Age-related Changes in GABA: Effects on Neural Distinctiveness and Variability

by

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Dedication

To my mother

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Abstract

Tens of millions of otherwise healthy people experience age-related cognitive and sensory impairments. Nevertheless, there are large individual differences in these declines and understanding the neural bases of individual differences during aging is imperative in designing future interventions to slow age-related cognitive impairments. The objective of this dissertation is to investigate the neurochemical bases of three such neural factors that may play a role, namely age-related changes in the distinctiveness of neural representations, age-related changes in the variability of neural signals, and age-related changes in the modulation of this variability in response to different visual stimuli.

Previous work in humans using functional Magnetic Resonance Imaging (fMRI) has found that neural distinctiveness in the ventral visual cortex in response to different stimulus categories (face vs. houses) is reduced in older adults compared to young adults (Park et.al.,2004), a phenomenon known as neural dedifferentiation that is also associated with poorer cognitive performance (Park et.al.,2010). In first study of the dissertation, I showed that this age-related neural dedifferentiation, measured using fMRI, extends to the auditory cortex and that individual differences in the brain's major inhibitory neurotransmitter (gamma-aminobutyric acid (GABA)), measured using MR Spectroscopy, are associated with individual differences in neural distinctiveness in older adults.

In a second study, I replicated previous research findings showing that neural variability (measured as standard-deviation in the fMRI BOLD signal, SD_{BOLD}) declines with age in most cortical regions of the brain. I also found that pharmacologically potentiating the activity of

GABA using lorazepam led to an increase in SD_{BOLD} particularly for older, poorer cognitive performers. These results provide the first evidence that GABA activity plays a causal role in individual differences in SD_{BOLD} and that in older adults it can be restored by targeting the GABAergic system.

In the third and final study, I examined the modulation of SD_{BOLD} (ΔSD_{BOLD}) during a visual task (viewing houses vs. faces). Previous research has found that SD_{BOLD} differs by cognitive states and that individuals who modulate SD_{BOLD} more in response to different task conditions perform better on a range of fluid processing tasks. Furthermore, variability seems to be upregulated to match the richness and complexity of perceptual inputs. I found that ΔSD_{BOLD} and ventrovisual GABA levels are significantly lower in older adults and lower GABA levels are associated with lower ΔSD_{BOLD} in both young and older adults. However, GABA-agonism can change ΔSD_{BOLD}: Older adults with lower baseline GABA levels experience a boost in ΔSD_{BOLD} on drug while those with higher GABA levels experience a reduction, consistent with an inverted-U account. Finally, I also found that individual differences in visual GABA levels and ΔSD_{BOLD} are both associated with individual differences in visual sensory function. These results are consistent with the hypothesis that age-related declines in GABA levels lead to a reduction in ΔSD_{BOLD}, which in turn is associated with visual function.

Across the three studies, this dissertation provides novel evidence that age-related differences in GABA play an important role in age-related changes in three different measures of neural function, all of which are linked to individual differences in behavior. This research suggests the promise of interventions targeting the brain's inhibitory systems to slow cognitive declines associated with aging.

Chapter 1 : Introduction

The world is facing an aging crisis – in 2019, 703 million individuals were over the age of 65, and that number is expected to reach a staggering 1.6 billion by 2050 (He et al., 2016). Unfortunately, aging is typically associated with substantial behavioral and cognitive declines, even in the absence of pathology. Consequently, millions of otherwise healthy people are already experiencing age-related behavioral impairments, and that number is only going to grow. However, some people age significantly more gracefully than others and understanding the neural factors that underlie those individual differences could lead to interventions to slow or even stop age-related behavioral impairments. This dissertation focuses on three neural factors that have been associated with individual differences in aging (fMRI-based distinctiveness, neural variability and stimulus-based change in variability) and the role that individual differences in the neurotransmitter GABA might play in these.

1.1 Aging World

The proportion and number of individuals who are over the age of 65 is increasing in almost every country in the world. The world-wide proportion of those over 65 has increased from 6% in 1990 to 9% in 2019 and is projected to increase to 16% by 2050. This increase in older adults can be attributed to both lower fertility rates as well as decreasing mortality rates. The global average life expectancy increased by 8 years between 1990 and 2020 and is projected to increase by an additional 4.5 years by 2050 (United Nations et al., 2020). This growth in life expectancy is one of the greatest accomplishments of modern-day medicine but the aging population is also one of the biggest challenges for the economy, families, and government.

Moreover, aging presents changes for individuals; it is typically accompanied by a decrease in physical and mental capacity along with a growing risk of disease. The promotion of healthy aging is critical in allowing people to age gracefully, maintain independent living into older ages, improve quality of life, increase life productivity, and reduce potential health costs.

1.2 Aging Brain and Cognition

Cognition — remembering, planning and organizing, making decisions, and much more— obviously depends on the brain. These cognitive abilities affect how well we do in everyday tasks and whether we can live independently. Unfortunately, aging is often accompanied by declines in cognition and sensory perception. For example, older adults may be slower at processing information, have difficulty recalling names, and get distracted more easily (Park et al., 2003). Using cognitive testing, previous research has found that speed of processing, problem solving, motor function and perception in all modalities decline with age even in the absence of pathology.

There are, however, large individual differences in these declines: some older adults experience severe cognitive declines that significantly impact their daily living and that are often early markers of pathology, while others experience only mild impairments and lead a relatively healthy life (Wilson et al., 2002). What causes these age-related declines in cognition? What makes some individuals age more gracefully than others? Understanding the age-related changes in underlying neural functioning might help us design ways for all individuals to have longer and healthier lives.

1.3 GABA and the Aging Brain

One neural factor that may be critical in age-related cognitive declines is a reduction in the brain's major inhibitory neurotransmitter, gamma-aminobutyric acid (GABA). A growing literature, in both animals and humans, indicates that GABA function declines with age through several mechanisms including reduction of GABA synthesis, GABA release, receptor density, receptor binding and GABA-ergic neuron density. For example, during normal aging in humans and rhesus macaques, genes related to GABA-ergic function are down-regulated at the level of both mRNA and protein, independent of neuronal or synaptic loss (Loerch et al., 2008). GABA-ergic interneurons in the prefrontal cortex and hippocampus degenerate or cease to express glutamic acid decarboxylase (GAD)-67, the GABA-synthesizing enzyme, in aged rats (Shetty and Turner, 1998; Stanley and Shetty, 2004; Stranahan et al., 2012). Similarly, the number of GABA-immunoreactive neurons declines with age in the striate cortex in cats (Hua et al., 2008), and in rats' inferior colliculus (Caspary et al., 1990). There are also age-related reductions in GABA receptors, baseline GABA, and GABA release in rats (Caspary et al., 1995).

Age-related reductions in GABA levels can also be measured in humans using magnetic resonance spectroscopy (MRS). Researchers have found GABA to be reduced in older adults compared to younger adults in the occipital cortex (Chalavi et al., 2018; Hermans et al., 2018; Chamberlain et al., 2021), in the frontal and parietal regions (Gao et al., 2013; Hermans et al., 2018), and in the supplementary motor area and sensorimotor cortex (Chalavi et al., 2018; Hermans et al., 2018; Cassady et al., 2019).

Although age-related GABA reductions are ubiquitous in the human brain, they are not uniform across different regions or lifespan. For example, (Porges et al., 2021), found that there is a non-linear relationship between age and GABA levels measured using MRS in humans

where GABA levels increase from age 10 to 20 and decline after the age of 40. Another study of 94 older adults found that age-related declines in GABA levels were more aggressive in frontal regions compared to posterior regions (Porges et al., 2017). Ling et al., 2005, found that in rats there was a significant loss of GAD in the auditory cortex through middle age, but age-related changes in GAD were only present in the oldest group in the parietal regions. Importantly, there are not only age and region-specific differences in GABA reduction but also meaningful individual differences in GABA levels.

Individual differences in GABA in specific cortical regions have been associated with individual differences in cognition (Porges et al., 2017; Hermans et al., 2018; Simmonite et al., 2018). Lower GABA levels in the motor cortex are also linked with sensorimotor performance (Cassady et al., 2019) while those in the auditory cortex have been linked to age-related decline in auditory function (Dobri and Ross, 2021). How does this age-related reduction in GABA level lead to cognitive declines?

1.4 Role of GABA levels in age-related neural changes

GABA is vital to the functioning of neural networks – it is associated with cortical plasticity (Jones, 1993; Hensch et al., 1998; Fagiolini et al., 2004), with the selectivity of stimulus representations (Leventhal et al., 2003; Shu et al., 2003; Rubin et al., 2017), with neural oscillations and connectivity (Fingelkurts et al., 2004; Bonifazi et al., 2009; Kapogiannis et al., 2013), and with information capacity (Shew et al., 2011; Puzerey and Galán, 2014), pattern complexity (Monteforte and Wolf, 2010; Lajoie et al., 2014; Agrawal et al., 2018) and the dynamic range of neural networks (Shew et al., 2009; Agrawal et al., 2018). In this dissertation, I investigated the role of GABA in three different fMRI-based neural measures that have been

found to decline with age and to be associated with individual differences in cognition: neural distinctiveness, brain signal variability, and modulation of brain signal variability.

1.5 Neural Distinctiveness

Study 1 focuses on the scope and cause of age-related decline in neural distinctiveness. Neuroimaging studies have repeatedly found that activation patterns evoked by different categories of visual stimulus are more similar (less distinctive or differentiated) in older adults than in younger adults, a phenomenon referred to as age-related neural dedifferentiation (Park et al., 2004, 2010; Voss et al., 2008; Carp et al., 2011). For example, Park et al., (2010) trained a support vector machine (SVM) to distinguish fMRI activation patterns evoked by faces from activation patterns evoked by houses and then tested its accuracy in classifying activation patterns on which it had not been trained. The classifier was significantly more accurate in distinguishing face patterns from house patterns in young compared with older adults, providing evidence that neural distinctiveness declines with age in the visual cortex. Following Haxby et al., (2001), Carp et al., (2011) assessed the similarity (correlation) of activation patterns evoked by faces, houses, and words in young and old adults. In young adults, activation patterns evoked by the same stimulus category (e.g., different face blocks) were much more similar than patterns evoked by different stimulus categories (e.g., face vs. house blocks), but this measure of neural distinctiveness also declined significantly with age.

Most previous studies of neural dedifferentiation have focused on the visual cortex, and so it remains unclear the extent to which dedifferentiation occurs in other cortical regions, such as the auditory cortex. I thus asked: Are activation patterns measured using fMRI in the auditory cortex less selective or distinctive in older compared with younger adults?

Evidence from animal research suggests that the answer to this question is likely to be yes. It has been shown that the receptive fields of individual neurons in the auditory cortex, like in the visual cortex, become less selective or differentiated with age. For example, Turner et al., (2005) reported that the receptive fields of auditory neurons are less selective to pure tones in older rats compared with younger rats. Likewise, neurons in the primary and secondary auditory cortex are less spatially tuned in older compared with younger macaques (Juarez-Salinas et al., 2010) and auditory frequency selectivity also declines with age in mice (Leong et al., 2011). Together, these results suggest that in many mammals, the neural selectivity of single neurons declines in the auditory cortex. Of course, the effects of age on neural selectivity as measured at the level of single neurons in animals could be very different from the effects on the distinctiveness of gross functional activation patterns measured by fMRI in humans. I therefore wanted to investigate whether activation patterns in the auditory cortex are less distinctive in older compared with younger adults, like they are in the visual cortex.

In study 1, I also investigated the neurochemical basis of individual differences in neural distinctiveness, and specifically, the role of GABA. Previous studies in animals have shown a causal link between GABA levels and selectivity at the level of single neurons. Leventhal et al. (2003) showed that the application of GABA or a GABA agonist increased the orientation selectivity of cells in the visual cortex of older rhesus monkeys. Conversely, application of a GABA antagonist decreased the orientation selectivity of cells in the visual cortex of young monkeys (Leventhal et al., 2003). GABA receptor antagonists have also been shown to broaden the frequency response of neurons in the inferior colliculus of chinchillas, making the cells' response less selective (Caspary et al., 2002). These animal-based findings suggest that inhibitory GABA levels play a causal role in maintaining the neural selectivity of single neurons

and raise the possibility that age-related declines in GABA levels might contribute to age-related declines in neural distinctiveness. I therefore wanted to investigate the association between GABA levels in the auditory cortex of each individual estimated using MRS and neural distinctiveness measured using fMRI.

1.6 Brain Signal Variability

Study 2 focuses on the causal role of age-related decline in GABA levels in age-related reductions of another neural measure: brain signal variability. Even during rest, brain signals as measured by fMRI vary considerably from moment-to-moment. This moment-to-moment brain signal variability is often treated as noise, but substantial evidence now demonstrates it is more than just noise. For example, the standard deviation of the fMRI BOLD signal (SD_{BOLD}) declines significantly with age (Garrett et al., 2010, 2011; Grady and Garrett, 2014) and this effect of age on SD_{BOLD} is robust to multiple vascular controls (Garrett et al., 2017). Furthermore, theoretical and computational models suggest that greater brain signal variability might confer a number of advantages, including higher complexity and flexibility, greater dynamic range and information transfer capacity, and stronger long-range functional connectivity (Li et al., 2006; Faisal et al., 2008; McIntosh et al., 2008, 2010; Shew et al., 2009, 2011; Garrett et al., 2010, 2011, 2013, 2013; Deco et al., 2011; Mišić et al., 2011; Vakorin et al., 2011; Beharelle et al., 2012). An age-related decline in SD_{BOLD} might thus underlie some of the behavioral impairments seen with aging. Consistent with this idea, previous research has found that SD_{BOLD} predicts individual differences in several cognitive abilities (Garrett et al., 2010, 2011, 2013; Grady and Garrett, 2014). But what is the neurochemical basis of individual differences in SD_{BOLD}?

Computational modelling and animal research suggest that GABA levels might play a critical role (Shew et al., 2009, 2011; Agrawal et al., 2018). Computational modelling suggests

that a neural network can sample a greater variety of states when inhibitory levels are optimal. Consistent with this idea, decreasing GABA activity pharmacologically in rats and monkeys reduced the number of states sampled by the cortical network (Shew et al., 2011). Since, brain signal variability and GABA levels are associated with several of same network properties - cortical plasticity, functional connectivity, and information capacity and transfer efficiency, I hypothesized a link between GABA and brain signal variability. To investigate this hypothesis, I manipulated GABA activity pharmacologically in healthy 25 older and 20 younger adults and examined the effect on SD_{BOLD}. I also investigated the relationship between baseline fluid processing abilities and the drug-induced change in SD_{BOLD} in older adults.

1.7 Modulation of Brain Signal Variability

In Study 3, I investigated the role of individual differences in visual GABA levels in stimulus-related change in visual variability and visual function. It has been hypothesized that the ability to modulate brain signal variability across distinct cognitive states reflects one's ability to tune internal neural dynamics to match the dynamics of the external world. Specifically, it has been suggested that brain signal variability is modulated to match the complexity of input stimuli. For example, activity might be suppressed in response to more common and simple stimuli, but would be more dynamic in response to rare, more differentiated/complex stimuli. The idea is that the brain reduces signal variability when stimulus input is more reducible (less feature rich or complex), thereby saving energy. Conversely, the brain upregulates signal variability when stimulus input is more differentiated (complex or feature rich). Thus, upregulation of variability when processing more complex stimulus is thought to reflect an optimal and well-functioning neural network (Garrett et.al.,2020).

I investigated stimulus-based changes in visual variability (ΔSD_{BOLD}) in response to a more feature-rich or complex category (houses) compared to a less feature-rich and complex category (faces). Computational modelling has confirmed that houses are indeed more feature-rich than faces, and previous fMRI studies have found that SD_{BOLD} in the visual cortex is upregulated in response to houses compared to faces. Moreover, individual differences in the degree of this upregulation predicted individual differences in visuo-cognitive abilities in older adults (Garrett et al., 2020). I investigated the role of GABA levels measured in the visual cortex in the modulation of brain signal variability (ΔSD_{BOLD}), whether ΔSD_{BOLD} can be changed by altering GABA activity pharmaceutically, and how GABA levels and ΔSD_{BOLD} relate to individual differences in visual function.

Together, the findings from these studies advance our understanding of the neural and neurochemical mechanisms underlying age-related cognitive and sensory declines and opens new avenues for pharmacological interventions to treat cognitive declines in healthy aging. Note that studies 1 and 2 have already been published (Lalwani et al., 2019, 2021) while study 3 is being prepared for submission.

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Chapter 2 : Neural Distinctiveness Declines with Age in Auditory Cortex and is associated with Auditory GABA Levels.

Abstract

Neural activation patterns in the ventral visual cortex in response to different categories of visual stimuli (e.g., faces vs. houses) are less selective, or distinctive, in older adults than in younger adults, a phenomenon known as age-related neural dedifferentiation. In this study, we investigated whether neural dedifferentiation extends to auditory cortex. Inspired by previous animal work, we also investigated whether individual differences in GABA are associated with individual differences in neural distinctiveness in humans. 20 healthy young adults (ages 18-29) and 23 healthy older adults (over 65) completed a functional magnetic resonance imaging (fMRI) scan, during which neural activity was estimated while they listened to music and foreign speech. GABA levels in the auditory, ventrovisual and sensorimotor cortex were estimated in the same individuals in a separate magnetic resonance spectroscopy (MRS) scan. Relative to the younger adults, the older adults exhibited both (1) less distinct activation patterns for music vs. speech stimuli and (2) lower GABA levels in the auditory cortex. Also, individual differences in auditory GABA levels (but not ventrovisual or sensorimotor GABA levels) were associated with individual differences in neural distinctiveness in the auditory cortex in the older adults. These results demonstrate that age-related neural dedifferentiation extends to the auditory cortex and suggest that declining GABA levels may play a role in neural dedifferentiation in older adults.

2.1 Introduction

Aging is often accompanied by declines in cognitive (Harada et al., 2013; Park et al., 2002; Salthouse, 1996) and sensory function (Fortunato et al., 2016). These declines have a significant negative impact on the daily lives of older individuals and are often early indicators of pathology. However, there are substantial individual differences in these declines: some older adults experience severe impairments while others do not (Christensen et al., 1999; Hultsch et al., 2002; Wilson et al., 2002). Understanding the neural bases of these individual differences may therefore be helpful in designing interventions that slow or halt some age-related impairments.

One neural factor that may play a role is an age-related decline in neural distinctiveness. Neuroimaging studies have repeatedly found that activation patterns evoked by different categories of visual stimulus are more similar (less distinctive or differentiated) in older adults than in younger adults (Carp et al., 2011b; Park et al., 2004; Voss et al., 2008), a phenomenon referred to as age-related neural dedifferentiation. For example, Park et al. (2004) reported that young adults exhibit much greater activation in the fusiform face area (FFA) when viewing faces than when viewing words or buildings. In contrast, older adults exhibited almost as much activity in the FFA when viewing words and buildings as they did when viewing faces. In other words, activity in the FFA was more specialized or distinctive in the young compared with the old. Likewise, activity in the parahippocampal place area was more specialized for buildings in the young compared with the old, and activity in the visual word form area was more specialized for words.

Similar results have been reported using multi-voxel pattern-based analysis (MVPA). For example, Park et al. (2010) trained a support vector machine (SVM) to distinguish fMRI

activation patterns evoked by faces from activation patterns evoked by houses and then tested its accuracy in classifying activation patterns on which it had not been trained. The classifier was significantly more accurate in distinguishing face patterns from house patterns in young compared with older adults, providing additional evidence that neural distinctiveness declines with age. Following Haxby et al., (2001), Carp et al. (2011b) assessed the similarity (correlation) of activation patterns evoked by faces, houses, and words in young and old adults. In young adults, activation patterns evoked by the same stimulus category (e.g., different face blocks) were much more similar than patterns evoked by different stimulus categories (e.g., face vs. house blocks) suggesting high neural distinctiveness. However, this measure of neural distinctiveness declined significantly with age. We use both the SVM- and similarity-based measures of neural distinctiveness in this study.

Individual differences in neural distinctiveness have also been associated with individual differences in behavior in older adults. For example, Park et al. (2010) assessed behavioral performance on a range of fluid processing tasks that tend to decline with age (WAIS Digit Symbol task, Dot Comparison task, Trail-making tasks A and B, and the Controlled Oral Association Task (verbal-fluency)). They found that individual differences in neural distinctiveness accounted for over 30% of the variance in fluid processing ability over and above age. Likewise, Koen, Hauck, & Rugg (2019) reported that neural distinctiveness in the parahippocampal place area was significantly correlated with recognition memory performance and with a latent fluency factor derived from the neuropsychological test battery.

Most previous studies of neural dedifferentiation have focused on the visual cortex, and so it remains unclear the extent to which dedifferentiation occurs in other cortical regions, such as auditory cortex. There is evidence that the receptive fields of individual neurons in auditory

cortex become less selective or differentiated with age. For example, Turner, Hughes, & Caspary (2005) reported that the receptive fields of auditory neurons are less selective to pure tones in older rats compared with younger rats. Frequency selective bandwidths of auditory neurons also get larger and receptive fields overlap more in older rats (Villers-Sidani et al., 2010). Likewise, neurons in primary and secondary auditory cortex are less spatially tuned in older compared with younger macaques (Juarez-Salinas et al., 2010) and auditory frequency selectivity also declines with age in mice (Leong et al., 2011). Together, these results suggest that in many mammals, the neural selectivity of single neurons declines in auditory cortex. Of course, effects of age on neural selectivity as measured at the level of single neurons in animals could be very different from effects on the selectivity of gross functional activation patterns measured by fMRI in humans. One goal of the present study is therefore to investigate whether activation patterns in auditory cortex are less selective or distinctive in older compared with younger adults, like they are in visual cortex.

Another neural factor that may contribute to age-related behavioral impairments is declines in the brain's major inhibitory neurotransmitter, gamma-aminobutyric acid (GABA). GABA levels measured using magnetic resonance spectroscopy (MRS) are reduced in older adults compared to younger adults in the occipital cortex (Chalavi et al., 2018; Hermans et al., 2018; Simmonite et al., 2018), in frontal and parietal regions (Gao et al., 2013; Hermans et al., 2018), and in supplementary motor area and sensorimotor cortex (Cassady et al., 2019; Chalavi et al., 2018; Hermans et al., 2018). Furthermore, individual differences in GABA in specific cortical regions have been associated with individual differences in some aspects of cognitive (Hermans et al., 2018; Porges et al., 2017; Simmonite et al., 2018) and motor (Cassady et al., 2019) performance. However, the results of the small number of human studies investigating

age-related changes in GABA levels in the auditory cortex are mixed (Chen et al., 2013; Profant et al., 2015). In the present study, we therefore also investigated whether older adults exhibit reduced GABA levels in the auditory cortex compared with young adults.

To date, age-related neural dedifferentiation and declines in GABA levels have been studied in isolation from one another. In the present study, we also test whether individual differences in GABA are associated with individual differences in neural distinctiveness and if this relationship is region specific. This work is motivated by previous studies in animals showing a causal link between GABA levels and neural selectivity. Leventhal et al. (2003) showed that the application of GABA or a GABA agonist increased the orientation selectivity of cells in the visual cortex of older rhesus monkeys. Conversely, application of a GABA antagonist decreased the orientation selectivity of cells in the visual cortex of young monkeys (Leventhal et al., 2003). GABA receptor antagonists have also been shown to broaden the frequency response of neurons in the inferior colliculus of chinchillas, making the cells' response less selective (Caspary et al., 2002). These animal-based findings suggest that inhibitory GABA levels play a causal role in maintaining the neural selectivity of single neurons and that age-related declines in GABA levels might therefore mediate age-related declines in neural selectivity.

Of course, selectivity at the level of individual neurons is quite different from selectivity at the level of fMRI activation patterns. Nevertheless, age-related declines in GABA could plausibly influence both. For example, many models of cortical processing assume that neural representations compete with each other and that more active representations inhibit less active representations in a kind of winner-take-all competition (Desimone and Duncan,1995; O'Reilly, 1998). Such competition between neural representations is presumably mediated by inhibitory interneurons using GABA. And if GABA levels decline with age, then winning neural

representations would be less able to inhibit other representations, potentially resulting in the kind of neural dedifferentiation observed with fMRI. We investigated this idea by measuring GABA levels and neural distinctiveness in the same individuals in the auditory cortex and assessing the relationship between these measures.

In sum, we combined fMRI and MRS to test the hypotheses that age-related dedifferentiation extends to the human auditory cortex, that auditory GABA levels decline with age, and that GABA levels are associated with neural distinctiveness in the auditory cortex of older adults.

2.2 Methods

2.2.1 Participants

Twenty young adults (8 males, mean age = 23.6, range 18 to 28 years) and 23 older adults (7 males, mean age = 69.91, range 65 to 81 years) adults participated in the study. Carp et al., 2011, found that the neural representations of visual stimuli are less distinct in older adults than in young adults (effect size: Cohen's d = 1.06). Assuming a similar effect size in the auditory modality, a sample of approximately 20 subjects per group would be required to achieve 90% power to detect an effect. All participants were right-handed, native English speakers with normal or corrected to normal vision. We excluded participants who used hearing aids or scored lower than 23 on the Montreal Cognitive Assessment (MOCA) (Carson et al., 2018). We ensured that none of our participants knew any of the foreign languages that were used as auditory stimuli for the fMRI task. All sessions took place at the University of Michigan's Functional MRI Laboratory, Ann Arbor, Michigan. Participants were recruited from Ann Arbor and the surrounding area.

2.2.2 Session Design

Eligible participants completed a functional MRI session and an MRS session on the same scanner on separate days within a few weeks of each other. These data were collected as a part of larger study called the Michigan Neural Distinctiveness or MiND study. Here, we only describe the portions of the study that are relevant to this experiment. Please refer to (Gagnon et al., 2019) for further details on the MiND study itself.

2.2.3 fMRI Session

We collected both structural and functional MRI data using a 3T General Electric Discovery Magnetic Resonance System with an 8-channel head coil at the Functional MRI Laboratory, University of Michigan, Ann Arbor, MI, USA. We obtained T1-weighted images using an SPGR (3D BRAVO) sequence with the following parameters: Inversion Time (TI) = 500 ms; flip angle = 15°; Field of View (FOV) = 256 x 256 mm. While the structural scan was being collected, each participant heard a trial version of the auditory stimuli and the volume was adjusted to ensure that each participant could comfortably hear the stimuli presented during the scan.

During the functional scans, T2*-weighted images were collected with a 2D Gradient Echo spiral pulse sequence with the following parameters: TR = 2000 ms; TE = 30 ms; flip angle = 90°; FOV = 220 x 220 mm; 43 axial slices with thickness = 3 mm and no spacing, collected in an interleaved bottom-up sequence. The total acquisition time for the functional scan was 6 minutes and 10 seconds with 185 volumes. E-Prime software was used to present auditory stimuli, which consisted of six 20-second blocks of foreign speech clips, six 20-second blocks of instrumental music clips, and twelve 10-second blocks of fixation between every pair of auditory blocks. The order of the speech and music blocks was pseudorandomized.

Each speech block consisted of a 20-second news segment in one of the following foreign languages: Creole, Macedonian, Marathi, Persian, Swahili and Ukranian. Each music block consisted of a 20-second segment of instrumental music from one of the following pieces: Bach Sinfonia No. 5, Smokey by Mountain, Bamboula by L.M Gottschalk, Spagnoletta Nuova by Fabritio Caroso, Kuhlau: Fantaisie for Solo Flute in D major (Op. 38, No. 3), and a violin rendition of the country song "When the right one comes along".

A fixation cross was presented on the screen for the entire duration of the task. To ensure that subjects were attending to the auditory presentation, target trials (guitar plucks) occurred randomly about once a minute during the task. The participants were instructed to press a button with their right index finger every time a target trial was presented. Sounds were presented through an MRI-compatible Avotec Conformal Headset.

2.2.4 MRS Session

MR Spectroscopy data was collected using the same scanner on a different day. During this second session, we first collected T1-weighted structural images using the same parameters as in the fMRI session. MRS data were acquired using a MEGA-PRESS sequence with the following parameters: TE=68ms (TE1=15ms, TE2=53ms), TR=1.8sec, 256 transients (128 ON interleaved with 128 OFF) of 4,096 data points; spectral width=5kHz, frequency selective editing pulses (14ms) applied at 1.9ppm (ON) and 7.46 ppm (OFF); total scan time about 8.5 minutes per voxel.

MRS data were collected from two 3cm x 3cm x 3cm voxels placed in the left and right auditory cortex (Figure 1), left and right ventrovisual cortex and left and right sensorimotor cortex (Figure S1). In order to ensure subject-level specificity, auditory voxels were placed to

overlap maximally with each participant's own functional activation maps (using a contrast of Speech + Music vs. Fixation) obtained from the fMRI run described previously.

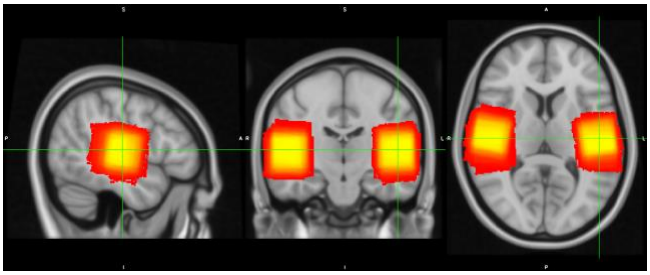

Figure 1. ***MRS voxel overlap across participants.*** *Brighter (yellow) colors representing more participant overlap and darker (red) colors representing less overlap*

2.2.5 Quantification of GABA levels

We used the Gannet 3.0 MATLAB toolbox to estimate GABA levels in each of the two (left and right auditory) MRS voxels. The time domain data was frequency- and phase-corrected using spectral registration. It was filtered with 3-Hz exponential line broadening and zero-filled by a factor of 16. GABA levels were computed by fitting a Gaussian model to the 3-ppm peak in the difference spectrum and quantified relative to water (fit with a Gaussian-Lorentzian model) in institutional units (Figure S2). This editing scheme results in significant excitation of coedited macromolecule (MM) signal, that have been reported to contribute approximately 45% to the edited signal at 3-ppm. Thus, we report all GABA values as GABA+ (i.e., GABA + MM) in the present study. There are substantial differences in the relaxation constants and water visibility between white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF). To account for these differences, a binary mask of the MRS voxels was created using Gannet's integrated voxel-to-image co-registration. Next, segmentation of the anatomical image was performed using the Segment function in SPM12 and the voxel fractions containing CSF, GM and WM were computed. From this procedure, a tissue-corrected GABA+ value was calculated for each participant. Since the signal in GM and WM have different strengths, an alpha tissue-corrected (fully corrected) GABA+ value was also computed for each participant. We also estimated levels

of N-acetylspartate (NAA) from LCModel (Provencher, 1993**)** to control for neural integrity differences within older adults.

2.2.6 fMRI Data Preprocessing

fMRI data were k-space despiked, reconstructed, and corrected for heartbeat and breathing using the RETROICOR algorithm. The initial five volumes were deleted and the data were then slice time corrected using the spm_slice_timing function from SPM. Motion correction was performed using the Freesurfer FSFAST processing stream. Freesurfer was used to resample the data into two-dimensional cortical surfaces (one for the left hemisphere and one for the right hemisphere) based on a white/gray matter segmentation of each subject's own high-resolution structural image computed using Freesurfer's recon-all function. The data were then spatially smoothed within each cortical surface using a 5-mm two-dimensional smoothing kernel.

2.2.7 ROI Selection

Because this was an auditory task we restricted our analysis to an anatomical mask containing the bilateral superior temporal gyrus, bank of the superior temporal sulcus, transverse temporal gyrus and supramarginal gyrus using cortical parcellation labels generated by FreeSurfer based on the Desikan-Killiany Atlas (aparc.annot). The resulting mask contained more than 37,000 vertices on the cortical surface (Figure 2). We obtained grey-matter thickness, volume and surface area estimates within this mask.

In order to ensure that only subject-specific, task-relevant vertices were analyzed, we then created a functional mask for each subject. Neural activation was estimated using a General Linear Model, fit with two box-car regressors (music vs. fixation and speech vs. fixation), convolved with a standard hemodynamic function (See Figure S3 for example contrast maps).

Beta values for each of the two regressors were obtained at each vertex. The functional mask was generated by selecting the most active vertices from both conditions in an alternating order (e.g., the most highly activated vertex for the music vs. fixation contrast, then the most highly activated vertex for the speech vs. fixation contrast, then the next most activated vertex for the music vs. fixation contrast, etc.). If the next most active vertex for a contrast had already been included in the functional mask, then the next most active voxel that had not already been included in the functional mask was added. This approach ensured that both conditions were equally represented in the functional mask. The functional mask selection was blind to whether the chosen vertex was selective for one condition or was activated by both conditions (Figure 2).

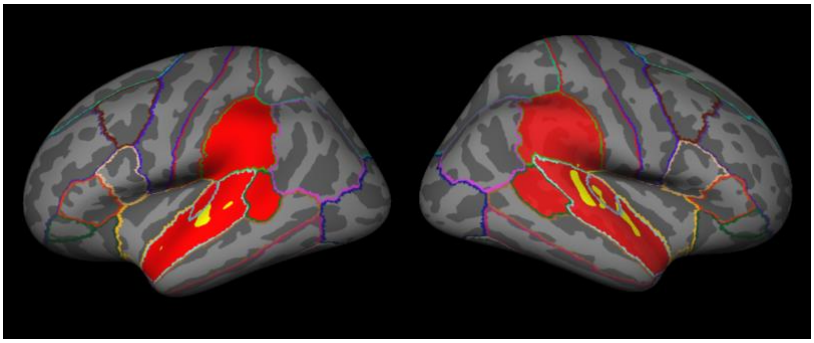

Figure 2. ***Participant-specific example mask.*** *Structural (in red) and functional (in yellow) masks. The functional mask was based on the 1400 most activated vertices from the music vs. fixation and foreign speech vs. fixation contrasts, under the constraint that an equal number of vertices were included from each contrast.*

Using a speech + music vs. fixation contrast, we calculated the total number of vertices across both hemispheres that were activated ($p<0.001$, uncorrected) during auditory perception for each subject. 95% of the subjects had greater than 1400 such vertices, so we chose an ROI-size of 1400 vertices as our default functional mask size. We also varied the ROI-size from small (1000 vertices) to very large (the entire anatomical mask) to ensure that any observed effects on neural distinctiveness did not depend on the size of the ROI.

2.2.8 Neural activation

In order to generate multiple independent activation patterns for use in multivoxel pattern analysis (MVPA), we then fit another General Linear Model that included separate box-car

regressors for each of the 12 task-blocks (6 music and 6 speech), convolved with a standard hemodynamic function. Fitting the model produced beta values at each vertex separately for each of the 12 blocks. Neural distinctiveness was computed using these beta values (activation maps) as described below.

2.2.9 SVM-based calculation of distinctiveness

Machine learning classifiers, such as linear-SVMs (support vector machines), find a hyperplane that maximally separates multidimensional datapoints into different categories based on labeled training data. Classification accuracy can then be assessed on new, untrained activation patterns. Following previous work (Park et.al., 2010), we used SVM accuracy as a proxy for neural distinctiveness; if accuracy in classifying activation patterns is high, then those patterns are considered distinctive. Conversely, if accuracy is low, then the distinctiveness of the patterns is low. We used a leave-one-pair-out cross-validation approach, in which the classifier was trained to fit 10 of the 12 activation maps (5 music and 5 speech) within the functional ROI and then was tested on the two left-out activation maps (1 music and 1 speech). This process was repeated leaving out each of 36 different activation map pairs and the average classification accuracy was used as a measure of neural distinctiveness or specificity. Classification accuracy of 50% is chance.

2.2.10 Correlation-based calculation of distinctiveness

We also used a correlation-based approach that produces a more continuous measure of neural distinctiveness and that avoids ceiling effects (Haxby et al., 2001; Park et al., 2010). For each subject, correlations between the activation maps for all unique pairs of blocks of the same type were computed within the functional ROI (e.g., music block 1 with music block 2, music block 3 with music block 6, speech block1 with speech block4, etc.). These correlations were

then averaged to produce a within-category correlation value. Likewise, correlations between activation maps for all unique pairs of blocks of different types were computed (e.g., music block 1 with speech block 2, music block 3 with speech block 6, speech block1 with music block4, etc.). These correlations were then averaged to produce a between-category correlation value. Neural distinctiveness was then defined as the difference between the average within-category correlation and average between-category correlation. This measure has a theoretical range of 2 to -2. This multivariate analysis reveals fine-grained differences in the distinctiveness of activation patterns rather than differences in the average activation between the two categories as a univariate method would.

2.3 Results

2.3.1 Neural Distinctiveness and Aging

Neural distinctiveness as measured by SVM classifier accuracy (Figure 3a) was significantly lower in older adults (mean = 85.9%) compared to young adults (mean = 96.3%), ($t(41) = -3.2$, $p = 0.005$ (based on 10,000 bootstraps). Likewise, when neural distinctiveness was computed based on pattern similarity/dissimilarity using the difference between within-category and between-category correlations (Figure 3b), older adults exhibited less distinctive activation patterns (mean = 0.27) than did young adults (mean = 0.39) ($t(41) = -2.04$, $p = 0.047$). In other words, using both measures the activation patterns for music and speech were more similar or confusable in older adults than younger adults.

One problem with the SVM-based measure of distinctiveness is that it is prone to ceiling effects. For example, the classifier was 100% accurate in classifying the activation patterns for 17 of the 43 participants. In contrast, the correlation-based measure can take on any real value between -2 and 2 and is much less susceptible to ceiling effects. The two measures were also

significantly correlated (r (41) = 0.43, p = 0.004). We therefore used the correlation-based measure for subsequent analyses.

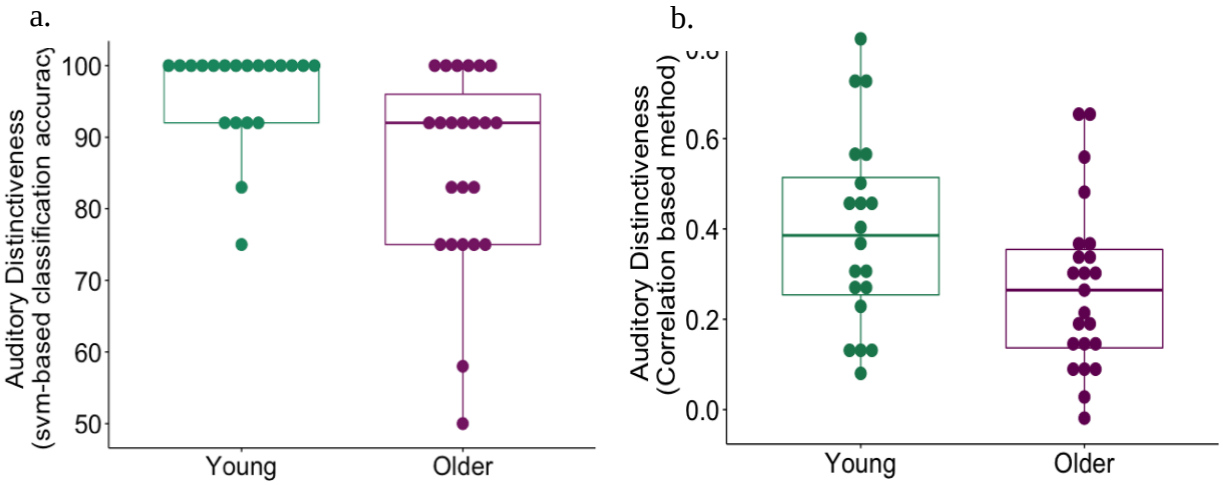

Figure 3. ***Auditory neural distinctiveness and Age.*** *(a) Neural distinctiveness based on the accuracy of an SVM classifier in distinguishing music from foreign speech (percent correct classifications). Distinctiveness was significantly lower in older adults (in purple) than young adults (in green) (t (41) = -3.2, p = 0.005 (based on 10,000 bootstraps)). (b) Neural distinctiveness based on the difference between within-condition similarity and between-condition similarity. Distinctiveness was again significantly lower in older adults (in purple) than young adults (in green) (t (41) = -2.04, p = 0.047).*

There was no significant difference between the number of activated vertices (p<0.001, uncorrected) within the anatomical mask for young and older adults in a music + speech vs. fixation contrast (t (41) = -0.64, p = 0.53). Furthermore, there was no significant difference in the mean (t (41) = -1.26, p=0.22) or peak (t (41) = -0.71, p=0.48) activation level between the two age groups for this contrast. Similarly, the total number of vertices activated during the music vs. fixation contrast (t (41) = -0.86, p = 0.39) and speech vs. fixation contrast (t (41) = 0.81, p = 0.42) did not differ between the two age-groups. Differences in distinctiveness between the two age groups were therefore not driven by differential activation levels between the groups, but rather by differences in the similarity/dissimilarity of neural activation patterns elicited by music and speech. In order to ensure that the effect of aging on neural distinctiveness was not due to the selection of a particular ROI size, we computed a pairwise t-test at every ROI size and found that

distinctiveness declined with age independent of ROI size selection (the effect was only marginally significant at the smallest ROI size) (Figure 4, Table S1). However, as ROI-size increased, the average distinctiveness values declined suggesting that the larger ROIs included task-irrelevant vertices that added noise to the distinctiveness measure.

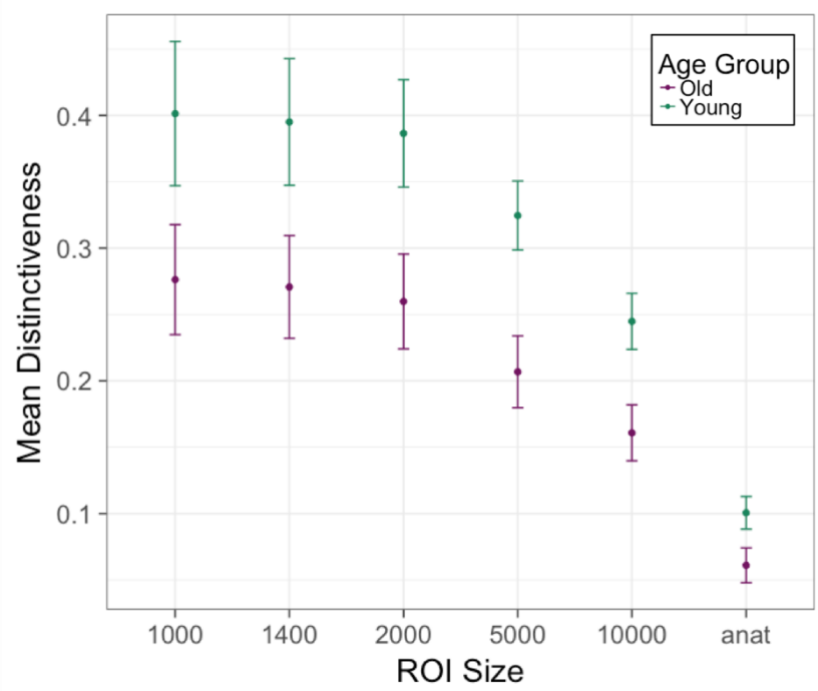

Figure 4. ***Neural distinctiveness and Age as a function of ROI size.*** *Distinctiveness was significantly lower in older adults (in purple) than younger adults (in green) for most ROI sizes (also see Table 1). The vertical axis is mean distinctiveness (measured as within-between difference) for each ROI size and group (young and older adults) with standard error bars. The horizontal axis is the ROI size (in number of vertices; "anat" refers to the entire anatomical mask of approximately 37,000 vertices).*

Somewhat surprisingly, we did not find any significant differences in mean activation levels in the left vs. right hemisphere, either in the speech vs. fixation contrast (t_{speech} (42) = 0.57, p = 0.57) or the music vs. fixation contrast (t_{music}(42) = 1.4, p = 0.14). Likewise, we did not observe significant differences in peak activation levels (t_{music} (42) = 1.01, p = 0.32; t_{speech} (42) = -0.22, p = 0.82) or in the total number of activated vertices (t_{music} (42) = 0.77, p = 0.44; t_{speech} (42) = -0.4, p = 0.7) between right and left hemispheres.

The observed age-related decline in neural distinctiveness could be due to changes in the ear, to changes in the brain, or both. In particular, peripheral changes in the ear that reduce auditory sensitivity could lead to reduced neural distinctiveness, independent of age-related changes in auditory cortex itself. To explore this issue, we analyzed participants' pure-tone threshold and its relationship to neural distinctiveness. The older adults exhibited higher pure-tone thresholds at frequencies above 2000 Hz ($t_{4000}(41) = 6.3$, $p = 1.4e-07$, $t_{8000}(41) = 5.9$, $p = 6.3e-07$). Nevertheless, neural distinctiveness was still significantly lower in the older vs. younger participants after controlling for average pure tone threshold ($t(41) = -2.04$, $p = 0.048$) and average pure tone threshold was not significantly associated with neural distinctiveness, whether analyzed in the whole sample ($r(41) = -0.04$, $p = 0.79$) or in the older group alone ($r(21) = -0.17$, $p = 0.43$). Furthermore, when neural distinctiveness was correlated with pure tone threshold at each individual frequency (125,500,1000,2000,4000,8000), none of the associations was significant (all p's > 0.25). One reason we may not have observed any associations is that greater than 90% of the power in our auditory stimuli were at frequencies below 2000 Hz (See Figure S6) where the effects of age on pure tone threshold were not significant ($t_{125}(41) = 1.18$, $t_{500}(41) = 0.5$, $t_{1000}(41) = 1.8$, $t_{2000}(41) = 1.9$) (See Figure S5). Although these results suggest that peripheral changes cannot completely explain age-related declines in auditory neural distinctiveness, pure tone threshold is just one (rather coarse) measure of peripheral hearing. So, it is still quite plausible that age-related changes in the ear contribute to age-related changes in auditory neural distinctiveness.

We also examined age-related changes in grey-matter thickness and surface area. Older adults exhibited significantly thinner grey-matter ($t(37.4) = -6.82$, $p = 4.7e-08$) and reduced surface area ($t(39.6) = -3.49$, $p = 0.001$) within the anatomically defined mask. Neural

distinctiveness was still significantly lower in the older adults even after controlling for changes in grey-matter thickness (r (41) =-0.32, p=0.037), but not after controlling for surface area (r (41) = -0.17, p= 0.28). These results indicate that changes in neural distinctiveness might be at least partially due to anatomical changes that accompany aging.

2.3.2 GABA+ Levels and Aging

Raw GABA+ levels were significantly lower in the auditory cortex in older adults (mean = 1.75) than in young adults (mean = 1.89) (t (41) =-2.78, p=0.008) (Figure 5). Raw GABA+ levels were also significantly lower in the sensorimotor cortex (t (41) = -3.18, p = 0.002) (reported in Cassady et.al., 2019) and ventrovisual cortex (t (41) = -2.87, p = 0.006).

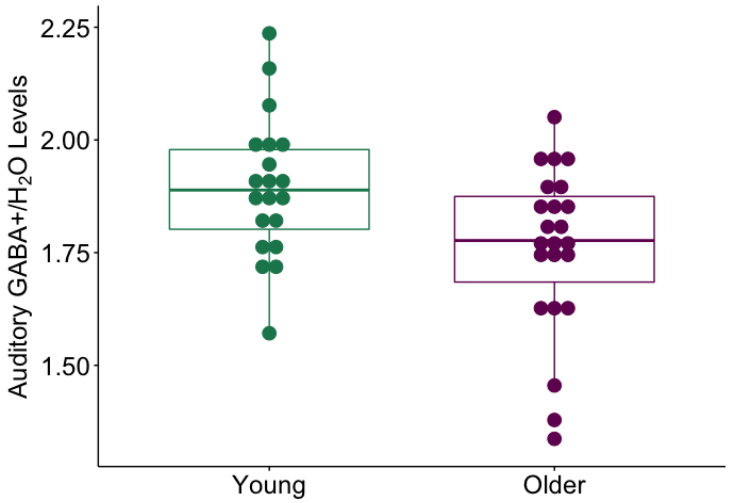

Figure 5. ***GABA and Age.*** *Raw GABA+/Water levels in the auditory cortex estimated by MRS were significantly lower in older adults (in purple) than young adults (in green) (t (41) = -2.6, p = 0.01).*

Raw GABA measures in the auditory cortex were not significantly correlated with GABA levels in the ventrovisual (r (43) = 0.25, p = 0.1) or sensorimotor cortex (r (43) = 0.17, p = 0.3) after controlling for age. Auditory GABA levels were also not significantly associated with average pure-tone threshold average, whether analyzed in the entire sample (r (43) = -0.22,

p = 0.16) or in older adults alone (r (21) = -0.26, p = 0.23), suggesting that individual differences in auditory GABA are not directly associated with peripheral auditory differences.

We also used an ANCOVA to investigate whether there were systematic differences between GABA levels across hemisphere and if this effect interacted with age. There was a significant main effect of age on GABA+ independent of hemisphere (F (1,41) = 7.5, p=0.009) but no main effect of hemisphere (F (1,41) = 0.008, p=0.93). There was also no significant interaction between hemisphere and age (F (1,41) = 0.19, p=0.66). Because there were no significant differences between the GABA+ estimates in the two hemispheres and because the two estimates were significantly correlated (r (41) = 0.52, p=0.0003), we averaged the GABA+ estimates from each hemisphere for further analysis.

There are differences in T1 and T2 relaxation time in the GABA signal that are dependent on the tissue from which it is measured. Since, GABA+ levels are estimated from relatively large voxels which contain grey matter, white matter and cerebrospinal fluid (CSF), it is important to estimate and correct for tissue composition differences between voxels. Gannet uses SPM-based registration to estimate the tissue composition within the voxel and correct them (Edden et.al., 2014; Harris et.al., 2015). These tissue-composition corrected GABA+ estimates were significantly (t (41) = -3.18, p = 0.003) lower in older adults (mean = 1.99) than young adults (mean = 2.18). However, there are also substantial GABA concentration differences between different tissues: CSF contains negligible amounts of GABA, while white matter has half the concentration of GABA compared to grey matter. Gannet also computes fully tissue-composition corrected GABA estimates that account for these concentration differences. Age did not have a significant main effect on these fully corrected GABA+ estimates (t (41) = 0.25, p =

0.8). These results indicate that completely accounting for structural changes with age like tissue composition might explain age differences in GABA+ estimates in auditory cortex.

2.3.3 GABA and Distinctiveness

Average raw GABA+ levels in the auditory cortex were positively correlated with neural distinctiveness in the older adults (r (21) = 0.54, p = 0.008) (Figure 6), but not the younger adults (r (18) = -0.18, p = 0.45) (Figure S4). This GABA-distinctiveness relationship was also region-specific: neither ventrovisual GABA (r (21) = 0.25, p = 0.25) nor sensorimotor GABA (r (21) = 0.19, p = 0.38) were significantly correlated with auditory distinctiveness in the older adults.

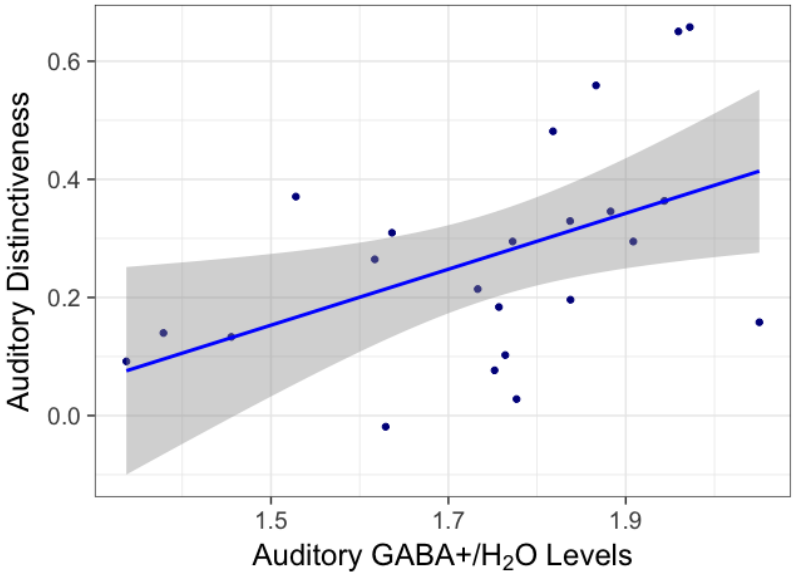

Figure 6. ***Auditory GABA levels and distinctiveness.*** *Individual differences in raw auditory GABA+/Water levels were significantly correlated with individual differences in auditory neural distinctiveness in older adults. (r (21) = 0.54, p = 0.008)*

Our primary measure of GABA was quantified relative to water, but we also analyzed GABA quantified relative to Creatine (Cr) to confirm the reliability of the results. GABA/Cr levels were also significantly lower in older adults compared to young (t (41) =3.44, p = 0.001), and were also correlated with auditory distinctiveness levels within the older adults (r (21) = 0.42, p = 0.04) but not within young adults (r (18) = 0.03, p = 0.9).

We also performed a hierarchical regression to examine whether the fully tissue-composition corrected auditory GABA+ levels explained significant variance beyond that explained by differences in age and other anatomical changes like grey matter volume, NAA (associated with neural integrity) and average pure tone threshold (to account for peripheral hearing differences). GABA levels explained significant variance beyond these other factors within the older sample ($F(1,17) = 5.37$, $p = 0.03$), while in the entire sample there was a trend ($F(1,37) = 3.75$, $p = 0.06$).

2.4 Discussion

The age-related neural dedifferentiation hypothesis posits that the neural representations of different stimuli become less distinct with age (Li et al., 2001). Most of the previous evidence for this hypothesis in humans has come from studies of visual cortex. In the present study, we showed that neural distinctiveness also declines with age in the auditory cortex, extending the scope of age-related neural dedifferentiation. This age-related decline in distinctiveness was independent of ROI-size, was present after controlling for peripheral hearing performance (pure-tone threshold), and was still present after controlling for grey matter thickness.

We also examined GABA levels in the auditory cortex and the relationship between GABA and distinctiveness. Consistent with previous animal research, we found that GABA levels decline with age in the auditory cortex and showed for the first time that individual differences in GABA levels are associated with individual differences in neural distinctiveness.

2.4.1 Age-related dedifferentiation

Previous research in animals provides direct evidence for age-related declines in neural selectivity or distinctiveness at the level of single neurons (Juarez-Salinas et al., 2010; Khouri et

al., 2011; Schmolesky et al., 2000). Neuroimaging studies in humans have also found that large-scale patterns of neural activation in ventral visual cortex become less distinct with age (Goh et al., 2010; Goh, 2011; Park et al., 2004). Similar findings have also been reported in motor cortex during left vs. right finger tapping (Carp et al., 2011a), in hippocampus during memory retrieval of different items (Giovanello and Schacter, 2012) and in posterior medial cortex for different emotion regulation strategies (Martins et al., 2015). Our study contributes to this growing body of literature by showing that age-related dedifferentiation also extends to auditory cortex.

A natural question is whether the observed declines in neural distinctiveness in auditory cortex are due to age-related changes in the peripheral auditory system, i.e. the ear, or whether they reflect more central changes in the cortex. Aging is accompanied by several changes in the ear, including the loss of hair cells, dysfunction of the stria vascularis, and stiffening of the basilar membrane (Ouda and Syka, 2012). Such changes in the peripheral auditory system could result in a noisier auditory input. And noisier information could plausibly produce less distinctive cortical representations, even if central auditory processing in the cortex itself has not changed dramatically.

To investigate these issues, we analyzed the pure tone thresholds of our young and old participants at frequencies ranging from 125 to 8000 Hz. And we did observe an increase in auditory thresholds at higher frequencies in the older adults (see Supplemental Figure S5). However, perhaps surprisingly, we did not find much evidence that this, admittedly coarse, measure of hearing influenced neural distinctiveness. Neural distinctiveness still declined with age after controlling for average pure tone threshold and the size of this effect was about the same as it had been without controlling for pure tone threshold. Also, average pure tone threshold was not significantly associated with neural distinctiveness. One reason we may not

have observed any associations is that 90% of the power in our auditory stimuli were at frequencies below 2000 Hz where the effects of age on pure tone threshold were not significant. Of course, pure tone threshold is just one (rather coarse) measure of peripheral hearing and it is still quite plausible that age-related changes in the ear contribute to age-related changes in auditory neural distinctiveness.

Another important issue is that the low-level auditory characteristics of our two categories of auditory stimuli were different. Most notably, the music stimuli have significantly more power at frequencies between 1000 and 1500 Hz than does the speech (see supplemental Figure S6). It is therefore quite plausible that neural distinctiveness was influenced by these low-level differences and not just by the difference in high-level category (speech vs. music).

Nevertheless, whether neural distinctiveness reflects low-level differences, high-level differences, or both, the critical findings in this paper are that neural distinctiveness declines with age and is significantly associated with GABA. And it seems difficult to attribute either of those between-subject effects to low-level differences between the categories (which contribute to within-subject differences). First, both effects were based on between-subject comparisons (old vs. young, lower GABA vs. higher GABA) and all the participants were presented with the same stimuli. And second, both stimulus categories were dominated by frequencies between 100 and 1500Hz (see supplemental Figure S6), where we did not see significant age differences in pure tone threshold (see supplemental Figure S5).

2.4.2 Age-related decline in GABA levels

Several animal studies have reported that levels of the inhibitory neurotransmitter GABA decline with age in the auditory system. For example, previous studies in animals have reported

declines in GABA in the inferior colliculus (Caspary et al., 1990; Gutiérrez et al., 1994; Ouda and Syka, 2012) and auditory cortex (Ling et al., 2005) of aging rats, as well as in the cochlea of aging mice (Tang et al., 2014). There is also an age-related decrease in the protein and mRNA levels of the most abundant $GABA_A$ receptor subunits in inferior colliculus and auditory cortex of rats (Caspary et al., 1990; Gutiérrez et al., 1994; Caspary et al., 2013). $GABA_B$ receptor binding in the inferior colliculus also declines with age in rats (Milbrandt et al., 1994).

Only a few human studies have investigated age-related changes in auditory GABA levels, and the results are mixed. Profant et al. (2015) did not observe a significant effect of age on GABA levels in auditory cortex. In contrast, Chen et al. (2013) did report a significant decline in GABA levels: in the right (but not the left) hemisphere before pure tone stimulation, and in both hemispheres after stimulation. Likewise, Gao et al. (2015) reported that older adults suffering from age-related hearing loss exhibited lower GABA levels in auditory cortex compared to other older adults. Consistent with these results, our study provides further evidence that auditory GABA levels decline significantly with age in older adults compared to younger adults. However, fully tissue-composition and concentration corrected GABA estimates did not show an age-related decline. This suggests that observed declines in GABA levels with age may be mediated by age-related changes in tissue composition. These observations might account for some of the apparent discrepancies in the previous literature.

The observed age-related declines in GABA are also consistent with the view that some age-related behavioral impairments may reflect an underlying deficit in inhibition (Hasher and Zacks, 1988; Lustig et al., 2007). These theories suggest that older adults have greater difficulty preventing irrelevant information from gaining access to attention than young adults as a result of impaired inhibitory function. Thus, older adults may be more susceptible to distraction and more

likely to choose a non-optimal response. Since GABA is the brain's major inhibitory neurotransmitter, age-related reductions in GABA could naturally explain the observed inhibitory deficit.

2.4.3 Auditory GABA is associated with neural distinctiveness

Leventhal et al. (2003) showed that the neural selectivity of orientation-specific cells in visual cortex declines with age. They also showed that the selectivity of individual neurons can be experimentally manipulated by the application of GABA, a GABA agonist, or a GABA antagonist. Specifically, visual neurons in older macaques that were not orientation-selective became selective after the application of GABA or the GABA agonist muscimol. Conversely, visual neurons in young macaques that were orientation-selective, became non-selective after the application of the GABA antagonist bicuculline. Together these results demonstrate that changes in GABA activity can cause changes in neural selectivity, at least in individual neurons in visual cortex. Researchers have reported similar findings in the auditory system. For example, the application of a GABA antagonist reduces the selectivity of cells to sinusoidally amplitude modulated (SAM) stimuli in the inferior colliculus of rats (Caspary et al., 2002), as well as the rate and direction selectivity of cells to FM sweeps in the auditory system of bats (Razak and Fuzessery, 2009).

Obviously, the selectivity of individual receptive fields might be quite different from the selectivity of the large-scale neural representations that can be measured using fMRI in humans. Nevertheless, age-related declines in GABA could plausibly influence both and so we decided to test whether individual differences in GABA were associated with individual differences in neural distinctiveness, and the results confirmed the prediction. Older participants with higher levels of auditory GABA, as measured by MRS, had significantly greater neural distinctiveness than did older adults with lower GABA levels, even after controlling for age, NAA (a marker of neural

integrity), and grey matter volume. These results are consistent with the hypothesis that age-related declines in GABA contribute to age-related neural dedifferentiation.

Furthermore, this relationship was region-specific: GABA estimates in ventrovisual and somatosensory cortex were not significantly associated with auditory distinctiveness. These results suggest that the observed GABA-distinctiveness relationship is probably not due to some confounding effect (increased variance with age, vascular changes with age) that would be present throughout the brain.

Animal research has shown a direct association between decline in auditory neural selectivity and age-related hearing loss (Khouri et al., 2011; Trujillo and Razak, 2013). If GABA levels influence neural distinctiveness, as our results suggest, then pharmacological treatments that target GABA could be a promising avenue for clinical research aimed at mitigating age-related hearing impairments.

2.5 Limitations

A key limitation of the current study is that it is correlational. We therefore cannot conclude that age-related changes in GABA cause changes in neural distinctiveness, only that they are related. Another limitation is that the study is cross-sectional rather than longitudinal. The observed age differences could therefore be influenced by cohort or period effects (Hofer et.al.,2002; Bowen et.al.,1999). Longitudinal studies also make it possible to observe the order of effects which can shed light on causal directionality. Finally, MRS estimates of GABA do not measure GABA activity, but GABA volume. Nor do they distinguish between intracellular and extracellular GABA. These shortcomings should presumably make it harder to observe

relationships between auditory GABA and auditory distinctiveness, so the fact that we did find a significant relationship suggests that the relationship may be fairly strong.

2.6 Conclusions

In sum, our findings show that neural dedifferentiation extends to the auditory cortex. Furthermore, they demonstrate that GABA levels in auditory cortex decline with age and that individual differences in GABA are associated with individual differences in neural distinctiveness. Together these findings are consistent with the hypothesis that age-related declines in GABA contribute to age-related declines in neural distinctiveness.

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Chapter 3 : Dynamic Recovery: GABA Agonism restores Neural Variability in Older, Poorer Performing Adults

Abstract

Aging is associated with cognitive impairment, but there are large individual differences in these declines. One neural measure that is lower in older adults and predicts these individual differences is moment-to-moment brain signal variability. Testing the assumption that gamma-aminobutyric acid (GABA) should heighten neural variability, we examined whether reduced brain signal variability in older, poorer performing adults could be boosted by increasing GABA pharmacologically. Brain signal variability was estimated using fMRI in 20 young and 24 older healthy human adults during placebo and GABA agonist sessions. As expected, older adults exhibited lower signal variability at placebo, and crucially, GABA agonism boosted older adults' variability to young adult levels. Furthermore, poorer performing older adults experienced a greater increase in variability on drug, suggesting that those with more to gain benefit the most from GABA system potentiation. GABA may thus serve as a core neurochemical target in future work on aging- and cognition-related human brain dynamics.

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3.1 Introduction

Functional magnetic resonance imaging (fMRI) has become a predominant method for non-invasively estimating brain activity in human beings. Most fMRI studies treat moment-to-moment variability in the blood oxygenation level dependent (BOLD) fMRI signal as noise, but recent research demonstrates that such variability is associated with better behavioral performance and is a more powerful predictor of cognitive abilities than mean BOLD signal (McIntosh et al., 2008; Garrett et al., 2011, 2013b; Grady and Garrett, 2014; Burzynska et al., 2015). Consistent with these findings, theoretical, experimental, and computational modelling work suggests that greater brain signal variability typifies younger, higher performing adults and well-functioning cortical networks capable of greater complexity and flexibility, increased dynamic range and information transfer, and stronger long-range functional connectivity (Li et al., 2006; Faisal et al., 2008; McIntosh et al., 2008, 2010; Shew et al., 2009; Deco et al., 2011; Garrett et al., 2011, 2013b; Misic et al., 2011; Vakorin et al., 2011; Beharelle et al., 2012; Grady and Garrett, 2014; Nomi et al., 2017). However, little is known about the underlying basis of performance-related deficits in brain signal variability, and even less is known about how to reverse these deficits.

Gamma-aminobutyric acid (GABA), the brain's major inhibitory neurotransmitter, plays a role in many of the same functions with which brain signal variability has been associated, including cortical plasticity (Jones, 1993; Hensch et al., 1998; Fagiolini et al., 2004), the synchronization of neural oscillations (functional connectivity) (Fingelkurts et al., 2004; Bonifazi et al., 2009; Kapogiannis et al., 2013), and the information capacity (Shew et al., 2011; Puzerey and Galán, 2014), efficiency (Sengupta et al., 2013; Zhou and Yu, 2018), pattern complexity (Monteforte and Wolf, 2010; Lajoie et al., 2014; Agrawal et al., 2018) and dynamic range (Shew

et al., 2009; Agrawal et al., 2018) of neural networks. Manipulating the strength of inhibitory connections in artificial neural networks also dramatically influences the number of different states that the network can sample (Agrawal et al., 2018). Crucially, decreasing GABA activity pharmacologically in healthy young rats and monkeys has been found to decrease network signal variability and to reduce the number of states that can be visited by the cortical network (Shew et al., 2011). Inspired by these results, and by previous work showing that older adults express lower GABA levels (Gao et al., 2013; Porges et al., 2017; Cuypers et al., 2018; Cassady et al., 2019; Chamberlain et al., 2019; Lalwani et al., 2019) and lower brain signal variability in a host of cortical regions (Garrett et al., 2011, 2013a; Grady and Garrett, 2014; Waschke et al., 2021; Grady and Garrett, 2018), we hypothesized that pharmacologically increasing GABA activity might causally reverse deficient brain signal variability levels in older adults.

Furthermore, given that lower brain signal variability is typically associated with poorer cognitive performance (even within older adults) (Garrett et al., 2011, 2013a; Grady and Garrett, 2014; Burzynska et al., 2015), we also hypothesized that GABA agonism-related upregulation of brain signal variability should be largest in poorer performing older adults. However, some studies suggest that region- and measure-specific brain signal variability can also be *higher* in older, poorer performing adults (e.g., Samanez-Larkin et al., 2010; Boylan et al., 2021). We thus examined whether there were any regions exhibiting higher signal variability with older adult age, and role of GABA agonism in the whole brain regardless of sign using multivariate partial least squares (PLS) (McIntosh et al., 1996).

In the current study, we analysed data from 21 young (ages 18-25) and 25 older (ages 65-85) adults who had previously participated in the Michigan Neural Distinctiveness (MiND) study (Gagnon et al., 2019). Specifically, we investigated 1) the effect of age on resting state brain

signal variability, 2) the effect of a pharmacological manipulation of GABA activity on brain signal variability, and 3) the association between individual differences in composite cognitive scores and changes in brain signal variability on drug compared to placebo.

3.2 Materials and Methods

This dataset was collected as part of the Michigan Neural Distinctiveness (MiND) study. Here we only describe the portions of the study that are relevant to this analysis. For details about the entire study protocol, see (Gagnon et al., 2019). The ethical approval for the study was granted by the Institutional Review Board of The University of Michigan (HUM00103117).

3.2.1 Participants

We analyzed data from 25 young (age 18-29 years) and 21 older (age 65 and above) male and female human adults who completed the entire MiND study and received the drug manipulation. All participants were recruited from Ann Arbor and the surrounding area, were right-handed, native English speakers, and had normal or corrected to normal vision. Participants completed an initial telephone screening interview and were determined to be eligible. We screened out participants who scored 23 or lower on the Montreal Cognitive Assessment (MOCA) (Carson et al., 2018). All sessions described took place at the University of Michigan's Functional MRI Laboratory, Ann Arbor. We present data collected during two sessions, each on a separate day.

3.2.2 Behavior Testing

Participants completed an extensive cognitive and behavioral task battery including tasks from the NIH Toolbox® for Assessment of Neurological and Behavioral Function (Weintraub et al., 2014). The NIH toolbox tasks are administered using an iPad, and the associated software

automatically generates a standardized composite cognitive score for each participant. Here, we provide a brief description (refer to (Weintraub et al., 2014) for details) of the tasks that contribute to this composite measure:

1. Pattern Comparison Processing Speed Test

   Two simple side-by-side pictures are presented on an iPad and participants are instructed to discern, as fast as they can, whether the two pictures are the same or different. Participants press buttons on the iPad screen to indicate their response. The score is calculated based on the number of items they correctly answer in 85 seconds.

2. List Sorting Working Memory Test

   Participants are presented with a few pictures from a specific category one at a time on the iPad. Participants are then asked to list the items in increasing order of size. Participants' response is marked correct if they list all of the items in the correct order.

3. Flanker Inhibitory Control and Attention Test

   Participants are presented with a row of arrows on the iPad and are instructed to indicate the direction of the middle arrow as quickly as they can. They press a left or right arrow button located on the iPad screen to indicate their response. The middle arrow may point in the same direction as the arrows surrounding it (congruent trials) or in the opposite direction (incongruent trials). There is a total of 20 trials, 40% of which are incongruent. The score is based on a combination of reaction time and accuracy.

4. Dimensional Change Card Sort Test

   In this task, participants are presented with one target image and two response images that match the target image on either shape or color. During each trial, participants are first presented with the word "SHAPE" or "COLOR" and are asked to choose the response image that matches the target image based on that dimension. There are 30 trials, and 23%

of these are color trials. The score computed for this task is also based on a combination of reaction time and accuracy.

5. Picture Sequence Memory Test

   Participants first must recall the order of 15 images displayed in sequence on the iPad screen. They move images on the screen to match the order they remember. Participants are then presented with 18 images, including the first 15 images and 3 new images presented in the middle of the sequence. Again, they are asked to recall the order of all images. The score is based on the total number of correct adjacent pairs recalled.

6. Picture Vocabulary Test

   Participants hear an audio recording of a word, and four pictures are displayed on the iPad screen. They are instructed to select the picture that best matches the meaning of the word they heard. This test uses the Computerized Adaptive Testing (CAT), whereby the difficulty of the next question is determined by the previous answer. A raw score is computed using Item Response Theory (IRT).

7. Oral Reading Recognition Test

   Participants are presented a word on the iPad screen and are asked to read the word out aloud. Using a pronunciation guide, the examiner scores the response as correct or incorrect. This test also utilizes CAT, and the score is computed using IRT.

3.2.3 fMRI Scans

Participants were given a low dose benzodiazepine (lorazepam) or a placebo pill approximately one hour before the scan on two separate days. The order of the sessions (on and off drug) was counterbalanced across participants. Participants were not told which pill they received on which day (they were blind to the drug administration order). During the drug

session, participants were administered a 0.5 or 1 mg oral dose of lorazepam (a benzodiazepine). The drug dosage (0.5 or 1mg) was randomly assigned across participants for two reasons: (1) in order to maximize the chances of including a dose strong enough to produce observable effects without producing significant sedation, and (2) to make it possible to analyze the effect of dosage. The participants were screened for use of medications that might interact with lorazepam or affect GABA levels. They also had no history of claustrophobia or mood disorders.

The functional scanning parameters (detailed below) were identical during both sessions. Functional MRI data was collected using a 3T General Electric Discovery Magnetic Resonance System with a volumetric quadrature bird cage head coil and 2 32-channel receive arrays. The functional scans were T2*-weighted images collected with a 2D Gradient Echo pulse sequence with the following parameters: TR = 2000 ms; TE = 30 ms; flip angle = 90°; FOV = 220 x 220 mm; 43 axial slices with thickness = 3 mm and no spacing, collected in an ascending sequential sequence (voxels were 3x3x3mm). The total acquisition time for the resting state functional scan was 8 minutes 10 seconds, with 245 volumes. Participants were instructed to relax, keep their eyes open and focus on a fixation cross presented for the duration of the scan. Using an eye-tracking system (in view mode only) we ensured that participants indeed had their eyes open and fixated. Heart rate was collected via a pulse oximeter placed on the left middle finger. We also obtained a T1-weighted image using the SPGR (3D BRAVO) sequence during this session, with the following parameters: Inversion Time (TI) = 500 ms; flip angle = 15°; FOV = 256 x 256 mm.

3.2.4 fMRI Data Preprocessing

The fMRI data were preprocessed and analyzed using a combination of FMRIB Software Library (FSL), SPM12 and MATLAB-based scripts. The first 5 volumes of each scan were discarded. Heart rate was collected via a pulse oximeter placed on the left middle finger and the

data were physio corrected during preprocessing. We performed 1st-level preprocessing using FSL-FEAT (Woolrich et al., 2001) with default parameters for motion correction, normalization, and smoothing (7mm). We used the SPM12 function spm_detrend to remove linear, quadratic and cubic trends in the time series and also applied a Butterworth filter (0.01-0.1Hz). We then ran FSL MELODIC to perform Independent Component Analysis (ICA). Three separate raters identified noise components through manual visual inspection (based on (Kelly et al., 2010)). These components reflected noise related to sinus activity, vascular and ventricle activations, and motion. We then removed the components identified as noise by at least two of the three raters using the FSL regfilt function. Subsequently, we performed linear registration of the functional and anatomical images of each participant and the MNI152 template using the FSL FLIRT function.

3.2.5 Quantification of Brain signal variability (SD_{BOLD})

After preprocessing the fMRI data, the standard deviation in the fMRI signal during the entire resting state scan was computed at each voxel for each participant and scanning session separately. We also computed $SD_{BOLD\text{-}Change}$ at each voxel as the difference between $SD_{BOLD\text{-}Drug}$ and $SD_{BOLD\text{-}Placebo}$. All analyses were restricted to voxels in a MNI152 grey matter volume mask.

3.2.6 Statistical Analysis

To examine the regional distribution of the effects on brain signal variability, we employed multivariate partial least squares (PLS) analyses (McIntosh et al., 1996).

1) We employed Behavior-PLS for investigating effects of age-group on $SD_{BOLD\text{-}Placebo}$. In this simple one-condition one-behavior PLS, a correlation matrix between age-group and each voxel's signal ($SD_{BOLD\text{-}Placebo}$) was first computed across subjects. This correlation matrix was then decomposed using singular value decomposition (SVD). This

resulted in a singular value (S) (reflecting the correlation strength), and brain voxel weights (V) (i.e., a weighting pattern across brain voxels that optimally expresses the correlation) – in this case, the original voxel-wise correlations with age, but scaled to be unit length. We then calculated individual "brainscores" by taking the dot product of the brain voxel weights and a given subject's brain measures. Thus, brainscores indicate the degree to which a subject expresses the multivariate spatial pattern captured by the latent variable.

2) We employed Task-PLS with two groups for investigating effects of drug on SD_{BOLD}.

   The task-PLS is similar to the behavior-PLS but involves a singular value decomposition of a between-subject covariance (COV) matrix instead of the correlation matrix. We first computed a COV matrix between drug and placebo conditions and each voxel's SD_{BOLD} within each age-group. Then using SVD we estimated a left singular vector of experimental condition weights (U) for each age-group, a right singular vector of brain voxel weights (V), and a diagonal matrix of singular values (S). This produced four latent variables. Only the first LV was significant and represented greater variability during the drug condition than during placebo in both age-groups. Brainscore was computed separately for each condition as the dot product of brain voxel weights and each subjects' $SD_{BOLD\text{-}Placebo}$ and $SD_{BOLD\text{-}Drug}$.

3) We employed simple Task-PLS for investigating effects of drug on SD_{BOLD} within each age-group separately.

   This is similar to the previously described Task-PLS except there are no age-groups. Thus, SVD results in only two latent variables. The first LV, representing greater variability during the drug condition compared to placebo, was significant in older adults. The LV in younger adults alone was not significant.

4) We employed rank-based Behavior-PLS with two groups for investigating effects of cognitive processing on $SD_{BOLD\text{-}Change}$

   Similar to the previously described one-condition one-behavior PLS, first a rank-based correlation matrix was computed between the composite cognitive scores and each voxel's signal ($SD_{BOLD\text{-}Placebo}$) across subjects within each age-group and then stacked into a single matrix. An SVD of this matrix results in two latent variables defined by singular value (S) and brain voxel weights (V). This PLS identifies a single latent space in the brain between the two groups that best captures the relationship between voxel signals and the behavioral measure for each of the groups. Thus, the relationship between $SD_{BOLD\text{-}Change}$ and cognitive processing could be different in older adults and younger adults. Brainscores were computed as a dot product between V and $SD_{BOLD\text{-}Change}$ at each voxel for each subject.

5) We employed a rank-based behavior PLS for investigating the effect of baseline composite cognitive performance on variability during all three conditions (placebo, drug and change)

   Like the previously described one-condition one-behavior PLS a rank-based correlation matrix is computed based on baseline composite cognitive task scores and each voxel's signal (SD_{BOLD}) across subjects for each of the three conditions. An SVD on this matrix resulted in three (equal to the number of conditions) latent variables defined by singular values (S), brain voxel weights (V) and behavior weights (U). Only one latent variable was significant. Brainscore for each condition was computed as the dot product between voxel weights (V) and variability in each condition ($SD_{BOLD\text{-}Placebo}$, $SD_{BOLD\text{-}Drug}$, $SD_{BOLD\text{-}Change}$) for each subject.

6) Finally, we employed a rank-based Behavior-PLS for investigating the effects of several cognitive processing tasks on $SD_{BOLD\text{-}Change}$ in older adults

It is very similar to the previously described one-condition one-behavior PLS. First a rank-based correlation matrix is computed based on each of the cognitive task scores and each voxel's signal ($SD_{BOLD\text{-}Placebo}$) across subjects. An SVD is performed on this matrix. In our model it resulted in seven (equal to the number of cognitive tasks) latent variables defined by singular values (S), brain voxel weights (V) and behavior weights (U). Only one latent variable was significant. All the behavior weights (U) of this LV were negative suggesting a negative correlation between change in variability and all cognitive tasks. Similar to Brainscore, a cognitive score was computed as the dot product between behavior weights (U) and cognitive score in each task for each subject.

For all the PLS models, significance of the detected relations was assessed using 1000 permutation tests of the singular value and the robustness of voxel saliences was computed using 1000 bootstrapped resamples. By dividing each voxel's mean salience by its bootstrapped standard error, we obtained "bootstrap ratios" (BSR) as normalized estimates of robustness. We thresholded the BSRs at a value of ≥3.00, which approximates a 99.9% confidence interval. We then used the Harvard Oxford Cortical Atlas to identify the regional identity of the significant clusters (presented in the Tables) in the cortical regions and the AAL atlas in the subcortical regions. All the other statistical analyses were conducted using R (Team, 2013). The lme4 package (Bates et al., 2007) was used to perform the linear mixed effects analyses while figures were plotted using the ggplot2 package (Wickham, 2016).

3.3 Results

3.3.1 Aging and brain signal variability

Consistent with previous results (Grady and Garrett, 2014), resting-state brain signal variability (operationalized as the standard deviation of BOLD fMRI signal; SD_{BOLD}) was

significantly lower in older vs. younger adults (Welch two sample t-test, $t(35.1) = 3.98$, $p = 0.0003$). There were two outliers (one young and one older adult) with a Cook's distance greater than 0.087 (4/sample size). Even after excluding these two subjects, brain signal variability was significantly lower in older adults (permuted p=0.02) compared to younger adults (Welch two sample t-test of Brainscore, $t(32.4) = 3.96$, $p = 0.0004$, See Figure 7 and Table S2 for details). All the results presented below are based on excluding the two outlier subjects.

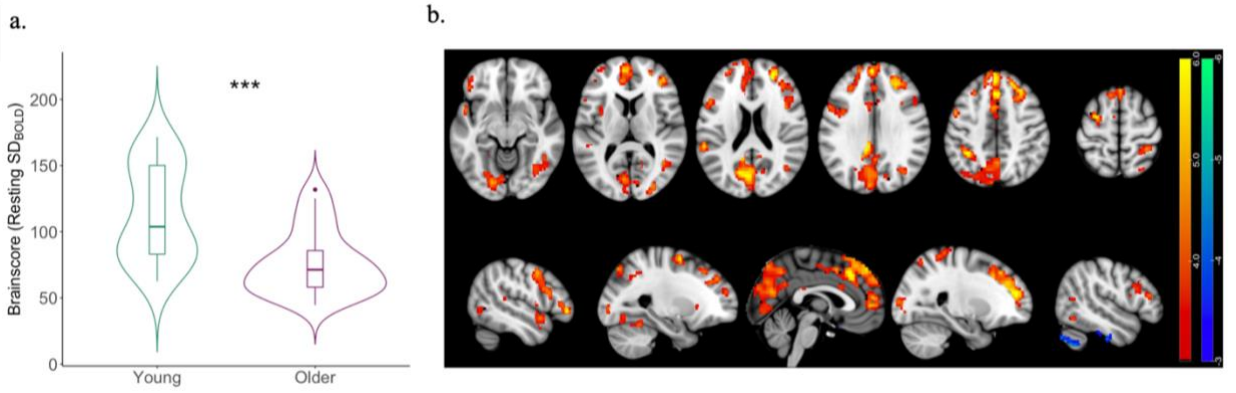

Figure 7. ***Effect of Age on SD_{BOLD}.*** *(a) Resting-state SD_{BOLD} at placebo is significantly lower in older adults (in purple) compared with younger adults (in green). (b) Spatial pattern expressing the effect of age. Yellow/red regions showed a reliable decrease in variability with age while blue regions showed a reliable increase. Bootstrap ratios increase from red to yellow and from dark to light blue and are thresholded at a value of ≥3.00.*

3.3.2 Increasing GABA activity boosts brain signal variability in older adults

We also investigated the effect of a small dose of a positive allosteric modulator (lorazepam) of that $GABA_A$ receptor on brain signal variability. We used PLS with younger and older adults as two separate groups and found one significant latent variable (permuted p<0.001) showing higher brain signal variability on drug compared to placebo. The spatial pattern indicated reliable increase in variability on drug in several regions including the cingulate gyrus, cerebellum, frontal, temporal, sensorimotor and occipital regions. No regions showed a reliable reduction in variability on drug compared to placebo. See Figure 8b for the full spatial extent of the effect and Table S3 for significant cluster details. We used a linear mixed effects model to investigate the effect of age-group, drug and the age-group x drug interaction on brain signal

variability estimates produced from the above model after accounting for the subjects as random effects. Brain signal variability was significantly larger on drug than on placebo ($F(1, 42) = 14.8$, $p = 0.0004$) and the age-group x drug interaction was also significant ($F(1, 42) = 5.6$, $p = 0.02$). On investigating the interaction further, we found that there was a significant increase in variability on drug within older adults ($t(23) = -4.04$, $p = 0.0005$), but not within younger adults ($t(19) = -0.97$, $p = 0.34$, See Figure 8a). Furthermore, variability was not significantly different between older adults on drug and younger adults on placebo ($t(42) = -1.6$, $p = 0.11$), consistent with the hypothesis that the drug restored the older adults' variability to young adult levels.

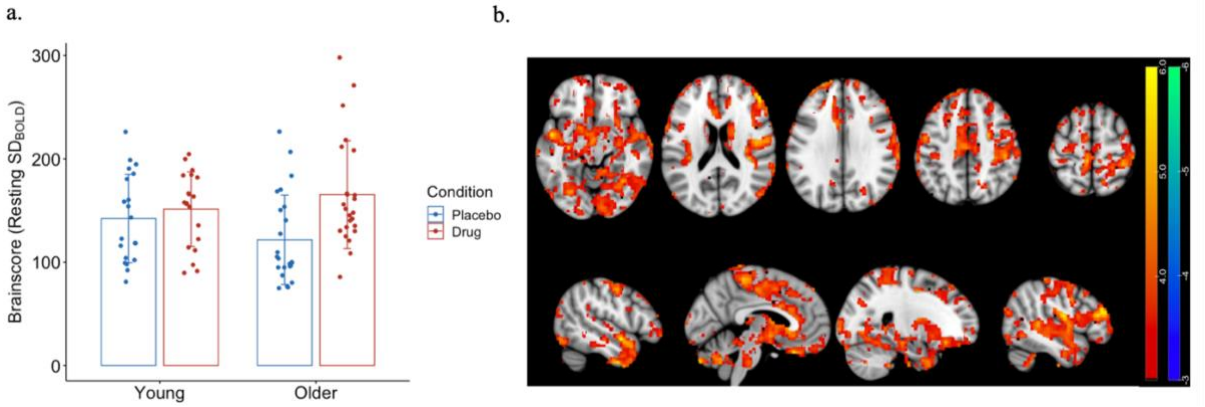

Figure 8. ***Effect of GABA agonism on SD_{BOLD}.*** *(a) SD_{BOLD} increases on drug significantly in older adults but not in younger adults. Error bars indicate standard deviation. (b) Spatial pattern expressing the effect of drug on variability. Yellow/red regions exhibited a reliable increase in variability on drug (bootstrap ratio increases from red to yellow). No regions showed a reliable decline in variability on drug (absence of blue/green regions). Bootstrap ratios are thresholded at a value of ≥3.00.*

Given that the drug effect was only robust within the older group, we then re-ran an older adult only PLS model to isolate relevant brain regions (see Figure 9a). We found that variability reliably increased on drug (permuted p<0.001) in several regions including the parahippocampal gyrus, fusiform cortex, sensorimotor regions, cerebellum, cingulate gyrus, frontal and the occipital regions. See Figure 9b for the full spatial extent of the effect and Table S4 for significant cluster details. No regions showed a reliable reduction on drug compared to placebo. As a further set of controls for this older-adult only PLS result, we used a linear mixed effects

model to account for main effects of session order, grey-matter volume, days between sessions (Mean: 16 days, Range: 2 to 79 days), dosage (0.5 vs. 1mg), self-reported drowsiness before and after the drug session, age (Mean: 69.9 Range:65 to 81), and drug on brain signal variability estimates from this model, as well as the age x drug interaction after accounting for subjects as random effects. Only the main effect of drug ($F(1, 23) = 16.62$, $p = 0.0005$) was significant and this remained the only significant effect after excluding one participant with a Cook's distance greater than 0.17 (4/number of older adults) ($F(1, 22) = 18.14$, $p = 0.0003$).

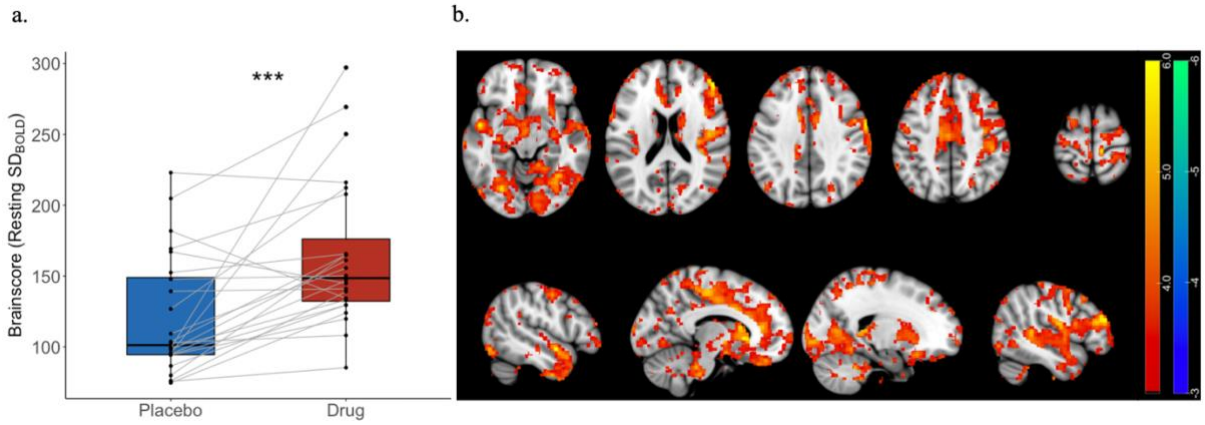

Figure 9. ***Effect of GABA agonism on SD_{BOLD} in older adults.*** *(a) Resting-state SD_{BOLD} in older adults was significantly higher on drug (in red) compared to placebo (in blue). (b) Spatial pattern expressing the effect of the drug on brain signal variability. Yellow/red regions exhibited a reliable increase in variability on drug vs. placebo while blue regions exhibited a reliable decrease. Bootstrap ratios were thresholded at a value of ≥ +-3.00.*

3.3.3 Increasing GABA activity leads to greater boost in brain signal variability in poorer performing older adults

To examine the relationship between cognitive performance and change in brain signal variability on drug, we obtained the composite cognition score from the NIH Toolbox for the Assessment of Neurological and Behavioral Function (Weintraub et al., 2014) and computed change in variability at each voxel ($SD_{BOLD\text{-}change} = SD_{BOLD\text{-}Drug} - SD_{BOLD\text{-}Placebo}$). Consistent with previous literature (Garrett et al., 2011, 2013a; Grady and Garrett, 2014; Burzynska et al., 2015), we found that the composite cognition scores were significantly lower in older adults compared

to young adults ($t(40.7) = 4.7$, $p < 0.001$). Using PLS with two age-groups modeled separately, we found a single significant latent variable (permuted p=0.03) that captured the relationship between change in brain signal variability on drug vs. placebo ($SD_{BOLD\text{-}change}$) and the composite cognitive score from the NIH battery. The relationship was negative and significant in older adults ($r(22) = -0.51$, *Bootstrap CI*: [0.46, 0.85], See Figure 10a) but not in younger adults ($r(18) = 0.17$, *Bootstrap CI*: [-0.1, 0.6]).

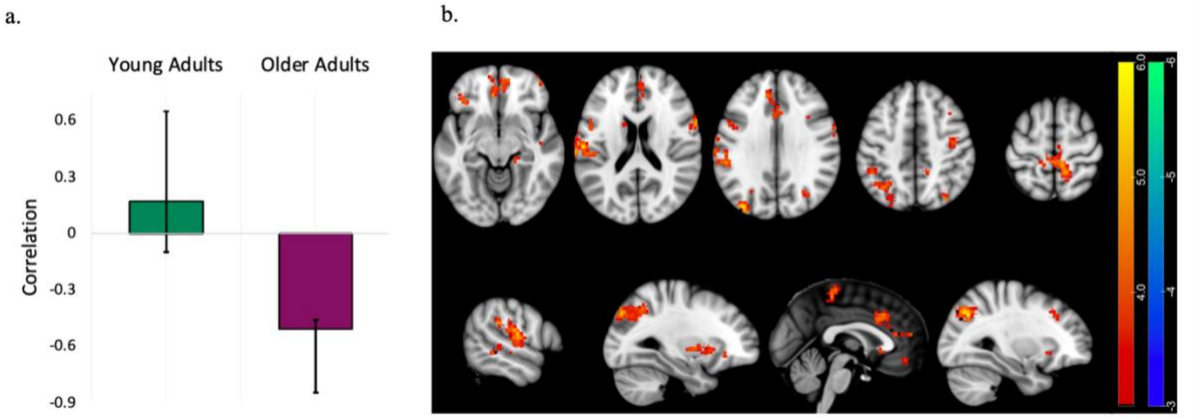

Figure 10. ***Cognition and boost in variability on drug****. (a) Change in SD_{BOLD} on drug is negatively correlated with composite cognitive score in older adults but not in younger adults. Error bars indicate bootstrapped 95% confidence intervals (see Methods). (b) Spatial pattern expressing the relationship between change in variability on drug and baseline composite cognitive score. Yellow/red regions exhibited a reliable negative relationship*

The age-group x cognition interaction (computed using ANOVA on the Brainscore estimates) was also significant ($F(1,40) = 7.2$, $p = 0.01$). There were no outliers based on Cook's distance. The negative association between cognitive performance and GABA drug-related change in variability in older adults suggests that poorer performers experience a greater drug-related boost in SD_{BOLD} than higher performers, an effect that was reliable in several brain regions (including the cingulate and middle frontal gyrus, thalamus, superior parietal lobule, sensorimotor, lateral occipital and temporal regions). See Figure 10b for the full spatial extent of the effect and Table S5 for significant cluster details.

These results support the hypothesis that older adults with poorer cognitive processing benefit more from the drug induced increase in GABA activity. Within older adults only, we further investigated the relationship between composite cognitive score and variability during all three conditions namely: 1) baseline variability ($SD_{BOLD\text{-}Placebo}$), 2) variability on drug ($SD_{BOLD\text{-}Drug}$), 3) change in variability on drug from baseline ($SD_{BOLD\text{-}Change}$) (see Figure 11a for the correlation between the composite cognitive measure and variability during each of the three conditions and Figure 11b for the spatial pattern of this effect and Table S6 for cluster details). We found that change in variability ($SD_{BOLD\text{-}Change}$) explained significant variance in cognition even after accounting for baseline variability ($SD_{BOLD\text{-}Placebo}$) ($F(1,21) = 17.4$, $p = 0.0004$) and variability on drug ($SD_{BOLD\text{-}Drug}$) ($F(1,21) = 9.1$, $p = 0.007$).

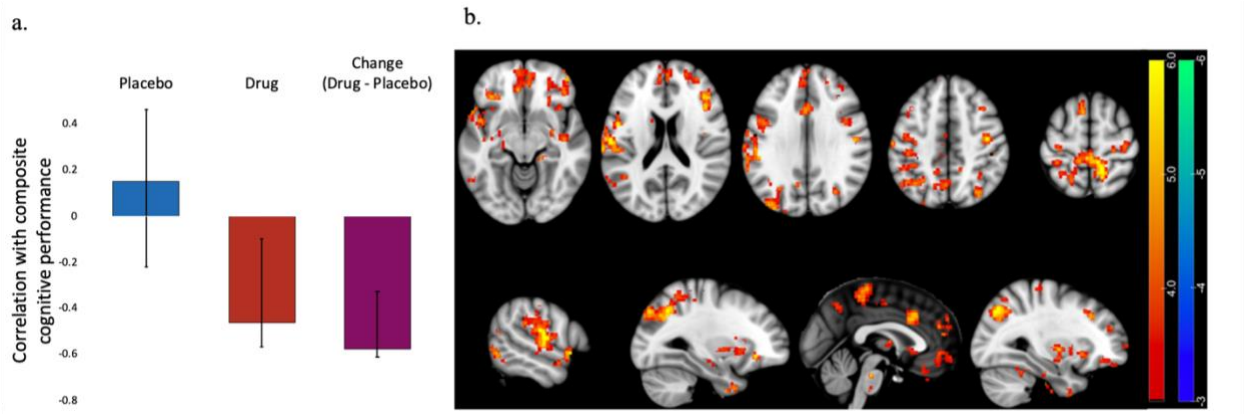

Figure 11. ***Cognition and SD_{BOLD} in older adults****. (a) Baseline variability is positively correlated with baseline cognitive performance (but not significantly), while variability on drug and change in variability from placebo to drug are negatively correlated with baseline cognitive performance. Error bars indicate bootstrapped 95% confidence intervals (see Methods). (b) Spatial pattern expressing the relationship between variability and baseline cognitive performance in all three conditions. Yellow/red regions exhibited a reliable effect (bootstrap ratio increases from red to yellow). No regions exhibited a reliable effect in the opposite direction (absence of blue/green regions). Bootstrap ratios were thresholded at a value of ≥ +-3.00.*

Finally, we examined the role of change in variability on drug and performance on the individual cognitive tasks (during baseline) that make up the composite cognitive score from the NIH Toolbox. We found that all the tasks were negatively associated (significantly in 5 out of 7 using 1000 bootstraps) with change in variability on drug (See Figure 12a). This relationship

was reliable in several regions of the brain including the middle frontal gyrus, superior parietal lobule, thalamus, parahippocampal gyrus, cingulum, sensorimotor, lateral occipital and temporal regions (See Figure 12c for the full spatial extent of the effect, and Table S7 for cluster details). See Figure 12b for the overall latent relationship between cognitive performance and $SD_{BOLD\text{-}change}$ derived from this PLS model ($r(22) = -0.58$, $p = 0.003$). This relationship was also significant, even after accounting for self-reported drowsiness before and after the on-drug scan, psychomotor vigilance score before and after the on-drug scan, age, and dosage (0.5 vs. 1mg);

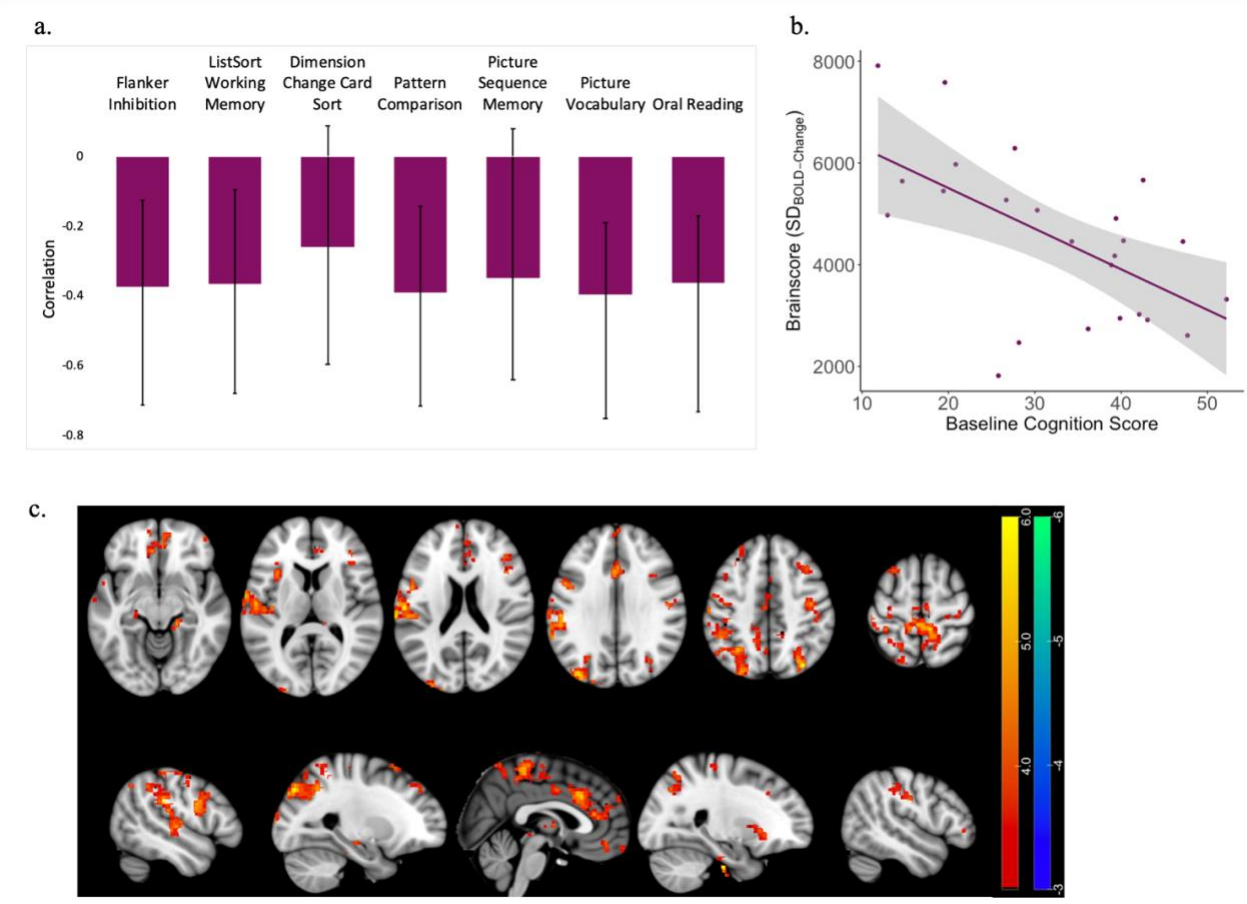

Figure 12. ***Cognition and boost in variability on drug in older adults****. (a) Drug-related change in SD_{BOLD} in older adults is negatively correlated with baseline performance on several cognitive tasks. Error bars indicate bootstrapped 95% confidence intervals (see Methods). (b) Drug-related change in SD_{BOLD} in older adults is negatively correlated with baseline cognitive-score. (c) Spatial pattern expressing the relationship between change in variability on drug and baseline cognitive performance. Yellow/red regions exhibit reliable effects (bootstrap ratio increases from red to yellow). No regions exhibited reliable effects in the opposite direction (absence of blue/green regions). Bootstrap ratios were thresholded at a value of ≥ +-3.00.cognitive processing (during baseline) showed the smallest changes on drug, while those with the lowest scores experienced a greater boost.*

($F(1,16) = 10.03$, $p = 0.006$). This correlation was negative suggesting that older adults with high cognitive processing (during baseline) showed the smallest changes on drug, while those with the lowest scores experienced a greater boost.

3.3.4 GABA-driven upregulation of brain signal variability in poorer performing older adults is present in regions showing robust age reductions in variability

To examine whether the drug related boost in brain signal variability was directly present in brain regions showing age-related reductions in SD_{BOLD}, we created a mask using clusters showing robust age-related declines in brain signal variability (see Figure 7 and Table S2). We then computed average brain signal variability within this mask during both the placebo and drug conditions. We found that brain signal variability in these regions was significantly higher on drug compared to placebo in older adults ($t(23)=3.7$, $p = 0.001$), effects similar to those found using the whole-brain PLS model $t(23) = 4.1$, $p = 0.0004$). Likewise, there was a negative correlation between composite cognitive score and average change in variability within the mask in older adults ($r(22) = -0.48$, $p = 0.02$), similar to that found using the whole-brain PLS model.

3.4 Discussion

In the present study, we found that brain signal variability was significantly lower in older adults than in younger adults, that increasing GABA activity pharmacologically increased brain signal variability in older adults to the level of healthy young adults, and that GABA-related boosts in variability were largest in the poorest performing older adults. These results provide evidence that GABA may provide a crucial basis for understanding associations between brain signal variability, aging, and cognition in humans.

Our finding that brain signal variability was significantly reduced in older adults (e.g., in the superior frontal regions, bilateral superior parietal lobule, and occipital, sensorimotor, and

auditory regions) is consistent with a number of previous studies. For example, fMRI signal variability in cortex is often found to be lower in older adults compared with young adults in various studies using fixation baseline periods as a resting-state proxy (see Grady and Garrett, 2014) and in those using entire resting-state data (Kielar et al., 2016; Nomi et al., 2017; Grady and Garrett, 2018). Age-related differences in resting-state variability are also robust to multiple vascular controls at the voxel level (Garrett et al., 2017). Further in line with previous work (e.g., (Garrett et al., 2011; Nomi et al., 2017), relatively few clusters (~6% of the overall spatial pattern in cerebellum and inferior temporal cortex; see Table S2) exhibited *greater* variability in the older adults. These results add to a growing body of literature suggesting that brain signal variability levels are lower overall in older vs. younger adults. However, other studies using different preprocessing pipelines, experimental designs, and variability measures such as the mean squared successive differences approach (e.g., Samanez-Larkin et al., 2010; Boylan et al., 2021) have found mainly positive effects between BOLD variability and adult age. Future work could investigate such differences by aggregating various datasets, performing direct comparisons of effects, and unifying the preprocessing pipelines.

GABA levels have been found to decline with age in human visual (Chamberlain et al., 2019), sensorimotor (Cassady et al., 2019; Cuypers et al., 2020), auditory (Lalwani et al., 2019), parietal (Gao et al., 2013), and frontal cortex (Porges et al., 2017), and are associated with individual differences in cognitive and sensorimotor abilities (Porges et al., 2017; Simmonite et al., 2018; Cassady et al., 2019; Levin et al., 2019). Moreover, the amplitude of low frequency fluctuations (ALFF), which is mathematically equivalent to SD_{BOLD} when computed on the exact same band-limited time series (median correlation greater than 0.92 in the present dataset), is also positively associated with GABA binding potential in healthy young adults (Nugent et al.,

2015). In the present work, we found that agonizing GABA activity led to an increase in cortical brain signal variability, especially in older, poorer performers.

So how does boosting GABA lead to higher moment-to-moment variability in brain activity? Computational modelling suggests several possible explanations. Having sufficient inhibitory activity to offset excitatory activity has been found to be crucial to allow artificial neural networks to operate near so-called "criticality," an operating point near the edge of instability where it is easier to switch from one network state to another (Shew et al., 2011; Deco and Jirsa, 2012; Poil et al., 2012; Agrawal et al., 2018). If inhibitory connections are too weak, then excitatory activity dominates and many neurons fire synchronously, resulting in redundant coding and deep attractor states that are very stable and harder to transition from (Agrawal et al., 2018). In this hyperexcited regime, the network only samples a few "synchronous" configurations and therefore does not exhibit as much variability. Conversely, if inhibitory connections are too strong, then fewer neurons fire which can also lead to a reduction in the number of states the network visits. Indeed, increasing GABA activity using propofol (a general anesthetic that also modulates activity at $GABA_A$ receptors) leads to a decrease in power spectral density (another measure of signal variance) in monkey ECoG recordings (Gao et al., 2017). In short, inhibitory connections should be strong enough to balance the excitatory connections, but not so strong as to dampen the entire network. Networks function at criticality and optimally when network dynamics are stabilized by sufficient inhibition (see Sadeh and Clopath, 2020, for review), i.e. there is an inverted-U relationship between brain dynamics and GABA that is similar to that observed with other neurotransmitters.

It is plausible that older adults with poorer cognitive performance may reside on the lower left half of a GABA-variability inverted–U function, while younger and better performing

older adults may be closer to the peak. Increasing GABA activity in older adults with worse cognitive performance should then allow their neural networks to operate nearer to criticality and visit different states more frequently, leading to increased brain signal variability. Conversely, younger adults and older adults with better cognitive performance would not be expected to show as much change in network dynamics/variability on drug. Consistent with these expectations, we found that older adults showed robust increases in variability through GABA agonism and reached levels comparable to younger adult's variability levels at placebo. Additionally, within older adults, change in variability explained significant variance in cognition even after accounting for variance explained by variability on placebo or drug alone. Finally, we found that the poorest performers were most likely to have their signal variability levels boosted on drug, and that association could not be attributed to individual differences in drowsiness, age, or drug dosage. These results provide the first evidence that brain signal variability can be restored by increasing activity of the GABAergic system, particularly for older, poorer cognitive performers.

3.5 Limitations and future work

It is important to note that this study was cross-sectional rather than longitudinal, and so the observed age differences could be influenced by cohort or period effects (Hofer et al., 2002). Another limitation was the absence of a middle-aged group, making it difficult to disentangle whether the age-related changes in variability were due to aging vs. maturation (Neuroscience, 2019). However, previous research has shown that brain signal variability increases during development (from ages 8 to 15) (McIntosh et al., 2010), and the role of individual differences in variability on cognition amongst older adults indicates that it is more likely to be an aging process. Additionally, we do not have behavioral data on-drug, and therefore cannot assess how

manipulating GABA activity affected behavior in our sample. However, promisingly, several drugs targeting the GABAergic system have already been shown to attenuate or even reverse features and symptoms of Alzheimer's Disease (see Guzmán et al., 2018, for review). Further research in healthy older adults is thus needed to assess whether cognitive function can also be jointly boosted with brain signal variability via GABA agonism. Finally, we need to better understand how the GABA system is associated with other candidate neurotransmitter systems that have also been proposed previously as plausible bases of moment-to-moment brain signal dynamics (e.g., noradrenaline, dopamine (Garrett et al., 2015; Alavash et al., 2018; Kosciessa et al., 2020)), and in particular, whether the inhibitory system is a more or less effective pharmacological target than the excitatory (glutamatergic) system in future work linking aging, cognition, and neural variability in humans.

3.6 Conclusion

Overall, we found that GABA agonism can increase brain signal variability in older poorer performing adults. These results suggest the critical role of the GABAergic system in neural variability and the importance of both in aging and cognition. Potentiating GABAergic signaling represents a potentially promising direction to pursue in efforts to mitigate age-related deficits in brain function and behavior.

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Chapter 4 : Modulation of Neural Variability: Aging-related Reduction, Neurochemical Cause, and Behavioral Consequences

Abstract

Neural responses in the sensory cortices are highly variable and recent computational work suggests that this rich structured variability is critical in encoding information about the stimulus. The ability to modulate this variability to match the dynamics of the external world, across different cognitive states or in response to different stimuli, reflects an underlying well-functioning flexible neural network. Specifically, moment-to-moment BOLD-signal variability in the visual cortex has been shown to scale with complexity of stimulus input and this modulation of variability (ΔSD_{BOLD}) has been associated with visuo-cognitive performance. Inspired by animal work, in the current study of 58 younger (ages 18-25) and 77 older (ages 65-85) adults, we utilized computational modelling, behavioral testing, fMRI, MR spectroscopy, and pharmacological intervention to examine the role of aging and GABA in individual differences in ΔSD_{BOLD}, and its behavioral implications. We found that participants had higher variability when passively viewing houses (a more complex stimulus as determined by an H_{MAX} computational model) vs. faces. This ΔSD_{BOLD} was smaller in older adults and associated with lower visual GABA levels. We manipulated GABA activity pharmaceutically and found that the drug-related shift in ΔSD_{BOLD} was associated with baseline GABA levels: participants with low baseline GABA levels exhibited a drug-related increase in ΔSD_{BOLD} while participants

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with high baseline GABA levels exhibited a drug-related decrease ΔSD_{BOLD} (consistent with an inverted-U account). Finally, higher GABA and greater ΔSD_{BOLD} were jointly associated with better performance on visual-discrimination tasks. Based on these results, we argue that age-related changes in GABA play a critical role in modulation of neural variability, which in turn influences behavior.

4.1 Introduction

Our sensory modalities are constantly exposed to a myriad of inputs that differ in complexity and familiarity across various dimensions. And yet, our neural networks can easily accommodate and process such heterogeneity in input. It has been postulated that an efficient neural network would encode complex stimuli with greater dynamic range but modulate neural dynamics to be lower when processing similar or redundant stimuli (Hermundstad et al., 2014; Orbán et al., 2016; Garrett et al., 2020). One neural measure that reflects neural dynamics and that scales with stimulus complexity is moment-to-moment variability in the fMRI signal (SD_{BOLD}). In particular, recent research has found that the fMRI signal in the visual cortex exhibits greater variability when people view more complex, feature-rich visual stimuli. Furthermore, this upregulation of variability when processing more complex stimuli (ΔSD_{BOLD}) was associated with better visuo-cognitive performance (Garrett et al., 2020). These results suggest that modulation of variability or ΔSD_{BOLD} is an index of the flexibility of an individual's neural networks and predicts individual differences in sensory processing. But what leads to individual differences in ΔSD_{BOLD} in the first place?

Gamma-aminobutyric acid (GABA), the brain's major inhibitory neurotransmitter, is one plausible candidate. GABA has been associated with cortical plasticity (Jones, 1993; Hensch et

al., 1998; Fagiolini et al., 2004), with pattern complexity (Monteforte and Wolf, 2010) and with the dynamic range (Shew et al., 2011; Agrawal et al., 2018) of neural networks. Furthermore, computational modelling has found that altering the strength of inhibitory connections in artificial neural networks can dramatically affect the number of different states that the network can sample (Agrawal et al., 2018). Similarly, decreasing GABA activity pharmacologically in healthy young rats and monkeys leads to a decrease in network signal variability and reduces the number of states visited by the cortical network (Shew et al., 2011). In recent work, we also found that resting-state SD_{BOLD} can be increased in older human adults by GABA agonism (Lalwani et al., 2021). The number of states that can be visited by the cortical network determines its dynamic range and in turn the ability to distinctly represent external stimulus (Buzsaki, 2006). We thus hypothesized that individual differences in visual GABA level might play a role in individual differences in visual ΔSD_{BOLD}.

In the current study of 58 younger (ages 18-25) and 77 older (ages 65-85) adults, we first utilized computational modelling to determine stimulus complexity, then we estimated ΔSD_{BOLD} using a visual fMRI task and visual GABA levels using MR spectroscopy. In a subset of participants, we used a very small dose of benzodiazepine to increase GABA activity and assessed visual ΔSD_{BOLD} on and off drug. In a different subset of participants, we collected performance on four visual discrimination tasks – faces-in-noise, buildings-in-noise, scenes-in-noise and objects-in-noise to compute a single latent visual performance score. We then tested (1) if ΔSD_{BOLD} was smaller in older adults, (2) if ΔSD_{BOLD} was associated with visual GABA levels, (3) if ΔSD_{BOLD} was different on vs. off the drug, and (4) whether GABA and ΔSD_{BOLD} were associated with performance on the visual-discrimination tasks.

4.2 Materials and Methods

This data was collected as part of the Michigan Neural Distinctiveness (MiND) study. Here we only describe the portions of the study that are relevant to this analysis. For details about the entire study protocol see (Gagnon et al., 2019). The ethical approval for the study was granted by the Institutional Review Board of The University of Michigan (HUM00103117).

4.2.1 Participants

We analyzed the data from 58 young (age 18-29 years) and 77 older (age 65 and above) adults who completed the entire MiND study before the start of COVID-19 pandemic. All participants were recruited from Ann Arbor and the surrounding area, were right-handed, native English speakers, and had normal or corrected to normal vision. We screened out participants who scored 23 or lower on the Montreal Cognitive Assessment (MOCA) (Carson et al., 2018). All the sessions described below took place at the University of Michigan's Functional MRI Laboratory, Ann Arbor, Michigan.

4.2.2 Power Analysis

Garrett et.al. (2020) found a correlation of r=0.47 between modulation of visual variability and behavioral performance. In our convenient sample, we had 80% power to detect a correlation of r=0.47 in each age-group alone.

4.2.3 Session Design

After completing an initial telephone screening interview and being determined eligible, all subjects participated in three sessions, each on a separate day. Session 1 only involved cognitive and behavioral testing, session 2 included behavioral testing and a functional magnetic

resonance imaging (fMRI) scan, and session 3 only involved a Magnetic Resonance Spectroscopy (MRS) scan (See Figure S7 for summary).

4.2.4 Behavioral Testing

A subset of participants (38 older and 36 younger adults) completed four visual tasks in noise, administered on a Dell laptop with a 15.6-inch screen using the Psychophysics Toolbox. All tasks consisted of trials in which a 500 ms fixation cross was followed by a black and white picture in dynamic Gaussian noise for 500 ms followed by a response screen. The next trial began after the response. The order of the stimulus presentation is pseudorandomized but is the same across participants. Each task begins with 4 practice trials with feedback and is followed by 50 scored trials without feedback. The tasks follow a staircase procedure – when a participant makes three correct responses in a row, the level of noise is increased. Following an incorrect response, the level of noise is decreased. There are total of 15 levels of Gaussian noise, and each task starts at the 5^{th} level of noise. The dependent measure is the average level of noise presented for the last 40 trials. Thus, a higher score represents better performance.

a. Buildings in Noise (BIN)

The stimulus picture is either a house (50% of trials) or an apartment (50% of trials). Participants need to press "1" with their left index finger if they think the picture was a house and "0" with their right index finger if they think the picture was an apartment. Stimuli were from Park et al., (2004).

b. Faces in Noise (FIN)

The stimulus picture is either a male (50% of trials) or female (50% of trials) face. Participants press "1" if they think the picture was a male face and "0" if they think the picture was a female face. Stimuli were from Gold et al., (1999).

c. Objects in Noise (OIN)

The stimulus picture is either an office item, such as a stapler (50% of trials), or a food item, such as a hamburger (50% of trials). Participants press "1" for an office item and "0" for a food item. Object stimuli were taken from Brady et al., (2008).

d. Scenes in Noise (ScIN)

The stimulus picture is either an urban (50% of trials) or nature (50% of trial) scene. Participants press "1" if they think the picture was an urban scene and "0" for a nature scene. Scene images are from Zhou et al., (2018).

Using the MATLAB function *factoran* we computed a single visual sensory factor based on these four tasks. The loadings of the tasks on the factor were 0.3,0.8, 0.5 and 0.2 respectively. Thus, higher factor scores reflect better performance.

4.2.5 fMRI Session

Functional MRI data was collected using a 3T General Electric Discovery Magnetic Resonance System with a volumetric quadrature bird cage head coil and 2 32-channel receive arrays. The functional scan parameters were as follows: T2*-weighted images using a 2D Gradient Echo pulse sequence; Repetition Time (TR) = 2000 ms; Echo Time (TE) = 30 ms; flip angle = 90°; Field of View (FOV) = 220 x 220 mm; 185 volumes; 43 axial slices; thickness = 3 mm, no spacing; and collected in an interleaved bottom-up sequence. The total acquisition time for the visual task scan was 6 minutes 10 seconds.

The task consisted of six 20-second blocks of images of male faces, six 20-second blocks of images of houses, presented in a pseudorandomized order and interleaved with twelve 10-second blocks of a fixation cross. Each block consisted of the stimulus presented for 500 ms with

an interstimulus interval (ISI) of 500 ms. The block order was the same for all the participants. This was a passive viewing task but to ensure participant attention, we presented rare target trials once every minute (6 in total). The target trial were images of female face for the face blocks, and images of apartment building for the house blocks. Participants were instructed to press a button with their right index finger every time they saw a target trial.

4.2.6 MRS Session

Magnetic resonance spectroscopy (MRS) scanning was completed on a different day using the same MRI scanner described above. This session lasted approximately 1.5 hours during which, we collected another T1-weighted structural image and MRS data. The T1-weighted image was obtained using the same parameters and sequence used during the fMRI session. The MRS data was obtained from 3cm x 3cm x 3cm voxels placed in the left and right ventrovisual cortex. The voxel placement was guided by the person-specific task-based fMRI activations, such that the voxels were centered roughly at the peak of activation for a face and house viewing vs. fixation contrast, separately for each participant. We used a MEGA-PRESS sequence with the following parameters to obtain MR spectra: TE=68ms (TE1=15ms, TE2=53ms), TR=1.8sec, spec. width=2kHz, Frequency selective editing pulses (14ms) applied at 1.9ppm (ON) & 7.46 ppm (OFF).

4.2.7 Drug session

A subset of participants (20 young and 25 older adults) underwent two functional MRI sessions instead of one, one after taking a low dose benzodiazepine (lorazepam) and one after taking a placebo pill. The functional scanning parameters were identical to those described above. The pills were given approximately 1 hour before the session and the order of the sessions (on and off drug) was counterbalanced across participants. During the drug session, participants

were administered a 0.5 or 1 mg oral dose of lorazepam (a benzodiazepine). The dosage was assigned randomly. Participants were not told which pill they received on which day (they were blind to the drug administration order).

4.2.8 fMRI Data Preprocessing

The fMRI data were preprocessed and analyzed using a combination of FMRIB Software Library (FSL), SPM12 and MATLAB-based scripts. The first 5 volumes of each scan were discarded. Heart rate was collected via a pulse oximeter placed on the left middle finger and the data was physio corrected during preprocessing. We performed 1st-level preprocessing using FSL-FEAT (Woolrich et al., 2001) with default parameters for motion correction, normalization, and smoothing (7mm). We used the SPM12 function spm_detrend to remove linear, quadratic and cubic trends in the time series and also applied a Butterworth filter (0.01-0.1Hz). We then ran FSL MELODIC to perform Independent Component Analysis (ICA). Three separate raters identified noise components through manual visual inspection as described in (Kelly et al., 2010). These components reflected noise related to sinus activity, vascular and ventricle activations, and motion. We then removed the components identified as noise by at least two of the three raters using the FSL regfilt function. Subsequently, we performed linear registration of the functional and anatomical images of each participant and the MNI152 template using the FSL FLIRT function.

4.2.9 Quantification of Brain signal variability (SD_{BOLD})

After preprocessing the fMRI data, the standard deviation in the fMRI signal during each of the conditions (faces and houses) was computed at each voxel for each participant. Modulation of variability (ΔSD_{BOLD}) was computed as the difference between the two conditions

($SD_{BOLD\text{-}HOUSES} - SD_{BOLD\text{-}FACES}$) at each of the voxels in a grey matter volume mask. Similarly, ΔSD_{BOLD} on drug was computed for the subset of participants who were administered lorazepam.

4.2.10 Quantification of GABA levels

We used Gannet 3.0 (Edden et al., 2014), a MATLAB based toolbox, to estimate GABA+/Water levels based on the MEGA-PRESS difference spectra in each of the MRS voxels. All the time-domain data were phase corrected and frequency corrected using spectral registration and filtered with 3-Hz exponential line broadening and zero-filled by a factor of 16. The GABA levels were scaled to water and expressed in institutional units by Gannet. Gannet quantifies GABA levels by fitting a five-parameter Gaussian model to the MR spectrum between 2.19 and 3.55 ppm while the water peak is modelled using a Gaussian-Lorentzian function. The MEGA-PRESS editing scheme also results in excitation of coedited macromolecules (MM), which can contribute up to 45% to the edited signal around 3ppm overlapping with the GABA peak. Thus, all GABA values are reported as GABA+ (i.e., GABA + MM) in the present study.

Gannet's integrated voxel-to-image co-registration procedure produces a binary mask of the MRS voxel. Using an SPM-based segmentation function, Gannet estimates the tissue composition (voxel fractions containing Cerebrospinal Fluid (CSF), Grey matter (GM) and white matter (WM)). Gannet then estimates a tissue-corrected GABA+ value that accounts for the fraction of grey matter, white matter, and CSF in each MRS voxel as well as the differential relaxation constants and water visibility in the different tissue types (Harris et al., 2015). Based on a quality control check of the spectra, we flagged and discarded three right and seven left GABA values. GABA measures between right and left ventrovisual voxel were correlated at $r(134) = 0.57$ ($p<0.0001$). Thus, we computed an average GABA+ measure for each participant.

One young participant had both right and left ventrovisual GABA values flagged and was excluded the analysis.

4.2.11 Statistical Analysis

To estimate the effects of task-condition on SD_{BOLD} and the effect of age and GABA on ΔSD_{BOLD} we employed multivariate partial least squares (PLS) analyses (McIntosh et al., 1996).

1. To estimate the effects of task-condition on SD_{BOLD} we used a task-PLS.
   In this method, first a between-subject covariance (COV) matrix is computed between house and face conditions and each voxel's SD_{BOLD}. Then a left singular vector of experimental condition weights (U) is estimated, along with a right singular vector of brain voxel weights (V) and a diagonal matrix of singular values (S). Significance of the detected relations is assessed using 1000 permutation tests of the singular value corresponding to the latent variable (LV). This resulted in two LVs of which only one was significant and represented greater variability during the house condition than during the face condition. *Brainscore* was computed separately for each condition as the dot product of brain voxel weights and each subjects' $SD_{BOLD\text{-}HOUSES}$ and $SD_{BOLD\text{-}FACES}$.
2. We employed rank-based Behavior-PLS for investigating effects of age and GABA levels on ΔSD_{BOLD} in a visual anatomical mask within our sample of 134 subjects (after excluding the one subject whose GABA estimates were flagged). The visual anatomical mask is a broad mask that contained the occipital pole, lingual gyrus, inferior division of lateral occipital cortex, the temporooccipital part of the inferior and middle temporal gyrus, temporal occipital fusiform cortex, occipital fusiform gyrus, and parahippocampal gyrus from the Harvard-Oxford atlas in FSL. A between-subject correlation matrix (CORR) was computed between each voxel's ΔSD_{BOLD} (i.e. $SD_{BOLD\text{-}HOUSES} - SD_{BOLD\text{-}}$

$_{FACES}$) within this mask and both – 1) MRS-based average ventrovisual GABA measures and 2) self-reported age (in years). Then, CORR was decomposed using singular value decomposition (SVD). This decomposition produced a matrix of behavior weights (U), a matrix of brain voxel weights (V), and a diagonal matrix of singular values (S). A single significant latent variable captured the activity pattern depicting the brain regions that show the strongest relationship of ΔSD_{BOLD} with both age and GABA. The behavior weights (U) of this LV suggest lower GABA levels and greater age were associated with lower ΔSD_{BOLD}.We obtained a summary measure of each participant's expression of this LV's spatial pattern (a within-person "*Brainscore*") by taking a dot product of the brain weights (V) with ΔSD_{BOLD} on placebo (and on drug for the subset that received the manipulation). This *Brainscore* was also used for investigating the role of ΔSD_{BOLD} on visual discrimination task.

In both models, we used a bootstrapping procedure (1000 bootstrapped resamples) to reveal the robustness of voxel weights. By dividing each voxel's weight by its bootstrapped standard error, we obtained "bootstrap ratios" (BSRs) as estimates of robustness. We thresholded BSRs at values of ±3.00 (~99.9% confidence interval). Statistical analyses were conducted using R (Team, 2013). Figures were plotted using the ggplot2 package (Wickham, 2016) and the lme4 package (Bates et al., 2007) was used to perform the linear mixed effects analysis.

4.3 Results

4.3.1 Variability and Visual task conditions

Following Garrett et al. (2020), we estimated the complexity of the face and house stimuli by presenting them to a biologically inspired computational model of visual processing

(H_{MAX}) (Riesenhuber and Poggio, 1999; Serre et al., 2005, 2007a, 2007b). Like Garrett et al. (2020), we found that H_{MAX} detected more visual features for houses than faces, consistent with the hypothesis that houses are more "feature-rich" (see Supplementary Materials).

Previous research indicates that variability in the BOLD signal during different task-conditions is modulated based on stimulus complexity. To evaluate this, the standard deviation in the fMRI BOLD signal (SD_{BOLD}) was computed during each task-condition (houses and faces) at each voxel for each participant after pre-processing the fMRI data (see Methods). Using a Partial Least Squares (PLS), we found a single significant latent variable ($p<0.001$) revealing higher variability when viewing houses than faces ($SD_{BOLD\text{-}HOUSES} > SD_{BOLD\text{-}FACES}$), particularly in ventral visual cortex (See Figure 13 for spatial extent and Table S8 for cluster details). This task-condition effect on SD_{BOLD} was significant in both older ($t(76) = 4.15$, $p < 0.001$, Cohen's $d = 0.47$) and younger adults ($t(57) = 3.43$, $p < 0.001$, Cohen's $d = 0.45$).

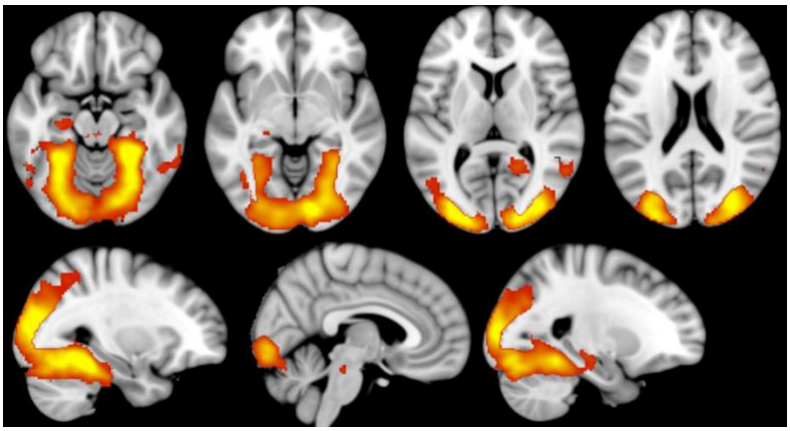

Figure 13. ***Spatial pattern of effect of task-condition on SD_{BOLD}.*** *Primary and extended visual cortex show reliably higher variability (Cohen's $f = 0.46$) during the house condition than face condition (no regions showed reliably lower variability). Bootstrap ratio are thresholded at a value of ≥3.00, which approximates a 99% confidence interval and increase from red to yellow.*

4.3.2 GABA+ levels and ΔSD_{BOLD}

Raw GABA+/H_2O levels measured using spectroscopy were significantly lower in older adults compared to younger adults ($t(131.9) = -6.6$, $p = 8e\text{-}10$, Cohen's $d = 1.1$). GABA+ levels

were also significantly lower in older adults compared to younger adults after correcting for tissue-composition differences ($t(131.9) = -3.13$, $p = 0.002$, Cohen's $d = 0.53$). These, results suggest that the observed age-related declines in GABA levels cannot be completely explained by differences in tissue composition. Raw GABA+ levels were tightly correlated with tissue-composition corrected GABA levels ($r(131) = 0.88$, $p < 2.2e\text{-}16$). All results presented below are based on the raw GABA+ levels but all the effects are similar when using tissue composition corrected GABA values.

To investigate the relationship between GABA levels and modulation of brain signal variability (ΔSD_{BOLD}) we used PLS within the voxels in the anatomically defined visual cortex in all subjects. We found a single significant latent variable ($p = 0.018$) capturing a positive correlation between ΔSD_{BOLD} and ventrovisual GABA levels and negative correlation between ΔSD_{BOLD} and age. The brain-score and brain-pattern computed using this latent factor was used for all further analysis. Both age ($F(1,129) = 54.73$, $p < 1.6e\text{-}11$, Cohen's $f = 0.62$) and GABA levels ($F(1,129) = 7.03$, $p = 0.009$, Cohen's $f = 0.21$) had a significant effect on this *Brainscore* even after accounting for gray-matter volume differences, but the Age x GABA interaction was not significant ($F(1,129) = 0.0004$, $p=0.98$) (Figure 14a). Higher GABA levels were associated with greater ΔSD_{BOLD} in both older and younger adults. The regions exhibiting a reliable relationship (using 1000 bootstraps) between ΔSD_{BOLD} and GABA levels were in the bilateral fusiform, calcarine and lingual cortex (See Figure 14b for spatial extent and Table S9 for cluster details). These results suggest that individual differences in GABA levels play a role in individual differences in the modulation of variability.

The effects were similar after excluding outliers as determined using Cook's Distance greater than 0.03 (=4/sample size). Similarly, *Brainscores* computed using a functional mask based on SD_{BOLD} –Task-Condition model were highly correlated with those computed using the anatomical mask (r(133) = 0.95, p<2.2e-16). We thus used the *Brainscores* based on the anatomical mask in all subjects for the subsequent analyses.

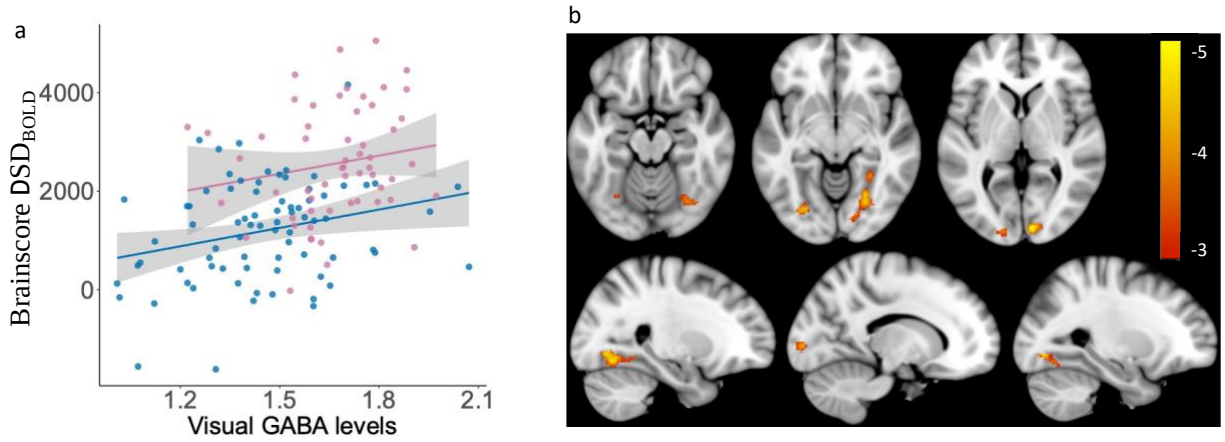

Figure 14. ***ΔSD$_{BOLD}$ and GABA+ levels.*** *(a) Higher ΔSD$_{BOLD}$ are associated with lower age (Cohen's f = 0.62) and greater GABA+ levels (Cohen's f = 0.21) in older (in blue) and younger adults (in pink). (b) Relationship between modulation of variability and GABA levels is robust in primary visual cortex and fusiform gyrus. Bootstrap ratios are thresholded at a value of ≥3.00, which approximates a 99% confidence interval and increase from red to yellow.*

4.3.3 Effect of GABA agonism on SD$_{BOLD}$

The relationship between GABA levels and ΔSD_{BOLD} is obviously correlational. To examine a more causal role of GABA, we administered lorazepam (a benzodiazepine known to potentiate the activity of GABA) in a subset of our participants (25 older and 20 younger adults). Masking by the same brain-pattern from the GABA–ΔSD_{BOLD} model shown in Figure 14, we estimated the influence of drug on $SD_{BOLD\text{-}FACES}$, $SD_{BOLD\text{-}HOUSES}$, and ΔSD_{BOLD}. There was a significant effect of drug on the baseline $SD_{BOLD\text{-}FACES}$ and $SD_{BOLD\text{-}HOUSES}$ (F (1,133) = 4.92, p = 0.03, Cohen's f = 0.17) but not on ΔSD_{BOLD} (F (1,44) = 0.04, 0.85). Strikingly however, consistent with an inverted-U account, we found that baseline GABA levels measured using MRS were negatively associated with the drug-related shift in ΔSD_{BOLD} (F (1,37) = 7.85, p =

0.008, Cohen's f = 0.42) across all subjects (see Figure 15), even after accounting for age, dosage, days between two sessions, order of sessions, and gray-matter volume differences. No outliers were determined using Cook's Distance greater than 0.088 (=4/sample size). As such, those with lower baseline GABA levels were more likely to upregulate ΔSD_{BOLD} on drug. The Age-Group – GABA levels interaction did not have a significant effect ($F(1,37) = 0.9$, $p = 0.36$) on ΔSD_{BOLD}. Notably, baseline GABA levels were not correlated with drug-related shift in baseline $SD_{BOLD\text{-}FACES}$ ($F(1,37) = 0.39$, $p = 0.5$) or $SD_{BOLD\text{-}HOUSES}$ ($F(1,37) = 1.2$, $p = 0.3$).

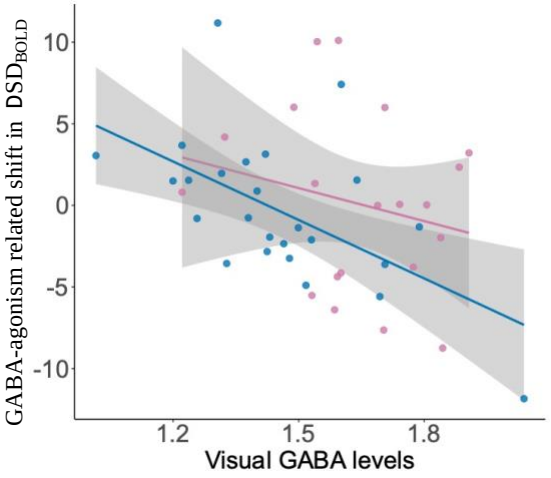

Figure 15. ***Drug-related shift ΔSD_{BOLD} is associated with GABA+ levels.*** *GABA agonism leads to a decrease in ΔSD_{BOLD} in participants with higher GABA levels, while those with lower GABA levels have a boost in ΔSD_{BOLD} (Cohen's f = 0.42) in both older (in blue) and younger (in pink) adults*

4.3.4 Variability, GABA+ and behavior

To investigate the role of ΔSD_{BOLD} and GABA levels in individual differences in behavior, a different subset of our participants (38 older and 36 younger adults) also completed four visual recognition tasks: Buildings-in-noise, Faces-in-noise, Objects-in-noise and Scenes-in-noise. Using factor analysis, we computed a single latent factor score based on all these tasks. All the tasks loaded positively on the latent factor (See methods for loadings) and a higher factor score reflected better performance. Ventrovisual GABA+ levels were significantly associated with this latent score even after controlling for age and gray matter volume ($F(1,69) = 9.7$, $p =$

0.003, Cohen's f = 0.34). The Age-Group x GABA interaction (F(1,69) = 0.74, p = 0.39) was not significant.

However, after excluding the four young outlier participants as determined using Cook's Distance greater than 0.054 (4/sample size) the Age-Group x GABA interaction (F(1,65) = 7.13, p = 0.01, Cohen's f = 0.3) was significant as was the effect of GABA (F(1,65) = 20.86, p = 2.3e-05, Cohen's f = 0.54). On examining the interaction further using Spearman's rank correlation (See Figure 16a), we found that GABA levels were more strongly associated with visual score in younger adults (rho(1,31) = 0.62, p = 0.0002) than older adults (rho(1,37) = 0.37, p = 0.02). Nonetheless, the relationship was significant in both age-groups, with and without outliers.

The *Brainscores* computed from the previously presented GABA–ΔSD_{BOLD} model were also significantly associated with this latent visual score (F(1,69) = 4.2, p = 0.04, Cohen's f = 0.21). The Age-Group x *Brainscore* interaction showed a trend towards significance (F(1,69) = 3.6, p = 0.06). On excluding the one young outlier subject as determined using Cook's Distance greater than 0.054 (4/sample size), the Age-Group x *Brainscore* interaction was significant (F(1,68) = 4.84, p = 0.03, Cohen's f = 0.23). On examining the two age-groups separately using Spearman's rank correlation we found that this relationship was significant only in older adults after controlling for age and gray matter volume (rho(37) = 0.41, p = 0.01) but not in younger adults (rho(34) = -0.01, p = 0.95) (See Figure 16b) with and without outlier.

To investigate whether brain signal variability explains any additional variance in visual function in older adults beyond that explained by GABA levels, we applied a hierarchical regression approach. We found that a model that included ΔSD_{BOLD} and GABA levels fit the behavioral factor significantly better than a model based on GABA levels alone (F(1,35) = 6.8, p

= 0.01, Cohen's f = 0.4). Thus, ΔSD_{BOLD} captures individual differences in visual sensory function beyond those related to differences in GABA levels.

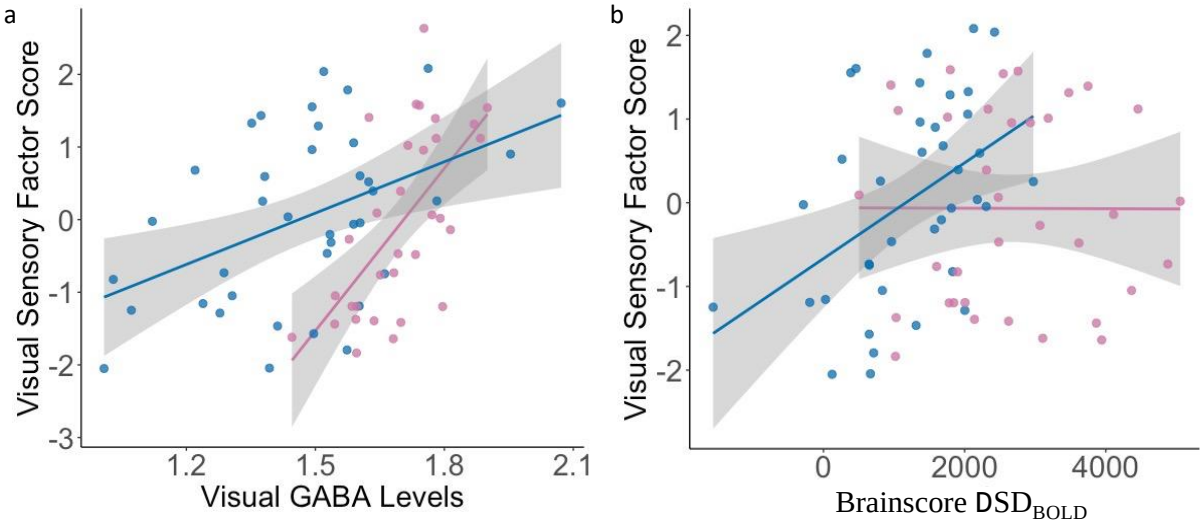

Figure 16. ***Visual sensory function, GABA+ levels, and ΔSD_{BOLD}.*** *(a) Greater visual sensory scores are significantly associated with higher GABA levels (Cohen's f = 0.54) in older adults (in blue) and younger adults (in pink). (b) Higher visual scores were also significantly associated with ΔSD_{BOLD} in older adults (rho(37) = 0.41)*

4.4 Discussion

We report five main findings. First, brain signal variability in the visual cortex is modulated during various task conditions (viewing of faces vs. houses). Second, this modulation of variability (ΔSD_{BOLD}) and MRS-based GABA measures in the ventrovisual cortex are both significantly lower in older adults than in younger adults. Third, individual differences in ΔSD_{BOLD} are significantly associated with GABA levels in the ventrovisual cortex (in both young and older adults). Fourth, these GABA-levels are negatively correlated with the drug-related shift in ΔSD_{BOLD}. Fifth, individual differences in both GABA levels and ΔSD_{BOLD} in the visual cortex are jointly associated with individual differences in visual function.

Consistent with prior research in older adults by Garrett et.al., 2020, we replicated the finding that BOLD signal variability (SD_{BOLD}) is modulated based on the complexity of visual stimuli. SD_{BOLD} in response to "feature-rich" house stimuli is greater than in response to simpler

face stimuli. These findings are consistent with the hypothesis that neural dynamics in the visual system exhibit greater dynamic range in response to more complex information (Van Hateren and H, 1992). Indeed, greater variance in stimulus features has been argued to drive salience in the visual system (Hermundstad et al., 2014) which in turn could lead to an increase in resource allocation. It is hypothesized that the visual network reduces resource allocation (narrowing neural dynamic range) when stimulus input is more reducible or less feature rich (like faces), while it upregulates the dynamic range for a more resource intensive processing when stimulus input is more differentiated or feature rich (like houses). Such upregulation of variability in response to the complexity of the input would reflect a well-adapted dynamic neural network (similar to the well-adapted organism discussed by (Marzen and DeDeo, 2017)), that modulates dynamic range based on the complexity of the input.

Our reported fMRI measures of SD_{BOLD} suggest that older adults are less able to modulate neural dynamics compared to younger adults. These results add to a growing body of literature suggesting that not only baseline brain signal variability but also the modulation of variability in response to task-demands is reduced in older adults compared to younger adults (Garrett et al., 2010, 2011, 2013). Moreover, previous work has found that modulation of variability in response to task-demand is associated with behavioral performance (Garrett et al., 2010, 2013). Modulation of variability in visual cortex in response to houses vs. faces also predicts individual differences on a variety of fluid processing tasks in older adults (Garrett et al., 2020). Thus, individual differences in ΔSD_{BOLD} are meaningful and have behavioral consequences. What is the neurochemical cause for these individual differences in variability modulation?

Computational modelling research suggests a potential role of the brain's inhibitory activity in modulation of brain signal variability. Having sufficient inhibitory activity to offset excitatory activity has been found to be crucial to allow artificial neural networks to operate near so-called criticality (Agrawal et al., 2018), allowing them to visit a variety of different network states and to exhibit higher baseline brain signal variability. Consistent with this hypothesis, previous research has found that pharmacologically manipulating GABA levels can alter neural dynamics in both rodents (Shew et al., 2011) and humans (Lalwani et al., 2021). However, inhibitory activity might also be critical in how flexible the underlying neural network is in response to different stimuli. Indeed, lower visual GABA levels in older adults have been associated with less differentiated response to different visual stimuli in primates (Leventhal et al., 2003) and humans (Chamberlain et al., 2021). GABA levels in the visual cortex have also been associated with better visual discrimination (Kurcyus et al., 2018).

We found that raw GABA+ levels declined with age in the ventrovisual cortex. These age-related declines in GABA were previously reported in earlier publications based on the Michigan Neural Distinctiveness (MiND) study (see Chamberlain et al., 2021). The main reason for including these results here was to investigate the relationship between GABA and ΔSD_{BOLD} (which the previous publications did not study). We replicated the previous finding of an age-related decline in ventrovisual GABA within the larger sample included in this study. We found that fully tissue-composition and concentration corrected GABA estimates also significantly declined with age and were tightly correlated with the uncorrected GABA levels in the visual cortex. This suggests that age-related changes in tissue composition might mediate but do not completely explain the observed age-related declines in GABA levels (Porges et al., 2017b).

We also found that GABA levels in the ventrovisual cortex were significantly associated with ΔSD_{BOLD} during visual task, suggesting that individual differences in GABA contribute to individual differences in ΔSD_{BOLD}. This relationship was also significant in older adults alone. The observed relationship between individual differences in GABA and ΔSD_{BOLD} was, of course, correlational. We therefore also manipulated GABA activity pharmacologically in order to establish a causal relationship between GABA activity and ΔSD_{BOLD}. We administered lorazepam, a benzodiazepine that is known to potentiate the action of GABA at $GABA_A$ receptors, thereby increasing inhibitory activity. We found that increasing GABA activity in this manner had effects on both baseline SD_{BOLD} and ΔSD_{BOLD}, particularly in the ventrovisual cortex. Participants with high GABA levels showed a decrease in ΔSD_{BOLD} on drug while those with lower GABA levels showed an increase in ΔSD_{BOLD} consistent with an inverted-U account in which there is an ideal level of GABA that allows the network to function optimally. Being above or below this optimal level would result in reduced flexibility of the neural network.

Individual differences in GABA levels are also behaviorally relevant. Previous research has found that GABA levels play a critical role in individual differences in sensory processing. For example, sensorimotor GABA levels predict motor function (Cassady et al., 2019; Levin et al., 2019), auditory GABA levels predicts hearing loss (Gao et al., 2015; Dobri and Ross, 2021), and fronto-parietal GABA levels predict cognitive function (Porges et al., 2017a). We extend these findings by showing that visual GABA levels are associated with visual sensory function. Moreover, ΔSD_{BOLD} explained additional variance in visual sensory performance beyond that explained by GABA levels in older adults. These results suggest that developing interventions targeting inhibitory systems and the modulation of variability might help slow sensory declines in healthy aging.

4.5 Limitations

The observed age differences in ΔSD_{BOLD} and GABA could be influenced by cohort or period effects as this was a cross-sectional rather than longitudinal study (Hofer et al., 2002). We also cannot assess the causal role of age-related changes in GABA on sensory function because we did not obtain both sensory processing measures and drug-related fMRI measures in the same set of participants. Additionally, spectroscopy estimates of GABA do not provide a direct measure of inhibitory activity itself (Stagg et al., 2011). However, this limitation should presumably make it harder to observe relationships between GABA and ΔSD_{BOLD}, so the fact that we did find a significant relationship suggests that the relationship may be relatively strong.

4.6 Conclusion

In summary, we found that a) brain signal variability in the visual cortex is modulated in response to visual stimulus complexity, b) this modulation (ΔSD_{BOLD}) is lower in older adults, c) GABA levels in visual cortex decline with age and are associated with individual differences in ΔSD_{BOLD}, d) GABA levels predict GABA agonism related shift in ΔSD_{BOLD} and e) ΔSD_{BOLD} and GABA levels are both associated with individual differences in visual sensory processing. Together, these results suggest that age-related declines in GABA levels contribute to age-related changes in modulation of variability, which in turn is associated with individual differences in visual processing.

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Chapter 5 : General Discussion

5.1 Summary

The human brain contains approximately 86 billion neurons that process sensory information and control behavior (Herculano-Houzel, 2009). This processing relies on an enormous number of computations done both within, and more importantly, between neurons at the synapses. It is this neural network comprising more than *(estimated)* 10^{14} synapses that results in the remarkable functional capacity and processing of the human brain (Tang et al., 2001). Neurotransmitters are the main currency of neuronal communications at these synapses, and it is estimated that about 40% of these synapses work with GABA. As the most abundant and major inhibitory neurotransmitter in the human brain, the critical role of GABA in neural functioning is hardly unexpected. GABA is associated with better inhibition performance (Quetscher et al., 2015), visual discrimination (Kurcyus et al., 2018), emotion regulation (Levar et al., 2017), motor performance (Cassady et al., 2019), fluid processing abilities (Simmonite et al., 2018) and general cognition (Porges et al., 2017). An imbalance in GABA levels is linked with several pathologies – Alzheimer's Disease (AD), Parkinson's, sleep disorders, epilepsy, anxiety, schizophrenia, depression, autism spectrum disorder, and movement disorders (See Kim and Yoon, 2017 for review).

In this dissertation, I investigated the role of GABA in three neural measures that have played an important role in the aging literature: fMRI-based neural distinctiveness, fMRI signal variability (SD_{BOLD}) and stimulus-based change in variability (ΔSD_{BOLD}). The findings from each study are summarized below.

In Study 1 (Lalwani et al., 2019), I showed that relative to younger adults, older adults exhibited both (1) less distinct activation patterns for music vs. speech stimuli and (2) lower GABA levels in the auditory cortex. Furthermore, individual differences in auditory GABA levels (but not ventral visual or sensorimotor GABA levels) were associated with individual differences in neural distinctiveness in the auditory cortex in older adults. These results demonstrate that age-related neural dedifferentiation extends to the auditory cortex and suggest that declining GABA levels play a role in neural dedifferentiation in older adults.

In Study 2 (Lalwani et al., 2021), I replicated previous research findings showing that SD_{BOLD} declines with age in most cortical regions of the brain, adding to the growing body of literature of age-related variability decline. Consistent with our hypothesis that GABA might play a role, we were successful in pharmacologically manipulating SD_{BOLD} by potentiating activity of GABA. We found that the effect of session order, dosage and age-group were not significant, but the effect of drug and the drug X age-group interaction were significant. On examining the interaction further, we found that the effect of drug was significant within older adults alone, but not within young adults alone. This suggests that changes in GABA activity play a causal role in changes in SD_{BOLD}. Moreover, we also found that in older adults, drug-related change in SD_{BOLD} depended on cognitive performance such that poorer performers experienced a greater drug-related boost than high-performers. These results provide the first evidence that brain signal variability can be restored by pharmaceutically targeting the GABAergic system, particularly for older, poorer cognitive performers.

In Study 3 (Lalwani et.al., in prep), I showed that SD_{BOLD} is upregulated in the visual cortex when passively viewing houses (more complex stimuli as determined by computational modelling) than faces. This modulation of SD_{BOLD} (ΔSD_{BOLD}) was significantly reduced in older

adults and was associated with lower MRS-based ventrovisual GABA levels. Moreover, these GABA levels were negatively associated with drug-related change in ΔSD_{BOLD}: older adults with lower GABA levels experienced a greater boost in ΔSD_{BOLD} while those with higher levels experienced a reduction in ΔSD_{BOLD} consistent with an inverted-U account. Finally, I also found that individual differences in visual GABA levels and ΔSD_{BOLD} were both associated with individual differences in visual sensory function. These results suggest that age-related declines in GABA levels contribute not only to age-related changes variability but also task-based modulation of variability, which in turn is associated with individual differences in sensory processing.

Together, these studies provide novel evidence that age-related reductions in GABA levels play a critical role in aging and age-related changes in three different neural measures across various regions of the brain. These measures are in turn linked with individual differences in cognition and sensory function. This research adds to the growing literature suggesting GABA's important role in healthy cortical functioning and as a potential therapeutic target in aging.

5.2 Limitations & Future Research

One main limitation of the studies presented in this dissertation is the use of a cross-sectional sample. Thus, differences between two age-groups could be influenced by cohort or period effects and should be interpreted with caution (Kraemer et al., 2000; Hofer et al., 2002). Moreover, cross-sectional analyses can imply the existence of an effect even when the true longitudinal indirect effect is zero (Maxwell et al., 2011). A longitudinal design can help elucidate the order and causal relationship of age-related differences explored in this dissertation.

Another limitation of Study 1 and 2 was a relatively smaller sample size. Although these studies were better powered than most previous studies and the sample size was determined to be sufficient by a power analysis, small samples in neuroimaging studies can lead to increased rates of false positive and inflated effects (Yarkoni, 2009; Button et al., 2013).

Our samples were also not representative of the world population (Henrich et al., 2010). In particular, Ann Arbor is an American university town and so our participants have higher-than-average family income and education, are native English speakers, and often participate in behavioral research studies. We also excluded participants who had glaucoma, breathing problems, allergy to benzodiazepines, were undergoing chemotherapy, or who had an immune system disorder, or kidney or liver disease (Gagnon et al., 2019). These additional exclusions were to avoid potential interactions with lorazepam, but this limits the generalization of our findings to a broader population. Future studies should thus recruit a larger and more diverse sample in order to verify these effects.

We used lorazepam to target GABA activity in the brain for Study 2 and 3. The primary motivation for doing so was that lorazepam (a benzodiazepine) is safe; it is FDA approved and regularly used in the treatment of anxiety. It has relatively small side effects with a single, oral dose. Unfortunately, lorazepam had three major shortcomings – 1) it acts on the whole-brain and cannot be targeted to specific regions, 2) it has a mild sedative effect – thus it is hard to ascertain if greater SD_{BOLD} and ΔSD_{BOLD} in older adults on drug could benefit behavior because participants get drowsy and 3) there are individual differences in drug-reactivity. Thus, future research needs to develop better ways of targeting the region-specific GABAergic systems. One possibility would be to use exercise or yoga regimens that have recently been shown to increase GABA levels in a safer and healthier fashion (Streeter et al., 2010; Li et al., 2017).

Computational research suggests that appropriate levels of inhibitory activity are crucial for neural networks to function optimally (e.g., to exhibit greater dynamic range (Shew et al., 2009), neural flexibility (Fagiolini et al., 2004), functional connectivity (Fingelkurts et al., 2004), and to be able to sample a larger number of attractor states (Agrawal et al., 2018)). Resting-state SD_{BOLD} has been hypothesized to be related to this property of neural networks. But how does the ability to sample a greater number of states during resting-state relate with neural functioning in response to stimuli? Animal research provides some clues. For example, in the absence of visual stimulation in cats, a single neuron's spontaneous activity is systematically related to its activity in the presence of a visual stimulus (Tsodyks et al., 1999). Similarly, multi-unit recordings in ferrets have found that spontaneous and evoked activities in the visual cortex in response to natural scenes are systematically related and become more similar with development (Berkes et al., 2011). Thus, neural network dynamics during rest potentially reflect how a neural network can accommodate an external stimulus. For instance, a neural network capable of visiting a greater number of states allows for different stimuli to be represented in distinct states. In line with this hypothesis, in visual cortex of monkeys, lower GABA levels are associated with increased firing rates, with reduced neural selectivity (e.g., different orientations represented by similar neural patters), and with reduced variability in neural responses (Schmolesky et al., 2000; Yang et al., 2009). Future research should examine the links between these neural measures empirically in humans and their joint and independent role in aging.

The only neurotransmitter that the present studies investigated was GABA. But optimal neural functioning depends on a balance between excitatory and inhibitory neurotransmitters along with neuromodulation that impacts the tuning of neural networks to these neurochemicals.

Future research should thus investigate the role of excitatory/inhibitory neurotransmitter balance in these age-related neural changes.

Similarly, the studies in this dissertation were focused on sensory function. However, age-related decline in memory is one of the most pervasive complaints by the elderly and is affecting millions of otherwise healthy older adults. It not only represents a significant public health impact in itself but has also been shown to be an important risk factor for Alzheimer's Disease (AD). The medial temporal lobe (MTL), especially the hippocampus, is critical to memory function. Unfortunately, it is vulnerable to healthy aging (Gallagher et al., 2006; Bettio et al., 2017) and is one of the structures to show the earliest pathological changes in Alzheimer's disease (AD) (Gómez-Isla et al., 1996; Price et al., 2001). Hippocampal dysfunction in mild cognitive impairment (MCI) patients is also a common precursor of AD (Petersen et al., 1999).

Future research could investigate whether neural dedifferentiation and changes in brain signal variability extend to the hippocampus, the role of excitatory/inhibitory neurotransmitter balance in these changes, and the behavioral consequences (especially for memory) of these neural changes, in both healthy older adults and in MCI patients. This research could lead to the development of preclinical markers for AD and open new avenues for early pharmacological interventions to slow or even reverse some of these neural changes.

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Appendix A: Supplementary Material for Chapter 2

Neural distinctiveness declines with age in auditory cortex and is associated with auditory GABA levels.

A.1 Supplementary Results

A.1.1 What guided the ROI size choice?

More than 95% of the participants had more than 1400 significantly activated vertices within the anatomical ROI. We therefore used 1400 vertices as a default ROI-size in many of the analyses. The 1 young and 1 older subject who had fewer than 1400 significantly activated vertices exhibited typical levels of neural distinctiveness (i.e. within one standard deviation of the average from all the subjects). Furthermore, the age effect on neural distinctiveness did not change when these subjects were excluded from the analysis ($t(33.97) = -2.182$, $p = 0.036$). Because these subjects did not exhibit any other differences from the rest of the participants, they were included in all the analyses.

A.1.2 Distinctiveness computed when primary auditory cortex is excluded

Transverse temporal gyrus (Heschl's gyrus) is the site of primary auditory cortex and is presumably involved in low-level feature processing of all auditory information independent of its category. Speech and music obviously have differences in their spectral and temporal properties, so the distinctiveness measured within primary auditory cortex could reflect low-level feature differences between the two stimulus categories. We therefore computed distinctiveness after excluding Heschl's gyrus from the ROI. Distinctiveness computed in the two different ROIs (with and without Heschl's gyrus) were highly correlated ($r(41) = 0.99$, $p<2.2e-16$) and neural distinctiveness was still significantly reduced in the older vs. younger participants even when

Heschl's gyrus was excluded (t (36.6) = -2.11, p = 0.042). Likewise, the relationship between GABA+ and neural distinctiveness in the older adults was still significant (r (21) = 0.46, p = 0.026).

A.1.3 Effect of age and GABA on distinctiveness is independent of hemispheric differences

Surprisingly enough, there were no significant differences in activation between the two hemispheres for either speech or music. Mean activation, peak activation, and the total number of activated vertices did not differ in left vs. right hemisphere in either the young or the older adults. We also performed an ANOVA with hemisphere as a within-subject factor and age, GABA levels, grey matter volume, NAA and pure tone average as between-subject factors to predict distinctiveness measured in separate functional masks of 700 vertices in the left and right hemisphere. We still found that age (F (1,37) = 4.3, 0.045) and GABA levels (F (1,37) = 5.54, p = 0.024) were significant predictors of distinctiveness even after accounting for these other factors.

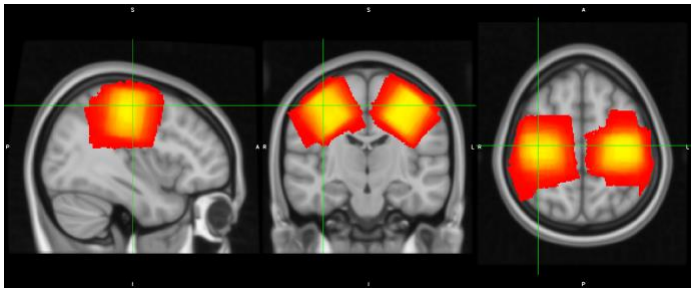
A.1.4 Neural distinctiveness computed within the GABA voxels

MRS voxels are large and ours included areas outside the functional region of interest (and outside the temporal lobe and auditory cortex). We therefore computed neural distinctiveness within the auditory GABA voxels, rather than within the functional ROI, so that the two measures would be more comparable. We found that neural distinctiveness within this larger region also declines with age (t (41) = -2.27, p = 0.029) and a hierarchical regression analysis revealed that GABA levels also account for significant variance in this measure of distinctiveness over and above that explained by age, grey matter volume, PTA, and NAA in older adults (F (1,17) = 6.87, p = 0.018). Furthermore, distinctiveness computed within the MRS

voxel was highly correlated with distinctiveness computed within the functional mask ($r = 0.63$, $p < 7.1*10^{\wedge}06$).

A.2 Supplementary Figures

(a)



(b)

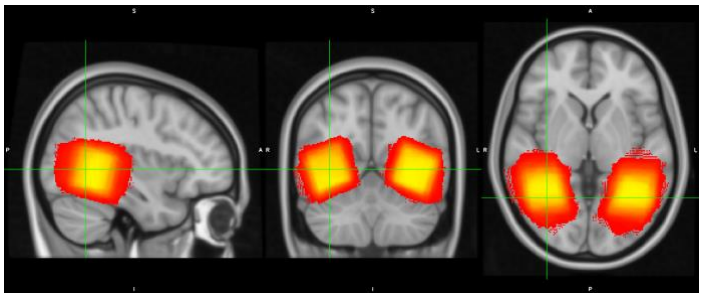


Figure S1. ***MRS voxel placement in the (a) sensorimotor and (b) ventrovisual cortex.*** *The color indicates the amount of overlap in the voxel placement across participants (yellow represents maximum overlap while red represents less overlap).*

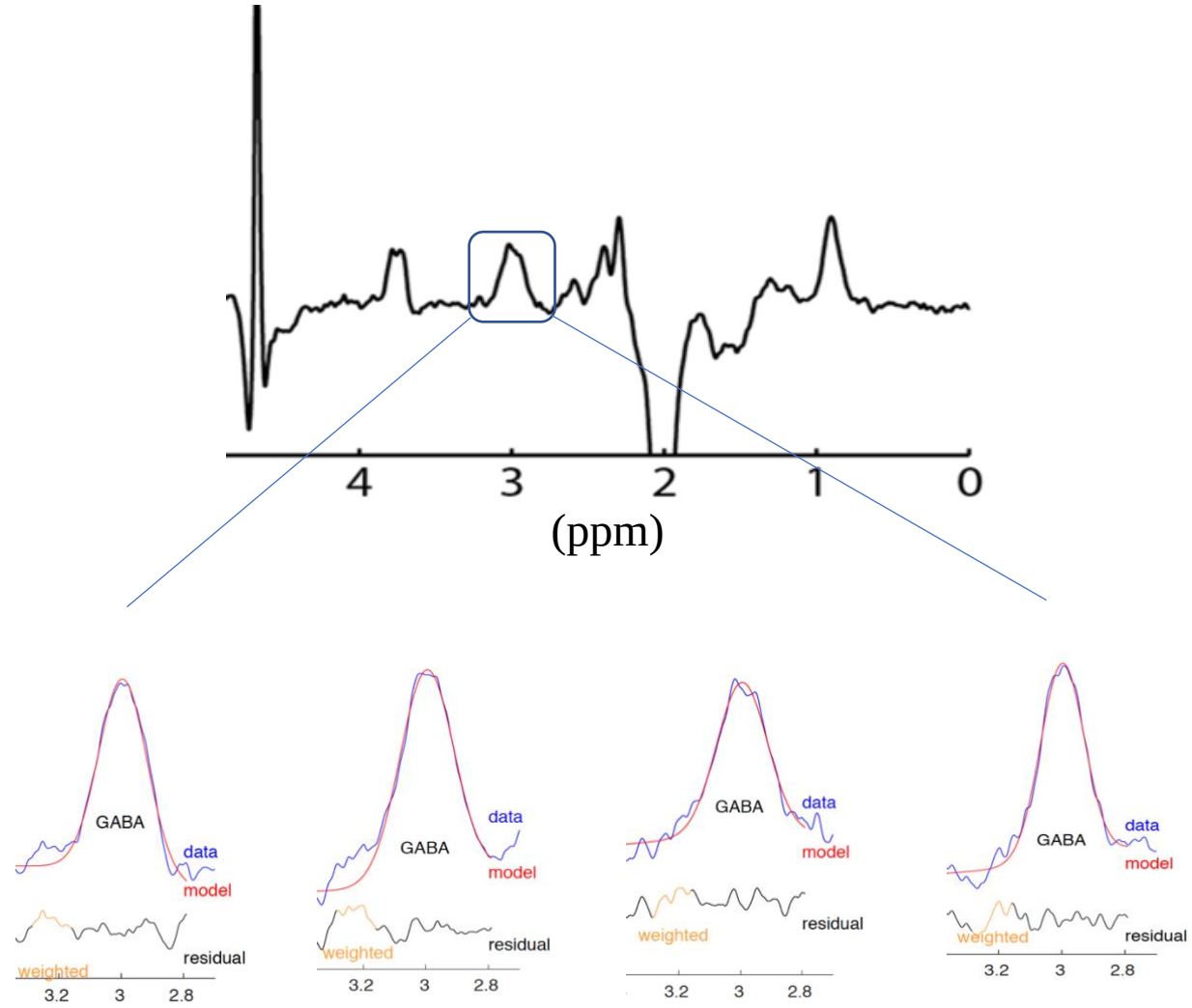
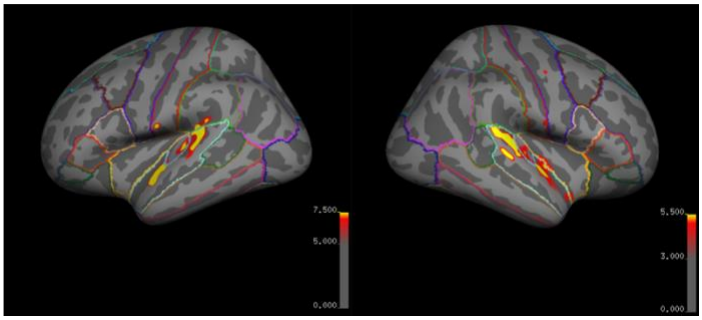


Figure S2. ***Example of an MRS spectrum.*** *The peak around 3ppm is associated with GABA. The enlarged figure shows a Gaussian model fit (dotted line) using Gannet to estimate the area under the curve i.e. GABA+ levels in older adult and young adult with high and low GABA levels each.*

(a)



(b)

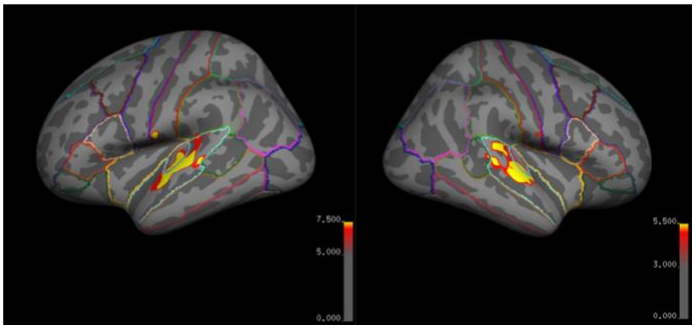


Figure S3. ***Participant-specific functional ROIs for the computation of neural distinctiveness.*** *(a) Heatmap of the significance of the music vs. fixation contrast. The scale is the negative log of the uncorrected significance value at each vertex (e.g. p=0.001 is displayed as 3 on the plot). The most significant areas are in yellow and less significant regions are in red. (b) Heatmap of the significance of the foreign speech vs. fixation contrast.*

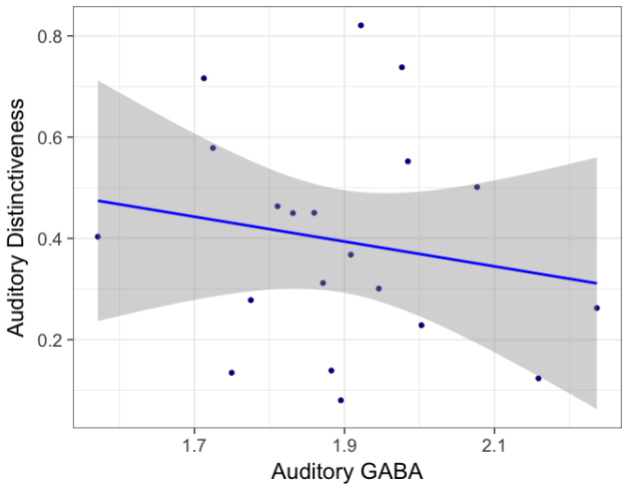


Figure S4. ***The relationship between raw auditory GABA+ levels and auditory neural distinctiveness in younger adults.*** *Individual differences in GABA+ were not significantly correlated with individual differences in neural distinctiveness in the younger adults. (r (18) = -0.18, p = 0.45)*

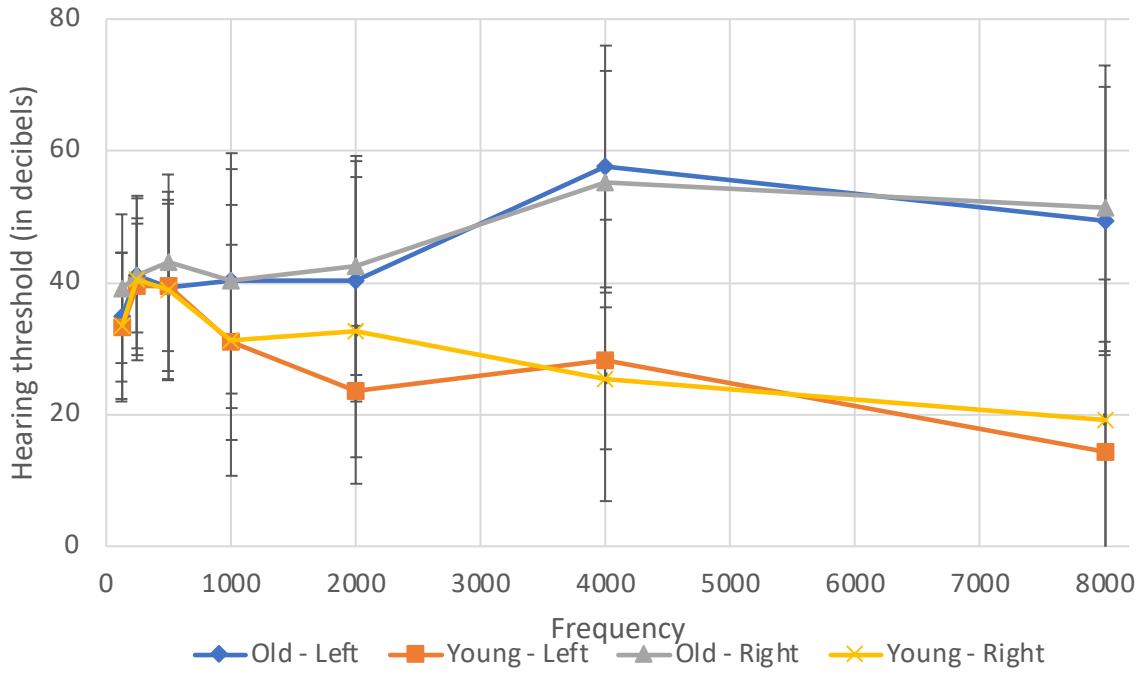


Figure S5. ***The pure tone threshold for young and old adults in the left and right ear.*** *Pure-tone average thresholds were significantly higher in older adults at frequency (t_{4000} (41) = 6.3, p = 1.4e-07); (t_{8000}(41) = 5.9, p = 6.3e-07)*

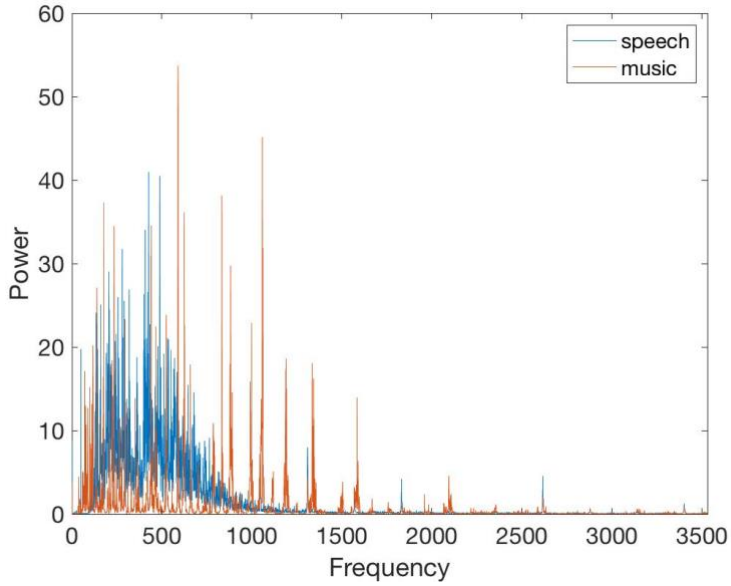


Figure S6. ***Power distribution for speech (blue) and music (red).*** *Speech and music have different frequency content from one another especially in higher frequency ranges.*

A.3 Supplementary Tables

Table S1. ***Neural distinctiveness in auditory cortex was reduced in older adults relative to younger adults independent of ROI size. Average distinctiveness also declined with increasing ROI size in both age groups.***

ROI Size (vertices)	Mean (Older adults)	Mean (Young Adults)	Student's t-value	p-value
1000	0.28	0.401	-1.86	0.071$^{\#}$
1400	0.27	0.395	-2.045	0.047*
2000	0.26	0.386	-2.36	0.023*
5000	0.21	0.35	-3.11	0.003**
10000	0.16	0.245	-2.80	0.007**
MRS voxel	0.04	0.08	-2.27	0.029*
Anatomical (~37000 vertices)	0.06	0.1	-2.18	0.035*

Appendix B: Supplementary Tables for Chapter 3

Dynamic recovery: GABA agonism restores neural variability in older, poorer performing adults

Cluster Number	MNI Co-ordinates			Peak Threshold (BSR)	Cluster Size (in 2mm voxels)	Cortical Region Label based on Harvard Oxford Cortical Atlas
	X	Y	Z			
1	2	26	44	8.90	5908	Bilateral Paracingulate Gyrus (Includes parts of Superior Frontal and Cingulate Gyrus)
2	-34	-46	40	7.80	5261	(L) Superior Parietal Lobule
3	-50	38	-2	6.99	414	(L) Frontal Pole
4	-28	-2	60	6.15	171	(L) Superior Frontal Gyrus
5	-58	-38	18	6.02	154	(L) Planum Temporale
6	36	-40	62	5.67	254	(R) Superior Parietal Lobule
7	-50	2	46	5.22	529	(L) Precentral Gyrus (Includes parts of Inferior Frontal Gyrus)
8	28	-86	7	5.08	157	(R) Lateral Occipital Cortex (inferior division)
9	42	-66	24	5.01	542	(R) Lateral Occipital Cortex (superior division)
10	40	10	22	4.61	337	(R) Inferior Frontal Gyrus
11	-50	6	-12	4.35	120	(L) Temporal Pole
12	-26	-52	-20	4.30	247	(L) Temporal Occipital Fusiform Gyrus
13	44	-60	54	4.27	105	(R) Lateral Occipital Cortex (superior division)
14	14	-72	26	3.95	136	(R) Cuneal Cortex
15	-42	-68	-2	3.77	176	(L) Lateral Occipital Cortex (inferior division)
16	-28	18	0	3.76	139	(L) Insular Cortex
17	-10	20	-26	-5.13	148	(L) Frontal Orbital Cortex (likely a sinus related artifact)
18	58	-16	-26	-5.13	265	(R) Inferior Temporal
19	54	-66	-36	-5.06	127	(R) Cerebelum_Crus2 (AAL atlas based)

Table S2. ***Brain regions that exhibited a reliable association between age and SD_{BOLD} on placebo.***

Cluster Number	MNI Co-ordinates			Peak Threshold (BSR)	Cluster Size (in 2mm voxels)	Cortical Region Label based on Harvard Oxford Cortical Atlas
	X	Y	Z			
1	34	-8	-30	7.14	27336	(R) Fusiform Cortex (Includes parts of Right Superior Temporal, Planum Temporale, Temporal pole, Inferior frontal, Inferior Temporal, Central Opercular etc.)
2	-28	-10	-34	6.75	8893	(L) Parahippocampal (Includes parts of Left Superior Temporal, Planum Polare, Temporal Pole, Inferior Temporal region etc.)
3	-38	-86	-38	6.43	970	Bilateral Cerebellum Crus2 (AAL atlas based)
4	14	-60	52	5.66	1733	(R) Lateral Occipital Cortex (Includes parts of Superior Parietal Lobule and Precuneus)
5	-8	-34	56	5.47	4931	(L) Postcentral Gyrus (Includes parts of bilateral Precentral Gyrus, Cingulum, Middle Frontal Gyrus, Supplementary Motor area etc.)
6	-62	4	8	5.12	192	(L) Precentral Gyrus
7	-52	-12	52	4.96	437	(L) Precentral Gyrus
8	44	-76	-40	4.72	161	(R) Cerebellum Crus2 (AAL atlas based)
9	-40	-28	40	4.70	351	(L) Postcentral Gyrus
10	-14	-102	6	4.70	155	Bilateral Occipital Pole
11	12	34	16	4.60	123	Bilateral Cingulate Gyrus
12	30	34	50	4.57	240	(R) Middle Frontal Gyrus

Table S3. ***Brain regions that exhibited a reliable increase in variability on drug compared to placebo, mainly in older compared to younger adults***

Cluster Number	MNI Co-ordinates			Peak Threshold (BSR)	Cluster Size (in 2mm voxels)	Cortical Region Label based on Harvard Oxford Cortical Atlas
	X	Y	Z			
1	-22	10	-26	6.94	7991	(L) Orbitofrontal Cortex (Includes parts of Left Temporal Pole, Superior Temporal Gyrus, Planum Temporalis etc.)
2	34	-8	-30	6.54	8189	(R) Temporal Fusiform Cortex (Includes parts of Right Superior Temporal, Parahippocampal, Temporal Pole, Middle Temporal Gyrus etc.)
3	36	-50	-20	6.49	7280	(R) Temporal Fusiform Cortex (Includes parts of Occipital Fusiform Cortex)
4	50	40	22	6.34	1465	(R) Frontal Pole
5	4	-86	-38	6.03	323	Bilateral Cerebellum Crus2 (AAL atlas based)
6	-6	-34	58	5.98	7844	(L) Precentral Gyrus (Includes parts of bilateral Precentral Gyrus, Supplementary Motor area, Superior Frontal Cortex, Cingulum etc.)
7	62	-2	36	5.89	1121	(R) Precentral Gyrus
8	14	-60	56	5.81	217	(R) Lateral Occipital Cortex (superior division) (Includes parts of Superior Parietal Lobule and Precuneus)
9	-26	-72	-10	5.79	906	(L) Occipital Fusiform Cortex
10	-26	46	42	5.34	587	(L) Frontal Pole
11	-14	-102	6	5.31	169	(L) Occipital Pole
12	24	4	-40	5.22	585	(R) Temporal Pole
13	-44	32	40	4.94	231	(L) Middle Frontal Gyrus
14	-62	4	8	4.83	202	(L) Precentral Gyrus
15	-50	-6	48	4.59	205	(L) Precentral Gyrus
16	-16	62	-18	4.59	365	(L) Frontal Pole
17	18	60	30	4.28	109	(R) Frontal Pole
18	-44	-70	38	4.13	129	(L) Lateral Occipital Cortex (superior division)
19	-50	44	-8	3.88	128	(L) Frontal Pole
20	-44	-68	-2	3.65	103	(L) Lateral Occipital Cortex (inferior division)

Table S4. ***Brain regions that exhibited a reliable increase in variability on drug compared to placebo in older adults alone***

Cluster Number	MNI Co-ordinates			Peak Threshold (BSR)	Cluster Size (in 2mm voxels)	Cortical Region Label based on Harvard Oxford Cortical Atlas
	X	Y	Z			
1	32	-74	44	8.06	286	(R) Lateral Occipital (superior division) (Includes parts of Right Superior Parietal Lobule)
2	64	12	18	6.84	150	(R) Precentral Gyrus
3	-36	-82	30	6.19	792	(L) Lateral Occipital (superior division) (Includes parts of Left Superior Parietal Lobule)
4	16	-40	2	6.11	195	(R) Cingulate Gyrus (posterior division)
5	20	4	0	5.93	221	(R) Pallidum (Based on AAL atlas) (Includes parts of Putamen)
6	-26	-8	2	5.91	357	(L) Pallidum (Based on AAL atlas) (Includes parts of Putamen)
7	-42	-44	40	5.77	1459	(L) Supramarginal Gyrus (superior division) (Includes parts of Left Superior Parietal Lobule, Lateral Occipital Cortex, Angular Gyrus etc.)
8	14	46	-20	5.69	561	(R) Frontal Pole
9	46	48	4	5.61	165	(R) Frontal Pole
10	2	22	34	5.53	305	Bilateral Cingulate Gyrus (anterior division)
11	12	-46	62	5.44	668	(R) Postcentral Gyrus (Includes parts of Precuneus)
12	-6	14	10	5.40	282	(L) Caudate (Based on AAL atlas)
13	-66	-40	-6	5.07	101	(L) Middle Temporal Gyrus
14	-48	2	26	5.00	142	(L) Precentral Gyrus
15	40	-12	46	4.77	103	(R) Precentral Gyrus
16	44	-16	-4	4.69	104	(R) Planum Temporale
17	-38	36	-7	4.57	108	(L) Frontal Orbital Cortex
18	2	34	16	4.16	159	Bilateral Cingulate Gyrus (anterior division)
19	40	14	34	4.14	180	(R) Middle Frontal Gyrus

Table S5. ***Brain regions that exhibited a reliable association between overall cognitive processing score and drug-related shifts in variability, mainly expressed within the older adult group***

Cluster Number	MNI Co-ordinates			Peak Threshold (BSR)	Cluster Size (in 2mm voxels)	Cortical Region Label based on Harvard Oxford Cortical Atlas
	X	Y	Z			
1	18	-40	4	10.22	621	(R) Cingulate Gyrus (posterior division) (Includes parts of Parahippocampal Gyrus)
2	20	4	0	9.27	1370	(R) Pallidum
3	12	-46	62	8.22	4322	(R) Postcentral Gyrus (Includes parts of left Postcentral Gyrus, and bilateral Precentral Gyrus, Precuneus, Lateral Occipital Cortex etc.)
4	-52	-28	28	7.78	2399	(L) Supramarginal Gyrus (anterior division) (Includes parts of Heschl's Gyrus, Central Opercular Cortex etc.)
5	30	-72	38	7.63	510	(R) Lateral Occipital (superior division) (Includes parts of Right Superior Parietal Lobule)
6	44	-16	-4	7.18	387	(R) Planum Polare (Includes parts of Heschl's Gyrus, Superior Temporal Gyrus)
7	40	32	18	7.1	252	(R) Middle Frontal Gyrus
8	2	20	38	6.63	260	Paracingulate Gyrus (posterior division) (Includes angular division of cingulate gyrus)
9	-58	10	-2	6.31	260	(L) Inferior temporal gyrus (pars opercularis)
10	32	28	36	6.18	400	(R) Middle Frontal Gyrus
11	-28	30	-6	6.12	259	(L) Frontal Orbital Cortex
12	-42	-60	4	5.98	164	(L) Lateral Occipital Cortex (inferior division)
13	-26	8	-42	5.58	161	(L) Temporal Pole
14	-8	16	58	5.53	207	(L) Superior Frontal Gyrus
15	12	46	-20	5.40	1406	(R) Frontal Pole
16	-36	-12	-24	5.35	234	(L) Parahippocampal Gyrus (anterior division) (Includes parts of Temporal Fusiform Cortex)
17	-36	8	14	5.33	467	(L) Central Opercular Cortex (Includes parts of Frontal Operculum Cortex)
18	26	6	-44	5.33	102	(R) Temporal Pole
19	22	54	22	4.82	245	(R) Frontal Pole

Table S6. ***Brain regions that exhibited a reliable association between cognitive processing score from the NIH Toolbox and brain signal variability measure during placebo and drug condition as well as drug related change in brain signal variability in older adults alone***

Cluster Number	MNI Co-ordinates			Peak Threshold (BSR)	Cluster Size (in 2mm voxels)	Cortical Region Label based on Harvard Oxford Cortical Atlas
	X	Y	Z			
1	32	-74	44	8.10	505	(R) Lateral Occipital (superior division) (Includes parts of Right Superior Parietal Lobule, Angular gyrus, Supramarginal Gyrus etc.)
2	-28	-80	40	7.40	1736	(L) Lateral Occipital (superior division) (Includes parts of Left Superior Parietal Lobule, Angular Gyrus, Supramarginal Gyrus etc.)
3	-54	-12	52	7.21	2695	(L) Precentral (Includes parts of Postcentral Gyrus, Heschl's Gyrus, Central Operculum and Supramarginal Gyrus etc.)
4	16	-40	0	7.11	237	(R) Cingulate Gyrus (posterior division)
5	24	-18	-36	6.33	242	(R) Parahippocampal Gyrus (anterior division)
6	-4	-34	62	5.96	1509	Precentral Gyrus (Includes parts of bilateral Precentral and Postcentral gyrus)
7	18	6	-4	5.87	217	(R) Pallidum
8	2	20	34	5.80	641	Bilateral Cingulate Gyrus (anterior division) (Includes parts of Paracingulate Gyrus)
9	-36	-12	-26	5.73	236	(L) Parahippocampal Gyrus
10	32	28	36	5.62	301	(R) Middle Frontal Gyrus
11	46	48	4	5.58	319	(R) Frontal Pole
12	-8	-6	2	5.39	117	(L) Thalamus
13	-34	16	8	5.25	184	(L) Frontal Operculum Cortex (Includes Central Operculum Cortex, Insular Cortex)
14	14	46	-20	5.21	622	Bilateral Frontal Pole (Includes parts of Frontal Medial Cortex and Paracingulate Gyrus)
15	-32	-92	20	5.19	102	(L) Occipital Pole
16	-26	16	62	4.75	238	(L) Superior Frontal Gyrus
17	40	-12	46	4.73	394	(R) Precentral Gyrus

Table S7. ***Brain regions that exhibited a reliable association between various cognitive processing tasks from the NIH Toolbox and drug-related shifts in brain signal variability in older adults alone***

Appendix C: Supplementary Material for Chapter 4

Modulation of neural variability: Aging-related reduction, neurochemical cause, and behavioral consequences

C.1 Supplementary Figure

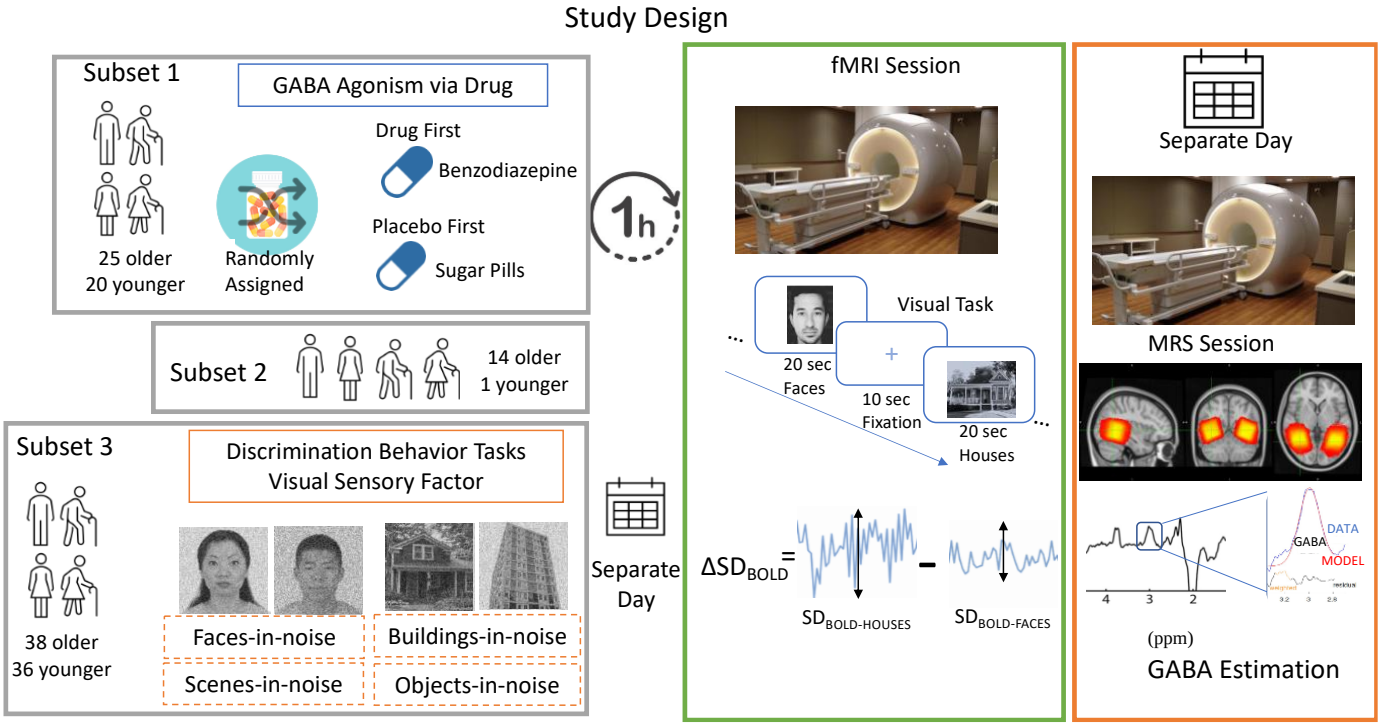


Figure S7. ***Chapter 4 Session Design and Participant Distribution.*** *All participants underwent an fMRI and MRS scanning sessions on separate days. One subset of participants (25 older and 20 younger) received an additional on-drug fMRI scan on a separate day. The order of on-drug and off-drug fMRI sessions was randomized. A different subset of participants (38 older and 36 younger) completed four visual discriminatory tasks on a separate day before fMRI testing. During the fMRI session participants completed a 6-minute visual task with pseudorandomized 20-second blocks of passively viewing houses and faces interleaved with 10-second fixation as shown in green panel. Change in variability (ΔSD_{BOLD}) is computed at every voxel as the difference between $SD_{BOLD\text{-}HOUSES}$ and $SD_{BOLD\text{-}FACES}$. Orange panel shows the MRS voxel overlap across participants with brighter (yellow) indicating maximum overlap and red showing the least, an example spectrum obtained, and that raw GABA+/water is estimated by fitting a Gaussian model to compute the area under the curve of 3ppm GABA peak.*

C.2 Supplementary Results

Physiological studies in non-human primates over recent decades have demonstrated that the receptive fields of neurons increase in both size and complexity as we move anteriorly along the ventral visual pathway. These insights are reflected in the biologically inspired, openly available HMAX feedforward model of visual recognition (code available at: http://maxlab.neuro.georgetown.edu/hmax.html). The two earliest layers in this model (S1 and C1) correspond to neurons in primary visual cortex (V1) and the next two layers (S2 and C2) correspond to neurons in extrastriate visual areas (V2/V4). Following Garrett et al. (2020), we used this model to objectively estimate the visual complexity of the two stimulus categories presented during our visual task (houses vs. faces). This model can also generate predictions about which cortical regions should be most sensitive to differences in stimulus complexity in our specific stimulus set. We focused our analyses on the C1 and C2 layers that aggregate the responses of cells in the S1 and S2 layers. To anticipate the results, we found that houses were more "feature-rich" than faces in both C1 (corresponding to V1) and C2 layers (corresponding to extrastriate regions), as described in detail below.

Layers in the first layer (S1) are modelled using Gabor functions with 16 different size filters (ranging from 7 to 37) corresponding to n x n pixel neighborhoods for four different orientations (Default: -45°, 0°, 45°, 90°). For each orientation and filter size, a model was fit to each image in overlapping windows (50% overlap) resulting in a simple cell response map for all positions within the input image. At the next layer (complex cells in C1), the maximum activity over S1 units corresponding to each orientation is computed separately. Since 16 filter sizes were used, taking the maximum over neighboring pairs of filters results in eight "scale bands". The scale band index corresponds to the spatial neighborhood of S1 cells over which outputs are

pooled. For each of the 8 scale-bands and 4 orientations, we calculated a median within-image C1 activation value for each image and then standardized them by computing z-scores. Using t-tests, we compared these within-image median values across the two stimulus categories. The results of these 8 (scales) × 4 (orientations) independent sample t-tests (Figure S8A and S8C) indicate that houses consistently produced a larger median C1 activation value than faces across all receptive field sizes.

In the third layer (S2), a template-matching approach is used. The receptive fields of the S2 cells correspond to a set of universal prototype templates derived from a library of naturalistic stimuli and their activation is computed based on the Euclidean distance between incoming C1 activity from all four orientations and the stored prototype for that S2 cell. For each prototype, an S2 map is computed across all positions at each of the 8 scale bands. The final layer (C2) then takes a global maximum over all scales and positions for each S2 map separately for different neighborhood (patch) sizes. We computed the median activation for each C2 neighborhood size separately and then standardized the results using z-scores. We then compared median activation by faces and houses using eight independent sample t-tests (one for each patch size). We found that house stimuli showed greater median activity compared with faces across different patch sizes (Figure S1B and S1D).

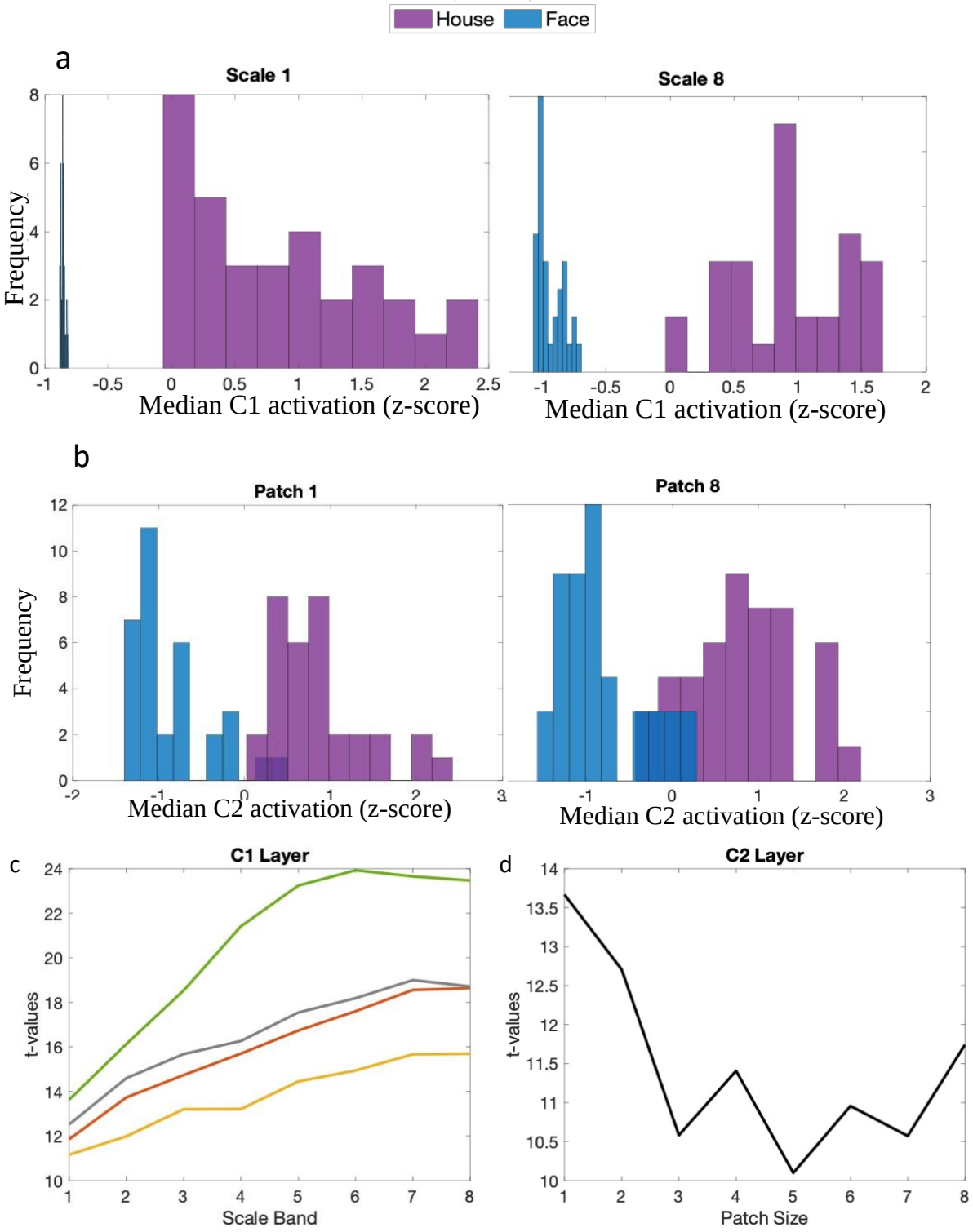


Figure S8. ***Example C1 and C2 activation distributions to house and face stimuli.*** *A) Z-scored median activation at C1 for all images in the two stimulus categories (faces in blue, houses in purple) for one orientation and two different scales. B) Z-scored median activation at C2 for all images in both stimulus categories for two different patch sizes. C) t-values comparing house vs. face median activation in layer C1 across four different orientations and 8 scale bands (from smallest to largest receptive field). D) t-values comparing house vs. face median activation in layer C2 for 8 different patch sizes. All p-values for the t-tests are less than 0.001.*

C.3 Supplementary Tables

Cluster Number	MNI Co-ordinates			Peak Threshold (BSR)	Cluster Size (in 2mm voxels)	Cortical Region Label based on Harvard Oxford Cortical Atlas
	X	Y	Z			
1	-28	-78	-6	5.36	118	(L) Occipital Fusiform Gyrus
2	10	-92	2	5.05	124	Bilateral Occipital Pole (Includes parts of Intracalcarine Cortex)
3	24	-70	-8	4.69	199	(R) Occipital Fusiform Gyrus (Includes parts of Lingual Gyrus, Temporal Occipital Fusiform Gyrus and Occipital Pole)

Table S8. ***Brain regions that exhibited a reliable relationship between GABA and ΔSD_{BOLD} within visual cortex in both young and older adults.***