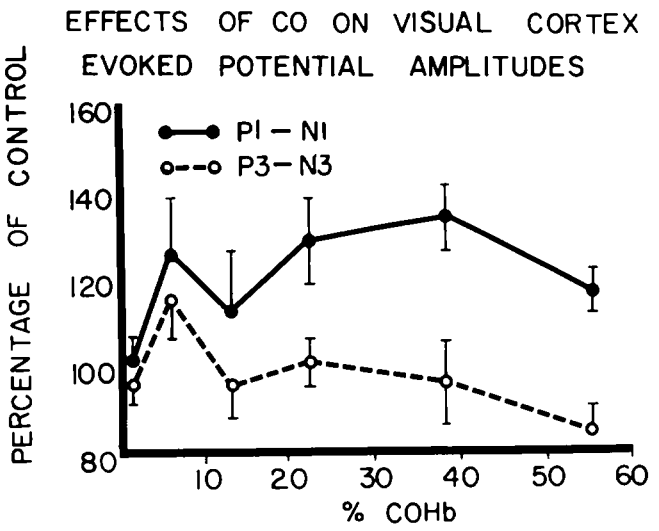

FIG. 4. Effects of different concentrations of CO upon amplitudes of the visual cortex evoked potential (P1-N1 and P3-N3 components).

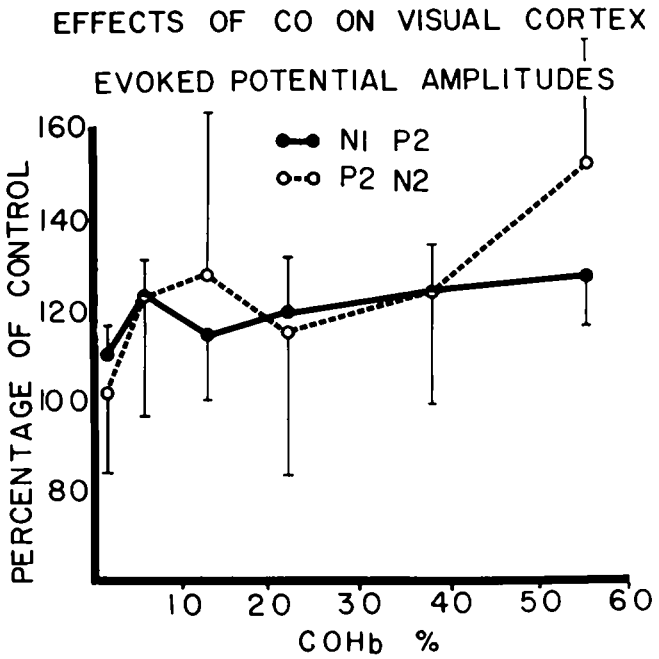

FIG. 5. Effects of different concentrations of CO upon amplitudes of N1-P2 and P2-N2 components of the visual cortex evoked potential.

component of the SC evoked potential follow the typical inverted U dose/effect curve. The failure of other peaks to conform to the same dose/effect relationship may reflect in part contribution of differentially sensitive neurons to different components. Alternatively, some peaks may have the true form of the curve masked by high variability.

The retina has traditionally been considered exquisitely sensitive to hypoxia [7]. However, the present experiments suggest that under some conditions other brain areas may be even more sensitive. The initial components of the evoked potential are those which are most sensitive to changed input parameters, and the input to SC which accounts for the initial components of the flash evoked potential comes exclusively from the retina [6]. Therefore, if the retina is more sensitive than SC to CO, the N1-P1 component should show changes at exposure levels at least as low as those at which changes occur with later components. Reference to Fig. 1 shows clearly that N1-P1 remains largely unaffected throughout the range of exposure levels tested. Since N1-P1 is one of the most stable components of the SC evoked potential [6], it appears unreasonable to argue that its sensitivity to CO is masked by high variability. Thus one must conclude that SC is more sensitive than the retina to CO.

The finding that amplitudes of at least some components increase during exposure to mild levels of CO hypoxia is of interest. For some time it has been known that when exposures to severe hypoxia are produced by inhalation of gas mixtures containing low oxygen content (hypoxic hypoxia) there is a short period of activation during which cortical evoked potential amplitudes increase before ultimately becoming depressed [1]. Several explanations might be proposed to account for the state of activation: greater sensitivity of inhibitory neurons to hypoxia [10], activation of carotid body chemoreceptors by low arterial O_2 pressure with subsequent reticular and cortical activation [4] or partial depolarization triggered by electrolyte shifts known to occur during hypoxic hypoxia [12]. Since the present paper demonstrates an activation stage resulting from CO hypoxia, and since chemoreceptors are presumably not activated at these CO concentrations, this explanation appears inadequate. Electrolyte shifts cannot account for the activation stage because they do not occur during CO exposures [5]. Thus the most plausible explanation is a release from inhibition produced by a greater sensitivity of inhibitory synapses to hypoxia.

It is important to discriminate between changed brain function and impaired brain function resulting from CO hypoxia. Clearly at 250 and 500 ppm CO the physiology of SC is different than at 0 ppm. But perhaps the change represents not a toxic effect, but a compensatory mechanism. This suggestion is supported by the work of Traystman [18], which has shown that in the dog brain blood flow increases with increasing COHb, and this increase is sufficient to maintain forebrain oxygen consumption constant until COHb levels reach about 40%. Above this level blood flow continues to increase, but oxygen consumption decreases. It is probably not coincidence that evoked potential latency begins to increase and amplitudes decrease at about this same level.

The data from this experiment appears to fall between the reports of Xintaras *et al.* [21] and Petajan *et al.* [15]. Xintaras' report suggests that one may observe changes in the SC evoked potential at 50 ppm CO. Reference to Fig. 1 suggests that there is no threshold CO level at which changes suddenly occur in the P3-N4 component. Thus, if enough animals were run at 50 ppm CO, a small but significant difference from control might be demonstrated. However, it is not possible to argue that Xintaras *et al.* [21] have already demonstrated this difference since their report is based upon qualitative observation of only a few animals and does not describe attempts to control the many variables which can lead to error of interpretation. No attempts to repeat the same procedures either within or between animals, or to describe any actual measurements made comparing exposed and preexposed evoked potentials within animals, were described, and consequently the

conclusions drawn regarding the sensitivity of SC evoked potentials to CO are unsupported. Thus, although the P3-N4 component of the SC evoked potential has been shown in the present study to be sensitive to low levels of CO, it is perhaps not quite as sensitive as originally suggested.

Petajan *et al.* [15] suggest that changes in evoked potential latency cannot be observed until exposure levels of 1500 ppm CO are used, but the present results show that even in the cortex such changes can be observed at 1000 ppm CO. This discrepancy may result from the different procedures used. The Petajan *et al.* [15] results are difficult to interpret, since the animals were placed in a darkroom 30 min before the exposure began, and the results are thus confounded by the process of dark adaptation.

Finally, it has been argued that the visual evoked potential does not reflect changes in brain function resulting from CO as well as either the auditory or somatosensory evoked potential [8]. Thus, the presumed sensitivity of the brain to CO would have been missed in the present study. Such an argument must be made carefully for two reasons. First, both somatosensory and auditory potentials evoked by peripheral stimulation require transmission along neural pathways which are embryologically part of the peripheral nervous system [14]. Thus it is not possible to attribute any changes observed directly to changes in central nervous system functions when natural stimuli are used. The retina, on the other hand, may be considered part of the central nervous system embryologically [14], with regard to anatomical structure of the vascular walls of retinal vessels [16], and with regard to the effect of hypoxia upon vessel diameter [3]. A comparative study of the relative sensitivities of different functional areas within the brain therefore remains to be accomplished. Secondly, a greater sensitivity of the auditory and somatosensory systems would be difficult to understand since experimentally determined changes in sensory function during hypoxia invariably favor a greater sensitivity of the visual system [19].

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