STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official title: Exploring the Effects of Corticosteroids on the Human Hippocampus using Neuroimaging

NCT number: NCT03896659

IRB Approved date: June 14, 2023

Form A

IRB #	STU 2018-0360

PROTOCOL FORM / RESEARCH DESCRIPTION

If an item does not apply to your research project, indicate that the question is "**not applicable**" – do not leave sections blank

Click once on the highlighted entry in each box to provide your response. Click the item number/letter or word, if hyperlinked, for detailed instructions for that question. If your response requires inserting a table, picture, etc, you may need to first delete the box that surrounds the answer and then insert your table or other special document.

1. Purpose and objectives. *List the purpose and objectives:*

This study will, for the first time in humans, examine the effects of cortisol on specific hippocampal subfields using state-of-the art multimodal high-resolution neuroimaging methods. This research study is translational in nature and will advance our understanding of the effects of stress and cortisol on the brain. This is a non-treatment study. The researchers are interested in whether hydrocortisone (medication form of a stress hormone cortisol) influences the brains of depressed vs. non-depressed people differently. The researchers are also interested in whether there are differences in the effects of hydrocortisone between males and females. The study has both mechanistic and clinical aims. The aims are as follows:

Mechanistic Aim 1: Determine the effect of cortisol on task-driven hippocampal activation using, for the first time, high-resolution (1.5 mm isotropic) functional MRI of hippocampal subfields (i.e., DG/CA3, CA1, subiculum).
Mechanistic Aim 2: Determine the effect of cortisol on hippocampal subfield volume using high-resolution structural MRI.
Mechanistic Aim 3: Assess changes in hippocampal metabolites NAA, Glu, Cho and mI with cortisol using 1HMRS (spectroscopy).
Clinical Aim 4: Determine the effect of cortisol on declarative and visuospatial memory.
Clinical Aim 5: Determine whether sex, stress, early life adversity, age, physical activity, GCR number or sensitivity, or changes in sleep during cortisol administration, predict hippocampal CS response, as assessed by memory testing and multimodal neuroimaging.
Exploratory Aim 6: Explore the effects of cortisol on resting state functional connectivity using whole brain connectomic mapping.
Exploratory Aim 7: Explore the impact of depressive symptoms on memory and hippocampal structure, activation, connectivity and 1HMRS outcomes in response to hydrocortisone administration.

2. Background.

- Describe past experimental and/or clinical findings leading to the formulation of your study.
- For research involving investigational drugs, describe the previously conducted animal and human studies.
- For research that involves FDA approved drugs or devices, describe the FDA approved uses of this drug/device in relation to your protocol.
- Attach a copy of the approved labeling as a product package insert or from the Physician's Desk Reference.

You may reference sponsor's full protocol or grant application (section number and/or title) or if none, ensure background includes references.

Please respond to all components of this item, or clearly indicate which components are not applicable.

a. Background

See grant application uploaded in section 4.1.3d of the Smartform. The background information can be found in Section 3 (Research Strategy, pages 68-69).

b. Current practice

N/A

3. Study Design.
Describe the study design (e.g., single/double blind, parallel, crossover, etc.) Consider inserting a scheme to visually present the study design.

The study involves a double-blind, placebo-controlled, crossover design of hydrocortisone in depressed and non-depressed (healthy controls) participants. Within each group (depressed and non-depressed), participants will be randomized to receive

hydrocortisone or placebo for 3 days, followed by MRI and cognitive testing on day 3. Participants will then undergo a 25-day washout of study medication and will then, again, receive hydrocortisone or placebo for 3 days, followed by MRI and cognitive testing. The order in which participants will receive hydrocortisone vs. placebo will be randomized by a study statistician.

4. Research Plan / Description of the Research Methods:

4.a. Provide a **comprehensive narrative** describing the **research methods**.

1) Provide the **order in which tests/procedures will be performed**,
2) Provide the **setting** for these events and a description of the **methods used to protect privacy** during the study.
3) Provide the **plan for data analysis** (include as applicable the **sample size calculation)**

Please respond to all components of this item, or clearly indicate which components are not applicable.

Informed consent and all study procedures, other than neuroimaging, will be conducted at BL8.224 or remotely through secure video conferencing software (e.g. Zoom, Teams). Neuroimaging will be done at the AIRC. When informed consent is conducted remotely, DocuSign will be used to obtain signatures. Please see the next page for the assessment schedule.

Methods used to protect privacy: Deidentified data will be stored in REDCap – a self-managed, secure, web-based solution that is designed to support data collection strategies for research studies. Only researchers associated with the study will have access to any protected health information (PHI). This information will not be stored on REDCap. Identifying information will be kept in a separate password-protected file only accessible to the PI and the research team on a need-to-know basis. Data will be analyzed by group, and subjects will be identified only by a subject ID number to protect their confidentiality. Researchers will destroy the personal identifiers upon completion of data collection for the study.

Data analysis plan:

Aim 1: Determine the effect of cortisol on task-driven hippocampal activation using, for the first time, high-resolution (1.5 mm isotropic) fMRI of hippocampal subfields (i.e., DG/CA3, CA1, subiculum). The design is a two-period crossover with randomized order of treatment conditions, and the primary interest is in the treatment effect on hippocampal subfield activation, estimated as the within-subjects difference in hippocampal subfield activation between the hydrocortisone and placebo treatment conditions. The index of hippocampal subfield activation is a beta-weight of the contrast of the task-active condition vs. the control condition (e.g. novel vs. familiar in the Novelty Detection task and lure correct rejections vs. false alarms in the Mnemonic Discrimination task). We will use linear mixed effects regression to estimate the following model of DG/CA3 activation, Y_{ijk}, of the *i*th subject in the *j*th group in the *k*th period, cast in a traditional longitudinal data setup.
$Y_{ijk} = \mu_i + \beta_c C_{ij0} + \gamma_t T_k + \gamma_c (C_{ijk} - C_{ij0}) + \varepsilon_{ijk}$, where the subject-level mean $\mu_i = \mu + S_i$ is comprised of an overall mean μ and a random subject effect S_i, the time and treatment condition indicators are T_k and C_{ijk}, and ε_{ijk} represents a random residual effect. The corresponding between-subjects sub-model is $Y_{ij0} = \mu_i + \beta_c C_{ij0} + \varepsilon_{ij0}$, where the parameter β_c is identified with the initial condition assignment, which is synonymous with group assignment and therefore represents the carry-over effect. The corresponding within-subjects sub-model is $\Delta Y_{ij} = Y_{ij1} - Y_{ij0} = \gamma_t + \gamma_c (C_{ijk} - C_{ij0}) + (\varepsilon_{ij1} - \varepsilon_{ij0})$, where the parameter γ_c is identified with the change in assigned condition and therefore represents the treatment effect.

Aim 2: Determine the effect of cortisol on hippocampal subfield volume using high-resolution structural MRI. Using the same randomized regression approach described above we will assess mechanistic aim 2.

Aim 3: Assess changes in hippocampal NAA, Glu, Cho and ml with cortisol using ^{1}HMRS. Similarly, changes in spectroscopy will be assessed using the randomized regression utilized in the previous aims.

Aim 4: Determine the effect of cortisol on declarative and visuospatial memory. We will estimate the treatment effects on declarative memory using the same linear mixed model setup as the primary aim.

Aim 5: Examine whether sex, stress, early life adversity, age, *physical activity, GCR number or sensitivity*, or changes in sleep during cortisol administration, predict hippocampal CS response, as assessed by memory testing and neuroimaging. We will assess treatment moderation by sex, stress, early life adversity, age, physical activity, and changes in sleep using the modeling setup described in the primary aim with additional terms added to the model. To assess the moderation of a dichotomous variable (i.e., sex), a group indicator (male vs. female) will be added to the regression model (

$Y_{ijk} = \mu_i + \beta_c C_{ij0} + \gamma_t T_k + (\gamma_c + \gamma_{cf} F_i)(C_{ijk} - C_{ij0}) + \varepsilon_{ijk}$), where the overall treatment effect $\gamma_c + \gamma_{cf} F_i$ is allowed to depend on an indicator F_i of sex. For continuous variables, baseline levels will replace the indicator of sex. Continuous moderator analyses will be refined entering and, adjusting for changes in moderators over time by including baseline and longitudinal levels in the model as $Y_{ijk} = \mu_i + \beta_c C_{ij0} + \beta_q Q_{ij0} + \gamma_t T_k + (\gamma_c + \gamma_{cq} Q_{ij0})(C_{ijk} - C_{ij0}) + \gamma_q (Q_{ijk} - Q_{ij0}) + \varepsilon_{ijk}$. Here, the overall treatment effect $\gamma_c + \gamma_{cq} Q_{ij0}$ is allowed to depend on baseline levels, and the term $\gamma_q (Q_{ijk} - Q_{ij0})$ adjusts for changes from baseline in the within-subjects sub-model.

Aim 6: **Explore the effects of cortisol on resting state regional functional connectivity, and *whole brain connectomics*.** As discussed in the primary aim, the treatment effects on resting state functional connectivity will be assessed using a randomized regression in a traditional longitudinal cast. The key indices of interest will be overall measures of network integrity (e.g., degree, centrality, rich club architecture, etc.).

Aim 7: Explore the impact of depressive symptoms on memory and hippocampal structure, activation, connectivity and [1]HMRS outcomes in response to hydrocortisone administration. *Depression as a moderator will be assessed similarly to the moderators proposed in aim 5. However, a dichotomous indicator variable representing baseline group assignment (depressed vs. healthy control) will also be included in the model.*

Sample size determination:

Aims 1, 2, 3, 4 and 6: Complete data from 168 (84 subjects per group) will provide 90% power to detect a treatment effect on hippocampal activation of magnitude 0.08 or greater (correspondingly, $d \geq 0.34$), based on the findings of Brown et al. (2013).[34] The magnitude of the treatment effect observed by Brown et al. (2013)[34] was 0.16. This is a very conservative estimate because we anticipate enrolling 188 (94 per group) expecting no more than 10% attrition. If attrition rates are abnormally high, at approximately 20%, the completer sample of 150 (75 per group) would provide 80% power and provide sufficient power to detect a statistical difference with an effect size of $d = 0.34$. Complete data from 168 (84 per group) subjects will provide 90% power to detect a treatment effect on hippocampal volume of magnitude 0.06 mm^3 or greater, based on the findings of Brown et al. (2015)[43] and to detect a treatment effect on declarative memory of magnitude 3.31 or greater, based on the findings of Brown et al. (2013).[34] The magnitude of the treatment effect on hippocampal volume observed by Brown et al. (2015)[43] was 0.12 mm^3, and the magnitude of the treatment effect on declarative memory observed by Brown et al. (2013)[34] was 4.25. To power the study for the spectroscopy aims, we used data from a study of patients receiving prednisone for medical illnesses vs. disease matched controls.[30] The participants were receiving corticosteroids for a longer duration than in the proposed study, but no studies examining more acute exposure were available. Based on the NAA/Cr values reported we expect a medium effect size (~$d = 0.54$). Assuming we have complete data on more than 68 (34 per group) participants we will have approximately 90% power to detect a significant difference in spectroscopy data. This is a conservative estimate given other effect sizes observed in similar work were much larger ($d = 0.84$) which would only need complete data from 40 subjects (20 per group) to observe 96% power. For clinical aim 4, complete data from 136 (68 per group) subjects will provide 90% power to detect a treatment effect on declarative memory of an effect size of $d = 0.38$ or greater, based on the findings of Brown et al. (2013).[34] In the case of high attrition rates (> 28%) we would still have over 90% power to observe a significant effect on memory changes. Although data are not available for a sample size estimate for the exploratory aim of rsFC, a sample size of 168 (84 per group) will provide 80% power for an effect size of $d = 0.30$ which is smaller than effect sizes observed in previous work referenced above.

Aims 5 and 7: Complete data from 48 participants (50% women) will provide 90% power to detect treatment moderation by sex of magnitude 0.16 for hippocampal activation, 0.12 mm^3 for hippocampal volume, and 6.63 for declarative memory (all corresponding to a medium effect size of $d = 0.68$).[43] We will assess treatment moderation in the same way but replacing the indicator of female sex with depressed status. Based on previous studies, a difference in declarative memory performance following CS administration between depressed people and controls has a large effect size ($d = 1.1$).[54] Using the conservative approach of powering the study for the lowest observed magnitude of $d = 0.34$ allows adequate power (≥ 90%) when assessing effects with higher magnitudes.

References for sample size calculations:

Brown ES, Lu H, Denniston D, et al. A randomized, placebo-controlled proof-of-concept, crossover trial of phenytoin for hydrocortisone-induced declarative memory changes. J Affect Disord. 2013;150(2):551-558.

Brown ES, Jeon-Slaughter H, Lu H, et al. Hippocampal volume in healthy controls given 3-day stress doses of hydrocortisone. Neuropsychopharmacology. 2015;40:1216-1221.

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Assessment Schedule

Instrument	Baseline (Visit 1)	Visit 2	2 days (Visit 3 occurs on Day 3)	Visit 3	Washout (25 days)	Visit 4	2 days (Visit 5 occurs on Day 3)	Visit 5
Informed consent	X							
Medical and psychiatric history	X							
Concomitant medication review	X	X		X		X		X
Physical exam	X							
SCID-CV for DSM *5*	X							
Quick Inventory of Depressive Symptomatology (QIDS-C)	X	X		X		X		X
Young Mania Rating Scale (YMRS)	X	X		X		X		X
Columbia Suicide Severity Rating Scale (C-SSRS)	X			X				X
Systematic Assessment for Treatment Emergent Event (SAFTEE)	X			X				X
Rey Auditory Verbal Learning Test (RAVLT)	X			X				X
Ruff Light Trail Learning (RULIT)	X			X				X
Volunteer Health Questionnaire	X							
Weekly Questionnaire		X		X		X		X
Prodromal Questionnaire – Brief Version (PQ-B)	X			X				X
Perceived Stress Scale (PSS-10) Childhood Trauma Questionnaire (CTQ) International Physical Activity Questionnaire (IPAQ) Treatment Impressions Inventory (TII)	X X X X							
Internal Status Scale (ISS)				X				X
Blood work (CBC, CMP, morning cortisol) CMP – 8.5 mL; CBC – 4 mL; Cortisol – 10 mL	X			X				X
Blood work (GCR analysis and epigenetics, 2 x 8.5 mL)	X							
Blood work (progesterone in naturally cycling women) Progesterone – 10 mL				X				X
Blood work (Glucocorticoid)	X			X				X
Vitals	X							
Electrocardiogram (ECG)	X			X				X
Urine pregnancy test (UPT)	X			X				X
Randomization		X						
Begin 3-day course of hydrocortisone or placebo		X				X		
Begin 3-day actigraphy monitoring		X				X		
Structural and functional MRI, spectroscopy				X				X
Follow-up with a clinician		X		X		X		X
Adverse events review		X		X		X		X
Exit survey								X
Approximate time/visit	**3 hr**	**45 m**		**3.5 hr**		**45 m**		**3.5 hr**

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4.b. List of the study intervention(s) being tested or evaluated under this protocol

☐ **N/A -** this study does not test or evaluate an intervention. Skip to item 4.d.

#	Study intervention(s) being tested or evaluated under the protocol *Add or delete rows as needed*	Affiliate Place a check next to institution(s) where the intervention will be performed	Local Standard Practice? Indicate whether the intervention is considered acceptable practice locally for applicable institutions
1	Hydrocortisone	☒ UTSW	☐ Yes
		☐ PHHS	☐ Yes
		☐CMC	☐ Yes
		☐THR	☐ Yes
		☐TSRH	☐ Yes
		☐ Other: _____	☐ Yes

4.c. Risk:Benefit Analysis of study interventions being tested or evaluated under this protocol

For each study intervention identified in section 6b above, complete a risk:benefit analysis table.

(Two tables are provided, copy & paste additional tables as needed or delete both tables if this study does not test an intervention)

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4.c. **Study Intervention #1** Hydrocortisone	
List each group exposed to this intervention on a separate line. (e.g., experimental, control, Arm A, Arm B, etc) **Or** state All Groups/Subjects	For each group, list the **benefits** of this intervention. (Benefits can be directly from the intervention or from a monitoring procedure likely to contribute to the subject's well being). If there are no benefits, state "none".
All subjects	All subjects will receive ongoing monitoring for their depressive symptoms. However, this is not a treatment study and the researchers are not evaluating the effectiveness of hydrocortisone in treating depression. The researchers are interested in whether a short 3-day dose of hydrocortisone has different effect on the brain and cognition in depressed vs. non-depressed individuals. Participants will also receive blood work, ECG, progesterone level testing, diagnostic interviews, psychiatric symptom assessment, cognitive assessments, and psychiatric care. Thus, we feel that the risks, although clearly present, are greatly outweighed by the benefits of this study.

If you are requesting a Waiver of Informed Consent, complete the table below.

If you have a consent form, **list the reasonably foreseeable risks in the consent form (and do not complete this section).**

List the risks according to the probability (likely, less likely or rare) and magnitude (serious or not serious).
(include: 1) expected adverse events; 2) rare and serious adverse events; 3) all other psychological, social, legal harms)
Do not delete frequency. Frequency must be estimated because it will assist you with determining which adverse events will require prompt reporting.

	Not serious	**Serious**
Likely These risks are expected to occur in more than **20** out of **100** subjects.	• N/A (listed in consent)	• N/A (listed in consent)
	Not serious	**Serious**
Less likely These risks are expected to occur in **5-20** subjects or less out of **100** subjects.	• N/A (listed in consent)	• N/A (listed in consent)
		Serious
Rare These risks are expected to occur in less than **5** subjects out of **100**		• N/A (listed in consent)

4.d. List ALL other research procedures or components not listed in table 4.b.
The combination of Tables 4b and 4d should account for all of the research procedures that will take place during this study.

Consider grouping similar procedures under a single component (e.g., blood work, CT = safety assessments)

#	Research component • **individual procedures** *example:* **Eligibility Assessments** • History and physical • Questionnaire • Laboratory tests *Add or delete rows as needed*	Column A **Local Standard Practice** Indicate the number of times each procedure will be performed as stipulated in the research plan **that would be performed if the participant were not participating in the study.**	Column B **Research Only** Indicate the number of times each procedure will be performed solely for research purposes *(meaning that the participant would not undergo the same number of procedures or would not undergo the procedure(s) at the same frequency if they were not participating in the study)*	Column D **Risks** **If you are requesting a Waiver of Informed Consent, complete the table below.** List the reasonably expected risks for each procedure or group of procedures under the following categories as appropriate: • Serious and likely; • Serious and less likely; • Serious and rare; • Not serious and likely; • Not serious and less likely
1	**Baseline (Visit 1)**			**N/A – not requesting a waiver of consent**
	Informed consent	0	1	
	Psychiatric/medical history	0	1	
	Physical exam	0	1	
	Concomitant medication review	0	1	
	SCID-CV for DSM *5*	0	1	
	RAVLT	0	1	
	RULIT	0	1	
	QIDS-C	0	1	
	YMRS	0	1	
	PSS-10	0	1	
	CTQ	0	1	
	C-SSRS	0	1	
	SAFTEE	0	1	
	IPAQ	0	1	
	Blood draw	0	1	
	ECG	0	1	
	Urine pregnancy test	0	1	
	PQ-B	0	1	
	TII	0	1	
2	**Visits 2 and 4**			
	QIDS-C	0	1	
	YMRS	0	1	
	Clinician follow-up	0	1	
	3-day actigraphy	0	1	
	3-day hydrocortisone vs. placebo	0	1	
3	**Visits 3 and 5**			
	RAVLT	0	1	
	RULIT	0	1	
	PQ-B	0	1	
	QIDS-C	0	1	
	YMRS	0	1	
	C-SSRS	0	1	
	SAFTEE	0	1	

	Internal Status Scale	0	1	
	Blood draw	0	1	
	ECG	0	1	
	Clinician follow-up	0	1	
	MRI (structural and functional)	0	1	
	1HMRS (spectroscopy)	0	1	
	Urine pregnancy test	0	1	
4	Visit 5			
	Exit survey	0	1	

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5. Safety Precautions. *(Describe safeguards to address the serious risks listed above.)*

a. Describe the procedures for protecting against or minimizing any potential risks for each of the more than minimal risk research procedures listed above.

The primary risks of this study are potential medication side effects to a brief 3 day exposure to the study medication and a possible delay in treating depression. PI Brown has extensive clinical and research experience with depressed patients, including the studies involving the use of hydrocortisone and neuroimaging. At all visits, the participants will also be assessed by a licensed clinical provider and monitored for side effects, worsening of mood symptoms, and suicidality.

Brief hydrocortisone administration appears to be safe and well tolerated. However, participants will be informed of all potential risks of brief corticosteroid exposure. The participants may experience a brief and reversible change in declarative memory while receiving hydrocortisone.

At the MRI scanner, participants will fill out an MRI screening form and will also be screened by the MRI technologist to ensure it is safe for the participant to undergo the scan. Participants will also be informed that they may stop the imaging session at any time, and participants with claustrophobia will be excluded during the pre-screen. The mock scanner will be available if needed to ensure participants feel comfortable in an enclosed space. Participants will be asked to press the call button in the scanner if they experience any discomfort, and will be immediately removed from the scanner. Both the MRI technologist and the research assistant will be present during the entire MRI session to monitor patient safety. A Rapid Response Team will be called should the patient experience a medical emergency.

All assessments will be performed by trained research personnel experienced in working with the research population involved in the study. Breaks will be utilized as needed to reduce participation burden and psychological stress. Blood will be drawn by a certified phlebotomist. A urine pregnancy test will be administered to all participants of childbearing potential, and pregnant or breastfeeding women will be excluded from the study.

Another risk will include premenopausal women. Participants will be asked to use an acceptable and effective form of birth control during the study. A hysterectomy or bilateral tubal ligation is acceptable because these women continue to have menstrual cycles. Women with an ovariectomy (“surgical menopause”) will be excluded. Women currently taking estrogen containing oral contraceptives will be excluded due to the possible effects of these medications on cortisol levels and the brain’s response to cortisol. Non-estrogen containing contraceptives including progestin-only pills (e.g. Micronor, Nor-QD, or Camilla), implants such as Nexplanon or Implanon that contain the progestin etonogestrel, ParaGard IUD (a copper-T device that contains no hormone), as well as barrier methods are acceptable.

The PI on the study is a board-certified psychiatrist and the study clinician team includes two psychiatrists, a psychiatric nurse practitioner and a psychiatric physician assistant, all with extensive experience managing clinical trial patients with mood disorders. Should a psychiatric emergency (e.g., active suicidal ideations with plan and intent) arise during the screening visit (or any other study visit), the subject will be assessed by a clinician, 911 will be called, and the subject will be escorted to the Clements Emergency Room or Parkland Psychiatric Emergency Room.

b. Where appropriate, discuss provisions for ensuring necessary medical or professional intervention in the event of adverse events, or unanticipated problems involving subjects.

Should a medical emergency arise during the study participation, 911 will be called and a participant will be escorted to Clements or Parkland Emergency Room. All investigators and clinicians on the study are experienced psychiatric care providers. Should a psychiatric emergency arise that require inpatient monitoring, 911 will be called and a participant will be escorted to the Clements Emergency Room or Parkland Psychiatric Emergency Room. In the event of an adverse event or an unanticipated problem that does not require emergency hospitalization, a participant will be carefully monitored by the PI and the PI’s team and the appropriate referrals will be arranged as needed for the participant. All UPIRSOs will be reported to the IRB board according to the specified guidelines.

c. Will the safeguards be different between/among groups?

☐ Yes ☑ No

N/A