Title of the study (one request per article):
Functional brain networks underlying self-regulation pre-puberty

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Wave (more options are possible):

- ☐ Rondom zw – 20 weeks
- ☐ Rondom zw – 30 weeks
- ☐ Rondom 0 – 5 mo
- ☐ Rondom 0 – 10 mo
- ☐ Rondom 3 (not available yet)
- ☐ Rondom 6 (not available yet)
- ☒ Rondom 9
- ☐ Rondom 12 (not available yet)
- ☐ Rondom 15 (not available yet)

We ask you to provide us with a clear background, methods section and data-analysis plan. These parts of the proposal will be publicly displayed for reference.

Background of the project (max. 500 words): Please provide a short background including the rational of your study as you would do in an introduction of the paper

In order to function adequately in everyday life, being able to exert control over your emotions, behaviour, and impulses is crucially important. This ability is commonly referred to as self-regulation. Self-regulation has been defined as the ability to monitor and modulate emotions, behaviour, and cognition, that in turn allows us to achieve goals and adapt to changing circumstances[1]. Self-regulation develops from early infancy until well into adulthood.

Self-regulation can be studied across development in terms of executive functions (Vink et al., in prep). Low-level executive functions such as inhibition develop in infancy and preschool years[3]. During middle childhood, children develop high-level executive functions, such as planning, problem solving, information processing and cognitive flexibility[2]. These high-level executive functions are funded on the integration of low-level functions. Then, during adolescence, the various executive functions are beginning to become integrated to support high-level *executive control* (also called cognitive control[4]). Executive control refers to the coordination of previously acquired low- and high-level executive functions such as working memory, inhibition, mental shifting, and information processing, which are then called upon as needed[5,6].

In the case of inhibition, it has been shown that while children at the end of childhood can inhibit prepotent responses, a low-level executive function, they become much more skilled in their inhibitory control during childhood and adolescence[7]. This improvement is associated with the rise of proactive response strategies that allow for more efficient processing by engaging inhibitory functions prior to the actual appearance of a stop-signal[8]. The true progress across childhood and adolescence is not better executive functions in itself, but rather a much more effective use of these functions due to their integration with other high-level executive functions such as planning. As such, the development of self-regulation is supported by the development of low-level executive functions early in life, and their subsequent integration later on.

This integration of executive functions, which allows for proactive inhibitory control, has been theorized to depend upon the establishment of frontal control over the rest of the brain, in particular subcortical regions[7,9]. Indeed, we have shown that the shift from low-level reactive to more higher-level proactive inhibition strategies was statistically linked to increased frontal activation as well as increased functional connectivity between frontal and subcortical regions[7].

However, we included predominantly adolescents and young adults in our prior study, so that we do not know how individual differences in the state of executive function development and brain maturation at the end of childhood are linked to levels of self-regulation. It may very well be that children who show higher levels of self-regulation are better able to engage relevant brain regions. This may be coupled with higher functional connectivity between regions that will begin to form brain networks. Consequently, measurements at this period in development may provide predictors of progress in adolescence and possibly outcome in adulthood. Given the impact of hormones during this time of development, such progress may prove to be different for boys and girls.

To address these questions, we will use functional MRI data acquired during resting-state and while subjects perform the Stop-Signal Anticipation Task (SSAT) (https://www.uu.nl/sites/default/files/inhibition_task_for_functional_mri.pdf). The SSAT will provide us with identification of the regions associated with inhibitory control, and a measure of relative activation in those regions[11-14]. Resting state fMRI allows us to provide an account, unaffected by task performance, of connectivity between these regions[15-18]. Finally, we will assess self-regulation by means of scores on the TMCQ.

Research question

How does self-regulation pre-puberty relate to brain activity and connectivity? We will investigate pre-pubescent individual differences in the state of the networks underlying inhibitory control and examine how they relate to measures of self-regulation. Finally, we examine how this relation is affected sex, age and/or hormonal development.

The usage of both task-related and resting state fMRI data serves as a cross-validation of the state of the fronto-striatal network. Task fMRI allows us to identify brain regions associated with inhibitory control, a key executive function underlying self-regulation. Resting state fMRI allows us to subsequently investigate connectivity between these areas unbiased by performance on a task (in children performance can vary and introduce noise).

Hypotheses

The central hypothesis is that children who show high levels of self-regulation will already show high levels of proactive inhibitory control. In the brain, this is paralleled by increased activation during proactive inhibition trials and increased connectivity during resting-state.

We furthermore expect to see an increase in proactive inhibitory control with age, both in terms of performance as well as in activation and connectivity. This effect of age likely is different for males and females. Adolescent males exhibit higher levels of sensation seeking and lower levels of impulse control than females, and those differences even appear during pre-adolescence[19]. Longitudinal studies have shown sex differences in the trajectory of brain development, with females reaching peak values of brain volumes earlier than males[19]. We therefore expect females to exhibit both better proactive and reactive inhibition performance (and associated brain measurements, outlined below) than males. Stages on the Tanner scale have been linked to hormonal development [20], and can subsequently be used as an indirect measurement of pubertal development in an additional analysis.

Specific hypotheses

Reactive inhibition

1. We expect reactive inhibition performance to increase with age, expressed as:

   a. Inverse correlation of stop-signal response time and age.
   b. Correlation of motor-cortex deactivation during reactive inhibition on the task and age.
2. As females are shown to reach peak brain development earlier than males, we expect girls to demonstrate better reactive inhibition than boys at an earlier age.
3. We expect that hormonal development, as indicated by Tanner stage, will also be linked to increases in reactive inhibition performance.

Proactive inhibition

1. We expect, on average, that subjects slow down their responses on go trials proportional to the level of stop-signal probability.
   a. We expect this effect to be predicted by age, with older children showing more slowing down than younger ones.
   b. As females are shown to reach peak brain development earlier than males, we expect girls to demonstrate more slowing down than boys.
   c. We expect that hormonal development, as indicated by Tanner stage, will also be linked to increases in slowing down.
   d. Children who show high levels of self-regulation will show higher levels of proactive inhibition as compared to children who show low levels of self-regulation.

2. We expect, on average, activation in brain areas related to proactive inhibition (rIFG, rIPC, striatum).
   a. We expect activation in these regions during proactive inhibition to be predicted by age, with older children showing more activation than younger ones.
   b. As females are shown to reach peak brain development earlier than males, we expect girls to demonstrate more activation than boys.
   c. We expect that hormonal development, as indicated by Tanner stage, will also be linked to increases in activation in these regions.
   d. Children who show high levels of self-regulation will show higher levels of activation as compared to children who show low levels of self-regulation.

3. We expect, on average, resting-stage connectivity between the striatum and rIFG.
   a. We expect this resting-state connectivity to be predicted by age, with older children showing more connectivity than younger ones.

b. As females are shown to reach peak brain development earlier than males, we expect girls to show more resting-state connectivity than boys.
c. We expect that hormonal development, as indicated by Tanner stage, will also be linked to increases in resting-state connectivity.
d. Children who show high levels of self-regulation will show higher levels of resting-state connectivity as compared to children who show low levels of self-regulation.

Methods Describe the methods as in the paper in which the data will be presented, according to the categories below, with a total **maximum** of 1500 words. For a description of task, methods etc. refer to the website, if possible.

Design of the study (for instance cross-sectional, longitudinal etc.; substantiate your choices)

Cross-sectional (longitudinal not yet available)

Study population and sample-size (entire population or a subset; substantiate your choices e.g. Provide a rationale for the requested sample-size, for instance using a power calculation)

Datasets from YOUth participants from the Rondom9 wave where participants have completed the anatomy scan, the inhibition fMRI experiment, the resting-state experiment, and the TMCQ questionnaire. We request the available data from all children (collected between March 14th, 2016 and April 30th, 2019, N>1000).

fMRI inhibition sets, with each set consisting of:

- fMRI inhibition scan + task data
- fMRI resting-state scan
- Anatomical scan (Required for fMRI co-registration purposes, not for a separate analysis)

For these subjects we also request:

- Age
- Sex
- Tanner scale
- TMCQ

Data processing and preparation (including necessary recoding of data etc.)

Image data for the task-related fMRI will be analyzed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm). In brief, preprocessing will involve slice timing correction, realignment correcting for motion, spatial normalization to the Montreal Neurological Institute template brain, and smoothing (8mm FWHM) to correct for inter-individual anatomical differences. Functional data will be co-registered to the anatomical image (T1-weighted), and this image will not be used for any additional analyses.

Behavioral data will be processed using MATLAB (statistics toolbox), and averages will be calculated per task condition. This will yield mean response times for go trials with three levels of stop-signal probability (zero, low and high probability). The duration between stop-cue and target at which the participant is able to attain a 50% accuracy is known as the Stop-Signal Response Time (SSRT), and will be used as a measurement of inhibition performance.

Resting-state data will be analyzed using the Resting-State fMRI Data Analysis Toolkit in combination with the Data Processing Assistant for Resting-State fMRI [21]. Scans are detrended to correct for general signal drift and band-pass filtered (0.08-0.1 Hz) to reduce low-frequency drifts and high-frequency noise.

Handling missing data (describe how you will detect and handle missingness in the data)

We will only use complete datasets. In other words, we will use data from subjects who have task data, task fMRI data, a resting state scan and an anatomical image. The reason for this is that if one part of a set is missing, the data cannot be analyzed. Imputation is not possible. Significant artefacts that yield one of the scans unusable will also lead to the exclusion of the subject.

Data analysis methods (including statistical design and statistical analysis plan. If it is not possible to provide a detailed statistical plan, as this does not fit in with the research questions formulated above, please explain.)

The fMRI data are modeled voxel-wise using a general linear model, in which the following events are included as regressors: successful stop trials, failed stop trials, and go trials with stop-signal probability >0%. Rest blocks are also modeled so that go trials with a 0% stop-signal probability served as baseline. For go trials with a stop-signal probability>0%, we also included two parametric regressors modeling response time and stop-signal probability level. The response time regressor will be included to control for variation in response speed independent from stop-signal probability effects. The realignment parameters are included to account for residual effects of head motion during scanning. A high-pass filter will be included to correct for low-frequency

drifts. For each participant, we will compute four contrast images: (1) activation in the motor cortex during go trials with a 0% stop-signal probability (to assess basic response execution), (2) activation during successful stop trials versus failed stop trials (to assess reactive inhibition), (3) activation during successful stop trials versus go trials in the 0% stop-signal probability context (also to assess reactive inhibition), and (4) the parametric effect of stop-signal probability on go-signal activation (to assess proactive inhibition). We will compute two contrasts for reactive inhibition because there is no consensus on which contrast is most appropriate for investigating reactive inhibition, and the contrasts may provide complementary information.

Intrinsic functional connectivity: resting-state

The striatum will be used as a seed, and the mean time series of all voxels within are extracted. Next, correlations maps are generated for each individual by correlating the mean time series data of the striatum with the time series data from each voxel in the brain. All correlations are normalized using Fishers z-transform. This will generate a map with values indicating the degree to which the temporal activation pattern per voxels is correlated with the seed region, and allows us to investigate the specific correlation between the striatal seed and regions in the frontal cortex.

Outcome variables

Behavioral outcome measures are stop-signal response time (SSRT) and response speed relative to stop-signal probability level. For imaging data, mean activation level, expressed as percentage of signal change, will be calculated per participant for each region of interest mentioned above. This will be done for task-related activation and intrinsic connectivity using resting-state. The TMCQ will provide us with a measure of the level of self-regulation.

Hypothesis testing

Reactive inhibition

a. A regression analysis with SSRT as a dependent variable (DV) and age as an independent variable (IV).
b. A regression analysis with activation in the motor cortex ROI as a DV and age as an IV.

In addition, the same analysis is performed with sex and Tanner scale as independent variables.

Proactive inhibition

1. A repeated measures analysis is used to test for the effect of stop-signal probability on response times.
    a. A regression analysis with response slowing as DV and age as IV.
    b. A regression analysis with response slowing as DV and sex as IV.
    c. A regression analysis with response slowing as DV and Tanner as IV.
    d. A regression analysis with response slowing as DV and TMCQ as IV

2. A t-test is used to test for significant brain activation in the rIFG, rIPC and Striatum ROIs, with a parametric modulation of the BOLD response by stop-signal probability.
    a. A regression analysis with activation in these ROIs and age as IV.
    b. A regression analysis with activation in these ROIs and sex as IV.
    c. A regression analysis activation in these ROIs and Tanner as IV.
    d. A regression analysis activation in these ROIs and TMCQ as IV.

3. After a connectivity analysis on the resting-state scan with the striatum ROI as a seed, A t-test is used to test for significant brain activation in the rIFG.
    a. A regression analysis with activation in the rIFG as DV and age as IV.
    b. A regression analysis with activation in the rIFG as DV and sex as IV.
    c. A regression analysis activation in the rIFG as DV and Tanner as IV.
    d. A regression analysis activation in the rIFG as DV and TMCQ as IV.

Planned subgroup analyses (if applicable. Substantiate your choices)

See above.

Planned sensitivity analyses (if applicable. Substantiate your choices)

Sensitivity analyses are analyses that you plan beforehand to test whether certain factors have a major influence on your results.

1. **Timeline and milestones (including dates of when to analyze/write up):**

Data-analysis – 3 months

Writing the paper - 3 months

2. **Output (e.g. article, report, etc.):**

Scientific paper submitted to a peer-reviewed international journal.

Paper presented at a scientific convention

3. **Proposed authors + affiliations (please note that the YOUth data access committee can request certain authors to be included):**

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