

# Modulation of locus coeruleus activity by novel oddball stimuli

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**Abstract** It has long been known from animal literature that the locus coeruleus (LC), the source region of noradrenergic neurons in the brain, is sensitive to unexpected, novel, and other salient events. In humans, however, direct assessment of LC activity has proven to be challenging due to its small size and difficult localization, which is why noradrenergic activity has often been assessed using more indirect measures such as electroencephalography (EEG) and pupil recordings. Here, we combined high-resolution functional magnetic resonance imaging (fMRI) with a special anatomical sequence to assess neural activity in the LC in response to different types of salient stimuli in an oddball paradigm (novel neutral oddballs, novel emotional oddballs, and familiar target oddballs). We found a significant linear increase of LC activity from standard trials, over familiar target oddballs, to novel neutral and novel emotional oddballs. Importantly, when breaking down this linear trend, only novel oddball stimuli led to robust activity increases as compared to standard trials, with no statistical difference between neutral and emotional ones. This pattern suggests that activity modulations in the LC in the present study were mainly driven by stimulus novelty, rather than by emotional saliency, task relevance, or contextual novelty alone. Moreover, the absence of significant activity modulations in response to target oddballs (which were reported in a recent study) suggests that the LC represents relative rather than absolute saliency of a stimulus in its respective context.

**Keywords** Locus coeruleus · fMRI · Oddball · Novelty · Saliency

## Introduction

The ability to detect and react to salient stimuli in the environment is crucial for survival in uncertain environments. One brain system that has been commonly linked to fast orienting and flexible behavior of foraging species is the noradrenergic system. Specifically, using neuronal recordings from the locus coeruleus (LC), the host region of noradrenergic neurons in the pons, it has been shown that these neurons respond with phasic activity bursts to salient events, including infrequent, novel, motivational, and emotional stimuli (Berridge and Waterhouse 2003; Sara 2009). This noradrenergic signal is thought to modulate neural activity in diverse cortical and subcortical target regions, thereby affecting sensory processing, attentional control, and long-term encoding of these events (Aston-Jones and Cohen 2005; Sara 2015; Sara and Bouret 2012). Due to challenges in assessing activity of noradrenergic neurons in the human brain, indirect measures have been used as proxies for noradrenergic activity, first and foremost certain event-related potential (ERP) components and pupil dilation (reviewed in Nieuwenhuis et al. 2011a). Importantly, changes in pupil dilation have recently been linked to activity modulations in the LC using fMRI (Murphy et al. 2014), further corroborating their assumed relationship. In addition to the covariation of pupil dilation and LC activity across time in both a resting period and a traditional oddball task, Murphy et al. (2014) showed a differential activity increase in the LC for oddball stimuli for the first time, i.e., for the prototypical event to trigger a noradrenergic orienting response (Nieuwenhuis et al. 2011a). Importantly, however,

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all oddballs acted as targets requiring an immediate motor response, leaving open as to whether neural activity in response to the oddball stimuli was specifically driven by the orienting response to infrequent events or (also) by the associated motor requirement (which was present in oddball but absent in standard trials). This is an important dissociation as activity in noradrenergic neurons increases at the moment of goal-directed responses (e.g., Bouret et al. 2012). Besides the detection of unexpected events, neural recordings in animals and results from indirect measures in humans (i.e., EEG and pupil dilation) have demonstrated that the noradrenergic system is implicated in processing other salient stimulus features, such as stimulus novelty and emotional content (Berridge and Waterhouse 2003; Nieuwenhuis et al. 2011a; Ranganath and Rainer 2003; Sara and Bouret 2012).

In the present study, we sought to illuminate the role of the human noradrenergic system in processing unexpected and other salient information by employing an oddball paradigm in which we manipulated additional properties of the oddball stimuli, i.e., stimulus novelty, emotional content, and task relevance, while keeping both the probability (infrequent) and response requirements (none) for all oddball stimuli equal. Specifically, participants performed a face-oddball task, in which a standard stimulus was presented in the majority of the trials (75%), randomly intermixed with infrequent oddball stimuli (25%). Importantly, oddball stimuli could either be novel faces with a neutral expression, novel faces with an emotional expression, or a familiar neutral face (which also served as a target stimulus). This allowed us to test for potential activity differences in the LC elicited by different types of salient events in the same paradigm, i.e., contextual novelty (common to all oddballs), stimulus novelty (common to both neutral and emotional novel oddballs), emotional saliency (unique to novel emotional oddballs), and task relevance (unique to the familiar target oddball). The fMRI protocol was adapted to meet the challenges associated with imaging brain structures that are fairly small and/or hard to localize on standard anatomical scans due to low (or no) intensity differences to the neighboring tissue (cf. Astafiev et al. 2010). Specifically, we implemented a high-resolution partial-head volume protocol at the level of the pons for better functional resolution, and acquired a neuromelanin-sensitive structural sequence on which the LC can be detected as hyperintensity in the pontine tegmentum (cf. Keren et al. 2009).

The main objective of the present study was to test whether neural activity in the human LC, as assessed by high-resolution fMRI, is responsive to unexpected (infrequent) events in the absence of any motor requirements, and in how far stimulus novelty and emotional content further modulate such responses.

## Materials and methods

**Participants** Data of 21 participants without a history of neurological or psychiatric diseases are reported in the present study (mean age = 23.7 years, age range = 18–39 years, 9 male, 4 left-handed). Two additional participants (male, 23 and 25 years) were excluded due to missing neuromelanin-sensitive anatomical scans (which were aborted by the scanner due to the specific absorption rate being exceeded). All participants gave written informed consent before participating in the study and the experimental procedures were approved by the Ethical Committee of the Ghent University Hospital.

**Oddball task** Participants performed an oddball task with face stimuli in the fMRI scanner. The task was appended to another unrelated experiment, for which participants were already in the fMRI scanner for about 40 min. The stimuli of the oddball task consisted of 252 repetitions of the same familiar neutral face (*standard trial*), 28 repetitions of another familiar neutral face (*target oddball*), 28 unfamiliar neutral faces (*novel neutral oddball*), and 28 unfamiliar fearful faces (*novel negative oddball*).

All stimuli were color photographs taken from the Radboud Face database (Langner et al. 2010) showing male and female faces including neck and shoulders (all seated in front of a grey background and wearing black sweaters). The neutral and negative oddball images both consisted of 50% male and 50% female faces of similar age. The standard face depicted a specific man similar in age to the oddball stimuli, and the target face depicted a specific teenage boy, both of which are considered similarly neutral as compared to the neutral oddball faces (according to the database). The only unique feature of the target stimulus was the slightly younger age to facilitate target recognition. All images were randomly intermixed, creating a baseline of a frequent standard face (75% of all trials) interspersed with oddball presentations (25% of all trials). The sequence of events was random, but the same for all participants. Each face was shown for 800 ms, and the stimulus onset asynchrony was fixed at 2000 ms, i.e., the fMRI scan repetition time. Note that while often very short durations are used in more basic versions of the oddball paradigm (e.g., 75 ms, Murphy et al. 2014), studies focusing on (conscious) emotional processes often use longer durations (e.g., 2000 ms, Fichtenholtz et al. 2004), which is why we chose for a medium duration in the present study. All face stimuli were presented in the center of a white background (visual angle  $5^{\circ} \times 6^{\circ}$ ), projected to a mirror mounted to the MR head coil. Throughout the experiment, a small black fixation dot was visible at all times, i.e., overlaid on each face stimulus (between nose and mouth) and displayed during the inter-trial-interval with the same coordinates. The participants' task was to keep their eyes on the fixation dot, silently count

the occurrence of the target face, and verbally report the total number of targets at the end of the experiment.

Before entering the fMRI scanner, participants were instructed about both the first unrelated experiment (not reported here) and the oddball task. After completion of the first experiment, participants were reminded of the oddball task via written instructions on the screen. After scanning, participants completed the Behavioral-Inhibition and Behavioral-Activation Scales (BIS-BAS, Carver and White 1994) to assess inter-individual differences in the sensitivity to novel information.

**fMRI acquisition** Data were acquired using a 3-T Siemens Magnetom Trio MRI system (Siemens Medical Systems, Erlangen, Germany) with a 32-channel head coil. Participants were instructed to minimize head movements during the scanning session. Before the functional scans, an anatomical T1-weighted 3D magnetization-prepared rapid acquisition with gradient echo sequence (T1-MPRAGE, time repetition TR = 1550 ms, time echo TE = 2.39 ms, TI = 900 ms, acquisition matrix =  $256 \times 256$ , field of view FoV = 220 mm, flip angle =  $9^\circ$ , voxel size =  $0.9 \times 0.9 \times 0.9$  mm) was acquired to enable coregistration and normalization. During the oddball task, T2\*-weighted echo-planar images (EPIs) were acquired in 22 slices as high-resolution partial-head volume with an interleaved scanning order covering the brainstem and upper part of the pons (TR = 2000 ms, TE = 30 ms, acquisition matrix =  $128 \times 128$ , FoV = 216 mm, flip angle =  $90^\circ$ , voxel size =  $1.7 \times 1.7 \times 2$  mm, no inter-slice gap). The first four functional volumes were discarded to allow a steady magnetization to be reached. Afterwards, an additional T1-weighted melanin-sensitive turbo-spin echo sequence was acquired as partial-head volume to allow for an individual localization of the LC (T1-TSE, TR = 559 ms, TE = 9.8 ms, acquisition matrix =  $384 \times 384$ , FoV = 192 mm, flip angle  $1/2 = 70^\circ/180^\circ$ , voxel size =  $0.5 \times 0.5 \times 3$  mm).

**Data analysis** Images were preprocessed and further analyzed using Statistical Parametric Mapping (SPM8; University College, London, UK). After coregistering all scans to the T1 template image provided by SPM, the T1-MPRAGE was spatially normalized to the template (resliced to isotropic 1 mm voxels). The same parameters were used to coregister and normalize the T1-TSE image (resliced to isotropic 1 mm voxels). Functional EPIs were slice-time corrected, and spatially realigned to the first acquired EPI. Next, EPIs were normalized based on the T1-derived normalization parameters, resliced to isotropic 1.5 mm voxels, and smoothed with an isotropic full-width half-maximum Gaussian kernel of 3.5 mm. A manual co-registration check confirmed a good alignment of the brainstem in all anatomical and functional images within and across participants.

After removing direct oddball repetitions from the sequence in order to avoid trial-by-trial repetition suppression (Henson and Rugg 2003), the fMRI analysis was based on 24 novel negative oddballs, 24 novel neutral oddballs, 26 familiar target oddballs, and 252 standard trials. Statistical analysis was performed in three steps. First, for each participant, blood-oxygen-level dependent responses were modeled by delta functions at stimulus onset, which were then convolved with a standard hemodynamic response function, along with temporal and dispersion derivatives (Friston et al. 1998). In addition to four experimental regressors reflecting stimulus type (negative oddball, neutral oddball, target oddball, standard trials), six motion regressors derived from the realignment procedure were included in the general linear model. Before model estimation, a high-pass temporal filter of 128 s was applied (Ashburner and Friston 1999). Second, the LC was segmented manually based on the individual T1-TSE images using MRICron ([www.mricron.com](http://www.mricron.com), Rorden and Brett 2000). In axial slice view, the LC can be detected as bilateral hyperintensity at the level of the pontine tegmentum in the vicinity of the 4th ventricle (Keren et al. 2009). All segmentations were performed by the same experienced researcher and were exclusively based on the anatomical scans, rendering the subsequent analysis of the functional data entirely non-circular (Kriegeskorte et al. 2009). Considering that the LC is the only area that displays hyperintensity on these scans at the level of the pontine tegmentum, anatomical specificity is assumed to be relatively high. Coordinates are reported based on the Montreal Neurological Institute (MNI) system. Third, the blood-oxygen-dependent (BOLD) parameter estimates (beta values) were extracted from the segmented region of interest for each individual participant (average of left and right LC) using the MarsBaR toolbox implemented in SPM (Brett et al. 2002), and submitted to a one-way repeated-measures ANOVA testing for the effect of stimulus type. Furthermore, correlation analyses were performed in order to test whether LC activity was modulated by inter-individual differences in the sensitivity to novel information.

It has been suggested that brainstem regions can suffer from physiological artifacts leading to small movements of the brainstem, which can be accounted for post hoc using respiratory and cardiac information. Such physiological denoising has been shown to improve task-related activations and reduce task-unrelated noise (cf. Harvey et al. 2008; Limbrick-Oldfield et al. 2011). While we were unable to record physiological data for denoising in the present study, we tested whether the observed activity pattern is specific for the LC instead, since one would assume that physiological artifacts would also influence activity in nearby areas, or brain structures with a similar anatomical constellation. In addition, despite using individual anatomical segmentations, it is possible that the observed activity modulations are not specific to the LC but reflect a generalized activity pattern across the

entire pons. To test whether activity modulations in the LC are primarily related to generalized regional activity and/or unspecific physiological noise, we extracted BOLD parameter estimates (beta values) from spheres in two control regions, i.e., a region in the anterior pons (12-mm sphere at [x y z: 1–20–34]) and the superior colliculi (bilateral 6-mm spheres at [–3–31–5] and at [5–31–5]). The anterior pons sphere is located opposite to the LC at the same height, while the superior colliculi spheres are located in the dorsal mesencephalon close to the cerebral aqueduct connecting 3rd and 4th ventricle. Since all three regions are assumed to be similarly affected by small brainstem movements (Harvey et al. 2008), one would assume that these structures would display a similar result pattern if the results in the LC were majorly driven by unspecific physiological noise. Moreover, the anterior pons region serves as additional control to test whether activity modulations in the LC are merely reflecting unspecific hemodynamic activity changes across a wider area.

## Results

All participants reported the number of target oddballs with high precision at the end of the experiment (mean target count = 27.6; target count range = 26–28), confirming that they paid close attention to the stimulus stream of the oddball task.

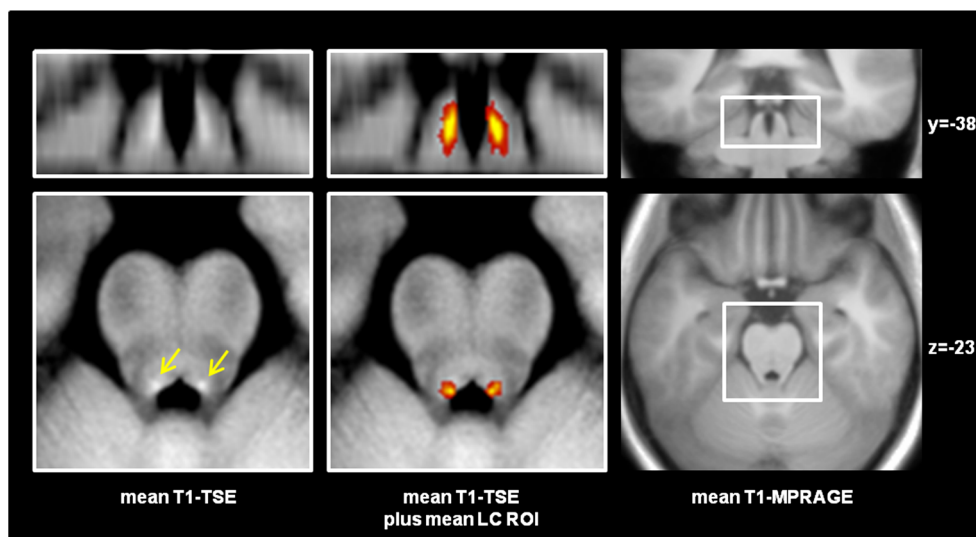
An illustration of LC localization based on T1-TSE scans is shown in Fig. 1. The dimensions of the averaged LC ROI with outer boundary MNI coordinates [posterior/anterior y = –41/–38; ventral/dorsal z = –19/–31; left/right x = –5/6] are consistent with a previously reported map of the human LC based on 44 individuals (Keren et al. 2009).

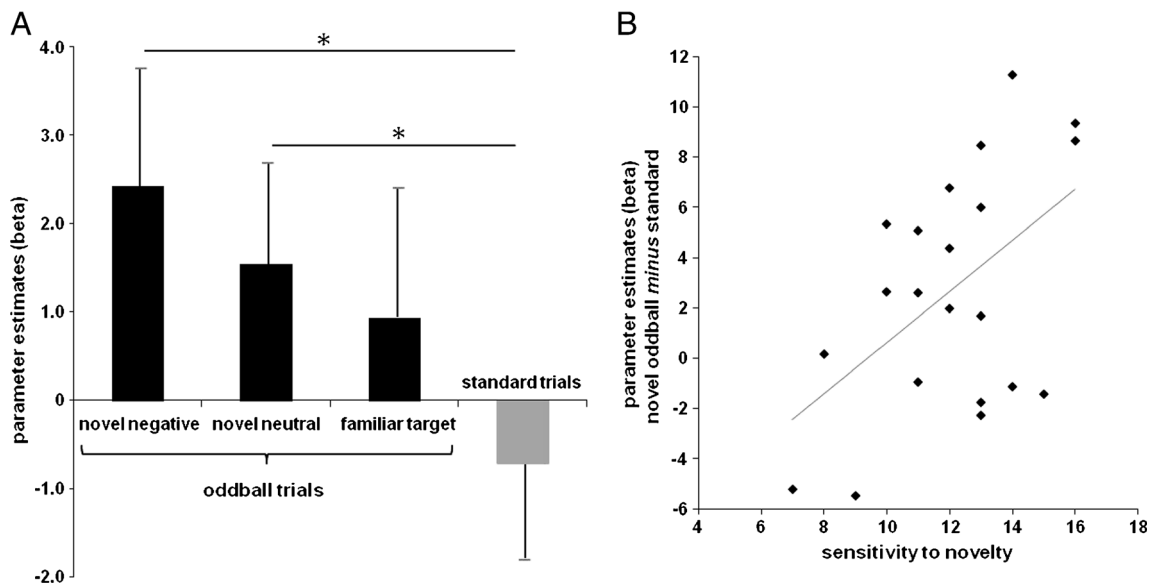
**LC BOLD parameter estimates** A repeated-measures ANOVA of the BOLD parameter estimates (beta values)

extracted from the individually segmented LC ROIs (left and right LC collapsed) revealed a main effect of *stimulus type* ( $F_{(2.1,41.1)} = 3.37$ ,  $p = .043$ , Greenhouse-Geisser-corrected degrees of freedom based on significant Mauchly's Test of Sphericity,  $p < .001$ ). Specifically, LC activity increased linearly (linear contrast:  $F_{(1,20)} = 6.78$ ,  $p = .017$ ) from standard trials, target oddballs, neutral oddballs, and novel negative oddballs (Fig. 2a). Post-hoc paired-sample t-tests revealed that both novel negative oddballs ( $t_{(20)} = 2.38$ ,  $p = .027$ , two-tailed) and novel neutral oddballs ( $t_{(20)} = 2.62$ ,  $p = .016$ , two-tailed) differed significantly from standard trials, while there was no significant difference between novel negative and novel neutral oddballs, and no difference between target oddballs and any of the other stimulus types (all  $p > .1$ ).

The same ANOVA was performed on BOLD parameter estimates (beta values) extracted from two control regions, namely the anterior pons and superior colliculi. None of these regions displayed an effect of *stimulus type* (anterior pons:  $F_{(1.5,29.2)} = 0.48$ ,  $p = .565$ , Greenhouse-Geisser-corrected degrees of freedom based on significant Mauchly's Test of Sphericity,  $p < .001$ ; SC:  $F_{(3,60)} = 3.37$ ,  $p = .273$ , sphericity assumed), suggesting that the activity modulations in the LC are relatively specific and neural in origin, rather than a reflection of physiological artifacts, which would be assumed to affect different brainstem regions in a similar fashion. To provide a more principled statistical test of whether activity patterns in the three regions are indeed different (cf. Nieuwenhuis et al. 2011b), a two-way repeated-measures ANOVA with factors *stimulus type* and *region* (LC, anterior pons, SC) was performed. The interaction between the two factors was marginally significant ( $F_{(3.1,61.6)} = 2.43$ ,  $p = .07$ , Greenhouse-Geisser-corrected degrees of freedom based on significant Mauchly's Test of Sphericity,  $p < .001$ ), indicating that the activity pattern likely indeed differed between regions. It

**Fig. 1** On the left, yellow arrows indicate the location of the LC on the mean T1-TSE (averaged across 21 participants). The same image is overlaid by the mean LC ROI (averaged across 21 participants) in the middle panel. Note that LC ROIs were created for each participant on the basis of the individual T1-TSE scans. For reference, the mean standard T1-weighted MPRAGE scan is shown on the right with z and y reference coordinates (MNI space)





**Fig. 2** **a** LC BOLD parameter estimates (beta, proportional to percent signal change) display a significant linear increase with lowest activity for standard trials and highest for oddball trials containing novel negative stimuli. Asterisks indicate significant post-hoc comparisons. Error bars represent the standard error of the mean. **b** The differential activity

increase for novel oddballs in the LC was correlated with inter-individual differences in the sensitivity to novel information across subjects. Note that difference scores were averaged across novel negative and novel neutral oddballs here (i.e., the two conditions that showed significant differences compared to standard trials)

should be noted that this test can be considered relatively conservative and that sensitivity might be limited due to the inclusion of two control regions and the fact that this interaction tests for *any* differences in the result pattern between regions rather than for a specific linear trend (which is only observed in the LC).

**Sensitivity to novelty** To relate the activity modulations in the LC in response to novel stimuli to well-known inter-individual differences in the sensitivity to novel information (or *novelty seeking*), the extracted BOLD response was correlated with the respective subscale of the BIS-BAS, i.e., *BAS fun* which has been shown to be highly correlated with *novelty seeking* as assessed by Cloninger's Tridimensional personality Questionnaire (Carver and White 1994; Cloninger 1987). To be able to pick up novelty-specific modulations, we computed the BOLD activity difference between all novel oddballs and standard trials (averaged across novel neutral and novel negative oddballs). We observed a significant correlation between novelty seeking and the LC response to *novel oddball stimuli* (Pearson's  $r = .51$ ,  $p < .02$ , two-tailed), suggesting that the LC response to novel oddball stimuli is related to increased individual sensitivity to novel information (Fig. 2b). The separate correlation analyses of the two types of novel oddballs revealed a significant correlation with novelty seeking for the *novel negative oddballs* ( $r = .52$ ,  $p < .02$ , two-tailed), but only a trend for *novel neutral oddballs* ( $p = .43$ ,  $p = .051$ , two-tailed). Of note, these correlation coefficients did not differ significantly from one another (Hotelling  $t$ -test  $p > .4$ ),

suggesting that emotional content of the novel stimulus does not further enhance the relationship with novelty seeking.

## Discussion

In this study, we used high-resolution fMRI to directly investigate human LC activity during the presentation of different types of salient events in an oddball task. To meet the technical challenges at the level of functional assessment (small diameter of the LC) and anatomical localization (low structural contrast on standard anatomical MR sequences), we combined a high-resolution fMRI protocol with neuromelanin-sensitive structural images that allowed for individual segmentations of the LC.

Based on this approach, we found first and foremost that the LC indeed displayed significantly higher activity for infrequent events compared to standard trials, despite the absence of an overt motor response, which is consistent with the role of the noradrenergic system in generating an orienting response to infrequent events (Murphy et al. 2014; Nieuwenhuis et al. 2011a). In addition, we observed differences across the different oddball categories in the form of a linear effect (novel emotional > novel neutral > familiar target > standard), possibly reflecting additive influences of stimulus novelty and emotional content. When further teasing apart this effect, we found that only novel oddballs lead to a significant activity increase in the LC – with no statistical difference between novel neutral and novel emotional trials. In contrast, familiar infrequent events (target oddballs) did not differ significantly from

standard trials. If we assume that this is not merely an issue of limited sensitivity, this is particularly interesting because these were the only task-relevant (but not *response*-relevant) items in the series. Together, these data suggest that in a series of different types of frequent and infrequent stimuli that do not require an immediate behavioral response, the noradrenergic system seems to give highest priority to any novel information (possibly regardless of emotional valence) as compared to contextual novelty or task relevance alone. The idea that the LC is primarily engaged in processing stimuli that were truly novel was further supported by the relationship between LC activity and individual sensitivity to novel information.

The present observations are in line with well-established findings in neuronal recordings from animals demonstrating that unexpected, novel, or in other ways salient events increase phasic firing of LC neurons (Berridge and Waterhouse 2003). More specifically, LC neurons seem to assign highest priority to events that are entirely unexpected, disrupt ongoing processing, or require actions under uncertainty in a motivational context (Aston-Jones et al. 1991; Bouret et al. 2012; Grant et al. 1988). Since all oddball events were equally contextually unexpected in the present study, differences among them can only be based on stimulus novelty and task relevance. Our data support the notion that stimulus novelty is prioritized (novel oddballs) over task relevance (familiar target oddballs) - at least when neither an explicit reward nor an overt response is attached to any of these stimuli. Moreover, the observation that the LC is particularly sensitive to novel information is consistent with previous research in animals investigating the role of noradrenergic neurotransmission during novelty exploration demonstrating that inhibition of LC neurons as well as blocking post-synaptic noradrenaline receptors disrupted exploratory behavior in freely-moving rats (Sara et al. 1995).

Importantly, the present findings seem to also corroborate previous results obtained using indirect measures of noradrenergic activity in humans. Similar relationships have been reported in humans using the amplitude of the P3 ERP component, in particular the “novelty P3”, as well as pupil size and skin conductance as proxy measures for changes in noradrenergic activity in response to alerting stimuli (Nieuwenhuis et al. 2011a; Ranganath and Rainer 2003). The novelty P3 is thought to arise through projections between LC and anterior cingulate cortex (Dien et al. 2003), whereas pupil and skin conductance responses are believed to be modulated by noradrenergic influences on the autonomous nervous system (Nieuwenhuis et al. 2011a). While all three measures are thought to be generally sensitive to salient stimuli, there could also be important distinctions. For example, it has been shown that the novelty P3 is more sensitive to stimulus novelty, while

the pupil is more responsive to contextual novelty within the same paradigm (Kamp and Donchin 2015). Considering this double dissociation, the activity modulations observed in the present study at the level of the LC seem to resemble the pattern of the novelty P3 rather than pupil size, and this notion is also consistent with the observation that the novelty P3 (Zheng et al. 2010) as well as the response in the LC (shown in the current study) is amplified in individuals that score high on scales assessing novelty seeking. Interestingly, in a typical oddball paradigm probing for contextual novelty in the absence of stimulus novelty, LC activity was associated with changes in pupil size (Murphy et al. 2014).

Consistent with the idea of a contextual coding of saliency, there is another important distinction to be made with regard to existing literature. In contrast to Murphy et al. (2014), infrequent target oddballs did not elicit a robust neural response in the LC compared to standard stimuli in the present study. As discussed above, this discrepancy may be partly explained by stimulus context, in that it is likely that the presence of other salient oddballs will reduce the relative saliency of a particular stimulus. Moreover, considering that goal-directed motor responses have been shown to increase LC activity in animals (Bouret et al. 2012), the absence versus presence of a target-related motor response may explain why the current study does not pick up a significant activity increase for target oddballs - in contrast to Murphy et al. (2014).

Our results also suggest that the LC codes for the relative rather than absolute saliency of an event, in that events that feature different levels of saliency (e.g., novel versus familiar information, emotional versus neutral content, response versus no response requirements), some may factor in more strongly depending on the current task context. Considering such global context effects, it seems feasible to assume that even oddball stimuli that are familiar, of neutral valence, and/or response-irrelevant could lead to increases in phasic LC activity if they are the *only* events in the task that differ from baseline. It has long been known that the prioritization of novel information has an important functional role in foraging organisms, both in terms of attentional orienting and long-term encoding (Lisman and Grace 2005; Ranganath and Rainer 2003; Nyberg 2005), and we present evidence that the initial encoding of novel events is subserved by the human noradrenergic system.

It is also important to consider the current findings in the light of previous fMRI studies that have reported activity modulations in the human LC in response to different types of salient events, including infrequent events (Murphy et al. 2014, discussed above), emotional stimuli and contexts (Liddell et al. 2005; Sterpenich et al. 2006) and conflicting stimuli (Kohler et al. 2016; Krebs et al. 2013). On the one

hand, these studies all seem to be consistent with work in animals on the role of the noradrenergic system in processing and memorizing salient information, and thus contribute to bridging the gap between neuronal recordings in animals and neuroimaging in humans. On the other hand, it is possible that some of these previous findings are partly underspecified for technical reasons. Specifically, the before-mentioned fMRI studies employed standard scan protocols with an in-plane spatial resolution between 2.7 and 3.5 mm<sup>2</sup>, which is larger than the diameter of the LC (Astafiev et al. 2010; Fernandes et al. 2012), and did not use anatomy-based segmentations of the LC on an individual-subject level, making influences from neighboring anatomical structures more likely. Here, we ameliorate these issues to some extent by adjusting functional and anatomical scan parameters. However, improved anatomical specificity may come at the cost of a lower signal-to-noise ratio - a direct consequence of high-resolution fMRI (see Kriegeskorte and Bandettini 2007), which could have limited the sensitivity of the present study. In terms of functional specificity, the observed activity pattern in the LC seems relatively distinctive when compared to two control areas (anterior pons and SC), in that only the LC displayed the unique linear trend with highest activity for novel oddball and lowest for standard stimuli. The dissimilarities between the patterns in the three regions suggests that activity modulations in the LC are not primarily driven by physiological noise or unspecific hemodynamic changes across the brainstem.

Finally, although previous studies observed both increases in pupil size (e.g., Bradley et al. 2008; Snowden et al. 2016) and noradrenergic activity modulations (e.g., Liddell et al. 2005; Sterpenich et al. 2006) in response to negative compared to neutral events, we did not find evidence for this notion in the present study in that emotional stimuli did not differ from neutral ones statistically. Whether the absence of an additional modulation due to emotional content of an already salient novel oddball stimulus is a reliable result or related to low sensitivity remains to be tested in future studies. In any event, based on their high target detection performance and the unpredictable nature of the task, it is unlikely that participants did not process the emotional information.

To conclude, the present study shows that the LC, the source region of noradrenergic innervation in the brain, “gives” highest priority to novel stimuli when presented amongst other salient events that are comparable in terms of event probability (infrequent) and motor requirements (none). That said, considering previous reports, we believe that such prioritization may be context-dependent, suggesting that the LC codes for the relative saliency of an event. Moreover, these findings complement reports on the underlying neural mechanisms of the orienting response using indirect measures with a more direct approach, providing a bridge between animal and human research in this domain.

### Compliance with ethical standards

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**Conflict of interest** Ruth M. Krebs, Haeme R. P. Park, K. Bombeke, and Carsten N. Boehler declare that they have no conflicts of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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