FINAL REPORT for the grant 150/2020: "A swing between the inner and the outer worlds: exploring the function of the frontal aslant tract with transcranial magnetic stimulation."

1. **BACKGROUND AND AIMS OF THE PROJECT:** The present work aimed at understanding the neural bases of the interactions between two main types of behavioural strategies: **internally-generated actions** and **externally-driven actions**. It is a common daily experience that we can interact with the world in two main modalities: either triggered/attracted by external cues or internal drives. The difference between the two modalities is substantial. In the case of externally-cued actions, the brain requires actual sensory evidence on a given target, and then uses it to plan a movement. In the second case, the brain establishes an internal goal that is translated into action, in the absence of external stimuli. The two systems (exogenous and endogenous action triggering) are well-known to be represented in different parts of the frontal lobes. The medial premotor cortex contains the machinery for internally-generated actions, while the lateral frontal cortex contains the machinery for externally-triggered, goal-directed actions (see (1,2) for data on non-human primates and (3) for a revision of human data on the topic). Behaviourally, the two processes are often mutually incompatible and the dynamic supporting the choice to commit to one of the two possible strategies (internal or external) is poorly understood. Recently a white matter bundle, the Frontal Aslant Tract (FAT) has been described to connect the medial frontal region to the lateral frontal region (4), therefore representing a potential anatomical substrate for the reciprocal cross-talk between the internal and the external actions systems. The aim of the present work is to investigate the potential role of the FAT in the interactions between internally guided and externally guided actions. IN the original project this general aim has been articulated in 3 sub-objectives that investigate the different hierarchical levels that are layered into motor behaviour, i.e. 1) action strategies (e.g. action initiation, choices and inhibition) 2) movements (upper limb goal-directed movements or orofacial speech-related movements) and 3) motor execution (kinematic parameters of velocity, acceleration, etc.). To do so we articulated the research in 3 separate packages regarding: a) actions, b) movements and c) kinematic parameters.

2. **FULFILLMENT OF THE ORIGINALLY PLANNED EXPERIMENTS.** We performed all 3 experiments that were planned in the original proposal, therefore the initial commitments are completely fulfilled. In addition, due to a serendipitous, completely unforseen finding (though of great scientific interest), we added an extra work package that has been fully acknowledged as funded by the grant in the publication.

Work package	planned	Experiment performed
Work Package 1: Investigating strategic choices in the FAT system (Experiment 1 of the present report)	YES	YES

Work package 2: investigating internal timing of internal actions in the FAT system (Experiment 2 of the present report)	**YES**	**YES**
Work package 3: investigating movement kinematics in the FAT system (Experiment 3 of the present report)	**YES**	**YES**
Work package 4: Re-definition of FAT anatomy (Experiment 1b of the present experiment)	NO	**YES**

[Note: all experiments were conducted following the Helsinki declaration principles on human experimentation. All experiments followed a protocol approved by the local ethical committee (protocol 2020_035). All participants were fully informed on the nature of the procedures and gave written consent].

3. EXPERIMENT 1: interaction between internally-driven and externally-driven action strategies is mediated by the FAT. In 19 healthy human volunteers, we applied online transcranial magnetic stimulation (TMS) to six spots along the medial and lateral terminations of the FAT, during the set period of a delayed reaction task. Such scenario can be solved using either predictive (internally driven and timed) or reactive (externally-driven) strategies. TMS increased the propensity toward reactive behaviour if applied to a specific portion of the IFG and increased predictive behaviour when applied to a specific SFG spot. The two active spots in the SFG and IFG were directly connected by a sub-bundle of FAT fibers as indicated by diffusion-weighted imaging (DWI) tractography. Since FAT connectivity identifies two distant cortical nodes with opposite functions, we propose that the FAT mediates mutually inhibitory interactions between SFG and IFG to implement a "winner takes all" decisional process. We hypothesize such role of the FAT to be domain-general, whenever competition occurs between internal predictive and external reactive behaviours. Finally, we also show that anatomic connectivity is a powerful factor to explain and predict the spatial distribution of brain stimulation effects.

3.1. Methods: We took advantage of a novel behavioural paradigms that allows us to test the propensity to internally-timed (predictive) way or a an externally-triggered (reactive) way. This task consists in a simple pre-cued reaction time (ready-set-go) with a set period of predictable duration as schematized in Figure 1.

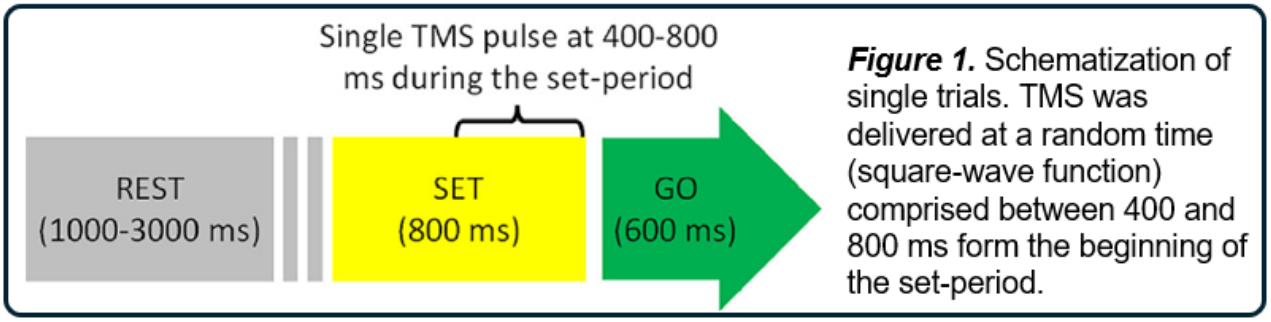

Figure 1. Schematization of single trials. TMS was delivered at a random time (square-wave function) comprised between 400 and 800 ms form the beginning of the set-period.

In this way, on a trial by-trial basis, participants can respond by predicting the GO-signal, and in this case the reaction time is very short (<170 ms) or by waiting for the GO-signal and responding to it and in this case the reaction time will be long (>170 ms). Each of these strategies are mutually exclusive and therefore a single trial, on the basis of reaction times can be classified either s reactive or predictive, as demonstrated by the distribution of response times shown in Figure 2.

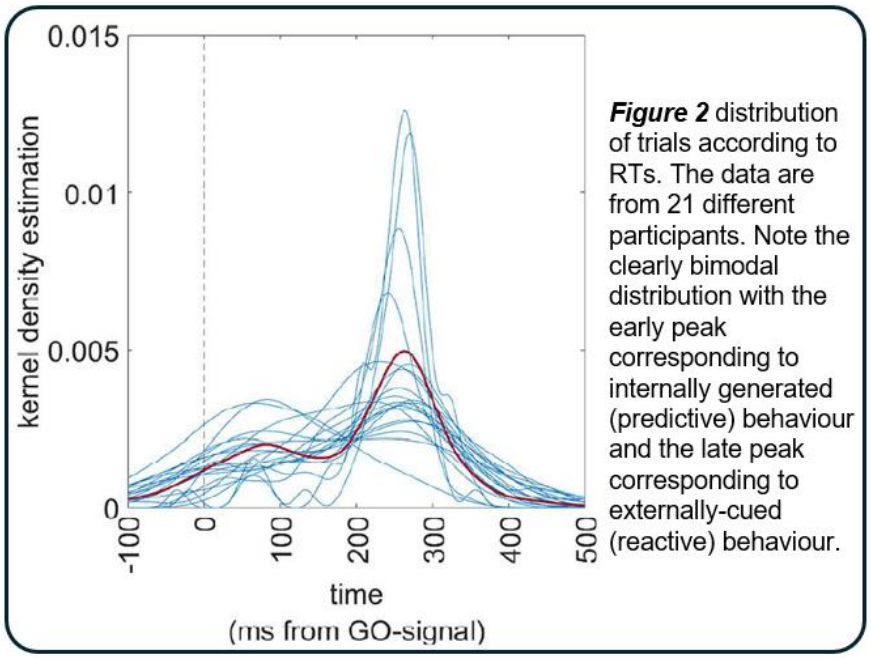

Figure 2 distribution of trials according to RTs. The data are from 21 different participants. Note the clearly bimodal distribution with the early peak corresponding to internally generated (predictive) behaviour and the late peak corresponding to externally-cued (reactive) behaviour.

TMS was applied in an event-related way, time locked to the SET period, but jittered over the 600-800 ms after the onset of the set-period, so that the TMS click could not be used as a reliable GO-signal. TMS was applied at 120% of resting motor threshold. Importantly, the localization of TMS was guided by individual tractography. We performed diffusion-weighted MRs in every participant, and dissected a tractographic image of the individual FAT. We then divided the FAT into 3 distinct parts, the posterior, the middle and the anterior one. In this way we defined 3 pairs of homologue IFG and SFG positions, that were reciprocally connected by each FAT sub-bundle. Figure 3 shows a representative subject. The 3 pairs of homologous cortical regions are referred to as P01-P02, P03-P04 and finally P05-P06. Odd-numbered spots are on the SFG and even-numbered spots are on the IFG. We applied TMS to each of the 6 cortical spots (P01-P06), in addition to a sham condition in which ineffective TMS was applied in a point equidistant to the active points. Data analysis consisted in determining the relative frequency of reactive trials on the total of actual trials. As can be observed in Figure 2, the behaviour of each individual is a mix of reactive and predictive trials, with considerable inter-individual variability in the proportion of reactive/predictive trials (note that trials can be classified as belonging to only one of the two strategies, as these are mutually-exclusive). Such “reactive index” is therefore distributed between 0 (only predictive behaviour) and 1 (only reactive behaviour) and was averaged within each of the 6 active TMS spots and the sham condition. The result was that each subject’s behavior was characterized by 7 mean values (P01-P06 + sham). We then conducted an ANOVA for repeated measures with 1 within-subjects factors (stimulation condition, 7 levels corresponding to the 6 active spots + sham). Planned comparisons were between sham and all the active spots.

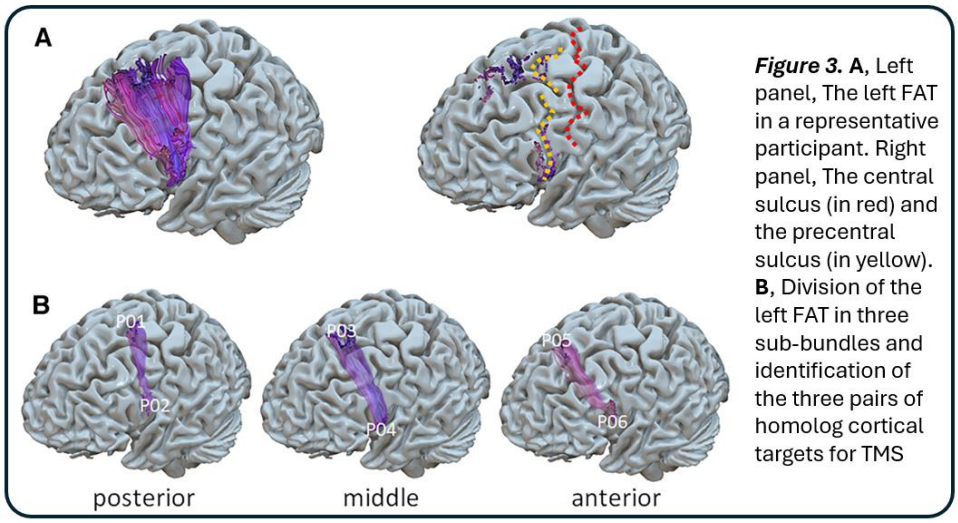

Figure 3. **A**, Left panel, The left FAT in a representative participant. Right panel, The central sulcus (in red) and the precentral sulcus (in yellow). **B**, Division of the left FAT in three sub-bundles and identification of the three pairs of homolog cortical targets for TMS

3.2. Results. The one-way ANOVA showed a main effect of the TMS factor [$F(6,108) = 5.61$, $p = 0.00{,}004$; partial $\eta^2 = 0.238$; observed power ($\alpha = 0.05$) = 0.996]. Planned comparisons between each of the six active TMS spots with the sham stimulation are summarized in Table 3 and showed that only the mean reactive index in the P03 and the P04 spots were different from the mean reactive index of Sham stimulation. In particular, (1) TMS over P03 induced significantly more predictive behaviour compared with Sham; and (2) TMS over P04 induced significantly more reactive behaviour compared with Sham. The results are illustrated in Figure 4.

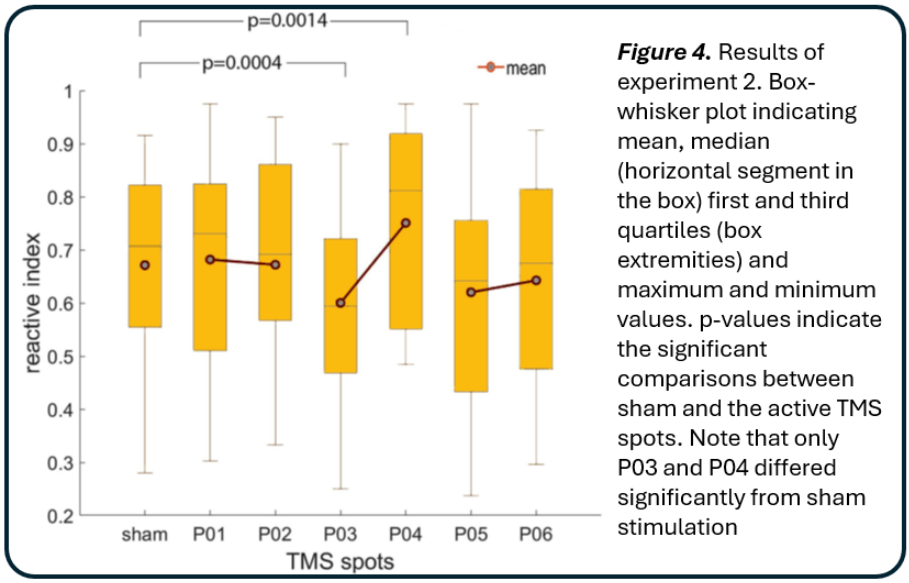

Figure 4. Results of experiment 2. Box-whisker plot indicating mean, median (horizontal segment in the box) first and third quartiles (box extremities) and maximum and minimum values. p-values indicate the significant comparisons between sham and the active TMS spots. Note that only P03 and P04 differed significantly from sham stimulation

3.3. Discussion. We confirm here our specific hypothesis was that the SFG and IFG have reciprocally opposite roles, with the SFG promoting internally-generated (predictive) behavior and the IFG promoting externally-cued (reactive) behaviour and that their interaction is mediated by the FAT. TMS delivered during the SET-period to a specific portion of the SFG, induced a bias toward predictive behaviour. The effective TMS spot was P03 (Figure 3). Stimulation of the IFG, specifically of the P04 spot, produced behavioural effects opposite to those over P03, i.e., an increase the propensity to perform reactive responses. We hypothesize that TMS induced a gain-of-function of the SFG, specifically in the capacity to serve as clock for internal timing of action. Similarly, we hypothesize that TMS induced transient facilitation of IFG, specifically in its capacity to code sensorimotor associations in rule-dependent behavior. We hypothesize that the propensity to choose one strategy over the other is mediated by a mechanism of reciprocal (mutual) inhibition, supporting a winner-takes-all decision process that is *supported by the direct*

connectivity by FAT fibers. The results also indicate that up to the actual time of movement, during the SET-period, both strategies are still available and present in parallel channels in the participant's motor system. If the commitment to one of the strategies was determined earlier on during neural processing, we would not be able to induce a strategy switch with TMS during the SET period.

4. EXPERIMENT 1B: a revision of the anatomy of the FAT in the superior frontal gyrus.

Repeated dissection of the FAT in the 19 experimental subjects + 4 pilot subjects allowed me to gain real world hands-on familiarity with the anatomy of the FAT. We observed that the claim that the FAT originates in the medial surface of the superior frontal lobe is actually incorrect, and that common observations in typical adult humans do not correspond to the textbook anatomy. The actual origin of the FAT is the convexity (crown) of the SFG and not the medial surface. This serendipitous finding was observed in the tractographic images of the 19 subjects that were scanned for Experiment 1. Though not present in the original project, we thought that this finding was highly pertinent to the project, because the anatomy of the FAT is the founding principle of the chain of hypotheses to be tested. We decided that given the importance of the observation we had to confirm it in an independent set of tractographies.

4.1. Methods. We therefore analysed data from 105 subjects obtained freely from the open access Human Connectome Project (HCP) database. We parcelled the cortex of the IFG, dorsal SFG and medial SFG in several regions of interest (ROIs) ordered in a caudal-rostral

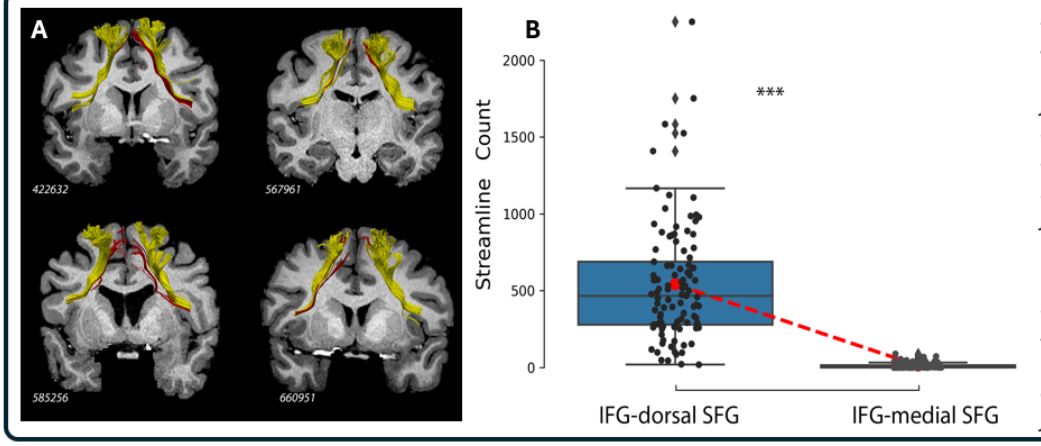

Figure 5: A. *Representative subjects. Fibers originating in the SFd are shown in yellow, fibers originating in the SFm are shown in red. The consistent predominant numerosity of SFG crown fibers (yellow) is clear at visual inspection.* ***B.*** *Quantitative comparison between the number of streamlines originating from the dorsal (convexity – crown) part of the SFG and from the medial part.*

direction, which served as seed locations for the generation of streamlines. Diffusion imaging data (DWI) was processed using a multi-shell multi-tissue CSD-based algorithm. We collected 150 preprocessed subjects' data from the HCP divided in a test (105 subjects) and a retest (45 subjects) datasets (5). We targeted the medial and dorsal walls of the SFG by ROIs images, as seed locations for the streamline generation. Additionally, we included ROIs over the inferior frontal gyrus (IFG) to restrict the streamline generation. We used Glasser's and JHU atlases to generate the ROIs images, which we preprocessed through a custom pipeline for registration and refinement into each subject's space. We processed the DWI data using a non-tensor deterministic multi-shell multi-tissue algorithm, and performed a quality check to remove false positives by Tractome

software. We generated various metrics data, including density maps, mean diffusivity, circular connectivity matrices, and fractional anisotropy (FA) tract profiles, along with the number of FAT streamlines generated starting from the dorsal wall of the SFG towards the IFG, and from the medial wall of the SFG towards the IFG.

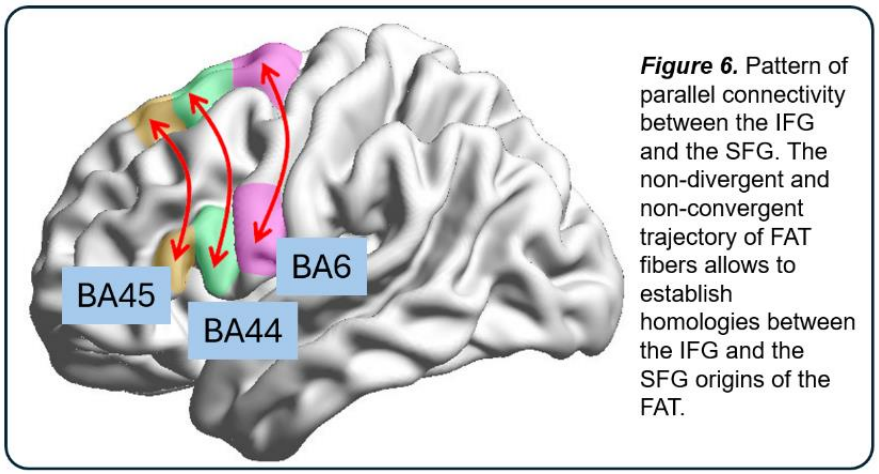

Figure 6. Pattern of parallel connectivity between the IFG and the SFG. The non-divergent and non-convergent trajectory of FAT fibers allows to establish homologies between the IFG and the SFG origins of the FAT.

4.2. Results. We showed that the number of streamlines originating from the dorsal wall of the SFG significantly exceeds those from the medial wall of the SFG (Figure 5). Connectivity patterns between ROIs indicated that FAT sub-bundles are segregated parallel circuits ordered in a caudal-rostral direction. Such high degree of coherence in the streamline trajectory allows to establish pairs of homologous cortical parcels in the SFG and IFG (as illustrated in Figure 6).

4.3. Conclusion. It is clear that the frontal aslant tract (FAT) originates in the dorsolateral portion of the SFG, in contrast to most of the previous scientific literature, in which the FAT endpoints are defined as coming from medial portions of the SFG. The significance of this distinction lies on an important function differentiation between the two systems mentioned above, as the convexity of the SFG marks the boundary between the “hot” system, related to temporal and spatial planning, and the “cold” system, related to executive control, potentially bearing both properties (3). This study contributes in solving uncertainties regarding the anatomical origins of the FAT offering crucial valuable perspectives about its functionality.

5. EXPERIMENT 2: Stimulation of the caudal origin of the frontal aslant tract (FAT) in the superior frontal gyrus impairs self-paced rhythmic movements independently from the effector.

Capitalizing on the results of Experiment 1 and, and coherently with thew the original general aims of the project, we investigated whether the cortical origins of the FAT posterior to the ones

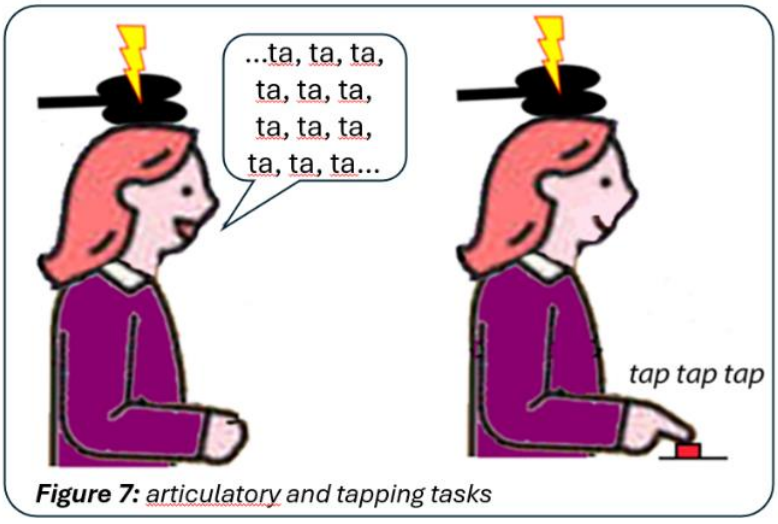

Figure 7: *articulatory and tapping tasks*

that mediated strategy switching in Experiment 1 could represent the actual execution of the motor plan. We have demonstrated in Experiments 1b and 1 that a) the Frontal Aslant Tract (FAT), a white matter bundle that connects the SFG and the IFG, is anatomically organized in relatively independent, parallel modules arranged in a caudal-cranial order (Figue 6) and b) that the **mid-portion of the FAT** is involved in **strategy selection**, by mediating competition between mutually-incompatible internally-timed and generated vs. externally-triggered behavior. According to our original hypothesis, the FAT has a caudal-cranial anatomical differentiation that reflects local specialization of function. We therefore aimed in Experiment 2, to demonstrate with focal TMS that the posterior portion of the dorsal origin of the FAT is involved in direct production of internally-generated movements. We investigated movements performed with different effectors (mouth and hand) to understand the level of abstraction of motor representations, in terms of effector-dependency. We used two well-established behavioural tasks that involve spontaneous production of rhythmic movements: a ***spontaneous* syllable production** task (syllable /ta/) and a **spontaneous finger tapping task**.***Methods*.** we asked 20 healthy human adult volunteers to perform in 2 separate sessions, two motor tasks involving A) self-paced repetitive speech (syllable) production or B) self-paced finger tapping (Figure 7). Before the experiment we acquired individual brain tractographies. We performed individual DW-MR and T1-weighted MRs in every participant. The DWI preprocessing was performed by TORTOISE toolkit, employing DIFFPREP and DRBUDDI for corrections of Gibbs ringing, thermal noise, motion, eddy current, and EPI distortions. We applied multi-shell, multi-tissue constrained spherical deconvolution (CSD) and estimated the response function using the Dhollander method to derive the WM fiber orientation distribution (fODF). Deterministic tractography was then computed based on CSD. Syllable or tapping tasks were performed in single trials lasting 4 seconds (subjects received the START and the STOP instruction but were encouraged to perform the task at a self-generated pace). Individual tractography-guided single pulse TMS (120% of resting motor threshold) was delivered randomly in the 1.5-3.5 seconds interval within the 4-seconds trial. TMS was aimed at 3 spots on the SFG convexity, on the cortex associated with the origin of the FAT, corresponding to the points P01, P03 and P05 of Experiment 1, that were renamed SFG1, SFG2 and SFG3 (See figure 9).

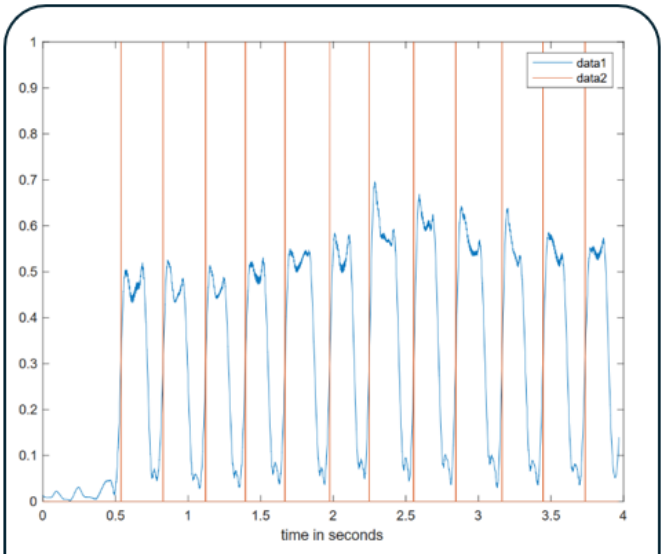

Figure 8: envelope of the rectified audio signal (blue) in the syllabel repetition task. The orange lines indicate automatic recognition and digitalization of syllable onset

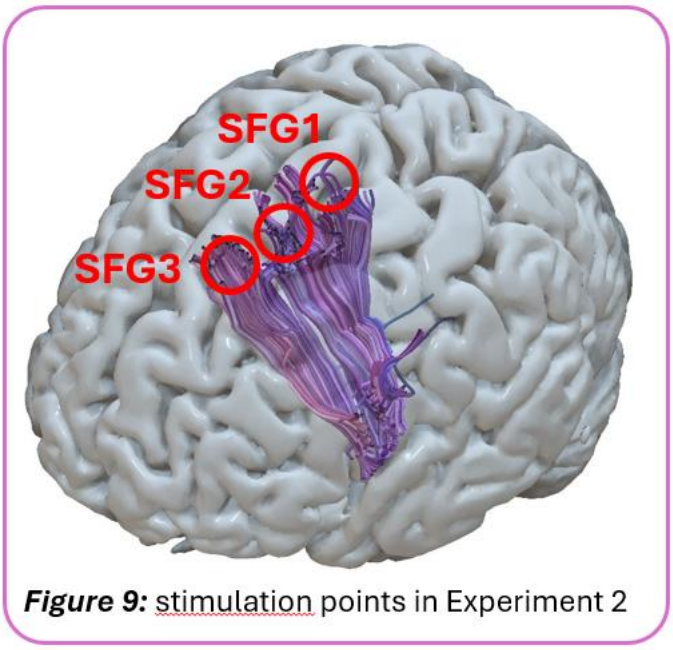

Figure 9: stimulation points in Experiment 2

Sham stimulation was delivered by tilting the coil by 90° and applying the stimulus to SFG2. Sixty trials per condition (2 tasks x 4 stimulation conditions) were recorded for a total of 480 trials per participant. Data analysis was based on a probabilistic account of the occurrence of motor events in the peri-TMS time interval. First of all, we digitalized the data from both tasks marking as events the syllable onsets (Figure 8) and the finger taps. We then aligned all events to the TMS pulse and divided the timeline in bins 200 ms wide, counting occurrences of motor events within 200 ms-wide bins. The raw count of events within each bin was baseline corrected by dividing it by the average value of the 5 bins pre-TMS (baseline period). The ratio was then log-transformed to fit the data to a normal distribution. We considered for analysis only 3 bins: the one before TMS, and the 2 after TMS. We analyzed the overall data by means of a 3-way ANOVA with the following factors: TASK (2 levels: SPEECH and TAPPING), TMS (4 levels: SFG1, SFG2, SFG3 and SHAM) and BIN (BIN-1, BIN1 and BIN2).

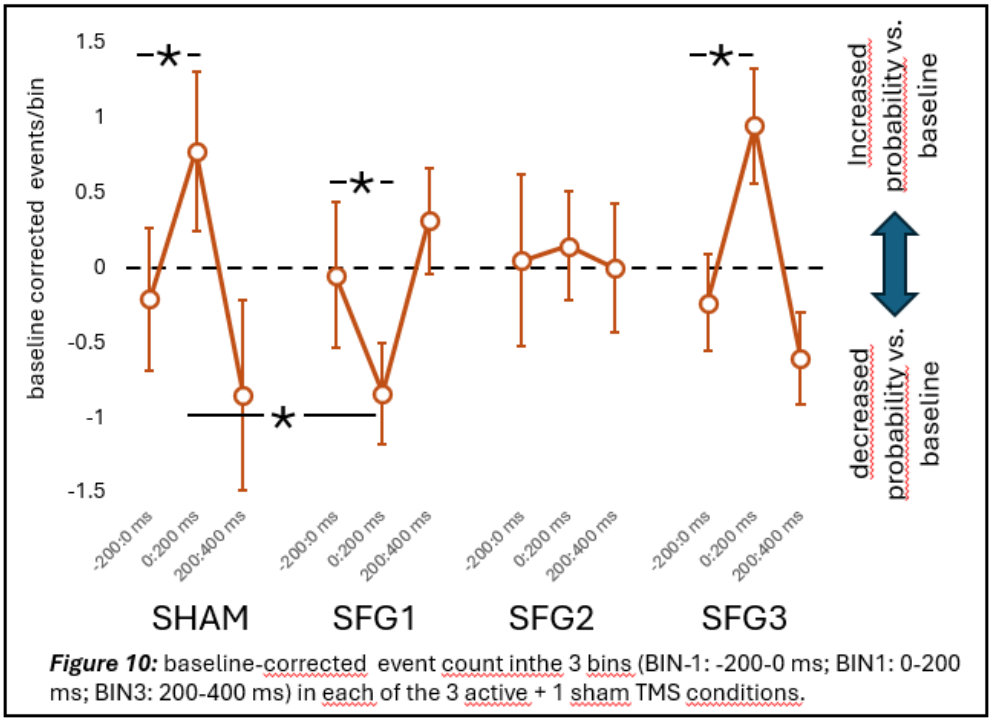

Figure 10: baseline-corrected event count inthe 3 bins (BIN-1: -200-0 ms; BIN1: 0-200 ms; BIN3: 200-400 ms) in each of the 3 active + 1 sham TMS conditions.

5.1. Results:

We observed a significant TMS*BIN interaction [F(6, 108)=4.05, p=0.001, eta-squared=0.18] illustrated in Fig. 10. Breakdown into 3 one-way ANOVAs (for each BIN) showed no main effect of TMS for bins -1 and 2 (p=0.1 and p=0.2 respectively), but a significant interaction of TMS*BIN (F(6, 120)=3.28, p=0.005) effect sizes were in the medium range (eta-square=0.14, observed alpha power=0.92). Post-hoc analyses indicated

that this was due entirely to the effects of stimulation over SFG1 that reduced the probability of the event in that specific BIN (i.e. in the 200 ms after TMS). The results indicate that TMS over a specific caudal portion of the SFG convexity (immediately rostral to the precentral sulcus) produced an increase in probability of a motor event, compared to sham stimulation and to active TMS of the 2 more rostral portions of the SFG. The pattern of behavioral effects of TMS was observed for both tapping and speech tasks without significant differences between them.

5.2. Discussion: We show that the cortex at the dorsal origin of the FAT is associated with rhythmic, self-paced behavior. Such role is commonly attributed to the neighboring supplementary motor area (SMA), however, on the present work we show that also the convexity of the SFG is necessary for internally generated actions. We also show that neural processes in the SFG convexity controlling self-paced rhythms are domain-general, being effector-independent.

6. EXPERIMENT 3. We investigated whether FAT-associated cortex controls low level motor features such as kinematic parameters of movement. We used a QualiSys optical motion capture system to measure the kinematic parameters of different types of movement, belonging to the internally generated or to the externally-triggered domains in ecological settings. We performed a first set of kinematic recordings in which subjects were asked to point towards given spatial targets or to grasp a set of objects. with different geometry. We tried several sets of motor tasks, looking for an appropriate temporal frequency of motor events that allows for the task to be spontaneous and ecological but at the same time to occur frequently enough to be affected by TMS: The final version of the motor tasks, which includes integrated spatially-oriented behavior and object-oriented behavior consists in continuous reaching towards specific portions of space, grasping small objects (Lego bricks) and assembling them (for around 20 minutes). The task can be accomplished with a spontaneous pace or with an external auditory cue that signals the start of the movements and therefore contains both an internally-driven component and an externally driven component. TMS, was delivered over the posterior and middle origins of the FAT (spots P01-P02 and P03-P04 in Figure 3) guided by individual tractography + sham stimulation. All tractography, neuronavigation and TMS/sham parameters were the same as in Experiments 1 and 2. Single-pulse TMS was delivered every 7+/-2 seconds. We examined the behavior of 20 subjects. Data processing was performed so that canonical kinematic parameters (movement time, velocity, acceleration and jerk of the fingers and wrist, maximal grip aperture) and time-frequency analyses of the movement's power spectrum are extracted. Each of the kinematic parameters were analyzed by means of an ANOVA with 2 within-subjects factors: TMS (5 levels: P01, P02, P03, P04 and Sham) and TASK (2 levels). The results failed to show any significant TASK*TMS interactions ($p>0.4$) on all ANOVAs performed on the dependent variables: a) peak wrist velocity during reaching, b) peak wrist acceleration, c) maximum grip aperture and b) peak velocity of grip aperture. Neither main effect was found to be significant ($p>0.2$ in all ANOVAs). Overall, despite

extensive investigation, the study of kinematic parameters failed to highlight significant kinematic markers of internal vs. external actions nor did we observe significant evidence that low level motor features are represented in the FAT circuitry.

7. GENERAL DISCUSSION AND CONCLUSIONS.

The present data show a complex role of the FAT Iin mediating the interaction between externally-triggered and internally-generated actions. Specifically we found a hierarchical and modular organization of the FAT (Figure 11 C) that is differentiated along two orthogonal dimensions: medial-lateral (figure 11 A) corresponding to an internal-external trigger of actions and caudal-cranial (figure 11B), corresponding to an action-movement complexity gradient.

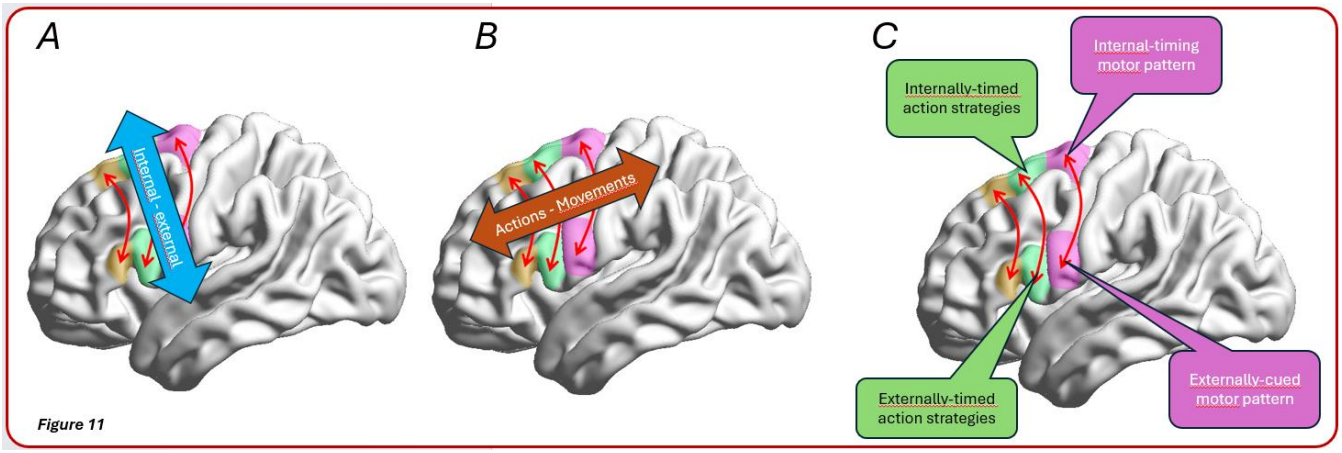

Figure 11

The findings therefore are fully compatible with the initial general hypothesis of the study. Additional information that we gained is that FAT circuitry seems to process effector-independent information. The middle portion elaborates action strategies and the posterior portion provides a general-purpose machinery for timing of movements. Apparently these functions may be borrowed by different action plans that use different effectors. Experiment 1 clearly supported our original claim that portions of the medial and lateral systems that are directly connected by the FAT support similar functions. In addition, we show here for the first time that individual information on anatomical connectivity (tractography) coupled with TMS can significantly increase the signal to noise ratio in spatial mapping of the cerebral cortex and provides a whole new way to interpret the functional mapping of the brain by non-invasive stimulation techniques.