Cortisol Stress Response and in Vivo PET Imaging of Human Brain Serotonin 1A Receptor Binding

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Abstract

Background: Abnormalities in the hypothalamic-pituitary-adrenal axis, serotonergic system, and stress response have been linked to the pathogenesis of major depressive disorder. State-dependent hyper-reactivity of the hypothalamic-pituitary-adrenal axis is seen in major depressive disorder, and higher binding to the serotonin 1A receptor is observed as a trait in both currently depressed and remitted untreated major depressive disorder. Here, we sought to examine whether a relationship exists between cortisol secretion in response to a stressor and serotonin 1A receptor binding throughout the brain, both in healthy controls and participants with major depressive disorder.

Methods: Research participants included 42 medication-free, depressed subjects and 31 healthy volunteers. Participants were exposed to either an acute, physical stressor (radial artery catheter insertion) or a psychological stressor (Trier Social Stress Test). Levels of serotonin 1A receptor binding on positron emission tomography with [11C]WAY-100635 were also obtained from all participants. The relationship between [11C]WAY-100635 binding and cortisol was examined using mixed linear effects models with group (major depressive disorder vs control), cortisol, brain region, and their interactions as fixed effects and subject as a random effect.

Results: We found a positive correlation between post-stress cortisol measures and serotonin 1A receptor ligand binding levels across multiple cortical and subcortical regions, independent of diagnosis and with both types of stress. The relationship between [11C]WAY-100635 binding and cortisol was homogenous across all a priori brain regions. In contrast, resting cortisol levels were positively correlated with serotonin 1A receptor ligand binding levels independently of diagnosis, except in the RN. There was no significant difference in cortisol between major depressive disorder participants and healthy volunteers with either stressor. Similarly, there was no correlation between cortisol and depression severity in either stressor group.
Conclusions: This study suggests that there may be a common underlying mechanism that links abnormalities in the serotonin system and hypothalamic-pituitary-adrenal axis hyper-reactivity to stress. Future studies need to determine how hypothalamic-pituitary-adrenal axis dysfunction affects mood to increase the risk of suicide in major depression.

Keywords: cortisol, PET imaging, serotonin, stress
of 2 stressors: (1) arterial line placement before a PET scan (n = 34) or (2) the TSST (n = 39). Participants from stress paradigm (1) had cortisol assayed in a blood sample drawn immediately after their arterial line was placed, while participants of paradigm (2) had salivary cortisol assayed in samples collected at multiple time-points during the TSST. The PET data presented here were previously reported in published studies (Parsey et al., 2006a, 2006b, 2010; Miller et al., 2009b), but the cortisol data have never been published. Subject selection was based on the availability of usable [11C]-WAY-100635 brain binding data and either plasma cortisol or TSST saliva cortisol stress sample measurements.

 Forty-two depressed participants (25 female, 17 male) aged 18 to 62 years who met DSM-IV criteria for MDD in a current major depressive episode (as assessed by doctoral- or masters’-level psychologists and reviewed in a consensus conference of research psychologists and psychiatrists), a 17-item Hamilton Depression Rating Scale (HDRS) score REF ≥16, and capacity to provide informed consent were included in the analysis. Depression severity was assessed with the 24-item HDRS and a 1.5-T Signa Advantage (General Electric Medical Systems, Milwaukee, WI) at a resolution of 1.5 × 9 × 1.0 mm.

PET images were acquired with an ECAT EXACT HR+ scanner (Siemens/CTI, Knoxville, TN) as previously detailed (Parsey et al., 2000). Briefly, after a 15-minute transmission scan, an injection of [11C]-WAY-100635 was administered over 30 seconds and then an emission scan of 110 minutes, consisting of 20 frames of increasing duration (3 × 20 seconds, 3 × 1 minute, 3 × 2 minutes, 2 × 5 minutes, 9 × 10 minutes), was obtained.

To correct for subject motion, each PET frame was registered to the eighth frame of the scan using the FMRIB linear image registration tool, version 5.0 (FMRIB Image Analysis Group, Oxford, UK). Brain regions of interest (ROIs) were chosen a priori based on areas of abundant [11C]-WAY-100635 binding (Hall et al., 1997) that included raphe nuclei (RN), anterior cingulate, cingulate, dorsal prefrontal cortex, hippocampus, insula, medial prefrontal cortex, parietal cortex, parahippocampal gyrus, occipital cortex, orbital cortex, temporal cortex, and amygdala. Cerbellar white matter was used as a reference region. All ROIs except for RN were identified on each individual’s T1-weighted MRI using a previously described automated algorithm (Milak et al., 2010). Due to their small size, RN were labeled using a standard space mask of the average location of the RN in 52 healthy subjects, which was created using [11C]-WAY-100635 voxel binding maps as previously described (Delorenzo et al., 2013). MRI T1 images were transformed into standardized 3D space using Advanced Normalization Tools (7), and the reverse transform was applied to the RN mask.

PET images were co-registered to the MRI images using the FMRIB linear image registration tool, optimized as previously described (Delorenzo et al., 2009). The average activity measured over the voxels within each ROI over the specified time frames through the course of the acquisition generated time activity curves.

Outcome Measure Estimation

Distribution volumes (V of [11C]-WAY-100635 were estimated for each ROI using kinetic analysis with an arterial input function and a 2-tissue compartment constrained model as previously described (Parsey et al., 2000). Time activity curves were fit with a 2-tissue compartment constrained model in which the K/k, ratio was constrained to that of the reference region (cerebellar white matter) for each ROI. BP, was calculated as (V – V)/f, where V is the volume of distribution in a specific ROI, V is the volume of distribution in the reference region, and f is the free fraction in plasma.

Cortisol Measurement

Blood cortisol was measured in blood samples drawn from 34 subjects immediately prior to PET scans and just after the arterial line was inserted. Given the diurnal variation of cortisol, all these blood samples were drawn within a 2-hour time window between 12 PM and 2 PM. In addition, we adjusted statistically for time of day when samples were drawn. Blood cortisol levels were ascertained by radioimmunoassay (Vecsei, 1979) after denaturation of the binding proteins by heat. Both blood and saliva cortisol levels were measured by radioimmunoassay with antibodies for cortisol:3-O-carboxymethylxime-BSA (MP Biochemicals). This was compared to cortisol standards (Sigma Chemical). Free and bound fractions were separated using anti-rabbit globulin serum and polyethylene glycol. All samples and standards were analyzed in duplicate.

Subjects who underwent the TSST, which took place between 2 PM and 6 PM, gave saliva samples approximately 10 minutes
All subjects

(n = 31) (n = 42) (n = 73)

Table 1. Demographic and Clinical Characteristics of Combined Study Sample

|                              | Healthy Volunteers (n=31) | MDD (n=42) | All subjects (n=73) | P value |
|------------------------------|--------------------------|------------|---------------------|---------|
| Age ± SD                     | 35.3 ± 13.3               | 38.2 ± 12.6| 37 ± 12.9           | .032    |
| Hamilton Depression Rating Scale (24-item) | 2.5 ± 3.2               | 18.9 ± 13.5| <.001               |         |
| Beck Depression Inventory    | 1.9 ± 3.8                 | 20.8 ± 16  | <.001               |         |
| Years of Education           | 15.7 ± 2.1                | 15.2 ± 2.5 | 15.45 ± 3.2         |         |
| Female                       | 17 (54.8)                 | 25 (59.5)  | 42 (57.5)           | .68     |
| Tobacco users                | 3 (9.7)                   | 5 (11.9)   | 8 (11)              | .76     |
| Prior exposure to antidepressants | N/A                    | 29 (69)    | 29 (39.7)           |         |
| Suicide attempts             | N/A                       | 16 (38.1)  | 16 (21.9)           |         |
| Past alcohol abuse           | N/A                       | 7 (16.6)   | 7 (9.6)             |         |
| Comorbid anxiety disorder    | N/A                       | 19 (45.2)  | 19 (26)             | .09     |
| Race/ethnicity               |                          |            |                     |         |
| Asian                        | 5 (16.1)                  | 2 (4.8)    | 7 (8.3)             | .10     |
| African American             | 7 (22.6)                  | 5 (11.9)   | 14 (16.7)           | .22     |
| Caucasian                    | 15 (48.3)                 | 31 (73.8)  | 55 (65.5)           | .02     |
| Hispanic                     | 4 (12.9)                  | 10 (23.8)  | 14 (16.7)           | .22     |

TSST

Salivary cortisol response to social stress was measured in 39 subjects not overlapping with the subjects with blood cortisol measures during the PET scan: 29 MDD patients and 10 healthy volunteers. The TSST was administered as previously described (Melhem et al., 2016). In brief, subjects were asked to give a 5-minute personal introduction speech, followed by 9 minutes of speeded mental arithmetic, while being watched by 1 observer known to the subject and a staff member who was unknown to the subject.

Statistical Analysis

The associations between the cortisol responses to the 2 stressors and 5-HT1A binding measured in our a priori ROIs were examined separately for each stressor, each analysis using linear mixed effects models with group (MDD vs control), cortisol, brain region, and their interactions as fixed effects and time, sex, and their interaction as predictors. Cortisol measurements were also compared between healthy volunteers and participants with MDD using an ANCOVA analysis, and, in MDD patients, were correlated with 24-item HDRS and BDI scores.

Results

Participant Characteristics

Demographic and clinical data are presented in Table 1 for all participants combined. Demographic and clinical data are presented for the separate samples of subjects with blood cortisol and those who underwent the TSST in Tables 2 and 3, respectively. Of 42 MDD participants, 29 had previous exposure to antidepressants and 16 had previously attempted suicide, indicating high illness burden. For those with previous antidepressant exposure, the mean time off antidepressants was 49 ± 74 weeks (range 2–312 weeks).

Cortisol Stress Response in Healthy Volunteer and Major Depression Groups

There was no statistically significant difference in blood cortisol levels measured following radial artery catheter placement between control and MDD groups when covarying for age, sex, and blood sample time of day (F=3.70; df=1,31; P=.064). Salivary cortisol values were log-transformed. Cortisol response during the TSST for each subject was defined as the difference between the maximum (log-transformed) value after the baseline and the (log-transformed) baseline value. Baseline cortisol was also log-transformed.

Given that cortisol has a diurnal cycle (Wüst et al., 2000), we adjusted blood cortisol measurements during the PET scan for time of day relative to 12:00 PM, even though the correlation between blood cortisol and time of day was not significant in this dataset (P=.091). This adjustment, performed to remove the time trend, was based on fitting a linear regression model to the cortisol data with time as the only predictor. Log-transformed baseline cortisol before the TSST was similarly adjusted, although the time trend was not statistically significant. Cortisol response during the TSST was adjusted by a time trend that differed between males and females, using an ANCOVA model with time, sex, and their interaction as predictors. Cortisol measurements were also compared between healthy volunteers and participants with MDD using an ANCOVA analysis, and, in MDD patients, were correlated with 24-item HDRS and BDI scores.

3-way interaction between region, group, and cortisol measure was significant, brain region-wise analyses were performed to test the association between cortisol, subject group, and binding. Covariates that were not significant in any region were dropped from region-wise analyses.
baseline cortisol and cortisol response during the TSST also did not differ significantly between control and MDD groups when covarying for age, sex, time of day, and the sex by time interaction (F = 0.91; df = 1, 33; P = .348, baseline cortisol: F = 2.51; df = 1, 34; P = .122).

### Cortisol Stress Response and Depression Severity

Within the MDD sample, we did not find a relationship between PET scan blood cortisol levels and the BDI score (F = 0.485; df = 1, 8; P = .506) or the 24-item HDRS score (F = 0.455; df = 1, 8; P = .518), covarying for age, sex, and time of day in each case. The salivary cortisol response to TSST, covarying for age, sex, time of day, and their interaction, was not correlated with either 24-item HDRS score (F = 0.56; df = 1, 23; P = .462) or BDI score (F = 0.01; df = 1, 22; P = .936).

### Cortisol Stress Response and Brain \[^{11}C\]WAY-100635 BPF

\[^{11}C\]WAY-100635 binding across the ROIs, selected a priori (Figure 1), was positively related with blood cortisol levels drawn immediately prior to the scan, after accounting for age, sex, and diagnosis (F = 6.40; df = 1, 29; P = .017). The relationship between cortisol levels and \[^{11}C\]WAY100635 binding was homogenous across a priori brain regions, as the interaction term for brain region was not significant (F = 0.67; df = 12, 384; P = .777). For region-wise results, see supplemental Table 1. There was no significant interaction between diagnosis and cortisol level on \[^{11}C\]WAY-100635 binding (F = 3.77; df = 1, 28; P = .062). There was also no main effect of diagnosis after removing the interaction (F = 0.01; df = 1, 34; P = .988).

Similarly, we found the time-adjusted salivary cortisol response during TSST was positively related with \[^{11}C\]WAY100635 binding (F = 7.34; df = 1, 34; P = .003). This was also homogeneous across all a priori brain regions (region by cortisol response interaction: F = 0.93; df = 11, 374; P = .516). For region-wise results, please see supplemental Table 2. There was no significant interaction between diagnosis and cortisol response on \[^{11}C\]WAY-100635 binding (F = 0.21; df = 1, 33; P = .649). There was also no main effect of diagnosis after removing the interaction (F = 0.01; df = 1, 34; P = .988). There was a significant age by region interaction (F = 2.31; df = 11, 396; P = .009) and a significant sex by region interaction

| Table 2. Demographic Information for Blood Cortisol Subjects Only |
|---------------------------------------------------------------|
| Healthy Volunteers | MDD | All subjects | P value |
| (n = 21) | (n = 13) | (n = 34) | |
| Age ± SD | 34.3 ± 14.1 | 38.1 ± 13.6 | 35.8 ± 13.18 | .44 |
| Hamilton Depression Rating Scale (24-item) | 1 ± 1.2 | 24.8 ± 8.2 | 28.5 ± 11.9 | <.001 |
| Beck Depression Inventory | 1.9 ± 5 | 15.9 ± 2.5 | 15.1 ± 3.3 | <.001 |
| Years of education | n (%) | n (%) | n (%) | P value |
| Female | 11 (52.4) | 10 (76.9) | 21 (61.8) | .31 |
| Tobacco users | 2 (9.5) | 1 (7.7) | 3 (8.8) | .8 |
| Prior exposure to antidepressants | N/A | 9 (69.2) | 9 (26.5) | |
| Suicide attempters | N/A | 8 (61.5) | 8 (23.5) | |
| Past alcohol abuse | N/A | 4 (30.7) | 4 (11.8) | |
| Comorbid anxiety disorder | N/A | 6 (46.2) | 6 (17.6) | |
| Race/ethnicity | | | | .88 |
| Asian | 4 (19) | 1 (7.7) | 5 (14.7) | .36 |
| African American | 5 (23.8) | 1 (7.7) | 6 (17.6) | .23 |
| Caucasian | 9 (42.9) | 7 (53.8) | 16 (47.1) | .53 |
| Hispanic | 3 (14.3) | 4 (30.8) | 7 (20.6) | .24 |

| Table 3. Demographics of TSST Subjects |
|----------------------------------------|
| Healthy Volunteers | MDD | All subjects | P value |
| (n = 10) | (n = 29) | (n = 39) | |
| Age ± SD | 38.2 ± 11.7 | 38.2 ± 12.6 | 38.8 ± 12 | .85 |
| Hamilton Depression Rating Scale (24-item) | 2.6 ± 3 | 18.7 ± 10.7 | 14.6 ± 4.8 | <.001 |
| Beck Depression Inventory | 1.9 ± 2.4 | 20.5 ± 10.8 | 15.6 ± 12.5 | <.001 |
| Years of education | n (%) | n (%) | n (%) | |
| Female | 6 (60) | 15 (51.7) | 21 (53.8) | .65 |
| Tobacco users | 1 (10) | 2 (6.9) | 3 (7.7) | .75 |
| Prior exposure to antidepressants | N/A | 8 (27.6) | 3 (10.3) | |
| Suicide attempters | N/A | 3 (10.3) | 3 (10.3) | |
| Past alcohol abuse | N/A | 13 (44.8) | 13 (44.8) | |
| Comorbid anxiety disorder | | | | .64 |
| Race/ethnicity | | | | .64 |
| Asian | 1 (10) | 1 (3.4) | 2 (5.1) | .41 |
| African American | 2 (20) | 4 (13.8) | 6 (15.4) | .63 |
| Caucasian | 6 (60) | 24 (82.8) | 30 (76.9) | .14 |
| Hispanic | 1 (10) | 6 (20.7) | 7 (17.9) | .44 |
indicating differential effects of these demographic variables on binding across brain regions.

Baseline Cortisol and Brain $[^{11}C]WAY-100635$ BP$_r$

The association between baseline salivary cortisol measured before the TSST and $[^{11}C]WAY100635$ binding differed by region and diagnostic group (baseline cortisol by region by group interaction: $F=2.41; df=11,374; P=.007$). To interpret the interaction, we ran posthoc region-wise analyses adjusted for age, sex, and diagnostic group. The effects of the interaction terms of diagnostic group with baseline cortisol on $[^{11}C]WAY100635$ binding were not significant in any of the region-specific models and were removed. The main effect of diagnosis was not significant in any region. Baseline cortisol and $[^{11}C]WAY100635$ binding...
were negatively correlated in all regions (Figure 3; see supplemental Table 3 for coefficients by ROI) except in the RN (b = 0.18; SE = 0.11; t = −1.71; P = .098).

Discussion

Here we show a positive correlation between cortisol levels after two different types of stressors and 5-HT$_{1A}$ receptor binding on PET using [$^{11}$C]WAY-100635. This effect was observed across multiple brain regions. The fact that this observation held under 2 different stress paradigms speaks to the strength of this relationship. Conversely, a negative correlation was found between baseline salivary cortisol and [$^{11}$C]WAY-100635 binding in all a priori regions, except RN. We previously reported that stress-responsive disorders like MDD and PTSD are associated with higher 5-HT$_{1A}$ binding (Parsey et al., 2006a, 2010; Sullivan et al., 2013), although other studies have found no difference or the opposite (Yates and Ferrier, 1990; Lowther et al., 1997; Sargent et al., 2000; Bonne et al., 2005; Sullivan et al., 2015; Mann et al., 2017). In this study, the relationship of 5-HT$_{1A}$ binding to post-stress cortisol in blood and salivary samples was independent of diagnosis, indicating a mechanism that operates comparably in healthy volunteers and in patients with mood disorders. Consistent with this observation, within the MDD group the severity of current major depression was not correlated with either 5-HT$_{1A}$ binding or post-stress cortisol in either blood or salivary measures. Higher brain 5-HT$_{1A}$ receptor ligand binding is a biological trait observed in medication-free major depression during acute depression and during remission (Parsey et al., 2006a, 2006b; Miller et al., 2009b; Parsey et al., 2010) and is transmitted in families (Milak et al., 2018). What remains to be determined is whether there is a causal link between higher 5-HT$_{1A}$ binding and HPA axis overactivity or responsivity in depression.

A negative correlation was found between baseline salivary cortisol and [$^{11}$C]WAY-100635 binding in all a priori regions, except RN. This is consistent with previous data in depression and social anxiety disorder (Lanzenberger et al., 2010). That baseline cortisol has a weaker or no correlation with 5-HT$_{1A}$ binding in RN may be explained by RN being a small structure with noisier quantification. Moreover, because of this small size, RN is susceptible to partial volume effects and underestimation of binding. Alternatively, presynaptic 5-HT$_{1A}$ receptors (autoreceptors) in RN may be functionally distinct from post-synaptic 5-HT$_{1A}$ receptors such that expression of the latter may be more strongly modulated by baseline cortisol levels. The latter explanation is consistent with the observation that adrenalectomy has no effect on 5-HT$_{1A}$ receptor binding in RN (Le Corre et al., 1997; van Gaalen et al., 2002). Furthermore, animal work has shown that different types of stressors can lead to increased serotonin secretion within parts of the RN, which would then act on 5-HT$_{1A}$ autoreceptors and differentially affect serotonin release in terminal fields (Adell et al., 1997, 2002). This may also explain why [$^{11}$C]WAY-100635 binding differs between the RN and terminal fields.

Previous animal work demonstrating an inverse relationship between cortisol levels and 5-HT$_{1A}$ receptor expression in cortical and hippocampal regions is consistent with our findings with basal salivary cortisol levels (Chalmers et al., 1993; Meijer and de Kloet, 1994; Flügge, 1995; Zhong and Ciarnello, 1995; Le Corre et al., 1997; Czyrak et al., 2002; Iyo et al., 2009). It is thought that cortisol-dependent transcriptional repression of 5-HT$_{1A}$ requires coactivation of both the glucocorticoid and mineralocorticoid receptors (Meijer et al., 2000; Ou et al., 2001).

However, it is important to note that in addition to possible species differences, the animal experiments differ markedly from our paradigm in that they were performed either in animals in the context of adrenalectomy, chronic steroid treatment, or chronic stress paradigms, or in cell culture. We did not measure baseline cortisol in our blood cortisol samples, which were taken after arterial line insertion prior to PET scan. Therefore, the post-stress blood cortisol measures represent a combination of the resting cortisol levels and the response to the stressor, and they cannot be disambiguated. We considered the potential contribution of circadian fluctuations in cortisol level by adjusting TSST cortisol level for time of day in the model.

HPA axis dysfunction has been linked to depression and suicide in many previous studies. Postmortem data from suicide completers shows that they tend to have heavier adrenal glands, higher tissue levels of CRF, which indicates oversecretion of CRF, and lower expression of CRF receptors in prefrontal cortex (Nemeroff et al., 1988; Arató et al., 1989; Szügyi et al., 1994). A subset of patients with depression, generally those with more severe illness, also demonstrate nonsuppression on dexamethasone suppression testing and are at higher risk of suicide (Caroff et al., 1983; Dratcu and Calil, 1989; Coryell and Schlesser, 2001; van Heeringen, 2003; Yerevanian et al., 2004; Pfennig et al., 2005; Kunugi et al., 2006). HPA axis dysfunction is also linked directly to serotonergic dysfunction and specifically to changes in 5-HT$_{1A}$ receptor levels. CRF directly affects the dorsal raphe, modulating serotonergic tone in the prefrontal cortex and nucleus accumbens (Lowry et al., 2000; Forster et al., 2008; Lukkes et al., 2008; Quadros et al., 2014). Several studies in animal models show that cortisol reduces 5-HT$_{1A}$ receptor expression in the hippocampus (Martire et al., 1989; Chalmers et al., 1993; Kuroda et al., 1994; Meijer and de Kloet, 1994; Zhong and Ciarnello, 1995). Our data add to this literature by demonstrating a functional relationship between 5-HT$_{1A}$ receptor binding levels and cortisol levels in response to an acute stressor in human subjects.

The study had several limitations. There were modest differences in racial/ethnic composition between our healthy volunteers and MDD group. Subjects in this study were a convenience sample, included on the grounds of having undergone [$^{11}$C]WAY-100635 imaging and having either a blood or salivary measure of cortisol available. We cannot determine whether these serotonin system relationships to baseline and post-stress cortisol levels will extend to more severe MDD, which is characterized by cortisol hypersecretion and dexamethasone resistance. However, we saw no relationship between MDD severity across the range that was present in our sample and cortisol measures. Finally, this cross-sectional study cannot demonstrate causal relationships. This would be more feasible to study in mouse models where pharmacological and genetic manipulations of HPA axis responsiveness are possible and the time frame to determine the relationship of developmental effects on adult phenotypes much shorter. To more fully characterize the relationship of depression status to HPA axis reactivity, a longitudinal study with repeated measurements in individual subjects during and between episodes of major depression would be required and would complement mouse developmental studies.

In summary, despite limitations, we found a positive correlation between 5-HT$_{1A}$ receptor and post-stress cortisol levels, independent of diagnosis. This suggests an underlying mechanism that links 5-HT$_{1A}$ receptor overexpression with HPA axis feedback dysfunction. Conversely, binding and resting cortisol are negatively correlated as reported in several rodent studies and likely involve different mechanisms including the mineralocorticoid receptor.
Such mechanisms are a combination of genetic vulnerability and environmental risk factors, such as early-life stress, which leads to epigenetic changes generating this biological phenotype.

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References
Adell A, Casanovas JM, Artigas F (1997) Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. Neuropsychopharmacology 36:735–741.
Adell A, Celada F, Abellán MT, Artigas F (2002) Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. Brain Res Brain Res Rev 39:154–180.
Arató M, Bánki CM, Bissette G, Nemeroff CB (1989) Elevated CSF CRF in suicide victims. Biol Psychiatry 25:355–359.
Badawy AA, Morgan CJ, Lovett JW, Bradley DM, Thomas R (1995) Decrease in circulating tryptophan availability to the brain after acute ethanol consumption by normal volunteers: implications for alcohol-induced aggressive behaviour and depression. Pharmacopsychiatry 28:93–97.
Bhagwagar Z, Montgomery AJ, Grasby PM, Cowen PJ (2003) Lack of effect of a single dose of hydrocortisone on serotonin(1A) receptors in recovered depressed patients measured by positron emission tomography with [11C]WAY-100635. Biol Psychiatry 54:890–895.
Bonne O, Bain E, Neumeister A, Nugent AC, Vythilingam M, Carson RE, Lynamuken BA, Eckelmann W, Herscovitch P, Drevets WC, Charney DS (2005) No change in serotonin type 1A receptor binding in patients with posttraumatic stress disorder. Am J Psychiatry 162:383–385.
Brown RP, Mason B, Stoll P, Brizer D, Koceis J, Stokes PE, Mann J (1986) Adrenocortical function and suicidal behavior in depressive disorders. Psychiatry Res 17:317–323.
Caroff S, Winokur A, Rieger W, Schweizer E, Amsterdam J (1983) Response to dexamethasone in psychotic depression. Psychiatry Res 8:59–64.
Chalmers DT, Kwak SP, Mansour A, Akil H, Watson SJ (1993) Corticosteroids regulate brain hippocampal 5-HT1A receptor mRNA expression. J Neurosci 13:914–923.
Coryell W, Schlesser M (2001) The dexamethasone suppression test and suicide prediction. Am J Psychiatry 158:748–753.
Cowen PJ (2010) Not fade away: the HPA axis and depression. Psychol Med 40:1–4.
Czyzak A, Mackowiak M, Choczyk A, Fijal K, Tokarski K, Bijak M, Wedzony K (2002) Prolonged corticosterone treatment alters the responsiveness of 5-HT1A receptors to 8-OH-DPAT in rat CA1 hippocampal neurons. Naunyn Schmiedebergs Arch Pharmacol 366:357–367.
Delorenzo C, Klein A, Mikhno A, Gray N, Zanaderigo F, Mann J (2009), A new method for assessing PET-MRI coregistration. In: SPIE medical imaging, pp 72592–72598. USA.
Delorenzo C, Delaporte L, Thapa-Chhetry B, Miller JM, Mann J, Parsey RV (2013) Prediction of selective serotonin reuptake inhibitor response using diffusion-weighted MRI. Front Psychiatry 4:5.
Dratcu L, Calil HM (1989) The dexamethasone suppression test: its relationship to diagnoses, severity of depression and response to treatment. Prog Neuropsychopharmacol Biol Psychiatry 13:99–117.
First MB, Spitzer RL, Gibbon M, Williams JB (2012). Structured clinical interview for DSM-IV axis I disorders (SCID-I), clinical version, administration booklet.
Flugge G (1995) Dynamics of central nervous 5-HT1A-receptors under psychosocial stress. J Neurosci 15:7132–7140.
Forster GL, Pringle RB, Moww NJ, Vuong SM, Watt MJ, Burke AR, Lowry CA, Summers CH, Renner KJ (2008) Corticotropin-releasing factor in the dorsal raphe nucleus increases medial prefrontal cortical serotonin via type 2 receptors and median raphe nucleus activity. Eur J Neurosci 28:299–310.
Hall H, Lundkvist C, Halldén C, Farde I, Pike VW, McCaron JA, Fletcher A, Cliffe IA, Barf T, Wikström H, Sedvall G (1997) Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]way-100635. Brain Res 745:96–108.
Iyo AH, Kieran N, Chandran A, Albert PR, Wicks I, Bissette G, Austin MC (2009) Differential regulation of the serotonin 1A transcriptional modulators five prime repressor element under dual repression-1 and nuclear-deformed epidermal autoregulatory factor by chronic stress. Neuroscience 163:1119–1127.
Jokinen J, Ouda J, Nordström P (2010) Noradrenergic function and HPA axis dysregulation in suicidal behaviour. Psychoneuroendocrinology 35:1536–1542.
Kirschbaum C, Pinke K, Hellhammer DH (1993) The ‘trier social stress test’ – a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28:76–81.
Kunugi H, et al (2006) Assessment of the dexamethasone/CRH test as a state-dependent marker for hypothalamic-pituitary-adrenal (HPA) axis abnormalities in major depressive episode: a multicenter study. Neuropsychopharmacology 31:212–220.
Kuroda Y, Watanabe Y, Albeck DS, Hastings NB, McEwen BS (1994) Effects of adrenocorticos and type I or type II glucocorticoid receptor activation on 5-HT1A and 5-HT2 receptor binding and 5-HT transporter mRNA expression in rat brain. Brain Res 648:157–161.
Laaris N, Haj-Dahmane S, Hamon M, Lanfumey L (1995) Glucocorticoid receptor-mediated inhibition by corticosterone of 5-HT1A autoreceptor functioning in the rat dorsal raphe nucleus. Neuropsychopharmacology 34:1201–1210.
Lanzenberger R, Wadsak W, Spindelegger C, Mitterhauser M, Aki-Meijer OC, de Kloet ER (1994) Corticosterone suppresses the expression of 5-HT1A receptor mRNA in rat hippocampus after adrenalectomy. Psychopharmacology (Berlin) 130:368–374.

Le Corre S, Sharp T, Young AH, Harrison PJ (1997) Increase of 5-HT7 (serotonin-7) and 5-HT1A (serotonin-1A) receptor mRNA expression in rat hippocampus after adrenalectomy. Psychopharmacology (Berlin) 130:368–374.

Leshc KP, Rupprecht R, Poten B, Müller U, Söhnel K, Fritze J, Schulte HM (1989) Endocrine responses to 5-hydroxytryptamine-1A receptor activation by ipsapirone in humans. Biol Psychiatry 26:203–205.

Lindy DC, Walsh BT, Roose SP, Gladis M, Glassman AH (1985) The effects of adrenalectomy and corticosterone on 5-HT1A and 5-HT1B receptors in the dorsal hippocampus and cortex of the rat. Neuroendocrinology 55:444–450.

Mendelson SD, McEwen BS (1992) Autoradiographic analyses of the effects of adrenalectomy and corticosterone on 5-HT1A and 5-HT1B receptors in the hippocampus and cortex of the rat. Neuroendocrinology 55:444–450.

Miklas MS, DeLorenzo C, Zanderigo F, Prabhakaran J, Kumar JS, Majo VJ, Mann JJ, Parsey RV (2010) In vivo quantification of human serotonin 1A receptor using 11C-CUMI-101, an agonist PET radiotracer. J Nucl Med 51:1892–1900.

Miklas MS, Pantazatos S, Rashid R, Zanderigo F, DeLorenzo C, Hesslergrave N, Ogden RT, Oquendo MA, Mulhern ST, Miller JM, Burke AK, Parsey RV, Mann JJ (2016) Higher 5-HT1A autoreceptor binding as an endophenotype for major depressive disorder identified in high risk offspring - A pilot study. Psychiatry Res Neuroimaging 276:15–23.
system regulation and suicidal behavior in depression. Biol Psychiatry 57:336–342.
Porter RJ, McAllister-Williams RH, Lunn BS, Young AH (1998) 5-hydroxytryptamine receptor function in humans is reduced by acute administration of hydrocortisone. Psychopharmacology (Berl) 139:243–250.
Porter RJ, McAllister-Williams RH, Jones S, Young AH (1999) Effects of dexamethasone on neuroendocrine and psychological responses to L-tryptophan infusion. Psychopharmacology (Berl) 143:64–71.
Porter RJ, Gallagher P, Watson S, Lunn BS, Young AH (2002) The effects of sub-chronic administration of hydrocortisone on hormonal and psychological responses to L-tryptophan in normal male volunteers. Psychopharmacology (Berl) 163:68–75.
Porter RJ, Gallagher P, Watson S, Young AH (2004) Corticosteroid-serotonin interactions in depression: a review of the human evidence. Psychopharmacology (Berl) 173:1–17.
Quadros IM, Hwa LS, Shimamoto A, Carlson J, DeBold JF, Miezek KA (2014) Prevention of alcohol-heightened aggression by CRF-R1 antagonists in mice: critical role for DRN-PFC serotonin pathway. Neuropsychopharmacology 39:2874–2883.
Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J, Gunn RN, Grasby PM, Cowen PJ (2000) Brain serotonin1a receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. Arch Gen Psychiatry 57:174–180.
Sham PC, MacLean CJ, Kendler KS (1994) A typological model of schizophrenia based on age at onset, sex and familial morbidity. Acta Psychiatr Scand 89:135–141.
Stetler C, Miller GE (2011) Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. Psychosom Med 73:114–126.
Sullivan GM, Ogden RT, Huang YY, Oquendo MA, Mann JJ, Parsley RV (2013) Higher in vivo serotonin-1a binding in post-traumatic stress disorder: a PET study with [11C]WAY-100635. Depress Anxiety 30:197–206.
Sullivan GM, Oquendo MA, Milak M, Miller JM, Burke A, Ogden RT, Parsley RV, Mann JJ (2015) Positron emission tomography quantification of serotonin(1A) receptor binding in suicide attempters with major depressive disorder. JAMA Psychiatry 72:169–178.
Szizethy E, Conwell Y, Forbes NT, Cox C, Caine ED (1994) Adrenal weight and morphology in victims of completed suicide. Biol Psychiatry 36:374–380.
Tauscher J, Verhoeff NP, Christensen BK, Hussey D, Meyer JH, Kecoevic A, Javanmard M, Kasper S, Kapur S (2001) Serotonin 5-HT1A receptor binding potential declines with age as measured by [11C]WAY-100635 and PET. Neuropsychopharmacology 24:522–530.
van Gaalen MM, Reul JH, Gesing A, Stenzel-Poore MP, Holsboer F, Steckler T (2002) Mice overexpressing CRH show reduced responsiveness in plasma corticosterone after a5-HT1A receptor challenge. Genes Brain Behav 1:174–177.
von Heeringen K (2003) The neurobiology of suicide and suicidality. Can J Psychiatry 48:292–300.
Vecsei P (1979). Glucocorticoids: cortisol, cortisone, corticosterone, compound S, and their metabolites. In: Methods of hormone radioimmunoassay. New York: Academic Press.
Wüst S, Wolf J, Hellhammer DH, Federenko I, Schommer N, Kirschbaum C (2000) The cortisol awakening response - normal values and confounds. Noise Health 2:79–88.
Yates M, Ferrier IN (1990) 5-HT1A receptors in major depression. J Psychopharmacol 4:69–74.
Yerevanian BI, Feusner JD, Koek RJ, Mintz J (2004) The dexamethasone suppression test as a predictor of suicidal behavior in unipolar depression. J Affect Disord 83:103–108.
Yin H, Galfalvy H, Pantazatos SP, Huang YY, Rosoklija GB, Dwork AJ, Burke A, Arango V, Oquendo MA, Mann JJ (2016) Glucocorticoid receptor-related genes: genotype and brain gene expression relationships to suicide and major depressive disorder. Depress Anxiety 33:531–540.
Young AH, Sharpley AL, Campling GM, Hockney RA, Cowen PJ (1994) Effects of hydrocortisone on brain 5-HT function and sleep. J Affect Disord 32:139–146.
Zhong P, Ciarcangelo RD (1995) Transcriptional regulation of hippocampal 5-HT1A receptors by corticosteroid hormones. Brain Res Mol Brain Res 29:23–34.