**Analysis Plan:** Antidepressant Discontinuation Effects on hippocampus dependent Episodic Memory Recall

1. Definitions
   1. **General definitions**
      * MDD = major depressive disorder
      * MDE = major depressive episode
      * ADM = antidepressant medication
      * HC = hippocampus
      * pg/sgACC = perigenual/ subgenual anterior cingulate
      * dlPFC = dorsolateral prefrontal cortex
      * FDR = false discovery rate
      * TFCE = threshold-free cluster enhancement
   2. **Subjects**
      * C = Healthy Controls
      * P = remitted patients with MDD and antidepressant treatment, randomly assigned to discontinuation group
      * 1W2= Patients who discontinued first, right after time point T1
      * 12W = Patients who discontinued second, right after time point T2
   3. **Time** **points**
      * T1 = first MRI session 🡪 C, 1W2 with ADM, 12W with ADM
      * T2 = second MRI session 🡪 1W2 without ADM, 12W with ADM
   4. **Overall study setting** (*2 x 2 Design (group x time)*)
      * 2 subject groups: C, P
      * 2 time points
        1. T1: C and P
        2. T2: P (1W2 without ADM, 12W with ADM)
      * Episodic Memory Task (Recall) with two task conditions
        1. Memory = face-profession-pairs
        2. Control = ears
   5. **Effects**
      * ME Recall = main effect (memory > control)
      * SE Memory = simple effect (memory)
      * SE Control = simple effect (control)
2. Task validation
   1. **Hypothesis**: Both groups (C + P) show significant activation in bilateral HC for following contrasts.
      * Contrasts: SE Memory, ME Recall during T1
      * If effect cannot be found across groups, task validation only in C
   2. **Methods**
      * One-sample t-tests, F-tests
      * Possible covariates to explain variance: age, gender, site
3. Task reliability
   1. **Hypothesis**: The patterns of neural activity did not differ in 12W between T1 and T2.
      * Contrasts: SE Memory, SE Control, ME Recall
      * ROIs: HC, ACC, (dl)PFC
   2. **Intra-Class-Correlation**: Fixed Effects
      * Paired t-tests
      * Power analysis to determine the significance of the result
4. Cross-sectional analysis

One major symptom of depression is cognitive impairment, especially during episodic memory [1, 2] which relies highly on the HC [3]. Since the HC is a highly sensitive region and depression is associated with a hyperactive hypothalamic-pituitary-adrenal axis, long-term exposure to glucocorticoids could explain HC deficits and the resulting symptoms of cognitive impairment [1, 4, 5].

HC volume reductions are repeatedly reported [6-8]. In contrast, only a few studies have investigated HC activity while recalling episodic memory. Their findings are heterogeneous which could be due to small sample sizes and varying inclusion criteria. Some studies report decreased HC activity in patients with depression [9-11] and others cannot find significant differences [12, 13]. Diminished activation in the ACC was also described [11, 13].

Concerning dlPFC activity, literature suggests diminished activation in cognitively impaired and symptomatic patients in contrast to increased activations in patients with less or without cognitive impairment [14]. This goes along with the common finding of prefrontal hyperactivation in Alzheimer’s research suggesting compensation of cognitive performance when hippocampal activation fails [15-17].

To explore HC function, we used an established memory task of Erk et al. (2010) where they demonstrated a diminished hippocampal activation in healthy controls with a higher genetic risk for depression during recall of previously encoded face-profession-pairs. These results were replicated in another sample of risk gen carriers, in healthy first-degree relatives of MDD and subjects with subjective memory impairment [16, 19, 20]. They also reported diminished activation in the pg/sgACC [18-20].

We assume we will see the same activation patterns in a group of remitted depressed patients as they have. We also assume we can replicate their finding of a negative correlation between HC activity and the anxiety- and depression-subscales from the SCL-90-R-Questionnaire [18, 19].

Therefore, we hypothesize:

* **Main hypothesis**: Pshow a diminished activation in the bilateral HC and pg/sgACC compared to C.
  + ME Recall (HC, pg/sgACC) C (T1) > P (T1)
    - Two-sample t-test with nuisance covariates: age, gender, site
    - FDR-corrected activations (p < 0.05) in *a priori* defined ROIs
  + Negative correlation between HC activity and anxiety- and depression- subscales of SCL-90-R questionnaire
* **Second hypothesis**: If there are no performance differences between groups, P might compensate decreased activation in HC by increasing activation in the dlPFC.
  + ME Recall (dlPFC) C (T1) < P (T1)
    - Two-sample-T-test with nuisance covariates: age, gender, site
    - FDR-corrected activations (p < 0.05) in *a priori* defined ROI
* If there are no differences between groups, this could be the result of the remitted status of P and protective effects of ADM.

1. Comparability analysis

* **Hypothesis**: Both 12W and 1W2 show neural activity in bilateral HC for following contrasts.
  + Contrasts: SE Memory, ME Recall
  + Time point: T1
  + Possible covariates to explain variance: age, gender, site

1. Discontinuation analysis

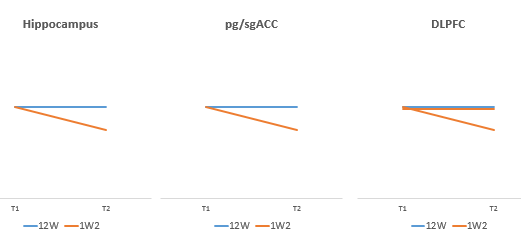
ADM can improve cognitive function to some extent [21], especially concerning psychomotor speed and delayed recall [22]. Unfortunately, fMRI studies examining the effects of ADM in cognition (in a non-emotional context) are scarce and reported results very heterogeneous, which could result from small sample sizes. While Robertson et al. (2007) found an elevated right dlPFC activity during recognition of attentional targets after treatment, Bremner et al. (2007) reported a decreased dlPFC and increased ACC activity during encoding of neutral words and paragraphs. No differences after treatment with ADM were found performing an n-back-task in Walsh et al. (2007).

As we purely want to test recall of episodic memory, it is not possible to draw strong conclusion from these findings as they explore various forms of memory that rely on different neural structures. Due to the HC primarily being responsible for episodic memory [3], working memory is still intact after hippocampal damage [26, 27]. Furthermore, the different stages of episodic memory (encode, recall, recognition) activate neural structures differently [28-31]. Thereby, recall is especially sensitive to hippocampal dysfunction as it is the most challenging. This could explain why studies of Erk and Kepa found significant activation differences only during recall of face-profession-pairs [16, 18-20, 32].

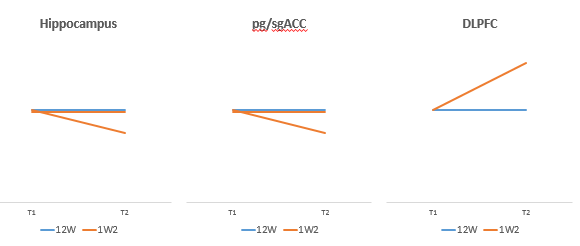
While task activation studies are scarce, there is compiling evidence that ADM is able to increase HC neurogenesis and protect its integrity in animal studies [33, 34]. Kepa et al. (2017) found increased HC activity in healthy controls with MYT1L (a brain-specific transcription factor that is required for neurogenesis) during recall of previously encoded face-profession-pairs compared to controls. We assume that ADM can also elevate HC activity through increasing neurogenesis and therefore result in cognitive improvements.

As there are – to our knowledge – no fMRI studies on discontinuation effects in cognitive performance, we hypothesize that by discontinuation we will observe the reverse effects of ADM described in the literature due to abolishing the protective effects of antidepressants:

* **Main hypothesis (Depression trait hypothesis)**: Due to losing the protective effects of ADM, remitted patients will show a depression-like pattern. Therefore, task-related activation in bilateral HC and pg/sgACC will decrease even more after antidepressant discontinuation. Furthermore, compensatory (increased) dlPFC activation will decrease or stay the same after discontinuation  
  + ME Recall (HC, pg/sgACC) 12W (T1=T2) > 1W2 (T1>T2)   
    ME Recall (dlPFC) 12W (T1=T2) >/= 1W2 (T1>/=T2)
    - 2 x 2 Design (group x time)
    - Two-sample t-tests, F-tests
    - FDR-corrected activations (p < 0.05) in *a priori* defined ROIs
    - Possible covariates to explain variance: age, gender, site



* **Alternative hypothesis (Compensation hypothesis)**: Assuming prefrontal areas are able to compensate for HC dysfunction in depression via hyperactivation (see above) this effect could still be detectable after discontinuation. Increasing dlPFC activity might therefore be accountable for missing performance differences. Furthermore, we suggest that the dlPFC might be even able to counteract decreases in HC and sg/pgACC activity.
  + ME Recall (HC, pg/sgACC) 12W (T1=T2) >/= 1W2 (T1>/=T2)   
    ME Recall (dlPFC) 12W (T1=T2) < 1W2 (T1<T2)
    - 2 x 2 Design (*group x time*)
    - Two-sample t-tests, F-tests
    - FDR-corrected activations (p < 0.05) in *a priori* defined ROIs
    - Possible covariates to explain variance: age, gender, site



* If there are no differences between groups, described activation alterations may not occur within 1-2 weeks after ADM discontinuation, but rather after a longer period of time. The time of tapering down might have an influence on this effect (long tapering down period vs. short tapering down period).
* **Exploratory Whole-Brain Analysis:** 
  + 2 x 2 Design (*group x time*)
  + TFCE-corrected
  + Possible covariates to explain variance: age, gender, site

Methods

**Preprocessing**:

* Brain extraction (via ANTs)
* Motion Correction to the middle volume (via FSL, MCFLIRT)
* Spatial Smoothing with 6-mm-kernel (via FSL)
* Grand Mean Intensity Normalization with a single scaling factor (via FSL)
* Registration (ANTs)
  + Linear Registration of EPI to (brain-extracted) T1
  + Nonlinear Registration of T1 to 2mm MNI
* ICA-based noise reduction (via ICA-AROMA)
* High-pass Temporal Filtering (cut-off 125 s)
* Linear Registration matrix and nonlinear warp image to bring preprocessed 4D data into MNI space (via FSL, FLIRT)
* Creating tsnr and tstd (for additional quality check)
* Slice Timing Correction

**First-level Analysis** (in FSL): General Linear Model (GLM)

* Block-related design
* Modulation of 2 regressors
  + Memory condition: face-profession-pairs
  + Control condition: ears
* Additional regressors for physiological signal
  + Breathing rate
  + Pulse

**Higher-level Analysis** (in FSL):

* ROI Analyses
  + Regions determined by Harvard-Oxford Subcortical/Cortical Atlas:
    - Left/Right Hippocampus
    - Cingulate Gyrus, anterior division
    - Middle Frontal Gyrus
  + False discovery rate (FDR) for multiple comparisons (p < 0.05)
* Covariates
  + Nuisance covariates: age, gender, site
  + Potentially interesting covariates
    - Anxiety- and depression- subscale of SCL-90-R questionnaire
      * Negative correlation between HC activity (P,T1)
    - Recall performance
      * Positive correlation between dlPFC activity (P, T1)
    - Cortisol levels
      * Negative correlation with HC activity (P, T1)
    - Severity factor
      * Negative correlation with HC und dlPFC activity (P, T1)
      * Negative correlation with dlPFC activity after discontinuation (1W2, T2)
    - ADM treatment duration before discontinuation
      * Positive correlation with HC activity after discontinuation (1W2, T2)
    - Medication load before discontinuation
    - Duration of tapering down (short vs. long tapering down period)
    - Hippocampal volume

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