Supplementary Information

Reduced GABAergic Cortical Inhibition in Aging and Depression

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1.0 Sensitivity Analyses

In order to examine the robustness of the reported SICI findings in patients with late-life depression, sensitivity analyses were performed to assess the influence of factors that may affect GABAergic functioning. In particular, the influence of benzodiazepine or antidepressant use, treatment resistance, late- or early-onset of depressive symptoms, and diagnosis of a comorbid anxiety disorder were examined. Evidence exists that antidepressant medication may enhance cortical inhibition, as measured using SICI (Eichhammer *et al*, 2003; Robol *et al*, 2004), and that benzodiazepines may similarly potentiate SICI (Paulus *et al*, 2008; Ziemann *et al*, 1996). Previous studies also indicate that abnormalities in the GABAergic system may differ in treatment-resistant patients with depression, as compared to non-treatment-resistant patients (Levinson *et al*, 2010; Price *et al*, 2009). Further, depression with onset in late-life may involve

different pathophysiological mechanisms than early-onset depression (McKinney and Sibille, 2013; Sibille, 2013). Lastly, trait-level anxiety has been associated with reduced cortical inhibition (Wassermann et al. 2001), thus is it possible that anxiety disorder comorbidity could influence the SICI results. Analyses of subgroups within the smaller sample size of younger adults with depression were not performed due to a lack of power; only older adults with depression and healthy controls were included in the sensitivity analyses. As a result, the sensitivity analyses primarily assessed the robustness of the observed age effect.

1.1 Methods

One-way ANOVAs, followed by independent t-tests, comparing SICI ratios (2ms interstimulus interval) between older adults with depression, older healthy adults and younger healthy adults were performed in the absence of potentially influential patient subgroups. In particular, analyses were performed including only (i) patients not taking antidepressants or benzodiazepines at the time of the study, (ii) treatment-resistant patients, i.e. with an ATHF (Antidepressant Treatment History Form) score ≥ 3, (iii) non-treatment-resistant patients, (iv) patients with late-onset depression (≥ 60 years of age), (v) patients with early-onset depression (< 60), and (vi) patients without a comorbid anxiety disorder. SICI ratios within subgroups were non-normally distributed, therefore SICI data was log-transformed to attain a normal distribution.

1.2 Results and Discussion

A total of 32 older adults with depression not taking antidepressants or benzodiazepines were included in the first subgroup analysis. The mean ± SD SICI ratio in older adults with depression not taking antidepressants or benzodiazepines was 0.6829 ± 0.5722. A significant main effect of group was observed for SICI ($F_{2,100} = 5.16$, $p = 0.007$). Post hoc analyses revealed significantly reduced cortical inhibition in antidepressant- and benzodiazepine-free older adults with depression compared to younger healthy controls ($t_{60} = 3.19$, $p = 0.002$).

A total of 69 treatment-resistant older adults with depression were included in the second subgroup analysis. In older patients with treatment-resistant depression, the mean ± SD SICI ratio was 0.6463 ± 0.4860. A significant main effect of group was observed for SICI ($F_{2,137} = 6.14$, $p = 0.003$). Cortical inhibition was significantly reduced in treatment-resistant older adults

with depression compared to younger healthy controls ($t_{97} = 3.60$, $p < 0.001$). Similarly, in the subgroup analysis of SICI in 23 non-treatment-resistant older adults with depression (SICI ratio = 0.6232 ± 0.4991), a significant main effect of group was observed ($F_{2,91} = 3.73$, $p = 0.028$), reflecting significantly reduced cortical inhibition in older adults with non-treatment-resistant depression compared to younger controls ($t_{51} = 2.34$, $p = 0.023$).

A total of 19 older adults with late-onset depression were included in the fourth subgroup analysis. The mean ± SD SICI ratio was 0.5773 ± 0.1973 in the subgroup of patients with late-onset depression. A significant main effect of group was observed for SICI ($F_{2,87} = 5.52$, $p = 0.006$). Cortical inhibition was significantly lower in older adults with late-onset depression than in younger controls ($t_{43} = 3.46$, $p < 0.001$). A significant main effect of group was also observed for SICI when older patients with early-onset depression were included (SICI ratio = 0.6615 ± 0.5352; $F_{2,141} = 4.94$, $p = 0.008$), and post hoc comparisons revealed significantly attenuated cortical inhibition in older patients with early-onset depression compared to younger healthy adults ($t_{101} = 3.12$, $p = 0.002$).

Lastly, 57 older adults with depression and without a comorbid anxiety disorder (SICI ratio = 0.666 ± 0.4832) were included in a subgroup analysis of SICI. A significant main effect of group was found ($F_{2,125} = 7.01$, $p = 0.001$). SICI was significantly lower in older depressed patients without a comorbid anxiety disorder diagnosis than in younger healthy controls ($t_{85} = 3.94$, $p < 0.001$).

1.3 Conclusion

For all subgroup sensitivity analyses, the main finding of impaired $GABA_A$ receptor-mediated inhibitory neurotransmission in older adults with depression compared to younger, healthy controls persisted. These results suggest that the findings reported in the full sample of older adults with depression are robust, and not dependent on these particular patient subgroups within the dataset.

2.0 Varying the Interstimulus Interval in SICI and ICF Paradigms

In a subset of study participants, SICI and ICF paradigms were performed with varying interstimulus intervals (ISIs) between the conditioning and test TMS pulses. In addition to the ISIs reported in the main text (2ms and 10ms), SICI was also performed with an ISI of 4ms

($SICI_{4ms}$), and ICF was performed with ISIs of 15ms (ICF_{15ms}) and 20ms (ICF_{20ms}). All three TMS measures were square-root transformed in order to attain a normal distribution. A two-way ANOVA with between-subject factors of age (≥60 and < 60 years) and diagnosis (depressed and not depressed) was then performed for each TMS measure.

For $SICI_{4ms}$, we found a significant main effect of diagnosis ($F_{1,108} = 4.83$, $p = 0.030$), and a non-significant main effect of age ($F_{1,108} = 3.78$, $p = 0.055$). The interaction between age and diagnosis was not significant ($F_{1,108} = 1.68$, $p = 0.20$). Planned pairwise comparisons revealed significantly lower $SICI_{4ms}$ in older adults with depression ($t_{62} = 3.07$, $p = 0.003$, $d_{Cohen} = 0.69$), older healthy adults ($t_{45} = 2.31$ p = 0.026, $d_{Cohen} = 0.69$) and younger adults with depression ($t_{53} = 2.43$, $p = 0.014$, $d_{Cohen} = 0.68$), as compared to younger healthy adults.

For ICF_{15ms}, we did not observe significant main effects of diagnosis ($F_{1,108} = 0.66$, $p = 0.42$) or age ($F_{1,108} = 0.52$, $p = 0.47$), and the diagnosis × age interaction was similarly non-significant ($F_{1,108} = 0.17$, $p = 0.69$). Main effects of diagnosis and age were also not significant for ICF_{20ms} ($F_{1,108} = 3.28$, $p = 0.073$ and $F_{1,108} = 3.62$, $p = 0.06$, respectively), and the diagnosis × age interaction was not significant ($F_{1,108} = 2.1$, $p = 0.15$)

Overall, the SICI and ICF findings were similar across ISIs. SICI was consistently reduced in older adults with depression, older healthy adults and younger adults with depression as compared to younger healthy controls when SICI was performed with ISIs of 2ms and 4ms. By contrast, ICF remained similar across groups with ISIs of 10ms, 15ms and 20ms.

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