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Mechanisms underlying cognitive enhancement and reversal of cognitive deficits in nonhuman primates by the ampakine CX717

R. E. Hampson,

Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Medical Center Boulevard, Winston-Salem, NC 27157-1083, USA

R. A. España,

Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Medical Center Boulevard, Winston-Salem, NC 27157-1083, USA

G. A. Rogers,

Medical School of the Americas, Nevis, West Indies

L. J. Porrino, and

Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Medical Center Boulevard, Winston-Salem, NC 27157-1083, USA

S. A. Deadwyler

Department of Physiology and Pharmacology, Wake Forest University Health Sciences, Medical Center Boulevard, Winston-Salem, NC 27157-1083, USA

Abstract

Rationale—Performance of cognitive tasks in nonhuman primates (NHPs) requires specific brain regions to make decisions under different degrees of difficulty or “cognitive load.”

Objective—Local cerebral metabolic activity ($[^{18}\text{F}]\text{FDG}$ PET imaging) in dorsolateral prefrontal cortex (DLPFC), medial temporal lobe (MTL), and dorsal striatum (DStr) is examined in NHPs performing a delayed-match-to-sample (DMS) task with variable degrees of cognitive load.

Materials and methods—Correlations between cognitive load and degree of brain metabolic activity were obtained with respect to the influence of the ampakine CX717 (Cortex Pharmaceuticals), using brain imaging and recordings of neuronal activity in NHPs and measures of intracellular calcium release in rat hippocampal slices.

Results—Activation of DLPFC, MTL, and DStr reflected changes in performance related to cognitive load within the DMS task and were engaged primarily on high load trials. Similar increased activation patterns and improved performance were also observed following administration of CX717. Sleep deprivation in NHPs produced impaired performance and reductions in brain activation which was reversed by CX717. One potential basis for this facilitation of cognition by CX717 was increased firing of task-specific hippocampal cells. Synaptic mechanisms affected by CX717 were examined in rat hippocampal slices which showed that *N*-methyl-D-aspartic acid-mediated release of intracellular calcium was reduced in slices from sleep-deprived rats and reversed by application of CX717 to the bathing medium.

Conclusions—The findings provide insight into how cognition is enhanced by CX717 in terms of brain, and underlying neural, processes that are activated on high vs. low cognitive load trials.

Keywords

Hippocampus; Prefrontal cortex; Striatum; AMPA receptor; NMDA receptor; Macaque; Temporal lobe; PET; Behavior; Calcium imaging

Introduction

In prior reports from this laboratory, the ability to both enhance and restore cognitive performance was demonstrated in nonhuman primates (NHPs) engaged in a complex short-term memory and recall task (Porrino et al. 2005; Deadwyler et al. 2007). Two different agents were employed to show that cognitive performance could be enhanced under normal testing conditions or “reversed” under conditions where performance was debilitated by an interposed period of sleep deprivation. In those same reports, we showed that enhancement and reversal by these agents involved changes in the activation pattern of certain brain regions that were (1) differentially engaged by performance of the task and (2) sensitive to performance reduction by depriving the animals of sleep. The three brain regions, dorsolateral prefrontal cortex (DLPFC), medial temporal lobe (MTL), and dorsal striatum (DStr), showed differential susceptibility to the effects of sleep deprivation and were selectively responsive to the two agents: an ampakine CX717 (Cortex Pharmaceuticals, Irvine, CA, USA) and orexin-A, the orexin-1,2 receptor agonist.

Activation patterns of these three brain regions consistently reflect changes in level of cognitive performance (Wolf et al. 2008; Pallesen et al. 2008; Tomasi et al. 2007; Gazzaley et al. 2004; Barcelo et al. 2000) and hence provide a basis for understanding how agents that enhance or disrupt performance alter correlated activation patterns in the same regions. Because of the extensive prior work in this area with a well-characterized NHP model of cognitive performance, it is now possible to delineate how the ampakine CX717 enhanced performance under normal and sleep-deprived conditions. In addition, insight into what underlying cellular processes and neural encoding mechanisms are altered to produce the observed changes in NHP glucose activation patterns that occur under these conditions is presented. Major insight into conditions whereby the cognitive enhancer CX717 increased performance accuracy in the delayed-match-to-sample (DMS) task is provided in this study by an assessment of activation patterns of these three brain regions during performance on trials with low vs. high cognitive load (Deadwyler et al. 2007). In addition, other experiments investigate (1) the actions of CX717 on neuronal firing of cells that encode critical stimulus features during performance of the task and (2) the potentiation and modulation of cellular and synaptic processes coupled to AMPA receptors affected by this ampakine (CX717).

Materials and methods

Subjects, training, and cognitive testing

Subjects—All procedures were approved by the Animal Care and Use Committee of Wake Forest University and performed by established practices described in the National Institutes of Health Guide for Care and Use of Laboratory Animals. Results reported were obtained from 13 adult male rhesus monkeys (*Macaca mulatta*) weighing 6.0–12.0 kg. Subjects were housed individually or in groups in stainless steel cages within temperature- and humidity-controlled colony rooms with lighting maintained on a 0600:1800 on/off schedule. NHPs were fed a diet of monkey chow supplemented by fresh fruit to maintain daily monitored body weight. Fluid intake was controlled in time and amount to provide a large volume of the required fluid intake (80 mL/kg) during daily behavioral testing sessions, with the difference delivered via ad libitum administration when testing was not

performed. Some animals ($n=10$) were utilized in prior studies employing similar methods and procedures to those reported in this study (Porrino et al. 2005; Deadwyler et al. 2007).

Multiimage delayed-match-to-sample task—Training and testing were the same as reported previously with very few exceptions except that different types of DMS trials were presented in some sessions vs. others in order to assess the effects of cognitive load on performance. Subjects were tested while sitting in a primate chair 1.5 m in front of an LCD front projection screen on which clip-art images were presented as stimuli in a visual DMS task (Hampson et al. 2004). Animals were trained to move a cursor tracked by a fluorescent marker attached to the back of the monkey's hand on the screen by positioning the hand within a two-dimensional coordinate system referenced to the chair counter. Stimuli consisted of clip-art images projected as 25 cm squares within a 3×3 position matrix onto a 1.0×1.0-m screen display and movement of the cursor into the image constituted a response in the task. Responses to appropriate stimuli were rewarded with diluted fruit juice delivered through a sipper tube placed near the animals' mouth. All animals were trained to a stable baseline of performance (mean 70% correct trials) on the DMS task. Trials consisted of sample, delay and match phases in which a single stimulus image was presented constituting the sample image followed by a delay period of variable duration (1–90 s) in which the screen was blank and a subsequent match phase in which the sample image and one to five other clip-art images were presented simultaneously on the screen in different locations. A response within 5.0 s on the same image in the match phase was followed immediately by juice delivery and screen blanking for an intertrial interval of 10 s. Sessions consisted of 120–300 trials and lasted approximately 60–90 min. Each individual image was selected from a set of 5,000 (that was changed monthly) and used only once per session to maintain trial-uniqueness and decrease interference or generalization of stimulus features. Trials varied with respect to both duration of delay (1–30 or 60 s) and number of images (two to six, sample and one to five, nonmatch images) while performance accuracy (mean percentage of correct choices) both within and across animals varied directly as a function of these two parameters (Fig. 1a).

Sleep deprivation procedures—Sleep deprivation procedures for NHPs were as described previously (Porrino et al. 2005; Deadwyler et al. 2007) and consisted of 30–36 h of continuous sleep prevention supervised by laboratory personnel. Animals were maintained in a cage separate from their home cage in a continuously lighted room and kept awake with videos, music, occasional treats, gentle cage shaking, and interaction with technicians until their usual daily testing time. Sleep deprivation sessions were conducted a minimum of 10 days apart, interleaved with normal alert sessions to allow full recovery of baseline performance levels (typically within 24–48 h) following the sleep deprivation session. Adult and juvenile Sprague–Dawley rats were sleep-deprived by housing in a slowly rotating (1.0 rpm) 45-cm diameter running wheel for 48 h while food and water were continuously available. This method is sufficient to maintain rats in a state of rapid eye movement (REM) and non-REM sleep deprivation (McCoy et al. 2007; McKenna et al. 2007).

Drug administration—The ampakine CX717 (Cortex Pharmaceuticals, Irvine, CA, USA) was administered in 10% w/v hydroxypropyl-beta-cyclodextrin and saline (0.45%) vehicle through an intravenous vascular access port chronically implanted in each monkey. CX717 was mixed as 1.5 mg/mL and administered within a dose range of 0.3 to 1.5 mg/kg to each monkey (weight range 8–11 kg). Vehicle injections (cyclodextrin and saline, 0.45%) were administered to all monkeys prior to nondrug sessions. CX717 was administered 10 min prior to the start of each drug session. Normal sessions in which CX717 was administered were interposed between days with vehicle-only (normal) sessions. Recovery of baseline

DMS performance was required prior to administration of CX717 in either sleep-deprived or nonsleep-deprived sessions. In sessions in which positron emission tomography (PET) scans were conducted, CX717 was administered 10 min prior to isotope injection.

Brain imaging

Measurement of local rates of cerebral glucose metabolism—Measurements of local rates of cerebral glucose metabolism (CMR_{glc}) were made in ten NHPs in different conditions including: baseline (no task) in which NHPs watched a video, the standard DMS task–vehicle (mixed trial sessions), DMS task–vehicle (high and low cognitive load trial sessions), DMS task+CX717, DMS task following sleep deprivation, and DMS task–sleep deprivation+CX717. All animals were acclimated to the PET scan procedures and, in most cases, scans were obtained from animals employed in prior studies (Porrino et al. 2005; Deadwyler et al. 2007). On the day of the scan, animals were placed in the testing room and the DMS task initiated. Following ten DMS trials, a 30-s injection of 3–5 mCi of [^{18}F]-2-deoxy-2-fluoro-D-glucose (FDG) was administered. Animals worked another 40 min as ^{18}F FDG was incorporated during performance of the DMS task, then anesthetized with ketamine (15 mg/kg, iv) and transported to the PET scanner. After the PET scan, animals were transported back to their home cages and continuously monitored until fully recovered. Under this procedure, incorporation and measurement of the metabolized tracer reflected activation of brain regions during 80–100 trials of DMS task performance in the task (Porrino et al. 2005).

A population-averaged FDG blood curve (calculated for NHPs) was scaled to the measured blood curve for the time period from injection to the end of the PET scan (Takikawa et al. 1993; Porrino et al. 2005) and data transformed to CMR_{glc} based on the operational equation (Sokoloff et al. 1977; Phelps et al. 1979). Rate constants (k_1 , k_2 , and k_3) and lumped constant as determined in monkey by Kennedy et al. (1978), along with a k_4 value used in human studies (Phelps et al. 1979) were applied. Scan data were acquired with a General Electric (GE) Advance NXi PET scanner with a resolution of 4 mm. As described previously (Porrino et al. 2005), scanning consisted of a 5-min transmission scan acquired in 2D mode, followed by a 10-min emission scan acquired in 3D mode. The image reconstruction of the 3D data used the 3D-reprojection method with full quantitative corrections. Scans were smoothed and the emission data corrected for attenuation and reconstructed into 128×128 matrices using a Hanning filter with a 4-mm cutoff transaxially and a ramp filter with an 8.5-mm cutoff axially.

Reconstructed images for each scan were coregistered to corresponding structural magnetic resonance (MR) images obtained on a 1.5-T MR scanner (GE Medical Systems, Milwaukee, WI, USA) using automated image registration (Woods et al. 1993) and then transformed spatially into a standard space with a customized FDG template (Porrino et al. 2005). Resultant images were smoothed using a 2 mm isotropic Gaussian kernel with a voxel size of 1×1×1 mm. PET data were analyzed with the Statistical Parametric Mapping (SPM99) software (<http://www.fil.ion.ucl.ac.uk/spm/>) implemented in MATLAB (MathWorks, Natick, MA, USA). Scans were normalized for differences in global activity by proportional scaling. Effects at each voxel were estimated according to the general linear model using the multisubject conditions and covariates option in SPM. Statistical maps were created for comparisons between all of the above-indicated experiment conditions. Exploratory analyses used a minimum voxel height (magnitude) threshold of $p < 0.01$ and a minimum cluster size of 50 voxels. A region of interest (ROI) analysis was conducted including the DLPFC, MTL, DStr, parietal cortex (precuneus), and thalamus selected based on our prior investigations in these same conditions (Porrino et al. 2005; Deadwyler et al. 2007). Spherical ROIs were constructed on a structural MR template using the MarsBaR toolbox

contained in SPM. Statistical significance for the four comparisons described above was determined with a threshold value of $p < 0.05$ corrected for the search volume. Areas of activation were displayed on a T1-weighted anatomic magnetic resonance imaging (MRI) template (Black et al. 2001). Regions were then identified with reference to standard atlases of the primate brain (Paxinos et al. 2003).

Electrophysiology

Hippocampal neuronal recording during DMS task performance—In a prior report (Hampson et al. 2004), single-unit recording from NHP hippocampus during performance of the DMS task was described and hippocampal cell types were identified that responded to particular features of the multiimage stimuli presented during the task. During similar testing sessions, the ampakine CX717 was administered and assessed for effects on task-related neural firing. Procedures for preparation and recording from NHPs were described in detail in that report and were identical to those employed in sessions in which CX717 was administered as described in this study.

Calcium imaging

Calcium imaging in hippocampal slices—Hippocampal slices were prepared from adult Sprague–Dawley rats following the National Institutes of Health guidelines. After rapid decapitation, the brain was removed quickly to a chilled (4°C) oxygenated (95% O₂, 5% CO₂) artificial cerebrospinal fluid (ACSF) solution containing (in millimolar) NaCl (126), NaHCO₃ (20), KCl (5), MgCl₂ (2), CaCl₂ (2.5), glucose (10), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (20). Transverse slices (150–300 μm) were cut with a vibratome (Leica VT1000S), transferred to a darkened Petri dish containing 13 μM calcium green-1 acetoxymethyl ester (Molecular Probes), solution in ACSF with 20% Pluronic F-127 in dimethyl sulfoxide, incubated at room temperature with oxygenation for 45 min, rinsed in fresh ACSF, and maintained in the dark at room temperature in oxygenated ACSF. Individual slices were then transferred to a temperature-controlled recording chamber for imaging (Warner Instruments; Hamden, CT, USA) and perfused continuously with oxygenated ACSF at a rate of 1.8 mL/min.

Confocal microscopy—Intracellular calcium was imaged in individual CA1 pyramidal cells using a Nikon E600FS upright microscope equipped with a ×60 water-immersion objective, a Hamamatsu-Orca-ER digital camera, and a Perkin Elmer Ultraview LCI (spinning disc confocal) system with Melles Griot 488 nm laser illuminator. Calcium green fluorescence images were acquired with 488 nm excitation wavelength. For testing drug-induced changes in intracellular calcium (Rogers et al. 2006), slices were perfused for 8 min following exchange of ACSF from a different reservoir, 10 min with drug+ACSF, then ACSF alone for 10 min. Exchange of experimental solutions was controlled via a solenoid valve switching system (Warner Instruments) at equal pressure to insure constant perfusion rates.

Image analysis—The Ultraview LC was utilized to employ laser confocal illumination to image 1 μm thick “slices” of tissue and scans of the tissue to ±25 μm depth of field (from the initial focal plane of the microscope) using a piezoelectric stepper on the microscope objective lens. Images were acquired and stored every 1.0 s which continued for a preprogrammed interval (typically 6–10 min). Analysis of intracellular calcium was performed via software provided and consisted of defining one to ten ROIs (150–200 μm diameters) containing identified neurons in the CA1 region of the slice. Computation of fluorescent densitometry of the ROIs was in relation to background levels over the same time period. Photobleaching of the fluorescent dye was compensated by linear regression of ACSF only control recordings to remove any change in the measurements over time. ROI

fluorescence changes indicative of intracellular calcium release were then plotted relative to change in fluorescence from the control level ($\Delta F/F\%$).

Drug preparation and administration—Drug solutions were prepared from the same ACSF solution and administered via a separate valve switching system. *N*-methyl-D-aspartic acid (NMDA) was added to the slice medium of in vitro calcium imaging studies at concentrations of 10–50 μM for establishment of baseline levels of increased fluorescence from which to compare other drug levels. The following agents: the ampakine CX717 (20 μM), AMPA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX; 20 μM), or NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM) were also administered either individually or in combination with NMDA. All solutions were prepared fresh daily from concentrated stock in purified water and diluted to final concentrations in bathing medium. Prior work demonstrated the effectiveness of ryanodine (10–50 μM) and dantrolene (10 μM) in these contexts to verify the nature of the calcium release from intracellular stores as via calcium-sensitive calcium channels in the endoplasmic reticulum (Zhuang et al. 2005).

Results

Relationship between cognitive workload and brain areas engaged during DMS performance

Differences in cognitive workload activate different brain regions—The effects of cognitive load on performance are shown in Fig. 1a in terms of the decrease in performance on trials with (1) increased number of nonmatch images and (2) increased duration of delay. This effect is shown in the series of delay graphs plotted separately for each type of trial as a function of the number of nonmatch images. It is possible for different trial types to have the same degree of cognitive load based on how these two parameters were combined on a particular trial. However, it was not possible for animals to learn or anticipate the cognitive load on a given trial since both the duration of delay and number of nonmatch images were imposed after the animal responded (i.e., encoded) the sample image. The two extremes with respect to low and high cognitive load trials are bracketed by the blue and red dashed boxes, respectively. Figure 1b shows the normal performance differences for high and low load trials compared to overall average performance when each type of trial was presented randomly with other trials in the same session under normal testing conditions (mixed sessions).

Figure 1c shows the results of ^{18}F FDG PET scans obtained under different testing conditions to determine the effects of cognitive load on local CMR_{glu} activity in three brain regions previously shown to be engaged during DMS task performance in NHPs under different conditions (Porrino et al. 2005; Deadwyler et al. 2007). The results of voxel-by-voxel SPM analyses are shown superimposed on MRIs of the particular brain regions where significant differences were detected. Significantly increased metabolic activity in mixed trial session relative to nontask conditions (see the “Materials and methods” section) is shown in the left set of panels in Fig. 1c (DMS task) for DLPFC (top), DStr (middle), and MTL (bottom). This can be contrasted with the same measures in sessions where only high load trials were presented as shown in the difference images comparing mixed trial sessions with sessions in which only high cognitive load trials were presented to the animals (Fig. 1c, right panel). The color codes for the degree of significance of the difference between scan images are listed at the bottom of Fig. 1c. It is clear that the high load trials activated more specific areas within the three regions than sessions with mixed trials, suggesting that trials of higher degree of difficulty require activation of additional subregions of the same areas in order to sustain performance with a sufficient number of correct responses.

This difference between high and low load trials is shown explicitly in Fig. 2 in the same monkeys performing in sessions with only high vs. only low load trials compared. The images on the left show increased activation on high vs. low load sessions within all three brain regions shown in Fig. 1, as well as in the thalamus (Thal) and visual cortex (VISCX). Interestingly, there was a significant difference in the activation of the ventromedial prefrontal cortex (VMPFC) on low load trials in the same sessions, indicating that this region may not become activated on more difficult trials but is activated when the other regions (DLPFC, DStr, and MTL) are not, on trials that are less demanding. The above imaging data strongly suggest that cognitive processing is controlled by differential activation of subareas in the three brain regions (DLPFC, DStr, and MTL) engaged during performance of the DMS task (Fig. 1a–c). The behavioral data from the same sessions in which the scans were obtained for high vs. low load sessions (Fig. 1b) confirm the fact that performance did not differ from when the same (high or low load) trials were presented with other trials in mixed sessions.

Facilitation of DMS performance by the ampakine CX717 activates high cognitive load brain regions—The above demonstration of specific brain regions activated by more difficult DMS trials was assessed in a previous study employing the ampakine CX717 (Porrino et al. 2005), a drug which facilitates transmission by decreasing AMPA receptor-gated channel inactivation of (Suppiramaniam et al. 2001; Arai et al. 2000, 2002). Figure 3c shows that performance was facilitated in a dose-dependent manner in NHPs ($n=9$) tested in mixed trial sessions (Fig. 1a,b). Vehicle was delivered every day for 6 days to establish a baseline, then CX717 was delivered in order of increasing dose on alternating DMS sessions (days) to avoid possible carryover effects as reported previously in rats (cf. Hampson et al. 1998a, b). The brain activation pattern from CX717 sessions (Fig. 3b) in which the mean performance was elevated across all trials (Fig. 3c) shows two of the same areas that exhibited increased activation on high load trials in Fig. 2, DLPFC and MTL. The increase in activation relative to vehicle sessions was significant for DStr also (not shown in Fig. 3) as reported in Porrino et al. (2005).

Reversal of deficits in cognitive performance produced by sleep deprivation engages high load brain regions—In order to be consistent with the above findings, it is necessary that when cognitive performance is decreased below normal levels, the areas that are responsible for performance on high cognitive load trials should exhibit decreased activation. A recent study from this laboratory demonstrated that the deficit previously reported in this task following sleep deprivation in NHPs (Porrino et al. 2005) was due to a reduction in performance on high cognitive load trials (Deadwyler et al. 2007). Figure 4 shows the brain activation pattern for normal alert performance with two superimposed arrows indicating possible connecting loops between MTL and DLPFC and between Thal and other cortical regions not identified as yet, to which such mutual activation would be functionally significant (Tomasi et al. 2007; Gazzaley et al. 2004; Bockova et al. 2007; Friedman and Goldman-Rakic 1994). Associated with the decreased performance during sleep deprivation, results from both studies demonstrated deficiencies in activation of DLPFC and DStr with compensatory increased activation of MTL as shown in Fig. 4 (sleep deprivation). The two “Xs” suggest that sleep deprivation impairs this connectivity or “network activation” on high cognitive load trials and, therefore, results in less activation than normal of DStr and DLPFC. We have hypothesized that MTL attempts to compensate for this loss of DLPFC activation by increasing activity under sleep-deprived testing conditions; however, this is apparently not sufficient to reverse the performance deficit produced by sleep deprivation (Porrino et al. 2005). This reciprocity between MTL and DLPFC was also observed when animals were tested in a sleep-deprived state but injected with CX717 prior to testing; activation of DLPFC is increased back to nearly its normal

status, and MTL activity is decreased (Choo et al. 2005) reflecting the lack of need for MTL attempts to compensate for DLPFC inactivation, presumably, on high cognitive load trials. The sleep deprivation+CX717 condition shown in Fig. 4 (right panel) reflects the fact that the DLPFC/MTL network was restored by the administration of the ampakine to sleep-deprived NHPs; however, the decrease in thalamic metabolism (Thal) in the same animals was not reversed by CX717. This lack of reversal of the decrease in thalamic metabolic activity by CX717 indicates the specificity of the drugs' action for the brain regions that were more frequently observed to be engaged by the increased cognitive demands of the task, namely, DLPFC, MTL, and DStr (Figs. 1, 2, and 3).

Neuronal basis for CX717 enhancement of cognitive performance

Effects of CX717 on MTL neuronal activity during cognitive processing in the DMS task—In prior investigations from this laboratory, an ampakine similar to CX717 (CX516) enhanced performance by rats in a hippocampal-dependent delayed-nonmatch-to-sample (DNMS) task (Hampson et al. 1998a) and did so by altering task-related hippocampal cell firing (Hampson et al. 1998b). In a more recent report (Hampson et al. 2004), it was shown that hippocampal cells in NHPs encode images in the DMS task described in this study in terms of *visual categories* such as pictures of people, specific colors, shapes, faces, etc. (Fig. 5a). This tendency was shown to influence the selection strategies of NHPs performing the task, in a manner that could narrow the number of elements chosen from sample images since the stimuli differ on every trial in the task (see the “Materials and methods” section). Moreover, it was shown that such categories were defined by hippocampal cells that fired whenever images with similar elements were presented in the sample and nonmatch phases of the DMS task (Fig. 5b, upper), thereby enhancing recognition of the match image from the variable number of nonmatch images and facilitating performance. *Category cells* have been detected in all NHPs from which recordings have been obtained in the task ($n=5$). In addition, other cells have been identified that only respond in the delay phase of the DMS task (delay cells) and presumably have a role in processing information related to the variable length of delays in trials with different degrees of cognitive load as shown in Figs. 1 and 2. These two hippocampal cell types influence encoding of the two main parameters that affect cognitive load in the DMS task, duration of delay, and number of nonmatch images. Figure 5b (lower) shows the effect on performance of a facilitating dose of CX717 (1.0 mg/kg) on firing of a *category* and a *delay* cell in the DMS task. The two cells were isolated and recorded for the first half of the session following vehicle injections (upper rasters and histograms) and then for the remainder of the same session following injections of CX717 (lower records). It is clear that firing of both cell types increased significantly, which correlated with improved performance on high cognitive load trials during the same session (not shown). Presumably, these neural correlates are the basis for the consistent activation of MTL in the task as evidenced by increased ^{18}F FDG uptake and modifications related to CX717 administration (Figs. 3 and 4).

Possible synaptic basis for CX717 enhancement of cognitive performance

Modulation of NMDA-mediated release of intracellular calcium in hippocampal neurons by CX717—The ultimate basis for enhancement of performance in cognitive tasks must eventually reside at the level of connectivity and circuits, incorporating changes that occur in cellular and synaptic processes that underlie increased cellular activation (i.e., firing) in circumstances where there is increased cognitive demand or load. The ampakine CX717 has been studied in several in vivo and in vitro test systems to delineate its mechanism of action related to glutamatergic transmission and interaction with AMPA receptor-mediated processes (Larson et al. 1995; Ingvar et al. 1997; Turrigiano and Nelson 1998; Lynch 2002; Takahashi et al. 2003). Figure 5 demonstrates that hippocampal neurons

responsible for encoding features of the DMS task increased firing rates in task-specific events concomitant with the improved performance produced by administration of CX717. As a basis for the CX717-related increased firing and imaging changes (Figs. 1, 2, 3, and 4) during the task, the enhancement of glutamatergic synaptic transmission via positive modulation of AMPA receptors is a likely explanation (Suppiramaniam et al. 2001; Umemiya et al. 1999; Jahn et al. 2006). Simply modulating instantaneous current flow through AMPA receptor-gated sodium channels, however, may not be sufficient for the sustained performance increases consistently shown with CX717 throughout the entire testing session.

Alternatively, it is possible that positive allosteric modulation of AMPA receptor-gated currents could lead to downstream changes in cellular processes that are coupled to increased depolarization via the activation synaptic events during the task (Wang et al. 2008; Whitney et al. 2008; Morton-Jones et al. 2008; Dravid and Murray 2004). Figure 6 illustrates changes in the NMDA receptor-mediated intracellular calcium release via calcium-sensitive calcium channels (CSCs). In prior work utilizing calcium-specific fluorescence dyes, we showed that in vitro NMDA application released intracellular calcium via ryanodine-sensitive CSCs located in the endoplasmic reticulum of individual hippocampal neurons (Zhuang et al. 2005; see also Morton-Jones et al. 2008; Huang et al. 2004). Confocal images with 488 nm laser excitation (right) were captured from cells in the CA1 cell layer of hippocampal slices (Fig. 6d) every second for 10 min and analyzed for percent change in fluorescence over time after normalization for photobleaching (Fig. 6b). The cellular images and changes associated with NMDA (10 μ M)-mediated intracellular calcium release from individual CA1 neurons in slices of hippocampus (illustrated in Fig. 6d) are shown in Fig. 6a,b. Vehicle applications did not produce an increase in calcium release (black dotted trace, Fig. 6a), but NMDA (10 μ M, green curve Fig. 6a) increased calcium fluorescence in the same CA1 cell images as shown in Fig. 6b (NMDA). NMDA +CX717 (20 μ M) markedly facilitated the release of calcium provoked by NMDA as indicated in Fig. 6a,c (blue curve and bar) and the cell images shown in Fig. 6b (NMDA +CX717). Other important control measurements are shown by curves and bars in Fig. 6c. These include: (1) the blockade of the additional increase produced by NMDA+CX717 when slices were pretreated with NBQX, the AMPA receptor antagonist (solid red trace and red bar), which shows that only the increase *above the NMDA alone level* was sensitive to AMPA receptor activation by the ampakine; (2) the suppression of NMDA-induced release of intracellular calcium (dotted orange trace and bar Fig. 6a,c) by the NMDA receptor antagonist CNQX, which also prevented the increase provoked by NMDA+CX717 (dotted purple trace and bar). It is clear that for all conditions of modulation of intracellular calcium release, NMDA actions were required (Goldberg et al. 2003; Huang et al. 2004; Morton-Jones et al. 2008; Dravid and Murray 2004), as noted by the lack of increased fluorescence for any of the above agents applied in the absence of NMDA (Fig. 6c).

Finally, it is important to know whether the above NMDA-mediated changes in calcium release are sensitive to the same type of environmental manipulations that affect cognition in NHPs such as sleep deprivation. To determine this, rats were deprived of sleep over a 48-h period by housing them in a continuously rotating wheel (McCoy et al. 2007), over which time electroencephalography was periodically monitored to verify loss of slow-wave sleep. Following this exposure, slices of hippocampus were extracted from both sleep-deprived and normal (alert) rats and the same NMDA-based calcium release measures (Fig. 6) applied in CA1 cells. Figure 7 shows the results of these assessments in hippocampal slices from alert (a and c) and sleep-deprived (b and d) rats exposed to the same concentrations of NMDA (10 μ M) or NMDA+CX717 (20 μ M). NMDA elicited an increase in intracellular calcium in alert animals which was increased significantly by addition of CX717 (NMDA +CX717) as shown for the mean over all animals (slices) in Fig. 7c. However, in slices

exposed to the same concentration of NMDA from sleep-deprived animals, significantly less calcium was released from identified CA1 cells in comparison to alert animals (gray bar in Fig. 7d). Thus, the effects of sleep deprivation perturbed the ability of NMDA to provoke release of the same amount of intracellular calcium as in cells from alert animals. However, addition of NMDA+CX717 was sufficient to increase the amount of calcium release to the same extent as in cells in slices from alert animals. Figure 7c,d shows no significant difference between slices from sleep-deprived vs. alert animals with respect to calcium release by NMDA+CX717, indicating that positive AMPA receptor modulation by the ampakine was intact and sufficient enough to override the desensitizing effects of sleep deprivation in reducing NMDA receptor-mediated calcium release. The results clearly demonstrate that sleep deprivation produced a significant reduction in the baseline NMDA-induced calcium release compared to alert animals that was reversed by CX717, which suggests that sleep deprivation directly affects a process that could account for CX717-enhanced cognition (Schmitt et al. 2005; Rumpel et al. 2005; Granger et al. 1996).

Discussion

The above findings demonstrate the potential for CX717 to enhance the release of intracellular calcium via NMDA-mediated processes (Figs. 6 and 7) and thereby magnify the effects of glutamatergic synaptic depolarization to: (1) enhance cellular responses to the events that provoke such depolarization; and perhaps more importantly, (2) provide a consistent change in the calcium levels to enhance firing in MTL and DLPFC cells (Fig. 5) thereby providing the necessary change in metabolic substrate to enhance performance on high cognitive load trials as demonstrated in Figs. 1, 2, 3, and 4. While several issues remain to be resolved before such a direct relationship can be demonstrated, there are several aspects of the above pattern of results that support such a conclusion. Primarily, the findings that sleep deprivation (a) altered cognitive performance on high cognitive load trials in NHPs (Fig. 4) and (b) was effective in suppressing NMDA-mediated calcium release in the rat hippocampus (Fig. 7), but the detrimental effects were reversed when, (c) CX717 was administered to the same sleep-deprived NHPs (Fig. 4) or the same hippocampal slices from sleep-deprived rats (Fig. 7).

Enhancement of cognitive performance in the DMS task by NHPs has been shown for other agents besides CX717 (Deadwyler et al. 2007); however, in that study, the effective drug, orexin-A, did not improve performance in alert, nonsleep-deprived, NHPs. Thus, performance under sleep-deprived conditions may be susceptible to a number of different facilitating influences, any one of which could improve performance under those physiological conditions. However, many studies have shown increases in cognitive performance related to increased arousal (Rogers et al. 2003; Thomas et al. 2000), which may or may not activate the same specific brain regions shown to be engaged by increased cognitive load under normal (nonsleep-deprived) conditions (Figs. 1 and 2). The three brain regions described in this study, DLPFC, MTL and DStr, which were engaged and modified during varying conditions of cognitive demand (Figs. 1, 2, 3, 4) in the DMS task, have been traditionally associated with executive processing, memory, and motor planning, respectively, in similar studies with NHPs (Meyers et al. 2008; Hampson et al. 2004; Porrino et al. 2005; Cahusac et al. 1993; Colombo and Gross 1994) and are primary candidates for these roles in human cognition as well (Robertson et al. 2001; Ridderinkhof et al. 2004; Koechlin et al. 2003; Kane and Engle 2002; Manns et al. 2003). The effectiveness of the ampakine CX717 in facilitating normal performance while increasing activation in these areas (Figs. 1 and 2) and reversing the influences of sleep deprivation on ^{18}F FDG incorporation in these same brain regions (Figs. 3 and 4) implicates glutamatergic AMPA receptor-mediated synaptic processes as critical not only for maintenance of cognitive

performance, but also for enhancement of cognition above normal levels (Buccafusco et al. 2004; Porrino et al. 2005).

The demonstration in this study that NMDA-triggered calcium release in CA1 neurons from rat hippocampal slices is responsive to allosteric modification of AMPA receptor-mediated glutamatergic synaptic transmission by an ampakine such as CX717 (1) verifies earlier work from this laboratory and others showing specificity of NMDA-triggered activation of ryanodine-sensitive CSCs (Zhuang et al. 2005; Huang et al. 2004; Morton-Jones et al. 2008), (2) validates the fact that NMDA receptor activation can be modulated by increased glutamatergic synaptic activity in hippocampal neurons (Li et al. 2006; Umekiya et al. 2001; Liu et al. 2004; Jahn et al. 2006), and (3) implicates NMDA-mediated release of intracellular calcium as a major factor in the maintenance and enhancement of cognitive performance (Boric et al. 2008; Raymond and Redman 2006; Lareo and Corredor 2004; Petit 1988). The linkage between NMDA receptor function and sleep deprivation was demonstrated by McDermott et al. (2006) showing reduced presence of glutamate-triggered single-channel activation in NMDA receptors in sleep-deprived rats (McDermott et al. 2006; Chen et al. 2006; Kopp et al. 2006). The persistence of changes produced by NMDA-mediated release of intracellular calcium and the multiple numbers of cellular and synaptic pathways capable of being altered by this release (Granger et al. 1996; Lynch 2002; Rumpel et al. 2005) would provide a basis for the increased ¹⁸FDG uptake in the three brain regions that were differentially engaged by the DMS task and altered by administration of CX717 as shown in Figs. 1, 2, 3, and 4.

Enhancement of cognition as indicated by improved performance in cognitively based tasks has been demonstrated in humans together with correlated brain changes (Daselaar et al. 2004). It is also clear, however, that not all such changes involve the same three brain regions, a factor dependent primarily on the nature of the task (Choo et al. 2005; Chee and Choo 2004; Drummond and Brown 2001). In NHPs, there are also dissociations between brain regions engaged by tasks incorporating increased cognitive demand when compared with structures activated under more habitual behavioral performance conditions (Malkova and Mishkin 2003; Freedman et al. 2003; Inoue et al. 2004). In particular with respect to the prefrontal cortex, Bachevalier and Mishkin (1986) noted in monkeys that lesions involving the VMPFC impaired object recognition but not spatial delayed response, while lesions of DLPFC impaired the spatial delayed response but not recognition. Furthermore, imaging studies in humans (Petrides et al. 2002; Walter et al. 2007) indicate VMPFC involvement in low cognitive load aspects of memory tasks, whereas DLPFC is activated when cognitive workload is high. The findings presented in the current study suggest that the same brain regions that show increased activation under normal testing conditions (Figs. 1 and 2) are also differentially affected by decreased capacity to perform, such as during sleep deprivation (Figs. 3 and 4). In addition, administration of a drug such as CX717 that can presumably increase task-related neural activity in those same regions (Fig. 5) was sufficient to enhance cognitive performance under both normal as well as adverse conditions (Figs. 1, 2, 3, and 4).

Therefore, given that one of the major factors influencing performance in the DMS task was cognitive load and since it was possible for these same brain regions to show changes in activity during (1) sessions with trials with different cognitive loads and (2) compensation for loss of function under adverse testing conditions (i.e., sleep deprivation), it is likely that the DLPFC and MTL are major nodes within a neural circuit that modulate cognition and its enhancement in the primate brain.

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Fig. 1.

Performance by NHPs on DMS task under conditions of high and low cognitive load. **a** DMS trial-by-trial performance curves show the mean ($\pm$ SEM $n=9$ animals) percentage of correct trials sorted by delay (1–30 s, 5 s blocks) and number of images (two to six) presented in the match phase of the task. *Dashed boxes* indicate delay and no. of image combinations for what are considered low cognitive load trials (*blue box*: 1–10 s, two to three images) vs. high cognitive load trials (*red box*: 21–30 s, five to six images). **b** DMS performance over the entire session ($n=3$ sessions) consisting of (1) randomly presented high, low, and normal cognitive load trials (mixed sessions) or sessions consisting of only low or high load trials (exclusive sessions) as defined in **a** ($n=5$ animals). Performance on low and high load trials within mixed sessions is segregated for comparison with performance in exclusive sessions comprised of only low or high load trials. There was no significant difference in performance as a function of trial type in either mixed or exclusive sessions. Asterisks: $*p<0.01$, $**p<0.001$ refer to comparison with overall mixed session performance (*All*). **c** PET images compare local CMR_{glc} in brain regions activated by performance of DMS task vs. sessions in which the animals watched a video and no task was performed. Images shown are differences obtained by subtraction of no task sessions from DMS sessions. *Left* PET images at the level of the dorsolateral prefrontal cortex (DLPFC), striatum (DStr) and medial temporal lobe (MTL) show increased metabolic activity of the respective brain areas plus activation of sensory motor cortex (SI) while performing the DMS task. *Right* differences in CMR_{glc} shown for the same brain regions between mixed trial sessions and sessions comprised exclusively of high cognitive load trials indicate significantly increased activation in DLPFC, MTL, and DStr, but not in SI. *Color scale* indicates degree of statistical significance as multiples of t statistic for change in CMR_{glc} from global mean (see the “Materials and methods” section)

Fig. 2.

PET images comparing CMR_{glc} in sessions composed exclusively of high cognitive load trials to sessions composed exclusively of low cognitive load trials. Difference images computed as in Fig. 1c. *Left* difference between low and high load sessions. The same three brain regions shown in Fig. 1 (*DLPFC*, *DStr*, and *MTL*) plus the thalamus (*Thal.*) and visual cortex (*VISCX.*) showed increased activation on high load vs. low load DMS trials ($n=8$). *Right* opposite comparison of high load session PET images subtracted from images in low load sessions indicated an area in the VMPFC with greater activation on low load DMS trials. This same region in the frontal cortex is shown in Fig. 1 for mixed trial sessions. Significance of CMR_{glc} differences between sessions indicated by the *color scale* (*lower right*) as multiples of the t statistic as in Fig. 1c

Fig. 3.

Effects of the ampakine CX717 on DMS performance. **a** CMR_{glc} during normal (mixed trial session) DMS task performance shown in Fig. 1c in which drug vehicle was injected 5 min prior to start of session. **b** Additional activation of DLPFC and MTL during performance of DMS task following intravenous injection of the ampakine CX717 (0.8 mg/kg). *Color scale* indicates degree of significance of changes in CX717 vs. vehicle DMS sessions. **c** Overall performance during CX717 and vehicle mixed trial sessions for four animals in which CMR_{glc} comparisons are shown in **b**. Mean percentage of correct ($\pm$ SEM) overall session trials on successive days when animals were administered either vehicle or 0.5–1.5 mg/kg CX717. ** $p < 0.001$, differences in performance relative to vehicle sessions

Fig. 4.

The ampakine CX717 reverses changes in brain region CMR_{glc} produced by sleep deprivation. PET scans at the level of DLPFC (*top*) and MTL/Thal (*bottom*) are shown for successive conditions of normal alert mixed trial DMS test sessions (*left*); different test sessions conducted following 30–32 h sleep deprivation (*center*); and sleep deprivation test sessions in which CX717 was administered (*right*). Effects of CX717 (0.8 mg/kg, iv) on CMR_{glc} in sleep-deprived animals ($n=9$) as reported in Porrino et al. (2005). *Color scales (inset right)* in PET scans indicate significant (t statistic) increases (yellow–orange) or decreases (blue) in CMR_{glc} for difference images of normal vehicle DMS sessions vs. no task condition (*left*); sleep-deprived DMS sessions vs. normal vehicle DMS sessions (*center*); and sleep-deprived+CX717 DMS sessions vs. sleep-deprived DMS sessions (*right*). Arrows indicate suggested neural processing “loops” from MTL to PFC and Thal to PFC disrupted by sleep deprivation. Decreased CMR_{glc} in DLPFC combined with increased CMR_{glc} in MTL following sleep deprivation and reversal by CX717 can be contrasted to lack of reversal in the Thal/PFC loop (Porrino et al. 2005)

Fig. 5.

Enhancement of neuronal firing in the hippocampus following the administration of CX717. Hippocampal category-selective neuron fires in response to multiple images within a category (Hampson et al. 2004). *Left* sample images presented in DMS task and response to other similar images (*underscored*) in the same category (*probes*). *Center* neural firing of a *category cell* during DMS task following vehicle or CX717 (1.0 mg/kg). *Upper* rastergram and trial-based histogram of firing of a *category cell* to images with a human face. Each *row of dots* is the occurrence of action potential reflecting cell firing on a single trial in which an image containing one or more faces was presented. *Red dots* indicate behavioral responses to the sample and match image presentations (*vertical lines*). Histogram below raster is the sum of spikes from the same cell across all trials in the session normalized in 250-ms bins to spikes per second (Hz). *Lower* recordings from same CA1 neuron after CX717 (1.0 mg/kg, iv) was administered during the session showed a significant increase in sample peak firing rate (vehicle, 15.2 ± 0.3 Hz; CX717, 20.3 ± 0.5 Hz; $F_{(1,1588)}=12.4$, $p<0.001$) and prolonged the duration of stimulus elicited discharge. *Upper right* rastergrams and trial-based histograms of a hippocampal *delay cell* which fired only during the delay phase (2–17 s on horizontal axis) of the DMS task. *Lower right* administration of CX717 (1.0 mg/kg I.V.) significantly increased the frequency of sustained firing during the delay phases of the task (vehicle, 9.3 ± 0.2 Hz; CX717, 18.7 ± 0.7 Hz; $F_{(1,1588)}=24.7$, $p<0.001$) as shown in the lower trial-based histogram (CX717). DMS trial events are indicated on the *horizontal axis*, start ring *arrow*, sample and match presentation indicated by *vertical lines* at 0.0 and 17 s, respectively. Juice reinforcement delivered at the time of correct match response. Single neurons in the hippocampus were recorded using tungsten electrodes (tip diameters 2–5 μ M) (Hampson et al. 2004)

Fig. 6.

Confocal fluorescent imaging of intracellular calcium in slices of rat hippocampus. **a** and **b** Changes detected by intracellular calcium green fluorescence (illustrated in **b**) relative to background fluorescence ($\Delta F/F$) produced by bath application of NMDA (10 μ M, *black bar on horizontal axis*). **b** Images of fluorescence for multiple CA1 cells in a single slice are shown before and after perfusion with different agents. *Colored traces (left)* indicate the mean time-dependent increase in fluorescence from multiple slices ($n=9$ slices; three to eight cells analyzed per slice) corresponding to the increase in intracellular calcium concentration following transient exposure to NMDA (10 μ M) in the bathing medium as shown in **b** (NMDA). Slices were exposed to NMDA alone (*green trace*), then to a combination of NMDA and one or more other agents known to modulate NMDA receptor function. Coadministration of the ampakine CX717 (20 μ M) with NMDA produced an increase in NMDA peak fluorescence (*blue trace*). Exposure with the AMPA receptor antagonist NBQX (20 μ M) blocked the increase in NMDA fluorescence produced by CX717 (*red trace*). Application with the NMDA receptor antagonist CNQX (20 μ M) decreased the NMDA-induced increase (*dashed red trace*) and also suppressed the increase produced by coadministration of CX717 (*dashed purple trace*). NMDA was delivered as a transient (100 s) perfusion in the bathing medium, and slices were exposed to normal medium (ACSF) before and after exposure to each agent. Slices were exposed to CX717, NBQX, and CNQX in combination with NMDA for 10 min after transient (6 min) exposure to NMDA alone. **c** Mean change in peak fluorescence (as shown in **a**) across all cells in which NMDA was coadministered with CX717, NBQX, and/or CNQX. *Asterisks* indicate significant differences with respect to NMDA alone (** $p < 0.001$). **d** Area of 300 μ m thick hippocampal slices (extracted from adult rats aged 45–65 days) showing CA1 cell layer in which pyramidal cells were imaged

Fig. 7.

Sleep deprivation induced decrease in NMDA-mediated intracellular calcium flux is reversed by CX717. **a** Mean change in calcium green fluorescence following exposure to NMDA (10 μ M) and NMDA+CX717 (20 μ M) in slices from alert 45- to 65-day-old rats ($n=3$ rats, three slices per rat, four to eight cells per slice). Slices were exposed to normal medium (5 min) before and after exposure to NMDA (solid line). Slices were exposed to CX717 (dashed line) in the bathing medium for 10 min prior to as well as during NMDA exposure. **b** Effects of NMDA and CX717 in slices from sleep-deprived (*Sleep Dep.*) rats ($n=3$). Traces indicate mean change in calcium green fluorescence in response to NMDA (solid line) and NMDA+CX717 (dashed line) in slices ($n=10$ slices, five to nine cells per slice) prepared immediately following 48 h sleep deprivation procedure. **c** Mean peak fluorescence indicating increased intracellular calcium concentration in response to NMDA (gray bar) and NMDA+CX717 (striped bar) in slices from alert (nonsleep-deprived) rats as described in **a**. * $p < 0.010$, significant increase NMDA+CX717 vs. NMDA alone. **d** Mean peak fluorescence for NMDA (gray bar) and NMDA+CX717 (striped bar) in slices from sleep-deprived (*Sleep Dep.*) rats shown in **b**. ** $p < 0.001$, significant decrease in NMDA elicited peak in slices from sleep-deprived vs. alert rats; ‡ $p < 0.001$, significant increase in NMDA+CX717 vs. NMDA alone in sleep-deprived slices. NMDA+CX717 fluorescence was not significantly different in alert vs. sleep-deprived slices