

# Neuroendocrine stress responses predict catecholamine-dependent working memory-related dorsolateral prefrontal cortex activity

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## Abstract

It is generally thought that the effect of acute stress on higher-order functions such as working memory is, for an important part, mediated by central catecholamine activity. However, little is known about the association between neuroendocrine stress responses and catecholamine-dependent working memory-related brain function in the *absence* of stress. Here, we investigate for the first time in healthy humans ( $n = 18$ ) how neuroendocrine responses to stress (cortisol and alpha-amylase) relate to fronto-parietal working memory activity changes in response to atomoxetine, a noradrenaline transporter inhibitor that selectively increases extracellular cortical dopamine and noradrenaline. We observed positive correlations between stress-induced cortisol (Pearson's  $r = 0.75$ ,  $P < 0.001$ ) and alpha amylase ( $r = 0.69$ ,  $P = 0.02$ ) increases and catecholamine-dependent working memory-related activity in dorsolateral prefrontal cortex. Stress-induced cortisol increases furthermore correlated with supramarginal gyrus working memory-related activity ( $r = 0.79$ ,  $P < 0.001$ ). Comparing high vs low stress responders revealed that these correlations were driven by *decreased* working memory activity on placebo and *greater* working memory activity increases following atomoxetine in high stress responders. These results further corroborate the notion that neuroendocrine responses to stress are an informative proxy of catecholamine function relevant to higher order functions and provide novel hints on the complex relationship between acute stress, catecholamine function and working memory.

**Key words:** dopamine; noradrenaline; cortisol; prefrontal cortex; working memory; stress; neuroimaging

## Introduction

Acute stress can trigger a global reallocation of processing resources from an executive control to a salience network, thereby impacting higher-order functions such as working

memory (Arnsten, 2009; Hermans *et al.*, 2014). It is thought that such stress-induced prioritizations of survival over executive control are, for an important part, mediated by central catecholamine activity (Arnsten, 2015). However, the relationship

Received: 13 January 2017; Revised: 28 August 2017; Accepted: 16 October 2017

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between neuroendocrine stress responses and catecholamine-dependent working memory-related brain function in the absence of stress has never been probed. In this study, we investigate how salivary cortisol (sCORT) and alpha-amylase (sAA) changes in response to a psychophysiological stressor relate to catecholamine-dependent working memory-related brain activity in the same individuals. The aim was to provide novel insights into how trait-like neuroendocrine stress responses may be informative of central catecholamine activity relevant to higher-order functions.

Working memory abilities are, for an important part, underlain by catecholamine function, most importantly dopamine and noradrenaline. Among others, projections from *locus coeruleus* (LC) and ventral tegmental area (VTA) to prefrontal cortex (PFC) play an important part in facilitating, tuning and, thereby, optimizing PFC-mediated circuits that support working memory, response inhibition and other executive functions (Robbins and Arnsten, 2009; Chandler et al., 2014). The presence of substantial inter-subject variation in the effect of catecholamine manipulations on higher-order functions (Cools and D'Esposito, 2011) suggests potential differences in baseline catecholamine function. Indeed, surrogate markers of catecholamine function such as allelic variation in genetic polymorphisms of the dopamine system (Mier et al., 2010; Cummins et al., 2012), working memory span (Mehta et al., 2000; Hernaus et al., 2017) and *in vivo* measures of dopamine function (Cools et al., 2009) relate to a wide range of executive functions and the neural correlates thereof.

Stress-related neuroendocrine activity measured in saliva or plasma might also be informative of central catecholamine function. This idea is motivated by the observation that acute stress fosters changes in PFC catecholamine release (Hermans et al., 2014; Arnsten, 2015; Vaessen et al., 2015) and (often) negatively impacts working memory abilities (Oei et al., 2006; Qin et al., 2009; Schoofs et al., 2009). Moreover, in the limited literature available, cortisol increases in response to an acute stress challenge correlated with stimulant- and stress-induced dopamine release (Pruessner et al., 2004; Wand et al., 2007). Thus, stress-related neuroendocrine activity might serve as an informative proxy of catecholamine-dependent PFC-mediated higher-order functions, although a formal relationship between the two has never been investigated.

To these aims, we interrogated the association between (i) neuroendocrine responses to a psychophysiological stressor and (ii) the effect of atomoxetine (ATX), a noradrenaline reuptake inhibitor, on working memory-related fronto-parietal activity, in healthy humans. At single doses, ATX increases cortical, but not striatal, dopamine and noradrenaline release (Bymaster et al., 2002). Thus, ATX's pharmacological action can be exploited to achieve regionally specific modulation of cortical dopamine and noradrenaline function in regions directly relevant for working memory (Goldman-Rakic, 1995) and stress processing (Arnsten, 2015). We expected that stress-induced increases in sCORT and sAA, two reliable indicators of catecholamine and neuroendocrine activity, would be predictive of ATX's effect on working memory-related activity in fronto-parietal regions.

## Materials and methods

### Participants

The total sample consisted of 19 healthy male right-handed non-smokers between 18–30 years recruited from a student population (mean age = 23.26, s.d. = 2.64). In this sample, we

have previously reported ATX-induced fronto-parietal functional connectivity increases during an N-back task (Hernaus et al., 2017). Here, we focus on the relationship between neuroendocrine responses to acute stress and catecholamine-dependent working memory activity. Participants with a psychiatric or neurological disorder, history of psychopharmacological treatment or current (recreational) drug (ab)use, high (>30) or low (<18) BMI and magnetic resonance imaging (MRI) contraindications were excluded. The presence of a psychiatric disorder was excluded using the Mini-International Neuropsychiatric Interview [MINI; (Sheehan et al., 1998)] and substance abuse was assessed using the Composite International Diagnostic Interview [CIDI; (Kessler and Ustun, 2004)]. A urine sample was screened for the presence of amphetamine, tetrahydrocannabinol, cocaine, opiates, methamphetamine and benzodiazepines prior to the placebo (PLC) and ATX session. The study was carried out in accordance with the Declaration of Helsinki (WHO, 2013) and was approved by the local medical ethics committee of Maastricht University Medical Centre (ref. no. NL53913.068.15; NTR number: NTR5679). All participants gave written informed consent prior to all testing days and were reimbursed for their participation.

### Study procedures and pharmacological challenge

We employed a three-session within-subject design (see Figure 1 for overview); during the first session, acute stress was induced using a validated psychophysiological stressor ('Acute stress induction'). The second and third session were conducted according to a double-blind randomized placebo-controlled crossover design, during which participants performed a working memory paradigm in a 3 T MRI environment. Eight participants received PLC first, followed by ATX; 11 participants received ATX first, then PLC.

All participants received an oral dose of ATX (Brand name: Strattera<sup>®</sup>) 60 mg and cellulose microcrystal (PLC) in capsule form between 12 and 4 pm on two separate days, separated in time by a minimum of 1 week. The minimum and maximum interval between MAST session and PLC/ATX sessions was 3 and 10 days, respectively.

Except for one participant that experienced nausea (excluded) and one that experienced a brief spell of dizziness, no significant side effects were reported during PLC or ATX sessions, which were monitored at -15, 90, 120 and 150 min post-dosing using a 10 cm Visual Analogue Scale (Bond and Lader, 1974).

### Acute stress induction

Acute psychophysiological stress was induced using the Maastricht Imaging Stress Task [MAST; (Smeets et al., 2012)]. The MAST reliably increases sCORT levels over and above other stressors such as the Cold Pressor Task and increases negative affect to a similar degree as the Trier Social Stress Task (Smeets et al., 2012). Participants were tested between 2 and 4 pm, when cortisol levels are relatively stable (Smyth et al., 1997), and were instructed to refrain from caffeine, nicotine and strenuous activity on the day of stress induction. They were additionally asked to consume a light meal at least 3 h before the start of the session.

The MAST consists of a 5-min preparation phase during which participants are briefed about the task at hand. What follows is a 10-min acute stress-induction phase, during which participants immerse their hand in cold water, which is

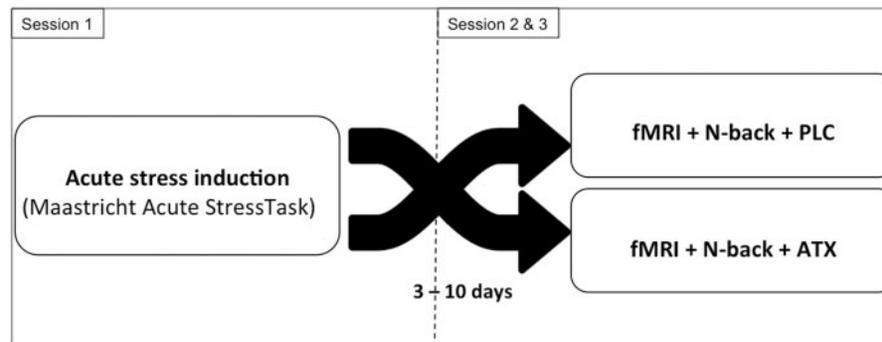


Fig. 1. Study Design. Following the acute stress induction session, participants were randomized according to a double-blind placebo-controlled cross-over design, in which PLC and ATX session were randomized.

alternated with blocks of mental arithmetic in combination with negative evaluative feedback. Specifically, during five trials that vary in duration from 60 to 90 s, participants immerse their non-dominant hand in cold water (2 °C; plexiglas box with an electric cooler and a circulation pump from JULABO Labortechnik, Seelbach, Germany). In between hand immersion trials, participants engage in mental arithmetic challenges. They are asked to count backwards as fast and accurately as possible in steps of 17 starting at a random 4-digit number for 45, 60 or 90 s. Whenever they count too slowly or make a mistake, they receive scripted negative evaluative feedback and are instructed to start over. To increase unpredictability and uncontrollability, participants are told that the order and duration of the hand immersion and mental arithmetic trials is randomly chosen by a computer and that they will be videotaped for analyses of their facial expressions, for which they provide fictitious informed consent.

#### Affective, physiological and neuroendocrine response to acute stress

**Negative affect.** Approximately 25 min prior to and immediately after the MAST, negative affect was assessed using the 'negative affect' subscale of the Positive and Negative Affect Schedule, state version [PANAS (Watson *et al.*, 1988)]. This scale consists of 10 items that are rated on a 5-point scale ranging from 'not at all' [1] to 'extremely' [5]. The MAST has previously been shown to reliably increase negative affect (Smeets *et al.*, 2012).

**Physiological response.** Systolic blood pressure and pulse rate were measured 20 min before and immediately after the MAST using an OMRON M4-I (OMRON Healthcare Europe B.V., Hoofddorp, The Netherlands) combined blood pressure and pulse rate meter.

**Neuroendocrine responses.** Saliva samples were collected at -20 (baseline), -10 (post-instructions), 0 (end of MAST), 10, 30 and 40 (post-MAST) min using Sarstedt cortisol Salivette® devices (Sarstedt, Etten-Leur, the Netherlands). SCORT and sAA—measures of, respectively, hypothalamus-pituitary-adrenal (HPA) axis and sympatho-adrenal-medullary (SAM) activity—were determined from these samples. All sCORT samples pre/post MAST were utilized. Given the rapid onset and decay of sAA following acute stress (Nater *et al.*, 2005), time points -20, 0 and 10 were used for sAA. The -10 min/post-instructions sample served as a substitute baseline sample in the event that analysis of the -20 min sample failed.

Saliva samples were stored at -20 °C until sCORT and sAA levels were determined. SCORT levels were determined by a commercially available luminescence immune assay kit (IBL, Hamburg, Germany). Mean intra- and inter-assay coefficients of variation are typically less than 5%, and the lower and upper detection limits were 0.015 mg/dl (0.41 nmol/l) and 4.0 mg/dl (110.4 nmol/l), respectively. SAA concentrations were determined using a commercially available kinetic reaction assay (Salimetrics, Penn State, PA, USA). Mean intra- and inter-assay coefficients of variation of the sAA analyses are typically less than 8% and 6%, respectively.

#### Working memory paradigm

The N-back task (Gevins and Cutillo, 1993) is a well-validated neuroimaging paradigm, previously shown to reliably increase fronto-parietal activity (Owen *et al.*, 2005) and sensitive to changes in catecholamine function (Apud *et al.*, 2007; Garrett *et al.*, 2015). Briefly, letters were presented for 1000 ms, followed by a 1000 ms fixation cross. Participants were instructed to detect target letters, which constituted letters that were the same as the letter presented one, two or three trials previously (i.e. 1-back, 2-back and 3-back condition). As a control condition, participants were asked to detect the letter 'X' (0-back). Participants responded to targets and distractors with right index finger and middle finger button presses, respectively. Prior to the testing phase, participants were trained on the n-back task until they exceeded 85% correct responses in every condition.

Each condition consisted of 44 letters, 11 to 12 of which were targets. Conditions were divided into blocks of 11 letters, which were presented in a pseudo-randomized order (3 to 4 targets per block). The total duration of the task was approximately 9 min. Different versions of the task were used for each session and task version was counterbalanced between participants (version 1-2  $n=10$ ; version 2-1  $n=9$ ; version 1 on ATX  $n=11$ ; version 2 on ATX  $n=8$ ). In line with the pharmacokinetic profile of ATX (Witcher *et al.*, 2003), all participants performed the n-back at 100 min ( $\pm 10$  min) after PLC/ATX intake.

#### Image acquisition

Images were acquired on a whole-body 3T MAGNETOM Prisma scanner (Siemens; Erlangen, Germany). 270 functional T2\*-weighted axial images approximately aligned to the AC/PC line were acquired over a 9-min period during which participants performed the n-back task. Each volume consisted of 35 3 mm-slices (0.3 mm slice gap) which were acquired in interleaved fashion. Acquisition protocol details were: TR=2000 ms,

TE = 30 ms, flip angle = 77°, FoV = 21.6 cm, GRAPPA acceleration factor = 2 and matrix size = 72 x 72. For co-registration purposes a T1-weighted high-resolution image (MPRAGE sequence) was acquired, consisting of 192 1 mm-sagittal slices acquired in interleaved fashion with TR = 2250 ms, TE = 2.21 ms, flip angle = 9°, FoV = 25.6 cm.

### Image preprocessing and fMRI contrasts

Image preprocessing steps for this sample have been published previously (Hernaus et al., 2017). Briefly, all image preprocessing steps and analyses were performed in Statistical Parametric Mapping 12 (SPM 12; <http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>). Functional images were motion corrected (first volume as a reference), co-registered to the individual T1-weighted image, warped to MNI space using SPM's T1 template and smoothed (6 mm FWHM). Time series were high-pass filtered (128 s) and serial correlations were accounted for using an AR(1) model. For all participants, motion was always below 2 mm or 1.5 degrees of motion.

For each session, a general linear model with four regressors of interest (0-, 1-, 2- and 3-back), their temporal derivatives and six motion regressors of no interest was constructed. A grey matter mask was constructed from the segmented images of all participants using SPM 12's segmentation algorithm (default settings), which was used as a second level inclusive mask.

For PLC and ATX sessions, a load-independent contrast image was created to reveal brain regions that showed increased activity in response to working memory demands, compared to the control condition (i.e. all N-back back conditions vs 0-back). The resulting load-independent contrast images were entered into a second level random effects analysis and always thresholded using a cluster-forming threshold of  $P < 0.001$ , 20 voxel minimal cluster size and a FWE-corrected cluster threshold of  $P < 0.05$ . We used a load-independent contrast because of two reasons. First, we have previously observed that ATX caused an upward shift in brain network-level integration for every condition of the N-back task, with only a small minority of regions shown a drug-by-load interaction (Shine et al.). This suggests that ATX had similar effects on brain function for every condition of the N-back task, making it unlikely that correlations with neuroendocrine function would be driven by specific N-back conditions. Secondly, we did not have any a priori hypotheses regarding load-dependent relationships with stress-related neuroendocrine function.

We selected the dlPFC as our primary region of interest (ROI) based on three important observations. First, abundant evidence exists for the role of the dlPFC in working memory processes (Goldman-Rakic, 1995). Secondly, acute stress modulates working memory-related activity in dlPFC (Qin et al., 2009; Arnsten, 2015). Third, this effect is mediated by genetic variation in catecholamine function as indexed by COMT genotype (Qin et al., 2012). This all suggest a close relationship between working memory-related activity in dlPFC and catecholamine function. We therefore settled on a 10 mm sphere in the right dlPFC, where previously COMT genotype-dependent effects of stress on working memory-related activity were identified (MNI coordinates:  $x = 30, y = 30, z = 42$ ) (Qin et al., 2012). Activity in this region was thresholded at  $P(\text{uncorrected}) = 0.01$  and a small volume correction (FWE) was applied to adjust the false-positive rate.

MarsBar (Brett et al., June 2–6, 2002) (v.44) was used to extract beta values from a 6 mm spherical ROI surrounding the peak

voxel. fMRI figures were prepared in bspmview (<http://www.bobspunt.com/bspmview/>).

### Statistical testing

Data were analyzed in SPSS (v23; IBM Corp, Arlington, NY). In order to validate our stress-induction approach, we compared negative affect, systolic blood pressure and pulse rate pre- and post-MAST using a paired samples two-tailed t-test. Changes in sCORT levels were investigated using a repeated measures general linear model with time (6 measures) as a within subjects factor. Additionally, peak stress-induced sCORT/sAA concentrations (i.e.  $\Delta$ sAA and  $\Delta$ sCORT) were calculated by subtracting the -20 baseline sample from maximum post-MAST sCORT/sAA concentrations. Greenhouse-Geisser sphericity-corrected values were reported when assumptions were violated.

In order to investigate how neuroendocrine responses to acute stress related to ATX-induced changes in working memory-related activity, we performed a whole-brain and ROI correlation analysis. To these aims, an area-under-the-curve cortisol value with respect to ground ( $AUC_g$ ) was calculated for every participant, reflecting total neuroendocrine activity (Pruessner et al., 2003). In line with Pruessner et al. (2003),  $AUC_g$  was defined as the total sum of two adjacent time points, divided by two and multiplied by measurement time [i.e.  $((t_0 + t_{\text{pre-stress}})/2) * 20 + ((t_{+10} + t_{+0})/2) * 10 + ((t_{+30} + t_{+10})/2) * 20 + ((t_{+40} + t_{+30})/2) * 10$ ]

The benefit of using an AUC measure is that it takes into account inter-individual differences, such as variation in baseline cortisol levels, the onset time of the stress-induced cortisol release and the width of the cortisol peak. In addition to a whole-brain and ROI correlation analysis, dlPFC beta values from the ATX > PLC contrast were extracted for every participant, in order to investigate correlations with  $\Delta$ sCORT and  $\Delta$ sAA. One participant with a studentized residual score 2 times greater than the critical cutoff was excluded for correlation analyses, leaving a final sample of 18 individuals.

## Results

### The MAST increases affective, physiological and hormonal measures of acute stress

Negative affect [ $t(18) = 5.37, P < 0.001$ ], systolic blood pressure [ $t(18) = 5.78, P < 0.001$ ] and pulse rate [ $t(18) = 2.21, P = 0.04$ ] increased significantly in response to the MAST (Figure 2A–C). Upon closer inspection, the effects of acute stress on negative affect were mostly driven by increases in 'Upset', 'Hostility', 'Distressed', 'Ashamed' and 'Irritability' (Supplementary Figure S1).

sCORT data were available for 91.22% (10 missing) of all timepoints.  $\Delta$ sCORT and  $AUC_g$  values could be calculated for all but one participant. For this particular participant, sCORT estimates of four samples (including baseline) were missing. Using all available samples, there was an overall main effect of time on sCORT [ $F(1.96, 23.348) = 17.06, P < 0.001$ ] and Bonferroni-corrected pairwise comparisons revealed that sCORT levels rose until  $t_{+30}$  min post-task (Figure 2D). At  $t_{+40}$ , sCORT levels seemed to decrease, but this decrease was not significantly different from  $t_{+30}$  ( $P = 0.20$ ).  $\Delta$ sCORT values were significantly greater than zero [ $t(17) = 7.15, P < 0.001$ ] (Figure 2D).

SAA data were available for 62% (29 missing) of all timepoints.  $\Delta$ sAA values were available for 11 participants.  $\Delta$ sAA

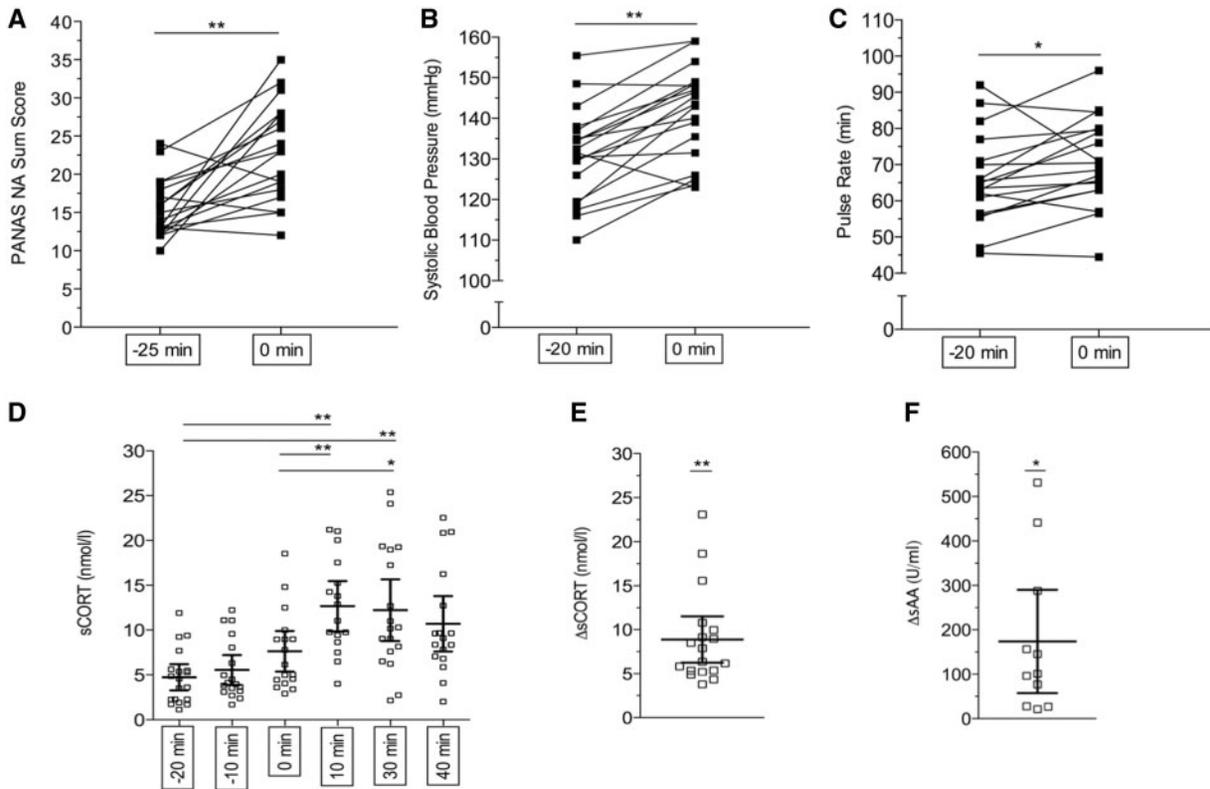


Fig. 2. Stress-induced changes at the affective (Panel A), physiological (Panels B and C) and neuroendocrine (Panels D-F) level. 0 min. marked the end of the MAST. \* $P < 0.05$ , \*\* $P < 0.001$ .

was significantly greater than zero [ $t(10) = 3.29$ ,  $P = 0.008$ ] (Figure 2F).

All in all, these results clearly demonstrate that the MAST was successful in inducing acute stress at the subjective, physiological and neuroendocrine level.

### Catecholamine-dependent supramarginal gyrus activity is predicted by neuroendocrine responses to acute stress

We have previously reported working memory-related activity increases on PLC and ATX in among others dlPFC, anterior insula and superior parietal lobule during the N-back task (Heraus et al., 2017).

A whole-brain correlation analysis revealed a large cluster of activity in the right supramarginal gyrus [ $T = 6.82$ , cluster size = 161, cluster  $P(\text{FWE-corrected}) < 0.001$ , peak voxel MNI coordinates:  $x = 54$ ,  $y = -42$ ,  $z = 50$ ], where ATX-induced changes in activity (i.e. ATX > PLC) correlated positively with  $AUC_g$  cortisol values ( $r = 0.79$ ,  $P < 0.001$ ; Figure 3A and B).

To gain insights into the nature of this correlation, we conducted an exploratory post-hoc stratification, creating low and high stress responder subgroups using the average  $AUC_g$  value (385.72). This resulted in 10 low responders and 7 high responders. Directly comparing ATX-induced activity changes between these groups revealed greater right supramarginal gyrus, left precentral gyrus and left dlPFC activity for high vs low responders (Figure 3C; Table 1). These results indicated that ATX induced greater activity increases in high compared to low responders. When looking at low and high responders separately, low responders showed decreased (i.e. more negative) ATX-induced activity in supramarginal gyrus, while there were no ATX-induced activity changes in this region for high responders

(Supplementary Figure S2A and B). On PLC, high vs low responders showed less activity in a fronto-parietal network including superior parietal lobule, postcentral gyrus and insula (Supplementary Table S1; Supplementary Figure S3).

No regions were identified where activity on PLC was greater than on ATX in the entire sample. Negative correlations between ATX-induced increases in working memory-related activity and neuroendocrine responses to acute stress were not observed.

### Catecholamine-dependent dorsolateral prefrontal cortex activity is predicted by neuroendocrine responses to acute stress

The ROI correlation analysis revealed that  $AUC_g$  correlated positively with ATX-induced increases in right dlPFC ( $T = 3.69$ , cluster size = 51,  $P(\text{small volume FWE-corrected}) = 0.036$ , peak voxel MNI coordinates =  $x = 34$ ,  $y = 26$ ,  $z = 44$ ) (Figure 4A and B; Pearson's  $r = 0.75$ ,  $P < 0.001$ ). Moreover, ATX-induced activity increases (peak voxel MNI coordinates:  $x = 26$ ,  $y = 26$ ,  $z = 34$ ) significantly correlated with  $\Delta sCORT$  Pearson's  $r = 0.54$ ,  $P = 0.03$ ) and  $\Delta sAA$  (Pearson's  $r = 0.69$ ,  $P = 0.02$ ) (Figure 4C and D).

### The relationship between catecholamine-dependent working memory activity and stress-induced neuroendocrine activity can not explained by working memory capacity

We previously reported that the effect of ATX on working memory-related brain activity was dependent on working memory capacity (Heraus et al., 2017). Neuroendocrine responses to stress were not associated with forward digit

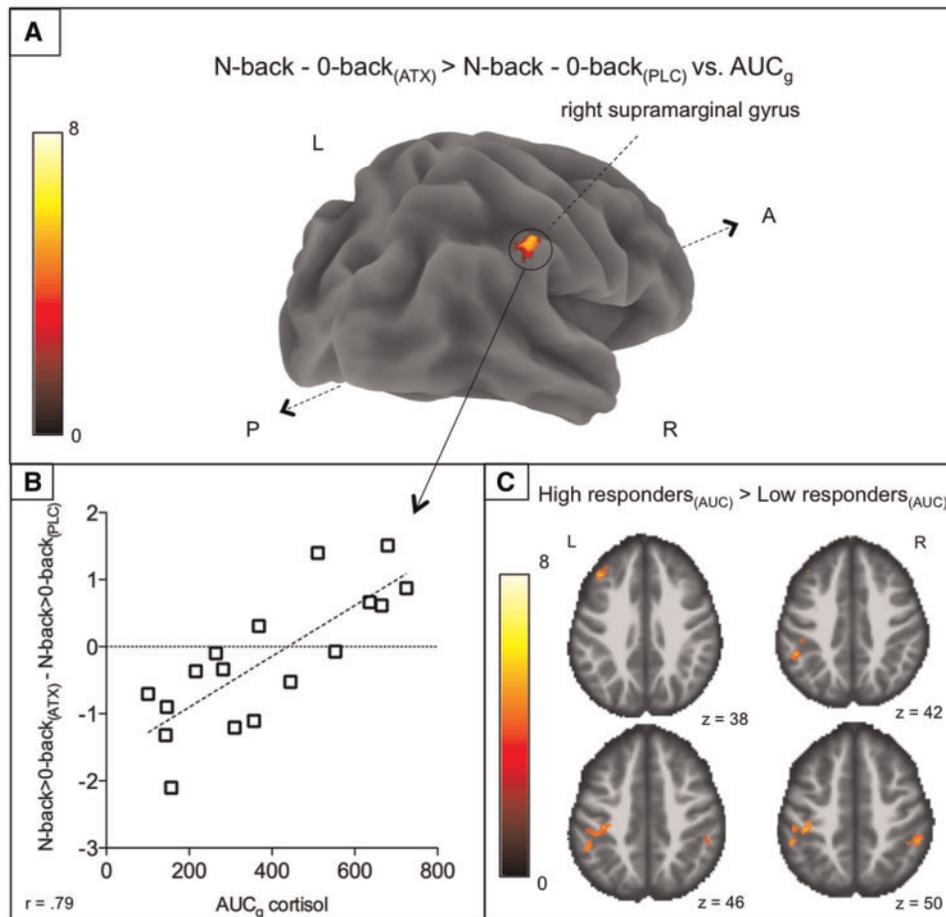


Fig. 3. (Panel A) A significant correlation was observed between salivary cortisol  $AUC_g$  and ATX-induced changes in right supramarginal gyrus (ATX > PLC; whole brain correlation analysis peak voxel MNI coordinates  $x = 54, y = -42, z = 50$ ; see main text for details). Panel B Visualization of the correlation between sCORT  $AUC_g$  and average activity in a 6 mm sphere surrounding the peak voxel (panel A). Panel C Post-hoc stratification: greater ATX-induced activity increases for high  $AUC_g$  responders vs low  $AUC_g$  responders ( $z =$  axial slice number; see Table 1 for regions).

Table 1. Whole brain analysis: ATX-induced working memory-related activity increases independent of load (ATX > PLC) for high vs low responders and groups separately (vs 0)

Nback-Xback(ATX) > Nback-Xback(PLC)	Region	Cluster $P$ (FWE-corrected) < 0.05	Cluster size	Peak t-value (uncorrected)	MNI Coordinates peak voxel		
					x	y	z
High > Low responders							
Left	Postcentral gyrus	<0.001	261	5.47	-42	-32	50
Left	Middle frontal gyrus	0.002	144	5.18	-42	30	38
Right	Supramarginal gyrus	0.044	81	5.03	52	-42	50
High responders > 0							
Left	Precentral gyrus	<0.001	90	4.87	-42	-16	64
Left	Postcentral gyrus	<0.001	218	4.40	-44	-34	48
Left	Supramarginal gyrs	0.013	52	3.74	-52	-44	50
Low responders < 0							
Left	Supramarginal gyrus	<0.001	20	3.46	52	-42	50

span (all  $P_s > 0.05$ ), our measure of working memory capacity. Moreover, adding working memory capacity as a covariate did not seem to affect the results. Associations between catecholamine-dependent dlPFC activity and neuroendocrine

response to stress remained significant, except for the association between ATX-induced changes in dlPFC activity and  $\Delta$ sCORT, which changed to trend-significant ( $P = 0.055$ ).

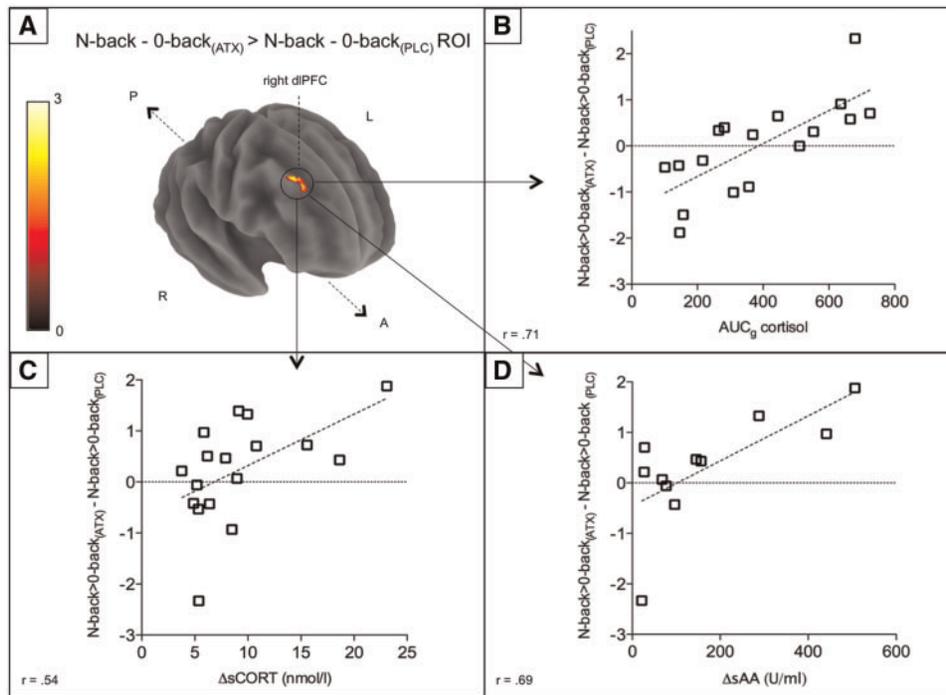


Fig. 4. (Panel A) ATX-induced changes in right dlPFC activity (ROI correlation analysis peak voxel MNI coordinates  $x = 36, y = 24, z = 44$ ; see text for details) correlated significantly with AUC<sub>g</sub> sCORT (Panel B). ATX-induced changes in right dlPFC activity (peak voxel coordinates:  $x = 26, y = 26, z = 34$ ) additionally correlated with ΔsCORT (Panel C) and ΔsAA (Panel D).

## Discussion

Our main aim was to interrogate the relation between stress-induced neuroendocrine activity and catecholamine-dependent working memory signalling in a non-stressful context. We observed that total stress-induced sCORT activity related positively to catecholamine-dependent parietal and dlPFC working memory activity. In dlPFC, an additional positive association between catecholamine-dependent working memory activity and peak sCORT and sAA levels was observed. Here, we discuss the potential mechanisms involved and the implications of these findings.

The association between stress-related neuroendocrine function and catecholamine-dependent fronto-parietal working memory-related brain activity might be attributable to major projections that mediate stress processing, as well as the effects of ATX on/and executive functions. Selective noradrenaline transporter blockade (here; reboxetine) modulates the firing rate of LC (Grandoso et al., 2004) and VTA (Linner et al., 2001) neurons, which has been co-observed with increased dopamine and noradrenaline release in terminal field projections, most notably PFC (Linner et al., 2001; Bymaster et al., 2002; Koda et al., 2010). Somewhat similarly, exposure to acute stress increases burst firing of LC (Gonon et al., 1983) and VTA (Holly and Miczek, 2016) neurons, consistent with increased frontal cortical catecholamine release in rodents (Finlay et al., 1995; Murphy et al., 1996), primates (Arnsten and Goldman-Rakic, 1998; Arnsten, 2015) and, for dopamine, humans (Vaessen et al., 2015). In PFC, glucocorticoid receptors, likely expressed on dopaminergic neurons (Barik et al., 2013), mediate stress-induced dopamine release and its concurrent effect on working memory performance (Butts et al., 2011). Thus, overlap in the neuropharmacological infrastructure underlying stress processing and executive functions may explain why stress-induced neuroendocrine

responses might be an informative proxy of catecholamine function relevant to higher-order functions. The observation that ATX increases sAA levels (Warren et al., 2017) further lends credibility to the notion that stress-related changes in sAA can perhaps be traced back to central catecholaminergic activity.

Interpretation of the direction of the observed associations remains speculative. Acute stress decreases working memory-related dlPFC activity (Qin et al., 2009) and subjective stress responses correlate negatively with dlPFC-amygdala functional connectivity (Quaedflieg et al., 2015). An important difference to note, however, is that these studies investigated stress-induced changes in brain function, while here we employed a correlation design. The positive association between stress-induced neuroendocrine activity and catecholamine-dependent working memory signalling may indicate that *high* stress responders showed low baseline working memory-related activity and, subsequently, showed *greater* activity increases following ATX. Indeed, our post-hoc stratifications showed exactly this: high compared to low stress responders showed less working memory-related activity on PLC and greater activity increases following ATX in a fronto-parietal network that included dlPFC. Moreover, we have previously shown that ATX-induced functional connectivity changes correlate with improved reaction time stability during the n-back task (Heraus et al., 2017). These results are noteworthy because they provide some degree of directionality to the observed neuroendocrine-working memory activity associations. Specifically, high acute neuroendocrine stress responses may be indicative of lower baseline cortical catecholamine function. ATX may have had beneficial effects on performance in these individuals, as evidenced by the previously-reported correlation with task performance.

The use of measures of stress-related neuroendocrine activity may be useful in improving the prediction of pharmacological treatment effects. For example, ATX is a common treatment

for attention deficit/hyperactivity disorder (ADHD), which often shows a heterogeneous clinical picture (Mostert et al., 2015). Stress-related cortisol responses, but not diurnal cortisol, are related to ADHD symptom severity (Pesonen et al., 2011), suggesting potential to stratify patient care based on stress sensitivity. Alternatively, the use of neuroendocrine stress responses may be useful in the context of depression, where stress plays a major role in symptom exacerbation (Harkness et al., 2014) and noradrenaline reuptake inhibitors such as reboxetine are commonly used. Finally, correlations between total and peak neuroendocrine activity and working-memory related brain function may reflect a trait-like configuration of the system. This may suggest that stress-induced changes in brain function are dependent on baseline stress sensitivity. Future studies investigating the effect of stress-induced changes on brain function may therefore wish to include baseline measure of stress sensitivity.

### Limitations

While we observed strong correlations between various measures of stress-induced neuroendocrine activity and catecholamine-dependent working memory activity, we did not gain insights into causal mechanisms behind this association. Although post-hoc stratification analyses did provide initial hints on the interaction between catecholamine and neuroendocrine function, future studies combining pharmacological challenges and stress-induction approaches will be able to more decisively elucidate such neurochemical interactions. Additionally, our post-hoc stratifications resulted in smaller groups and should therefore be regarded as preliminary. Importantly, however, these analyses were not imperative for the main conclusions of this manuscript and served to narrow down potential mechanisms at play. A final limitation may be that assessments of stress-related SAM activity were only available in a subgroup of our sample. While the observed correlations with sAA contribute to the generalizability of our results and further implicate the noradrenergic system, the smaller sample size warrants caution when interpreting the nature of these associations.

### Conclusions

Our results show that neuroendocrine responses to stress are associated with atomoxetine-induced activity increases in dlPFC and supramarginal gyrus. These associations might be driven by *decreased* working memory-related activity at baseline and *greater* ATX-induced activity increases in high vs low stress responders. These results underscore the utility of stress-induced neuroendocrine activity as a marker of frontal cortical catecholamine function, which could be useful to improve understanding of inter-individual differences in stress susceptibility, working memory performance and pharmacological treatment response. Future studies are necessary in order to tease apart the exact mechanisms involved in these associations; studies that combine pharmacological manipulation with acute stress induction will be essential to address this question.

### Acknowledgements

We are indebted to Dr. Tom Smeets for advice on the use of the psychophysiological stress paradigm. We thank Wendy Beuken for her involvement in the administrative aspects of this study.

### Funding

DH's research is funded by a Kootstra post-doctoral fellowship and a Brains Unlimited pioneer grant (grant no: S.2015.1.02). TvA's research is funded by NIMH, and a ZonMW VIDI grant (grant no: 91712394). CW is funded by a Netherlands Organization for Scientific Research (NWO) Rubicon grant (grant no: 446-15-003, 2015).

### Supplementary data

Supplementary data are available at SCAN online.

*Conflict of interest.* None declared.

### References

- Apud, J.A., Mattay, V., Chen, J., et al. (2007). Tolcapone improves cognition and cortical information processing in normal human subjects. *Neuropsychopharmacology*, *32*(5), 1011–20.
- Arnsten, A.F. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Review of Neuroscience*, *10*(6), 410–22.
- Arnsten, A.F.T. (2015). Stress weakens prefrontal networks: molecular insults to higher cognition. *Nature Neuroscience*, *18*(10), 1376–85.
- Arnsten, A.F., Goldman-Rakic, P.S. (1998). Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. *Archives of General Psychiatry*, *55*(4), 362–8.
- Barik, J., Marti, F., Morel, C., et al. (2013). Chronic stress triggers social aversion via glucocorticoid receptor in dopaminergic neurons. *Science*, *339*(6117), 332–5.
- Bond, A., Lader, M. (1974). The use of analogue scales in rating subjective feelings. *British Journal of Psychology*, *47*(3), 211–8.
- Brett, M., Anton, J., Valabregue, R., Poline, J. (2002). Region of interest analysis using an SPM toolbox, In: Presented at the 8th International Conference on Functional Mapping of the Human Brain, Sendai, Japan.
- Butts, K.A., Weinberg, J., Young, A.H., Phillips, A.G. (2011). Glucocorticoid receptors in the prefrontal cortex regulate stress-evoked dopamine efflux and aspects of executive function. *Proceedings of the National Academy of Science USA*, *108*(45), 18459–64.
- Bymaster, F.P., Katner, J.S., Nelson, D.L., et al. (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder. *Neuropsychopharmacology*, *27*(5), 699–711.
- Chandler, D.J., Waterhouse, B.D., Gao, W.J. (2014). New perspectives on catecholaminergic regulation of executive circuits: evidence for independent modulation of prefrontal functions by midbrain dopaminergic and noradrenergic neurons. *Frontiers in Neural Circuits*, *8*, 53.
- Cools, R., D'Esposito, M. (2011). Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biological Psychiatry*, *69*(12), e113–25.
- Cools, R., Frank, M.J., Gibbs, S.E., Miyakawa, A., Jagust, W., D'Esposito, M. (2009). Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. *Journal of Neuroscience*, *29*(5), 1538–43.
- Cummins, T.D., Hawi, Z., Hocking, J., et al. (2012). Dopamine transporter genotype predicts behavioural and neural

- measures of response inhibition. *Molecular Psychiatry*, *17*(11), 1086–92.
- Finlay, J.M., Zigmond, M.J., Abercrombie, E.D. (1995). Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: effects of diazepam. *Neuroscience*, *64*(3), 619–28.
- Garrett, D.D., Nagel, I.E., Preuschhof, C., et al. (2015). Amphetamine modulates brain signal variability and working memory in younger and older adults. *Proceedings of the National Academy of Science USA*, *112*(24), 7593–8.
- Gevens, A., Cutillo, B. (1993). Spatiotemporal dynamics of component processes in human working memory. *Electroencephalography and Clinical Neurophysiology*, *87*(3), 128–43.
- Goldman-Rakic, P.S. (1995). Cellular basis of working memory. *Neuron*, *14*(3), 477–85.
- Gonon, F., Buda, M., De Simoni, G., Pujol, J.F. (1983). Catecholamine metabolism in the rat locus coeruleus as studied by in vivo differential pulse voltammetry. II. Pharmacological and behavioral study. *Brain Research*, *273*(2), 207–16.
- Grandoso, L., Pineda, J., Ugedo, L. (2004). Comparative study of the effects of desipramine and reboxetine on locus coeruleus neurons in rat brain slices. *Neuropharmacology*, *46*(6), 815–23.
- Harkness, K.L., Theriault, J.E., Stewart, J.G., Bagby, R.M. (2014). Acute and chronic stress exposure predicts 1-year recurrence in adult outpatients with residual depression symptoms following response to treatment. *Depress Anxiety*, *31*(1), 1–8.
- Hermans, E.J., Henckens, M.J.A.G., Joels, M., Fernandez, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in Neurosciences*, *37*(6), 304–14.
- Hernaus, D., Casales Santa, M.M., Offermann, J.S., Van Amelsvoort, T. (2017). Noradrenaline transporter blockade increases fronto-parietal functional connectivity relevant for working memory. *European Neuropsychopharmacology*, *27*(4), 399–410.
- Holly, E.N., Miczek, K.A. (2016). Ventral tegmental area dopamine revisited: effects of acute and repeated stress. *Psychopharmacology (Berl)*, *233*(2), 163–86.
- Kessler, R.C., Ustun, T.B. (2004). The World Mental Health (WMH) survey initiative version of the World Health Organization (WHO) Composite International Diagnostic Interview (CIDI). *International Journal of Methods Psychiatry Research*, *13*(2), 93–121.
- Koda, K., Ago, Y., Cong, Y., Kita, Y., Takuma, K., Matsuda, T. (2010). Effects of acute and chronic administration of atomoxetine and methylphenidate on extracellular levels of noradrenaline, dopamine and serotonin in the prefrontal cortex and striatum of mice. *Journal of Neurochemistry*, *114*(1), 259–70.
- Linner, L., Endersz, H., Ohman, D., Bengtsson, F., Schalling, M., Svensson, T.H. (2001). Reboxetine modulates the firing pattern of dopamine cells in the ventral tegmental area and selectively increases dopamine availability in the prefrontal cortex. *Journal of Pharmacology and Experimental Therapeutics*, *297*(2), 540–6.
- Mehta, M.A., Owen, A.M., Sahakian, B.J., Mavaddat, N., Pickard, J.D., Robbins, T.W. (2000). Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. *Journal of Neuroscience*, *20*(6), RC65.
- Mier, D., Kirsch, P., Meyer-Lindenberg, A. (2010). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Molecular Psychiatry*, *15*(9), 918–27.
- Mostert, J.C., Onnink, A.M., Klein, M., et al. (2015). Cognitive heterogeneity in adult attention deficit/hyperactivity disorder: a systematic analysis of neuropsychological measurements. *European Neuropsychopharmacology*, *25*(11), 2062–74.
- Murphy, B.L., Arnsten, A.F., Goldman-Rakic, P.S., Roth, R.H. (1996). Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proceedings of the National Academy of Sciences USA*, *93*(3), 1325–9.
- Nater, U.M., Rohleder, N., Gaab, J., et al. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology*, *55*(3), 333–42.
- Oei, N.Y.L., Everaerd, W.T.A.M., Elzinga, B.M., Van Well, S., Bermond, B. (2006). Psychosocial stress impairs working memory at high loads: an association with cortisol levels and memory retrieval. *Stress—the International Journal on the Biology of Stress*, *9*(3), 133–41.
- Owen, A.M., McMillan, K.M., Laird, A.R., Bullmore, E. (2005). N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. *Human Brain Mapping*, *25*(1), 46–59.
- Pesonen, A.K., Kajantie, E., Jones, A., et al. (2011). Symptoms of attention deficit hyperactivity disorder in children are associated with cortisol responses to psychosocial stress but not with daily cortisol levels. *Journal of Psychiatric Research*, *45*(11), 1471–6.
- Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*, *28*(7), 916–31.
- Pruessner, J.C., Champagne, F., Meaney, M.J., Dagher, A. (2004). Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [<sup>11</sup>C]raclopride. *Journal of Neuroscience*, *24*(11), 2825–31.
- Qin, S., Hermans, E.J., van Marle, H.J., Luo, J., Fernandez, G. (2009). Acute psychological stress reduces working memory-related activity in the dorsolateral prefrontal cortex. *Biological Psychiatry*, *66*(1), 25–32.
- Qin, S., Cousijn, H., Rijpkema, M., et al. (2012). The effect of moderate acute psychological stress on working memory-related neural activity is modulated by a genetic variation in catecholaminergic function in humans. *Frontiers in Integrative Neuroscience*, *6*, 16.
- Quaedflieg, C.W.E.M., van de Ven, V., Meyer, T., et al. (2015). Temporal dynamics of stress-induced alternations of intrinsic amygdala connectivity and neuroendocrine levels. *PLoS One*, *10*(5), e0124141.
- Robbins, T.W., Arnsten, A.F. (2009). The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. *Annual Review of Neuroscience*, *32*(1), 267–87.
- Schoofs, D., Wolf, O.T., Smeets, T. (2009). Cold pressor stress impairs performance on working memory tasks requiring executive functions in healthy young men. *Behavioural Neuroscience*, *123*(5), 1066–75.
- Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., et al. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59* (Suppl 20), 22–33, quiz 34–57.
- Shine, J.M., van den Brink, R.L., Hernaus, D., Nieuwenhuis, S., Poldrack, R.A. Catecholaminergic manipulation alters dynamic network topology across behavioral states. *BioRxiv*, 169102. doi: <https://doi.org/10.1101/169102>.
- Smeets, T., Cornelisse, S., Quaedflieg, C.W., Meyer, T., Jelicic, M., Merckelbach, H. (2012). Introducing the Maastricht Acute Stress Test (MAST): a quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses. *Psychoneuroendocrinology*, *37*(12), 1998–2008.

- Smyth, J.M., Ockenfels, M.C., Gorin, A.A., et al. (1997). Individual differences in the diurnal cycle of cortisol. *Psychoneuroendocrinology*, *22*(2), 89–105.
- Vaessen, T., Hernaus, D., Myin-Germeys, I., van Amelsvoort, T. (2015). The dopaminergic response to acute stress in health and psychopathology: a systematic review. *Neuroscience and Biobehavioural Reviews*, *25*, 241–51.
- Wand, G.S., Oswald, L.M., McCaul, M.E., et al. (2007). Association of amphetamine-induced striatal dopamine release and cortisol responses to psychological stress. *Neuropsychopharmacology*, *32*(11), 2310–20.
- Warren, C.M., van den Brink, R.L., Nieuwenhuis, S., Bosch, J.A. (2017). Norepinephrine transporter blocker atomoxetine increases salivary alpha amylase. *Psychoneuroendocrinology*, *78*, 233–6.
- Watson, D., Clark, L.A., Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology*, *54*(6), 1063–70.
- Witcher, J.W., Long, A., Smith, B., et al. (2003). Atomoxetine pharmacokinetics in children and adolescents with attention deficit hyperactivity disorder. *Journal of Child and Adolescent Psychopharmacology*, *13*(1), 53–63.