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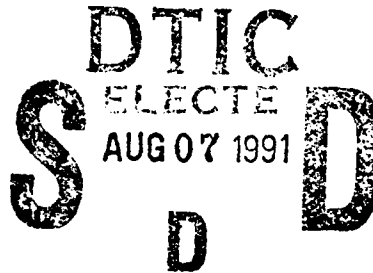


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INTRODUCTION

The Naval Medical Research and Development Command (NMRDC) is the primary Navy R&D organization responsible for managing research and development programs concerning the health, safety, readiness, and effective operational performance of Navy and Marine Corps personnel. The NMRDC organization consists of eight echelon-4 laboratories and five echelon-5 detachments, staffed by military and civilian scientists and located in the continental U.S. and overseas. The main research topic areas studied by NMRDC scientists include combat casualty care and combat dentistry, infectious diseases and AIDS, diving and submarine medicine, aviation medicine and human performance, and environmental and occupational medicine.

NMRDC views the Independent Research (IR) program as unique among research programs, with its central focus on fostering the in-house laboratory investigators' scientific creativity, enthusiasm, and pride in conducting top-notch biomedical research in support of the Fleet. Through the IR program, NMRDC strives to provide investigators with a mechanism for pursuing their novel research ideas and approaches, for broadening their expertise in state-of-the art and emerging technologies and for initiating new efforts in research areas that are compatible with projected Navy biomedical needs. NMRDC believes the opportunities provided by the IR program are critical for stimulating and maintaining the creative and innovative thinking of Navy scientists, and can only result in a heightened return for the investment of the Navy's precious basic research dollars.



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5000	Naval Submarine Medical Research Laboratory, Groton, CT
6000	Naval Health Research Center, San Diego, CA
7000	Naval Aerospace Medical Research Laboratory, Pensacola, FL

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"Best" Independent Research Project of FY90

Nomination Rationale

Cold-Induced Amnesia was initiated in the Naval Medical Research and Development Command's (NMRDC) FY90 Independent Research (IR) Program to elucidate the neurochemical and physiological mechanisms underlying the decrements to working memory that are induced by exposure to moderate or severe cold (here termed cold-induced amnesia). Such memory impairment has been reported by Navy personnel who are commonly exposed to cold temperatures, yet clearly must be able to function effectively in increasingly complex and high tech operational environments. *Cold-induced Amnesia* proposed to determine the effects of temperature on specific neurotransmitters and neurohormones in vivo, in brain regions whose physiological integrity is known to be critical for normal memory function, and to relate observed neurophysiological changes to alterations in working memory and behavioral performance. The ultimate transition goal of this study is to provide advanced biomedical therapies for the prevention and treatment of cold-induced memory impairment in Navy personnel.

Cold-Induced Amnesia was chosen as NMRDC's best IR work of FY90 because of the outstanding scientific quality and progress of the research (adjudged by both internal and external review), the expected impact in enhancing the performance of Navy personnel and in advancing neuroscience research at large, and the high degree to which this project exemplifies the goals and objectives of the Chief of Naval Research's IR program.

From the outset, *Cold-Induced Amnesia* was viewed as a well designed, state-of-the-art approach to an operationally relevant aspect of cold weather medicine not previously addressed in ongoing studies in the Thermal Stress Program at the Naval Medical Research Institute (NMRI). It was NMRDC's judgement that the work was not only important to Navy medicine, but also within the capabilities of the young investigator team, and would significantly expand NMRI's technical base for mission-oriented studies in environmental medicine. This judgement was validated during a scientific peer review (September, 1990) where this work was rated the best of thirty-one projects, and by an expert peer group's unanimous selection (March, 1990) of the follow-on effort, *Cold-Induced Amnesia: Neurobiological Mechanisms* for funding in NMRDC's FY92-FY95 core research program.

The comments of the unbiased, neuroscience and thermal stress experts who reviewed this research are perhaps most convincing of its overall value and outstanding scientific quality. These experts' enthusiastic reaction to the work is described in their summarized comments: "This research undertaking is extremely complex, as the investigators are basically addressing the neurotransmitter basis of memory, a scope even broader than that principally described (the memory effects of cold exposure). Given the current state of knowledge about the brain, the investigators are aggressively entering an area that has been one of science's 'great black holes'. Although there is a significant level of risk in the project, there is also a high degree of confidence that the NMRI investigators, who have established an excellent network with outside experts, will be successful in their efforts. The project's research merit is outstanding; the work is unique, soundly designed, and very innovative, having great intellectual potential in terms of basic research in hypothermia and memory, both to the civilian and military medical communities. The multidisciplinary research approach is first-rate, blending cognitive and behavioral measures with state-of-the-art neurological approaches, including indwelling microdialysis and microthermister techniques. These are excellent approaches for measuring neurotransmitter release in the brain's hippocampus during cold and during behavioral events. Such data have not been previously obtainable in the absence of such advanced neurophysiological technology."

Progress in the project's first year has been impressive. The rat was established as a reliable model of human cold-induced amnesia through the use of a behavioral performance procedure that measures the ability to remember specific events over a short period of time. Rats and humans demonstrated a similar decay of short term memory over time, and, notably, a strikingly similar increase of memory decay during cold exposure. Impairment of short term memory in rats performing a behavioral task under cold conditions correlated with

a reduced rise in hippocampal brain temperature that was observed when rats performed the same task under thermoneutral conditions. Cold exposure caused the release of stress hormones in the rat brain, and these hormones were shown to cause a short term memory impairment similar to that induced by cold temperature. These data suggest that cold-induced memory loss involves a disruption of these important neurohormonal systems. Administration of the amino acid, tyrosine, significantly reduced the magnitude of memory loss caused by cold stress. These research findings have already been published in the peer-reviewed scientific literature and have been presented at multiple scientific meetings (see below). The following paper *Hippocampal and Body Temperature Changes in Rats During Delayed Matching-to-Sample Performance in a Cold Environment* describes the most recent findings of this outstanding research effort.

In the outyears, what will *Cold-Induced Amnesia* contribute to the operational Navy? NMRDC and expert external advisors are convinced of the likelihood of the scientific success of the work, and "the implications for enhancing military effectiveness in cold environments is great. This work may be the foundation for strategies for the pharmacological prevention of cold-induced amnesia in the field, obviously experienced by Marine Corps, surface and diving personnel, and by Navy SEALs. Notably, cold-induced amnesia is not being addressed by investigators in academic institutions. It is important to point out, also, that although this research is presently focused on the effects of cold-exposure on brain function and memory, the fundamental questions raised in this work address neurotransmitter release and brain temperature functions during stress, and results will be applicable to stress effects in general, not just to the effects of cold stress. This breadth of application is a definite virtue of the work."

Cold-Induced Amnesia clearly represents the Navy's IR Program at its best. The project afforded some of the Navy's best environmental and behavioral neuroscientists the opportunity to acquire, for their own professional development and for their laboratory, new technological capabilities to address formerly unanswerable questions in neuroscience and cold-weather medicine. Their research is of the highest quality, applauded by experts in the DoD and university biomedical research communities. Clearly, their positive experience in the IR program has encouraged these investigators to pursue and competitively acquire funding for their novel research questions and approaches in NMRDC's core research program. NMRDC proudly and sincerely congratulates Stephen Ahlers, John Thomas, John Schrot, and Donna Berkey for their most outstanding accomplishments and research contributions to NMRDC's FY90 IR program.

Publications

Thomas, J. R.; Ahlers, S.T.; Schrot J., 1991. Cold-induced impairment of delayed matching in rats. *Behavioral and Neural Biology*. 55:19-30.

Ahlers, S.T.; Thomas, J.R.; Berkey, D.L., 1991. Hippocampal and body temperature changes in rats during delayed matching-to-sample performance in a cold environment. *Physiology and Behavior*. 50 (accepted for publication)

Presentations

Ahlers, S. T.; Berkey, D.; Thomas, J.R., Analysis of brain and core temperature during performance on a delayed match task in a cold environment. Annual Meeting of the Federation of American Societies for Experimental Biology, Washington, DC, 1990.

Thomas, J. R.; Ahlers, S.T.; Schrot, J., Impairment of delayed matching accuracy in rats by cold stress. Annual Meeting of the Eastern Psychological Association, Philadelphia, PA, 1990.

Thomas, J. R.; Ahlers, S. T.; Shurtleff, D., Thermal stress modulates scheduled-controlled behavior. Annual Meeting of the Eastern Psychological Association, New York, NY, 1991

Ahlers, S. T.; Shurtleff, D.; Thomas, J. R., Pretreatment with tyrosine reduces a behavioral deficit produced by central administration of corticotropin releasing factor (CRF). Annual Meeting of the Eastern Psychological Association, New York, NY, 1991

Shurtleff, D.; Thomas, J. R.; Ahlers, S. T., Cold stress and delayed matching-to-sample: the effects of tyrosine. Annual Meeting of the Eastern Psychological Association, New York, NY, 1991

Hippocampal and Body Temperature Changes in Rats During Delayed Matching-to-Sample Performance in a Cold Environment

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Abstract

In order to study the effects of temperature changes induced by cold stress on impairment of working memory, telemetry thermistor probes were implanted into the hippocampal region of the brain and into the peritoneal cavity of rats. Temperatures in these regions were monitored while rats performed on a delayed matching-to-sample (DMTS) task at ambient temperatures of 23°C and 2°C. Matching accuracy was significantly impaired during exposure to 2°C indicating a marked impairment of short-term or working memory. Temperature in the hippocampus increased 2°C during exposure to 23°C but only 1°C when the environmental temperature was 2°C. Body temperature showed a similar but less pronounced pattern in that cold exposure attenuated the increase in temperature observed when animals performed the DMTS task. These results suggest that cold-induced impairment of working memory may be associated with subtle temperature changes in the brain.

Background

Exposure to cold stress which produces hypothermia has been demonstrated to impair retention on a variety of tasks (2, 4, 10, 24, 28, 29, 30). In general, most studies examining the effects of cold stress on memory in animal models have emphasized situations in which relatively profound decrease in body temperature, i.e. on the order of 2-3°C or greater, impairs retention. Many of these studies have tended to involve one trial inhibitory (passive) avoidance conditioning and have emphasized the effects of cold stress on long-term memory processes in which the resulting amnesia is observed 24 hours after exposure to conditioning and thermal insult.

Recent studies have demonstrated impairment of short-term or working memory processes in humans (3, 12, 16, 32) and animals (31) exposed to moderately cold environmental conditions in which there was no demonstrable change in body temperature. For example, using a delayed matching-to-sample (DMTS) procedure in rats, Thomas et al. (31) demonstrated that exposing rats to an ambient cold stress of 2°C impaired accuracy to correctly match the stimulus as the delay interval between presentation of the sample and comparison stimuli increased. Since cold stress impaired accuracy at the long delays of 8 and 16 secs but did not decrease accuracy at a 2 sec delay interval, Thomas et al. concluded that cold stress decreased the ability to maintain stimulus information in working memory over time. A salient feature of the performance decrement observed by Thomas et al. was that it occurred in the absence of any observable decrease in colonic temperature. Although this would suggest that a change in body temperature was not a necessary condition to produce cold-induced impaired performance on working memory tasks of this type, it is possible that measurement of body temperature was potentially confounded by the stress involved in obtaining colonic temperature in rats (5, 11). Recent research by Berkey et al. (5) using implanted telemetry thermistors in the peritoneal body cavity demonstrated that measurement of colonic temperature via insertion of a rectal probe can by itself significantly raise body temperature.

Objective

The aim of the present experiment was to elucidate the relationship of brain and body temperature to cold-induced impairment of short-term (working) memory. Long-Evans rats were implanted with chronic indwelling thermistor probes into the peritoneal cavity and into the dentate gyrus region of the hippocampal

formation. Placement of thermistor probes into the hippocampus was based on studies which have shown that this structure is important for normal working memory (13,14). The use of the telemetry probes allowed for precise measurement of hippocampal and intra-peritoneal (body) temperature changes during exposure to experimentally controlled environmental temperatures and reduced the potential confounding influences of handling stress.

Methods

Subjects

Subjects were six male Long-Evans rats maintained over the course of the study at 85% of their free-feeding weight of approximately 340-360 g. The animals were individually housed in hanging cages in an air controlled unit. Water was available ad libitum in the home cage.

Apparatus

Rats performed in a three lever operant chamber 24.1 cm by 30.4 cm by 26.6 cm. Two response levers were mounted on the front wall, 5.0 cm above the grid floor and 3.8 cm from either of the side walls. A food tray was mounted 1.2 cm above the grid floor and in the center of the front wall equidistant from each of the levers. The tray was connected by a short tube to a pellet feeder located behind the front wall which could dispense 45 mg food pellets. A small light with a white lens cover was mounted 5.0 cm above both the right and left levers. A third response lever with a light located above it was located on the back wall, 5.0 cm above the floor. A speaker located behind the front wall was used for presentation of a 2800 Hz tone at approximately 40 dB. A house light was mounted on the top of the front wall. A radiotelemetry receiver (Mini-Mitter Co., Inc, Sunriver, OR) was attached to the side wall of the test chamber. Frequency data was transmitted from the receiver to a microcomputer system located outside of the environmental chamber. All baseline and cold exposure sessions were conducted with the rat cage housed inside a temperature controlled environmental chamber with internal dimensions of 61.0 cm by 71.1 cm by 121.9 cm. Experimental events were controlled and recorded by a second microcomputer system.

Sessions were conducted five days per week (M-F) with sessions terminating after completion of 180 trials or 60 minutes, whichever occurred first. The house light was illuminated during all sessions. At the start of each trial the correct lever was cued by illumination of the light over one of the two levers on the front wall. The rat was required to press the lever under the illuminated light. A response on the cued lever turned off the light and started a delay interval. A response on the non-cued lever also turned off the light but was followed by a 5 sec inter-trial interval and the start of the next trial. A trial occurrence was recorded only if the rat correctly responded on the cued lever. At the start of the delay the light was illuminated over the single lever on the back wall. The delay lasted for either 2, 8, or 16 secs. A random order of delay intervals was presented in each session with the following constraints. Within a block of 60 trials, each delay appeared 20 times. Half of the trials at a particular delay interval began with the left light illuminated on the front wall and the other half began with the right light illuminated. No more than two trials with the same delay could occur consecutively. The first response on the back wall lever following the completion of the delay resulted in turning off the back wall light, sounding a 2800 Hz tone, and illuminating both lights over the two front wall levers. Responding during the delay was maintained on a fixed-interval schedule. The maintenance of responding on the back wall lever functioned to prevent the development of position bias or the adoption of simple mediating response patterns, such as standing in front of the appropriate front wall lever. The fixed-interval requirement also ensured that the rat was always positioned centrally in the back of the chamber at the termination of the delay interval. Following illumination of the two front wall lights and tone onset, a response on the previously cued front wall lever was recorded as a correct matching response. A correct matching response produced a food pellet and turned off both front panel lights. If a response was made on the previously non-cued front panel lever (an incorrect matching response), both front panel lights were turned off. Following either a correct or an incorrect matching response, a 5 sec inter-trial interval preceded the beginning of the next trial. During the inter-trial interval only the house light was illuminated. Daily sessions were conducted to establish stable performance on the matching procedure.

Implantation of Thermistor Probes

Once stable performance on the delay matching task was reached and maintained for several weeks, rats were implanted with body (intra-peritoneal) and hippocampal thermistor probes (Mini-Mitter Co., Inc, Sunriver, OR) under surgical anesthesia induced by sodium pentobarbital (40 mg/kg, ip). Hippocampal thermistors consisted of a plastic cylindrical shaped probe 1.3 mm in length and 1.2 mm in diameter. The top portion of the thermistor probe consisted of a cap which could be removed to allow battery placement. Extending from the bottom end of the hippocampal transmitter was a 9 mm 26 gauge cylindrical probe which transmitted temperature from the tip of the probe. The sterilized probe was surgically placed using aseptic surgical techniques into the dentate gyrus region of the hippocampal formation (Bregma; AP -4.8, L 2.6, V 5.0) using stereotaxic coordinates from Paxinos and Watson (25). Hippocampal thermistor probes were secured to the skull by dental cement attached to four stainless steel bone screws implanted to a depth of approximately 1 mm. Skin surrounding the thermistor probe was closed with wound clips which were removed approximately 7 days after surgery. At the end of the experiment animals with hippocampal thermistor probes rats were euthanized with CO₂ and the brains extracted to corroborate the placement of the cannula. Proper placement of the hippocampal thermistor probes was confirmed for all subjects.

Implantation of the body (intra-peritoneal) thermistor probes was also accomplished using aseptic surgical procedures. Body thermistors consisted of a plastic cylindrical shaped probe 1.8 mm in length and 1.2 mm in diameter and were coated with wax to prevent contamination. Once the rat was anesthetized with sodium pentobarbital (40 mg/kg, ip) in order to achieve a surgical plane of anesthesia, it was placed on its back and the abdominal region was shaved and then wiped with betaine and alcohol. A 3 cm incision was made vertically along the lateral aspect of the abdomen into the peritoneal cavity. The sterilized thermistor probe was then placed into the peritoneal cavity and the inner layers sutured with dissolvable chromic suture and the skin layers with non-dissolvable suture; the outer sutures were removed after ten days.

Because the frequency characteristics of the telemetry probes do not allow for simultaneous measurement of more than one probe at a time, only one thermistor was utilized at a time and probe conditions were counterbalanced. Rats 1-3 were initially implanted with hippocampal probes while rats 4-6 were implanted with intra-peritoneal body probes. Once the temperature measures for each animal was recorded during 23°C and 2°C sessions (see below), probe conditions were reversed. Hippocampal thermistor probes were inactivated by removal of the battery prior to implantation of the body probes. Body probes were removed from the peritoneal cavity at the same time that hippocampal thermistors were implanted. Two animals initially implanted with hippocampal thermistors died during implantation of the intra-peritoneal thermistor probes. As a result, a total of six subjects contributed to the hippocampal thermistor measurements while only four subjects contributed to the body temperature measurements. Thermistor probes were calibrated before and after implantation.

Cold Exposure

Rats were placed into the chamber 30 minutes before the start of each session. During this pre-session time all lights in the chamber were off. At the end of this 30 minute period, the house light was illuminated and the session started. The 30 minute pre-session placement in the chamber was in effect for all sessions, both cold and non-cold. During all non-cold sessions the environmental chamber was programmed at 23°C. The subjects were cold exposed once a week (either W or Th) for two weeks with the environmental chamber programmed at 2°C. The session immediately before or just after a cold exposure session served as a 23°C baseline comparison session for that exposure. Telemetry frequency data were sampled every 10 secs and converted by the computer to degrees Celsius. Temperature measurements were averaged over 4 minute intervals. All exposures were performed between the hours of 0730-1200 in order to minimize potential confounding influences from circadian variations in body temperature.

Results

Matching Response Accuracy

No decrements in performance compared to pre-surgery baseline performance were observed after subjects were implanted with the hippocampal or intra-peritoneal body probes indicating that implantation of these probes did not impair memory performance. In addition, because rats exhibited the same effects to the environmental temperatures regardless of what type of probe was implanted, behavioral data from sessions in which rats were tested under body and hippocampal probe conditions were pooled for analysis of the behavioral data.

Figure 1 shows the response accuracy of mean performance on the matching procedure at each of the three delay intervals during exposure to 23°C and 2°C. Analysis indicated a significant decline in matching response accuracy with increasing delay for both temperature conditions ($F(2,10) = 31.58, p < .001$) indicating decreased retention of the stimulus with increasing delay. Although the main effect of temperature on response accuracy was not significant ($F(1,5) = 3.59, p > 0.1$), there was a highly significant temperature exposure by delay interaction ($F(2,10) = 12.77, p < .002$) reflecting a steeper decline in performance accuracy in the cold exposed condition as the delay between the sample and test stimuli increased. Matching response accuracy during exposure to 2°C was significantly impaired at the 2 sec, ($t(6) = 3.84, p < .02$), 8 sec ($t(6) = 3.63, p < .02$) and 16 sec ($t(6) = 4.41, p < .01$) delays. Overall, these results indicate that cold exposure significantly impaired working memory performance and that the magnitude of the effect was more severe as the interval between the sample and test stimuli increased.

Accuracy in pressing the lever underneath the sample stimulus at the beginning of each trial was not impaired during cold exposure ($t(6) = 1.33, p < 0.1$). When rats were exposed to 23°C the mean sample accuracy was 93.2%. During exposure to an ambient temperature of 2°C sample accuracy was 92.3%. Analysis of the latency to respond to either the sample or test stimuli indicated no systematic differences for the three delays or between the temperature treatment conditions.

Hippocampal Temperature

Mean hippocampal temperature of rats is depicted in Figure 2. Temperature in the hippocampus in rats exposed to 23°C was relatively unchanged during the first 30 minutes and then was observed to increase markedly while the rats performed the DMTS task. Towards the end of the session the hippocampal temperature was asymptotic at a temperature around 39°C. When the temperature in the chamber was 2°C, hippocampal temperature increased in the first 30 minutes and increased slightly during the remainder of the session. Temperature in the hippocampus remained at a lower asymptotic level of approximately 38°C compared to hippocampal temperature at 23°C. Analysis of these data indicated a significant treatment effect of temperature ($F(1,22) = 44.84, p < .0001$) and a significant effect of temperature over the course of the session ($F(1,22) = 20.38, p < .0001$). The temperature by time interaction ($F(1,22) = 5.24, p < 0.001$) was also significant. Pairwise analysis indicated significant increases in hippocampal temperature at the 4 ($t(6) = 5.46$), 8 ($t(6) = 4.51$), 12 ($t(6) = 2.66$), 16 ($t(6) = 3.52$), and 20 ($t(6) = 2.58$) minute time points prior to the beginning of the DMTS task. There were no differences in hippocampal temperature at time zero. Pairwise analysis of temperature during performance of the task indicated that hippocampal temperature in animals performing the task at ambient temperatures of 2°C were significantly less at 44 minutes into the session and at all time points thereafter.

Body Temperature

The mean body temperature of rats measured by the intra-peritoneal thermistor is shown in Figure 3. Body temperature of rats exposed to 23°C rose steadily throughout the first half of the 90 minute session and remained asymptotic at approximately 39°C. Analysis indicated no differences in body temperature at time zero between the two conditions. During exposure to an ambient temperature of 2°C, body temperature increased initially but reached a lower asymptotic level of approximately 38.2°C, slightly higher than the mean level observed in the hippocampus. Analysis indicated a significant effect of temperature condition

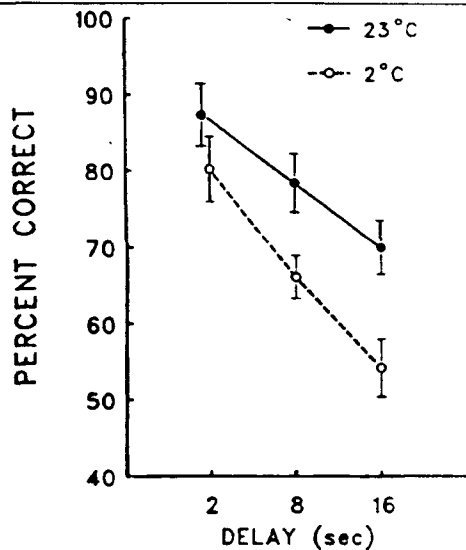
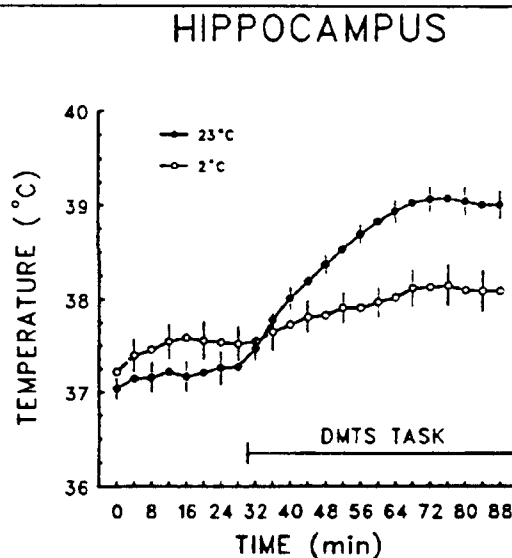


Figure 1. Mean matching response accuracy on the delayed matching-to-sample (DMTS) procedure at delay intervals of 2, 8, and 16 seconds during exposure to ambient temperatures of 2°C and 23°C (mean SE in brackets).

Figure 2. Mean changes in temperature obtained from telemetry probes implanted into the hippocampus. Temperature data were averaged over four minute periods during exposure to ambient temperatures of 2°C and 23°C. Rats performed on the delayed matching-to sample task (DMTS) 30 minutes after the exposure period began.



BODY

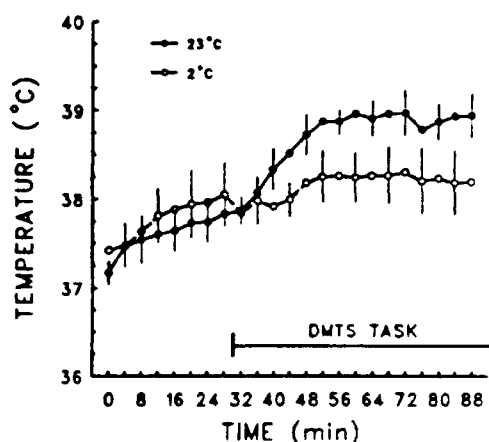


Figure 3. Mean changes in body temperature obtained from telemetry probes implanted into the peritoneal cavity. Temperature data were averaged over four minute periods during exposure to ambient temperatures of 2°C and 23°C. Rats performed on the delayed matching to sample task (DMTS) 30 minutes after the exposure period began.

($E(1,22) = 13.87, p < .001$) and a significant effect of temperature over the course of the exposure session ($E(22) = 4.91, p < .0001$). Pairwise analysis indicated no significant differences in body temperature in the 2°C and 23°C treatment conditions at any time point. There was a trend towards greater differences in the temperature conditions during the last 45 minutes of the session. This was reflected in the observation that most of these comparisons were significant at a level $p < 0.1$. Statistical comparison of the body and hippocampal probe conditions for animals that completed both conditions did not reveal any significant differences during exposure to 23°C or 2°C.

Discussion

Impairment of working memory during exposure to 2°C appears not to result from hypothermia in which temperature drops below a normal resting value. Rather, the memory decrement is apparently related to a reduction of a slight, but significant, increase in hippocampal and body temperature normally observed when rats perform the DMTS task. An unexpected outcome of the present study was the observation that hippocampal and body temperature increased during performance on the memory task. In our previous demonstration of cold-induced impairment in rats, assessment of colonic temperature before and after performance of the DMTS task did not reveal any change in temperature when performing the test at 23°C (31). However, these observations were based upon the use of colonic temperature assessment with standard rectal probes. In the rat, the measurement of colonic temperature involves temporarily restraining the animal in order to obtain a stable value. It has been shown that this procedure alone can significantly increase body temperature when the determination of normal resting temperature is made by telemetry thermometry in which body temperature is obtained without the potential confounding influences involving excessive handling and stress (5, 11). In addition to the potential for restraint stress to overshadow temperature measurements, it is also possible that colonic temperature is slower to respond and/or less responsive than thermal changes measured from other regions such as the peritoneal cavity or hippocampus. The sensitivity of thermistor probes may also influence whether changes in temperatures are observed.

When rats performed the DMTS task under normal thermal conditions of 23°C, accuracy decreased as the delay between the sample stimulus and comparison stimuli increased. Matching accuracy during non-cold sessions was approximately 88% at the 2 sec delay and approximately 70% for the 16 sec delay. This decreasing linear slope function reflects the normal rate of forgetting of information in working memory on this task. The pattern in which accuracy decreases as a function of delay is similar to performance reported in studies with rats employing similar procedures and delay values (13, 35). An interaction was shown between exposure to the cold environment and retention across the delay intervals. Exposure to the cold produced a slight impairment in accuracy at the 2 sec delay and substantially larger impairments at the 8 and 16 sec delays. The observation of impaired performance at the 2 sec interval suggests that cold stress may have impaired initial acquisition or possibly that cold stress produces rapid degradation of the information in working memory, evident even at a 2 sec interpolated delay interval. The further decline in performance accuracy at the 8 and 16 sec delays observed in the environment cold may have been influenced both by weakened initial acquisition or accelerated forgetting of information in working memory. In our previous study (30), accuracy in the cold was not impaired at the 2 sec delay in which the mean accuracy in the 2°C and 23°C conditions was approximately 95%. Cold stress in that study appeared to produce a specific impairment in the ability to maintain stimulus information in working memory since the subjects performance was equated at a 2 sec interval and accuracy in the cold exposed condition was decremented only at the 8 and 16 sec delays. In the present experiment however, accuracy at the 2 sec interval for the 23°C control condition was at 88% and was decreased to approximately 80% during cold exposure. These data suggest that under conditions in which performance levels at short delay intervals are slightly lower, a potential contributing factor to cold-induced decrements in working memory may be a decrease in initial acquisition of the target stimulus. It is important to note that no impairment of acquisition was revealed in response to the sample stimulus. The accuracy to correctly respond to the sample stimulus for the 23°C was 93.2% and 92.3% for the 2°C exposure condition.

During exposure to the cold environment which produced impaired performance on the DMTS task, rats exhibited significant temperature changes. Generally, the pattern of body and hippocampal temperature changes were similar in that temperature increased approximately 2°C from the beginning to the end of the

90 minute exposure session in which the ambient temperature was 23°C. The pattern in both probe conditions was also similar during exposure to the 2°C environment in that cold exposure attenuated the increase normally observed when animals were performing on the task. These data are consistent with the findings of Lewis et al. (20) demonstrating a high correlation between body (colonic) and brain temperature under conditions which produced profound hypothermia and amnesia in rats.

Despite the overall similarity of hippocampal and body temperature under the two environmental temperature conditions, the temperature response in the regions did appear to show subtle differences over the course of the 90 minute exposure session. When rats were exposed to the 23°C ambient environment, body temperature increased steadily during the initial 30 minute period before the onset of the DMTS task whereas hippocampal temperature was relatively unchanged. During exposure to 2°C, body temperature increased at approximately the same rate as it did during exposure to the 23°C ambient temperature condition. In the hippocampus, exposure to the cold environment significantly increased temperature prior to the onset of the DMTS task. Hippocampal and body temperature both increased rapidly and reached a peak of 39°C when rats performed the DMTS task at 23°C. The temperature increase in the hippocampus appeared to be more rapid given that temperature was unchanged prior to the onset of the DMTS task.

Although slight, the differential responsiveness in temperature in the hippocampus and peritoneal cavity suggest some unique thermal dynamics in these regions. The pattern in which the brain displays greater dynamic temperature changes than in the peritoneal cavity are consistent with the findings of Blumberg and Moltz (6, 7). These investigators demonstrated dynamic temperature changes in the medial preoptic anterior hypothalamus are a result of rapid heat exchange through inspiration in the nose which provides for a more rapid exchanges of heat in the ventral brain (7). The findings of the present study would also support the observation that heat loss is greater in the brain. This cooling effect in the brain could possibly contribute to neurophysiological alterations which may underlie cold-induced impairment of working memory.

The increase in temperature observed during the DMTS task at 23°C may result from increased activity required to perform the DMTS task requirements rather than from a specific memory component of the task. Increases in body temperature have been observed to occur in a variety of conditions in which rats have been excessively handled (6), or exposed to aversive conditioned stimuli (34). Such increases may reflect a general increase in sympathetic activity (34) or stress-induced alteration in the thermoregulatory "set point" (19, 21). In addition, it is important to note that the increase in temperature observed when animals performed during the DMTS task was correlated with optimal performance whereas a reduction in the observed increase in temperature was correlated with impaired performance. Although several studies have reported impairment of memory with increased body temperature (1, 22, 23, 27), the magnitude of temperature increase required to induce memory loss has typically been reported to be 3-4°C above normal, less than the 2°C increase observed in the present experiment. It would appear that the increase in body and brain temperature during performance of the task reflects optimal physiological temperature for performance of this task.

The attenuation of the slight increase in brain temperature produced by cold exposure may alter neurophysiological or neurochemical functioning of the hippocampus or related structures that are important for normal working memory. Studies have shown that cold exposure can produce changes in brain function reflected in modulations of neural activity (4, 18, 33). Cold exposure which results in severe hypothermia and severe memory loss has also been reported to induce seizure activity in the hippocampus (17). In addition to neurophysiological changes, the slight decrease in temperature observed during exposure to cold may produce changes in neurotransmitter systems that are important for normal working memory. For example, exposure to cold stress may reduce cholinergic activity in the brain. Impairments obtained with cold exposure in the present study are similar to the accelerated forgetting of information attributable to an anticholinergic drug reported for rats on a similar delay matching task (13). In any case, the potential contribution of alterations in temperature gradients in the body or brain which may either directly or indirectly disrupt neurophysiological functioning and thus contribute to cold-induced impairment of DMTS performance remains to be empirically determined. Further analysis of additional anatomical sites within other regions of the hippocampus and in other brain structures is necessary in order to determine whether the temperature changes recorded from the hippocampus are uniquely correlated with specific requirements of the DMTS task.

In summary, the findings of the present study suggest that cold-induced impairment of working memory may result from subtle temperature changes in subcortical structures important to memory. It has been proposed that rather subtle temperature gradients in selective brain regions may underlie cold-induced performance decrements observed in humans (26). Additional analysis using local cooling of specific areas (9) together with the recording of temperature from other brain regions will further elucidate the contribution of brain and body cooling to cold-induced impairment of working memory.

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Biological Effects of Locally Administered Cytokines

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Abstract

Cytokines (CK), a diverse group of secreted cellular molecules that are proving to be potent modifiers of the immune response, are currently being used clinically to enhance immunity against cancers; tumors; and bacterial, viral and fungal infections. Treatments with these immune modulators, however, are associated with many negative side effects, which are due in large part to the need for high systemic doses to achieve effective CK concentrations at the infection- or wound-site. Local administration of CK might avoid the toxic effects of high level systemic dosing and CK delivered directly to the target site might actually be more effective. In order to test these hypotheses, we have established an *in vivo* murine model for the local delivery of CK. This unique model uses implanted blocks of agarose infused with CK to administer CK to specific tissues *in vivo*. Recovering the agarose blocks allows us to study the kinetics and phenotypes of cells (neutrophils, lymphocytes, and macrophages) attracted by the CK and entering the blocks as well as the functions (phagocytosis and CK production) of these cells harvested from the blocks and subsequently cultured *in vitro*. This CK-agarose block model provides a new capability for studying CK control of local immune responses that are important for the elimination of microbial infections, the control of tumor growth and the enhancement of wound repair.

Background

A diverse group of secreted cellular molecules, the cytokines (CK), have recently been investigated as modifiers of immune and inflammatory responses. CK, also called lymphokines, monokines, interleukins (IL) and interferons (IFN), are produced by a wide variety of cells and play a role in cellular communication. These heterogeneous molecules are low molecular weight (< 80 kDa) glycoproteins that are produced transiently and locally, act in a paracrine and autocrine manner, and are extremely potent.

CK have been used clinically to enhance the immune competence of individuals against certain tumors or infections. Interleukin-2 (IL-2), a T-cell product, has been shown to enhance the immunogenicity of vaccines (1), to limit the replication of bacteria (2), to increase cytotoxicity against viruses (3-5), and to affect the growth of fungi *in vivo* (6,7). Highly purified or recombinant IL-2 (rIL-2), in combination with immune killer cells, has been used as an anti-cancer or anti-tumor agent in humans (8,9). Treatment with recombinant human tumor necrosis factor (rTNF- α) has been shown to enhance resistance to *Listeria monocytogenes* infection in mice in a dose-dependent manner. Combined administration of suboptimal doses of rTNF- α and rIL-1- α has resulted in significant enhancement of resistance beyond that obtained with either CK alone (10). Interferon gamma (IFN- γ), a product of antigen-activated T-cells, has been shown to alter the course of chronic infection of *Mycobacterium intracellulare* in mice (11) and to suppress the production of HIV in an infected cell line (12).

Despite the promising clinical utility of CK therapy, treatments with these immune modulators are restricted due to association with many side effects such as fever, headache, nausea, rash, diarrhea, fluid retention and eosinophilia. These negative effects may be attributable to inappropriate dosages and routes of administration (13-17). Among the many limitations in using these potent materials *in vivo*, the most important is that, in order to reach effective local concentrations, the systemic doses required approach toxic levels. Further, diffusion and half-life considerations make systemic administration of CK a less than ideal mode of delivery.

Since CK function naturally under local conditions, it seems likely that regional administration of these agents might avoid the problems encountered with systemic dosing, and that CK delivered directly to the target site might actually be more effective. In order to test this hypothesis, we have established an *in vivo* murine model for the local application of CK which will mimic the direct production of CK at an infection- or wound-site. This approach will enable us to evaluate the local administration of combinations of various CK which are known to regulate each other. The aim of such treatment would be the reconstitution or augmentation of naturally occurring, local immune responses.

Methods

Mice

BALB/cByJ female mice, 4-6 weeks old, were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were screened by Charles River Testing Services for serological evidence of infection by sendai, mouse hepatitis, pneumonia, encephalitis, cytomegalovirus, reovirus, and *Mycoplasma pulmonis*. No evidence of infection was seen during this study. Mice were housed in laminar flow cages in animal facilities at the Naval Medical Research Institute (Bethesda, MD) for 10-15 days before being used for these experiments. Standard laboratory animal chow and water were provided *ad libitum*.

Tissue culture media

Hanks balanced salt solutions (HBSS), phosphate buffered saline (PBS) and the media used throughout these experiments were purchased from JRH Biosciences (Lenexa, KS). Dulbecco's modified Eagle's medium (MEM) or RPMI 1640 were supplemented with 10% heat-inactivated (56° C, 60 min) fetal calf serum (JRH Biosciences, Lenexa, KS), 2mM L-glutamine, 0.1 mM nonessential amino acids, 5×10^{-5} M 2-beta mercaptoethanol, 100 u/ml penicillin, 100 ug/ml streptomycin (Gibco Laboratories, Grand Island, NY), and 50 ug/ml gentamicin sulfate (M. A. Bioproducts, Walkersville, MD). Supplemented modified media are hereafter referred to as complete MEM (CMEM) and complete RPMI (CRPMI).

Antibodies and lymphokines

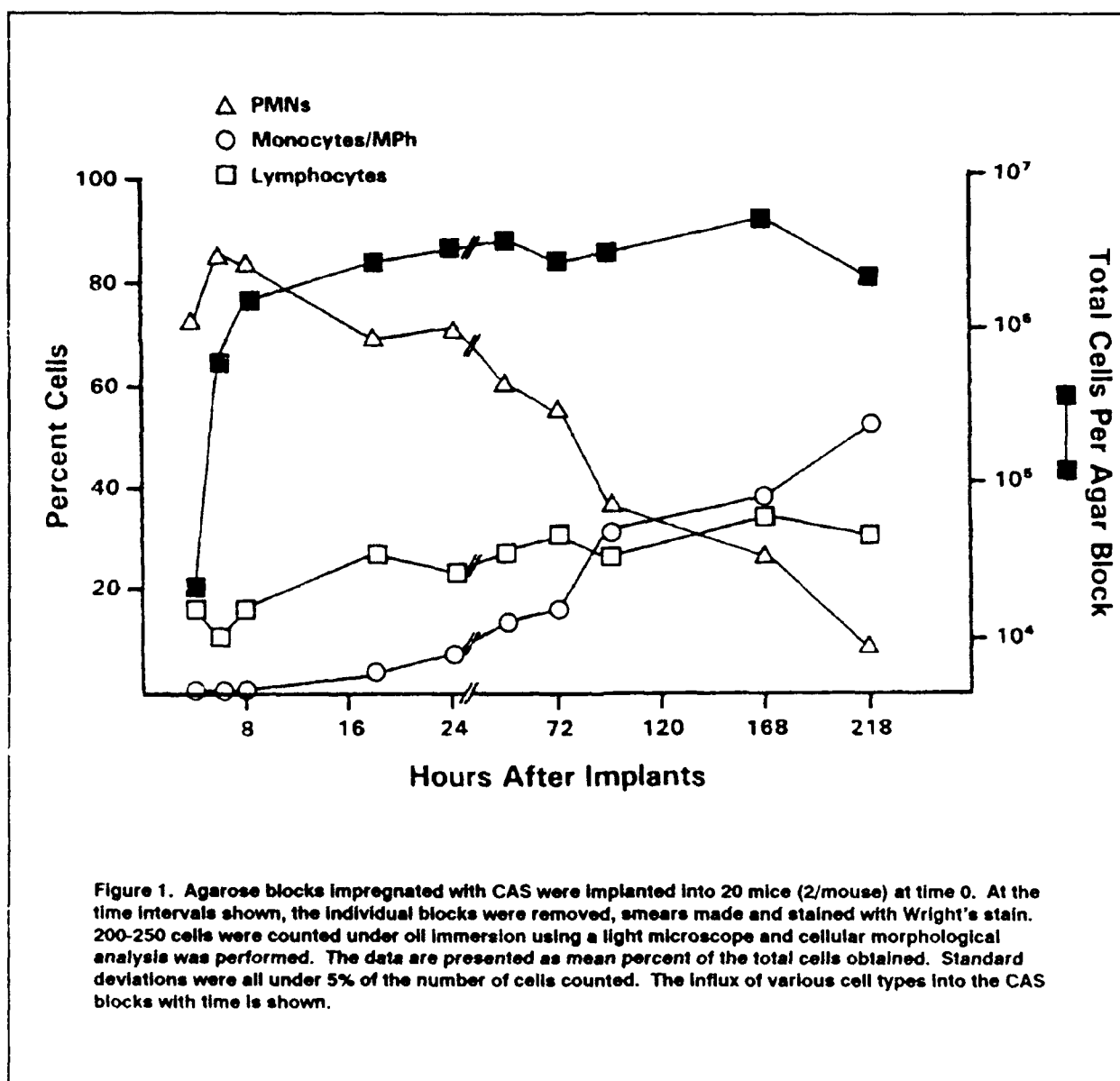
Fluoresceinated monoclonal antibodies for flow microfluorometry analysis were bought from Becton Dickinson (Mountain View, CA). Recombinant CK to establish bioassay standard curves were obtained from Genzyme Corp., Boston, MA.

Preparation of crude cytokine

Crude CK were prepared by a modification of published procedures (18,19). In brief, Sprague Dawley male rats, 400 g (Taconic Farms, Germantown, MD), were killed by CO₂ inhalation. The spleens were aseptically removed and cut into small pieces with bent scissors in a Petri dish containing 10-15 ml HBSS. The cells were forced through the grid with the plunger of a 10 ml plastic syringe into a 50 ml polypropylene centrifuge tube. The cells were washed twice with HBSS by centrifuging for 10 min at 400 x g. Cells were counted and adjusted to 5×10^6 /ml in CMEM. Concanavalin A (Sigma Chemicals, St. Louis, MO) was added to a final concentration of 5 ug/ml of cell suspension. One hundred fifty milliliters of cell suspension was added to 150 cm² flasks (Corning Co., Corning, NY) and incubated in a horizontal position at 37°C, 5% CO₂, for 48 h. At the end of the incubation, media from all the flasks were collected, and freed of cells and cell debris by centrifuging at 4°C, 10 min at 400 x g. Supernatants were collected, aliquoted in 15 ml tubes and stored at -20°C until used. This supernatant is referred to as the Con A supernatant (CAS). Analysis of CK concentration by bioassay revealed 250 units/ml IL-2, 12 units/ml IL-6, 6000 units/ml TNF, < 1 unit/ml M-CSF, positive reactions for IL-5 and gamma interferon (not quantitated). No detectable levels of IL-1 were found.

In vivo model to study local effects of cytokines

A murine in vivo model for the local administration and production of CK was developed using CAS. A 2% suspension of type VII agarose and 0.5% gelatin (Sigma Chemicals, St. Louis, MO) was autoclaved for 15 min at 121°C, 15 lbs pressure. The suspension was cooled to 40°C in a water bath, and an equal volume of agarose was mixed with CAS or recombinant interleukins. A block of 6 x 6 mm was prepared on a square gridded Petri dish using 100 ul of the agarose-CK suspension. After the agarose had hardened, the blocks were placed subcutaneously into the mice (one block in each side). A small incision was made in the lateral flank of anaesthetized mice (Methoxyflurane Metofane Pitman-Moore Inc, Washington Crossing, NJ). A pocket was made on each side with a pair of blunt scissors, and the block was inserted at least 1.0-1.5 cm away from the incision. The cut was closed with 9 mm stainless steel clips (Clay Adams, Parsippany, NJ). As a control, agar-containing medium with Con A was implanted into the mice. The kinetics of influx of the cells to the blocks were studied from 4 h to 9 days. Each block was harvested in CRPMI. A single cell suspension was made using a plastic transfer pipet, counted in hemocytometer and expressed as cells/block. Morphologically, cells were analyzed by Wright's staining, and phenotypic analysis was done by flow microfluorometry. In some experiments, cells were kept in culture for a prolonged time (see below).



Functional analysis of the cells recovered from the blocks

Cells harvested from the CAS blocks at 7 days after implantation were maintained in culture in CRPMI for 64 days. The culture medium was changed at 4-6 day intervals. The spent medium was frozen in aliquots at -70°C and the levels of IL-1, IL-2, IL-6, TNF, and IFN-g were determined.

To determine phagocytic capacity, 1×10^6 block-harvested cells in 300 μl CMEM were seeded in 8 chamber titertek slides (Nunc Inc., Naperville, IL). After 2 h of incubation, unattached cells were removed by vigorous washing with HBSS. 2×10^7 beads in 0.5 ml was added and incubated for an additional 2 h. Slides were washed, fixed in 1% paraformaldehyde and mounted in 90% buffered glycerol. Using a confocal microscope, the number of phagocytized beads was counted. Phagocytic Activity Index was calculated as (Number of internalized beads/Total beads attached) $\times 100$ (20).

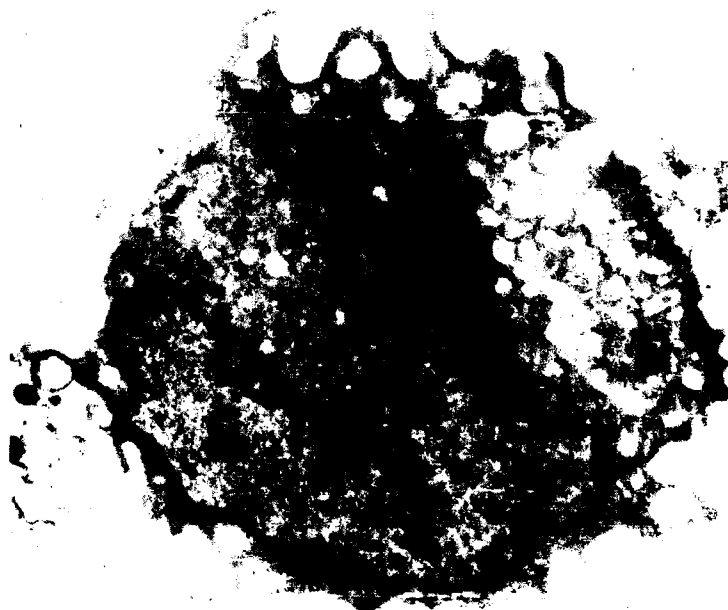
Bioassay for CK was carried out according to published procedures. The IL-1 assay was performed on A375 human melanoma cells obtained from Dr. K. Matsushima (Fort Detrick, MD). An IL-6 dependent B cell hybridoma line B9 was obtained from Dr. R. Asofsky (NIH). The cells were maintained in the laboratory in CRPMI supplemented with 1-2% P388D1 supernatant. P388D1 is a murine macrophage-like cell line which can be induced to produce IL-6 (20). TNF bioassay was performed using L929 cells (ATCC, Rockville, MD) in a modification of previously published procedures (21). A modification of the MTT dye uptake assay (22) was used instead of [^3H]-thymidine uptake or crystal violet staining as an indication of cell growth in the IL-1, 6 or TNF assays. IL-2 biologic activity was determined by the IL-2 concentration-dependent stimulation of proliferation of a cloned murine T helper lymphocyte line HT-2 obtained from Dr. T. Jerrells (Walter Reed Army Institute of Research, Washington, DC). A commercially available ELISA kit was used for quantitative determinations of IFN-g (Genzyme, Boston, MA).

Results

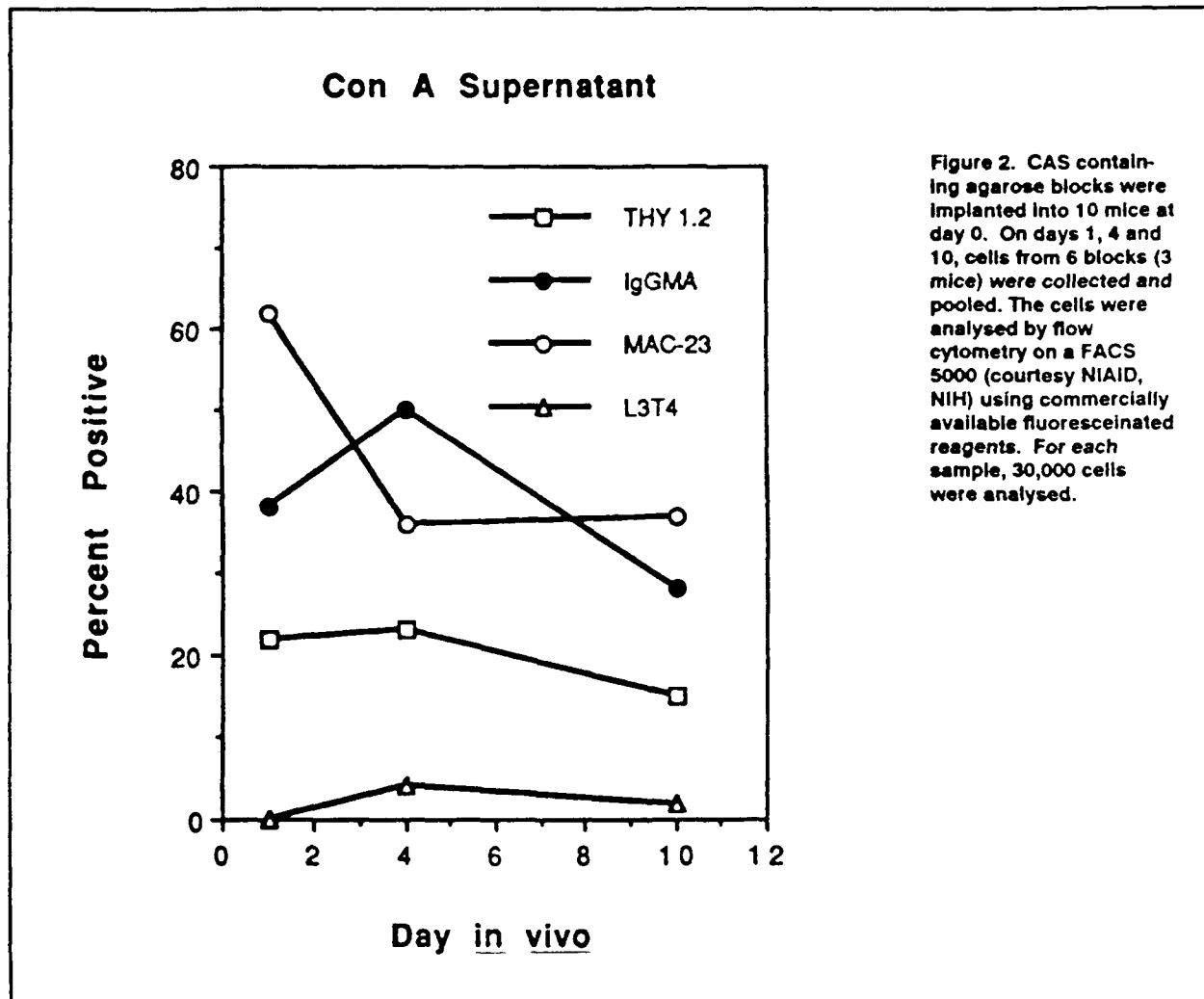
CAS-agarose blocks

Agarose blocks containing CAS implanted subcutaneously into mice began to attract cells into the block within 4 h (Figure 1). Similar blocks containing CMEM + Con A did not attract cells at any time *in vivo*. The total number of cells per block increased until 24-36 h at which time the block was saturated with as

Photo 1. Transmission electron micrograph of a macrophage-like cell isolated from CAS impregnated blocks on day 6 post implantation. Cells were fixed in Nakane's fixative and embedded into Epon for cutting and staining with osmium tetroxide. All EM work was done courtesy of Dr. Y-H. Kang, Pathophysiology Department, NMRI.



many as 8×10^6 cells. The number of cells/block remained constant thereafter, but the cell population changed significantly. Morphological analysis by Wright's staining revealed that the initial cell type attracted at early hours (4-8 h) were 80% polymorphonuclear (PMN) cells, followed at 18-24 h by lymphocytes (LC) and macrophage (MPH) lineage cells. The initial PMN influx was reduced to 30% by 96 h when LC and MPH constituted 30% each of the total cells. A cell population, unidentifiable by Wright's staining, appeared at 72h and remained constant at about 10% of the total population. These large cells had acentric nuclei which, by electron microscopy, appeared to be "C" shaped and contained abundant rough endoplasmic reticulum in their cytoplasm. This appearance is consistent with secretory function (Photo #1).



Cell surface phenotype analysis of lymphoid cells harvested at various intervals was done by flow microfluorometry using fluorescein-conjugated monoclonal antibodies. Granulocytes were excluded from the analysis. The data presented in Figure 2 show that Thy 1.2 + cells constituted 20% of the total lymphoid pool at day 1 and remained unchanged until day 10. Only a small fraction of these cells were L3T4 + (helper). B cells, determined by anti IgG, IgM and IgA antibody surface staining, comprised 38% at day 1, peaked to 45% at day 4 and then dropped to 38% at day 10. Mac 2,3 + cells showed a sharp decline from 62% at day 1 to 35% at day 4 and then remained unchanged.

Functional analysis of the cells recovered from the blocks

Cells harvested 7 days after implantation of CAS blocks into mice were maintained in culture in CRPMI at 2×10^5 cells/ml for 64 days. Spent medium was collected at 4-7 day intervals and replaced by fresh medium containing no exogenous CK. The cell free supernatant collected at various times was analyzed for CK secretion (Table 1). Bulk cultures carried for long periods of time changed their CK secretion patterns. IL-2 was secreted from day 14 to 39 and at day 43 switched to TNF-alpha secretion for 11 days (day 54 of culture). The IL-1 and IFN-g were produced transiently between days 14 and 18, whereas IL-6 was present from day 4 to day 54. No detectable levels of any CK were found in the supernatant collected after 64 days of *in vitro* culture. This differential secretion of CK suggests that either 1) different cell subpopulations were being selected for in the bulk cultures, 2) a maturational event was taking place over time, or 3) the production of one CK is regulating the synthesis and release of other CK.

Table 1

Days in Culture	Cytokines assayed in culture supernatants				
	IL-1	IL-2	IL-6	TNF	IFN-g
4	-	-	+	-	-
14	-	+	+	-	+
18	+	+	+	+/-	+
27	-	+	+	-	-
34	-	+	+	-	-
39	-	+	+	-	-
43	-	-	+	+	-
49	-	-	+	+	-
54	-	-	+	+	-
60	-	-	+/-	-	-
64	-	-	-	-	-

The phagocytic capacity of adherent cells recovered from CAS impregnated blocks at different intervals *in vivo* was determined by allowing the cells to ingest 1 μ fluoresceinated beads *in vitro*. During the first 4-6 h very few adherent cells were present (Table 2). At 8 h, the Phagocytic Activity Index (PAI) averaged 1.7 (± 2.5) and increased to 8.8 (± 2.9) by the cells recovered after 96 h of block implantation. The change in PAI reflects an activation of adherent cells within the blocks *in vivo*. This was also evident by the foamy cytoplasm and active membranes of the cells. The large standard deviation observed reflects the wide variation in macrophage activation state, since the number of beads taken up varied markedly from cell to cell.

Table 2

Hours Post Implantation	Phagocytosis Index ^a Mean $\pm$ Std. Dev.
4	No adherent cells recovered
6	0.8 $\pm$ 1.4
8	1.7 $\pm$ 2.5
18	3.3 $\pm$ 3.1
24	8.6 $\pm$ 5.1
48	4.5 $\pm$ 8.2
72	3.4 $\pm$ 3.9
96	8.8 $\pm$ 2.9

^a Adherent cells were incubated with 1 μ fluoresceinated beads. Slides were read using a confocal microscope (courtesy Mr. C. Odeyale). Results are expressed as Phagocytic Activity Index calculated as: (Number of internalized beads/Total beads attached) x 100.

Discussion

The results reported in this communication clearly show that agarose blocks containing cytokines can be used to study the kinetics of cellular influx during a local perturbation of the immunologic steady-state. The agarose model has several advantages over existing methods currently in use. Most importantly, the control blocks do not induce a foreign body reaction such as is seen with gelatin sponge models (23). Another major advantage over subcutaneous injection of CK-containing supernatants is that the cells can be recovered from the agarose blocks and studied for their immunological phenotype and functions *in vitro*. We plan to develop this model for investigating local immune responses and the role of cytokines in wound repair.

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Electrophysiological And Cognitive Evaluation Of Abstinent Acute Alcoholics

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Abstract

Event-related potential (ERP) component parameters were used as dependent measures in an evaluation of the functional aspects of cognition in acute alcoholics. Previous ERP studies indicated that chronic alcoholic subjects differ in unique ways from nonalcoholics in their brain electrical responses to stimuli. However, a longitudinal study has not been conducted to determine whether these differences persist. Two groups (10 alcoholics and 10 nonalcoholics) of age-matched volunteer subjects were used. The alcoholic group was diagnosed according to DSM-III-R criteria and consisted of U.S. Navy and Marine Corps enlisted men. The nonalcoholic group was composed of nine males and one female. All subjects participated in sessions one (pretreatment of the alcoholic group) and two (three months later), and seven subjects from each group completed the third session (nine months after session one). Each subject completed 300 artifact-free trials of a binaural auditory "oddball" task. The WAIS-R was administered as an additional measure of cognitive functioning. Overall, P50 amplitude, N200 amplitude and latency, and P300 amplitude and latency appeared to differ between groups over the posttreatment period. However, these differences were reduced over the nine-month posttreatment time period. A group main effect for the verbal WAIS-R subtest scaled scores was found in the first session (before treatment), but no differences were found after treatment. The initial results of this study support the idea that alcoholism has a deleterious effect upon the ERPs of human subjects. The reduced P300 amplitude, and the lack of an "oddball" effect (i.e., no difference in amplitude between rare and frequent tones) for alcoholics replicate the results of other ERP studies with alcoholics.

Background

Numerous studies have documented a difference in brain electrical activity between alcoholics and controls (Begleiter, Porjesz, & Chou, 1981; Ehlers & Shuckit, 1990; Hill, Steinhauer, Zubin, & Baughman, 1988; Patterson et al., 1989; Porjesz & Begleiter, 1983; Skerchock & Cohen, 1984; Spitzer & Newman, 1987). These studies have demonstrated that the neurophysiologically measured (e.g., computerized axial tomograph / [CAT], electroencephalography [EEG]) cognitive activity of alcoholics varies significantly from that of nonalcoholics. Testing with neuropsychological instruments, such as the Wechsler Adult Intelligence Scale (WAIS) and the Raven Progressive Matrices, have also shown that alcoholics are cognitively dysfunctional (Goodwin & Hill, 1975; Jones & Parsons, 1972; Parsons & Leber, 1982; Patterson, Williams, McLean, Smith, & Schaffer, 1987; Ryan & Butters, 1983). Clearly, research studies have shown that alcoholism produces cognitive impairment (Grant, 1987). Although alcoholics treated by the Navy are given formal treatment and abstain from alcohol, no cognitive testing is done to ensure that they are cognitively fit to return to duty. One reason for this is the choice of an appropriate metric which could be used to assess global cognitive ability. However, past research has demonstrated the sensitivity of the event-related potential (ERP) as a tool for measuring various aspects of cognitive performance. Using ERP component parameters as a dependent measure enables the functional aspects of cognition (e.g., filtering, discrimination, memory encoding, memory retrieval, and decision making) to be observed (Donchin, Kramer, & Wickens, 1986). The performance of many Navy jobs are highly correlated with the ability to perform these covert behaviors. Therefore, the ERP seems to be a promising chronometric measure to assess operator capability.

Most electrophysiological studies of alcoholics have investigated a positive component that occurs at approximately 300 ms poststimulus (P300). P300 has been characterized as a valuable tool in the study of human information processing, and more specifically, as a reflection of memory updating (Duncan-Johnson, 1981). ERP studies that have compared alcoholics and nonalcoholics have found significantly smaller P300 amplitudes and longer latencies in alcoholics (Patterson et al., 1987; Pfefferbaum, Horvath, Roth, Clifford, & Kopell, 1980; Porjesz & Begleiter, 1985; Porjesz, Begleiter, & Sammuely, 1982; Skerchock & Cohen, 1984). Porjesz and Begleiter (1985) have provided a review of their studies that have demonstrated that P300 was attenuated in alcoholics after two to five years of abstinence. However, their subjects were older chronic alcoholics unlike the patient population normally of concern to the Navy. Their studies used "chronic" alcoholics with a mean age of approximately 36 years who had used alcohol in excessive quantities for at least seven continuous years (Ellis & Oscar-Berman, 1989). Therefore, one plausible interpretation of their results is that chronic alcoholics may have sustained brain damage due to their heavy, long-term use of alcohol. A great deal of research effort has investigated the long-term effects of alcoholism, but little is known regarding the changes in brain activity of younger "acute" alcoholics normally found in the military setting.

Although the literature suggests that the P300 continues to be attenuated in abstinent alcoholics (Porjesz & Begleiter, 1985), a longitudinal study has not been conducted. This paper describes the initial phase of a long-term, longitudinal study to investigate whether acute alcoholic subjects demonstrate "recovery" of cognitive function with abstinence. If an electrophysiological rebound exists in abstinent alcoholics and it is correlated with cognitive performance, then the ERP technique may be a useful tool for measuring fitness for duty.

Methods

Subjects

Two groups (10 alcoholics and 10 nonalcoholics) of age-matched subjects participated in the study. The alcoholics were all awaiting treatment at the Navy Alcohol Rehabilitation Center at NAS Miramar, CA. They were diagnosed according to DSM-III-R criteria and consisted of U. S. Navy enlisted men with a mean age of 25.7 years ($SD = 6.8$) and mean education level of 11.7 years ($SD = .67$). Eight of the alcoholic subjects had a positive family history for alcoholism. The nonalcoholic comparison group consisted of individuals who did not have a history of alcohol or drug abuse. The group was composed of 9 males and one female ($M = 28.7$ years, $SD = 4.3$) and a mean education level of 13.4 years ($SD = 1.95$). Polich, Burns, and Bloom (1988) found no significant differences between male and female alcoholics and nonalcoholics using an auditory ERP paradigm.

Procedure

All subjects completed 300 artifact-free trials of an auditory "oddball" task. Two tones were delivered binaurally at 70 dB nHL with a constant 50 dB nHL white background noise. A 1500 Hz tone, with a 2 ms ramp and a 22 ms duration, was presented on 20% of the trials (rare tone). A 750 Hz tone, with a 2 ms ramp and a 22 ms duration, was presented on 80% of the trials (frequent tone). The WAIS-R was administered as an additional measure of cognitive functioning.

Apparatus

ERPs were amplified ($\times 20,000$), filtered (bandwidth 1-30 Hz), averaged, and stored on a Nicolet Compact Four Electrodiagnostic System (C-4). ERPs were sampled for 100 ms prestimulus and for 800 ms poststimulus (640 Hz sampling rate). Stimuli were delivered binaurally via a Telephonics headset (TDH-39p). Grass gold cup electrodes were attached at Fz, Cz, and Pz in accordance with the International 10-20 system. All electrodes were attached with electrode paste. Linked mastoids served as reference. All impedances were below 5 kohm. Beckman biopotential electrodes were attached supra to the left and right eyes to serve as ground and to monitor eye movement. Trials with EOG amplitude greater than 100 microvolts were automatically rejected.

Results

Age and education, in number of years, were subjected to separate one-way analyses of variance. Although no significant difference between the groups were found for age, education was significantly different ($F(1,18) = 6.75, < p.05$). A separate three-way (Group [2] X Site [3] X Stimuli [2]) MANOVA was calculated for each of the three sessions for the electrophysiological components of interest (P50, N100, P200, N200, and P300). Verbal, performance, and full scale IQ scores were analyzed with two-way, score by group (2), MANOVAs with education used as a covariate. No differences were found between the groups for the number of auditory targets detected. ERP components that showed a significant difference between groups in the first session data were analyzed in sessions two and three in order to test for changes over sessions (within group changes). The statistically significant results are reported below. In sessions one and two, there were 10 subjects in each group and for session three, there were seven. Six subjects (three from each group) were unable to continue participation due to operational commitments. The grand mean ERP waveforms for each group, each session, and targets and nontargets at Cz are shown in Figure 1. Although the alcoholic group's mean P3 target amplitude and P3 amplitude difference is smaller (for all three sessions) the overall morphology of the waveform appears to become more similar to the comparison group over sessions.

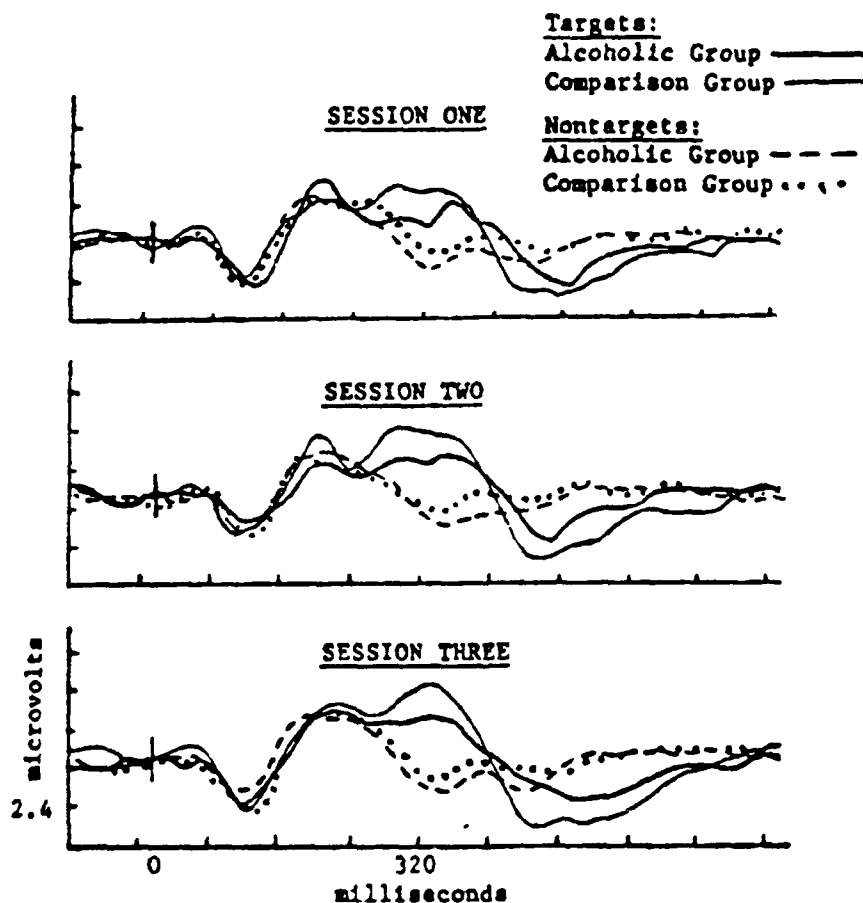


Figure 1. Grand Mean ERP Waveforms for Both Groups and Stimuli at Cz for each session

P50 Component

Session One: A Group X Stimulus interaction was found for P50 amplitude ($F(1,18) = 7.75, p < .05$). An examination of the Group means shows a larger Nontarget amplitude for Alcoholics while the Comparison group displayed a larger Target amplitude.

Session Two and Three: No significant results were found.

N200 Amplitude

Session One: This session revealed a Group X Stimulus interaction ($F(1,18) = 5.40, p < .05$). Results indicate that the Alcoholic group had a similar response to Target and Nontarget stimuli. The comparison group had a larger response to Nontarget stimuli than did the Alcoholic group.

Session Two: The analysis revealed a Group X Site interaction ($F(2,36) = 3.69, p < .05$). Alcoholics had a larger response at Fz while the Comparison group had a larger response at Cz and Pz.

Session Three: No significant differences were found. The Group X Stimulus interaction found in the first session approached significance, $p = .08$.

N200 Latency

Session One: A significant Group X Stimulus interaction was found for N200 latency ($F(1,18) = 7.99, p < .05$). The group means show that the Alcoholics had a longer response to Target stimuli and a shorter response to Nontarget stimuli.

Session Two: The Group X Stimulus interaction approached significance in this session, $p = .08$.

Session Three: The Group X Stimulus interaction found in the first session was again significant in this session ($F(1,18) = 7.99, p < .05$). An examination of the means revealed the same relationship as found in session one.

P300 Amplitude

Session One: A significant interaction was found for Group X Stimulus ($F(1,18) = 4.77, p < .05$). The Alcoholic group had a larger Nontarget amplitude while the Comparison group had a larger Target amplitude.

Session Two: The analysis revealed a similar significant Group X Stimulus interaction ($F(1,18) = 6.06, p < .05$) to that found in session one.

Session Three: No significant differences were found.

P300 Latency

Session One: The analysis revealed a significant Group X Stimulus interaction ($F(1,18) = 5.71, p < .05$). The Alcoholic group had a longer response to Target stimuli and a shorter response to Nontarget stimuli.

Session Two: No significant effects were found.

Session Three: A significant effect of Group ($F(1,12) = 5.21, p < .05$) was found. The Alcoholics group had shorter latencies. The same Group X Stimulus interaction found in session one was displayed in this session. The Alcoholic group had longer Target latencies and shorter Nontarget latencies.

WAIS-R

Session One: A significant effect of groups was found for the verbal tests ($F(1,17) = 4.64, p < .05$). The alcoholic group displayed overall lower verbal scores.

Session Two: The analysis revealed an effect that approached significance, $p < .08$.

Session Three: No significant differences between groups were found.

All ERP amplitude and latency values were correlated with WAIS-R subtests, Verbal IQ, Performance IQ, and Full Scale IQ scores. Although no trends appear to dominate in the correlations, the early components (P50 and N100) comprise most of the significant correlations for the Alcoholic Group and the later components (P200, N200, and P300) comprise most of the significant correlations for the Comparison Group (see Tables 1, 2, and 3). The Information subtest score was significantly correlated with P50 amplitude in sessions one and two for the Alcoholic Group (see Tables 1 and 2). The Performance IQ was significantly correlated with N200 latency in sessions two and three for the Comparison Group (see Tables 2 and 3).

Table 1

WAIS-R and ERP Correlations for Session One

SESSION ONE			
<u>Test</u>	<u>Component</u>	<u>Site</u>	<u>r</u>
<u>Alcoholic Group</u>			
Information	p50 tar amp	Cz	.78**
	N1 tar	Cz	-.81*
Comprehension	p3 tar lat	Fz	.81*
	p2 nt lat	Fz	.80*
Performance IQ	p50 nt lat	Fz	-.86*
<u>Comparison Group</u>			
Digit Span	N2 nt amp	Cz	-.79*
	P3 tar amp	Cz	.82*
Picture Arrangement	N1 nt lat	Fz	-.81*
	P2 tar amp	Cz	.82*
	P2 nt amp	Pz	.78*
	P3 tar amp	Cz	.81*
	P3 tar amp	Pz	.87*
Verbal IQ	N1 tar amp	Fz	-.77*
	N2 nt amp	Cz	.82*

*p.<.01 ** p.<.001

Note: tar = target; nt = nontarget; amp = amplitude; lat = latency

Table 2

WAIS-R and ERP Correlations for Session Two

SESSION TWO

<u>Test</u>	<u>Component</u>	<u>Site</u>	<u>r</u>
<u>Alcoholic Group</u>			
Information	P50 tar amp	Fz	-.81*
	N1 tar amp	Fz	-.92**
Similarities	P50 nt lat	Fz	.92**
Picture Completion	P50 nt amp	Fz	-.79*
Verbal IQ	N1 tar ammp	Fz	-.79*
Full Scale IQ	N1 tar amp	Fz	-.89**
<u>Comparison Group</u>			
Similarities	P50 nt amp	Pz	.78*
Digit Span	N1 nt amp	Fz	-.78*
Picture Completion	P50 nt amp	Fz	.77*
	P50 nt amp	Cz	.78*
Digit Symbol	N2 nt lat	Fz	.92**
	P3 tar amp	Fz	.84*
	P3 tar amp	Cz	.83*
	P3 nt lat	Cz	.91**
Block Design	P3 nt lat	Fz	.86*
Performance IQ	N2 tar lat	Fz	-.77*
	N2 nt lat	Fz	.82*
	P3 tr amp	Fz	.90**
	P3 nt lat	Fz	.72*

*p < .01 **p < .001

Note: tar = target; nt = nontarget; amp = amplitude; lat = latency

Table 3**WAIS-R and ERP Correlations for Session Three****SESSION THREE**

<u>Test</u>	<u>Component</u>	<u>Site</u>	<u>r</u>
<u>Alcoholic Group</u>			
Arithmetic	P50 tar lat	Cz	-.92*
Block Design	P50 tar lat	Cz	-.93*
	N1 tar lat	Pz	-.90*
	P3 tar amp	Fz	-.91*
Performance IQ	P50 nt amp	Fz	.93*
<u>Comparison Group</u>			
Similarities	N2 nt lat	Cz	-.91*
	N2 nt lat	Pz	-.88*
Arithmetic	N1 nt lat	Cz	.88*
Picture Arrangement	P50 tar lat	Pz	-.90*
	P2 tar amp	Cz	-.92*
	P2 nt amp	Cz	-.91*
	P2 tar amp	Pz	-.88*
	P2 nt amp	Pz	-.92*
Object Assembly	P2 tar amp	Pz	-.91*
Digit Symbol	N2 tar lat	Pz	-.90*
Performance IQ	N2 tar lat	Pz	-.92*

* p.<.01 ** p.<.001

Note: Tar = target; nt = nontarget; amp = amplitude; lat = latency

Discussion

The results of this study support the notion that alcoholism has a deleterious effect upon the ERPs of human subjects. The reduced amplitude for N200 and P300 and the lack of an "oddball" effect, (i.e., no difference in amplitude between rare and frequent tones) for alcoholics replicate the results of other ERP studies using chronic alcoholics (Patterson et al., 1987; Porjesz & Begleiter, 1983; Porjesz et al., 1982). These results were found even though the subject population in the present study was concerned with acute alcoholics. It appears that the initial differences observed in the electrophysiological data were the same for acute alcoholics as shown in previous research testing chronic alcoholics. However, certain ERP component parameters (P50, N200, P300), with abstinence, may change over time in acute alcoholics. These results are very different to the past findings of Porjesz and Begleiter, (1990) who have suggested that few changes are found in alcoholics after two to five years of abstinence. Although one must be cautious in the interpretation of the results with such a low study population ($n = 7$), the results suggest that there may be a "recovery" of function in acute alcoholics postabstinence.

Windle and Blane (1989) previously reported an association between verbal ability and drinking behavior in a national sample of young adult males. They found that nondrinkers had higher verbal ability (as measured by their Armed Services Vocational Aptitude Battery or "ASVAB" verbal subtests). Lower verbal ability was predictive of alcohol-related problem behavior. The WAIS-R verbal subtests differences found in this study, though significantly higher for the nonalcoholic group, were within normal limits for both groups. Although education was used as a covariate, the comparison group had significantly more years of education than the alcoholic group. These group differences may have had an impact on the verbal subtests scores. Although differences did exist between groups in the correlations of ERPs and WAIS-R scores, no significant trend was observable.

These results may provide insight for additional monitoring techniques to assess the progress of acute alcoholics and their cognitive ability prior to returning to their jobs. The present results are concerned with 3/4 of the data of the current research project. An attempt will be made to re-test all subjects ($n = 10$) in the fourth quarter of this year.

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FY90 PROJECT SUMMARIES

0061 Cloning and Characterization of Protease-Type Enzymes from Treponema Denticola Associated with Periodontal Disease

T. L. Mapes

Periodontal disease is a common health problem in Navy and Marine Corps personnel. The bacterium, Treponema denticola, has been implicated as a causative agent of periodontal disease; however, the mechanisms by which this organism injures periodontal tissue is unknown. This study theorized that treponemal organisms possess protein-destroying enzymes which can weaken the bony tissue surrounding the teeth, and proposed to isolate these enzymes through gene cloning. Initial work resulted in the identification of a I. denticola/restriction endonuclease combination effective for cloning the I. denticola protease-type enzymes. Using this combination, four isolates which demonstrated trypsin-like activity were isolated from a gene bank of 6000 isolates. During these experiments, a previously unknown I. denticola plasmid was discovered and was used to create a new shuttle vector which will greatly expand the ability to study the pathogenicity of I. denticola as it relates to periodontal disease.

0062 Characterization of Surface Antigens of Treponema Denticola

A. M. Nilius

Periodontal infections are a serious problem in Navy and Marine Corps personnel. The anaerobic bacterium I. denticola is thought to be a causative agent of periodontal disease; however, the bacterium's surface antigens, which may play a role in pathogenesis, are not well characterized. This study proposed to characterize the antigens on the bacterial cell surface, since surface molecules often modulate host responses during infection and may be directly harmful to the host. Surface antigens have been extracted from two treponemal species, I. denticola and I. vincentii using a variety of biochemical techniques and have been analyzed using both immune serum and monoclonal antibodies. Significantly, lipooligosaccharide antigens purified from I. denticola were determined to be responsible for the serovar-specificity of I. denticola strains. The role of lipooligosaccharides in I. denticola variability has never before been described.

1366 Cellular Control Mechanisms of Bone Biosynthesis

M. C. Falk

Treatments to improve bone repair and the development of new bone graft materials are important priorities for improved combat casualty care. The objective of this study was to acquire a clear understanding of the factors which initiate and sustain bone formation, specifically, of the nutritional, growth, and hormonal factors which stimulate the proliferation of osteoprogenitor cells and induce their differentiation into bone-forming cells (osteoblasts). A new cell culture system was developed to produce bone from isolated bone cells. In this system, bone is produced twice as rapidly and in 100 times greater amount than ever reported. The layer of bone propagated in culture is thick enough to be peeled intact from the culture dish, and may provide the basis for a new graft material. During this research, a vital link between bone resorption and bone maturation was identified with the discovery of a new bone-specific protease. A new microvascular cast technique was adapted to study angiogenesis (blood vessel formation) and the role of various angiogenesis factors were investigated.

1376

Biochemical Decompression Following High Pressure Hydrogen Exposures Using Microbial Extracts

G. J. Imbert

Navy deep saturation diving currently requires long decompression schedules to allow the diver's tissues to eliminate sufficiently the dissolved breathing gases which can cause decompression sickness. Theoretically, decompression could be made safer and faster if the solubilized tissue gases could be chemically converted to an innocuous compound *in vivo*. Although this would not be possible with traditional diving gases, such as helium or nitrogen, it may be possible with hydrogen gas, now used in some deep diving operations. This study was designed to determine if anaerobic bacterial hydrogenase enzymes can be used to oxidize dissolved hydrogen to water *in vivo*. A candidate hydrogenase has been purified from the bacterium, *Alkaligenes eutrophica*, and a sensitive assay system for this enzyme has been developed. Additionally, techniques for the large-scale production of *Alkaligenes eutrophica* have been established. Experiments to develop an animal model for *in vivo* enzymatic decompression have identified the incidence of decompression sickness in animals exposed to helium-oxygen atmospheres at the depths planned to be used in the hydrogen diving experiments.

1378

Effects of Chronic Cold Exposure on the Cellular Distribution of Human Thyroid Hormones

M. M. D'Alesandro

Long term exposure to cold environments has been shown to be associated with changes in the concentration of a thyroid hormone, triiodothyronine (T^3), in the serum. The mechanism for this change is unknown, yet the observation itself could be important in understanding the adaptation of Navy personnel to cold operational environments. This study investigated the effects of cold air exposure and daily T^3 supplementation on T^3 nuclear receptor binding characteristics of human mononuclear leukocytes (MNL). First, a receptor-binding assay using MNL was developed to enable assessment of the kinetics of thyroid hormone binding. MNL were obtained from cold-exposed human subjects who had been given either placebo or T^3 . The experimental results suggested that MNL from cold-exposed, T^3 -supplemented individuals decreased in T^3 /receptor affinity while substantially increasing in the number of receptor binding sites per cell. It has been estimated that the number of nuclear T^3 receptor binding sites per cell increased by more than 200%.

1379

Design of a New Shuttle Expression Vector

H. Chang

The recovery of military personnel from illness or injury could be enhanced by stimulating cellular reparative processes, theoretically through the use of exogenous factors or by altering cellular genes to become more active. Genetic manipulation of mammalian cells is commonly accomplished through the use of retroviruses; however, sufficient problems remain with the retrovirus system to justify the development of alternate systems, especially those with strong, inducible promoters in eukaryotic cells. The goal of this project was to create a better vehicle for the control of genes which have been transferred into mammalian cells. Initial efforts focused on identifying the most suitable constructs of antibiotic resistance and heat shock vectors to be used in the transfection experiments.

L. H. Lee

Navy and Marine Corps personnel work with a wide variety of operational chemicals, some of which could produce delayed neurotoxic effects, especially after long term, low level exposure. Early detection of neurologic injury, at a cellular level, offers the possibility of rapid intervention and treatment to prevent severe and lasting adverse effects. This study is investigating the hypothesis that, due to the similarities between the blood-brain and blood-testis barriers, an initial toxic insult to brain cells would be paralleled by early injury to sperm. Laboratory rats are exposed to various Navy-relevant chemicals, subjected to a series of behavioral tests, and analyzed for damage to epididymal sperm, through the use of videomicrographic techniques. Two compounds known to have immediate central nervous system effects, halothane and trimethylolpropanephosphate, have been studied and shown not to have testicular effects, as measured by decreased sperm motility. Potential alterations in sperm morphology, however, are still under investigation. Other Navy-relevant toxic agents, including styrene, triethyl tin, ethylene glycol monomethyl ether and methyl ethyl ketone are being tested in the final year of this study.

R. J. Hartzman

The medical management of immune system-injured Navy personnel could be greatly enhanced with improved methods for monitoring and controlling the proliferation and differentiation of immune system cells. This study investigated the functional and structural events surrounding the activation of a critical immune cell type, the T lymphocyte. A digital imaging system, the Tracor Northern Imaging System, was used to study the binding of the T cell receptor complex of the T lymphocyte to the major histocompatibility complex of an antigen-presenting cell and to identify changes in intracellular free calcium. Recent efforts have enabled the investigators to develop techniques for observing three or more colors of fluorescence emission from labelled thymocytes, to quantitatively analyze image data, and to use a resonance energy transfer imaging system to probe the nuclear structure of interphase peripheral blood lymphocytes.

S. T. Ahlers

Impairment of learning and memory resulting from hypothermia is a serious threat to military operations carried out in cold weather. Recent research has indicated, however, that even moderate cold exposure may disrupt memory in ways not associated with a decline of core temperature. The aim of this project is to examine the contribution of cold exposure to subtle changes in brain temperature and disruptions of neurochemical function in regions of the brain that are critical for memory formation and expression. Initial progress was the development of a rat model of cold-induced amnesia. It was demonstrated that both the decay of the animal's memory over time and its cold-induced performance decrement during a short-term memory task were similar to those observed using human subjects. Analysis of the rat's core temperature and brain temperature (measured using implanted microtransmitter thermistors implanted in the brain) indicated that cold-induced amnesia is associated with subtle changes in temperature in subcortical structures of the brain's hippocampus. Such information on the mechanisms by which cold exposure disrupts normal memory will aid in the development of technologies for the treatment and prevention of cold-induced performance decrements.

1384

The Role of Lymphokines in the Generation and Maintenance of Enteric Immune Responses in Mice

F. M. Rollwagen

Enteric (intestinal) diseases continue to be a major threat to the health and operational effectiveness of deployed Navy personnel. This project is investigating the role of immune cell products, called lymphokines, on the establishment and control of the immune response to the enteric pathogen *Campylobacter coli*. The investigators have developed a novel *in vivo* technique which uses implantable agarose blocks containing specific lymphokines to recruit and trap the immune cell types responding to the localized lymphokines. The data from these experiments have clearly demonstrated a differential migration of immune cell types into the agarose blocks when various lymphokines are used. Additionally, it has been shown that interfering with the *in vivo* production of lymphokines by administration of antibodies to specific immune cell types has a dramatic influence on the generation of anti-*C. coli* antibodies by mice and on the animals' ability to clear the *C. coli* infection. It is thought that the introduction of antibody to specific immune cells cripples the migration and maturation of these cells that are critical to an effective immune response.

1385

Role of the CD4 Receptor in Cellular Signalling

C. H. June

Following chemical, radiological, or biological insult to military personnel, survival depends on the successful and adequate activation of the immune system, specifically, of the T lymphocyte. This study focused on elucidating the mechanism of T lymphocyte activation through specific T cell receptors. Real time analyses were performed on the effects of "T cell receptor" or "CD4 receptor" ligation on intracellular calcium and tyrosine kinase activity. Results showed that the tyrosine kinase pathway was activated in a variety of cells after ligation of both T cell receptors, within five seconds of receptor ligation. To date, this is most rapid activation of a T cell biochemical signal transduction pathway ever reported. This new insight lays the foundation for new ways to manipulate T cell activation pharmacologically. Specific inhibitors and activators of tyrosine kinase should be excellent candidates for new classes of immunosuppressants and immune adjuvants useful for either augmenting or inhibiting the cellular immune response.

1387

The Therapeutic Value of Lipid X in the Management of Gram-negative Sepsis

E. A. Suba

Gram negative sepsis remains a serious clinical problem with a high mortality rate in both combat casualties and in peace-time health care. The primary objective of this study was to determine if non-toxic analogues of lipid A (the toxic moiety of bacterial endotoxin) might be used to interfere with the physiological events that lead to vascular contractile dysfunction. Investigators treated isolated samples of rat aorta with endotoxin or endotoxin analogues. Protein kinase C (an enzyme believed to mediate the reduced vascular responsiveness observed during septic shock) was isolated from these aortae and compared with the enzyme which had been extracted from normal aortic tissue. Results showed that protein kinase C is activated by *in vitro* exposure of rat aortae to endotoxin and its analogues, and suggested that the biological action of endotoxin can be manipulated by agonists or antagonist (such as lipid A or lipid X).

2107

Evaluation of Electrical Impedance Plethysmography in the Non-Invasive Physiologic Assessment of Patients with Shock Caused by Dengue Hemorrhagic Fever

N. D. Witham

Shock is a frequent, major complication in military personnel suffering from wounds, other trauma, and infections, and can potentially lead to death or severe disability. The physiologic parameters of shock (intravascular dehydration, bleeding, etc.) are difficult to assess, especially in a field hospital situation. This study investigated the efficacy of a non-invasive technique, electrical impedance plethysmography, in assessing the pathophysiologic causes of shock and in monitoring the effect of anti-shock treatment regimens. Electrical impedance values were used to determine cardiac output, stroke volume, and total body water content and distribution for dengue patients with and without shock, and for non-ill controls. The potential for transitioning this technique to combat casualty care treatment in forward medical treatment facilities was carefully evaluated.

2108

Biological Basis of Cross-Reaction Between Human T-Cell Lymphotropic Virus Type 1 (HTLV-1) and Plasmodium falciparum

C. G. Hayes

Recently, it has been observed that antibodies produced against the protozoan agent of malaria, Plasmodium falciparum, crossreact with certain polypeptides of the human pathogenic retrovirus, the human T cell lymphotropic virus type I (HTLV-I). This observation is particularly interesting, because understanding the molecular mechanisms responsible for this phenomenon could help explain how P. falciparum is able to avoid immune elimination and could prove useful in managing malaria, a disease of historic and current military importance. Investigators, using specific immunological tests, have shown that a P. falciparum-infected red blood cell antigen preparation blocked both anti-malaria and anti-HTLV-1 reactivity in the sera of people living in a malaria-endemic areas of the Philippines. The HTLV-1 reactivity also was blocked by the HTLV-1 lysate, but anti-malaria reactivity was not blocked by this latter preparation. These results indicate that antibodies formed during infection with P. falciparum in this population can also cross-react with some HTLV-1 proteins. Initial results suggest that a similar phenomenon may also occur in some areas of Indonesia.

3086

Immunodependence of Chemotherapy of Urinary Schistosomiasis

S. Morcos

Urinary schistosomiasis, a disease caused by the parasite Schistosoma haematobium, is endemic in the Middle East and Africa and is currently treated with either praziquantel or metrifonate. Recent studies have demonstrated that the efficacy of praziquantel is severely impaired in immunodeprived mice, indicating that an immune component is necessary for the drug to be maximally effective. The objective of this study was to determine whether treatment of S. haematobium infection with either drug is dependent upon the host immune capabilities and whether efficacy of treatment of immunodeprived animals can be restored through immunologic therapy. Preliminary *in vitro* experiments demonstrated that high concentrations of praziquantel damage the surface of the skin stage schistosomula of S. mansoni, but fail to kill the skin stage larvae in the presence of serum from either normal or schistosome-infected mice. Further work using S. haematobium and older development stages of both schistosomal species is being continued in a core work unit.

3087

Development of an Antigen Capture Enzyme Immuno-assay for the Early Diagnosis of Tuberculous Meningitis Based on *In Vivo* Expressed Antigens

K. A. Kamal

Tuberculous (TB) meningitis is frequently a fatal disease, mostly because the difficulties associated with diagnosis delay the initiation of appropriate anti-bacterial therapy. There is a pressing need for the development of rapid, sensitive and specific diagnostic tests to allow earlier diagnosis and improved patient prognosis. The objective of this project is to identify and isolate antigens that are unique to mycoplasmal organisms during active human infection (theoretically, these antigens, obtained from diseased human body fluids can be different from antigens extracted from cultured bacteria). Electrophoresis and immunoblotting techniques are being used to detect multiple antigens in human pleural fluids and cerebral spinal fluid (CSF) samples from human pulmonary tuberculosis and tuberculous meningitis patients, respectively. CSF samples from sources other than TB meningitis were negative, indicating that the detected antigens were truly specific to TB infection. Such antigens will be used to prepare specific monoclonal antibodies, which will in turn be used in sensitive enzyme-linked Immunosorbent assays for the early detection of TB meningitis infection.

5109

Event Related Potentials Reflect Different Stages in Learning to Discriminate Complex Auditory Stimuli

C. L. Schlichting

A central problem of Navy sonarmen is to detect ship sounds from the vast number of noises present in the ocean. With practice, sonarmen become quite skilled at this task, but it is not clear which characteristics of the signal they are using to make their decisions. The purpose of this study was to determine which sonar signal characteristics are critical to ship detection, by analyzing evoked-related potential patterns which are developed as the brain reacts to auditory signals, and as an individual proceeds through different phases of learning sonar detection. Electrophysiological methods, such as dipole source localization and cortical imaging techniques, were used to develop topographical maps of the brain's electrical activity during acoustical stimulation and at time-intervals known to correspond to the times required for basic sensory and attentional processing. The effects of gender and age on the distribution of such neuroelectrical activities also were studied, as such effects reflect changes in central inhibitory and excitatory processes which may be related to those that occur during the learning process. Results showed that greater subject age was associated with a decrease in the amplitude and a prolongation of the latency of the attention-related neuroelectric wave components N1 and P300, and also with a decrease in the level of activity in the centro-parietal areas of the brain. Greater age, however, related to an increase in the electrical activity in the frontal areas of the brain during auditory discrimination. This same pattern of change is found during discrimination learning and may reflect a shift in the type of cognitive processes required to maintain performance.

6041

Effects of Skin Blood Flow on Bioelectrical Impedance

M. B. Beckett

Dehydration of deployed personnel can be a significant problem during Navy and Marine Corps operations; thus, field-applicable techniques for monitoring the status of body hydration are of military importance. Bioelectrical impedance analysis (a technique which measures the body's resistance after a harmless, imperceptible current is sent through the body) is useful for predicting total body water content, but is influenced by a number of extraneous variables, including body posture, electrode placement, and ambient temperature. This project investigated the effect of changes in skin blood flow on the measurement of bioelectrical impedance. Skin blood flow was modulated by immersing the subject's right hand in water at 5, 15, and 35 degrees C and bioelectrical impedance, skin blood flow, and skin temperature were measured on the subject's left limbs. Results showed that skin blood flow and resistance responses were significantly and inversely correlated at all water temperatures. However, the magnitude of these responses were slight and had a relatively small impact on the prediction of body water (the largest resistance observed, 3.5 ohm, predicted a difference of only 0.4 liter body water).

6042

Evaluation of Cognitive Performance Following Substance Abuse

D. A. Kobus

Alcohol abuse severely impacts an individual's ability to perform. In dealing with the alcoholism problem, the Navy has focused on epidemiology and developing methods of intervention, and has established alcoholism rehabilitation centers (ARC) to support recovering alcoholics. Although abstinence allows for substantial cognitive recovery, the concern that cognitive capacity may not return to pre-abuse levels remains and some cognitive deficits may even be permanent. The purpose of this study is to determine if event-related potentials (ERP) can be used as objective criteria for evaluating the levels of cognitive performance in recovering alcoholics. Longitudinal (one-year) studies are being conducted on ARC-enrolled alcoholics in order to identify the ERP component and cognitive performance changes that occur during rehabilitation. Results have already shown that interactions exist between groups for P50, N200, and P300 amplitudes and a group main effect was found for P300 latency. A discriminant analysis based upon the ERP component values correctly classified 100% of the subjects as alcoholic or non-alcoholic. In addition, examination of the P50 component indicated that alcoholics may have an impaired ability to gate stimuli. Ultimately, this research may establish an objective method for tracking cognitive rehabilitation, aiding the diagnosis of alcoholism, and testing the functional efficiency of an alcoholic's basic selective attention mechanisms.

S. Makeig

The military has long been interested in developing tests of the cognitive capabilities of its personnel who often have to fulfill crucial responsibilities under conditions ranging from extreme boredom to crisis. Electrophysiological methods, such as the electroencephalogram and event-related potential technique, have been useful tools in assessing brain electrical activities and correlating them to cognitive performance. This project focuses on a new electrophysiological approach, the steady-state response (SSR), and is assessing its utility for elucidating the psychophysiology of attention and its breakdown. SSR responses and behavioral data are being obtained from groups of normal subjects and from volunteers suffering from known attentional disorders (depressives and schizophrenics). It is hypothesized that minute-scale fluctuations will be found in the SSR patterns of subjects with attentional disorders and that the size and frequency of these fluctuations correlate with degraded performance both in these patients and in normal volunteers exposed to stressful operating conditions.

L. G. Meyer

This project was designed to develop a physiologically accurate mathematical model of the endocrine and circulatory response to cold based on both theoretical biophysics and physiological data from research on humans and primates. This computer model would simulate the physiological response of both men and women, at rest or during exercise, and at different cold air temperatures. Body type, body mass, body composition, metabolic rate, and physical fitness are considered as factors affecting body temperature response, circulating hormone levels, and body fluid shifts in a dry, cold environment. To date, the equations for the model have been developed and the model is now being constructed. This computerized model will enhance other mathematical models used to simulate human thermal behavior under various conditions, will aid in thermoregulation research by reducing the cost, time, and hazards involved in human experimentation, and will strengthen the prediction of performance and survival capabilities during Navy and Marine Corps operations in cold environments.

FY90 - FY91 PROJECT FUNDING

WORK UNIT IDENTIFICATION	TITLE	PRINCIPAL INVESTIGATOR	PHONE	FY 90		FY 91		APPLICABILITY CODES	
				Funding \$K	In-House Labor \$K	Funding \$K	In-House Labor \$K	1	2
0061	Cloning and Characterization of Protease-Type Enzymes from <i>Trisponema</i> <i>Denticola</i> Associated with Periodontal Disease	T.L. Mapes	(312)688-5347	39.5	19.5	0	0	PMD	FSO
0062	Characterization of Surface Antigens of <i>Trisponema Denticola</i>	A.M. Nilius	(312)688-5647	50	23	58.2	0	PMD	FSO
1366	Cellular Control Mechanisms of Bone Biosynthesis	M.C. Falk	(301)295-2029	87.4	0	0	0	PMD	FSO
1376	Biochemical Decompression Following High Pressure Hydrogen Exposures Using Microbial Extracts	G.J. Imbert	(301)295-3196	78	19.2	0	0	PMD	FSO
1378	Effects of Chronic Cold Exposure on the Cellular Distribution of Human Thyroid Hormones	M.M. D'Alessandro	(301)295-0777	10	0	0	0	PMD	FSO
1379	Design of a New Shuttle Expression Vector	H. Chang	(301)295-1837	20	0	0	0	PMD	FSO
1380	Research into Semen Analysis as a Sensitive Indicator of Neurotoxicity	L.H. Lee	(513)255-6056	26	0	9.1	0	PMD	FSO
1382	Visualization of Early Events in T-Lymphocyte Recognition and Activation	R.J. Hartzman	(301)295-1837	44.6	20	0	0	PMD	FSO
1383	Cold-Induced Amnesia	S.T. Ahlers	(301)295-0066	134.5	24	94.5	0	PMD	FSO
1384	The Role of Lymphokines in the Generation and Maintenance of Enteric Immune Responses in Mice	F.M. Rollwagen	(301)295-1045	95	5	87.2	0	PMD	FSO
1385	Role of the CD4 Receptor in Cellular Signalling	C.H. June	(301)295-1121	84	14.4	0	0	PMD	FSO

FY 90 - FY91 PROJECT FUNDING

WORK UNIT IDENTIFICATION	TITLE	PRINCIPAL INVESTIGATOR	PHONE	FY 90		FY 91		APPLICABILITY CODES	
				Funding \$K	In-House Labor \$K	Funding \$K	In-House Labor \$K	1	2
1386	Endotoxin Blocking Agents and Their Mechanisms	S. Gartner	(301)295-1381	18	0	0	0	PMD	FSO
1387	The Therapeutic Value of Lipid X in the Management of Gram-negative Septic Shock	E.A. Suba	(301)295-1553	22	0	0	0	PMD	FSO
1388	Protection of CD4 Lymphocytes Against HIV-1 Infection by Chromosomal Integration of DNA Complementary to the HIV-1 Pol-encoded Enzymes	M. Carl	(301)295-0306	0	0	81.7	0	PMD	FSO
1389	Use of Oligonucleotides in the Treatment of Infectious Disease	A.M. Churilla	(301)295-0306	0	0	132.5	0	PMD	FSO
1390	Rapid Detection and Identification of Bacteria by Characterization of Their Conserved Heat Shock Protein Genes	G.A. Dasch	(301)295-0025	0	0	72.6	0	PMD	FSO
1391	The Role of recA Gene in Antigenic Variation of Campylobacter	P. Guerry	(301)295-1514	0	0	65.4	0	PMD	FSO
1392	Studies on Purified Cord Blood Hematopoietic Stem Cells (CD34 + Cells)	S. Kessler	(301)295-1121	0	0	90.3	0	PMD	FSO
1393	Isolation and Characterization of Gamma Interferon Inducible Genes and Gene Products from a Human Monocytic Cell Line	A.L. Richards	(301)295-0021	0	0	72.6	0	PMD	FSO
1394	Isolation of Mls Loci and Characterization of Human T-cell Responses to These Determinants	J. Ryan	(301)295-1897	0	0	54.5	0	PMD	FSO
1395	Cloning and Characterization of Enteroregative <i>Escherichia coli</i> Heat Stable Enterotoxins (EAST)	S.J. Savarino	(301)295-0021	0	0	70.8	0	PMD	FSO

FY90 - FY91 PROJECT FUNDING

WORK UNIT IDENTIFICATION	TITLE	PRINCIPAL INVESTIGATOR	PHONE	FY 90		FY 91		APPLICABILITY CODES	
				Funding \$K	In-House Labor \$K	Funding \$K	In-House Labor \$K	1	2
2107	Evaluation of Electrical Impedance Plethysmography in the Non-Invasive Physiologic Assessment of Patients with Shock Caused by Dengue Hemorrhagic Fever	N.D. Witham	*63-2-7323776	3	0	0	0	PMD	FSO
2108	Biological Basis of Cross-Reaction Between Human T-Cell Lymphotropic Virus Type 1 (HTLV-I) and Plasmodium falciparum	C.G. Hayes	(301)663-7193	57	15.3	68.1	0	PMD	FSO
3086	Immunodependence of Chemotherapy of Urinary Schistosomiasis	S. Morcos	*20-2-2841381	30	0	0	0	PMD	FSO
3087	Development of an Antigen Capture Enzyme Immuno-assay for the Early diagnosis of Tuberculous Meningitis Based on In Vivo Expressed Antigens	K.A. Kamal	*20-2-2841381	30	0	27.2	0	PMD	FSO
5109	Event Related Potentials Reflect Different Stages in Learning to Discriminate Complex Auditory Stimuli	C.L. Schlichting	(203)449-2529	57	40	0	0	PMD	FSO
6041	Effects of Skin Blood Flow on Bio-electrical Impedance	M.B. Beckett	(619)524-4517	40	20.6	0	0	PMD	FSO
6042	Evaluation of Cognitive Performance Following Substance Abuse	D.A. Kobus	(619)553-8419	50	16	60.8	0	PMD	FSO
6043	Steady-State Responses and Attentional Disorders	S. Makeig	(619)553-8376	57	31	68.5	0	PMD	FSO
7048	Development of a Model to Predict the Endocrine and Circulatory Responses to Cold	L.G. Meyer	(904)452-4301	80	39	0	0	PMD	FSO

* overseas phone number

COMPLETIONS AND TERMINATIONS

WORK UNIT IDENTIFICATION	TITLE	PRINCIPAL INVESTIGATOR	CODE C-COMPLETION T-TERMINATION	REASON FOR TERMINATION
0061	Cloning and Characterization of Protease-Type Enzymes from <u>Treponema Denticola</u> Associated with Periodontal Disease	T.L. Maples	C	
1366	Cellular Control Mechanisms of Bone Biosynthesis	M.C. Falk	C	
1376	Biochemical Decompression Following High Pressure Hydrogen Exposures Using Microbial Extracts	G.J. Imbert	C	
1378	Effects of Chronic Cold Exposure on the Cellular Distribution of Human Thyroid Hormones	M.M. D'Alesandro	C	
1379	Design of a New Shuttle Expression Vector	H.Chang	C	
1382	Visualization of Early Events in T-Lymphocyte Recognition and Activation	R.J. Hartzman	C	
1385	Role of the CD4 Receptor in Cellular Signalling	C.H. June	C	
1386	Endotoxin Blocking Agents and Their Mechanisms	S.Gartner	T	Transfer of the principal investigator
1387	The Therapeutic Value of Uplid X in the Management of Gram-Negative Septic Shock	E.A. Suba	C	
2107	Evaluation of Electrical Impedance Plethysmography in the Non-Invasive Physiologic Assessment of Patients with Shock Caused by Dengue Hemorrhagic Fever	N.D. Witham	C	

COMPLETIONS AND TERMINATIONS

WORK UNIT IDENTIFICATION	TITLE	PRINCIPAL INVESTIGATOR	CODE C-COMPLETION T-TERMINATION	REASON FOR TERMINATION
3086	Immunodependence of Chemotherapy of Urinary Schistosomiasis	S. Morcos	C	
5109	Event Related Potentials Reflect Different Stages in Learning to Discriminate Complex Auditory Stimuli	C.L. Schlichting	C	
6041	Effects of Skin Blood Flow on Bio-Electrical Impedance	M.B. Beckett	C	
7048	Development of a Model to Predict the Endocrine and Circulatory Responses to Cold	L.G.Meyer	C	

TRANSITIONS

WORK UNIT IDENTIFICATION	TITLE	PRINCIPAL INVESTIGATOR	PROGRAM ELEMENT	FY91 \$(K)	SPONSOR
0061	Cloning and Characterization of Protease-Type Enzymes from <i>Treponema Denticola</i> Associated with Periodontal Disease	T.L. Maples	61102A	139	Army
1376	Biochemical Decompression Following High Pressure Hydrogen Exposures Using Microbial Extracts	G.J. Imbert	61153N	651	ONR
1378	Effects of Chronic Cold Exposure on the Cellular Distribution of Human Thyroid Hormones	M.M. D'Alesandro	62233N	425	ONT
1382	Visualization of Early Events in T-Lymphocyte Recognition and Activation	R.J. Hartzman	61153	200	ONR
1385	Role of the CD4 Receptor in Cellular Signaling	C.H. June	61153	200	ONR
1387	The Therapeutic Value of Lipid X in the Management of Gram-Negative Septic Shock	E.A. Suba	61153	114	ONR
3086	Immunodependence of Chemotherapy of Urinary Schistosomiasis	S. Morcos	61102A	785	Army

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