



## Short Communication

## Norepinephrine transporter blocker atomoxetine increases salivary alpha amylase



Christopher M. Warren<sup>a,b,\*,1</sup>, Ruud L. van den Brink<sup>a,b</sup>, Sander Nieuwenhuis<sup>a,b</sup>,  
Jos A. Bosch<sup>c,d,e</sup>

<sup>a</sup> Institute of Psychology, Leiden University, Leiden 2333 AK, Netherlands

<sup>b</sup> Leiden Institute for Brain and Cognition, Leiden University, Leiden 2300 RC, Netherlands

<sup>c</sup> Department of Clinical Psychology, University of Amsterdam, Amsterdam 1018 WT, Netherlands

<sup>d</sup> Mannheim Institute of Public Health, Heidelberg University, Mannheim 68167, Germany

<sup>e</sup> Department of Medical Psychology, Academic Medical Center Amsterdam, Amsterdam, 1105 AZ, Netherlands

## ARTICLE INFO

## Article history:

Received 17 August 2016

Received in revised form

22 December 2016

Accepted 23 January 2017

## Keywords:

Atomoxetine

Alpha amylase

Noradrenaline

Sympathetic

Yohimbine

Locus-coeruleus

Noradrenergic

## ABSTRACT

It has been suggested that central norepinephrine (NE) activity may be inferred from increases in salivary alpha-amylase (SAA), but data in favor of this proposition are limited. We administered 40 mg of atomoxetine, a selective NE transporter blocker that increases central NE levels, to 24 healthy adult participants in a double-blind, placebo-controlled cross-over design. Atomoxetine administration significantly increased SAA secretion and concentrations at 75–180 min after treatment (more than doubling baseline levels). Consistent with evidence that elevation in central NE is a co-determinant of hypothalamic-pituitary-adrenal axis activity, salivary cortisol also approximately doubled at the same time points. Moreover, changes in salivary cortisol positively correlated with SAA ( $0.44 < \rho < 0.56$ ), bolstering the position that the origin of the changes in SAA reflect central NE. This work points toward the potential value of SAA as an inexpensive and non-invasive procedure to obtain information about activation of the central NE system.

© 2017 Elsevier Ltd. All rights reserved.

## 1. Introduction

Norepinephrine (NE) is one of the most widely distributed neuromodulators in the mammalian brain, playing a central role in many cognitive processes, and disturbances of the NE system have been implicated in a variety of neuro-psychiatric conditions (Berridge and Waterhouse, 2003). However, a recognized limitation to further development of functional theory and translation into clinical practice is the absence of practical markers of central NE activity for use in human subjects.

Ehlert et al. (2006) have proposed salivary alpha amylase (SAA) as a candidate marker of central NE activity. SAA is a digestive enzyme that is released by the salivary glands in response to stimulation by the local sympathetic nerves. This activity, in turn, has the

central locus coeruleus-norepinephrine system as a main determinant (Dunn et al., 2004). In a placebo-controlled design, Ehlert et al. (2006) observed copious release of SAA in 13 participants administered with yohimbine, an  $\alpha$ 2-adrenergic receptor antagonist that increases peripheral and central NE. Based on the observation that yohimbine-induced increases in SAA did not correlate with peripheral release of plasma NE, Ehlert et al. reasoned that SAA more likely reflects the actions of central NE activity. While this is an intriguing and potentially important findings, currently it reflects the only evidence to support the notion of SAA as a marker of central NE.

Complicating the interpretation of the above data is that yohimbine also has strong parasympathetic effects on the salivary glands, through blocking of inhibitory  $\alpha$ 2 receptors on pre- and post-ganglionic salivatory parasympathetic neurons (Samuels and Szabadi, 2008). This effect is evident in a large enhancement of salivary flow rate (e.g. Bagheri et al., 1997), which was also observed by Ehlert and colleagues. These parasympathetic effects present a potential confound for at least two reasons: first, release of SAA is partially under parasympathetic control, i.e., independent of central regulation of the local sympathetic nerves (Bosch et al., 2011; Proctor and Carpenter, 2007). Second, such parasympathetic effects on SAA release are difficult to detangle from sympathetic effects,

\* Corresponding author at: Institute of Psychology, Leiden University, Leiden 2333 AK, The Netherlands.

E-mail addresses: [c.m.warren@fsw.leidenuniv.nl](mailto:c.m.warren@fsw.leidenuniv.nl), [cwarren2@bowdoin.edu](mailto:cwarren2@bowdoin.edu) (C.M. Warren).

<sup>1</sup> Present address: Bowdoin College, Department of Psychology and Program in Neuroscience, Brunswick, ME 04011, United States.

as both autonomic branches co-determine SAA release in a non-additive, interactive manner (Proctor and Carpenter, 2007; Bosch et al., 2011; Nagy et al., 2015). Thus, SAA release by yohimbine involves a mix of sympathetic/NE and parasympathetic/cholinergic effects (Berntson et al., 2016).

A possible further complication is that the inference that SAA release may involve the actions of central NE was based on negative evidence: i.e., the lack of an association with peripheral catecholamines (Ehlert et al., 2006). However, an absent or weak association between measures of peripheral sympathetic-adrenomedullary (SAM) activity is a rather common observation, although there exist little doubts about their shared central determinants, and the interpretation of a non-association is thus somewhat uncertain (Bosch et al., 2011, 2009). Moreover, later reanalysis of the same data showed that peripheral noradrenaline and SAA were, in fact, highly correlated (Ditzen et al., 2014). Thus the proposal by Ehlert et al. (2006) would seem to benefit from additional, positive validation.

In light of the points detailed above, the present study sought to examine the relationship between central NE and SAA using atomoxetine, a selective NE transporter blocker that raises central NE while exhibiting few side effects compared to other noradrenergic drugs (Bymaster et al., 2002; Chamberlain et al., 2007).

We administered 40 mg of atomoxetine to 24 healthy adult participants in a randomized, double-blind, placebo-controlled, cross-over design to determine if NE reuptake inhibition would increase SAA release. Relevant to the concerns raised above, we also predicted that increases in SAA release would positively correlate with elevated salivary cortisol. Underlying this prediction is the observation that central (but not peripheral) NE activity is associated with release of cortisol (Bosch et al., 2009); a glucocorticoid stress hormone that is released upon activation of the hypothalamic-pituitary-adrenal (HPA) axis, which is anatomically and functionally distinct from the SAM system (Bosch et al., 2009; Hill et al., 2003). This effect of central NE is mediated, in part, by noradrenergic inputs to the hypothalamus (Hill et al., 2003). Consistent with the notion that both SAA and salivary cortisol increases are driven by a common central NE determinant, Chamberlain et al. (2007) demonstrated that a 60-mg dose of atomoxetine increases salivary cortisol, and a similar effect has been shown for other NE reuptake inhibitors (Hill et al., 2003).

## 2. Methods

### 2.1. Participants

Twenty-four young adult participants (age range 19–26, 5 male) were recruited through campus advertisement as part of a larger neuroimaging study (van den Brink et al., 2016). Participants were screened by a physician for physical health, drug contraindications, and other exclusion criteria (see Supplementary material). Thirteen of the female participants reported currently using oral contraception. Participants were paid 135 euros for two sessions of 210 min each. All participants gave informed consent, and the study was approved by the Leiden University medical ethics committee.

### 2.2. Procedure

Atomoxetine and placebo were administered in separate sessions in randomized order, spaced one week apart, and scheduled at the same time of day. Atomoxetine was administered orally as a single, encapsulated 40-mg pill, and ingested with water. The placebo consisted of carrier only and was identical in appearance to the active drug. Saliva samples were collected five times per session, beginning immediately before taking the pill ( $T = 0$ ), at  $T = 30$ ,

$T = 75$ ,  $T = 105$ , and  $T = 180$  min after pill ingestion. These time points are consonant with the pharmacokinetics of atomoxetine, which reaches a plateau in blood plasma levels after approximately 90 min and remains elevated for up to 9 h (Sauer et al., 2005). In between samples, participants intermittently rested and participated in a resting-state neuroimaging study. We note that undergoing fMRI assessment, which was part of the larger study, may have provoked a stress response in some of our subjects, which might have interacted with the effects of the drug (see Supplementary material). However, our counterbalanced, randomized, cross-over design would be expected to adequately eliminate any potential of a confounding effect of stress on SAA.

### 2.3. Saliva sample collection and processing

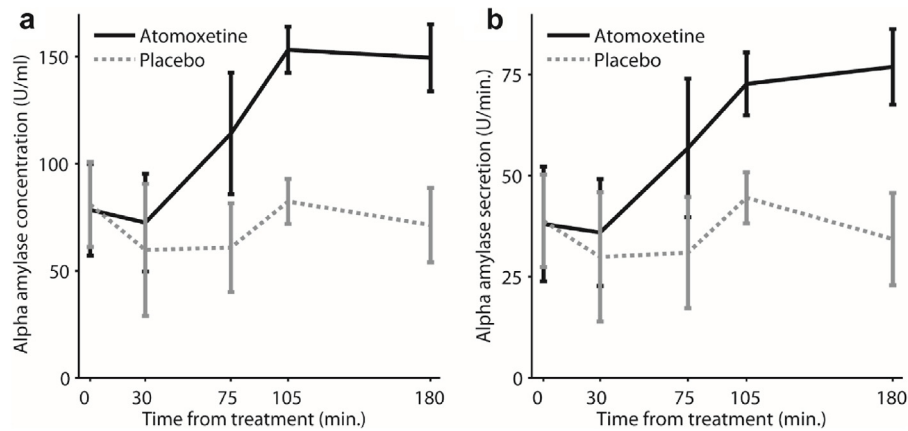
Whole saliva was collected using the spitting method, whereby participants were asked to let saliva collect passively on the floor of their mouth, and to spit the accumulated saliva into a polypropylene tube every minute over a three-minute period (Beltzer et al., 2010; Nagy et al., 2015). This method does not elicit confounding flow rate effects (e.g., through mechanical or gustative stimulation) and avoids biasing effects of absorbent materials (Bosch et al., 2011). Fresh samples were stored on ice for a maximum of two hours, homogenized using a vortex mixer, and centrifuged at room temperature for 4 min at  $4000 \times g$  to eliminate debris and bacteria. The clear supernatant was aliquoted and frozen at  $-60^\circ\text{C}$  until the assay procedure. Samples were assayed for alpha amylase using a quantitative kinetic determination kit (IBL, Hamburg, Germany), essentially as described elsewhere (Nagy et al., 2015). The assay has a sensitivity of 12.5 U/ml, and an intra-assay variability (CV%) of 3.3%. Samples were assayed for cortisol using a competitive enzyme-linked immunosorbent assay, according to the manufacturer's instructions (IBL, Hamburg, Germany), with a sensitivity of 0.045  $\mu\text{g/mL}$ , and intra-assay variability (CV%) of 2.5%. All samples of the same participant were assayed simultaneously.

### 2.4. Statistical analysis

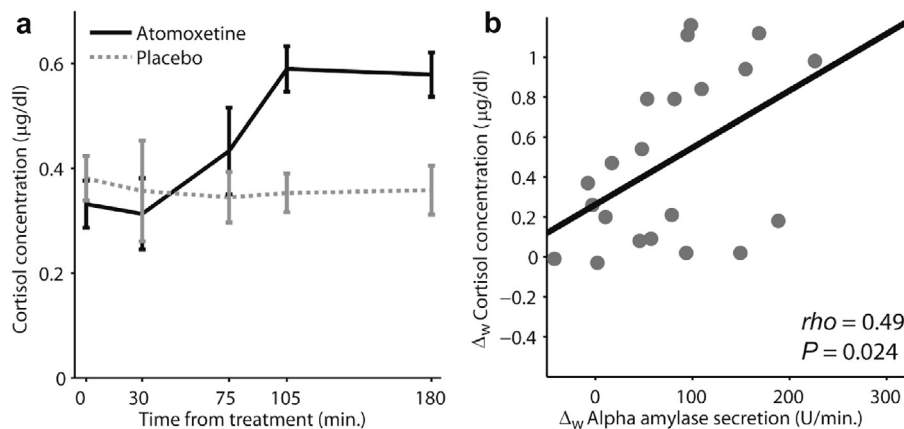
Saliva data were analysed using a mixed ANOVA with treatment and time as within-subjects factors and treatment order as a between-subjects factor. Including use of oral contraception and sex as categorical variables in the omnibus ANOVA yielded no significant effects of these variables, and did not meaningfully change the reported effects of treatment. Missing data points (e.g., insufficient saliva) were estimated through interpolation by taking the mean of the immediately preceding and subsequent time point (Chamberlain et al., 2007). In the case of a missing baseline ( $T = 0$ ), the baseline value from the other session was used.<sup>2</sup> In all other instances, data points were entered as missing. For SAA, 10/240 points were interpolated and 9/240 were missing, resulting in 21 participants being included in the omnibus ANOVA. For cortisol, 3/240 points were interpolated and 10/240 point were missing, resulting in 22 participants being included in the omnibus ANOVA.

In examining the relationship between drug effects on SAA and salivary cortisol, we computed difference ( $\Delta$ ) scores in two ways. To examine within-session drug effects (drug effect over time), difference scores were calculated by subtracting the baseline ( $T = 0$ ) measure in the atomoxetine condition from the peak value after

<sup>2</sup> In repeated-measures ANOVAs, a single missing data point will result in the elimination of all data from that subject from the analysis. Consequently, ANOVAs without imputation resulted in a large reduction in total N. As a result, some of the interactions reported did not reach significance, although effect sizes remained nearly identical. The analysis of simple main effects, most central to supporting the conclusions of this paper, remained significant without imputation.



**Fig. 1.** Effect of atomoxetine on salivary alpha amylase (SAA). Time course of treatment and placebo effects on (a) SAA concentrations and (b) SAA secretion (concentration x flow rate). Error bars reflect SEM.



**Fig. 2.** (a) Effect of atomoxetine on salivary cortisol and (b) relationship between treatment effects on salivary measures of alpha amylase and cortisol. Error bars reflect SEM. Delta values were calculated by subtracting baseline from peak values after treatment.

treatment. To examine between-session drug effects (difference between drug and placebo) difference scores were calculated by subtracting mean concentrations from samples 3–5 (for which significant treatment effects were observed, see below) in the placebo session from those in the atomoxetine session.

### 3. Results

#### 3.1. Salivary alpha amylase concentration and secretion

Atomoxetine increased SAA concentration over time, as compared to the placebo condition, yielding a significant time by treatment interaction,  $F(4,72)=9.03$ ,  $p<0.001$  (Fig. 1a). This interaction was largely driven by a steep increase in SAA in the atomoxetine condition. Post-hoc analyses showed that this increase was significantly greater than placebo at  $T=75$  ( $t(22)=3.66$ ,  $p=0.001$ ), and remained larger through to the final sample,  $T=105$  ( $t(22)=3.43$ ,  $p=0.002$ ), and  $T=180$  ( $t(20)=5.97$ ,  $p<0.001$ ).

The above findings were replicated after adjusting for salivary flow rate [SAA secretion=SAA concentration X flow rate] (see Supplementary material). Atomoxetine also increased SAA secretion, resulting in a significant time by treatment interaction,  $F(4,72)=5.49$ ,  $p=0.001$  (Fig. 1b). As with the concentration data, the effect of atomoxetine on SAA secretion was significantly different from placebo at  $T=75$  ( $t(22)=2.89$ ,  $p=0.009$ ), and held through  $T=105$  ( $t(22)=2.60$ ,  $p=0.016$ ), and  $T=180$  ( $t(20)=4.37$ ,  $p<0.001$ ).

#### 3.2. Cortisol concentrations

Atomoxetine likewise increased salivary cortisol over time relative to the placebo condition, yielding a significant time by condition interaction,  $F(4,84)=8.01$ ,  $p<0.001$  (Fig. 2a). Post-hoc analyses again showed that treatment effects on salivary cortisol concentration became apparent at  $T=75$  ( $t(21)=2.55$ ,  $p=0.018$ ),  $T=105$ , ( $t(21)=3.57$ ,  $p=0.002$ ), and at  $T=180$  ( $t(21)=4.16$ ,  $p<0.001$ ).

#### 3.3. Relationship between salivary alpha amylase and cortisol

We were interested in whether the drug-induced effect on SAA would correlate with the drug-induced effect on cortisol. The Spearman rank correlation ( $\rho$ ) between within-session treatment effects on SAA secretion and cortisol concentration was significant,  $\rho=0.49$ ,  $p=0.024$  (Fig. 2b). Additional figures presented in the online supplement demonstrate a robust relationship between SAA and salivary cortisol: the relationship was also significant when SAA secretion was replaced by SAA concentration,  $\rho=0.44$ ,  $p=0.047$ , (Fig. S1a), and when correlating between-session treatment effects,  $\rho=0.52$ ,  $p=0.017$  (Fig. S1b).

### 4. Discussion

The present study aimed to investigate the effects of the NE transporter blocker atomoxetine on SAA. The results showed, for

the first time, that atomoxetine enhances SAA concentration and secretion. These results present a complimentary test of the idea, first advanced by Ehlert et al. (2006), that SAA measures can be used to infer central NE activity. The current results also replicated and extended work from Chamberlain et al. (2007), showing that atomoxetine increased salivary cortisol at a dose lower than used previously (40 mg vs. 60 mg). In line with predictions, the atomoxetine-induced changes in SAA concentration and secretion correlated with changes in salivary cortisol, comparing both within-session and between-session treatment effects. This robust association between biological markers that reflect two physiologically and anatomically distinct stress systems, but share central NE as a common determinant, presents strong convergent evidence that increases in SAA reflect elevations in central noradrenergic activity.

The current study also improved upon prior work by Ehlert et al. (2006) by using a drug that has very limited, if any, acute cholinergic/parasympathetic effects. Ehlert et al. used yohimbine, a drug with strong cholinergic potency, which has even led to its use as a drug to alleviate pathological dry mouth symptoms (Bagheri et al., 1997). Atomoxetine is regarded as a highly selective drug with few side effects (Bymaster et al., 2002; Chamberlain et al., 2007). However, prolonged use of NE transporter blockers has been reported to increase the sensation of dry mouth and possibly also reduced salivation in approximately 20% of users (Samuels and Szabadi, 2008). These effects were observed, however, after four weeks of daily doses of 60 mg/day to 120 mg/day. We are not aware of similar reports after acute administration of a lower dose of atomoxetine, and our data provided little evidence of salivation effects. Therefore, confounding parasympathetic effects enhancing SAA secretion directly or via augmentation of peripheral sympathetic/noradrenergic effects, seem unlikely (cf. Bosch et al., 2011).

The absence of any increase in salivary flow rate in this study rules out an effect of cholinergic/parasympathetic nerves on SAA. This implies that increased central NE is mediating the increase in SAA predominantly through activation of the sympathetic nerves that innervate the saliva glands, which is supported by extensive neuro-anatomical data showing dense projections of relevant centers (e.g., locus coeruleus) onto pre-ganglionic sympathetic nuclei (Samuels and Szabadi, 2008).

## 5. Conclusions

This work points toward the potential value of SAA as an inexpensive and non-invasive procedure to obtain information about activation of the central NE system. The work implies that SAA may be used to mark endogenous fluctuations in central noradrenergic activation in a manner similar and complimentary to pupil dilation (c.f. Warren et al., 2016), thus providing a valuable tool for testing theories of the function and malfunction of the noradrenergic system. Further research, validating these findings in other contexts, and using other manipulations, possibly complemented with additional assays of glandular proteins sensitive to autonomic activity, (cf. Proctor and Carpenter, 2002), seems both warranted and necessary to bring this potential to fruition (Bosch et al., 2011; Nagy et al., 2015).

## Contributors

CMW, SN, and JAB designed the study. CMW and RLvdB collected the data. CMW, RLvdB, and JAB processed the data. CMW analyzed the data. CMW and RLvdB made the figures. All authors contributed to writing the paper, and approved the final version. This work was supported by a Consolidator Grant of the European Research Council.

## Conflicts of interest

None.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.psyneuen.2017.01.029>.

## References

- Bagheri, H., Schmitt, L., Berlan, M., Montastruc, J.L., 1997. A comparative study of the effects of yohimbine and anetholtrithione on salivary secretion in depressed patients treated with psychotropic drugs. *Eur. J. Clin. Pharmacol.* 52 (5), 339–342.
- Beltzer, E.K., Fortunato, C.K., Guaderrama, M.M., Peckins, M.K., Garramone, B.M., Granger, D.A., 2010. Salivary flow and alpha-amylase: collection technique, duration, and oral fluid type. *Physiol. Behav.* 101 (2), 289–296.
- Berntson, G.G., Cacioppo, J.T., Bosch, J.A., 2016. From homeostasis to alldynamic regulation. In: Cacioppo, J.T., Berntson, G.G., Tassinari, L.G. (Eds.), *Handbook of Psychophysiology*, 4th edition. Cambridge University Press, Cambridge, pp. 379–400.
- Berridge, C.W., Waterhouse, B.D., 2003. The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res. Rev.* 42 (1), 33–84.
- Bosch, J.A., de Geus, E.J., Carroll, D., Goedhart, A.D., Anane, L.A., van Zanten, J.J.V., Helmerhorst, E.J., Edwards, K.M., 2009. A general enhancement of autonomic and cortisol responses during social evaluative threat. *Psychosom. Med.* 71 (8), 877–885.
- Bosch, J.A., Veerman, E.C., de Geus, E.J., Proctor, G.B., 2011.  $\alpha$ -Amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet! *Psychoneuroendocrinology* 36 (4), 449–453.
- Bymaster, F.P., Katner, J.S., Nelson, D.L., Hemrick-Luecke, S.K., Threlkeld, P.G., Heiligenstein, J.H., Morin, S.M., Donald, M.S., Gehlert, D.R., Perry, K.W., 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.
- Chamberlain, S.R., Muller, U., Cleary, S., Robbins, T.W., Sahakian, B.J., 2007. Atomoxetine increases salivary cortisol in healthy volunteers. *J. Psychopharmacol. (Oxf.)* 21, 545–549.
- Ditzen, B., Ehlert, U., Nater, U.M., 2014. Associations between salivary alpha-amylase and catecholamines—a multilevel modeling approach. *Biol. Psychol.* 103, 15–18.
- Dunn, A.J., Swiergiel, A.H., Palamarchouk, V., 2004. Brain circuits involved in corticotropin-releasing factor–norepinephrine interactions during stress. *Ann. N. Y. Acad. Sci.* 1018 (1), 25–34.
- Ehlert, U., Erni, K., Hebisch, G., Nater, U., 2006. Salivary  $\alpha$ -amylase levels after yohimbine challenge in healthy men. *J. Clin. Endocrinol. Metab.* 91 (12), 5130–5133.
- Hill, S.A., Taylor, M.J., Harmer, C.J., 2003. Acute reboxetine administration increases plasma and salivary cortisol. *J. Psychopharmacol.* 17 (3), 273–275.
- Nagy, T., van Lien, R., Willemsen, G., Proctor, G., Efting, M., Fülöp, M., Bárdos, G., Veerman, E.C.I., Bosch, J.A., 2015. A fluid response: Alpha-amylase reactions to acute laboratory stress are related to sample timing and saliva flow rate. *Biol. Psychol.* 109, 111–119.
- Proctor, G.B., Carpenter, G.H., 2002. Neural control of salivary S-1gA secretion. *Int. Rev. Neurobiol.* 52, 187–212.
- Proctor, G.B., Carpenter, G.H., 2007. Regulation of salivary gland function by autonomic nerves. *Auton. Neurosci.* 133 (1), 3–18.
- Samuels, E.R., Szabadi, E., 2008. Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part II: physiological and pharmacological manipulations and pathological alterations of locus coeruleus activity in humans. *Curr. Neuropharmacol.* 6 (3), 254–285.
- Sauer, J.M., Ring, B.J., Witcher, J.W., 2005. Clinical pharmacokinetics of atomoxetine. *Clin. Pharmacokinet.* 44 (6), 571–590.
- Warren, C.M., Eldar, E., van den Brink, R.L., Tona, K.D., van der Wee, N.J., Giltay, E.J., van Noorden, M.S., Bosch, J.A., Wilson, R.C., Cohen, J.D., Nieuwenhuis, S., 2016. Catecholamine-mediated increases in neural gain improve the precision of cortical representations? *J. Neurosci.* 36 (21), 5699–5708.
- van den Brink, R.L., Pfeffer, T., Warren, C.M., Murphy, P.R., Tona, K.D., van der Wee, N.J., Giltay, E.J., van Noorden, M.S., Rombouts, S.A., Donner, T.H., Nieuwenhuis, S., 2016. Catecholaminergic neuromodulation shapes intrinsic MRI functional connectivity in the human brain. *J. Neurosci.* 36 (30), 7865–7876.