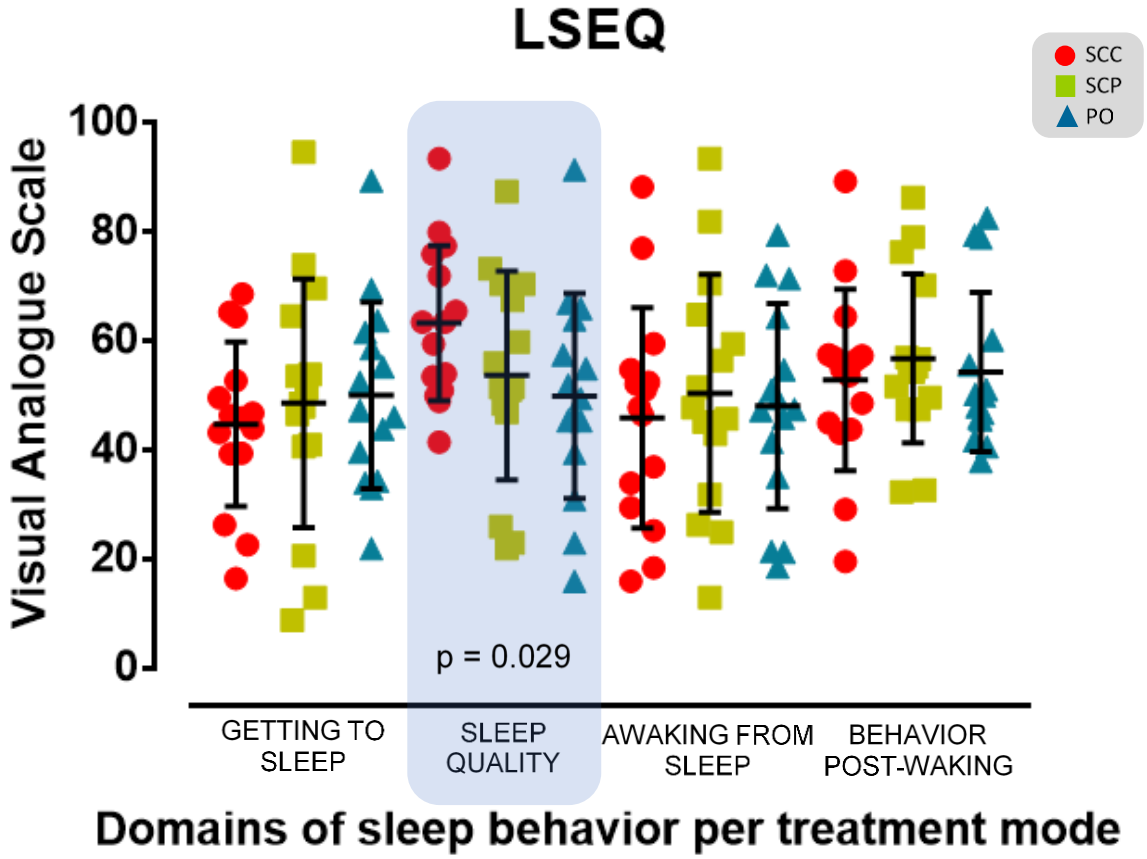

LSEQ
SCC
SCP
PO
Visual Analogue Scale
100
80
60
40
20
0
p = 0.029
GETTING TO SLEEP
SLEEP QUALITY
AWAKING FROM SLEEP
BEHAVIOR POST-WAKING
Domains of sleep behavior per treatment mode

Fig. S1

Self-perceived sleep behaviour. Deviations from the self-perceived normality of night sleep, as assessed by the Leeds sleep evaluation questionnaire (LSEQ), across the three treatment groups. LSEQ evaluates 4 domains of sleep behaviour; easiness for getting to sleep, quality of sleep, easiness of awakening and behaviour after awakening. The sleep that participants were asked to evaluate was the one they experienced during the night of the fourth day / early morning of the fifth (last) day after starting each pharmacological intervention. The scatter plot depicts the raw (untransformed) values; improvements in the sleep behavior take values < 50 and deteriorations > 50, as each subject was instructed to consider the middle of the visual analogue scale (50) his subjective "normal" or "usual". A notable interaction between cortisol dynamics and quality of sleep was observed ($p < 0.05$).

PO: per os hydrocortisone treatment group, SCC: subcutaneous-continuous hydrocortisone infusion group, SCP: subcutaneous-pulsatile hydrocortisone infusion group

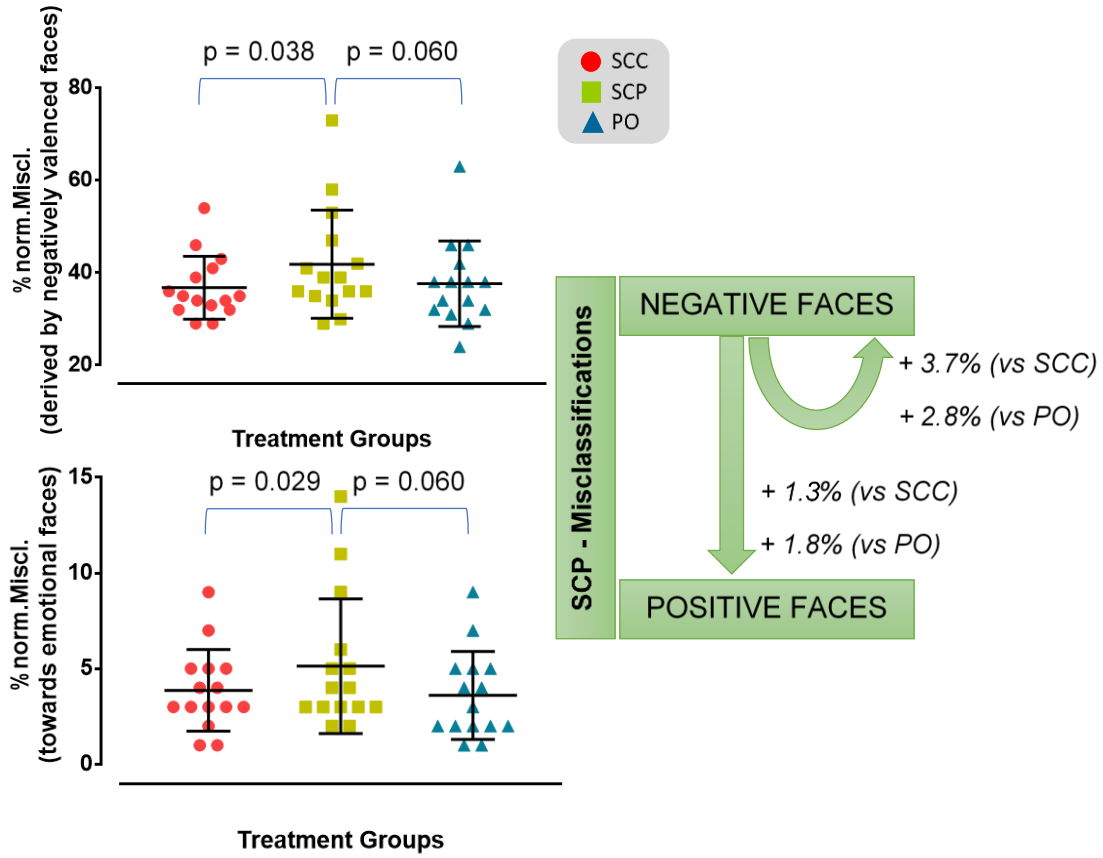

p = 0.038
p = 0.060
SCC
SCP
PO
% norm.Miscl.
(derived by negatively valenced faces)
80
60
40
20
Treatment Groups
p = 0.029
p = 0.060
% norm.Miscl.
(towards emotional faces)
15
10
5
0
Treatment Groups
SCP - Misclassifications
NEGATIVE FACES
+ 3.7% (vs SCC)
+ 2.8% (vs PO)
+ 1.3% (vs SCC)
+ 1.8% (vs PO)
POSITIVE FACES

Fig. S2

Post-hoc behavioural data on emotional face recognition task (FERT). The upper scatter plot shows each treatment group's %normalized number of misclassifications deriving from negatively valenced faces (with the corresponding mean and standard deviation). Normalization was performed by dividing the actual number of misclassifications by the maximum possible number that could have been derived from. Subject undergoing the SCP are more likely [$F(1.478, 20.698) = 6.633$, $p = 0.010$] to misclassify negative emotional faces compared to when undergoing the other 2 modes of hydrocortisone replacement (the mean difference with the SCC group is 5.1% with 95% CI 0.3-9.9%, $p = 0.038$ and the mean difference with the PO group is 4.2% with 95% CI -0.2% to 8.6%, $p = 0.060$). The lower scatter plot shows each treatment group's %normalized number of misclassifications directed towards emotional faces (with the corresponding mean and standard deviation). Normalization was performed by dividing the actual number of misclassifications by the maximum number of emotional faces. Subject undergoing the SCP tend, on average, to misclassify towards emotional faces with a higher frequency [$F(1.665, 23.312) = 6.522$, $p = 0.008$] compared to the other groups (the mean difference with the PO group is 1.4% with 95% CI 0.1-2.7%, $p = 0.029$ and the mean difference with the SCC group is 1.3% with 95% CI 0-2.6%, $p = 0.060$). Overall, subjects on the SCP show a greater number of misclassifications compared to the other groups, deriving from negatively-valenced faces being misclassified towards other negative or positive faces. More details on the categorization of the various kinds of misclassifications can be found in Figure S18.

PO: per os hydrocortisone treatment group, SCC: subcutaneous-continuous hydrocortisone infusion group, SCP: subcutaneous-pulsatile hydrocortisone infusion group

		COPE mean	COPE SD	Z-statistic	99.9% CI Min.	99.9% CI Max.	%BOLD signal change (mean)	%BOLD signal change (SD)	%extent ROI
Right Amygdala	FEAR	26.21	14.51	4.70	7.28	66.71	0.25	0.14	0.69
	HAPPY	22.15	11.09	3.95	6.97	50.09	0.21	0.11	0.51
	SAD	21.40	10.41	3.66	7.65	48.82	0.20	0.10	0.64
Right Insula	FEAR	21.88	8.74	4.74	6.84	42.03	0.19	0.08	0.40
	HAPPY	16.08	5.90	3.29	5.89	28.31	0.14	0.05	0.26
	SAD	16.04	6.03	4.11	6.51	28.20	0.14	0.06	0.32
Right Dorsal Striatum	FEAR	17.62	7.26	4.60	3.61	47.28	0.17	0.06	0.92
	HAPPY	13.96	4.71	3.78	3.97	32.71	0.14	0.05	0.72
	SAD	14.26	4.98	3.93	3.84	34.15	0.14	0.05	0.78
Right Orbitofrontal Cortex	FEAR	19.79	7.23	3.92	8.02	39.49	0.21	0.07	0.42
	HAPPY	15.21	3.97	3.03	7.67	23.80	0.15	0.03	0.15
	SAD	15.02	4.12	3.58	7.22	24.60	0.15	0.03	0.20
Right Nucleus Accumbens	FEAR								0.02
	HAPPY								0.00
	SAD								0.00
Right Hippocampus	FEAR	10.71	5.81	3.11	4.76	28.01	0.11	0.06	0.13
	HAPPY	10.72	6.01	2.96	4.84	28.10	0.11	0.06	0.08
	SAD	8.53	5.90	2.49	1.20	28.10	0.09	0.06	0.11
Anterior Cingulate Cortex	FEAR	16.48	5.93	3.52	6.57	43.53	0.14	0.05	0.69
	HAPPY	16.69	5.42	2.75	7.52	30.16	0.15	0.05	0.07
	SAD	13.13	6.21	2.23	2.95	30.16	0.11	0.05	0.11
Posterior Cingulate Cortex	FEAR	20.13	6.38	3.54	8.32	34.68	0.19	0.06	0.12
	HAPPY	17.27	3.28	2.73	10.51	22.83	0.16	0.03	0.04
	SAD	14.61	4.48	2.37	4.43	22.83	0.14	0.05	0.08
Left Hippocampus	FEAR								0.01
	HAPPY								0.00
	SAD								0.00

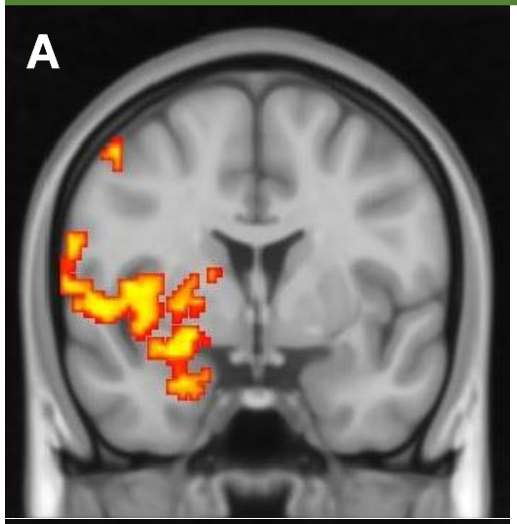
Left Nucleus Accumbens	FEAR								0.11
	HAPPY								0.01
	SAD								0.06
Left Orbitofrontal Cortex	FEAR	21.64	7.78	3.91	6.01	42.72	0.20	0.07	0.20
	HAPPY	16.01	3.97	3.25	8.51	25.83	0.14	0.04	0.08
	SAD	13.72	5.10	2.78	3.86	25.83	0.12	0.05	0.11
Left Dorsal Striatum	FEAR	18.73	5.97	4.76	6.00	45.57	0.18	0.06	0.96
	HAPPY	13.72	3.98	3.67	6.05	27.41	0.13	0.04	0.82
	SAD	13.26	4.26	3.56	4.02	27.41	0.13	0.04	0.88
Left Insula	FEAR	20.81	9.30	4.20	7.06	42.19	0.17	0.08	0.36
	HAPPY	17.39	4.19	3.52	9.70	24.35	0.15	0.04	0.15
	SAD	15.16	5.35	4.01	5.25	25.76	0.13	0.05	0.20
Left Amygdala	FEAR	19.74	9.28	3.78	5.68	46.81	0.18	0.09	0.45
	HAPPY	20.04	5.21	2.64	12.52	29.82	0.19	0.06	0.06
	SAD	11.09	6.74	1.75	1.09	29.81	0.10	0.07	0.23

Table S3

The table shows the responsiveness of this study's predefined regions of interest (ROIs) to viewing fearful, happy and sad faces. For each ROI and valence of facial expression the following data are provided; the mean and standard deviation (SD) of the contrast of parameter estimate (COPE, arbitrary values), its 99.9% confidence intervals (CI), the corresponding Z-statistic and two effect sizes (the mean and SD of the %BOLD signal change contrasting baseline, and the %extent of the ROI, which elicited a stimulus-driven response). We can group the ROIs to four categories: (i) ROIs which are widely (> 15%) and consistently activated by all emotional valences (right amygdala, right dorsal striatum, right insula, right orbitofrontal cortex, left dorsal striatum, left insula), (ii) ROIs which are mainly responsive to fearful faces (anterior cingulate cortex, left orbitofrontal cortex, left amygdala), (iii) ROIs which are weakly responsive to emotional faces (right hippocampus, posterior cingulate cortex), and (iv) ROIs which are irresponsive to our experimental setting (nucleus accumbens bilaterally and the left hippocampus). In these areas, either no voxel or a very small number of voxels (0-10) was engaged to the emotional face presentation, thus no group statistics are shown.

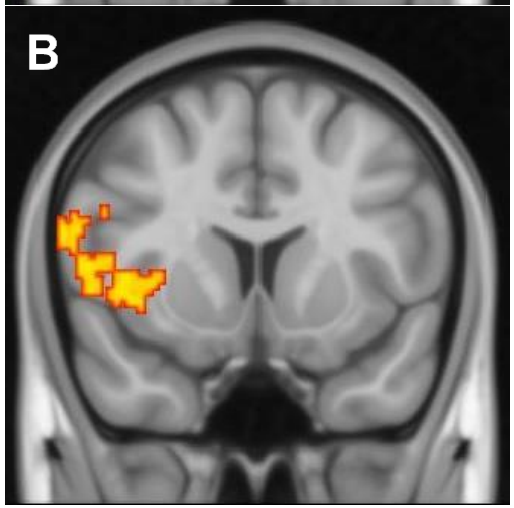
BOLD: blood oxygen-level dependent

Subcutaneous-continuous **VS** subcutaneous-pulsatile infusion



Happy > Sad

Pairwise comparisons for the specified contrast (whole-brain analysis, Z > 2.3, p < 0.05, cluster-corrected)						
Brain Regions	p-value	Cluster size (No voxels)	Z-value	Coordinates of local maxima		
				X	Y	Z
Right IFG (p. operc.) / Precentral gyrus	< 0.001	2191	**3.48**	**50**	**8**	**12**
Right amygdala			3.42	28	2	-14
Right Precentral Gyrus			3.41	66	4	12
Right Insular Cortex			3.41	36	10	-14
Right Postcentral Gyrus / COC			3.37	64	-16	18
Right Superior Parietal Lobule	= 0.0032	789	**3.22**	**32**	**-54**	**42**
Right Postcentral Gyrus			3.06	60	-6	40
Right Angular Gyrus			3.04	36	-54	42
Right Supramarginal Gyrus (p)			3.01	44	-44	54



Fear > Sad

Pairwise comparisons for the specified contrast (whole-brain analysis, Z > 2.3, p < 0.05, cluster-corrected)						
Brain Regions	p-value	Cluster size (No voxels)	Z-value	Coordinates of local maxima		
				X	Y	Z
Right Postcentral gyrus / COC	< 0.0001	2180	**3.75**	**60**	**-8**	**16**
Right Postcentral / Precentral gyrus			3.69	64	-4	34
Right COC			3.47	52	-6	10
Right Insular Cortex			3.47	42	6	-2
Right COC / Insular Cortex			3.47	44	-8	10

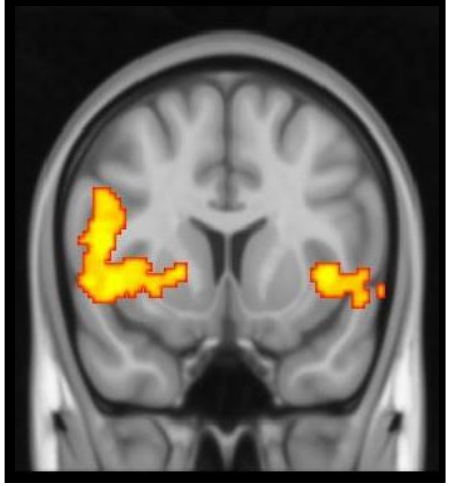
Fig. S4

(*A*) Whole-brain statistical map (family wise error-corrected, Z-threshold > 2.3, p < 0.05), depicting two clusters of brain regions where emotional discrimination between happy and sad faces shows significant differences in the pairwise comparison between the subcutaneous-continuous and -pulsatile hydrocortisone infusion groups. The statistical map has been rendered onto the standard MNI152 brain. The brain regions presented in the Table (and their coordinates in the MNI152 space) correspond to each cluster's local maxima (Z-value), based on the Harvard-Oxford Cortical Atlas. (*B*) Whole-brain statistical map (family wise error-corrected, Z-threshold > 2.3, p < 0.05), depicting one cluster of brain regions where emotional discrimination between fearful and sad faces shows significant differences in the pairwise comparison between the subcutaneous-continuous and -pulsatile hydrocortisone infusion groups. The statistical map has been rendered onto the standard MNI152 brain. The brain regions presented in the Table (and their coordinates in the MNI152 space) correspond to the cluster's local maxima (Z-value), based on the Harvard-Oxford Cortical Atlas.

COC: central opercular cortex, IFG (p. operc.): inferior frontal gyrus, pars opercularis

Subcutaneous-continuous infusion **VS** oral treatment

Happy > Sad



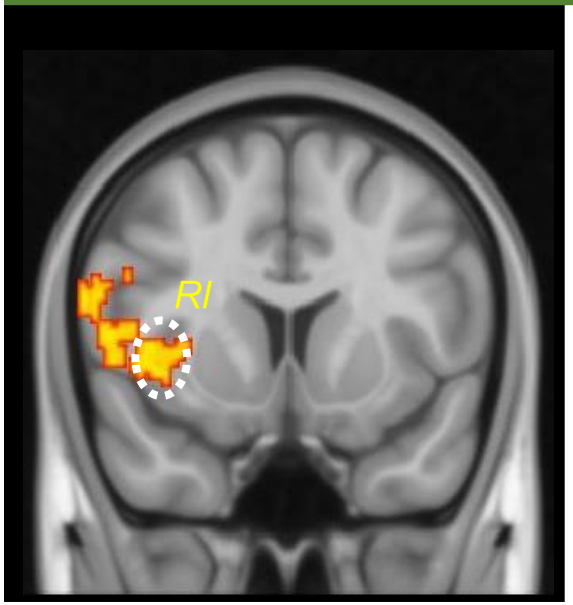
Pairwise comparisons for the specified contrast (whole-brain analysis, Z > 2.3, p < 0.05, cluster-corrected)						
Brain Regions	p-value	Cluster size (No voxels)	Z-value	Coordinates of local maxima		
				X	Y	Z
Right Insular Cortex	< 0.0001	2076	**3.92**	**42**	**6**	**-2**
Right IFG (p. operc.)			3.89	50	14	14
Right Frontal Pole / OFC			3.74	26	36	-12
Right Insular Cortex			3.65	40	-6	-10
Right IFG (p. operc.)			3.53	56	16	2
Right OFC			3.52	50	26	-8
Left LOC (i)	< 0.0001	1314	**3.44**	**-44**	**-76**	**-6**
Left LOC (s) / Occipital Pole			3.33	-28	-88	16
Left LOC (i)			3.31	-42	-74	2
Left LOC (s) / (i) / Occipital Pole			3.29	-32	-88	10
Left TOFC / OFG			3.20	-32	-62	-14
Left Lingual Gyrus / OFG			3.19	-16	-74	-10
Right LOC (i) / (s)	= 0.0007	980	**3.57**	**38**	**-78**	**8**
Right LOC (s)			3.43	44	-76	24
Right LOC (s)			3.31	40	-80	24
Right LOC (i)			3.28	54	-66	-6
Right LOC (s) / (i)			3.23	48	-78	14
Right LOC (s) / (i)			3.03	46	-70	18
Left COC / Insular Cortex	= 0.0047	746	**3.65**	**-40**	**12**	**2**
Left Insular Cortex			3.31	-40	-6	-4
Left Insular Cortex			3.30	-38	**4**	2
Left Planum Polare			3.13	-44	-10	-6
Left Insular Cortex / COC			3.02	-44	6	-2
Left IFG (p. operc.) / (p. triang)			3.01	-52	18	-4

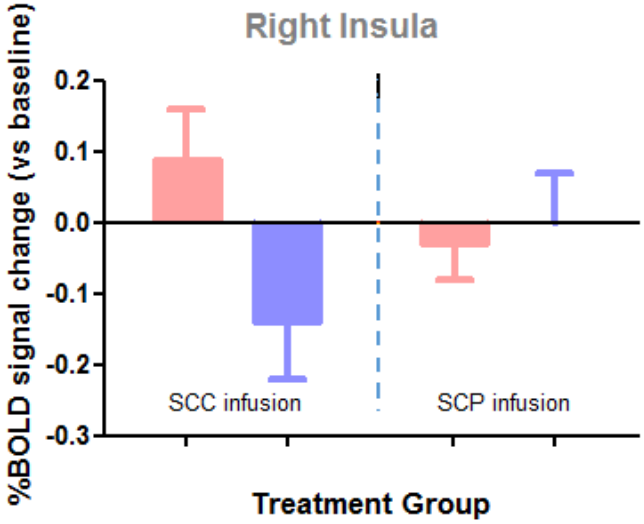
Fig. S5

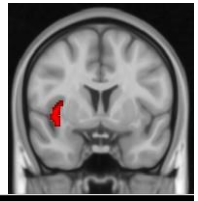
Whole-brain statistical map (family wise error-corrected, Z-threshold > 2.3, $p < 0.05$), depicting two (of the total 4) clusters of brain regions where emotional discrimination between happy and sad faces shows significant differences in the pairwise comparison between the subcutaneous-continuous infusion and oral hydrocortisone administration groups. The statistical map has been rendered onto the standard MNI152 brain. The brain regions presented in the Table (and their coordinates in the MNI152 space) correspond to each cluster's local maxima (Z-value), based on the Harvard-Oxford Cortical Atlas.

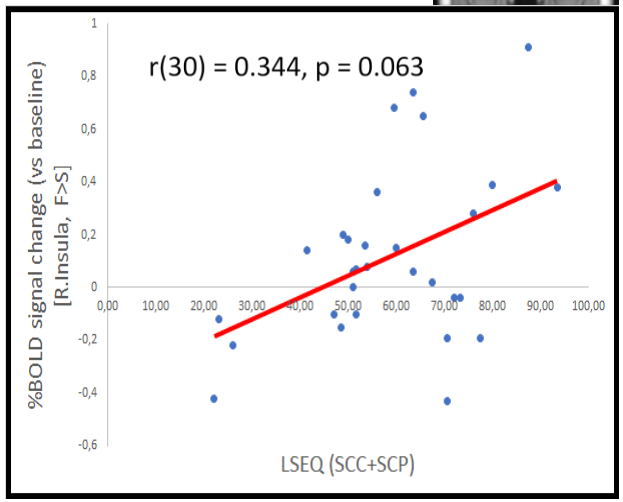
COC: central opercular cortex, (i): inferior, IFG: inferior frontal gyrus, LOC: lateral occipital cortex, OFC: orbitofrontal cortex, OFG: occipital fusiform gyrus, (p. operc.): pars opercularis, (p. triang): pars triangularis, (s): superior, TOFC: temporal occipital fusiform cortex

Subcutaneous-continuous VS subcutaneous-pulsatile infusion









Resting Perfusion

ROI	M.	S.D.	DIFF.	S.D. DIFF.	Post-hoc paired-samples t-test	
RIns[1]	65.9 67.9	12.1 11.8	-2	13.1	t(14) = -0.592 z = 0.227	p = 0.563 p = 0.820

Fig. S6

Whole-brain statistical map (FWE-corrected, Z-threshold = 2.3, $p < 0.05$), depicting one cluster of brain regions where emotional discrimination between fearful and sad faces shows significant differences in the pairwise comparison between the subcutaneous-continuous and -pulsatile hydrocortisone infusion. The statistical map has been rendered onto the standard MNI152 brain. One region of interest (ROI) was included in this cluster; right insula (RI). ROI analysis of the %BOLD signal changes to presentations of fearful (pink bars) and sad (blue bars) facial expressions (versus resting condition) per treatment group is presented for this brain region. These %BOLD signal changes are not confounded by non-specific differences in the neural reactivity, neural coupling or resting perfusion in this ROI. Graph bar represents mean +/- standard deviation (S.D.).

It is worth noting that the difference in the %BOLD signal change from baseline of the right insula between viewing fearful and sad faces among the SCC and SCP shows a moderate positive correlation (Spearman's rank order test) with the self-perceived quality of sleep, as assessed by the Sleep Leeds Evaluation Questionnaire (SLEQ). The poorer the quality of sleep, the greater the difference in the insular %BOLD signal changes between fearful and sad face presentation.

One ([1]) unusual value was detected in the ASL dataset referring to the right insula perfusion (values higher or lower than 3 x studentized residuals); this value was retained in the dataset, but a post-hoc Wilcoxon singed-rank test was performed in addition to the corresponding paired-samples t-test, and both results are presented in the Table.

BOLD: blood oxygen level dependent, DIFF.: difference, FWE: family-wise error, M.: mean, MNI: Montreal Neurological Institute, SCC: subcutaneous-continuous hydrocortisone, SCP: subcutaneous pulsatile hydrocortisone

Subcutaneous-continuous **VS** subcutaneous-pulsatile infusion

Pairwise comparisons for the specified contrast (whole-brain analysis, Z > 2.3, p < 0.05, cluster-corrected)						
Brain Regions	p-value	Cluster size (No voxels)	Z-value	Coordinates of local maxima		
				X	Y	Z
Left Postcentral gyrus	= 0.0301	436	**3.48**	**-58**	**-6**	**24**
Left Postcentral gyrus			3.37	-54	-6	16
Left Precentral gyrus			3.31	-60	2	38
Left Precentral gyrus			3.27	-58	0	34
Left Precentral gyrus			3.12	-50	-6	36
Left COC			3.05	-58	-6	12

Fig. S7

Whole-brain, between-group analysis (family wise error-corrected, Z > 2.3, P < 0.05) using the contrast VISUAL STIMULATION (vs baseline) didn't show any significant BOLD signal variations between the different modes of hydrocortisone replacement in any areas implicated in visual processing (occipital and temporal lobes) or any of our predefined regions of interest (see main body of text). Nevertheless, there is one cluster of cortical brain regions around the left central sulcus (frontoparietal region) showing significant variation in the BOLD signal responses underlying non-emotional visual processing between the subcutaneous-continuous and -pulsatile hydrocortisone infusion groups. The brain regions presented in the Table (and their coordinates in the MNI152 space) are based on the Harvard-Oxford Cortical Atlas and correspond to the cluster's local maxima with the highest Z-values.

BOLD: blood oxygenated-level dependent

		ROIs	MEAN	SD	MEAN.DIFF	SD.DIFF	**Paired-samples t-test**	
SCC vs SCP	Happy > Sad	R. Amygdala	31.7 36.2	15 19.9	-4.5	19.8	t(14) = -0.889	p = 0.389
		R. Striatum	61.3 64.1	7,9 10.6	-2.8	7.4	t(14) = -1.468	p = 0.164
		R. Insula[1]	66.7 67.4	13.4 11.8	-0.7	10.9	t(14) = -0.236 z = 0.398	p = 0.817 p = 0.691
SCC vs PO		R. Insula[2]	71 74.9	15.7 16.6	-3.9	10.8	t(14) = -1.414 z = 1.478	p = 0.179 p = 0.139
		R. Striatum[1]	56.3 60.1	12.5 10.3	-3.8	10	t(14) = -1.472 z = 1.708	p = 0.163 p = 0.088
		R. Orbitofrontal cortex[2]	67.8 70.2	12.4 11.9	-2.4	15	t(14) = -0.619 z = 0.739	p = 0.546 p = 0.460
		L. Insula	63.2 68.9	12.6 12.9	-5.7	11.6	t(14) = -1.921	p = 0.075

Table S8

Regional resting perfusion of brain regions of interest, which showed a treatment-dependent, differential %BOLD signal response during the emotional stimulation fMRI experiment (see Fig. 5, 6 and S6). The mean scores (MEAN) and the mean difference values (MEAN.DIFF) are expressed in mL/100 g tissue/min. One ([1]) or two ([2]) unusual values were detected in some arterial spin labelling datasets (values of studentized residuals higher or lower than 3); they were retained in the datasets, but a Wilcoxon singed-rank test was performed in addition to the corresponding paired-samples t-test, and both results are presented in the Table.

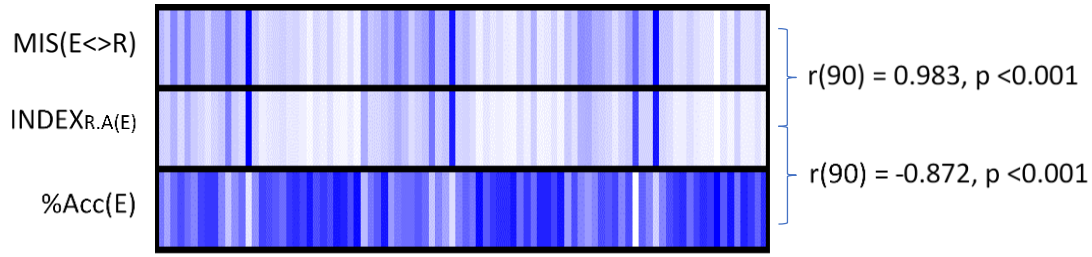
PO: per os treatment group, ROIs: regions of interest, SCC: subcutaneous-continuous hydrocortisone infusion group, SCP: subcutaneous pulsatile hydrocortisone infusion group, SD: standard deviation, SD.DIFF: standard deviation of the difference

Given emotional valence (eg. sadness)

The subject identifies correctly the given emotional valence shown on the screen (A).

The subject misidentifies the given emotional valence shown on the screen as anything else (B).

The subject misclassifies any other emotional valence or non-emotional faces shown on the screen as the given emotional valence (C).



$$INDEX_{R.A(E)} = \frac{B + C}{A}$$

- The numerator of the index shows the frequency of the perceptual bias of the individual involving the given emotion.
- The frequency of the perceptual bias is normalized by the number of correct identifications of the given emotion by the subject.
- The higher the index, the stronger the perceptual bias (ambiguity) related to the given emotion.

Fig. S9

In order to define the ambiguity in recognizing emotional faces we created an index derived from the data of the FERT. For this index, we divided the number of misclassifications from or towards each valence (sadness and happiness) of emotional faces (i.e. the sum of the number of non-emotional faces and emotional faces not expressing each given valence, which were wrongly recognized as that given valence, and the number of faces expressing that given valence which were wrongly recognized as something else) by the number of emotional faces of that valence correctly recognized as such. The higher the index, the higher the degree of the perceptual bias related to each emotional valence. This index was chosen, because of its concurrent very high (absolute) correlation coefficient with both, the %accuracy scores (for correctly identifying) and the number of misclassifications involving the corresponding emotional valence, two pieces of information not necessarily reflecting the same underlying neural processes, although strongly reciprocally correlated. In other words, the neural mechanisms, related to the sensitivity of the brain to identify a given emotion from all faces expressing it in different intensities, do not necessarily completely overlap with those resolving the perceptual ambiguity between that given emotion and other relevant cues. The index we've constructed takes into account both processes (in the denominator and numerator respectively). More details on the categorization of the various kinds of misclassifications, their origin and direction, can be found in Figure S18.

%Acc(E): percentage accuracy for recognizing a given emotion E (these data are presented in Fig. 4), FERT: face emotion recognition task, $INDEX_{R.A(E)}$: index of the recognition ambiguity (of a given emotion E), MIS(E<>R): misclassifications involving a given emotion E (either from anything else -R- to the given emotion E or *vice versa*)

Quality of Sleep (assessed by the LSEQ)	**FERT – Accuracy in recognizing emotional faces[1]**			
	%Accuracy (positive valence)		*%Accuracy (negative valence)*	
	-0.232		-0.209	
	[-0.059]		[0.024]	
	FERT – Misclassifications of emotional faces[1]			
	%Norm. Origin (positive valence)	*%Norm. Origin (negative valence)*	*%Norm. Destination (positive valence)*	*%Norm. Destination (negative valence)*
	-0.038	**-0.465****	0.253	0.256
	[0.066]	**[-0.366]***	[0.261]	[0.090]
	FDOT – Vigilance scores[1]			
	Masked-Fearful	*Unmasked-Fearful*	*Masked-Happy*	Unmasked-Happy
	0.009	0.031	0.193	0.140
	IFEPT/fMRI – Valence-related DIFF. %BOLD signal change of ROIs in SCC and SCP[2]			
	Right Amygdala (H-S)	*Right Striatum (H-S)*	*Right Insula (H-S)*	*Right Insula (F-S)*
	[0.118]	[0.281]	[0.294]	[0.344]
	IFEPT/fMRI – Valence-related DIFF. %BOLD signal change of ROIs in SCC and PO[2]			
	Right Striatum (H-S)	*Right OFC (H-S)*	*Right Insula (H-S)*	*Left Insula (H-S)*
	[0.068]	[0.224]	[0.082]	[0.160]
	N-back[3]			
	%Accuracy (2back)	*%Accuracy (3back)*	*DIFF. %Accuracy (3back-2back)*	
	0.049	-0.177	-0.198	

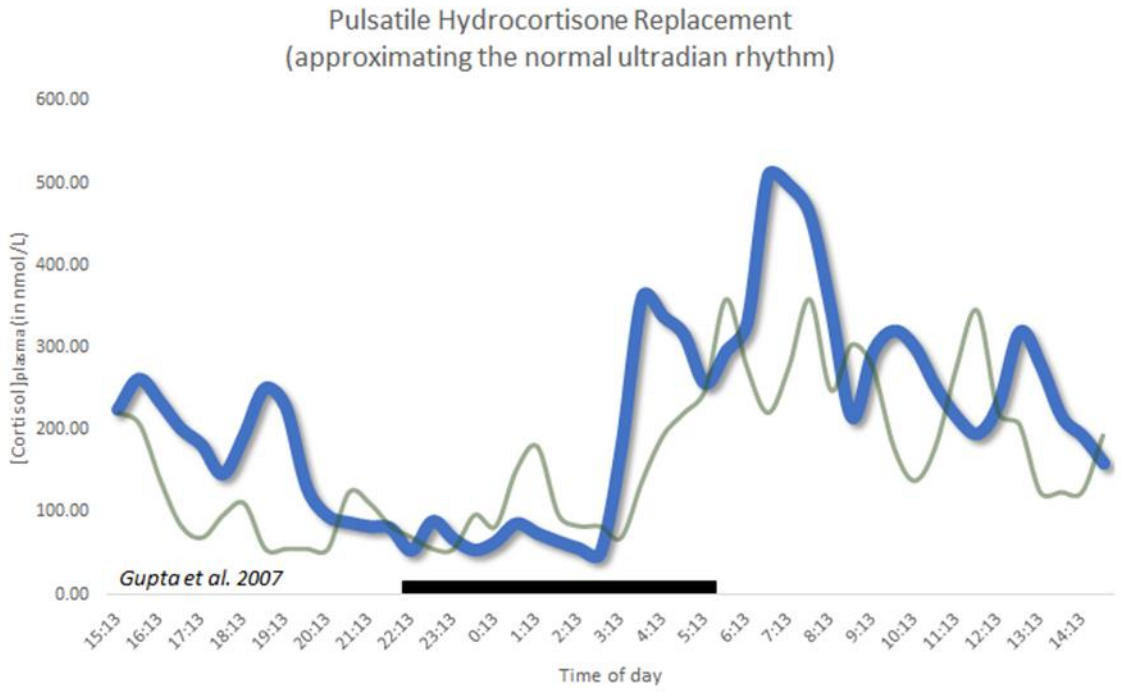
Table S10

Correlations between the self-perceived quality of sleep (as assessed by LSEQ) and the rest of the neurobehavioural outcome measures; neural responses to emotional stimulation, cognitive and behavioural tasks. Pearson's product-moment correlation test (correlation coefficient values outside brackets, df=N-2) and/or Spearman's rank-order correlation test (correlation coefficient values inside brackets, df=N) was used.

[1] N=45, [2] N=30, [3] N=39

* $p < 0.05$, ** $p < 0.01$

BOLD: blood oxygen-level dependent, df: degrees of freedom, DIFF.: difference in, (F-S): contrast referring to the differential neural processing of fearful and happy faces, FDOT: emotional face-related attentional bias task, FERT: face emotion recognition task, fMRI: functional magnetic resonance imaging, (H-S): contrast referring to the differential neural processing of happy and sad faces, IFEPT: implicit facial expression processing task, LSEQ: Leeds Sleep Evaluation Questionnaire, N: number of subjects in a given group, OFC: orbitofrontal cortex, PO: per os treatment group, ROIs: regions of interest, SCC: subcutaneous-continuous infusion group, SCP: subcutaneous-pulsatile infusion group



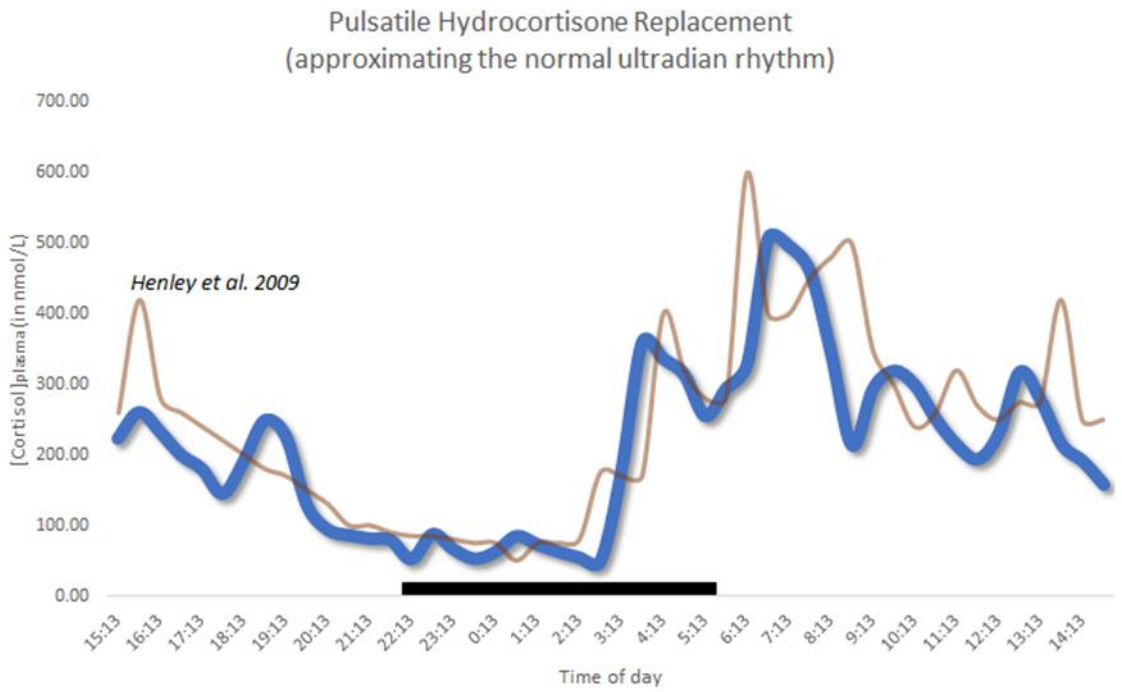


Fig. S11

24-hour biochemical profile of plasma cortisol in a subject receiving metyrapone treatment and subcutaneous-pulsatile hydrocortisone replacement (*60*). This mode of metyrapone-induced block of cortisol biosynthesis and concurrent hydrocortisone replacement tries to approximate the physiological (normal circadian and underlying ultradian) profile of endogenous cortisol secretion (blue line). The profile is replicated twice in this figure to be overlain by the 24-hour biochemical profiles of plasma cortisol of male individuals, as created by the uninterrupted, endogenous activity of the hypothalamic-pituitary-adrenal axis, reproduced by the data of Gupta S et al. (*38*) (green line, top) and Henley DE et al. (*34*) (brown line, bottom). The thick black line signifies the period of the day during the night sleep.

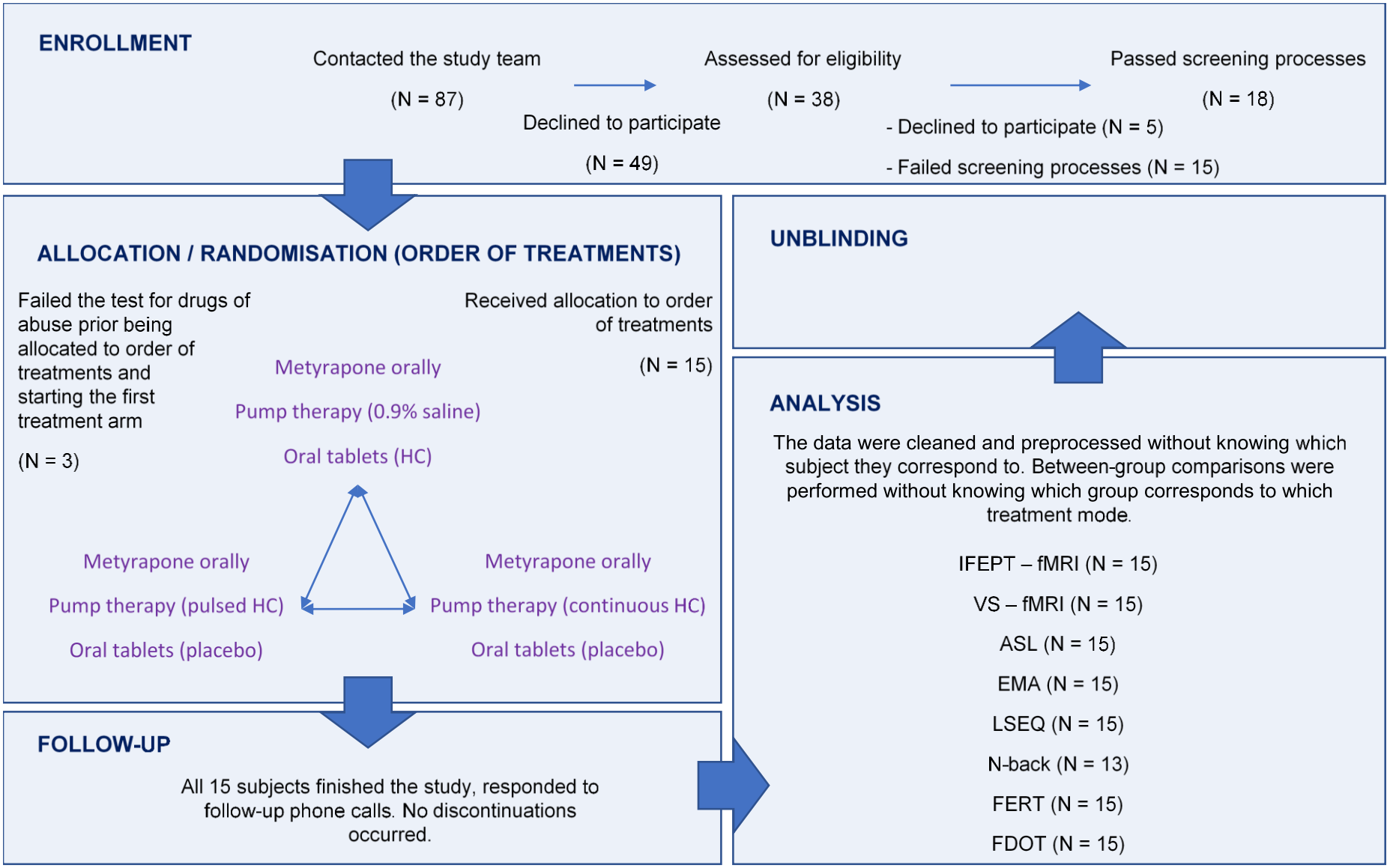

ENROLLMENT
Contacted the study team
(N = 87)
Declined to participate
(N = 49)
Assessed for eligibility
(N = 38)
- Declined to participate (N = 5)
- Failed screening processes (N = 15)
Passed screening processes
(N = 18)
ALLOCATION / RANDOMISATION (ORDER OF TREATMENTS)
Failed the test for drugs of abuse prior being allocated to order of treatments and starting the first treatment arm
(N = 3)
Received allocation to order of treatments
(N = 15)
Metyrapone orally
Pump therapy (0.9% saline)
Oral tablets (HC)
Metyrapone orally
Pump therapy (pulsed HC)
Oral tablets (placebo)
Metyrapone orally
Pump therapy (continuous HC)
Oral tablets (placebo)
FOLLOW-UP
All 15 subjects finished the study, responded to follow-up phone calls. No discontinuations occurred.
UNBLINDING
ANALYSIS
The data were cleaned and preprocessed without knowing which subject they correspond to. Between-group comparisons were performed without knowing which group corresponds to which treatment mode.
IFEPT – fMRI (N = 15)
VS – fMRI (N = 15)
ASL (N = 15)
EMA (N = 15)
LSEQ (N = 15)
N-back (N = 13)
FERT (N = 15)
FDOT (N = 15)

Fig. S12

CONSORT flow diagram containing all data according to latest suggestions (http://www.consort-statement.org/consort-statement/flow-diagram).

ASL: arterial spin labelling, EMA: ecological momentary assessment, FDOT: emotional face-related attentional bias task, FERT: face emotion recognition task, fMRI: functional magnetic resonance imaging, HC: hydrocortisone, IFEPT: implicit facial expression processing task, LSEQ: Leeds sleep evaluation questionnaire, N: number of subjects, VS: visual stimulation (flashing checkerboard)

PARTICIPANTS	AGE	ETHNICITY	DEGREE OF RIGHT-HANDEDNESS[1]	SMOKING HABBITS[2]	ALCOHOL CONSUMPTION HABBITS[3]	CAFFEINE CONSUMPTION HABBITS[4]	WEIGHT	HEIGHT	BMI
1	33	Greece	+1.0	0	0	0	65	165	23.9
2	29	Nigeria	+1.0	0	0	0	68	177	21.7
3	25	Canada	+0.7	0	0	0	87	183	26.0
4	26	United Kingdom	+0.9	0	0	0	105	183	31.4
5	28	India	+0.4	0	3	0	88	187	25.2
6	22	United Kingdom	+0.9	0	10	1	67	184	19.8
7	30	United Kingdom	+0.6	0	3	2	86	189	24.1
8	31	Chile	+0.8	0	8	2	73	177	23.3
9	29	United Kingdom	+0.9	0	0	0	81	192	22.0
10	25	United Kingdom	+0.7	0	14	1	100	188	28.3
11	32	United Kingdom	+0.4	0	4	1	83	183	24.8
12	20	United Kingdom	+0.5	0	16	2	76	190	21.1
13	23	United Kingdom	+0.6	0	10	0	77	185	22.5
14	21	United Kingdom	+0.7	0	9	3	81	176	26.1
15	21	Australia	+0.3	0	10	2	66	179	20.6
MEAN	**26.3**	**-**	**+0.7**	**0**	**6**	**1**	**80.2**	**182.5**	**24.1**
SD	**4.2**	**-**	**0.2**	**0**	**5**	**1**	**11.5**	**6.7**	**3**

Table S13

[1] the degree of handedness was assessed by the Edinburgh Handedness Inventory,

[2] cigarettes per day

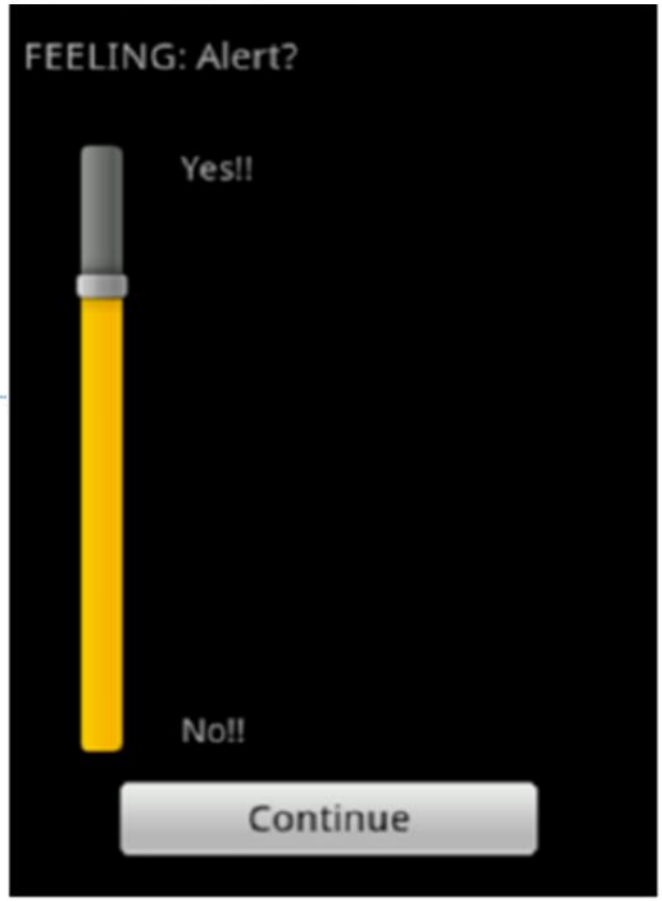
[3] units per week

[4] cups per day

BMI: body mass index

Questions about self-perceived reactivity and feelings of well-being

VAS_Mood_1: Feeling right now… Alert?
VAS_Mood_2: Feeling right now… Energetic?
VAS_Mood_3: Feeling right now… Happy?
VAS_Mood_4: Feeling right now… Enthusiastic?
VAS_Mood_5: Feeling right now… Sad?
VAS_Mood_6: Feeling right now… Upset?
VAS_Mood_7: Feeling right now… Irritable?
VAS_Mood_8: Feeling right now… Stressed?
VAS_Mood_9: Feeling right now… Unmotivated?



Item	Factor 1	Factor 2	Communality
Energetic	0.92[a]	- 0.15	0.87
Enthusiastic	0.85 [a]	- 0.27	0.79
Happy	0.69 [a]	- 0.46 [a]	0.69
Alert	0.85 [a]	- 0.16	0.75
Irritable	- 0.43 [a]	0.63 [a]	0.58
Sad	- 0.17	0.86 [a]	0.74
Stressed	- 0.39	0.68 [a]	0.63
Unmotivated	- 0.72 [a]	0.25	0.58
Upset	- 0.15	0.89 [a]	0.81

[a] factor loadings retained

Fig. S14

A series of 9 questions about self-perceived reactivity and feelings of well-being (top left part of the figure) appear at multiple, random time-points throughout each day (of each treatment arm) in the form of a visual analogue scale on the screen of the android phones given to each subject (right part of the figure). Participants need to move the slider up or down, to the appropriate level (0-100, with 0 being the absolute negative response and 100 the absolute positive). Principal component analysis was used to reduce the 9 self-perceived reactivity and wellbeing items to a lower number of variables and to identify empirically related groups of variables. Two factors, positive mood and motivation (factor 1) and negative mood (factor 2), were extracted based on the examination of the eigenvalues, the scree plot and the interpretability of the factors. A varimax rotation to the factor loading matrix was applied to achieve a simpler loading pattern. Only rotated factor loadings with a magnitude of 0.4 or greater were retained for the computation of the factor scores (bottom left part of the figure). The factor scores are a weighted sum of the loaded factors for each participant. The mean scores from all factor values, corresponding to the positive and negative affect, collected per subject per treatment mode were calculated.

TECHNICALITIES OF SUPPORTING fMRI SCANS	ANATOMICAL MRI SCAN	Z-SHIMMING SCAN	FIELD MAPPING SCAN
Volumes	1	7	1
Pulses sequence type	3D gradient echo	Echo planar	Gradient echo
Field of view (mm)	240	192	224
Matrix size (mm^2)	256 x 256	64 x 64	64 x 64
Slice thickness (mm)	0.9	3	3
Acquisition orientation	S > C1.6 > T-0.4	T > C-30.0 > S2.3	T > C-30.0 > S2.3
Whole brain?	YES	YES	YES
Bandwidth (Hz/Px)	210	2298	521
Order of acquisitions	Single shot	Interleaved	Interleaved
Echo time (TE, in ms)	2.25	30	5.19 / 2.73
Repetition time (TR, in ms)	1800	3000	488
Flip angle (degrees)	9^o	87^o	60^o

Table S15

Technical image properties of the supporting MR sequences used for improving the accuracy of data acquisition, and the analysis pre- and post-processing steps (like for instance the registration of the images to standard space). These pieces of information are provided following the guidelines for good practices in reporting fMRI studies, as proposed by Poldrack et al. (*78*). The technical image properties of the functional MR sequences used for data acquisition can be found in Kalafatakis et al. (*60*).

C: coronal, D: dimensional, MR: magnetic resonance (imaging), S: sagittal, T: transverse (axial)

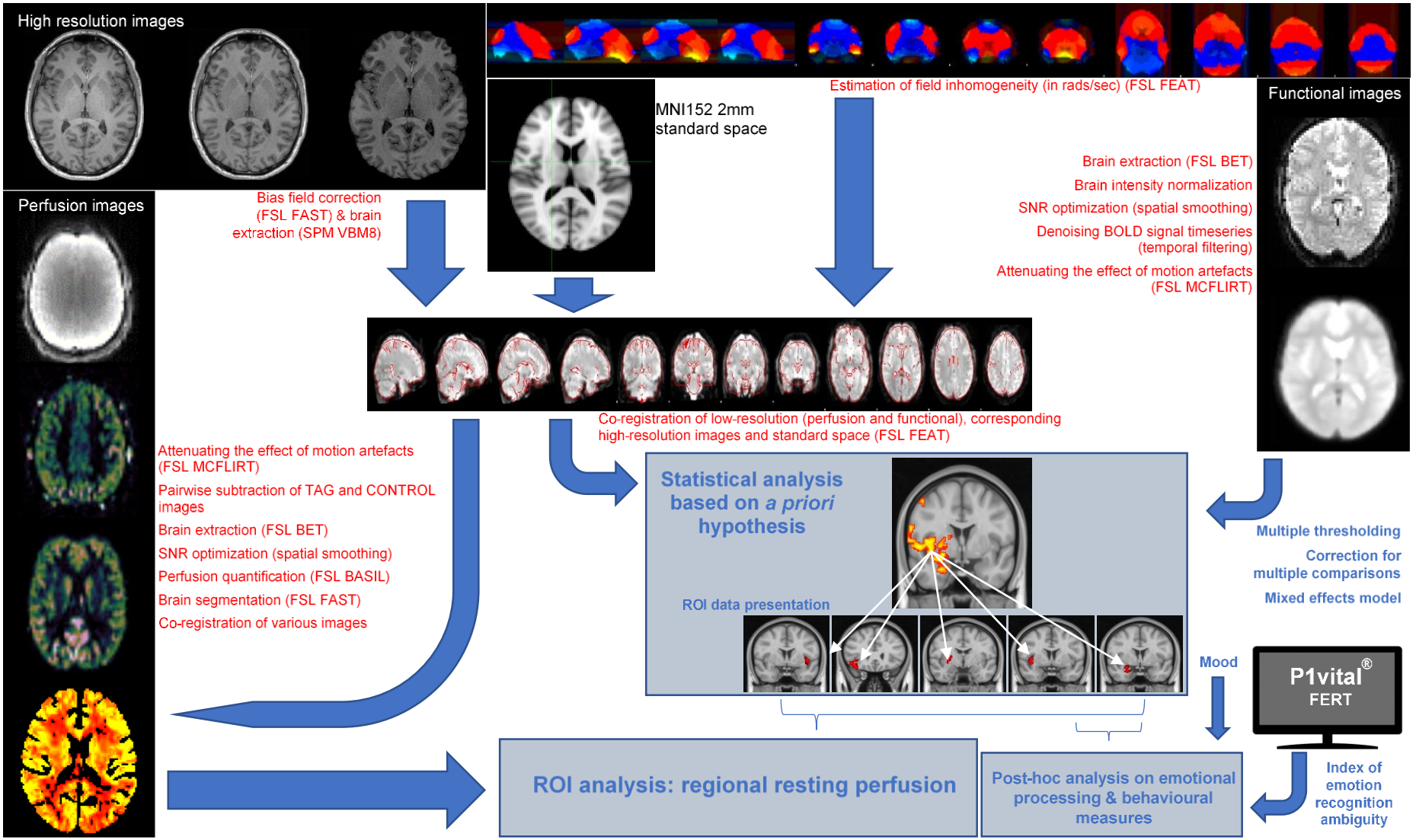

High resolution images
Perfusion images
Bias field correction (FSL FAST) & brain extraction (SPM VBM8)
MNI152 2mm standard space
Estimation of field inhomogeneity (in rads/sec) (FSL FEAT)
Functional images
Brain extraction (FSL BET)
Brain intensity normalization
SNR optimization (spatial smoothing)
Denoising BOLD signal timeseries (temporal filtering)
Attenuating the effect of motion artefacts (FSL MCFLIRT)
Co-registration of low-resolution (perfusion and functional), corresponding high-resolution images and standard space (FSL FEAT)
Attenuating the effect of motion artefacts (FSL MCFLIRT)
Pairwise subtraction of TAG and CONTROL images
Brain extraction (FSL BET)
SNR optimization (spatial smoothing)
Perfusion quantification (FSL BASIL)
Brain segmentation (FSL FAST)
Co-registration of various images
Statistical analysis based on a priori hypothesis
ROI data presentation
Multiple thresholding
Correction for multiple comparisons
Mixed effects model
Mood
P1vital®
FERT
Index of emotion recognition ambiguity
ROI analysis: regional resting perfusion
Post-hoc analysis on emotional processing & behavioural measures

Fig. S16

Outline of the pipeline of the neuroimaging analysis. More details on the functional neuroimaging data analysis in relation to the scientific questions under investigation can be viewed in Fig. S17.

The high-resolution, anatomical, T1-weighted images were used for spatially normalizing the low-resolution functional and perfusion images, and for anatomical localization. They were pre-processed to fit into the co-registration process with the functional and perfusion images, and standard space. Bias field correction has been applied, before removing the non-brain tissue.

The functional image pre-processing steps consisted of (i) brain intensity normalization, (ii) 3D motion correction, (iii) B0 unwarping with assistance from the B0 fieldmap images, (iv) brain extraction, (v) spatial smoothing, (vi) temporal high pass filtering, and (vii) co-registration of functional images with corresponding high-resolution anatomical images and with MNI152 standard space.

For each individual/session fMRI dataset, a regression analysis was performed using a general linear model fitting the temporal evolution corresponding to the paradigm (IFEPT or visual stimulation). A fraction of the temporal derivative of the blurred original waveform was added to the model. Temporal filtering was also applied. The form of the hemodynamic response function convolution method to be applied to the basic waveform was the Gamma variate. In the case of the IFEPT three different effects were modelled (original exploratory variables); visual exposure to (i) fearful human faces, (ii) happy human faces and (iii) sad human faces. In the case of the visual stimulation task, one effect was modelled; the visual exposure to the flashing checkerboard.

For the resting state perfusion images, pre-quantification processing involved (i) construction of a pseudo-4D dataset by stacking up the different ASL flow images along the existing time axis in order of their T1 values, (ii) motion correction, (iii) pairwise subtraction of TAG and CONTROL volumes to generate time-averaged perfusion-weighted images, (iv) brain extraction, (v) generation of transformation matrices between the brain-extracted high-resolution brain image and the M0 calibration image, and (vi) spatial normalisation to reduce arterial contamination.

For the session-level quantification of brain perfusion, the head coil image was divided by a pre-scan normalized version to give an estimate of the receive coil sensitivity map. This map was used to correct the perfusion and head coil calibration images to prevent bias in the final quantitative parameter maps. The explicit expected bolus arrival time was set to 1.3 seconds. Signal calibration was performed using CSF as a reference. The mean CSF signal within the ventricles was calculated and corrected for the T1 and T2$*$ of CSF (4.3 seconds and 400 ms respectively), to determine its equilibrium magnetization, M0,CSF. This value was corrected for the T2$*$ (50 ms) and relative proton density of blood, the density of brain tissue, and the inversion efficiency of the ASL pulse train (a = 85%), to obtain an estimate of the effective equilibrium magnetization of blood, M0,b. Absolute values of brain perfusion in mL/100g tissue/min were eventually created. In that process, it was taken into consideration that the T2$*$ of blood depends on whether the labelled water still resides in the vascular compartment, or whether it has exchanged into tissue, a feature proportional to the permeability-surface area for water, which is smaller in white matter than in grey matter. Post-quantification processing involved B0 unwarping with assistance from the B0 fieldmap images for subsequent co-registration of perfusion images with corresponding high-resolution anatomical images and the latter with MNI152 standard space.

ASL: arterial spin labelling, BOLD: blood oxygen-level dependent, CSF: cerebrospinal fluid, FERT: face emotion recognition task, FSL: Software Library for Neuroimaging Analysis developed by the University of Oxford, IFEPT: implicit facial expression processing task, ROI: regions of interest, SPM: Statistical Parametric Mapping (University College London)

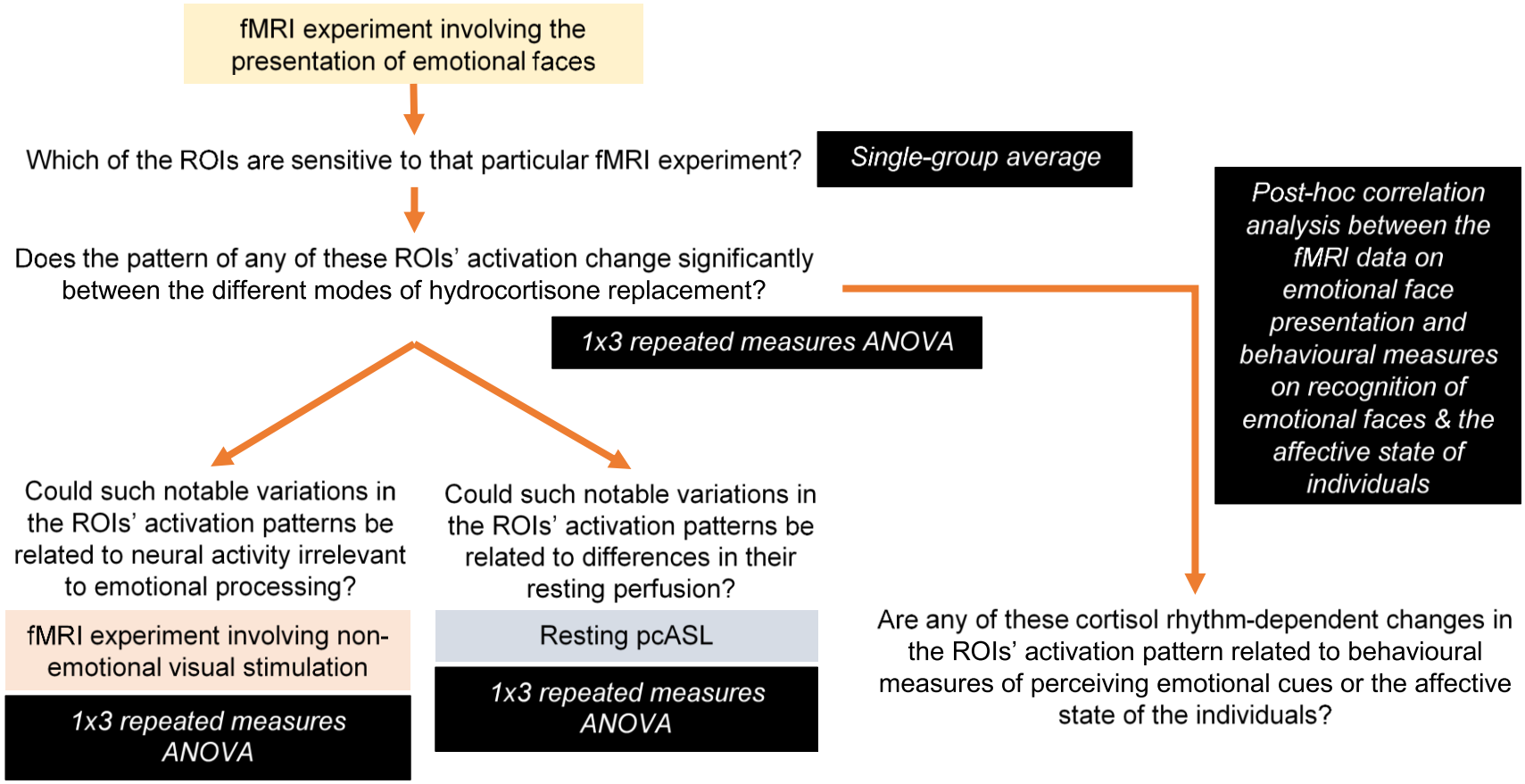

fMRI experiment involving the presentation of emotional faces
Which of the ROIs are sensitive to that particular fMRI experiment?
Single-group average
Does the pattern of any of these ROIs' activation change significantly between the different modes of hydrocortisone replacement?
1x3 repeated measures ANOVA
Post-hoc correlation analysis between the fMRI data on emotional face presentation and behavioural measures on recognition of emotional faces & the affective state of individuals
Could such notable variations in the ROIs' activation patterns be related to neural activity irrelevant to emotional processing?
fMRI experiment involving non-emotional visual stimulation
1x3 repeated measures ANOVA
Could such notable variations in the ROIs' activation patterns be related to differences in their resting perfusion?
Resting pcASL
1x3 repeated measures ANOVA
Are any of these cortisol rhythm-dependent changes in the ROIs' activation pattern related to behavioural measures of perceiving emotional cues or the affective state of the individuals?

Fig. S17

Functional and perfusion neuroimaging-related scientific questions asked in the context of this study on different glucocorticoid daily rhythms. The different neuroimaging methods recruited to answer these questions are evident in the colored boxes, and the corresponding statistical approaches are mentioned in italics, inside black boxes.

For the statistical analysis of the fMRI data acquired during the presentation of emotional faces we produced individual session/subject level maps of activity indicating which brain regions were responding to the emotional face recognition (contrasting the baseline, resting state condition). Various contrasts were used representing either each separate emotional valence processing (fear, happy, sad), or emotion discrimination processing (fear > happy, fear > sad, happy > sad, and vice versa).

To test whether the task of the emotional face presentation was inducing the (expected) activation of the ROIs, we've performed a whole-brain single group average, carried out by using a mixed effects model, by inputting all 45 individually-analyzed fMRI datasets. This type of analysis produced thresholded z-score maps showing whose brain regions' mean activation in response to each valence of emotional faces was significantly different from resting condition.

For the between-group comparisons, whole-brain group level analyses (separately for the emotional and the visual stimulation experiments) were carried out using a mixed effects model. Each group-level analysis produced thresholded z-score brain region clusters highlighting between-treatment groups' statistically significant variations in the activation pattern per contrast used (1x3 repeated measures ANOVA, FEAT is fitting such a mixed effects model with ordinary least squares, requiring the assumption of compound symmetry). In all cases, corrections for multiple comparisons were performed at the cluster level using Gaussian random field theory (minimum $z > 2.3$, cluster p threshold < 0.05). Only data from the predefined ROIs, if contained in contrasts that exhibit a cluster-corrected, between-treatment groups' statistically significant variance, are presented in full detail. Both, the contrast of parameter estimate (arbitrary values) and two measures of the effect size (%BOLD signal change values and extent of the ROIs' activation) with standard deviations are shown in the corresponding Graphs, according to latest guidelines for reporting fMRI data.

For the statistical analysis of the fMRI data acquired during the non-emotional visual stimulation we produced individual session/subject level maps of activity indicating which brain regions were responding to the visual stimulation (contrasting the baseline, resting state condition). In the context of these individual/session-level timeseries analyses, pre-whitening was applied.

Regional perfusion values were extracted from these ROIs, which were contained in the family wise error-corrected clusters, exhibiting statistically significant variance of their %BOLD signal responses in the emotional face fMRI experiment between the treatment groups. The influence of the different cortisol rhythms on the absolute perfusion of these ROIs was evaluated with a paired-samples t-test. Tests for detecting outliers and normality in the distribution of data have been used and taken into consideration. One or two genuinely unusual values were detected in some ASL datasets (values of studentized residuals higher or lower than 3); they were retained in the datasets, but a Wilcoxon singed-rank test was performed in addition to the corresponding paired-samples t-test (and the outcome of both tests is provided in Table S8). This test can be considered as the nonparametric equivalent to the paired-samples t-test. Two-tailed tests were performed for all analyses and p was set to 0.05.

ANOVA: analysis of variance, %BOLD: percent blood oxygen level-dependent, fMRI: functional neuroimaging, pcASL: pseudo-continuous arterial spin labelling, ROI(s): region(s) of interest

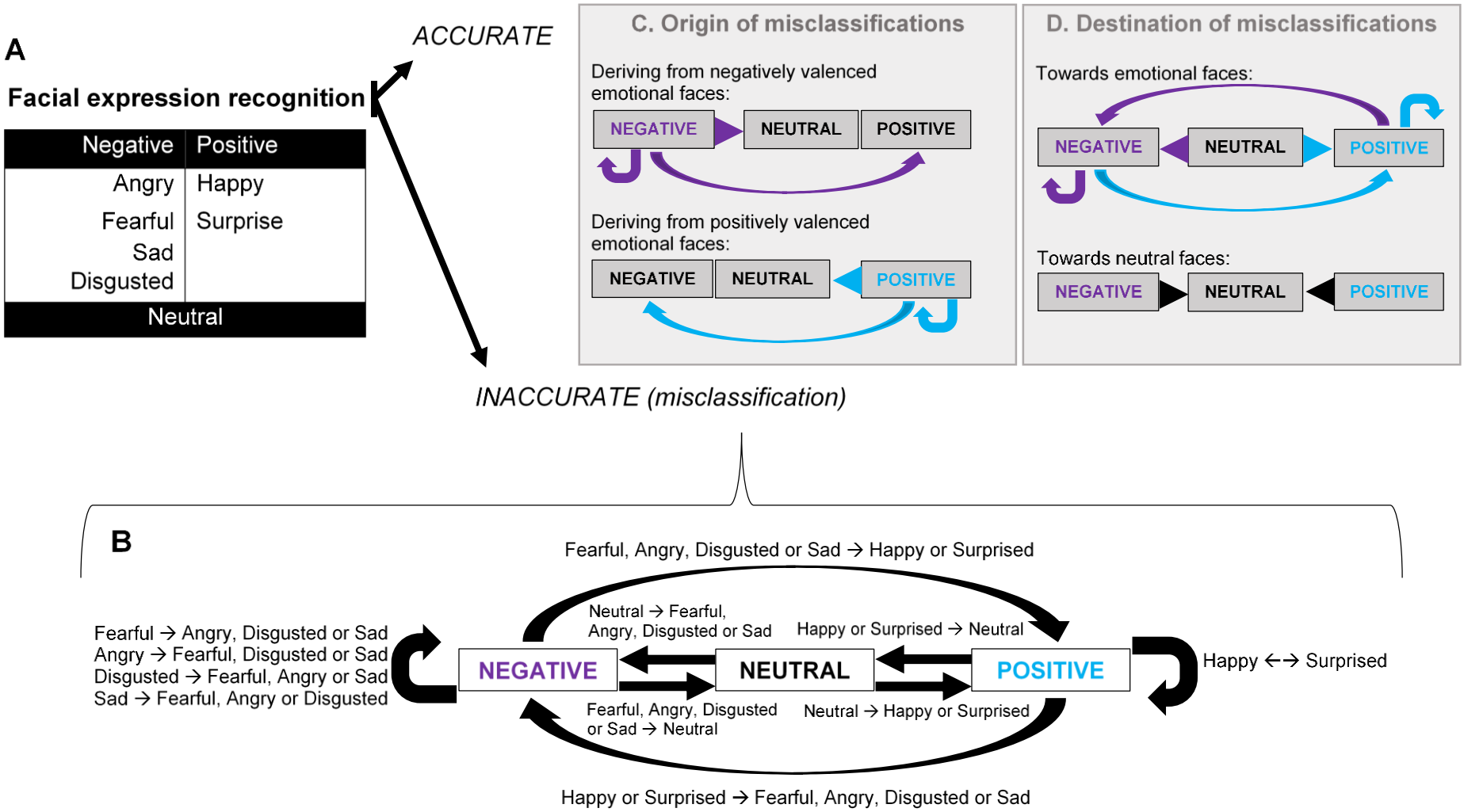

A
Facial expression recognition
Negative
Positive
Angry
Happy
Fearful
Surprise
Sad
Disgusted
Neutral
ACCURATE
INACCURATE (misclassification)
C. Origin of misclassifications
Deriving from negatively valenced emotional faces:
NEGATIVE
NEUTRAL
POSITIVE
Deriving from positively valenced emotional faces:
NEGATIVE
NEUTRAL
POSITIVE
D. Destination of misclassifications
Towards emotional faces:
NEGATIVE
NEUTRAL
POSITIVE
Towards neutral faces:
NEGATIVE
NEUTRAL
POSITIVE
B
Fearful, Angry, Disgusted or Sad → Happy or Surprised
Neutral → Fearful, Angry, Disgusted or Sad
Happy or Surprised → Neutral
Fearful → Angry, Disgusted or Sad
Angry → Fearful, Disgusted or Sad
Disgusted → Fearful, Angry or Sad
Sad → Fearful, Angry or Disgusted
NEGATIVE
NEUTRAL
POSITIVE
Happy ←→ Surprised
Fearful, Angry, Disgusted or Sad → Neutral
Neutral → Happy or Surprised
Happy or Surprised → Fearful, Angry, Disgusted or Sad

Fig. S18

(*A*) During the face emotion recognition task (FERT), every participant was instructed to identify whether the various faces displayed on a computer screen express an emotion (and if yes, determine its valence by pressing the corresponding button from six different options; angry, fear, sad, disgust, happy, surprise) or not (by pressing another button labelled as neutral). According to the taxonomy followed in earlier studies using the same psychological techniques (see literature provided in the main paper), we've divided the different emotional valences to two main categories; negative emotions (angry, fear, sad, disgust) and positive emotions (happy, surprise). For each face displayed, participants could have either identified the emotional valence (if present) accurately or not. (*B*) In the latter case, participants would have misclassified the face displayed. In this part of the figure, all combinations of possible misclassifications are presented, where the reader can also follow the pathway between the origin (what would have been the accurate answer) and the destination (what was the actual answer) of each misclassification. (*C*) In this part of the figure, the reader can see the kind of groups, referring to the origin of misclassifications, that were formed, to conduct the analysis of variance between the treatment conditions. (*D*) In this part of the figure, the reader can see the kind of groups, referring to the destination of misclassifications, that were formed, to perform the conduct of variance between the treatment conditions.

Negative mood ratings (assessed via EMA)	**FERT – Accuracy in recognizing emotional faces[1]**			
	%Accuracy (positive valence)		*%Accuracy (negative valence)*	
	-0.133		0.133	
	FERT – Misclassifications of emotional faces[1]			
	%Norm. Origin (positive valence)	*%Norm. Origin (negative valence)*	*%Norm. Destination (positive valence)*	*%Norm. Destination (negative valence)*
	0.047	0.233	-0.033	0.265
	FDOT – Vigilance scores[1]			
	Masked-Fearful	*Unmasked-Fearful*	*Masked-Happy*	Unmasked-Happy
	0.073	0.049	0.104	-0.101
	LSEQ (quality of sleep)[1]			
	0.127			
	N-back[2]			
	%Accuracy (2back)	*%Accuracy (3back)*	*DIFF. %Accuracy (3back-2back)*	
	-0.014	0.049	0.051	

Table S19

Correlations between the ratings on negative mood (as assessed via EMA techniques) and the rest of the neurobehavioural outcome measures; sleep quality, cognitive and behavioural tasks. Spearman's rank-order correlation test (correlation coefficient values shown, df=N) was used. No significant correlations have been specified.

[1] N=45, [2] N=39

df: degrees of freedom, DIFF.: difference in, EMA: ecological momentary assessment, FDOT: emotional face-related attentional bias task, FERT: face emotion recognition task, IFEPT: implicit facial expression processing task, LSEQ: Leeds Sleep Evaluation Questionnaire, N: number of subjects in a given group