Locus Coeruleus Engagement Drives Network Connectivity Dynamics In Humans And Rats

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Abstract:

The locus coeruleus (LC) projects broadly throughout the brain, serving as the main source of norepinephrine and consequently driving arousal, attention and task performance. However, the arousal-performance relationship is non-monotonic, with low and high LC engagement associated with poorer task performance than intermediate LC activity. Signs of this "Yerkes-Dodson" LC-performance curve have been observed in both humans and animals, but its underlying computational mechanisms remain poorly understood. We hypothesized that LC's role in driving performance is due largely to its effect on neural noise, i.e. variability in innervated network activity. As a preliminary test, using two existing fMRI datasets we examined how LC engagement impacted BOLD and functional connectivity variability and dynamics in resting state and attentional networks in humans and rats. LC engagement changed (a) BOLD variability in a network-specific manner (humans), and (b) dynamic functional connectivity state switching speed between LC and thalamus (rats). These results provide preliminary cross-species evidence suggesting that LC's computational role in regulating performance may rest largely on its role in regulating neural variability.

Keywords: Locus coeruleus; fMRI; cross-species comparisons; functional connectivity

Introduction

The locus coeruleus (LC) circuit is the main source of norepinephrine in the brain; it projects broadly throughout the entire brain and consequently is deeply related to cognitive functions related to attention (Song et al., 2017). In normal cognition, the relationship between arousal and task performance has been explained by the Yerkes-Dodson curve: Moderate LC tonic firing rates correspond to optimal task performance, while low and high LC firing rates are associated with poor performance due to inattention or distractibility, respectively (Aston-Jones & Cohen, 2005). Despite observed correlations between LC engagement and attention, the underlying mechanisms driving changes in network dynamics as a function of arousal are not well understood (Sara, 2009).

To approach this challenge, in a cross-species approach (humans and rats) we capitalized on experimental paradigms that actively manipulated LC engagement. This allowed us to examine LC's effect on noise in various brain networks as a primary factor underlying the Yerkes-Dodson curve, i.e. variability in BOLD and functional connectivity (FC) (humans), and in the speed of state-switching via dynamic FC (rats). In two existing fMRI datasets, we examined BOLD in resting state and task-related networks (humans; 3T) and LC-thalamic FC (rats; 7T).

We found that LC up-regulation significantly impacts BOLD signal variability in humans in a network-specific manner, and that connectivity between LC and certain thalamic nuclei significantly increases in rats as a function of LC engagement. These results suggest a strong role for LC engagement in driving neural noise and variability,

providing preliminary insights into the computational mechanisms underlying the non-monotonic Yerkes-Dodson relationship between task performance and arousal.

Methods

Humans

We reanalyzed an existing data set collected on a Siemens 3T MAGNETOM Prisma Fit (TR=2000ms, TE=25ms, FA=90°, FOV=192mm^3, voxel size=3mm^3) at the University of Southern California. BOLD data were collected while human subjects completed five one-minute resting state epochs interspersed with five 18-second blocks where they brought their hand to their chest and squeezed a squeeze-ball at maximum grip force ("stressor") throughout the block to stimulate sympathetic nervous activity and up-regulate LC (Nielsen & Mather, 2015). 78 total subjects completed this experiment: 43 "active" subjects squeezed when prompted, while 35 "control" subjects brought their hand to their chest but did not squeeze. BOLD data were motion corrected and the BOLD signal extracted and normalized (z-scored) in four networks to examine activity and resting state connectivity: default mode network (DMN), fronto-parietal control network (FPCN), dorsal attention network (DAN), and salience network (SN). We selected DMN (a resting state network) and DAN (an attention network) because squeezing ought to invoke a transition from the resting state into a task-positive state (Greicius & Menon, 2004); FPCN because it is linked to DAN and regulates perceptual attention (Dixon et al., 2018); and SN because it determines which stimuli are most deserving of attention (Menon & Uddin, 2010). Details of the nodes making up each network have previously been described elsewhere (Deshpande, Santhanam, & Hu, 2011; Raichle, 2011). We computed the mean and variance of the normalized BOLD signal for all nodes in a network, during each of the resting state and squeezing blocks. Within-network static FC was found by performing pairwise Pearson correlations of BOLD signal fluctuations across time between each pair of nodes within each network in each of the resting state (30 TRs) and squeezing (9 TRs) blocks (Friston, 2011; Rogers, Morgan, Newton, & Gore, 2007). For each subject, the BOLD means, BOLD variances, and connectivity magnitudes were averaged within block types to produce two values for each subject: one for resting state, and one for squeezing.

Rats

As with the human data, we capitalized on a pilot data set collected in rats as part of a different study. Six rats were conditioned to fear an auditory tone, and resting state data were collected before and after this tone was presented in a 7T Bruker scanner (TR=1000ms, TE=15ms, FA=60°, FOV=32x32x20mm, voxel size=0.5x0.5x1mm^3). One rat's data were excluded due to excessive motion. The data were despiked (the first 10 frames were removed as well as any with large motion) and temporally filtered (0.01Hz to 0.1Hz), and the first and last two slices were removed due to inconsistent FOV coverage between animals. The first 20 seconds of BOLD in each run was discarded to allow the magnetization to reach steady state (this step is unnecessary in our human data because the Siemens scanner completes it automatically). BOLD was extracted from thalamic nuclei using manually labeled histology slices which were then normalized to a structural atlas space, and normalized by dividing by the absolute value of the integral of the BOLD curve across time. We focused on ventral posteromedial nucleus (VPM), medial dorsal thalamus (MD), anterodorsal nucleus (AD), anteromedial nucleus (AM), and ventral posterolateral nucleus (VPL) because these thalamic nuclei are also present in humans, the LC innervates the thalamus, and the thalamus acts as a sensory relay center to the rest of the brain (Morel, Magnin, & Jeanmonod, 1997; Vertes, Linley, & Hoover, 2015). The static FC between LC and each nucleus was found using the same method as in humans. The dynamic functional connectivity (dFC) was found via an overlapping sliding time window approach (window size 10s, overlapping 9s). The first derivative of this dynamic connectivity time series was estimated from the difference in dFC between each point. We computed the mean absolute value of this "first derivative" as a metric of the speed with which FC between LC and each thalamic nucleus changed across the resting state epoch.

Statistical Analyses & Expected Results

We expected the BOLD signal and within-network static FC of FPCN, DAN, and SN in humans to increase during squeezing compared to resting state for subjects who squeezed; conversely, we expected DMN to exhibit the opposite phenomenon (Greicius & Menon, 2004). In rats, connectivity between LC and the thalamic nuclei was expected to increase after the tone associated with fear had been presented due to up-regulation of LC. To examine these hypotheses, for humans we planned a 2x2x4 mixed design ANOVA with a between-subjects factor group (squeeze/control) and within-subjects factors block type (resting state vs. squeeze) and network (DMN, FPCN, DAN, SN); for rats we planned two-tailed paired t-tests to compare dFC elements before and after tone presentation.

Results

Humans

No significant differences in mean BOLD signal or within-network static FC were observed for DMN, FPCN, DAN, or SN (statistics not shown) between either block type or group. However, we observed greater variability in BOLD signal during the squeezing blocks compared to the resting state blocks (Figure 1). The 2x2x4 mixed design ANOVA

resulted in significant main effects of group ($F(1,76) = 19.5$, $p < 0.01$), block type ($F(1,76) = 22.026$, $p < 0.001$), and network ($F(3,228) = 10.470$, $p < 0.01$). However, this test also revealed a significant 3-way interaction among group, block type, and network ($F(3,228) = 5.369$, $p = 0.001$), making the interpretation of main effects difficult. As a result, we conducted two step-down mixed design ANOVAs (2 group x 4 network), one within the resting state blocks and one within the squeeze blocks. These tests revealed that within the squeeze blocks there is a main effect of network ($F(3,228) = 9.060$, $p < 0.001$), a main effect of group ($F(1,76) = 16.80$, $p < 0.001$), and a significant interaction between network and group ($F(3,228) = 4.375$, $p = 0.005$). In contrast, within the resting state blocks there was a trending effect of group only ($F(1,76) = 2.966$, $p = 0.089$), but no other significant effects.

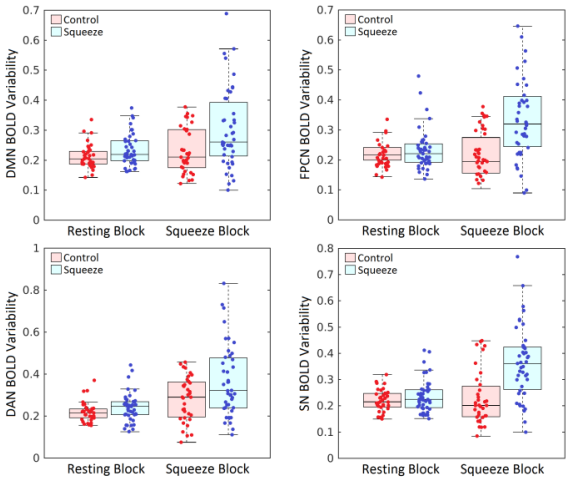

Figure 1: **Average BOLD signal variance** across resting state and squeezing blocks for DMN, FPCN, DAN, and SN. Squeezing, and consequent LC engagement, significantly changed BOLD signal variability as a function of network.

We also conducted step-down t-tests to further explore these interactions. Subjects who squeezed showed significantly higher BOLD variability during squeezing blocks compared to resting state blocks in DMN, FPCN, DAN, and SN ($t_{DMN}(84) = -3.154$, $p = 0.002$; $t_{FPCN}(84) = -4.276$ $p = <.001$; $t_{DAN}(84) = -4.438$, $p <.001$; $t_{SN}(84) = -5.404$, $p <.001$). Subjects who squeezed also exhibited higher BOLD variability during squeezing blocks for DMN, FPCN, DAN, and SN compared to control subjects ($t_{DMN}(76) = 2.891$, $p = 0.005$; $t_{FPCN}(76) = 4.514$, $p <.001$; $t_{DAN}(76) = 2.514$, $p = 0.014$; $t_{SN}(76) = 4.678$, $p <.001$). This suggests that squeezing increases the variability of brain network activity across time. Interestingly, control subjects exhibited higher DAN BOLD variability during squeezing blocks compared to resting state blocks ($t_{DAN}(68) = -3.2400$, $p = 0.0019$), likely because they brought their arm up to their chest when prompted to squeeze—exactly as the squeezing subjects did—but did not squeeze, which might have triggered a small increase in LC engagement. Finally, DMN variability during even the resting state blocks of the subjects who squeezed was significantly higher than that of the control group ($t_{DMN}(76) = 2.0851$, $p = 0.0404$). DMN is primarily active during resting state, so these results suggest that squeezing is causing a distinct disruption in the activity of even this network, again possibly due to an up-regulation of LC that lasts beyond the acute squeezing phase.

Rats

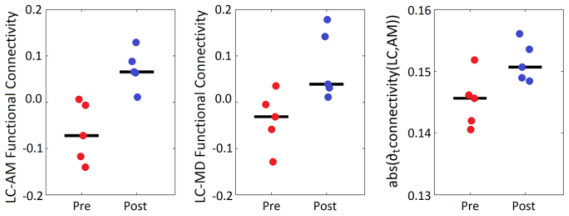

Figure 2: **Static and dynamic FC analysis**. Boxplots illustrating the static connectivity and absolute value of the first derivative of dFC between LC and MD, and between LC and AM. Presenting the tone associated with fear caused a significant increase in both parameters for LC-AM, but only in static FC for LC-MD.

Significant changes in static FC before and after tone presentation (preStressor and postStressor, respectively) were not found between LC and all thalamic nuclei previously mentioned (statistics not shown). Only LC-MD and LC-AM connectivity exhibited significant increase postStressor ($t_{LC\text{-}MD}(8), = -2.7247$, $p = 0.0261$; $t_{LC\text{-}AM}(8) = -3.9411$, $p = 0.0043$, Figure 2). MD and AM are involved in memory which is consistent with the idea that the rats are remembering the foot-shock associated with the tone. Furthermore, the mean of the absolute value of the first derivative of dFC significantly increased postStressor for AM but not for MD ($t_{LC\text{-}AM}(8), = -2.5847$, $p = 0.0324$); this again suggests that LC engagement significantly increases neural activity variability in LC innervated targets consistent with the results from the human analyses.

Discussion & Future Directions

Overall, our results suggest an important role for LC in driving variability in brain response across time. We observed changes in BOLD variability for resting state and attention networks in humans, and an increase in the absolute value of the derivative of dFC in rats in network-specific manners for both species. The increase in first derivative likely reflects increased connectivity state switching due to LC engagement, akin to the increased variability in BOLD seen in the humans. These effects were particularly strong in the humans while squeezing.

These findings suggest that LC engagement increases the noisiness of neural network dynamics, which in turn should impact the signal-to-noise ratio of the perceptual system overall and drive subjects to move "upwards" along the Yerkes-Dodson curve (Aston-Jones & Cohen, 2005; Guedj,

Meunier, Meunier, & Hadj-Bouziane, 2017; Yu & Dayan, 2005). In this model, the LC may indirectly modulate DMN, FPCN, DAN, and SN via LC's connection to the thalamus (Vertes et al., 2015). MD and AD are deeply involved with sensory-related functions, but are not as well understood as the posterior thalamus (Vertes et al., 2015). Our results indicate that the LC could be "switching" portions of the thalamus on and off, as has been suggested by others (Rodenkirch, Liu, Schriver, & Wang, 2019). Unfortunately, we are currently unable to perform the same analyses in humans, because LC's small size makes it difficult to localize with whole-brain coverage sequences available in human neuroimaging; ongoing work is actively working towards addressing these challenges.

Despite these limitations, the LC-thalamus connectivity changes in rats are consistent with the increases in variability in the DMN, FPCN, DAN, and SN in humans, despite the suboptimalities in paradigms due to our reliance on existing datasets. Ongoing work is seeking to perform dFC analyses in humans using a new paradigm with longer resting state blocks and at a higher spatial resolution. Future work will combine resting state dFC with the variability analyses explored here using approaches optimized for such questions, including hidden Markov models (Vidaurre, Smith, & Woolrich, 2017).

Our results provide preliminary evidence that active LC manipulation paradigms, in conjunction with investigation of BOLD variability and FC across time, may allow for convergent, cross-species investigation of the non-monotonic LC-performance relationship. Future studies will resolve differences between the experimental paradigms piloted here to facilitate a cross-species approach to characterize the computational mechanisms underlying LC's functional role in driving arousal.

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