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AN EXPERIMENTAL BRAIN MISSILE WOUND: ASCERTAINING PATHOPHYSIOLOGY AND EVALUATING TREATMENTS TO LOWER MORTALITY AND MORBIDITY

ANNUAL/FINAL REPORT

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An Experimental Brain Missile Wound: Ascertaining Pathophysiology and Evaluating Treatments to Lower Mortality and Morbidity

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Cerebral ischemia does not occur following a brain wounding provided that ICP is < 60 mmHg. Rather focal hyperperfusion occurs.

Mechanical and chemical control of CBF are disturbed following brain wounding but these disturbances have wide regional variations.

The missile-wounded brain may show severe CBF reductions with even a mild fall in MABP. No reflow follows cerebral ischemic occurring after frain wounding.

Brain wounding is associated with decreased brain stem and hypothalamic biogenic amines especially epinephrine. This seems to be a part of a generalized stress response.

Free radicals appear in the brain within minutes of brain injury.

Experimental brain wounding; Cerebral blood flow; Brain biogenic amines; Free radicals after brain wounding;
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Unclassified Unclassified Unclassified Unlimited

SUMMARY

This brain wound project has shown the following:

1) a brain missile wound may kill by producing apnea. This missile-induced apnea may be reversed provided respiratory support is provided. Drug therapy to reverse apnea should be sought.

2) mild vasogenic edema occurs with missile wounding but this does not require specific treatment.

3) immediately following brain wounding extremely large increases in CSF prostaglandins occur. These biologically active molecules may cause physiologic effects at a distance from the missile wound.

4) a brain missile wound does not cause cerebral ischemia provided that the ICP is not > 60 mmHg. Rather than ischemia, a transient increased rCBF occurs particularly about the missile track

5) both mechanical and chemical CBF regulation are profoundly affected following a brain missile wound but the effect is not uniform throughout the brain; rather marked regional variations occur.

6) even a slight drop in mean arterial blood pressure after brain wounding may cause a profound decrease in CBF. Furthermore, once a decreased CBF has occurred following MABP reduction, restoration of MABP with blood may not improve CBF: the missile-wounded brain is subject to NO REFLOW. **It is imperative that blood pressure be maintained in brain wounded soldiers.**

7) brain wounding causes a change in hypothalamic and brain stem biogenic amines particularly a decrease in epinephrine. The so-called "Cushing" response appears to be a variant of a generalized stress response. An extreme disordering of biogenic amines in the brain stem does not appear to account for death following brain wounding.

8) GM1 ganglioside suggestively improved the extent and rapidity of neurologic recovery following brain missile wounding. Further tests with this drug are warranted.

9) free radicals are formed within minutes of traumatic brain injury. To combat their action our reserach suggests that antioxidant therapy should be started within a few minutes of brain injury.

FOREWORD: In conducting the research described in this report, the investigators adhered to the "Guide for the care and use of laboratory animals", prepared by the committee on care and use of laboratory animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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TABLE OF CONTENTS

PAGE

TABLE OF CONTENTS

PAGE

This report summarizes the high points of research done under contract DAMD17-86-C-6098 from April 1986 through November 1989 when research funding was terminated through Congressional action apparently instigated by the Honorable Robert Livingston (R-LA) acting at the behest of animal activists. Methodological details and data from all experiments have been provided in the following yearly reports: DAMD17-83-C-3145 of 31 December 1985; DAMD17-86-C-6098 of 21 September 1987, 27 April 1989, 26 October 1990 and 9 May 1991, (more than 500 pages). To provide continuity of thought our major findings from our 1983 to 1985 contract will also be presented in this report.

BACKGROUND

Brain wounding accounts for almost half of all single-wound combat deaths among soldiers. No essential change has occurred in the post operative neurosurgical mortality of brain wounds from WWII when it ranged from 11-14% to Vietnam where the post operative mortality was 10-12% (17). Too few Americans received brain wounds in Desert Storm to draw conclusions about current mortality. In the civilian community more than 16,000 individuals die each year from brain gunshot wounds (18). Thus, brain wounding is a severe problem for all segments of American society.

Virtually nothing is known about brain wounding from missiles in modern pathobiologic terms. Fewer than 25 papers have ever been written concerning animal-based brain wound experiments where cerebral and systemic reactions to a missile wound have been studied. At this point one sixth of these have come from our laboratory.

METHOD

In our first contract period from 1983 to 1985 (see report DAMD17-83-C-3145, 31 December 1985) we refined the brain wound model of Gerber and Crockard (24,47) and developed our laboratory model simulating a non-lethal wound to the brain. This brain wound model has been used in all experiments discussed below. Three to 5 kg cats were anesthetized with i.p. and i.v. pentobarbital (35-40mg/kg) and, after appropriate surgical and other procedures, were placed in a stereotaxic frame. The thoroughly anesthetized cats (feeling no pain) were then wounded in the right cerebral hemisphere through the intact skull with a 2mm, 31.7 mg steel sphere fired at 240-390 m/sec. This produced a brain wound by a missile having either 0.9, 1.4 or 2.7 Joules (J). Steel spheres have been frequently employed in basic, experimental ballistic research (58,157). The lower missile energies we selected were designed to simulate those of a fragment wound. Figure 1 shows the characteristic wound produced in the right cerebral hemisphere.

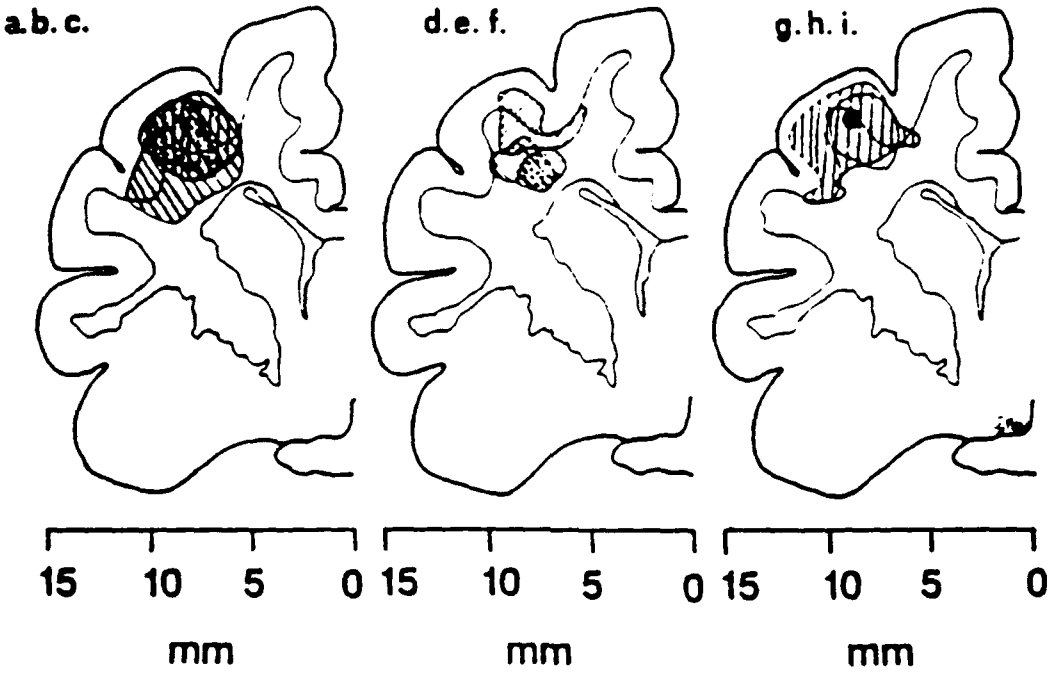


Figure 1: Drawings of coronal brain sections 14 mm caudal to the right frontal tip showing characteristic missile track locations and sizes in nine cats (a to i). Only occasionally did missile tracks deviate from this region.

Statistics: Appropriate statistics were done in all experiments. These are well defined in the individual yearly reports in which these experiments are contained and in published papers developed from these experiments.

SIGNIFICANT FINDINGS

A. Contract DAMD17-83-C-3145 (1983-1985)

1) Respiratory Dysfunction

We determined that post wounding respiratory dysfunction accounted for the majority of deaths following this right hemisphere brain missile wound and that the probability of a fatal apnea was missile-energy dependent being 14% with a 0.9 J missile, 40% with 1.4 J missile and ~70% with a 2.4 J missile. Importantly, the apnea resulting from brain wounding would often spontaneously resolve up to two hours after wounding if the animal were provided respiratory support. The significant clinical ramification of this finding is that possibly soldiers with fragment wounds to their brain die not from an intrinsically fatal brain wound per se but from missile wound-induced apnea which might reverse itself over time provided respiratory support were given (as by CPR). Of course, if the brain wound-induced apnea could reverse by itself (ie if the brain stem cardiorespiratory centers were only transiently disturbed and not permanently destroyed by energy transfer from the missile) the possibility also exists that drug therapy might be found which would enhance

the recovery of temporarily deranged respirations thereby lowering the mortality of soldiers with brain wounds. No work on this concept of trying to reverse trauma-induced apnea has been carried out but it has civilian as well as military applications not only for missile wounding but for closed head injury as well (18).

2) Brain Edema

In our initial work we also evaluated post wound brain edema formation and found it to be vasogenic in nature, not too severe, and confined to the wounded hemisphere (19). The edema peaked 24-48 hours after wounding, then receded and resolved in a week. The amount of edema formation was the same whether the missile had 0.9 J or 1.4 Js of energy. We concluded that, clinically, after fragment wounding, provided that brain hypoxia or ischemia did not occur, the amount of brain edema to be expected was mild, self limited and would not require specific treatment as by steroids. No information exists on the augmentation of missile-wound caused brain edema by simultaneous hemorrhagic hypotension or hypoxia but this would be worthy of study because it might directly affect how individuals with brain wounds are handled. Soldiers in combat are particularly prone to multiple wounds and hemorrhagic shock because of concomitant major vascular injury so the possibility of shock occurring with a brain wound is real.

3) CSF Prostaglandins

Because brain injury is associated with prostaglandin formation and because prostaglandins themselves may cause additional tissue, vascular, or neural effects, we evaluated the appearance of prostaglandins in the CSF after brain wounding. Within 5 minutes of wounding enormous amounts of multiple prostaglandins ($F1\alpha$, Thromboxane, E_2 and D_2) were seen in cisternal CSF. As CSF circulates widely throughout the brain, prostaglandins contained in CSF would be widely dispersed and these biologically potent prostaglandins could, theoretically, alter neural or vascular function in the brain at distances quite far removed from the actual wound site.

B. Contract DAMD17-86-C-6098 (1986-1991: experimentation terminated November 1989 by order of Congress)

Clearly, normal brain function requires an adequate circulation. We have been able to find only a few prior studies investigating cerebral blood flow (CBF) after missile wounding but in none was regional cerebral blood flow (rCBF) studied (25,33,92). Because of the importance of CBF for brain function, from 1986-1989 we undertook an exhaustive study of total and regional CBF and how its mechanical and chemical regulation might be affected by brain wounding. Microspheres and the reference syringe withdrawal method were used to measure CBFs in all experiments in this report.

1) Regional cerebral blood flow after brain wounding (DAMD17-86-C-6098, 21 September 1987)

Our initial experiments evaluated rCBF changes (~30 brain regions) including 3 concentric zones about the wound track, figure 2 A,B.

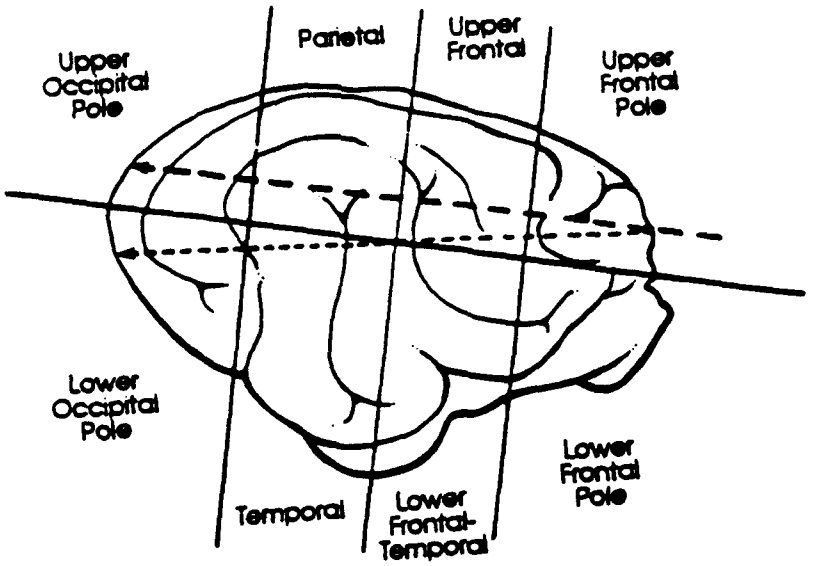


Figure 2A: Brain dissection scheme and missile trajectories. The heavier line depicts the usual missile trajectories. The lighter line indicates less frequent trajectories.

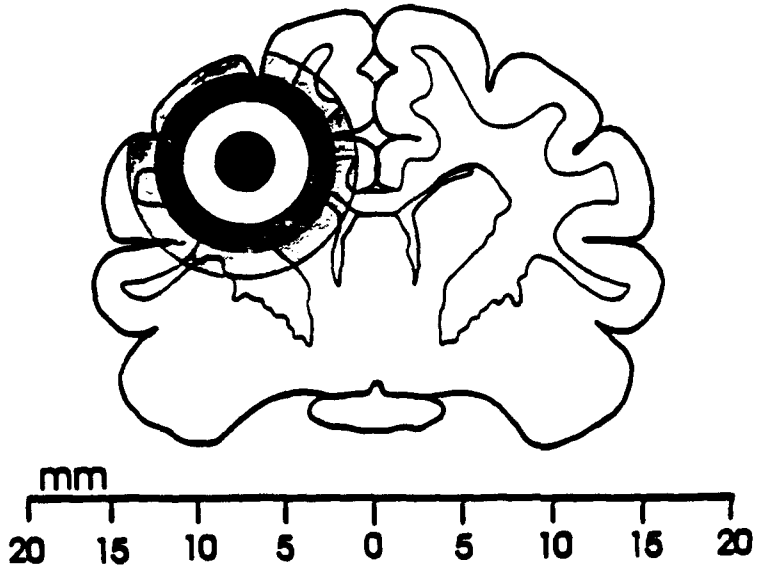
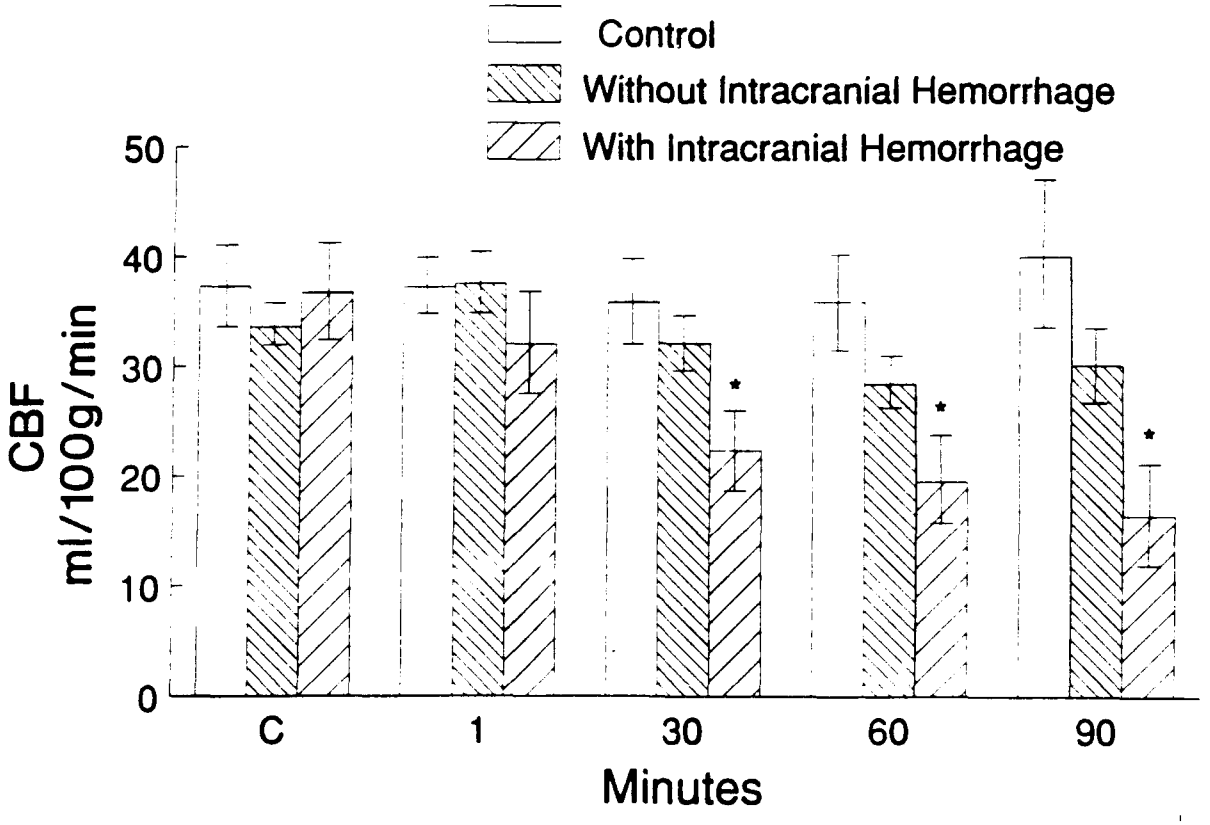


Figure 2B: The usual wound track measured 1-2 min in radius. The "inner core" extended 2.0-4.0 mm outward from the center of the missile track, the "middle core" extended 4.0-5.5, and the "outer core" 5.5-7.0.

We observed no brain ischemia in any region caused by missile wounding whether the brain wound was created by a missile having an energy of 0.9, 1.4 or 2.4 J. Considering all brain wounded animals together, after wounding there was a general tendency for total and regional CBF to decrease but not to an ischemic level (<15ml/100g/min in the cat, 62). We attributed the generalized, mild CBF decrease to the increase in intracranial pressure (ICP) caused by missile wounding and a concomitant loss of CBF autoregulation.

In animals where the post wounding ICP remained ~40 mmHg and their cerebral perfusion pressures (CPP) were greater than 75 mmHg, no CBF reductions occurred. CCP= mean arterial blood pressure (MABP) - ICP. CBF decreases after wounding were more likely to occur when the post wounding ICP was 60 mmHg or greater and the CPPs were consequently reduced to ~50 mmHg owing to intracranial clots or brain swell... figure 3.



* p<0.05 compared to control period and to cats without hemorrhage

Figure 3: Control unwounded cats had no change in whole brain CBF. Brain wounded cats with lower ICP elevations (~40mmHg) also maintained CBF. If ICP rose to greater than 60 mmHg following wounding significant CBF reductions were likely to occur.

In order to avoid the obfuscating effect of those animals with high post wounding ICPs and consequent generalized CBF reductions we then analyzed regional CBF data from animals which had lower post wounding ICPs and which maintained CBF. By this means we ascertained the effects of brain wounding alone on rCBF (as opposed to brain wounding plus elevated ICP).

In cats which maintained normal post wounding CBF two periods of discrete, post wounding hyper perfusion occurred: the first, within a minute of wounding, appeared in the brain about the missile entry site in the right frontal lobe and also in the left occipital lobe as though somehow the effects of missile energy deposit were transmitted to the coup and contra coup areas of the brain preferentially, Figure 4. This early, discrete hyperperfusion occurred when the wounded animal's MABP rose and we concluded that the effects of missile wounding disturbed specific, regional precapillary vasoconstrictors causing CBFs in these areas to become transiently MABP dependent (ie rCBF autoregulation was lost).

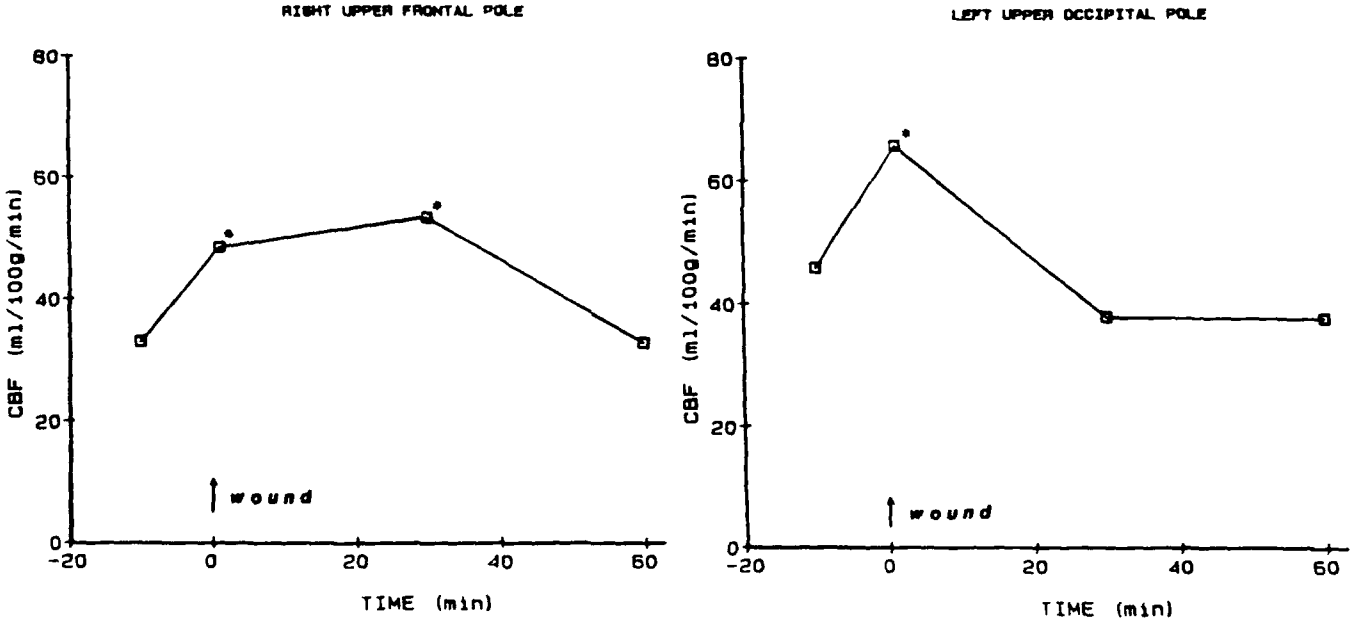


Figure 4: In the first post wounding minute rCBF rose at the missile entry site in the right frontal pole and in the contracoup left occipital pole, * $p<0.05$ compared to control (-10 min).

Thirty minutes after wounding hyperperfusion occurred solely about the wound track (including the right frontal pole) and we attributed this to local vasoactive tissue factors activated consequent to crushing of brain tissue by the transiting missile, Figure 5. The amount of hyperperfusion was greatest closest to the missile track itself.

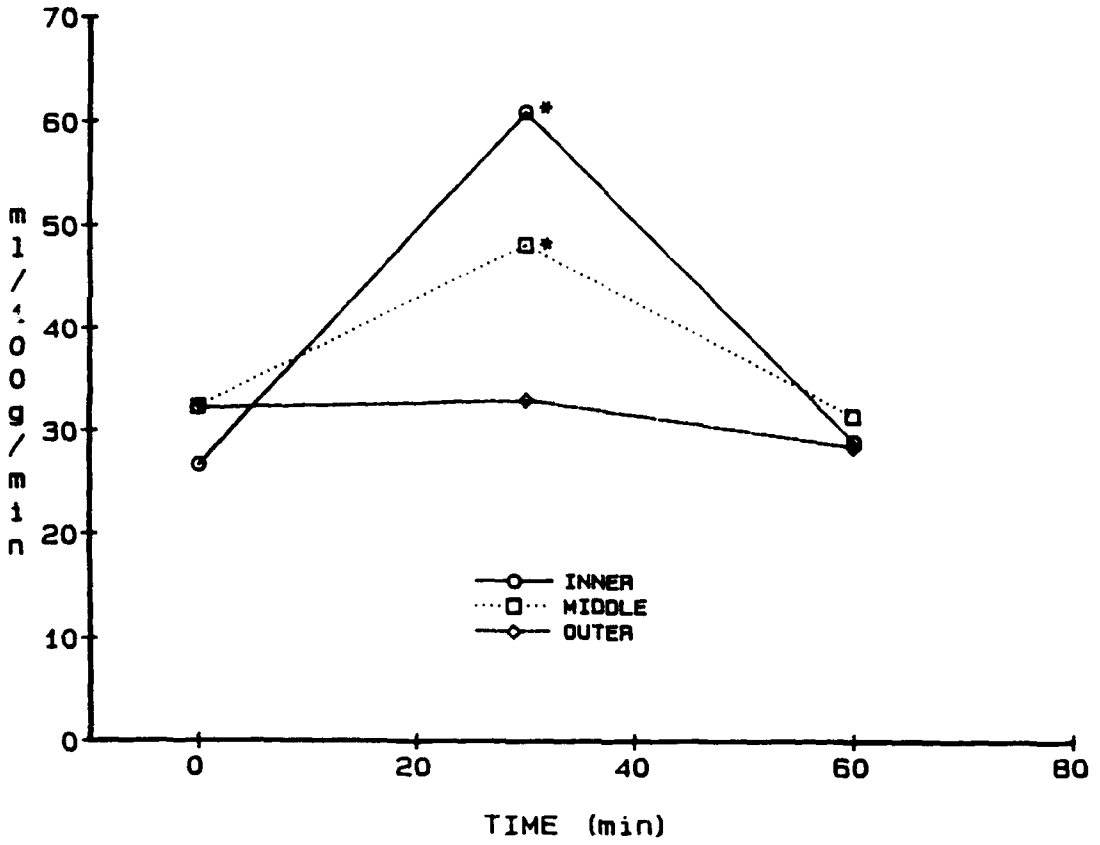


Figure 5: Hyperperfusion occurred about the wound missile track 30 minutes after wounding. The amount of hyperperfusion appeared significantly greater closer to the missile track than further away from it. (* $p<0.05$ vs control; + $p<0.05$ vs middle; # $p<0.05$ vs outer>

We concluded that if intracranial pressure were not elevated (as by an associated intracranial clot) and the CPP were above 60-70 mmHg, brain wounding would not be expected to be associated with cerebral ischemia. Any neurologic deficits observed after wounding were the result of the mechanical effects of wounding and were not caused by or potentiated by concomitant rCBF deficits. This knowledge is important because, intuitively, one might expect brain wounding would cause some degree of ischemia and that drugs used to treat stroke might also be useful in treating brain wounds. Such is unlikely to be the case if the intended drug's main purpose is to improve a reduced CBF as would be desirable in stroke. Drugs with other properties must be sought to ameliorate the neurologic defects caused by brain missile wounding.

Comments on missile energy and observed pathobiological events

The effect of missile energy deposit upon the small and compact brain stem producing apnea was unequivocally missile-energy dependent (p 2). Other physiologic changes which we have measured consequent to brain wounding were not correlated with missile energy eg: the amount of brain edema formation, rise in rCBF in specific brain areas after wounding, and the level of CSF prostaglandin increase after missile injury. We hypothesize that: 1) the lack of correlation of these physiologic variables with missile energy was because of the extremely narrow energy windows which we had to use to produce a relatively non-fatal brain wound; 0.9J to 1.4J. 2) Pathobiologically, the cerebral hemispheres are much larger than the brain stem and are able to absorb energy more readily than the compact medulla. They, thus, reacted to these closely spaced missile energies as to a single energy.

2) Mechanical regulation of CBF after brain wounding

Because we often saw a mild CBF reduction following brain wounding in the above experiments, suggesting a loss of CBF autoregulatory control, and because trauma and other CNS insults are known to impair CBF autoregulation (26,93,94,96,107) we undertook a detailed study of rCBF autoregulation specifically following a brain missile wound. To our knowledge no such study had ever been undertaken.

Mechanical CBF regulation occurs when CBF is maintained in the face of a decreasing MABP (130). Classically, CBF remains normal as MABP is lowered by bleeding to ~60 mmHg owing to relaxation of cerebral resistance vessels. This phenomenon defines classical CBF autoregulation. Below this level of hemorrhagic hypotension CBF falls along with MABP: the brain can no longer regulate its blood flow because precapillary arterioles can not dilate further to decrease resistance and maintain flow (Flow= Pressure/Resistance). CBF may also be attenuated by elevations of ICP which may reduce the CPP (107). Brain wounding affects the brain stem,, increases ICP, and may reduce CPP (18,23,25,33,47,92). One may anticipate that brain wounding would adversely affect CBF autoregulation but the extent and degree of such an impairment has never been investigated.

Hemorrhagic hypotension to lower MABP with the simultaneous measuring of CBF has been the standard means by which mechanical CBF regulation has been tested (93,130). This time-honored physiological technique coincidently happens to mimic the clinical situation wherein a soldier might get a brain wound as well as another wound (eg femoral artery) leading to significant blood loss. Multiple fragment wounds are commonly seen in combat. More than 90% of wounded soldiers in VII Corps during Desert Storm were so injured (unpublished data gathered by Dr. Carey). Militarily, it would be very important to know how the missile-wounded brain handles its blood flow in the face of substantial blood loss.

In the experiments on mechanical CBF regulation we also decided to see whether the reinfusion of shed blood would improve any observed CBF derangements. This would mimic an exsanguinated, brain-wounded soldier receiving a blood transfusion to restore MABP and hopefully CBF.

The non-wounded brain autoregulated CBF (i.e. maintained CBF) down to a mean MABP of ~48mmHg because cerebrovascular resistance appropriately and significantly decreased from 3.6 to 1.6 resistance units. Reinfusion of blood did not alter CBF nor was it associated with a rise in ICP, Figure 6.

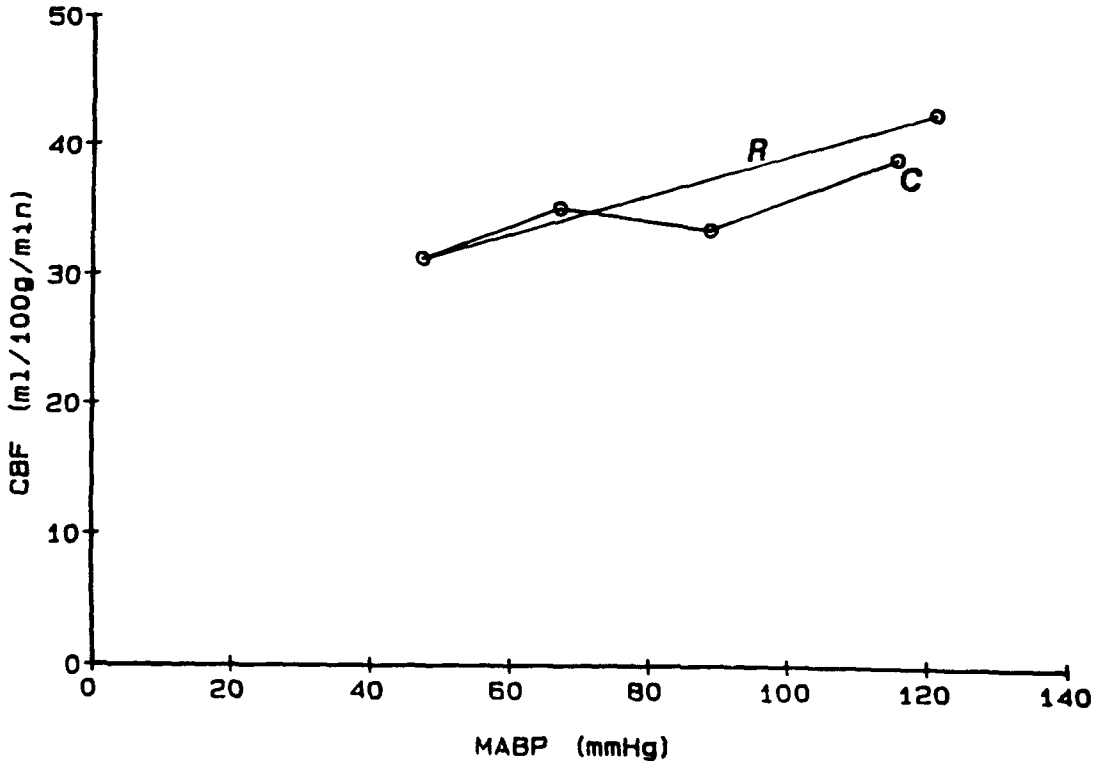


Figure 6: Mechanical CBF autoregulation was intact in unwounded cats; CBF did not significantly change even when MABP was reduced to 48 mmHg forty five minutes after bleeding began. Once MABP was reduced to 48 mmHg blood was reinfused over 30 minutes. Because ICP was not elevated CPP was always greater than 40 mmHg.

We measured mechanical CBF regulation in 10 cats after brain wounding and found that CBF autoregulation was severely deranged; not only about the wound track but widely throughout the brain. Mean blood flows (thick line, Figure 7) showed a significant and inexorable fall from control levels with but slight or moderate decreases in MABP.

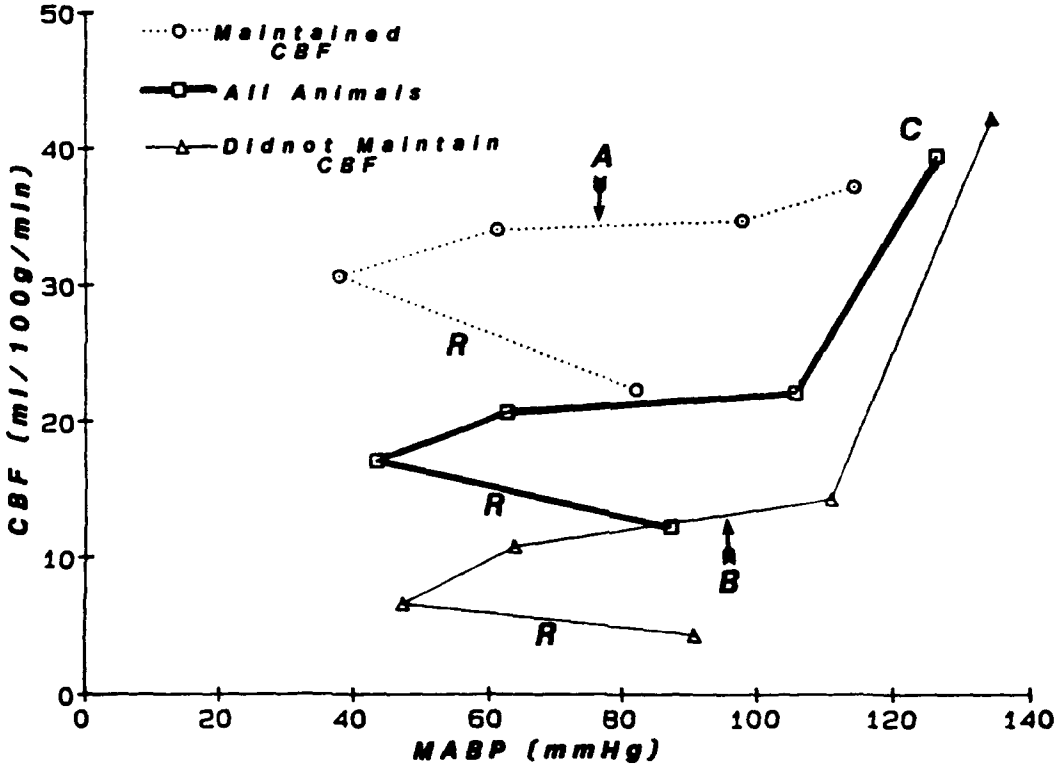


Figure 7: As a group (thick line) brain wounded cats showed a defect in mechanical CBF regulation, wherein CBF appeared MABP dependent. Within this group, however, two distinct post wounding CBF patterns were evident. Group A maintained flow while group B did not. To the right, C indicates the control CBF(s). R indicates blood reinfusion, which took place from 45 to 75 mins after wounding. Final CBF was at 90 min. On each line the first point to the left of control is 5 min after wounding and the second is 20 min. CPPs (mmHg) were: control, 120; 5 min, 46; 20 min, 23; 45 min, 12; 90 min, 27.

The clinical significance of this finding is that if an individual sustains a brain wound and also incurs blood loss so that MABP goes down, even modestly, CBF may be dangerously reduced, even to ischemic levels. Therefore, in brain-wounded individuals, MABP must be closely monitored and assiduously maintained to prevent irreparable cerebral ischemia.

Further analysis of the CBF-MABP responses in the 10 cats in our experiments revealed that 4 brain wounded cats were able to maintain CBF despite hemorrhagic hypotension (A line, Figure 7) while 6 demonstrated an extremely precipitous fall in CBF with even slight MABP decreases (B line, Figure 7). Cats able to maintain flow had mean post wounding ICPs of ~40 mmHg and CPPs of ~60 mmHg. Animals unable to maintain flow had mean post wounding ICPs of ~60 mmHg and CPPs ~40mmHg. We hypothesize that the group A cats were able to vasodilate as MABP fell. They thus maintained autoregulatory ability to a falling MABP. Group B brain-wounded animals which had a large CBF fall to even slight MABP decreases clearly had lost all autoregulatory control and we hypothesize that their precapillary vasodilatory mechanisms were inoperative, possibly as a consequence of their higher ICPs and reduced CPPs.

Upon reinfusion of shed blood neither group of animals exhibited an improvement in CBF. This failure is most easily comprehended in the group B animals which became overtly ischemic. The "no-reflow" phenomenon (5,63) has been seen following percussion injury of the brain, cerebral ischemia, increased ICP, and hypovolemic shock (11,51,94,122,144) and has been attributed to several causes as cerebral edema compressing capillaries or to intravascular sludging (27,45). The failure of blood reinfusion to improve CBF in the group A animals which maintained CBF is more difficult to understand because mechanisms presumably activated by cerebral ischemia would not have come into play (because these animals were not ischemic).

In both groups, whether CBF was maintained or not, the ability to control ICP was lost. When blood reinfusion raised MABP in either group increased systemic vascular pressure was transmitted to the intracranial space. This raised the ICP and drastically reduced the CPP. The reduced CPP might have accounted for the failure of group A cats to improve flow. We hypothesize that possibly in group A animals an autoregulatory derangement was present that prevented dilated precapillary arterioles from constricting again in response to rising MABP. Consequently, the systemic blood pressure was transmitted directly into the cerebral capillary bed and thence into the brain parenchyma which greatly increased ICP.

Because brain wounding is associated with increased ICP we evaluated the effect of elevating ICP alone on CBF in otherwise normal cats. ICP was increased by infusing mock CSF into the cisterna magna. During these experiments CPP was kept in the 30-35 mmHg range. Under these circumstances CBF fell somewhat as ICP was raised but with blood reinfusion systemic arterial pressure was not transmitted intracranially and CBF was restored, Figure 8.

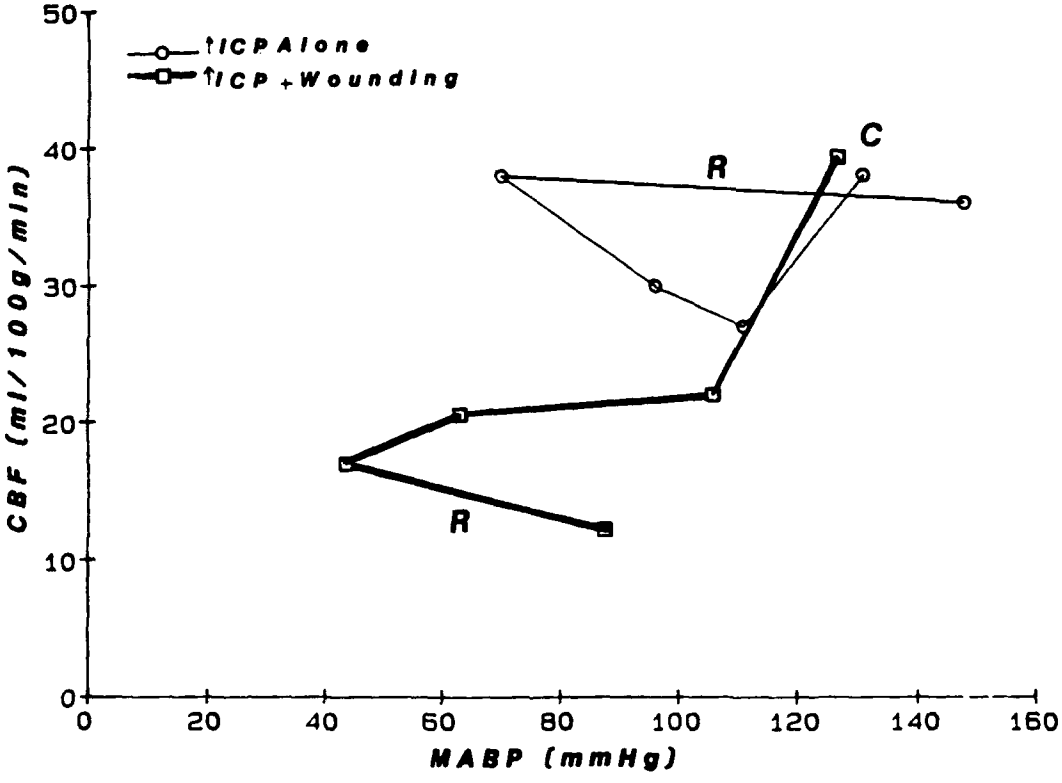


Figure 8: The effect of increased ICP alone (thin line and circles) or brain wounding with increased ICP (thick line and squares) on CBF. Wounding severely disturbed mechanical CBF regulation while elevated ICP alone did not.

These experiments lead us to conclude that the autoregulatory disturbances seen following brain wounding are not entirely as a result of ICP increases because ICP increases alone up to levels seen with brain wounding did not result in drastic CBF reductions. Furthermore, such an ICP increase by itself did not result in an autoregulatory disturbance so severe that, upon reinfusion, systemic blood pressure was transmitted to the intracranial space reducing CPP.

The critical clinical point is that following brain wounding, once systemic hypotension has occurred and CBF has decreased, restoration of MABP by blood infusion is unlikely to improve CBF and reverse or prevent cerebral ischemia. This doubly emphasizes the critical need for maintenance of MABP following brain wounding. This is not an idle or trivial point because one brain wounded American soldier seen by Dr. Carey in the recent Desert Storm operation had a BP of 70/0 when first seen at the 31st CASH Superimposed widespread cerebral ischemia unrelieved by restoration of MABP might have contributed to his poor neurologic outcome.

3) Chemical regulation of CBF following brain wounding

Chemical regulation of CBF is defined as the ability of $PaCO_2$ and PaO_2 to alter CBF. Carbon dioxide is a very potent vasodilator of cerebral arterioles and normally, hypercapnia increases CBF by diminishing cerebral vascular resistance (CVR). Hypocapnia causes cerebral vasoconstriction and decreases CBF (10,54,72,128). Arterial hypoxemia (PCO_2 < 60 mmHg > 50 mmHg) increases CBF while arterial hyperoxygenation has been shown to decrease CBF about 10-15% (73,87,103). Increase CBF with hypercarbia and hypoxia (as is usually seen with respiratory distress) may be viewed as protective to the brain because increased CBF caused by the elevated $PaCO_2$ and decreased PaO_2 would provide more oxygen per unit time to a brain threatened by oxygen lack.

CO_2 freely penetrates into the cerebral extracellular space, dissociates, and decreases the extracellular and perivascular pH which, in turn, affects the CVR (78,89,113). Decreasing extracellular pH leads to a delayed vasodilation of precapillary arterioles while increasing pH causes vasoconstriction (84,131). Hypoxic cerebral vasodilitation is thought to be primarily related to an increase in adenosine followed by increased K^+ concentrations (84).

Mild arterial hypercapnia of 50-60 mmHg and severe hypoxia of PaO_2 < 40 mmHg both increase the cerebral glycolytic rate, leading to tissue lactoacidosis (112). This also, in turn, decreases pH and promotes vasodilitation. Thus, superimposed metabolic factors also may lead to cerebral vasodilation and add to the vasodilatory effect of an increased PCO_2.

Hypoventilation and/or transient apnea are common features of traumatic brain injury, including brain missile wounding, (18,22,24,26,155). If chemical regulation of CBF is severely impaired by missile wounding, hypoventilation-induced hypercapnia may not be effective in increasing CBF and providing increased oxygen to the brain. Loss of chemical CBF autoregulation following missile wounding may, thus, be regarded as a serious event further degrading post-injury brain homeostasis.

The induction of hypocapnia is a preferred initial treatment of patients with acutely increased intracranial pressure (ICP), (66,96,104,131). Clinical and experimental studies have shown that acute and prolonged hyperventilation reduces trauma-induced increases in the ICP (10,66,96) by reducing CBF and intracranial blood volume (10).

The specific questions which these experiments on the chemical regulation of CBF sought to answer were: 1- Is the response of cerebral arterioles to changes in $PaCO_2$ and PaO_2 impaired after a brain missile wound? If so, is the impairment focal or generalized? Does the missile-wounded brain respond to hypercapnia and hypoxia with a protective CBF increase? 2- Is the vasoconstrictive response of hypocapnia preserved after missile wounding so that a hypocapnia-induced reduction in CBF could lower ICP? 3- Would any vasoconstrictive effect of hypocapnia be enhanced after wounding? This might lead to excess CBF reductions leading to ischemia if hyperventilation were employed.

In these experiments all cats were anesthetized with pentobarbital, paralyzed with pancuronium bromide, intubated, and placed on a respirator so that various gas mixtures could be given. All experiments were designed as a test-retest paradigm for each animal. The cat's response in the unwounded state served as a control for the wounded condition.

a) <u>Response to increasing arterial PCO_2</u>

We evaluated the effect of raising arterial PCO_2 from 29 to 54 mmHg in normal cats. As expected, rCBFs significantly increased in all 12 brain structures examined; total CBF rose from 34 to 72 ml/100g/min.* Following the hypercapnic challenge mean CVR appropriately decreased from ~4 to ~2 resistance units allowing

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* In addition to reporting the total CBF change in response to a given arterial PCO_2, the alteration of any observed CBF change may also be expressed in terms of "<u>reactivity</u>" defined as the unit change in CBF per unit change in arterial PCO_2. Total reactivity to hypercapnea in these normal cat brains averaged 38/25 or 1.5 ml/100g/minute/mmHg (see appendix 1). Owing to the large increases in PaO_2 employed to test the response to hyperoxia, > 280 mmHg, reactivity units to increased PaO_2 were not calculated.

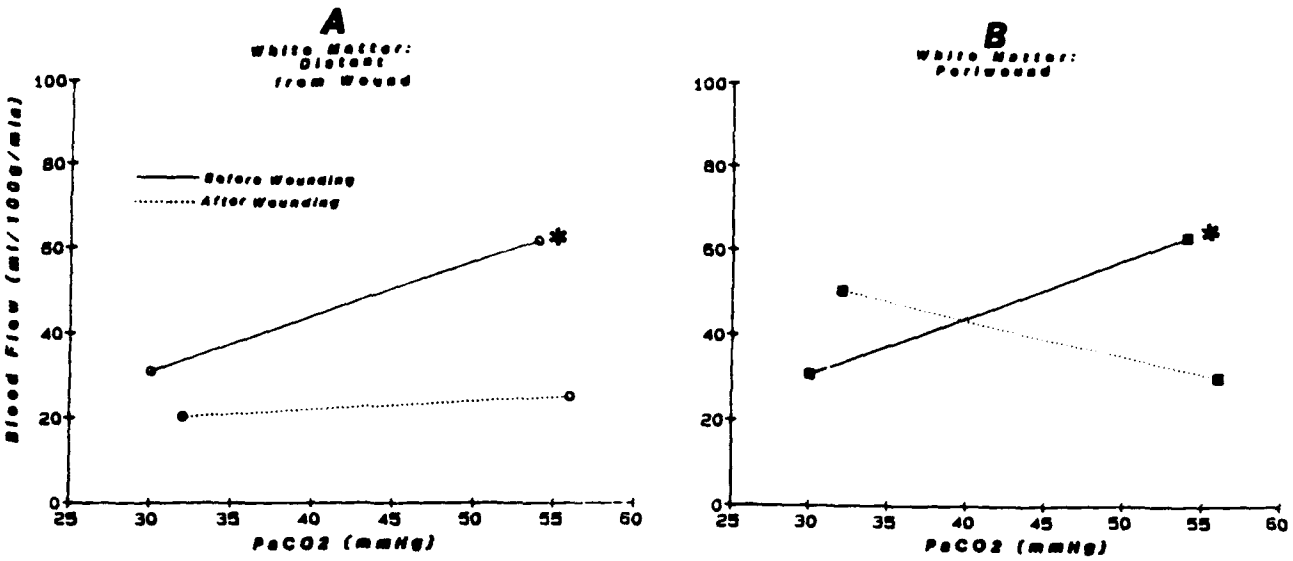
the CBF increase. After wounding, when arterial PCO_2 was raised from 32 to 56 mmHg, cats failed to show any changes in either regional or total CBF. No CVR changes occurred and total cerebral vascular reactivity was almost abolished being reduced to 0.01 ml/100g/min/mmHg.

Brain tissue about the wound track* responded paradoxically to the increased PCO_2: its blood flow decreased from 48 to 28 ml/100g/min corresponding to a reactivity of -0.83 ml/100g/min/mmHg. This periwound flow decrease probably resulted from an actual local CVR increase in response to hypercarbia. It was unlikely to have resulted from a "steal" of blood flow from non-reactive vasculature about the wound track to more normally reactive brain blood vessels because our results indicate a virtual lack of dilitation in response to increased $PaCO_2$ anywhere in the brain after wounding, Figures 9A to 9D, 10A to 10D.

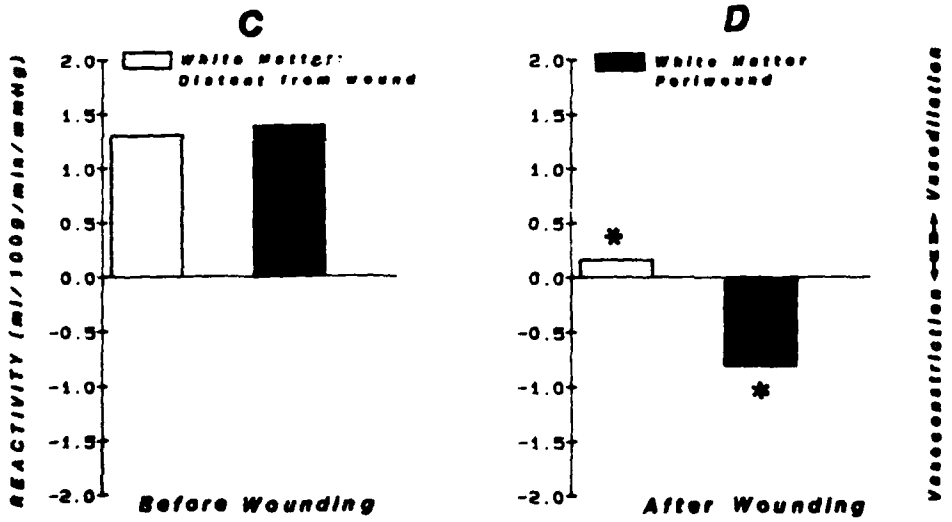
Impaired vascular reactivity to hypercapnia has been also demonstrated in cats subjected to fluid percussion injury and other experimentally induced cerebral trauma (6,94,141,161). Lewelt et al (94) studied the effect of hypercapnia on cerebrovascular reactivity in two separate groups of cats sustaining either mild or severe fluid percussion injury. Severely traumatized cats had a greatly attenuated CBF in response to hypercapnia of 54 mmHg, (about the same $PaCO_2$ level obtained in our hypercapnic trials). In their experiments ICPs increased from a control of 12 mmHg to 44 mmHg immediately after severe injury. The increased ICP declined rapidly, however, and was at control levels during post-percussion hypercapnic trial when cerebral vascular reactivity was noted to be impaired. Their mildly concussed cats showed no substantial increase in ICP at any time after injury but nevertheless demonstrated impaired CVR responses to increased $PaCO_2$. It thus appears unlikely that the impaired chemical responses to hypercapnia noted by Lewelt et al (94) and by us were caused by transient increases ICP alone. In our experiments after brain wounding, however, a static ICP increase of 34-39 mmHg occurred and the effect of such a long standing ICP increase is unknown. In our unwounded cats an ICP increase below 60 mmHg did not significantly affect mechanical CBF autoregulation (39,40,52,68,164), but the effect of sustained intracranial hypertension on chemical regulation of CBF is entirely unknown.

- -

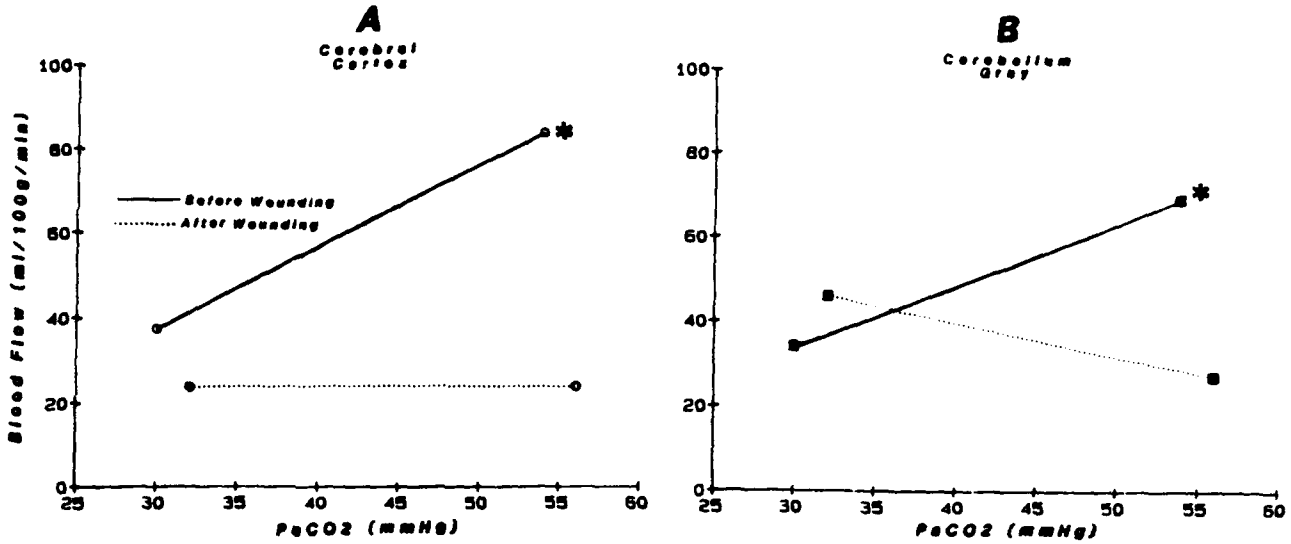
* In this report brain tissue about the wound track is often called "periwound" tissue. In all CBF experiments control values for "periwound" tissues indicate CBF in these tissues before injury i.e. in brain destined to become periwound in location after missile passage. White matter taken from sites away from the wound track is called "distant" white matter.



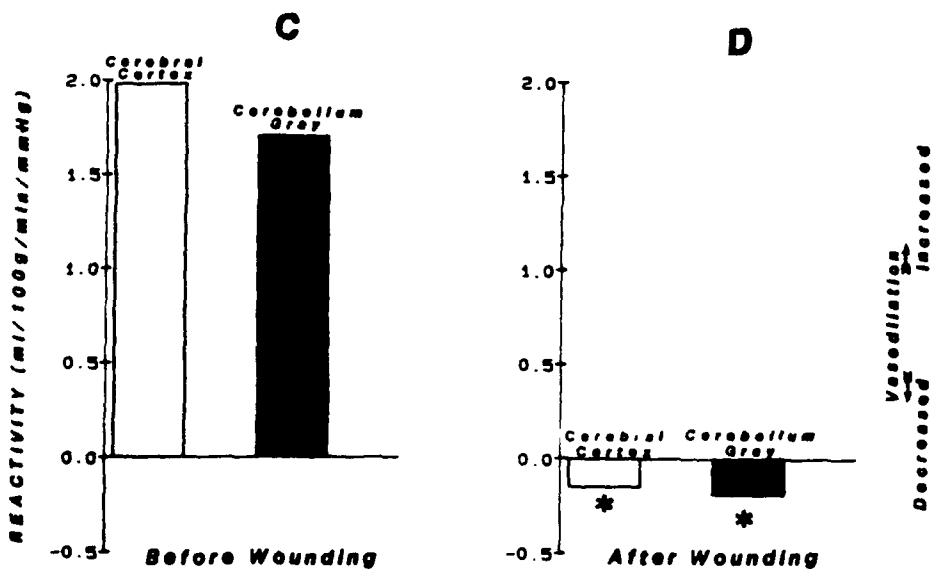
Figures 9 A and B: Before wounding hypercapnia significantly increased rCBF in brain white matter. After wounding it did not increase rCBF in either brain area. Periwound rCBF tended to decrease as $PaCO_2$ increased (9B).



Figures 9 C and D: Vascular reactivity in both brain areas became significantly less after wounding compared to their prewound levels. The post wounding reactivity of the periwound white was also significantly reduced compared to the post wounding reactivity of white matter distant from the wound (compare both bar graphs, 9D). This indicates a paradoxical hypersensitivity of damaged brain to hypercapnia.



Figures 10 A and B: Before wounding increasing arterial $PaCO_2$ significantly increased CBF in cerebral cortex and cerebellar gray matter. After wounding this effect was abolished in the cerebral cortex. Furthermore, increasing $PaCO_2$ tended to decrease cerebellar gray rCBF.



Figures 10 C and D: After wounding vascular reactivity in both cerebral cortex and cerebellar gray was significantly decreased compared to their prewound levels. The negative values indicate slight vasoconstriction.

b) Response to decreasing arterial PCO_2

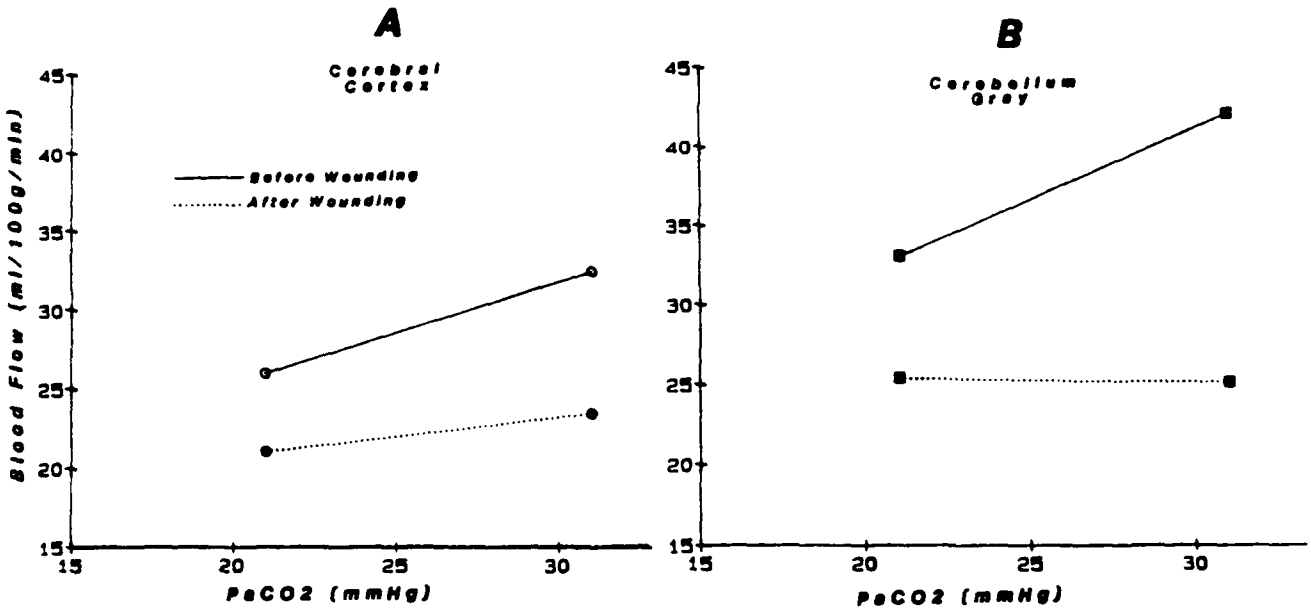
In our experiments hyperventilating normal cats and reducing arterial PCO_2 from 31 to 21 mmHg decreased total CBF from 31 to 26 ml/100g/min (-18%). Total vascular reactivity was -0.50 ml/100g/min/mmHg (CBF = -5 ml/100g/min; $PaCO_2$ = 10). After brain wounding total CBF also significantly decreased from 23.1 to 20.5 ml/100g/min with normoxic hypocapnia. Vascular reactivity was suggestively but insignificantly reduced to -0.26 ml/100g/min/mmHg.

While total CBF showed a significant diminution in response to hypocapnia after wounding, marked regional variations occurred, Figures 11A to D; 12A to D.

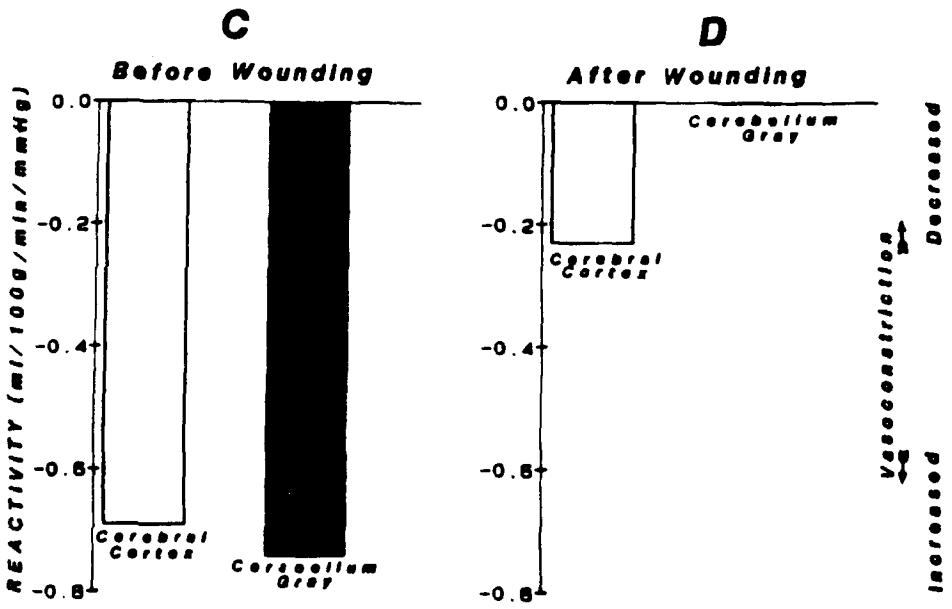
1) Cerebellar and brain stem blood flow decreases normally seen with hypocapnia were virtually abolished following brain wounding, Figures 11B. This total loss of vasoconstrictive ability after wounding suggests that posterior circulation vessels have a fundamentally different response to chemical stimuli mediating vasoconstriction than do anterior circulation vessels.

2) In contrast to the rest of the brain, damaged brain about the wound track exhibited an increased response to hypocapnia. Before wounding, brain in the region of what was to become the missile track demonstrated a 14 to 18% rCBF decrease when arterial PCO_2 was reduced from 31 to 21 mmHg. Afterwards, a similar arterial PCO_2 reduction caused periwound rCBF to decrease significantly by 24 to 27%. Vascular reactivity about the wound track fell from -0.44 to -0.77 ml/100g/min/mmHg indicating enhanced vasoconstriction. This decreased reactivity (-0.77 ml/100g/min/mmHg) was also significantly greater than the post-wounding reactivity in the distant white matter (-0.35 ml/100g/min/mmHg). Reasons why periwound white matter would show an increased reactivity to the lowered $PaCO_2$ remain obscure. Since an increase in periwound vascular reactivity also occurred during the post-wounding hypercapnic trials, it seems that blood vessels in the periwound tissues do not react to chemical stimuli as does the vasculature in the rest of the brain. Presumably, yet to be determined vasoactive factors from damaged brain diffuse into surrounding tissues and alter the vascular response to chemical stimuli. It would appear that the greatest potential for damage to the brain tissue because of impairment of chemical regulation is greatest about the wound track because rCBF tends to decrease with either hypercapnia or hypocapnia.

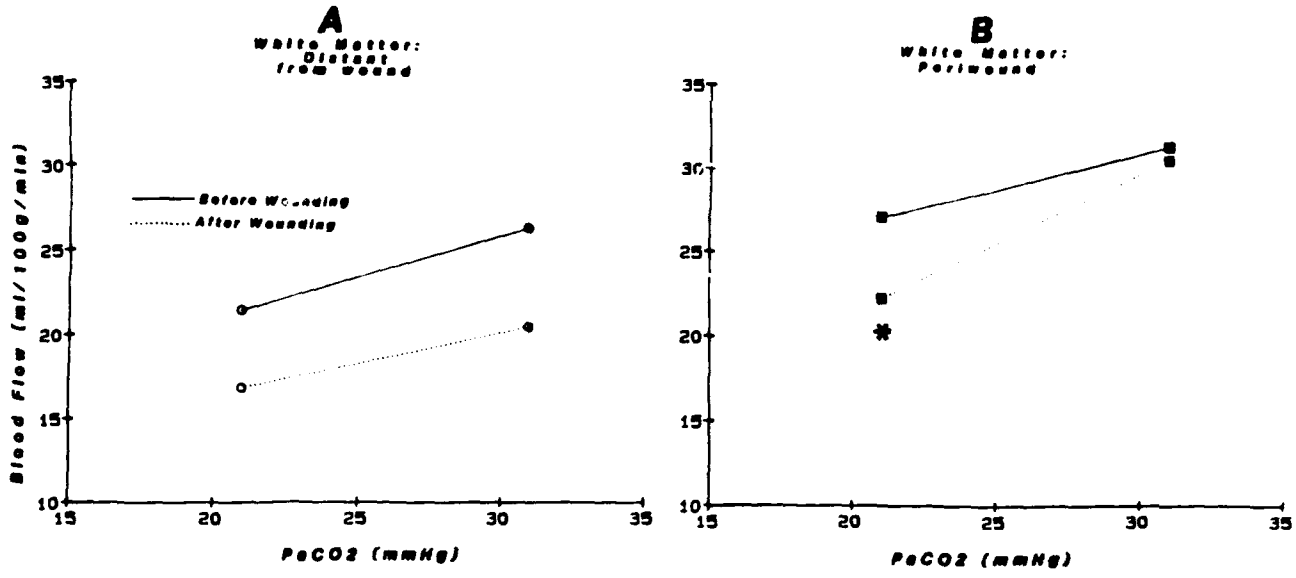
3) After brain wounding the ICP rose to 53 mmHg and hyperventilation, wherein the arterial PCO_2 was reduced from 32 to 21 mmHg, failed to lower the ICP even though CBF fell from 31 to 23 ml/100g/min. The failure of hyperventilation to lower ICP may possibly be explained by the diminished post wounding cerebral vascular reactivity to decreasing $PaCO_2$. After brain wounding it would appear that cerebral blood vessels cannot constrict sufficiently to decrease cerebral blood volume enough to lower ICP. The explanation does not consider any effect on CSF production or absorption. Clinically, data from brain-injured children indicate that hyperventilation cannot control ICP levels of greater than 60 mmHg (114). Possibly, the ICP of 53 mmHg observed in our cats after wounding approached the level where hyperventilation becomes ineffective. The limits to the effectiveness of hyperventilation as an ICP-reducing therapy following brain wounding should be investigated because if an elevated ICP has reached a level where hyperventilation is unlikely to be effective in lowering it, other means of reducing ICP should be immediately employed (67).



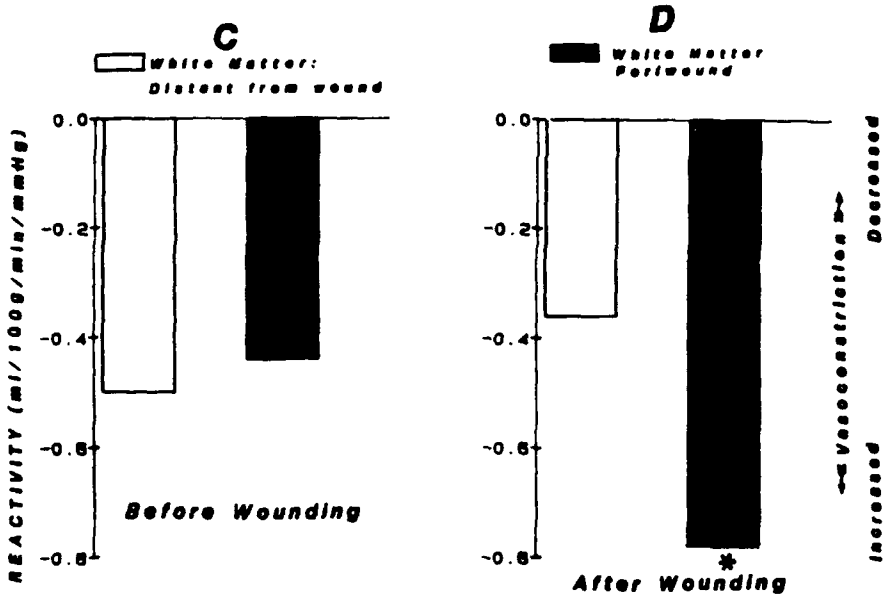
Figures 11 A and B: Before wounding decreasing $PaCO_2$ tended to decrease rCBF in cerebral cortex and cerebellum gray. After wounding, no reduction in rCBF of cerebellum gray matter occurred (B).



Figures 11 C and D: After wounding the reactivity of cerebellar gray to hypocapnia was lost suggesting no cerebellar vasoconstriction at all (D).



Figures 12 A and B: Before wounding decreasing $PaCO_2$ tended to decrease rCBF in brain white matter (distant and periwound). After wounding, hypoxia significantly decreased periwound blood flow but not distant white matter flow (B).



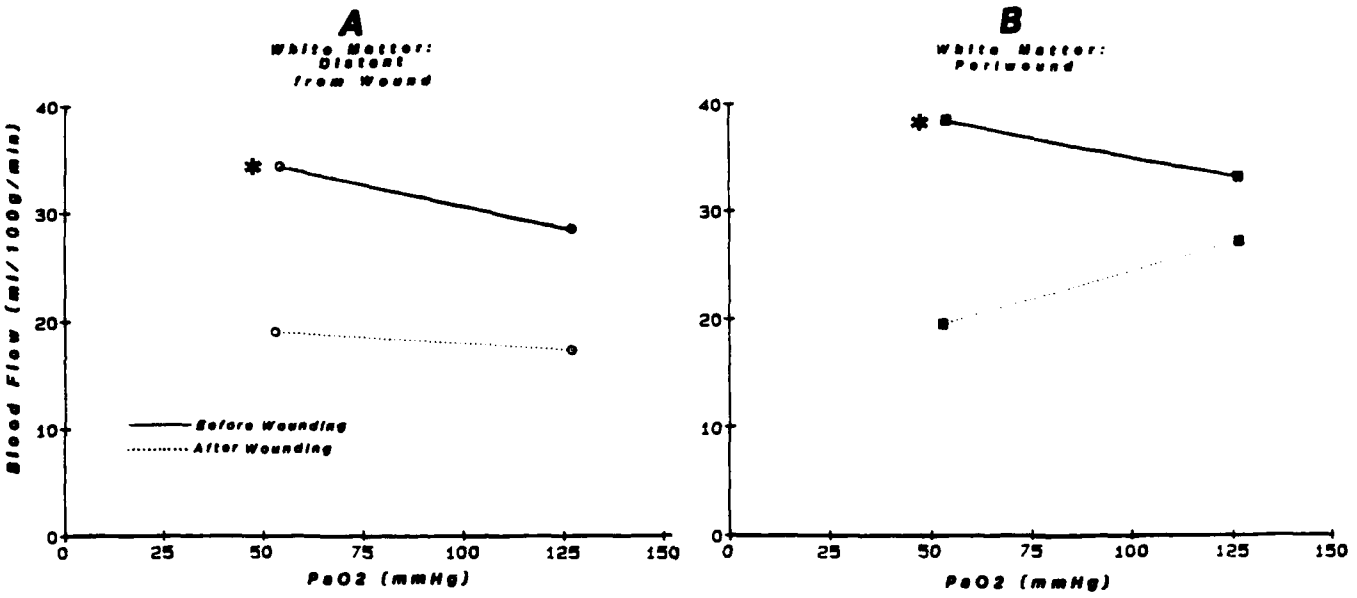
Figures 12 C and D: After wounding periwound vascular reactivity to a lower $PaCO_2$ became significantly more decreased compared to prewounding. Somehow wounding enhanced periwound vasoconstriction effect of hypocapnia in periwound tissue. This enhanced periwound was also significantly greater than distant white matter vasoconstriction after wounding (D).

c) Response to decreasing arterial PO_2

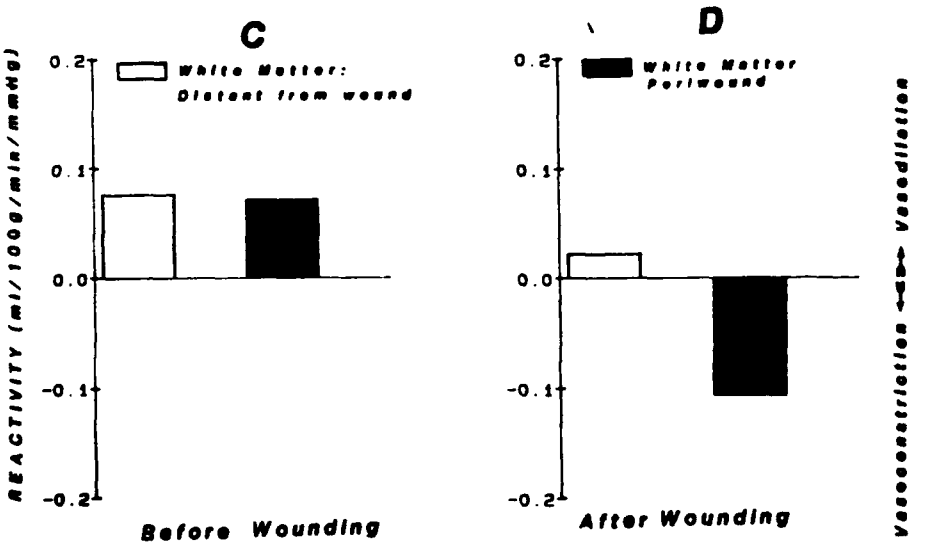
In normal cats, breathing 10% O_2 reduced arterial PO_2 from 127 mmHg to 54 mmHg. Under this circumstance total CBF significantly increased (35%) from 33 to 46 ml/100g/min. CVR tended to decrease but not significantly. After wounding, all cats failed to show any increase in total or regional CBFs following hypoxia which reduced arterial PO_2 to 53 mmHg. Cortical tissue adjacent to the wound track showed a significant and paradoxical decrease in CBF from 34 to 17 ml/100g/min, Figures 13A to D; 14A to D.

Impaired cerebrovascular reactivity to hypoxia has been demonstrated in cats subjected to fluid percussion injury and other experimentally induced cerebral trauma (6,94,141,160,161). In Lewelt et al's (94) study mentioned above (p 15) severely brain traumatized cats had a greatly attenuated CBF response to profound hypoxemia while their ICPs were at control levels. The ICPs in mildly concussed cats were never substantially increased at any time after injury yet these animals, too, demonstrated impaired vasodilitation to a decreased PaO_2. These results suggest that impaired cerebrovascular reactivity to hypoxia was not caused by an ICP increase alone. The mean levels of ICP and CPP observed in our wounded cats before hypoxic trial were 60 and 79 mmHg respectively. They were further increased to 69 and 87 mmHg with hypoxia. These static ICP levels observed in our experiments after wounding were considerably above those observed by Lewelt et al (94) and conceivably these ICP elevations alone could have impaired the vascular reactivity to hypoxia in our cats. Cecil et al (20), however, demonstrated in hypoxic lambs that increases of ICP to about 60 mmHg did not affect cerebral vasodilation in response to severe hypoxemia of 30 mmHg. This suggests that possibly an ICP increase alone into the 60 mmHg range may not be sufficient to abolish rCBF responses to hypox

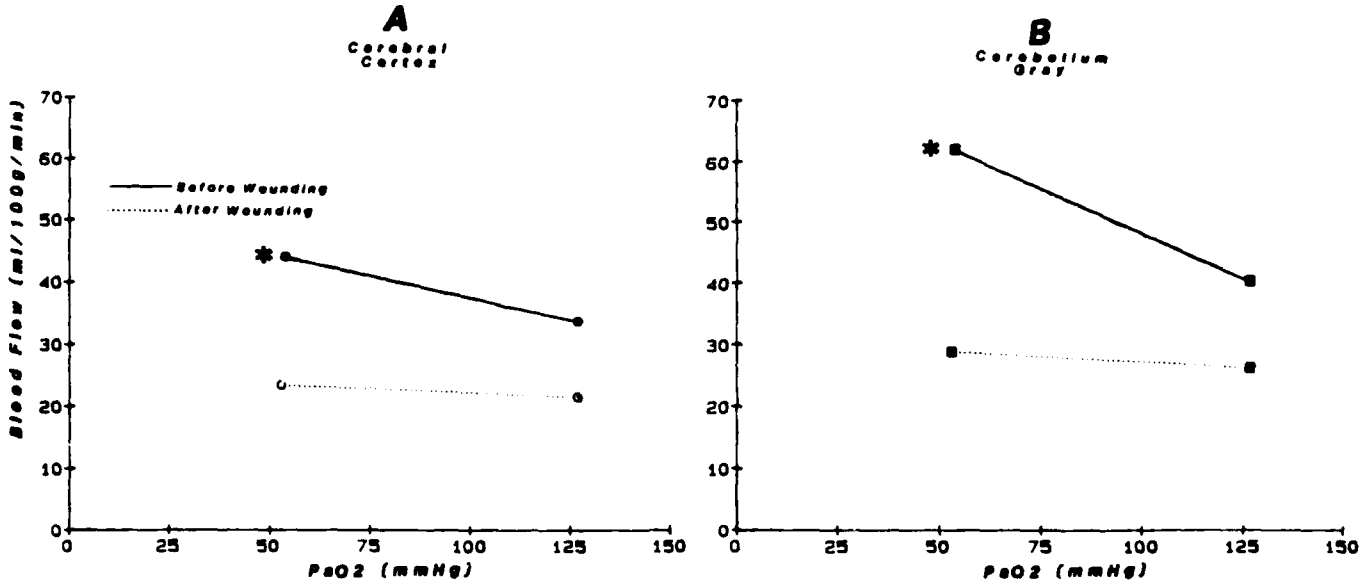
Our experiments have shown that after sustaining a missile wound the feline brain is totally incapable of chemical CBF regulation to hypoxia or hypercapnia. If these results can be extrapolated to the human brain wound situation they strongly suggest that the missile-wounded brain will not be able to increase CBF should hypoxia-hypercapnia supervene from any respiratory embarassment. Not being thus able to be supplied with additional oxygen by virtue of increased CBF the missile wounded brain is under great jeopardy from any hypoxia. Such an event must be avoided at all costs.



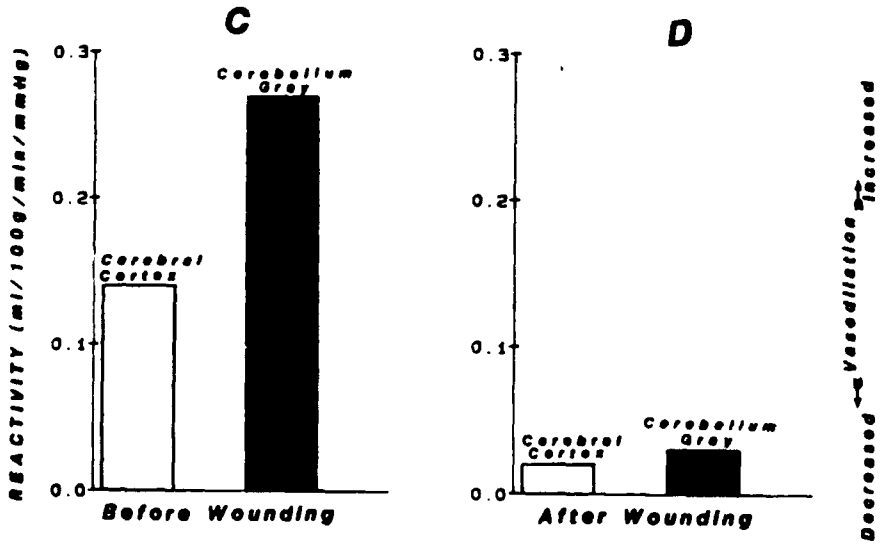
Figures 13 A and B: Before wounding, hypoxia ($PaO_2 \simeq 55$ mmHg) significantly increased rCBF in brain white matter. After wounding, hypoxia did not increase rCBF in brain white matter, (distant or periwound).



Figures 13 C and D: After wounding, vascular reactivity to hypoxia in both areas appeared greatly, but not significantly attenuated. The negative reactivity of the periwound white matter (D) suggests a paradoxical hypersensitivity of damaged brain to hypoxia.



Figures 14 A and B: Before wounding hypoxia, (PaO_2 ≃ 55 mmHg) significantly increased rCBF in cerebral cortex and cerebellar gray. After wounding hypoxia did not increase rCBF in either brain area.

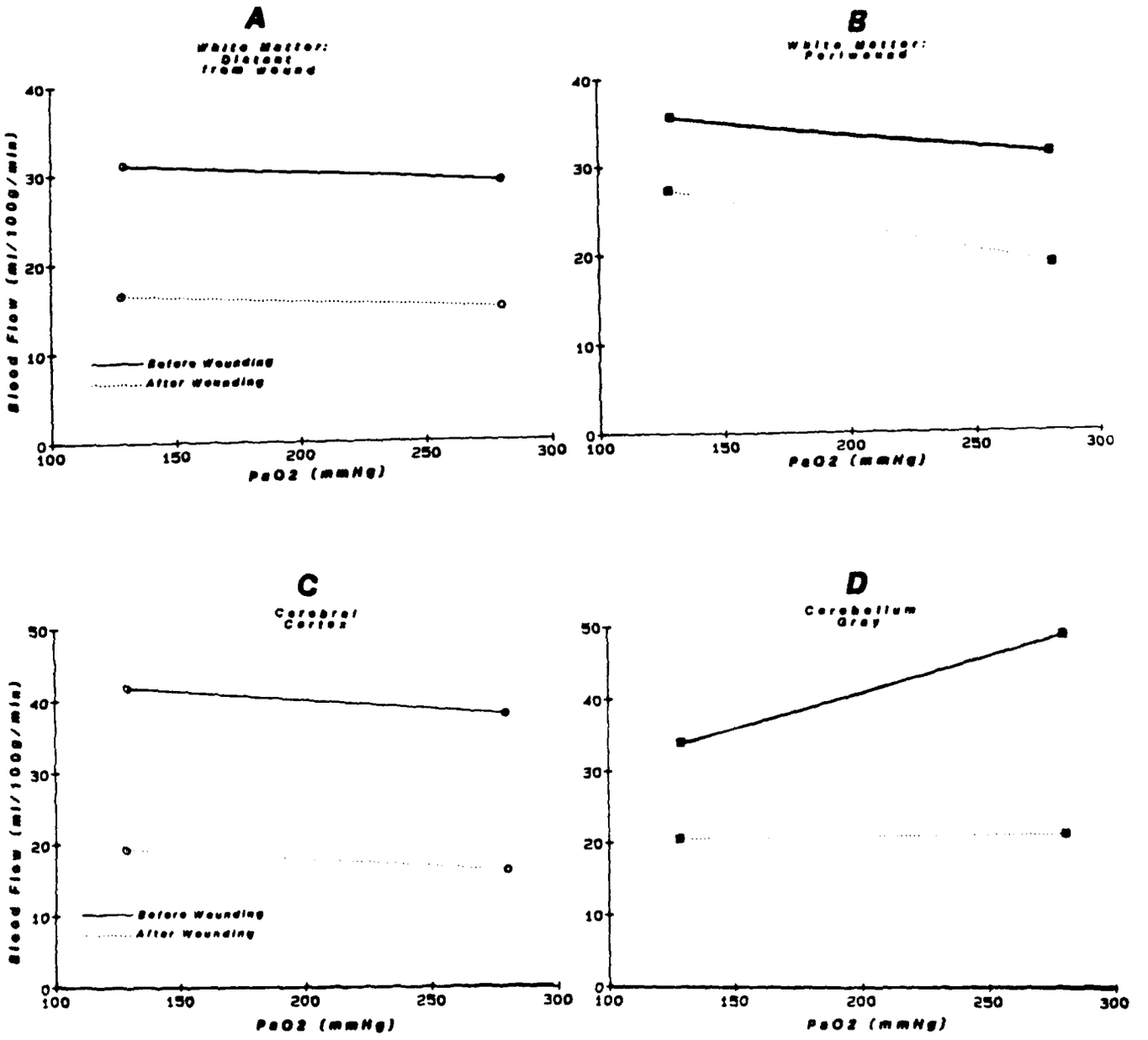


Figures 14 C and D: After wounding, vascular reactivity to hypoxia in both brain areas appeared greatly, but not significantly attenuated.

d) Response to increasing arterial PO_2

In contrast to other investigations which have indicated a mild CBF reduction with increased PaO_2, CBFs as evaluated in 9 cats by us showed no change when the PaO_2 was increased to > 280 mmHg for 10 min. Unexpectedly, this level of hyperoxia caused a significant CBF increase in brain stem and cerebellar blood flows (from 25 to 32 ml/100g/min and 33 to 48 ml/100g/min) again suggesting that basivertebral vascular control to chemical stimuli differs from that of the anterior circulation.

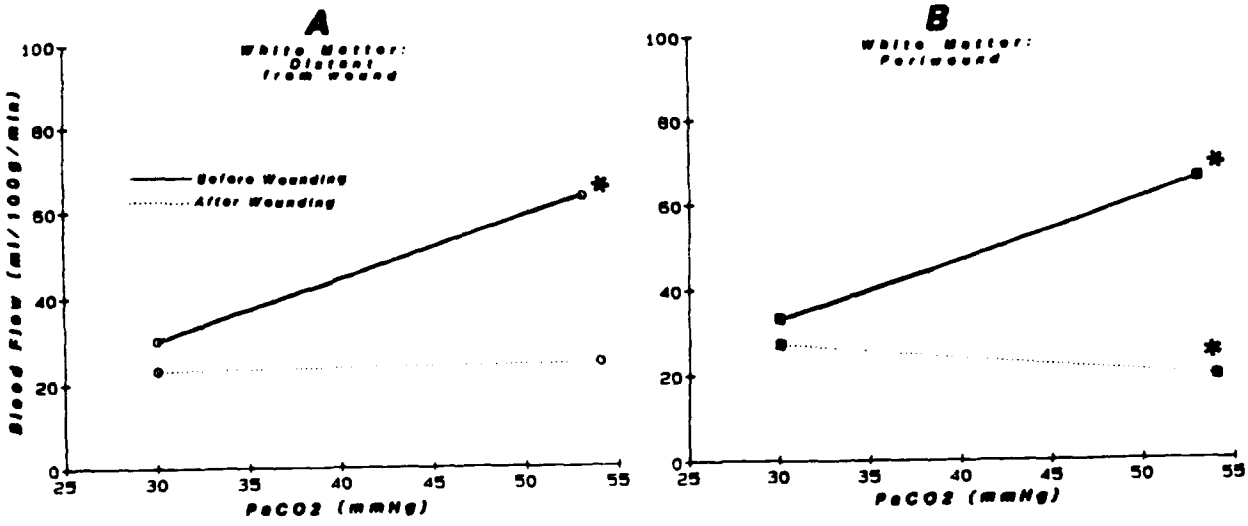
Owing to post wounding deaths only 5 of 9 wounded cats completed both pre and post wound hyperoxic challenges. In these animals total CBF decreased slightly but significantly from 19.5 to 17.0 ml/100g/min to a hyperoxic challenge after wounding. Since this response did not occur before wounding it may be considered abnormal. The brain stem and cerebellar flow increases, so prominent in the normal brain when hyperoxia was induced, were totally absent after wounding, Figures 15A to D.



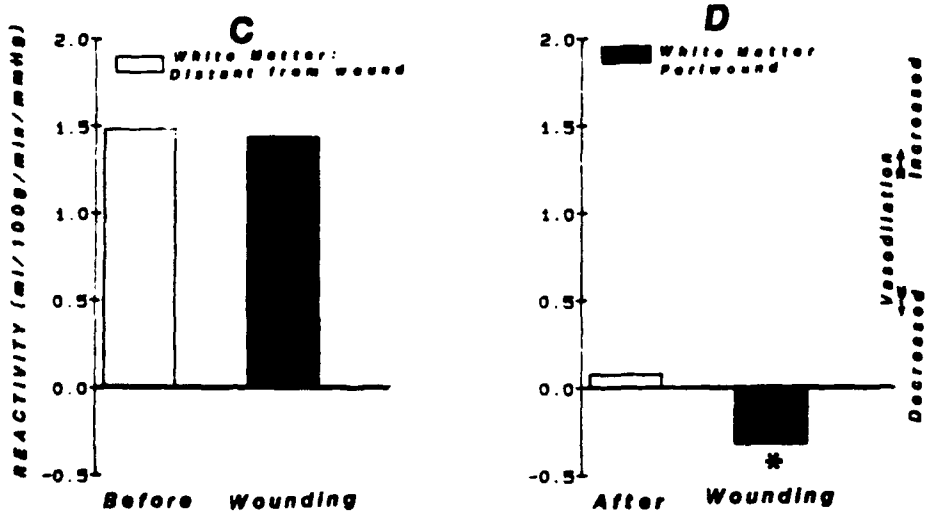
Figures 15 A to D: Before wounding 10 minutes of hyperoxia (PaO_2 > 280 mmHg) did not alter rCBF in white matter or cerebral cortex (A to C). The pre-wounding rCBF in cerebellum (D) was suggestively increased by hyperoxia. After wounding rCBF tended to decrease in periwound white matter and the suggestive rise in cerebellar blood flow was completely abolished.

e) Response to simultaneously increased arterial PO_2 and PCO_2

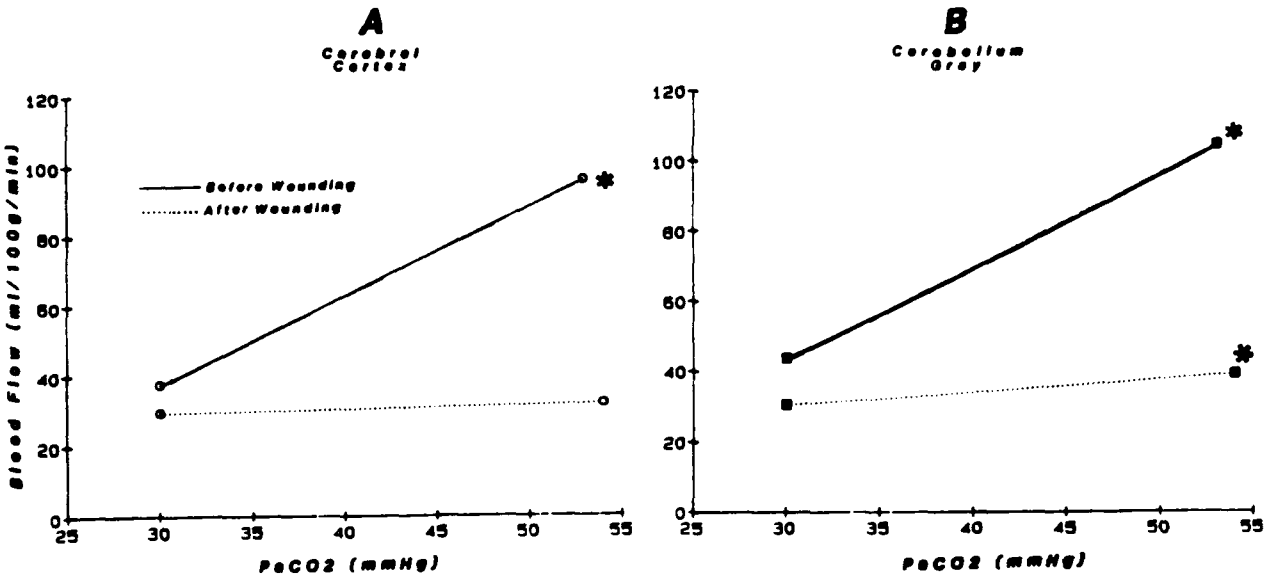
In applying either increased arterial PO_2 or PCO_2 to the normal brain two different effects were noted: hypercapnia increased total CBF while hyperoxia showed no effect. Following wounding, hyperoxia significantly reduced total CBF. Hypercapnia had no effect. These two chemical stimuli seemed to have somewhat opposite effects and what was particularly disturbing was that the vasoconstrictor effect of hyperoxia appeared to be enhanced following brain wounding (see d. above). This could have clinical ramifications because oxygen is often given to patients following brain injury and increasing PaO_2 could theoretically lead to vasoconstriction and CBF reductions in brain areas that might have marginal blood flows. We endeavored to see whether the post wounding enhanced vasoconstrictive effect of hyperoxia could be ameliorated by the simultaneous administration of CO_2. Accordingly, we measured CBF in normal brains and missile wounded brains when PaO_2 was increased to >400 mmHg and $PaCO_2$ was elevated to 54 mmHg. When the normal brain was exposed to these arterial gas tensions total CBF rose significantly indicating that the vasodilatory effect of the elevated $PaCO_2$ predominated. Whereas virtually all rCBFs in brain-wounded animals tended to show insignificant flow decreases when subject to hyperoxia this effect was prevented after wounding when hypercapnia was induced along with hyperoxia. In fact rCBFs all tended to increase slightly save the periwound tissue which still demonstrated a significant flow decrease from 27 to 19 ml/100g/min. Why all brain areas tended to show a flow increase after wounding when subject to hyperoxia plus hypercapnia but showed no post wound flow increase when the $PaCO_2$ alone was elevated is unknown. Cerebellar gray tissue exhibited a significant flow increase after wounding when exposed to hypercapnia and hyperoxia suggesting that in the cerebellum after wounding the effect of O_2 chemical control predominates over CO_2 chemical control. We infer this because after wounding increased $PaCO_2$ tended to decrease cerebellar CBF; while both before and after wounding increased PaO_2 tended to increase cerebellar CBF, Figures 16A to D; 17A to D.



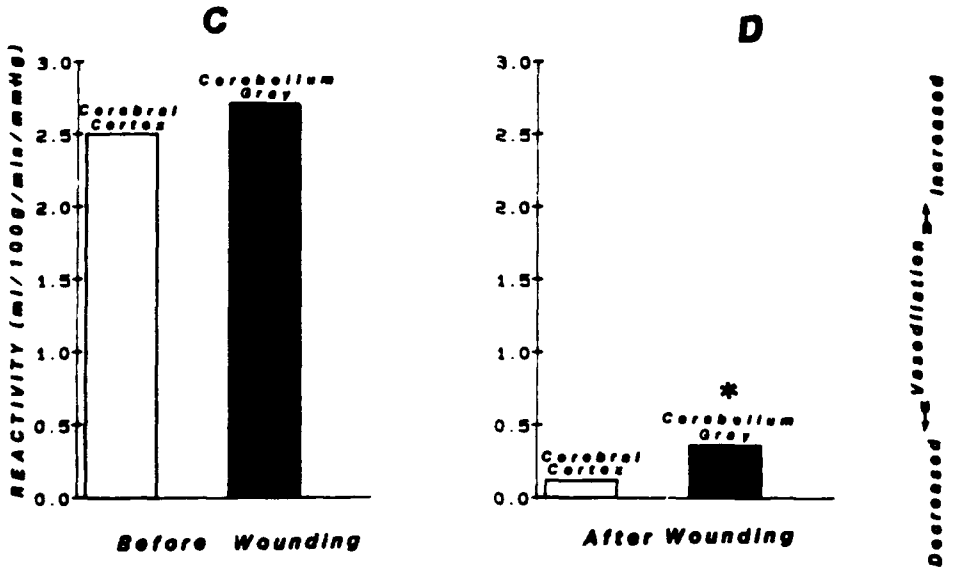
Figures 16 A and B: Before wounding, increasing $PaCO_2$ during hyperoxia significantly increased the rCBF in white matter distant from wound and periwound white matter. After wounding it did not increase rCBF in distant white matter (A) while it significantly decreased rCBF in periwound white matter (B).



Figures 16 C and D: After wounding the vascular reactivity to hyperoxia-hypercapnia was greatly attenuated in both brain areas, showing a significant vasoconstriction (D) in periwound white matter. This pattern resembles that observed with hypercapnia alone indicating that the $PaCO_2$ effect predominates.

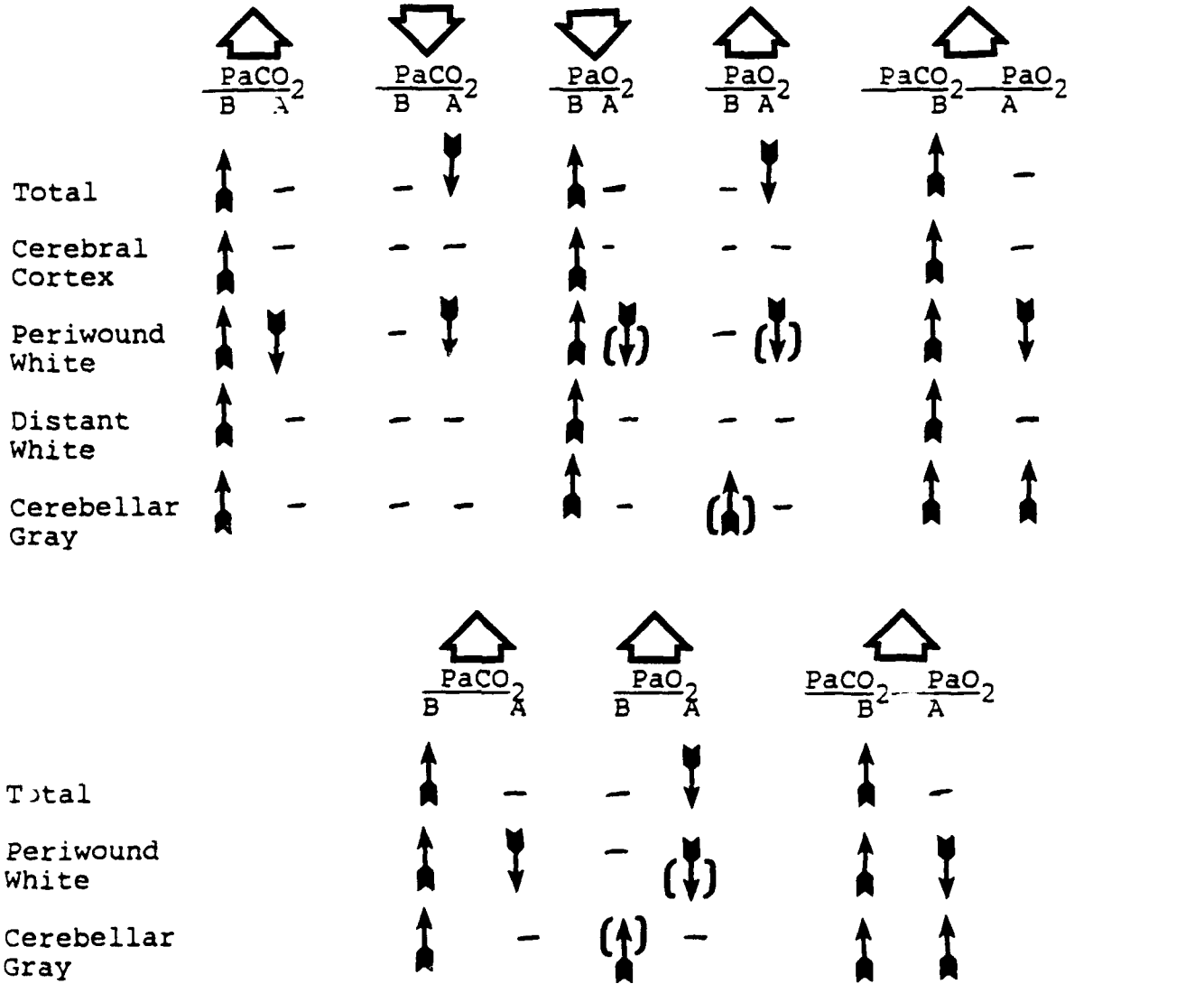


Figures 17 A and B: Before wounding increasing $PaCO_2$ and PaO_2 significantly increased rCBF in cerebral cortex and cerebellum gray. After wounding, hyperoxic hypercapnia increased cerebellar rCBF alone.



Figures 17 C and D: After wounding vascular reactivity to increased PaO_2 and $PaCO_2$ was greatly reduced in the cerebral cortex and cerebellum but despite the reduced vasodilation a post wounding flow increase occurred in the cerebellum (D). Note that post wounding reactivity remained positive with hyperoxia (indicating some vasodilation) but became negative with hypercapnia alone, (D) indicating mild vasoconstriction.

Table 1: A brief schematic summary of significant ($p<0.05$) rCBF changes to chemical stimuli before and after wounding



B = before wounding A = after wounding

( ) suggestive change ($p>0.05$)

— = no change = flow increase = flow decrease

Possible brain stem effects consequent to wounding which could alter the chemical regulation of CBF:

While local tissue factors, other mediators, or innate differences in neural control of vascular beds may be implicated in these observed CBF changes to chemical stimuli following brain injury, brain stem dysfunction after wounding may be another factor. Brain stem perturbation, manifested by cardiovascular and respiratory changes, is seen with brain wounding (7,23,24,25,47) The locus coerceus or other brain vasoregulatory centers lying just below the floor of the IV ventricle and implicated in chemical CBF regulation (129) could be impaired by missile energy transfer and contribute to the observed loss of chemical autoregulatory control following brain wounding.

f) Summary

These experiments reiterate what has been known for many years from closed head injury experiments: namely that after a brain missile wound mechanical and chemical CBF regulation mechanisms are disturbed. Consequently, the missile wounded brain is at great risk to the effect of hemorrhage and hypoxia. Importantly, for military purposes these experiments denote the kind of mechanical and chemical autoregulatory loss to be expected specifically after missile wounding. For instance 1) following a brain missile wound and hemorrhagic hypotension, marked CBF reductions may occur with only slight MABP reductions. Brain ischemia, once having occurred from blood loss and MABP reduction may not be reversed with blood reinfusion. **IT IS IMPERATIVE THAT BLOOD PRESSURE BE MAINTAINED IN THE BRAIN-WOUNDED INDIVIDUAL.** 2) The loss of chemical CBF regulation following brain wounding is not uniform but heterogeneous throughout the brain. Periwound tissue appears profoundly affected and tissue about the wound track is apt to show flow decreases to both hypercapnia and hypoxemia. 3) The basivertebral vascular system appears to respond to chemical CBF autoregulatory stimuli differently than the anterior circulation. 4) The effect of elevated $PaCO_2$ on CBF appears to predominate over that of an elevated PaO_2 both before and after wounding. **To provide maximal brain blood flow and oxygenation possibly some thought should be given to administering CO2 with oxygen to patients with brain injury. This would avoid any vasoconstrictive effects of oxygen alone.** Before any such clinical application of this proposed therapy, however, further laboratory experimentation is necessary.

4) Determining why cats died following brain wounding

Brain missile wounding may affect cardiac and respiratory functions (18,24,25,26,92,121) even though the missile wound does not involve the brain stem directly. A missile wound of the cerebral hemisphere may even cause immediate death from apnea (4,18,26,47,61). A brain missile wound may also cause additional intracranial phenomena as hematoma and brain swelling which may raise ICP. These associated conditions, if moderate, may merely aggravate mechanical damage to the brain caused by missile passage. If severe, associated ICP elevations and CBF reductions may cause widespread cerebral ischemia or failure of brain energy metabolism. This chain of events may further depress medullary function and cause additional cardiorespiratory abnormalities. If respiratory and cardiac circulatory functions are severely impaired either primarily as a consequence of missile energy deposit itself or secondarily because of associated intracranial effects, widespread "secondary" cerebral damage may occur which might greatly aggravate initial missile damage or lead to death.

In addition to stereotyped "brain stem" effects, brain wounding is associated with potentially life threatening systemic changes as transient hypertension in cats and monkeys and delayed hypotension which occurs in monkeys (18,23,24,47). Very large elevations in plasma catecholamines also occur after brain wounding. (J. S. Soblosky, see page). Decreased cardiac output (CO) has also been observed after experimental brain wounding and it has been suggested that a post wounding reduction in CO accounts for CBF failure and death.

From the above it is clear that death from apnea following a missile wound could have been from a multiplicity of causes: 1- from primary brain stem damage caused by missile energy transfer; 2- from secondary associated intracranial events such as increased ICP; 3- from systemic changes as CO decrease or 4- from a combination of several factors.

In order to determine just what factors did account for death we evaluated multiple physiologic variables in 15 spontaneously breathing, pentobarbital anesthetized cats before and up to 90 min after brain wounding in the right cerebral hemisphere by a 1.4J missile. Physiological variables evaluated were: total and rCBF and CO (microsphere technique); arterial blood, pH, PO_2 and PCO_2; MABP, ICP, CPP, EKG, heart rate (HR) and EEG. Respiratory frequency (f), tidal volume (V_t) and ventilation (V) were recorded during each flow measurement and periodically throughout the experiment. Four unwounded cats served as controls.

Unwounded cats showed no significant changes in any physiologic variables measured during a 100 min experimental period. Four brain wounded cats survived a 90 min post-wounding period and these had only a transient brain stem effect including a brief, 50% increase in MABP concurrently with a temporary, 50% reduction in respiratory frequency and heart rate. Non-survivors

(11/15) lived from 1 to 41 min after wounding and after wounding had persistently unstable blood pressures, rates of breathing and heart rates possibly indicating persisting brain stem damage, Figure 18.

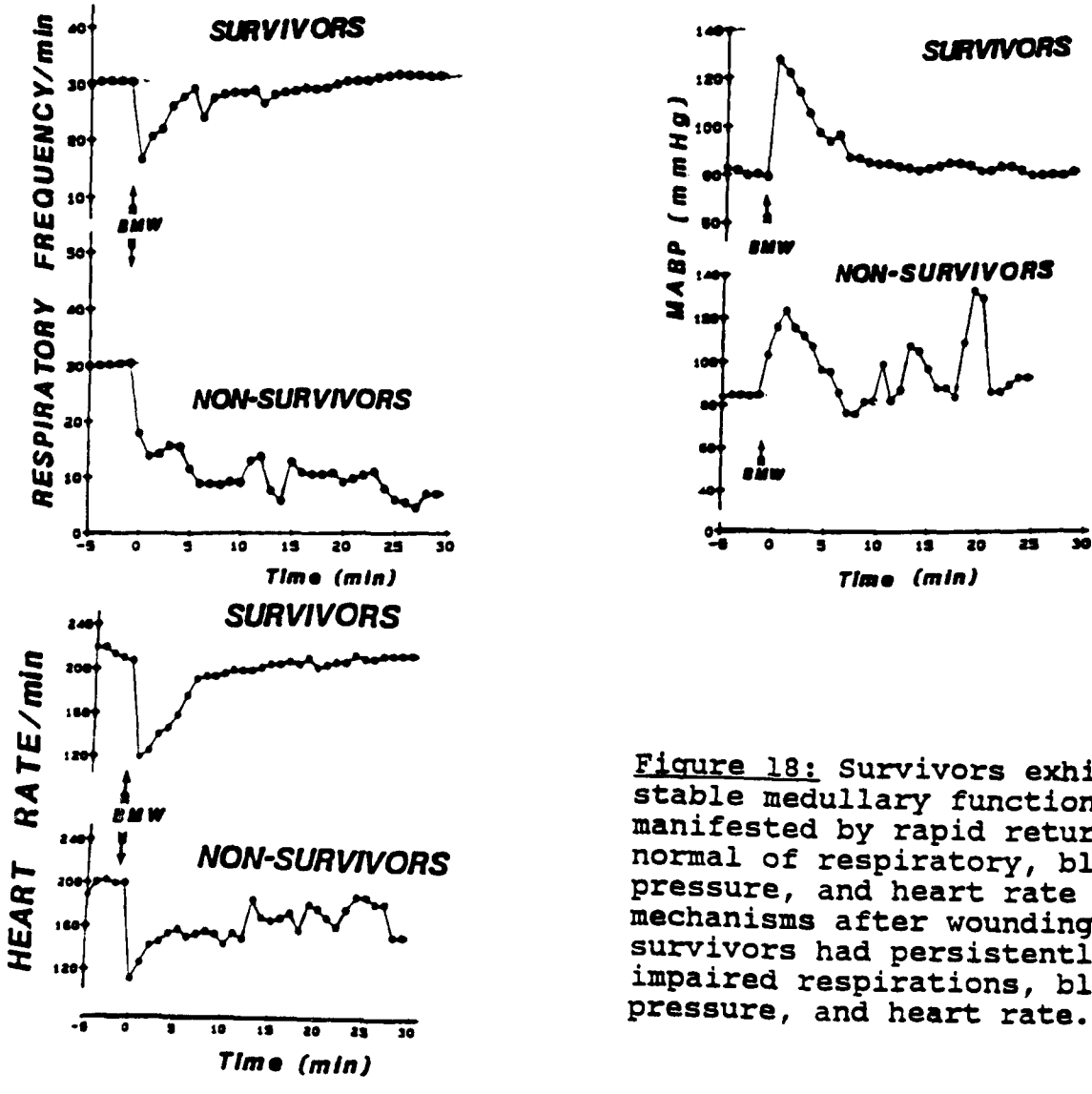


Figure 18: Survivors exhibited stable medullary function manifested by rapid return to normal of respiratory, blood pressure, and heart rate mechanisms after wounding. Non-survivors had persistently impaired respirations, blood pressure, and heart rate.

Causes of death following an experimental brain missile wound:

In this feline model of brain wounding the immediate cardiorespiratory, brain stem effects of missile injury appear stereotyped and similar to that noted in many other species sustaining brain injury, open or closed. No one or two physiologic factors consistently appeared to account for the appearance of sustained apnea after wounding. Rather, fatal apnea appeared to result from an interplay of many factors including: 1-

loss of CBF autoregulation, coupled with CO decrease, leading to cerebral ischemia (a- Table 2). 2- indirect damage to brain stem respiratory centers from missile energy (b- Table 2); 2- loss of CBF autoregulation, coupled with CO decrease, leading to cerebral ischemia (b- Table 2); 3- ICP increase or CPP decrease affecting respirations (c,d- Table 2); and 4- cardiac arrest, (e- Table 2).

Table 2: Changes in Physiological Variables and Causes of Death in 5 Non-surviving Cats After Brain Missile Wounding

VARIABLES	CAT NUMBER AND TIME OF LAST MEASUREMENT (min)					MEAN±SE
	1(5)	2(20)	3(5)	4(5)	5(20)	
PRESSURES (mmHg):						
MABP	141	85	120	71	120	107±10
ICP	27	10	90	84	61	54±13
CPP	114	75	30	-13	59	53±17
BLOOD FLOW (ml/100g/min):						
TOTAL CBF	11	23	57	31	55	35±7
BRAIN STEM	8	23	44	23	32	26±5
CO (ml/min/kg)	67	91	53	144	149	101±16
RESPIRATORY PARAMETERS:						
f/min	2	14	43	16	5	16±6
V (lit/min)	0.16	0.46	0.89	0.14	0.54	0.44±0.1
P_aO_2 (mmHg)	40	-	47	56	72	54±5
P_aCO_2 (mmHg)	58	-	71	52	52	58±3
pH	7.17	-	7.05	7.14	7.20	7.14±0.02
NUMBER OF TRANSIENT APNEA	4	5	5	6	1	4.2±0.7
CAUSE AND TIME OF DEATH (min)	APNEA (8)	APNEA (25)	APNEA (8)	APNEA (10)	CARDIAC ARREST (26)	-
	a	b	c	d	e	

Basically, the cats which survived brain wounding appeared to maintain all aspects of physiologic function (HR, MABP, f, CBF, CO, V) while animals which died tended to exhibit multiple cardiorespiratory and other physiologic abnormalities. Factors accounting for the differences between these groups should be sought because knowledge and manipulation of these critical pathophysiologic events may decrease the mortality of brain wounds.

5) BRAIN BIOGENIC AMINES

Our brain injury experiments including wounding and increasing intracranial pressure (ICP) were associated with brain stem effects consisting of systemic arterial hypertension plus changes in heart rate and respirations. Alterations in blood pressure have been correlated with changes in biogenic amines (norepinephrine, NE; epinephrine, EPI; dopamine, DA; serotonin, 5-HT) in the nucleus tractus solitarius (12,76) rostral and caudal ventrolateral medulla (14,71,127) locus coeruleus (13), hypothalamus (117,118,144) and dorsal raphe nucleus (35.43). A few studies have determined that brain trauma alters biogenic amines in gross brain areas but none have studied post-traumatic changes in biogenic amines in specific hypothalamic and brain stem nuclei which relate to observed post-traumatic brain stem effects (36,64,75).

We examined hypothalamic and brain stem biogenic amines by high pressure liquid chromatography following a 2.4 J missile wound to the right cerebral hemisphere in our pentobarbital anesthetized cats. Wounds of this energy produce profound brain stem effects and cause death 70% of the time from apnea (18). We wished to know whether such a high energy wound induced extremely disorganized changes in brain stem and hypothalamic biogenic amines which could account for the fatal effects of such a high energy missile.

Because brain missile wounding is also associated with increased ICP we also measured hypothalamic and brain stem biogenic amine changes after artificially increasing ICP (120-140 mm Hg) by intracisternal infusion of mock CSF to see whether the pattern of biogenic amine changes associated with missile wounding was intrinsically different than biogenic amine changes associated with increased ICP alone.

Both brain wounded cats and those with mock CSF infusions to increase ICP had significant decreases (47-74%) in epinephrine (EPI) levels in the posterior hypothalamus, nucleus tractus solitarius, area A1C1, locus coeruleus and raphe nuclei. Brain wounded cats also had significant EPI reduction (31%) in the anterior hypothalamus as well. Cats whose ICP were raised by mock CSF infusion also had reduced EPI in the anterior hypothalamus but these reductions did not reach significance (Fig. 19).

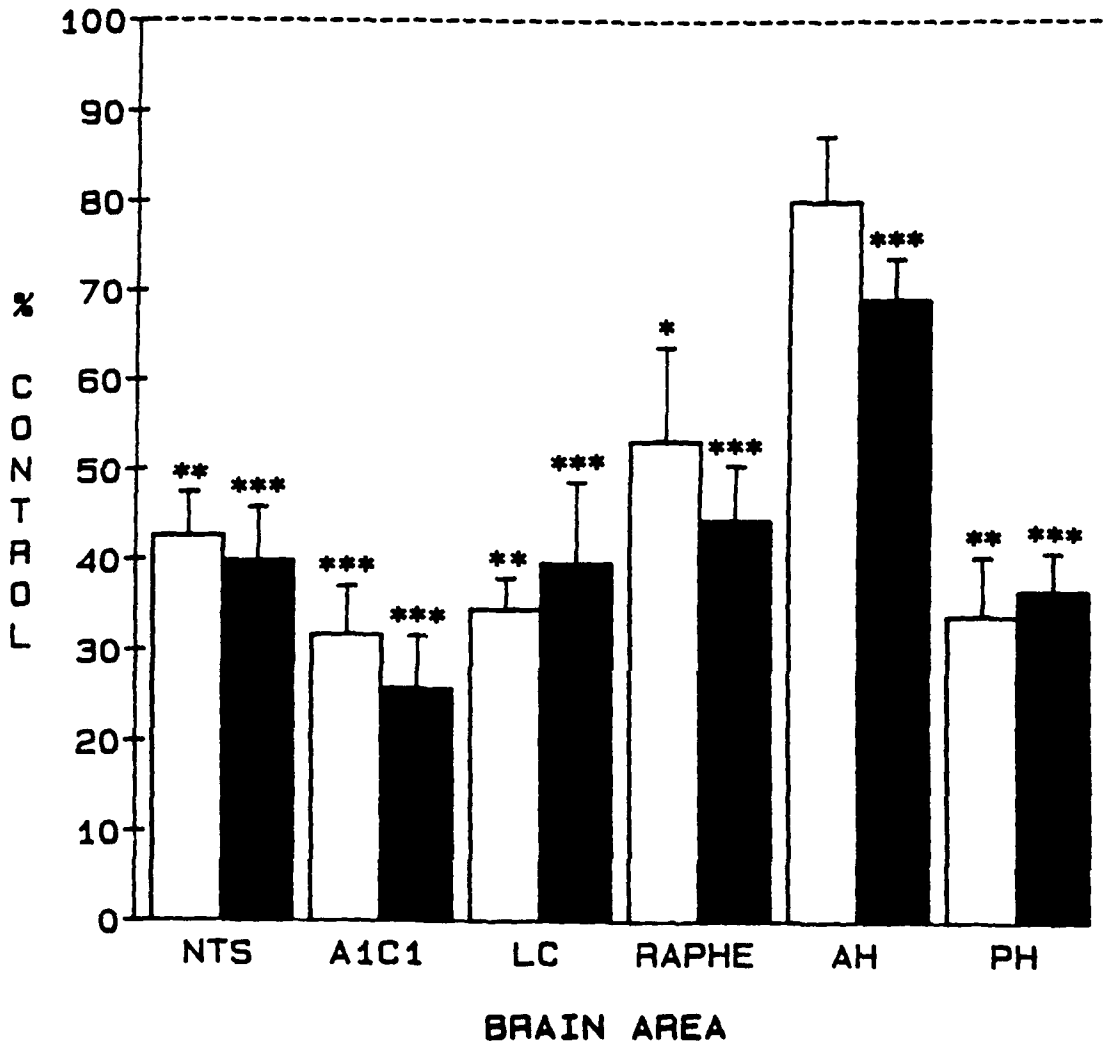


Fig. 19. The effect of artificially-induced increase in ICP (open bars) and brain missile wounding (solid bars) on epinephrine levels in the nucleus tractus solitarius (NTS), area A1C1, locus coeruleus (LC), raphe nuclei, anterior hypothalamus (AH) and posterior hypothalamus (PH) 6 mins. after wounding and/or elicitation of the pressor response. Values (Mean ± SEM) are expressed as percentage of control data. Artificially-induced increases in ICP n=6; brain wounding n=8. * $p<.05$, ** $p<.01$, *** $p<.001$, ANOVA.

Brain wounding with attendant, brief intracranial pressure differences between supra- and infratentorial compartments might be expected to cause brain stem displacement, while mock CSF infusions into the cisterna magna would not. In the latter case the induced ICP rise would have occurred relatively much more slowly and the resultant pressure increase would have been distributed evenly within the cranium. Since hypothalamic and brain stem EPI changes occurred with increased ICP alone (without brain stem displacement) it would appear that the ICP increase associated with brain wounding was the adequate stimulus to cause the post-wounding biogenic amine changes. Possibly, brain stem displacement caused by wounding might have enhanced these changes but brain stem distortion would not appear to be a primary factor.

Norepinephrine (NE) was decreased in the posterior hypothalamus in response to wounding and ICP increase, but was unaffected by either stimulus in the anterior hypothalamus, locus coeruleus or raphe nuclei. The fact that both EPI and NE decreased in the posterior hypothalamus may be of particular significance because the posterior hypothalamus supposedly mediates the vasopressor response and application of EPI or NE to the posterior hypothalamus will increase MABP (70,117,119,126,148). We cannot be certain if the depletions found in our experiments were caused by increased EPI or NE utilization or decreased neuronal functioning. Likewise we cannot definitively say whether EPI and NE changes represent causative, vasopressor changes or represent attempts at vasopressor compensation.

The levels of NE and DA and its metabolites (DOPAC and HVA) did not show consistent changes paralleling EPI suggesting a dissociation between the EPI and the NE and DA systems. Brain wounding significantly reduced NE, DA and HVA in the nucleus tractus solitarius and area A1C1. Increasing ICP alone decreased NE, DA and HVA in these two areas but insignificantly. Again, it would appear that mechanisms involved with wounding augmented these biogenic amine changes. Since DA is a precursor to NE in noradrenergic neurons and because the levels of DA in the nucleus tractus solitarius and area A1C1 are small, the observed DA decreases might have basically resulted from NE reductions.

Serotonin (5-HT) and its metabolite 5-HIAA as well as DA and HVA were significantly reduced (22% to 37%) in the raphe nuclei in response to either brain wounding or increased ICP alone but these results are difficult to interpret relative to cardiovascular changes because discrete raphe nuclei were not sampled.

The most basic effect of an increase in ICP is a massive increase in sympathetic nerve activity causing a sympathetic pressor response (16,44,77,100,116,140). We have demonstrated that ICP elevations and the pressor response are also associated with biogenic amine changes (particularly EPI and NE) in the hypothalamus and brain stem.

Generalized activation of the sympathetic nervous system is also the main component of the classic stress response also associated with an MABP increase. Both foot shock and immobilization stress have shown EPI and/or NE decreases in the hypothalamus, nucleus tractus solitarius, area A1C1 and the locus coeruleus (48,85,135-137,142). Thus, from the similarity of patterns of biogenic amine responses in the hypothalamus and brain stem from either stress or increased ICP we hypothesize that the response to increased ICP and brain wounding is a variant of a generalized stress response. Though the peripheral manifestations of an ICP increase (brady- instead of tachycardia and respiratory slowing instead of tachypnea) differ somewhat from somatic stress owing to direct brain stem stimulation or distortion, the hypothalamic and brain stem biogenic amine changes are remarkably similar as though the hypothalamus and brain stem are "hard wired" to react to all stress, whether it be from an increase in ICP or other source, in a specific, stereotypic fashion.

Despite the severe brain injury and/or large increases in ICP in our experiments, all hypothalamic and biogenic amines were not totally depleted. In so far as the monoaminergic system was concerned the pattern of a generalized stress response was preserved indicating the basic integrity of this system remained intact. Only selective, organized alterations occurred in the EPI system overall and the 5-HT system in the raphe nuclei. This suggests that a severe brain wound likely to cause early death from apnea does not do so by totally disrupting the hypothalamic and brain stem biogenic amine system. If this system remains basically intact following severe brain injury, discrete alterations in the biogenic amine system which in the future are shown to be physiologically important may be susceptible to pharmacologic therapy.

6) PLASMA CATECHOLAMINES

Plasma catecholamines (CAs) have been shown to be elevated by brain trauma including experimental fluid percussion (134). The plasma CAs are of interest because high circulating levels of plasma CAs may have deleterious effects on the cardiovascular and pulmonary systems, basal metabolic rate and neuronal function in the CNS (21). Circulating CAs do not normally cross the blood-brain barrier (BBB) and enter the brain but a missile wound to the brain does break down the BBB and plasma CAs may enter the brain. Such abnormal entry could affect local microcirculation and directly affect brain neuronal function (98). Therefore, delineating the effect of missile wounds to the brain on plasma CAs may be of importance because this systemic effect of brain wounding may, in turn, affect brain function particularly in the area where the BBB is damaged.

An increase in intracranial pressure (ICP) alone, without brain injury, has been shown to increase the levels of plasma CAs (50,133). Since a missile wound to the brain may cause dramatic elevations in ICP, adjunct experiments were performed to determine the contributions, if any, of increased intracranial pressure (ICP) on the plasma CAs and the time course of the CA response. Additionally, in order to determine if the physiological and biochemical responses to brain wounding may be a function of trajectory angle, in a few experiments our standard anterior to posterior (AP) trajectory was changed to a transverse trajectory.

We ascertained:

A. A missile wound to the brain caused IMMEDIATE elevations in plasma CAs, even though the ICP increase may not have been enough to evoke a significant rise in MABP (Figs. 20 & 21). The time course of the plasma CA elevations suggest that this effect may not be the result of merely a large increase in ICP. In our model a 0.9 J brain wound which increases ICP by only 10-15 mm Hg caused an immediate MABP rise and a large CA elevation. By contrast, ICP had to be raised in excess of 80 mm Hg by mock CSF infusion to elicit a systemic pressor response and plasma CA increase.

B. An increase in ICP alone, without injury, caused only delayed elevations in plasma CAs if the ICP were increased enough to elicit a rise in MABP (Figs. 22 & 23). Even when an immediate increase in MABP occurred the plasma CA elevations were still delayed. It thus appears that the initial MABP rise does not depend upon plasma CAs.

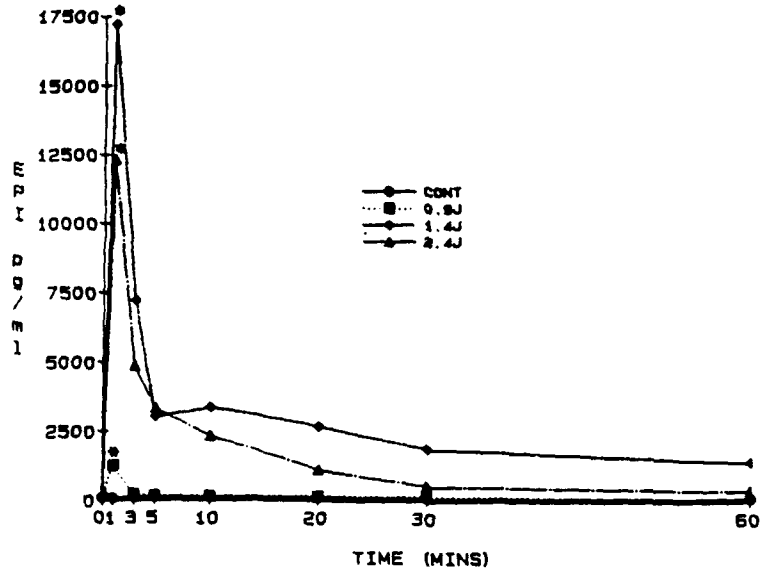


Fig. 20. Effect of brain missile wounding on plasma epinephrine levels for 60 mins. after wounding at 0.9 J (square), 1.4 J (diamond), or 2.4 J (triangle). Controls (circles) were surgically prepared but uninjured. Values are Mean ± SEM, n=3. * p<.05, paired t-test.

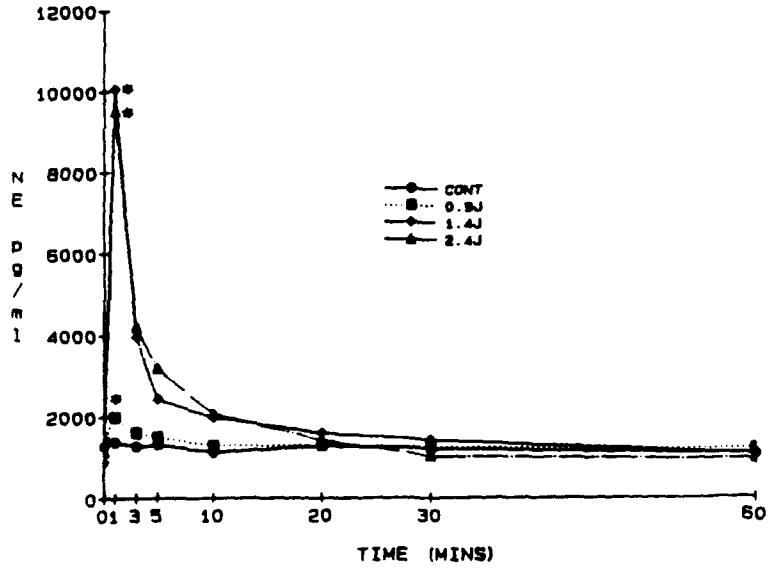


Fig. 21. Effect of brain missile wounding on plasma norepinephrine levels for 60 mins. after wounding at 0.9 J (square), 1.4 J (diamond), or 2.4 J (triangle). Controls (circles) were surgically prepared but uninjured. Values are Mean ± SEM, n=3. * p<.05, paired t-test.

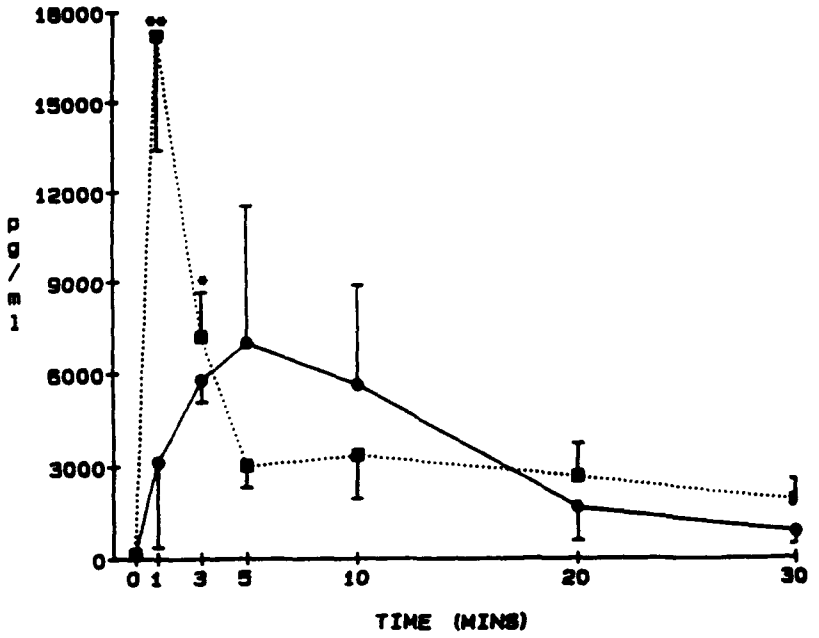


Fig. 22. Effect of brain missile wounding at 1.4 J (squares) and artificially-induced increases in ICP (circles) on plasma epinephrine levels for 30 mins. after brain wounding and/or elicitation of the pressor response. Values are Mean ± SEM, n=3, * p<.05, ** p<.01, paired t-test.

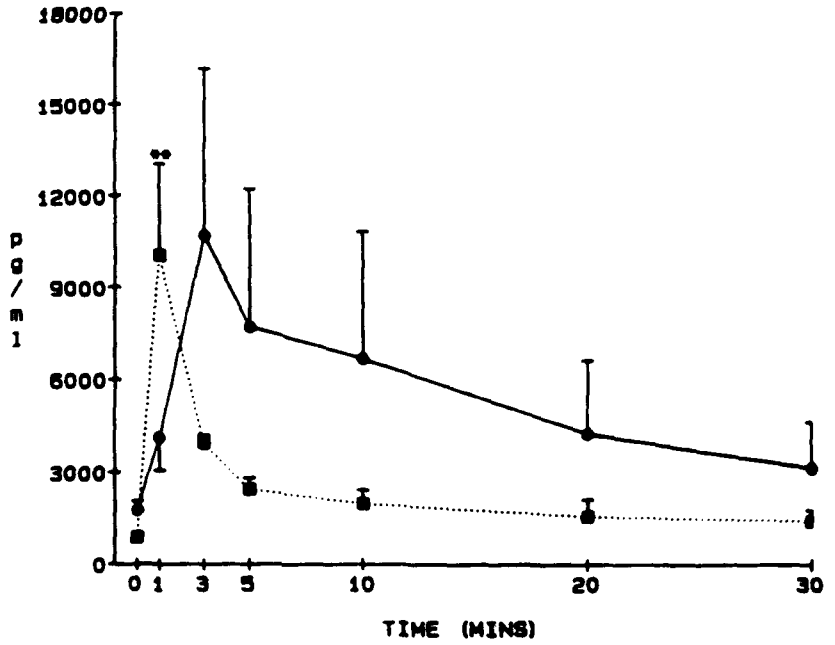


Fig. 23. Effect of brain missile wounding at 1.4 J (squares) and artificially-induced increases in ICP (circles) on plasma norepinephrine levels for 30 mins. after brain wounding and/or elicitation of the pressor response. Values are Mean ± SEM, n=3, ** p<.01, paired t-test.

C. Four animals sustained a transverse wound and two exhibited immediate rises in plasma CAs after wounding. Those which showed immediate CA rises also had large increases in ICP (152 and 153 mm Hg) immediately after wounding and corresponding immediate increases in MABP. The two cats which did not have plasma CA increases had immediate post-wounding ICPs of 38 and 73 mm Hg and they did not have a large MABP rise.

We infer that the transverse trajectory appeared to have a different effect on MABP and plasma CA responses than did the AP trajectory because only two of four transversely injured animals responded with elevations in MABP and plasma CAs. By contrast nine of nine AP injured animals responded with immediate plasma CA increases even if they were wounded with as little as 0.9J and ICP rose only to 18 mm Hg! This may mean that the peripheral CAs, local brain distortion or the direction of the forces applied to the brain stem are more important than ICP increase. Furthermore it may take more force from a side trajectory to effect the same brain stem sympathetic response irrespective of any ICP increase.

The origin of the immediate plasma CA elevations may be the result of pressure forces acting on the brain stem (e.g. displacement) and activating medullary sensitive areas (34,60,150) as mildly suggested from the results using a transverse trajectory. It is also possible that brain areas involved in the sympathoadrenal response also partake in the response, but if they do, it probably is not caused by merely increasing ICP.

It is also important to note that ballistic literature indicates that with missile transit there is an extremely rapid (msec) large pressure wave that occurs intracranially (58). We are unable to reliably quantify either its duration or magnitude in our model but we know that it occurs. How this extremely rapid pressure fluctuation affects either brain or plasma CAs, if at all, is presently unknown and must await further experimentation.

7) EFFECT OF GM-1 GANGLIOSIDE ON RECOVERY OF FUNCTION AFTER A MISSILE WOUND TO THE BRAIN

One of the purposes of the brain wound model which we developed was to create a standard neurologic injury in order to evaluate whether specific drugs would improve the speed and extent of neurologic recovery. Much work has been and is being done using rat models (1 gm. brain) to test recovery but we felt and feel that it would be very useful to use a more complex cat brain (20 gm.) and to evaluate how drugs affected recovery specifically from missile wound injury.

Dr. Soblosky developed a very sophisticated cat neurological/behavioral recovery paradigm. The first drug he evaluated was GM-1 ganglioside.

Gangliosides are sialic acid-containing glycosphingolipids found in high concentrations in the CNS, especially in synaptic membranes. GM-1 ganglioside treatment has been demonstrated to significantly enhance behavioral and neurochemical recovery from discrete neurotoxic and mechanical lesions (1,2,38,53,69,99,138,139,151-153). Moreover, the GM-1 gangliosides can be given exogenously and do not possess any known toxicological effects (59). Therefore, the encouraging data from the aforementioned discrete lesions experiments, the ease in which treatment can be started and continued, and the lack of toxicological effects all made GM-1 gangliosides an ideal drug to initially test in our brain wounding model. The present study was undertaken in order to determine if GM-1 gangliosides treatment would affect behavioral recovery specifically from a missile wound of the brain.

A population of cats (both sexes) were matched according to weight into pairs to preclude weight as a factor in the behavioral motor tests, especially beam balance performance. Each pair of cats was injured (0.9 J) then randomly assigned to either the control group or drug treatment group. Control cats received saline (I.P) and drug-treated cats received GM-1 ganglioside (20 mg/kg, I.P.), beginning approximately 10 mins. after injury, then daily for the next 10 days. When an injection day coincided with a test day, the injection was given one hour or more prior to testing. The cats were tested by a "blind" rater, i.e. the rater did not know to which of two groups the cats were assigned to. Testing began third day post-injury, then every third day thereafter for 30 days; then weekly for 5 weeks (65 days post-injury). The cats were scored according to the criteria previously described in detail in our Annual report dated April 27, 1989.

Because of inadvertent pregnancies, three cats had to be eliminated from the GM-1 ganglioside treatment group along with one atypically affected cat. This resulted in an n=7 for the control group and only an n=3 for the GM-1 ganglioside treated group. Therefore, the results are not conclusive because the

total number of subjects (n) for each group is too small to apply the appropriate statistical evaluation at this time. The data, however, are encouraging. Beam balance performance appeared to be enhanced in the GM-1 ganglioside-treated cats (Fig. 24) and the GM-1-treated cats ultimately appeared to have a better recovery with non-visual placing (days 37-65) (Fig. 25). Visual placing performance appeared to be improved due to GM-1 ganglioside treatment (days 3-12) (Fig. 26).

The accelerated recovery in beam balance performance of GM-1-treated cats may be related to their initially better visual placing performance or to their ultimately better non-visual placing performance, or both.

Because further testing was precluded by U.S. Representative Bob Livingston's stopping our project we could not continue to the point of achieving statistical significance. Nevertheless, the results are very encouraging and suggest that GM-1 gangliosides may speed up and even enhance recovery of neural functioning following a missile wound to the brain. Further testing is definitely warranted.

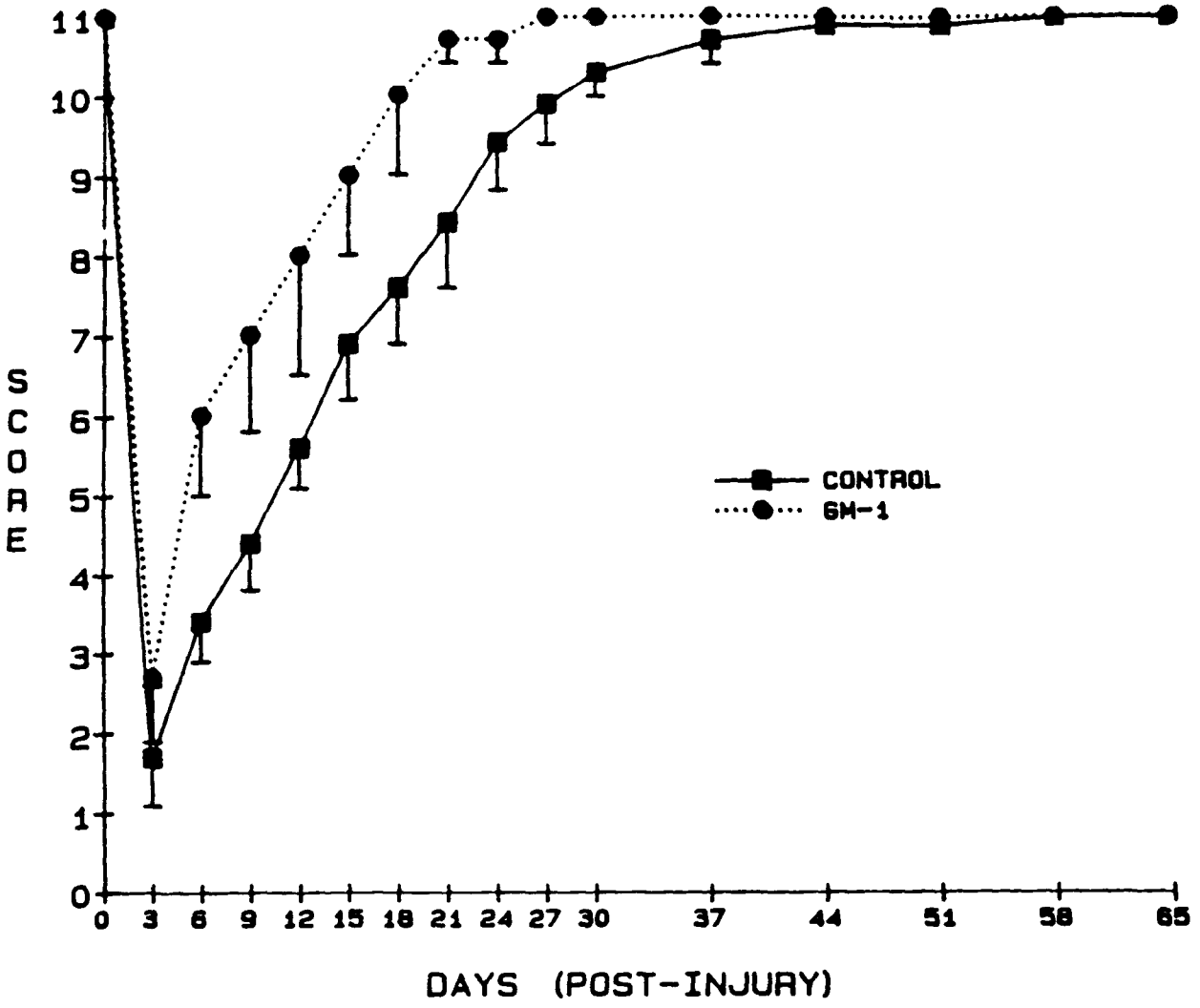


Fig. 24. Effect of GM-1 ganglioside treatment (20 mg/kg I.P., 10 days) on the Beam Balance performance of cats injured at 0.9 J (circles). Control animals were similarly injured but received saline. Values are Mean ± SEM, GM-1 n=3, control n=7.

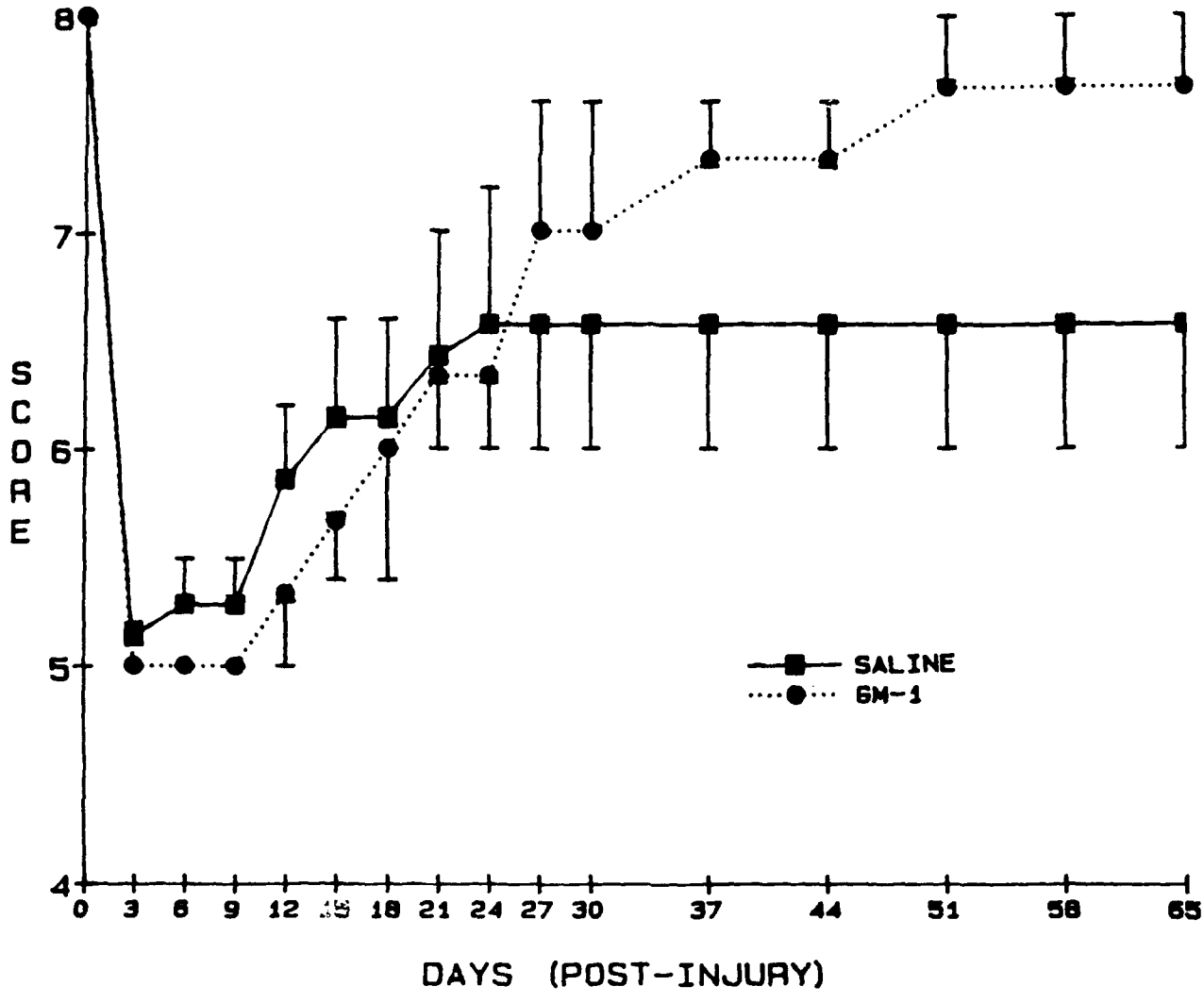


Fig. 25. Effect of GM-1 ganglioside treatment (20 mg/kg I.P., 10 days) on Non-Visual Placing performance of cats injured at 0.9 J (circles). Control animals were similarly injured but received saline. Values are Mean ± SEM, GM-1 n=3, control n=7.

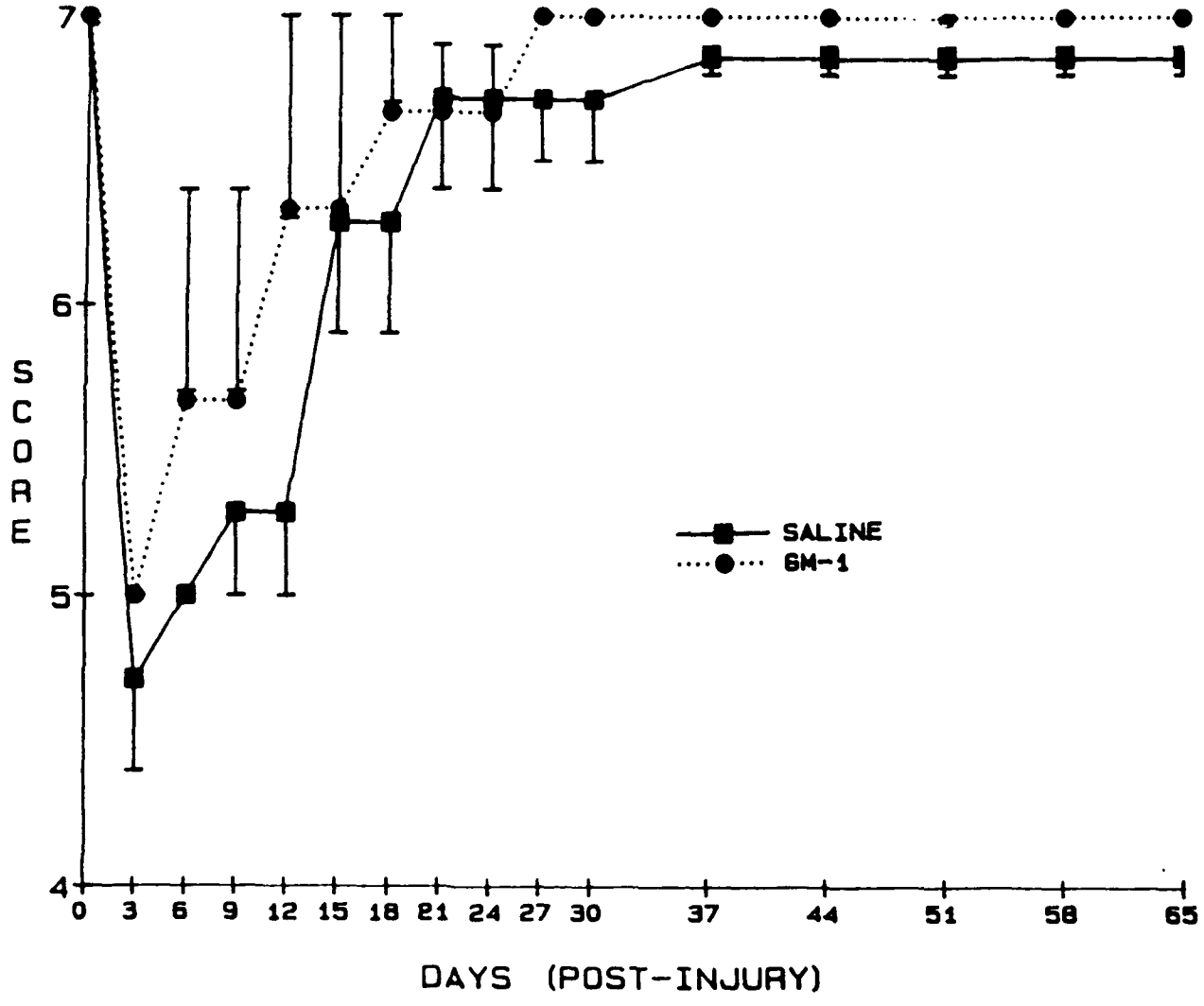


Fig. 26. Effect of GM-1 ganglioside treatment (20 mg/kg I.P., 10 days) on Visual Placing performance of cats injured at 0.9 J (circles). Control animals were similarly injured but received saline. Values are Mean ± SEM, GM-1 n=3, control n=7.

8) Generation of Free Radicals After Traumatic Brain Injury

In recent years it has been hypothesized that secondary neuronal and cerebrovascular damage following traumatic brain injury may be caused by an increase in oxidative reactions initiated by free radical formation (56,65,115,159). Free radicals after brain trauma may be generated by: 1) the arachidonic acid cascade (37,79,83,159); 2) increased leakage of superoxide from mitochondrial electron transport (55,65,125) (Superoxide is the primordial species leading to oxidative damage of membrane phospholipids, proteins and nucleic acids); 3) enhanced activities of xanthine oxidase (65,74,102); 4) autooxidation of catecholamines; and 5) activation of neutrophils (65,149). These oxidative reactions are presumably mediated by hydrogen peroxide and the hydroxyl radical (generated by the Fenton reaction) (125). Lipid peroxidation initiated by the active oxygen species may be a major mechanism for damage in the lipid- rich tissues of the CNS (29,30,55). Antioxidants, such as ascorbate and alpha-tocopherol as well as enzymes, such as superoxide dismutase, can serve to protect the CNS from damage caused by free radical reactions (6,65,74,97,124)

We felt it very important to begin to examine the occurrence of free radicals in the brain after missile wounding. We were particularly interested in the time course of free radical generation because if free radicals were massively generated within seconds of brain wounding antioxidant therapy might be of no avail. Conversely, if free radicals were formed many minutes or hours later, interceding with antioxidants might be expected to help ameliorate neural damage caused by free radicals.

Free radical reactions have been analyzed by several indirect methods. Evidence of lipid peroxidation reflecting oxidative stress in injured tissue can be obtained by measuring breakdown products of peroxidated poly-unsaturated fatty acids: the thiobarbituric acid reactions (7,28) or by increased production of malondialdehyde (65,162). Consumption of major antioxidants, alpha-tocopherol or ascorbate, also provides indirect evidence of free radical reaction (8,9,109,120,147). The superoxide radical reduces nitroblue tetrazolium (NBT) ion to its insoluble blue form and this provides a simple, direct method of detecting superoxide radicals (80).

We elected to measure free radical formation after brain injury as directly as possible and collaborated with Drs. William Pryor and Daniel Church from the Biodynamic Institute at LSU in Baton Rouge. These scientists are internationally known for their work in electron spin reasonance (ESR) spectroscopy and spin trapping which can directly measure free radicals. This technique is complex, and before ascertaining the occurrence of free radicals in missile-wounded cats we elected to work out the technique in rats sustaining brain damage by means of a 95 gm weight dropped 60 cm onto the right skull convexity. All rats were anesthetized by ether and their scalps remained intact.

Spin trapping with ESR measures free radical species in picomole concentrations. Superoxide, hydroxyl and lipid radicals are so short lived, however, and are present at such low steady-state concentrations that for ESR detection they must be joined to or "trapped" by a non-radical organic compound (spin trap) which forms a relatively more stable and measurable radical called the "spin adduct" (free radical + spin trap) (86). In these studies we used lipid soluble alpha-phenyl-N-t-butyl nitrone (PBN) as the spin trap. The peak heights of the free radical species detected by ESR (measured in arbitrary units) is taken as a measure of the amount of free radical being produced.

In the experiments described below we used ether - anesthetized male Sprague-Dawley rats (250-300 g) which were infused i.v. before brain injury with 1 ml of either 0.1M PBN or saline. Twenty-nine rats were then placed under a weight drop device for brain injury while 14 served as uninjured controls. We used 5 experimental groups:

I- 7 PBN-infused control rats; decapitated 60' after infusion
II- 11 PBN-infused injured rats; decapitated 60' after infusion
III- 7 saline-infused control rats; decapitated 60' after infusion
IV- 6 saline-infused injured rats; decapitated 60' after infusion
V- 12 PBN-infused injured rats; decapitated 5'after injury

After completion of the experiment and decapitation, brains were removed within one minute, processed appropriately, and analyzed by ESR to determine the presence of any free radicals being formed.

We also measured tissue ascorbate concentrations by the ascorbate oxidase assay to provide an indirect measure of free radical formation in our injury paradigm.

No rats in groups 1 to IV sacrificed ~60 minutes after injury showed evidence of any spin adducts for peroxy radicals. Ascorbyl free radical signals were, however, observed in all rats sacrificed at 60 minutes whether injured or not, Figure 27. Four of 12 injured rats sacrificed 5 minutes after brain injury (Group V) showed a carbon centered PBN-spin adduct providing direct evidence of tissue lipid peroxidation at this early time point, Figure 28. Brain ascorbate concentrations were significantly lower in brain injured rats than in uninjured controls, Table 3.

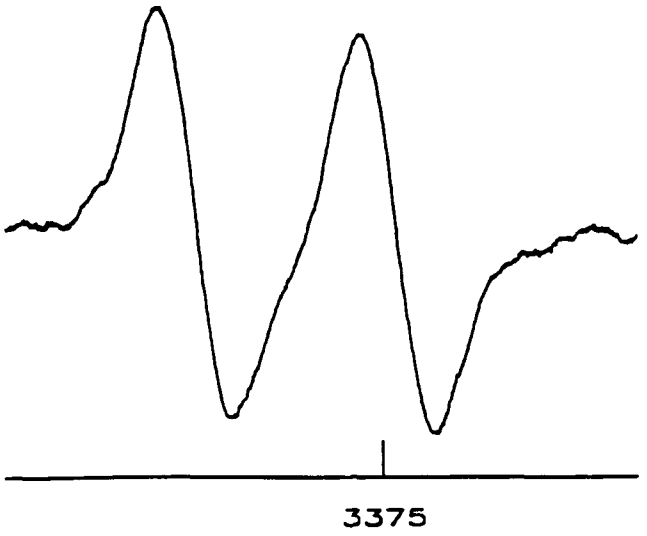


Figure 27: An ascorbyl signal radical observed in the brain (Folsch extract) from a saline-infused rat. The same signal with various degree of intensity appeared in saline and PBN-infused rats from both uninjured and injured groups. Instrument settings: Gain (GN) = 1E6; modulation amplitude (MA) = 1.0; Microwave power (MW) = 20; microwave frequency (FR) = 9.4; Centerfield (CF) = 3375; Sweep width (SW) = 10; Sweep time (TI) = 200; Time constant (TC) = 5000.

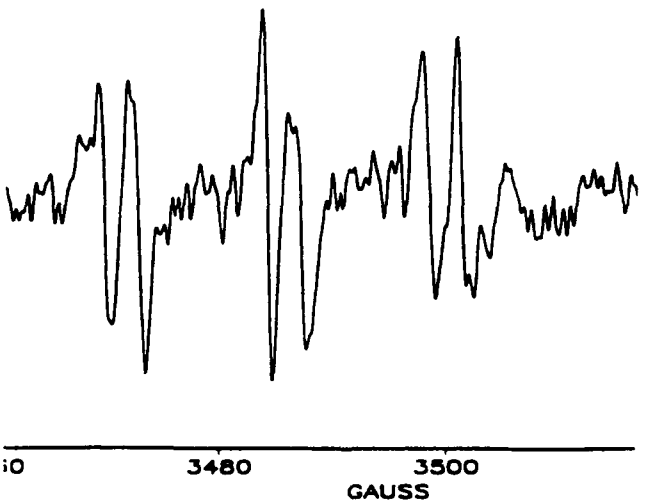


Figure 28: A typical PBN spin-adduct observed in the ESR spectra of a lipid extract prepared from a rat 5 min after TBI. Instrument settings: Gain (GN) = 2.5E6; Modulation amplitude (MA) = 10E-1; Microwave power (MW) = 20, Centerfield (CF) = 3495; Sweep width (SW) = 100; Sweep time (TI) = 1000; Time constant (TC) = 1000.

Table 3: Brain ascorbate concentration (AA), the ESR peak height (PH) of the ascorbyl signal and the ratio of the peak height and the brain ascorbate concentration (PH/AA) in control and brain injured rats

Values are mean ± SE

GROUP	BRAIN ASCORBATE CONCENTRATION (AA) (nM/g)	PEAK HEIGHT OF ASCORBYL SIGNAL (PH)	OXIDATIVE STRESS INDEX PH/AA
Uninjured Saline & PBN Combined (n=9)	860 ± 41	263 ± 44	0.300 ± 0.04
Injured Saline & PBN (n=6)	655 ± 75*	317 ± 21	0.506 ± 0.05*

*Significant, $p<0.05$; uninjured rats were compared with injured rats using an unpaired t-test

The brain has a large pool of ascorbic acid approximately 99% of which is in the reduced form and thus able to act as an antioxidant.(41,46) In these experiments ESR detected ONLY ascorbyl radicals at 60 minutes in both control and brain injured rats. We infer from this that some oxidative stress occurred in all brain tissues whether injured or not during animal sacrifice and tissue extraction. The brain ascorbate concentration, however, in injured rats was significantly less than that seen in controls at at the same time. This difference suggests oxidative stress. In another group (V) 4 out of 12 rats sacrificed 5 mins after trauma showed an ESR signal indicating the presence of a carbon-centered radical. This suggests that, following brain injury, oxidative stress from free radical formation occurred quite early.

Drs. Church and Pryor hypothesize that after traumatic brain injury tissue ascorbate is consumed and oxidized into an ascorbyl radical as it acts as an antioxidant. The peak height (PH) of the ascorbyl signal measured by ESR indicates the amount of ascorbate being changed into the ascorbyl. With oxidative stress the brain ascorbate concentration (AA) should decrease and the ascorbyl PH increase. Figure 22 suggests that, indeed, this happens in our model.

As mentioned above oxidative stress in tissues can be indicated by measuring either the ascorbyl signal by ESR or by determining tissue ascorbic acid concentrations. By measuring both simultaneously, however, one gets a more accurate picture of why tissue ascorbate is low; if oxidative stress is truly occurring the appearance of an ascorbyl radical indicates the consumption of ascorbate and its transformation into ascorbyl. Thus depicting the kinetics of ascorbate utilization. We, therefore, feel it is useful to combine peak height ascorbyl measurements with tissue ascorbate concentrations to form a PH/AA ratio. This maneuver normalizes the peak ascorbyl radical height for differing amounts of residual tissue ascorbate (units: signal height in arbitrary units/ tissue ascorbate concentration). We designate this PH/AA ratio as an "oxidative stress index." This ratio was significantly larger in our brain-injured rats than in controls, Table 3.

Although we demonstrated oxidative stress 60 minutes following injury, we did not show the generation of lipid peroxyl radicals (PBN-spin adduct signals) at this time point. Two major possibilities may account for this: 1- time course of free radical generation; 2- the occurrence of seizures.

The time course of free radical generation is very critical for the detection of free radical signals by ESR. We used a 60 minute period in these experiments because superoxide production has been reported for at least 1 hour after brain injury (65,80,81). In our experiments, however, this time period may have fallen outside the period of free radical generation and maintenance. PBN-trapped peroxyradicals, formed earlier, could have been metabolized or substituted ascorbate by 60 minutes following injury. This may well have been the case in our model because 4 of 12 rats in which ESR analysis was done within 5 minutes of injury demonstrated the presence of PBN spin adducts. The relatively small number of rats showing early spin adducts was not a significant number, howver. It could have been because at the 5 min sacrifice time the formation of carbon-centered peroxy radicals could have been just starting increasing, peaking or decreasing. In future experiments the time course of free radical generation after brain injury should clearly be delineated starting within minutes up to 2 hours after traumatic brain injury.

In another set of experiments peroxy radical signals which developed in the rat brain during exposure to hyperbaric oxygenation were significantly reduced following convulsions (154). Reasons why convulsions should reduce free radical signals are unknown. Recurrent seizures were observed in three of our brain injured animals after withdrawal of anesthesia.

Conclusions

1) The present data indicate that there is a definite increased oxidative stress in the brain after traumatic injury as determined by in vivo ESR spectroscopy and measurements of the endogenous ascorbate antioxidant.

2) While either peak height (PH) ascorbyl radical measurements determined by ESR or residual tissue ascorbate levels (AA) may be used to measure the oxidative stress we feel the PH/AA ratio provides a superior measure.

3) Knowledge of the time course of free radical generation following brain injury is critical in making treatment decisions. If free radicals are formed very early after brain injury (within seconds to minutes) then the successful initiation of any type of antioxidant treatment would be difficult. If free radicals continue to be formed later after injury (e.g. for several hours) then antioxidant agents may ameliorate the extent of oxidative damage which free radicals may cause. Although early generation of free radicals is suggested, our results do not conclusively prove early or late free radical generation.

4) We feel it very important to begin to examine the time course of oxidative stress by free radicals in the brain after missile wounding so that a rational approach for the most optimal therapy of brain missile wounding may be provided.

DIRECTIONS FOR FUTURE MISSILE WOUND RESEARCH

1) Apnea occurring after missile injury may prove fatal. The pathobiologic reasons for this apnea should be delineated and therapy to reverse it sought.

2) Our experiments have clearly demonstrated that no reflow may occur in the missile-wounded brain. Future experiments should delineate the exact physiologic conditions which lead to post-wounding no reflow, its pathobiologic substrate, and the treatment of post-wounding no reflow.

3) Experiments should answer whether, after wounding, hypercapnia-hyperoxia combined provides more benefit to the brain than hyperoxia alone.

4) The time course of free radical generation after brain wounding should be delineated to provide optimal treatment.

5) Specific neurochemical effects of brain wounding must be delineated to ensure the most rational future treatment strategies for this specific type of brain injury.

6) Drug testing should continue in order to find the best drugs maximize the extent of neural recovery following missile injury.

EPILOGUE

Dr. Michael E. Carey's research on experimental brain wounding has been conducted from 1983 to 1989 in an attempt to bring knowledge concerning the pathobiology of a brain missile wound into the 21st century. This must be done if more effective treatments for a brain wound are to be developed. When Dr. Carey's research began fewer than 25 papers on brain wounding using experimental, anesthetized animals had ever been written, world wide. (For comparison from 1983 to 1989 about 3000 papers were written concerning animal-based research for stroke).

Animal activists, with no concern for the American soldier or the American people, acting through Representative Robert Livingston (RLa), have stopped this research through legislative action.

That the federal government should mandate that American Servicepersonnel fight for America's interests and, at the same time, withhold research on the best way to treat their wounds is unconscionable.

The United States Army to its great credit has courageously attempted to improve wound treatment for the U.S. soldier through basic research on wounds. Animal activists on the other hand, have persistently disregarded the welfare of wounded American soldiers by stopping valuable wound research : 1) Brain wound research conducted by Dr. Michael E. Carey stopped in 1989; 2) bone and tendon wound research at the Letterman Army Institute of Research, stopped in 1989; 3) muscle wound research at the Uniformed Services University of the Health Sciences in Besthesda, M.D., stopped in 1984. All 3 aforementioned research projects were stopped without any hearings to which the involved researchers, other scientists, Army medical representatives, or even wounded Veterans could express their views. Only animal activists were heard.

Will basic, advanced, wound research to help U.S. soldiers and civilians be possible in the future? Not if animal activists and their benighted aims continue to prevail. Only 2 hopes remain: 1) that enough Congresspeople awake to the pernicious actions of the animal activists and realize how they are working directly against our servicepersonnel- those who give so much; 2) that the American people awaken to know how the animal activists are trying to destroy the development of modern care for their loved ones. One or both groups must take action if more advanced wound care is to develop.

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Appendix 1

We define reactivity (R) to hypercapnia as:

$$\frac{dCBF}{dPaCO_2}$$

where: dCBF = Normocapnic CBF - Hypercapnia CBF
and, $dPaCO_2$ = Normocapnic $PaCO_2$ - Hypercapnic $PaCO_2$

In this formula vascular reactivity which increases CBF has a positive value (vasodilation) and that which decreases CBF a negative one (vasoconstriction).

To differentiate between vasodilatory a : vasoconstrictive values of reactivity during hypocapnia, we defined activity to hypocapnia as:

$$\frac{-dCBF}{dPaCO_2}$$

This will allow us to present R values in response to hypocapnia as negative numbers indicating vasoconstriction.

Reactivity to hypoxia was defined as:

$$\frac{-dCBF}{dPaO_2}$$

where $dPaO_2$ = normoxic PaO_2 - Hypoxic PaO_2

In this formula too, positive values indicate vasodilation and negative values indicate vasoconstriction.

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