Age-related changes in the cerebral substrates of cognitive procedural learning

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Abstract

Cognitive procedural learning occurs in three qualitatively different phases (cognitive, associative and autonomous). At the beginning of this process, numerous cognitive functions are involved, subtended by distinct brain structures such as the prefrontal and parietal cortex and the cerebellum. As the learning progresses, these cognitive components are gradually replaced by psychomotor abilities, reflected by the increasing involvement of the cerebellum, thalamus and occipital regions. In elderly subjects, although cognitive studies have revealed a learning effect, performance levels differ during the acquisition of a procedure. The effects of age on the learning of a cognitive procedure have not yet been examined using functional imaging. The aim of this study was therefore to characterize the cerebral substrates involved in the learning of a cognitive procedure, comparing a group of older subjects with young controls. For this purpose, we performed a positron emission tomography activation study using the Tower of Toronto task. A direct comparison of the two groups revealed the involvement of a similar network of brain regions at the beginning of learning (cognitive phase). However, whereas the engagement of frontal and cingulate regions persisted in the older group as learning continued, it ceased in the younger controls. We assume that this additional activation in the older group during the associative and autonomous phases reflected compensatory processes and the fact that some older subjects failed to fully automate the procedure.

MESH Keywords Age Factors ; Aged ; Aging ; Brain Mapping ; Cerebral Cortex ; physiology ; radionuclide imaging ; Cognition ; physiology ; Female ; Humans ; Learning ; physiology ; Male ; Middle Aged ; Multivariate Analysis ; Neuropsychological Tests ; Photic Stimulation ; Positron-Emission Tomography ; methods ; Reference Values

INTRODUCTION

Procedural learning describes the encoding procedures which underlie motor, verbal or cognitive skills (assessed, for example, by means of the rotor test, mirror reading or Tower of Hanoi (TH) respectively). Cognitive procedural learning is characterized by three phases (cognitive, associative and autonomous) and the beginning of the learning process in particular has been found to involve many cognitive components, including intellectual capacities, working and episodic memory, and executive functions (Anderson, 2000 ; Ackerman, 1988 ; Beaunieux et al., 2006). These studies showed that learning a new cognitive procedure draws on processes which are highly controlled in the (initial) cognitive phase but which become increasingly automatic in the (final) autonomous phase. During the (intermediate) associative phase, as the skill improves with practice, this cognitive involvement is considerably reduced. So much so, in fact, that the autonomous phase does not require the intervention of any of the cognitive functions involved in the cognitive phase, and is essentially characterized by the intervention of procedural memory per se (Anderson, 2000).

Few studies have investigated cognitive procedural learning in older adults. Generally speaking, when older subjects learn a new cognitive procedure, they rarely reach the performance level of their younger counterparts after a similar amount of practice, although they do show a significant learning effect, which eventually allows them to catch up with the younger subjects (Charness & Campbell, 1988 ; Vakil & Agmon Ashkenasi, 1997 ; Davis & Bernstein, 1992 ; Peretti et al., 2002). Using the TH task, Brennan et al. (1997) , found differences between young adults and elderly adults on a four-disk task. More recently, Head et al. (2002) examined the impact of age-related differences in tregional cerebral volumes and cognitive resources on the acquisition of the TH task (8 trials of a computerized 4-disk version). Cognitive results revealed that older adults were slower and less efficient than their younger counterparts in the early stage of learning and they remained slower throughout the learning session. Results also revealed that in the early stage of learning (first trial), speed and efficiency scores were associated with age, prefrontal cortex volume and working memory. No such correlations were found with performances at the end of learning. The authors suggested that age differences in cognitive procedural learning were largely confined to the early learning stage.

To date, imaging studies have never been used to identify the cerebral substrates of the three learning phases for a cognitive procedure in older subjects and the few that have looked at these substrates in younger subjects (Haier et al., 1992 ; Poldrack et al., 1999) have focused mainly on motor procedures (Grafton et al., 1994 ; Seitz et al., 1990 ; Sakaï et al., 1998 ; Doyon et al., 2002 ; 2003). Haier et al. (1992) reported increased cerebral blood flow associated with improved novices' performances on the Tetris game, and showed that the

greatest improvement in performances was related to the largest metabolic glucose decreases in a number of areas after practice. Examining probabilistic category learning in healthy young adults, Poldrack et al. (1999) used a weather prediction task to demonstrate increases and decreases in the activation of a neural network which included the caudate nucleus and the prefrontal and parietal cortices. In a previous positron emission tomography (PET) study, we used the Tower of Toronto (TT) task to highlight the cerebral substrates involved in the three learning phases of this task, taking into account the performance levels of each subject (Hubert et al., 2007). In the first part of our protocol, we extracted predictors for the lengths of the three phases in order to select appropriate subjects for the PET activation study. A conjunction analysis revealed that only the right orbitofrontal cortex was involved in all three learning phases. The cognitive phase activated a frontoparietal network and the cerebellum (suggesting the use of problem-solving strategies and the correction of inappropriate moves, respectively), both of which became less active as learning progressed. The associative phase was characterized by the activation of the occipital regions (suggesting the intervention of mental imagery), right thalamus and caudate nucleus (both playing a major role in cognitive procedural learning). Lastly, during the autonomous phase, new regions became involved, including the left thalamus and an anterior part of the cerebellum (consistent with the fact that performances in this phase are determined by psychomotor abilities). The aim of the present study was thus to delineate the brain areas activated during the learning of this same cognitive procedure in a sample of older subjects and to compare these regions with those identified in the younger group.

METHOD

Subjects

Twelve healthy subjects (six women and six men; mean age = 65 years, range from 60 to 73 years; S.D. = 4.5) took part in this study. They were selected on the basis of cognitive predictors established using the same methodology as that described in Hubert et al. (2007) . In order to select subjects with similar lengths of phase, predictors were calculated. It was important for the lengths of the learning phases to be broadly similar for all our selected subjects, in order to allow brain activity to be recorded in each phase (i.e. cognitive, associative, autonomous) in the PET study. Using data from 50 other elderly subjects (not included in the PET study), we found that the length of the cognitive phase was determined by inhibition (Stroop), intellectual processes (block design subtest of the WAIS) and working memory capacity (forward digit span), while psychomotor (Tower of London transfer task) and episodic memory capacities (California verbal learning test) predicted the length of the associative phase (see Hubert et al., 2007 for details). These predictors were thus used to select 12 additional older subjects for the PET protocol of the present study.

The length of each phase was calculated using the following equation: Y (length of the phase in seconds) = origin + (β) Task 1 + (β) Task 2, etc… The magnitude of the β coefficient makes it possible to compare the relative contributions of each independent variable to the prediction of the dependent variable. The length of the cognitive phase was calculated using the following equation: Y (length of the cognitive phase) = 3091.21 + (−47.17) Stroop + (− 30.3) block design + (−230.02) forward digit span. Similarly, we estimated the length of the associative phase using the following equation: Y (length of the associative phase) = 4.40 + (834.08) Tower of London transfer task + (−76.23) California verbal learning test. The length of the autonomous phase corresponded to the number of trials remaining once the subject had left the associative phase and was thus determined by the length of the first two phases. Once the older subjects had performed the 40 trials of the TT task, we ensured that the length of each phase was approximately similar for each subject. Although the older subjects did not all find the optimum solution (i.e. start of the associative phase) after the same number of trials, they did discover it before the PET acquisitions of the associative phase. We can thus conclude that the predictors were reliable.

The protocol was approved by the regional ethics committee and all the subjects gave their informed written consent before taking part in the study. A health questionnaire was used to screen all the subjects for any history of neurological or psychiatric conditions, head injury and alcohol or drug abuse. Because the procedural task involved the processing of colors, participants were also screened for color blindness using the Ishihara Test (Ishihara, 1997). Lastly, we made sure that none of the participants were familiar with the TT problem. All were right-handed, as determined by the Edinburgh questionnaire (Oldfield, 1971). In order to test the effect of age, we compared the PET data of the older group with those obtained in 12 young subjects. The results from these young subjects have already been reported (Hubert et al., 2007).

Procedural task: The TT problem

The TT task consisted of a rectangular base and three pegs. Four different-colored disks were used: one black, one red, one yellow and one white. The TT disks were initially stacked on the leftmost peg, with the darkest one at the bottom and the lightest one on top. The task consisted in rebuilding this configuration on the rightmost peg, obeying the following two rules: only one disk may be moved at a time and a darker disk may never be placed on top of a lighter one. The subjects were required to solve 8 blocks of 5 consecutive trials, with a 5-minute break between each block. The TT device was connected to a computer which recorded the completion time and the number of moves per trial for each subject. The minimum number of moves for the 4-disk TT task is 15. As we gave a clue for the first move, this move (and the time it took) was not taken into account. The optimum solution was thus 14 moves.

We chose the TT because this task has become a useful neuropsychological assessment tool for cognitive procedural learning. The consistency of this task (same materials and same rules throughout the learning process; Ackerman, 1988) allows proficiency to be developed across the three phases described in the ACT model. Moreover, from the standpoint of rehabilitation, Pitel et al. (2006) have suggested that the Tower of Hanoi task (isomorphic problem of the TT) could be regarded as a predictive sign of the effectiveness of an errorless learning paradigm featuring new complex cognitive procedures.

PET activation paradigm

Since the TT protocol requires subjects to move disks, the PET technique was more appropriate than fMRI. A computerized version of the TT was not used because the motor component of this kind of learning is crucial and determines performance levels in the autonomous phase. The paradigm was the same as that performed by younger subjects and described in detail in Hubert et al. (2007) . Briefly, once the subject had been positioned in the scanner and the $H_2{}^{15}O$ had been administered, he or she was required to perform the procedural learning using the right arm (40 trials of the TT). A mirror was positioned above the subject's head, so that the latter could see his/her movements filmed and displayed on a TV screen placed in front of the scanner.

Each subject underwent 12 consecutive scans (injections of $H_2{}^{15}O$) during a single PET session lasting 2 hours, including 4 resting scans and 2 repetitions of 4 different experimental conditions. The activation paradigm began and ended with 2 scans at rest. The purpose of the scans at rest was to compare brain activation before and after learning (data not shown in the present paper). The first condition corresponded to the motor reference task (the subjects had to move the TT disks one by one regardless of their color). The trials selected for the PET acquisitions were the same as those retained in Hubert et al. (2007) for younger subjects, i.e. the two "cognitive acquisitions" corresponded to trials 1 to 2 and 3 to 5. The following 6 trials were performed without any PET scans. Several studies have shown that when older subjects learn a new cognitive procedure, they rarely reach the performance level of their younger counterparts after a similar amount of practice (although older subjects present a significant learning effect; Charness & Campbell, 1988 ; Vakil & Agmon Ashkenasi, 1997 ; Davis & Bernstein, 1992 ; Peretti et al., 2002). Accordingly, to ensure that older subjects had found the optimum solution (i.e. start of the associative phase) before the PET acquisitions of the associative phase, we decided to delay the PET acquisition of this second phase. The 2 "associative acquisitions" therefore took place between trials 12 to 15 and 16 to 19 (instead of trials 9 to 17 in the younger group). The subjects then performed 11 trials of the TT problem without any PET scans. For the 2 "autonomous acquisitions", the subjects were scanned during trials 31 to 35 and 36 to 40. As in the study by Hubert et al. (2007) , there was a slight between-subject difference in the numbers of trials completed during the 90 seconds of the PET acquisition. For example, in the first acquisition of the cognitive phase, some subjects were able to complete two trials within the 90 seconds whereas other subjects were only able to completed one and a half. The priority in this experiment was to ensure that all subjects were in the same learning phase during the PET acquisitions.

Data acquisition (See Hubert et al., 2007 , for further details)

Behavioral data acquisition

We recorded the number of moves and the time required to complete each trial of the TT task.

PET data acquisition

Measurements of the regional distribution of radioactivity were performed using a Siemens ECAT HR+ PET camera with full-volume acquisition allowing the reconstruction of 63 planes. The duration of each scan was 90 s. Approximately 5 mCi of $H_2{}^{15}O$ were administered as a slow bolus, for a total injected dose of ~70 mCi. The interval between injections was 6 min. 40 s.

Image handling and transformation

We used Statistical Parametric Mapping software (SPM5, Wellcome Department of Cognitive Neurology) implemented in the MATLAB environment. Briefly, the 8 scans corresponding to the 4 experimental conditions were first realigned and spatially normalized onto the MNI PET template. The data were then smoothed (using a 12-mm Gaussian filter) and scaled to an overall CBF grand mean of 50 ml/100g/min. We used a gray matter threshold of 80% of the whole brain mean.

Data analysis

Behavioral data analysis

In order to assess the learning effect and the group effect, the behavioral data obtained with the TT were first processed using a multivariate analysis of variance (MANOVA), with performance of the 40 trials as the repeated measure and group as a between-subject factor. In order to assess the effect of age upon the three phases, we also performed a MANOVA on the time taken to solve the problem in each phase (i.e. trials 1 to 10 for the cognitive phase, trials 12 to 22 for the associative phase and finally trials 30 to 40 for the autonomous phase). For clarity's sake, we used the term "autonomous phase" for both groups, even though we cannot say for sure whether the older subjects actually completed this final phase.

PET scan analysis

Conjunction analysis

In order to identify the cerebral substrates involved in each of the three learning phases in the elderly subjects, we used a conjunction analysis based on the recently proposed "valid conjunction inference with the minimum statistic" (Nichols et al., 2005). In this test, each comparison in the conjunction is individually significant, which corresponds to the valid test for a "logical AND".

Subtraction analyses for each learning phase

Firstly, in order to pinpoint the brain structures associated with each learning phase in the older group, we carried out three planned comparisons of means: cognitive vs. reference, associative vs. reference, autonomous vs. reference. Only significant increases are reported for these comparisons.

Secondly, in each peak of significant activity in each of these three contrasts, we also extracted the level of activity for each condition (cognitive, associative and autonomous). The activation level of these regions was then subjected to a repeated-measures analysis of variance in order to observe the time course of activation during learning (with phases as the repetition factor). Post-hoc analyses were conducted using Fisher's LSD test.

A proportional scaling model was used and analysis was performed on a voxel-by-voxel basis. The results of the t statistic (SPM {t}) were then turned into a normal standard distribution (SPM {z}). The significant cut-off was set at $p < .001$ uncorrected for multiple comparisons (the same threshold as that used in Hubert et al., 2007). Only activation clusters with a size larger than 100 voxels were considered. Anatomical/cytoarchitechtonic location of significant activation was based on the SPM 5 MNI template. All the coordinates listed in the sections below are the SPM 5 coordinates.

Correlational analyses

In order to study the link between the older subjects' activation and performance levels, we assessed the correlations between the activation level of the regions involved in the cognitive phase and the mean times of the TT trials performed during this phase. Only significant correlations ($p < .05$) were retained. Because of the lack of variability in the mean times for the associative and autonomous phases, we did not assess the correlations for these 2 phases.

Effect of age

Lastly, in order to assess the effect of age, the older subjects' activations were compared with those of the younger subjects, by contrasting both groups for each experimental condition. This allowed us to unravel the regions of higher or lower activity in aging. For all these comparisons, the SPM5 "masking" routine (function inclusive) was used to search for rCBF changes with learning (e.g. cognitive phase– reference task of the older subjects vs. cognitive phase–reference task of the younger ones), but only in those areas found to be activated in the corresponding contrast (e.g. cognitive phase of the older subjects–reference task of the older subjects). This masking procedure makes it possible to restrict the comparison analysis to the relevant voxels of interest (i.e. to search for greater activity in older subjects compared with young ones solely in areas that are significantly activated in the former), and consequently to exclude differences that could arise in brain areas not significantly involved in the task.

RESULTS

Behavioral data: assessing cognitive procedural learning

A MANOVA was carried out with performance on the 40 trials as the repeated measure and group as a between-subject factor. First, in terms of the number of moves (Figure 1A), the analysis yielded a significant trial effect ($F(39, 858) = 5.07$; $p < .001$), a significant group effect ($F(1, 22) = 39.93$; $p < .001$) and a significant interaction between learning [ou trial??] and group ($F(39, 858) = 2.33$; $p < .001$). There was an overall decrease in the number of moves needed to solve the problem across the 40 trials and a deleterious effect of age. Second, the MANOVA carried out on the time taken to complete each TT trial (Figure 1B) yielded similar results, i.e. a trial effect on mean performance levels ($F(39, 858) = 16.37$; $p < .001$), such that completion times decreased across skill acquisition trials. The results also revealed a significant group effect ($F(1, 22) = 20.8$; $p < .001$) and a significant interaction between these two factors ($F(39; 858) = 2.16$; $p < .001$).

Regarding the MANOVAs conducted on each phase, the effect of age on time was significant for both the cognitive ($p < .001$) and associative ($p < .001$) phases, but not for the autonomous one ($p < .08$). During the first two phases only, we also observed a significant trial effect ($p < .001$). In addition, analysis revealed a significant interaction between group and trial factors, but only during the associative phase ($p < .024$).

PET data

PET scan comparisons

In 3 subjects, either for technical reasons or due to poor comprehension of the requests, one of the two “cognitive acquisitions” had to be excluded from the analysis. We thus obtained 21 acquisitions of the cognitive phase instead of the expected 24.

Conjunction analysis

The conjunction analysis revealed that the bilateral frontal cortex extending to the anterior cingulate cortex (middle and superior frontal gyri; BA 9, 46, 24/32, 10) was activated in all three learning phases (cf. Table I).

Subtraction analyses for each learning phase

Using a t-test, three planned comparisons were carried out between each of the three learning phases and the reference task (motor task). Significant increases were found in all these comparisons. (cf. Tables II , III , IV and Figure 2).

Cognitive phase vs. reference task

The cognitive phase was associated with extensive bilateral activation of the cerebellum (Crus 1 & Crus 2), precuneus and angular regions, prefrontal cortex, anterior cingulate cortex, right temporal cortex and insula (cf. Table II).

Associative phase vs. reference task

A comparison of the associative phase with the reference task showed bilateral activation of the prefrontal cortex, insula, left precuneus and calcarine region, right thalamus, cerebellum and parietal cortex (cf. Table III).

Autonomous phase vs. reference task

A comparison of the autonomous phase with the reference task revealed bilateral activation of the calcarine and lingual regions, prefrontal cortex, anterior cingulate cortex, right cerebellum, right thalamus and right temporal region (cf. Table IV).

The results of the repeated-measures analysis of variance revealed that numerous regions showed a principal effect of learning on the activation level ($p < .05$; cf. Figure 2). Concerning the cognitive phase, the post-hoc analyses revealed two kinds of activation profiles. First, some regions (left cerebellum, right precuneus, right cerebellum, right angular and right temporal cortex; BA37) were recruited significantly more during the cognitive phase than during the other two phases. Second, other regions (left middle frontal cortex and right temporal cortex; BA20) displayed greater involvement during the cognitive phase than during the autonomous phase, but activation levels during the cognitive and associative phases were similar. Regarding the remaining regions that were significantly involved in the cognitive phase (as listed in Table II), they showed equivalent levels of activity during all three phases.

Concerning the associative phase, the post-hoc analyses also revealed two different profiles, with the right thalamus being more heavily involved during the associative phase than during the other two phases, while activation in the bilateral calcarine sulcus was greater in the associative phase than the cognitive phase, but not the autonomous phase.

During the autonomous phase, the lingual/calcarine regions and the cerebellum (vermis 8) appeared to be more highly activated than during the cognitive phase, although their level of activation did not significantly differ from that during the associative phase. The right temporal cortex (BA 21) was more highly activated during the autonomous phase than during the other two ones.

Correlational analyses

We analyzed the correlations between the different regions highlighted during the cognitive phase and the mean time taken to complete the TT. Results revealed two significant correlations. There were negative correlations with the cerebellum ($r = -67$; $p < .05$), in that the quicker a subject solved the problem, the more this region was activated. Results also revealed a positive correlation with the activation level of the middle cingulate gyrus ($r = +71$; $p < .05$): the older subjects who activated this region the most were those who performed least well, taking longer to complete the TT.

Effect of age

Direct between-group comparisons between young and elderly subjects failed to show any significant difference in the ‘younger > older' contrast, while the reverse contrast showed a higher level of activity in elders in the right and left frontal cortex, extending to the middle and anterior cingulate gyrus. These differences were only found for the associative and autonomous phases, while no significant differences were observed for the cognitive phase. These findings are displayed in Figure 3 and listed in Table V .

DISCUSSION

In the present PET study, we compared brain activity in young and elderly adults while they performed 40 trials of the TT. Our findings for the older group revealed the involvement of several brain regions shown to be recruited during cognitive procedural learning in young subjects (Hubert et al., 2007), together with additional regions thought to reflect the effect of age on cognitive procedural learning.

Behavioral results confirmed the deleterious effect of age on cognitive procedural performance levels but also a beneficial effect of trial repetition on performances on the TT task in aging. However, the interaction between group and trial factors during the associative phase revealed that the two groups did not learn the procedure in the same way. This interaction reflects a higher rate of improvement in the older subjects' performances during the associative phase, which can be explained by lower performances (more time and moves required to solve the problem) by this group during the cognitive phase. These data are consistent with several studies highlighting an effect of age on performance levels during the learning of motor (Wright & Payne, 1985), verbal (Hashtroudi et al., 1991) or cognitive procedures (Davis & Bernstein, 1992). Our results showed that the older subjects were capable of attaining the performance levels of the younger group. To obtain this result, we administered a far higher number of trials (40) than in other studies. In the light of Beaunieux et al.'s findings (2006) , we assumed that the 8 trials of the TH proposed in Head et al.'s study (2002) were not sufficient to automate the procedure. However, even though the older subjects had caught up with their younger counterparts after these forty trials, we cannot be sure that the procedure was fully automated by then.

Concerning the imaging data, the conjunction analysis revealed a bilateral activation of the prefrontal cortex in our group of elderly subjects, whereas activation was right-sided only in the young subjects (Hubert et al., 2007). Based on a comprehensive meta-analysis of imaging data, Cabeza (2002) has proposed a model, termed "hemispheric asymmetry reduction in older adults" or HAROLD, according to which prefrontal activity during the implementation of episodic memory processes becomes less lateralized with increasing age, reflecting compensatory processes. This phenomenon has also been demonstrated for semantic memory, working memory, perception and inhibitory control (Dolcos et al., 2002) and our findings indicate that the HAROLD model may also encompass procedural learning. It is worth noting that procedural learning (i.e. the process by which a procedure is encoded in procedural memory) involves different processes, including executive functions, episodic memory and working memory (Beaunieux et al., 2006).

Concerning the subtraction analyses of each learning phase, the elder subjects' patterns of activation were very similar to those of the younger group. The cognitive phase was associated with extensive bilateral activation of the prefrontal cortex, cerebellum, angular/precuneus regions and anterior cingulate gyrus. As in younger subjects, activation of the frontoparietal network may reflect the use of strategies in problem-solving, while activation of the posterior lobe of the cerebellum (Crus 1 and Crus 2) may be related to cognitive processes, such as error detection and the correction of inappropriate moves (Van Mier & Petersen, 2002). The first correlation result (quicker subjects activate the cerebellum more) confirms the key role of this area in regulating motor processes in cognitive tasks. Lastly, the activation of the anterior cingulate gyrus may reflect the attentional load required by the task. The direct between-group comparison did not reveal any significant difference in activation, suggesting that elderly and young subjects used the same strategies and thus the same cognitive components to tackle the procedure at this stage of learning. The difference in procedural performance levels characterizing this first phase can be attributed to a decline in these cognitive components, for although older subjects used the same cognitive components as younger ones, the decline in these components induced a difference in procedural performance levels in favor of the younger subjects. Thus, the absence of significant cerebral differences during the cognitive phase suggests that, in most cases, the older subjects did not try to compensate for their difficulties. The second correlation result, however, showing greater activation of the middle cingulate in poor performers, revealed the expected heterogeneity of the older sample and confirmed that some older individuals need to activate a wider network of brain regions than young ones.

The activations observed during the associative phase revealed that older subjects activated the same regions as the younger controls, but also some additional ones. According to Denis (1985) , this second learning phase is characterized by the creation and control of mental images. Bilateral activation of the calcarine and lingual regions suggests the involvement of mental imagery (Kosslyn et al., 1996) in order to anticipate suitable future moves while making the preceding ones. In younger subjects, the activation of these occipital regions was also present during the autonomous phase. Another region common to both groups was the right thalamus, which was significantly more highly activated in this intermediate phase, but new regions were also recruited by the older group. The between-group comparison highlighted the activation of the left frontal cortex, including the cingulate (middle and anterior) gyrus and the right prefrontal cortex in older subjects. The involvement of additional areas can be interpreted in two ways. First, older subjects may still have been in the cognitive phase (activation of the cingulate gyrus and the right prefrontal cortex was observed in the cognitive phase in both age groups). However, this hypothesis does not fit in well with the behavioral data showing that older subjects had already found the optimum solution to the problem prior to the PET acquisitions of the associative phase (see Method section; Subjects), and it is the discovery of this optimum solution that marks the beginning of the associative phase (Hubert et al., 2007). Moreover, a study of a younger group (Hubert et al., 2007) has already shown that the activation of the right thalamus observed in the older group during this second phase is confined to the associative phase The second, more plausible, interpretation is consistent with current compensation models of reduced asymmetry (see above). Whereas younger subjects drew on the right prefrontal cortex during the associative phase, the older subjects triggered the bilateral activation of the prefrontal region and, more precisely, the BA9 and BA46 - areas which have been shown to play a role in

working memory and problem solving in numerous neuroimaging studies (Cabeza & Nyberg, 2000 ; Unterrainer & Owen, 2006). Our results are also consistent with many studies on aging. Often, when age-related changes in prefrontal cortex activity are observed, the older subjects also perform more poorly than young subjects on working memory and episodic memory tasks (Anderson et al., 2000 ; Cabeza et al., 2000 ; Rypma et al., 2001). However, even in the absence of behavioral differences (as in the autonomous phase in our study), studies have reported differences in prefrontal cortex activation in young vs. old subjects (Rypma & D'Esposito, 2000). Thus, additional prefrontal and cingulate activation in the older group, during both the associative and autonomous phases, suggests that these areas were still at work during the final step of procedural learning. However, we cannot exclude the possibility that the older subjects may not have fully automated the procedure after 40 trials. At the end of the learning process, we can assume that even though the older subjects had found the optimum solution and were able to complete the trials as quickly as the younger ones, they had not attained the same level of automation as the latter. This kind of result has already been observed in a study comparing the procedural performances of alcoholic patients and normal subjects. Using a correlational analysis, Pitel et al. (2007) , showed that even though patients "managed to attain the same level of performance […] they were still at a controlled stage of acquisition, and automation of the cognitive procedure would have required many further trials". Similarly, we can assume that older subjects were still at a controlled stage at the end of the 40 trials. In this study, we chose a threshold of $p < .001$ uncorrected. Because our results suggest that the older group did not automate the procedure to the same extent or in the same way as the younger subjects, we expected to observe the activation of additional areas in the younger group, reflecting the automation of the procedure. We thus carried out further analyses at the threshold of $p < .005$ (data not shown), which revealed supplementary activation of the left thalamus and bilateral gyrus rectus in the younger vs. older group. We had previously shown that these regions are specifically activated during the autonomous phase in young subjects (Hubert et al, 2007). This supplementary activation also suggests that the older group did not attain the same procedural performance level as the younger ones in terms of automation, even though the cognitive data failed to reveal any differences in the number of moves or completion times during the autonomous phase ($p < .08$). We also performed complementary analyses (data not shown) in order to take execution speed into account, as the moves take longer at the beginning than at the end of the learning process. Thus, we added speed scores (i.e. number of moves divided by the total time per trial) as a nuisance variable in the PET analyses. The PET results were very similar to those obtained without this nuisance variable (as previously shown in Hubert et al., 2007), indicating that the rCBF measures were not influenced by the speed at which the moves were made.

Overall, our results are consistent with previous imaging studies of motor or perceptual-motor learning in elderly subjects (Raz et al., 2000 ; Kennedy et al., 2005). In a correlational study, Kennedy et al. (2005) showed that greater prefrontal cortex activity was associated with better performances on the mirror drawing task and demonstrated that this correlation was strengthened in the later stages of learning. Using probabilistic category learning, Fera et al. (2005) found that healthy older adults use the same neural network as young adults, albeit to a different degree, to achieve equivalent learning and performances. They suggested that healthy older adults may use some brain regions, such as the parietal cortices, as a compensation mechanism for their caudate nucleus and prefrontal cortex deficiency (Kalpouzos et al., in press). In keeping with Wu and Hallett (2005) , our results revealed, that older subjects required more brain activity than younger subjects to reach the same performance level. Interestingly, a similar network of brain regions was recruited by both groups during the cognitive phase, whereas this phase was characterized by the greatest differences in the cognitive data (more time and moves required to solve the problem). One explanation for this intriguing finding may be that the older subjects' brains made no attempt to compensate for their lower performances by using other cerebral substrates or other cognitive resources. By contrast, during the autonomous phase, although the older subjects performed at the same level as the younger ones (same time taken to complete the TT), they displayed increased activation of the prefrontal cortex. At the present time, based on the existing data reported in the literature, it is still difficult to fully understand the phenomenon of "under-activation" or "over-activation" in older adults. In the light of episodic and working memory studies, Persson and Nyberg (2006) have suggested that age-related increases in cerebral activation reflect a compensatory response to a detrimental process. However, as the impairment worsens, under-activation seems to be the typical pattern, with few or no signs of compensation. In the case of cognitive procedural learning, we can assume that 1) the absence of over-activation in the older group during the cognitive phase reflects the absence of compensation, and 2) the additional activation observed during the associative and autonomous phases reflects compensatory processes. The older subjects certainly seem to achieve the same performance levels as the younger ones, but the hypothesis that they do not fully automate the procedure cannot be rejected either. Even though the performance levels of the two groups did not differ significantly, we can "see" in Figure 1 that the time and number of moves required for the older group to solve the TT did not quite reach an asymptotic performance level.

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References:

Bibliography

- Ackerman PL . 1988 ; Determinants of individual differences during skill acquisition: cognitive abilities and information processing . J Exp Psychol Gen . 117 : 288 - 318
- Anderson JR . Editor: Anderson JR . 2000 ; Skill acquisition . Learning and Memory: An Integrated Approach . 2 New York John Wiley & Sons ; 304 - 337

- Anderson ND , Lidaka T , Cabeza R , Kapur S , McIntosh AR , Craik FI . 2000 ; The effects of divided attention on encoding and retrieval-related brain activity: a PET study of younger and older adults . J Cogn Neurosci . 12 : 775 - 92
- Beaunieux H , Hubert V , Witkowski T , Pitel AL , Rossi S , Danion JM , Desgranges B , Eustache F . 2006 ; Which processes are involved in cognitive procedural learning? . Memory . 14 : 521 - 539
- Brennan M , Welsh MC , Fisher CB . 1997 ; Aging and executive function skills: an examination of a community-dwelling older adult population . Percept Mot skills . 84 : 1187 - 1197
- Cabeza R , Nyberg L . 2000 ; Neural bases of learning and memory: functional neuroimaging evidence . Curr Opin Neurol . 13 : 415 - 421
- Cabeza R , Anderson ND , Houle S , Mangels JA , Nyberg L . 2000 ; Age related differences in neural activity during item and temporal-order memory retrieval: a positron emission tomography study . J Cogn Neurosci . 12 : 197 - 206
- Cabeza R . 2002 ; Hemispheric asymmetry reduction in older adults: the HAROLD model . Psychol Aging . 17 : 85 - 100
- Charness N , Campbell JID . 1988 ; Acquiring skill at mental calculation in adulthood: a task decomposition . J Exp Psychol Gene . 117 : 115 - 129
- Davis HP , Bernstein PA . Editor: Squire LR , Butters N . 1992 ; Age-related changes in explicit and implicit memory . Neuropsychology of Memory . 249 - 261
- Denis M . 1985 ; Visual imagery and the use of mental practice in the development of motor skills . Can J Appl Sport Sci . 10 : 4S - 16S
- Dolcos F , Rice HJ , Cabeza R . 2002 ; Hemispheric asymmetry and aging: right hemisphere decline or asymmetry reduction . Neuroscience and Behavioral Review . 26 : 819 - 825
- Doyon J , Song AW , Karni A , Lalonde F , Adams MM , Ungerleider LG . 2002 ; Experience dependent changes in cerebellar contributions to motor sequence learning . PNAS . 99 : 1017 - 1022
- Doyon J , Penhume V , Ungerleider LG . 2003 ; Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning . Neuropsychologia . 41 : 252 - 262
- Fera F , Weickert TW , Goldberg TE , Tessitore A , Hariri A , Das S , Lee S , Zoltick B , Meeter M , Myers CE , Gluck MA , Weinberger DR , Mattay VS . 2005 ; Neural mechanisms underlying probabilistic category learning in normal aging . J Neurosci . 25 : 11340 - 11348
- Grafton ST , Woods RP , Tyszka M . 1994 ; Functional imaging of procedural motor learning: relating cerebral blood flow with individual subjects' performance . Hum brain mapp . 1 : 221 - 234
- Haier RJ , Siegel BV , MacLachlan A , Soderling E , Lottenberg S , Buchsbaum MS . 1992 ; Regional glucose metabolic changes after learning a complex visuospatial/motor task: a positron emission tomographic study . Brain res . 570 : 134 - 143
- Hashtroudi S , Chrosniak LD , Schwartz BL . 1991 ; Effects of aging on priming and skill learning . Psychol aging . 6 : 605 - 15
- Head D , Raz N , Gunning-Dixon F , Williamson A , Acker JD . 2002 ; Age related differences in the course of cognitive skill acquisition: the role of regional cortical shrinkage and cognitive resources . Psychol Aging . 17 : 72 - 84
- Hubert V , Beaunieux H , Chételat G , Platel H , Landeau B , Danion JM , Viader F , Desgranges B , Eustache F . 2007 ; The dynamic network subserving the three phases of cognitive procedural learning . Hum Brain Mapp . 28 : 1415 - 29
- Ishihara S . 1997 ; Tests for Colour Blindness . Tokyo Kanehara Shuppan Co. Ltd ;
- Kalpouzos G , Chételat G , Baron JC , Landeau B , Mevel K , Godeau C , Barré L , Constans J-M , Viader F , Eustache F , Desgranges B . 2007 ; Voxel-based mapping of brain gray matter volume and glucose metabolism profiles in normal aging . Neurobiol Aging . in press
- Kennedy KM , Raz N . 2005 ; Age, sex and regional brain volumes predict perceptual-motor skill acquisition . Cortex . 41 : 560 - 569
- Kosslyn SM , Thompson WL , Kim IJ , Rauch SL , Alpert NM . 1996 ; Individual differences in cerebral blood flow in area 17 predict the time to evaluate visualized letters . J Cogn Neurosci . 8 : 78 - 82
- Lahy JM . 1978 ; Test d'attention concentrée [Concentrated attention test] . Paris EAP ;
- Nichols T , Brett M , Andersson J , Wager T , Poline JB . 2005 ; Valid conjunction inference with the minimum statistic . NeuroImage . 25 : 653 - 660
- Oldfield RC . 1971 ; The assessment and analysis of handedness: the Edinburgh Inventory . Neuropsychologia . 9 : 97 - 113
- Peretti CH , Danion JM , Gierski F , Grange D . 2002 ; Cognitive skill learning and aging. a component process analysis . Arch clin neuropsy . 17 : 445 - 459
- Persson J , Nyberg L . 2006 ; Altered brain activity in healthy seniors: what does it mean? . Progress in brain research . 157 : 45 - 56
- Pitel AL , Beaunieux H , Lebaron N , Joyeux F , Desgranges B , Eustache F . 2006 ; Two case studies in the application of errorless learning techniques in memory impaired patients with additional executive deficits . Brain Injury . 20 : 1099 - 1110
- Pitel AL , Witkowski T , Vabret F , Guillery-Girard B , Desgranges B , Eustache F , Beaunieux H . 2007 ; Effect of episodic and working memory impairments on semantic and cognitive procedural learning at alcohol treatment entry . Alcohol Clin Exp Res . 31 : 238 - 248
- Poldrack RA , Prabhakaran V , Seger CA , Gabrieli JDE . 1999 ; Striatal activation during acquisition of a cognitive skill . Neuropsychology . 13 : 564 - 574
- Raz N , Williamson A , Gunning-Dixon F , Head D . 2000 ; Neuroanatomical and cognitive correlates of adult age differences in acquisition of a perceptual-motor skill . Microsc Res Tech . 51 : 85 - 93
- Rypma B , Prabhakaran V , Desmond JE , Gabrieli JD . 2001 ; Age differences in prefrontal cortical activity in working memory . Psychol aging . 16 : 371 - 84
- Rypma B , D'Esposito M . 2000 ; Isolating the neural mechanisms of age-related changes in human working memory . Nat Neurosci . 3 : 509 - 15
- Sakaï K , Hikosaka O , Miyauchi S , Takino R , Sasaki Y , Pütz B . 1998 ; Transition of brain activation from frontal to parietal areas in visuomotor sequence learning . J Neurosci . 18 : 1827 - 1840
- Seitz RJ , Roland PE , Bohm C , Greitz T , Stone-Elander S . 1990 ; Motor learning in man: a positron tomographic emission study . Neuro Report . 1 : 57 - 66
- Tzourio-Mazoyer N , Landeau B , Papathanassiou D , Crivello F , Etard O , Delcroix N . 2002 ; Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain . NeuroImage . 15 : 273 - 289
- Unterrainer JM , Owen AM . 2006 ; Planning and problem solving: from neuropsychology to functional neuroimaging . J Physiol Paris . 99 : 308 - 317
- Vakil E , Agmon-Ashkenazi D . 1997 ; Baseline performance and learning rate of procedural and declarative memory tasks: younger versus older adults . J gerontol: psychol sci . 52 : 229 - 234
- Van Mier H , Petersen SE . 2002 ; Role of the cerebellum in motor cognition . Annals of the New York Academy of Sciences . 978 : 334 - 353
- Wright BM , Payne RB . 1985 ; Effects of aging on sex differences in psychomotor reminiscence and tracking proficiency . J Gerontol . 40 : 179 - 184
- Wu T , Hallett M . 2005 ; The influence of normal ageing on automatic movements . J Physiol . 562 : 605 - 615

Figure 1

Performance in terms of the number of moves and time taken per trial in the TT task. The results show a significant learning effect across the 40 trials, a significant group effect and a significant interaction between learning and groups in terms of moves and time.

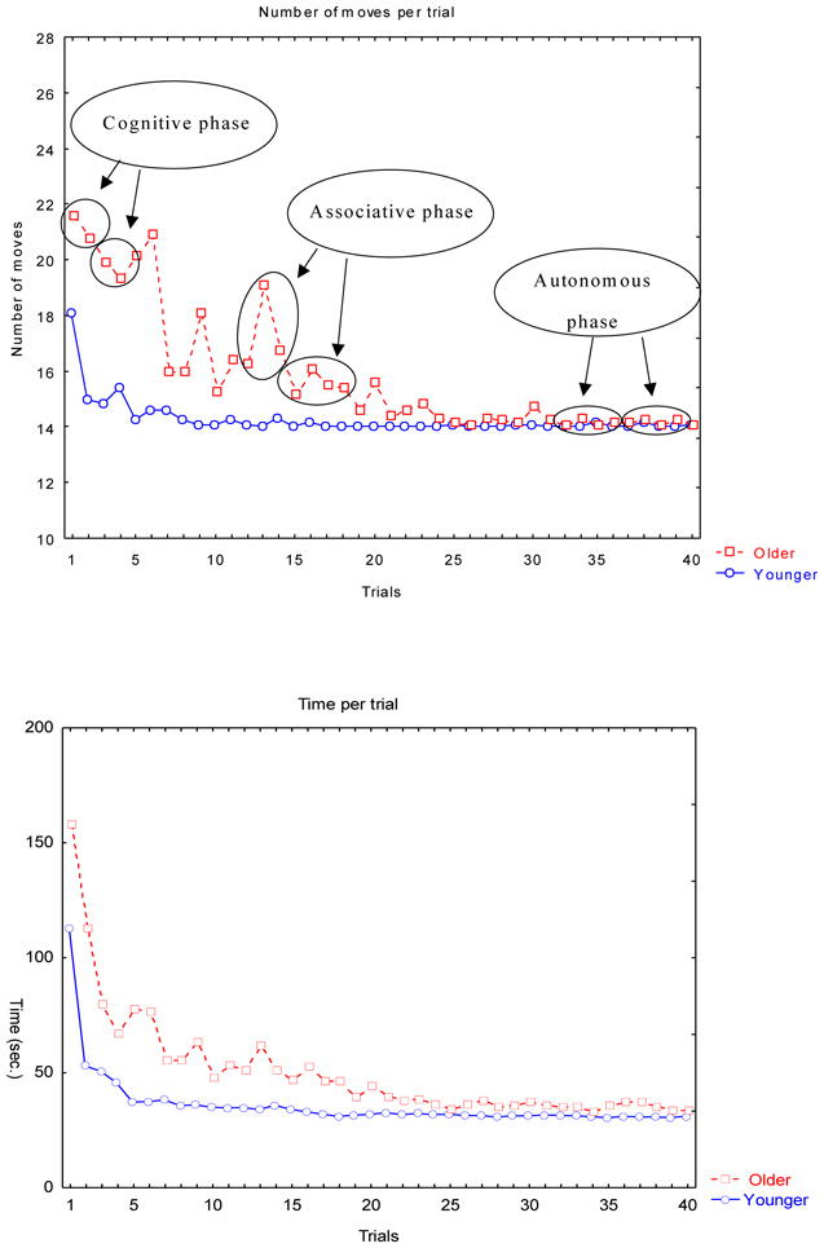

Figure 2

PET scan comparisons

Brain activation during the 3 phases of cognitive procedural learning (TT task). Significantly activated regions at the threshold of p <.001 uncorrected for multiple comparisons and time course of activation across learning. The plots represent the relative contribution of the different conditions of our paradigm, according to the “effects of interests” for selected peaks (The plots represented in this figure correspond to the regions in bold type. All the regions cited under this first region displayed the same changes in activation). The first, second and third histograms correspond to the cognitive, associative and autonomous phases respectively. For clarity's sake, the plot of the reference condition is not represented, but the corresponding activations of this condition were always below those in the three experimental conditions.

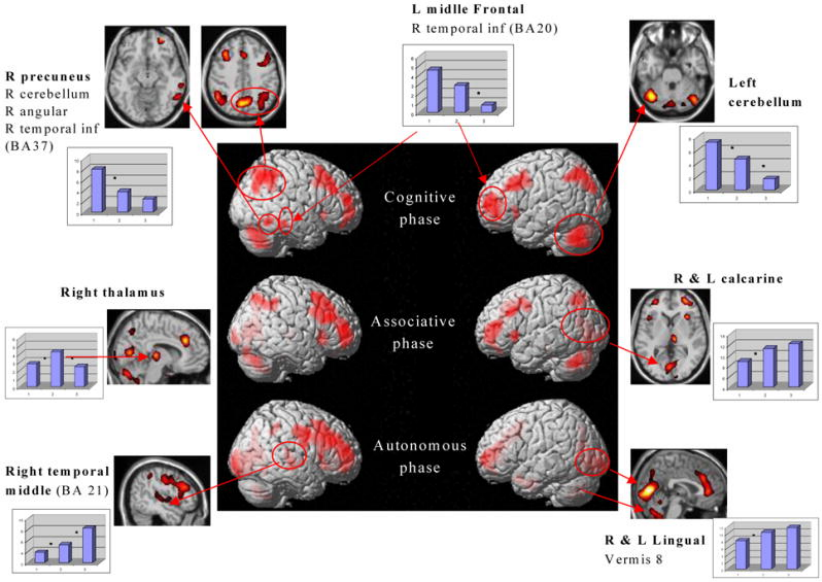

Figure 3

Between-group comparisons of the activations in the three learning phases. The most highly-activated regions in the older group are shown in red. Comparisons did not reveal any supplementary activations for the younger group.

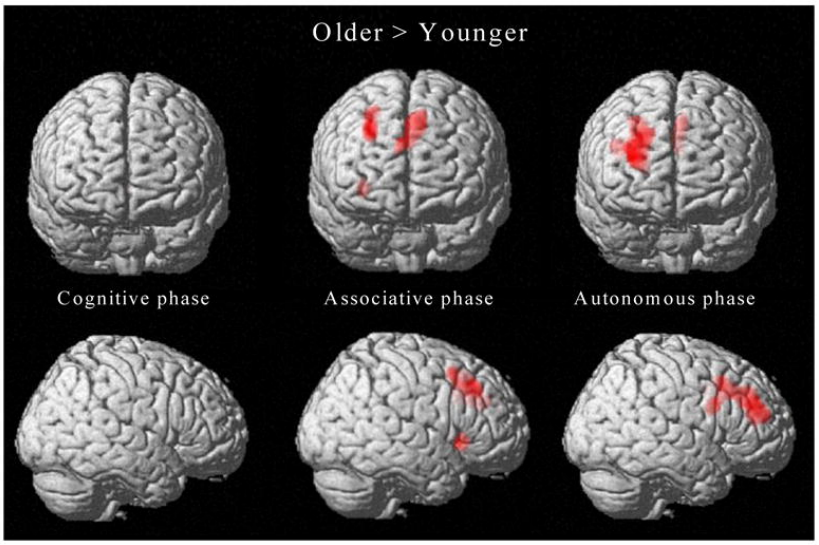

Table I

Brain regions common to all three learning phases vs. reference tasks

Anatomical region		z score	Cluster extent (k)	%	coordinates		
					x	y	z
Frontal Mid R	**(BA 9)**	5.54	952	72.58	30	16	46
Frontal Mid R	**(BA 46)**	4.75	603	58.04	36	50	14
Frontal Mid Orb R				18.91			
Frontal Sup R				12.11			
Frontal Sup Medial L	**(BA24/32)**	4.69	481	27.03	−2	26	36
Anterior cingulate gyrus L				19.33			
Anterior cingulated gyrus R				16.42			
Middle cingulate gyrus R				16.22			
Middle cingulate gyrus L				14.76			
Frontal Mid L	**(BA 10)**	4.67	261	66.28	−28	50	10
Frontal Sup L				18.01			
Frontal Sup Orb L				13.79			

Note: Areas significantly activated at p <.001 uncorrected for multiple comparison. Anatomical location of significant activation and approximate Brodmann areas was as described in the Method section. Stereotaxic coordinates shown here are those listed in SPM5. Labels and percentages were obtained using the aal toolbox (Tzourio-Mazoyer et al., 2002).

Table II

Brain regions activated during the cognitive phase vs. reference task

Anatomical region		z score	Cluster extent (k)	% cluster	coordinates		
					x	y	z
Cognitive phase vs. Reference							
Cerebellum Crus 2 L		6.77	1601	31.36	−38	−64	−36
Cerebellum Crus 1 L				51.34			
Precuneus R	**(BA 7)**	6.59	3363	23.52	8	−68	50
Precuneus L				24.77			
Parietal Inf L				19.03			
Occipital Mid L				11.89			
Parietal Sup L				10.35			
Frontal Mid L	**(BA 10)**	6.40	1362	52.42	−30	52	10
Frontal Mid Orb L				12.11			
Frontal Inf Tri L				11.97			
Frontal Sup L				11.89			
Cerebellum Crus 2 R		5.83	1345	17.25	38	−62	−42
Cerebellum Crus 1 R				42.90			
Cerebellum Crus 2 L				11.23			
Angular R	**(BA 39)**	5.70	1747	47.05	48	−56	46
Parietal Inf R				31.77			
Frontal Mid R	**(BA 9)**	5.54	1380	77.61	30	16	46
Frontal Mid L	**(BA 44)**	5.37	1137	46.17	−44	24	34
Precentral L				28.67			
Frontal Inf Tri L				14.34			
Frontal Mid Orb R	**(BA11)**	5.13	879	20.36	28	54	−10
Frontal Mid R				55.18			
Frontal Sup Orb R				10.69			
Middle cingulate gyrus L	**(BA 24)**	4.91	680	10.44	−2	26	38
Frontal Sup medial L				26.91			
Middle cingulate gyrus R				20.88			
Anterior cingulate gyrus L				15.15			
Anterior cingulate gyrus R				12.06			
Temporal Inf R	**(BA20)**	4.72	117	64.96	64	−34	−18
Temporal Mid R				35.04			
Temporal Inf R	**(BA37)**	4.40	185	96.76	56	−56	−16
Cerebellum 9 R		4.30	140	59.29	2	−54	−42
Vermis 9				25			
Insula R	**(BA47)**	4.03	123	60.16	34	20	−6
Insula L	**(BA47)**	3.89	171	68.42	−38	20	0
Frontal Inf Tri L				25.15			

Note. Same as in Table I

Table III

Brain regions activated during the associative phase vs. reference task

Anatomical region		z score	Cluster extent (k)	% cluster*	coordinates		
					x	y	z
Associative phase vs. Reference							
Middle cingulate gyrus R	**(BA 32)**	6.34	1484	15.70	−4	26	38
Frontal Sup Medial L				22.91			
Anterior cingulate gyrus L				19.68			
Anterior cingulate gyrus R				19.20			
Frontal Mid R	**(BA 8)**	6.14	3760	51.14	28	14	48
Frontal Sup R				12.37			
Frontal Inf Tri R				11.30			
Frontal Inf Oper R				7.47 (20.09%)			
Frontal Mid Orb R				5.45 (20.2%)			
Insula R	**(BA47)**	5.61	365	57.81	34	20	−2
Calcarine L	**(BA 17)**	5.58	4918	11.39	2	−78	8
Precuneus L				18.06			
Parietal Inf R				6.3 (23.05%)			
Vermis 7				2.7 (68.56%)			
Vermis 8				1.4 (28.40%)			
Vermis 9				1.28 (36.21%)			
Thalamus R		5.47	831	26.71	8	−24	2
Frontal Mid L	**(BA 10)**	5.22	1079	47.64	−30	50	6
Frontal Mid Orb L				15.48			
Frontal Inf Tri L				14.74			
Frontal Sup Orb L				12.88			
Cerebellum Crus 1 L		5.13	797	65.75	−36	−62	−34
Cerebellum Crus 2 L				18.07			
Precentral L	**(BA 6)**	4.68	718	35.38	−38	2	40
Frontal Mid L				40.81			
Frontal Inf Tri L				15.74			
Insula L	**(BA 47)**	4.56	219	48.86	−40	20	0
Frontal Inf Tri L				37.44			
Frontal Inf Orb L				10.05			
Cerebellum Crus 1 R		4.52	257	85.99	42	−70	−32
Parietal Inf L	**(BA 7)**	4.35	262	49.24	−34	−64	46
Angular L				25.95			
Parietal Sup L				24.43			
Calcarine R	**(BA 18)**	4.16	239	46.86	14	−96	−4
Lingual R				18.41			

* Percentage of the region included in the cluster shown in brackets (only for regions where % cluster is < 10%)

Table IV

Brain regions activated during the autonomous phase vs. reference task

Anatomical region		z score	Cluster extent (k)	% cluster *	coordinates		
					x	y	z
Autonomous phase vs. Reference							
Lingual L	**(BA 17)**	6.71	3924	11.80	2	−76	6
Calcarine L				25.41			
Calcarine R				19.88			
Lingual R				15.55			
Frontal Mid R	**(BA 46)**	6.13	5062	38.96	32	48	16
Precentral R				10.59			
Frontal Sup R				10.55			
Frontal Mid L	**(BA 10)**	5.26	2855	10.86	−24	52	10
Anterior cingulate gyrus L				20.39			
Anterior cingulate gyrus R				16.50			
Frontal Sup medial L				12.54			
Vermis 8		4.46	410	32.93	4	−68	−40
Cerebellum 9 R				24.88			
Cerebellum 8 R				10.73			
Vermis 9				9.27 (21.84%)			
Thalamus R		4.34	153	92.16	10	−26	4
Parietal Inf R	**(BA 40)**	3.85	141	68.09	38	−48	38
Supramarginal R				12.77			
Temporal Mid R	**(BA 21)**	3.77	332	10.24	56	−20	−6
Temporal Sup R				68.07			
Insula R				10.24			

Table V

Comparisons of the activation between the 2 age groups. Older > Younger subjects comparison.

Anatomical region	z score	Cluster extent (k)	Coordinates		
			x	y	z
Cognitive phase vs. Reference					
		No suprathreshold clusters			
Associative phase vs. Reference					
Frontal Sup Medial L (BA 32) Middle Cingulate Gyrus L & R Supp Motor Area L Anterior Cingulate Gyrus L & R	5.47	557	−4	24	42
Frontal Mid R (BA 9/46) Frontal Sup R	4.39	312	28	30	38
Insula R	4.32	108	32	20	−2
Autonomous phase vs. Reference					
Frontal Mid R (BA 9/46) Frontal Sup R	5.16	837	28	42	22
Middle Cingulate Gyrus L (BA 32) Supp Motor Area L Frontal Sup Medial L	4.35	215	−4	16	42
Frontal Inf Oper R (BA 44) Frontal Inf Tri R	4.06	114	40	10	28

Note: Areas significantly more activated in the older group than in the younger one at $p < .001$ uncorrected for multiple comparison (cluster level) cut-off. Stereotaxic coordinates shown here are those listed in SPM5.