Arousal increases neural gain via the locus coeruleus-noradrenaline system in younger adults but not in older adults

Tae-Ho Lee1,2,3, Steven G. Greening4,1,2,4, Taiji Ueno5, David Clewett6,7, Allison Ponzo2, Michiko Sakaki8 and Mara Mather1,2,6,8

In younger adults, arousal amplifies attentional focus to the most salient or goal-relevant information while suppressing other information. A computational model of how the locus coeruleus-noradrenaline system can implement this increased selectivity under arousal and a functional magnetic resonance imaging (fMRI) study comparing how arousal affects younger and older adults’ processing indicate that the amplification of salient stimuli and the suppression of non-salient stimuli are separate processes, with ageing affecting suppression without affecting amplification under arousal. In the fMRI study, arousal increased processing of salient stimuli and decreased processing of non-salient stimuli for younger adults. By contrast, for older adults, arousal increased processing of both low- and high-salience stimuli, generally increasing excitatory responses to visual stimuli. Older adults also showed a decline in locus coeruleus functional connectivity with frontoparietal networks that coordinate attentional selectivity. Thus, among older adults, arousal increases the potential for distraction from non-salient stimuli.

The arousal system helps the brain and body to coordinate action during threatening situations. Physiological arousal fluctuates moment by moment in response to events, such as thoughts, loud noises, effort and emotions. During an arousal response, the locus coeruleus (LC), a small nucleus in the brainstem, releases noradrenaline throughout most of the brain via its extensive network of axons. Noradrenaline increases the gain on neural activity, so that highly active neurons become more excited, whereas less-active neurons get suppressed1,2. Consistent with this, people notice and encode perceptually salient or goal-relevant stimuli even more under arousal while neglecting stimuli that do not stand out3. For instance, if people hear an emotional sound, such as a baby crying or a tone previously associated with getting a shock, in the next few seconds, they notice salient visual stimuli even more and non-salient stimuli even less than they would otherwise4,5.

Although these behavioural findings suggest that noradrenaline released during arousal affects neural representations differently depending on their priority or salience, it is not yet known how this interaction of arousal and salience occurs. The glutamate amplifies noradrenergic effects (GANE) model posits that phasic LC activity leads to amplified activity in selective cortical sites2. These hotspots emerge when, somewhere in the cortex, strongly active synapses and non-salient stimuli even less than they would otherwise4,5.

In addition to these hotspots of amplified activity under arousal, the GANE model also outlines several mechanisms that suppress less-active representations under arousal. First, the low levels of noradrenaline released at regions where no hotspots emerge cause the suppression of activity in non-hotspot regions. This is owing to the differential actions of α-2A and β-adrenergic receptors. The β-adrenergic receptors involved in the excitatory hotspot feedback loop have a low affinity for noradrenaline and so are activated only with the high levels of noradrenaline that are triggered when local high levels of glutamate interact with nearby LC varicosities under phasic arousal. By contrast, α-2A noradrenergic receptors have a high affinity for noradrenaline and so are activated at relatively low levels of noradrenaline. Furthermore, whereas β-adrenergic receptors tend to be excitatory, α-2A adrenergic receptors typically have inhibitory effects. α-2A receptors are highly prevalent both as autoreceptors at LC varicosities and as heteroreceptors on other neurons, leading to broad-scale inhibitory effects of arousal and noradrenaline on neural activity.

In addition, GABA (γ-aminobutyric acid) receptors could contribute to greater suppression of less-salient representations via a couple of mechanisms. First, high glutamatergic activity at local hotspots should activate nearby GABAergic interneurons that suppress competing weaker representations in the same local network. Second, attention networks in frontoparietal regions’ coordinate activity across disparate cortical representations via long-range glutamatergic projections to other brain regions that stimulate local GABAergic neurons and via long-range GABAergic projections. These frontoparietal attention networks help coordinate selectivity across the cortex. Because LC-noradrenaline activity stimulates these brain regions10–12, the GANE model proposed that
frontoparietal brain regions contribute to the increased inhibition of low-priority information under arousal.

Thus, the GANE model posits that the downstream inhibitory and excitatory effects of arousal on perception and attention have distinct mechanisms. In the current study, we tested this hypothesis by comparing younger and older adults, as there are reasons to believe that the inhibitory effects of arousal will decline more in ageing than the excitatory effects. Ageing is associated with more decline in α-2A receptor function than in β-receptor function, as reflected in decreased α-2 adrenergic receptor density in contrast to increased β-adrenergic receptor density in older rhesus monkeys4,5,14 and decreased gene expression differences in the α-2A receptor gene but not in β-receptor genes in older humans6,15,16. Furthermore, GABA function declines with age17. Fast-spiking interneurons use more energy than most other neurons, leaving them especially vulnerable to metabolic and oxidative stress in ageing18. In animals, age-related loss of GABAergic interneurons is greater than the loss of other neurons19,20 (see also ref. 21 for consistent findings in humans) and GABA function also declines more than glutamate function22. In addition, the frontoparietal networks activated by the LC–noradrenaline system (for a review, see ref. 23) that help to implement inhibition and selective processing across disparate cortical regions show age-related changes in functional connectivity that are associated with age-related declines in cognitive performance23–26. On the basis of these age-related vulnerabilities of the inhibitory mechanisms of GANE, we predict that arousal suppresses processing of less-salient information less effectively in older adults than in younger adults.

We used functional magnetic resonance imaging (fMRI) and computational modelling to test this prediction. We adapted a paradigm that we previously used with younger adults4 to compare the activation of salient and non-salient visual stimuli under arousal in younger adults versus older adults. We measured parahippocampal place area (PPA) activity while participants viewed a pair of images: one scene image that was either high or low priority compared with the other, with both images being presented simultaneously (Fig. 2). The high-priority options were both perceptually salient and goal relevant (that is, participants had to indicate the location of the perceptually salient object). We focused on scene-associated activation in the PPA because it exhibits greater category specificity than most other category-selective cortical regions27. Before each pair of images was presented, we manipulated arousal by playing a tone that was conditioned to predict a shock (CS+) or no shock (CS–). We measured skin conductance and pupil dilation to assess arousal. After confirming our hypothesis of age-related differences in how salience and arousal influence PPA activity, we evaluated whether the neurochemical mechanisms associated with the GANE model could explain the pattern of observed fMRI effects. For this, we implemented GANE in a neural network model and then examined how age-related declines in inhibitory mechanisms influence attention under arousal in this model. We then examined how arousal and place image salience on each trial influenced functional connectivity dynamics among the LC, PPA and frontoparietal network for younger versus older adults.

**Results**

**fMRI study. Fear-conditioning effectiveness.** In the fMRI experiment, younger adults (n = 28) and older adults (n = 24) first completed a fear-conditioning task in which they learned associations between a CS+ tone and shock and associations between a CS– tone and the lack of shock during functional imaging. (See ‘Methods’ for more task details.) During the fear-conditioning task, the CS+ tone increased arousal, as indicated by skin conductance, pupil diameter and brain activation patterns (see Supplementary Results and Supplementary Figs. 1 and 2). CS+ tones continued to increase arousal during the subsequent spatial detection task involving the conditioned tones (see Supplementary Results and Supplementary Fig. 3).

**PPA region-of-interest results during the spatial detection task.** After fear conditioning, participants completed the main task, a spatial detection task with each trial starting with a CS+ or CS– tone, followed by a place–object image pair (Fig. 2). The task for participants was to quickly indicate whether the high-salience image was on the right or the left via a button press. Based on previous studies3,4 and our model, we expected that arousal would enhance processing of salient stimuli. We examined the effects of picture saliency on stimulus-specific brain activation by tracking activation in individually determined PPA regions of interest (ROIs; Fig. 3a) in response to the place images when they were salient versus non-salient. These ROI results are the critical result that we use to assess the activation levels...
of the scene representation when it is salient versus non-salient. A mixed-effects analysis of variance (ANOVA) on the extracted PPA per cent signal changes for the target processing with arousal condition (2: CS+, CS−) × place saliency type (2: salient place target, non-salient place target) × hemisphere (2: left, right) × age group (2: younger, older) as factors yielded no main effects, but did reveal an arousal condition × place saliency type × age group interaction, $F(1, 27) = 6.12$, $P = 0.017$, $\eta^2_p = 0.19$, indicating that arousal and saliency interacted differently for younger adults versus older adults.

To examine these different arousal-by-saliency interactions for each age group, we conducted separate repeated-measures ANOVAs for younger adults and older adults. For the younger group, there was a significant cross-over arousal condition × place saliency type interaction, $F(1, 27) = 6.35$, $P = 0.018$, $\eta^2_p = 0.19$. Compared with CS− tones, CS+ tones amplified PPA activation when a place image was salient (PPA % signal change $M_{CS+} = 0.325$ versus $M_{CS−} = 0.294$; planned comparison $t(27) = 1.84$, $P = 0.038$, one-tailed) but not when the place image was non-salient ($M_{CS+} = 0.268$ versus $M_{CS−} = 0.283$; planned comparison $t(27) = 1.02$, $P = 0.159$, one-tailed; Fig. 3b). There was no main effect of the arousal condition, $F(1, 27) = 0.35$, $P = 0.557$, $\eta^2_p = 0.013$; thus, in younger adults, the effect of arousal depended on the saliency of the place image.

By contrast, for the older group, there was only a main effect of the arousal condition, $F(1, 23) = 4.99$, $P = 0.036$, $\eta^2_p = 0.178$, indicating that CS+ trials generally increased PPA activity ($M_{CS+} = 0.174$ versus $M_{CS−} = 0.151$) regardless of saliency type (Fig. 3c). There was no arousal-by-saliency interaction, $F(1, 23) = 1.11$, $P = 0.303$, $\eta^2_p = 0.046$. In addition, there were no significant effects of hemisphere in any of these analyses.

Thus, as expected for younger adults, arousal interacted with saliency to increase the gain on perceptual processing during high-arousal moments. By contrast, older adults showed no selectivity in the impact of arousal. For older adults, arousal increased activation associated with the presented place images regardless of their salience.

**GANE model simulation.** Although the fMRI results confirm our primary hypothesis regarding age-related changes to the effect of arousal on perceptual processing (as reflected in the PPA results) and provide evidence for the involvement of the LC–noradrenaline system, they cannot directly evaluate whether neurochemicals specified in the GANE model could have produced the observed effects. To address this, an auto-encoder neural network was used to instantiate GANE while considering all behavioural elements in the task (Fig. 4a). Its input, intermediary and output layers each have 80 processing units and they are connected by links (see Supplementary Methods for more detail). Each unit in each layer represents a unique stimulus within that layer. A processing unit in a neural network simulation is a neuron-like object that is intended to represent a small population of neurons. The activation strength of these processing units in the intermediary layer during the task was used as an approximate measure of brain activation and compared with PPA fMRI ROI results.

As described above, during the behavioural task, participants were required to indicate which of two presented stimuli were more salient. To enable the model to complete the same task, the model was first trained and values of connection weights linking units were determined to generate a stronger signal for a salient stimulus and a weaker signal for a non-salient stimulus in the output units when they received two inputs with different activation strengths in the input layer. Next, the model completed the main task, during which it received a stronger value for one input unit (that is, a salient stimulus) and a weaker value for another input unit (that is, a non-salient stimulus). The activation of these units propagated to the intermediary layer units, whose activation strengths were determined not only by these incoming inputs but also by current arousal and noradrenaline levels. The resultant activations from the intermediary layer propagated to the output layer units. Stronger signals in the output units are considered as stronger attention to the corresponding input stimulus. As the fMRI study probed the brain activity during such a behaviour, we also investigated the activity of the intermediary layer units during the time when the model achieved such an input–output mapping. The effect of arousal induced by CS+ was also modelled. To incorporate the local noradrenergic effects that GANE posits, we assigned a unique noradrenaline parameter to each unit. On each trial, this noradrenaline
parameter starts with a low baseline value of $1.0 \times 10^{-4}$ mol per litre noradrenaline (based on the baseline noradrenaline level observed in previous physiological studies of approximately 1 nM in the cortex\(^6\)). Immediately after an arousing event, there is a unit-specific noradrenaline release depending on the unit’s activation level. If the unit’s noradrenaline value exceeds a threshold high enough to activate $\beta$-adrenergic receptors ($7 \times 10^{-8}$ (refs \(33^{,34}\))), this leads to an excitatory feedback loop to allow for additional glutamate and noradrenaline release\(^7\), resulting in our hypothesized noradrenaline hotspots. The activation of $\beta$-adrenergic receptors also leads to the activation of GABAergic signals and suppresses other competing units\(^8\). The unit-specific value of noradrenaline then becomes smaller and smaller as time elapses after the event, simulating the noradrenaline reuptake process\(^9\). This model simulates the arousal-by-salience interaction (Fig. 4b) that is seen both in the current study (Fig. 3b) and in our previous research with younger adults\(^4\).

Modelling GANE changes in older adults. We examined several ways to simulate the effects of age-related declines in inhibitory mechanisms in the model (Fig. 4c, panels 1–4). First, we modified the reuptake rate to be lower (based on less $\alpha$-2A inhibition of noradrenaline release). This change had no effect on the greater excitation of high-salience units under arousal but abolished the inhibitory effect of arousal on low-salience units. Moderate GABA impairment also eliminated the inhibition of low-salience units under arousal. Combining both of these impairments in one model or making the GABA impairment more extreme led to indiscriminate excitation of units regardless of their salience (Fig. 4c, panels 3 and 4). In summary, these models indicate that impairment of basic inhibitory mechanisms, whether due to decreased function in either GABA or $\alpha$-2A receptors or both, could reduce how much arousal inhibits low-salience items without affecting how much arousal excites high-salience items, as shown in our fMRI data (Fig. 3c).

Effects of arousal on the frontoparietal network and LC functional connectivity. Returning to the fMRI analyses, the remaining results shed further light on how arousal affects network dynamics and LC functional connectivity.

Whole-brain voxel-wise analysis. We examined the overall brain activity differences on arousing versus non-arousing trials during the main detection task to see whether arousal amplified activity in the frontoparietal network regions associated with attentional selectivity. When the interaction between the arousal condition and age group was examined in a whole-brain analysis, significant differences in the right frontoparietal network region, including the dorsolateral prefrontal cortex, inferior frontal gyrus, inferior parietal lobule and dorsal premotor cortex extending to the frontal eye field, were identified (Fig. 5a, Supplementary Fig. 4 and Supplementary Table 2). These regions are involved in attentional inhibition, selection and control\(36^{,37}\). The significant interaction arose because, in younger adults, arousal during the task increased the activation of these attentional selection regions, whereas in older adults, arousal did not significantly affect these frontoparietal regions (Fig. 5b and Supplementary Fig. 5). Furthermore, we found that the mean activation in these regions was significantly correlated with pupil diameter changes (CS+ minus CS– during the post-tone period) in younger adults, $r(25) = 0.615$, $P = 0.001$, 95% CI $n = 5,000$ bootstrap = 0.214–0.828, but not in older adults, $r(15) = 0.231$, $P = 0.371$, 95% CI $n = 5,000$ bootstrap = –0.182 to 0.692 (Fig. 5c). There was no statistical difference in correlation coefficients between age groups. In summary, the results suggest that arousal changes indexed by pupil size modulate frontoparietal attentional processes more for younger adults than for older adults.

PPA functional connectivity analysis. In addition to PPA activation levels examined in the earlier ROI analyses, we also examined PPA–LC functional connectivity. In the GANE model, local cortical noradrenaline hotspots can only emerge both where there is high glutamatergic activity and when the LC is active. This is because the NMDA (N-methyl-d-aspartate) receptors on the LC varicosities in the cortex are only activated by glutamate when the LC is simultaneously depolarized. Thus, the GANE model predicts increased blood-oxygen-level-dependent (BOLD) coupling between the LC and the PPA when the participant is in a high-arousal state and viewing a salient place stimulus. For these analyses, one important question is whether the BOLD coupling seen in fMRI occurs at a similar timescale as the release of noradrenaline. LC–noradrenaline axons are slower than the typical axon conduction rate, conducting impulse activity on the order of 0.20–0.86 metres per second\(^18\). Although this is slow for neural transmission, this is fast enough to act on a trial-by-trial basis in our study where trials lasted for a few seconds. Furthermore, a rat study shows a relatively tight 0.01 second on/1 second off) for 20 seconds, the cerebral blood flow in the frontoparietal cortex started increasing within the first 3 seconds of the stimulation and continued to increase during the stimulation duration. When the LC stimulation period ended, the cerebral blood flow immediately started declining on the contralateral side, whereas there was a few-second delay in cerebral blood flow decline on the ipsilateral side. Thus, current evidence suggests that BOLD responses to LC activation can occur quickly enough to be detected in a trial-by-trial design.
We examined the functional connectivity of PPA seed regions (individually located for each participant), comparing CS+ and CS− trials for the salient place condition and the non-salient place condition for each age group. Given our a priori prediction of LC involvement in arousal–salience interactions based on our GANE model simulation and the small size of the LC (see Fig. 6a for the location of the LC), we focused our investigation on the brainstem region, aligned using a brainstem-weighted registration process40. Both younger and older adults showed greater PPA–LC functional connectivity during arousing trials than during non-arousing trials (Fig. 6b, left panel), a main effect that was seen during trials with salient places but not during trials with non-salient places (Fig. 6b, middle panel). This led to significant arousal-by-salience interactions in clusters overlapping the LC for both groups (Fig. 6b, right panel). There were no significant clusters within the LC for the three-way interaction of arousal, saliency and age.

According to the GANE model, the PPA should have high levels of glutamatergic activity during viewing salient stimuli, and those high levels of glutamate should allow for stimulation of more local noradrenaline release (which in turn stimulates more glutamate release) if the LC is phasically activated (Fig. 1). Thus, it is during conditions of high glutamate levels in the PPA and high phasic activity in the LC that coordinated bursts in activity should occur in the two regions. Thus, the arousal-by-saliency interactions in functional connectivity between these regions support the GANE model hotspot mechanism, indicating that LC activity during arousal is more coordinated with activity in a cortical representational area when that cortical area is representing something salient than non-salient.

In addition, the finding that the arousal-by-salience interaction was significant for PPA–LC functional connectivity, not only for younger adults who showed the behavioural arousal-by-salience effect but also for older adults who did not show behavioural selectivity, is quite interesting and suggests that the hotspot excitatory mechanism in which highly activated representations become even more active under arousal will fail to yield selectivity without intact inhibitory contributions. This scenario of intact noradrenaline-glutamate interactions that fail to lead to selective enhancement of salient stimuli is represented by our modelling, as depicted in Fig. 4c, with the strong GABA impairment model in the rightmost panel. That modelling scenario indicates that an increase in activation under arousal for salient representations will not yield a selective benefit for salient representations in the presence of an impairment in inhibitory mechanisms.

Using the same individually defined PPA ROIs, we also examined PPA functional connectivity with cortical regions in a whole-brain analysis. This allowed us to see whether arousal influenced the strength of functional connectivity between the PPA and frontoparietal regions. There was an age-by-arousal interaction of functional connectivity within parietal regions (Fig. 6c, lower left panel). When examined independently, younger adults had an arousal-by-salience interaction in functional connectivity with the PPA in frontoparietal network regions. This arousal-by-salience interaction reflected greater PPA–frontoparietal functional connectivity.
Fig. 5 | Age differences in the effects of arousal on frontoparietal activity and how the frontoparietal effects relate to pupil dilation. a, b. Whole-brain analysis results of the arousal-by-age group interaction (a) and the extracted percentage signal change (CS+ minus CS–) within the frontoparietal network clusters for younger adults (YA; n = 28) and for older adults (OA; n = 24) (b). Although error bars are included for the graph, it should not be interpreted inferentially. c. A scatter plot illustrating the relationship between the percentage signal change in the frontoparietal network region during the detection phase and pupil diameter changes during the post-tone period for each age group. *P = 0.001, 95% CI from non-parametric testing with 5,000 bootstrapped samples (YA, n = 27 and OA, n = 17). For distributions of individual data points for b, see Supplementary Fig. 5.

During CS+ than CS– trials only when the displayed place stimulus was salient. By contrast, older adults showed no differential cortical functional connectivity with the PPA that was dependent on salience or arousal. These findings suggest that arousal had a bigger effect on how the frontoparietal network modulated activity in the place area for younger adults than for older adults.

**Frontoparietal network functional connectivity.** To see whether there was also an age-by-arousal interaction in how the LC interacted with the frontoparietal network, we used a bilateral mask of the frontoparietal network (Fig. 6a from ref. 41) as the seed region, applied to activity within the brainstem mask (with brainstem-optimized alignment, as detailed above). The frontoparietal seed region had significantly more functional connectivity with the LC during CS+ trials than during CS– trials for both younger and older adults, but this effect was significantly stronger in younger adults, as indicated by significant age-by-arousal interaction effect clusters that overlap this effect was significantly stronger in younger adults, as indicated (Fig. 6d). Thus, in summary, significant age-related differences were seen in the functional connectivity pathways between the LC and the frontoparietal network regions and between the frontoparietal network regions and the PPA (Fig. 7).

**Analyses to check for potential age-related confounds.** Older adults may respond less specifically to places in the PPA due to age-related dedifferentiation. Representational similarity analyses (see Supplementary Fig. 6 and Supplementary Results) indicate that this was not the case in our data set. Another possible account of our findings is that younger adults were more likely than older adults to shift their gaze to salient items, especially under arousal. Analyses of gaze biases indicated that this was not the case (see Supplementary Fig. 7 and Supplementary Results).

**Discussion**

Under emotionally intense or cognitively demanding situations that elevate arousal, it can be beneficial to focus on whatever is most salient or important at that moment and ignore everything else. In this study, we tested a theoretical model of how arousal influences cortical processing (GANE3) and how these processes differ in older adults. We predicted that arousal would amplify salient stimuli similarly in younger and older adults but that arousal would suppress non-salient stimuli only in younger adults. To test this, we adapted an fMRI paradigm that we had previously used with younger adults4, in which one of two competing categorical stimuli had greater perceptual salience. We found that younger adults showed the expected increased gain under arousal, as indicated by greater activation of highly salient representations and less activation of competing less-salient representations. By contrast, older adults showed no increase in selectivity under arousal. Instead, they showed greater activation of both salient and non-salient stimuli under arousal. Thus, our findings suggest that, for older adults, arousal is less effective at highlighting only stimuli that stand out most and instead increases distractibility from multiple strongly activated representations.

We used neural network simulation to test whether these findings are consistent with the GANE model. The neural network model of GANE that we outline in this paper provides a computational model of how the LC–noradrenaline system can simultaneously
upregulate and downregulate processing of different stimuli depending on their salience. In this model, in younger adults, activation of the LC under arousal increases the gain on cortical neural activity by increasing the activation of highly active representations while also increasing the suppression of not-very-active representations. The activation of highly active representations is amplified as depolarization of LC neurons allows NMDA receptors on the LC axons that pass through cortical regions to respond to high levels of glutamate in a particular cortical milieu and release more noradrenaline in that local region (Fig. 1). At these sites where highly active representations result in the release of high levels of glutamate, glutamate–noradrenaline interactions create hotspots of even further amplified glutamatergic activity. At the same time, LC–noradrenaline activity amplifies inhibitory mechanisms via increased α-2A and GABAergic inhibition during LC activation.

Within our model, we simulated several different scenarios involving age-related decline in α-2A receptor activity and GABAergic processing inhibitory mechanisms. These simulations yielded intact excitatory components of the LC–noradrenergic effects in older adults, but a lack of the countervailing inhibitory components seen in younger adults. Two scenarios (Fig. 4c, panels 3 and 4) not only eliminated inhibition of low-salience representations but reversed it to yield excitation of low-salience representations under arousal. Thus, the modelling indicated that age-related impairments in basic neural inhibitory mechanisms could lead to age differences in processing non-salient information while not affecting processing of salient information, supporting the notion that the excitatory and inhibitory effects of arousal are dissociable.

Furthermore, our fMRI functional connectivity analyses help to discriminate between potential mechanisms underlying the age-related changes. The GANE hotspot mechanism predicts that activity in the LC should be most coordinated with a particular cortical region when two factors coincide: (1) that cortical region is strongly activated and (2) the LC is activated. Using individually defined PPA as seed regions confirmed this prediction; the LC was significantly more functionally connected to the PPA on trials when the place stimulus was salient and there was an arousing CS+ tone. This arousal-by-salience interaction in LC–PPA functional connectivity was significant for both younger and older adults. Thus, the direct interactions between the LC and the cortical representation were similarly modulated by arousal and salience for younger and older adults, suggesting that this pathway was responsible for the increased excitation of the salient stimulus representation under arousal seen in both younger and older adults. By contrast, age-by-arousal interactions were found in the interactions of the frontoparietal network with both the LC and the PPA. Arousal activated the frontoparietal network less in older adults than in younger adults and the frontoparietal network was less involved in modulating activity in the PPA under arousal. Frontoparietal network regions engage in long-range communication across cortical networks to activate local GABA activity (for example, refs 7,8); thus, a reduction in frontoparietal activation under arousal would decrease the ability of arousal to amplify reactivity of GABA (as in Fig. 4c, panel 4).

These findings raise the question of why, during brief bursts of arousal, the LC increases its coordination with the frontoparietal network less among older adults than among younger adults. Previous findings reveal age differences in the frontoparietal network activity and functional connectivity that are associated with age-related declines in cognitive performance15–28. Thus, it is possible that at least part of the reduced effect of arousal on this network...
lies in the declines in the frontoparietal network itself that make it less sensitive to modulatory influences, such as noradrenaline release. But contrary to this notion are findings that the LC–frontoparietal functional connectivity is greater during rest among older participants than among younger participants (although the sample only included those 18–49 years of age). This suggests another possibility: tonically elevated baseline cortical levels of noradrenaline among older adults make arousal inductions less able to increase the global levels of noradrenaline in ways that stimulate the frontoparietal network. Blockade of the noradrenaline transporter increases frontoparietal functional connectivity, which suggests that increasing the general cortical noradrenaline levels increases frontoparietal activity. If the α-adrenergic receptors in the frontoparietal network are already activated by higher circulating levels of noradrenaline in older adults, small global increases in noradrenaline levels may not have much impact. By contrast, high noradrenaline levels still seem to have an effect on β-adrenergic excitatory processes in older adults, as indicated by intact arousal-by-saliency LC–PPA functional connectivity interactions in older adults (Fig. 6b), which, based on the GANE model, depend on β-adrenergic activity.

Our findings not only advance the understanding of the basic mechanisms of selectivity under arousal but also those underlying age-related decline in selectivity. The GANE computational model outlined here provides a framework for thinking about how local cortical interactions of noradrenaline and glutamate can lead to hotspots of increased neural activation under arousal. The functional connectivity analyses from the fMRI study help to provide information about the broader context of which brain regions beyond the local site that represents the stimulus are involved. In particular, the functional connectivity findings point to an important role of the frontoparietal network in coordinating the suppression of competing representations across disparate regions. In the original presentation of the GANE model, a potential role of the frontoparietal cortex was suggested based on the strong noradrenergic influences over this network, but it was not the main focus. The findings here suggest that the LC interactions with the frontoparietal cortex are an important component of the phenomenon of increased selectivity under arousal. Furthermore, our findings of arousal-by-saliency interactions in the LC–PPA functional connectivity support the GANE hotspot model in which cortical regions with high glutamatergic activity show further amplified activity when the LC is simultaneously activated. These findings replicated in older adults and there were no age differences in the strength of this direct LC–PPA functional connectivity, indicating that this aspect of LC function is still intact in late life, allowing for greater excitation of high-salience stimuli under arousal.

In general, older adults are worse at inhibiting irrelevant information. For instance, older adults activate representations of whatever is the focus of their attention as much as younger adults but fail to suppress the representations they should be ignoring. Our findings indicate that age differences in the likelihood of suppressing less-salient competing information are particularly pronounced under arousal. This raises the interesting question of whether arousal-induced activation of the LC–noradrenaline system contributes to laboratory findings of age differences under arousal. Our model and findings suggest that the more engaged (and therefore the more the LC is probably activated) participants are during a task, the more marked the age differences in the ability to inhibit irrelevant information should be. The LC is activated by a wide range of circumstances, including threatening or exciting situations, cognitive load and novelty. Focusing on what is most salient during these moments may often be advantageous even if it means neglecting some less-salient information. Our findings suggest that, due to age-related changes in inhibitory mechanisms, older adults cannot rely on increases in selective attention during these potentially high-stake moments.

Methods

GANE fMRI experiment. Participants. Twenty-eight healthy younger adults (M_age = 24.39 years, age range = 18–34; 9 females) and 24 healthy older adults (M_age = 66.95 years, age range = 55–75; 9 females) participated in the current study. There were no significant differences between groups in terms of intellectual level (M_Academic: younger adults = 16.85 years versus older adults = 16.38 years; M_Wechsler Test of Adult Reading: younger adults = 43.96/50 versus older adults = 39.75/50). Participants had normal or corrected-to-normal visual acuity. Participants provided informed consent approved by the University of Southern California Institutional Review Board and were paid for their participation. Procedures conformed to the human subject ethical guidelines.

MRI data acquisition and preprocessing. MRI data were acquired on a Siemens 3 T Magnetom Trio with a liquid crystal display projector (1,024 × 768 pixels at 60 Hz) onto a rear screen that was the head of participants and viewed using a mirror attached to a 32-channel matrix head coil. High-resolution structural images (MPRAGE) were acquired first; repetition time (TR) = 1,950 ms; echo time (TE) = 2.26 ms; flip angle (FA) = 7°; 1-mm isotropic voxel; field of view (FOV) = 256 mm. Next, functional images were acquired with gradient-echo echo-planar T2*-weighted imaging. Each functional volume consisted of 41 interleaved (no skip) 4-mm axial T2*-weighted slices; TR = 2,000 ms; TE = 25 ms; FA = 90°; matrix size = 64 × 64; FOV = 256 mm. The fear-conditioning run, each of the spatial detection task and the PPA localizer run were acquired with 180, 180 and 256 echo planar imaging volumes, respectively. An additional T1-weighted fast-spin echo sequence was administered (TR = 750 ms, TE = 12 ms, FA = 120, 1 average, 11 axial slices, FOE = 220 mm, bandwidth = 220 Hz per pixel, slice thickness = 2.5 mm, slice gap = 3.5 mm, in-plane resolution = 0.43 mm², scan duration = 1 min and 53 s).
During preprocessing, we discarded the first three volumes to account for equilibration effects. fMRI data processing was carried out using FEAT (fMRI Expert Analysis Tool) version 6.00, which is part of FSL (FMRIB Software Library). The following steps were applied: motion correction using MCFLIRT (Motion Correction FMRIB’s Linear Image Registration Tool) and slice-timing correction using Fourier-space time-series phase-shifting; non-brain removal using the Brain Extraction Tool (BET)\(^1\) spatial smoothing using a Gaussian kernel of full width at half maximum of 5 mm; grand-mean intensity normalization of the entire 4D data set by a single multiplicative factor; independent component analysis denoising using MELODIC ICA\(^2\) (ref. \(^3\)) and an automated toolbox\(^4\) (an average of 15.54 components were removed from each participant); registration to high-resolution structural and standard Montreal Neurological Institute (MNI) 2-mm brain using FLIRT\(^5\). For brainstem-targeted connectivity analysis, we performed an additional registration step to optimize brainstem alignment (for more details, please see “PPA and frontoparietal network functional connectivity with brainstem regions”).

Stimuli and apparatus. Two tones (300 Hz and 800 Hz) served as Cs. We used 270 house/building place images obtained from several websites and 240 colour photographs of various real-world objects obtained from a previously published set of object stimuli\(^6\). All stimuli were grey-scaled and normalized to the mean luminance of all images. In the main spatial detection task, one object and one place image were randomly selected from the stimuli pool (each participant saw 160 object and 160 place stimuli from the larger pool of stimuli). The mild electric shock used as an unconditional stimulus was delivered to the third and fourth fingers of the left hand using the shock stimulation box (Medtronic, model 5740; Coulobrides Ltd.); each stimulus was associated with a ground-based audio signal. The PsychoToolbox extension\(^7\) of MATLAB 2010b (The MathWorks Corp.) controlled stimuli presentation and data collection.

Spatial detection task. After the fear-conditioning task (see Supplementary Methods for details), participants performed a simple spatial detection task (Fig. 2). A trial began with simultaneous onset of a fixation cross and either the CS+ or CS− tone. The tone played for 0.7 s, then the fixation cross remained on the screen for 2 s after the tone ended. Next, a place–object image pair was presented in two placeholder frames simultaneously for 0.6 s (4.3° × 4.3°; 11.5° eccentricity). The salient image had a higher contrast level (80%) than the non-salient image (20%), and to further increase its salience, it was framed by a yellow border for 0.1 s. Participants were asked to identify the location of the salient image by pressing a left or right button. The intertrial interval was randomly jittered (2.5, 3.5, 4.5 and 5.5 s). Each place image was randomly paired with one of the object images, with unique pictures shown on each trial; locations were also randomly determined. Across five runs, 160 trials were presented. During each run, 16 CS+ trials (8 place salient and 8 place non-salient) and 16 CS− trials appeared in a random order. To minimize extinction, three additional CS+ shock trials were presented randomly in each run with the constraint that shocks did not occur on consecutive trials. Other than the shock and a subsequent 10-s blank interval, these booster trials were identical to the main trials and were excluded from further analysis.

We instructed participants to fixate their eyes on the fixation point that was always in the middle of the screen during the task. We took into account stimulus size and eccentricity when choosing the two cue image locations, so that participants could see both sides simultaneously even when their gaze was directed at the fixation point. Both younger and older adults successfully maintained their gaze on the task-irrelevant information (see Supplementary Fig. 7). Details on skin conductance and pupil dilation measures during the tasks are in the Supplementary Methods (see associated Supplementary Figs. 1 and 3).

PPA ROI analysis for the spatial detection task. We first estimated stimulus-dependent changes in the BOLD signal for each participant using a general linear model with regressors for the target stimulus and their temporal derivatives for each saliency condition (that is, when a place image was salient versus non-salient) as a function of the arousal condition (CS+, CS−). Motion parameters, booster shock trials and target-onset timing were included in the design matrix as covariates of no interest. A group-level analysis (random effects) was also performed (random effects with the FLAME1 + 2 model; Z > 2.3 with a corrected cluster significance threshold of P = 0.05, one-tailed).

Whole-brain voxel-wise analysis for the spatial detection task. In this analysis, we focused on whether emotional arousal had different effects on brain activity in younger versus older adults (that is, the interaction arousal condition × age group). To do so, a standard general linear model was performed to estimate the BOLD signal for the tone onset and their temporal derivatives as a function of the arousal condition (CS+, CS−) regardless of saliency conditions. Motion parameters, booster shock trials and target-onset timing were included in the design matrix as covariates of no interest. A group-level analysis (random effects) was also performed (random effects with the FLAME1 + 2 model; Z > 2.3 with a corrected cluster significance threshold of P = 0.05, one-tailed).

PPA functional connectivity with frontoparietal regions. To characterize dynamic inter-regional interactions, a beta series correlation analysis\(^8\) was performed using least squares estimation (see the ‘least squares — separate model’\(^9\)) where each single-level general linear model included regressors for the current trial, all other remaining events and all other non-interest events (that is, nuisance regressor; motion parameters, booster shock trials, error trials and tone-onset timing). Finally, the extracted mean activation (that is, the mean parameter estimates) of each trial from the individual ROI masks were used to compute correlations between the seed’s signal and the signal of all other voxels in the whole brain, thus generating condition-specific correlation matrices for each ROI. Correlation matrices were converted into Z scores using the Fisher’s r-to-z transformation. Condition-dependent changes in functional connectivity were assessed using random-effects analyses, which were thresholded at the whole-brain level using clusters determined by Z > 2.3 and a cluster significance threshold of P = 0.05 (corrected, one-tailed). As our interest was how the PPA interacted with frontoparietal networks as a function of place salience, arousal level and age, we examined the three-way arousal (CS+, CS−) × salience (place salient, place non-salient) × age group (younger, older) interaction.

PPA and frontoparietal network functional connectivity with brainstem regions. To optimize brainstem signal measures for analyses examining functional connectivity between cortical seed regions and the LC, we conducted a separate registration process for the target brainstem region. Images were registered to a 2-mm standard-space MNI image using the following steps: (1) registering each participant’s functional scan to their high-resolution anatomical scan using an affine transformation with 6 d.f.; (2) registering each participant’s high-resolution anatomical scan to the MNI standard-space 2-mm brain template using an affine transformation with 12 d.f.; and (3) performing a follow-up anatomical-to-standard affine registration with 12 d.f. and applying a linearized brainstem mask (Harvard-Oxford atlas at 50% probability) as a reference weight\(^\text{10}^\text{10}\). Then, we used the same beta series correlation analysis method as outlined above, with the mean parameter estimates for the PPA and the frontoparietal networks extracted from data processed using the standard whole-brain alignment process. Condition-specific seed correlation maps were produced for the relationship between cortical seed signals and the signals in voxels within the brainstem mask. Given our a priori prediction of LC involvement in arousal–salience interactions based on our GANE model simulation and the small size of the LC, we applied voxel-based thresholding combined with false discovery rate correction (q < 0.05) based on the statistical map within the brainstem mask (from the Harvard-Oxford atlas).

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability. The code associated with the neural network simulation and with the experimental tasks are publicly available at https://osf.io/ztw8j/.

Data availability. The behavioural and summarised data from the current study are available at https://osf.io/zw8j/. The MRI data are available at the OpenNeuro repository at https://openneuro.org/datasets/ds00142.

Received: 2 May 2017; Accepted: 29 March 2018; Published online: 7 May 2018

References
1. Aston-Jones, G. & Cohen, J. D. An integrative theory of locus coeruleus—norepinephrine function: adaptive gain and optimal performance. Annu. Rev. Neurosci. 28, 403–450 (2005).
2. Mather, M., Clewett, D., Sakaki, M. & Harley, C. W. Norepinephrine ignites local hotspots of neuronal excitation: how arousal amplifies selectivity in perception and memory. Behav. Brain Sci. 39, e200 (2016).
3. Mather, M. & Sutherland, M. R. Arousal-biased competition in perception and memory. Perspect. Psychol. Sci. 6, 114–133 (2011).
mediated long-lasting potentiation in vivo using microdialysis and intracerebroventricular norpamine. Brain Res. 710, 293–298 (1996).
32. Salgado, H., Kohn, G. & Treviño, M. Noradrenergic ‘tone’ determines dichotomous control of cortical spike-timing-dependent plasticity. Sci. Rep. 2, 417 (2012).
33. Ferrero, J. J. et al. β-Adrenergic receptors activate exchange protein directly activated by cAMP (Epac), translocate Munc13-1, and enable the Rab3A- Rim1 interaction to potentiate glutamate release at cerebrocortical nerve terminals. J. Biol. Chem. 288, 31370–31385 (2013).
34. Nai, Q., Dong, H.-W., Hayar, A., Linster, C. & Emms, M. Noradrenergic regulation of GABAergic inhibition of main olfactory bulb mitral cells varies as a function of concentration and receptor subtype. J. Neurophysiol. 101, 2472–2484 (2009).
35. Amara, S. G. & Kuhar, M. J. Neurotransmitter transporters: recent progress. Annu. Rev. Neurosci. 16, 73–93 (1993).
36. Nee, D. E., Wager, T. D. & Jonides, J. Interference resolution: insights from a meta-analysis of neuroimaging tasks. Cogn. Affect. Behav. Neurosci. 7, 1–17 (2007).
37. Scolari, M., Seidl-Rathkopf, K. N. & Kastner, S. Functions of the human frontoparietal attention network: evidence from neuroimaging. Curr. Opin. Behav. Sci. 1, 32–39 (2015).
38. Bertrand, C. W. & Waterhouse, B. D. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res. Rev. 42, 33–84 (2003).
39. van den Dolder, X., Baau, L., Lamberts, S. P. J. & Hamel, E. Locus coeruleus stimulation recruits a broad cortical neuronal network and increases cortical perfusion. J. Neurosci. 33, 3390–3401 (2013).
40. Napadow, V., Dhond, R., Kennedy, D., Hui, K. K. & Makris, N. Automated brainstem co-registration (ABC) for MRI. Neuroimage 32, 1113–1119 (2006).
41. Laird, A. R. et al. Behavioral interpretations of intrinsic connectivity networks. J. Cogn. Neurosci. 23, 4022–4037 (2011).
42. Zhang, S., Hu, S., Chao, H. H. & Li, C.-S. R. Resting-state functional connectivity of the locus coeruleus in humans: in comparison with the ventral tegmental area/substantia nigra pars compacta and the effects of age. Cereb. Cortex 26, 3413–3427 (2016).
43. Gannon, M. & Wang, Q. Complex noradrenergic dysfunction in Alzheimer’s disease: low norpamine input is not always to blame. Brain Res. https://doi.org/10.1016/j.brainres.2018.01.001 (2018).
44. Healey, M. K., Hasher, L. & Campbell, K. L. The role of suppression in resolving interference: evidence for an age-related deficit. Psychol. Aging 28, 721–728 (2013).
45. Gazzaley, A., Cooney, J. W., Rissman, J. & D’Esposito, M. Top-down suppression deficit underlies working memory impairment in normal aging. Nat. Neurosci. 8, 1298–1300 (2005).
46. Mitchell, K. J., Johnson, M. R., Higgins, J. A. & Johnson, M. K. Age differences in brain activity during perceptual versus reflective attention. Neuroreport 21, 293–297 (2010).
47. Smith, S. M. et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23, S208–S219 (2004).
48. Jenkinson, M., Bannister, P., Brady, M. & Smith, S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17, 825–841 (2002).
49. Smith, S. M. Fast robust automated brain extraction. Hum. Brain Mapp. 17, 143–155 (2002).
50. Beckmann, C. F. & Smith, S. M. Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE Trans. Med. Imaging 23, 137–152 (2004).
51. Tolh, J. et al. Automatic independent component labeling for artifact removal in fMRI. Neuroimage 39, 1227–1245 (2008).
52. Brady, T. F., Konkle, T., Alvarez, G. A. & Oliva, A. Visual long-term memory has a massive storage capacity for object details. Proc. Natl Acad. Sci. USA 105, 14325–14329 (2008).
53. Brainard, D. H. The psychophysics toolbox. Spatial, Vis. 10, 433–436 (1997).
54. Pelli, D. G. The VideoToolbox software for visual psychophysics: transforming numbers into movies. Spatial, Vis. 10, 437–442 (1997).
55. Epstein, R. & Kanwisher, N. A cortical representation of the local visual environment. Nature 392, 598–601 (1998).
56. Epstein, R. The cortical basis of visual scene processing. Vis. Cogn. 12, 954–978 (2005).
57. Grill-Spector, K. & Malach, R. fMR-adaptation: a tool for studying the functional properties of human cortical neurons. Acta Psychol. 107, 293–321 (2001).
58. Erez, Y. & Yovel, G. Clutter modulates the representation of target objects in the human occipitotemporal cortex. J. Cogn. Neurosci. 26, 490–500 (2014).
59. Altmann, C. F., Debuellius, A. & Kourti, Z. Shape saliency modulates contextual processing in the human lateral occipital complex. J. Cogn. Neurosci. 16, 794–804 (2004).
Acknowledgements
This work was supported by grants from the National Institute on Aging RO1AG025340 awarded to M.M., JSPS KAKENHI 16H03750 and 15K21062 awarded to T.U., and JSPS KAKENHI 16H05959, 16KT0002 and 16H02053 and European Commission CIG618600 awarded to M.S. We thank C. Cho for assistance with Figs. 1 and 7. The funders had no role in the conceptualization, design, data collection, analysis, decision to publish or preparation of the manuscript.

Author contributions
T.-H.L. and M.M. designed the study. T.-H.L., S.G.-G. and A.P. acquired the data. Data were analysed by T.-H.L. with S.G.-G., D.C. and M.M. Modelling was conducted by T.U. and M.S. All the authors contributed to the preparation of the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41562-018-0344-1.
Reprints and permissions information is available at www.nature.com/reprints.
Correspondence and requests for materials should be addressed to M.M.
Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Experimental design

1. Sample size
   Describe how sample size was determined.
   Based on previous studies examining the arousal impact induced by fear-conditioning on human behavior in which the number of participants varied between 20 and 40 participants (Lee, Sakaki et al., 2014; Lee, Baek, Lu & Mather, Lee, Greening, Mather 2015), we planned to collect between 20 and 30 participants for each age group.

2. Data exclusions
   Describe any data exclusions.
   For fMRI data, we excluded one older adult (OA) participant due to a neurological issue that was detected by a neurologist hired at the University of Southern California. For pupil data, due to technical failure, the data was not successfully collected for one YA and three OAs. An additional four OA participants’ data were excluded from the analyses due to data loss of more than 25% of total. For SCR data, recording could not be completed for one YA and one OA participant due to a technical failure.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   N/A; we did not conduct a replication study. However, the results for the younger adults were consistent with our prior findings (Lee, Sakaki et al., 2014).

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   See below

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   Our main manipulation of arousal was done on a trial-by-trial basis; thus there were no group allocations requiring blinding. We did compare younger and older adults; but of course it was not possible to blind participants or experimenters interacting with them to which age group they were in.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- [x] The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- [x] A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [x] A statement indicating how many times each experiment was replicated
- [x] The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- [x] A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- [x] The test results (e.g. \(P\) values) given as exact values whenever possible and with confidence intervals noted
- [x] A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- [x] Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

7. Software
Policy information about availability of computer code

Describe the software used to analyze the data in this study.

For the brain data, all analyses were carried out using FSL (FMRIB's Software Library) and PyMVPA toolbox, which are open-source fMRI analysis packages. The simulation and modelling was carried out using our own in-house code that will be uploaded to the OSF page (project title: 2015 fear conditioning fMRI and aging project) upon publication.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials and reagents
Policy information about availability of materials

8. Materials availability
Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Stimuli have been uploaded to the publicly shared OSF page for this project (https://osf.io/zw8aj/).

9. Antibodies
Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

N/A

10. Eukaryotic cell lines
a. State the source of each eukaryotic cell line used.
N/A

b. Describe the method of cell line authentication used.
N/A
c. Report whether the cell lines were tested for mycoplasma contamination.
N/A
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.
N/A
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
Provide details on animals and/or animal-derived materials used in the study.

N/A

Policy information about studies involving human research participants

12. Description of human research participants
Describe the covariate-relevant population characteristics of the human research participants.

Twenty-eight healthy younger adults (YA; Mage = 24.39 years, age range = 18 – 34; 9 females) and 24 healthy older adults (OA; Mage = 66.95 years, age range = 55 – 75; 9 females) participated in the current study. There were no significant differences between groups in terms of intellectual level (Meducation: YA = 16.85 vs. OA = 16.38 years; MWechsler Test of Adult Reading: YA = 43.96 / 50 vs. OA = 39.75 / 50). Participants had normal or corrected-to-normal visual acuity. Participants provided informed consent approved by the University of Southern California Institutional Review Board and were paid for their participation.
MRI Studies Reporting Summary

**Experimental design**

1. **Describe the experimental design.**
   
   It consisted of two tasks; first, a fear conditioning in which one tone was classically conditioned to a mild electrical shock and another tone was not; second, a spatial detection task in which place/household object pairs were presented and participants how to indicate via button press which of the images in the pair was most perceptually salient. Both were assessed using event-related fMRI.

2. **Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.**
   
   Fear conditioning: one event-related run with 36 trials (12 CS+ with shock, 12 CS+ without shock, 12 CS- tones). Each trial was 0.7 s followed by jittered trial intervals (8, 8.5, 9 s).
   
   Detection task: five event-related runs and each run was consisted of 32 task trials (12 CS+, 12 CS-) and three shock booster trials. Each task trial was 3.3s with jittered intervals (2.5, 3.5, 4.5, 5.5s) and the booster trial was 0.7 s with 10 s trial intervals.
   
   PPA Localizer: one block-designed run consisted of 18 blocks (six place blocks, six object blocks, six scrambled object blocks). Each block lasted 14.4 s with 12 trials, and each trial was presented 1 s followed by 0.2 blank screen). Each block was spaced by 10 s intervals.

3. **Describe how behavioral performance was measured.**
   
   We measured both reaction time and accuracy. We also measured skin conductance response, pupil dilation, and the blood-oxygenation-level-dependent signal using fMRI (BOLD epil).
### Acquisition

4. Imaging
   
   a. Specify the type(s) of imaging.
   
   Functional and structural MRI.
   
   b. Specify the field strength (in Tesla).
   
   3T

   c. Provide the essential sequence imaging parameters.
   
   High resolution structural images (MPRAGE):
   repetition time (TR) = 1950 ms; echo time (TE) = 2.26 ms; flip angle (FA) = 7°; 1-mm isotropic voxel; field of view (FOV) = 256 mm.
   Echo-planar T2*-weighted images (BOLD epi):
   41 interleaved slices, slice-thickness = 4 mm (no slice gap); TR = 2000 ms; TE = 25 ms; FA = 90°; matrix size = 64 X 64; FOV = 256 mm. The fear conditioning run, each run of the spatial detection task, and the PPA localizer run were acquired with 180, 160 and 256 EPI volumes respectively.
   Neuromelanin-weighted images (T1-weighted fast-spin echo sequence):
   TR = 750 ms, TE = 12 ms, FA = 120, 1 average, 11 axial slices, field of view = 220 mm, bandwidth = 220 Hz/Px, slice-thickness = 2.5 mm, slice gap = 3.5 mm, in-plane resolution = 0.43 mm2, scan duration = 1 minute and 53 seconds.

   d. For diffusion MRI, provide full details of imaging parameters.
   
   N/A

5. State area of acquisition.

   Whole brain.

### Preprocessing

6. Describe the software used for preprocessing.

   FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL [FMRIB’s Software Library]. The following preprocessing steps were applied; motion correction using MCFLIRT; slice-timing correction using Fourier-space time-series phase-shifting; non-brain removal using BET; spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; ICA denoising using MELODIC ICA2 and an automated toolbox. A multivoxel pattern analysis on the localizer run data was carried out using PyMVPA toolbox.

7. Normalization

   a. If data were normalized/standardized, describe the approach(es).

   linear registration (rigid body) to high resolution structural and standard Montreal Neurological Institute (MNI) 2-mm brain using FLIRT.

   b. Describe the template used for normalization/transformation.

   MNI 2-mm brain template

8. Describe your procedure for artifact and structured noise removal.

   Motion correction and add motion regressors with motion outliers on the GLM model. Individual ICA denoising combined with automated toolbox and visual inspection based on a set of explicit criteria outlined in a training manual. As is typical, investigators were blind to how ICA noise component selections affected result outcomes.

9. Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

   In addition to regular motion correction, we identified outlier volumes in terms of signal change using fsl_motion_outlier function. The default threshold was used to define an outlier (the 75th percentile + 1.5 times the InterQuartile Range). Then we added outlier volumes in the model as a nuisance regressor with six motion parameters.
10. Define your model type and settings. A standard two-stage mixed-effects analysis was performed. For the fear-conditioning task, the general linear model (GLM) of the BOLD signal using CS tone type (CS+, CS-) as the regressor was estimated at the first (fixed) level with a double-gamma hemodynamic response function. Motion parameters and timeline demarcating trials involving an electrical shock were included in the design matrix as covariates of no interest. Data were combined across participants using random-effects at the group level (FLAME 1+2 model; Z > 2.3 with corrected cluster p = .05, one-tailed). A main effect of Arousal term (i.e., CS+ > CS- irrespective of group) was examined.

For the detection task, we first estimated stimulus-dependent changes in BOLD signal for each participant using a GLM with regressors for target stimulus and their temporal derivatives for each saliency condition (i.e., when place image was salient vs. when place image was not salient) as a function of arousal condition (i.e., CS+ and CS-). Motion parameters, booster shock trials, error trials and tone onset timing were included in the design matrix as covariates of no interest. The effects of each regressor were estimated over five functional runs (fixed-effects; one YA and OA completed four runs, and one OA finished three runs due to time issues). We conducted a region of interest (ROI) analysis to probe how emotional arousal interacted with stimulus saliency for each Age Group. An additional standard GLM (two-stage mixed-effects analysis) at the whole brain level was performed to estimate the BOLD signal for the tone onset and their temporal derivatives as a function of arousal condition (CS+, CS-) regardless of saliency conditions. Motion parameters, booster shock trials, and target onset timing were included in the design matrix as covariates of no interest. Finally, a group-level analysis (random-effects) was also performed (random-effects with FLAME1+2 model; Z > 2.3 with corrected cluster p = .05, one-tailed).

11. Specify the precise effect tested. We generally were testing age x salience x arousal interactions using 3-way ANOVAs.

12. Analysis

a. Specify whether analysis is whole brain or ROI-based. Both

b. If ROI-based, describe how anatomical locations were determined. Individual functional localizer for main ROI (i.e., PPA). Previously published frontoparietal network mask (Laird et al., 2011).

13. State the statistic type for inference. (See Eklund et al. 2016.)

For whole-brain analysis, cluster-wise inference based on FSL’s FLAME1+2 was applied with a cluster-defining threshold of Z = 2.3 and a corrected cluster threshold of P = 0.05

For random-effect model at the group level (FLAME1+2), Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>2.3 and a (corrected) cluster significance threshold of P=0.05.

14. Describe the type of correction and how it is obtained for multiple comparisons.
15. Connectivity  

a. For functional and/or effective connectivity, report the measures of dependence used and the model details.

For the connectivity analysis, we adopted beta-series correlation approach, in which single trial betas were correlated for experimental condition and group. To estimate single-trial betas, we used a least squares estimation [LS-S model] where we iteratively ran a first-level general linear model (GLM) to individually estimate the beta coefficient for each trial one by one. Each GLM included regressors for the current trial being estimated, all other remaining events, and all other non-interest events (i.e., nuisance regressor; motion parameters, booster shock trials, error trials and tone onset timing). Finally, mean trial-wise parameter estimates were extracted from the individual seed ROI mask, which formed a seed beta-series that was correlated with the beta-series and signal of all other voxels in the whole brain, thus generating condition-specific seed correlation maps. Correlation magnitudes were converted into z scores using the Fisher’s r-to-z transformation. Condition-dependent changes in functional connectivity were assessed using random effects analyses, which were thresholded at the whole-brain level using clusters determined by Z > 2.3 and a cluster significance threshold of p = .05 (corrected; one-tailed).

b. For graph analysis, report the dependent variable and functional connectivity measure.

N/A

16. For multivariate modeling and predictive analysis, specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.

For our multivariate modeling we used a representational similarity analysis approach. Our independent measure was the correlation between a vector of voxel-wise activity produced during place versus object viewing extracted independently from the PPA and the LOC. Voxel-wise feature extraction was from group-level masks of the PPA and LOC, this masking procedure also served to reduces the dimensionality of the data. We quantified the similarity differences using a 2x2 ANOVA after first transforming the Pearson’s r values to z values using a Fisher transformation.