Marqueurs neurophysiologiques de la cognition et du vieillissement : Études par stimulation magnétique transcrânienne

Marielle Young-Bernier

Thèse soumise à la
Faculté des études supérieures et postdoctorales
dans le cadre des exigences
du programme de doctorat en philosophie - Psychologie clinique

École de psychologie
Faculté des sciences sociales
Université d'Ottawa

© Marielle Young-Bernier, Ottawa, Canada, 2015

REMERCIEMENTS

D'abord et avant tout, je tiens à remercier mes superviseurs, Patrick Davidson et François Tremblay, qui m'ont accompagné et guidé tout au long de ce processus. Sans leur support, patience, temps et encouragements, ce projet n'aurait pu être possible.

Un sincère merci à Annick Tanguay pour son aide indispensable au cours des deux dernières années de ce projet. Merci aussi à Yael Kamil, Travis Davidson, Sabah Master, Francisca Avila-Ramirez et Héloïse Drouin pour leur aide au niveau du recrutement et la collecte de données.

Je remercie aussi mes parents, Denis Bernier et Lucille Young, qui m'ont toujours encouragé à persévérer et à poursuivre mes objectifs. Finalement, je dois le plus grand remerciement à mon conjoint, Patrick Savoie, pour son support constant et sa patience tout au long de ce processus.

RÉSUMÉ

Le système cholinergique joue un rôle important au niveau de l'attention, de la mémoire et des fonctions exécutives. Les processus corticaux sous-tendant ces fonctions cognitives reposent sur des mécanismes de plasticité synaptique, tels que la potentialisation à long terme (PLT), qui font aussi appel à la transmission cholinergique. La diminution de l'activité cholinergique et de la plasticité synaptique lors du vieillissement normal semble contribuer au déclin des fonctions cognitives chez les personnes âgées. Les études comprises dans cette thèse portent sur un marqueur de l'activité cholinergique dérivé de la stimulation magnétique transcrânienne (SMT), soit l'inhibition afférente à courte latence (*short-latency afferent inhibition*, en anglais; SAI). Les modifications liées à l'âge dans les niveaux de SAI ont été examinées chez des jeunes adultes et des personnes âgées en santé. Celles-ci ont ensuite été mises en lien avec la performance à des tests psychomoteurs et cognitifs évaluant l'attention, la mémoire, et les fonctions exécutives. Nous avons aussi examiné comment ces variations liées à l'âge dans les niveaux de SAI influencent la susceptibilité d'exprimer de la plasticité de type PLT dans le cortex moteur en réponse à un protocole de stimulations répétitives intermittentes par bouffées thêta (SiBT).

Nos résultats ont d'abord montré que le conditionnement afférent des réponses évoquées par la SMT était sélectivement moins efficace à 20 ms chez les personnes âgées que chez les jeunes adultes, indiquant ainsi une réduction sélective du SAI avec l'âge (Études 1 et 3/4). Cette diminution des niveaux de SAI suggère un déclin de l'activité cholinergique avec l'âge. Des déclins associés au vieillissement normal ont ensuite été notés dans de nombreuses tâches examinant les fonctions psychomotrices et cognitives (Études 1-3). À cet égard, nous avons montré que les variations liées à l'âge dans les

niveaux de SAI étaient significativement corrélées à la performance à des tests psychomoteurs complexes (Études 1 et 3), ainsi qu'à un score global reflétant la mémoire épisodique (Étude 2). Toutefois, les variations dans les niveaux de SAI n'étaient pas associées aux fonctions exécutives (Étude 2) ou à l'attention (Étude 3). Finalement, les niveaux de SAI mesurés chez les jeunes et les personnes âgées étaient peu indicateurs de la susceptibilité à exprimer de la plasticité synaptique de type PLT suite au protocole SiBT. En fait, nos résultats ont plutôt mis en évidence la présence d'une grande variabilité interindividuelle dans les réponses à ce protocole de stimulations répétitives visant la plasticité corticale (Étude 4).

Dans l'ensemble, nos travaux suggèrent un déclin parallèle de l'activité cholinergique et de certaines fonctions cognitives lors du vieillissement normal. La diminution de l'efficacité du système cholinergique dans le cortex moteur, telle que reflétée dans les niveaux de SAI, semble être liée au déclin des fonctions psychomotrices et de la mémoire avec l'âge. Toutefois, des liens n'ont pu être clairement établis avec la susceptibilité individuelle à exprimer de la plasticité synaptique. D'autres études seront nécessaires afin de clarifier davantage la relation entre la diminution de l'activité cholinergique avec l'âge, telle que mesurée *in vivo* avec la SMT, et le déclin des performances cognitives.

LISTE DES ABRÉVIATIONS

ACh	Acétylcholine
AChE	Acétylcholinestérase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionique
APB	Muscle court abducteur du pouce
ChAT	Choline acétylcholinestérase
DLT	Dépression a long terme
GABA	Acide γ-Aminobutyrique
FDI	Premier muscle dorsal interosseux de la main
IRM	Imagerie par résonance magnétique
MA	Maladie d'Alzheimer
NMDA	N-methyl-D-asparate
PEM	Potentiel évoqué moteur
PLT	Potentialisation à long terme
SAA	Stimulation associative appariée
SAI	Inhibition afférente à courte latence (*Short latency afferent inhibition*)
SBT	Stimulations par bouffées thêta
SiBT	Stimulations intermittentes par bouffées thêta
SET	Stimulation électrique transcrânienne
SMT	Stimulation magnétique transcrânienne
SMTr	Stimulation magnétique transcrânienne répétée
TEMP	Tomographie d'émission monophotonique
TEP	Tomographie par émission de positons

TABLE DES MATIÈRES

CHAPITRE I. INTRODUCTION ET REVUE DE LA LITTÉRATURE

Introduction

En 2011, environ 15% des canadiens âgés de plus de 65 ans souffraient de démence ou avaient fait l'objet d'un diagnostic de déficit cognitif léger, qui est souvent considéré comme un stade transitoire entre le vieillissement normal et la démence. On s'attend à ce que ce nombre double d'ici les vingt prochaines années (Société Alzheimer Canada, 2012). Pour cette raison, la caractérisation des changements neurophysiologiques et cognitifs sous-jacents à la démence a suscité beaucoup de travaux visant à identifier des pistes de traitements possibles pour ces troubles neurodégénératifs. Toutefois, la caractérisation du vieillissement normal s'avère aussi importante. En effet, le vieillissement cérébral « normal » est lui aussi associé à des altérations structurales et physiologiques ainsi qu'à des changements cognitifs, tels que des déclins au niveau de l'attention, de la mémoire et des fonctions exécutives (e.g., Drag & Bieliauskas, 2010).

Des altérations affectant les systèmes de neurotransmission lors du vieillissement contribuent aux déclins des fonctions cognitives avec l'âge. Parmi ceux-ci, une diminution de l'efficacité du système cholinergique semble avoir un impact particulièrement notable sur le vieillissement cognitif normal et pathologique, notamment dans la maladie d'Alzheimer (MA; e.g., Bartus, 2000; Bartus, Dean, Beer, & Lippa, 1982; Terry & Buccafusco, 2003). Le système cholinergique joue un rôle dans les fonctions cognitives en influençant, entre autre, la plasticité synaptique au niveau cortical (e.g., Hasselmo, 2006; Mitsushima, Sano, & Takahashi, 2013).

La stimulation magnétique transcrânienne (SMT) permet d'explorer *in vivo* et de façon sécuritaire et minimalement invasive la physiologie du cortex cérébral chez l'humain. Des techniques relativement récentes faisant appel à la SMT sont centrales à ce projet de thèse. D'abord, la SMT permet l'étude d'un circuit dans le cortex moteur

primaire qui est impliqué dans l'inhibition intra-corticale et dont l'efficacité synaptique repose sur la transmission cholinergique (Di Lazzaro et al., 2000; Di Lazzaro et al., 2002; Tokimura et al., 2000). Au moyen de cette technique, il est donc possible de dériver un marqueur *in vivo* de l'activité cholinergique dans le cortex moteur chez l'humain, ce qui ouvre la porte à l'étude des modifications de ce système de neurotransmission pendant le vieillissement normal ou pathologique (e.g., démences de type Alzheimer ou vasculaire, déficit cognitif léger). L'exploration *in vivo* de processus liés à la plasticité corticale (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005; Stefan, Kunesch, Cohen, Benecke, & Classen, 2000), qui reposent en partie sur la transmission cholinergique (Rasmusson; 2000), s'avère aussi possible au moyen de la SMT par l'entremise de protocoles de stimulations répétitives (i.e., SMTr).

1. Changements neurophysiologiques associés au vieillissement

Plusieurs changements surviennent au niveau structural et physiologique dans le cerveau humain vieillissant. Entre autre, le volume total du cerveau diminue avec l'âge et il en est de même pour la majorité des régions du cortex (Fjell, McEvoy, Holland, Dale, & Walhovd, 2014; Raz et al., 2005). Toutefois, la vitesse de détérioration des régions du cortex cérébral est hétérogène (Fjell et al., 2013). En effet, l'atrophie corticale semble suivre une progression antérieure-postérieure, ce qui implique que le cortex frontal se détériore le plus rapidement, suivi de près par le lobe temporal, alors que la corrélation entre l'âge et l'atrophie des lobes pariétaux et occipitaux est plus faible (Fjell et al., 2014; Raz & Rodrigue, 2006). La relation entre l'atrophie de la matière grise et l'âge est particulièrement importante dans les régions du cortex préfrontal et orbitofrontal ainsi que dans les gyri parahippocampique et fusiforme des régions limbiques et temporales

(Hogstrom, Westlye, Walhovd, & Fjell, 2013). En plus des changements prenant place au niveau de la matière grise, les altérations structurales du cortex associées à l'âge se caractérisent par une détérioration de la matière blanche, surtout dans la région préfrontale (Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009), ainsi que par un élargissement des ventricules (Fjell et al., 2013).

D'autres altérations structurales, telles que la dégénérescence neurofibrillaire, la formation de plaques amyloïdes et les maladies microvasculaires, sont aussi associées au vieillissement normal (Price et al., 2009; Warsch & Wright, 2010). Bien qu'ils aient une influence sur la cognition, ces changements structuraux peuvent être présents en l'absence de déficits détectables au plan cognitif (e.g., Knopman et al., 2003; Villeneuve & Belleville, 2010).

Les principaux systèmes de neurotransmission du cortex cérébral (i.e., cholinergique, dopaminergique, GABAergique, glutaminergique, sérotoninergique, noradrénergique) subissent aussi des changements importants lors du vieillissement. Ces changements s'expriment notamment par une réduction du nombre de projections de ces systèmes ainsi que par une diminution de la densité de leurs récepteurs (e.g., Backman, Nyberg, Lindenberger, Li, & Farde, 2006; Fidalgo, Ivanov, & Wood, 2013; Rissman, De Blas, & Armstrong, 2007; Robertson, 2013; Segovia, Porras, Del Arco, & Mora, 2001; Sunderland, 2005). La caractérisation des altérations affectant le système cholinergique s'avère particulièrement importante puisqu'il semble constituer l'un des substrats anatomiques sous-tendant les changements cognitifs associés au vieillissement normal et pathologique (Dumas & Newhouse, 2011). En effet, le vieillissement est associé à une

diminution de l'innervation cholinergique et celle-ci est encore plus prononcée chez les patients atteints de la MA (Mesulam, 2004a).

1.1 Organisation du système cholinergique

L'acétylcholine (ACh) est synthétisée dans le neurone présynaptique à partir de la choline, qui provient de la diète alimentaire. L'enzyme choline acétyltransférase (ChAT) permet la synthèse de l'ACh alors que l'acétylcholinestérase (AChE) la dégrade dans la fente synaptique. L'ACh agit sur deux classes de récepteurs cholinergiques dans le cerveau, soit les récepteurs muscariniques (M1-M5) et nicotiniques (types alpha et bêta). Les récepteurs muscariniques de type M1 sont les plus nombreux dans le système nerveux central (Mash, White, & Mesulam, 1988) et se situent principalement sur les neurones pyramidaux, qui constituent donc une cible importante des projections cholinergiques au niveau cortical (Yamasaki, Matsui, & Watanabe, 2010).

La majorité des projections cholinergiques proviennent de noyaux cellulaires situés dans la formation réticulée pontique (e.g., noyau pédonculo-pontin) ainsi que dans le prosencéphale antéro-basal (e.g., noyau basal de Meynert; Scarr, Gibbons, Neo, Udawela, & Dean, 2013). Les projections du système pédonculo-pontin latéral se terminent principalement sur le thalamus, le mésencéphale et d'autres régions du tronc cérébral (McKinney & Jacksonville, 2005; Scarr et al., 2013). Pour leur part, les noyaux cellulaires du prosencéphale antéro-basal innervent l'hippocampe (i.e., groupes cellulaires Ch1 et Ch2 du noyau septal médian et du bras vertical de la bande diagonale de Broca), le bulbe olfactif (groupe Ch3), ainsi que l'amygdale et chacune des régions du cortex cérébral (groupe Ch4 du noyau basal de Meynert; Mesulam, 2013; Selden, Gitelman, Salamon-Murayama, Parrish, & Mesulam, 1998).

1.2 Altérations du système cholinergique lors du vieillissement

Plusieurs changements biochimiques et structuraux affectent le système cholinergique lors du vieillissement. Ceux-ci entraînent une diminution de l'innervation cholinergique et de l'efficacité de la transmission synaptique de ce système dans le cerveau. Au niveau biochimique, le vieillissement affecte la synthèse de l'ACh, son relâchement dans la fente synaptique et l'affinité des récepteurs qui permettent la recapture de la choline dans le neurone présynaptique (Cuello, 2006). Au niveau structural, les recherches animales montrent, entre autre, une atrophie et une perte des corps cellulaires dans le noyau basal de Meynert ainsi qu'une atrophie des boutons synaptiques cholinergiques (Mesulam, Mufson, & Rogers, 1987; Turrini et al., 2001).

Le complexe cellulaire Ch4 semble être particulièrement affecté par le vieillissement et la MA (revue par Mesulam, 2013). Plus spécifiquement, le noyau basal de Meynert montre une vulnérabilité à l'accumulation de protéines tau hyperphosphorylées, ce qui mène à la formation d'enchevêtrements neurofibrillaires. Ces enchevêtrements sont présents en grand nombre dans la région du prosencéphale antéro-basal des patients ayant un diagnostic de déficit cognitif léger et leur densité est encore plus grande chez les patients atteints de la MA. Toutefois, leur présence est moins importante chez les personnes âgées en santé, qui ne présentent pas de déclin cognitif. L'accumulation d'enchevêtrements neurofibrillaires dans le noyau basal a un impact sur le fonctionnement des neurones cholinergiques (e.g. perte de projections vers le cortex, diminution du nombre de terminaux pré-synaptiques de l'ACh) et mène éventuellement à leur mort (Mesulam, 2012, 2013).

La transmission cholinergique est aussi affectée par l'accumulation de la protéine β-amyloïde dans le cortex lors du vieillissement (Schliebs & Arendt, 2011) ainsi que par une atteinte au fonctionnement du système vasculaire cérébral (Sarter & Bruno, 2004). Toutefois, de nombreuses questions demeurent en ce qui a trait à la contribution des enchevêtrements neurofibrillaires, des plaques amyloïdes et des dommages microvasculaires sur la diminution de l'activité cholinergique, ainsi qu'à leur ordre d'apparition dans le vieillissement.

1.3 Rôles fonctionnels du système cholinergique et neuromodulation

Le système cholinergique a longtemps été considéré comme étant un système de neuromodulation ayant une organisation topographique diffuse dans lequel l'ACh est relâché dans l'espace extra-cellulaire et active des récepteurs se retrouvant à une certaine distance du bouton synaptique (e.g., Picciotto, Higley, & Mineur, 2012; mais voir Zaborszky et al., 2013). À cet égard, les études animales ont montré que les projections du système cholinergique innervent chacune des couches cellulaires ainsi que l'ensemble des régions du cortex cérébral (Eckenstein, Baughman, & Quinn, 1988). De plus, la densité des projections cholinergiques est moins grande que celle des neurones ciblés par ce neurotransmetteur dans le cortex, ce qui suggère un mode de transmission diffus de l'ACh (e.g., Yamasaki et al., 2010). La transmission diffuse et tonique de l'ACh permet de moduler les niveaux ambiants de ce neurotransmetteur dans l'espace extracellulaire de façon simultanée à travers une grande région du cortex (e.g., Parikh, Kozak, Martinez, & Sarter, 2007; Sarter, Lustig, & Taylor, 2012; Yamasaki et al., 2010). Une dysfonction de ce système de neurotransmission peut donc affecter l'activité de plusieurs régions

corticales (Hasselmo & Sarter, 2011). Ce mode de transmission est impliqué dans l'attention et la mémoire (voir ci-bas; Hasselmo & Sarter, 2011).

Des études récentes suggèrent aussi la présence d'un mode de transmission cholinergique plus dynamique, qui exercerait une influence sur des régions corticales plus restreintes (Sarter, Parikh, & Howe, 2009). Dans le cortex préfrontal, ce mode de transmission dynamique de l'ACh influencerait la capacité de « détection » de stimuli, permettant ainsi une réponse comportementale lors de tâches d'attention (Parikh et al., 2007; Sarter, Lustig, Howe, Gritton, & Berry, 2014; Sarter et al., 2009). Les modes de transmission tonique et dynamique de l'ACh agissent de concert afin de moduler l'activité corticale, mais leurs interactions et leurs rôles plus spécifiques dans la cognition demeurent encore méconnus (Hasselmo & Sarter, 2011).

1.4 Système cholinergique et plasticité

L'ACh joue un rôle important dans l'excitabilité corticale et la neuroplasticité (Gu, 2002; Mesulam, 2004b; Rasmusson, 2000). Plus précisément, la transmission cholinergique jouerait un rôle permissif sous-tendant la capacité de réorganisation des cartes corticales lors de l'apprentissage par l'entremise de son action facilitatrice sur des mécanismes synaptiques, dont la potentialisation à long terme (PLT). La plasticité PLT correspond à des altérations durables dans l'excitabilité neuronale. Elle repose sur des changements dans l'activité des récepteurs N-methyl-D-aspartate (NMDA), suite à leur activation par le glutamate, qui influencent ensuite la perméabilité des récepteurs α-amino-3-hydroxy-5-methyl-4-isoxazolepropionique (AMPA) en permettant une augmentation de la concentration du Ca^{2+} (calcium) intracellulaire (Thickbroom, 2007). La PLT se produit dans la majorité des synapses excitatrices du cerveau et elle constitue

l'un des processus cortical à la base des nouveaux apprentissages et de la mémoire (Malenka & Bear, 2004).

En ce qui à trait au système cholinergique, une analyse de la littérature effectuée par Rasmusson (2000) révèle que l'ACh contribue à l'induction de la plasticité en facilitant les mécanismes PLT en participant à la dépolarisation du neurone post-synaptique, facilitant ainsi l'ouverture des canaux NMDA et l'influx de Ca^{2+} pour changer la perméabilité des récepteurs AMPA, ou en régulant la libération d'autres neurotransmetteurs au niveau pré-synaptique (i.e., facilitation de la libération de glutamate ou réduction de la transmission GABAergique, qui a une action inhibitrice sur ces processus). Toutefois, l'ACh pourrait aussi jouer un rôle indépendant dans l'initiation de la plasticité (i.e., sans activer les canaux NMDA) par l'entremise de son action directe sur le Ca^{2+} ou en participant à d'autres mécanismes intracellulaires. Des études animales montrent que les agonistes cholinergiques ont pour effet de rehausser la réponse des neurones pyramidaux de l'hippocampe et de faciliter la PLT dans cette région, tandis que les antagonistes diminuent cette dernière. Des études *in vitro* ont aussi permis de montrer une modulation cholinergique des processus PLT prenant place dans les autres régions du cortex, dont dans les différentes couches corticales du cortex moteur (Rasmusson, 2000).

La PLT dépend de l'activité simultanée des neurones pré- et post-synaptiques (i.e., concepts de plasticité Hebbiens), ce qui a pour effet de renforcer les synapses situées entre les neurones impliqués et d'augmenter l'excitabilité corticale (Cooke & Bliss, 2006). À cet égard, l'ACh pourrait aussi jouer un rôle de focalisateur des signaux dans les processus PLT. Plus précisément, l'activité cholinergique faciliterait l'analyse de l'information parvenant aux réseaux neuronaux en améliorant la qualité du signal afférent

tout en diminuant l'importance des bruits de fonds (i.e., augmentation du rapport « signal-bruit »; Kuo, Grosch, Fregni, Paulus, & Nitsche, 2007). Par exemple, la modulation de l'activité des récepteurs muscariniques suite à l'administration d'agonistes cholinergiques facilite la réactivité des neurones, et donc l'efficacité corticale, aux stimulations dans les aires primaires visuelle et somatosensorielle (Rasmusson, 2000).

1.5 Cortex moteur

Malgré la vulnérabilité accrue du cortex préfrontal et des lobes temporaux aux effets du vieillissement (Fjell et al., 2014; Raz et al., 2005), il faut souligner que le cortex moteur, qui est la cible de nombreuses études SMT en raison de la possibilité d'y mesurer l'impact direct de la stimulation, est lui aussi affecté par des changements structuraux importants. En effet, les aires primaires motrices sont atrophiées (Salat et al., 2004; Ziegler et al., 2010) et la morphologie du sillon central (ou scissure de Rolando), qui contient les cartes motrices et sensorielles, change de façon significative lors du vieillissement (Li et al., 2011). Des plaques amyloïdes ainsi que des enchevêtrements neurofibrillaires sont aussi présents dans le cortex moteur des patients atteints de la MA (Suva et al., 1999).

Quant à l'ACh, les études animales et chez l'humain ont montré la présence de nombreux récepteurs muscariniques dans le cortex moteur, suggérant ainsi que cette région est la cible d'une grande densité de projections cholinergiques (Geula & Mesulam, 1996; Lidow, Gallager, Rakic, & Goldman-Rakic, 1989). De plus, tout comme dans les autres régions du cortex cérébral et du système limbique, les projections cholinergiques vers le cortex moteur sont majoritairement extrinsèques et proviennent du prosencéphale antéro-basal, alors que les interneurones cholinergiques y sont seulement présents en petit

nombre (Mesulam, 1990; von Engelhardt, Eliava, Meyer, Rozov, & Monyer, 2007). Tel que mentionné ci-haut, cette organisation anatomique supporte l'idée selon laquelle la libération d'ACh a un effet diffus sur l'activité du cortex cérébral. À cet égard, chez le rongeur, les changements toniques de l'activité cholinergique dans le cortex moteur sont corrélés à ceux prenant place dans le cortex préfrontal lors d'une tâche d'attention nécessitant la détection de stimuli (Parikh et al., 2007).

En ce qui à trait aux processus de plasticité, l'ACh permet la réorganisation des cartes corticales motrices lors d'apprentissages moteurs chez l'animal (Conner, Chiba, & Tuszynski, 2005; Rasmusson, 2000). En effet, le fait de bloquer l'innervation cholinergique du cortex moteur a pour effet de diminuer le potentiel de plasticité corticale et perturbe significativement l'apprentissage moteur (Conner, Kulczycki, & Tuszynski, 2010). Le cortex moteur est donc lui aussi affecté de façon importante par le vieillissement et l'activité du système cholinergique. Par contre, les projections du cortex moteur chez l'humain semblent être moins affectées par la MA que celles des régions temporales et frontales (Geula & Mesulam, 1996).

2. Vieillissement et déclin cognitif

Le vieillissement normal s'accompagne généralement d'un déclin des fonctions cognitives dans divers domaines, incluant l'attention, les fonctions exécutives, la mémoire, ainsi que la vitesse psychomotrice et de traitement de l'information (e.g. Davidson & Winocur, 2010; Drag & Bieliauskas, 2010; Salthouse, 2000). Par contre, les habilités verbales et les connaissances sémantiques sont habituellement préservées (Park & Reuter-Lorenz, 2009).

En ce qui concerne les capacités attentionnelles, les personnes âgées éprouvent généralement davantage de difficultés dans des tâches d'attention sélective et divisée que les jeunes adultes, tandis que les résultats sont plus mitigés au niveau de l'impact du vieillissement sur l'attention soutenue (Drag & Bieliauskas, 2010; Staub, Doignon-Camus, Despres, & Bonnefond, 2013). En ce qui concerne le déclin des fonctions exécutives lié à l'âge, il se traduit surtout par des déficits au niveau de la planification, de la mémoire de travail, de la résolution de problèmes, de la flexibilité mentale, de l'inhibition des réponses indésirables ainsi que par une difficulté à implémenter les stratégies nécessaires pour compléter de nombreuses tâches (Drag & Bieliauskas, 2010). Finalement, la mémoire épisodique est plus affectée lors du vieillissement que la mémoire sémantique et la mémoire procédurale, qui s'avèrent plutôt préservées. Plus précisément, l'encodage d'informations en mémoire est surtout affecté lorsqu'un effort accru et l'implémentation de stratégies pour faciliter l'apprentissage sont nécessaires. Un déclin des performances au niveau du rappel sera surtout évident lorsqu'un niveau d'effort élevé est requis pour retrouver l'information déjà encodée, ainsi que lorsqu'il est nécessaire de séparer cette information du matériel qui n'est pas pertinent (Drag & Bieliauskas, 2010).

Il importe de souligner que les déclins cognitifs associés au vieillissement sont généralement variables d'un individu à l'autre (e.g., Gunstad et al., 2006; Ylikoski et al., 1999). En effet, certaines personnes âgées ont des performances comparables aux jeunes adultes alors que d'autres démontrent une diminution marquée de leur performance cognitive globale. De plus, pour certaines, les difficultés semblent se limiter davantage à des domaines spécifiques de la cognition (Glisky, 2007).

2.1 Rôles du système cholinergique dans l'attention

Le système cholinergique participe de façon importante aux fonctions cognitives de par sa capacité à moduler le traitement de l'information dans diverses régions du cortex (Sarter, Hasselmo, Bruno, & Givens, 2005). Plus précisément, l'ACh joue un rôle critique dans la régulation de l'attention en facilitant la détection des stimuli sensoriels grâce à l'augmentation de la réactivité corticale dans les aires réceptrices (Davidson & Marrocco, 2000). Plus précisément, l'activité des récepteurs nicotiniques de l'ACh permet de focaliser les ressources sur les stimuli pertinents en facilitant l'activation des couches cellulaires recevant l'information afférente du thalamus (Hasselmo & Sarter, 2011). La modulation de l'activité corticale par l'activité des récepteurs muscariniques du système cholinergique, que l'on retrouve principalement sur les interneurones GABAergiques (acide γ-aminobutyrique) dans l'ensemble du cortex, contribue aussi aux fonctions attentionnelles (Hasselmo & Sarter, 2011). À cet égard, l'ACh facilite l'activité GABAergique inhibitrice pour diminuer l'influence des sources de distractions ou de « bruit » dans l'information afférente, permettant donc d'optimiser la capacité de détection des stimuli (Hasselmo & Sarter, 2011; Sarter et al., 2005). Le système cholinergique participe donc aux processus attentionnels de traitement de l'information ascendant (*bottom-up,* en anglais) ainsi que descendant (*top-down*, en anglais), ce qui facilite l'augmentation du rapport « signal-bruit ». L'ACh exerce alors sa plus grande influence lorsque les tâches sont complexes et qu'il est difficile de discriminer l'information pertinente de celle qui n'est pas nécessaire pour compléter une tâche (Dumas & Newhouse, 2011; Sarter et al., 2005).

Ces rôles du système cholinergique dans l'attention permettent, entre autre, la flexibilité mentale, l'inhibition des réponses indésirables et la planification de comportements adaptés (Floresco & Jentsch, 2011; Hasselmo & Sarter, 2011; Picciotto, Meenakshi, & Jentsch, 2002). Ces processus se rapportent aux fonctions exécutives, ce qui supporte ainsi un rôle de l'ACh dans les fonctions cognitives de haut niveau (e.g., Behl et al., 2007; Behl, Lanctot, Streiner, Guimont, & Black, 2006).

2.2 Rôles du système cholinergique dans la mémoire

Comme souligné précédemment, le système cholinergique participe aussi au remodelage des circuits neuronaux liés à l'apprentissage et à la mémoire. En effet, la libération d'ACh facilite l'encodage de nouvelles informations en facilitant leur transmission au niveau de l'hippocampe grâce à une potentialisation accrue (Hasselmo & Giocomo, 2006; Hasselmo & Sarter, 2011). L'ACh pourrait aussi promouvoir l'encodage en augmentant les oscillations à rythme thêta (4–8 Hz) dans l'hippocampe, durant lesquelles l'apprentissage est généralement favorisé (Hasselmo, 2006)

Les projections corticales cholinergiques influencent aussi l'encodage en facilitant l'allocation des ressources attentionnelles aux stimuli pertinents de l'environnement externe (Hasselmo & McGaughy, 2004; Hasselmo & Sarter, 2011). À cet égard, les études chez l'humain ont permis de démontrer que des déclins en ce qui à trait à la capacité de contrôler l'attention mènent à des déficits d'encodage (e.g., Naveh-Benjamin, Cowan, Kilb, & Chen, 2007).

3. Neuroimagerie et techniques d'exploration

L'imagerie par résonance magnétique (IRM) a grandement fait avancer les connaissances sur le système cholinergique humain et a permis de mieux comprendre son influence sur de nombreuses fonctions cognitives. L'IRM structurelle permet, entre autre, d'examiner de façon indirecte la dégénérescence du système cholinergique liée à l'âge en examinant les changements de volume et de l'intégrité structurale de la région du prosencéphale antéro-basal. Cette technique a permis de démontrer une diminution progressive et disproportionnée (lorsque comparée à l'atrophie globale de la matière grise) du volume de cette région au cours du vieillissement normal, ainsi qu'une atrophie encore plus prononcée chez les patients atteints de la MA (Grothe, Heinsen, & Teipel, 2013; Grothe, Heinsen, & Teipel, 2012). Le volume du prosencéphale antéro-basal est aussi associé au statut cognitif global ainsi qu'à la performance à des tests de mémoire chez les patients ayant un diagnostic de déficit cognitif léger (Grothe et al., 2010). Toutefois, bien que cet indice soit lié à un score d'intelligence globale, il n'est pas significativement associé à la mémoire ou aux fonctions exécutives chez les personnes âgées en santé (Wolf et al., 2014). Cette absence de relation entre la cognition et les changements structuraux observés dans le système cholinergique lors du vieillissement normal (mais pas pathologique) pourrait suggérer qu'un certain degré de dégénérescence de cette structure est nécessaire avant que des déficits fonctionnels ne se manifestent (Wolf et al., 2014; voir aussi Schliebs & Arendt, 2006). Toutefois, un indice quelque peu différent de l'intégrité du prosencéphale antéro-basal, obtenu grâce au rapport de transfert d'aimantation, suggère qu'une altération de cette structure lors du vieillissement normal est associée au déclin de la performance globale à des tâches de mémoire verbale et de

mémoire de travail (Duzel et al., 2010). D'autres études permettront donc de clarifier l'influence de l'intégrité de cette structure du système cholinergique sur la cognition dans le contexte du vieillissement normal.

L'IRM fonctionnelle permet aussi d'étudier l'influence du système cholinergique sur la cognition par l'entremise de l'administration d'antagonistes (e.g., scopolamine) et d'agonistes cholinergiques (e.g., nicotine), ou d'inhibiteurs de l'AChE (e.g., donepezil, galantamine, rivastigmine). De telles études ont permis d'établir que la modulation de l'activité du lobe temporal médial et du cortex préfrontal suite à l'administration d'agonistes cholinergiques a pour effet de faciliter l'apprentissage et l'encodage d'informations en mémoire. Par ailleurs, des niveaux élevés d'ACh diminuent l'activité de l'hippocampe lors du rappel d'informations déjà emmagasinées en mémoire (revue par Bentley, Driver, & Dolan, 2011). Quant aux capacités attentionnelles, une modulation de l'activité des régions fronto-pariétales suite à l'administration d'agonistes cholinergiques est associée à une meilleure performance à des tâches d'attention (Bentley et al., 2011), alors que les antagonistes ont pour effet de diminuer l'activité des régions frontales permettant l'orientation aux stimuli de l'environnement et le contrôle exécutif de l'attention (Thienel, Kellermann et al., 2009; Thienel, Voss et al., 2009).

L'étude du système cholinergique humain peut aussi se faire par l'entremise de la tomographie par émission de positons (TEP) et la tomographie d'émission monophotonique (TEMP). Grâce à l'utilisation de traceurs, les études par TEP ont permis de démontrer une réduction de l'activité corticale de l'AChE chez les patients dans les stades initiaux et plus avancés de la MA (Herholz, 2008), ainsi qu'un lien entre ce déclin et la performance à une batterie de tests neuropsychologiques chez des patients atteints

d'un déficit cognitif léger (Haense et al., 2012). Par contre, les résultats des études par TEP et TEMP chez la personne âgée en santé sont variables. Elles montrent une diminution de la ChAT au niveau des récepteurs muscariniques et un déclin de l'affinité des récepteurs cholinergiques retrouvés dans diverses régions corticales (e.g. Lee et al., 1996; Mitsis et al., 2009; Zubieta et al., 2001), tandis que les études effectuées avec des traceurs de l'AChE ne démontrent pas un déclin marqué de l'activité de cette enzyme avec l'âge (Bohnen, Muller, Kuwabara, Constantine, & Studenski, 2009; Kuhl et al., 1999; Namba et al., 1999). La distribution des récepteurs nicotiniques cholinergiques semble aussi demeurer stable lors du vieillissement et cet indice n'est pas associé au déclin des fonctions cognitives avec l'âge (Ellis et al., 2009).

En résumé, les techniques de neuroimagerie ont grandement contribué aux connaissances sur les changements qui prennent place dans la transmission cholinergique avec l'âge et sur le rôle de ce système dans le déclin des fonctions cognitives lors du vieillissement humain. Toutefois, les études pharmacologiques effectuées chez les jeunes adultes et les patients atteints de la MA, à qui on administre des drogues qui bloquent ou facilitent l'activité cholinergique, permettent uniquement d'étudier les effets aigus de ces drogues et non le déclin progressif et à long terme du système cholinergique, tel qu'encouru lors du vieillissement normal. Il semble donc particulièrement important d'étudier le système cholinergique sans influencer son activité par des drogues. De plus, les études IRM et par TEP et TEMP peuvent s'avérer coûteuses et invasives pour le participant. À cet égard, une technique relativement récente, qui fait appel à la SMT, permet d'étudier le système cholinergique *in vivo* chez l'humain, et ce de façon

sécuritaire et minimalement invasive (Di Lazzaro et al., 2000; Nardone, Golaszewski, Ladurner, Tezzon, & Trinka, 2011; Tokimura et al., 2000).

3.1 Stimulation magnétique transcrâninenne

La SMT est une méthode d'exploration neurophysiologique permettant d'examiner l'intégrité des circuits corticaux. Elle consiste en la stimulation du cerveau humain grâce à une bobine placée sur le scalp à travers laquelle un bref courant de haute intensité (i.e., 5 kA) est introduit. Ceci produit un champ magnétique de forte intensité (environ 2 à 2.5 Teslas) et de courte durée (100-200 μsec). En traversant le crâne, le champ électrique, associé au champ magnétique, permet d'induire un courant électrique dans le tissu nerveux qui est capable de dépolariser les membranes cellulaires des neurones de la région stimulée (profondeur maximale ~2 cm; George, Lisanby, & Sackeim, 1999). Une bobine en « figure de 8 », dans laquelle le courant est concentré au point d'intersection des deux bobines, permet de stimuler une région focale du cerveau. Cette bobine est généralement orientée selon un axe postéro-antérieur, ce qui permet le recrutement des neurones pyramidaux (Lefaucheur, 2005).

Plus précisément, lorsque la SMT est appliquée au niveau du cortex moteur primaire, elle active préférentiellement les neurones pyramidaux des couches corticales II et III, dont la disposition tangentielle à la surface corticale les rend plus facilement excitables. Par l'entremise de connexions intra-corticales, l'activation des cellules des couches supérieures II et III mène à l'activation subséquente des neurones pyramidaux de la couche V, qui appartiennent à la voie cortico-spinale (Di Lazzaro, 2013). La SMT active donc de façon trans-synaptique les neurones de la voie cortico-spinale. À cet égard, elle se distingue de la stimulation électrique transcrânienne (SET; *transcranial*

electrical stimulation, en anglais), qui permet de stimuler directement les neurones pyramidaux de la couche V (i.e., sans passer par des interneurones corticaux). La SET induit donc des ondes directes (*D-waves*, en anglais), qui ont une courte latence (2.0-2.6ms; Di Lazzaro, 2013; Di Lazzaro et al., 2012).

Pour sa part, la SMT induit une série d'ondes indirectes (*I-waves*, en anglais). Ces dernières ont une plus longue latence que les ondes directes, ce qui reflète probablement l'activation trans-synaptique des cellules de la couche V par l'entremise de l'activité des cellules des couches supérieures II et III. Le nombre et la grandeur des ondes indirectes dépend de l'excitabilité des neurones pyramidaux activés par la SMT (Di Lazzaro et al., 2012; Terao & Ugawa, 2002). En effet, lorsque la SMT est appliquée à une faible intensité, une seule onde indirecte précoce (I1) est évoquée et celle-ci reflète l'activation monosynaptique des cellules cortico-spinales par les neurones des couches II et III. Une intensité de stimulation plus élevée augmente la taille de l'onde I1 et génère des ondes plus tardives, soit les ondes I2, I3 et I4. Ces dernières proviennent de la réactivation répétée des cellules pyramidales de la couche V par l'entremise d'un circuit de projections récurrentes avec les cellules des couches II et III. Ce circuit est modulé par une série d'interneurones inhibiteurs GABAergiques (Di Lazzaro et al., 2012). Le GABA est le principal neurotransmetteur inhibiteur dans le cortex et permet le maintien d'un équilibre entre l'excitabilité neuronale et l'inhibition de cette activité, ce qui est essentiel au fonctionnement cérébral normal (Scarr et al., 2013).

La propagation des ondes indirectes précoce (I1) et tardives (I2, I3, I4) dans la voie cortico-spinale se traduit ultimement par l'induction d'une brève réponse musculaire, nommée potentiel évoqué moteur (PEM). Les caractéristiques du PEM, dont

son amplitude et sa latence, peuvent être enregistrées dans les muscles ciblés par la SMT grâce à un appareil d'électromyographie. L'amplitude des PEMs est utilisée comme une mesure de l'excitabilité de la région ciblée, qui correspond généralement au premier muscle dorsal interosseux (FDI) de la main ou au muscle court abducteur du pouce (APB).

3.2 Inhibition afférente à courte latence

La SMT permet donc d'explorer l'excitabilité de différents circuits intra-corticaux régulant l'activité du cortex moteur, ainsi que ses projections cortico-spinales. Ces études sont basées notamment sur l'utilisation de paradigmes de stimulations à impulsions doubles. Dans ces paradigmes, la réponse à la SMT est généralement conditionnée au préalable par une stimulation sous-liminaire, ce qui permet de tester des circuits facilitateurs et inhibiteurs (Reis et al., 2008). Un de ces paradigmes implique le pairage de la SMT à une stimulation afférente d'un nerf périphérique controlatéral au site de la stimulation corticale. Lorsque cette stimulation afférente est administrée à des intervalles spécifiques, les PEMs évoqués par la SMT peuvent être inhibés (diminution de la taille du PEM) ou facilités (augmentation de l'amplitude du PEM).

Lorsqu'un intervalle de 18-20 ms est introduit entre la stimulation électrique périphérique du nerf médian au poignet et la stimulation magnétique corticale, la volée afférente issue du nerf donne lieu à des interactions inhibitrices dans le cortex moteur (Tokimura et al., 2000). Ceci se traduit par une diminution de l'amplitude du PEM conditionné lorsque comparé à la réponse non-conditionnée. Ce phénomène, nommé inhibition afférente à courte latence (*short latency afferent inhibition*, en anglais; SAI) dépend de la modulation cholinergique de certains circuits inhibiteur du cortex (Di

Lazzaro et al., 2000; Di Lazzaro et al., 2002). Plus précisément, l'ACh modulerait les circuits intra-corticaux activés par la stimulation périphérique par l'entremise de son action sur des groupes d'interneurones GABAergiques qui, eux, influencent l'activité des neurones pyramidaux de la couche V (Di Lazzaro et al., 2004; Di Lorenzo et al., 2013). L'excitabilité accrue des cellules GABAergiques mènerait ensuite à la suppression des ondes indirectes tardives (voir section précédente) et donc à la diminution de l'amplitude des PEMs enregistrés dans le muscle cible (e.g., FDI; Di Lazzaro et al., 2012).

Le SAI est considéré un marqueur de l'activité cholinergique car il est sensible au blocage des récepteurs muscariniques de l'ACh lors de l'administration de scopolamine (Di Lazzaro et al., 2000). Il est aussi modulé par l'activation des récepteurs cholinergiques nicotiniques suite à l'administration de nicotine à des non-fumeurs (Grundey et al., 2013). La mise en évidence du rôle crucial de l'activité cholinergique dans la modulation de l'excitabilité de ce circuit inhibiteur provient aussi du fait que les niveaux de SAI sont réduits chez les patients atteints de la MA, qui est souvent considéré comme étant un modèle de déficience chronique du système cholinergique (Di Lazzaro, Pilato, Dileone, Profice, Marra et al., 2008; Marra et al., 2012; Nardone, Bergmann, Kronbichler et al., 2008; Sakuma, Murakami, & Nakashima, 2007). Par contre, chez ces patients, les niveaux de SAI peuvent être rétablis grâce aux inhibiteurs d'acétylcholinestérase (Di Lazzaro et al., 2002).

Les variations dans les niveaux de SAI ont aussi été examinées chez diverses autres populations cliniques. À cet égard, une diminution des niveaux de SAI a été notée chez les patients atteints de la démence à corps de Lewy (Di Lazzaro et al., 2007; Marra et al., 2012), de la démence vasculaire (Di Lazzaro, Pilato, Dileone, Profice, Marra et al.,

2008; Nardone, Bergmann, Tezzon, Ladurner, & Golaszewski, 2008), de la sclérose en plaques (Cucurachi, Immovilli, Granella, Pavesi, & Cattaneo, 2008), du syndrome de Wernicke-Korsakoff (Nardone et al., 2010), ainsi que chez les individus atteints de la maladie de Parkinson qui présentent des déficits cognitifs légers (Yarnall et al., 2013). Les niveaux de SAI sont aussi diminués chez les individus ayant un diagnostic de déficit cognitif léger (Nardone et al., 2012; Tsutsumi et al., 2012; mais voir Sakuma et al., 2007). Chacune de ces conditions impliquent un déficit cholinergique. Par contre, aucun changement significatif dans les niveaux de SAI n'a été observé chez les patients de la démence fronto-temporale, laquelle est considérée comme une forme non-cholinergique de démence (Di Lazzaro et al., 2006).

Peu d'études ont examiné le SAI dans le contexte du vieillissement normal et celles-ci n'ont pas révélées de différences d'âge dans ce marqueur de l'activité cholinergique (Degardin et al., 2011; Oliviero et al., 2006), ce qui vient à l'encontre des résultats d'études qui ont montré une dégénérescence de ce système de neurotransmission avec l'âge (revue ci-haut). Ces études ont toutefois fait appel à un protocole de stimulation basé sur l'obtention d'un PEM de taille fixe (e.g., 1mV) pour chaque participant afin de déterminer l'intensité « test » de stimulation (généralement exprimée en terme de pourcentage de la puissance de sortie). Bien que cette façon de procéder soit répandue, une telle approche mène à l'utilisation d'une intensité de stimulation variable d'un participant à l'autre en raison de la présence d'une grande variabilité inter-individuelle dans l'amplitude des PEMs. Par exemple, afin d'obtenir un PEM de 1mV, il peut être nécessaire d'utiliser une intensité de 40% chez un individu mais une intensité de 55% chez un autre. À cet égard, Garry et Thomson (2009) ont montré que l'utilisation

d'une intensité basée sur une valeur fixe de PEM lors de protocoles à impulsions doubles peut mener à une sur-stimulation, masquant ainsi les effets d'inhibition. Il est toutefois possible de contrer ce problème en utilisant le seuil moteur (i.e., intensité de stimulation minimum requise pour éliciter des PEMs ayant une taille de plus de 50 μV dans au moins la moitié des essais d'une série consécutive de dix stimulations; Rossini et al., 1994) de chaque individu comme base pour déterminer l'intensité « test » de la stimulation. Cette approche permet de prendre en compte la variabilité inter-individuelle et de diminuer ses effets. Un protocole de SMT basé sur une intensité fixe de stimulation serait particulièrement important dans les études portant sur le vieillissement où la variabilité inter-individuelle est plus grande (Pitcher, Ogston, & Miles, 2003). Une telle approche pourrait donc mener à des résultats qui diffèrent de ceux décrits par Degardin et al. (2011) ainsi que par Oliviero et son équipe (2006).

3.3 Stimulation répétitive et plasticité

La SMT permet aussi d'explorer *in vivo* les mécanismes impliqués dans la plasticité corticale par l'entremise de protocoles de stimulations répétitives (i.e., SMTr). À cet égard, deux protocoles ont fait l'objet de nombreuses études, soit la stimulation par bouffées thêta (SBT; Huang et al., 2005) et la stimulation répétitive associative appariée (SAA; Stefan et al., 2000). Ces protocoles permettent d'induire des changements d'excitabilité corticale qui persistent plusieurs minutes après la période d'induction (Hoogendam, Ramakers, & Di Lazzaro, 2010; F. Muller-Dahlhaus, Ziemann, & Classen, 2010). Ces changements sont dépendants de l'activation des récepteurs NMDA, qui sont essentiels à l'expression de la plasticité synaptique (Huang, Chen, Rothwell, & Wen, 2007; Stefan, Kunesch, Benecke, Cohen, & Classen, 2002; Teo, Swayne, & Rothwell,

2007). Selon la fréquence et l'intervalle utilisé entre les stimulations, la SBT et la SAA permettent d'étudier des processus s'apparentant à la PLT ou à la dépression à long terme (DLT), qui sont tous les deux à la base des nouveaux apprentissages et de la mémoire (Hoogendam et al., 2010).

La SBT se prête bien à l'étude des changements d'excitabilité corticale qui prennent place lors du vieillissement en raison de la courte durée de ce protocole et de l'utilisation d'une intensité de stimulation plus faible que pour la SAA. Lorsqu'appliquée de façon intermittente, la SBT (i.e., SiBT) mène généralement à une facilitation des PEMs (Huang et al., 2005). La SiBT consiste en l'administration de trains de trois impulsions magnétiques livrées à une fréquence de 50 Hz à toutes les 200 ms pendant une période de 2 secondes. Ces élans de stimulations sont administrés à 20 reprises et sont séparés par une période de 8 secondes de repos, et ce pour un total de 600 stimulations. La SBT simule les protocoles utilisés dans de nombreuses études *in vitro* et animales qui permettent d'induire de la plasticité synaptique. Elle tente aussi de reproduire le rythme naturel thêta (4-8 Hz) de décharge des neurones de l'hippocampe (Hoogendam et al., 2010), durant lequel le rythme d'apprentissage et la plasticité sont accrus (Griffin, Asaka, Darling, & Berry, 2004).

Pour sa part, la SAA se base davantage sur les concepts de plasticité Hebbiens. Tout comme le SAI, ce protocole consiste en l'administration d'une stimulation électrique au nerf médian avant l'impulsion de la SMT (Stefan et al., 2000). Lorsque cet intervalle est de 25 ms, ces deux stimulations arrivent simultanément au niveau des neurones pyramidaux du cortex moteur primaire, ce qui a pour effet de renforcer les

synapses entre les neurones impliqués et donc d'augmenter l'excitabilité corticale (Hoogendam et al., 2010).

La plasticité attribuable aux protocoles SBT et SAA reposent sur la modulation sélective des ondes indirectes tardives générées par la SMTr (Di Lazzaro, 2013). En effet, la stimulation répétée permet d'augmenter l'excitabilité des neurones pyramidaux des couches corticales II et III, qui établissent des connexions avec les cellules cortico-spinales de la couche V. Ce gain d'excitabilité se traduit par une augmentation dans la taille et le nombre d'ondes tardives (I2, I3, I4), ce qui mène à une plus grande excitation de la voie cortico-spinale et donc à l'augmentation de la taille des PEMs enregistrés dans les muscles de la main (Di Lazzaro, 2013). Ce phénomène est aussi rendu possible par la diminution de l'activité des connections GABAergiques inhibitrices sur les cellules de la couche V (Di Lazzaro et al., 2012).

Une diminution de l'efficacité des mécanismes se rapportant à la PLT avec l'âge a été démontrée chez l'humain grâces aux protocoles SAA (Fathi et al., 2010; Muller-Dahlhaus, Orekhov, Liu, & Ziemann, 2008; Tecchio et al., 2008) et SBT (Di Lazzaro, Pilato, Dileone, Profice, Oliviero et al., 2008; Freitas et al., 2011), ce qui est en accord avec les résultats d'études animales (e.g., Blau et al., 2012; Kelly et al., 2006; Lynch, Rex, & Gall, 2006). De plus, la plasticité corticale suite à la SAA est diminuée chez les patients atteints de la MA (Battaglia et al., 2007), ce qui supporte un rôle de l'activité cholinergique dans les processus de type PLT. À cet égard, l'administration d'un antagoniste cholinergique diminue significativement la capacité d'induire de la plasticité corticale de type PLT chez les jeunes adultes (Korchounov & Ziemann, 2011), alors que la prise d'un agoniste ou d'un inhibiteur d'AChE a pour effet d'augmenter et prolonger

les effets de la SBT et de la SAA (Kuo et al., 2007; Swayne, Teo, Greenwood, & Rothwell, 2009; Thirugnanasambandam et al., 2011).

La plasticité synaptique a donc été examinée grâce à la SMT dans le contexte du vieillissement normal ou d'une modulation pharmacologique de l'activité du système cholinergique, mais la relation entre ces facteurs n'a pas été examinée dans le cadre d'une seule étude. La combinaison de ces éléments pourrait permettre une meilleure compréhension de l'influence de l'intégrité du système cholinergique sur les processus de plasticité qui sont à la base des apprentissages et de la mémoire.

Objectifs du présent travail

L'objectif général de cette thèse était d'explorer les liens entre un marqueur neurophysiologique de l'activité cholinergique dérivé de la SMT et certaines mesures comportementales des changements cognitifs et psychomoteurs accompagnant le vieillissement. Plus spécifiquement, nous avons cherché à :

1) Déterminer si l'âge affecte la modulation des réponses à la SMT suivant la stimulation afférente à différents intervalles, dont à 20 ms (i.e., SAI).
2) Déterminer si les variations de SAI liées à l'âge sont associées à des déclins au plan psychomoteur ainsi qu'à des changements cognitifs dans les domaines de la mémoire, des fonctions exécutives et de l'attention.
3) Déterminer si les variations liées à l'âge dans les niveaux de SAI peuvent prédire la susceptibilité individuelle à exprimer de la plasticité de type PLT en réponse à la SiBT.

L'hypothèse principale de ce projet de thèse était qu'une diminution des niveaux de SAI avec l'âge serait liée au déclin des performances cognitives et psychomotrices

associé au vieillissement normal, ainsi qu'à une moins grande susceptibilité à exprimer de la plasticité synaptique de type PLT suite à la SiBT.

CHAPITRE II. ARTICLES DE RECHERCHE

Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition

Marielle Young-Bernier, Patrick Davidson and François Tremblay

Young-Bernier, M., Davidson, P. S.R., & Tremblay, F. (2012). Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition with age. *Neurobiology of Aging, 33*(4), 835.e1-835.e11.

Authors' contributions: MYB participated in the design of the study, performed the experiments, analysed the data and drafted earlier versions of the manuscript. PD participated in the design of the study and in editing the manuscript. FT conceived the study, performed the experiments, helped with data analysis, and contributed to the writing of the final version of the manuscript.

Abstract

Changes in motor cortical excitability were examined in two groups of participants, young (18-30 years of age, n=25) and senior (65-82 years of age, n=31), using paired-pulse afferent stimulation with transcranial magnetic stimulation (TMS). Motor evoked potentials (MEPs) elicited by TMS at suprathreshold intensity (120% motor threshold) were first recorded in unconditioned trials (TMS alone) and then in conditioned trials, in which TMS pulses were preceded by median nerve stimulation at three different inter-stimulus intervals (ISI: 20, 50, and 200 ms). Conditioning of MEP responses revealed a similar pattern of modulation in the two age groups, with two periods of inhibition at 20 and 200 ms ISIs, separated by a period in which MEPs tended to return to baseline at a 50 ms ISI. Afferent-induced inhibition at the short interval (i.e., SAI: 20 ms ISI), was selectively reduced in seniors, with half of them showing either low or no MEP suppression. Age-associated changes in SAI level were also good predictors of performance on tests of processing speed and dexterity. The selective decrease in SAI exhibited by many seniors is consistent with reported alterations in intra-cortical inhibition with age. Our observations also highlight the potential value of SAI, as a putative marker of central cholinergic activity, in predicting declines in motor and cognitive function with age.

1. Introduction

In recent years, the development of non-invasive brain stimulation techniques based on transcranial magnetic stimulation (TMS) has opened new windows on the neurophysiological correlates of cortical aging *in vivo*. The use of paired-pulse paradigms in the motor cortex, in particular, has allowed investigators to assess the excitability of cortical circuits mediating inhibition and facilitation. One such paradigm involves pairing peripheral nerve stimulation with cortical stimulation to condition motor responses evoked by TMS. When applied at inter-stimulus intervals (ISI) of approximately 20 ms, afferent nerve conditioning leads to suppression of the subsequent motor evoked potentials (MEPs) elicited by suprathreshold TMS, a phenomenon referred to as short latency afferent inhibition (SAI). In contrast, at slightly longer ISIs (i.e., 40-70 ms) before TMS, afferent conditioning tends to produce MEP facilitation rather than inhibition (Devanne et al., 2009; Komori et al., 1992). Another period of late inhibition is induced when afferent conditioning is evoked using long ISIs (i.e., 180-220 ms) a phenomenon referred to as "long latency afferent inhibition" (LAI). These afferent-evoked periods of inhibition and facilitation are considered to be cortical in origin, reflecting changes in the excitability of intra-cortical circuits elicited by afferent projections to the motor cortex either directly through thalamo-cortical projections (SAI) or via cortico-cortical connections (LAI) from adjacent somatosensory cortex (Bikmullina et al., 2009; Chen et al., 1999; Tokimura et al., 2000). In the case of SAI, the observation that afferent conditioning at ISIs of approximately 20 ms attenuates corticospinal volleys elicited by TMS provides direct evidence for the cortical origin of the inhibition (Tokimura et al., 2000). Further evidence of the cortical origin of SAI comes from combined electroencephalography-TMS experiments, showing attenuation of the cortical N100

potential at ISI corresponding to SAI (Bikmullina et al., 2009). At the physiological level, SAI is thought to involve cholinergic transmission because it is selectively reduced or abolished by scopolamine, which blocks muscarinic acetylcholine receptors (Di Lazzaro et al., 2000). In LAI, the inhibition appears to be mediated at the cortical level but through γ-aminobutyric acid (GABA) receptors rather than cholinergic ones (Sailer et al., 2002). The two forms of afferent inhibition are also differently affected by central nervous diseases, such as Parkinson's disease (Sailer et al., 2003).

Although TMS-derived markers of motor cortex inhibition have been investigated in clinical populations with dementia and motor disorders (Di Lazzaro et al., 2002; Nardone et al., 2010; Orth et al., 2005), relatively few studies (Oliviero et al., 2006; Peinemann et al., 2001; Smith et al., 2009) have examined how these markers are affected by normal aging and whether these alterations could be related to declining motor and/or cognitive performance. Yet, this is crucial, given that the motor cortex undergoes similar structural changes to those seen in other cortical regions in aging (Salat et al., 2004) and is also affected by neuropathological changes in Alzheimer's disease (Suvà et al., 1999). In addition, recent observations point to a strong association in aging between motor and cognitive functions, notably executive control and perceptual speed (Voelcker-Rehage et al., 2010). Evidence for an age-related reduction in the excitability of circuits mediating short interval intra-cortical inhibition (SICI) in response to paired-pulse cortical stimuli was obtained by Peinneman et al. (2001), but these findings were not supported by subsequent studies (Smith et al., 2009). In a study comparing SICI and SAI levels in young and older subjects, Olivero et al. (2006) failed to detect any age differences. Such inconsistencies regarding aging effects on motor cortical inhibition

could reflect methodological differences. In this regard, one potential confound among studies relying on paired-pulse protocols has been the selection of test intensity. In the majority of such studies, the test intensity is adjusted to elicit unconditioned MEPs of a given amplitude (e.g., 1 mV), regardless of individual susceptibility to magnetic stimulation. Although such a procedure (i.e., using varying TMS intensities to obtain constant MEP sizes) could be justified when examining relatively homogenous groups of subjects (e.g., healthy young adults), it can lead to potential problems when assessing individuals from different age groups or with different characteristics (e.g. patients vs. healthy controls), where individual variations in resting motor threshold (RMT) can be substantial. The issue of test intensity in paired pulse inhibition protocols was raised recently by Garry and Thomson (2009). They compared SICI levels induced at different test intensities (90 to 150% MT), both at rest and during contraction. Their results showed that SICI was strongly influenced by test intensity, virtually disappearing at very strong intensities (i.e., <130% RMT), irrespective of the MEP size or changes in excitability. The authors concluded that controlling for TMS levels, using moderately suprathreshold intensity (110-120% MT) for test intensity, was indeed critical when probing changes in intra-cortical excitability with paired pulse TMS paradigms (Garry and Thomson, 2009).

In the present study, we investigated age-related changes in motor cortex excitability using paired-pulse afferent stimulation to modulate subsequent motor responses evoked by TMS. In line with the recent report of Garry and Thompson (2009), we elected to use a constant test intensity approach, as opposed to constant MEP size, to examine potential age differences in afferent induced modulation of TMS responses. We

were particularly interested in determining whether SAI levels, as a putative marker of central cholinergic activity, would be associated with age-related changes in specific tests of motor and cognitive performance. In this paper, we describe our observations of age-related changes in afferent induced inhibition and their relationships with changes in motor performance with age.

2. Methods

The Research Ethics Boards of the University of Ottawa and the Élisabeth Bruyère Research Institute approved the study procedure in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained before the experimental session. All assessments were performed in a controlled laboratory environment. All participants received a minimal honorarium for their participation.

2.1 Participants

Two groups of participants were recruited from the local community through newspaper advertisements for this study (Table 1): young adults (18-30 years), including many undergraduate students, and community-dwelling seniors (>65 years). Prior to the experimental session, all completed a medical questionnaire to ensure no contra-indications to TMS (e.g., history of seizures, skull metallic implants) and no antecedents of major conditions likely to affect their performance (e.g., stroke, Parkinson's Disease, severe hand arthritis, multiple sclerosis, acute/chronic pain in the upper extremities). The seniors were further screened for undiagnosed peripheral neuropathies in the hand using a graduated Rydel–Seiffer tuning fork, a reliable and valid tool for assessing sensory nerve function (Martina et al., 1998; Pestronk et al., 2004). All seniors exhibited vibration

extinction thresholds that exceeded the norms for their age group (i.e., ≥ 6.5, range, 6.5-8). All participants were also screened with the Montreal Cognitive Assessment (MoCA) for possible mild cognitive impairment. Although some seniors (5/31) scored slightly below the recommended cutoff (i.e., >26), they were deemed eligible for the study based on the interview and given that they were alert and cooperative and showed good cognitive function on the other tasks. The demographic characteristics of the participants, along with some baseline TMS measures of corticospinal excitability, are shown in Table 1.

2.2 Motor performance

Participants underwent extensive behavioral testing to assess motor and cognitive functions but only motor tests will be reported here. For this assessment, a battery of four timed motor tests was administered (order counterbalanced): 1) dexterity pegboard test, 2) simple reaction time (RT), 3) choice RT, 4) go/no go task. All tests were performed with the dominant hand (2 left-handed) with participants comfortably seated at a table. Finger dexterity was assessed with the Grooved Pegboard Test (GPT, Lafayette Instrument Co., Lafayette, IN, USA) using a stop watch to measure the time to insert the 25 pegs into the board. Participants were instructed to ignore dropped pegs and to go as quickly as possible. The remaining tests were administered using a MOART panel with the accompanying PsymSoft II software (Lafayette Instrument Co.). For simple RT, participants were asked to release a button by lifting their index finger in response to a go cue (green light). In the choice RT task, participants were asked to place four fingers (index and middle finger of both hands) over keys on the panel and to release the appropriate key in response to a go cue that appeared on top of one key. For go/no-go,

participants were instructed to release a button in response to a go cue (green light) and to refrain from releasing it in the presence of the no-go cue (red light). On all three RT tasks, 20 trials were performed.

2.3 EMG recording and TMS

Electromyographic (EMG) activity was recorded using small auto-adhesive surface electrodes (1-cm diameter, Ag-AgCl) placed over the first dorsal interosseous (FDI) muscle of the right hand in a belly-tendon montage. EMG signals were amplified (100-500 μV per division) and filtered (bandwidth, 5 Hz to 1 kHz) with a polygraph amplifier (RMP-6004, Nihon-Kohden Corp., Nihon Kohden America, Foothill Ranch, CA, USA). Signals were digitized at 2 kHz sampling rate using custom software on a PC running Microsoft Windows XP (Microsoft, Canada Co., Mississauga, ON, Canada) equipped with a digital/analogue acquisition card (PCI-6023, National Instrument Corp., Austin, TX, USA).

TMS was administered with participants comfortably seated in a recording chair. Magnetic stimulation was delivered via a Magstim Rapid^2 stimulator (Magstim Company Ltd, Wales, UK) connected to a figure-eight coil (90-mm inside loop diameter). To determine the optimal site to evoke MEP's in the contralateral hand muscles, participants were fitted with a Waveguar EEG cap (ANT North America Inc., Madison, WI, USA) with labels at prefrontal and parietal sites to assist in coil positioning. Movements of the head were restrained with a U-shape neck cushion. With the coil held ~45° in the mid-sagittal plane, the approximate location of the hand motor area on the left hemisphere was explored in ~1 cm steps until reliable MEP's could be evoked in the target muscle (FDI). This site was then marked with a 0.5-cm diameter circular sticker to ensure

consistent coil positioning. The coil was held in place manually over the hotspot by the same experimenter (FT) for all participants. Following this procedure, the motor threshold was determined using the method of Mills and Nithi (1997). Starting from supra-threshold intensity, the stimulator's output was gradually decreased in 1% steps until no MEP could be evoked for 10 consecutive stimuli. This TMS intensity corresponded to the lower threshold value. From this point, the intensity was gradually increased until MEP's > 50 μV in amplitude could be evoked in 10/10 consecutive stimuli. This intensity was recorded as the upper threshold value. The RMT was defined for each participant as the median intensity between the upper and lower threshold values.

2.4 Conditioning afferent stimulation

Conditioning afferent stimulation was produced by applying single 200 μs electrical pulses at the median nerve at the wrist through bipolar electrodes connected to a S88 Stimulator (Grass Technologies, Astro-Med, Inc, West Warwick, RI, USA). The conditioning intensity was adjusted to evoke a minimal visible twitch of the thenar muscles (Di Lazzaro et al., 2000; Tokimura et al., 2000). The effect of conditioning afferent stimulation on test MEP was studied at three different ISIs: 20, 50, and 200 ms for all participants. A subset of participants (young, n=12; seniors, n=28) was also tested with an additional ISI of 25 ms. As stated above, we elected to use a constant intensity protocol to assess MEP modulation in response to afferent conditioning. Thus, for both unconditioned and conditioned trials the test intensity was set at 120% above each individual's RMT. Beside the advantage of eliminating large inter-individual differences

in test intensity (which could affect results), a fixed intensity was more suitable for testing seniors, given the expected reduction in MEP size with age (Oliviero et al., 2006).

For testing, unconditioned MEPs were first recorded by eliciting 15 MEPs at rest at the test intensity (i.e., 120% RMT). Then, conditioned MEPs were recorded by pairing median nerve stimulation with TMS pulses at the selected ISIs (i.e., 20, 25, 50 and 200 ms). Each ISI was tested in a separated block, in which 15 conditioned MEPs were recorded consecutively, with a 5 s rest between trials. The order of testing with the different ISIs was counterbalanced across the participants. The testing session ended with our recording of another series of 10 unconditioned MEPs at the test intensity, making a total of 25 MEPs for this condition. During testing, muscle activity from the FDI was constantly monitored on an oscilloscope at high gain to ensure that full relaxation was maintained. Trials in which unwanted contractions were present were aborted or eliminated and repeated, if necessary. At the end of each block of trials for both unconditioned and conditioned series, a 1-minute pause was taken before proceeding to the next block.

2.5 Analysis of behavioral data

Performance in the GPT was determined as the time to complete the task in "s". The performance in the three RT tasks (simple, choice and go/no go) was derived from the PsymSoft II software (Lafayette Instrument Co.) output, which provided both single trial and averaged data for the 20 trials in ms. Each individual's performance was scrutinized to eliminate trials with response time longer than 2 SD from the mean. This represented respectively 6% and 5% of the trials (n=1100; note that motor performance data were not available for one young adult [subject A106] due to technical difficulties,

so the total number of trials was 1100 for each test [20 trials X 55 subjects]) in the simple and choice RT tasks but not in the "go/no go" task. Such outlier trials (1-2/block of 20 trials) were detected in a large proportion of the participants in the two age groups (young, 20/24; senior, 28/31) and were not specific to any combination of factors related to age or gender. After elimination of outliers, mean response times were computed for each RT tasks to reflect individual performance.

2.6 Analysis of MEP data

MEP amplitude (peak-to-peak) and latency for both conditioned and unconditioned trials were measured off-line and then averaged to derive mean values for each participant. The level of MEP amplitude modulation produced by afferent conditioning was expressed as a percentage of the unconditioned MEP response (i.e., % $MEP_{conditoned}/MEP_{unconditioned}$).

2.7 Statistical analysis

Independent t-tests were used to examine differences in baseline measures of excitability between age groups (i.e., RMT, resting MEP amplitude and latency, intensity for conditioning afferent stimulation), using adjusted *p* values to account for multiple comparisons (i.e., p=0.0125). Repeated measures analyses of variance (3 X 2 X 2, ANOVA) were used to detect main effect and interactions with intervals (20, 50, 200 ms) as the within-subjects factor and gender (female vs. male) and age group (young vs. senior) as the between-subjects factors. Note that a similar analysis (i.e., 4 X 2 X 2 ANOVA) was performed for the subset of participants (n=40) tested with the additional 25 ms interval. Upon detection of significant main effect or interactions, Bonferroni post-

hoc tests were performed. An ANOVA was also performed within each group to compare variations in MEP amplitude at the different intervals. Relationships between MEP afferent induced modulation and performance in the motor tests were examined using linear regression analyses. Significance level was set at $p<0.05$ to detect main effect and interactions. All statistical tests were performed using SPSS software version 17.0 for Windows® (Chicago, IL, USA). GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) was used for graphing and to prepare illustrations.

3. Results

3.1 Age differences in baseline measures of excitability.

The procedure was well tolerated by both age groups and no participants experienced adverse effects while undergoing TMS. As shown in Table 1, senior participants exhibited, on average, higher RMTs than the young, and this difference was reflected in the corresponding test intensity used to assess afferent conditioning. Note, however, that this difference in RMT was only marginally significant (t_{54} =2.1, p=0.04) at the adjusted p value. Seniors also exhibited much lower resting unconditioned MEP values than the young participants (t_{54} =3.1, p=0.003). Likewise, MEP resting latency was significantly prolonged in seniors compared to young participants (t_{54} =3.8, $p<0.01$). On the other hand, conditioning intensity levels used for median nerve stimulation were comparable in the two age groups. The potential confounding effects of these pre-existing differences in corticospinal excitability are addressed below.

3.2 Effect of afferent conditioning on MEP amplitude modulation

Although recordings of MEP amplitude modulation at the specific intervals could be obtained in all participants, one outlier (a senior) was removed from analysis due to abnormally high levels of facilitation at 50 ms ISI (Grubb's test, $p<0.01$, $z=7.25$). As shown in Figure 1A, the two age groups exhibited the expected pattern of modulation in response to afferent conditioning, with two periods of inhibition at the short and long ISIs, with a tendency to return close to preconditioning values at the middle interval. In fact, the only noticeable difference between the two groups was at the 20 ms ISI, in which seniors exhibited less SAI than their younger counterparts. Representative individual examples of recordings of MEP modulation induced by afferent conditioning are shown for the two age groups in Figure 1B.

The ANOVA showed the large main effect of "ISI" on MEP amplitude modulation ($F_{2,50}=30.1$, $p<0.001$), owing to the significant differences between MEP conditioning at 50 ms and the two periods of inhibition at 20 and 200 ms ($p<0.001$). Within-group comparisons showed that MEP modulation at 50 ms was significantly (Bonferroni post-hoc comparisons) different from the inhibition seen at 20 and 200 ms ISIs in each age group, although the magnitude of this effect was larger in the young group ($p<0.001$, Figure 1 A). The ANOVA also revealed a significant "Age group x ISI" interaction ($F_{2,50}=8.4$, $p=0.001$), whereas no main effect or interaction was detected for "Gender" ($F_{2,50}<2.0$, $p>0.50$). The interaction with age reflected the significant difference found in the post-hoc comparison ($t=4.6$, $p<0.001$) between SAI levels measured in young and senior participants (17.6±3.1% vs. 52.2±6.4 %). No such age difference was detected for the 50 and 200 ms ISI's ($p>0.30$). In the subset of participants tested at the

additional 25 ms ISI (n=40, not shown in Figure 1A), afferent conditioning still led to MEP inhibition but at a lower level than that seen at 20 ms (overall means = 55 vs. 36 %, respectively). No significant difference was observed, however, between the two age groups at this specific ISI (seniors, 61.2± 12%, young, 47.9 ± 7.6%, t=1.0, p=0.32). In fact, adding the 25 ms interval in the ANOVA led to similar a conclusion as before, i.e. a significant "Age group x ISI" interaction ($F_{3,34}$=7.89, p<0.001) reflecting the selective age difference observed at 20 ms ISI.

3.3 Influence of RMT, MEP size, MEP latency and advancing age

As noted above, the fact that the young and senior groups differed on baseline measures of corticospinal excitability prompted a secondary analysis to examine the potential confounding effects of these factors on the observed afferent induced modulation at the different ISIs. A series of analyses of covariance (ANCOVA) was thus performed in which RMT, resting MEP amplitude, and MEP latency were each examined as a covariate, leaving ISI as the within-subjects and age group as the between-subjects factors. These analyses failed to detect any significant main effect or interaction attributable to any of the covariates ($F_{1,52}$ < 1.5, p>0.20), while still showing the significant "Age group X ISI" interaction ($F_{2,51}$ >5, p<0.01). The relatively low impact of age differences in TMS baseline measures of corticospinal excitability on afferent induced inhibition can be further appreciated when looking at Figure 2 (A-C), in which the distribution of individual SAI levels measured in the two groups is represented as a function of RMT values (low vs. high), resting MEP size (small vs. large) and MEP resting latency (early vs. late). The age difference in SAI level was relatively well preserved in spite of the differences seen in threshold intensity, resting MEP size, and

latency. Finally, Figure 2D shows the relationship between age and SAI levels for all participants. As expected, variations in SAI level were strongly related to age. It can be seen that a subset of participants (16/55), which consisted mainly of seniors (14/16), exhibited particularly low level of inhibition (MEP_{cond}≥50% suppression) and, in some cases, even facilitation (MEP_{cond} ≥100%). However, as evident in Figure 2D, this subgroup of seniors with low SAI showed no particular distribution with respect to age. Indeed, in the senior group, SAI levels were unrelated to age (r=0.07, p=0.73).

3.4 Relationships between SAI and motor performance

Figure 3 illustrates the relationships found between MEP modulation measured at the SAI interval and performance on the different motor tests after combining data from the two age groups (similar analyses performed at the other ISI's failed to show any significant relationships). As evident in Figure 3 A and B, SAI levels proved to be good predictors of performance in both the dexterity and choice RT tests, explaining 32% and 17% of the variance, respectively. Variations in SAI were also significant predictors for the go/no-go task, although the strength of this relationship (10% of the variance explained) was weaker than for the two previous tasks. On the other hand, performance in the simple RT test was unrelated to variations in SAI levels observed in the two groups. The same analysis was repeated on the same pool of data but excluding the subgroup of participants (n=16) with either low or absent SAI (see Figure 2D, above). This analysis led basically to the same conclusion: SAI levels were good predictors (p<0.05) of performance in the dexterity (r^2=0.19) and choice RT (r^2=0.13) tasks, but not for the go/no-go (r^2=0.06) and simple RT (r^2=0.07) tasks. This indicated that this subgroup did

not have an undue influence on the relationships between SAI and performance in the tests.

4. Discussion

The present study investigated the neurophysiological correlates of aging in the human motor cortex using afferent stimulation to condition motor responses evoked by TMS. It revealed four main findings. First, both young and senior participants exhibited a pattern of modulation consistent with previous reports on afferent-induced modulation of MEP responses (Devanne et al., 2009; Tokimura et al., 2000). Second, although the pattern of modulation was similar in the two age groups, senior participants exhibited a selective decrease in MEP inhibition at the shortest ISI, corresponding to the classical SAI period. Third, this difference in SAI level was largely unaffected by pre-existing age differences in baseline TMS measures of excitability (i.e., RMT, MEP size, and latency). Finally, variations in SAI level proved to be good predictors of performance in specific timed motor tests. Before addressing the significance of the present findings for the neurophysiology of aging, we discuss some possible confounding factors.

4.1 Afferent-induced modulation of MEPs

In this study, we examined modulation of TMS-evoked responses by paired afferent stimulation using constant suprathreshold intensity for all participants rather than trying to control for MEP size using varying intensities. As stressed above, this approach was adopted on the basis of previous reports examining the impact of test intensity on TMS markers of cortical inhibition (Garry and Thomson, 2009) and also on the basis that such an approach could help to clarify whether age differences truly exist. With this approach, the observed modulation in response to afferent conditioning followed the

expected pattern in the two groups, albeit with a difference in the level of induced inhibition at the shortest ISI. Our secondary analysis (see Results) showed that potential confounding effects of pre-existing age differences in RMT, MEP amplitude, and MEP latency, did not affect the amplitude modulation evoked in response to paired afferent stimulation. In this respect, the present observations are in line with previous reports, showing, for instance, that even large variations in test MEP size (e.g., from 0.2 to 1 mV) have no significant effect on afferent induced inhibition, either for SAI (Udupa et al., 2009) or LAI (Sailer et al., 2002). Interestingly, our findings with regard to the minimal impact of unconditioned MEP amplitude on afferent-evoked inhibition are entirely consistent with the findings of David-Jurgens and Dinse (2010), who investigated age effects on paired-pulse inhibition in the rat somatosensory cortex. As in the present study, these authors showed that paired-pulse suppression of neuronal responses was reduced with age and that the age-related reduction in the unconditioned response did not affect the amplitude of the second conditioned response.

4.2 Age-related changes in cortical excitability and afferent-induced modulation of MEP

In agreement with previous studies, our group of seniors displayed age-related differences in basic measures of corticospinal excitability, such as elevated RMT, reduced MEP size, and prolonged onset latency. Elevations of RMT with age, although an inconsistent finding across TMS studies (Rossini et al., 2007), could reflect structural alterations such as an increase in the skull-cortex distance owing to cortical thinning in the frontal motor areas (Stokes et al., 2007) or even alterations in white matter integrity, as shown recently by Klöppel et al. (2008). Reduced MEP size and increased onset latency are common findings in older individuals and are thought to reflect alterations in

the integrity of the corticospinal pathway leading to asynchronous temporal discharge of volleys elicited by TMS along with a reduction in their number and size as they reach spinal motoneurons (Rossini et al., 2007). Other alterations in the peripheral nervous system affecting motor units (Aagaard et al., 2010) might also contribute to age-related changes in MEP amplitude and latency. Interestingly, our observations on age-related changes in basic corticospinal excitability measures are in agreement with those of Olivero et al. (2006), who also observed a marginal increase in RMT (~14%) along with comparable age-related reductions (~55%) in resting MEP amplitude. In this regard, our group of seniors could be considered representative of the expected changes in basic motor cortical excitability measures associated with normal aging. In the same vein, our seniors also exhibited the expected pattern of MEP modulation in response to afferent conditioning, which was in fact very similar to the one observed in the young group, albeit for the difference in SAI. In both age groups, the two periods of early and late inhibition (i.e. SAI and LAI) were separated by a period of recovery at the middle interval, in which MEPs were significantly less depressed by afferent conditioning. We did not observe, as reported recently by Devanne et al. (2009), a clear MEP facilitation at the 50 ms ISI, although ~one-third of the participants in each age group (young, n=8; senior, n=10) showed such facilitation. It is not clear as to why this facilitation was absent in the majority of participants, even the young ones, but the fact that we used a constant intensity approach to assess MEP modulation might have contributed to the discrepancy. For the 200 ms ISI, both groups of participants exhibited the characteristic LAI period, with a comparable amount of MEP reduction (~50%) in the two age groups. It is worth noting that LAI of a similar magnitude was reported previously in young

(Sailer et al., 2002) and healthy older adults (Sailer et al., 2003). Altogether, these observations indicate that the overall pattern of MEP modulation induced by afferent nerve conditioning was relatively well preserved in our group of healthy seniors, with the exception of SAI, which was selectively decreased.

4.3 Age differences in SAI

The main finding of the present study is the large age difference found in MEP modulation at the 20 ms ISI. As mentioned above, the selectivity of this effect for the shortest ISI suggests a specific age-dependant alteration in the cortical circuits mediating SAI in the motor cortex.

Although the reduction in SAI level with age reported here is at odds with the report of Oliviero et al. (2006), we used a somewhat different experimental approach. First, our observations came from a larger sample of participants (i.e., n=55 vs. 42), including a larger group of seniors. Second, as emphasized above, modulation in response to paired-pulse afferent stimulation was assessed using a constant intensity protocol, as opposed to the constant MEP size used by Oliviero et al. Finally, and more importantly, Oliviero et al. used monophasic pulses for TMS, whereas biphasic pulses were used in the present study. This latter factor is critical given that the type of stimulus waveform can have profound influences on TMS measures of corticospinal excitability (Kammer et al., 2001; Sommer et al., 2006). Interestingly, Peinemenan et al. (2001), who also used biphasic stimulation to measure SICI, were able to demonstrate, as in the present study, a significant reduction in paired-pulse inhibition with age.

In many respects, our observations on SAI are very similar to those of Peinememan et al. (2001) on age-related changes in SICI. Like these authors, we

observed relatively large inter-individual variations in the two age groups, although young subjects clearly exhibited less variability and more consistent MEP inhibition than seniors. In this latter group, the greater variability stemmed from the fact that approximately half of the individuals showed either low or no inhibition in response to afferent conditioning at 20 ms. Older participants in Peinemann et al.'s study exhibited the same pattern with about only half of them showing the expected pattern of MEP inhibition at short ISIs, and the remaining ones showing clear facilitation (see Figure 2 in Peinemann et al., 2001). Also in line with Peinemann et al. (2001), we found that SAI levels were clearly different between the two age groups. However, low SAI level was not necessarily associated with older age in seniors, indicating that advancing age by itself was not a strong predictor of afferent induced inhibition. In sum, the present findings converge with those of Peinemann et al. (2001) in supporting the notion that the excitability of intra-cortical circuits mediating inhibition in the motor cortex is reduced in healthy aging, although the extent of this reduction may vary greatly from one individual to another. The reduced intra-cortical inhibition with age could reflects alterations in fast GABAergic transmission, which is mediated primarily by the $GABA_A$ receptor subtype (Rissman and Mobley, 2011). Indeed, $GABA_A$ receptors are involved in mediating both SAI (through cholinergic modulation) and SICI in the motor cortex (Di Lazzaro et al., 2005), making them susceptible to age-dependant reductions in GABAergic transmission (Rissman and Mobley, 2011). Recent evidence from rodent models of aging points to specific alterations in $GABA_A$ receptors expression in the motor cortex. For instance, Yu et al. (2006) showed that the expression of $GABA_A$ receptor sub-units α3 and α5 was markedly reduced in the motor and somatosensory cortices of aged rats, while spared in

other cortical and sub-cortical regions. A subsequent investigation by the same group (Schmidt et al., 2010) showed that reduced expression of $GABA_A$ subunit α5 in the cortex was associated with decreased functional inhibition, as evidenced by reduced cortico-cortical paired-pulse inhibition in aged animals. Thus, alterations in fast $GABA_A$ neurotransmission appear to be a likely cortical mechanism to explain the age-associated decrease in central motor inhibition that we and others have found using TMS paired-pulse protocols.

4.4 SAI, cholinergic function, and motor performance

With regards to SAI, the fact that this form of intra-cortical inhibition has been linked to cholinergic transmission, as first demonstrated by Di Lazzaro et al. (2000), can provide insights into how age-related changes in neuromodulation could affect central motor system functioning. The motor cortex receives dense cholinergic inputs from the nucleus basalis of Meynert, notably through muscarinic receptors (Selden et al., 1998) and thus easily could be affected by impaired cholinergic modulation (Pennisi et al., 2011). In this respect, the selective decrease in SAI found here in seniors points to an age-dependant alteration in cholinergic modulation of intra-cortical inhibition in the motor cortex. Previous TMS studies examining the link between SAI and impaired cholinergic modulation have focused primarily on patients with various form of dementias such as Alzheimer's disease or dysexecutive syndromes, in which SAI was found to be either significantly reduced or abolished (Di Lazzaro et al., 2004; Nardone et al., 2010; Nardone et al., 2006). In these investigations, healthy seniors served primarily as age-matched controls and the reported variations on SAI levels were simply considered to be "normal". In the absence of explicit comparisons with younger people, it

is difficult to comment on any potential age effects in aforementioned studies. It is worth noting, however, that the average level of SAI exhibited by our group of healthy seniors falls within the range of Di Lazzaro et al.'s (2002, 2004) healthy seniors (i.e., 52% vs. 45 %, respectively), whereas SAI levels measured in their AD patients were considerably lower (i.e., >85%) indicating cortical hyperexcitability. This raises the issue as to whether the subgroup of participants showing evidence of cortical hyperexcitability (i.e., low level or absent SAI) could be considered to be "abnormal". This would be a premature conclusion at this stage, given that no normative data on SAI levels exist in the healthy population. Besides, comparisons among existing studies remain difficult because of methodological differences in testing protocols. As stressed before, TMS measures of intra-cortical inhibition are inherently variable in nature, a factor which is often amplified with age, and thus the large differences we observed among individuals might just be a reflection of the normal variability within each age group.

It remains that most of the individuals showing evidence of cortical hyperexcitability (i.e. low or absent SAI) consisted of seniors, which suggests a major impairment in cholinergic-dependant intra-cortical motor inhibition. In the same vein, low SAI levels can also be indicative of cognitive decline in light of the importance of cholinergic systems in many cognitive domains (e.g., Robbins and Roberts, 2007), as well as their likely role in the pathology of Alzheimer's disease (Kása et al., 1997; Perry, 1980). However, our seniors consisted largely of healthy community dwelling individuals with no evident signs of cognitive decline. In fact, the majority of those exhibiting markedly low SAI (12/14) showed MoCA scores above the recommended clinical cutoff score. We are currently exploring the issue of the possible relationship between SAI and

cognitive performance further. For example, it may be that SAI levels must cross a critical threshold before significant cognitive impairment (i.e., dementia) becomes evident (Edginton and Rusted, 2003; Fredrickson et al., 2008; Hodges et al., 2009; Little et al., 1998; Robbins et al., 1997). Yet, in the present study, we provide to our knowledge the first compelling evidence of the potential link between SAI, as a putative marker of age-dependant changes in central cholinergic function, and behavioral measures of functional decline in motor performance. Indeed, our regression analysis showed that variations in SAI level in the two age groups were linearly related to performance on tests assessing fine motor execution and processing speed. The fact these linear relationships were still apparent for dexterity and choice RT, even after we eliminated participants with suspected cortical hyperexcitability, further strengthens the conclusion that age-related variations in motor performance were well reflected in the fine modulation of SAI levels measured in the two age groups. Interestingly, SAI proved to be particularly helpful in predicting performance on the three complex tasks (GPT, choice RT, and go/no-go) rather than the simple RT task. This is consistent with the putative role of SAI as a cholinergic marker since all three tasks involve processes linked with executive controls (e.g. attentional selection, decision making, monitoring of performance), which are critically dependent on cholinergic modulation (Sarter et al., 2005). The strong relationship between SAI and performance in the GPT is particularly compelling since this test has been shown recently to correlate strongly with various domains of cognitive function in healthy old adults, including memory, processing speed, and executive functioning (Ashendorf et al., 2009). In line with this, the GPT was shown to be highly sensitive to nigro-striatal innervation integrity in patients with PD, reflecting its

dependence, at least in part, on dopaminergic modulation (Bohnen et al., 2007). Thus, the GPT appears to be a sensitive marker of declining function across several domains of motor and cognitive performance, which seems to underlie its dependence on processes affected by cholinergic and dopaminergic neuromodulation. The fact that reduced SAI level in seniors was accompanied by correspondingly lower performance in the GPT would fit a model of age-related dysfunction in cholinergic modulation. A decline in cholinergic modulation in healthy aging was also proposed recently by Düzel et al. (2010) who examined the relationship between structural integrity of basal forebrain region (where most cortical cholinergic projections originate) and cognitive decline in aged persons. Their results showed that age-associated change in basal forebrain integrity, and presumably cholinergic modulation, correlated with declining function across various cognitive domains combining verbal learning, verbal long-term memory, and working memory. For these authors, such an observation provided clues as to why age-related declines are often correlated across different domains.

In conclusion, the present study provides further neurophysiogical evidence that motor cortical inhibitory processes are altered in normal aging. Our results, in particular, show that a specific form of paired-pulse inhibition, referred to as SAI, is selectively reduced with age. We also provide compelling evidence for the functional impact of age-related changes in SAI at the functional level in terms of explaining changes in dexterity and speed of processing with age. As SAI is known to be dependent upon cholinergic modulation, we interpret our findings as further evidence that central cholinergic neuromodulation is affected in normal aging.

Acknowledgements: The authors wish to thank all participants for their time and patience during testing. Special thanks to Sabah Master, Yael Kamil, and Travis Davidson for help with data collection and analysis. This work will serve as a partial fulfillment for a doctoral thesis in clinical psychology by MYB. This work was supported by a student award from the Canadian Institutes of Health Research to MYB, a Research Development Grant from the Faculty of Social Sciences of the University of Ottawa to FT and PD, and a Discovery grant from the Natural Sciences and Engineering Research Council of Canada to PD.

References

Aagaard, P., Suetta, C., Caserotti, P., Magnusson, S.P., & Kjær, M. (2010). Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scan J Med Sci Spor, 20*, 49-64.

Ashendorf, L., Vanderslice-Barr, J.L., & McCaffrey, R.J. (2009). Motor tests and cognition in healthy older adults. *Appl Neuropsychol, 16*, 171-6.

Bikmullina, R., Kičić, D., Carlson, S., & Nikulin, V. (2009). Electrophysiological correlates of short-latency afferent inhibition: a combined EEG and TMS study. *Exp Brain Res, 194,* 517-26.

Bohnen, N.I., Kuwabara, H., Constantine, G.M., Mathis, C.A., & Moore, R.Y. (2007). Grooved pegboard test as a biomarker of nigrostriatal denervation in Parkinson's disease. *Neurosci Lett, 424*, 185-9.

Chen, R., Corwell, B., & Hallett, M. (1999). Modulation of motor cortex excitability by median nerve and digit stimulation. *Exp Brain Res, 129*, 77-86.

David-Jurgens, M., & Dinse, H.R. (2010). Effects of aging on paired-pulse behavior of rat somatosensory cortical neurons. *Cereb cortex, 20*, 1208-16.

Devanne, H., Degardin, A., Tyvaert, L., Bocquillon, P., Houdayer, E., Manceaux, A., Derambure, P., & Cassim, F. (2009). Afferent-induced facilitation of primary motor cortex excitability in the region controlling hand muscles in humans. *Eur J Neurosci, 30*, 439-48.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Marra, C., Daniele, A., Ghirlanda, S., Gainotti, G., & Tonali, P.A. (2004). Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 75,* 555-9.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M.A., Di Giovanni, S., Zito, G., Tonali, P., & Rothwell, J.C. (2000). Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. *Exp Brain Res, 135,* 455-61.

Di Lazzaro, V., Oliviero, A., Tonali, P.A., Marra, C., Daniele, A., Profice, P., Saturno, E., Pilato, F., Masullo, C., & Rothwell, J.C. (2002). Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology, 59*, 392-7.

Di Lazzaro, V., Pilato, F., Dileone, M., Tonali, P., & Ziemann, U. (2005). Dissociated effects of diazepam and lorazepam on short-latency afferent inhibition. *J Physiol, 569,* 315-23.

Düzel, S., Münte, T.F., Lindenberger, U., Bunzeck, N., Schütze, H., Heinze, H.-J., & Düzel, E. (2010). Basal forebrain integrity and cognitive memory profile in healthy aging. *Brain Res, 1308*, 124-36.

Edginton, T., & Rusted, J.M. (2003). Separate and combined effects of scopolamine and nicotine on retrieval-induced forgetting. *Psychopharmacology (Berl.), 170*, 351-7.

Fredrickson, A., Snyder, P.J., Cromer, J., Thomas, E., Lewis, M., & Maruff, P. (2008). The use of effect sizes to characterize the nature of cognitive change in psychopharmacological studies: an example with scopolamine. *Hum Psychopharmacol, 23*, 425-36.

Garry, M., & Thomson, R. (2009). The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. *Exp Brain Res 193*, 267-74.

Hodges, D.B., Jr., Lindner, M.D., Hogan, J.B., Jones, K.M., & Markus, E.J. (2009). Scopolamine induced deficits in a battery of rat cognitive tests: comparisons of sensitivity and specificity. *Behav Pharmacol, 20*, 237-51.

Kammer, T., Beck, S., Thielscher, A., Laubis-Herrmann, U., & Topka, H. (2001). Motor thresholds in humans: a transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. *Clin Neurophysiol, 112*, 250-8.

Kása, P., Rakonczay, Z., & Gulya, K. (1997). The cholinergic system in Alzheimer's disease. *Prog Neurobiol, 52*, 511-35.

Klöppel, S., Bäumer, T., Kroeger, J., Koch, M.A., Büchel, C., Münchau, A., & Siebner, H.R. (2008). The cortical motor threshold reflects microstructural properties of cerebral white matter. *Neuroimage, 40*, 1782-91.

Komori, T., Watson, B.V., & Brown, W.F. (1992). Influence of peripheral afferents on cortical and spinal motoneuron excitability. *Muscle Nerve, 15*, 48-51.

Little, J.T., Johnson, D.N., Minichiello, M., Weingartner, H., & Sunderland, T. (1998). Combined nicotinic and muscarinic blockade in elderly normal volunteers: cognitive, behavioral, and physiologic responses. *Neuropsychopharmacol, 19*, 60-9.

Martina, I.S.J., van Koningsveld, R., Schmitz, P.I.M., van der Meche, F.G.A., & van Doorn, P.A. (1998). Measuring vibration threshold with a graduated tuning fork in normal aging and in patients with polyneuropathy. *J Neurol Neurosurg Psychiatry, 65*, 743-7.

Mills, K.R., & Nithi, K.A. (1997). Corticomotor threshold to magnetic stimulation: normal values and repeatability. *Muscle Nerve, 20*, 570-6.

Nardone, R., Bergmann, J., De Blasi, P., Kronbichler, M., Kraus, J., Caleri, F., Tezzon, F., Ladurner, G., & Golaszewski, S. (2010). Cholinergic dysfunction and amnesia in patients with Wernicke–Korsakoff syndrome: a transcranial magnetic stimulation study. *J Neural Transm, 117*, 385-91.

Nardone, R., Marth, R., Ausserer, H., Bratti, A., & Tezzon, F. (2006). Reduced short latency afferent inhibition in patients with Down syndrome and Alzheimer-type dementia. *Clin Neurophysiol, 117*, 2204-10.

Oliviero, A., Profice, P., Tonali, P.A., Pilato, F., Saturno, E., Dileone, M., Ranieri, F., & Di Lazzaro, V. (2006). Effects of aging on motor cortex excitability. *Neurosci Res, 55*, 74-7.

Orth, M., Amann, B., Robertson, M.M., & Rothwell, J.C. (2005). Excitability of motor cortex inhibitory circuits in Tourette syndrome before and after single dose nicotine. *Brain, 128*, 1292-300.

Peinemann, A., Lehner, C., Conrad, B., & Siebner, H.R. (2001). Age-related decrease in paired-pulse intracortical inhibition in the human primary motor cortex. *Neurosci Lett, 313,* 33-6.

Pennisi, G., Ferri, R., Lanza, G., Cantone, M., Pennisi, M., Puglisi, V., Malaguarnera, G., & Bella, R. (2011). Transcranial magnetic stimulation in Alzheimer's disease: a neurophysiological marker of cortical hyperexcitability. *J Neural Transm, 118*, 587-98.

Perry, E.K. (1980). The cholinergic system in old age and Alzheimer's disease. *Age Ageing, 9*, 1-8.

Pestronk, A., Florence, J., Levine, T., Al-Lozi, M.T., Lopate, G., Miller, T., Ramneantu, I., Waheed, W., & Stambuk, M. (2004). Sensory exam with a quantitative tuning fork: rapid, sensitive and predictive of SNAP amplitude. *Neurology, 62*, 461-4.

Rissman, R.A., & Mobley, W.C. (2011). Implications for treatment: GABAA receptors in aging, Down Syndrome and Alzheimer's Disease. *J Neurochem, 117*, 623-31.

Robbins, T.W., & Roberts, A.C. (2007). Differential regulation of fronto-executive function by the monoamines and acetylcholine. *Cereb Cortex, 17* Suppl 1, i151-60.

Robbins, T.W., Semple, J., Kumar, R., Truman, M.I., Shorter, J., Ferraro, A., Fox, B., McKay, G., & Matthews, K. (1997). Effects of scopolamine on delayed-matching-to-sample and paired associates tests of visual memory and learning in human subjects: comparison with diazepam and implications for dementia. *Psychopharmacology (Berl.), 134*, 95-106.

Rossini, P.M., Rossi, S., Babiloni, C., & Polich, J. (2007). Clinical neurophysiology of aging brain: From normal aging to neurodegeneration. *Prog Neurobiol, 83*, 375-400.

Sailer, A., Molnar, G.F., Cunic, D.I., & Chen, R. (2002). Effects of peripheral sensory input on cortical inhibition in humans. *J Physiol, 544*, 617-29.

Sailer, A., Molnar, G.F., Paradiso, G., Gunraj, C.A., Lang, A.E., & Chen, R. (2003). Short and long latency afferent inhibition in Parkinson's disease. *Brain, 126*, 1883-94.

Salat, D.H., Buckner, R.L., Snyder, A.Z., Greve, D.N., Desikan, R.S.R., Busa, E., Morris, J.C., Dale, A.M., & Fischl, B. (2004). Thinning of the Cerebral Cortex in Aging. *Cereb Cortex, 14*, 721-30.

Sarter, M., Hasselmo, M.E., Bruno, J.P., & Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Rev, 48*, 98-111.

Schmidt, S., Redecker, C., Bruehl, C., & Witte, O.W. (2010). Age-related decline of functional inhibition in rat cortex. *Neurobiol Aging, 31*, 504-11.

Selden, N.R., Gitelman, D.R., Salamon-Murayama, N., Parrish, T.B., & Mesulam, M.M. (1998). Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. *Brain, 121* (Pt 12), 2249-57.

Smith, A., Ridding, M., Higgins, R., Wittert, G., & Pitcher, J. (2009). Age-related changes in short-latency motor cortex inhibition. *Exp Brain Res, 198*, 489-500.

Sommer, M., Alfaro, A., Rummel, M., Speck, S., Lang, N., Tings, T., & Paulus, W. (2006). Half sine, monophasic and biphasic transcranial magnetic stimulation of the human motor cortex. *Clin Neurophysiol, 117*, 838-44.

Stokes, M.G., Chambers, C.D., Gould, I.C., English, T., McNaught, E., McDonald, O., & Mattingley, J.B. (2007). Distance-adjusted motor threshold for transcranial magnetic stimulation. *Clin Neurophysiol, 118*, 1617-25.

Suva, D., Favre, I., Kraftsik, R., Esteban, M., Lobrinus, A., & Miklossy, J. (1999). Primary motor cortex involvement in Alzheimer disease. *J Neuropath Exp Neur, 58*, 1125-34.

Tokimura, H., Di Lazzaro, V., Tokimura, Y., Oliviero, A., Profice, P., Insola, A., Mazzone, P., Tonali, P., & Rothwell, J.C. (2000). Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J Physiol, 523* Pt 2, 503-13.

Udupa, K., Ni, Z., Gunraj, C., & Chen, R. (2009). Interactions between short latency afferent inhibition and long interval intracortical inhibition. *Exp Brain Res, 199*, 177-83.

Voelcker-Rehage, C., Godde, B., & Staudinger, U.M. (2010). Physical and motor fitness are both related to cognition in old age. *Eur J Neurosci, 31*, 167-76.

Yu, Z.-Y., Wang, W., Fritschy, J.-M., Witte, O.W., & Redecker, C. (2006). Changes in neocortical and hippocampal GABAA receptor subunit distribution during brain maturation and aging. *Brain Res, 1099*, 73-81.

Table 1

Characteristics of participants in the two age groups (mean ± sd).

	Young (n=25)	Senior (n=31)
Age (years)	22.5 ± 3.5	70.3 ± 3.8
Gender	11♂, 14♀	13♂, 18♀
Hand Dominance (L/R)	2/23	1/30
MoCA[1]	28.4 ± 1.6	27.3 ± 2.3
Resting MT (% output)	65.8 ± 11.3	72.6 ± 12.7
Test MT (% output)	77.8 ± 13.2	86.2 ± 15.8
Resting MEP amplitude (μV)	960.0 ± 776.2	427.2 ± 540.6*
Resting MEP latency (ms)	22.2 ± 1.9	24.0 ± 1.9*
Intensity MNS[2]	63 ± 18	73 ± 17

[1] Scores on the Montreal Cognitive Assessment test. Note that 5/31 seniors showed MoCA scores below the cut-off score of 26 (range: 21-25).

[2] Conditioning intensity for median nerve stimulation (MNS)

*Significant difference at adjusted *p*-values (p=0.0125) for multiple comparisons

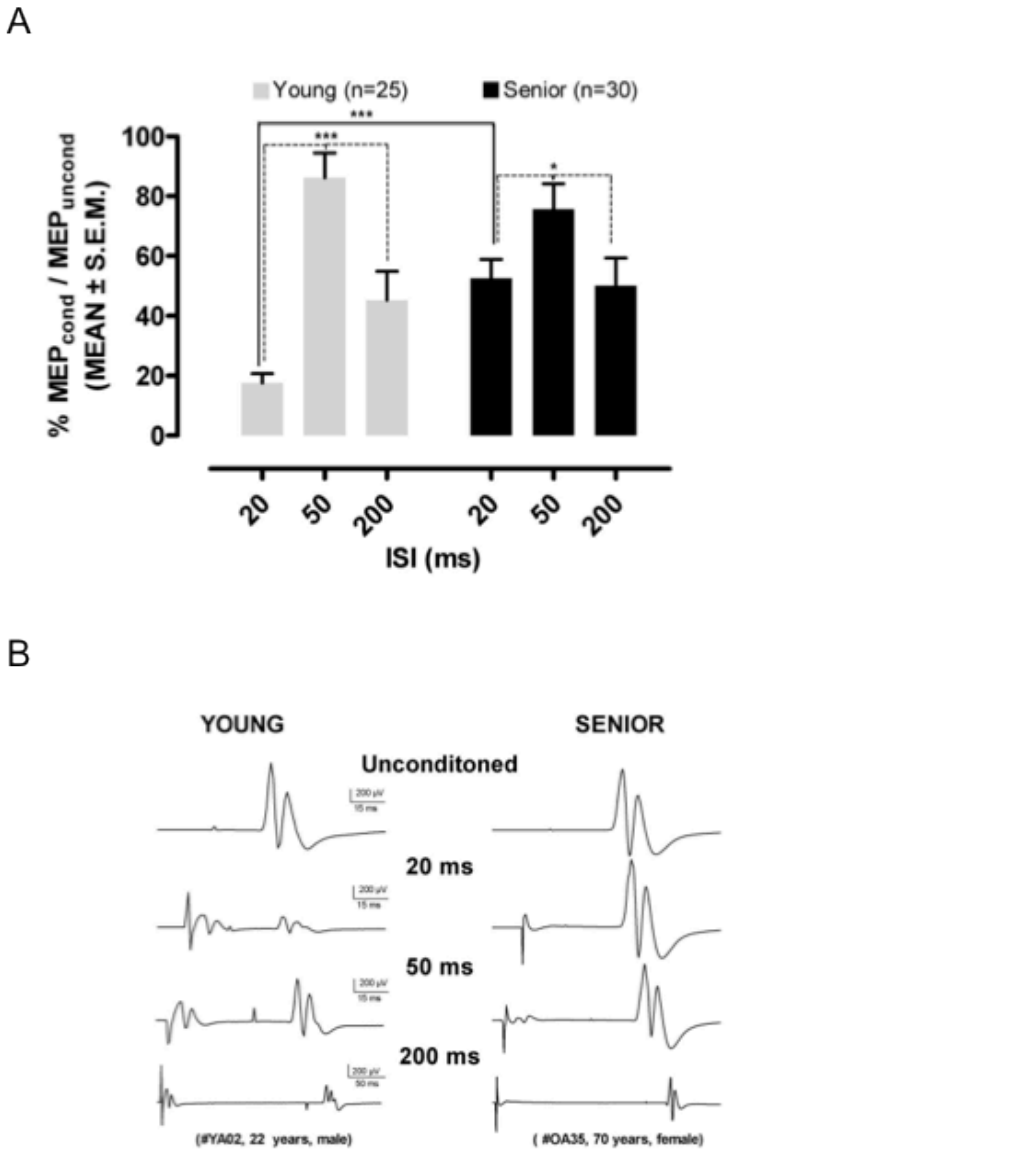

Figure 1. (A) Modulation of motor evoked potentials (MEP) amplitude induced by afferent conditioning at the different inter-stimulus intervals (ISIs) in the two groups of participants. Each value represents an average computed in each age group of the conditioned MEP size expressed as percentage of the unconditioned MEP size. The asterisks denote significant effects (*$p<0.05$, ***$p<0.01$) detected both within- and between-groups in the analysis of variance. Note the selective reduction in afferent-induced MEP inhibition at 20 ms in the senior group, which contrasted with the marked inhibition seen in the young group at the same interval. (B) Representative individual examples of recordings of MEP modulation

induced by afferent conditioning for the two age groups. Note the difference in the time scale for the 200 ms interval. All traces represent an average of 15 responses, except for unconditioned trials (n=25).

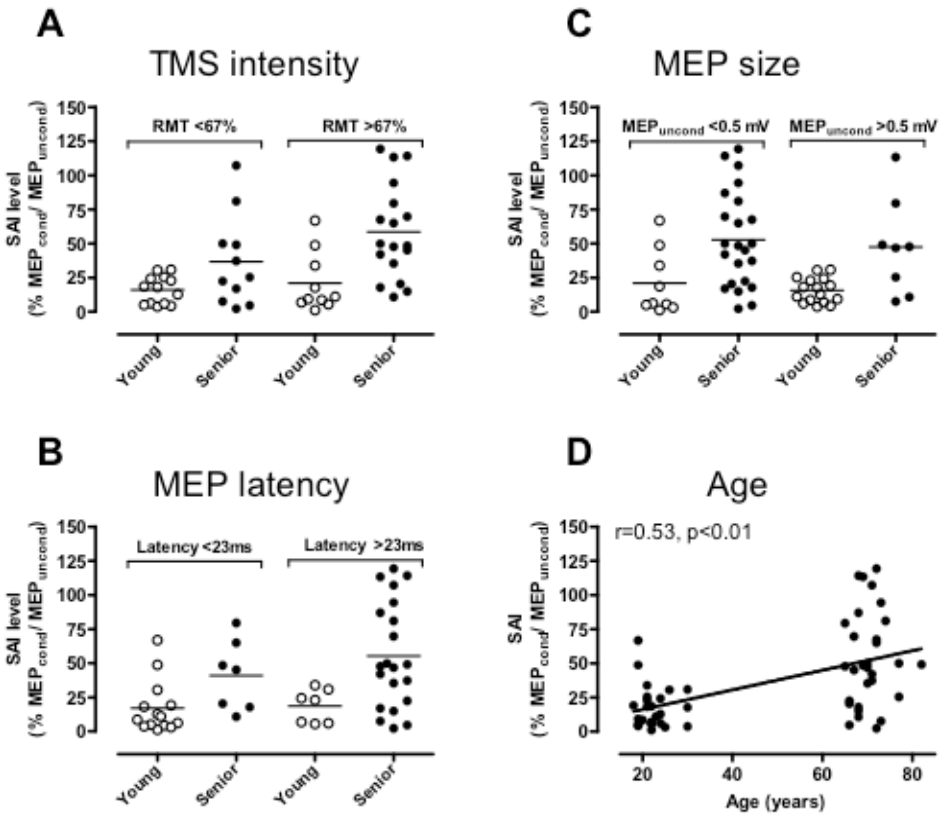

Figure 2. (A-C). Distribution of individual levels of short latency afferent inhibition (SAI) measured in the two age groups as a function of differences in basic measures of corticospinal excitability at rest (i.e., pre-conditioning trials). To ease comparisons, the distributions have been split into two categories for each measure using the specified value as the dichotomizing factor. The horizontal bar in each distribution represents the mean. Note that age differences in SAI were relatively unaffected by differences in basic measures of corticospinal excitability. (D) Association between age and SAI levels measured in the two age groups. RMT: resting motor threshold. MEP_{uncond}: motor evoked potential unconditioned.

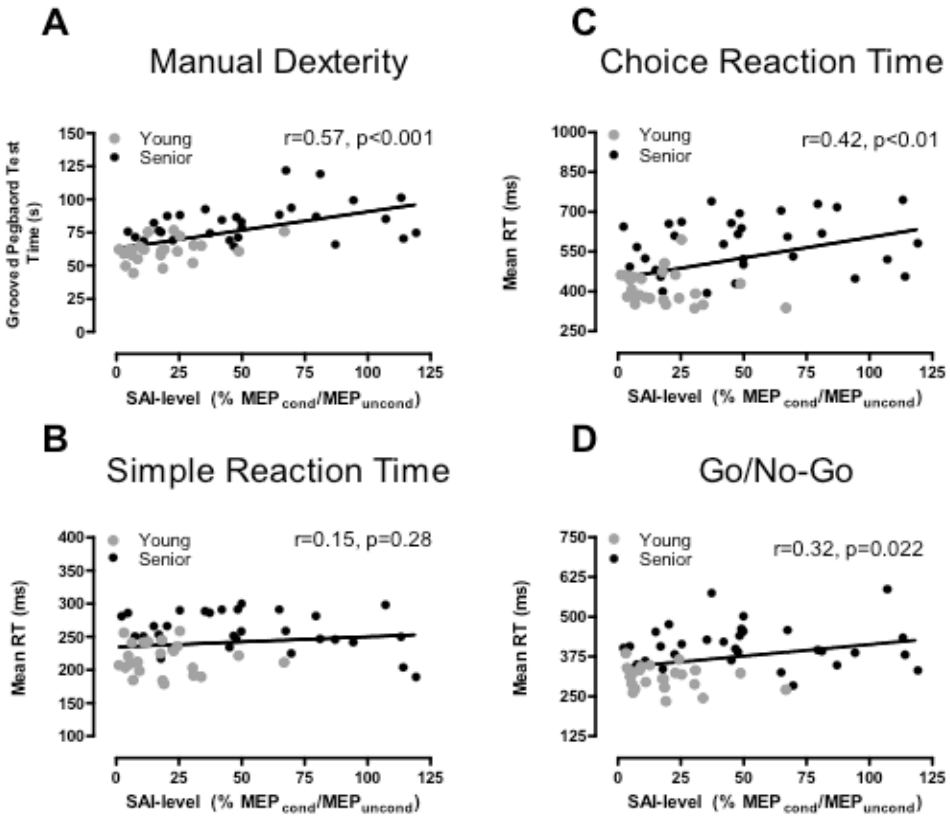

Figure 3. **(**A-D) Scatter plots showing the associations between short latency afferent inhibition (SAI) levels and measures of motor performance in young adults and seniors. Note that "r" and "p" values in each plot are given for pooled data from the two age groups. Three outlier data points, as revealed by the Grubb's test ($p<0.01$), were discarded from the correlational analysis: GPT (senior; $z=4.0$), simple RT (senior; $z=3.8$), and go/no-go task (young adult; $z=3.7$). Note also that data was not available for one young adult because of technical difficulties.

Associations between a neurophysiological marker of central cholinergic activity and cognitive functions in young and old adults

Marielle Young-Bernier, Yael Kamil, François Tremblay and Patrick Davidson

Young-Bernier, M., Kamil, Y., Tremblay, F., & Davidson, P. S. R. (2012). Associations between a neurophysiological marker of central cholinergic activity and cognitive functions in young and old adults. *Behavioral and Brain Functions, 8*:17.

Authors' contributions: MYB participated in the design of the study, carried out the cognitive and behavioural testing, performed the statistical analyses, and drafted the manuscript. YK participated in the cognitive testing. FT conceived of the study, participated in its design, and helped with the behavioural testing. PD conceived of the study, participated in its design, helped with the statistical analyses and drafted the manuscript.

Abstract

Background: The deterioration of the central cholinergic system in aging is hypothesized to underlie declines in several cognitive domains, including memory and executive functions. However, there is surprisingly little direct evidence regarding acetylcholine's specific role(s) in normal human cognitive aging. *Methods:* We used short-latency afferent inhibition (SAI) with transcranial magnetic stimulation (TMS) as a putative marker of cholinergic activity *in vivo* in young (n=24) and older adults (n=31). *Results:* We found a significant age difference in SAI, concordant with other evidence of cholinergic decline in normal aging. We also found clear age differences on several of the memory and one of the executive function measures. Individual differences in SAI levels predicted memory but not executive functions. *Conclusion:* Individual differences in SAI levels were better predictors of memory than executive functions. We discuss cases in which the relations between SAI and cognition might be even stronger, and refer to other age-related biological changes that may interact with cholinergic activity in cognitive aging.

1. Background

Normal aging is associated with declines in several cognitive domains, most notably episodic memory and executive functions (for reviews, see P. S. R. Davidson & Winocur, 2010; Drag & Bieliauskas, 2010; Glisky, 2007; Park & Reuter-Lorenz, 2009). These cognitive deficits are associated with myriad brain changes, including structural and functional deterioration of prefrontal, basal ganglia, and medial temporal areas and their interconnections. However, establishing a link between these changes and cognitive decline in normal aging has proven surprisingly difficult (Park & Reuter-Lorenz, 2009; Salthouse, 2011).

Alterations in two classic neurotransmitter systems have drawn considerable attention in cognitive aging: dopamine (Bäckman, Lindenberger, Li, & Nyberg, 2010) and acetylcholine. For decades, acetylcholine (ACh) was thought of primarily as a memory-related neurotransmitter, but this view has recently been revised, with ACh now thought to play an equally if not more crucial role in executive functions (for reviews, see Floresco & Jentsch, 2011; Hasselmo & Sarter, 2011; Picciotto, Meenakshi, & Jentsch, 2002). The integrity of cortical cholinergic inputs appears to be critical for modulating attention, by enhancing responsiveness to sensory inputs to facilitate cue detection and orienting (M. C. Davidson & Marrocco, 2000; for a review, see Hasselmo & Sarter, 2011). Cholinergic neuromodulation may also play an important role in executive functions by selectively enhancing task-relevant inputs via bottom-up thalamic processes, while suppressing irrelevant stimuli via top-down prefrontal modulation (Sarter, Hasselmo, Bruno, & Givens, 2005; for other perspectives, see Yu & Dayan, 2002, 2005). This cholinergic-dependent interaction between bottom-up and top-down processes appears to be affected by aging, leading to difficulty in task-switching, handling

competition among several possible responses, and suppressing unwanted responses (Sarter et al., 2005). In memory, optimal levels of ACh may facilitate encoding by increasing the influence of inputs into the hippocampus through enhanced potentiation (Hasselmo & Giocomo, 2006; Hasselmo & Sarter, 2011), and/or by providing the attentional "glue" to bind together disparate elements of an episode into a unified memory trace (Botly & De Rosa, 2007, 2008).

Experimental and correlational animal studies, as well as computational modelling, have yielded much information on the role of the cholinergic system in cognition. However, the extent to which age-related changes in cholinergic neuromodulation contribute to cognitive decline in normal human aging remains unclear. There are at least three reasons for this: First, making inferences from animal and computational models to humans has sometimes proven surprisingly difficult (e.g., Arnsten & Robbins, 2002; Graef, Schonknecht, Sabri, & Hegerl, 2011). Second, much of what we infer about the role of ACh in cognitive aging comes from studies in which Alzheimer's patients are treated with cholinesterase inhibitors, including donepezil, galantamine, and rivastigmine (e.g., Behl, Lanctot, Streiner, Guimont, & Black, 2006). Unfortunately, these patients can be difficult to test and they experience other confounding factors including significant structural and functional brain changes. Third, manipulation of ACh via agonist and antagonist drugs (e.g., scopolamine) has produced a vast amount of data, but strictly speaking this line of research tells us more about acute effects than it does about the long term decline in cholinergic activity seen in normal aging. There is thus a need to further examine the *in vivo* contribution of age-related alterations in central cholinergic function to declines in human cognition.

Recent advances in the field of non invasive brain stimulation have yielded new opportunities to examine the neurophysiological correlates of aging using markers of cortical excitability that can be linked with relative confidence to specific neurotransmitter systems (Reis et al., 2009). One such marker involves pairing afferent nerve stimulation with transcranial magnetic stimulation (TMS) of the motor cortex to modulate motor responses evoked in contralateral hand muscles (Chen et al., 2008). When applied at short intervals (e.g., 18-20 milliseconds [ms]) before TMS pulses, afferent nerve stimulation typically leads to a period of inhibition of the motor evoked potentials (MEPs). This *short-interval afferent inhibition* (SAI) is mediated at the cortical level through cholinergic-dependent $GABA_A$ receptor activation (Di Lazzaro et al., 2002). The implication of cholinergic action in mediating SAI is supported by *in vivo* observations of its reduction or even abolition by administration of a selective muscarinic cholinergic receptor blocker (scopolamine) in healthy participants (Di Lazzaro et al., 2000). Further, SAI is lower than expected in Alzheimer's patients but restored by cholinesterase inhibitors (Di Lazzaro et al., 2002). SAI is also reduced in other disorders characterized by cholinergic dysfunction, including Lewy body dementia (Di Lazzaro et al., 2007), multiple sclerosis (Cucurachi, Immovilli, Granella, Pavesi, & Cattaneo, 2008), and Wernicke–Korsakoff syndrome (Nardone et al., 2010), but it is normal in frontotemporal dementia, a non-cholinergically mediated form of dementia (Di Lazzaro et al., 2006). Together, these observations provide strong evidence that SAI is a cholinergic-dependent marker of motor intra-cortical excitability.

Given the clear decline in cholinergic modulation with age (Bartus, 2000; Gallagher & Colombo, 1995), one would predict that SAI would be altered in healthy

older adults. Yet, very few studies have examined this issue. Oliviero et al. (2006) compared SAI levels in healthy young and older adults and found no age differences. More recently, Degardin et al. (2011) performed a similar study and reached a similar conclusion. However, as we and others (Garry & Thomson, 2009) have argued previously, the use of varying test intensities to obtain a constant MEP size across participants might have contributed to masking any age effects in the two studies above. In line with this, we recently found a large and selective decrease in SAI in healthy seniors when we used a constant TMS test intensity approach (Young-Bernier, Davidson, & Tremblay, 2012). Further, we found that age-related variations in SAI explained a substantial proportion of the variance in timed motor tasks assessing processing speed.

This study constitutes an extension of our previous findings; data were derived from the same sample of participants as already described (Young-Bernier et al., 2012). In the present study, we examined possible relationships between SAI, as a putative marker of cholinergic-dependent cortical inhibition, and cognition in young and older healthy adults. Because mean differences between young and older adult groups are often small, especially relative to the extensive variability that can be seen among healthy older adults (e.g., some perform much more poorly than young people, whereas others are indistinguishable from the young (Gunstad et al., 2006), we capitalized on the individual-differences approach used by Glisky and colleagues (Glisky & Kong, 2008; Glisky, Rubin, & Davidson, 2001). This approach allows the characterization of each participant's long-term memory and executive functions using neuropsychological testing to construct aggregate scores reflecting performance across several tasks in each domain (for details, see Section 2.4). We hypothesized that age-related differences in SAI levels

would be associated with age-related differences in memory and executive functions. For memory, several investigators have emphasized ACh's putative role in binding information in memory (Botly & De Rosa, 2008), which we assessed using a canonical measure of paired associate learning (Verbal Paired Associates from the Wechsler Memory Scale-III; WMS-III; Wechsler, 1997). We also examined face recognition from the WMS-III because recent studies have also described cholinergic modulation of face-memory-related activity in the fusiform gyrus (Sperling et al., 2002). Given the emphasis in the recent literature on the crucial role of ACh in modulating executive functions (Behl et al., 2007; Behl et al., 2006; Swartz, Sahlas, & Black, 2003), we also expected correlations between SAI and our aggregate executive function measure.

2. Method

2.1 Participants

The present data were derived from the same group of participants previously described (Young-Bernier et al., 2012), with minor differences in the current sample (i.e. one young adult was excluded from the present study because of incomplete cognitive data). We analyzed data from 24 young adults (age range=18 to 30 years; M=22.52, SD=3.49; 14 females) and 31 community-dwelling older adults (age range=65 to 82 years; M=70.29, SD=3.81; 18 females). The two age groups were similar in education (young: M=16.08 years, SD=1.89; older adults: M=16.19, SD=2.83). All participants were fluent English and/or French speakers with normal or corrected-to-normal vision (one participant was blind in one eye, but had no difficulty with the visual tasks) and hearing, and were screened for depression (two participants were taking anti-depressants but their depression screening scores, TMS, and cognitive data were normal), dementia,

psychiatric or neurological disorders, drug or alcohol abuse, and counter-indications to TMS. Participants' medications were not altered for testing, with many older adults taking drugs related to vascular health (e.g., hypertension, statins cholesterol lowering drugs). None of the participants were taking neuroactive drugs such as neuroleptics, however one young adult and one older adult were taking antidepressants (as mentioned above, their TMS data were normal). Vascular risk factors were assessed for each participant and consisted of a cumulative score of 6 factors: body mass index with obesity defined as being greater than 30kg/m^2, current smoking status, lack of physical activity, type-2 diabetes, history of hypertension, and history of cardiac symptoms (Kuczynski, Jagust, Chui, & Reed, 2009; Wiederkehr, Laurin, Simard, Verreault, & Lindsay, 2009). Vascular risk factors for participants ranged from 0 to 3 (M=0.44) with the maximum possible score being 6, suggesting generally good vascular health. All participants also completed the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005). Although some older adults (5/31) scored slightly below the recommended cutoff (i.e., >26), they were deemed eligible for the study based on the interview and their good performance on the other tasks, and on recent evidence that this cut-off may be too high (Rossetti, Lacritz, Cullum, & Weiner, 2011). The results of five additional participants were discarded because they did not meet inclusion criteria and thirteen more (including 6 older adults) because of incomplete testing (10 could not be reached for a second testing session resulting in missing TMS-SAI data and 3 decided to stop before completion). The Research Ethics Boards of the University of Ottawa and Bruyère Continuing Care approved the study procedure in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from each participant before the

experimental session and all volunteers received a minimal honorarium to defray expenses for participation.

2.2 TMS procedure for short-afferent inhibition

The TMS procedure has been reported in detail previously (Young-Bernier et al., 2012). In brief, motor evoked potentials (MEP) were recorded using small pairs of auto-adhesive surface electrodes (10 mm diameter, Ag-AgCl) placed over the first dorsal interosseous (FDI) muscle of the right hand. Electromyographic signals were amplified (100-500 mV/div) and filtered (bandwidth, 10 Hz to 1 kHz) with a polygraph amplifier (RMP-6004, Nihon-Kohden Corp.; BNC-2090, National Instrument Corp.). Magnetic stimulation was delivered with a Magstim Rapid^2 stimulator (Magstim Co. Dyfed, UK) connected to a figure-eight coil (90-mm inside loop diameter), held ~45° in the mid-sagittal plane. The resting motor threshold (RMT) was determined using the method of Mills and Nithi (Mills & Nithi, 1997): the RMT was defined for each participant as the median intensity between the upper and lower threshold values. The test TMS intensity was fixed at 120% RMT for both unconditioned and conditioned trials. Conditioning afferent stimulation was produced by applying 200 μs electrical pulses (S88 Stimulator, Grass Technologies, Astro-Med, Inc, West Warwick, RI 02893 U.S.A.) on the median nerve at an intensity just above the motor threshold to evoke a minimal visible twitch of the thenar muscles (Di Lazzaro et al., 2000; Tokimura et al., 2000). SAI was measured by applying afferent stimulation 20 ms before the TMS pulse over the motor cortex. Other inter-stimulus intervals (ISI; 25, 50 or 200 ms; see Young-Bernier et al., 2012) were also investigated. Unconditioned MEP amplitude was first determined for each participant by eliciting 15 MEPs at rest (120% RMT). Following the same procedure, blocks of trials

were made for each conditioned interval (order was counterbalanced across participants). Trials for which unwanted contractions were present were eliminated and repeated if necessary.

2.3 Analysis of MEP data

Mean individual values for conditioned and unconditioned MEP responses were measured off-line by averaging the amplitude (peak-to-peak) and latency of each trial. SAI level was determined in each participant in terms of percent of unconditioned MEP responses (i.e.% $MEP_{Conditioned}$ / $MEP_{Unconditioned}$).

2.4 Memory and executive functions

Participants underwent neuropsychological testing in a quiet, well-lit room, in their language of choice. We created two composite *z* scores for each individual, based on previous factor analyses (Glisky & Kong, 2008; Glisky et al., 2001). The first factor score reflects long-term memory and is comprised of five scores: the Logical Memory I, Faces recognition I, and Verbal Paired Associates I subtests of the WMS-III, Visual Paired Associates II from the Wechsler Memory Scale–Revised (WMS-R; Wechsler, 1987), and Long Delay Cued Recall from the California Verbal Learning Test-II (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2000). The second factor score, reflecting executive function, is made up of the number of categories achieved on the computerized Wisconsin Card Sorting Test (Kongs, Thompson, Iverson, & Heaton, 2000), the total number of words produced to the cues *F*, *A*, and *S* on a phonemic fluency test (Spreen & Benton, 1977), and the Backward Digit Span and Mental Control measures from the WMS–III. In previous studies involving only older adults, the executive function factor had also

included Mental Arithmetic from the Wechsler Adult Intelligence Scale—Revised (WAIS-R; Wechsler, 1981, but see Glisky & Kong, 2008) reported that this measure did not load significantly on the executive function factor in their young adults. Therefore, we omitted this measure from the executive function *z* score in both groups to allow for direct age group comparisons.

2.5 Statistical methods

Independent *t*-tests, with adjusted *p* values for multiple comparisons (i.e. p=0.0125), were used to examine age group differences on baseline measures of excitability. Mixed analysis of variance (ANOVA) and independent *t*-tests were used to examine differences between age groups on cognitive performance. We adjusted *p* values to correct for multiple comparisons in the between-group *t*-tests on the cognitive tasks (p=0.05/9, that is, p=0.0056). We used Pearson's correlations to examine associations among SAI levels and memory and executive function scores. All statistical tests were performed using the PASW software version 18.0 for Windows® (Chicago, IL, USA). Figures were prepared with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

3. Results

3.1 TMS and SAI

The TMS procedure was well tolerated and no participants experienced adverse effects. A thorough analysis of the physiological data has been reported previously (Young-Bernier et al., 2012; see Table 1 for baseline TMS measurements). Briefly, young adults generally exhibited marked MEP suppression in response to afferent

conditioning leading to high levels of SAI (18.13±15.74). In contrast, seniors exhibited more variable afferent-induced inhibition with a substantial proportion of subjects (14/31) showing either low or absent inhibition ($MEP_{cond} \geq 50\%$ suppression). Accordingly, SAI levels estimated in seniors (51.36±34.62) were significantly lower than in young adults ($p<0.001$).

3.2 Age differences in cognition

The young adults performed significantly better on several of the memory and executive function tasks than the older adults did (ANOVA: main effect of Age: $F_{1,51}=6.86$, $p=0.01$, significant Age X Task interaction: $F_{7, 357}=3.22$; $p=0.003$[1]). At the adjusted p value, post-hoc t tests showed that the young significantly outperformed the older adults on memory for Verbal Paired Associates I ($t_{53}=4.03$, $p=0.0002$) and Faces I ($t_{52}=3.89$, $p=0.0003$), and number of categories on the Wisconsin Card Sorting Test ($t_{52}=4.10$, $p=0.0001$). Although the two age groups could not be compared on the Visual Paired Associates II measure using parametric methods because of ceiling effects in the young adults, a Chi-Squared analysis suggested a significant advantage for the young adults (range of scores of 4 to 6 in older adults; $\chi^2_2=9.82$, $p=0.007$). The factor scores, by definition, reflected the individual test scores: The young had significantly higher scores than the older adults on the memory factor z score ($t_{53}=4.53$, $p<0.0001$), but the groups were not significantly different from one another on the executive function factor z score ($t_{53}=1.65$, $p=0.11$). The mean levels of performance on the individual cognitive tasks and the factor scores are shown in Table 2.

[1] Three older adults each did not complete one cognitive measure (Faces I, Wisconsin Card Sorting Test and Visual Paired Associates II); their factor z scores were calculated by computing the mean of the remaining tests.

3.3 Correlations between SAI and cognition

When we performed an analysis across all individuals (Clark, Loftus, & Hammond, 2011; Duzel et al., 2010; but see Baxter & Gallagher, 1996; Lazic, 2010), SAI significantly predicted the memory factor score (r=-0.31, p=0.02), whereas it did not predict the executive function z score (r=-0.09, p=0.51; see Figure 1). The correlation between SAI and memory was modest in size (r^2=10%), and when we examined the correlation separately within each age group it failed to obtain significance. Although in the young group alone a significant correlation between SAI levels and the executive function z score emerged in our initial analysis (r=-0.56, p=0.004), visual inspection suggested that this was driven by two data points; indeed, if we deleted these two cases the correlation was rendered non-significant.

Based on the hypotheses outlined at the end of the introduction, we also examined associations between SAI and specific individual subtest scores. First, we found a significant correlation between SAI and Verbal Paired Associates I (r=-0.35, p=0.008), a canonical measure of memory binding, although this correlation became non-significant when we examined each age group on its own ($r \leq |0.21|$). Note that although Visual Paired Associates II is also a canonical measure of this ability, it was not explored further because of the ceiling-level scores in data, especially for the young adults. Second, we found a significant correlation between SAI and memory for faces (Faces I; r=-0.31, p=0.02), although, again, it disappeared when analyses were performed separately within each age group ($r \leq |0.17|$).

We also performed all analyses while excluding the five older adults who had MoCA scores lower than the recommended cutoff. This did not yield any changes in the results.

4. Discussion

Deficits in central cholinergic activity are thought to underlie age-related cognitive decline, but evidence regarding the specific role(s) of ACh in human cognitive aging is still scarce. We investigated the relation of SAI, a putative neurophysiological marker of cholinergic activity, to memory and executive functions in aging.

4.1 Age differences in SAI

Consistent with reports of impaired cortical inhibition with age (Peinemann, Lehner, Conrad, & Siebner, 2001), as a group, our senior participants exhibited reduced intra-cortical inhibition, as reflected in the overall decrease in afferent-induced inhibition. The fact that SAI has been linked with cholinergic activity in the motor cortex in pharmacological and patient studies (e.g., Di Lazzaro et al., 2004; Di Lazzaro et al., 2000; Di Lazzaro et al., 2002; but see below) provides further converging *in vivo* evidence of a decline in central cholinergic function in normal human aging (e.g., Mesulam, Shaw, Mash, & Weintraub, 2004 for reviews, see Bartus, 2000; Dumas & Newhouse, 2011).

4.2 Associations between SAI and cognition

The young adults outperformed their older counterparts on several measures of memory, consistent with numerous previous reports (P. S. R. Davidson & Winocur,

2010; Drag & Bieliauskas, 2010; Glisky, 2007; Park & Reuter-Lorenz, 2009). Although memory was clearly impaired in the older adults, executive function was not. This finding is concordant with a similar study to ours (Glisky & Kong, 2008), which noted that others too have found this pattern. For example, Lamar and Resnick (2004) reported no age differences in verbal fluency, mental control, and digit span, which were included in the present executive function factor score.

SAI predicted individual performance in memory, although, contrary to expectations, it did not predict executive functioning. These results are consistent with some studies (Thienel et al., 2009), but not with others (Behl et al., 2007; Behl et al., 2006; Swartz et al., 2003) and may stem from the poor vascular health of the patients included in those studies. (This issue will be discussed further below.) The associations between SAI and memory are also consistent with Duzel et al. (2010), who recently reported that a magnetic resonance imaging estimate of the structural integrity of the basal forebrain (the major source of cholinergic input into the cortex and hippocampus) predicted verbal memory in a mixed sample of young and older adults.

In the present study, SAI levels explained approximately 10% of the variance in memory. Although this is comparable in size to the explanatory power of Duzel et al.'s (2010) measure of basal forebrain integrity, we suspect that the relation between SAI and cognition might be even stronger under different circumstances. First, pharmacological studies indicate that ACh must decline past a certain threshold before changes in cognition are detectable (Edginton & Rusted, 2003; Fredrickson et al., 2008; Hodges, Lindner, Hogan, Jones, & Markus, 2009; Little, Johnson, Minichiello, Weingartner, & Sunderland, 1998; Robbins et al., 1997). Although we studied a representative group of

older adults, only a small number of them exhibited relatively low SAI levels. Given that cholinergic function declines with age, one future possibility would be to recruit older seniors (i.e., over 80 years of age) with the expectation that stronger correlations with cognition would emerge. Also, one important putative cause of cholinergic decline in aging is microvascular damage to the ascending cholinergic pathways from the midbrain to the cortex (Mesulam, Siddique, & Cohen, 2003; Roman, 2005). Our older participants were in relatively good vascular health. Were we to focus on recruiting people in poorer vascular health, we might find stronger correlations between cholinergic function and cognition (Behl et al., 2007; Swartz et al., 2003).

Second, it is possible that cholinergic modulation supports only relatively specific aspects of memory and executive functions and that these processes were not optimally assayed or taxed by the current neuropsychological battery. A general assertion is that for ACh to be significantly implicated in cognitive tasks, these tasks must be difficult and require effortful attention (Dumas & Newhouse, 2011; Sarter et al., 2005). The tasks in the current study all fit this description. However, based on techniques that can target specifically the cholinergic system in animals (e.g., the immunotoxin 192 IgG-saporin), it has recently been argued that ACh is particularly important for certain memory functions, including encoding more so than retrieval, and remembering relational and contextual information in particular (Botly & De Rosa, 2008; Easton, Fitchett, Eacott, & Baxter, 2011). Consistent with the strong involvement of Ach in attention, studies have also suggested that the cholinergic system is more important for strategic and effortful processing of information to be remembered rather than when it is automatic (Rusted, Trawley, Heath, Kettle, & Walker, 2005). Regarding executive functions, cholinergic

activity may be especially important for task-switching, handling competition among possible responses, and suppressing unwanted responses (Sarter et al., 2005). Although we did measure several of these putative processes (e.g., memory binding with the visual and verbal paired associates subtests; switching and suppression with the Wisconsin Card Sorting Test), we are currently developing a new battery to probe some of these memory and executive sub-processes more specifically. Combined with our previous observation of an association between SAI and complex motor tasks (i.e. Grooved Pegboard Test, complex reaction times, go/no-go) but not with simple reaction times in aging (Young-Bernier et al., 2012), this study suggests that SAI may be a better predictor of memory than executive functions, but an even stronger indicator of motor performance and information processing speed.

Third, recent microdialysis studies have described phasic cholinergic release during attention-related tasks in rats (Parikh, Kozak, Martinez, & Sarter, 2007; Parikh & Sarter, 2008). These studies suggest that indices of relatively tonic ACh levels (including SAI, positron emission tomography, and magnetic resonance spectroscopy) in the brain will need to be supplemented with methods that have higher temporal resolution when they become available in humans. Finally, like most studies, this one was cross-sectional. Complementary longitudinal studies of within-subject changes must be completed to yield a more complete understanding of the relationship between the onset and course of cholinergic dysfunction and cognitive decline in normal and pathological aging (e.g., (Shinotoh et al., 2000 cf. Nilsson, Sternang, Ronnlund, & Nyberg, 2009; Salthouse, 2009).

Strong evidence that SAI is a reliable marker of cholinergic function comes from pharmacological and patient studies (Di Lazzaro et al., 2004; Di Lazzaro et al., 2000; Di Lazzaro et al., 2002), but gamma-aminobutyric acid (GABA), dopamine, and serotonin may also contribute to the signal (e.g., Di Lazzaro, Oliviero et al., 2005; Martorana et al., 2009). For example, as we have noted previously (Young-Bernier et al., 2012), our older adults showed greater inter-individual variability in SAI than did our young adults, with approximately half the seniors exhibiting either poor or absent intra-cortical inhibition. These older adults were indistinguishable from the other seniors in terms of age and vascular health, and there was no evidence that these individuals were in a preclinical stage of dementia. One possibility, however, is that these individual differences in intra-cortical inhibition are related to variability in changes in motor cortex $GABA_A$ receptors in aging (Di Lazzaro, Pilato, Dileone, Tonali, & Ziemann, 2005; Yu, Wang, Fritschy, Witte, & Redecker, 2006). Future pharmacological and neuroimaging work must verify that SAI is strongly, although perhaps not exclusively, reflective of activity in the cholinergic system.

5. Conclusion

We found that individual differences in episodic memory could be explained in part by SAI, a putative marker of central cholinergic functioning. However, cholinergic decline is only one of many brain changes that occur in aging (Dennis & Cabeza, 2008; Raz & Rodrigue, 2006; Yankner, Lu, & Loerch, 2008). The goal of future research on the biological bases of cognitive aging should be to combine multiple methods to increase explanatory power, for example by combining multiple neuroimaging methods (e.g., Kalpouzos, Persson, & Nyberg, 2012; Van Petten et al., 2004) with genetic information

(e.g., Duzel et al., 2010; Ryan et al., 2011). The short afferent inhibition marker of cholinergic integrity reported in this study is a minimally-invasive, relatively inexpensive, significant predictor of cognition. Combining it with neuroimaging, genetic, and other cognitive neuroscience methods should prove useful in future studies.

Acknowledgements: We thank our participants for their time and patience during testing, and Héloïse Drouin, Sabah Master, and Travis Davidson for help with data collection and analysis. This work will serve as a partial fulfillment for a doctoral thesis in clinical psychology by MYB. This work was supported by student awards from the Canadian Institutes of Health Research to MYB and from the Natural Sciences and Engineering Research Council of Canada to YK, a Research Development Grant from the Faculty of Social Sciences of the University of Ottawa to FT and PD, and a Discovery grant from the Natural Sciences and Engineering Research Council of Canada to PD. These funding sources played no role in the design or administration of the study, the analysis or interpretation of the results, or the decision to submit for publication.

References

Arnsten, A. F., & Robbins, T. W. (2002). Neurochemical modulation of prefrontal cortical function in humans and animals. In D. T. Stuss & R. T. Knight (Eds.), *Principles of Frontal Lobe Function* (pp. 51-84). New York, NY: Oxford University Press.

Bäckman, L., Lindenberger, U., Li, S.-C., & Nyberg, L. (2010). Linking cognitive aging to alterations in dopamine neurotransmitter functioning: Recent data and future avenues. *Neuroscience & Biobehavioral Reviews, 34*(5), 670-677.

Bartus, R. T. (2000). On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Exp Neurol, 163*(2), 495-529.

Baxter, M. G., & Gallagher, M. (1996). Neurobiological substrates of behavioral decline: models and data analytic strategies for individual differences in aging. *Neurobiol Aging, 17*(3), 491-495.

Behl, P., Bocti, C., Swartz, R. H., Gao, F., Sahlas, D. J., & Lanctot, K. L. (2007). Strategic subcortical hyperintensities in cholinergic pathways and executive function decline in treated Alzheimer patients. *Arch Neurol, 64*(2), 266-272.

Behl, P., Lanctot, K. L., Streiner, D. L., Guimont, I., & Black, S. E. (2006). Cholinesterase inhibitors slow decline in executive functions, rather than memory, in Alzheimer's disease: a 1-year observational study in the Sunnybrook dementia cohort. *Curr Alzheimer Res, 3*(2), 147-156.

Botly, L. C., & De Rosa, E. (2007). Cholinergic influences on feature binding. *Behav Neurosci, 121*(2), 264-276.

Botly, L. C., & De Rosa, E. (2008). A cross-species investigation of acetylcholine, attention, and feature binding. *Psychol Sci, 19*(11), 1185-1193.

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaucheur, J. P., & Magistris, M. R. (2008). The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol, 119*(3), 504-532.

Clark, J., Loftus, A., & Hammond, G. (2011). Age-related changes in short-interval intracortical facilitation and dexterity. *NeuroReport, 22*(10), 499-503.

Cucurachi, L., Immovilli, P., Granella, F., Pavesi, G., & Cattaneo, L. (2008). Short-latency afferent inhibition predicts verbal memory performance in patients with multiple sclerosis. *J Neurol, 255*(12), 1949-1956.

Davidson, M. C., & Marrocco, R. T. (2000). Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. *J Neurophysiol, 83*(3), 1536-1549.

Davidson, P. S. R., & Winocur, G. (2010). Aging and Cognition. In G. F. Koob, M. Le Moal, & R. F. Thompson (Eds.), *Encyclopedia of Behavioral Neuroscience, Volume 1* (pp. 20-26). Oxford: Academic Press.

Degardin, A., Devos, D., Cassim, F., Bourriez, J. L., Defebvre, L., & Derambure, P. (2011). Deficit of sensorimotor integration in normal aging. *Neurosci Lett*, *498*(3), 208-212.

Delis, D. C., Kramer, J., Kaplan, E., & Ober, B. A. (2000). *The California Verbal Learning Test* (second ed.). San Antonio, TX: Psychological Corporation.

Dennis, N. A., & Cabeza, R. (2008). Neuroimaging of healthy cognitive aging. In F. I. M. Craik & T. A. Salthouse (Eds.), *Handbook of Aging and Cognition, 3rd Edition* (pp. 1-54). Mahwah, NJ: Eribaum.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., & Marra, C. (2004). Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 75*(4), 555-559.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M. A., Di Giovanni, S., & Zito, G. (2000). Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. *Exp Brain Res, 135*(4), 455-461.

Di Lazzaro, V., Oliviero, A., Saturno, E., Dileone, M., Pilato, F., & Nardone, R. (2005). Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans. *J Physiol, 564*(Pt 2), 661-668.

Di Lazzaro, V., Oliviero, A., Tonali, P. A., Marra, C., Daniele, A., & Profice, P. (2002). Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology, 59*(3), 392-397.

Di Lazzaro, V., Pilato, F., Dileone, M., Saturno, E., Oliviero, A., & Marra, C. (2006). In vivo cholinergic circuit evaluation in frontotemporal and Alzheimer dementias. *Neurology, 66*(7), 1111-1113.

Di Lazzaro, V., Pilato, F., Dileone, M., Saturno, E., Profice, P., & Marra, C. (2007). Functional evaluation of cerebral cortex in dementia with Lewy bodies. *Neuroimage, 37*(2), 422-429.

Di Lazzaro, V., Pilato, F., Dileone, M., Tonali, P. A., & Ziemann, U. (2005). Dissociated effects of diazepam and lorazepam on short-latency afferent inhibition. *J Physiol, 569*(Pt 1), 315-323.

Drag, L. L., & Bieliauskas, L. A. (2010). Contemporary review 2009: cognitive aging. *J Geriatr Psychiatry Neurol, 23*(2), 75-93.

Dumas, J. A., & Newhouse, P. A. (2011). The cholinergic hypothesis of cognitive aging revisited again: Cholinergic functional compensation. *Pharmacol Biochem Behav, 99*(2), 254-261.

Duzel, S., Munte, T. F., Lindenberger, U., Bunzeck, N., Schutze, H., & Heinze, H. J. (2010). Basal forebrain integrity and cognitive memory profile in healthy aging. *Brain Res, 1308*, 124-136.

Easton, A., Fitchett, A. E., Eacott, M. J., & Baxter, M. G. (2011). Medial septal cholinergic neurons are necessary for context-place memory but not episodic-like memory. *Hippocampus, 21*, 1021-1027.

Edginton, T., & Rusted, J. M. (2003). Separate and combined effects of scopolamine and nicotine on retrieval-induced forgetting. *Psychopharmacol, 170*(4), 351-357.

Floresco, S. B., & Jentsch, J. D. (2011). Pharmacological enhancement of memory and executive functioning in laboratory animals. *Neuropsychopharmacol, 36*(1), 227-250.

Fredrickson, A., Snyder, P. J., Cromer, J., Thomas, E., Lewis, M., & Maruff, P. (2008). The use of effect sizes to characterize the nature of cognitive change in psychopharmacological studies: an example with scopolamine. *Hum Psychopharmacol, 23*(5), 425-436.

Gallagher, M., & Colombo, P. J. (1995). Ageing: the cholinergic hypothesis of cognitive decline. *Curr Opin Neurobiol, 5*(2), 161-168.

Garry, M. I., & Thomson, R. H. (2009). The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. *Exp Brain Res, 193*(2), 267-274.

Glisky, E. L. (2007). Changes in Cognitive Function in Human Aging. In D. R. Riddle (Ed.), *Brain aging: Models, methods and mechanisms* (pp. 4-20). Boca Raton, FL: CRC Press.

Glisky, E. L., & Kong, L. L. (2008). Do young and older adults rely on different processes in source memory tasks? A neuropsychological study. *J Exp Psychol Learn Mem Cogn, 34*(4), 809-822.

Glisky, E. L., Rubin, S. R., & Davidson, P. S. (2001). Source memory in older adults: an encoding or retrieval problem? *J Exp Psychol Learn Mem Cogn, 27*(5), 1131-1146.

Graef, S., Schonknecht, P., Sabri, O., & Hegerl, U. (2011). Cholinergic receptor subtypes and their role in cognition, emotion, and vigilance control: An overview of preclinical and clinical findings. *Psychopharmacol, 215*(2), 205-229.

Gunstad, J., Paul, R. H., Brickman, A. M., Cohen, R. A., Arns, M., & Roe, D. (2006). Patterns of cognitive performance in middle-aged and older adults: A cluster analytic examination. *J Geriatr Psychiatry Neurol, 19*(2), 59-64.

Hasselmo, M. E., & Giocomo, L. M. (2006). Cholinergic modulation of cortical function. *J Mol Neurosci, 30*(1-2), 133-135.

Hasselmo, M. E., & Sarter, M. (2011). Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacol, 36*(1), 52-73.

Hodges, D. B., Jr., Lindner, M. D., Hogan, J. B., Jones, K. M., & Markus, E. J. (2009). Scopolamine induced deficits in a battery of rat cognitive tests: comparisons of sensitivity and specificity. *Behav Pharmacol, 20*(3), 237-251.

Kalpouzos, G., Persson, J., & Nyberg, L. (2012). Local brain atrophy accounts for functional activity differences in normal aging. *Neurobiol Aging, 33,* 623.e1-623.e13.

Kongs, S., Thompson, L. L., Iverson, G. L., & Heaton, R. K. (2000). *Wisconsin Card Sorting Test-64 Card Version*. Lutz, FL: Psychological Assessment Resources.

Kuczynski, B., Jagust, W., Chui, H. C., & Reed, B. (2009). An inverse association of cardiovascular risk and frontal lobe glucose metabolism. *Neurology, 72*(8), 738-743.

Lamar, M., & Resnick, S. M. (2004). Aging and prefrontal functions: dissociating orbitofrontal and dorsolateral abilities. *Neurobiol Aging, 25*(4), 553-558.

Lazic, S. E. (2010). The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis? *BMC Neurosci, 11*, 5.

Little, J. T., Johnson, D. N., Minichiello, M., Weingartner, H., & Sunderland, T. (1998). Combined nicotinic and muscarinic blockade in elderly normal volunteers: cognitive, behavioral, and physiologic responses. *Neuropsychopharmacol, 19*(1), 60-69.

Martorana, A., Mori, F., Esposito, Z., Kusayanagi, H., Monteleone, F., & Codeca, C. (2009). Dopamine modulates cholinergic cortical excitability in Alzheimer's disease patients. *Neuropsychopharmacology, 34*(10), 2323-2328.

Mesulam, M. M., Shaw, P., Mash, D., & Weintraub, S. (2004). Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. *Ann Neurol, 55*(6), 815-828.

Mesulam, M. M., Siddique, T., & Cohen, B. (2003). Cholinergic denervation in a pure multi-infarct state: observations on CADASIL. *Neurology, 60*(7), 1183-1185.

Mills, K. R., & Nithi, K. A. (1997). Corticomotor threshold is reduced in early sporadic amyotrophic lateral sclerosis. *Muscle Nerve, 20*(9), 1137-1141.

Nardone, R., Bergmann, J., De Blasi, P., Kronbichler, M., Kraus, J., & Caleri, F. (2010). Cholinergic dysfunction and amnesia in patients with Wernicke-Korsakoff syndrome: a transcranial magnetic stimulation study. *J Neural Transm, 117*(3), 385-391.

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., & Collin, I. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society, 53*(4), 695-699.

Nilsson, L. G., Sternang, O., Ronnlund, M., & Nyberg, L. (2009). Challenging the notion of an early-onset of cognitive decline. *Neurobiol Aging, 30*(4), 521-524; discussion 530-523.

Oliviero, A., Profice, P., Tonali, P. A., Pilato, F., Saturno, E., & Dileone, M. (2006). Effects of aging on motor cortex excitability. *Neurosci Res, 55*(1), 74-77.

Parikh, V., Kozak, R., Martinez, V., & Sarter, M. (2007). Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron, 56*(1), 141-154.

Parikh, V., & Sarter, M. (2008). Cholinergic mediation of attention: contributions of phasic and tonic increases in prefrontal cholinergic activity. *Ann N Y Acad Sci, 1129*, 225-235.

Park, D. C., & Reuter-Lorenz, P. (2009). The adaptive brain: aging and neurocognitive scaffolding. *Annu Rev Psychol, 60*, 173-196.

Peinemann, A., Lehner, C., Conrad, B., & Siebner, H. R. (2001). Age-related decrease in paired-pulse intracortical inhibition in the human primary motor cortex. *Neurosci Lett, 313*(1-2), 33-36.

Picciotto, M. R., Meenakshi, A., & Jentsch, J. D. (2002). Acetylcholine. In K. L. Davis, D. Charney, J. T. Coyle, & C. Nemeroff (Eds.), *Neuropsychopharmacology: The Fifth Generation of Progress* (pp. 3-14). Philadelphia, PA: Lippincott Williams & Wilkins.

Raz, N., & Rodrigue, K. M. (2006). Differential aging of the brain: patterns, cognitive correlates and modifiers. *Neurosci Biobehav Rev, 30*(6), 730-748.

Reis, H. J., Guatimosim, C., Paquet, M., Santos, M., Ribeiro, F. M., & Kummer, A. (2009). Neuro-transmitters in the central nervous system & their implication in learning and memory processes. *Curr Med Chem, 16*(7), 796-840.

Robbins, T. W., Semple, J., Kumar, R., Truman, M. I., Shorter, J., & Ferraro, A. (1997). Effects of scopolamine on delayed-matching-to-sample and paired associates tests

of visual memory and learning in human subjects: comparison with diazepam and implications for dementia. *Psychopharmacol, 134*(1), 95-106.

Roman, G. C. (2005). Cholinergic dysfunction in vascular dementia. *Curr Psychiatry Rep, 7*(1), 18-26.

Rossetti, H. C., Lacritz, L. H., Cullum, C. M., & Weiner, M. F. (2011). Normative data for the Montreal Cognitive Assessment (MoCA) in a population-based sample. *Neurology, 77*(13), 1272-1275.

Rusted, J. M., Trawley, S., Heath, J., Kettle, G., & Walker, H. (2005). Nicotine improves memory for delayed intentions. *Psychopharmacology (Berl), 182*(3), 355-365.

Ryan, L., Walther, K., Bendlin, B., Lue, L., Walker, D. G., & Glisky, E. (2011). Age-related differences in white matter integrity and cognitive function are related to APOE status. *Neuroimage, 54,* 1565-1577.

Salthouse, T. A. (2009). When does age-related cognitive decline begin? *Neurobiol Aging, 30*(4), 507-514.

Salthouse, T. A. (2011). Neuroanatomical substrates of age-related cognitive decline. *Psychol Bull*, *137,* 753-784.

Sarter, M., Hasselmo, M. E., Bruno, J. P., & Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Rev, 48*(1), 98-111.

Shinotoh, H., Namba, H., Fukushi, K., Nagatsuka, S., Tanaka, N., & Aotsuka, A. (2000). Progressive loss of cortical acetylcholinesterase activity in association with cognitive decline in Alzheimer's disease: a positron emission tomography study. *Ann Neurol, 48*(2), 194-200.

Sperling, R., Greve, D., Dale, A., Killiany, R., Holmes, J., & Rosas, H. D. (2002). Functional MRI detection of pharmacologically induced memory impairment. *Proc Natl Acad Sci U S A, 99*(1), 455-460.

Spreen, O., & Benton, A. L. (1977). *Neurosensory Center Comprehensive Examination for Aphasia, Revised edition*. Victoria, BC: University of Victoria Neuropsychology Laboratory.

Swartz, R. H., Sahlas, D. J., & Black, S. E. (2003). Strategic involvement of cholinergic pathways and executive dysfunction: Does location of white matter signal hyperintensities matter? *J Stroke Cerebrovasc Dis, 12*(1), 29-36.

Thienel, R., Kellermann, T., Schall, U., Voss, B., Reske, M., & Halfter, S. (2009). Muscarinic antagonist effects on executive control of attention. *Int J Neuropsychopharmacol, 12*(10), 1307-1317.

Tokimura, H., Di Lazzaro, V., Tokimura, Y., Oliviero, A., Profice, P., & Insola, A. (2000). Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J Physiol, 523 Pt 2*, 503-513.

Van Petten, C., Plante, E., Davidson, P. S., Kuo, T. Y., Bajuscak, L., & Glisky, E. L. (2004). Memory and executive function in older adults: relationships with temporal and prefrontal gray matter volumes and white matter hyperintensities. *Neuropsychologia, 42*(10), 1313-1335.

Wechsler, D. (1981). *Weschler Adult Intelligence Scale-Revised*. New York: Psychological Corporation.

Wechsler, D. (1987). *Wechsler Memory Scale-Revised*. New York: Psychological Corporation.

Wechsler, D. (1997). *Wechsler Memory Scale-III*. San Antonio, TX: Psychological Corporation.

Wiederkehr, S., Laurin, D., Simard, M., Verreault, R., & Lindsay, J. (2009). Vascular risk factors and cognitive functions in nondemented elderly individuals. *J Geriatr Psychiatry Neurol, 22*(3), 196-206.

Yankner, B. A., Lu, T., & Loerch, P. (2008). The aging brain. *Annu Rev Pathol, 3*, 41-66.

Young-Bernier, M., Davidson, P.S., & Tremblay, F. (2012). Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition with age. *Neurobiol Aging 33***,** 835.e1-835.e11.

Yu, A. J., & Dayan, P. (2002). Acetylcholine in cortical inference. *Neural Netw, 15*(4-6), 719-730.

Yu, A. J., & Dayan, P. (2005). Uncertainty, neuromodulation, and attention. *Neuron, 46*(4), 681-692.

Yu, Z. Y., Wang, W., Fritschy, J. M., Witte, O. W., & Redecker, C. (2006). Changes in neocortical and hippocampal GABAA receptor subunit distribution during brain maturation and aging. *Brain Res, 1099*(1), 73-81.

Table 1

Hand dominance and baseline measures of excitability in the two age groups (mean ± SD).

	Young (n=24)	Senior (n=31)
Hand Dominance (L/R)	2/22	1/30
Resting MT (% output)	66.00 ± 11.55	72.55 ± 12.71
Test MT (% output)	79.17 ± 13.82	86.97 ± 15.15
Resting MEP amplitude (μV)	926.61 ± 774.34	427.22 ± 540.59*
Resting MEP latency (ms)	22.27 ± 1.88	24.03 ± 1.87*
Intensity MNS[1]	64.17 ± 1.80	72.87 ± 1.72

Key: MEP, motor evoked potential; MNS, median nerve stimulation; MT, motor threshold.

[1] Conditioning intensity for median nerve stimulation (MNS)

*Significant difference at adjusted *p*-values (p=0.0125) for multiple comparisons (see (Young-Bernier et al., 2012) for a more elaborate analysis of these age differences).

Table 2

Cognitive performance in the two age groups (mean ± SD)

	Young Adults (n = 24)	Older Adults (n = 31)
Logical Memory I	30.46 ± 4.04	29.00 ± 6.77
Visual Paired Associates II	6.00 ± 0.00	5.50 ± 0.77 **
Verbal Paired Associates I	26.63 ± 5.59	19.00 ± 7.84 **
Faces I	38.71 ± 4.31	34.67 ± 3.34 **
CVLT-II Long-Delay Cued Recall[1]	13.67 ± 1.81	12.39 ± 2.70
Verbal Fluency (FAS) Test	40.25 ± 9.81	41.00 ± 12.20
Backward Digit Span	7.67 ± 2.67	7.42 ± 2.80
Wisconsin Card Sorting Test	4.25 ± 0.85	2.83 ± 1.51 **
Mental Control	27.13 ± 4.74	26.39 ± 4.10
Memory factor (*z* score)	0.39 ± 0.39	-0.31 ± 0.68 ***
Executive function factor (*z* score)	0.16 ± 0.50	-0.12 ± 0.71

[1] California Verbal Learning Test-II

**Significant difference at adjusted p-values (p=0.006) for multiple comparisons

*** $p < 0.001$

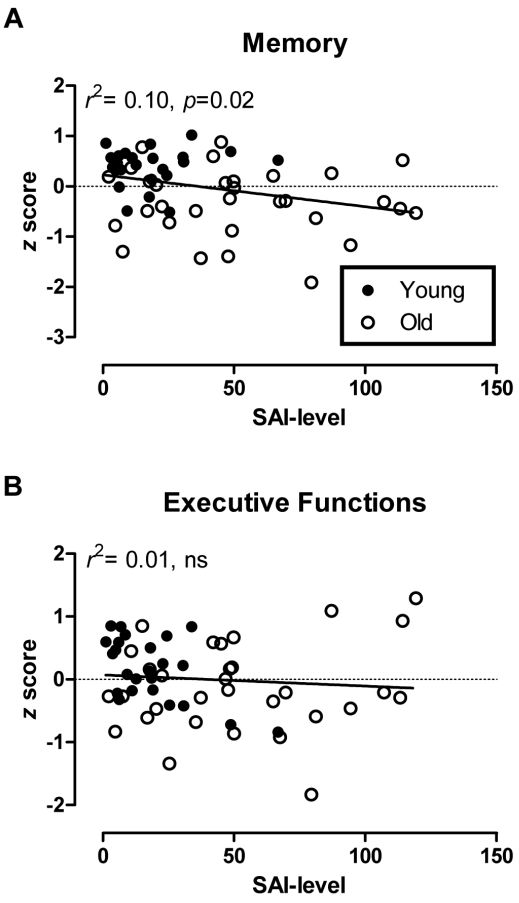

Figure 1. Scatter plots showing the associations between SAI levels and composite z scores of (A) memory and (B) executive functions. SAI levels correspond to the modulation of motor evoked potentials (MEP) induced by afferent conditioning at an inter-stimulus interval (ISI) of 20 ms (% Conditioned MEP / Unconditioned MEP).

Age differences in reaction times and in a neurophysiological marker of cholinergic activity

Marielle Young-Bernier, Annick Tanguay, François Tremblay and Patrick Davidson

Manuscript submitted to the Canadian Journal on Aging.

Author's contributions: MYB participated in the design of the study, carried out the data collection, analyzed the data, and drafted the manuscript. AT participated in the collection and analysis of data. FT participated in the design of the study, aided with data collection and in editing the manuscript. PD participated in the design of the study and in drafting and editing the manuscript.

Abstract

The deterioration of the cholinergic system in aging is hypothesized to contribute to age-related declines in attention. We investigated potential age differences in performance on the Attention Network Test (ANT) and intra-individual variability in speed (RT-IIV) on go/no-go and choice reaction time tasks in young and healthy older adults. We also asked whether short-latency afferent inhibition (SAI), a neurophysiological marker of central cholinergic activity obtained via transcranial magnetic stimulation, might be correlated with performance. *Results:* Older adults were slower on the ANT and exhibited greater RT-IIV than young adults on the choice RT task, but there were no age differences on the ANT network scores (alerting, orienting, and executive control). SAI was diminished in older adults, but it was not significantly correlated with performance. *Conclusion:* It may only be in cases of severe cholinergic dysfunction that relations with attention emerge. Other brain mechanisms may also be stronger predictors of these attentional functions.

1. Introduction

The cholinergic system supports many cognitive functions, including aspects of attention, likely at least in part through its role in facilitating the detection of relevant sensory cues while filtering out irrelevant information (i.e., increasing the signal-to-noise ratio; Furey, 2011; Hasselmo & Sarter, 2011; Sarter, Hasselmo, Bruno, & Givens, 2005). As such, the deterioration of the cholinergic system in aging (e.g., Dumas & Newhouse, 2011) is hypothesized to be associated with declines in attention.

The Attention Network Test (ANT; Fan, McCandliss, Sommer, Raz, & Posner, 2002) has been used widely to study three putative attention networks: The *alerting* network underlies the maintenance of an alert state, the *orienting* network involves the selection of sensory information to guide attention to particular stimuli, whereas the *executive control* network corresponds to the detection and resolution of conflicts. Though interactions between these networks have been described (e.g., Wang et al., 2014), they may rely on at least partially dissociable neuroanatomical networks and neurotransmitter systems (Fan, McCandliss, Fossella, Flombaum, & Posner, 2005; Petersen & Posner, 2012). A highly-cited study with rhesus monkeys using a task similar to the ANT suggested that the cholinergic system might be especially important for orienting (Davidson & Marrocco, 2000). However, evidence from human experiments only partially supports this suggestion, as the administration of cholinergic antagonists reduces brain activity in regions supporting the orienting and executive control networks but only disrupts performance on the latter (Thienel, Kellermann et al., 2009; Thienel, Voss et al., 2009). Additionally, administration of nicotine (a cholinergic agonist) to non-smokers appears not to affect alerting or executive control on the ANT, but may paradoxically have mild detrimental effects on orienting (Kleykamp, Jennings, Blank, &

Eissenberg, 2005; Wignall & de Wit, 2011). In contrast, effects of normal aging on the ANT have been found often in alerting, rarely in orienting, and inconsistently in executive control (Gamboz, Zamarian, & Cavallero, 2010; Jennings, Dagenbach, Engle, & Funke, 2007; Knight & Mather, 2013; Mahoney, Verghese, Goldin, Lipton, & Holtzer, 2010; Westlye, Grydeland, Walhovd, & Fjell, 2011; Zhou, Fan, Lee, Wang, & Wang, 2011). A similar (but more extreme) pattern of deficits on the ANT has been described in patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD), a cholinergic form of dementia (Fernandez-Duque & Black, 2006; Martella et al., 2014; Van Dam et al., 2013). MCI patients with vascular damage, likely involving decreased cholinergic integrity, have also been reported to exhibit reduced orienting on the ANT (Fernandez et al., 2011).

Attentional control can also be examined through intra-individual variability in reaction times (RT-IIV; MacDonald, Li, & Backman, 2009). RT-IIV appears to increase in normal aging, and increases further still in MCI and AD (Duchek et al., 2009; Dykiert, Der, Starr, & Deary, 2012; Phillips, Rogers, Haworth, Bayer, & Tales, 2013). This increased variability has been ascribed in part to changes in neurotransmission at the cortical level, with dopamine having garnered the most interest (MacDonald et al., 2009). However, given acetylcholine's (ACh) role in increasing the signal-to-noise ratio, the deterioration of the cholinergic system in aging has also been hypothesized to contribute to the greater variability seen in normal aging and may well underlie the additional declines seen in MCI and AD (MacDonald, Nyberg, & Backman, 2006).

1.1 The present study

Here, we examined performance on three attentional tasks in young and older adults. On the ANT, for the reasons outlined above, we expected age differences in the alerting and possibly also the executive control network scores. On the two other RT tasks (a 4-choice RT and a go/no-go task), we predicted that older adults would display greater RT-IIV than their younger counterparts, especially on the former due to its greater difficulty (Dykiert et al., 2012).

We also examined the relationship between performance on the behavioral tests and a marker of central cholinergic activity derived from transcranial magnetic stimulation (TMS). When paired with afferent nerve stimulation at an interval of approximately 20 ms (Tokimura et al., 2000), TMS of the motor cortex leads to the inhibition of the motor evoked potential (MEP) which is referred to as *short-latency afferent inhibition* (SAI). The level of afferent inhibition, as reflected in the modulation of MEP amplitudes, is dependent on cholinergic modulation of intra-cortical excitability in the motor cortex through the activation of $GABA_A$ receptors (Di Lazzaro et al., 2002). We have previously demonstrated that SAI is significantly reduced in normal aging when MEPs are evoked using a constant intensity approach (Young-Bernier, Davidson, & Tremblay, 2012). In addition, we found that age-related variations in SAI level were more strongly associated with differences in memory and motor speed (RTs) than with executive functions (Young-Bernier, Davidson et al., 2012; Young-Bernier, Kamil, Tremblay, & Davidson, 2012). However, few studies have examined the relationship between SAI and attentional processes in normal aging. As outlined above, the literature is discordant as to the specific role(s) of the cholinergic system in alerting, orienting, and executive control. We asked whether central cholinergic activity, as indexed by SAI,

would be associated with any of the three attention networks from the ANT (i.e., alerting, orienting, and executive control). Given ACh's possible role in modulating intra-individual variability in speeded performance (MacDonald et al., 2006), it seemed reasonable to predict that SAI would be associated with RT-IIV.

2. Methods

2.1 Participants

Thirty-three young (22.4 ± 3.2 years; 20 females) and 31 healthy older adults (70.2 ± 4.9 years; 18 females) were recruited for this study. The sample size was determined to allow detection of large-size effects (1-β≈0.80) in age-group differences in both SAI (Young-Bernier, Davidson et al., 2012) and behavioral performance on the ANT (e.g., Jennings et al., 2007; Zhou et al., 2011). Estimated power to detect large correlations (ρ= 0.5) between the behavioral and TMS data for n=31 was 0.84. Prior to participation, subjects were screened for psychiatric or neurological disorders and contraindications to TMS. Older adults were also screened with the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005). Although some seniors scored below the recommended cutoff of 26 points (n=10, MoCA scores of 23-25), they were deemed eligible for the study based on the interview and published evidence that this cutoff may be too high (Rossetti, Lacritz, Cullum, & Weiner, 2011). Participant's medication schedules were not altered for testing, with many older adults taking drugs related to vascular health (i.e., hypertension, statins cholesterol lowering drugs). None of the participants were taking neuroactive drugs such as neuroleptics, however one young and one older adult were taking antidepressants but their TMS data was within normal limits. Results from five additional young and one older adult were discarded due to incomplete

data. The Research Ethics Boards of the University of Ottawa and the Bruyère Research Institute approved the study procedure in accordance with the principles of the Declaration of Helsinki. Participants provided written consent and received a minimal honorarium for their participation.

2.2 ANT testing

Participants completed the Attention Network Test (ANT; JAVA version) on a 17-inch color computer screen following the parameters described by Fan et al. (2002). Participants were instructed to quickly indicate the direction (right or left) of a target arrow on the screen. The target was either preceded by no cue or a central, double or spatial cue (i.e., asterix). On some trials, the target arrow was flanked between 4 arrows that were pointing in the same (congruent) or opposite (incongruent) direction. Each participant completed a practice session of 24 trials (with auditory feedback on performance) followed by 3 blocks of 96 trials (no feedback).

Median RTs on the valid trials were averaged across the 3 blocks to reduce the effect of outliers and inherent skew in RTs. Performance on the ANT was examined using both the median RTs for each cue and flanker conditions and the attention network scores. With regards to these scores, we followed the methodology recently suggested by Wang et al. (2014) to compute "purer" attention network scores and diminish the possible influence of irrelevant cues or flankers and inter-network interactions when calculating network scores. This method also accounts for slowing with age by adjusting each score by the relevant baseline RT. The ANT networks scores (expressed in terms of percentage) were thus computed as follows with the median RTs:

$$\text{Alerting} = (RT_{\text{double-cue congruent}} - RT_{\text{no-cue congruent}}) / RT_{\text{no-cue congruent}}$$

$$\text{Orienting} = (RT_{\text{spatial-cue congruent}} - RT_{\text{center-cue congruent}}) / RT_{\text{center-cue congruent}}$$

$$\text{Executive control} = (RT_{\text{no-cue incongruent}} - RT_{\text{no-cue congruent}}) / RT_{\text{no-cue congruent}}$$

2.3 RT testing

Participants also completed 20 trials each of a go/no-go and 4-choice RT task on a MOART panel (Lafayette Instrument, Co.). For go/no-go, participants were asked to release a key with their dominant index finger in response to a go-cue (green light) but withhold responses to a no-go cue (red light). In the choice RT task, they were instructed to place four fingers (index and middle finger of both hands) over keys and to release the appropriate key in response to a go-cue that appeared on top of one finger. Performance was derived from the PsymSoft II software (Lafayette Instrument, Co.). For the go/no-go task, mean RTs and standard deviations were computed for each participant by averaging their performance on correct go-cue trials (mean number of correct go trials = 15 ± 2; accuracy for go and no-go trials = 97%). Similarly, only correct trials on the choice RT task (mean number of correct trials = 17.7 ± 1.6, accuracy = 89%) were used to compute the mean RT and standard deviation values for each participant. Coefficients of variation were also derived for each participant (CV = SD/mean RT) to examine IIV while adjusting for general slowing with age.

2.4 TMS and Short Afferent Inhibition (SAI)

The procedures used for TMS and measurement of SAI are similar to the ones described in our previous report (Young-Bernier, Davidson et al., 2012). In this study, magnetic stimulation was delivered with a Magstim 200 stimulator (Magstim Co. Dyfed,

UK) connected to a focal coil (70-mm loop diameter). The coil was manually held in place by the same experimenter (FT) for all participants and MEPs were recorded in the first dorsal interrosseus muscle (FDI) using small auto-adhesive surface electrodes (Ag/AgCl, Kendall Medi-Trace™ 130). Electromyographic signals were amplified (AB-621G Bioelectric amplifier, Nihon-Kohden Corp., Irvine, CA, USA), digitized at a rate of 2 kHz (BNC-2090, National Instrument Corp., Austin, TX, USA) and further relayed to a laboratory computer running custom software to control acquisition. The resting motor threshold (RMT) was determined using the maximum likelihood strategy for estimating motor thresholds (Awiszus, 2003) with the Motor Threshold Assessment Tool 2.0 software (available at http://www.clinicalresearch.org/sorfware.htm). Test TMS intensity was fixed at 120% RMT for both unconditioned and conditioned trials. Conditioning afferent stimulation was produced by applying 200 μs electrical pulses (S88 Stimulator, Grass Technologies, Astro-Med, Inc, West Warwick, RI 02893 U.S.A.) on the median nerve 20 ms before TMS at an intensity just above the motor threshold to evoke a minimal visible twitch of the thenar muscles (Di Lazzaro et al., 2000; Tokimura et al., 2000). Unconditioned MEP amplitude in the FDI was first determined for each participant by eliciting 15 MEPs at rest (120% RMT). Following the same procedure, 15 MEPs were elicited at the conditioned 20 ms interval. Mean individual values for conditioned and unconditioned MEP responses were measured off-line by averaging the amplitude of each trial. SAI was expressed as percent of unconditioned responses (i.e., $MEP_{Conditioned} / MEP_{Unconditioned} \times 100$).

2.5 Statistical analysis

Mixed analysis of variance (ANOVA) and independent *t*-tests were used to examine age group differences in SAI levels and cognitive performance. Pearson's correlations were used to examine associations between SAI levels and performance on the ANT and RT tasks. Statistical significance was set at p=0.05. Statistical analyses were performed with SPSS (Chicago, IL, USA) and we used G*Power for power analyses (Faul, Erdfelder, Lang, & Buchner, 2007). Figures were prepared GraphPad Prism (San Diego, CA, USA).

3. Results

3.1 Age differences in performance on the ANT

Mean performance on the ANT and RT tasks is shown in Table 1. Mean accuracy was very high on the ANT (98%). A mixed 2 (young vs. older adults) x 4 (cue type) x 3 (flanker type) ANOVA on median RTs from the ANT revealed main effects of group, cue, and flanker type (p<0.001). There was a significant flanker by group interaction ($F_{2, 124}$ = 25.09, p<0.001) and cue by flanker interaction ($F_{6, 372}$ = 13.71, p<0.001; see below). The cue by group interaction ($F_{3, 186}$=1.82, p>0.05) and the three-way interaction between cue, flanker, and group ($F_{6, 372}$=1.27, p>0.05) were not significant. The group by flanker interaction was still significant when including only congruent and incongruent flankers ($F_{1, 62}$=7.05, p=0.01) and was explained by a slightly stronger effect of incongruent flankers for older adults (η_P^2 =0.93) compared to young adults (η_P^2 =0.90). Main effects of cue and flanker and cue by flanker interactions were still present when both age groups were examined individually (p<0.001).

To further explore the cue by flanker interaction, we performed two 2 x 2 ANOVAs to examine the influence of alerting (no cue versus double cue) and orienting (center versus spatial cue) on the flankers (congruent versus incongruent flanker). Both ANOVAs showed main effects of cue type ($F_{1, 63}$>29.48, p<0.01) and flanker type ($F_{1, 63}$>455.58, p<0.01). Not surprisingly, RTs were faster with the double cues and spatial cues than when no cues were provided, while performance was slower with incongruent than congruent flankers. More specifically, the greatest benefit from the alerting cue was present when flankers were congruent and conflict resolution was therefore not required (Cohen's d=2.91) than with incongruent flankers (Cohen's d=0.84). In contrast, the spatial cue was of most benefit when the flankers were incongruent (Cohen's d=5.61) than congruent (Cohen's d=3.72; for similar results, see Gamboz et al., 2010; Jennings et al., 2007).

Age differences in ANT network scores were examined using the traditional computation method (Fan et al., 2002). This method revealed significant age-group differences for the orienting and executive control effect, with older adults showing greater benefit from the spatial cue (t_{62}=2.22, p=0.03) but being more slowed by incongruent flankers than young adults (t_{62}=2.66, p=0.01). There were no age differences on the alerting network. However, a different pattern of results emerged when RTs were adjusted for relevant baseline to account for slowing with age (Westlye et al., 2011). In this case, we found significant age-group differences for alerting and executive control network scores with older adults showing less benefit from alerting cues (t_{62}=2.05, p=0.04) but, surprisingly, being less affected by incongruent flankers than young adults

(t_{62}=2.13, p=0.04). There were no age differences on the orienting network. (Additional information on these analyses can be made available upon request.)

As discussed in the Methods section, to further examine age differences in performance, we followed the methodology proposed by Wang et al. (2014) because it reduces the possible influence of inter-network interactions when calculating the ANT network scores. Thus, three additional mixed ANOVAs (2 x 2) were performed on the median RTs to examine the effect of age (young versus older adults) on the relevant cue/flanker conditions for each attention network (i.e., alerting: $RT_{double\text{-}cue\ congruent}$ and $RT_{no\text{-}cue\ congruent}$; orienting: $RT_{spatial\text{-}cue\ congruent}$ and $RT_{center\text{-}cue\ congruent}$; executive control : $RT_{no\text{-}cue\ incongruent}$ and $RT_{no\text{-}cue\ congruent}$). All these ANOVAs revealed main effects of age group and cue or flanker type, but group by cue/flanker interactions were non-significant (p>0.09). With regards to the network scores (again, computed according to Wang et al., 2014), *t*-tests revealed no age-group differences when performance was adjusted for relevant baseline RTs to account for slowing with age (p>0.11).

3.2 Age differences in performance on the RT tasks

Regarding the RT tasks, young adults were significantly faster than the older adults and they exhibited less intra-individual variability on the choice task than the older adults (t_{62}=*-3.46, p*=0.001). Go/no-go mean-adjusted RT-IIV was similar across both groups.

3.3 TMS and SAI

Data from one young adult were excluded from all analysis involving SAI due to abnormally poor MEP inhibition in response to afferent conditioning when compared to

the other young adults (Grubb's test, $p<0.01$, $z=2.95$). Afferent conditioning led to a marked inhibition of MEP responses in young adults whereas older adults exhibited more variable responses, with many showing poor or even absent inhibition. Accordingly, SAI was significantly reduced in the older group compared to the young ($t_{61}=-3.78$, $p=0.001$; see Table 1). Further analysis revealed that about half (17/31) of the participants in the older group exhibited abnormally low levels of SAI, corresponding to ≥ 2SD from the mean level observed in the young group (i.e., 46.7%). There were no age differences between the older adults displaying "abnormally" low level of SAI (n=17) and the seniors with "normal" SAI levels (i.e. within 2SD from the mean in young; $t_{29}=1.40$, $p=0.17$).

3.4 Correlation between SAI and performance

When we performed an analysis across all individuals, SAI was significantly correlated with intra-individual variability in choice RTs ($r^2=0.10$, $p=0.01$) and was marginally associated with mean speed on this task ($r^2=0.05$, $p=0.08$). However, when we examined these correlations separately within each age group, they failed to obtain significance. SAI was also only weakly associated with performance on the three ANT (computed according to Wang et al., 2014) network scores and individual variability on the go/no-go RT task within both age groups ($r<0.30$, $p>0.05$; Figure 1).

We conducted an exploratory follow-up analysis to explore the possibility that only those seniors with "abnormal" SAI levels would be different from the young, but three-group between-subjects ANOVAs examining performance on the ANT and RT-IIV in young adults, older adults with "abnormal" SAI levels (≥2SD SAI young adults; n=17) and seniors with more efficient afferent inhibition (<2SD SAI young adults; n=14) did not reveal differences in performance over and above age-related changes on most tasks.

Having said this, we did note that on choice RT-IIV, although the older adults with "normal" (Coefficient of variation; CV M =0.28±0.13) and "abnormal" SAI (CV M =0.31±0.08) did not differ from one another ($t<1$, $p>0.05$, Cohen's d=0.28), the seniors with "abnormal" SAI were further away from the young adults (CV M =0.22±0.09, Cohen's d = 1.06) on this score than were the seniors with "normal" SAI (Cohen's d = 0.54; Figure 2).

4. Discussion

Older adults were slower than young adults on all three tasks and exhibited greater RT-IIV than young adults on the choice RT task (although not on go/no-go). The latter finding is consistent with the suggestion of Dykiert et al. (2012) that older adults appear to have greater difficulty sustaining their attention when tasks are relatively more demanding.

We also replicated our previous finding of reduced SAI in the older adults compared to the young in a largely independent sample (Young-Bernier, Davidson et al., 2012), consistent with myriad other evidence of cholinergic decline in normal aging (e.g., Duzel et al., 2010; Grothe, Heinsen, & Teipel, 2012). However, there were no significant group differences on the ANT network scores after adjusting for general slowing with age (scores computed according to Wang et al., 2014), suggesting that at least some healthy older adults can benefit from simple environmental cues to the same level as young adults to improve their attention functions and that they have efficient executive and inhibitory control. This result is in contrast to previous reports of age-effects on alerting and executive control on the ANT (Gamboz et al., 2010; Jennings et al., 2007; Knight & Mather, 2013; Mahoney et al., 2010; Westlye et al., 2011; Zhou et al., 2011)

and may be attributed to our use of a computation method that reduced the possible influence of irrelevant cues or flankers and of inter-network interactions on the network scores (Wang et al., 2014). [Indeed, we found that age differences on the network scores are largely dependent upon the type of computation method used. When we followed the original method (Fan et al., 2002), we found that older adults benefited more from orienting cues (surprisingly) than young adults but their performance was more affected by incongruent cues. However, when these network scores were adjusted for slowing with age (Westlye et al., 2011), we found a significantly reduced alerting effect, but paradoxically increased executive control in older adults compared to young adults]. The absence of age differences in orienting on the ANT has been described consistently, but, given that aging involves clear deterioration of the cholinergic system (as evidenced in the present study by the age-related difference in SAI levels, commensurate with myriad other data; e.g., Duzel et al., 2010; Grothe et al., 2012), this observation is seemingly at odds with the idea that the cholinergic system influences orienting abilities (for further discussion, see below).

Overall, the parallel age differences in SAI, ANT reaction times, and RT-IIV could be taken as evidence that cholinergic declines underlie older adults' reduced attentional functions. However, the case would have been stronger if we had found the expected correlations between SAI and performance within our older adults. This may have to do with statistical power. Our sample sizes were sufficient to detect the large age differences we observed in SAI, in RT-IIV on the choice task, and in RTs on the ANT (i.e., Cohen's *d* ranging from 0.84 to 2.97) and to detect large correlations between the neurophysiological and behavioral data. More subtle relationships, if indeed they exist,

might be uncovered with more high-powered studies. The low to moderate reliability of the ANT network scores (Macleod et al., 2010) might also have impacted power and our ability to find age-related differences on this task or associations with SAI. As such, including a larger number of ANT trials to increase the reliability of the scores derived from this measure might be a direction for future studies (Ishigami & Klein, 2010). Similarly, increasing the number of RT trials could allow for more reliable measures of IIV, though previous studies have also examined RT IIV with comparable number of trials to the tasks included here (e.g., Finkel & McGue, 2007; Hultsch, MacDonald, & Dixon, 2002).

Alternatively, a certain threshold in SAI may need to be reached before declines in cholinergic efficiency translate to detectable changes at the behavioral level (e.g., Robbins et al., 1997; Schliebs & Arendt, 2006). For example, although healthy older adults and even MCI patients are relatively unimpaired on the orienting index from the ANT, Fernandez and colleagues reported that MCI patients who also have significant subcortical vascular damage (likely involving decreased cholinergic integrity) exhibit impaired orienting on the ANT (Fernandez et al., 2011). It is therefore possible that we would have found stronger relationships between SAI and performance if we had included more older adults with substantial cholinergic declines (including patients with MCI and early AD). To add a further level of complexity to the possible association between SAI and cognitive performance, Nardone et al. (2012) recently showed that although SAI levels are correlated with performance on measures of attention/executive functions and verbal memory in patients with MCI, this marker of cholinergic activity is only reduced in patients with amnesic multi-domain MCI but not in other MCI subtypes

(i.e., single domain or non-amnesic multi-domain MCI). These results suggest that the disease process may need to be more advance for SAI levels to be further reduced in patients with MCI when compared with healthy controls. Longitudinal studies in older adults and patients with MCI and AD will allow for a better understanding of the complex relationship between cholinergic and cognitive functions.

Another possibility is that the tasks used in this study may not have sufficiently taxed attentional resources to show strong associations between SAI and declining performance with age (Dumas & Newhouse, 2011; Sarter et al., 2005). Increasing the cognitive load, for example by including a divided attention component (Sarter & Turchi, 2002), may be a direction for future studies. Finally, declines in SAI levels may need to be associated with additional age-related changes, including microvascular damage and/or reduced dopaminergic activity, to show a relationship with attention (Backman, Nyberg, Lindenberger, Li, & Farde, 2006; Fernandez et al., 2011; MacDonald et al., 2009). In future studies, combining SAI, as a non-invasive marker of cholinergic activity, with neuroimaging and other methods, including perhaps EEG markers of dopaminergic activity (Lackner, Bowman, & Sabbagh, 2010), might provide a better understanding of how various anatomical, physiological, and neurotransmission changes interact to influence cognitive performance in normal aging.

Acknowledgements: We thank our participants for their time and patience during testing, and Francisca Avila Ramirez and Travis Davidson for their help with data collection. This work will serve as a partial fulfillment for a doctoral thesis in clinical psychology by MYB. This work was supported by a student award from Fonds de Recherche du Québec – Santé to MYB, a Research Development Grant from the Faculty of Social Sciences of

the University of Ottawa to FT and PD, and a Discovery grant from the Natural Sciences and Engineering Research Council of Canada to PD.

References

Awiszus, F. (2003). TMS and threshold hunting. *Suppl Clin Neurophysiol, 56*, 13-23.

Backman, L., Nyberg, L., Lindenberger, U., Li, S. C., & Farde, L. (2006). The correlative triad among aging, dopamine, and cognition: current status and future prospects. *Neurosci Biobehav Rev, 30*(6), 791-807.

Davidson, M. C., & Marrocco, R. T. (2000). Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. *J Neurophysiol, 83*(3), 1536-1549.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M. A., Di Giovanni, S., & Zito, G. (2000). Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. *Exp Brain Res, 135*(4), 455-461.

Di Lazzaro, V., Oliviero, A., Tonali, P. A., Marra, C., Daniele, A., & Profice, P. (2002). Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology, 59*(3), 392-397.

Duchek, J. M., Balota, D. A., Tse, C. S., Holtzman, D. M., Fagan, A. M., & Goate, A. M. (2009). The utility of intraindividual variability in selective attention tasks as an early marker for Alzheimer's disease. *Neuropsychology, 23*(6), 746-758.

Dumas, J. A., & Newhouse, P. A. (2011). The cholinergic hypothesis of cognitive aging revisited again: Cholinergic functional compensation. *Pharmacol Biochem Behav, 99*(2), 254-261.

Duzel, S., Munte, T. F., Lindenberger, U., Bunzeck, N., Schutze, H., & Heinze, H. J. (2010). Basal forebrain integrity and cognitive memory profile in healthy aging. *Brain Res, 1308*, 124-136.

Dykiert, D., Der, G., Starr, J. M., & Deary, I. J. (2012). Age differences in intra-individual variability in simple and choice reaction time: systematic review and meta-analysis. *PLoS One, 7*(10), e45759.

Fan, J., McCandliss, B. D., Fossella, J., Flombaum, J. I., & Posner, M. I. (2005). The activation of attentional networks. *Neuroimage, 26*(2), 471-479.

Fan, J., McCandliss, B. D., Sommer, T., Raz, A., & Posner, M. I. (2002). Testing the efficiency and independence of attentional networks. *J Cogn Neurosci, 14*(3), 340-347.

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods, 39*(2), 175-191.

Fernandez, P. J., Campoy, G., Garcia Santos, J. M., Antequera, M. M., Garcia-Sevilla, J., & Castillo, A. (2011). Is there a specific pattern of attention deficit in mild cognitive impairment with subcortical vascular features? Evidence from the Attention Network Test. *Dement Geriatr Cogn Disord, 31*(4), 268-275.

Fernandez-Duque, D., & Black, S. E. (2006). Attentional networks in normal aging and Alzheimer's disease. *Neuropsychology, 20*(2), 133-143.

Finkel, D., & McGue, M. (2007). Genetic and environmental influences on intraindividual variability in reaction time. *Exp Aging Res, 33*(1), 13-35.

Furey, M. L. (2011). The prominent role of stimulus processing: cholinergic function and dysfunction in cognition. *Curr Opin Neurol, 24*(4), 364-370.

Gamboz, N., Zamarian, S., & Cavallero, C. (2010). Age-related differences in the attention network test (ANT). *Exp Aging Res, 36*(3), 287-305.

Grothe, M., Heinsen, H., & Teipel, S. J. (2012). Atrophy of the cholinergic Basal forebrain over the adult age range and in early stages of Alzheimer's disease. *Biol Psychiatry, 71*(9), 805-813.

Hasselmo, M. E., & Sarter, M. (2011). Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacol, 36*(1), 52-73.

Hultsch, D. F., MacDonald, S. W., & Dixon, R. A. (2002). Variability in reaction time performance of younger and older adults. *J Gerontol B Psychol Sci Soc Sci, 57*(2), P101-115. doi: 10.1093/geronb/57.2.P101

Ishigami, Y., & Klein, R. M. (2010). Repeated measurement of the components of attention using two versions of the Attention Network Test (ANT): stability, isolability, robustness, and reliability. *J Neurosci Methods, 190*(1), 117-128.

Jennings, J. M., Dagenbach, D., Engle, C. M., & Funke, L. J. (2007). Age-related changes and the attention network task: an examination of alerting, orienting, and executive function. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn, 14*(4), 353-369.

Kleykamp, B. A., Jennings, J. M., Blank, M. D., & Eissenberg, T. (2005). The effects of nicotine on attention and working memory in never-smokers. *Psychol Addict Behav, 19*(4), 433-438.

Knight, M., & Mather, M. (2013). Look out-it's your off-peak time of day! Time of day matters more for alerting than for orienting or executive attention. *Exp Aging Res, 39*(3), 305-321.

Lackner, C. L., Bowman, L. C., & Sabbagh, M. A. (2010). Dopaminergic functioning and preschoolers' theory of mind. *Neuropsychologia, 48*(6), 1767-1774.

MacDonald, S. W., Li, S. C., & Backman, L. (2009). Neural underpinnings of within-person variability in cognitive functioning. *Psychol Aging, 24*(4), 792-808.

MacDonald, S. W., Nyberg, L., & Backman, L. (2006). Intra-individual variability in behavior: links to brain structure, neurotransmission and neuronal activity. *Trends Neurosci, 29*(8), 474-480.

Macleod, J. W., Lawrence, M. A., McConnell, M. M., Eskes, G. A., Klein, R. M., & Shore, D. I. (2010). Appraising the ANT: Psychometric and theoretical considerations of the Attention Network Test. *Neuropsychology, 24*(5), 637-651.

Mahoney, J. R., Verghese, J., Goldin, Y., Lipton, R., & Holtzer, R. (2010). Alerting, orienting, and executive attention in older adults. *J Int Neuropsychol Soc, 16*(5), 877-889.

Martella, D., Manzanares, S., Campoy, G., Roca, J., Antunez, C., & Fuentes, L. J. (2014). Phasic and tonic alerting in mild cognitive impairment: A preliminary study. *Exp Gerontol, 49*, 35-39.

Nardone, R., Bergmann, J., Christova, M., Caleri, F., Tezzon, F., Ladurner, G. (2012). Short latency afferent inhibition differs among the subtypes of mild cognitive impairment. *J Neural Transm, 119*(4), 463-471.

Nasreddine, Z. S., Phillips, N. A., Bedirian, V., Charbonneau, S., Whitehead, V., & Collin, I. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society, 53*(4), 695-699.

Petersen, S. E., & Posner, M. I. (2012). The attention system of the human brain: 20 years after. *Annu Rev Neurosci, 35*, 73-89.

Phillips, M., Rogers, P., Haworth, J., Bayer, A., & Tales, A. (2013). Intra-individual reaction time variability in mild cognitive impairment and Alzheimer's disease: gender, processing load and speed factors. *PLoS One, 8*(6), e65712.

Robbins, T. W., Semple, J., Kumar, R., Truman, M. I., Shorter, J., & Ferraro, A. (1997). Effects of scopolamine on delayed-matching-to-sample and paired associates tests of visual memory and learning in human subjects: comparison with diazepam and implications for dementia. *Psychopharmacol, 134*(1), 95-106.

Rossetti, H. C., Lacritz, L. H., Cullum, C. M., & Weiner, M. F. (2011). Normative data for the Montreal Cognitive Assessment (MoCA) in a population-based sample. *Neurology, 77*(13), 1272-1275.

Sarter, M., Hasselmo, M. E., Bruno, J. P., & Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Rev, 48*(1), 98-111.

Sarter, M., & Turchi, J. (2002). Age- and dementia-associated impairments in divided attention: psychological constructs, animal models, and underlying neuronal mechanisms. *Dement Geriatr Cogn Disord, 13*(1), 46-58.

Schliebs, R., & Arendt, T. (2006). The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. *J Neural Transm, 113*(11), 1625-1644.

Thienel, R., Kellermann, T., Schall, U., Voss, B., Reske, M., & Halfter, S. (2009). Muscarinic antagonist effects on executive control of attention. *Int J Neuropsychopharmacol, 12*(10), 1307-1317.

Thienel, R., Voss, B., Kellermann, T., Reske, M., Halfter, S., & Sheldrick, A. J. (2009). Nicotinic antagonist effects on functional attention networks. *Int J Neuropsychopharmacol, 12*(10), 1295-1305.

Tokimura, H., Di Lazzaro, V., Tokimura, Y., Oliviero, A., Profice, P., & Insola, A. (2000). Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J Physiol, 523 Pt 2*, 503-513.

Van Dam, N. T., Sano, M., Mitsis, E. M., Grossman, H. T., Gu, X., & Park, Y. (2013). Functional neural correlates of attentional deficits in amnestic mild cognitive impairment. *PLoS One, 8*(1), e54035.

Wang, Y. F., Cui, Q., Liu, F., Huo, Y. J., Lu, F. M., & Chen, H. (2014). A New Method for Computing Attention Network Scores and Relationships between Attention Networks. *PLoS One, 9*(3), e89733.

Westlye, L. T., Grydeland, H., Walhovd, K. B., & Fjell, A. M. (2011). Associations between regional cortical thickness and attentional networks as measured by the attention network test. *Cereb Cortex, 21*(2), 345-356.

Wignall, N. D., & de Wit, H. (2011). Effects of nicotine on attention and inhibitory control in healthy nonsmokers. *Exp Clin Psychopharmacol, 19*(3), 183-191.

Young-Bernier, M., Davidson, P.S., & Tremblay, F. (2012). Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition with age. *Neurobiol Aging 33***,** 835.e1-835.e11.

Young-Bernier, M., Kamil, Y., Tremblay, F., & Davidson, P.S. (2012). Associations between a neurophysiological marker of central cholinergic activity and cognitive functions in young and older adults. *Behav Brain Funct, 8*, 17.

Zhou, S. S., Fan, J., Lee, T. M., Wang, C. Q., & Wang, K. (2011). Age-related differences in attentional networks of alerting and executive control in young, middle-aged, and older Chinese adults. *Brain Cogn, 75*(2), 205-210.

Table 1

Mean (standard deviation) short afferent inhibition (SAI) level (% MEPcond/MEPuncond) and performance (ms) on the Attention Network Test and the reaction time tasks in the two age groups.

		Young Adults	**Older Adults**	**Cohen's *d***
Short Afferent Inhibition (SAI)		20.43 (13.12)	74.83 (80.31)	0.95
Attention Network Test				
Flanker	*Cue*			
Congruent	No	556.39 (74.76)	787.10 (86.71)	2.85
	Center	531.06 (82.97)	762.71 (72.56)	2.97
	Double	523.64 (67.55)	751.35 (88.73)	2.89
	Spatial	496.03 (63.22)	711.32 (84.92)	2.88
Incongruent	No	673.30 (111.93)	926.65 (102.23)	2.36
	Center	669.03 (97.29)	927.84 (108.81)	2.51
	Double	659.70 (98.67)	918.87 (107.12)	2.52
	Spatial	603.24 (92.19)	851.39 (106.40)	2.49
Neutral	No	524.67 (63.40)	685.84 (81.43)	2.21
	Center	477.36 (66.98)	665.71 (77.88)	2.59
	Double	474.27 (68.66)	647.32 (79.97)	2.32
	Spatial	452.00 (62.97)	621.06 (78.65)	2.37
Network Effects (% relative to relevant baseline)				
Alerting		-5.73 (4.76)	-4.44 (5.53)	0.25
Orienting		-6.10 (5.62)	-6.78 (5.93)	0.12

Executive Control		20.89 (8.95)	17.85 (5.74)	0.40
Reaction Times Tasks				
Choice	Mean	420.21 (55.19)	582.11 (127.38)	1.65
	Standard deviation	92.87 (44.08)	181.25 (87.79)	1.27
	Coefficient of variation	0.22 (0.09)	0.30 (0.10)	0.84
Go/no-go	Mean	381.02 (88.58)	461.85 (65.95)	1.04
	Standard deviation	76.52 (35.26)	92.20 (33.31)	0.46
	Coefficient of variation	0.20 (0.08)	0.20 (0.06)	0.00

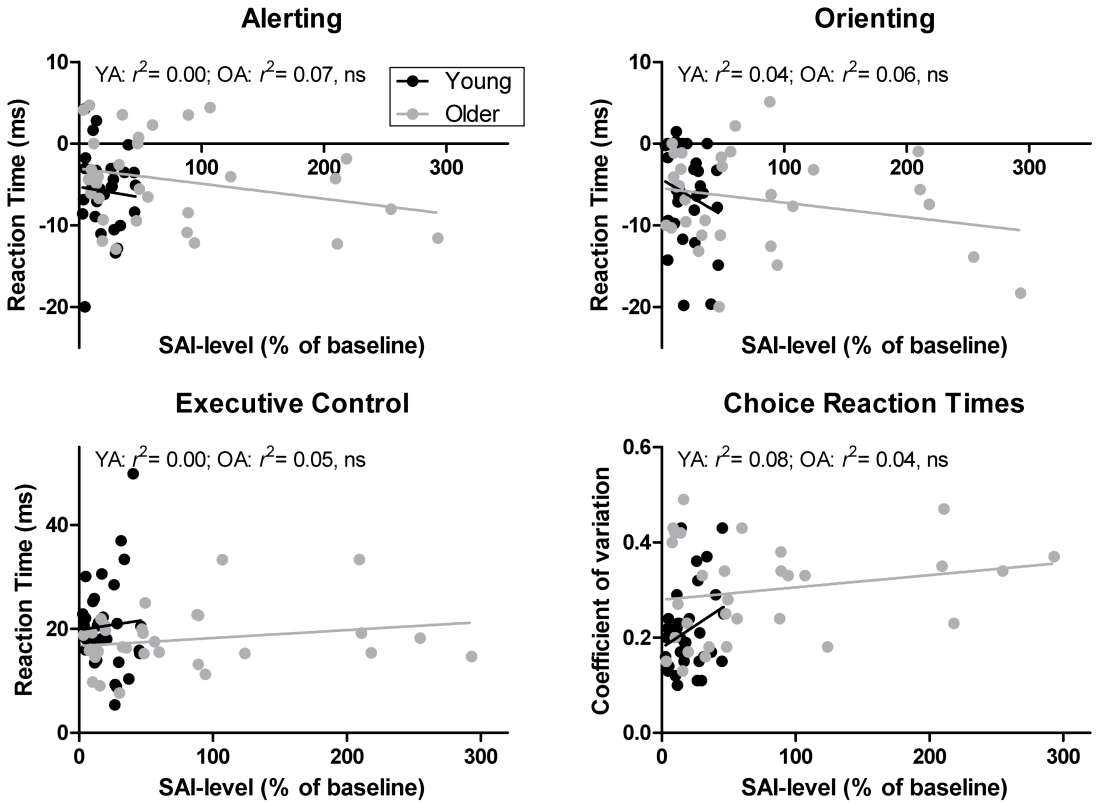

Figure 1. Associations between short-latency afferent inhibition (SAI) and performance on the Attention Network Test (ANT) and intra-individual variability (IIV) on the choice reaction time task in young (YA) and older adults (OA).

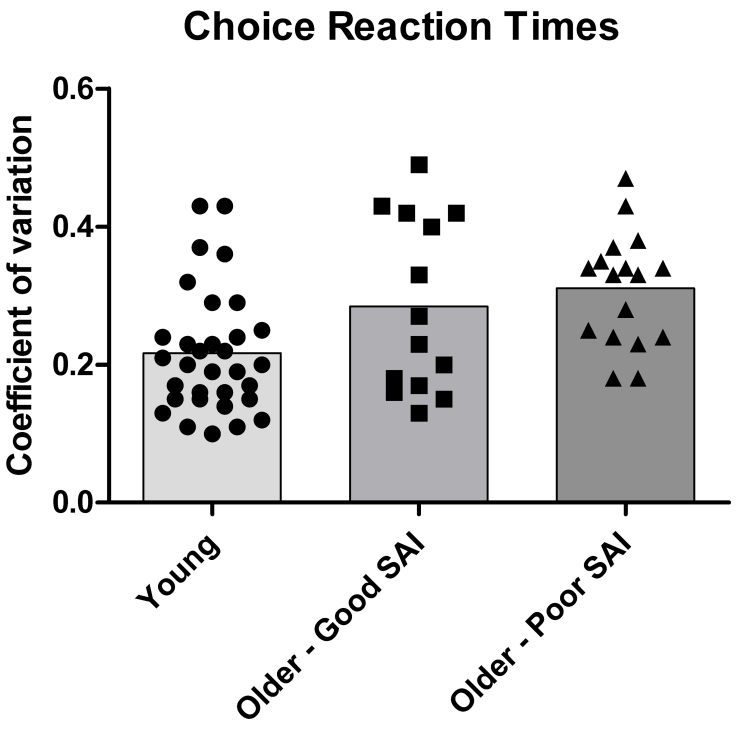

Figure 2. Association between short-latency afferent inhibition (SAI) and variability in choice reaction times in the young and older adults with "normal" (i.e., <2SD SAI young adults) and "abnormal" SAI levels (i.e., ≥2SD SAI young adults).

Short-latency afferent inhibition is a poor predictor of individual susceptibility to rTMS-induced plasticity in the motor cortex of young and older adults.

Marielle Young-Bernier, Annick Tanguay, Patrick Davidson and François Tremblay

Young-Bernier, M., Tanguay, A.N., Davidson, P.S.R. and Tremblay, F. (2014). Short-latency afferent inhibition is a poor predictor of individual susceptibility to rTMS-induced plasticity in the motor cortex of young and older adults. *Frontiers in Aging Neuroscience*, 6:182.

Authors' contributions: MYB participated in the design of the study, carried out the data collection, analyzed the data, and drafted the manuscript. AT participated in the collection and analysis of data. PD participated in the design of the study and editing the manuscript. FT conceived the study, aided with data collection and in drafting and editing the manuscript.

Abstract

Cortical plasticity, including long-term potentiation (LTP)-like plasticity, can be assessed non-invasively with repetitive transcranial magnetic stimulation (rTMS) protocols. In this study, we examined age differences in responses to intermittent theta burst stimulation (iTBS) in a group of 20 young and 18 healthy older adults. Because the cholinergic system plays a role in the neural processes underlying learning and memory, including LTP, we also investigated whether short latency afferent inhibition (SAI), a neurophysiological marker of central cholinergic activity, would be associated with age-related differences in LTP-like plasticity induced by iTBS. *Methods:* SAI was first assessed by examining the modulation of motor evoked potentials (MEPs) in response to median nerve conditioning 20 ms prior to TMS. Participants then underwent iTBS (3 pulses at 50 HZ every 200 ms for 2 s with 8 s between trains, repeated 20 times). MEP responses (120% RMT) were assessed immediately after iTBS and 5, 10, and 20 min post-application. *Results:* Responses to iTBS were quite variable in both age groups, with only approximately 60% of the participants (n=13 young and 10 older adults) showing the expected facilitation of MEP responses. There were no significant age group differences in MEP facilitation following iTBS. Although older adults exhibited reduced SAI, individual variations were not associated with susceptibility to express LTP-like induced plasticity after iTBS. *Conclusion:* Overall, these results are consistent with reports of high inter-individual variability in responses to iTBS. Although SAI was reduced in older adults, consistent with a deterioration of the cholinergic system with age, SAI levels were not associated with LTP-like plasticity as assessed with iTBS.

1.Introduction

Non-invasive transcranial magnetic stimulation (TMS) can be used to explore the neurophysiological mechanisms underlying synaptic plasticity in the human motor cortex through various repetitive protocols (rTMS). One of these protocols, theta burst stimulation (TBS; Huang et al., 2005), has gained particular attention as it is relatively short in duration, uses a low intensity of stimulation, and can induce lasting changes in brain excitability that are very similar to those described in *in vitro* studies in terms of long-term potentiation (LTP) and long-term depression (LTD; Huang et al., 2007; Teo et al., 2007). When applied in an intermittent pattern, TBS (i.e., iTBS) generally leads to the facilitation of motor evoked potentials (MEPs) and induces LTP-like plasticity in the motor cortex.

Initial reports on iTBS revealed robust facilitation of brain excitability (Huang et al., 2005), but considerable inter-individual variability has more recently been described with up to 50% of participants not exhibiting the expected facilitation of MEP responses (e.g., Player et al., 2012; Hamada et al., 2013; Vallence et al., 2013; Hinder et al., 2014; Lopez-Alonso et al., 2014). Factors such as genetics, voluntary motor activity, sex, and physical exercise all contribute to this variability (Ridding & Ziemann, 2010). Of importance to this study, aging has also been associated with a reduced modulation of brain excitability by TBS and other rTMS plasticity-inducing protocols, including paired associative stimulation (PAS; Muller-Dahlhaus et al., 2008; Tecchio et al., 2008; Fathi et al., 2010; Freitas et al., 2011). Only one study has examined age effects on iTBS responses in a small group of participants but, although a slight reduction in LTP-like plasticity with age was described, results were non-significant (Di Lazzaro et al., 2008b).

The deterioration of the cholinergic system in aging is thought to contribute to age-related changes in learning and memory due to the critical role of cholinergic innervations in modulating cortical plasticity and LTP-like processes (Rasmusson, 2000). Pharmacological studies have supported an effect of acetylcholine on responses to plasticity-inducing rTMS protocols. Indeed, cholinergic agonists, such as nicotine and the cholinesterase inhibitor rivastigmine, tend to increase and prolong facilitatory iTBS and PAS effects (Kuo et al., 2007; Swayne et al., 2009; Thirugnanasambandam et al., 2011; but see Korchounov & Ziemann, 2011). In contrast, the administration of a cholinergic antagonist to young adults reduces LTP-like plasticity following PAS (Korchounov & Ziemann, 2011). PAS-induced LTP-like plasticity is also reduced in Alzheimer's disease (AD), which is often considered a model of chronic deficient central cholinergic activity (Battaglia et al., 2007). The effect of chronic age-related changes in cholinergic integrity on responses to iTBS, as opposed to the acute effects of cholinergic agonists and antagonists on acetylcholine's levels in the brain, has not been examined in a healthy population.

Central cholinergic activity can be examined using TMS by applying a contralateral conditioning stimulation to the median nerve 18-20 ms prior to the TMS pulse. This pairing generally leads to the inhibition of MEPs and is called short-latency afferent inhibition (SAI; Di Lazzaro et al., 2000; Tokimura et al., 2000). SAI levels are significantly reduced by scopolamine, a muscarinic cholinergic antagonist, in young healthy adults (Di Lazzaro et al., 2000) and can be improved with acetylcholinesterase inhibitors in patients with AD (Di Lazzaro et al., 2002). Using a constant TMS test

intensity protocol, we have previously shown that SAI is also reduced in normal aging (Young-Bernier et al., 2012; but see Oliviero et al., 2006; Degardin et al., 2011).

In this study, we investigated age-related differences in the modulation of cortical excitability following iTBS in young and healthy older adults. Given the cholinergic system's role in the underlying processes supporting plasticity, we also examined whether SAI levels, as a neurophysiological marker of cholinergic activity, are associated with individual responses to iTBS and could explain part of the inter-individual variability in plasticity-inducing TMS protocols.

2. Methods

2.1 Participants

Young adults (n=20; age range = 22.3 ± 3.2 years; 13 females) and healthy older adults (n=18; age range = 70.1 ± 5.6 years; 9 females) were recruited from the local community. (The participants included in this study are part of a larger cohort; their SAI levels and performance on measures of attention are described elsewhere). Participants were screened for psychiatric or neurological disorder and contraindications to TMS. Older adults also completed the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005) to screen for possible mild cognitive impairment. Although some older adults scored below the recommended cutoff of 26 points (n=4, MoCA scores of 23-25/30), they were deemed eligible for the study based on the interview and evidence that this cutoff may be too high (Rossetti et al., 2011). Participant's medications were not altered for testing, with many older adults taking drugs related to vascular health (i.e., hypertension, statins cholesterol lowering drugs). None of the participants were taking neuroactive drugs such as neuroleptics, however one young adult and one older adult

were taking antidepressants but their TMS data were within normal limits. Data from five additional young adults were not included in this report because they could not complete the TMS assessment for their resting threshold was particularly high making stimulation uncomfortable. The institutional Research Ethics Boards approved this study. Participants provided informed consent and received a minimal honorarium to defray expenses for their participation.

2.2 MEP recordings: Determination of the hotspot and resting motor threshold

MEPs were recorded using small auto-adhesive surface electrodes (Ag/AgCl, Kendall Medi-Trace™ 130) placed over the first dorsal interosseous (FDI) and abductor pollicis brevis (APB) muscles of the right hand in a belly-tendon montage. Electromyographic signals were amplified and filtered with a time constant of 0.03 s and a low-pass filter of 1 kHz (AB-621G Bioelectric amplifier, Nihon-Kohden Corp., CA 92610). Signals were digitized at a rate of 2 kHz (BNC-2090, National Instrument Corp. Austin, TX, USA) and relayed to a laboratory computer running custom software to control acquisition. TMS was administered with participants comfortably seated in a recording chair. Movements of the head were restrained with a U-shape neck cushion. Single pulse magnetic stimulation was delivered via a Magstim 200 stimulator (Magstim Co. Dyfed, UK) connected to a figure-of-eight coil (70-mm loop diameter). The coil was held approximately 45° in the mid-sagittal plane and the approximate location of the hand motor area on the left hemisphere was explored in approximately 1-cm steps until reliable MEPs could be evoked in the target muscle. This site was then marked with a circular sticker to ensure consistent coil positioning. The coil was held in place manually over the hotspot by the same experimenter (FT) for all participants. Following this procedure, the

resting motor threshold (RMT) was determined for both the FDI and APB using the maximum likelihood strategy for estimating motor thresholds (Awiszus, 2003; TMS Motor Threshold Assessment Tool 2.0; Brain Stimulation Laboratory, Medical University of South Caroline, USA, http://www.clinicalresearch.org/sorfware.htm). This method has been shown to produce similar results to Mills and Nithi's method (1997), while requiring a smaller number of stimulations to determine the motor threshold (Mishory et al., 2004).

2.3 Baseline measures of corticospinal excitability

Test TMS intensity was fixed at 120% RMT for both the FDI and APB muscles. Baseline MEP amplitude in the FDI was first determined for each participant by eliciting 15 MEPs at rest. The same procedure was followed for the APB muscle after completion of the SAI protocol. Trials for which unwanted muscle contractions were present were eliminated and repeated.

2.4 Short Afferent Inhibition (SAI)

For SAI, we used a protocol similar to the one described by Tokimura et al. (2000) and Di Lazzaro et al. (2000). Conditioning afferent stimulation consisted in the application of 200 μs electrical pulses (S88 Stimulator, Grass Technologies, Astro-Med, Inc, West Warwick, RI 02893 U.S.A.) on the median nerve at an intensity just above the motor threshold to evoke a minimal visible twitch of the thenar muscles. SAI was induced by applying conditioning afferent stimulation 20 ms before the TMS pulse over the motor cortex (120% RMT_{FDI}). This conditioning interval was shown to be optimal to evoke MEP inhibition in several studies (e.g., Tokimura et al., 2000; Fischer and Orth,

2011). Fifteen MEPs were elicited at the conditioned 20ms interval and recorded from the FDI muscle.

2.5 Intermittent Theta-Burst Stimulation (iTBS)

TBS was delivered via a Magstim Rapid^2 stimulator (Magstim Co. Wales, UK) connected to a figure-of-eight coil (90-mm inside loop diameter). We followed the iTBS procedure described by Huang et al. (2005). The iTBS protocol consisted of three pulses delivered at a frequency of 50Hz (i.e., 1 burst) and applied every 200 ms for 2 s (10 bursts). This was repeated every 10 s for a total duration of 190 s and 600 pulses. The effect of iTBS was assessed by monitoring changes in the APB because some participants had taken part in an earlier pilot study based on the PAS protocol in which the APB was the target muscle. Stimulation intensity was set at 80% APB active motor threshold (AMT). The latter was determined while participants exerted a light tonic contraction by pinching a soft exercise ball between their thumb and index finger (~15-20% maximum contraction). Blocks of 15 MEPs at 120% RMT_{APB} were elicited immediately after and 5, 10, and 20 minutes post-iTBS and recorded from the APB muscle.

2.6 Analysis of MEP data

Mean individual MEP responses for each condition (i.e., baselines, SAI, and post-iTBS intervals) were determined off-line by averaging the amplitude (peak-to-peak) and latency of each trial. Data for SAI and iTBS were expressed as percent of baseline MEP amplitude for the relevant target muscle (i.e., SAI = % $MEP_{Conditioned}$ / $MEP_{Resting\ FDI}$; iTBS response = % $MEP_{Interval\ post\text{-}iTBS}$ / $MEP_{Resting\ APB}$). Based on previous studies (e.g., Hamada et al., 2013; Hinder et al., 2014; Lopez-Alonso et al., 2014), responders to the

iTBS protocol were defined as the individuals who exhibited the expected facilitation of normalized to baseline MEP responses (>100%) when responses across the four post-iTBS time intervals were averaged.

2.7 Statistical methods

Independent *t*-tests, with adjusted *p* values for multiple comparisons (i.e. p=0.01) were used to examine differences in baseline measures of excitability between the two age groups. A repeated measure analysis of variance (ANOVA) was used to detect main effect and interactions with post-iTBS time intervals (0, 5, 10, and 20 minutes) as the within-subjects factor and age group (young vs. older adults) or response to iTBS (responders vs. non-responders) as the between-subjects factor. The Fisher's Exact Test for a 2x2 contingency table was used to examine differences in the number of responders to the iTBS protocol (i.e., mean MEP amplitude post-iTBS > 100%) in each group. We used *t*-tests to examine age group differences in SAI level. Pearson's correlations were used to examine associations among SAI levels and responses to iTBS. Significance level was set at $p = 0.05$. Analyses were performed with SPSS version 21.0 (Chicago, IL, USA) and figures were prepared with GraphPad Prism version 5.00 (San Diego, CA, USA).

3. Results

3.1 Age differences in baseline measures of excitability

Baseline TMS measures of excitability in both age groups are reported in Table 1. Overall, older adults exhibited a trend towards decreased cortical excitability (e.g.,

elevated resting MT) but age-related differences were only significant in regard to an increased latency of MEP responses in seniors ($p<0.001$).

3.2 Responses to iTBS and age differences

As shown in Figure 1, inter-individual responses to iTBS were quite variable. Mixed ANOVAs revealed no main effect of time interval on iTBS responses in the young ($F_{3,57} = 0.97$, $p=0.41$) and older adults ($F_{3,51} = 1.78$, $p=0.16$). Overall changes in MEP amplitude ranged from -40% to 218% in young adults and from -65% to 261% in older adults. Only 13/20 young adults (65%) and 10/18 older adults (56%) exhibited the expected increase in MEP amplitudes following iTBS when their responses across the four time intervals were averaged (i.e., $\%MEP_{post\text{-}iTBS} >100\%$). A Fisher's exact test analysis did not reveal any difference in the ratio of responders in young and older adults ($p=0.74$). Baseline RMT and AMT were negatively associated with overall iTBS responses in older adults ($r=-0.55$ and -0.74, respectively; $p<0.02$) but not in young adults ($r<-0.41$), while there were no significant correlations with baseline MEP latency.

Qualitatively, both age groups exhibited MEP facilitation after iTBS and there was a trend for MEP amplitudes to return to baseline levels more quickly in older adults (after approximately 10 minutes) than in young adults, who were still showing facilitation 20 minutes post-iTBS (Figure 2). However, these group differences did not reach significance as a repeated measures ANOVA did not reveal a main effect of time ($F_{3,108} = 0.36$, $p=0.79$) or age group ($F_{1,36} = 0.80$, $p=0.38$) on iTBS responses and time interval by group interaction ($F_{3,108} = 2.08$, $p=0.11$). An additional repeated measures ANOVA did not yield any main effect or interaction between time and age group when only the responders to iTBS were included in the analysis (n=23, $p>0.14$).

3.3 Variations in SAI in relation to responses to iTBS

Data from one older adult was excluded from all analysis involving SAI due to an abnormally high MEP facilitation in response to afferent conditioning (Grubb's test, $p<0.01$, $z=3.32$). Young adults exhibited significantly deeper levels of SAI than older adults (19.43±12.13% vs. 42.07±34.79%, respectively; $p=0.01$). SAI measures in the older group were also characterized by higher variability with four seniors showing either low or absent inhibition (i.e., SAI≥50%) and two even showing facilitation in response to afferent conditioning. As illustrated in Figure 3, age-related variations in SAI levels were only poor predictors of corresponding variations in mean responses to iTBS. Similar non-significant associations were found when both groups were examined separately (young: $r^2=0.02$, $p=0.61$; older adults: $r^2=0.03$, $p=0.54$). SAI levels were also weakly associated with iTBS responses at the various time-intervals examined within the young and older adults ($r < -0.37$, $p>0.05$). There were no significant differences in SAI levels between responders and non-responders to iTBS in both the young ($t_{18}=1.30$, $p=0.21$) and older groups ($t_{15}=0.67$, $p=0.52$). Furthermore, mean MEP modulation after iTBS in the four older adults with low SAI levels was similar to the other seniors (SAI<50%). However, this subgroup of older adults exhibited less facilitation 5-minutes post-iTBS, but this effect was not present at the other test intervals.

4. Discussion

In this study, we examined age-related differences in LTP-like plasticity induced by iTBS and their relationship with SAI, a marker of cholinergic activity in the motor cortex. Three main observations emerged from our results. First, individual responses to

iTBS were quite variable as only 60% of participants exhibited the expected pattern of MEP facilitation. Second, there were no significant age group differences in iTBS-induced facilitatory effects. Finally, while older adults exhibited lower levels of SAI than younger adults, age-related variations in SAI were not associated with individual susceptibility to iTBS-induced plasticity. The significance of our results and possible confounding factors are discussed below.

4.1 iTBS : Variability and aging

Consistent with recent reports of large inter-individual variability in iTBS responses (e.g., Player et al., 2012; Hamada et al., 2013; Vallence et al., 2013; Hinder et al., 2014; Lopez-Alonso et al., 2014), we found that susceptibility to express LTP-like plasticity following iTBS was variable in both young and older adults. Only about 60% of participants displayed the expected overall facilitation of MEP amplitudes and the number of responders to iTBS was similar in both age groups (i.e., 65% of young adults and 58% older adults). Accordingly, we did not find a significant difference between young and older adults in terms of MEP facilitation following iTBS, even after sorting participants to retain only the "responders" (mean post-iTBS modulation >100%). In this respect, our results are in line with those of Di Lazzaro et al. (2008b) who also examined age effects on responses to iTBS in a small sample of participants but did not find LTP-like plasticity to be significantly reduced in older adults. In contrast, cTBS has been shown to lead to smaller and shorter lasting inhibitory effects in aging (Freitas et al., 2011) and may thus be more reliably affected by age. We did not find significant age differences in the duration of iTBS effects in this study, but given that the young adults were still displaying MEP facilitation at 20 minutes, it is possible that an effect of age

would have been found if we had examined later time intervals. However, this seems unlikely given that peak MEP modulation seems to occur between 5 and 20 minutes post-iTBS in healthy young adults (e.g., Swayne et al., 2009; Li Voti et al., 2011; Player et al., 2012; Cardenas-Morales et al., 2013; Lopez-Alonso et al., 2014; but see Hinder et al., 2014).

The other TMS studies that have investigated age effects on LTP/LTD-like plasticity have relied on the PAS protocol (Muller-Dahlhaus et al., 2008; Tecchio et al., 2008; Fathi et al., 2010). In general, these studies have reported significant age-related reductions in LTP-like plasticity after PAS, but differences between stimulation protocols may in part explain why we did not find similar age differences with iTBS. Indeed, both protocols do not attempt to produce plasticity in the same way: iTBS relies on low intensity bursts of stimulation and simulates natural cortical theta and gamma rhythms (Cardenas-Morales et al., 2010) while PAS is dependent upon Hebbian synaptic strengthening following the synchronous activation of neurons by nerve and TMS stimulations (Stefan et al., 2000). Recent studies have also reported small correlations between MEP modulation by iTBS and facilitatory PAS, suggesting that the mechanisms on which they rely to induce LTP-like plasticity, while both being dependent on NMDA receptors, may only partially overlap (Player et al., 2012; Vallence et al., 2013; Lopez-Alonso et al., 2014).

Additionally, in these studies, the test and stimulation intensity was based on MEP size, a procedure that can lead to overstimulation (Garry & Thomson, 2009), especially in seniors. In the present study, the stimulation intensity was based on individual motor thresholds, which could have contributed to mitigate age differences in

responses to iTBS. In agreement with this, we found negative correlations between overall iTBS responses and RMT and AMT in seniors, suggesting that stimulation intensity can influence the magnitude of MEP modulation by rTMS. However, as others, we did not find significant age differences in these measures, suggesting that cortical atrophy (and therefore increased distance between the coil and stimulation site) did not significantly contribute to reduced cortical plasticity in aging (Fathi et al., 2010; Freitas et al., 2011; but see Muller-Dahlhaus et al., 2008). Although we found age differences in the latency of baseline MEPs, these were not associated with iTBS responses. Still, it remains possible that the stimulation intensity used for our iTBS protocol was not optimal and this might explain why the overall effect of iTBS on motor cortical excitability was not significant in our study, which is in contrast to previous reports (e.g., Huang et al., 2005; Iezzi et al., 2008). On the other hand, the intensity of stimulation might not be the sole explanatory factor as other recent reports addressing the issue of inter-individual variability in responses to rTMS protocols have used the constant MEP size approach to monitor changes in excitability, and yet, they also failed to report a significant iTBS effect in young adults (Player et al., 2012; Hamada et al., 2013; Vallence et al., 2013; Lopez-Alonso et al., 2014). Clearly, there is a need to better delineate the influence of TMS parameters on the variability in responses to plasticity-inducing protocols.

Many other factors have also been shown to influence responses to TBS. Of particular interest, voluntary muscle activity before or during TBS (Huang et al., 2008) can reverse and abolish plasticity effects, while contractions immediately after iTBS leads to greater MEP modulation (Huang et al., 2008; Iezzi et al., 2008). These studies

suggest that baseline cortical excitability might greatly influence TBS and should thus be monitored more carefully in future studies. Controlling for other factors such as genetics (Cheeran et al., 2008; but see Li Voti et al., 2011; Mastroeni et al., 2013) and diurnal variations related to the time of day the testing took place (Sale et al., 2007; but see Lopez-Alonso et al., 2014) may also prove useful in maximizing the characterization of cortical plasticity as induced by TBS in aging. Another interesting line of questioning was raised by a recent study, which suggests that high inter-individual variability in TBS responses might be more closely related to differences in the population of interneurons recruited by rTMS than to intrinsic neuronal capacity for plasticity (Hamada et al., 2013). Indeed, when late I-waves were easily recruited by an anterior-posterior directed TMS current, participants were more susceptible to exhibit long-lasting TBS responses. The differential recruitment of interneurons as described by Hamada et al. (2013) accounted for approximately 50% of the variability in TBS responses. Whether similar effects may also be found in aging should be investigated.

4.2 Relationship between iTBS and SAI

The main contribution of this study relates to our observation of a null correlation between individual responses to iTBS and SAI levels, a marker of central cholinergic activity in the motor cortex. This lack of relationship was present despite clear reductions in SAI with age, which is consistent with our previous results in an independent sample (Young-Bernier et al., 2012; but see Oliviero et al., 2006; Degardin et al., 2011) and other evidence of a deterioration of the cholinergic system in normal aging (e.g., Mesulam et al., 2004; Duzel et al., 2010; Dumas and Newhouse, 2011; Grothe et al., 2012).

Both iTBS and SAI have been shown to be preferentially dependent upon the recruitment of late I-waves, suggesting that they both involve similar cortical circuits (Di Lazzaro et al., 2004; Di Lazzaro et al., 2008b). Accordingly, SAI levels can be increased and partially restored by cTBS in patients with AD, but they are not modulated by iTBS or PAS in young and healthy older adults (Di Lazzaro et al., 2011; Di Lorenzo et al., 2013). A significant decline in cholinergic activity may thus need to be present for strong associations between cortical plasticity and SAI levels to be present. Such potential effects could have been masked by the fact that only four of the older adults included in this study exhibited an absence or reduction of cortical inhibition (SAI≥50%). Although this subgroup did not differ from the other older adults in regards to mean levels of MEP modulation following iTBS, they tended to exhibit less facilitation at 5 minutes post-iTBS. This could suggest that cholinergic activity contributes to the early build-up of cortical plasticity. We might be able to shed more light on this issue and find the expected relationship between SAI and LTP-like plasticity in a larger group of older adults with reduced SAI levels or if we investigated TBS effects in patients with AD, vascular damage or other diseases in which the deterioration of cholinergic transmission is a major pathologic component.

Our results suggest that age-related declines in cholinergic activity (as indexed by SAI) do not significantly contribute to the modulation of MEP amplitudes by iTBS or to the duration of these effects. These results are in contrast with pharmacological studies that have demonstrated increased and prolonged iTBS and PAS induced LTP-like plasticity following the administration of cholinergic agonists but reduced effects by antagonists (Kuo et al., 2007; Swayne et al., 2009; Korchounov & Ziemann, 2011;

Thirugnanasambandam et al., 2011). Discrepancies between the results of these studies and ours might be related to our use of SAI levels to examine chronic age-related changes in cholinergic activity as opposed to the effects of phasic alterations in cortical cholinergic availability by drugs. As discussed above, these differences could also be related to the fact that most of these pharmacological studies were performed with PAS as opposed to iTBS (i.e., Kuo et al., 2007; Korchounov & Ziemann, 2011; Thirugnanasambandam et al., 2011). In this regard, Kuo et al. (2007) argued that rivastigmine leads to more LTP-like plasticity following PAS than tDCS due to acetylcholine's role in increasing the signal-to-noise ratio and enhancing the detection of afferent sensory inputs. The influence of iTBS appears to be more diffuse than the synapse-specific PAS effects and different influences of the cholinergic system on LTP-like responses could thus be expected following each of these protocols.

Additionally, declines in cholinergic activity are only one of many changes taking place in central neurotransmission during normal aging (e.g., Yankner et al., 2008) and may thus not be sufficient to predict age-related differences in responses to plasticity-inducing rTMS protocols. For example, age-related changes in dopamine (Backman et al., 2006) may also contribute to the modulation of cortical plasticity (Kuo et al., 2008; Korchounov & Ziemann, 2011).

Finally, although there is ample reason to think of SAI as a good marker of cholinergic activity [SAI is reduced by scopolamine in healthy adults (Di Lazzaro et al., 2000), it is decreased in patient populations with deficient cholinergic activity (e.g., AD, Lewy Body disease, vascular dementia; Di Lazzaro et al., 2007b; Di Lazzaro et al., 2008a), and it can be rescued by acetylcholinesterase inhibitors in AD patients (Di

Lazzaro et al., 2002)], SAI may also be modulated at the cortical level in part by $GABA_A$ receptors. The efficiency of this motor cortical inhibition circuit is influenced by benzodiazepines (Di Lazzaro et al., 2007a), consistent with a role of the GABAergic system in controlling acetylcholine release in the cortex (Giorgetti et al., 2000). Also, SAI is decreased in patients with obsessive-compulsive disorder (Russo et al., 2014) and posttraumatic stress disorder (Rossi et al., 2009), which are both psychiatric conditions involving a GABAergic imbalance but with limited cholinergic involvement. The age-related reductions in SAI described in this study may thus reflect not only declines in cholinergic activity but also alterations in GABAergic transmission, though this system appears to be relatively spared in aging (Rissman et al., 2007).

In summary, the present study provides further evidence of high inter-individual variability in responses to iTBS in both young and healthy older adults. We also found evidence of declines in SAI levels with age, but these were not associated with iTBS responses. Together, these results suggest that chronic changes in cholinergic neuromodulation as they occur in normal aging do not significantly contribute to the inter-individual variability in LTP-like plasticity induced by iTBS.

Acknowledgements: We thank our participants for their time and patience during testing, and Francisca Avila Ramirez and Travis Davidson for their help with data collection. This work will serve as a partial fulfillment for a doctoral thesis in clinical psychology by MYB. This work was supported by a student award from Fonds de Recherche du Québec – Santé to MYB, a Research Development Grant from the Faculty of Social Sciences of

the University of Ottawa to FT and PD, and a Discovery grant from the Natural Sciences and Engineering Research Council of Canada to PD.

References

Awiszus, F. (2003). TMS and threshold hunting. *Suppl Clin Neurophysiol* 56**,** 13-23.

Backman, L., Nyberg, L., Lindenberger, U., Li, S.C., & Farde, L. (2006). The correlative triad among aging, dopamine, and cognition: current status and future prospects. *Neurosci Biobehav Rev* 30**,** 791-807.

Battaglia, F., Wang, H.Y., Ghilardi, M.F., Gashi, E., Quartarone, A., Friedman, E., & Nixon, R.A. (2007). Cortical plasticity in Alzheimer's disease in humans and rodents. *Biol Psychiatry 62***,** 1405-1412.

Cardenas-Morales, L., Nowak, D.A., Kammer, T., Wolf, R.C., & Schonfeldt-Lecuona, C. (2010). Mechanisms and applications of theta-burst rTMS on the human motor cortex. *Brain Topogr 22***,** 294-306.

Cardenas-Morales, L., Volz, L.J., Michely, J., Rehme, A.K., Pool, E.M., Nettekoven, C., Eickhoff, S.B., Fink, G.R., & Grefkes, C. (2013). Network Connectivity and Individual Responses to Brain Stimulation in the Human Motor System. *Cereb Cortex 24*, 1697-1707.

Cheeran, B., Talelli, P., Mori, F., Koch, G., Suppa, A., Edwards, M., Houlden, H., Bhatia, K., Greenwood, R., & Rothwell, J.C. (2008). A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. *J Physiol 586***,** 5717-5725.

Degardin, A., Devos, D., Cassim, F., Bourriez, J.L., Defebvre, L., Derambure, P., & Devanne, H. (2011). Deficit of sensorimotor integration in normal aging. *Neurosci Lett 498*, 208-212.

Di Lazzaro, V., Dileone, M., Pilato, F., Capone, F., Musumeci, G., Ranieri, F., Ricci, V., Bria, P., Di Iorio, R., De Waure, C., Pasqualetti, P., & Profice, P. (2011). Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. *J Neurophysiol 105***,** 2150-2156.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., Insola, A., Tonali, P.A., & Rothwell, J.C. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clin Neurophysiol 115***,** 255-266.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M.A., Di Giovanni, S., Zito, G., Tonali, P., & Rothwell, J.C. (2000). Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. *Exp Brain Res 135***,** 455-461.

Di Lazzaro, V., Oliviero, A., Tonali, P.A., Marra, C., Daniele, A., Profice, P., Saturno, E., Pilato, F., Masullo, C., & Rothwell, J.C. (2002). Noninvasive in vivo assessment

of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology 59*, 392-397.

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Marra, C., Ranieri, F., Quaranta, D., Gainotti, G., & Tonali, P.A. (2008a). In vivo functional evaluation of central cholinergic circuits in vascular dementia. *Clinical Neurophysiology 119*, 2494-2500.

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Oliviero, A., Mazzone, P., Insola, A., Ranieri, F., Meglio, M., Tonali, P.A., & Rothwell, J.C. (2008b). The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. *J Physiol 586*, 3871-3879.

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Ranieri, F., Ricci, V., Bria, P., Tonali, P.A., & Ziemann, U. (2007a). Segregating two inhibitory circuits in human motor cortex at the level of GABAA receptor subtypes: a TMS study. *Clin Neurophysiol 118*, 2207-2214.

Di Lazzaro, V., Pilato, F., Dileone, M., Saturno, E., Profice, P., Marra, C., Daniele, A., Ranieri, F., Quaranta, D., Gainotti, G., & Tonali, P.A. (2007b). Functional evaluation of cerebral cortex in dementia with Lewy bodies. *Neuroimage 37*, 422-429.

Di Lorenzo, F., Martorana, A., Ponzo, V., Bonni, S., D'angelo, E., Caltagirone, C., & Koch, G. (2013). Cerebellar theta burst stimulation modulates short latency afferent inhibition in Alzheimer's disease patients. *Front Aging Neurosci 5*:2.

Dumas, J.A., & Newhouse, P.A. (2011). The cholinergic hypothesis of cognitive aging revisited again: Cholinergic functional compensation. *Pharmacol Biochem Behav 99*, 254-261.

Duzel, S., Munte, T.F., Lindenberger, U., Bunzeck, N., Schutze, H., Heinze, H.J., & Duzel, E. (2010). Basal forebrain integrity and cognitive memory profile in healthy aging. *Brain Res 1308*, 124-136.

Fathi, D., Ueki, Y., Mima, T., Koganemaru, S., Nagamine, T., Tawfik, A., & Fukuyama, H. (2010). Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. *Clin Neurophysiol 121*, 90-93.

Fischer, M., & Orth, M. (2011). Short-latency sensory afferent inhibition: conditioning stimulus intensity, recording site, and effects of 1 Hz repetitive TMS. *Brain Stimul 4*, 202-209.

Freitas, C., Perez, J., Knobel, M., Tormos, J.M., Oberman, L., Eldaief, M., Bashir, S., Vernet, M., Pena-Gomez, C., & Pascual-Leone, A. (2011). Changes in cortical plasticity across the lifespan. *Front Aging Neurosci 3*:5.

Garry, M.I., & Thomson, R.H. (2009). The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. *Exp Brain Res 193*, 267-274.

Giorgetti, M., Bacciottini, L., Giovannini, M.G., Colivicchi, M.A., Goldfarb, J., & Blandina, P. (2000). Local GABAergic modulation of acetylcholine release from the cortex of freely moving rats. *Eur J Neurosci 12*, 1941-1948.

Grothe, M., Heinsen, H., & Teipel, S.J. (2012). Atrophy of the cholinergic Basal forebrain over the adult age range and in early stages of Alzheimer's disease. *Biol Psychiatry 71*, 805-813.

Hamada, M., Murase, N., Hasan, A., Balaratnam, M., & Rothwell, J.C. (2013). The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex 23*, 1593-1605.

Hinder, M.R., Goss, E.L., Fujiyama, H., Canty, A.J., Garry, M.I., Rodger, J., & Summers, J.J. (2014). Inter- and Intra-individual Variability Following Intermittent Theta Burst Stimulation: Implications for Rehabilitation and Recovery. *Brain Stimul 7,* 365-371.

Huang, Y.Z., Chen, R.S., Rothwell, J.C., & Wen, H.Y. (2007). The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin Neurophysiol 118*, 1028-1032.

Huang, Y.Z., Edwards, M.J., Rounis, E., Bhatia, K.P., & Rothwell, J.C. (2005). Theta burst stimulation of the human motor cortex. *Neuron 45*, 201-206.

Huang, Y.Z., Rothwell, J.C., Edwards, M.J., & Chen, R.S. (2008). Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. *Cereb Cortex 18*, 563-570.

Iezzi, E., Conte, A., Suppa, A., Agostino, R., Dinapoli, L., Scontrini, A., & Berardelli, A. (2008). Phasic voluntary movements reverse the aftereffects of subsequent theta-burst stimulation in humans. *J Neurophysiol 100*, 2070-2076.

Korchounov, A., & Ziemann, U. (2011). Neuromodulatory neurotransmitters influence LTP-like plasticity in human cortex: a pharmaco-TMS study. *Neuropsychopharmacology 36*, 1894-1902.

Kuo, M.F., Grosch, J., Fregni, F., Paulus, W., & Nitsche, M.A. (2007). Focusing effect of acetylcholine on neuroplasticity in the human motor cortex. *J Neurosci 27*, 14442-14447.

Kuo, M.F., Paulus, W., & Nitsche, M.A. (2008). Boosting focally-induced brain plasticity by dopamine. *Cereb Cortex 18*, 648-651.

Li Voti, P., Conte, A., Suppa, A., Iezzi, E., Bologna, M., Aniello, M.S., Defazio, G., Rothwell, J.C., & Berardelli, A. (2011). Correlation between cortical plasticity,

motor learning and BDNF genotype in healthy subjects. *Exp Brain Res 212*, 91-99.

Lopez-Alonso, V., Cheeran, B., Rio-Rodriguez, D., & Fernandez-Del-Olmo, M. (2014). Inter-individual variability in response to non-invasive brain stimulation paradigms. *Brain Stimul 7*, 372-380.

Mastroeni, C., Bergmann, T.O., Rizzo, V., Ritter, C., Klein, C., Pohlmann, I., Brueggemann, N., Quartarone, A., & Siebner, H.R. (2013). Brain-derived neurotrophic factor--a major player in stimulation-induced homeostatic metaplasticity of human motor cortex? *PLoS One 8*, e57957.

Mesulam, M.M., Shaw, P., Mash, D., & Weintraub, S. (2004). Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. *Ann Neurol 55*, 815-828.

Mills, K.R., & Nithi, K.A. (1997). Corticomotor threshold is reduced in early sporadic amyotrophic lateral sclerosis. *Muscle Nerve 20*, 1137-1141.

Mishory, A., Molnar, C., Koola, J., Li, X., Kozel, F.A., Myrick, H., Stroud, Z., Nahas, Z., & George, M.S. (2004). The maximum-likelihood strategy for determining transcranial magnetic stimulation motor threshold, using parameter estimation by sequential testing is faster than conventional methods with similar precision. *J ECT 20*, 160-165.

Muller-Dahlhaus, J.F., Orekhov, Y., Liu, Y., & Ziemann, U. (2008). Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Exp Brain Res 187*, 467-475.

Nasreddine, Z.S., Phillips, N.A., Bedirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J.L., & Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society 53*, 695-699.

Oliviero, A., Profice, P., Tonali, P.A., Pilato, F., Saturno, E., Dileone, M., Ranieri, F., & Di Lazzaro, V. (2006). Effects of aging on motor cortex excitability. *Neurosci Res 55*, 74-77.

Player, M.J., Taylor, J.L., Alonzo, A., & Loo, C.K. (2012). Paired associative stimulation increases motor cortex excitability more effectively than theta-burst stimulation. *Clin Neurophysiol 123*, 2220-2226.

Rasmusson, D.D. (2000). The role of acetylcholine in cortical synaptic plasticity. *Behav Brain Res 115*, 205-218.

Ridding, M.C., & Ziemann, U. (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J Physiol 588*, 2291-2304.

Rissman, R.A., De Blas, A.L., & Armstrong, D.M. (2007). GABA(A) receptors in aging and Alzheimer's disease. *J Neurochem 103***,** 1285-1292.

Rossetti, H.C., Lacritz, L.H., Cullum, C.M., & Weiner, M.F. (2011). Normative data for the Montreal Cognitive Assessment (MoCA) in a population-based sample. *Neurology 77***,** 1272-1275.

Rossi, S., De Capua, A., Tavanti, M., Calossi, S., Polizzotto, N.R., Mantovani, A., Falzarano, V., Bossini, L., Passero, S., Bartalini, S., & Ulivelli, M. (2009). Dysfunctions of cortical excitability in drug-naive posttraumatic stress disorder patients. *Biol Psychiatry 66***,** 54-61.

Russo, M., Naro, A., Mastroeni, C., Morgante, F., Terranova, C., Muscatello, M.R., Zoccali, R., Calabro, R.S., & Quartarone, A. (2014). Obsessive-compulsive disorder: a "sensory-motor" problem? *Int J Psychophysiol 92***,** 74-78.

Sale, M.V., Ridding, M.C., & Nordstrom, M.A. (2007). Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. *Exp Brain Res 181***,** 615-626.

Stefan, K., Kunesch, E., Cohen, L.G., Benecke, R., & Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain 123* Pt 3**,** 572-584.

Swayne, O.B., Teo, J.T., Greenwood, R.J., & Rothwell, J.C. (2009). The facilitatory effects of intermittent theta burst stimulation on corticospinal excitability are enhanced by nicotine. *Clin Neurophysiol 120***,** 1610-1615.

Tecchio, F., Zappasodi, F., Pasqualetti, P., De Gennaro, L., Pellicciari, M.C., Ercolani, M., Squitti, R., & Rossini, P.M. (2008). Age dependence of primary motor cortex plasticity induced by paired associative stimulation. *Clin Neurophysiol 119***,** 675-682.

Teo, J.T.H., Swayne, O.B., & Rothwell, J.C. (2007). Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. *Clin Neurophysiol 118***,** 1648-1651.

Thirugnanasambandam, N., Grundey, J., Adam, K., Drees, A., Skwirba, A.C., Lang, N., Paulus, W., & Nitsche, M.A. (2011). Nicotinergic impact on focal and non-focal neuroplasticity induced by non-invasive brain stimulation in non-smoking humans. *Neuropsychopharmacology 36***,** 879-886.

Tokimura, H., Di Lazzaro, V., Tokimura, Y., Oliviero, A., Profice, P., Insola, A., Mazzone, P., Tonali, P., & Rothwell, J.C. (2000). Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J Physiol 523* Pt 2**,** 503-513.

Vallence, A.M., Kurylowicz, L., & Ridding, M.C. (2013). A comparison of neuroplastic responses to non-invasive brain stimulation protocols and motor learning in healthy adults. *Neurosci Lett 549***,** 151-156.

Yankner, B.A., Lu, T., & Loerch, P. (2008). The aging brain. *Annu Rev Pathol 3***,** 41-66.

Young-Bernier, M., Davidson, P.S., & Tremblay, F. (2012). Paired-pulse afferent modulation of TMS responses reveals a selective decrease in short latency afferent inhibition with age. *Neurobiol Aging 33***,** 835.e1-835.e11.

Table 1

Baseline measures of cortical excitability (mean ± SD) in the two age groups.

	Young adults (n = 20)	Older adults (n = 18)
Baseline measurements for iTBS (APB muscle)		
Resting MT (% output)	43.0±8.6	47.6±10.8
MEP amplitude (μV)	560.9±408.2	569.5±484.6
MEP latency (ms)	21.3±0.3	24.1±2.8*
AMT (% output)	63.9±11.1	64.1±8.9
Baseline measurements for SAI (FDI muscle)		
Resting MT (% output)	42.6±9.3	46.2±11.2
MEP amplitude (μV)	898.2±826.5	507.6±484.9
MEP latency (ms)	21.0±1.6	24.4±3.0*
Intensity MNS (V)	6.3±2.1	7.3±1.8

Abbreviations: AMT: active motor threshold; APB: abductor pollicis brevis; FDI: first dorsal interosseous; iTBS: intermittent theta burst stimulation; MEP: motor evoked potential; MNS: median nerve stimulation (intensity of); MT: motor threshold; SAI: short latency afferent inhibition

*Significant difference at adjusted *p*-value (p=0.01) for multiple comparisons.

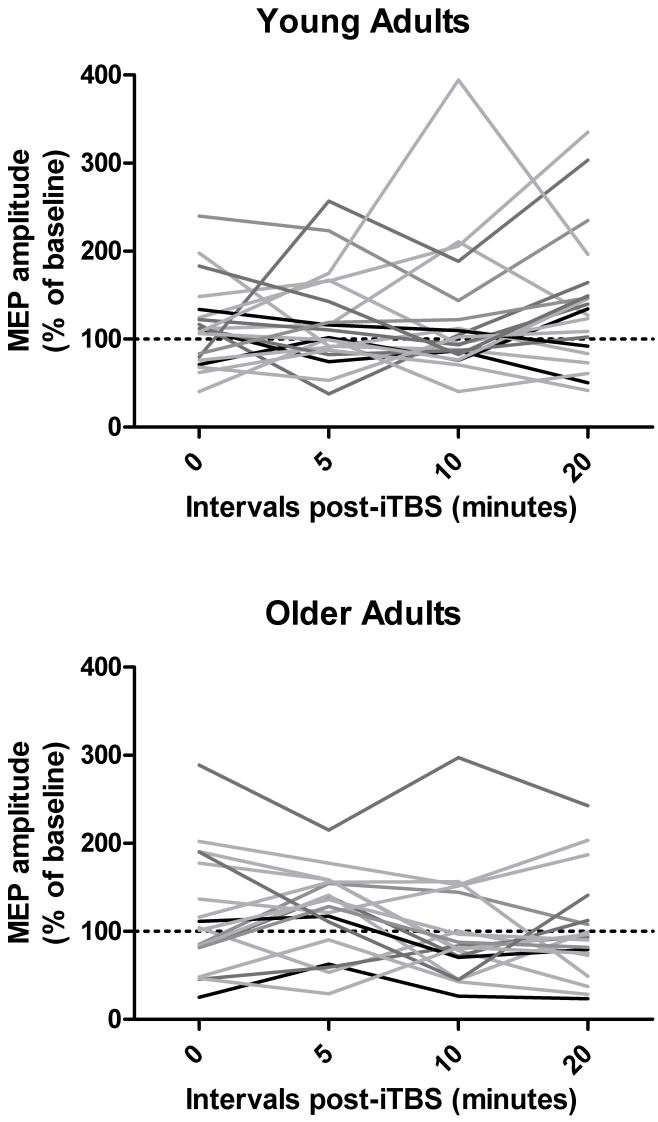

Figure 1. Individual responses to intermittent theta burst stimulation (iTBS) in young and older adults at 0, 5, 10, and 20 minutes post-iTBS.

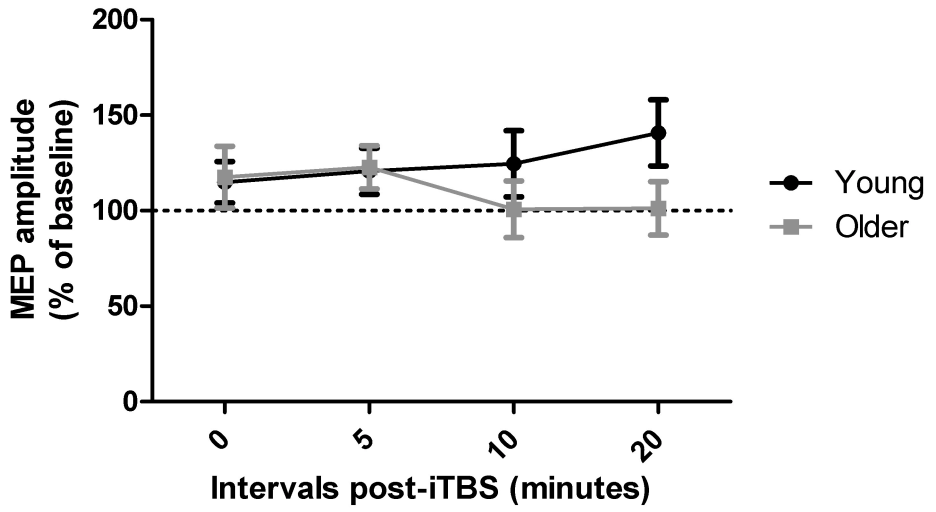

Figure 2. Effect of intermittent theta burst stimulation (iTBS) at times 0, 5, 10, and 20 minutes following the procedure in young and older adults.

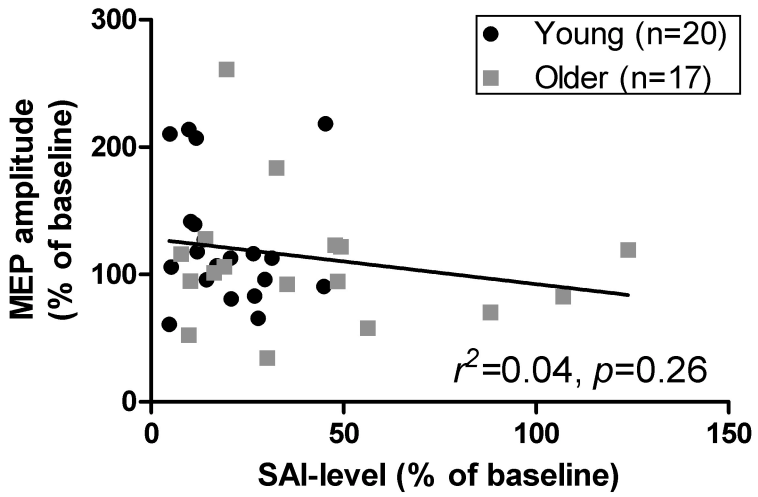

Figure 3. Association between short latency afferent inhibition (SAI) levels and mean modulation of MEP amplitudes (time intervals 0, 5, 10, and 20 minutes) following intermittent theta burst stimulation (iTBS) in young and older adults.

CHAPITRE III. DISCUSSION GÉNÉRALE ET CONCLUSION

Les études comprises dans cette thèse sont les premières à démontrer une réduction des niveaux de SAI, un marqueur neurophysiologique de l'activité cholinergique obtenu par l'entremise de la SMT, chez les personnes âgées en santé par rapport aux jeunes adultes (mais voir Degardin et al., 2011; Oliviero et al., 2006). Ce résultat a d'ailleurs été répliqué à l'intérieur de deux groupes de participants indépendants (i.e., études 1/2 et 4). Cette réduction spécifique de l'inhibition intra-corticale motrice chez les personnes âgées, suite à la modulation afférente des réponses SMT par une stimulation périphérique livrée à un intervalle de 20 ms (i.e., SAI), indique une diminution de l'efficacité du système cholinergique avec l'âge. Ceci est en accord avec de nombreuses études qui suggèrent une dégénérescence de ce système de neurotransmission au cours du vieillissement normal (e.g., Dumas & Newhouse, 2011; Duzel et al., 2010; Grothe et al., 2012).

Par la suite, nous avons établi des liens entre la diminution de l'activité cholinergique, telle que reflétée dans les niveaux de SAI, et le déclin des performances à des tâches psychomotrices ainsi qu'à des tests de mémoire avec l'âge. Par contre, bien que les personnes âgées aient démontré une plus grande variabilité dans leurs temps de réaction et donc un contrôle attentionnel plus variable que les jeunes adultes, leur performance à des tâches examinant les fonctions exécutives et l'attention était généralement préservée. Cette performance n'était pas significativement associée aux niveaux individuels de SAI. Finalement, étant donné le déclin parallèle de l'activité cholinergique et de la performance à certaines tâches lors du vieillissement, les mécanismes de plasticité synaptique sous-jacents à l'apprentissage et à la mémoire ont été examinés grâce à la SMTr. Toutefois, la possibilité d'induire des changements

d'excitabilité corticale se rapportant à la plasticité synaptique de type PLT, par le biais du protocole SiBT, n'était pas associée à l'âge ou aux niveaux de SAI. Nos résultats ont plutôt mis en évidence la présence d'une grande variabilité interindividuelle au sein des réponses à ce protocole de stimulations répétitives visant la plasticité corticale.

Les corrélations obtenues entre les niveaux de SAI et la performance psychomotrice et cognitive étaient dans la direction attendue, soit une inhibition moins efficace des PEMs suite à la modulation afférente des réponse à la SMT (i.e., niveaux de SAI élevés) était liée à une performance plus pauvre à certaines tâches. Toutefois, ces corrélations se sont avérées non-significatives lorsqu'elles furent examinées séparément à l'intérieur des deux groupes d'âge. De plus, bien que certaines personnes âgées aient démontrées un niveau de SAI anormal (i.e., inhibition pauvre ou inexistante équivalente a >2 écart type des niveaux de SAI obtenus chez les jeunes adultes, ou SAI ≥50% des réponses PEMs non-conditionnées), leur performance aux tâches cognitives n'était pas significativement différente de celle des aînés ayant démontrés des niveaux de SAI se rapprochant davantage de ceux des jeunes adultes. Étant donné de nombreuses évidences du rôle important de l'ACh dans la cognition (e.g,, Dumas & Newhouse, 2011; Hasselmo, 2006; Hasselmo & Sarter, 2011; Sarter et al., 2005), la force des associations retrouvées entre cet indice de l'activité cholinergique et la performance s'est donc avérée moins importante qu'anticipé. Puisque le système cholinergique semble être davantage mobilisé lorsqu'un effort attentionnel important est requis (e.g., Sarter et al., 2005), il est possible que le niveau de complexité de certaines des tâches examinées n'était pas suffisamment élevé pour permettre l'identification d'associations fortes entre le SAI et la cognition. Il faut aussi noter que le SAI se rapproche davantage d'un indice de l'activité

tonique et diffuse du système cholinergique central, qu'au mode de transmission dynamique et plus spécifique de l'ACh. Bien que des interactions entre ces deux modes semblent être nécessaires afin de supporter la cognition, des études récentes suggèrent que la neuromodulation lente de l'ACh serait plus fortement associée aux demandes en ce qui à trait au contrôle attentionnel et à l'effort requis afin de maintenir un certain niveau de performance, qu'à cette performance en tant que telle (Sarter et al., 2014). Pour sa part, la libération dynamique de l'ACh dans le cortex préfrontal serait plus directement liée à l'efficacité de la performance (Sarter et al., 2014). Les études futures permettront de clarifier le rôle de ces deux modes de transmission cholinergique dans la cognition, et ce d'abord chez le rongeur mais éventuellement chez l'humain lorsque les instruments de recherche le permettant auront été développés.

L'absence de fortes corrélations entre le SAI et la cognition chez les personnes âgées pourrait aussi être attribuable au fait que la diminution de l'activité cholinergique lors du vieillissement n'est pas un phénomène isolé et se produit dans le contexte de nombreux autres changements, tels que l'atrophie globale du cortex (Fjell et al., 2014; Raz et al., 2005), la déposition de β-amyloïde (Grothe et al., 2013) et la réduction de l'activité de la dopamine et d'autres systèmes de neurotransmission (e.g., Backman et al., 2006). L'étude conjointe du système cholinergique avec celle d'autres altérations physiologiques associées à l'âge pourrait donc fournir un portrait plus complet du vieillissement et des interactions entre ces changements. Certains facteurs de protection liés à l'hypothèse de la réserve cognitive pourraient aussi contribuer à masquer des associations entre le SAI et la performance cognitive et psychomotrice. À cet égard, les participants âgés inclus dans les études présentées ci-haut étaient généralement bien

éduqués et étaient actifs au plan social ainsi que physique, ce qui suggérerait qu'ils bénéficiaient tout probablement d'une bonne réserve cognitive (Villeneuve & Belleville, 2010).

Les études pharmacologiques et celles effectuées chez diverses populations cliniques ont permis de bien établir le rôle du SAI en tant que marqueur de l'activité cholinergique (e.g., pour une revue, voir Nardone et al., 2011). Toutefois, ce type d'inhibition intra-corticale est aussi modulé par l'activation des récepteurs $GABA_A$ du système de neurotransmission GABAergique (voir section 3.2 de l'introduction; Di Lazzaro, Oliviero, Saturno et al., 2005; Di Lazzaro, Pilato, Dileone, Tonali, & Ziemann, 2005). Les niveaux de SAI peuvent aussi être affectés par l'administration de dopamine chez les patients atteints de la MA et de la maladie de Parkinson (Martorana et al., 2009; Sailer et al., 2003). Par contre, l'administration de quetiapine, qui a un effet antagoniste au niveau des récepteurs de divers systèmes de neurotransmission (i.e., dopamine, sérotonine, histamine et catécholamines) mais n'a pas d'affinité avec les récepteurs muscariniques cholinergique, ne semble pas moduler les niveaux de SAI chez les jeunes adultes en santé (Di Lazzaro, Oliviero, Saturno et al., 2005). Ceci semble donc indiquer un rôle limité de ces neurotransmetteurs dans la régulation du SAI chez les individus en santé (Nardone et al., 2011). Les études futures permettront de mieux caractériser l'influence du système GABAergique et possiblement dopaminergique sur le SAI. L'établissement d'associations entre le SAI et d'autres indices de la dégénérescence cholinergiques obtenus grâce à la TEP ou à l'IRM (e.g., traceurs de l'activité AChE, indice volumétrique du prosencéphale antéro-basal) pourrait aussi permettre de renforcer

le rôle de ce marqueur à titre d'indicateur de l'intégrité du système cholinergique lors du vieillissement normal.

Le potentiel clinique du SAI a fait l'objet de peu d'études, toutefois celui-ci semble être prometteur. En effet, l'augmentation des niveaux de SAI suite à l'administration d'une seule dose de rivastigmine (inhibiteur d'AChE) est associée au maintient ou à l'amélioration des performances cognitives sur une période d'un an chez des patients atteints de la MA, mais pas chez ceux n'ayant pas démontré un rétablissement du SAI avec ce traitement (Di Lazzaro, Oliviero, Pilato et al., 2005). La reproduction d'une telle étude dans le contexte du vieillissement normal pourrait avoir des implications cliniques importantes. Notamment, ceci pourrait permettre de déterminer si les niveaux de SAI peuvent contribuer à l'identification des personnes âgées pouvant bénéficier de traitements cholinergiques précoces pour promouvoir le maintien des fonctions cognitives ou possiblement retarder l'apparition de déclins cognitifs associés au vieillissement.

À cet égard, bien que les études transversales comprises dans ce projet de thèse contribuent à la compréhension des changements neurophysiologiques et cognitifs prenant place avec l'âge, la mise en place d'études longitudinales pourrait s'avérer une avenue importante pour les projets futurs. En effet, une étude longitudinale permettrait une compréhension plus complète des changements qui prennent place avec le temps dans l'efficacité de la transmission cholinergique chez un individu ainsi que sur leurs impacts dans la cognition et les processus d'excitabilité corticale qui se rapportent à la plasticité synaptique. Cette approche permettrait par ailleurs de clarifier si le SAI doit dépasser un certain seuil critique avant que des changements cognitifs puissent être

détectés dans la performance à des tests neuropsychologiques. Ultimement, une telle étude pourrait permettre de déterminer si le SAI serait en mesure de contribuer à l'identification des individus à risque de développer un trouble cognitif léger ou une démence de type cholinergique, telle que la MA. D'ici là, une alternative moins coûteuse consisterait à limiter l'étude du SAI et l'impact du déclin de l'activité cholinergique sur la cognition et la plasticité synaptique à un groupe de personnes âgées appartenant à une tranche d'âge plus restreinte (e.g., les personnes âgées ayant entre 73 et 75 ans). Cette approche permettrait d'augmenter la taille de l'échantillon des personnes âgées et donc le pouvoir statistique, tout en diminuant l'effet du facteur « âge » sur la performance psychomotrice et cognitive, ainsi que sur l'excitabilité corticale se rapportant à la plasticité synaptique. Dans un tel cas, les relations obtenues entre les niveaux de SAI et la performance seraient plus facilement attribuables au déclin de l'activité cholinergique, ce qui permettrait une meilleure compréhension de l'impact de la détérioration de ce système de neurotransmission sur le fonctionnement cognitif.

Pour conclure, les études comprises dans cette thèse sont les premières à démontrer une diminution significative d'un marqueur de l'activité cholinergique lors du vieillissement normal à l'aide d'une technique de SMT minimalement invasive et peu coûteuse. Ces études ont aussi permis d'établir des liens entre la réduction de l'activité cholinergique et le déclin de certaines fonctions psychomotrices et cognitives avec l'âge. Grâce au SAI et à la SMT, il est souhaitable que des études futures permettront de préciser encore davantage l'effet de la dégénérescence du système cholinergique sur les fonctions cognitives et les mécanismes de plasticité sous-jacents à la cognition, tout en

clarifiant le potentiel clinique de ce marqueur neurophysiologique dans le contexte du vieillissement normal.

RÉFÉRENCES

Alzheimer Society of Canada (2012). "A new way of looking at the impact of dementia in Canada".

Backman, L., Nyberg, L., Lindenberger, U., Li, S. C., & Farde, L. (2006). The correlative triad among aging, dopamine, and cognition: current status and future prospects. *Neurosci Biobehav Rev, 30*(6), 791-807.

Bartus, R. T. (2000). On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Exp Neurol, 163*(2), 495-529.

Bartus, R. T., Dean, R. L., 3rd, Beer, B., & Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science, 217*(4558), 408-414.

Battaglia, F., Wang, H. Y., Ghilardi, M. F., Gashi, E., Quartarone, A., Friedman, E. (2007). Cortical plasticity in Alzheimer's disease in humans and rodents. *Biol Psychiatry, 62*(12), 1405-1412.

Behl, P., Bocti, C., Swartz, R. H., Gao, F., Sahlas, D. J., Lanctot, K. L. (2007). Strategic subcortical hyperintensities in cholinergic pathways and executive function decline in treated Alzheimer patients. *Arch Neurol, 64*(2), 266-272.

Behl, P., Lanctot, K. L., Streiner, D. L., Guimont, I., & Black, S. E. (2006). Cholinesterase inhibitors slow decline in executive functions, rather than memory, in Alzheimer's disease: a 1-year observational study in the Sunnybrook dementia cohort. *Curr Alzheimer Res, 3*(2), 147-156.

Bentley, P., Driver, J., & Dolan, R. J. (2011). Cholinergic modulation of cognition: Insights from human pharmacological functional neuroimaging. *Prog Neurobiol, 94*(4), 360-388.

Blau, C. W., Cowley, T. R., O'Sullivan, J., Grehan, B., Browne, T. C., Kelly, L. (2012). The age-related deficit in LTP is associated with changes in perfusion and blood-brain barrier permeability. *Neurobiol Aging*, *33*(5), 1005 e.23-e.35.

Bohnen, N. I., Muller, M. L., Kuwabara, H., Constantine, G. M., & Studenski, S. A. (2009). Age-associated leukoaraiosis and cortical cholinergic deafferentation. *Neurology, 72*(16), 1411-1416.

Conner, J. M., Chiba, A. A., & Tuszynski, M. H. (2005). The basal forebrain cholinergic system is essential for cortical plasticity and functional recovery following brain injury. *Neuron, 46*(2), 173-179.

Conner, J. M., Kulczycki, M., & Tuszynski, M. H. (2010). Unique contributions of distinct cholinergic projections to motor cortical plasticity and learning. *Cereb Cortex, 20*(11), 2739-2748.

Cooke, S. F., & Bliss, T. V. (2006). Plasticity in the human central nervous system. *Brain, 129*(Pt 7), 1659-1673.

Cucurachi, L., Immovilli, P., Granella, F., Pavesi, G., & Cattaneo, L. (2008). Short-latency afferent inhibition predicts verbal memory performance in patients with multiple sclerosis. *J Neurol, 255*(12), 1949-1956.

Cuello, A. C. (2006). Cholinergic synaptic terminations in the cerebral cortex, trophic factor dependency, and vulnerability to aging and Alzheimer's pathology. In E. Giacobini & G. Pepeu (Eds.), *The brain cholinergic system in health and disease* (pp. 33-46). Abingdon, Oxon England: Informa Healthcare.

Davidson, M. C., & Marrocco, R. T. (2000). Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. *J Neurophysiol, 83*(3), 1536-1549.

Davidson, P. S. R., & Winocur, G. (2010). Aging and Cognition. In G. F. Koob, M. Le Moal, & R. F. Thompson (Eds.), *Encyclopedia of Behavioral Neuroscience, Volume 1* (pp. 20-26). Oxford: Academic Press.

Degardin, A., Devos, D., Cassim, F., Bourriez, J. L., Defebvre, L., Derambure, P. (2011). Deficit of sensorimotor integration in normal aging. *Neurosci Lett*, *498*(3), 208-212.

Di Lazzaro, V. (2013). Biological effects of non-invasive brain stimulation. *Handb Clin Neurol, 116*, 367-374.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Marra, C. (2004). Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. *J Neurol Neurosurg Psychiatry, 75*(4), 555-559.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Marra, C. (2005). Neurophysiological predictors of long term response to AChE inhibitors in AD patients. *J Neurol Neurosurg Psychiatry, 76*(8), 1064-1069.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M. A., Di Giovanni, S., Zito, G. (2000). Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. *Exp Brain Res, 135*(4), 455-461.

Di Lazzaro, V., Oliviero, A., Saturno, E., Dileone, M., Pilato, F., Nardone, R. (2005). Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans. *J Physiol, 564*(Pt 2), 661-668.

Di Lazzaro, V., Oliviero, A., Tonali, P. A., Marra, C., Daniele, A., Profice, P. (2002). Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology, 59*(3), 392-397.

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Marra, C., Ranieri, F. (2008). In vivo functional evaluation of central cholinergic circuits in vascular dementia. *Clinical Neurophysiology, 119*(11), 2494-2500.

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Oliviero, A., Mazzone, P. (2008). The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. *J Physiol, 586*(16), 3871-3879.

Di Lazzaro, V., Pilato, F., Dileone, M., Saturno, E., Oliviero, A., Marra, C. (2006). In vivo cholinergic circuit evaluation in frontotemporal and Alzheimer dementias. *Neurology, 66*(7), 1111-1113.

Di Lazzaro, V., Pilato, F., Dileone, M., Saturno, E., Profice, P., Marra, C. (2007). Functional evaluation of cerebral cortex in dementia with Lewy bodies. *Neuroimage, 37*(2), 422-429.

Di Lazzaro, V., Pilato, F., Dileone, M., Tonali, P. A., & Ziemann, U. (2005). Dissociated effects of diazepam and lorazepam on short-latency afferent inhibition. *J Physiol, 569*(Pt 1), 315-323.

Di Lazzaro, V., Profice, P., Ranieri, F., Capone, F., Dileone, M., Oliviero, A. (2012). I-wave origin and modulation. *Brain Stimul, 5*(4), 512-525.

Di Lorenzo, F., Martorana, A., Ponzo, V., Bonni, S., D'Angelo, E., Caltagirone, C. (2013). Cerebellar theta burst stimulation modulates short latency afferent inhibition in Alzheimer's disease patients. *Front Aging Neurosci, 5*, 2.

Drag, L. L., & Bieliauskas, L. A. (2010). Contemporary review 2009: cognitive aging. *J Geriatr Psychiatry Neurol, 23*(2), 75-93.

Dumas, J. A., & Newhouse, P. A. (2011). The cholinergic hypothesis of cognitive aging revisited again: Cholinergic functional compensation. *Pharmacol Biochem Behav, 99*(2), 254-261.

Duzel, S., Munte, T. F., Lindenberger, U., Bunzeck, N., Schutze, H., Heinze, H. J. (2010). Basal forebrain integrity and cognitive memory profile in healthy aging. *Brain Res, 1308*, 124-136.

Eckenstein, F.P., Baughman, R.W., Quinn, J. (1988). An anatomical study of cholinergic innervation in rat cerebral cortex. *Neuroscience* 25**,** 457-474.

Ellis, J R., Nathan, P. J., Villemagne, V. L., Mulligan, R. S., Ellis, K. A., Tochon-Danguy, H. J. (2009). The relationship between nicotinic receptors and cognitive

functioning in healthy aging: An in vivo positron emission tomography (PET) study with 2-[(18)F]fluoro-A-85380. *Synapse, 63*(9), 752-763.

Fathi, D., Ueki, Y., Mima, T., Koganemaru, S., Nagamine, T., Tawfik, A. (2010). Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. *Clin Neurophysiol, 121*(1), 90-93.

Fidalgo, S., Ivanov, D. K., & Wood, S. H. (2013). Serotonin: from top to bottom. *Biogerontology, 14*(1), 21-45.

Fjell, A. M., McEvoy, L., Holland, D., Dale, A. M., & Walhovd, K. B. (2014). What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. *Prog Neurobiol*, *117*, 20-40.

Fjell, A. M., Westlye, L. T., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I. (2013). Critical ages in the life course of the adult brain: nonlinear subcortical aging. *Neurobiol Aging, 34*(10), 2239-2247.

Floresco, S. B., & Jentsch, J. D. (2011). Pharmacological enhancement of memory and executive functioning in laboratory animals. *Neuropsychopharmacol, 36*(1), 227-250.

Freitas, C., Perez, J., Knobel, M., Tormos, J. M., Oberman, L., Eldaief, M. (2011). Changes in cortical plasticity across the lifespan. *Front Aging Neurosci, 3*:5.

Garry, M. I., & Thomson, R. H. (2009). The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. *Exp Brain Res, 193*(2), 267-274.

George, M. S., Lisanby, S. H., & Sackeim, H. A. (1999). Transcranial magnetic stimulation: applications in neuropsychiatry. *Arch Gen Psychiatry, 56*(4), 300-311.

Geula, C., & Mesulam, M. M. (1996). Systematic regional variations in the loss of cortical cholinergic fibers in Alzheimer's disease. *Cereb Cortex, 6*(2), 165-177.

Glisky, E. L. (2007). Changes in Cognitive Function in Human Aging. In D. R. Riddle (Ed.), *Brain aging: Models, methods and mechanisms* (pp. 4-20). Boca Raton, FL: CRC Press.

Griffin, A. L., Asaka, Y., Darling, R. D., & Berry, S. D. (2004). Theta-contingent trial presentation accelerates learning rate and enhances hippocampal plasticity during trace eyeblink conditioning. *Behav Neurosci, 118*(2), 403-411.

Grothe, M., Heinsen, H., & Teipel, S. (2013). Longitudinal measures of cholinergic forebrain atrophy in the transition from healthy aging to Alzheimer's disease. *Neurobiol Aging, 34*(4), 1210-1220.

Grothe, M., Heinsen, H., & Teipel, S. J. (2012). Atrophy of the cholinergic Basal forebrain over the adult age range and in early stages of Alzheimer's disease. *Biol Psychiatry, 71*(9), 805-813.

Grothe, M., Zaborszky, L., Atienza, M., Gil-Neciga, E., Rodriguez-Romero, R., Teipel, S. J. (2010). Reduction of basal forebrain cholinergic system parallels cognitive impairment in patients at high risk of developing Alzheimer's disease. *Cereb Cortex, 20*(7), 1685-1695.

Grundey, J., Freznosa, S., Klinker, F., Lang, N., Paulus, W., & Nitsche, M.A. (2013). Cortical excitability in smoking and not smoking individuals with and without nicotine. *Psychopharmacology (Berl) 229***,** 653-664.

Gu, Q. (2002). Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. *Neuroscience, 111*(4), 815-835.

Gunning-Dixon, F. M., Brickman, A. M., Cheng, J. C., & Alexopoulos, G. S. (2009). Aging of cerebral white matter: a review of MRI findings. *Int J Geriatr Psychiatry, 24*(2), 109-117.

Gunstad, J., Paul, R. H., Brickman, A. M., Cohen, R. A., Arns, M., Roe, D. (2006). Patterns of cognitive performance in middle-aged and older adults: A cluster analytic examination. *J Geriatr Psychiatry Neurol, 19*(2), 59-64.

Haense, C., Kalbe, E., Herholz, K., Hohmann, C., Neumaier, B., Krais, R. (2012). Cholinergic system function and cognition in mild cognitive impairment. *Neurobiol Aging*, *33*(5), 867-877.

Hasselmo, M. E. (2006). The role of acetylcholine in learning and memory. *Curr Opin Neurobiol, 16*(6), 710-715.

Hasselmo, M. E., & Giocomo, L. M. (2006). Cholinergic modulation of cortical function. *J Mol Neurosci, 30*(1-2), 133-135.

Hasselmo, M. E., & McGaughy, J. (2004). High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation. *Prog Brain Res, 145*, 207-231.

Hasselmo, M. E., & Sarter, M. (2011). Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacol, 36*(1), 52-73.

Herholz, K. (2008). Acetylcholine esterase activity in mild cognitive impairment and Alzheimer's disease. *Eur J Nucl Med Mol Imaging, 35 Suppl 1*, S25-29.

Hogstrom, L. J., Westlye, L. T., Walhovd, K. B., & Fjell, A. M. (2013). The structure of the cerebral cortex across adult life: age-related patterns of surface area, thickness, and gyrification. *Cereb Cortex, 23*(11), 2521-2530.

Hoogendam, J. M., Ramakers, G. M., & Di Lazzaro, V. (2010). Physiology of repetitive transcranial magnetic stimulation of the human brain. *Brain Stimul, 3*(2), 95-118.

Huang, Y. Z., Chen, R. S., Rothwell, J. C., & Wen, H. Y. (2007). The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin Neurophysiol, 118*(5), 1028-1032.

Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron, 45*(2), 201-206.

Kelly, K. M., Nadon, N. L., Morrison, J. H., Thibault, O., Barnes, C. A., & Blalock, E. M. (2006). The neurobiology of aging. *Epilepsy Res, 68 Suppl 1*, S5-20.

Knopman, D. S., Parisi, J. E., Salviati, A., Floriach-Robert, M., Boeve, B. F., Ivnik, R. J. (2003). Neuropathology of cognitively normal elderly. *J Neuropathol Exp Neurol, 62*(11), 1087-1095.

Korchounov, A., & Ziemann, U. (2011). Neuromodulatory neurotransmitters influence LTP-like plasticity in human cortex: a pharmaco-TMS study. *Neuropsychopharmacology, 36*(9), 1894-1902.

Kuhl, D. E., Koeppe, R. A., Minoshima, S., Snyder, S. E., Ficaro, E. P., Foster, N. L. (1999). In vivo mapping of cerebral acetylcholinesterase activity in aging and Alzheimer's disease. *Neurology, 52*(4), 691-699.

Kuo, M. F., Grosch, J., Fregni, F., Paulus, W., & Nitsche, M. A. (2007). Focusing effect of acetylcholine on neuroplasticity in the human motor cortex. *J Neurosci, 27*(52), 14442-14447.

Lee, K. S., Frey, K. A., Koeppe, R. A., Buck, A., Mulholland, G. K., & Kuhl, D. E. (1996). In vivo quantification of cerebral muscarinic receptors in normal human aging using positron emission tomography and [11C]tropanyl benzilate. *J Cereb Blood Flow Metab, 16*(2), 303-310.

Lefaucheur, J. P. (2005). [Transcranial magnetic stimulation: applications in neurology]. *Rev Neurol (Paris), 161*(11), 1121-1130.

Li, S., Xia, M., Pu, F., Li, D., Fan, Y., Niu, H. (2011). Age-related changes in the surface morphology of the central sulcus. *Neuroimage, 58*(2), 381-390.

Lidow, M. S., Gallager, D. W., Rakic, P., & Goldman-Rakic, P. S. (1989). Regional differences in the distribution of muscarinic cholinergic receptors in the macaque cerebral cortex. *J Comp Neurol, 289*(2), 247-259.

Lynch, G., Rex, C. S., & Gall, C. M. (2006). Synaptic plasticity in early aging. *Ageing Res Rev, 5*(3), 255-280.

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD: an embarrassment of riches. *Neuron, 44*(1), 5-21.

Marra, C., Quaranta, D., Profice, P., Pilato, F., Capone, F., Iodice, F. (2012). Central cholinergic dysfunction measured "in vivo" correlates with different behavioral disorders in Alzheimer's disease and dementia with Lewy body. *Brain Stimul, 5*(4), 533-538.

Martorana, A., Mori, F., Esposito, Z., Kusayanagi, H., Monteleone, F., Codeca, C. (2009). Dopamine modulates cholinergic cortical excitability in Alzheimer's disease patients. *Neuropsychopharmacology, 34*(10), 2323-2328.

Mash, D. C., White, W. F., & Mesulam, M. M. (1988). Distribution of muscarinic receptor subtypes within architectonic subregions of the primate cerebral cortex. *J Comp Neurol, 278*(2), 265-274.

McKinney, M., & Jacksonville, M. C. (2005). Brain cholinergic vulnerability: relevance to behavior and disease. *Biochem Pharmacol, 70*(8), 1115-1124.

Mesulam, M. M. (1990). Human brain cholinergic pathways. *Prog Brain Res, 84*, 231-241.

Mesulam, M. M. (2004a). The cholinergic innervation of the human cerebral cortex. *Prog Brain Res, 145*, 67-78.

Mesulam, M. M. (2004b). The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? *Learn Mem, 11*(1), 43-49.

Mesulam, M. M. (2012). Cholinergic aspects of aging and Alzheimer's disease. *Biol Psychiatry, 71*(9), 760-761.

Mesulam, M. M. (2013). Cholinergic circuitry of the human nucleus basalis and its fate in Alzheimer's disease. *J Comp Neurol, 521*(18), 4124-4144.

Mesulam, M. M., Mufson, E. J., & Rogers, J. (1987). Age-related shrinkage of cortically projecting cholinergic neurons: a selective effect. *Ann Neurol, 22*(1), 31-36.

Mitsis, E. M., Cosgrove, K. P., Staley, J. K., Bois, F., Frohlich, E. B., Tamagnan, G. D. (2009). Age-related decline in nicotinic receptor availability with [(123)I]5-IA-85380 SPECT. *Neurobiol Aging, 30*(9), 1490-1497.

Mitsushima, D., Sano, A., & Takahashi, T. (2013). A cholinergic trigger drives learning-induced plasticity at hippocampal synapses. *Nat Commun, 4*, 2760.

Muller-Dahlhaus, F., Ziemann, U., & Classen, J. (2010). Plasticity resembling spike-timing dependent synaptic plasticity: the evidence in human cortex. *Front Synaptic Neurosci, 2*, 34.

Muller-Dahlhaus, J. F., Orekhov, Y., Liu, Y., & Ziemann, U. (2008). Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Exp Brain Res, 187*(3), 467-475.

Namba, H., Iyo, M., Fukushi, K., Shinotoh, H., Nagatsuka, S., Suhara, T. (1999). Human cerebral acetylcholinesterase activity measured with positron emission tomography: procedure, normal values and effect of age. *Eur J Nucl Med, 26*(2), 135-143.

Nardone, R., Bergmann, J., Christova, M., Caleri, F., Tezzon, F., Ladurner, G. (2012). Short latency afferent inhibition differs among the subtypes of mild cognitive impairment. *J Neural Transm, 119*(4), 463-471.

Nardone, R., Bergmann, J., De Blasi, P., Kronbichler, M., Kraus, J., Caleri, F. (2010). Cholinergic dysfunction and amnesia in patients with Wernicke-Korsakoff syndrome: a transcranial magnetic stimulation study. *J Neural Transm, 117*(3), 385-391.

Nardone, R., Bergmann, J., Kronbichler, M., Kunz, A., Klein, S., Caleri, F. (2008). Abnormal short latency afferent inhibition in early Alzheimer's disease: a transcranial magnetic demonstration. *J Neural Transm, 115*(11), 1557-1562.

Nardone, R., Bergmann, J., Tezzon, F., Ladurner, G., & Golaszewski, S. (2008). Cholinergic dysfunction in subcortical ischaemic vascular dementia: a transcranial magnetic stimulation study. *J Neural Transm, 115*(5), 737-743.

Nardone, R., Golaszewski, S., Ladurner, G., Tezzon, F., & Trinka, E. (2011). A Review of Transcranial Magnetic Stimulation in the in vivo Functional Evaluation of Central Cholinergic Circuits in Dementia. *Dement Geriatr Cogn Disord, 32*(1), 18-25.

Naveh-Benjamin, M., Cowan, N., Kilb, A., & Chen, Z. (2007). Age-related differences in immediate serial recall: dissociating chunk formation and capacity. *Mem Cognit, 35*(4), 724-737.

Oliviero, A., Profice, P., Tonali, P. A., Pilato, F., Saturno, E., Dileone, M. (2006). Effects of aging on motor cortex excitability. *Neurosci Res, 55*(1), 74-77.

Parikh, V., Kozak, R., Martinez, V., & Sarter, M. (2007). Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron, 56*(1), 141-154.

Park, D. C., & Reuter-Lorenz, P. (2009). The adaptive brain: aging and neurocognitive scaffolding. *Annu Rev Psychol, 60*, 173-196.

Picciotto, M.R., Higley, M.J., & Mineur, Y.S. (2012). Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron 76***,** 116-129.

Picciotto, M. R., Meenakshi, A., & Jentsch, J. D. (2002). Acetylcholine. In K. L. Davis, D. Charney, J. T. Coyle, & C. Nemeroff (Eds.), *Neuropsychopharmacology: The Fifth Generation of Progress* (pp. 3-14). Philadelphia, PA: Lippincott Williams & Wilkins.

Pitcher, J. B., Ogston, K. M., & Miles, T. S. (2003). Age and sex differences in human motor cortex input-output characteristics. *J Physiol, 546*(Pt 2), 605-613.

Price, J. L., McKeel, D. W., Jr., Buckles, V. D., Roe, C. M., Xiong, C., Grundman, M. (2009). Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease. *Neurobiol Aging, 30*(7), 1026-1036.

Rasmusson, D. D. (2000). The role of acetylcholine in cortical synaptic plasticity. *Behav Brain Res, 115*(2), 205-218.

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A. (2005). Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb Cortex, 15*(11), 1676-1689.

Raz, N., & Rodrigue, K. M. (2006). Differential aging of the brain: patterns, cognitive correlates and modifiers. *Neurosci Biobehav Rev, 30*(6), 730-748.

Reis, J., Swayne, O. B., Vandermeeren, Y., Camus, M., Dimyan, M. A., Harris-Love, M. (2008). Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. *J Physiol, 586*(2), 325-351.

Rissman, R. A., De Blas, A. L., & Armstrong, D. M. (2007). GABA(A) receptors in aging and Alzheimer's disease. *J Neurochem, 103*(4), 1285-1292.

Robertson, I. H. (2013). A noradrenergic theory of cognitive reserve: implications for Alzheimer's disease. *Neurobiol Aging, 34*(1), 298-308.

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., et al. (1994). Non- invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clini- cal application. Report of an IFCN committee. *Electroencephalogr. Clin. Neurophysiol.* 91, 79–92.

Sailer, A., Molnar, G. F., Paradiso, G., Gunraj, C. A., Lang, A. E., & Chen, R. (2003). Short and long latency afferent inhibition in Parkinson's disease. *Brain, 126*(Pt 8), 1883-1894.

Sakuma, K., Murakami, T., & Nakashima, K. (2007). Short latency afferent inhibition is not impaired in mild cognitive impairment. *Clinical Neurophysiology, 118*(7), 1460-1463.

Salat, D. H., Buckner, R. L., Snyder, A. Z., Greve, D. N., Desikan, R. S., Busa, E. (2004). Thinning of the cerebral cortex in aging. *Cereb Cortex, 14*(7), 721-730.

Salthouse, T. A. (2000). Aging and measures of processing speed. *Biol Psychol, 54*(1-3), 35-54.

Sarter, M., & Bruno, J. P. (2004). Developmental origins of the age-related decline in cortical cholinergic function and associated cognitive abilities. *Neurobiol Aging, 25*(9), 1127-1139.

Sarter, M., Hasselmo, M. E., Bruno, J. P., & Givens, B. (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Rev, 48*(1), 98-111.

Sarter, M., Lustig, C., Howe, W. M., Gritton, H., & Berry, A. S. (2014). Deterministic functions of cortical acetylcholine. *Eur J Neurosci, 39*(11), 1912-1920.

Sarter, M., Lustig, C., & Taylor, S. F. (2012). Cholinergic contributions to the cognitive symptoms of schizophrenia and the viability of cholinergic treatments. *Neuropharmacology, 62*(3), 1544-1553.

Sarter, M., Parikh, V., & Howe, W. M. (2009). Phasic acetylcholine release and the volume transmission hypothesis: time to move on. *Nat Rev Neurosci, 10*(5), 383-390.

Scarr, E., Gibbons, A. S., Neo, J., Udawela, M., & Dean, B. (2013). Cholinergic connectivity: it's implications for psychiatric disorders. *Front Cell Neurosci, 7*, 55.

Schliebs, R., & Arendt, T. (2006). The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. *J Neural Transm, 113*(11), 1625-1644.

Schliebs, R., & Arendt, T. (2011). The cholinergic system in aging and neuronal degeneration. *Behav Brain Res*, *221*(2), 555-563.

Segovia, G., Porras, A., Del Arco, A., & Mora, F. (2001). Glutamatergic neurotransmission in aging: a critical perspective. *Mech Ageing Dev, 122*(1), 1-29.

Selden, N. R., Gitelman, D. R., Salamon-Murayama, N., Parrish, T. B., & Mesulam, M. M. (1998). Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. *Brain, 121 (Pt 12)*, 2249-2257.

Staub, B., Doignon-Camus, N., Despres, O., & Bonnefond, A. (2013). Sustained attention in the elderly: what do we know and what does it tell us about cognitive aging? *Ageing Res Rev, 12*(2), 459-468.

Stefan, K., Kunesch, E., Benecke, R., Cohen, L. G., & Classen, J. (2002). Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *J Physiol, 543*(Pt 2), 699-708.

Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R., & Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain, 123 Pt 3*, 572-584.

Sunderland, T. (2005). Neurotransmission in the aging central nervous system. In C. Salzman (Ed.), *Clinical geriatric psychopharmacology* (pp. 64-85). Philadelphia, PA: Lippincott Williams & Wilkins.

Suva, D., Favre, I., Kraftsik, R., Esteban, M., Lobrinus, A., & Miklossy, J. (1999). Primary motor cortex involvement in Alzheimer disease. *J Neuropathol Exp Neurol, 58*(11), 1125-1134.

Swayne, O. B., Teo, J. T., Greenwood, R. J., & Rothwell, J. C. (2009). The facilitatory effects of intermittent theta burst stimulation on corticospinal excitability are enhanced by nicotine. *Clin Neurophysiol, 120*(8), 1610-1615.

Tecchio, F., Zappasodi, F., Pasqualetti, P., De Gennaro, L., Pellicciari, M. C., Ercolani, M. (2008). Age dependence of primary motor cortex plasticity induced by paired associative stimulation. *Clin Neurophysiol, 119*(3), 675-682.

Teo, J. T. H., Swayne, O. B., & Rothwell, J. C. (2007). Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. *Clin Neurophysiol, 118*, 1648-1651.

Terao, Y., & Ugawa, Y. (2002). Basic mechanisms of TMS. *J Clin Neurophysiol, 19*(4), 322-343.

Terry, A. V., Jr., & Buccafusco, J. J. (2003). The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. *J Pharmacol Exp Ther, 306*(3), 821-827.

Thickbroom, G. W. (2007). Transcranial magnetic stimulation and synaptic plasticity: experimental framework and human models. *Exp Brain Res, 180*(4), 583-593.

Thienel, R., Kellermann, T., Schall, U., Voss, B., Reske, M., Halfter, S. (2009). Muscarinic antagonist effects on executive control of attention. *Int J Neuropsychopharmacol, 12*(10), 1307-1317.

Thienel, R., Voss, B., Kellermann, T., Reske, M., Halfter, S., Sheldrick, A. J. (2009). Nicotinic antagonist effects on functional attention networks. *Int J Neuropsychopharmacol, 12*(10), 1295-1305.

Thirugnanasambandam, N., Grundey, J., Adam, K., Drees, A., Skwirba, A. C., Lang, N. (2011). Nicotinergic impact on focal and non-focal neuroplasticity induced by non-invasive brain stimulation in non-smoking humans. *Neuropsychopharmacology, 36*(4), 879-886.

Tokimura, H., Di Lazzaro, V., Tokimura, Y., Oliviero, A., Profice, P., Insola, A. (2000). Short latency inhibition of human hand motor cortex by somatosensory input from the hand. *J Physiol, 523 Pt 2*, 503-513.

Tsutsumi, R., Hanajima, R., Hamada, M., Shirota, Y., Matsumoto, H., Terao, Y. (2012). Reduced interhemispheric inhibition in mild cognitive impairment. *Exp Brain Res, 218*(1), 21-26.

Turrini, P., Casu, M. A., Wong, T. P., De Koninck, Y., Ribeiro-da-Silva, A., & Cuello, A. C. (2001). Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: synaptic pattern and age-related atrophy. *Neuroscience, 105*(2), 277-285.

Villeneuve, S., & Belleville, S. (2010). [Cognitive reserve and neuronal changes associated with aging]. *Psychol Neuropsychiatr Vieil, 8*(2), 133-140.

von Engelhardt, J., Eliava, M., Meyer, A. H., Rozov, A., & Monyer, H. (2007). Functional characterization of intrinsic cholinergic interneurons in the cortex. *J Neurosci, 27*(21), 5633-5642.

Warsch, J. R., & Wright, C. B. (2010). The aging mind: vascular health in normal cognitive aging. *Journal of the American Geriatrics Society, 58 Suppl 2*, S319-324.

Wolf, D., Grothe, M., Fischer, F. U., Heinsen, H., Kilimann, I., Teipel, S. (2014). Association of basal forebrain volumes and cognition in normal aging. *Neuropsychologia, 53*, 54-63.

Yamasaki, M., Matsui, M., & Watanabe, M. (2010). Preferential localization of muscarinic M1 receptor on dendritic shaft and spine of cortical pyramidal cells and its anatomical evidence for volume transmission. *J Neurosci, 30*(12), 4408-4418.

Yarnall, A. J., Rochester, L., Baker, M. R., David, R., Khoo, T. K., Duncan, G. W. (2013). Short latency afferent inhibition: a biomarker for mild cognitive impairment in Parkinson's disease? *Mov Disord, 28*(9), 1285-1288.

Ylikoski, R., Ylikoski, A., Keskivaara, P., Tilvis, R., Sulkava, R., & Erkinjuntti, T. (1999). Heterogeneity of cognitive profiles in aging: successful aging, normal aging, and individuals at risk for cognitive decline. *Eur J Neurol, 6*(6), 645-652.

Zaborszky, L., Csordas, A., Mosca, K., Kim, J., Gielow, M.R., Vadasz, C., & Nadasdy, Z. (2013). Neurons in the Basal Forebrain Project to the Cortex in a Complex Topographic Organization that Reflects Corticocortical Connectivity Patterns: An Experimental Study Based on Retrograde Tracing and 3D Reconstruction. *Cereb Cortex*. [Epub ahead of print].

Ziegler, D. A., Piguet, O., Salat, D. H., Prince, K., Connally, E., & Corkin, S. (2010). Cognition in healthy aging is related to regional white matter integrity, but not cortical thickness. *Neurobiol Aging, 31*(11), 1912-1926.

Zubieta, J. K., Koeppe, R. A., Frey, K. A., Kilbourn, M. R., Mangner, T. J., Foster, N. L. (2001). Assessment of muscarinic receptor concentrations in aging and Alzheimer disease with [11C]NMPB and PET. *Synapse, 39*(4), 275-287.