

Carbohydrate intake improves cognitive performance of stress-prone individuals under controllable laboratory stress

C. R. Markus*, G. Panhuysen, L. M. Jonkman and M. Bachman

Department of Psychonomics, Utrecht University, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands

(Received 1 October 1998 – Revised 4 June 1999 – Accepted 24 June 1999)

Cognitive performance has been found to decline after exposure to stress, particularly in stress-prone subjects. The present study investigated whether a carbohydrate-rich, protein-poor (CR/PP) diet, which may enhance cerebral serotonin function in stress-prone subjects due to increases in the available tryptophan, improves the performance of stress-prone subjects after exposure to acute laboratory stress. Twenty-two high-stress-prone (HS) subjects and twenty-one low-stress-prone (LS) subjects aged between 19 and 26 years performed a memory scanning task after controllable and uncontrollable stress, following either a CR/PP diet or a protein-rich, carbohydrate-poor (PR/CP) isoenergetic diet. Uncontrollable stress reduced feelings of control ($F(1,38)$ 9.30; $P=0.004$), whereas pulse rate and skin conductance increased after both stress tasks ($F(1,38)$ 78.34; $P=0.0005$ and $F(1,37)$ 83.16; $P=0.0004$). Diet, stress-proneness and stress-controllability interacted ($F(1,36)$ 9.46; $P=0.004$) in such a way that performance in HS subjects was better with the CR/PP diet than with the PR/CP diet, but only after controllable stress. As the CR/PP diet has been found to increase the plasma tryptophan:large neutral amino acids ratio, indicating an increased availability of cerebral tryptophan and, thus, higher serotonin levels, it appears that there may be an increased availability of brain serotonin in HS subjects after controllable laboratory stress.

Carbohydrate: Cognitive performance: Stress

It is common knowledge that stress can have a detrimental effect on cognitive performance (Mandler, 1984; Smith, 1990). Marked examples are found following traumatic stress experiences (Bremner *et al.* 1992; Marmar *et al.* 1994), chronic life events (Cohen *et al.* 1980) and short-lived acute stressors (Hockey & Hamilton, 1983; Loeb, 1986; Smith, 1990; Kramer *et al.* 1991).

The onset of cognitive disturbances following stress may depend on the interaction between the intensity of the stressor and an emotional disposition to become aroused (Eysenck, 1982). For instance, Sorg & Whitney (1992) found that highly anxious subjects performed worse than non-anxious subjects in a reading span task under stress, whereas they performed better than control subjects during a non-stressful condition. Comparable effects were demonstrated by Stelmack *et al.* (1984) in a study in which anxious subjects showed improved performance relative to controls in a moderate stress situation, but not in an intense stress situation. As anxiety reflects an emotional susceptibility for stress, these findings suggest that in subjects with chronic stress experiences (stress-prone subjects), acute stress may

lead to poor cognitive information processing. However, this relationship has not been thoroughly investigated. Moreover, the neurophysiological mechanisms mediating the effects of stress on cognitive performance are not well understood.

A possible factor mediating the negative effect of stress on cognitive performance is biochemical imbalances in the brain. In particular, increased serotonin (5-hydroxytryptamine) activity in the brain is a well-established consequence of stress (Joseph & Kennett, 1983; Stanford, 1993) and decreased serotonin function has been demonstrated in patients with mood disturbance (Delbende *et al.* 1992) and cognitive disorder (Altman & Normile, 1988). Hence, an increased serotonin function may be a prerequisite to cope with stress (Anisman & Zacharko, 1991; Deakin, 1991; Deakin & Graeff, 1991) and to maintain control over cognitive information processing (Spoont, 1992). Accordingly, differences in stress-related cognitive disturbances between stress-prone subjects and controls may partly be attributed to differences in serotonin function. If chronic stress in stress-prone subjects results in frequently elevated

Abbreviations: CR/PP, carbohydrate-rich, protein-poor; CS, controllable stress; HS, high-stress prone; LNAA, large neutral amino acids; LS, low-stress prone; PCQ, perceived controllability questionnaire; PR/CP, protein-rich, carbohydrate-poor; RT, reaction time; Trp, tryptophan; US, uncontrollable stress.

* **Corresponding author:** Dr Rob Markus, present address TNO Nutrition and Food Research Institute, Division of Target Organ Toxicology, Utrechtseweg 48, PO Box 360, 3700 AJ Zeist, The Netherlands, fax +31 30 6960264, email Markus@voeding.tno.nl

levels of cerebral serotonin activity, serotonin release may be exhausted, resulting in the depletion of available tryptophan and brain serotonin concentrations (Tuiten *et al.* 1995; Markus *et al.* 1998). As a consequence, the serotonergic system in stress-prone subjects may be overloaded during confrontations with actual stressors, bringing already low concentrations below functional requirements and resulting in poor performance.

Serotonin levels in the brain can be increased by the intake of a carbohydrate-rich, protein-poor (CR/PP) diet. In contrast to a protein-rich, carbohydrate-poor (PR/CP) diet, a CR/PP diet raises the plasma ratio tryptophan (Trp; the precursor of serotonin): the sum of the other large neutral amino acids (LNAA), giving tryptophan the advantage in the competition for access to the brain thus increasing brain tryptophan and serotonin (Fernstrom & Wurtman, 1971*a,b*; Fernstrom & Wurtman, 1972; Fernstrom *et al.* 1973; Curzon, 1985; Wurtman, 1987). This dietary effect on the plasma Trp:LNAA ratio has been well documented (e.g. Rosenthal *et al.* 1989; Christensen & Redig, 1993; Pijl *et al.* 1993; Markus *et al.* 1998).

The relationship between brain serotonin function and cognitive performance, and between a CR/PP diet and serotonin availability, has inspired a broad range of dietary studies based on the assumption that carbohydrates may improve cognitive performance through changes in brain serotonin concentration (Young, 1991). However, dietary studies yield inconsistent findings, revealing that carbohydrates may either improve performance (Kanarek & Swinney, 1990), deteriorate performance (Spring *et al.* 1982/83) or show no effect at all (Lieberman *et al.* 1986; Lloyd *et al.* 1996; for a review see Bellisle *et al.* 1998).

A crucial factor in explaining the inconsistent findings in the literature may be that stress-proneness interacts with serotonin levels. A high-carbohydrate diet may improve performance in stress-prone subjects, particularly under conditions of actual stress, by preventing a critical decline in serotonin functioning. A recent study (Markus *et al.* 1998) investigated whether a CR/PP diet improved cognitive performance in stress-prone subjects after uncontrollable laboratory stress. Although the diet significantly increased the plasma Trp:LNAA ratio, and in stress-prone subjects prevented a stress-induced increase in depression and a cortisol response, no effects were found on performance in a Sternberg memory scanning task (Sternberg, 1969, 1975). It was assumed by the authors that due to low memory load conditions (one, two, three and four different digits) the memory scanning task might have been too low to reveal an effect. However, although hypothetical, the absence of a dietary effect on performance after laboratory stress might also be caused by the use of a highly uncontrollable stressor. The uncontrolled stress may have depleted the extra brain serotonin concentrations supplied by the CR/PP diet.

The present study was designed to test whether a CR/PP diet improves performance on the memory scanning task (Sternberg, 1969, 1975) after laboratory stress in stress-prone subjects, and whether this depends on the controllability of the stressor. High- and low-stress-prone subjects were randomly assigned to a CR/PP diet or a PR/CP diet condition. During two experimental days subjects successively

consumed the diet, were exposed to laboratory stress, and performed the Sternberg memory scanning task. During one day, laboratory stress was uncontrollable, on the other day a controllable version was used. Changes of pulse rate and skin conductance from baseline were measured as physiological indices of the stress response. It was expected that the CR/PP diet would improve memory scanning only in stress-prone subjects, depending on the controllability of the stressor.

Methods

Subjects

Utrecht University social science students ($n = 331$) filled in the inadequacy scale of the Dutch personality inventory (measuring neuroticism; Luteijn *et al.* 1975), and a questionnaire concerning personal details. Neuroticism is closely related to negative affectivity (Luteijn & Bouman, 1988), the disposition to see events as alarming and to experience aversive emotional states (Watson & Clark, 1984), and also to stress-proneness (Gallagher, 1990; Wells & Matthews, 1994). In accordance, subjects high in neuroticism frequently experience stress (Bolger & Schilling, 1991; Deary & Matthews, 1993). Twenty-two subjects (six males and sixteen females) were selected from the highest quartile of inadequacy scores for the high-stress-prone (HS) group (mean score 25, range 20–37), twenty-one subjects (five males and sixteen females) from the lowest quartile of inadequacy scores were selected for the low-stress-prone (LS) group (mean score 3, range 1–5). Subjects' ages ranged from 19 to 26 years (mean age 22.5 years). Exclusion criteria for participation were chronic and current illness, medication, or irregular diets. All subjects selected for the experiment were of normal weight for height (BMI between 20 and 25 kg/m²). All subjects participating in the experiment signed a letter of informed consent. The protocol was approved by the ethics committee of the clinical health department of Utrecht University.

Procedure

Equal numbers of both HS and LS subjects were randomly assigned to a CR/PP diet or a PR/CP diet condition, consisting of breakfast, lunch and one between-meal snack. On each of two experimental days, subjects completed a demanding stress-inducing mental arithmetic task. During one day, a controllable version of the stress task (controllable stress, CS) was presented, and this was alternated with an uncontrollable version (uncontrollable stress, US) on the other day. The order of presentation of CS and US was counterbalanced between subjects. The experimental days were separated by a 4-week interval in order to account for the menstrual phase of the female subjects. Women participated during their mid-late follicular phase (days 4–10).

On each experimental day, two subjects arrived at the laboratory at 09.00 hours (first subject) and at 10.00 hours (second subject), were allowed to read in a study room, and received breakfast at 09.00–09.30 or 10.00–10.30 hours, a between-meal snack at 10.15–10.30 or 11.15–11.30 hours

and lunch at 11.00–11.30 or 12.00–12.30 hours, according to their dietary condition. Subjects did not eat or drink anything during the 11 h before their arrival. At 1.5 h after lunch the subject was brought into a laboratory room, seated in front of a computer screen, and instructed about the experiment. The electrodes for skin conductance and the finger sensor for pulse-rate measurements were attached, and during the following 10 min baseline physiological recordings were made. The subject was then exposed to a computer-assisted battery of experimental tasks (MINDS; Brand & Houx, 1992). The battery consisted of (1) a CS or US version of the stress task (Peters *et al.* 1998), (2) a self-constructed questionnaire measuring experiences of control (perceived controllability questionnaire, PCQ), and (3) a Sternberg (1969, 1975) memory scanning task. Pulse-rate and skin conductance were recorded continuously until the end of the experiment. Subjects spent approximately 22 min on the stress task, 4 min on the PCQ and 10 min on the memory scanning task.

Diets

Two different diets were used; a CR/PP and a PR/CP diet. The diets were composed by an authorized dietitian of the Academic Hospital Utrecht (AZU, The Netherlands) and complied with the reference daily energy intake for peer groups of both men and women. As shown in Table 1, the total amounts of energy and fat in the two diets were approximately equal (the difference of 515 kJ is negligible in regard to behavioural consequences). In order to keep the reducing effect of protein on the increase of the plasma Trp : LNAA ratio small, the amount of protein in the CR/PP diet did not exceed 5 % of the total amount of dietary energy (Yokogoshi & Wurtman, 1986).

Both diets were identical to meals used in a previous study with similar groups (Markus *et al.* 1998). In the former study, the plasma Trp : LNAA ratio was significantly increased by 42 % during the CR/PP diet compared with the PR/CP diet ($P < 0.0001$).

Perceived controllability questionnaire

Experience of control refers to a subjective sensation of the ability to influence the outcome of self-directed actions and is commonly accompanied by increased feelings of mastery and control. Control experiences can be influenced by, among other factors, manipulating the difficulty of a task,

the chance of successful performance, and the expectations about the outcome. In order to measure the effectiveness of manipulating the controllability of the stress task (CS *v.* US), a computerized questionnaire was designed for the constructs 'experiences of control' and 'effort'. This questionnaire comprised seven questions examining subjects' experiences of control (e.g. 'I could control the number of successes during the task'; 'I feel incompetent to perform well') and seven questions measuring the amount of effort required by the task (e.g. 'performing this task took me a lot of energy' and 'the task required much effort'). Subjects were asked to rate their level of agreement on an eight-point interval scale ranging from 'strongly agree' to 'strongly disagree'.

Factor analysis was performed on the total sample scores, for which the results of factor extraction (by principal component analysis with Varimax, rotation; SPSS 7.5.2 for Windows, SPSS Inc., Chicago, IL, USA) revealed an optimal factor structure of two factors. Both scales revealed strong reliability in the range of 0.85 (Cronbach's α).

Two additional questions (e.g. 'I have confidence in performing well during the following task') tested the effect of stress controllability on expectations of competence (mastery). Subjects were asked to rate their level of agreement on an interval scale ranging from 1 (strongly agree) to 8 (strongly disagree).

Laboratory stress task

A mental arithmetic task performed under noise stimulation was used as a stress-inducing task and was offered in a controllable version (CS) and an uncontrollable version (US) (Peters *et al.* 1998). Subjects were given eighteen successive 1 min trials in which they had to solve mental arithmetic problems under time constraint, while at the same time different levels of noise (either 65, 70 or 80 dB) were presented through a headphone. During each trial, multiple choice arithmetic questions were presented on a computer screen one at a time for which a specified number of sums (called the criterion) had to be solved correctly. Subjects were told that their performance would control the intensity of noise presented to them during the task. If they failed the criterion, the computer would set the noise level to be present during the next trial; however, if they met the criterion they could choose the noise level for the following trial. Before the actual test, subjects were given two practice trials in which they became familiar with the task and the noise levels. The credibility of the task, as well as

Table 1. Compositions of the carbohydrate-rich, protein-poor (CR/PP) diet and the protein-rich, carbohydrate-poor (PR/CP) diet used in the present study

Diet	Foods	Carbohydrate		Protein		Fat		Energy	
		(g)	(% energy)	(g)	(% energy)	(g)	(% energy)	(kJ)	(kcal)
CR/PP	Bread, margarine, jam, marmalade, tea, black coffee, sugar, candy bar, grape juice	219.1	66.2	11.9	3.6	44.3	30.1	5555	1323
PR/CP	Bread, margarine, cheese, roast beef, tea, black coffee, high-fat milk, peanuts	123.1	41.0	81.2	27.0	42.2	32.0	5039	1200

motivation, was enhanced by providing the subjects with constant on-screen feedback of the criterion in a particular trial, the number of sums already solved correctly, and the time left for that trial.

Controllability was manipulated by altering the total number of sums to be solved in each trial (the criterion). For each subject, as well as each trial, a new criterion was established based on the average calculation time needed for previous trials. In the CS condition, controllability was accomplished by manipulating the criterion so that all subjects had successes in most of the trials, and could thus choose the noise intensity for the following trial. One restriction was that two of the louder intensities had to be selected three times each, but the order of appearance was determined by the subjects themselves. In the US condition subjects continued to fail on each trial and, thus, could not choose the intensity of the noise. The criterion was always set at one sum above what subjects could manage.

Memory task

Cognitive performance refers to the observable outcome of mental computations involved in the perception, recognition and encoding of a stimulus (input level) as well as the preparation and organization of a response (output level). A Sternberg memory scanning task (Sternberg, 1969, 1975) was used as a cognitive performance test since this task allows for subtracting different levels of mental processing. This task consisted of four sub-tasks, corresponding to memory sets of three, four, five and six different consonants. Each sub-task started with a presentation on the computer screen of the particular set to be memorized, as long as each subject needed, after which sixty trials followed. In thirty trials the presented letter belonged to the memorized set, in the other thirty trials this was not the case. Every first ten trials in each sub-task were used for practice and, therefore, not used in the analysis. In each trial the probe letter was presented at the centre of the screen for 1 s (machine-paced; with an interval range of 1 s). The order of presentation of the sixty letters was randomized. Subjects were instructed to decide as quickly as possible whether the presented letter did or did not belong to the memory set by pressing a red button (No) or a green button (Yes) with the preferred hand. Specific button boards were made for both left- and right-handed subjects.

Measure of electrodermal activity

Skin conductance was measured using Ag–AgCl electrodes (surface 5 mm²), filled with adhesive solid gel (ARBO H91, Braunschweig, Germany). These electrodes were bipolarly placed at the thenar palmar and hypothenar palmar sites of the non-preferred hand of the subject. Tonic skin conductance was measured by a constant voltage system (0.5 V). Conductance values were recorded with a sampling rate of 2 Hz, starting from a 10 min baseline rest period until the end of the experiment.

Measure of pulse rate

Reflection-photoplethysmography was used to measure changes in peripheral pulse rate at the volar surface of the

middle finger of the non-preferred hand. The signal was transduced by Sat-TrakTM signal processing (sampling rate 65 Hz), based on the quadrature division multiplexing technique, using the pulse oximeter (SensorMedics Corporation, Bilthoven, The Netherlands). With a sampling rate of 1 Hz, pulse rate samples were collected and stored in the computer. Pulse rate recording started from a 10 min baseline rest period until the end of the experiment.

Experimental design and statistical analysis

The design of our study included diet (CR/PP *v.* PR/CP diet) and stress-proneness (HS *v.* LS) as between-subjects factors. To test the effect of the order of stress-controllability (CS first followed by US, *v.* the opposite order) and sex, these variables were also taken as between-subjects factors in a preliminary analysis. The within-subjects factors were: stress-controllability (CS *v.* US) and memory-load (the four memory sets with three, four, five and six different consonants that had to be memorized during the memory scanning task). Dependent variables were: laboratory-stress (increases in pulse rate and skin conductance measures before and after the laboratory stress task) and the results for the PCQ and memory scanning. The definitions of the main variables used in the present study are summarized in Table 2.

The results for pulse rate, skin conductance, the PCQ and memory scanning were analysed by means of repeated-measures multivariate ANOVA (General Linear Model: SPSS 7.5 for Windows). For pulse rate and skin conductance (measured before, during and after the stress task) as well as for the scores on the four different consecutive memory sets of the memory scanning task, multivariate ANOVA were performed with first- and second-order polynomial contrasts (testing linear and quadratic effects). Only significant multivariate results were further examined by univariate tests. Huynh-Feldt and Greenhouse-Geisser corrected *P* values, their corresponding epsilons (ϵ) as well as the original, uncorrected degrees of freedom are reported when the sphericity assumption was not met. The study was designed to detect a large effect size (μ^2 0.20) for a power of 0.80 at α 0.05. All significant effects on memory scanning are represented with their corresponding power estimations. As order of stress-controllability only contributed to the scores on the PCQ, these scores were analysed with order of stress-controllability as a covariate. Since there were no effects of sex ($P=0.84$), sex was not included in the final analysis. All statistics were evaluated at a significant level of 5% (two-tailed).

Results

Perceived controllability questionnaire

As expected, there were significant effects of stress-controllability on the scores of the PCQ ($F(2,37)$ 10.26, $P=0.001$). As shown in Fig. 1, in all subjects the mean scores on the subscale of experience of control significantly increased after CS (42.57 (SD 7.29)) compared with US (22.0 (SD 6.81)) ($F(1,38)$ 9.30, $P=0.004$), and there was a significant reduction in the mean scores on effort after CS

Table 2. Definitions of the main variables used in the present study, together with outcome expectations

Variable	Definition	Expectation
Diet	A two-level between-subjects factor: a carbohydrate-rich, protein-poor diet or a protein-rich, carbohydrate-poor diet (CR/PP v. PR/CP)	Only the CR/PP diet increases brain serotonin synthesis, by raising the plasma tryptophan : large neutral amino acid ratio
Stress-proneness	A two-level between-subjects factor: subjects with low chronic stress (LS) v. high chronic stress (HS)	In HS subjects the brain benefits more from a rise in brain serotonin synthesis due to a chronic stress-induced serotonin shortage
Stress-controllability	Changes in the controllability of the stress-inducing task: controllable stress (CS) v. uncontrollable stress (US)	US reduces experiences of control, causing more mental stress, and is accompanied by a profound utilization of cerebral serotonin
Laboratory-stress	Increase in pulse rate and skin conductance measured before and after the stress-inducing task	Increases in mental and physiological stress accompanied by a rise in cerebral serotonin synthesis and activity
Memory-load	The number of items memorized during memory scanning	A higher memory load is accompanied by increases in reaction time

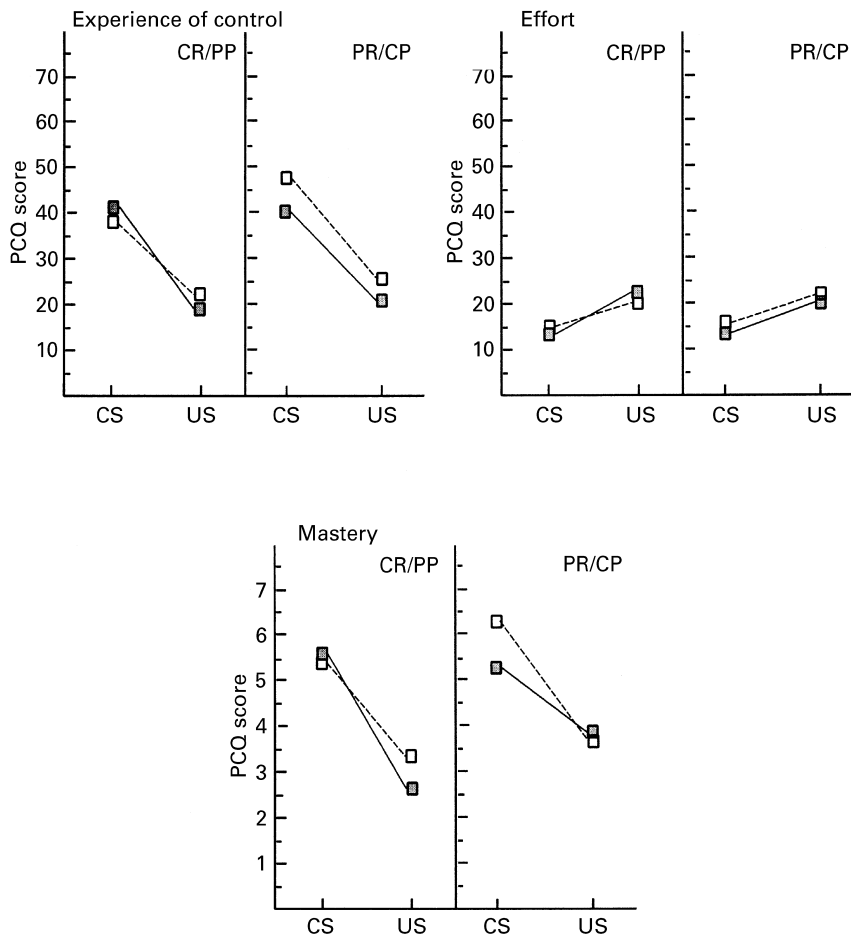


Fig. 1. Significant effects of stress-controllability ($P=0.001$) on the scores of the perceived controllability questionnaire (PCQ), indicating greater experience of control ($P=0.004$) and mastery ($P<0.001$), and lower effort ($P<0.001$), after controllable stress (CS) compared with uncontrollable stress (US), in subjects consuming a carbohydrate-rich, protein-poor (CR/PP) diet and a protein-rich, carbohydrate-poor (PR/CP) diet. (■), High-stress-prone subjects; (□), low-stress-prone subjects.

(14.79 (SD 3.71)) compared with US (20.95 (SD 2.39)) ($F(1,38) 17.48, P < 0.001$). Multivariate ANOVA did not reveal any differences in the effect of stress-controllability between HS and LS subjects, nor was there an effect of diet.

ANOVA also revealed a significant effect of stress-controllability on feelings of mastery ($F(1,37) 42.88, P < 0.001$). As shown in Fig. 1, experienced competence was lower after the US task (3 (SD 1.47)) than after the CS task (6 (SD 1.40)) (with the scores of diet and stress-proneness combined). There were no differences between HS and LS subjects, nor was there an effect of diet. These results indicate a successful manipulation of the controllability of the stress task.

Psycho-physiological measures

Multivariate ANOVA revealed a significant effect of laboratory-stress on changes in pulse rate and skin conductance (Wilks's $F(2,37) 40.23, P < 0.001$). No significant effects were found for diet, stress-proneness or stress controllability.

As shown in Fig. 2, the laboratory stress task increased mean pulse rate from 74.72 (SD 10.44) to 84.0 (SD 12.83) ($F(1,38) 78.34, P < 0.001$) and skin conductance from 7.0 (SD 3.13) to 11.27 (SD 4.75) ($F(1,37) 83.16, P < 0.001$) with the scores of diet, stress-proneness and stress-controllability combined.

Cognitive performance: memory scanning

It was predicted that the HS group within the CR/PP diet condition would perform better after laboratory stress compared with the PR/CP condition, depending on the controllability of the stress task (CS *v.* US). In the LS group this effect was not expected. To test this, the mean reaction time (RT) across the four subtasks was analysed as an overall measure of performance efficiency. Multivariate ANOVA revealed an effect of stress-controllability (Wilks' $F(1,36) 31.32, P < 0.001$; power 1.0) and a significant three-way interaction effect of diet \times stress-proneness \times stress-controllability (Wilks' $F(1,36) 9.46, P = 0.004$; power 0.85), indicating that in the HS group only the mean RT was

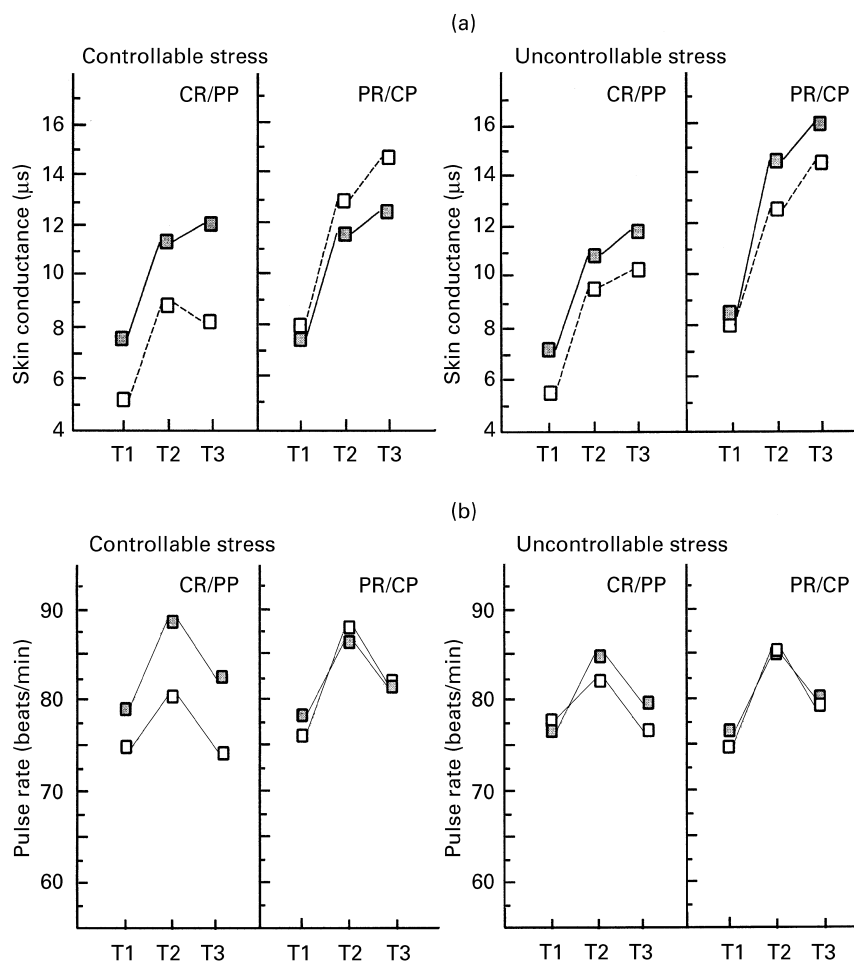


Fig. 2. Significant effects of controllable and uncontrollable stress on changes in (a) skin conductance ($P < 0.001$) and (b) pulse rate ($P < 0.001$) before (T1), during (T2) and after (T3) the period of stress, in subjects consuming a carbohydrate-rich, protein-poor (CR/PP) diet and a protein-rich, carbohydrate-poor (PR/CP) diet. (■), High-stress-prone subjects; (□), low-stress-prone subjects.

faster in the CR/PP diet condition (649 (SD 106) ms) than in the PR/CP diet condition (780 (SD 170) ms) after CS, whereas after US these differences were not found (see Fig. 3(a)). Further univariate testing revealed that RT in these subjects significantly improved after CS compared with US during the CR/PP diet condition ($t = 2.36$; d.f. 11, $P = 0.038$), whereas the apparent decline in RT across both stress conditions during the PR/CP diet was not significant ($t = 1.54$, d.f. 11, $P = 0.22$). Furthermore, the apparent difference in RT in HS subjects between CS and US during the PR/CP diet condition was not significant ($P > 0.60$). In LS subjects no effects of diet or stress-controllability were found.

It was further explored whether in the HS group the increase in RT as a function of memory load was less during a CR/PP day than during a PR/CP day, depending on the

controllability of the stress task (CS v. US). This slope (the rate at which RT increases with memory load) is interpreted by Sternberg (1969, 1975) as an indicator of the time needed for scanning an item in short-term memory. As a near significant effect of order of stress-controllability (Wilks' $F(3,34) = 2.76$, $P = 0.06$) was found on this slope, a multivariate ANOVA was performed with order of stress-controllability as a covariate. However, analysis only revealed a significant effect of memory-load on RT (Wilks' $F(3,34) = 7.91$, $P < 0.001$; power 0.98). Further univariate analysis showed that this effect mainly originated from the second polynomial contrast ($F(1,36) = 8.69$, $P < 0.001$; $\epsilon = 0.588$; power 0.91), reflecting a quadratic effect, even though the linear contrast was also significant ($F(1,36) = 4.09$; $P = 0.05$; power 0.51). As can be seen in Fig. 3(b), this quadratic effect

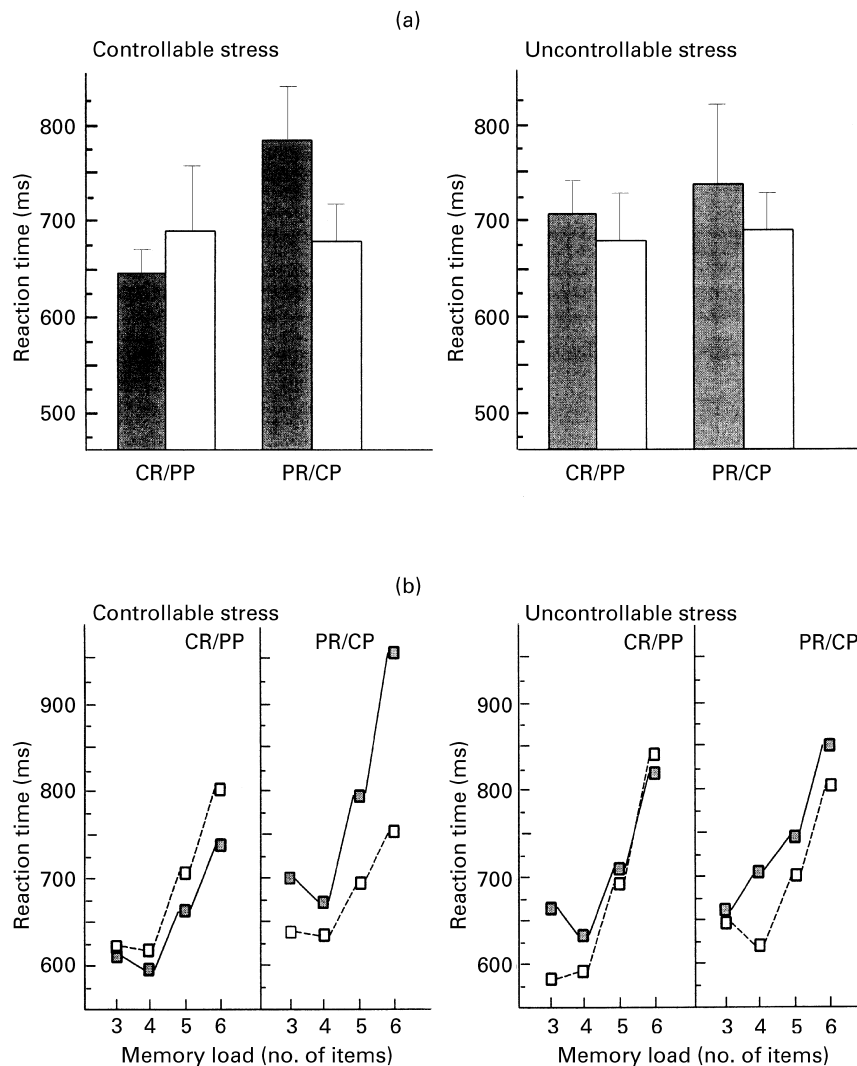


Fig. 3. (a) Reaction times of high-stress-prone subjects (■, $n = 22$) and low-stress-prone subjects (□, $n = 21$) receiving a carbohydrate-rich, protein-poor (CR/PP) diet or a protein-rich, carbohydrate-poor (PR/CP) diet during periods of controllable and uncontrollable stress. Values are means with standard deviations represented by vertical bars. There was a significant diet \times stress-proneness \times stress-controllability interaction ($P = 0.004$). (b) Reaction times of high- (■) and low- (□) stress-prone subjects on the two diets as a function of memory load. Reaction time increased significantly during the last three memory sets of four, five and six items ($P < 0.001$).

seemed to be caused by a learning effect during the first memory set and may have obscured the linear increase in RT during the following three memory sets. To test this, a second multivariate analysis was performed without the first memory set. Again there was a significant effect of memory-load (Wilks' $F(2,35)$ 6.62, $P=0.004$; power 0.89), without an order of stress-controllability effect (Wilks' $F(2,35)$ 0.52, $P=0.60$). This time, the effect of memory-load only originated from a linear increase in RT ($F(1,36)$ 9.31, $P<0.001$; ϵ 0.692; power 0.92).

It was expected that subjects in the HS group would make fewer errors on memory scanning after laboratory stress in the CR/PP diet condition than in the PR/CP condition. Fig. 4(a) shows the mean number of errors across the four sub-tasks. Only in HS subjects did the mean number of errors

appear to be lower during a CR/PP diet than during the PR/CP diet after CS, and, to a lesser extent, after US. Note that HS subjects within the CR/PP diet condition also made fewer errors than LS subjects after CS, and that this was reversed within the PR/CP diet condition. However, these latter observations were not significant ($P>0.25$).

Fig. 4(b) presents the number of errors as a function of memory load. Multivariate ANOVA revealed an effect of memory-load (Wilks' $F(3,35)$ 4.09, $P<0.01$; power 0.85). Further polynomial analysis revealed that this effect originated from a linear increase in the number of errors as a function of memory load ($F(1,37)$ 9.59, $P<0.001$; ϵ 0.75; power 0.85), indicating that all subjects made more errors when more items had to be memorized (see Fig. 4). In HS subjects, there appeared to be marked increase in errors

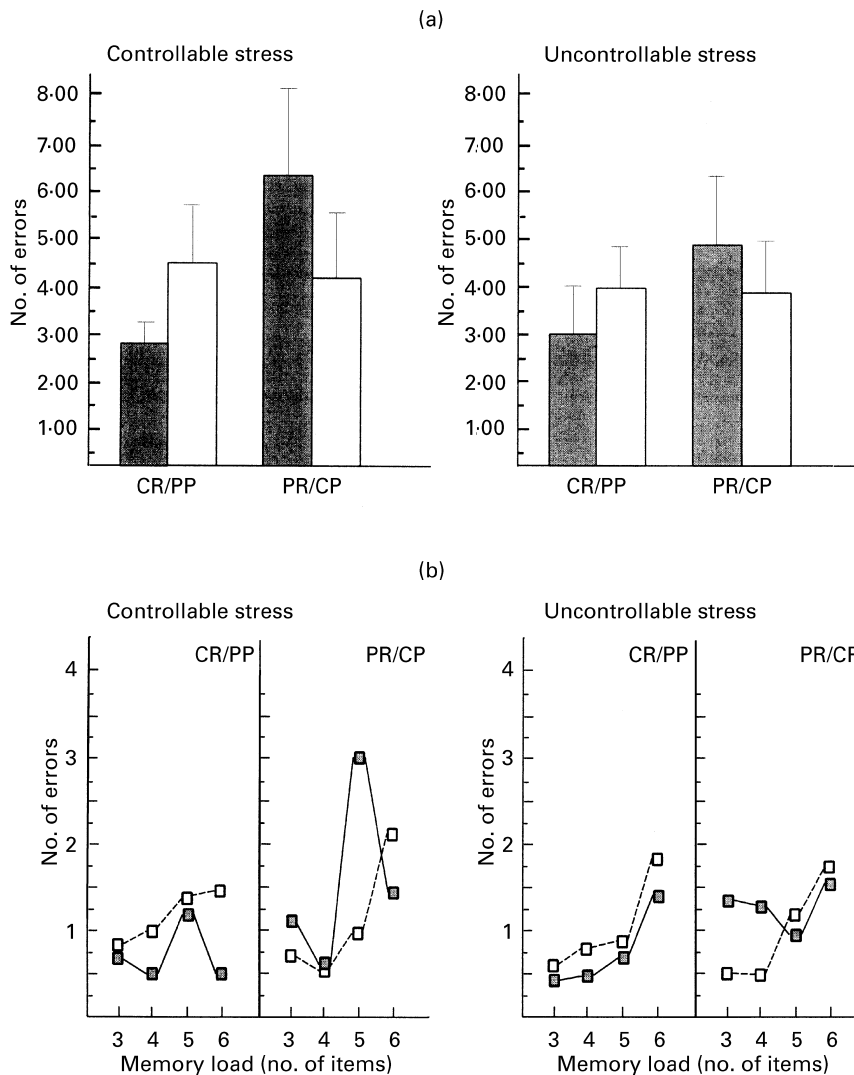


Fig. 4. (a) Number of errors by high-stress-prone subjects (■, n 22) and low-stress-prone subjects (□, n 21) receiving a carbohydrate-rich, protein-poor (CR/PP) diet or a protein-rich, carbohydrate-poor (PR/CP) diet made during a memory scanning task under conditions of controllable and uncontrollable stress. Values are means with standard deviations represented by vertical bars. (b) Number of errors made by high- (■) and low- (□) stress-prone subjects on the two diets as a function of memory load. There was a significant increase in the number of errors at the higher memory loads ($P<0.001$).

when five items had to be memorized, followed by a recovery during the memory set of six items, particularly after CS. However, this latter interaction was not significant ($P > 0.85$).

Discussion

The present study investigated whether a CR/PP diet in HS subjects improves memory scanning following acute stress, depending on the controllability of the stressor. This research question was based on the assumption that in HS subjects there is a chronic stress-induced serotonin deficiency in the brain. As the CR/PP diet previously has been found to increase the plasma Trp : LNAA ratio (Markus *et al.* 1998), a measure indicative of increases in brain tryptophan and serotonin (Fernstrom & Wurtman, 1971*b*; Fernstrom *et al.* 1973), it was assumed that this diet would enhance serotonin function in HS subjects, thereby reducing the negative effects of stress on memory scanning. Accordingly, the present findings reveal that after controllable but not uncontrollable stress, memory scanning in HS subjects improved with a CR/PP diet compared with a PR/CP diet. No such differences were found for LS subjects.

Internal validity

We believe that the effects of diet manipulation on performance in the present study were not caused by differences in food intake, expectations about the food, or insufficient statistical power. Dietary intake was under continuous observation in the laboratory to ensure that all subjects consumed the whole diet and that no other foods were taken. Regarding food expectancies, the literature indicates that it is unlikely that a subject will hold personal expectancies about the behavioural effects of nutrients like carbohydrates (Christensen *et al.* 1985; Spring *et al.* 1987; Rosenthal *et al.* 1989). Moreover, food-related expectancies do not represent a meaningful factor involved in the effect of dietary manipulation on human behaviour (Spring *et al.* 1987). Furthermore, in the present experiment none of the subjects was aware of the fact that the dietary condition was a factor involved in the study, as was established by a brief interview of each subject at the end of the last experimental day. With regard to the statistical power we have to mention that in order to detect a large effect of dietary manipulation on performance with a power of 0.82 at an α level of 0.05, a group size of seventeen subjects is required. This was exceeded in the present study. Furthermore, all significant multivariate effects were accompanied by power values above 0.80. Moreover, all non-significant findings were accompanied by probabilities between 0.22 and 0.89.

Controllability manipulation of the stress task

Scores on the PCQ (measuring experience of control) revealed a successful manipulation of the controllability of the stress task. Although pulse rate and skin conductance significantly increased during both the CS and US conditions, indicating an enhancement of general arousal and effort, there was only a significant reduction in experienced control and mastery during the US task. Similarly, in a study

by Peters *et al.* (1998) lower control experiences as well as increases in cortisol were found exclusively after a US condition. Based on these findings, the US version in the present study may be considered less controllable than the CS version.

Dietary effects on performance

Concerning memory scanning, multivariate analysis showed a significant three-way interaction effect of diet \times stress-proneness \times stress-controllability on the mean RT across all four memory sets ($P = 0.004$). Further testing indicated that this interaction effect originated from faster memory scanning in HS subjects during the CR/PP diet condition after CS compared with US ($P = 0.038$), instead of slower performance during the PR/CP diet condition after CS compared with US ($P = 0.22$). This means that the CR/PP diet in HS subjects improved memory scanning after controllable laboratory stress. There was also a significant linear increase in RT as a function of memory load particularly across the last three memory sets. The first memory set was thought to be subjected to a learning effect, as it caused a quadratic change at the start of the memory task particularly during the first experimental day. The slope of the RT is regarded as an index of a cognitive comparison process involved in scanning an item in short-term memory, whereas the intercept is a measure of both input and output stages of information processing regardless of memory load (Sternberg, 1996, 1975). As there were no dietary effects on the slope of RT, the three-way interaction effect on the mean RT across all subtasks may indicate that HS subjects benefited from a CR/PP diet regarding both input and output stages of information processing after laboratory stress, but only when the stressor was experienced as controllable.

In a previous study, a dietary effect on cognitive performance in HS subjects was not found after high uncontrollable laboratory stress (Markus *et al.* 1998). It was assumed by the authors that due to low memory load conditions, this task might have been too easy to reveal a dietary effect. However, the present results do not confirm such an explanation as we still did not find a dietary effect on memory scanning after US with memory loads of three, four, five and six items. Rather, the effect of a CR/PP diet on performance after acute stress in HS subjects seems to depend on the controllability of the stressor.

Studies that have investigated the direct effects of carbohydrate consumption on performance have yielded inconsistent findings; either showing an improvement (Kanarek & Swinney, 1990), a deterioration (Spring *et al.* 1982/83) or no effect at all (Lieberman *et al.* 1986; Lloyd *et al.* 1996). The results of the present study show that the effect of carbohydrate consumption on performance may be mediated indirectly by stress-proneness during an actual confrontation with stressors that differ in controllability.

Conclusion: explaining the dietary effects on performance

The question remains why memory scanning after stress in HS subjects improves during a CR/PP diet only when the preceding stressor is controllable. First, since the CR/PP diet

used in the present study has previously been found to increase the plasma Trp:LNAA ratio (Markus *et al.* 1998), it is reasonable to assume that in the present study also the CR/PP diet may have increased the supply of available tryptophan and the brain serotonin concentration. Second, because a mental stress-induced increase in cerebral serotonin activity will be accompanied by an enhanced breakdown of this neurotransmitter, there might be more serotonin available after CS than after US. Chronic stress in HS subjects ultimately may induce a shortage of brain serotonin. Because the serotonergic system becomes more sensitive under chronic stress because of compensatory receptor sensitization (Kennett *et al.* 1985; Adell *et al.* 1988; Cancela & Molina, 1990), an increased availability of serotonin after CS during the CR/PP diet condition particularly in HS subjects may have profound beneficial effects on performance. In support of this line of reasoning, the CR/PP diet has previously been found to improve stress coping during high uncontrollable laboratory stress only in HS subjects (Markus *et al.* 1998), suggesting a more enhanced serotonergic functioning in HS subjects than in LS subjects. However, to find evidence for the involvement of the brain serotonergic system as suggested, it is necessary to use a more direct measure of cerebral tryptophan and serotonin alterations.

References

- Adell A, Garcia-Marquez C, Armario A & Gelpi E (1988) Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their responses to a further acute stress. *Journal of Neurochemistry* **50**, 1678–1681.
- Altman HJ & Normile HJ (1988) What is the nature of the role of serotonergic nervous system in learning and memory: prospects for development of an effective treatment strategy for senile dementia. *Neurobiology of Aging* **9**, 627–638.
- Anisman H & Zacharko RM (1991) Multiple neurochemical and behavioral consequences of stressors: implications for depression. In *Psychopharmacology of Anxiolytics and Antidepressants*, pp. 57–82 [SE File, editor]. New York, NY: Pergamon.
- Bellisle F, Blundell JE, Dye L, Fantino M, Fletcher RJ, Lambert J, Roberfroid M, Specter S, Westenhöver J & Westerterp-Plantenga MS (1998) Functional food science and behaviour and psychological functions. *British Journal of Nutrition* **80**, Suppl. 1, S173–S193.
- Bolger N & Schilling EA (1991) Personality and the problems of everyday life: the role of neuroticism in exposure and reactivity to daily stressors. *Journal of Personality* **59**, 355–386.
- Brand N & Houx PJ (1992) MINDS: Towards a computerized test battery for health psychological and neuropsychological assessment. *Behavioural Research Methods, Instrumentation and Computers* **24**, 385–389.
- Bremner JD, Southwick SM, Brett E, Fontana A, Rosenheck R & Charney DS (1992) Dissociation and posttraumatic stress disorder in Vietnam combat veterans. *American Journal of Psychiatry* **149**, 328–332.
- Cancela LM & Molina VA (1990) Reduced apomorphine-induced sedation following chronic stress. *European Journal of Pharmacology* **134**, 117–119.
- Christensen L & Redig C (1993) Effect of meal composition on mood. *Behavioral Neuroscience* **107**, 346–353.
- Christensen L, White B & Krietsch K (1985) Failure to identify an expectancy effect in nutritional research. *Nutrition and Behavior* **2**, 149–159.
- Cohen S, Evans G, Krantz G & Stokals D (1980) Physiological, motivational, and cognitive effects of noise in children. *American Psychologist* **35**, 231–245.
- Curzon G (1985) Effects of food intake on brain transmitter amine precursors and amine synthesis. In *Psychopharmacology and Food*, pp. 59–70 [M Sandler and T Silverstone, editors]. Oxford: Oxford University Press.
- Deakin JWF (1991) Depression and 5-HT. *International Clinical Psychopharmacology* **3**, 23–28.
- Deakin JWF & Graeff FG (1991) 5-HT and mechanisms of defence. *Journal of Psychopharmacology* **5**, 305–315.
- Deary L & Matthews G (1993) Personality traits are alive and well. *Psychologist* **6**, 299–311.
- Delbende C, Delarue C, Lefebvre H, Tranchand-Bunel D, Szafrarczyk A, Mocaer E, Kamoun A, Jegou S & Vaudry H (1992) Glucocorticoids, transmitters and stress. *British Journal of Psychiatry* **160**, Suppl. 15, 24–34.
- Eysenck MW (1982) *Attention and Arousal*. Berlin: Springer-Verlag.
- Fernstrom JD, Larin F & Wurtman RJ (1973) Correlations between brain tryptophan and plasma neutral amino acids levels following food consumption in rats. *Life Sciences* **13**, 517.
- Fernstrom JD & Wurtman RJ (1971a) Brain serotonin content: physiological dependence on plasma tryptophan levels. *Science* **173**, 149–152.
- Fernstrom JD & Wurtman RJ (1971b) Brain serotonin content: increase following ingestion of carbohydrate diet. *Science* **174**, 1023–1025.
- Fernstrom JD & Wurtman RJ (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science* **178**, 414–416.
- Gallagher DJ (1990) Extraversion, neuroticism and appraisal of stressful academic events. *Personality and Individual Differences* **11**, 1053–1058.
- Hockey GR & Hamilton P (1983) The cognitive patterning of stress states. In *Stress and Fatigue in Human Performance*, pp. 331–363 [GRJ Hockey, editor]. New York, NY: John Wiley & Sons.
- Joseph MH & Kennett GA (1983) Stress-induced release of 5-HT in the hippocampus and its dependence on increased tryptophan availability: an in vivo electrochemical study. *Brain Research* **270**, 251–257.
- Kanarek RB & Swinney D (1990) Effects of food snacks on cognitive performance in male college students. *Appetite* **14**, 15–27.
- Kennett GA, Dickinson SL & Curzon G (1985) Enhancement of some 5-HT-dependent behavioral responses following repeated immobilisation in rats. *Brain Research* **330**, 253–263.
- Kramer TH, Buckhout R, Fox P, Widman E & Tusche B (1991) Effects of stress on recall. *Applied Cognitive Psychology* **5**, 483–488.
- Lieberman HR, Caballero B & Finer N (1986) The composition of lunch determines afternoon plasma tryptophan ratios in humans. *Journal of Neural Transmission* **65**, 211–217.
- Lloyd HM, Rogers PJ & Hedderley DI (1996) Acute effects on mood and cognitive performance of breakfast differing in fat and carbohydrate content. *Appetite* **27**, 151–164.
- Loeb M (1986) *Noise and Human Efficiency*. Chichester: John Wiley & Sons.
- Luteijn F & Bouman TK (1988) The concepts of depression, anxiety and neuroticism in questionnaires. *European Journal of Personality* **2**, 113–120.
- Luteijn F, Starren J & van Dijk H (1975) *Nederlandse Persoonlijheids Vragenlijst (Dutch Personality Inventory)*. The Netherlands: Swets & Zeitlinger.
- Mandler G (1984) *Mind and Body; Psychology of Emotion and Stress*. New York, NY: Norton.

- Markus CR, Panhuysen G, Tuiten A, Koppeschaar H, Fekkes D & Peters M (1998) Does carbohydrate-rich, protein-poor food prevent a deterioration of mood and cognitive performance of stress-prone subjects when subjected to a stressful task? *Appetite* **31**, 49–65.
- Marmar CR, Weiss DS, Schlenger WE, Fairbank JA, Jordan BK, Kulka RA & Hough RL (1994) Peritraumatic dissociation and posttraumatic stress in male Vietnam theater veterans. *American Journal of Psychiatry* **151**, 902–908.
- Peters ML, Godaert GLR, Ballieux RE, van Vliet M, Willemsen JJ, Sweep CGJ & Heijnen CJ (1998) Cardiovascular and catecholamine response to experimental stress: effects of mental effort and controllability. *Psychoneuroendocrinology* **23**, 1–17.
- Pijl H, Koppeschaar H, Cohen AF, Iestra JA, Schoenmaker HC, Frölich M, Onkenhout W & Meinders AE (1993) Evidence for brain serotonin-mediated control of carbohydrate consumption in normal weight and obese humans. *International Journal of Obesity* **17**, 513–520.
- Rosenthal NE, Genhart MJ, Caballero B, Jacobsen FM, Skwerer RG, Coursey RD, Rogers S & Spring B (1989) Psychobiological effects of carbohydrate- and protein-rich meals in patients with seasonal affective disorder and normal controls. *Biological Psychiatry* **25**, 1029–1040.
- Smith A (1990) Stress and information processing. In *Stress and Medical Procedures*, pp. 58–79 [M Johnston and L Wallace, editors]. Oxford: Oxford University Press.
- Sorg BA & Whitney P (1992) The effect of trait anxiety and situational stress on working memory capacity. *Journal of Research in Personality* **26**, 235–241.
- Spont MR (1992) Modulatory role of serotonin in neural information processing: implications for human psychopathology. *Psychological Bulletin* **112**, 330–350.
- Spring B, Chiodo J & Bowen DJ (1987) Carbohydrates, tryptophan and behavior: a methodological review. *Psychological Bulletin* **102**, 234–256.
- Spring B, Maller O, Wurtman J, Digman L & Gozolino L (1982/83) Effects of protein and carbohydrate meals on mood and performance: interactions with sex and age. *Journal of Psychiatric Research* **17**, 155–167.
- Stanford SC (1993) Monoamines in response and adaptation to stress. In *Stress, From Synapse to Syndrome*, pp. 24–30 [SC Stanford and P Salmon, editors]. London: Academic Press.
- Stelmack RM, Wieland LD, Wall MU & Plouffe L (1984) Personality and the effects of stress on recognition memory. *Journal of Research in Personality* **18**, 164–178.
- Sternberg S (1969) Memory-scanning: mental processes revealed by reaction-time experiments. *American Scientist* **57**, 421–457.
- Sternberg S (1975) Memory scanning: new findings and current controversies. *Quarterly Journal of Experimental Psychology* **27**, 1–32.
- Tuiten A, Panhuysen G, Koppeschaar H, Fekkes D, Pijl H, Frölich M, Krabbe P & Everaerd W (1995) Stress, serotonergic functioning, and mood in users of oral contraceptives. *Psychoneuroendocrinology* **20**, 1–11.
- Watson D & Clark LA (1984) Negative affectivity: the disposition to experience aversive emotional states. *Psychological Bulletin* **96**, 465–490.
- Wells A & Matthews G (1994) *Attention and Emotion: A Clinical Perspective*. London: Lawrence Erlbaum Associates Publishers.
- Wurtman RJ (1987) Nutrients affecting brain composition and behavior. *Integrative Psychiatry* **5**, 226–257.
- Yokogoshi H & Wurtman RJ (1986) Meal composition and plasma amino acid ratios: effects of various proteins or carbohydrates, and of various protein concentrations. *Metabolism* **35**, 837–842.
- Young SN (1991) Some effects of dietary components (amino acids, carbohydrate, folic acid) on brain serotonin synthesis, mood, and behaviour. *Canadian Journal of Physiology and Pharmacology* **69**, 893–903.