Supporting Information

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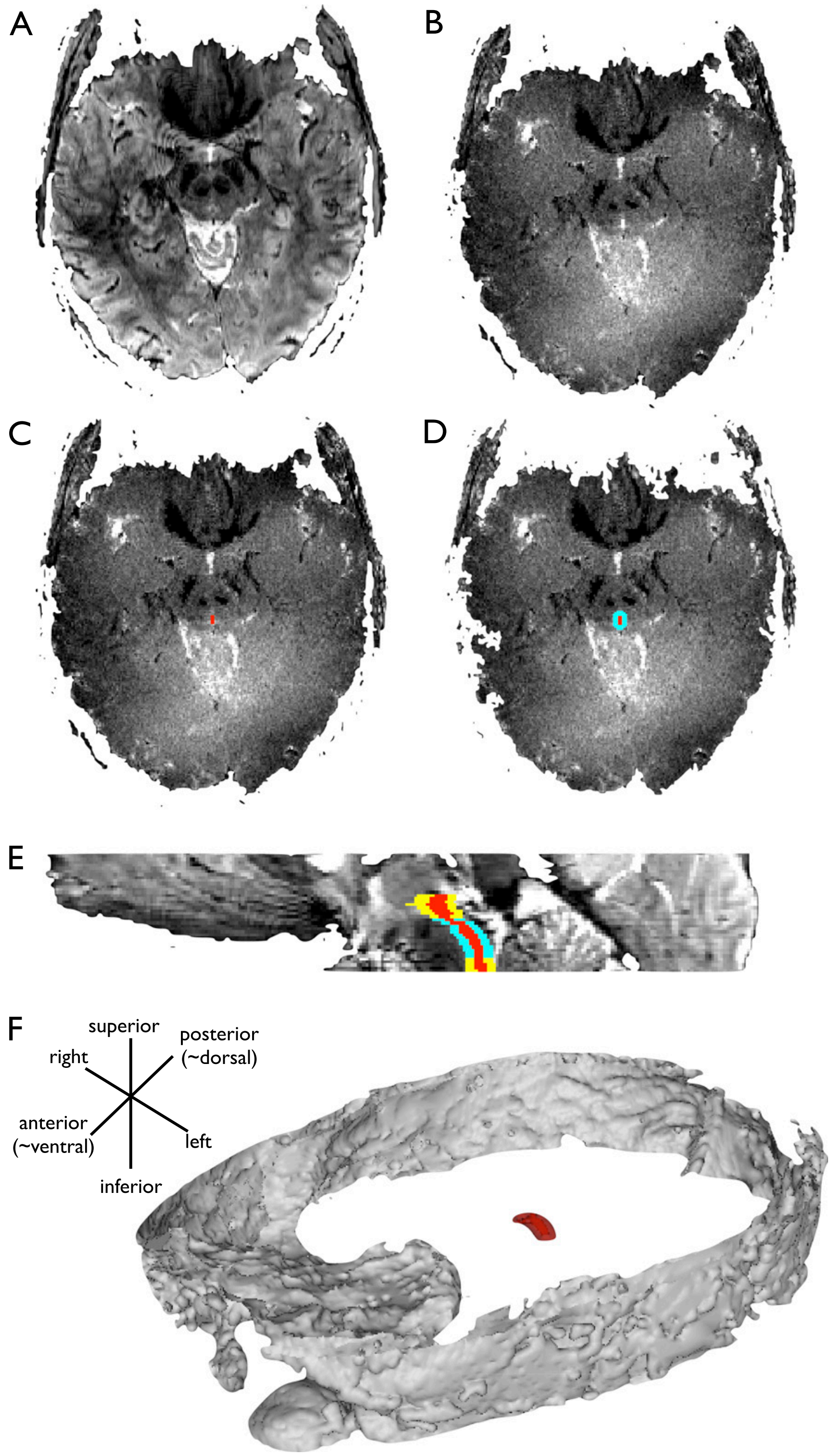

Fig. S1. Isolating the periaqueductal gray (PAG). PAG masks were generated directly from the functional data. Standard imaging procedures introduce partial-volume effects that merge signal from the aqueduct with signal from the PAG because of large voxel sizes, imprecise normalization, and smoothing

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procedures. The figure illustrates how ultra-high resolution 7-T neuroimaging and a procedure for generating targeted masks can be used to isolate signal from the PAG. (*A*) The mean image of a single functional run illustrates the advantage of high-resolution scanning at 7 T by clearly showing the PAG and other subcortical structures including the red nucleus and substantia nigra. (*B*) The variability image of the functional scan clearly distinguishes the aqueduct (in white) from the surrounding PAG (in gray). (*C*) A mask is generated for aqueduct as shown in red using the variability image for each functional run. (*D*) The PAG is identified as surrounding the aqueduct, shown in blue, using a dilation of three voxels (2.25 mm) for each functional run. Masks were inspected visually and edited manually as needed. (*E*) A sagittal slice showing the aqueduct in red and PAG in blue (yellow regions extended beyond the PAG and were removed manually). (*F*) An example of a PAG mask for one run depicted in 3D.

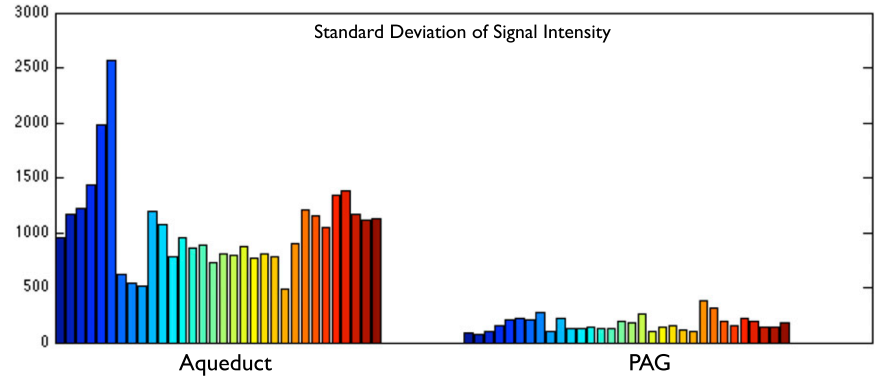

Fig. S2. Comparison of signal variability in the PAG and the aqueduct. The SD of signal is plotted for 32 functional runs for signal averaged within masks for the aqueduct and the PAG. On average and across runs, the SD of signal in the aqueduct is 6.11 times greater than in the PAG (or 36 times greater in variance). Signal in the aqueduct can mask findings because of added noise and also can create artifactual observations attributed to the PAG because of the influence of heart rate on cerebrospinal fluid flow (1).

1. Dagli MS, Ingeholm JE, Haxby JV (1999) Localization of cardiac-induced signal change in fMRI. *Neuroimage* 9(4):407–415.

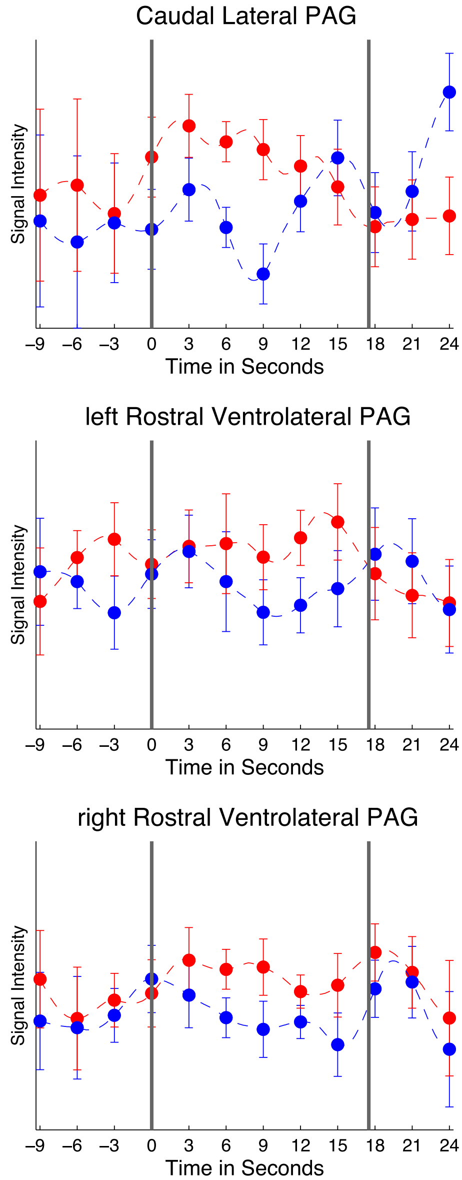

Fig. S3. Sample mean time-course plots from single voxels in the PAG. Raw signal intensity was extracted from each functional scan for each subject for the three peak voxels: the caudal lateral PAG (*Top*), the left rostral ventrolateral PAG (*Middle*), and the right rostral ventrolateral PAG (*Bottom*). The first gray bar at time point 0 marks the onset of the block of images (either aversive or neutral), which lasted for 17.5 s; the second bar marks the offset of the block. SE bars for within subject effects show the variability of the differences between conditions over time points. The plots demonstrate that, on average, the increase in signal intensity is greater during aversive-image viewing (red lines) than during neutral-image viewing (blue lines). Images were presented in separate blocks, but activity is overlaid here for ease of comparison. Intriguingly, the averaged hemodynamic responses appear slightly different among the regions, a finding that may be of interest for future investigations that optimize detecting the properties of the hemodynamic response to various kinds of affective stimuli.

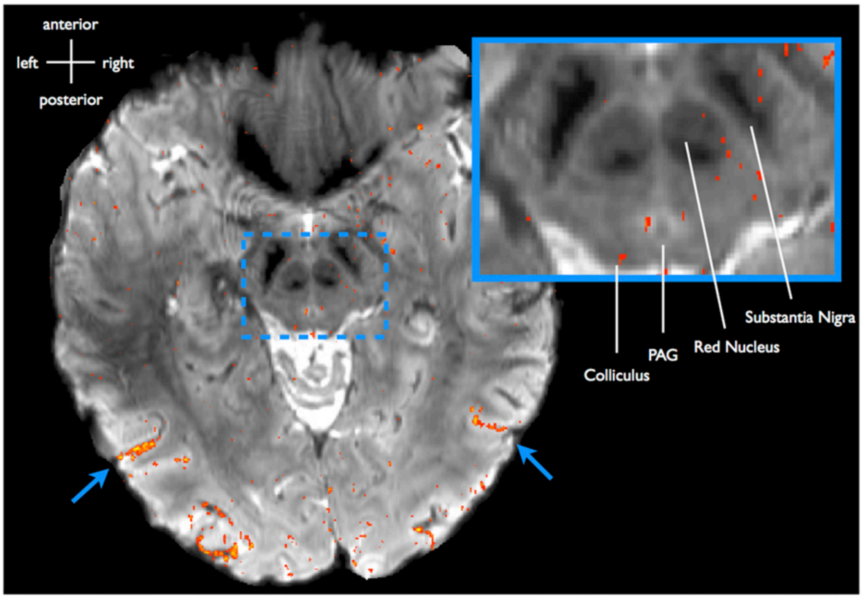

Fig. S4. Functional activity in brainstem nuclei as revealed by neuroimaging at 7 T. The figure illustrates the greater precision obtained when using high-field strength and high-resolution imaging. The figure is provided for illustrative purposes only and does not reflect the precise methods used to acquire signal from the PAG specifically (which are illustrated in Fig. S1). The underlay is the mean functional image for a single participant. The overlay is functional activity during the viewing of aversive relative to neutral images for that participant (uncorrected, $P < 0.05$). Blue arrows point to functional activations that track closely with the particular morphology of sulci and gyri of the participant's lateral occipital and occipitotemporal cortex. The enlarged view in the blue box shows separation among brainstem nuclei directly in the functional scans. The colliculi, the PAG, the red nucleus, and substantia nigra are visible landmarks that also may be used to triangulate activation in adjacent nuclei. The functional parameters were tuned to maximize signal in the PAG. Adjustments would be required to maximize signal in the red nucleus and substantia nigra (which are higher in iron content and may improve with a reduced echo time) and possibly in other brainstem nuclei.

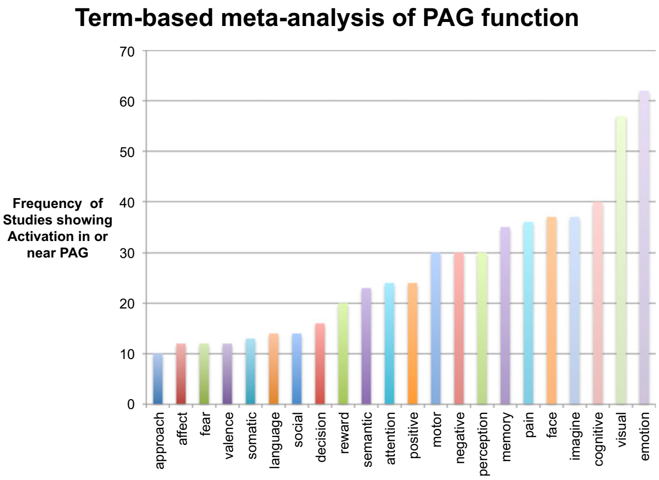

Fig. S5. Term-based meta-analysis of PAG function from functional MRI and PET studies. Neuroimaging studies routinely show activity in the vicinity of the PAG across a broad array of domains, suggesting that regulation of homeostasis may be important to a broad range of psychological events. We used a term-based summary map from a database of more than 6,000 neuroimaging studies (NeuroSynth) (1) and identified 145 studies that showed activation in or near the PAG. NeuroSynth indexes the psychological content domains on which these studies focused based on the frequency counts of words. The bar plot shows the number of articles that focused on various kinds of psychological content and also show activity in or near PAG. Of the 145 total studies, studies examining "emotion" were most frequent; however, studies investigating a variety of other psychological contents also frequently engage voxels in the vicinity of the PAG. The standard neuroimaging methods used in these studies cannot pinpoint whether the PAG is in fact frequently engaged in these domains.

1. Yarkoni T, Poldrack RA, Nichols TE, Van Essen DC, Wager TD (2011) Large-scale automated synthesis of human functional neuroimaging data. *Nat Methods* 8(8):665–670.

Table S1. Pattern matrix from factor analysis

Measure	Factor 1	Factor 2	Factor 3
Angry	−0.19	**0.89**	−0.21
Aroused	**0.73**	−0.25	0.28
Disgusted	**0.82**	0.18	0.12
Sad	0.18	0.03	**0.89**
Scared	**0.72**	0.32	0.01
PAG, left rostrallateral	−0.09	−0.11	**0.83**
PAG, left caudal ventrolateral	−0.33	**-0.85**	−0.07
PAG, right caudal ventrolateral	**-0.73**	0.44	0.34

The table presents the pattern matrix from a factor analysis involving participants' self-reported emotional experiences and neural activity in PAG clusters for aversive vs. neutral images. A principle components extraction with direct oblimin rotation was performed on these measures. Three factors were extracted with eigenvalues above 1, accounting for 35, 24, and 18% of the variability, respectively, indicating that variability was spread fairly evenly, given the principle components extraction method. Communalities were in the moderate to high range (0.62–0.88), indicating no Heywood cases. Factor loadings at 0.72 and above are in bold. Instead of loading similarly across factors or uncovering a single-factor solution, the analysis suggests that the PAG clusters loaded on separate factors. Moreover, each factor comprised activity in a PAG cluster and a self-report measure of emotion. Factor 1 involved the left caudal ventrolateral PAG and the emotions disgust, arousal, and fear. Factor 2 involved the right caudal ventrolateral PAG and the emotion anger. Factors 1 and 2 suggest that feelings of disgust, arousal, fear, and anger emerge as activity in ventrolateral passive coping columns diminishes. Factor 3 involved the rostral lateral PAG and sadness. Although sadness is considered a prototypically low-arousal emotion, studies also have identified high-arousal and approach-oriented forms of sadness, which may be more likely to occur with the aversive images used here (see main text). As such, Factor 3 suggests that more feelings of sadness may relate with greater activity in the ventrolateral active coping column. In general, these findings suggest that mappings between subjective emotional experiences, active-/passive-coping responses, and activity in PAG subregions may vary with the particular experimental contexts involved and are unlikely to involve simple one-to-one formulations of specific emotions with active and passive behavioral coping strategies in nonhuman animals.

Table S2. Correlation between emotional experience and PAG subregions

	Angry	Activated	Disgusted	Sad	Scared
Angry					
Activated	−0.38				
Disgusted	−0.04	0.45			
Sad	−0.17	0.51	0.36		
Scared	−0.03	0.29	0.37	0.02	
PAG, left rostral lateral	−0.28	−0.10	0.08	**0.53**	0.00
PAG, left caudal ventrolateral	**−0.62**	0.06	−0.38	−0.22	−0.25
PAG, right caudal ventrolateral	0.48	**−0.53**	−0.14	0.10	−0.39

The table presents the correlation matrix (obtained using robust correlations for outlier correction) between emotional experience reports and between emotional experience with PAG subregions identified from the voxelwise analyses. Correlations in bold were significant at $P < 0.05$ (uncorrected). The factor analysis indicates that variability in activity in PAG subregions loaded on three separate factors. Each factor also was associated with different emotional experience reports. The results suggest that although subregions of PAG were only millimeters apart, they appear to operate in different functional circuits based on showing low intercorrelations among each other (as described in the main text) and differential patterns of factor loadings and correlations with the emotional experience reports. Greater activity in the rostral lateral region was correlated with self-reported sadness in response to the aversive images ($r = 0.53$, $P < 0.05$), whereas activity in the caudal ventrolateral regions were not (left caudal ventrolateral: $r = 0.10$, $P < 0.4$; right caudal ventrolateral: $r = -0.22$, $P < 0.3$). Alternatively, greater activity in the left caudal ventrolateral region was negatively correlated with self-reported anger ($r = -0.62$, $P < 0.05$), and activity in the right caudal ventrolateral region was negatively correlated with arousal to the aversive images ($r = -0.53$, $P < 0.05$). Activity in the right rostral lateral region was not associated with self-reported anger ($r = -0.28$, $P < 0.3$) or with arousal ($r = -0.10$, $P < 0.4$; Table S1). Robust regressions were used for outlier correction.