Age-Related Neural Dedifferentiation in the Sensorimotor System and its Behavioral Consequences

by

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DEDICATION

To my parents and my sister,
for their unconditional love and support all these years
Acknowledgments

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Abstract

Tens of millions of people experience age-related declines in sensorimotor functioning, but the neurobiological mechanisms underlying this decline are not well understood. Previous studies have linked age-related behavioral declines to decreases in neural differentiation (i.e., dedifferentiation), including decreases in the distinctiveness of neural activation patterns and in the segregation among large-scale neural networks. However, no studies to date have explored the relationship between these two neural measures and whether they explain the same aspects of sensorimotor behavior. Furthermore, no studies have explored the potential neurochemical substrates of age-related neural dedifferentiation. The present research sought to explore the neural and behavioral mechanisms of age-related neural dedifferentiation in the sensorimotor system using multi-voxel pattern analysis to examine the distinctiveness of sensorimotor neural representations, and graph theoretical analysis to examine the segregation of resting state sensorimotor networks in young and older adults. Study 1 demonstrated that these two measures of neural dedifferentiation are related. Segregation also predicted individual differences in sensorimotor performance, particularly in older adults, whereas distinctiveness did not, suggesting that segregation may be a more sensitive predictor of age-related declines in sensorimotor behavior. Study 2 extended this investigation by exploring potential neurochemical causes of age-related dedifferentiation in the sensorimotor system. Using MR spectroscopy to measure the inhibitory neurotransmitter, gamma aminobutyric acid (GABA), the results revealed that sensorimotor network segregation is linked to sensorimotor GABA levels and that these levels decline with age. Furthermore, individual differences in GABA predicted sensorimotor performance and this relationship was mediated by network segregation. These findings link age-related differences in network segregation to age-related differences in GABA levels and sensorimotor performance. In general, they suggest a neurochemical substrate of age-related dedifferentiation at the level of large-scale brain networks. Taken together, the findings from this dissertation advance our understanding of the neural and neurochemical mechanisms that underlie age-related declines in sensorimotor functioning. These findings may help lead to the
development of targeted interventions and treatments to combat age-related sensorimotor behavioral decline.
Normal aging is associated with declines in cognitive and sensorimotor performance, even in the absence of significant disease. For instance, measures of fluid processing abilities, such as speed of processing and executive function, tend to decline with age compared with measures of crystallized knowledge (Salthouse, 1996; Park et al., 2002). Aging is also linked to declines in sensorimotor function, such as fine motor dexterity, gait and tactile sensitivity (Seidler et al., 2010; Zhang et al., 2011). However, while older adults demonstrate poorer performance than young adults on average, some older adults continue to perform relatively well while others exhibit more significant impairments (Cabeza et al., 2002; Reuter-Lorenz, 2002). Understanding the causes of such individual differences is a central challenge in the field. Developing such an understanding will allow researchers to design targeted interventions to help extend work productivity and facilitate activities of daily living for older adults. One possibility is that age-related behavioral deficits are at least partially attributable to age declines in the specificity of neural representations. Li et al. (Li and Lindenberger, 1999; Li et al., 2000, 2001; Li and Sikstrom, 2002; Li and Rieckmann, 2014) proposed that age-related impairments in neurotransmission lead to decreases in the signal to noise ratio of neural signals. As a result, neural activation patterns in response to different mental states become less distinctive with increasing age. Using a computational model, Li et al. (2000) simulated this hypothesis and
demonstrated that such age-related neural dedifferentiation could account for many aspects of age-related declines in behavior.

Subsequent work in non-human primates provided direct evidence for neural dedifferentiation in the aging brain. Schmolesky and colleagues recorded neural activity from cells in primary visual cortex of young and old macaque monkeys. They found that neural receptive fields were more selective in young monkeys than in old monkeys (Schmolesky et al., 2000). This group has reported comparable findings across visual modalities (Liang et al., 2010), and in cats (Hua et al., 2006) and rats (Wang et al., 2006).

Extending this line of work to humans, several studies have demonstrated age-related neural dedifferentiation of perceptual (Park et al., 2004, 2010), motor (Carp et al., 2011), and cognitive representations (Carp et al., 2010). In one of the first studies, Park et al. (2004) used functional magnetic resonance imaging (fMRI) to demonstrate that neural activation patterns evoked by faces, places, and words were significantly more distinctive in young adults compared to older adults. In particular, activity in the fusiform face area (FFA) was more selective to faces, activity in the parahippocampal place area (PPA) was more selective to places, and activity in the visual word form area (VWFA) was more selective to words.

More recently, Seidler and colleagues provided evidence for motor cortex dedifferentiation in older adults (Seidler et al., 2010). Specifically, they used transcranial magnetic stimulation (TMS) to demonstrate that contralateral motor cortical representations for the dorsal interosseous (FDI) muscle are more spatially extensive in older than younger adults, suggesting reduced
*intra*hemispheric distinctiveness (Bernard and Seidler, 2012). In another study, they demonstrated that older adults exhibited greater recruitment of ipsilateral motor cortex than young adults during a unimanual motor task, suggesting reduced *inter*hemispheric distinctiveness (Langan et al., 2010).

These previous neuroimaging studies focused on age differences in mean localized activation. However, such measures may not capture information encoded across multi-voxel activation patterns (Haynes and Rees, 2006; Norman et al., 2006). Differences in activation patterns that cannot be detected by univariate analyses may, however, be discriminable by multivariate techniques. Utilizing multi-voxel pattern analysis (MVPA), Carp et al. (2011) measured age differences in the distinctiveness of neural activation patterns induced by left vs. right hand finger tapping in young and older adults. They found that neural distinctiveness was reduced among older adults compared to younger adults throughout the motor control network, including primary motor cortex (M1), supplemental motor area (SMA), insula, and cerebellum. Another study investigated age differences in neural representations in the context of verbal and spatial working memory tasks, and found reduced distinctiveness in the activation patterns associated with verbal encoding and retrieval vs. spatial information in older adults (Carp et al., 2010).

Age-related reductions in neural distinctiveness are typically reported in localized brain areas, but most cognitive and sensorimotor functions require the interaction of multiple large-scale brain networks. It is therefore logical to ask whether age-related neural dedifferentiation also occurs at the network level. And the answer appears to be yes.
It is well established that brain activity at rest is organized into mainly segregated functional networks in young adults (Buckner et al., 2013). Areas within these different networks reveal spontaneous yet correlated activity; these correlations are highly spatially structured, following known anatomical networks, and thus are hypothesized to reflect functional connectivity of the human brain (Biswal et al., 1995; Fox et al., 2005; Damoiseaux et al., 2006; Fox and Raichle, 2007). Furthermore, functional connectivity strength has been correlated with behavior in a network-specific manner (Greicius et al., 2004; Raichle and Mintun, 2006; Wang et al., 2007; Smith et al., 2009; Kwak et al., 2010; Langan et al., 2010).

And accumulating evidence points to age differences in the organization of these functional brain networks (Damoiseaux, 2017). This organization is typically investigated using graph theoretical analysis of resting state functional connectivity data. In graph theory, a network is a set of nodes (i.e., regions of interest; ROIs) with edges (i.e., correlations) between them (Bullmore and Sporns, 2009). These ROIs can be defined previously or using data-driven techniques. Using this framework, multiple measures of brain networks can be calculated. One common measure used in graph theory is network segregation, defined as the scaled difference between within-network and between-network connectivity. Numerous studies have demonstrated that older age is associated with less segregated (i.e., dedifferentiated) networks (Betzel et al., 2014; Cao et al., 2014, 2014; Chan et al., 2014; Geerligs et al., 2015; Song et al., 2014., Iordan et al., 2018).

Several of these studies also observed associations between segregation and cognitive function, such that participants with less segregated networks demonstrated worse cognitive performance (Damoiseaux et al., 2008; Wang et al., 2010; Chan et al., 2014; Geerligs et al., 2015). For
instance, Chan and colleagues found that the magnitude of network segregation is predictive of long-term memory function, independent of an individual’s age (Chan et al., 2014). Fewer studies have investigated the relationship between age-related differences in network segregation and sensorimotor behavior. One such study conducted by King and colleagues found that age-related declines in bimanual motor performance are associated with decreased segregation of several large-scale resting state brain networks (King et al., 2017). Overall, these findings suggest that such age-related decreases in network segregation may contribute to age-related declines in cognitive and sensorimotor performance.

The studies reviewed so far focused on dedifferentiation of neural activation patterns or dedifferentiation of resting state brain networks. An important open question is whether these different measures are actually measuring the same underlying construct and whether they predict the same aspects of age-related declines in performance. Previous work has found a relationship between the brain’s large-scale functional organization at rest and its functional recruitment during task performance. For instance, Langan and colleagues found that reduced interhemispheric resting state connectivity in older adults was associated with a decreased ability to inhibit activity in the non-dominant hemisphere during unimanual motor performance (Langan et al., 2010). Another study conducted by Chan and colleagues found that individual differences in functional connectivity were related to differences in brain activity during two independent tasks (Chan et al., 2017). These findings suggest that age-related neural dedifferentiation of resting state networks may be linked to dedifferentiation of task-based activation patterns.
Another important open question is what causes age-related neural dedifferentiation. Previous animal studies have linked neural dedifferentiation to changes at the neurotransmitter level. In particular, a series of animal studies conducted by Leventhal and colleagues suggest that age differences in the brain’s major inhibitory neurotransmitter, gamma aminobutyric acid (GABA), may play an important and potentially causal role in age-related neural dedifferentiation (Wang et al., 2006; Schmolesky et al., 2000; Leventhal et al., 2003; Hua et al., 2006). In one seminal study, Leventhal and colleagues measured the receptive fields of single cells in the visual cortex of young and old macaque monkeys (Leventhal et al., 2003). The receptive fields of neurons in the young monkeys were more selective to specific orientations and directions than were the receptive fields of neurons in the old monkeys, consistent with age-related neural dedifferentiation. However, the application of GABA, or a GABA agonist, made the neurons in the old monkeys much more selective, similar to neurons in the young monkeys. Conversely, the application of a GABA antagonist to the visual neurons of young macaques disrupted the selectivity of neurons, making their response similar to that of neurons in the old monkeys. These results demonstrate that manipulations of GABA cause changes in neural selectivity, so it is at least possible that age-related declines in GABA contribute to age-related neural dedifferentiation and associated behavioral declines in humans.

Consistent with this hypothesis, more recent studies in humans have linked individual differences in GABA levels to differences in perceptual and motor performance across healthy young adults. For instance, Edden and colleagues observed that better orientation discrimination performance is predicted by higher GABA levels in primary visual cortex (Edden et al., 2009). In addition, Boy and colleagues found that GABA levels in SMA are associated with individual
differences in subconscious motor performance (Boy et al., 2010). Finally, Puts et al. (2011) reported that higher GABA levels in sensorimotor cortex correlate with better performance during a vibrotactile discrimination task (Puts et al., 2011).

Prior work has also found a relationship between GABA levels and functional connectivity within specific resting state networks. For instance, Stagg and colleagues demonstrated that resting state functional connectivity strength within the motor network was associated with GABA levels in primary motor cortex (Stagg et al., 2014). Similarly, Kapogiannis and colleagues observed a relationship between GABA levels in posteromedial cortex and the strength of default mode network functional connectivity (Kapogiannis et al., 2013). While these studies have linked individual differences in functional connectivity to differences in GABA levels, no studies to date have explored whether this relationship varies with age. Furthermore, no studies have examined GABA levels, network segregation and/or neural distinctiveness, and sensorimotor behavior within the same individuals in order to explore the potential links between all three.

The present research sought to address these limitations and gaps in understanding. In this dissertation, I conducted two studies to investigate age-related neural dedifferentiation in the sensorimotor system. In study one, I investigated the relationship between age-related neural dedifferentiation of large-scale resting state brain networks and dedifferentiation of neural activation patterns during (sensorimotor) task performance. I also collected a battery of sensorimotor behavioral measures in older and younger adults to determine whether these two measures of neural dedifferentiation explain the same aspects of sensorimotor behavior. This
study was recently submitted for publication and the chapter describing it corresponds to the submitted manuscript.

In study two, I explored the relationship between sensorimotor cortex GABA levels and sensorimotor network segregation in a subset of the same participants. I also applied a mediation analysis to investigate whether individual differences in GABA levels predict differences in sensorimotor behavior, and whether segregation mediates this relationship. This study was recently published in Neuroimage and the chapter describing it corresponds to the published article.

The findings from these studies advance our understanding of the neural and neurochemical mechanisms underlying age-related declines in sensorimotor functioning, which could ultimately lead to the development of targeted interventions to prolong functional independence for older adults.

*Neuroimaging Methods*

*Multi-voxel pattern analysis*

A central challenge in cognitive neuroscience is determining how mental representations map onto patterns of neural activity. Functional MRI is a powerful method to address this question. While a participant performs a task, researchers can acquire estimates of local blood flow from tens of thousands of specific neuroanatomical locations within a matter of seconds. However,
more recent studies argue that traditional univariate analyses that focus on mean regional activation often fail to detect information encoded in multi-voxel activation patterns (Haxby et al., 2001; Kamitani and Tong, 2005). Multi-voxel pattern analysis (MVPA) methods have been shown to provide higher sensitivity than traditional univariate measures for two main reasons (Norman et al., 2006). First, univariate analyses threshold the significance of each brain voxel individually, such that information from subthreshold voxels is lost. On the other hand, MVPA can incorporate information from voxels that do not show significant responses based on single-voxel significance testing. Second, conventional univariate methods typically use spatial averaging that likely blurs out fine-grained spatial patterns that may discriminate between experimental conditions (Kriegeskorte et al., 2006). In contrast, MVPA typically does not involve spatial averaging of voxel responses. Rather, it uses pattern-classification methods to extract the signal that is present in the pattern of response across multiple voxels, even if the voxels considered individually may not be significantly responsive to any of the experimental conditions.

The studies included in this dissertation illustrate how MVPA can be used to distinguish between cognitive states. Specifically, we performed MVPA to measure the distinctiveness of sensorimotor (i.e., right hand vs. left hand movement) representations. Based on the work of Haxby and colleagues, neural distinctiveness was defined as the difference between the average within-condition Pearson correlation (i.e., the average of the correlations between left hand patterns and correlation between right hand patterns) and the average between-condition correlation (i.e., the average of the correlations between left-hand and right-hand patterns)
(Haxby et al., 2001). This approach was used instead of alternative classification methods (e.g., support vector machines) to avoid ceiling effects in classifier accuracy (Carp et al., 2011b).

Graph theoretical analysis

Brain functional connectivity refers to the coherence between temporal fluctuations in the blood oxygen level dependent (BOLD) signal across brain regions (Damoiseaux, 2017). Functional connectivity measures are frequently obtained from resting state fMRI data, in which participants are not presented with a task but are instructed to relax and let their mind wander. Whole-brain approaches such as graph theoretical analyses have been used with resting state data to examine the large-scale organization of functional brain networks. Some of these networks include default mode, executive control, attention, salience, sensorimotor, and visual networks (Damoiseaux et al., 2006; Heuvel et al., 2008; Zuo et al., 2010). In graph theory, a network is a set of nodes with edges between them (Bullmore and Sporns, 2009). Using this model, multiple measures of brain networks can be calculated. One common measure used in graph theory (and in this dissertation) is network segregation. This is defined as the difference between mean within-network connectivity and mean between-network connectivity, as a proportion of mean within-network connectivity (Chan et al., 2014). In this dissertation, we performed graph theoretical analysis to measure sensorimotor segregation and its relationship with sensorimotor GABA levels and sensorimotor performance.

Magnetic resonance spectroscopy
Magnetic resonance spectroscopy (MRS) is a method that enables the measurement of neurotransmitters in the human brain non-invasively. MRS was traditionally applied within clinical contexts. However, in recent years there has been increasing interest in using MRS to study the healthy human brain. Although the majority of cortical neurons are glutamatergic, about one sixth of cortical neurons are GABAergic inhibitory interneurons (Buzsáki et al., 2007). Several research groups have used MRS to study the role of inhibitory processes in normal brain function and in the pathophysiology of disease. Among a diverse set of methods for studying GABA and GABAergic mechanisms, MRS is the only technique that allows direct, non-invasive detection of endogenous GABA in the human brain in vivo.

MRS is the in vivo application of nuclear magnetic resonance (NMR) spectroscopy, and it is applied with magnetic resonance imaging (MRI) scanners. MRS identifies radiofrequency signals that are emitted from hydrogen nuclear spins in tissue metabolites. These nuclear spins have chemically specific frequencies that are determined by the chemical environment of the hydrogen spins. MRS signals are thus separated in the MR spectrum along chemical lines, referred to as the chemical shift dimension (Puts and Edden, 2012). Chemical shift is reported in field-independent units, ppm (or parts per million of the proton frequency). GABA is present in the human brain at a concentration of about 1mM, which is extremely low compared to some of the more concentrated metabolites. Because of the limited dispersion of signals along the chemical shift axis, researchers use different approaches to reduce signal overlap in order to extract GABA signals. One common approach is to use spectral editing methods, which make it possible to separate GABA signals from the rest of the spectrum. The GABA signal at 3.0 ppm is coupled to the signal at 1.9 ppm. In contrast, most signals at 3.0ppm are not coupled to 1.9 ppm,
which makes it possible to separate the signals on this basis. This editing scheme, called J-difference editing, is used with the method most commonly applied in MRS research, the MEGA-PRESS method (Mescher et al., 1998). In recent years, investigators have used MRS to study individual differences in GABA concentration and its relationship to inhibition-dependent cognitive processes. For instance, in this dissertation, we examined age differences in sensorimotor cortex GABA levels, and its relationship with neural dedifferentiation and sensorimotor behavior.
References


Fox MD, Raichle ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci 8:700–711.


Chapter II: Network segregation varies with neural distinctiveness in sensorimotor cortex and predicts sensorimotor performance

Abstract

Normal aging is associated with declines in sensorimotor function. Previous studies have linked age-related behavioral declines to decreases in neural differentiation (i.e., dedifferentiation), including decreases in the distinctiveness of neural activation patterns and in the segregation among large-scale neural networks at rest. However, no studies to date have explored the relationship between these two neural measures and whether they explain the same aspects of behavior. To investigate these issues, we collected a battery of sensorimotor behavioral measures in older and younger adults and estimated (a) the distinctiveness of neural representations in sensorimotor cortex and (b) sensorimotor network segregation in the same participants. Consistent with prior findings, sensorimotor representations were less distinct and sensorimotor resting state networks were less segregated in older compared to younger adults. We also found that participants with the most distinct sensorimotor representations exhibited the most segregated sensorimotor networks. However, only sensorimotor network segregation was associated with individual differences in sensorimotor performance, particularly in older adults. These novel findings link network segregation to neural distinctiveness, but also suggest that network segregation may play a larger role in maintaining sensorimotor performance with age.
Introduction

Aging is associated with extensive declines in sensorimotor function including fine motor control, gait, and balance (Seidler et al., 2010). Although these declines can reflect changes in the peripheral sensorimotor system (Cole et al., 1999), they also likely reflect changes in the central nervous system, such as alterations in brain structure and function (Seidler et al., 2010). A central challenge, then, is to advance our understanding of the neural mechanisms that underlie age-related declines in sensorimotor function. Doing so could lead to new interventions to extend work productivity and facilitate a variety of daily life activities in older adults.

Previous research has found that neural representations are less selective, or distinctive, in older compared with younger adults. This phenomenon is often referred to as age-related neural dedifferentiation (Li and Lindenberger, 1999), reflecting the fact that neural activity in response to different stimulus categories is less differentiated in older adults. For example, in young adults, the neural activation patterns evoked by looking at pictures of faces are quite different from those evoked by looking at pictures of houses. However, these activation patterns are more similar (i.e., less distinct or less differentiated) in older adults (Park et al., 2004). Furthermore, older adults who exhibit less distinct neural representations often perform significantly worse on a range of cognitive (Park et al., 2010) and motor tasks (Bernard and Seidler, 2012) than older adults whose neural representations are more distinct.

Accumulating evidence also points to age differences in the organization of functional brain networks (Damoiseaux, 2017). This type of organization is most frequently investigated using
resting-state functional connectivity (Biswal et al., 1995) and more recently, using graph theoretical analyses (Bullmore and Sporns, 2009). In graph theory, a network is a set of nodes with edges between them. Using this framework, one can calculate multiple measures of brain networks. One common measure is network segregation, defined as the scaled difference between within-network connectivity and between-network connectivity. A number of studies have demonstrated that older age is associated with less segregated (i.e., dedifferentiated) networks (Damoiseaux, 2017). Further, less segregated networks predict poorer cognitive (Chan et al., 2014) and sensorimotor (King et al., 2018) performance.

Given that both neural distinctiveness and network segregation decline with age and that both are associated with behavior, a natural question is whether these two measures of neural dedifferentiation are actually measuring the same underlying construct and whether they predict similar aspects of behavior. One reason to suspect that they do is that previous work has found a relationship between the brain’s functional connectivity at rest and its functional recruitment during task performance. For instance, Langan and colleagues found that reduced resting state connectivity between motor cortices in older adults was associated with greater activity in the non-dominant motor cortex during unimanual motor performance (Langan et al., 2010). Chan et al. recruited participants across the adult life span (from ages 20-89 years), and demonstrated that reduced differentiation between network-specific connector and non-connector nodes measured at rest correlated with reduced differentiation of connector vs. non-connector nodes during visual and semantic task performance (Chan et al., 2017). These findings suggest that network topology observed at rest may constrain functional activity of brain areas during motor, visual and
semantic task performance, and that network segregation might be closely related to neural distinctiveness.

To address the relationship between neural distinctiveness and network segregation in the sensorimotor domain, we measured both in the same participants. We also collected a battery of sensorimotor behavioral measures to examine the relationship between the neural measures and performance.

We explored three questions. First, do older adults exhibit reduced sensorimotor distinctiveness and sensorimotor network segregation relative to young adults? Second, are less distinct sensorimotor representations associated with less segregated sensorimotor networks? Third, are both neural distinctiveness and network segregation associated with sensorimotor performance and does either explain significant behavioral variance over and above the other?

**Methods and materials**

**Participants**

We collected data from 25 younger adults (age range 19 to 29 years; 16 females) and 46 older adults (age range 65 to 81 years; 28 females), some of whom overlap with our previous study (Cassady et al., 2019). All participants were right-handed, native English speakers. They were screened to ensure they were not taking any medications with psychotropic effects, and were free of any MRI safety contraindications. Participants were also screened for cognitive impairments using the Montreal Cognitive Assessment (Brenkel et al., 2017), and only those with scores ≥23
were included in this study. Two younger adult participants were excluded from further analyses because of excessive head motion in the MRI scanner (more than 2 mm or 2 degrees in any axis). A detailed explanation of the study was provided, and written informed consent was obtained from all participants. The study was approved by the Institutional Review Board of the University of Michigan.

**Experimental Design and Statistical Analysis**

All participants completed two separate test sessions: an imaging session during which we collected task-based and resting-state fMRI data and a behavior session during which we collected sensorimotor behavioral data. The order of the fMRI and behavioral sessions was counterbalanced across participants. All data was obtained within an average period of 24 days.

To examine age differences in neural distinctiveness, network segregation and behavior between young and older adults, we performed independent sample t-tests. To assess the relationship between distinctiveness, segregation, and behavior, we performed partial correlations analyses across all participants (controlling for age and gray matter (GM) volume differences). We further explored these relationships within each age group separately using bivariate correlation analyses. For all statistical analyses, data points greater than three standard deviations above or below the group mean were excluded. SPSS software was used for all statistical analyses (SPSS Inc., Chicago IL).

**Sensorimotor assessments**
We used a National Institute of Health sensorimotor test battery that includes tests of fine motor dexterity (tested with the 9-hole pegboard dexterity test), grip strength, and endurance (measured with a 2-minute walk endurance test). Please refer to Cassady et al., 2019 for details of the sensorimotor assessments. A summary of age-group means (and standard errors) for each behavioral measure is included in Table 1. Participants’ scores for all tests were submitted to an exploratory factor analysis in order reduce the dimensionality of the data. Please refer to the supplemental material for factor analysis model coefficients across all participants (Table 2).

**Table 1.** Mean and standard error of demographics and behavioral measures across all participants, and separately in the older and younger adult groups. GS = Grip strength.

<table>
<thead>
<tr>
<th>Behavioral measure</th>
<th>All participants</th>
<th>Old adults</th>
<th>Young adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>53.4 ± 2.8</td>
<td>70.4 ± 0.6</td>
<td>22.9 ± 0.6</td>
</tr>
<tr>
<td>MoCA</td>
<td>27.6 ± 0.2</td>
<td>27.3 ± 0.3</td>
<td>28.2 ± 0.3</td>
</tr>
<tr>
<td>Dexterity</td>
<td>102.1 ± 1.1</td>
<td>98.4 ± 1.2</td>
<td>109 ± 1.1</td>
</tr>
<tr>
<td>GS</td>
<td>99 ± 1.4</td>
<td>97.2 ± 1.7</td>
<td>102.4 ± 2.5</td>
</tr>
<tr>
<td>Endurance</td>
<td>90.6 ± 1.5</td>
<td>86 ± 1.7</td>
<td>99.3 ± 2</td>
</tr>
</tbody>
</table>

**Table 2.** Model coefficients from factor analysis across all participants. GS = Grip strength.

<table>
<thead>
<tr>
<th>Behavioral measure</th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexterity dominant</td>
<td>-0.04</td>
<td><strong>0.63</strong></td>
</tr>
<tr>
<td>Dexterity non-dominant</td>
<td>-0.11</td>
<td><strong>1.03</strong></td>
</tr>
<tr>
<td>GS dominant</td>
<td><strong>1.03</strong></td>
<td>-0.1</td>
</tr>
<tr>
<td>GS non-dominant</td>
<td><strong>0.96</strong></td>
<td>-0.08</td>
</tr>
<tr>
<td>Endurance</td>
<td><strong>0.52</strong></td>
<td>0.35</td>
</tr>
</tbody>
</table>

*MRI data acquisition*
Structural and functional brain images were obtained using a GE Signa 3-Tesla MRI scanner, located at the University of Michigan Functional Magnetic Resonance Imaging Laboratory. A 16-rod bird cage head coil was used for all participants, and movement was minimized by using head cushions and Velcro straps. During each participant’s scanning session, we acquired T1-weighted structural images, high-resolution structural images using spoiled 3D gradient-echo acquisition (SPGR), and T2*-weighted functional images. Functional images were obtained using a single-shot gradient-echo (GRE) reverse spiral pulse sequence. The field of view was 220 x 200 mm, the voxel size was 3 x 3 x 4 mm (40 axial slices), the TR (repetition time) was 2 seconds, and the TE (echo time) was 30 ms.

**Resting state fMRI preprocessing and analysis**

Preprocessing of the resting state fMRI data was performed with the Statistical Parametric Mapping 8 software (SPM8; [www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)). Preprocessing steps included slice-time correction, realignment, segmentation of structural images, normalization into standard Montreal Neurological Institute (MNI) space, and spatial smoothing using a Gaussian kernel of 8mm full width at half-maximum. To detect and reject head motion artifacts in the scanner, we used the Artifact Detection Toolbox (ART; [https://www.nitrc.org/projects/artifact_detect](https://www.nitrc.org/projects/artifact_detect)).

We performed additional denoising on the resting state data with the CONN toolbox. The data were first filtered using a temporal band-pass filter of 0.008 to 0.09 Hz to examine the frequency band of interest and to exclude higher frequency sources of noise. For additional noise reduction,
the anatomical component-based noise correction method, aCompCor (Behzadi et al., 2007), was used.

The signals from all ROIs (see below for ROI definitions) were extracted from the unsmoothed functional images to avoid potential “spillage” of the BOLD signal from nearby regions. Residual head motion parameters (three rotations, three translations, and six parameters representing their first-order temporal derivatives) and signals from white matter (WM) and cerebrospinal fluid (CSF) were regressed out during the calculation of functional connectivity maps.

We performed an ROI-to-ROI first-level functional connectivity analysis using the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). To do so, we first created (5mm diameter sphere) ROIs using MNI coordinates published in Power et al. (Power et al., 2011). We used all coordinates from this previous study except for those that belonged to “subcortical” and “undefined” networks, leaving us with 214 ROIs. Each ROI was labeled according to this published functional network map, which included ten networks (including Hand sensorimotor, Visual, Mouth sensorimotor, Auditory, Default, Frontal-parietal control, Ventral attention, Cingulo-opercular control, Dorsal attention, and Salience networks). For each participant, the resting state time series within each ROI was then extracted from the unsmoothed functional images and the mean time series was computed. Next, the cross-correlation between each ROI’s time course with every other ROI’s time course was calculated, producing a 214 x 214 correlation matrix for each participant. Fisher’s r-to-z transformation was then used to convert correlation coefficients (i.e., graph edges) into z-values. Last, negatively-weighted edges were
set to zero in each participant’s correlation matrix to avoid potential misinterpretation of negative edge weights (Chan et al., 2014, 2017).

Network segregation was calculated to examine within-network correlations in relation to between-network correlations. As introduced in Chan et al. (2014), network segregation was defined as the difference in mean within-network connectivity and mean between-network connectivity as a proportion of mean within-network connectivity:

\[
\text{Network segregation} = \frac{Z_w - Z_b}{Z_w}
\]

where \(Z_w\) is the mean Fisher z-transformed correlation between ROIs within the same network and \(Z_b\) is the mean Fisher z-transformed correlation between ROIs of one network with all ROIs in other networks (Chan et al., 2014).

**Task-based fMRI design, preprocessing and analysis**

During the task-based fMRI session, participants performed one (6-minute) run of a sensorimotor task while blood oxygenation-level dependent (BOLD) data were collected. For this task, participants were instructed to tap their left thumb (six blocks per run), right thumb (six blocks per run), or to fixate at a crosshair stimulus in the center of the visual display (rest blocks; twelve blocks per run). The left/right tapping conditions were cued by flashing arrows that pointed to the left and to the right of the visual display. Each block consisted of the stimulus presented for 500ms with a 500ms inter-stimulus interval. The order of the experimental blocks...
was randomized and interleaved with rest blocks. Each experimental block lasted 20 seconds; each rest block lasted 10 seconds. Stimuli were presented using E-Prime (Psychology Software Tools, Pittsburgh, PA) and displayed using a back-projection system. Participant responses were collected via a Celeritas 5-button fiber optic response unit so that we could ensure that participants were following the instructions of the task.

FreeSurfer and FSFAST were used to perform the preprocessing and first-level analyses of the task-based fMRI data (http://surfer.nmr.mgh.harvard.edu/). Surface-based methods as implemented in the FreeSurfer environment were used to reconstruct the cortical surface from the T1-weighted anatomical image. Preprocessing procedures included slice-timing correction, motion correction, and spatial smoothing using a Gaussian kernel with full width at half maximum of 5 mm. Given that the resting state data were preprocessed in MNI space (see above), we also analyzed the task-based data in MNI space to allow direct comparison with the volume-based resting state data. For this procedure, we performed the same preprocessing steps (except for the denoising steps) for the task-based data as for the resting state data.

Neural distinctiveness was measured using multi-voxel pattern analysis (MVPA) in functionally-defined regions of interest (ROIs). Neural responses were first estimated by fitting a General Linear Model, implemented in FSFAST. Responses in the left- and right-hand blocks were modeled using a block design. The model included separate regressors for each of the experimental blocks convolved with a canonical hemodynamic response function.
Using FreeSurfer’s Cortical Parcellation technique (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 2004), we created bilateral anatomical masks in each participant that included precentral gyrus, postcentral gyrus, and supramarginal gyrus. Estimates of gray matter volume were also computed in each of these anatomical regions to account for age differences in brain structure. Next, we used custom MATLAB code to define each participant’s functional ROI. To do so, we first sorted the vertices within each participant’s anatomical mask based on activation level for left hand tapping (experimental condition 1) vs. rest. We then sorted the vertices within the anatomical ROI for right hand tapping (experimental condition 2) vs. rest. Finally, the functional ROI was defined by alternating between the two sorted lists, adding the most active voxel for condition 1 that had not already been included to the functional ROI, then adding the most active voxel for condition 2 that had not already been included, then the next most active voxel for condition 1, and so on. This procedure was continued until we reached our target functional ROI size of 2000 vertices (see Figure 1). This approach has the advantage of including voxels that are activated by both condition 1 and condition 2 without biasing the ROI to have more voxels associated with one condition vs. the other.
Figure 1. Functional mask (size = 1000 vertices in each hemisphere) from a representative older adult participant used for calculating neural distinctiveness of sensorimotor representations evoked from right vs. left hand finger tapping.

We then used the activation estimates within each participant’s functional ROI to measure the distinctiveness of multi-voxel representations for the two experimental tasks (i.e., left vs. right hand tapping). Inspired by Haxby and colleagues (Haxby et al., 2001), neural distinctiveness was defined as the difference between the average within-condition Pearson correlation (i.e., the average of the correlations between left hand patterns and the correlations between right hand patterns) and between-conditions (i.e., the average of the correlations between left-hand and right-hand patterns). Higher scores indicate greater distinctiveness whereas lower scores indicate less distinctiveness. This approach was used rather than alternative classification methods (i.e., support vector machines) to avoid ceiling effects in classifier accuracy (Carp et al., 2011a).

To allow direct comparison with the volume-based resting state data, we also analyzed the task-based data in MNI space using the Power et al. (2011) sensorimotor hand ROIs as an anatomical mask for each subject. We used each participant’s activation estimates within this mask to measure the distinctiveness of multi-voxel representations for the experimental task (i.e., left vs. right hand tapping).

Results

Age differences in sensorimotor performance
The factor analysis of all sensorimotor behavioral measures identified two factors: (1) grip strength and endurance and (2) fine motor dexterity. These two sensorimotor factors were used in all further statistical analyses. Given that grip strength and endurance reflect gross motor performance whereas dexterity reflects fine motor performance, we refer to the grip strength/endurance factor as “gross motor performance” and to the dexterity factor as “fine motor performance”.

Significant age differences were observed in both gross ($t(68)=2.69, p=.009$; Figure 2A) and fine ($t(68)=5.01, p<.001$; Figure 2B) motor performance, with older adults exhibiting worse performance than younger adults. To test whether there were sex differences in these results, we performed follow-up ANCOVAs using age group as the independent variable, behavior as the dependent variable, and sex as a covariate. After controlling for sex, we still observed significant age differences in both gross ($F(1, 67)=14, p<.001$) and fine ($F(1, 67)=24.74, p<.001$) motor performance.

*Age differences in network segregation*

We found that sensorimotor network segregation was significantly reduced in older compared to younger adults, $t(69)=2.55, p=.013$ (See Figure 2C). To test whether sex influenced these results, we performed a follow-up ANCOVA using sex as a covariate in the model. After controlling for sex, we still found that sensorimotor segregation was significantly reduced in older compared to younger adults, $F(1, 67)=6.7, p=.012$. 
The distinctiveness of activation patterns evoked by left vs. right hand movement was significantly lower in older compared to younger adults, $t(62)=2.29, p=.025$ (See Figure 2D). Further, this effect was still observed after controlling for sex, $(F(1, 61)=5.11, p=.027)$.

**Figure 2.** Significant age differences were observed in A) a summary measure of gross motor performance ($t=2.69, p=.009$); B) fine motor performance ($t=5.01, p<.001$); C) sensorimotor network segregation ($t=2.55, p=.013$); and D) neural distinctiveness of sensorimotor representations ($t=2.29, p=.027$) between young (blue) and older (red) adults.

To test whether the (surface-based) functional ROI size influenced these results, we performed a repeated measures ANOVA with a Greenhouse-Geisser correction using ROI size as the within-
subjects factor (using ten functional ROI sizes of 50, 100, 200, 300, 400, 600, 1000, 2000, 5000, and 10,000 vertices) and age group as the between-subjects factor. The results revealed a significant within-subject effect of ROI size on neural distinctiveness, $F(1.68, 107.71)=47.41$, $p<.001$. Specifically, activation patterns for left vs. right hand movement were more distinctive at smaller ROI sizes and less distinctive at larger ROI sizes, $F(1, 64) = 54.97$, $p<.001$ (See Figure 3). More importantly, the age differences in neural distinctiveness that we observed did not vary with ROI size (i.e., there was not a significant ROI size x age group interaction ($F(1.68, 107.71)=.70$, $p=.48$). Neural distinctiveness was also significantly reduced in older compared to younger adults using the Power sensorimotor anatomical mask after processing the task-based data in MNI space, $t(66)=2.1$, $p=.041$.

**Figure 3.** Neural distinctiveness as a function of ROI size in young (blue) and older (red) adults. Sensorimotor cortical activation patterns for right vs. left hand movement were more distinctive at smaller ROI sizes and less distinctiveness at larger ROI sizes.
Controlling for age and gray matter (GM) volume, we observed a positive relationship between sensorimotor network segregation and neural distinctiveness across all participants, $r(59) = .36$, $p = .004$ (See Figure 4A). This finding was also observed when examining the older adult group alone ($r(42) = .36$, $p = .02$; Figure 4B), and there was a trend toward a positive relationship in the younger adult group ($r(22) = .40$, $p = .064$). Controlling for age and GM volume within each age group separately did not change the results (Old: $r(38) = .37$, $p = .02$; Young: $r(18) = .39$, $p = .086$). We transformed the correlations to $z$-scores to compare between the groups, but found no significant differences between the age groups in the relationship between sensorimotor network segregation and neural distinctiveness, $p = .87$.

**Figure 4.** A) Relationship between sensorimotor network segregation and neural distinctiveness of sensorimotor representations across all participants (accounting for age and GM volume differences; $r = .36$, $p = .004$) and B) within each age group separately (Old: $r = .36$, $p = .02$; Young: $r = .40$, $p = .064$).
We also observed a positive relationship between sensorimotor network segregation and neural distinctiveness across all participants using the volume-based (rather than surface-based) distinctiveness measure, $r(60)=.42, p=.001$. This finding was also observed when examining the older adult group alone ($r(45)=.47, p=.001$), and there was a trend toward a positive relationship in the younger adult group ($r(23)=.37, p=.086$). Once again, there were no significant differences between the age groups in the relationship between sensorimotor network segregation and this measure of neural distinctiveness, $p=.65$.

**Relationship between network segregation and behavior**

Controlling for age and GM volume, we observed a positive relationship between sensorimotor network segregation and gross motor performance across all participants, $r(61)=.26, p=.037$ (See Figure 5A). This finding was also observed in the older adult group alone ($r(45)=.36, p=.014$; Figure 5B), but not in the younger adult group ($r(25)=.09, p=.67$). Controlling for age and GM volume within each age group separately did not change the results (Old: $r(40)=.37, p=.02$; Young: $r(20)=.065, p=.77$)

We also observed a significant relationship between sensorimotor network segregation and fine motor performance across all participants after controlling for age and GM volume, $r(61)=.31, p=.014$ (See Figure 5C). Examining this relationship with the two age groups separately, we found a significant association within the older adult group ($r(45)=.36, p=.015$; Figure 5D), but not within the younger adult group ($r(25)=.07, p=.75$). Controlling for age and GM volume
within each age group separately did not change the results (Old: $r(40)=.39, p=.01$; Young: $r(20)=.1, p=.68$).

**Figure 5.** A) Relationship between sensorimotor network segregation and gross motor performance across all participants (accounting for age and GM volume differences; $r=.26, p=.037$) and B) within each age group separately (Old: $r=.36, p=.014$; Young: $r=.09, p=.67$). C) Relationship between sensorimotor network segregation and fine motor performance across all participants (accounting for age and GM volume differences; $r=.31, p=.014$) and D) within each age group separately (Old: $r=.36, p=.015$; Young: $r=.07, p=.75$).

To examine whether either or both of the two relationships above were significantly different between the age groups, we transformed the correlations to z-scores to compare between the
groups. We observed no significant differences between the age groups in either the relationship between gross (p=.28) or fine (p=.24) motor performance and network segregation.

In order to test whether network segregation explains behavioral variance over and above the variance explained by neural distinctiveness, we performed partial correlation analyses between segregation and both gross and fine motor performance while controlling for neural distinctiveness. We observed a significant positive relationship between segregation and gross motor performance across all participants (also controlling for age and GM volume differences; r(59)=.30, p=.021), and within the older adult group alone (r(39)=.32, p=.04), but not the young adult group alone (r(19)=.26, p=.25). Similarly, we observed a significant positive relationship between segregation and fine motor performance across all participants after controlling for age and GM volume (r(59)=.32, p=.012) and observed a similar relationship within the older adult group (r(39)=.33, p=.036), but not within the young adult group (r(19)=.27, p=.24).

**Relationship between neural distinctiveness and behavior**

Controlling for age and GM volume differences, we did not observe an association between neural distinctiveness and gross motor performance within the whole group (r(59)=-.037, p=.78; Figure 6A) or within either age group separately (Old: r(42)=.23, p=.15; Young: r(22)=-.24, p=.28; Figure 6B). Controlling for age and GM volume within each age group separately did not change the results (Old: r(38)=.1, p=.55; Young: r(18)=-.42, p=.07). Note that these correlations in the young group were negative. Follow-up analyses revealed a trend toward an age group difference in this relationship, p=.087. We did observe a trend toward a positive relationship
between neural distinctiveness and fine motor performance across all participants, \( r(59) = .21, p = .098 \) (See Figure 6C). However, this relationship was not significant within either age group separately (Old: \( r(42) = .24, p = .12 \); Young: \( r(22) = .044, p = .85 \); Figure 6D). Controlling for age and GM volume within each age group separately did not change the results (Old: \( r(38) = .25, p = .12 \); Young: \( r(18) = .03, p = .91 \)). Follow-up analyses revealed no significant group differences in this relationship, \( p = .47 \).

Figure 6. A) Relationship between neural distinctiveness of sensorimotor representations and gross motor performance across all participants (accounting for age and GM volume differences; \( r = -.037, p = .78 \)) and B) within each age group separately (Old: \( r = .23, p = .15 \); Young: \( r = -.24, p = .28 \)). C) Relationship between neural distinctiveness and fine motor performance across all participants (accounting for age and GM volume differences; \( r = .21, p = .098 \)) and D) within each age group separately (Old: \( r = .24, p = .12 \); Young: \( r = .044, p = .85 \)).
To allow direct comparison with the volume-based resting state data, we repeated the analyses above using a volume-based (rather than surface-based) measure of neural distinctiveness within the same ROI used for the segregation analyses. Once again, we did not observe an association between neural distinctiveness and gross motor performance either within the whole group ($r(60)=.033, p=.80$) or within either age group separately (Old: $r(44)=.24, p=.12$; Young: $r(23)=-.17, p=.45$). Follow-up analyses showed no significant group differences in this relationship, $p=.13$. We also did not observe an association between this volume-based measure of neural distinctiveness and fine motor performance either within the whole group ($r(60)=.083, p=.52$) or within either age group separately (Old: $r(44)=.048, p=.76$; Young: $r(23)=.045, p=.84$). Follow-up analyses showed no significant group differences in this relationship either, $p=.99$.

In order to test whether neural distinctiveness explains behavioral variance over and above the variance explained by sensorimotor segregation, we performed partial correlation analyses between distinctiveness and both gross and fine motor performance while controlling for segregation. We did not observe an association between distinctiveness and gross motor performance, either across all participants (also controlling for age and GM volume differences; $r(59)=-.14, p=.29$), or within either age group separately (old: $r(39)=.11, p=.51$; young: $r(19)=-.33, p=.15$). Similarly, we did not observe an association between distinctiveness and fine motor performance, either across all participants ($r(59)=.076, p=.56$), or within either age group separately (old: $r(39)=.12, p=.45$; young: $r(19)=-.071, p=.76$).
Predicting behavior from models that include both network segregation and neural distinctiveness

We also performed multiple regression analyses to predict sensorimotor performance from both segregation and distinctiveness. When analyzing gross sensorimotor performance across all participants (including age and GM volume as nuisance covariates), the overall model was significant ($F(4,59)=6.74, p<.001$), and segregation was a significant predictor ($t(59)=2.38, p<0.05$) but distinctiveness was not ($t(59)=-1.06, p=.29$). Performing this analysis within the old group alone produced similar results: the overall model was significant ($F(2,39)=3.24, p=.05$), segregation was a significant predictor ($t(39)=2.03, p<0.05$) but distinctiveness was not ($t(39)=0.70, p=0.48$). The overall regression model was not significant in the younger group alone.

When analyzing fine sensorimotor performance across all participants (again including age and GM volume as nuisance covariates), the overall multiple regression model was significant ($F(4,59)=9, p<.001$), and segregation was a significant predictor ($t(59)=2.59, p<0.05$) but distinctiveness was not ($t(59)=.59, p=0.56$). When performing this analysis within the old group alone, the overall model was significant ($F(2,39)=3.29, p<.05$), segregation was a marginally significant predictor ($t(39)=1.98, p=0.055$) and distinctiveness was not ($t(39)=0.82, p=0.42$). The overall regression model was again not significant in the younger group alone.

Discussion
The present study examined potential links between sensorimotor neural distinctiveness, sensorimotor network segregation, and sensorimotor behavior. Consistent with previous findings, older adults exhibited reduced sensorimotor neural distinctiveness and reduced sensorimotor (resting state) network segregation relative to younger adults. New to the present study, participants with the most distinct sensorimotor representations exhibited the most segregated sensorimotor networks. Further, sensorimotor network segregation was associated with individual differences in sensorimotor performance, particularly in older adults, whereas sensorimotor neural distinctiveness was not. These findings link, for the first time, sensorimotor network segregation to sensorimotor neural distinctiveness. They also suggest that sensorimotor network segregation may be a more sensitive predictor of age-related declines in sensorimotor behavior.

*Age differences in network segregation and neural distinctiveness*

We found that sensorimotor network segregation is significantly lower in older adults than in younger adults. Although we focused exclusively on the sensorimotor network in this study, previous studies have reported significant age differences in several sensorimotor and association networks, suggesting that age-related reductions in network segregation occur at the whole-brain (i.e., multiple network) level.

We also demonstrated that older adults have significantly less distinctive neural representations in sensorimotor cortex than younger adults. This finding is consistent with previous studies that reported age differences in neural distinctiveness involving motor (Carp et al., 2011a), visual
(Park et al., 2004, 2010; Carp et al., 2011b), auditory (Lalwani et al., 2018), and memory representations (Carp et al., 2010; Koen et al., 2018). Together, these findings suggest that age differences in neural distinctiveness are not limited to sensorimotor cortex, but rather are a general feature of the aging brain.

**Network segregation is related to neural distinctiveness**

Our study is the first to show that resting state sensorimotor network segregation varies with the distinctiveness of task-based activation patterns in sensorimotor cortex. This finding is consistent with previous data suggesting a relationship between the brain’s large-scale functional organization at rest and its functional recruitment during task performance (Langan et al., 2010; Chan et al., 2017).

In the context of aging, age differences in resting state organization may provide a network-based explanation for the commonly observed finding of age-related neural dedifferentiation of task-evoked activity. Or perhaps other neural factors influence the differentiation of both resting state networks and task-evoked activity. For instance, previous work by our group indicates that lower levels of the inhibitory neurotransmitter, gamma aminobutyric acid (GABA), are associated with reduced network segregation (Cassady et al., 2019) and reduced neural distinctiveness (Lalwani et al., 2018). Reduced white matter integrity (Burzynska et al., 2010) and amyloid deposition (Buckner et al., 2009; Mormino et al., 2011) also disrupt the organization of functional networks and brain activity. Future work could employ multimodal imaging to investigate potential interactions between brain structure, function, and chemistry.
Network segregation (but not neural distinctiveness) is associated with sensorimotor performance

The present results demonstrate that sensorimotor network segregation (but not sensorimotor neural distinctiveness) is associated with sensorimotor performance, and that this relationship is particularly strong in older adults. Specifically, older individuals with less segregated networks exhibit worse sensorimotor performance (both gross and fine) than older adults with more segregated networks. Importantly, this relationship remained significant even after controlling for neural distinctiveness, both in partial correlation and multiple regression analyses. In contrast, although there was a trend toward a positive relationship between neural distinctiveness and fine motor performance across all participants, this relationship was not observed within either age group separately. These findings suggest that network segregation may be a more sensitive predictor of age-related declines in sensorimotor performance than neural distinctiveness.

One potential explanation concerns the regions of interest studied. Many investigations of neural dedifferentiation of task-based activation patterns (including this one) are restricted to localized regions of interest. Given that most sensorimotor functions require the interaction of multiple large-scale distributed brain networks, it is likely that regions outside of these localized ROIs are contributing to the associated declines in behavior. Because network segregation inherently examines dedifferentiation at a multiple network level, this may be one reason why segregation is a better predictor of sensorimotor performance compared to neural distinctiveness.
Another possibility is that resting state measures of fMRI provide a more stable, “trait-like” measure of brain function compared to task-based measures, which are less stable and more “state-like”. Because of the relative stability of resting state functional connectivity (e.g., Iordan et al., 2018), many studies use it as a trait measure. In contrast, functional activity inherently provides a transient, state-dependent measure of brain characteristics. For instance, Cole and colleagues demonstrated that functional connectivity between brain regions at rest is more informative for predicting individual differences in fluid intelligence compared to task-evoked activity of functional regions in isolation (Cole et al., 2012). These findings, in addition to those from the present study, suggest that trait-based measures such as resting state functional connectivity may be more sensitive in predicting individual differences in behavior compared to task-based state measures such as neural distinctiveness.

Limitations

Interpretation of the present results is restricted by several limitations that could be addressed in future studies. For one thing, our sample was cross sectional and therefore we can only make inferences about age differences rather than longitudinal changes that occur with age. Future longitudinal designs could examine age-related changes in neural and behavioral measures as well as the relationship between them over time.

Furthermore, the present study employed a simple unimanual thumb tapping task. We were therefore unable to examine the effects of aging on the neural representations of more realistic,
complex movements or of different individual movements. Future studies that use more complex sensorimotor tasks could add additional insight into age-related neural dedifferentiation of sensorimotor representations.

Conclusions

The present study examined the relationship between sensorimotor network segregation, neural distinctiveness of sensorimotor representations, and sensorimotor behavior in young and older adults. Consistent with previous studies, we found that sensorimotor networks are less segregated and sensorimotor representations are less distinct in older relative to young adults. We also discovered that less segregated networks are associated with less distinct representations. Finally, we found that less segregated networks predict worse sensorimotor performance, particularly within older adults, whereas neural distinctiveness is not associated with performance. These findings link network segregation to neural distinctiveness and suggest that segregation is a more sensitive predictor of age-related declines in sensorimotor behavior.
References


Chapter III: Sensorimotor network segregation declines with age and is linked to GABA and to sensorimotor performance

Abstract

Aging is typically associated with declines in sensorimotor performance. Previous studies have linked some age-related behavioral declines to reductions in network segregation. For example, compared to young adults, older adults typically exhibit weaker functional connectivity within the same functional network but stronger functional connectivity between different networks. Based on previous animal studies, we hypothesized that such reductions of network segregation are linked to age-related reductions in the brain’s major inhibitory transmitter, gamma aminobutyric acid (GABA). To investigate this hypothesis, we conducted graph theoretical analyses of resting state functional MRI data to measure sensorimotor network segregation in both young and old adults. We also used magnetic resonance spectroscopy to measure GABA levels in the sensorimotor cortex and collected a battery of sensorimotor behavioral measures. We report four main findings. First, relative to young adults, old adults exhibit both less segregated sensorimotor brain networks and reduced sensorimotor GABA levels. Second, less segregated networks are associated with lower GABA levels. Third, less segregated networks and lower GABA levels are associated with worse sensorimotor performance. Fourth, network segregation mediates the relationship between GABA and performance. These findings link age-related differences in network segregation to age-related differences in GABA levels and sensorimotor performance. More broadly, they suggest a neurochemical substrate of age-related dedifferentiation at the level of large-scale brain networks.
Introduction

Advanced age is typically associated with declines in sensorimotor functioning. Such declines affect the ability of older adults to perform activities of daily living and maintain their functional independence (R. D. Seidler et al., 2010). Part of this decline is associated with impairments in the peripheral sensorimotor system, including motor unit reorganization (Galganski, Fuglevand, & Enoka, 1993) or reduced functioning of cutaneous afferents. But evidence also suggests that some of these age-related declines in behavior are related to changes in the brain, including alterations in neurochemistry, gray matter atrophy, and changes in the functional organization of large-scale brain networks (Ferreira & Busatto, 2013; Goh, 2011; Langan et al., 2010; Raz & Rodrigue, 2006; R. D. Seidler et al., 2010; R. Seidler et al., 2015). Understanding these brain-behavior relationships is important for our efforts to prolong the functional independence of older adults as our society continues to age.

Studies in young adults have demonstrated the existence of multiple segregated functional brain networks. Regions within these networks exhibit spontaneous yet correlated activity, and thus are thought to be functionally connected (Biswal, Yetkin, Haughton, & Hyde, 1995; Buckner, Krienen, & Yeo, 2013). Studies have found that connections within these functional networks are quite dense, whereas connections between different networks are more sparse. This organization
is considered to benefit specialized or segregated information processing in different brain systems (Bullmore & Sporns, 2012). Several studies have investigated the effect of age on functional connectivity by measuring differences in correlated brain activity within and between brain networks at rest. Many of these studies have found that older adults exhibit weaker functional connectivity between brain regions within the same functional network but stronger functional connectivity between regions belonging to different networks. In other words, their functional networks are less segregated (i.e., a type of age-related dedifferentiation) (M. Y. Chan, Park, Savalia, Petersen, & Wig, 2014; Damoiseaux, 2017; Geerligs, Renken, Saliasi, Maurits, & Lorist, 2015).

Many studies have also found that less segregated brain networks are associated with worse cognitive performance, independent of age (M. Y. Chan et al., 2014; Damoiseaux et al., 2008; Geerligs et al., 2015; Wang et al., 2010). Only a few studies have investigated the relationship between network segregation and sensorimotor behavior, but one such study found that reduced segregation of several large-scale resting state brain networks was associated with poorer bimanual motor performance (King et al., 2018). In sum, the evidence to date suggests that age-related changes in functional connectivity may contribute to age-related declines in cognitive and sensorimotor performance.

An important open question is what causes age-related reductions in network segregation, or neural dedifferentiation. Previous animal studies have linked neural dedifferentiation to changes at the neurotransmitter level. In particular, studies by Leventhal and colleagues suggested that age differences in the brain’s major inhibitory neurotransmitter, gamma-aminobutyric acid
(GABA), may play an important and potentially causal role in age-related dedifferentiation (i.e., reductions in the specificity of neural activity). More specifically, they demonstrated that manipulations of GABA levels led to changes in the orientation-selectivity of neurons in the visual cortex (Leventhal, Wang, Pu, Zhou, & Ma, 2003). The application of GABA or a GABA agonist made visual cortex neurons in old monkeys more orientation-selective, thereby making them similar to neurons in young monkeys. In contrast, the application of a GABA antagonist reduced the orientation-selectivity of visual cortex neurons in young monkeys, thereby making them similar to neurons in old monkeys. These results demonstrate that manipulations of GABA cause changes in neural selectivity in animals, raising the possibility that age declines in GABA might contribute to age-related neural dedifferentiation and associated behavioral declines in humans.

Consistent with this hypothesis, more recent studies in humans have linked individual differences in GABA levels to performance variations across healthy young adults (Boy et al., 2010; Edden, Muthukumaraswamy, Freeman, & Singh, 2009; Puts, Edden, Evans, McGlone, & McGonigle, 2011). For example, Edden et al. found that orientation discrimination performance is predicted by GABA levels in primary visual cortex (Edden et al., 2009). In addition, Puts et al. demonstrated that GABA levels in sensorimotor cortex correlate with tactile discrimination thresholds (Puts et al., 2011). Previous work has also found that reactivity in the GABA system plays an important role in motor learning and learning-related brain activity. For instance, Stagg et al. (2011) demonstrated that participants in whom motor cortex (M1) GABA levels decreased the most following transcranial direct current stimulation (i.e., greatest GABA reactivity), also exhibited the most learning in a motor task (Stagg, Bachtiar, & Johansen-Berg, 2011).
Studies have also observed a relationship between functional connectivity within specific resting state networks and GABA levels in young adults. For example, Stagg and colleagues demonstrated that functional connectivity strength within the motor resting state network is related to GABA levels in primary motor cortex (Stagg et al., 2014). A similar relationship was also reported between GABA levels in the posteromedial cortex and the strength of default mode network connectivity (Kapogiannis, Reiter, Willette, & Mattson, 2013). While these studies linked individual differences in functional connectivity to differences in GABA levels, no studies have investigated whether this relationship varies with age. Moreover, no studies have examined GABA levels, network segregation, and sensorimotor behavior within the same participants in order to explore the potential links between all three levels.

In the present study, we specifically addressed these gaps. We performed graph theoretical analysis of resting state functional MRI data to measure sensorimotor network segregation and used magnetic resonance spectroscopy (MRS) to measure GABA levels in the sensorimotor cortex. We also collected a battery of sensorimotor behavioral measures to determine whether network segregation and/or GABA levels are associated with individual differences in performance. All three datasets were collected within the same participants, making it possible to examine associations between all the measures.

We tested three hypotheses: 1) The sensorimotor resting state brain network would be less segregated and sensorimotor cortex GABA levels would be reduced in older compared to young adults; 2) lower levels of GABA would be associated with less segregated networks, independent
of age; and 3) lower levels of GABA and less segregated networks would be associated with worse sensorimotor performance, independent of age.

**Materials and methods**

**Participants**

Twenty-two young adults (age range 19 to 29 years; 13 females) and 23 older adults (age range 65 to 81; 12 females) were recruited for this study. All participants were right-handed, native English speakers. We screened participants to ensure they were not taking any medications with psychotropic effects, and free from any other MRI safety contraindications. Participants were also assessed for cognitive impairment using the Montreal Cognitive Assessment (MoCA) and only those with scores ≥23 were included in the study (Brenkel, Shulman, Hazan, Herrmann, & Owen, 2017). Data from one younger and one older adult were excluded due to voluntary withdrawal from the study before completion of the protocol. Behavioral data from one additional older adult participant was excluded due to technical issues related to behavioral task assessments. All study procedures were reviewed and approved by the University of Michigan Institutional Review Board. All participants provided detailed written consent for their involvement in this study.

**Study design**

Participants completed three separate testing sessions: one resting state fMRI session, one MRS session, and one behavioral session. The order of the fMRI and behavioral sessions was
counterbalanced across participants, but the fMRI session always occurred prior to the MRS session so that we could use the fMRI activation to guide the placement of the MRS voxels. All measurements were acquired from participants within an average period of 25 days.

**Sensorimotor assessments**

We used a sensorimotor test battery that included both motor and somatosensory components. The motor measures included a 9-hole pegboard dexterity test, grip strength, and a 2-minute walk endurance test. The somatosensory (tactile) measures included vibrotactile simple and choice reaction time (RT), static and dynamic vibrotactile detection thresholds, and a functional tactile object recognition test (fTORT). Detailed descriptions of each task are provided in the supplemental materials, as are the age-group means (and standard errors) for each behavioral measure (Table S1). In addition, covariance matrices across all behavioral measures are included in the supplemental materials for all participants (Table S2), for the older adult group alone (Table S3), and for the younger adult group alone (Table S4). Given the wide range of sensorimotor measurements, participant scores for all tests were submitted to a factor analysis to identify behavioral factors that reflect general sensorimotor functioning. Please refer to supplemental material for factor analysis model coefficients across all participants (Table S5), within the older adult group (Table S6), and within the younger adult group (Table S7).

**MRI data acquisition**
Anatomical and functional brain images were acquired using a GE Discovery MR750 3-Tesla MRI scanner located at the University of Michigan Functional Magnetic Resonance Imaging Laboratory. A GE 8-channel head coil was used, and participant movement was minimized by stabilizing the head with cushions and Velcro straps. Imaging sessions included the acquisition of T1-weighted anatomical images, high-resolution anatomical images using spoiled 3D gradient-echo acquisition (SPGR), and T2*-weighted functional images. Functional images were acquired using a single-shot gradient-echo (GRE) reverse spiral pulse sequence. The field of view was 220 x 220 mm, with a voxel size of 3 x 3 x 4 mm (40 axial slices), a TR (repetition time) of 2 seconds, and a TE (echo time) of 30 ms. The duration of the resting state scan was eight minutes.

**MRS data acquisition**

MRS data were acquired on the same scanner on a different day. Data were collected from 30 mm x 30 mm x 30 mm voxels placed in the left and right sensorimotor cortex (See Figure 7A). The placement of voxels in each participant corresponded to the region of maximal sensorimotor activity in that same individual from their previous task-based fMRI session (not included in the present study). Briefly, participants performed motor (finger tapping on right vs. left hands) and somatosensory (vibrotactile stimulation to right vs. left hands) tasks in the MRI scanner. The MRS voxel was subsequently placed in each participant to maximize overlap with fMRI activation from both of these tasks. Participants were not instructed to do any task during MRS data acquisition.
Figure 7. A) Sensorimotor cortex MRS voxel overlap across all participants, with brighter (red) colors representing more participant overlap and darker (blue) colors representing less overlap. B) Edited MR spectra from a representative younger adult participant (black) demonstrating a clearly resolved peak for GABA+ at 3ppm, with the fitted GABA+ model in red.

GABA-edited MR spectra were acquired using a MEGA-PRESS sequence (Edden & Barker, 2007; Mescher, Merkle, Kirsch, Garwood, & Gruetter, 1998) with the following acquisition parameters: TE = 68 ms (TE1 = 15 ms, TE2 = 53 ms); TR = 1.8 s; 256 transients (128 ON interleaved with 128 OFF) of 4.096 data points; spectral width = 5 kHz; frequency selective editing pulses (14 ms) applied at 1.9 ppm (ON) and 7.46 ppm (OFF); total scan time, approximately 8.5 min per voxel. The MRS voxels were acquired serially.

Resting state fMRI data preprocessing

Resting state data preprocessing was performed with the Statistical Parametric Mapping 8 software (SPM8; www.fil.ion.ucl.ac.uk/spm). Preprocessing steps included slice-time correction, realignment, segmentation of structural images, normalization into standard Montreal Neurological Institute (MNI) space and spatial smoothing using a Gaussian kernel of 8 mm full width at half-maximum (FWHM). Because functional connectivity measurements are influenced
by head motion in the scanner (Van Dijk, Sabuncu, & Buckner, 2012), we detected and rejected motion artifacts using the artifact detection toolbox (ART; http://www.nitrc.org/projects/artifact_detect). Specifically, an image was defined as an outlier if the head displacement in the x, y, or z direction was greater than 0.5 mm from the previous frame, if the rotational displacement was greater than 0.02 radians from the previous frame, or if the global mean intensity of an image was greater than 3 standard deviations from the mean image intensity for the entire resting scan. Outliers in the global mean signal intensity and motion were subsequently included as nuisance covariates in the first level general linear model (GLM). A total of 36 volumes were removed from four participants during preprocessing (8 volumes in one older adult, 2 volumes in another older adult, 20 volumes in one young adult, and 6 volumes in a second young adult). The difference between the age groups was not statistically significant, t(41)=.76, p=.45).

Additional denoising on the resting state data was performed with the CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). The resting state data were filtered using a temporal band-pass filter of 0.008 to 0.09 Hz in order to examine the frequency band of interest and to exclude higher frequency sources of noise such as heart rate and respiration. For noise reduction, we used the anatomical component-based noise correction method aCompCor, which models the influence of noise as a voxel-specific linear combination of multiple empirically estimated noise sources by deriving principal components from noise regions of interest (ROIs) and including them as nuisance parameters in the first level GLM (Behzadi, Restom, Liao, & Liu, 2007). Specifically, the anatomical image for each participant was segmented into white matter (WM), gray matter (GM), and cerebrospinal fluid (CSF) masks. To minimize partial voluming effects
with GM, the WM and CSF masks were eroded by one voxel. The eroded WM and CSF masks were then used as noise ROIs. Residual head movement parameters (three rotations, three translations, and six parameters that represent their first-order temporal derivatives) and signals from WM and CSF were regressed out during the computation of functional connectivity maps.

First-level functional connectivity analysis

First-level ROI-to-ROI functional connectivity MRI (fcMRI) analysis was performed with the CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). For this analysis, we created ROIs (using 5mm diameter spheres) using coordinates published in Power et al. (See MNI coordinates in Table S8) (Power et al., 2011). We used all coordinates from this previous study except for those belonging to “subcortical” and “undefined” networks, which left us with 214 ROIs. Each ROI was labeled according to this published functional network map, which was defined by consensus of network assignments using community detection of resting state functional connectivity across multiple thresholds. The final network labels of each ROI are depicted in Figure 8. For each participant, the resting state fMRI time series within each of the 214 ROIs was extracted from the unsmoothed functional images (to avoid potential “spillage” of the BOLD signal from nearby regions/ROIs), and the mean time course was computed. The cross-correlation of each ROI’s time course with every other ROI’s time course was computed, creating a 214 x 214 ROI-to-ROI correlation matrix. Correlation coefficients (i.e., graph edges) were converted into z-values using Fisher’s r-to-z transformation (Zar, 1996). The resulting Fisher z-transformed correlation matrix is a fully connected, relatedness graph. Negatively weighted edges were set to zero in each correlation matrix to avoid potential misinterpretation of
negative edge weights (M. Y. Chan et al., 2014; Micaela Y. Chan, Alhazmi, Park, Savalia, & Wig, 2017) (we also performed the same analyses with inclusion of both positively and negatively weighted edges, and observed similar findings (See Table S9)). Thus, the final connectivity matrix for each participant was a 214 x 214 z-matrix with the diagonal removed and the negative values set to zero.

**Figure 8.** 214 regions of interest were created using coordinates defined by Power et al. (2011) to produce ten resting state networks of interest.

**Graph theoretical analysis**

Following Chan et al. (2014), network segregation was defined as the difference of mean within-network connectivity and mean between-network connectivity divided by the mean within-network connectivity:

\[
\text{Network segregation} = \frac{z_w - z_b}{z_w}
\]
where $\bar{Z}_w$ is the mean Fisher z-transformed correlation between ROIs within each network and $\bar{Z}_b$ is the mean Fisher z-transformed correlation between ROIs of one network to all ROIs in other networks (M. Y. Chan et al., 2014).

**MRS analysis**

GABA levels from both right and left sensorimotor cortices were measured using the GABA analysis toolbox, Gannet (Edden, Puts, Harris, Barker, & Evans, 2014). All time domain data were frequency- and phase-corrected using spectral registration and filtered with a 3-Hz exponential line broadening and zero-filled by a factor of 16 (Near et al., 2015). The 3-ppm GABA peak in the difference spectrum was fit using a Gaussian model and quantified relative to water (fit with a Gaussian-Lorentzian model) in institutional units (See Figure 7B). This editing scheme results in significant excitation of coedited macromolecule (MM) signal, which has been reported to contribute approximately 45% to the edited signal at 3-ppm (Mullins et al., 2014). Thus, all GABA values are reported as GABA+ (i.e., GABA + MM) in the present study. To control for the differential relaxation constants and water visibility between WM, GM and CSF, a binary mask of the MRS voxel was created using Gannet’s integrated voxel-to-image coregistration. Next, segmentation of the anatomical image was performed using the Segment function in SPM and the voxel fractions containing CSF, GM and WM were computed (Harris, Puts, Barker, & Edden, 2015). From this procedure, a tissue-corrected GABA+ value was calculated for each participant. GABA+ levels from right and left sensorimotor cortices were averaged together before any statistical analyses. Thus, all reported GABA+ measures are reported as the mean value across both right and left sensorimotor cortices. We also collected
measures of signal-to-noise (SNR) and fit error values for both voxels and for all participants to examine any age differences in these measures.

To control for age differences in brain structure, we measured the GM volume of bilateral precentral, postcentral, and supramarginal gyri in each participant. FreeSurfer was used to acquire these GM measurements, with each gyrus defined by an automated cortical parcellation technique (Dale, Fischl, & Sereno, 1999; B. Fischl et al., 2004; Bruce Fischl & Dale, 2000).

Statistical analysis

To test for group differences in network segregation, GABA+ levels, and behavior between young and older adults, we performed independent sample t-tests on these data. To explore the relationship between network segregation, GABA+ levels and behavior, we performed partial correlation analyses with bootstrapping (using 1000 samples, 95% confidence intervals) across all participants (controlling for age and GM volume differences). We also investigated this relationship specifically within each age group using bivariate correlation analyses. For all analyses, outliers greater than three standard deviations above or below the mean were excluded. SPSS software was used for all statistical analyses (SPSS Inc., Chicago IL).

Results

Age differences in sensorimotor performance
An exploratory factor analysis of the sensorimotor behavioral measures identified two factors, one corresponding to grip strength and one corresponding to all of the other sensorimotor measures (Table S5). These two sensorimotor factors were used in all further statistical analyses. Significant age differences were observed in the general sensorimotor factor (t(39)=7.24, p<.001; Figure 9A), whereas there was no significant effect of age on the grip strength factor (t(39)=1.24, p=.22; Figure 9B). To test whether gender influenced these results, we performed follow-up ANCOVAs using age group as the independent variable, behavior as the dependent variable, and gender as a covariate. We still observed a significant effect of age on both the sensorimotor performance factor (F(1, 37)=49.89, p<.001) and the grip strength factor F(1, 37)=4.44, p=.042 after controlling for gender. Because we observed significant age differences in years of education (t(40)=2.51, p=.017) between the two groups, we tested whether education influenced the observed age effects on behavior. Controlling for education in follow-up ANCOVAs, we still observed a significant effect of age on the sensorimotor performance factor (F(1, 38)=41.97, p<.001, but not on the grip strength factor, (F(1, 38)= 1.66, p=.21).

We also performed a factor analysis on each age group separately (see supplementary tables S6 and S7). Although the factor structures were somewhat different between the two age groups, the sample sizes in each group were relatively small and we therefore decided to use the factors from the entire sample for most of the subsequent analyses. We also examined the relationship between our neural measures with each individual behavioral measure to ensure that the results were not an artifact of differences in factor structure (see tables S10 & S11).

*Age differences in network segregation*
We found that sensorimotor network segregation was significantly reduced in older compared to younger adults, $t(41)=2.23, p=.031$ (See Figure 9C). As a follow-up analysis, we investigated age differences in mean network segregation (averaged across all 10 networks) and found that this was also significantly reduced in older relative to younger adults, $t(40)=4.84, p<.001$. We also examined the effect of age in each network individually and found significant age differences in five out of the ten networks examined. These included the Sensorimotor hand ($t(41)=2.23, p=.031$), Auditory ($t(41)=2.23, p=.031$), Sensorimotor mouth ($t(39)=4.35, p<.001$), Cingulo-opercular control ($t(41)=3.7, p=.001$) and Dorsal attention ($t(41)=2.28, p=.028$) networks. Furthermore, the p-value for three additional networks was between 0.05 and 0.10 (Visual: $p=.099$, Ventral attention: $p=0.059$, Salience: $p=.057$). It is important to note that these post-hoc analyses were not corrected for multiple comparisons, and therefore these findings should be interpreted with caution.

To test whether gender influenced these results, we performed a follow-up ANCOVA using gender as a covariate in the model. We still observed significant age differences in sensorimotor network segregation, even after controlling for gender, $F(2, 39)=4.55, p=.039$. We also tested whether level of education influenced the observed age effect on network segregation. Controlling for education in a follow-up ANCOVA, the effect of age on sensorimotor network segregation was not quite significant, $F(1, 38)=3.99, p=.053$.

*Age differences in GABA levels*
Tissue-corrected GABA+ levels (controlling for the differential relaxation constants and water visibility between WM, GM and CSF in the MRS voxels) in sensorimotor cortex were significantly reduced in older compared to younger adults, $t(40)=4.97, p<.001$ (See Figure 9D). Sensorimotor GABA+ levels were still significantly reduced in older relative to younger adults after controlling for gender ($F(1, 38)=24.80, p<.001$) and education ($F(1, 39)=18.64, p<.001$). We did not observe any significant age differences in SNR or fit error, either when examining left (SNR: $t(40)=1.43, p=.16$; fit error: $t(40)=1.11, p=.28$) or right (SNR: $t(39)=1.74, p=.09$; fit error: $t(39)=-.21, p=.84$) voxels separately, or averaged together (SNR: $t(41)=1.72, p=.093$; fit error: $t(41)=.83, p=.41$).
Figure 9. Age differences in A) a summary measure of general sensorimotor performance (t=7.24, p<.001); B) grip strength (t=1.24, p=.22); C) sensorimotor network segregation (t=2.23, p=.031); and D) sensorimotor GABA+ levels (t=4.97, p<.001) between young (blue) and older (red) adults. On each box, the central line indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers.

Relationship between network segregation and GABA levels

Controlling for age and gray matter (GM) volume differences, we observed a positive relationship between sensorimotor network segregation and GABA+ levels across all subjects, \( r(38)=.41, p=.008 \), bootstrap confidence interval \([.078, .65]\) (See Figure 10A). This finding was also observed when examining the older adult group alone \( r(21)=.45, p=.042 \); Figure 10B), but did not reach significance in the younger adult group \( r(21)=.37, p=.10 \). To explore whether these correlations were significantly different between the two age groups, we transformed the correlations to z-scores to compare between groups. Results revealed no significant differences between the two groups in the relationship between sensorimotor network segregation and GABA+ levels, \( p=.77 \).

To examine whether education level influenced these results, we performed a follow-up partial correlation analysis that included age (years), GM volume, and education level as covariates. Controlling for these variables, we still observed a significant positive relationship between sensorimotor network segregation and GABA+ levels, \( r(37)=.42, p=.008 \), confidence interval \([.095, .65]\). We also tested whether the association between sensorimotor network segregation and GABA+ levels was significant when analyzed using a non-parametric permutation test that makes no assumptions about the statistical distribution. To do this, we randomly shuffled the
within- and between-network connection labels 10,000 times. We then computed the association between GABA and sensorimotor network segregation based on that random shuffling. We found that the association with the true segregation value ($r=.496$) was within the top 1% (actually in the top 0.15%) of all segregation values.

In order to assess the specificity of the relationship between segregation and GABA+ levels in the sensorimotor system across the whole group, we examined the relationship between mean network segregation (averaged across all 10 networks) and GABA+ levels. Controlling for age and GM volume differences, we observed a significant positive relationship between these measures, $r(37)=.43, p=.007$, confidence interval $[.059, .69]$. This finding was also observed when examining the older adult group alone ($r(22)=.54, p=.011$), but did not reach significance in the younger adult group ($r(21)=.15, p=.53$) (but the strength of this relationship was not significantly different in the two groups, $p=.17$). We also examined the relationship between sensorimotor segregation and GABA+ levels measured in ventral visual cortex (VVC) and auditory cortex (controlling for age and GM volume differences). We observed no significant relationships, either between sensorimotor segregation and GABA+ levels in VVC ($r(38)=.03, p=.86$, confidence interval $[-.36, .38]$), or sensorimotor segregation and GABA+ levels in auditory cortex ($r(38)=.12, p=.46$, confidence interval $[-.30, .47]$).
Controlling for age and GM volume differences, we observed a positive relationship between sensorimotor network segregation and sensorimotor performance across all subjects, $r(37)=.38$, $p=.016$, confidence interval $[.011, .67]$ (See Figure 11A). These findings were also observed when examining the older adult group alone ($r(20)=.50$, $p=.026$; See Figure 11B), but did not reach significance in the younger adult group ($r(21)=.18$, $p=.44$). There was also a trend toward a positive relationship between sensorimotor network segregation and grip strength across all participants ($r(37)=.27$, $p=.093$, confidence interval $[-.028, .51]$; See Figure 11C). Examining this grip strength relationship within the two age groups separately, we observed a significant relationship within the older adult group ($r(20)=.51$, $p=.021$; See Figure 11D), but not within the younger adult group ($r(21)=.13$, $p=.59$). To explore whether there were significant age differences in these relationships, we again transformed the correlations to z-scores to compare
between groups. We found no significant group differences, either on the sensorimotor performance measure \( (p=.28) \) or on the grip strength measure \( (p=.20) \). Please refer to supplemental materials for (uncorrected) associations between sensorimotor network segregation and individual behavioral measures (Table S10).

To examine whether education level influenced these results, we performed follow-up partial correlation analyses that included age (years), GM volume, and education level as covariates. Controlling for these variables, we still observed a significant positive relationship between sensorimotor network segregation and the sensorimotor performance factor \( (r(36)=.39, p=.017, \text{ confidence interval } [-.012, .67]) \) but only a trend toward a positive relationship between sensorimotor network segregation and the grip strength factor \( (r(36)=.27, p=.097, \text{ confidence interval } [-.028, .55]) \)
Figure 11. A) Relationship between sensorimotor network segregation and sensorimotor performance across all participants (accounting for age and GM volume differences; $r=.38$, $p=.016$) and B) within each age group separately (Old: $r=.50$, $p=.026$; Young: $r=.18$, $p=.44$). C) Relationship between sensorimotor network segregation and grip strength across all participants (accounting for age and GM volume differences; $r=.27$, $p=.093$) and D) within each age group separately (Old: $r=.51$, $p=.021$; Young: $r=.13$, $p=.59$).

**Relationship between GABA levels and behavior**

Controlling for age and GM volume differences, we observed a positive relationship between GABA+ levels and sensorimotor performance across all participants ($r(37)=.32$, $p=.046$, confidence interval [.013, .56]; Figure 12A). This finding was also observed when examining the
older adult group alone (r(20)=.48, p=.031; Figure 12B), but was not significant in the younger adult group (r(21)=-.14, p=.55). Follow-up analyses revealed a trend toward an age group difference in this relationship, p=.05. We did not observe an association between GABA+ levels and grip strength within the whole group (r(37)=.21, p=.19, confidence interval [-.12, .49]; Figure 12C) or within either age group separately (Old: r(20)=.23, p=.33; Young: r(21)=.20, p=.38; Figure 12D). Not surprisingly, follow-up analyses revealed no significant group differences in this relationship, p=.93. Please refer to supplemental materials for (uncorrected) associations between sensorimotor GABA+ levels and individual behavioral measures (Table S11).

To test whether education level influenced these results, we performed follow-up partial correlation analyses that included age (years), GM volume, and education level as covariates. Controlling for these variables, we still observed a trend toward a positive relationship between sensorimotor GABA+ levels and the sensorimotor performance factor (r(36)=.32, p=.05, confidence interval [-.022, .55]) but no significant relationship between sensorimotor GABA+ levels and the grip strength factor (r(36)=.21, p=.20, confidence interval [-.11, .46]).
Figure 12. A) Relationship between sensorimotor GABA+ levels and sensorimotor performance across all participants (accounting for age and GM volume differences; $r=.32, p=.046$) and B) within each age group separately (Old: $r=.48, p=.03$; Young: $r=-.14, p=.55$). C) Relationship between sensorimotor GABA+ levels and grip strength across all participants (accounting for age and GM volume differences; $r=.21, p=.19$) and D) within each age group separately (Old: $r=.23, p=.33$; Young: $r=.20, p=.38$).

Mediation analysis

In order to further explore the potential role of network segregation as a mechanistic link between GABA levels and the sensorimotor performance factor, we performed a mediation analysis on the MR and behavioral data (we did not perform this analysis for the grip strength...
factor because GABA+ was not significantly associated with grip strength). In this analysis, GABA+ level was the independent variable (X), with sensorimotor network segregation as the mediator (M) and sensorimotor performance as the dependent variable (Y). Mediation was performed using regression with bootstrapping (Hayes & Scharkow, 2013; Hesterberg et al., 2005) to determine whether network segregation accounted for significant variance in the GABA-performance association.

Controlling for age and GM volume differences, we found that network segregation mediates the link between GABA+ and sensorimotor performance, with a percentage mediation ($P_M$) of 70%. This resulted from a significant indirect effect ($c$) of greater magnitude than the direct effect ($c'$), which itself was not significant ($ab=.13$, confidence interval [.0037, .41]; See Figure 13). Performing this analysis within each age group separately, we found no evidence for a mediation effect within either group alone. We also performed another mediation analysis using mean network segregation as the mediator variable, rather than sensorimotor network segregation. Controlling for age and GM volume differences, we found no significant mediation effect ($ab=.07$, confidence interval [-.026, .29]).
Figure 13. Sensorimotor network segregation mediates the link between sensorimotor GABA+ levels and sensorimotor performance across all participants (accounting for age and GM volume differences). The mediator accounted for 70% of the total effect (PM=.70).

Discussion

Previous studies have investigated the relationship between network segregation and behavior (M. Y. Chan et al., 2014; King et al., n.d.), GABA+ levels and behavior (Edden et al., 2009; Puts et al., 2011; Stagg et al., 2011), and within-network functional connectivity and GABA+ levels (Kapogiannis et al., 2013; Stagg et al., 2014). In the present study, we investigated whether older and younger adults differ with regard to sensorimotor network segregation, GABA+ levels, and sensorimotor performance, and for the first time we examined the association between all of these variables within the same participants. There were four main findings. First, relative to young adults, old adults exhibited less segregated sensorimotor brain networks and reduced sensorimotor GABA+ levels. Second, less segregated networks were associated with lower GABA+ levels. Third, less segregated networks and lower GABA levels were associated with worse sensorimotor performance. Fourth, network segregation mediated the relationship between
GABA+ and performance. These findings suggest that age-related reductions of GABA+ are a neurochemical substrate of age-related dedifferentiation at the level of large-scale brain networks. We now discuss each of these findings in turn.

*Resting state networks are less segregated in older adults*

Our results revealed significant age differences in the organization of large-scale resting state brain networks, such that the networks of older adults were less segregated than those of their younger counterparts. This finding was observed both in the sensorimotor network specifically, when segregation was averaged across all ten networks, and in five of the ten networks individually (uncorrected). Overall, these findings are consistent with several previous studies that also observed less segregated functional networks in older adults (Betzel et al., 2014; Cao et al., 2014; M. Y. Chan et al., 2014; Damoiseaux, 2017; Geerligs et al., 2015; Song et al., 2014).

The age differences in network segregation observed in this study are consistent with the neural dedifferentiation hypothesis of aging, which posits that aging results in reduced functional specificity of localized brain regions during task performance (J. Carp, Gmeindl, & Reuter-Lorenz, 2010; J. Carp, Park, Hebrank, Park, & Polk, 2011; Joshua Carp, Park, Polk, & Park, 2011; Li & Lindenberger, 1999; D. C. Park et al., 2004; J. Park, Carp, Hebrank, Park, & Polk, 2010). The present results are consistent with the hypothesis that neural dedifferentiation also occurs on the level of large-scale resting state functional networks. Such age-related dedifferentiation of resting state functional networks could potentially be linked to dedifferentiation of task-based activation patterns; future studies could address this question.
We also found that GABA+ levels in sensorimotor cortex were reduced in older relative to younger adults. These age differences in GABA+ levels were evident even after accounting for tissue differences in gray matter, white matter, and cerebrospinal fluid, suggesting that the effects were not due simply to cortical atrophy (Hermans et al., 2018; Maes et al., n.d.). This finding is consistent with previous work that has reported age-related reductions in GABA+ levels in frontal and parietal cortices (Gao et al., 2013; Porges et al., 2017). However, this finding is inconsistent with recent studies that have found no significant age differences in sensorimotor GABA levels (Hermans et al., 2018; Maes et al., 2017). For instance, Hermans and colleagues found that M1 GABA levels did not significantly differ between young and older adults. In addition, Maes et al. reported that age-related differences in sensorimotor and occipital GABA levels are driven by bulk tissue changes. However, after correcting for voxel composition, they observed a trend toward an age-related difference in sensorimotor but not occipital GABA levels, suggesting some degree of disproportionality in GABA levels within tissue fractions as a function of age in sensorimotor cortex only (Maes et al., 2017). The discrepancy between these findings may be due to differences in the specific cortical region studied, MRS voxel placement, or preprocessing procedures. Future studies should aim to disentangle these inconsistent findings by examining a variety of cortical regions and MRS placement and preprocessing methods in a larger sample size.

The reported decline in GABA+ levels in older adults may reflect the loss of GABAergic interneurons during normal aging. This is consistent with several animal studies that have shown
a reduction in GABAergic neurons with old age (Hua, Kao, Sun, Li, & Zhou, 2008; Stanley, Fadel, & Mott, 2012). Previous work has also provided evidence for a relationship between GABA levels and genes encoding for glutamic acid decarboxylase (GAD), a transaminase involved in the production of GABA (Marenco et al., 2010). Thus, the current findings may also be indicative of reduced GABA production with aging.

*Network segregation is related to GABA*

Our findings revealed a positive relationship between GABA+ levels and sensorimotor network segregation, such that participants with lower GABA+ levels showed less segregated networks. This observation is contrary to some previous reports that have found a negative relationship between GABA+ levels and functional connectivity (Bachtiar, Near, Johansen-Berg, & Stagg, 2015; Kapogiannis et al., 2013; Stagg et al., 2014). However, these previous studies only examined *within*-network functional connectivity in young adult participants. In contrast, our findings demonstrated a positive relationship between GABA+ levels and network segregation that was stronger in the older adult group.

Consistent with our finding, Antonenko et al. found a negative relationship between motor network connectivity and sensorimotor GABA+ levels, but only in young-older adults (i.e., 50-63 years old). In fact, when looking solely within an old-older adult group (i.e., >63 years old), there was a trend toward a positive relationship (Antonenko et al., 2017). Such an age-related dissociation may reflect disrupted neuronal functioning in older adults.
In addition to the relationship between sensorimotor GABA+ and sensorimotor segregation, there was also a significant relationship between sensorimotor GABA+ and mean network segregation (averaged across all 10 networks). It is therefore reasonable to ask whether the connection between GABA+ and network segregation is regionally specific or not. We believe that the data suggest that it is. For example, sensorimotor segregation was not correlated with GABA+ levels outside of sensorimotor cortex (i.e., VVC and auditory cortex). Furthermore, although sensorimotor segregation significantly mediated the association between sensorimotor GABA and sensorimotor behavior, mean network segregation did not (and sensorimotor GABA+ levels were related to sensorimotor performance whereas GABA+ levels outside of sensorimotor cortex were not ($p$s>.25)).

But if the GABA-segregation relationship is regionally specific, then why would sensorimotor GABA+ levels be associated with mean network segregation? We believe the answer is simple and relatively uninteresting: It turns out that sensorimotor network segregation is highly correlated with mean network segregation in both age groups (Old: $r$=.63, $p$=.002; Young: $r$=.67, $p$=.001). It is therefore not surprising that a variable like sensorimotor GABA+ that is associated with sensorimotor segregation would also be associated with mean segregation. Nevertheless, future studies should aim to test the regional specificity of the relationship between segregation and GABA in larger sample sizes.

Taken together, our results link, for the first time, network segregation to GABA+ levels, and thereby suggest a neurochemical substrate for age-related dedifferentiation at the level of large-scale resting state brain networks.
Age differences in segregation & GABA are associated with sensorimotor performance

We also found that less segregated sensorimotor networks were associated with worse sensorimotor performance. This finding is in line with previous investigations that have observed a relationship between segregation and cognitive (M. Y. Chan et al., 2014) and motor (King et al., n.d.) performance. Our study extends these findings by revealing a relationship between sensorimotor performance and segregation within the sensorimotor network. Specifically, sensorimotor network segregation was correlated with a summary measure of somatosensory and motor abilities in addition to grip strength.

Our data also revealed a positive correlation between sensorimotor GABA+ levels and sensorimotor performance. This finding is consistent with previous studies linking local GABA+ levels in motor cortex to motor performance (Boy et al., 2010), GABA+ levels in visual cortex to orientation discrimination performance (Edden et al., 2009), and GABA+ levels in sensorimotor cortex to tactile discrimination thresholds (Puts et al., 2011). Overall, these findings confirm a principal role of sensorimotor GABAergic inhibition in sensorimotor function.

It is also worth noting that nearly all the neural and neural-behavioral associations observed in this study appeared to be stronger within the older compared to the younger adult group, although these effects were not statistically significant. Future research with larger sample sizes will be necessary to determine whether these relationships are indeed driven by the older adult population. Nonetheless, these findings provide preliminary evidence for age-related dissociations in the link between GABA+, network segregation and sensorimotor performance.
They also point to the GABA-segregation relationship as a potential factor in explaining why some older adults age gracefully while others do not.

*Segregation mediates the link between GABA and sensorimotor performance*

Our findings also demonstrate for the first time that sensorimotor network segregation mediates the relationship between GABA+ levels and sensorimotor performance. One possible explanation for this finding is that age-related reductions in sensorimotor GABA levels lead to less segregated neural networks, which in turn lead to declines in sensorimotor behavior. Such a causal relationship between age declines in GABA and age-related reductions in neural specificity in visual cortex has been reported in previous animal studies (Leventhal et al., 2003). Furthermore, one recent human study reported that GABA+ levels in somatosensory cortex correlate with perceptual acuity, and that this relationship is mediated by the tuning of activity in somatosensory cortex (Kolasinski et al., 2017). Although we cannot make causal inferences from the data in the present study, our findings from the mediation analysis do provide preliminary evidence for directionality in the relationship between GABA, network segregation and behavior.

Given the relationship that we found between GABA+, network segregation and behavior, it is plausible that neuronal inhibition (mediated by GABAergic interneurons) may influence sensorimotor behavior at the network level. For instance, studies have proposed a role for beta and gamma oscillations in facilitating the synchrony of neural firing for somatosensory (Bessaih, Higley, & Contreras, 2018) and motor (van Wijk, Beek, & Daffertshofer, 2012) behavior.
Moreover, functional connectivity of the motor resting state network is linked to fluctuations in the power of beta oscillations (Brookes et al., 2011), which in turn have been linked to GABA activity (Hall et al., 2011). Thus, GABA may influence sensorimotor performance via modulation in the power of sensorimotor oscillations.

Limitations

A central challenge in network analyses concerns the different parcellation and/or clustering approaches that are used to obtain different network measures. Although some studies have reported consistent results between parcellation/clustering techniques (Cao et al., 2014; M. Y. Chan et al., 2014), these comparisons are not comprehensive. Thus, there is currently no consensus regarding what the canonical network organization approach should be. However, using an independent dataset to define ROIs and assign network labels to these ROIs (as performed in the present study) allows an examination of network segregation in a relatively unbiased manner. One problem with this approach is that the Power et al. (2011) parcellation scheme used here was based on a sample of young adults, which could potentially influence the results. Chan et al. compared the results across three different parcellation schemes (i.e., using Power (2011)-defined, age cohort-defined, and subject-defined communities) and found no significant differences across system label definitions and edge densities (M. Y. Chan et al., 2014), but this is still an important issue to consider in future studies.

The MRS technique used to measure GABA+ levels also has some limitations. The voxels used for MRS data acquisition, although fairly standard in the MRS literature (Mullins et al., 2014),
are quite large (30 x 30 x 30 mm), and were most likely not limited to sensorimotor cortex. In addition, at 3T, the MEGA-PRESS sequence results in significant excitation of coedited macromolecule (MM) signal, which has been shown to contribute ~45% to the edited signal at 3-ppm (Mullins et al., 2014; Puts et al., 2011). Accounting for MM contamination is an area of considerable on-going efforts and therefore will likely be addressed in future work.

Another limitation of the present study is that our findings were derived from cross-sectional comparisons. Therefore, we can only draw inferences about age-related differences, not age-related change (only longitudinal studies can measure change) (Maxwell & Cole, 2007). In addition, cohort and period effects hamper the interpretation of differences in neural and behavioral measures between groups (Damoiseaux, 2017) and the associations between neural and behavioral measures may suffer from the issue of cross-sectional mediation (Cole & Maxwell, 2003; Lindenberger, von Oertzen, Ghisletta, & Hertzog, 2011; Shrout, 2011). Longitudinal study designs mitigate these concerns and allow the evaluation of age-related changes in addition to the relationship between neural and behavioral measures, independent of age. Importantly, longitudinal studies also make it possible to identify the order of certain age-related changes, which may help to illuminate causal relationships. Determining directionality is critical for the early detection of age-related neural and behavioral changes and the design of potential targeted interventions.

Finally, we used behavioral factors derived from the entire sample, because the sample size in the two groups was relatively small. Nevertheless, it is worth noting that the covariance matrices were somewhat different in the two groups (See Tables S3 and S4), so it is possible that the
factors derived from the whole group were biased toward variance in one group more than the other.

Conclusions

Our findings provide evidence that 1) resting state networks are less segregated and sensorimotor GABA+ levels are reduced in older relative to younger adults; 2) less segregated sensorimotor networks are linked to lower GABA+ levels, 3) lower GABA+ levels and less segregated networks are associated with worse sensorimotor performance, and 4) the GABA-performance relationship is mediated by network segregation. Although these relationships were observed across all ages (controlling for age and GM volume difference), these associations appeared to be stronger in the older adult group specifically. These results suggest that resting state network segregation may be an important factor in distinguishing older adults who maintain their sensorimotor abilities from those who do not, and that age-related reductions in GABA may play a critical role.
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Chapter IV: Summary and Discussion

In this dissertation, I investigated age-related neural dedifferentiation in the sensorimotor system and its behavioral consequences. In the first chapter, I provided a general background on age-related neural dedifferentiation, including a review of previous studies that have investigated either dedifferentiation of task-based activation patterns or of large-scale resting state functional connectivity networks. I also provided the motivation for studying the relationship between these two measures of neural dedifferentiation as well as potential neurochemical causes of age-related neural dedifferentiation.

In the second chapter, I investigated the relationship between age-related neural dedifferentiation of large-scale resting state networks and dedifferentiation of neural activation patterns during (sensorimotor) task performance. Using multi-voxel pattern analysis to examine the distinctiveness of sensorimotor neural representations, and graph theoretical analysis to examine the segregation of resting state sensorimotor networks, I found that these two measures of neural dedifferentiation are significantly correlated. I also found that segregation predicts individual differences in sensorimotor performance, particularly in older adults, whereas distinctiveness does not, suggesting that segregation may be a more sensitive predictor of age-related declines in sensorimotor behavior.
In the third chapter, I further investigated age differences in resting state network segregation and specifically studied its relationship with sensorimotor performance and with levels of the inhibitory neurotransmitter, gamma aminobutyric acid (GABA) and to. I found that older adults have less segregated networks than young adults and that individuals with less segregated sensorimotor networks exhibit worse sensorimotor performance. Using MR spectroscopy to measure the inhibitory neurotransmitter GABA, I also found that network segregation is linked to sensorimotor GABA levels and that these levels decline with age. Furthermore, individual differences in GABA predict sensorimotor performance and this relationship is mediated by network segregation. These findings link age-related differences in network segregation to age-related differences in GABA levels and sensorimotor performance. In general, they suggest a neurochemical substrate of age-related dedifferentiation at the level of large-scale brain networks.

In sum, I have provided novel evidence 1) for a relationship between age-related neural dedifferentiation of task-based activation patterns and that of large-scale neural networks in the sensorimotor system, 2) that segregation predicts individual differences in sensorimotor performance in older adults whereas distinctiveness does not, and 3) that individual differences in sensorimotor cortex GABA levels predict individual differences in sensorimotor performance and that sensorimotor segregation mediates this relationship. Overall, these findings suggest that sensorimotor network segregation and neural distinctiveness in the sensorimotor system are related, but that segregation may be a more sensitive predictor of age-related declines in sensorimotor behavior. They also suggest that age declines in GABA may be an underlying cause of age-related neural dedifferentiation in the sensorimotor system. More broadly, the
findings from this dissertation advance our understanding of the neural and neurochemical mechanisms that underlie age-related declines in sensorimotor functioning.

**Limitations**

One main limitation of the studies presented in this dissertation is the relatively small sample size. Meta-analytic research demonstrates that neuroimaging studies are often underpowered (Yarkoni, 2009), which can lead to increased rates of false positive and inflated effects (Ioannidis, 2005, 2008). Although the present studies were better powered than most previous studies in the area, future studies should aim to recruit larger sample sizes of both young and older adult populations in order to verify these effects.

The studies in this dissertation were also conducted using atypical samples of both young and older adult participants (Henrich, Heine, & Norenzayan, 2010). For instance, young adult participants were mainly recruited from the University of Michigan and thus most likely enjoyed relatively high socioeconomic status and cognitive ability. Similarly, the older adults were mainly recruited from the University town of Ann Arbor, with higher average family incomes and cognitive abilities relative to the general public. Therefore, caution should be taken in generalizing the findings to a broader population.

Another limitation of the present studies is that our findings were produced from cross-sectional comparisons between young and older adults. Thus, we can only draw conclusions about age-related *differences* rather than age-related changes (Maxwell & Cole, 2007). Cohort and period effects also constrain the interpretation of differences in neural and behavioral measures between
the two age groups (Damoiseaux, 2017). In addition, the associations between neural and behavioral measures may be affected by cross-sectional mediation (Cole & Maxwell, 2003; Lindenberger, von Oertzen, Ghisletta, & Hertzog, 2011; Shrout, 2011). Longitudinal designs are a clear solution to this problem as they allow investigators to determine the order of certain age-related changes, which may help to elucidate causal relationships.

The present studies also utilized an a priori parcellation scheme (i.e., Power, 2011) to examine measures of network segregation. Although some studies have reported consistent findings with different parcellation techniques, these comparisons are not comprehensive and there is currently no gold standard for what the best network parcellation approach should be. One particular problem with the Power (2011) atlas is that it was based on a sample of young adults, which could potentially influence the findings from the present studies. However, previous work has found no significant age differences on network segregation using three different parcellation schemes (i.e., Power 2011-defined, age cohort-defined, and subject-defined parcellations). Nonetheless, this is still an important issue for future studies to explore.

To address these limitations, future studies should replicate the experiments documented here using 1) larger sample sizes, 2) representative samples, 3) longitudinal designs, and 4) a diverse set of brain network parcellation techniques.

**Future directions**
The present studies focused on the neural and behavioral mechanisms of age-related neural dedifferentiation in the sensorimotor system. However, it remains unclear whether the findings from these studies apply to different domains such as dedifferentiation in the visual or auditory system or in higher order cognitive (e.g., episodic, working memory) domains. One recent study conducted by Koen and colleagues demonstrated that older adults have less distinct episodic memory representations compared to young adults, and that such dedifferentiation was associated with worse memory performance (Koen, Hauck, & Rugg, 2019). Similarly, Chan and colleagues found that the segregation of association networks was predictive of episodic memory performance (Chan, Alhazmi, Park, Savalia, & Wig, 2017). Future studies could investigate whether these two measures of dedifferentiation (i.e., distinctiveness and segregation) in the episodic memory system are related and whether they explain the same age-related declines in episodic memory.

Future research could also investigate the effect of beta-amyloid and neurofibrillary tau tangle pathology on age-related neural dedifferentiation and episodic memory in healthy older adults and older adults along the Alzheimer’s disease (AD) spectrum. A loss of episodic memory is one of the hallmarks of age-related cognitive decline and is a major risk factor for dementia (Hedden & Gabrieli, 2004). Before the symptoms of dementia occur in typical-onset sporadic AD, amyloid and tau tangles appear throughout the brain, developing in the episodic memory system and tracking closely with age-related cognitive declines (Nelson et al., 2012). No studies to date have investigated whether age-related neural dedifferentiation in the episodic memory system is related to the development and spread of amyloid and tau pathology. By studying this question in healthy and pathological older adults, researchers can advance our understanding of the
pathogenesis of AD and its similarities to and differences from normal aging. Developing such an understanding could lead to the identification of those who could benefit from treatment in the earliest pre-symptomatic stages of AD.

Although we cannot make causal inferences from the results in the present studies, our findings from the mediation analysis (in chapter three) do provide preliminary evidence for directionality in the relationship between GABA, network segregation and behavior. In order to test whether GABA is indeed a neurochemical substrate of age-related neural dedifferentiation from a causal standpoint, future studies could manipulate GABA either pharmacologically or through the use of transcranial brain stimulation (TMS). By demonstrating that the manipulation of GABA levels affects (or does not affect) neural dedifferentiation and behavior, one could provide evidence to either support or refute the hypotheses from the present studies. However, recent studies have found no clear relationship between MRS-assessed measures of GABA levels and TMS measures of synaptic GABA-A and GABA-B activity (C. J. Stagg et al., 2011). In fact, it is likely that MRS-assessed GABA, which is sensitive to the total amount of GABA within a voxel, more closely reflects extra-synaptic rather than synaptic GABA activity (Stagg et al., 2014). To test the GABA-dedifferentiation-behavior link more directly and precisely, future studies could combine optogenetics with large-scale extracellular recordings in animals to activate and silence GABAergic interneurons and then quantify some measure of neural dedifferentiation and behavior.
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