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Time-dependent cerebrovascular responses to a seizure induced by the non-cholinergic convulsant, pentylenetetrazole (PTZ, 50 mg/kg), were examined in terms of regional cerebral blood flow (rCBF), regional cerebrovascular permeability-surface area products (rPS) for sucrose, and brain vascular space (BVS), as well as in terms of several peripheral cardiovascular parameters, including cardiac output and blood gases. Cerebrovascular measurements in 16 brain regions were performed at 1 hr, 15-18 hr and 1 week post-seizure, and were carried out in unanesthetized, unrestrained male Sprague-Dawley rats. Mean arterial blood pressure was elevated at 4 min (37%) and 12 min (13%) after seizure onset, returning to baseline levels by 2 hours. Significant changes were observed 1 hr after seizures in pH, PaO_2 and $PaCO_2$: -11%, +27% and +15%, respectively. Thereafter, the blood gas pattern was normal. Cardiac outputs were unaffected in all post-seizure groups. There were no significant differences in rPS in any brain region, although non-significant permeability increases were observed in 14 of 16 brain regions by 24 hrs, and in all 16 regions at 1 week post-seizure. At 1 hr post-seizure, rCBF was reduced significantly in the frontal cortex (-18%) and piriform cortex-amygdala (-27%): both, regions known to be involved in seizure processes. Such decreases occurred in the face of reduced pH and elevated $PaCO_2$ levels: both, stimulators of rCBF. RCBF were not significantly affected at the other time periods. BVS declined in most brain regions by an average of 5% within 1 hr post-seizure, significantly in the cerebellum (-18%). In contrast, by 15-18 hr post-seizure, BVS was elevated in 14 of 16 brain regions by an average of 10%, significantly in the olfactory bulb (+29%). Detailed histological examination failed to demonstrate delayed neuronal or axonal degeneration in any brain region at any time period. The data show that unique, acute and lingering regional changes in cerebrovascular physiology follow a single convulsive exposure to PTZ and suggest that disturbances in brain physiology may persist for relatively long times even in the absence of neuropathology.

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Regional blood-brain barrier responses to central cholinergic activity

TABLE OF CONTENTS

SUMMARY

The overall goal of our research is to determine the role of changes in cerebrovascular functions (i.e., regional cerebral blood flow, regional blood-brain barrier permeability, brain vascular space) following exposure to the anticholinesterase soman in producing damage to the central nervous system (CNS). The importance of soman-produced convulsive seizures per se in damaging the CNS is also being studied. The non-organophosphate convulsant pentylenetetrazole (PTZ) is being used as a reference compound, against which to compare microvascular and histopathological changes following convulsant and subconvulsant exposure to soman.

APPROACH

The irreversible anticholinesterase soman produces toxic effects on the central nervous system, even when administered in a single subcutaneous injection (McDonough et al., 1986; Shih, 1982). These include tremor, convulsions and respiratory failure, with long-term behavioral changes in those animals which survive the initial insult. The toxic action of soman is probably due to the accumulation of acetylcholine (ACh) at central synapses leading to hyperactivity of the cholinergic system. Soman also causes lesions and degeneration throughout the nervous system (Honchar et. al., 1983; McDonough et al., 1986), with the most extensive damage found in limbic system structures (i.e., piriform cortex, amygdala, entorhinal cortex and hippocampus). Since cholinergic agents are known to alter regional cerebrovascular permeability, and since muscarinic cholinergic receptors and cholinesterase activity are known to be associated with CNS vasculature, we have hypothesized that soman-induced pathology, and that produced by anticholinesterases in general, may be partly due to changes in cerebrovascular function.

Initial experiments have focused on characterizing seizures produced by pentylenetetrazole (PTZ) and their effects on cerebrovascular function. Parenteral administration of PTZ produces, sequentially, myoclonus, clonic and then tonic seizures. Seizures are monitored electrographically by chronically implanted epidural electrodes. Their time of onset and duration are dose-dependent. The mechanisms underlying PTZ seizures are only partially known, but may involve the GABA, noradrenergic and adenosine systems (Burley & Ferrendelli, 1984; Wasterlain, 1989). Specifically, PTZ blocks GABA receptor function, probably by blocking the benzodiazepine site linked to the $GABA_a$ receptor (Wasterlain, 1989). These PTZ seizures are of interest because they are well characterized and not mediated by the cholinergic system. Therefore, they provide an excellent seizure model for comparison with soman-induced seizures.

In subsequent studies, the effects of PTZ seizures on mean arterial blood pressure (MABP), cardiac outputs, hematocrit, regional cerebrovascular permeability (rPS), regional cerebral blood flow (rCBF), and brain vascular space (BVS) were characterized. The cerebral microvasculature (and its

blood-brain barriers) occupies a central position in the regulation of the internal environment of the brain and spinal cord. Short- and long-term effects on both the structure and function of the central nervous system would be likely to follow traumatic events (i.e., convulsive seizures) to the microvasculature. Blood flow and permeability changes have been reported to follow drug-induced seizures (Greig & Hellmann, 1983; Ingvar, et al., 1984; Ruth, 1984; Ziylan & Ates, 1989; Johansson & Linder, 1978). However, it is unclear to what extent anesthesia used in some of these studies may have affected the results, since such procedures are known to dramatically alter blood flow and permeability measures (Goldman & Sapirstein, 1973), as well as the neuroexcitatory effects of convulsants. Also, it is unclear whether such circulatory changes can be produced with subconvulsive doses of seizure-inducing agents.

Therefore, cerebrovascular functions were characterized in unanesthetized, unrestrained rats. Sixteen brain regions were examined, including the amygdala, piriform cortex, and hippocampus. These limbic regions were of particular interest because they show the largest decreases in glucose metabolism following limbic seizures (Ben-Ari et al., 1981). It is the piriform cortex-amygdala complex which appears to be critical for the development of limbic seizures (McIntyre & Racine, 1986). It is also the site at which neuronal cell loss and glial proliferation occur following prolonged seizures (Engel, 1983), including those produced by cholinergic agents (i.e., oxotremorine, pilocarpine, carbachol, physostigmine [Olney et al., 1983]). These are some of the same regions affected by soman-induced convulsions (McDonough et al., 1986).

Pentylenetetrazole seizures produce pathological changes in the central nervous system. These changes include swelling of astrocytic processes, especially around capillaries, which appears to interfere with capillary function (De Robertis, 1969). Subconvulsant, as well as convulsant doses of PTZ are sufficient to alter glial processes (Rodin et al., 1979). Neuronal processes typically appear intact (Rodin et al., 1979). We have examined histologically representative brain samples from rats 3 days following PTZ seizures of moderate severity. Combined cupric-silver and Fink-Heimer procedures were used to document potential pathological changes following a single PTZ convulsion.

MATERIALS AND METHODS

The following isotopes and drugs were used in these studies: ^{3}H-sucrose (14 Ci/mmol): NEN Products/Dupont, Boston, MA; ^{14}C-sucrose (350 mCi/mmol): ICN, Irvine, CA; ^{14}C-iodoantipyrine (58 mCi/mmol): NEN Products, Dupont, Boston, MA; pentylenetetrazole (PTZ): Sigma Chemicals, St. Louis, MO.

Dose-response relationships: pentylenetetrazole. Fifty-two male Sprague-Dawley rats (400-450 g) were surgically anesthetized (pentobarbital, 45mg/kg, ip) and implanted with chronic, epidural, tripolar electrodes for determining seizure thresholds. The animals were given at least 1 week to

recover from surgery. They were then injected intraperitoneally with pentylenetetrazole (50 mg/ml in saline). Convulsive seizures were scored behaviorally and recorded electrographically.

Circulation methods: A procedure was developed which permits the simultaneous measurements of regional permeabilities and blood flows to multiple regions of conscious, unrestrained rats. This has been accomplished by combining three methods, as follows: Blood flow was measured using a new version of our own method (Goldman & Sapirstein, 1973) which employs the fractionation of a bolus of ^{14}C-iodoantipyrine, and includes a correction for the time-dependent efflux of the tracer from brain tissues; Rapoport's method was used for measurement of brain permeability, employing ^{3}H-sucrose as a diffusion-limited marker (Rapoport et al., 1980); A "dye"-dilution technique was used for the measurement of cardiac output, in which either isotope replaces conventional dyes (Sapirstein, 1958). Blood flows and permeabilities were measured in 16 brain regions, as follows: olfactory bulb, olfactory tubercle, occipital cortex, parietal cortex, frontal cortex, piriform cortex-amygdala, basal ganglia, hippocampus, inferior and superior colliculus, hypothalamus, septal area, and callosal white matter, midbrain (including thalamus), pons & medulla, and cerebellum. Cardiac output as well as pH, $PaCO_2$ and PaO_2 of the arterial blood and hematocrit were monitored at the same time. Blood pressure was monitored in a subset of 4 animals during a typical moderate seizure (<10 min duration) and for several hours thereafter. Catheterization and handling of these animals were identical to those in which cerebrovascular measurements were determined.

The blood flow technique: The method (Goldman & Sapirstein, 1973) assumes that the indicator of flow is completely extracted and that the tissue reservoir into which the indicator flows is sufficiently large compared to the influx rate so that the efflux is not significant during the period of measurement, i.e., about 15 sec after intravenous delivery or about 11 sec after peak arterial concentrations have been reached in the brain. As we have previously described in detail (Goldman & Sapirstein, 1973), under these conditions the rather complex Kety equation and the associated Sokoloff method (Sakurada et al., 1978) can be simplified considerably. The revised equation, which is the basic equation of the indicator-fractional technique (Sapirstein, 1958), is written:

$$U(i)/I = F(i)/CO \text{ when } 6<T<30 \text{ sec} \qquad [1]$$

where $U(i)$ = tissue uptake of indicator
I = the injected dose of indicator
$F(i)$ = the blood flow in the tissue
CO = the cardiac output

Equation [1] states, in effect, that when a highly diffusible indicator, such as iodoantipyrine, is administered in a single intravenous injection and the killing time is short, then the pattern of indicator distribution in the brain will be the same as the pattern of the fractional distribution of the cardiac output.

The most recent modification of the indicator fractionation technique recognizes the fact that the rate of efflux of iodoantipyrine is great enough at T=15 sec so as to reduce tissue content significantly and thereby the estimate of blood flow; the error is proportional to the blood flow (Eckman et al., 1975, Raichle et al., 1976). At the killing time of 15 sec after intravenous injection of these tracers we have found that rCBF is underestimated by about 17% in unanesthetized, unrestrained rats (unpublished observations).

Cardiac output measures: Additionally, integration of the arterial concentration of the reference indicator, iodoantipyrine, can be used to calculate the cardiac output (CO) by indicator-dilution according to the equation:

$$CO = J/\int_o^T Ca' \, dt \qquad [2]$$

where J is the amount of indicator injected, and Ca' is the 15-sec integrated arterial concentration of the indicator, corrected for recirculation.

Regional cerebral permeability: Regional permeability of sucrose is estimated by the method of Rapoport, which is published in detail elsewhere (Ohno et al., 1978; Rapoport et al., 1980). Its integration with our regional blood flow method for use in the conscious, unrestrained rat is described in the protocol below. The estimate of permeability, the PS (s^{-1}), for sucrose is the product of cerebrovascular permeability P ($cm \cdot s^{-1}$) and the capillary surface area S, estimated to be 240 $cm^2 \cdot g^{-1}$ or cm^{-1} in the rat (Crone, 1963). Radiolabeled sucrose is injected intravenously, and the arterial plasma concentration is monitored until the animal is killed 10 min later, time T, so that only a small amount of the poorly diffusible tracer is accumulated in the brain. Brain concentration remains insignificant relative to plasma concentration, and back diffusion from brain to plasma can be ignored in this time period. Under these circumstances, brain uptake of the tracer can be given, as follows:

$$dC_{brain}/dt = PS \cdot C_{plasma} \qquad [3]$$

Integration of equation [3] to time T gives PS in terms of $C_{brain}(T)$ and the plasma concentration integral

$$PS = C_{brain}(T) \,/\, \int_o^T C_{plasma} \, dt \qquad [4]$$

C_{brain} in equation [4] represents parenchymal (extravascular) brain concentration of the tracer at the time of death, T = 10 min, and equals net brain concentration minus intravascular content of the tracer. The latter term is the product of the whole blood concentration and the regional blood volume. In our protocol, regional blood volume, BVS, is defined as the sucrose space at 15 sec after intravenous injection. Under the conditions of these experiments, mean sucrose space was determined separately in subsets of animals for each experimental condition utilizing a modification of Rapoport's method (Rapoport et al., 1980).

Experimental protocol: The protocol involved the surgical implantation of catheters, one in a femoral vein, another in the opposite femoral artery, 3 days before measurements so that the experiments would be conducted in unanesthetized, unrestrained animals. On the day of the experiment, the stored catheters were freed from a neck pouch and arterial blood samples were collected for hematocrit and blood gas assays. A bolus containing 10-20 uCi ^{3}H-sucrose was administered intravenously. This was followed by collection of 19 arterial samples over a 10-min period to determine the integrated content of sucrose. In the last minute, a bolus of ^{14}C-iodoantipyrine, 4 uCi, was injected, and 15 one-second arterial samples were collected for determination of the cardiac output. Animals were then killed with a rapid injection of a saturated solution of KCl. Brains were quickly removed and dissected on a chilled steel plate. Tissue and blood contents of both tracers were extracted (>98%) in Bray's scintillation cocktail and counted in a quench-correcting scintillation spectrometer (Packard 4230). Tissue uptake of sucrose (corrected for intravascular content) relative to the integrated plasma content of sucrose served as a measure of the permeability-surface area product, the PS (Rapoport et al., 1980). Tissue content of iodoantipyrine relative to the integrated blood content of iodoantipyrine was used to estimate the regional blood flow. Regional brain vascular contents (BVS) of the sucrose marker at the time of death, i.e., the 15 sec sucrose spaces, were determined in separate subsets of identically treated animals in each experimental group by means of a modified version of the method described by Rapoport (Rapoport et al., 1980).

Electrophysiological recording: Rats were anesthetized (45 mg/kg, pentobarbital, ip) and implanted bilaterally with cortical screw-type recording electrodes over the frontal and parietal cortex, as well as with an additional reference electrode. Care was taken to avoid damaging the dura or cortex. Connecting leads were soldered to the screw-electrodes and attached to a connecting socket. The entire assembly was anchored to the skull by acrylic cement. Field-Effect transistors (FETS) were used to minimize movement artifact. Recording leads were connected through a mercury swivel to allow relatively unrestricted movement of the implanted animals during recording. Animals were allowed 1 week to recover from surgery before drug treatment.

Prepared animals were placed in a sound-attenuated, electrically shielded recording chamber. Leads from a Grass Model 7 polygraph (Grass Instruments, Quincy, MA) were attached to the

recording electrodes, and 5 min of baseline EEG was recorded. Animals were then injected with drug. One hour of continuous EEG recording was carried out, followed by intermittent recording, until seizure activity was terminated or the death of the animal occurred.

Histopathology: The histopathological technique chosen for this project, the Fink-Heimer technique, is a sensitive, selective silver-impregnation procedure which permits the visualization of degenerating axons of all calibers, both myelinated and unmyelinated. While this procedure has been performed on selected sections from several specimens, we have additionally processed much of our experimental material by the cupric-silver procedure. This latter technique, when used in conjunction with the Fink-Heimer, demonstrates both neuronal and fiber (axonal) degeneration. This approach permits a much more complete, rapid and sensitive indication of degeneration throughout the brain.

Coded brains from representative samples of each experimental group were examined for axonal and neuronal degeneration. Such animals were deeply anesthetized with sodium pentobarbital (50-60 mg/kg, ip) and sacrificed by transcardiac perfusion with 100 ml of phosphate-buffered saline containing 0.5% procaine hydrochloride, followed by 150-250 ml of 10% buffered formalin. Just prior to perfusion, each animal received 500 units of heparin directly into the left ventricle. Following perfusion, brains were removed and hardened in additional volumes of fixative for a period of 7-20 days. Several of the brains from each experimental group were embedded in paraffin, sectioned at 10 microns in the frontal plane and stained with cresyl violet. The remaining brains were sectioned in the frontal plane on a freezing microtome at 10-12 microns and processed for degenerating neurons and axons.

Data analysis: Dose-response calculations (ED_{50}, confidence limits) were performed with the Litchfield-Wilcoxon method (1949). Regional cerebral permeabilities, blood flows, vascular spaces and cardiovascular parameters were analyzed by means of univariate and multivariate ANOVA's (BMDP Statistical Software, Inc., Los Angeles, CA).

RESULTS

Dose-response relationships: The intraperitoneal ED_{50} convulsant dose of PTZ in the Sprague-Dawley rats employed in our studies was 42 mg/kg (95% confidence limits, 36.2 - 48.5 mg/kg); the maximum subconvulsive dose was 26 mg/kg. The dose selected for subsequent studies was 50 mg/kg, since it produced convulsions in a majority of animals (70%) and was not lethal in our population. Potentially lethal seizure episodes at high dose levels (>60 mg/kg) were terminated with sodium pentobarbital (40 mg/kg, ip). No animal was injected more than one with this convulsant drug.

There was a stereotypical progression of behaviors during a moderate PTZ convulsive seizure. The actual seizure was preceded by a period of depressed locomotion. The mean latency to seizure following the intraperitoneal injection was 67 ± 4 sec after an intraperitoneal injection. A seizure progression usually began with multiple tonic jerks (forelimb clonus) of the forelimbs and head, accompanied by epileptiform EEG spikes. Within 10 sec of the onset of forelimb clonus the ictal period began as the animal exhibited loss of balance and opisthotonus typical of generalized seizures. Often, the loss of balance was preceded by rearing and bilateral forelimb clonus. During the ictal period, animals exhibited various combinations of bilateral hindlimb clonus, clonus of all four limbs, or unilateral clonus, usually of the forelimbs. Chewing and salivation occurred throughout the ictal period. Convulsions persisted for an average of 247 ± 14 sec.

Animals having more severe seizures demonstrated short running fits with bursts of violent jumping from 1 to 2 feet off the floor of the chamber. The majority of these animals had multiple seizures, interspersed with varying periods of violent tonic jerks of the forelimbs and head, which were also accompanied by EEG spikes. Although the duration of ictal periods were highly variable in this group, the approximate mean duration was 60 min. The animals which attempted to explore interictally did so only with great effort, and appeared unable to support themselves. Interictal EEG spindling was seen in the 4 to 6-Hz range. Results from studies of animals treated with subconvulsive doses of pentylenetetrazole are nearing completion and will be reported at a later time.

Blood pressure: The standard dose of PTZ adopted for these studies produced (usually) a single moderate seizure (50 mg/kg) within 70 sec after intraperitoneal injection. In a group of four animals, MABP was 119 ± 6 mmHg during a baseline period. A blood pressure spike of 165 ± 4 mmHg, lasting less than 9 sec, was observed at the onset of the convulsion. MABP rose steadily to 139 ± 6 mmHg by 2 min, to 163 ± 9 mmHg by 4 min, and decreased to 134 ± 23 mmHg at 12 min after injection; baseline values were reestablished within 2 hr.

Blood Parameters (Table 1): Predictably, significant changes were observed 1 hr after seizures in pH, PaO_2 and $PaCO_2$. The pH increased by 11% ($F = 69.86$, $df = 1,92$, $p<.001$), PaO_2 by 27% ($F = 107.90$, $df = 1,94$, $p<.001$) and $PaCO_2$ by 15% ($F = 58.17$, $df = 1,94$, $p<.001$) compared to placebo-injected control animals. This would be expected shortly after a period of intense skeletal muscular activity and reduced respiration rate associated with seizures. By the next day and at 1 week after convulsions the blood gas pattern was normal. In contrast, throughout this time period, cardiac outputs, body weights, and hematocrits remained unchanged in all groups compared to control animals.

Histology: Representative samples from each of the various experimental groups were examined, as follows: unseized controls and post-seizure (PTZ) groups at 1, 15-18 hr and 1 week after induction of a <5-min seizure. The combined cupric-silver and Fink-Heimer procedures are very sensitive and readily demonstrate axonal and neuronal degeneration. Such changes were not observed in samples from any group of moderately seized rats in any brain region.

Permeability-Surface Area Products (rPS), (Table 2): There were no significant time-dependent differences in rPS in any of the brain regions. Nevertheless, it should be noted that at 1 hr post-seizure, increased permeabilities relative to respective controls were observed in 14 of the 16 regions examined, whereas 1 week later permeabilities were reduced in all 16 regions. Large variances which usually accompany such estimates (Rapoport et al., 1980), prevented individual regional differences from reaching acceptable levels of statistical significance.

Effects of Metrazol on regional cerebral blood flow (Table 3, Figure 1): Convulsant doses of pentylenetetrazole (50 mg/kg, ip) clearly affected the blood flow to only two brain regions in unanesthetized, unrestrained rats: the frontal cortex and piriform cortex-amygdala. Blood flow decreased by 18% in the frontal cortex ($F = 6.67$, $df = 1,36$, $p<.014$) and by 27 % in the piriform cortex-amygdala ($F = 19.61$, $df = 1,36$, $p<.001$) compared to controls. This cerebrovascular effect was detectable at 1 hour post-seizure only. Decreased blood flows were unexpected since they occurred in the face of reduced arterial pH and elevated pCO_2 values (Table 1); both, stimulators of cerebral perfusion. These findings are of particular interest because the frontal cortex and piriform cortex-amygdala are both known to be important for seizure processes (McIntyre and Racine, 1968).

BVS (Table 4, Figure 2) declined in most brain regions by an overall average of 5% within 1 hr post-seizure. However, compared to placebo-injected control animals, the overall reduction was only significant in the cerebellum where it declined by 18% ($F = 5.02$, $df = 1,22$, $p<.035$). However, by 15-18 hr post-seizure, BVS had increased in 14 of 16 brain regions by an average of 10%, significantly in the olfactory bulbs by +29% ($F = 7.51$, $df = 1,22$, $p<.01$). This BVS trend continued in the 1 week post-seizure animals with an overall increase of 10%. Due to large variances, high specific regional differences in olfactory tubercle and callosal white matter were not significant.

DISCUSSION

The results of the present series of experiments have demonstrated that PTZ seizures can alter cerebrovascular function. The seizures produced by the dose of PTZ used in these experiments (i.e., 50 mg/kg, ip) were moderate in severity, typically with only a single primary convulsive seizure

episode lasting between 2-4 min. Seizure onset was marked by sharp, transient blood pressure spike which was followed by a more sustained MABP increase. The magnitude of the increase was substantial, rising from a baseline level of 119 mm to more than 160 mm, a mean increase of almost 40% by the end of the seizure episode; it subsided thereafter. If sustained for more than 10 min, increases in MABP of this magnitude are usually sufficient to open the blood-brain barrier (Ingvar et al., 1984; Johansson & Linder, 1978). With the moderate PTZ seizures in the present studies, the increases in MABP were transient, returning to baseline values within 2 hr. More severe PTZ seizures were accompanied by elevated MABP for more than 1 hr. Such seizures, however, were often lethal.

Under the conditions of the present experiments, regional cerebral permeability was only slightly increased 1 hr after a seizure, and the magnitude of the increase did not reach statistical significance for any individual brain region. Permeability did, however, increase in 14 of 16 brain regions by an average of 14%. It is likely that more severe, prolonged seizures would have led to greater changes in permeability, as demonstrated in a previous study following very severe seizures (Ingvar et al., 1984). Studies carried out in the late 1940's and 1950's indicated that seizures produced by soman and other anticholinesterases also were associated with increased blood-brain barrier permeability (Greig & Mayberry, 1951; Greig & Holland, 1949). However, these earlier studies gave conflicting results due, in part, to outdated methodologies, and this line of work was abandoned and largely forgotten. Therefore, the ongoing studies of soman-induced seizures on permeability, employing more sensitive and quantitative methods, should be more enlightening in this regard. It will be of interest to compare the results from these ongoing studies with earlier research.

In most brain regions, regional cerebral blood flow was unchanged at 1 hr after a PTZ seizure, with the notable exceptions of the frontal cortex and piriform cortex-amygdala. The decrease in these two regions was 18% ($p<.005$) for the frontal cortex and 27% ($p<.001$) for the piriform cortex-amygdala. This observation is important because it demonstrates diminished flow, and possibly diminished function, in two brain regions critical for seizure induction. Support for diminished function comes from studies indicating a decrease in regional glucose metabolism in the mesial-temporal lobe following seizures (Ben-Ari et. al., 1981). The piriform cortex-amygdala also appears to be a generator of epileptiform activity in a variety of seizure models (McIntyre and Racine, 1986). In addition, the piriform cortex-amygdala is the site of major pathology following prolonged seizures, including those produced by soman (McDonough et al., 1986; Engel, 1983). In the present experiments, it is noteworthy that the decreased regional blood flows at 1 hr occurred in the face of reduced arterial pH and elevated pCO_2 levels -- both, stimulators of cerebral blood flow -- which may have obscured even larger declines in regional flows and functions. Furthermore, the effects of the moderate seizures on blood flow encountered in the present studies were transient, i.e., by 15-18 hr and thereafter, regional blood flow had returned to control levels. The

possibility exists, therefore, that decreased flow to these critical brain regions may contribute to or mediate the known pathology, particularly following prolonged and severe epileptiform activity.

As mentioned above, PTZ seizures reduced arterial pH (-11%), elevated pCO_2 (+15%), and substantially increased the PaO_2 (+27%) 1 hr after a seizure termination. These changes in blood parameters appeared to occur in response to the period of intense skeletal activity and reduced respiration rate during the ictal phase. These parameters returned to baseline levels by 15 hr after seizure termination. At no time interval measured were cardiac outputs significantly altered from control values. Therefore, the peripheral hemodynamics were apparently not responsible for the regional cerebral blood flow changes described above. Similarly, no time-dependent changes in arterial blood hematocrits or body weights were observed.

Perhaps the most unexpected finding in the present series of studies is that brain vascular space (BVS) was modified by PTZ seizures in a biphasic manner. BVS declined in all brain regions by an average of 5% 1 hr after seizure followed by an overall increase of 10% during the following day and week. Regional BVS in the present experiments was estimated from a knowledge of the plasma space labeled by ^{3}H-sucrose and the assumption that the hematocrits in the brain tissue and the arterial blood were the same. Thus, BVS provided an estimate of the total blood volume of the brain vasculature system. We know from studies of head trauma in rats that the BVS is reduced under conditions which elevate intracranial pressure and produce cerebral edema (Goldman, paper in preparation, 1989). Therefore, we include alterations in BVS as well as blood flow and permeability, as indices of changes and potential damage in the central nervous system. The fact that PTZ seizures produce time-dependent swings in the BVS suggest potential seizure-induce damage. Such changes might presage delayed neuropathology following more prolonged, severe convulsive seizures. It is essential to determine if soman-induced seizures also alter estimates of BVS, and if there is a relationship to seizure severity and neuropathology.

Interestingly, three animals that had initially seized at a dose of 45-50 mg/kg, which were retested 1-2 weeks later, seized at a far lower dose than had been found to be effective (i.e., 25 mg/kg) in our rat population. This suggests that naive rats may be sensitized, or kindled (Goddard et al., 1969), by pentylenetetrazole following an initial convulsive dose of the drug. This interesting observation of a 1- to 2-week sensitization period appeared to parallel some of the time-dependent cerebrovascular changes noted above. In this regard, kindling has been reported with PTZ treatment, but only following repeated administration (Rodin et al., 1979; Diehl et al., 1984). Carbachol kindling also has been reported (Wasterlain, 1989), linking the cholinergic system to an increase in the sensitivity to convulsions. It will be important to determine whether treatment with anticholinesterases, such as soman, also sensitize animals to convulsants.

These results with PTZ are being compared to the effects of convulsive and subconvulsive doses of soman. It is expected that there will be large changes in cerebrovascular function following soman-induced seizures. We expect that some changes in cerebrovascular functions will be similar following soman and PTZ seizures, and would probably be attributable to the seizure activity per se. However, we argue that soman, as an esterase inhibitor, will have some selective actions on cerebrovascular function. Thus, the magnitude and pattern of changes produced by soman should be distinct from that produced by PTZ. Such studies are ongoing and the results will be given in subsequent reports.

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	Control	1 hr	15-18 hr	1 week
Cardiac Output $ml/min \cdot kg^{-1}$	387 ± 10	405 ± 18	400 ± 12	372 ± 20
Blood Conditions				
pH	7.43 ± .01	7.35 ± .01 **	7.41 ± .01	7.39 ± .01
PaO_2 mmHg	88 ± 2	112 ± 2 **	89 ± 2	87 ± 1
$PaCO_2$ mmHg	40 ± 1	46 ± 1 **	40 ± 1	42 ± 1
Hematocrit	0.48 ± .01	.47 ± .01	.47 ± .01	.47 ± .00
Body Weight (g)	401 ± 3	390 ± 5	397 ± 6	395 ± 4
Number of animals	27	23	19	17

Table 1. Cardiac output, body weight and blood parameters, expressed as means ± SEM, in unseized control rats, 1 hr, 15-18 hr, and 1 week post-seizure, **P<.001.

Brain Region	Control	1 hr	15-18 hr	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory, Bulb	14.06 ± 1.02 (10)	16.03 ± 1.50 (8)	15.69 ± 1.71 (7)	12.65 ± 1.06 (5)
Tubercle	6.56 ± 1.12 (10)	8.29 ± 1.60 (8)	6.14 ± 1.30 (6)	5.79 ± 1.01 (5)
Cortex, Occipital	5.74 ± 0.66 (10)	6.24 ± 1.18 (8)	4.90 ± 0.99 (6)	4.51 ± 1.13 (5)
Parietal	5.17 ± 0.75 (10)	7.44 ± 1.89 (8)	3.80 ± 0.79 (6)	3.94 ± 0.79 (5)
Frontal	5.58 ± 0.64 (10)	6.74 ± 0.94 (8)	4.39 ± 0.88 (7)	5.00 ± 0.90 (5)
Piriform-Amygdala	5.02 ± 0.39 (8)	5.64 ± 0.74 (7)	5.00 ± 0.77 (4)	3.54 ± 0.48 (4)
Basal Ganglia	4.95 ± 1.03 (8)	5.88 ± 0.84 (6)	5.48 ± 0.74 (3)	4.09 ± 1.65 (3)
Hippocampus	4.93 ± 0.59 (8)	5.78 ± 0.94 (6)	4.21 ± 0.93 (4)	4.51 ± 1.13 (5)
Colliculus, Inferior	6.95 ± 0.72 (8)	6.07 ± 0.98 (7)	5.30 ± 1.61 (5)	4.38 ± 0.98 (4)
Superior	4.99 ± 0.58 (8)	5.80 ± 0.55 (6)	4.32 ± 1.33 (4)	4.96 ± 0.07 (3)
Hypothalamus	6.40 ± 1.14 (10)	7.18 ± 1.14 (8)	4.65 ± 0.87 (7)	5.92 ± 1.65 (3)
Septal Area	5.87 ± 1.29 (7)	6.28 ± 1.35 (5)	7.43 ± 2.50 (3)	4.15 ± 0.62 (3)
White Matter	5.82 ± 0.95 (6)	6.58 ± 1.19 (4)	10.98 ± 0.54 (2)	4.39 ± 1.45 (2)
II. Midbrain and Hindbrain				
Midbrain	5.27 ± 0.93 (10)	5.15 ± 1.07 (8)	3.53 ± 0.51 (6)	3.28 ± 0.82 (5)
Pons & Medulla	9.43 ± 0.91 (10)	10.19 ± 0.90 (8)	7.55 ± 0.95 (7)	7.75 ± 0.96 (5)
Cerebellum	7.29 ± 0.51 (10)	7.90 ± 0.37 (8)	7.44 ± 1.10 (7)	6.95 ± 0.71 (5)

Table 2. Permeability-surface area products (rPS) in unseized control rats, 1 hr, 15-18 hr, and 1 week post-seizure, expressed as $ml \cdot g^{-1} \cdot sec^{-1} \times 10^{6}$, mean ± SEM. Numbers of tissue samples are shown in parentheses.

Brain Region	Control	1 hr	15-18 hr	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory, Bulb	1.42 ± .04	1.40 ± .06	1.48 ± .22	1.34 ± .09
Tubercle	1.45 ± .05	1.36 ± .04	1.44 ± .22	1.39 ± .07
Cortex, Occipital	1.45 ± .04	1.45 ± .08	1.45 ± .21	1.48 ± .07
Parietal	1.79 ± .05	1.80 ± .09	1.78 ± .27	1.74 ± .09
Frontal	1.51 ± .04	1.24 ± .09 *	1.52 ± .22	1.48 ± .07
Piriform-Amygdala	1.36 ± .04	0.99 ± .04 **	1.40 ± .21	1.37 ± .06
Basal Ganglia	1.34 ± .04	1.25 ± .06	1.36 ± .23	1.40 ± .06
Hippocampus	1.18 ± .04	1.14 ± .07	1.19 ± .17	1.25 ± .07
Colliculus, Inferior	1.66 ± .05	1.66 ± .07	1.66 ± .22	1.68 ± .07
Superior	1.56 ± .05	1.53 ± .06	1.50 ± .21	1.60 ± .07
Hypothalamus	1.39 ± .05	1.32 ± .06	1.42 ± .20	1.21 ± .18
Septal Area	1.17 ± .03	1.17 ± .05	1.25 ± .18	1.17 ± .05
White Matter	1.07 ± .03	0.94 ± .06	1.02 ± .16	1.00 ± .06
II. Midbrain and Hindbrain				
Midbrain	1.42 ± .04	1.39 ± .05	1.41 ± .20	1.40 ± .07
Pons & Medulla	1.25 ± .04	1.25 ± .05	1.28 ± .19	1.27 ± .07
Cerebellum	1.28 ± .04	1.25 ± .06	1.20 ± .16	1.31 ± .05
Number of animals	15	13	7	7

Table 3. Regional cerebral blood flow (rCBF) in unseized control rats, 1 hr, 15-18 hr, and 1 week post-seizure, expressed as ml/min · g^{-1} wet weight tissue, mean ± SEM. Comparisons between control and treatment groups: **P<.001; *P<.005.

Brain Region	Control	1 hr	15-18 hr	1 week
I. Forebrain + Corpora quadrigemina				
Olfactory, Bulb	2.03 ± .11	2.21 ± .14	2.62 ± .20 **	2.14 ± .32
Tubercle	1.92 ± .16	2.02 ± .19	2.03 ± .25	2.65 ± .45
Cortex, Occipital	1.37 ± .06	1.32 ± .09	1.52 ± .13	1.50 ± .17
Parietal	1.04 ± .10	1.10 ± .08	1.16 ± .10	1.22 ± .09
Frontal	1.11 ± .12	1.16 ± .07	1.31 ± .16	1.23 ± .22
Piriform-Amygdala	1.02 ± .06	0.99 ± .06	1.06 ± .09	1.04 ± .19
Basal Ganglia	0.81 ± .10	0.67 ± .03	0.92 ± .11	0.88 ± .21
Hippocampus	0.74 ± .06	0.71 ± .05	0.87 ± .08	0.87 ± .13
Colliculus, Inferior	1.50 ± .10	1.47 ± .10	1.59 ± .15	1.85 ± .33
Superior	0.94 ± .10	0.91 ± .05	0.98 ± .09	1.07 ± .17
Hypothalamus	1.16 ± .07	1.10 ± .07	1.33 ± .13	1.27 ± .24
Septal Area	1.04 ± .10	0.93 ± .08	1.02 ± .09	1.26 ± .40
White Matter	0.86 ± .09	0.79 ± .09	1.04 ± .07	1.13 ± .16
II. Midbrain and Hindbrain				
Midbrain	0.98 ± .07	0.85 ± .04	1.01 ± .10	0.98 ± .17
Pons & Medulla	2.11 ± .11	1.81 ± .14	1.94 ± .17	1.92 ± .32
Cerebellum	1.85 ± .12	1.51 ± .08 *	1.87 ± .21	1.75 ± .23
Number of animals	15	13	10	4

Table 4. Regional brain vascular space in unseized control rats, 1 hr, 15-18 hr, and 1 week post-seizure, expressed as ml/100 g wet weight tissue, mean ± SEM. *P<.05; **P<.02.

REGIONAL CEREBRAL BLOOD FLOW DIFFERENCES EXPRESSED
AS PERCENT CHANGE FROM MEAN, NON-SEIZED CONTROL VALUES

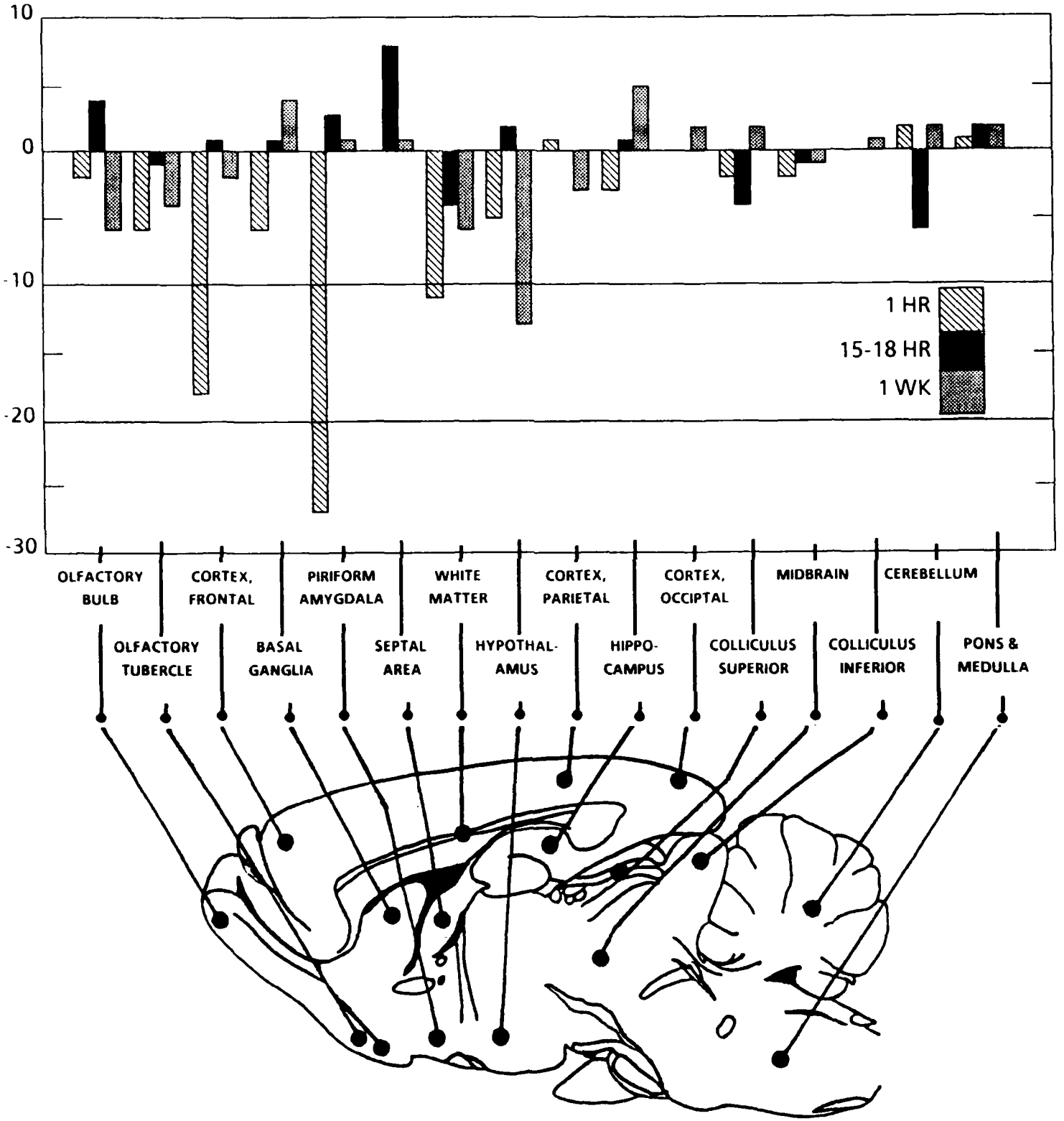

Figure 1

BRAIN VASCULAR SPACE DIFFERENCES WITH TIME AFTER A SHORT-TERM PTZ SEIZURE (< 10 MIN) EXPRESSED AS PERCENT CHANGE FROM NON-SEIZED CONTROL VALUES

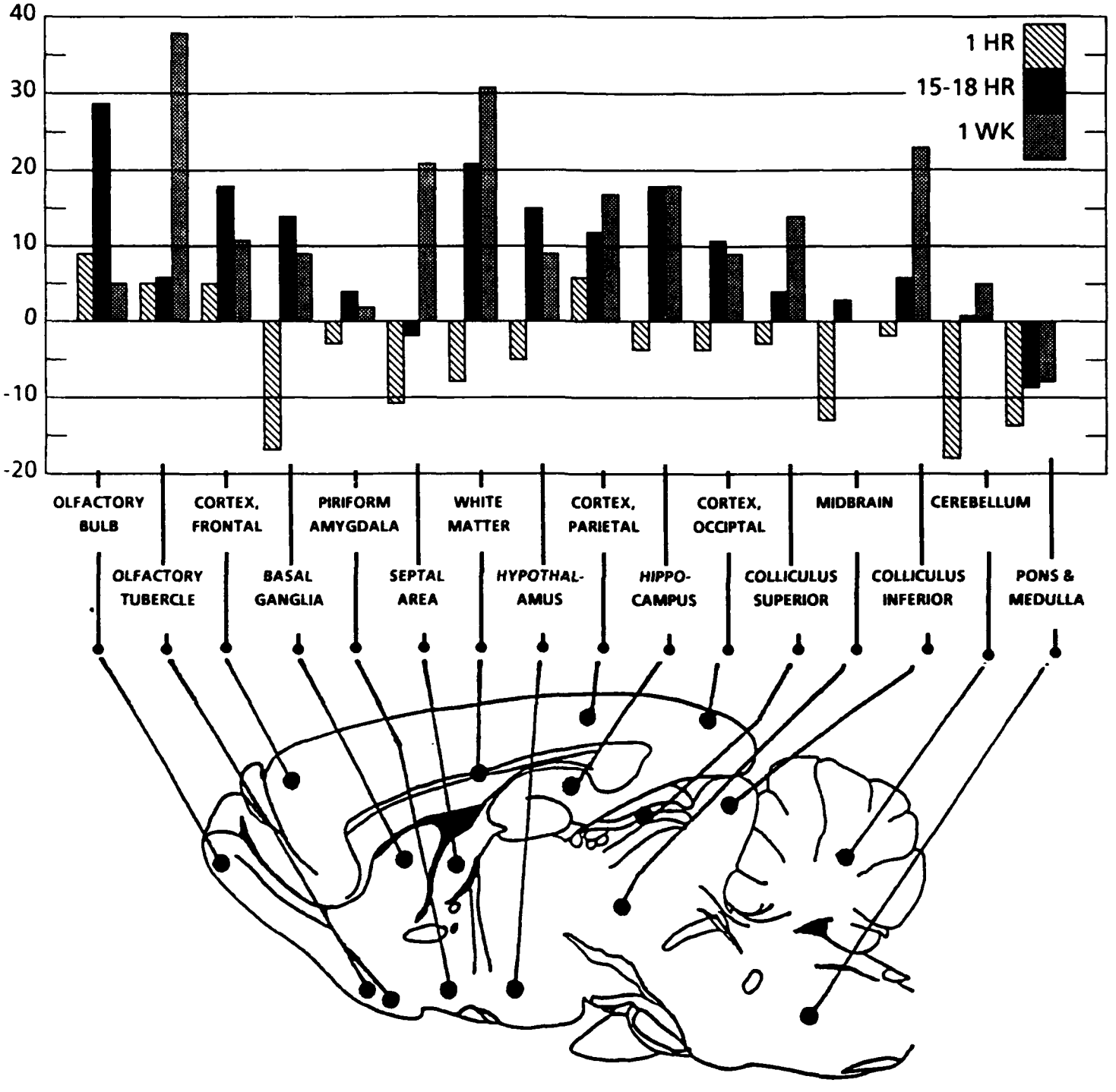

Figure 2