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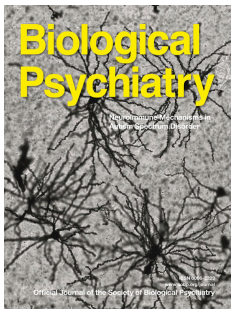

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Cortical paired associative stimulation influences response inhibition: cortico-cortical and cortico-subcortical networks

Short title: Paired associative stimulation and response inhibition

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Abstract

Background: The ability to stop a suboptimal response is integral to decision making and is commonly impaired across psychiatric disorders. Cortical paired associative stimulation (cPAS) is a form of transcranial magnetic stimulation in which paired pulses can induce plasticity at cortical synapses. Here we used cPAS protocols to target cortico-cortical and cortico-subcortical networks by using different intervals between the paired pulses in an attempt to modify response inhibition.

Methods: Twenty-five healthy volunteers underwent 4 cPAS sessions in random order 1 week apart: right inferior frontal cortex (IFC) stimulation preceding right pre-supplementary motor area (pre-SMA) stimulation by 10 or 4 milliseconds; pre-SMA stimulation preceding IFC stimulation by 10 or 4 milliseconds. Subjects were tested on the stop signal task along with the delay discounting task as control at baseline (randomized across sessions and cPAS protocol), and after each cPAS session.

Results: The stop signal reaction time showed a main effect of cPAS condition when controlling for age ($F(3,57)=4.05$, $p=0.01$). Younger subjects had greater impairments in response inhibition when the pre-SMA pulse preceded the IFC pulse by 10 msec. In older individuals, response inhibition improved when the IFC pulse preceded the pre-SMA pulse by 4 msec. There were no effects on delay discounting.

Conclusion: cPAS modified response inhibition through age-dependent long-term potentiation and depression-like plasticity mechanisms via putative cortico-cortical and cortico-subcortical networks. We show for the first time the

capacity for cPAS to modify a cognitive process highly relevant to psychiatric disorders.

Main text

Introduction

The ability to stop a suboptimal action –reaching for a cookie, walking into a bar, or perhaps inhibiting an intrusive thought – is integral to our decision making processes. Impairments in response inhibition, a form of impulsivity, cuts dimensionally across pathological disorders such as substance addiction, eating disorders, obsessive compulsive disorder and Parkinson's disease(1, 2). Here we use cortical paired associative stimulation (cPAS), a transcranial magnetic stimulation protocol often applied to the motor domain, to ask if cPAS can be applied to the cognitive domain to influence response inhibition.

PAS is a protocol in which repetitive low-frequency paired stimulation can induce changes in excitability by spike-timing dependent plasticity(3) (STDP)-like mechanisms. The original PAS protocol (paired peripheral-cortical stimulation) delivered an electrical stimulus to a peripheral nerve just prior to a magnetic stimulus to the contralateral primary motor cortex(3). The repeated pairing or associative process can increase or decrease corticospinal excitability depending on the relative timing of the interaction of stimuli in the cortex(4). These results are explained in terms of STDP from studies at the cellular level where synaptic potentiation (long term potentiation, LTP) occurs when an excitatory pre-synaptic action potential is repeatedly followed by a post-synaptic action potential, whereas synaptic depression (long term depression, LTD) occurs when the order of firing is reversed(5). Consistent with STDP features, plasticity induction by PAS tends to be rapid, reversible and persists beyond

stimulation. Furthermore, drugs interacting with NMDA receptors interfere with PAS-induced plasticity, further implying a LTP-like mechanism(3, 6).

Cortical PAS (cPAS) is a protocol in which repetitive low-frequency pairs of cortical stimuli (e.g. over parietal and primary motor cortices) can induce changes in cortical excitability. Most studies have focused on the motor domain, given the reliability of the motor evoked potential (MEP) as an outcome measure. Thus, studies have demonstrated the capacity for cPAS to influence MEP when the conditioning TMS pulse is applied in regions interconnected with M1, such as contralateral M1(7), ventral premotor cortex(8), supplementary motor area(9) and posterior parietal cortex(10). Cortico-subcortical interactions can also be modulated using PAS. A PAS protocol pairing M1 TMS stimulation with subthalamic nucleus (STN) deep brain stimulation enhanced MEPs at 3 and 25 milliseconds, presumed to be related to stimulation of hyperdirect and indirect pathways respectively(11). Outside the motor cortex, a cPAS protocol involving right inferior parietal cortex and dorsolateral prefrontal cortex (DLPFC) with a 10 millisecond interval modulated DLPFC high frequency oscillatory activity in a direction-specific manner, potentially reflecting LTP and LTD-like effects (12). Here we propose to evaluate whether cPAS could influence cognitive networks involved in reactive inhibition and modify a behavioural measure.

Reactive inhibition or stopping involves a frontostriatal network including the right inferior frontal cortex (IFC), pre-supplementary motor area (pre-SMA), caudate, and STN(2, 13-15). The STN is a nucleus within the indirect pathway

receiving significant cortical hyperdirect projections and plays a central role in inhibitory mechanisms. Several theories have been made regarding the precise roles and information flow between regions: the right IFC has been suggested to be relevant to attentional salience towards the stop signal(16) and the pre-SMA to STN output as critical to inhibition(2, 17), with effective connectivity suggesting that the right IFC precedes the pre-SMA temporally(18) (**Figure 1B)**. In the present experiments we used cPAS to change the effectiveness of the connections in this network and tested whether this had an effect on stopping behaviour.

Our design was based on the original M1 PAS model(19). In this, a TMS pulse to M1 activates interneurons in the cortex that receive inputs from the periphery and provide excitation to the corticospinal neurons that project to spinal cord. If peripheral inputs arrive just before the TMS pulse is given, then they excite the cortical interneurons and increase the probability that they will discharge in response to the TMS pulse. Repeated pairing of the pulses gradually increases the inter-neuronal response to TMS and the result is enhanced activity in the corticospinal output to spinal cord. In our present experiment we envisage preSMA as equivalent to M1 and the preSMA output to STN as equivalent to the corticospinal output of M1. Input to preSMA from right IFC is equivalent to the peripheral input to M1. Thus, if right IFC inputs are active prior to preSMA TMS, we expect the preSMA-STN connection to be strengthened. If the right IFC inputs arrive after preSMA TMS, then the connection to STN will be weakened.

In order to allow for an estimated cortico-cortical conduction time of about 8ms, we stimulated the right IFC 10 milliseconds before or after the pre-SMA **(Figure 1)**. In hypothesis 1 **(Figure 2A)**, stimulation of the right IFC 10 milliseconds before pre-SMA is presumed to facilitate the pre-SMA to STN connections whereas the opposite timing in hypothesis 2 **(Figure 2B)** should impair the pre-SMA to STN connection.

As compared to the polysynaptic fronto-striatal circuitry involving prefrontal-striatal-globus pallidus-STN pathway, hyperdirect monosynaptic glutamatergic connections from the pre-SMA and IFC to the STN have been suggested to provide a rapid signal from the cortex to STN relevant to fast reactive processes(20, 21). STN single unit recordings show that successful stops are associated with enhanced neuronal firing following the stop signal whereas unsuccessful stops or Go signals are unchanged from baseline neuronal firing(22, 23). The second element of this study, then, asked whether it might be possible to produce "subcortical" PAS at the level of the STN.

The logic of the design is that TMS of right IFC and preSMA will activate the hyperdirect pathway to STN. As with cortical PAS, we hypothesize that if right IFC input arrives first at STN, then it will increase the chance that preSMA inputs discharge STN neurons. Repeated pairing at this interval will therefore increase the strength of preSMA-STN connections. Reversing the order of the inputs would mean that preSMA inputs arrived first and therefore that PAS would strengthen right IFC-STN connections.

Although we do not know the relative conduction times from right IFC-STN and preSMA-STN, we assume, given their approximately equal distance, that they will also be equal. To allow for the uncertainty, we used timings of +4ms (right IFC prior to preSMA) (Hypothesis 3, **Figure 2C**) or -4ms (preSMA prior to right IFC) (Hypothesis 4, **Figure 2D**) (Figure 1) for our subcortical PAS. Note that given the brevity of the interval, any cortico-cortical effects would differ from those under hypotheses 1 and 2. Thus, assuming an approximate cortico-cortical conduction time of 8ms, stimulation of right IFC before preSMA would mean that cortical input to preSMA from right IFC would arrive after preSMA TMS, and thus would reduce the strength of preSMA-STN connectivity (as compared with the increase expected if the interval was 10ms).

As a control outcome measure, we also used a delayed discounting paradigm, a form of impulsivity in which subjects choose between immediate small rewards and larger delayed rewards. Delay discounting is associated with different cortical neural networks from inhibitory function including dorsolateral and ventromedial prefrontal cortices(24). We thus hypothesized effects of cPAS on response inhibition but not on delay discounting.

Methods and Materials

Subjects

Thirty healthy individuals were enrolled; 5 were excluded (2 fell asleep during some TMS sessions; 1 withdrew; 1 had regular cannabis use; 1 was missing both baseline and experimental condition data). Twenty-five individuals above 18 years (15 men; with no major neurological or psychiatric disorders and medication-free) were analyzed.

Study protocol

The study consisted of four experimental sessions of cPAS over the right IFC and the right preSMA **(Figure 1D)**. Prior to the PAS intervention, subjects were tested on one baseline stop signal task (SST) and Monetary Choice Questionnaire (MCQ) to test response inhibition and delay discounting respectively; the order of the single baseline test was randomized across the 4 sessions. Subjects underwent four stimulation conditions in a randomized order followed by post-PAS SST and MCQ testing (25). The post-PAS tests were within the 30 minutes in which PAS is presumed to be active(3). The sessions were at least seven days apart. On the first day subjects completed the Beck Depression Inventory and impulsive behavior scale (UPPS-P)(26) and were assessed with the Mini International Psychiatric Inventory.

Subjects were recruited via the University College London healthy volunteer pool. All participants provided written informed consent. Experimental procedures were approved by the University College London Ethics Committee.

TMS navigation

cPAS TMS was delivered by two Magstim 2002 machines and two 70mm figure-of-eight coils (The Magstim Company Ltd., United Kingdom). Coil 1 was positioned over the right IFC at a 20 degree angle to the coronal plane with the handle pointing anteriorly, coil 2 over the right preSMA, perpendicular to the midline (**Figure 1a**). The exact coil positions were guided by neuronavigation (Brainsight, Rogue Research Inc., Canada). Target sites (x,y,z in mm Montreal Neurological Institute (MNI) coordinates: right IFC: 48, 16, 16 and right preSMA: 10, 10, 60) (**Figure 1b**) were based on a meta-analysis on response inhibition and imaging(27). Pulse intensity was 120% of resting motor threshold (RMT) (minimum intensity producing low intensity responses (≥50 mV) of the first dorsal interosseous muscle of the left hand in five out of ten consecutive trials).

Each cPAS experimental session contained 100 pairs of stimuli at 0.2 Hz (8.3 min duration). The four experimental conditions differed in the interstimulus interval of the paired pulses: IFC stimulation precedes preSMA stimulation by 10ms (IFC+10) or by 4ms (IFC+4); preSMA stimulation precedes IFC stimulation by 4ms (preSMA+4) or by 10ms (preSMA+10) (**Figure 1c and 2**). The cortico-cortical conduction time was presumed to be 8ms based on facilitatory MEP excitation from other M1 ccPAS protocols: ventral premotor (8ms)(8), M1 (8-10ms)(7) and parietal (8ms most consistent; 2-6ms observed but more variable)(28-30). Other ccPAS protocols such as cerebellum to M1 implicate cortico-subcortical connections(31).

Stop signal reaction time task

Response inhibition was assessed with the SST (Cambridge Cognition, Cambridge, UK). The subject responded to an arrow (Go signal) pointing either right or left, by pressing one of two buttons with the right or left index finger. If an audio tone (Stop signal) was present, the subject had to withhold the response. The task uses a staircase model for the stop signal delay by changing the delay by 50 milliseconds depending on stop performance of the previous trial, thus allowing determination of a stop signal delay at an equal number of successful and unsuccessful stops. The primary outcome measure is the stop signal reaction time (SSRT) (=median reaction time on Go trials – stop signal delay)(32). A lower SSRT indicates less time is required to cancel a motor response, and thereby more efficient inhibition.

Monetary choice questionnaire

In the MCQ, subjects chose between a small immediate and larger delayed monetary outcome. The primary outcome parameter is the k value with higher values indicating greater delay discounting.

Data analysis

We first examined the SSRT and Go reaction times for normality of distribution (Shapiro-Wilkes test $p>0.05$), sphericity (Mauchly's test $p>0.05$) and outliers (>3 standard deviations from group mean).

To examine the main hypothesis of effects of the 4 stimulation conditions on response inhibition we used a repeated-measures ANOVA with the within-subject factor of experimental condition with age as a covariate. We examined

the variable of SSRT corrected for baseline (or, the difference between post- and pre-PAS SSRT). We show an interaction between experimental condition and age in keeping with previous observations of an effect of age on PAS plasticity effects(33-35) and on response inhibition(36-39). As the main effect was significant, we then assessed post-hoc comparisons between experimental conditions covaried for age.

Of the 25 subjects, 3 had missing data (each subject was missing a value from one condition: IFC+4, preSMA+4 or preSMA+10; or 3 missing values from 125). To deal with the missing data, we first conducted the main repeated measures ANOVA analysis with list-wise deletion, thus including 22 subjects. We then used multiple imputation using regression to provide estimates of the missing data (which draws from a distribution for the missing data over 5 imputations), then conducted the repeated measures ANOVA analysis over all 5 data sets along with pooling the data.

To further illustrate these findings and the effect of age, we then ran independent t-tests comparing the difference between post- and pre-PAS SSRT for different experimental conditions between low and high age groups (split by median age=25 years old). To ask which age group was driving the effect, we then ran paired t-tests comparing post-PAS SSRT versus pre-PAS SSRT separately for low and high age groups. For both analyses, we conducted line-wise deletion and multiple imputation analyses. We also assessed the relationship between age and the difference between post- and pre-PAS SSRT using Pearson's correlation coefficient.

We conducted a repeated measures ANOVA analyses for RMT. We also conducted repeated measures ANOVAs for order of experimental condition testing, proportion of successful stops, reaction time on Go trials and the K value of the MCQ comparing experimental conditions as within-subject factors with age as a covariate examining the differences between post- and pre-PAS values.

To understand the effects of baseline SSRT on post-PAS SSRT, we conducted repeated measures ANOVA with the within-subjects factor of experimental condition with baseline SSRT as a covariate. The significance level was set to 0.05. All statistical tests were performed using SPSS 25 (IBM, White Plains, NY, USA).

Results

Demographic information

The sample included 10 women and 15 men (mean age *M*=26.77, *SD*=5.54 (range 20-39 years); Beck depression inventory *M*=6.21, *SD*=5.43; UPPS-P scores for negative urgency, premeditation, perseverance, sensation seeking, and positive urgency *M*=2.91, *SD*=0.50, *M*=2.01, *SD*=0.42, *M*=1.87, *SD*=0.47, *M*=2.68, *SD*=0.25, and *M*=2.83, *SD*=0.45).

TMS settings

The mean RMT was M=43.42, *SD*=8.4 (p>0.05 across conditions); the mean stimulation conditioning intensity was *M*=52.56, *SD*=10.65.

cPAS effects on stop signal reaction time

The data was normally distributed without outliers and fulfilled sphericity assumptions. We first describe the SSRT analysis with listwise deletion of missing data (N=22) and then with multiple imputation to estimate the missing values (N=25). We show a main effect of experimental condition (F(3,57)=4.05, p=0.01) on SSRT **(Figure 3A)** and an interaction between age and experimental condition (*F*(3, 57)=3.51 *p*=0.02) (listwise deletion). These interactions with age are in keeping with the known effect of age on PAS plasticity (33, 34) and response inhibition (36, 37). As there was a main effect of condition, we then assessed post-hoc comparisons with the covariate of age: preSMA+10 had higher SSRT compared to IFC+10 (p=0.014), IFC+4 (p=0.001) and preSMA+4 (p=0.04); IFC+4 had lower SSRT compared to preSMA+4 (p=0.007) and IFC+10 (p=0.02) with no other differences between conditions (p>0.05).

We repeated the analysis with multiple imputation and show similarly a main (F(3,69)=3.51-5.46, p=0.02-0.002) and an interaction effect (F(3,69)=3.18-4.56, p=0.03-0.006) across all 5 iterations. The pooled means were IFC+10=3.07; IFC+4=2.15; preSMA+4=4.41; preSMA+10=17.71. We repeated the main posthoc analyses: preSMA+10 had a higher SSRT than IFC+10 (p=0.04-0.005) and IFC+4 had a lower SSRT than preSMA+4 (p=0.01-0.005) across all 5 iterations.

To further examine the difference in SSRT with respect to age, we then conducted a median split and compared the two groups (age in years *M*=22.43, *SD*=1.79 and *M*=31.83, *SD*=3.74). We show differences in stimulation effect for IFC+4 (linewise deletion: *t*=3.59, *p*=0.002; pooled: t=3.45, p=0.001) and preSMA+10 (linewise deletion: *t*=2.28, *p*=0.03; pooled: t=2.44, p=0.02) with young individuals showing impaired SSRT and older showing enhanced SSRT **(Figure 3B)**.

To ask which groups were driving the effect, we compared SSRT experimental condition versus baseline separately for the low and high age groups. In the young age group, the preSMA+10 showed significantly impaired SSRT versus baseline with multiple imputation (t=-2.20, p=0.03) with a trend observed with linewise deletion (t=2.02, p=0.07). In the older age group, the IFC+4 condition showed significantly improved SSRT versus baseline with both multiple imputation (t=2.55, p=0.01) and linewise deletion (t=2.55, p=0.03).

There were also significant correlations between age and cPAS effect in the IFC+4 and preSMA+10 conditions (IFC+10: R^2=0.092, p=0.142; IFC+4: R^2=0.392, p= 0.001; pre-SMA+4: R^2=0.054, p= 0.273; pre-SMA+10: R^2=0.258, p=0 .013 **(Figure 4).**

Baseline SSRT when tested as a covariate did not have a significant effect on SSRT (main effect: F(3,57)=0.25, p=0.86; interaction effect: F(3,57)=0.52, p=0.67).

cPAS effects on other stop signal task parameters

Proportion of inhibition was close to 50% **(Table 1)** and did not differ across conditions (main effect: (F(3,66)=0.60, p=0.62); interaction: F(3,66)=0.54, p=0.66)). The Go reaction time did not differ between stimulation conditions (main effect: F(3,66)=0.34, p=0.79; interaction: F(3,66)=0.37, p=0.74) **(Table 1)**.

Monetary choice questionnaire

There was no effects of stimulation condition on delay discounting measured using K-values in the MCQ (main effect: F(3,48)=1.40, p=0.25; interaction: F(3,48)=1.18, p=0.33) **(Table 1).**

Discussion

We investigated for the first time whether cPAS can modulate inhibitory cognitive networks and behavior, varying the timing of cPAS stimulation to probe specific cortico-cortical and cortico-subcortical connections involved in inhibition. We found a significant effect of cPAS condition on reactive inhibition (SSRT), but, crucially, the influence varies as a function of age. Specifically, younger individuals showed a greater impairment in inhibition following a cPAS protocol in which the pre-SMA pulse preceded the right IFC pulse by 10 msec **(Figure 2B)**. In contrast, older individuals showed improvements in inhibition when the right IFC pulse preceded the pre-SMA pulse by 4 msec **(Figure 2C)**. Critically we did not show any effect on delay discounting, an impulsivity measure that implicates different fronto-striatal networks from that targeted by our cPAS protocol(40).

The main effect of cPAS condition when covaried with age supported our general hypothesis. The interaction with age has been shown in other PAS protocols in the motor domain, which demonstrate substantial individual differences as a function of age(33, 34). We will discuss the putative mechanisms of cPAS-induced cortico-subcortical plasticity in the older subgroup, and the mechanisms of the cortico-cortical plasticity effects observed in the younger participants, before speculating as to our age-dependent effects. We emphasize that all of our conclusions related to the anatomical substrates of the observed behavioral phenomena are based on presumed anatomical locations derived from meta-analyses and not from contemporaneous measures in the subjects whose behavior was measured in this study. Thus, we must maintain caution regarding

these conclusions, pending a direct test of these substrates via contemporaneous behavioral and functional imaging measures.

Cortico-subcortical plasticity induced by cPAS

We hypothesized that stimulation of right IFC-STN 4ms before preSMA-STN would increase the strength of the preSMA-STN connection. The right IFC has been suggested to relate to attentional mechanisms(16), while the function of the pre-SMA is thought to relate to the stopping process(2, 17). Both functional MRI studies(41) and single-unit recording studies(22, 23) implicate the STN in inhibition. Functional connectivity of the IFC to STN has correlates with SSRT(42). However, the pre-SMA to STN connection seems critical for reactive inhibition(17); therefore, right IFC stimulation might prime or facilitate the pre-SMA to STN connection, improving reactive inhibition. This also follows the anatomical conformation of the two regions: the IFC is located more ventrally than the pre-SMA. Based on rules of ventral internal capsule tract outputs, ventral tracts will exit earlier than dorsal tracts, suggesting that stimulation of right IFC tracts to STN should precede that of pre-SMA tracts to STN(43). Note that with this timing there is the possibility of some cortico-cortical interaction. However, as explained in the Introduction if this occurred it would be the opposite of the subcortical interaction, and at +4ms would be expected to reduce the effectiveness of the preSMA input to STN. Given that reactive inhibition improves we conclude that the cortico-cortical effect is weaker than the subcortical effect at least in older subjects (see below), resulting in a stronger preSMA input to STN.

Here we presume an effect at the level of the STN, which has been particularly implicated in reactive inhibition. Equally, an effect could be observed at the level of the striatum (**Figure S1**); however, if inhibitory caudate output of both the indirect and direct pathways were enhanced, these effects might cancel and not necessarily change thalamocortical output.

Cortico-cortical plasticity induced by cPAS

We also hypothesized that cPAS might modulate cortico-cortical plasticity by improving response inhibition when right IFC stimulation preceded pre-SMA stimulation by 10msec. Our results did not support this hypothesis. However, our findings did support the hypothesis of impairment in response inhibition when the right IFC pulse occurred 10 msec after the pre-SMA pulse particularly in younger participants. Thus, stimulation of the IFC to pre-SMA input after stimulation of the pre-SMA appears to impair the primary post-synaptic pre-SMA to STN output (Hypothesis 2), suggestive of an LTD-like effect.

Our failure to find an improvement in response inhibition with IFC+10 is unexpected, but it may simply be that there is an asymmetry in the effectiveness of the cPAS protocol such that the inhibitory interactions are stronger than the facilitatory ones. A similar asymmetry was seen in the original M1 PAS in which the facilitatory effect was larger and more reliable than the inhibitory effect(3). We have not discussed plasticity at the IFC-preSMA connection, which is also theoretically plausible.

Age-dependence of effects

We observe a strong interaction effect of age with our effect of interest on cPAS condition. Most simply, this effect may relate to general age-related differences in response inhibition: younger participants, who show faster SSRTs, may show a ceiling effect, only receptive to potential impairments following intervention; the slower SSRTs of older participants might be receptive to possible improvements, but show a floor effect to potential impairments. We do not observe an effect of baseline inter-individual SSRT on the effects of cPAS condition, suggesting that this explanation of a baseline effect by itself is insufficient; however, an interaction between baseline and age remains plausible. SSRT declines with much older age(38) but in a large online study demonstrating a linear age-related impairment in SSRT, the impairment started between the ages of 18-29 and 30-39(39). Furthermore, young adults (20-42 years) as compared to adolescents (10-17 years) showed greater right IFC activation during successful inhibition(37), a finding that might explain why right IFC stimulation preceding pre-SMA might facilitate inhibition in our older participants.

An alternative explanation might lie in the relationship between neurodevelopmental processes and cPAS. Myelination of the prefrontal cortex and synaptic pruning of unnecessary prefrontal synaptic connections, both mechanisms underlying efficacy of communication, are understood to persist until the age of 25(44, 45). In the case of synaptic pruning, greater aberrant and excessive synaptic inputs or outputs in younger individuals might impair the precision of the signal from IFC to the same STN neurons targeted by the pre-SMA; thus, the potentiating effects of cPAS (LTP) might not occur to their full

extent, and the inhibitory effects (LTD) might appear more prominent as observed. The effects of myelination and synaptic pruning on conduction times might have an effect in younger individuals but is less likely to have a marked effect in the age range tested.

Age effects on cortical plasticity have been demonstrated(46). Practice-dependent plasticity(47) and modulation of task-specific activity in motor networks show age-related effects(48). Age effects have also been observed with PAS involving median nerve stimulation and MEP in those above 60 years and in post-menopausal women(33, 34) although may also be apparent in those above 31(35), suggesting impaired LTP processes with aging. Mechanisms postulated to underlie these age-related effects on plasticity include changes in neurochemistry, neurotrophic factors, and excitability of cortical inhibitory circuits(33-35).

Future directions and limitations

Future studies should delineate the neural mechanisms of cPAS and its relationship with age. Baseline effects could be further tested using an interval unlikely to have a PAS effect. Testing other intervals is also indicated.

Conclusions

We show for the first time that cPAS can influence a cognitive process outside of the motor domain. The effect appears specific to inhibitory networks on response inhibition with no effect on delay discounting, an impulsivity measure that implicates different networks, implying network target specificity. cPAS

appears to influence cortico-cortical and cortico-subcortical pathways possibly via LTD- and LTP-like processes, but is critically dependent on age. Nevertheless, given that response inhibition is impaired dimensionally across psychiatric disorders, this represents a novel means of non-invasive neuromodulation of an important cognitive process. In particular, the capacity to improve SSRT via priming pre-SMA to STN function in older individuals is potentially a critical therapeutic modality.

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Tables

Parameter	Baseline M (SD)	IFC+10 M (SD)	IFC+4 M (SD)	preSMA+4 M (SD)	preSMA+10 M (SD)
SST Go RT	347,27 (48,82)	341,79 (49,24)	340,68 (42,74)	342,35 (60,79)	333,5 (34,03)
SST successful stop (%)	46 (6.12)	48 (6.40)	48 (6.55)	47 (5.51)	47 (8.2)
MCQ	0.0139 (0.0147)	0.0138 (0.0171)	0.0135 (0.0133)	0.0164 (0.0235)	0.0149 (0.0146)

Table 1: Stop signal task and monetary choice scores at baseline and for each stimulation condition. Scores were reported as mean (M) and standard deviation (SD). Abbreviations: SST=Stop signal task; RT=reaction time; MCQ=K values of monetary choice questionnaire

Figure Legends

Figure 1: Study design

(A) Coil position and orientation over the right inferior frontal cortex (IFC) and the right presupplementary motor area (preSMA) (B) Illustration of target areas: right IFC (blue): x,y,z = 48, 16, 16 mm and right preSMA (red) x,y,z = 10, 10, 60 mm (Montreal Neurological Institute coordinates). The tracts displayed are from the right IFC and preSMA (green), preSMA and subthalamic nucleus (STN) (red) and right IFC and STN (blue). The bottom right image shows the STN (yellow) with tracts from the preSMA and right IFC. For tractography analysis, a standardized structural connectome derived from diffusion weighted imaging data of healthy subjects from the Human Connectome project was used as described elsewhere(49). (C) Pulse sequence with inter stimulus interval (ISI) of the different stimulation conditions. (D) Example illustration of protocol for one subject: Subjects were tested 4 times 7 days apart. The single baseline test (stop

signal task (SST) and Monetary Choice Questionnaire (MCQ)) occurred prior to the paired associative stimulation (PAS) with the order randomized across sessions (here shown in Session 2). Only a single baseline test was conducted to avoid excessive repeated testing of the same task. The order of the PAS protocols was randomized across sessions. Subjects were then tested on the SST and MCQ following PAS.

Figure 2: Illustration of hypotheses

The illustrations show the hypothesized effects of the paired associative stimulation conditions with the first stimulation pulse (green 1) preceding the second stimulation pulse (green 2) by either 10 milliseconds (presumed cortico-cortical effect) or 4 milliseconds (presumed cortico-subcortical effect). The red indicates the presumed post-synaptic output neuron target. (A) **Hypothesis 1**: Stimulation of the right inferior frontal cortex (IFC) 10 milliseconds before the pre-supplementary motor area (pre-SMA) presumes the IFC to pre-SMA input facilitates the pre-SMA to STN post-synaptic output activity (in bold red), thus improving response inhibition via a long-term potentiation-like effect. This effect was not demonstrated. (B) **Hypothesis 2**: Stimulation of the pre-SMA 10 milliseconds before the IFC presumes that stimulation of the IFC to pre-SMA input after pre-SMA stimulation impairs the pre-SMA to STN post-synaptic output activity (in dotted red), thus impairing response inhibition via a long-term depression-like effect. This effect was demonstrated in younger individuals. (C) **Hypothesis 3**: Stimulation of the IFC 4 milliseconds before the pre-SMA is presumed to be too brief for a cortico-cortical effect but presumes the IFC input to STN potentiates STN post-synaptic output by strengthening the pre-

SMA to STN input (in bold red), thus improving response inhibition. This hypothesis assumes input of the IFC to the STN precedes input from the pre-SMA. This effect was demonstrated in older individuals. (D) **Hypothesis 4:** Stimulation of the pre-SMA 4 milliseconds before the IFC presumes the opposite (in dotted red) of Hypothesis 3 along with an impairment of response inhibition. This effect was not demonstrated.

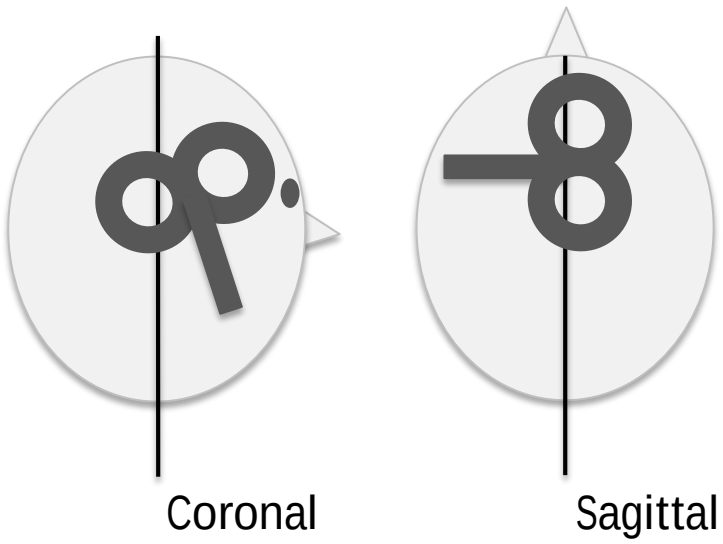
Figure 3: Effect of cortical paired associative stimulation on stop signal reaction time

(A) The graph represents the repeated measures ANOVA comparing the within-subjects factor of stop signal reaction time (SSRT) for each experimental condition – baseline SSRT (the difference between post- and pre-PAS SSRT) covaried with age (main effect: $p=0.01$). The data with linewise deletion of subjects with missing data is shown here. Post-hoc analyses covaried with age: $^{**}p<0.005$, $^{*}p<0.05$. (B) To further illustrate the effects of age, the graph represents SSRT experimental condition – baseline SSRT separated by the median age (25 years old) for each experimental condition. Independent t-tests on the median split: $^{**}p<0.005$, $^{*}p<0.05$. Comparing SSRT experimental condition versus baseline SSRT for each group showed the age group differences were driven by the improvement in SSRT in older individuals in IFC+4 and the worsening in SSRT in younger individuals in pre-SMA+10. Paired t-tests: § $p<0.05$).

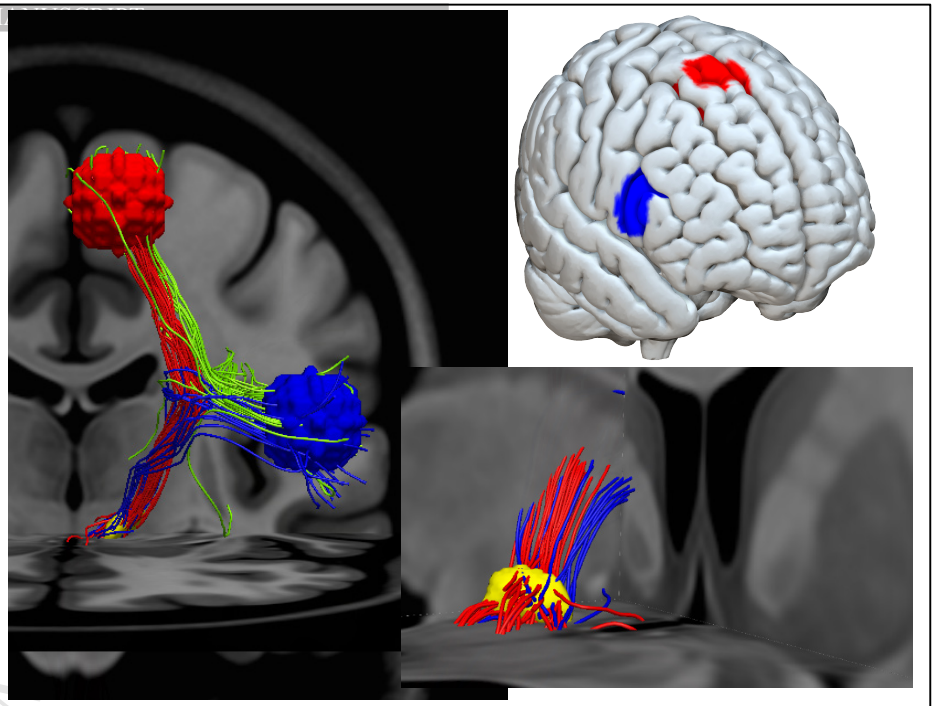
Figure 4: Correlation of age and cortico-cortical paired associative stimulation effect

The correlations show the stop signal reaction time (=SSRT experimental condition - baseline) as a function of age: (A)IFC+10 condition (B)IFC+4 condition (C)pre-SMA+4 condition (D)pre-SMA+10 condition.

A



B



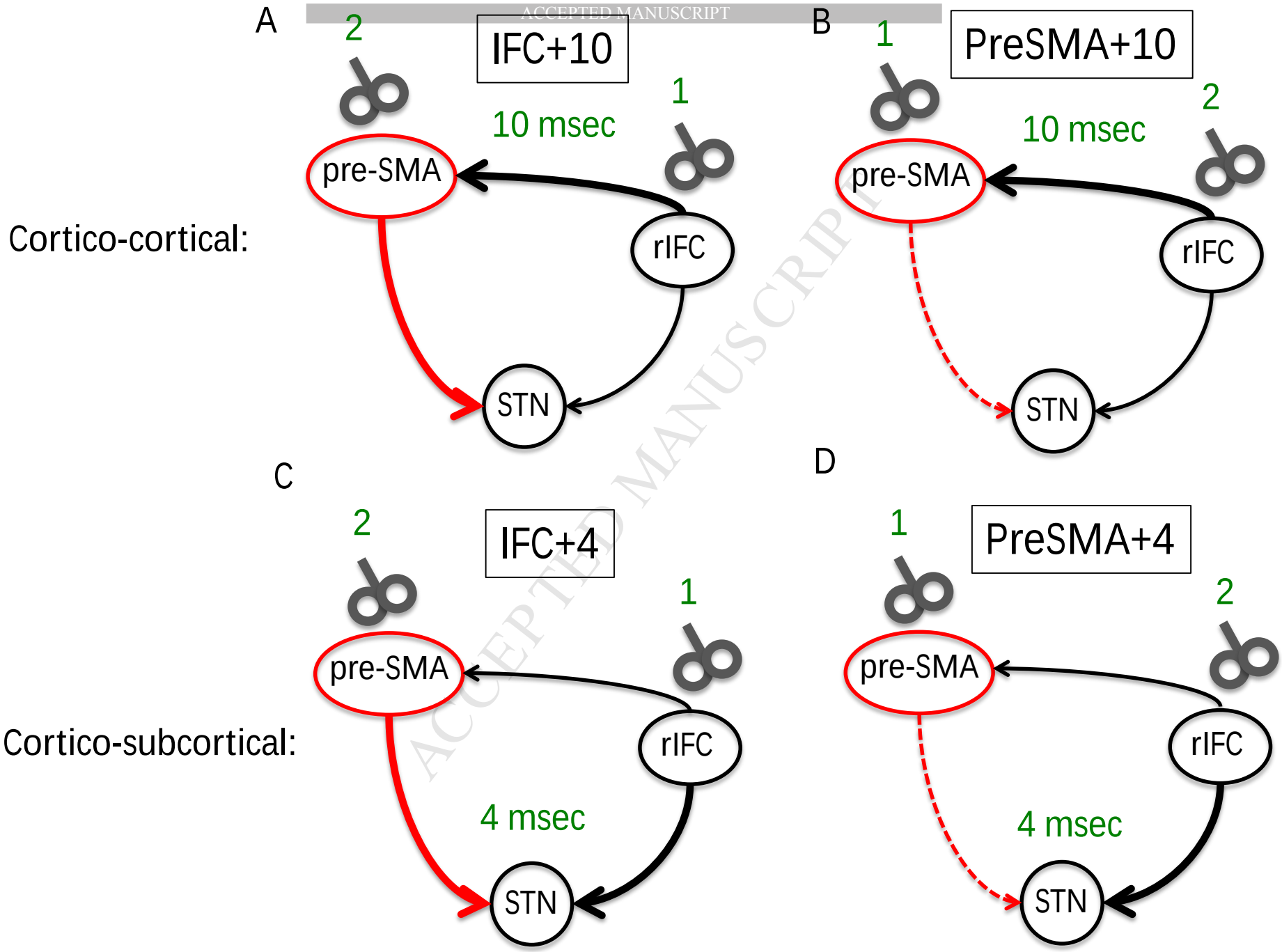
C

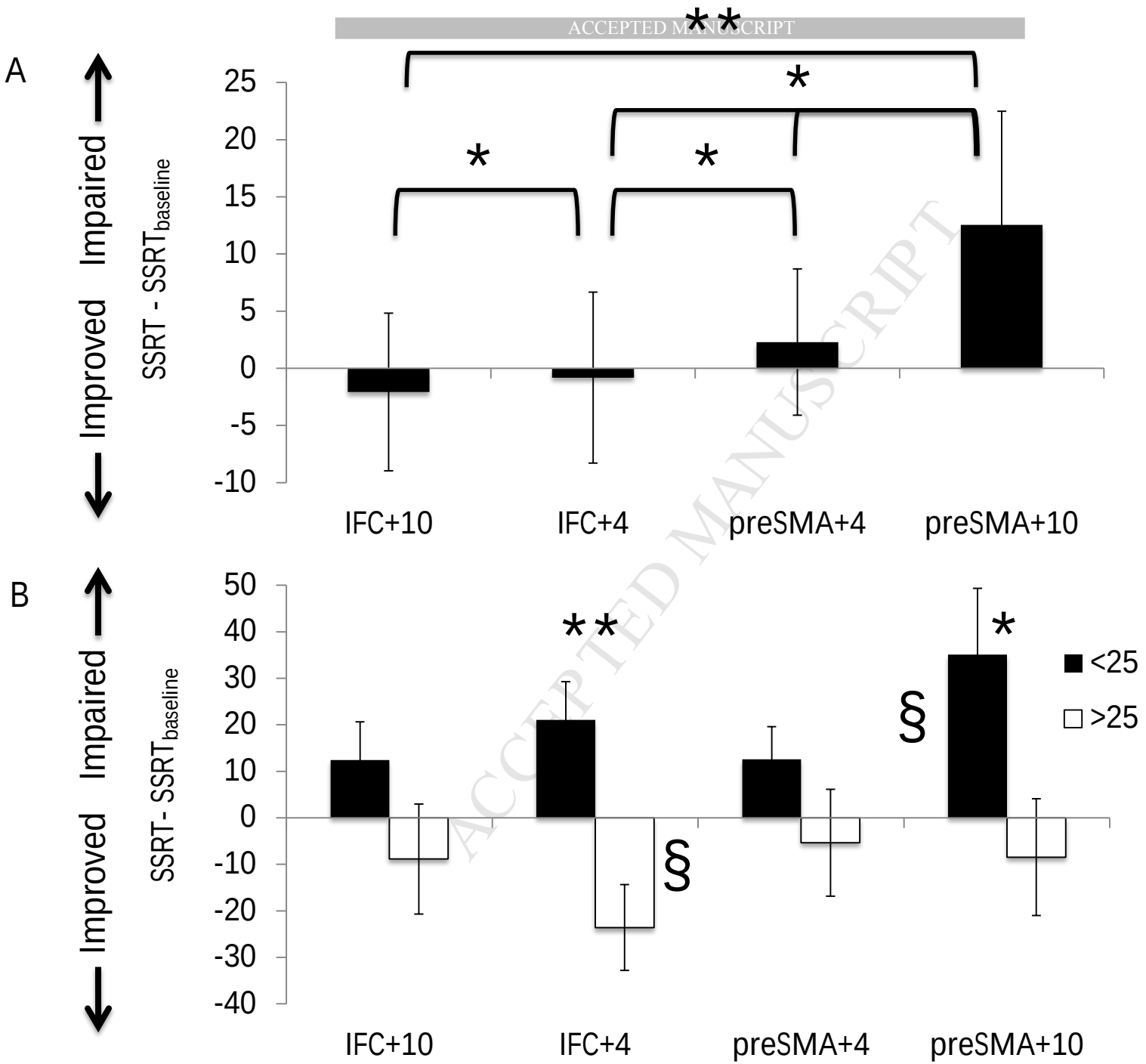
IFC+10	I--------10ms ISI--------I
IFC+4	I----4ms ISI----I
preSMA+4	I----4ms ISI----I
preSMA+10	I--------10ms ISI--------I

I = IFC pulse
I = preSMA pulse

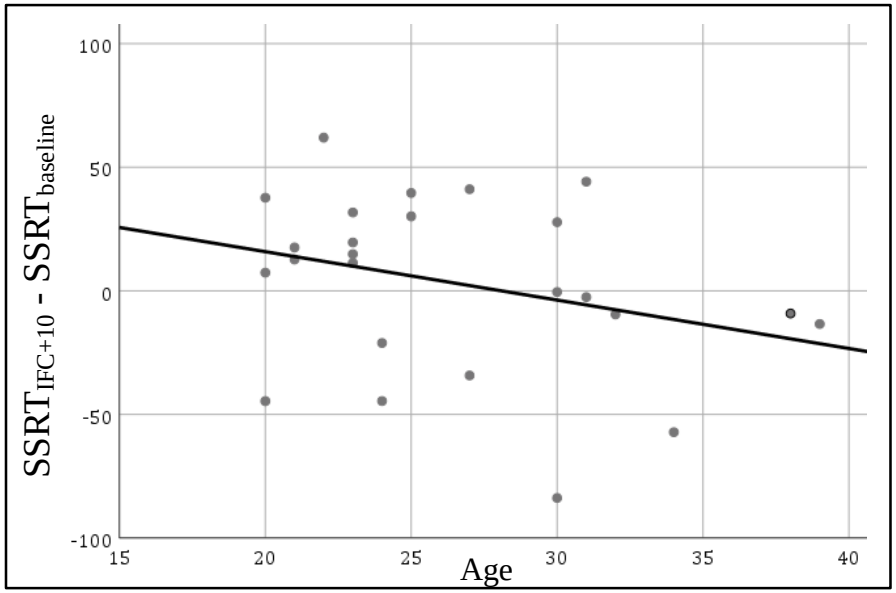
D

Session:	**Baseline test:**	**PAS:**	**Post-PAS test:**
Session 1		preSMA+4	SST, MCQ
Session 2	Baseline SST, MCQ	IFC+10	SST, MCQ
Session 3		preSMA+10	SST, MCQ
Session 4		IFC+4	SST, MCQ

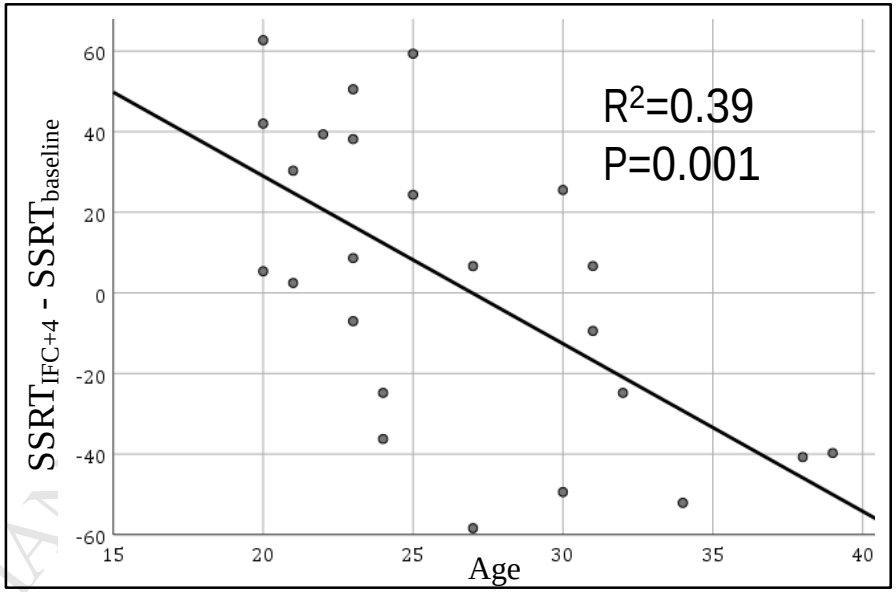




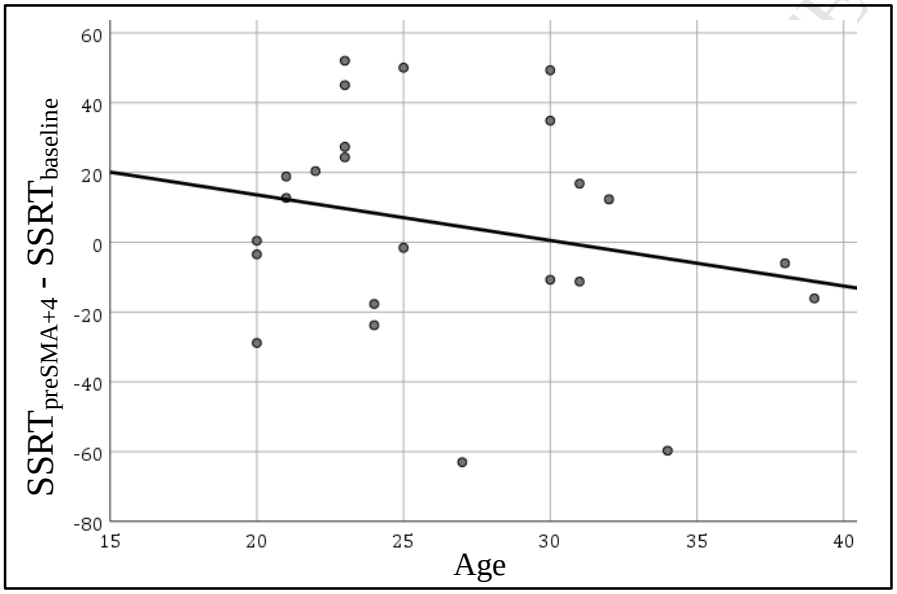
IFC+10



IFC+4



preSMA+4



preSMA+10