The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain

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Objective: We developed a protocol for inducing moderate psychologic stress in a functional imaging setting and evaluated the effects of stress on physiology and brain activation. **Methods:** The Montreal Imaging Stress Task (MIST), derived from the Trier Mental Challenge Test, consists of a series of computerized mental arithmetic challenges, along with social evaluative threat components that are built into the program or presented by the investigator. To allow the effects of stress and mental arithmetic to be investigated separately, the MIST has 3 test conditions (rest, control and experimental), which can be presented in either a block or an event-related design, for use with functional magnetic resonance imaging (fMRI) or positron emission tomography (PET). In the rest condition, subjects look at a static computer screen on which no tasks are shown. In the control condition, a series of mental arithmetic tasks are displayed on the computer screen, and subjects submit their answers by means of a response interface. In the experimental condition, the difficulty and time limit of the tasks are manipulated to be just beyond the individual's mental capacity. In addition, in this condition the presentation of the mental arithmetic tasks is supplemented by a display of information on individual and average performance, as well as expected performance. Upon completion of each task, the program presents a performance evaluation to further increase the social evaluative threat of the situation. **Results:** In 2 independent studies using PET and a third independent study using fMRI, with a total of 42 subjects, levels of salivary free cortisol for the whole group were significantly increased under the experimental condition, relative to the control and rest conditions. Performing mental arithmetic was linked to activation of motor and visual association cortices, as well as brain structures involved in the performance of these tasks (e.g., the angular gyrus). **Conclusions:** We propose the MIST as a tool for investigating the effects of perceiving and processing psychosocial stress in functional imaging studies.

Objectif : Nous avons mis au point un protocole afin de provoquer un stress psychologique moyen en contexte d'imagerie fonctionnelle et nous en avons évalué l'effet sur la physiologie et l'activation cérébrale. **Méthodes :** Dérivée de l'épreuve de provocation mentale Trier, la tâche du stress d'imagerie de Montréal (MIST) consiste en une série de provocations sous forme de calcul mental informatisé comportant des éléments de menace d'évaluation par la société et intégrées dans le programme ou présentées par le chercheur. Pour pouvoir étudier séparément les effets du stress et du calcul mental, l'épreuve MIST comporte trois conditions d'essai (repos, contrôle, contexte expérimental) qu'il est possible de présenter en bloc ou selon un concept relié aux événements et d'utiliser avec l'imagerie par résonnance magnétique fonctionnelle (IRMf) ou la tomographie par émission de positrons (TEP). Au repos, les sujets regardent un écran d'ordinateur statique sur lequel aucune tâche n'apparaît. En condition contrôlée, on affiche à l'écran de l'ordinateur une série de tâches de calcul mental et les sujets soumettent leurs réponses au moyen d'une interface de réponse. En contexte expérimental, on manipule la difficulté des tâches et le temps qui y est consacré pour qu'elles dépassent à peine la capacité mentale du sujet. Dans cette condition, on complète la présentation des tâches de calcul mental en affichant de l'information sur le sujet et son rendement moyen, ainsi que sur le rendement attendu. Lorsque chaque tâche est terminée, le programme présente une évaluation du rendement afin d'accroître encore davantage la menace d'évaluation par la société que présente la situation. **Résultats :** Au cours de deux études indépendantes pendant lesquelles on a utilisé la TEP et d'une troisième où l'on a utilisé l'IRMf, pour un total de 42 sujets, les concentrations de

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cortisol libre dans la salive pour tout le groupe étaient beaucoup plus élevées en contexte expérimental qu'en contexte de contrôle et au repos. On a établi un lien entre le calcul mental et l'activation des cortex moteur et d'association visuelle, ainsi que des structures cérébrales qui interviennent dans l'exécution de ces tâches (p. ex., le gyrus angulaire). **Conclusions :** Nous proposons l'épreuve MIST

Introduction

Associated with adverse effects on cardiovascular, immune, metabolic and psychological variables, stress is considered to have a significant impact on population health.[1] Investigating situations and variables that are perceived as stressful might therefore allow researchers to uncover some of the mechanisms that link stress to adverse health. In humans, one of the key physiologic markers of stress is activation of the hypothalamic–pituitary–adrenal (HPA) axis, with the accompanying secretion of corticotropin-releasing hormone from the hypothalamus, adrenocorticotropic hormone (ACTH) from the pituitary and cortisol from the adrenal cortex. Psychosocial stress has been identified as among the most powerful type of stress in activating the HPA axis.[2] In a recent review, social evaluative threat was identified as one of the key components of psychosocial stress.[3]

To investigate stress in the laboratory, paradigms that have been used effectively in the past include the Trier Social Stress Test,[4] consisting of public speaking and oral mental arithmetic, and the Trier Mental Challenge Test,[5] consisting of computerized mental arithmetic with negative feedback. Dependent measures obtained in conjunction with these tests usually include physiologic or endocrinologic markers of stress, e.g., changes in heart rate or in ACTH and cortisol levels. Direct assessment of changes in brain activation in response to stress has so far been impossible because of a lack of appropriate protocols to induce and measure stress in functional imaging environments. To overcome this limitation, we have developed the Montreal Imaging Stress Task (MIST). Based on the Trier Mental Challenge Test, the MIST comprises a series of computerized mental arithmetic tasks with an induced failure component. The protocol was developed to fit the constraints of the imaging environment in terms of stimulus content and duration of presentation and to incorporate social evaluative threat components, which are built into the program and brought on by the investigator.

In this article, we describe the MIST protocol and summarize its effects on physiologic and brain activation changes previously investigated in 3 independent studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI).

Methods

Subjects

For all studies, subjects were recruited by posting flyers at local university buildings and through advertisement in local newspapers. Upon initial contact, a telephone interview was conducted to establish demographic information and medical history. Exclusion criteria consisted of past or current depression, past head injuries with subsequent loss of consciousness and any known past occurrences of dysregulation of the HPA, the hypothalamic–pituitary–gonadal axis or the hypothalamic–pituitary–thyroid axis.

In study 1, 10 healthy young individuals (1 woman and 9 men, 21–30 years of age) were invited to participate in a PET study to investigate dopamine release as indicated by [^{11}C]raclopride receptor binding in the ventral striatum. In study 2, 10 healthy male college students (20–25 years of age) were recruited to participate in a PET study to investigate the effects of acute stress perception and processing in the human brain on cerebral blood flow using [^{15}O]H_2O. Finally, in study 3, we recruited 22 healthy young men (20–24 years of age) to investigate the effects of acute stress perception using fMRI.

Montreal Imaging Stress Task

The MIST protocol consists of a training session, conducted outside the imaging unit, and a test session, during which the functional images are acquired. Depending on the imaging method used, up to 3 different conditions (rest, control and experimental) are implemented.

The principal component of the MIST is a computer program that displays a mental arithmetic task, a rotary dial for submission of a response, a text field that provides feedback on the submitted response ("correct," "incorrect" or "timeout") and 2 performance indicators, one for the individual subject's performance and one for average performance of all subjects (Fig. 1). In the experimental condition, a time limit is enforced for each task; the elapsed time is displayed by a progress bar moving from left to right on the computer screen, with the exact time allowed for each task depending on the subject's previous performance (Fig. 1).

The program was developed using the SuperCard application for Mac OS X (Solutions Etcetera, Pollock Pines, Calif.). The basic algorithm of the program creates mental arithmetic tasks using up to 4 numbers ranging from 0 to 99 and up to 4 operands (+ for addition, – for subtraction, * for multiplication and / for division). The algorithm has been designed to automatically create tasks for which the solution will be an integer between 0 and 9, such that a single keystroke is needed for the response. A difficulty gradient for the mental arithmetic is built into the algorithm, with 5 different categories. In the 2 easiest categories, only tasks with 2 or 3 one-digit integers are created, and the operands are limited to + or – (example: 2 + 9 – 7). In the medium-difficulty categories, tasks with up to 4 integers are created, with up to 2 of these integers in the 2-digit range, and the * operand is also allowed (example: 3 * 12 – 29). Finally, in the fifth and most difficult category, tasks with 4 integers are created, the * and / operands are used, and all numbers may be in the 2-digit range (example: 12 * 12 / 8 – 9).

A subject selects a number on the rotary dial either by pressing the left or right arrow keys on the keyboard (during the training session, outside of the imaging unit) or by pressing the left or right mouse buttons on a scanner-compatible USB keyboard response box (when inside the scanner). Pressing the left arrow or left mouse button moves the highlighted number on the rotary dial of the program's user interface counterclockwise, whereas pressing the right arrow key or mouse button moves the highlighted number on the rotary dial of the program's user interface clockwise (see Fig. 1). Pressing the down arrow key or the middle mouse button submits the highlighted number on the rotary dial as the subject's response to the arithmetic task. This response is then compared with the correct answer for the task, and the appropriate feedback ("correct" or "incorrect") is presented in the feedback field of the computer screen. If no response is recorded within the time limit, the response "timeout" is displayed.

In the training session (outside the imaging unit), the subject's ability to perform mental arithmetic is assessed by recording the average time needed to solve problems at various difficulty levels. For this purpose, no time limit is enforced, and no time progress bar is shown on the screen. In addition, no performance indicators (for the subject's own performance or the average performance of all subjects) are displayed. However, the recorded time is used to set a default time limit for the experimental condition. The testing session must be at least 2 minutes long for the program to determine the average time the subject takes to perform mental arithmetic in the different categories; usually, the subject is given 5 minutes to practise the program before the imaging session.

During each experimental session (inside the imaging unit), the program is set to a time limit that is 10% less than the subject's average response time; this approach induces a high failure rate. In addition, the program continuously records the subject's average response time and the number of correct responses. If the subject answers a series of 3 consecutive mental arithmetic tasks correctly, the program reduces the time limit to 10% less than the average time for the 3 correctly solved tasks. Conversely, if the subject answers a series of 3 consecutive tasks incorrectly, the program increases the time limit for the following tasks by 10%. As such, under experimental conditions, a range of about 20% to 45% correct answers is enforced. Individual runs last between 2 and 6 minutes, depending on the scanning method (PET or fMRI) and the paradigm (receptor-binding or cerebral blood flow for PET scanning, event-related or block design for fMRI). During the experimental sessions, the colour bar at the top of the screen shows 2 performance indicators, for the subject's own performance and average performance of all subjects. The average performance arrow appears in the green area, at the right of the screen, while the subject's individual performance usually appears in the red area, to the left of the screen. Between experimental runs, the investigator informs the subject about his or her performance, reminding him or her that the average performance is about 80%–90% correct answers. The subject is then reminded that there is a required minimum performance and that his or her individual performance must be close or equal to the average performance of all subjects if his or her data are to be used in the study. The subject is told about the need for standard performance across users to allow the data to be grouped and is reminded about the correct use of the response box for submitting responses. Finally, the subject is told that all of the people in the scanner room (investigator, assistants, MR technicians) are following the subject's performance on a second monitor in the control room of the scanning environment. The investigator then leaves the room, and the next run of mental arithmetic is initiated.

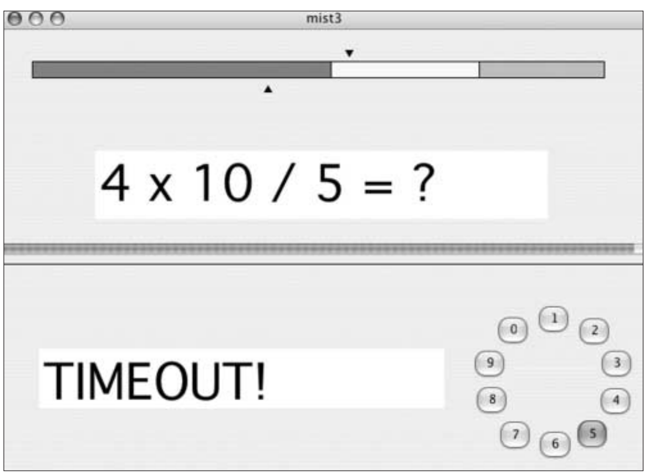

Fig. 1: Graphical user interface of the Montreal Imaging Stress Task (MIST). From top to bottom, the figure shows the performance indicators (top arrow = average performance, bottom arrow = individual subject's performance), the mental arithmetic task, the progress bar reflecting the imposed time limit, the text field for feedback, and the rotary dial for the response submission.

During the control condition, the program tries to match for any cerebral activation caused by the mental arithmetic aspects of the task, but without the stress components. For that purpose, mental arithmetic is presented with the same level of difficulty and at the same frequency as during the experimental sessions, but no time restriction is enforced, and individual performance and average users' performance are not displayed. To match the frequency of mental arithmetic tasks, the time between tasks is varied as a function of the time limit imposed during the experimental condition, so that the total number of tasks presented per condition is identical. Feedback ("correct" or "incorrect") is still shown after each task, but because of the absence of a time limit, average performance increases to about 90%. In between runs, which last between 2 and 6 minutes (i.e., the same as in the experimental condition; see above), the investigator tells the subject to try and perform the task as quickly and accurately as possible, but also states that his or her performance is not being evaluated because this is a control condition.

Finally, during the rest condition, which records a baseline state of cerebral activation, the interface of the computer program remains on the screen, but no tasks are shown. Subjects are told not to move the mouse until the next mental arithmetic task appears. The rest condition varies between 1 and 3 minutes, depending on the imaging method and paradigm used.

Saliva sampling procedure

Cortisol is acquired from saliva samples collected during imaging. Because of the constraints of the neuroimaging environment, a special saliva sampling procedure has been developed.

In the fMRI environment, access to the subject is limited, since he or she is confined to a cylindrical tube. To collect the saliva samples, the scanner bench is moved outside of the cylindrical tube far enough to allow the subject's head to be reached, but the bench is not moved completely out of the scanner. This way, the subject's position remains memorized by the scanner, and the subject can be returned to his or her original position inside the scanner for subsequent runs, without having to repeat localizer and structural scans. The subject is told that a salivette will be put into his or her mouth for the purpose of saliva sampling, but is instructed to refrain from chewing on the salivette (to avoid head movement). With sterile gloves, the investigator then places the sterile salivette into the subject's mouth. Without chewing, a period of 2 minutes is usually sufficient to saturate the salivette with up to 3 mL of saliva. During this time, the investigator provides feedback to the subject, as described above. After 2 minutes, the subject is asked to expel the salivette using the tip of the tongue, so that the investigator can remove it from the subject's lips and place it back into the sterile plastic tube. The investigator or a technician then moves the subject back to the original position in the scanner. The investigator then leaves the room in the case of subsequent scanning runs. This procedure is repeated for each saliva sample obtained during the scanning.

In the PET environment, the investigator has better access to the subject. With sterile gloves, the investigator puts the salivette into the subject's mouth without moving the bench. The salivette is allowed to saturate with saliva for about 2 minutes, at which point the subject expels the salivette using his or her tongue, and the investigator places it back into the sterile tube. This procedure is repeated for each saliva sample obtained during scanning.

Study design

In the first PET study, [^{11}C]raclopride was used as a tracer for investigation of dopamine release from the basal ganglia, and subjects were scanned twice on 2 separate days. On the day of scanning under the experimental condition, the 10 participants completed a 5-minute test session before the scan and five 6-minute runs of the MIST, with 2-minute feedback intervals between the runs, while being scanned for [^{11}C]raclopride binding. In the PET environment, mental arithmetic tasks are presented on a monitor that is placed outside of the scanner above and in front of the subject's head, at an angle of about 30° and about 150 cm away. The MIST program is operated using a 3-button mouse placed beneath the subject's right hand. During scanning under the rest condition (on the separate day), the subjects simply kept their eyes closed. The order of rest and stress scanning days was counterbalanced across subjects. For each scan, 8–10 mCi of [^{11}C]raclopride was injected into the left antecubital vein over a period of more than 1 minute. The subjects were engaged in mental arithmetic continuously from 10 minutes before to 28 minutes after the [^{11}C] raclopride injection, except for the 2-minute feedback blocks between each 6-minute math task block. Furthermore, starting at the time of injection of the PET tracer, saliva samples were taken every 12 minutes throughout the experiment. Cortisol shows a diurnal rhythm, with peak levels in the morning (shortly after the time of awakening), steadily declining levels during the day and a nadir around midnight. To control for circadian changes in cortisol levels, we tested all subjects at a similar time, in the late afternoon.

In the second PET study, [^{15}O]H_2O was used as a tracer for investigation of changes in cerebral blood flow in response to the MIST protocol. Because of the short half-life of [^{15}O]H_2O, changes in cerebral blood flow are detectable for about 1 minute, and injections can be repeated at 10-minute intervals. Thus, in this study, we implemented a third, control condition (user interface of the MIST program without any tasks being shown), and repeated each condition 3 times: 3 consecutive sessions of the experimental (stress) condition, 3 consecutive sessions of the control condition and 3 consecutive rest sessions. During the rest sessions, subjects were asked to lie still and look at the empty screen. The tracer was injected at approximately the 1st, 12th and 23rd minutes within each condition. The order of the experimental, control and rest conditions was counterbalanced among subjects. In the event that the stress condition was not the last condition to be completed, an extra 20-minute period of rest was added after the experimental condition to avoid elevated cortisol levels having any effect on further testing. The [^{15}O]H_2O bolus technique was used to measure regional blood flow.[6] Individual conditions were initiated 1 minute before injection of the tracer, to ensure that the subject was engaged in the task during the measurement period. The subject continued with the task for an additional 4 minutes after the 1-minute scan. This was followed by 4 minutes of rest with the eyes open. The whole procedure was repeated 9 times, 3 times for each condition. Saliva samples were obtained before and after the rest condition, before and after mental arithmetic without stress (control condition), and before and after mental arithmetic with stress (experimental condition), for a total of 6 samples. The time between saliva samples within each condition was approximately 30 minutes. Again, to control for the circadian rhythm of cortisol, all subjects were tested in the early afternoon.

In the fMRI study, the 3 conditions (rest, control and experimental) were arranged in a block paradigm, with the 3 conditions alternating throughout 3.5 minutes. Each run consisted of 2 rest, 2 control and 2 experimental conditions, and lasted a total of 7 minutes. Between runs, the investigator gave the subject negative verbal feedback about his or her performance and insisted on the importance of calculations being performed accurately. In this study, the rest condition showed the program interface without showing a task or any of the feedback elements, and subjects were instructed to not close their eyes. In the fMRI paradigm, the scanning session started with a high-resolution T1-weighted anatomic scan (1 × 1 × 1 mm) for a period of 15 minutes. During the next 27 minutes, T2*-weighted volumes with blood oxygenation level-dependent (BOLD) contrast were obtained for fMRI scanning according to the Mosaic 64 sequence. A total of 7 saliva samples were taken, starting at 30 minutes before the onset of the 3 fMRI runs and continuing until 40 minutes after the MIST runs. The samples were taken 20 minutes apart, except for the last 2 measurements (outside of the scanner), which were taken 10 minutes apart. All subjects were tested at a similar time, in the late afternoon.

In all 3 studies, after completion of the test session, the subjects were debriefed and told that the task had been specifically designed to be out of reach of their mental capacity and that the study was not an assessment of their ability to perform mental arithmetic. In all studies, the saliva samples were analyzed for cortisol using a time-resolved fluorescence immunoassay.[7] Intra- and inter-assay variability were less than 10% and 12%, respectively.

PET and fMRI data acquisition and analysis

In both PET studies, the images were acquired with a CTI-Siemens HR+ 63-slice tomography unit (Siemens AG, Erlangen, Germany) operating in 3-dimensional acquisition mode, yielding images with approximate resolution of 4.6 mm full width at half maximum. An MRI scan for anatomic registration of PET data was acquired for each subject on separate days (for technical specifications of the MRI scanner, see below). The PET frames were summed, coregistered with the individual MRI scans and transformed into standardized stereotaxic space[8] by means of an automated feature-matching algorithm to an average brain template.

For the fMRI study, subjects were scanned on a 1.5-T Siemens Magnetom Vision Scanner (Siemens AG, Erlangen, Germany). Twenty-eight axial slices 4 mm thick were acquired in an interleaved design in an angle along the long axis of the hippocampus (in-plane resolution 4 × 4 mm; field of view 256 mm, TR 2.5 seconds, flip angle 90°, TE 50 ms). Each run consisted of 168 acquisitions. The fMRI images were processed with a 6-mm Gaussian smoothing kernel and were motion corrected with alignment to the third frame of each run.

Voxelwise [^{11}C]raclopride binding potential was calculated to generate statistical parametric images of change in binding in the first PET study.[9] The statistical *t*-map threshold was calculated by using the random field theory,[10] a method that corrects for multiple comparisons. We calculated that a threshold of $t = 4.01$ would be equivalent to $p = 0.05$ corrected for multiple comparisons.

For analysis of the [^{15}O]H_2O PET study, the in-house software package *dot* was used for the statistical analysis of images.[11] Three comparisons (experimental versus control, experimental versus rest and control versus rest) were conducted to determine task-specific changes in regional cerebral blood flow (rCBF). The critical *t* values were found to be 4.5 ($p < 0.05$, corrected) with a 14-mm FWHM blurring kernel.

Finally, statistical analysis of fMRI images was performed with the in-house software packages *fmristat* and *multistat*.[12] As with the [^{15}O]H_2O PET study, 3 comparisons (experimental versus control, experimental versus rest and control versus rest) were analyzed and *t* maps generated. Statistical analysis revealed a *t*-map threshold of $t > 4.5$ ($p < 0.05$, corrected) for individual peaks.

Statistical analysis of endocrine data

In the [11C]raclopride PET study, a 2-factor (condition by time) mixed-design analysis of variance (ANOVA), with the 6 cortisol samples as dependent variables, was conducted to establish the difference in cortisol levels between the experimental and rest conditions. In the second PET study, a 2-factor (condition by time) mixed-design ANOVA, with the 6 cortisol samples as dependent variables, was conducted to investigate the effect of the 3 conditions on cortisol levels. In the fMRI study, a 1-factor (time) repeated-measures ANOVA, with the 7 cortisol samples as dependent variables, was conducted to investigate the effects of the 3 conditions on cortisol levels.

Results

In the [^{11}C]raclopride PET study, we found a significant difference in cortisol levels between the 2 scanning sessions (experimental versus rest) ($F = 43.9$, df = 8, $p < 0.001$) (Fig. 2). Because the order of experimental and rest scans was counterbalanced between subjects, we also tested whether the order of the presentation influenced the cortisol response to the stress task. The corresponding 2-factor (order by time) mixed-design ANOVA, with the cortisol levels as dependent variables, failed to show a significant effect for the order of the presentation ($F < 1$, $p > 0.20$).

In the examination of the effects of the stress condition on brain activity, PET data revealed that [^{11}C]raclopride binding potential in the bilateral ventral striatum was significantly lower during the stress session than the rest session, which implies a task-related increase of extracellular levels of dopamine. These results have been reported in detail elsewhere.[9]

In the [^{15}O]H_2O PET study, statistical analysis revealed a significant increase in saliva cortisol levels in response to the experimental sessions ($F = 12.46$, $p < 0.001$), which implies that cortisol levels changed significantly over time (Fig. 3). Post hoc analysis with Tukey's highly significant difference test revealed that the post-stress level was significantly

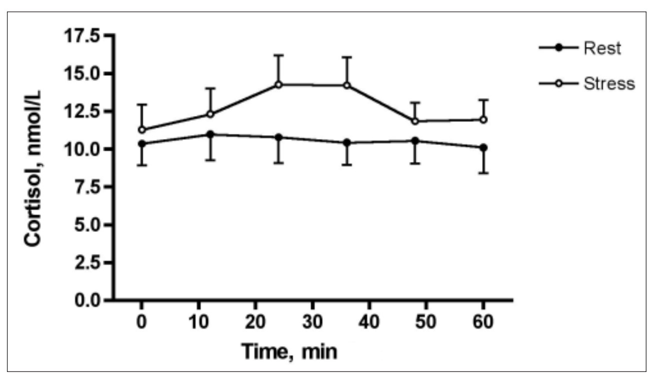

Fig. 2: Cortisol response to the MIST compared with the rest condition in positron emission tomography (PET) study 1. Ten subjects were scanned twice on 2 separate days. Six saliva samples were taken during both the experimental (stress) condition and the rest condition, starting at the time of injection of the PET tracer (time 0) and every 12 minutes thereafter throughout the experiment. The values represent cortisol levels as mean and standard error.

higher than the pre-stress level, which suggests that the stress condition led to a significant increase in cortisol secretion. The counterbalancing of rest, control and experimental conditions across subjects did not seem to affect cortisol responses to the different conditions ($F < 1$, $p > 0.20$). To examine changes in rCBF in response to the 3 different conditions, we investigated the contrasts experimental minus control, experimental minus rest and control minus rest, to assess the effects of stress, of both stress and mental arithmetic, and of mental arithmetic, respectively. We observed significant activation in the area of the visual association cortex and the angular gyrus, as a result of both mental arithmetic and stressful mental arithmetic. In comparing the experimental or control condition with the rest condition (to examine changes in brain activity as a result of doing mental arithmetic per se), we observed additional activation in the motor cortex and the frontal cortex. The threshold for the significant t value in these comparisons was $t > 4.5$ and $t < -4.5$, respectively.

Finally, in the fMRI study, statistical analysis revealed that the stressful mental arithmetic also led to a significant rise in cortisol in the overall group ($F = 4.34$, $p < 0.05$; Fig. 4). In terms of induced changes in brain activity, patterns similar to those of the previous PET study were observed. In comparing the experimental condition with the rest condition, we observed activation of the visual association cortices, angular cortex, sensory cortex, motor cortex, thalamus and caudate nucleus, which represents the main effect of performing the task on changes in brain activation (Fig. 5). When examining the effect of mental arithmetic on brain activity (by comparing the control condition with the rest condition), we found activation in the posterior cingulate, angular, motor and visual association cortices. The threshold for significant t values in these comparisons was $t > 4.5$.

The main effect of stress (experimental minus control condition) will be reported in detail elsewhere (manuscript in preparation).

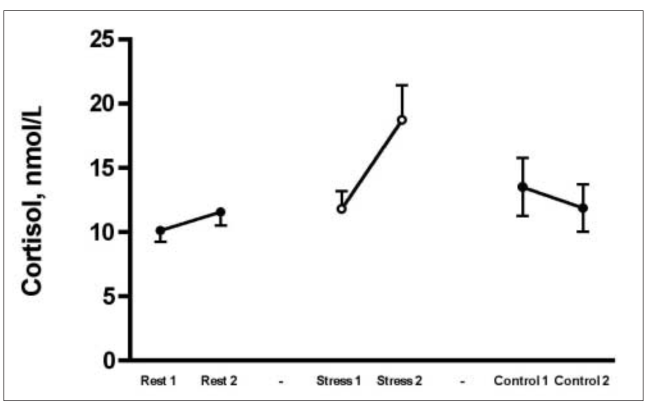

Fig. 3: Cortisol response to the MIST compared with control and rest conditions in PET study 2. Ten subjects underwent testing under 3 conditions on the same day: rest, control and experimental (stress), with 3 sessions for each condition. The order of sessions was counterbalanced between subjects (not shown). Six saliva samples were collected during the experiment, one before and one after each condition (time 1 and time 2). The values represent cortisol levels as mean and standard error.

Discussion

We investigated the capacity of a computerized stress task, the MIST, to induce psychosocial stress in the context of functional imaging in 3 independent studies. In all 3 studies, the participants were young, healthy college students. The subjects underwent scanning sessions during which they were asked to complete difficult mental arithmetic problems presented on a computer screen, while being given immediate negative feedback on their performance by the computer program, followed by negative feedback provided directly by the investigator between scanning runs. These experimental sessions were counterbalanced with control or rest conditions, which consisted of mental arithmetic performed without negative feedback or looking at the user interface, respectively. Examination of the accompanying cortisol levels indicates that the MIST led to a significant increase in cortisol levels for the whole group in all 3 studies, suggesting that the task is indeed perceived as stressful.

To the best of our knowledge, this is the first report of an imaging paradigm investigating cortisol stress responses with the capability of inducing elevations in cortisol levels. A number of conclusions can be drawn from these studies. First, it is possible to induce psychosocial stress in the context of a scanning environment. Despite the absence of humans during the presentation of the task, constant feedback from the computer program, combined with intermittent feedback from the investigator, and the reminder that the investigator and colleagues are monitoring the subject's performance even when outside the room seem to create a social evaluative threat situation capable of inducing an overall cortisol stress response. At the same time, it is possible that at least the fMRI studies might in general be perceived as stressful by

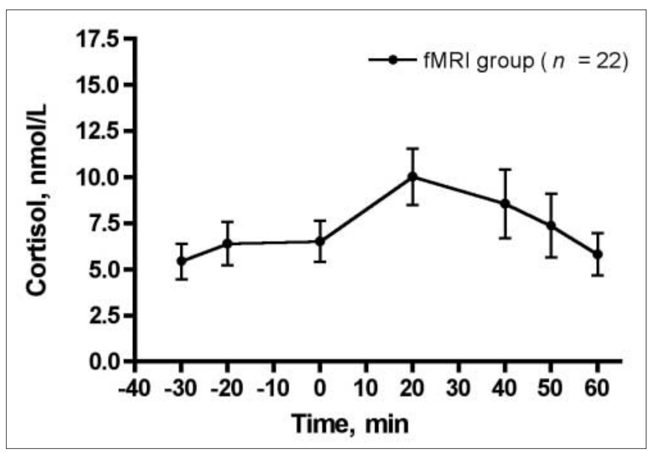

Fig. 4: Cortisol response to the MIST in the functional magnetic resonance imaging (fMRI) (study 3). Twenty-two subjects were exposed to 3 MIST fMRI runs. Each run consisted of 2 rest, 2 control and 2 stress conditions (ABCABC design). A total of 7 saliva samples were obtained, starting at 30 minutes before the onset of the 3 MIST runs (time 0) and continuing until 40 minutes after the MIST runs. The samples were taken 20 minutes apart, except for the last 2 measurements (obtained with the subject outside the scanner), which were taken 10 minutes apart. The values represent cortisol

the subjects. Since no other reported studies have systematically investigated the cortisol response to an fMRI scanning task, we cannot exclude the possibility that it is the fMRI environment per se that led to elevations in cortisol levels. In the fMRI environment, the subject is immobilized during the entire scan, and complete enclosure inside the scanner is sometimes associated with claustrophobia. On the other hand, the cortisol responses observed during the fMRI scanning session were no higher than those observed during the PET scanning sessions, which makes a general stress effect of the fMRI environment unlikely. Nonetheless, in future studies, cortisol levels during other fMRI tasks should be assessed, to exclude that possibility. The PET environment is probably not stressful on its own, since the scanning we performed on control days did not lead to elevations in cortisol levels. Because of the less restricted scanning environment for PET, the subject is likely to feel less constraint as well.

Second, although the stress task used here has proved effective in inducing a stress response, it is probably less capable as a stressor than classic laboratory tasks. The elevations in cortisol, although significant, were modest (in the range of 50%–100% elevation relative to baseline). The widely used Trier Social Stress Test has repeatedly induced 2- to 4-fold elevations of cortisol levels.[4] This difference in response magnitude is likely due to a number of factors, including absence of the investigator during the MIST, difference in the nature of the task (mental arithmetic versus public speaking), and the greater number of distractions in the MIST (scanning noise, imaging-related instructions from the MR technician). Compared with previous studies that have used computerized mental arithmetic as a stressor, however, we observed similar elevations of cortisol levels as a result of the stress task, which suggests that the social evaluative threat component was effectively induced even in the absence of personnel during the task.[5]

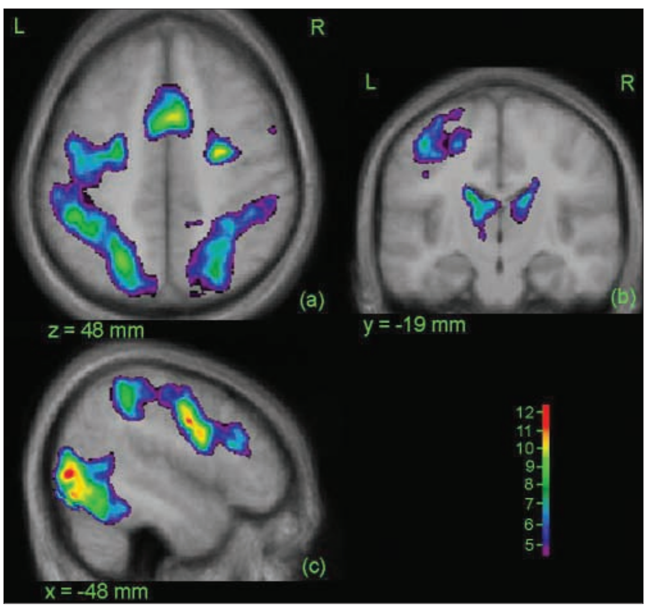

Fig. 5: Statistical parametric map displaying significant activations with blood oxygenation level-dependent (BOLD) signal as a result of performing the MIST in study 3 (n = 22). Areas significantly activated as a result of performing the MIST include the visual association cortex, the sensory and motor cortices, the angular gyrus, the thalamus and the cingulate gyrus, as shown here in (a) horizontal, (b) coronal and (c) sagittal slices. All areas shown exceed the threshold for statistical significance of $t > 4.5$.

The functional imaging activations we observed are consistent with previous studies involving mental arithmetic, including activation of the visual association cortex for the processing of visual stimuli, activation of the motor cortex as a result of operating the response box, activation of the angular cortex as a result of mental arithmetic and activation of the cerebellum.[13] The direct effects of acute stress on changes in brain activation will be reported in detail elsewhere (manuscript in preparation).

Taken together, the results presented here lead us to propose the MIST as a useful and versatile tool for investigating the effects of stress perception and processing on physiologic and brain activation changes in functional neuroimaging studies.

Competing interests: None declared.

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