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Intrinsic Fluctuations within Cortical Systems Account for Intertrial Variability in Human Behavior

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SUMMARY

The resting brain is not silent, but exhibits organized fluctuations in neuronal activity even in the absence of tasks or stimuli. This intrinsic brain activity persists during task performance and contributes to variability in evoked brain responses. What is unknown is if this intrinsic activity also contributes to variability in behavior. In the current fMRI study, we identify a relationship between human brain activity in the left somatomotor cortex and spontaneous trial-to-trial variability in button press force. We then demonstrate that 74% of this brain-behavior relationship is attributable to ongoing fluctuations in intrinsic activity similar to those observed during resting fixation. In addition to establishing a functional and behavioral significance of intrinsic brain activity, these results lend new insight into the origins of variability in human behavior.

INTRODUCTION

Historically, there have existed two alternate perspectives for understanding brain function (Llinas, 2001). The first conceptualizes the brain as an input-output system primarily driven by interaction with the external world. The second suggests that the brain operates on its own, intrinsically, with external factors modulating rather than determining the operation of the system. The former perspective has motivated the majority of neuroscience research, but accumulating evidence is emphasizing the importance of the latter.

Support for the intrinsic perspective on brain function comes from studies of spontaneous brain activity, or activity present even in the absence of task performance or stimuli. These studies span the gamut of techniques from fMRI BOLD (Biswal et al., 1995; Fox et al., 2005; Greicius et al., 2003; Hampson et al., 2002; Lowe et al., 1998) and optical imaging (Arieli et al., 1996; Kenet et al., 2003) to electrical recordings of various types (Arieli et al., 1996; Fiser et al., 2004; Hahn et al., 2006; Kenet et al., 2003; MacLean et al., 2005; Petersen et al., 2003; Shu et al., 2003b; Tsodyks et al., 1999). Because this spontaneous activity can be observed during rest (Biswal et al., 1995; Fiser et al., 2004; Fox et al., 2005; Greicius et al., 2003), under anesthesia (Arieli et al., 1996; Kenet et al., 2003; Kiviniemi et al., 2003; Petersen et al., 2003; Tsodyks et al., 1999; Vincent et al., 2007), and in vitro (MacLean et al., 2005; Shu et al., 2003b), it is not thought to depend on sensory input and therefore is considered intrinsic to neuronal systems. This intrinsic brain activity is not random noise, but specifically correlated between related neurons (Tsodyks et al., 1999), cortical columns (Kenet et al., 2003), and within widely distributed neuroanatomical systems (Biswal et al., 1995; Fox et al., 2005; Greicius et al., 2003; Hampson et al., 2002; Lowe et al., 1998).

Studies of intrinsic activity in the human brain have examined correlations in slow (<0.1 Hz) spontaneous fluctuations of the blood oxygen level-dependent (BOLD) signal of fMRI (Biswal et al., 1995; Fox et al., 2005; Greicius et al., 2003; Hampson et al., 2002; Lowe et al., 1998). One highly reproducible example of this intrinsic organization is the observation that spontaneous BOLD fluctuations in the left somatomotor cortex (SMC) are specifically correlated with fluctuations in the right SMC as well as with those in medial motor areas (Biswal et al., 1995; Fox et al., 2006b; Lowe et al., 1998).

Importantly, this intrinsic activity does not disappear with the administration of stimuli or during task performance. Rather, it continues and can account for a significant fraction of the intertrial variability routinely observed in evoked neuronal responses in animals (Arieli et al., 1996; Petersen et al., 2003; Shu et al., 2003a) and BOLD responses in humans (Fox et al., 2006b). An important unanswered question is whether these fluctuations in ongoing intrinsic activity are manifested functionally as fluctuations in behavior.

Several previous studies have examined the relationship between human brain activity and intertrial variability in behavior (Boly et al., 2007; Ergenoglu et al., 2004;

Grill-Spector et al., 2004; Pessoa et al., 2002; Pessoa and Padmala, 2005; Ress et al., 2000; Ress and Heeger, 2003; Sapir et al., 2005; Wagner et al., 1998). These studies have shown, for example, that differences in regional brain activity can predict whether a given stimulus will be seen or missed (Ress et al., 2000), remembered or forgotten (Wagner et al., 1998), or result in a correct or incorrect response (Sapir et al., 2005). What is unclear from these studies is the source of the variability underlying these brain-behavior relationships. Most studies have attributed their findings to intertrial variability in task-related factors such as attention or anticipation (Pessoa et al., 2002; Ress et al., 2000; Sapir et al., 2005; Wagner et al., 1998). Recently, we proposed an alternative mechanism, suggesting that intrinsic fluctuations in brain activity such as those observed during rest continue during task performance and contribute to intertrial variability in behavior (Fox et al., 2006b). While attention and anticipation have been shown to influence both brain activity and behavior under controlled conditions (Brefczynski and DeYoe, 1999; Drevets et al., 1995; Kastner et al., 1999; Posner, 1980; Posner and Petersen, 1990; Tootell et al., 1998), the behavioral significance of fluctuations in ongoing intrinsic activity remains to be demonstrated.

To test the hypothesis that fluctuations in ongoing intrinsic activity contribute to variability in behavior, we examined BOLD activity within the human somatomotor system both during rest and during a simple right-hand button-press task in which the force of each button press was recorded. This experimental design was used for three reasons. First, brain activity in the left SMC has previously been shown to relate to force output (Cramer et al., 2002; Dettmers et al., 1995), suggesting a suitable behavioral measure for the current investigation. Second, the left SMC and right SMC are coherent in their spontaneous activity but differentially activated by a right-handed button press (Biswal et al., 1995; Cramer et al., 2002; Dettmers et al., 1995; Fox et al., 2006b; Lowe et al., 1998), facilitating a separation of spontaneous and task-related activity in the left SMC (Fox et al., 2006b). Finally, our experimental design allows us to rule out confounding factors such as attention, arousal, and anticipation by examining the spatial distribution of the BOLD-behavior effect, correlations with reaction time, and the influence of interstimulus interval.

RESULTS

fMRI BOLD data were acquired in 17 normal right-handed subjects under two conditions: resting while maintaining visual fixation on a crosshair and during a button-press task. In the task condition, subjects were asked to press a button as quickly as possible with their right index finger when the crosshair dimmed. The force of each button press was measured with a custom-built optical transducer. Each subject performed 80 trials pressing with an average force of 9.34 N and a within-run standard deviation (trial-to-trial variability) of 2.34 N. The average reaction time was 365 ms with an average within run standard deviation of 58 ms. For each subject, we identified a left SMC region significantly activated by right-handed button presses (Figures 1A and 1B). We then identified a right SMC region significantly correlated with the left SMC in its spontaneous activity during the resting runs (Figures 1C and 1D; Biswal et al., 1995; Fox et al., 2006b). This procedure identifies two regions of interest strongly correlated in their spontaneous activity (Figures 1C and 1E) but differentially activated by right hand button presses (Figure 1A). The locations of the left SMC and right SMC regions of interest across our population of 17 subjects are displayed in Supplementary Figure 1.

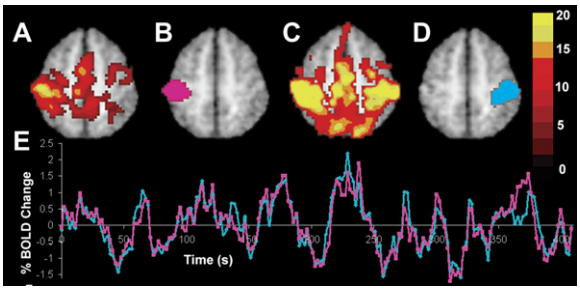

Figure 1. Identification of Subject-Specific SMC ROI and an Example of Intrinsic BOLD Fluctuations during Resting Fixation, Illustrated for One Selected Subject

(A) Z score map showing voxels significantly activated following right-hand button presses.

(B) Left SMC ROI derived from the map shown in (A).

(C) Z score map showing voxels significantly correlated with the left SMC during resting fixation.

(D) Right SMC ROI derived from the map shown in (C).

(E) BOLD time courses from the left SMC (magenta) and right SMC (cyan) during a single run of resting fixation.

Intrinsic Activity Accounts for Variability in Left SMC BOLD Responses

Average BOLD activity following a button press was extracted for both the left SMC (Figure 2A) and right SMC (Figure 2B) along with the average within-run standard deviations. The large standard deviations indicate pronounced trial-to-trial variability, with the mean evoked response accounting for 20.2% of the total left SMC variance and only 2.88% of the total right SMC variance. Thus, the majority of the left SMC activity and almost all of the right SMC activity following a button press was due to factors other than the deterministic evoked response.

We have previously reported that much of the variance in left SMC BOLD responses can be accounted for by coherent spontaneous activity as measured in the right SMC (Fox et al., 2006b). However, this previous study was a reanalysis of data not collected for this purpose and included complicating features such as video stimuli, a non-motor cognitive task, and button-press responses not synchronized with scanner acquisitions. Our first step therefore was to confirm the previous finding in the present data set. As in our original report, we used the signal

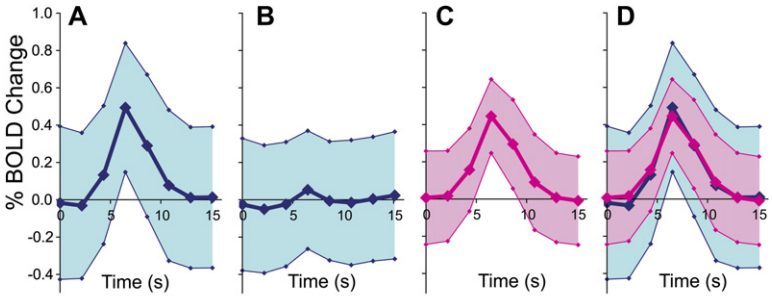

Figure 2. Coherent Spontaneous Activity Accounts for a Significant Fraction of the Trial-to-Trial Variance in Left SMC BOLD Responses

(A) The average BOLD response in the left SMC (thick line) following a right index-finger button press plus or minus the average within-run standard deviation (i.e., trial-to-trial variability within a run).

(B) Corresponding activity in the right SMC.

(C) Left SMC activity after subtraction of spontaneous activity as measured in the right SMC.

(D) Comparison of left SMC activity before (blue) and after (pink) removal of coherent spontaneous activity (i.e., an overlay of graphs [A] and [C]). Much of the variance in the left SMC can be attributed to ongoing spontaneous activity within the somatomotor system.

from the right SMC as an estimate of ongoing spontaneous activity in the left SMC. We subtracted the right SMC activity from the left SMC after weighting by a regression coefficient derived from the resting-state data (Figure 2C). Comparing left SMC responses before and after removal of right SMC activity (Figure 2D), we observed a 59.3% reduction in noise ($p < 0.0005$), a 19.4% reduction in signal ($p < 0.005$), and a 109.4% increase in signal-to-noise ratio ($p < 0.0005$). The increase in signal to noise is important as it rules out the possibility that a small task-evoked response in the right SMC accounts for the reduction in left SMC variance (Fox et al., 2006b).

These results replicate our previous finding (Fox et al., 2006b) and establish our experimental approach, showing that left SMC activity following right-handed button presses (Figure 2A) can be partitioned into coherent spontaneous activity (monitored in the right SMC; Figure 2B) and activity specific to the left SMC (Figure 2C). In the next section of this article, we will again apply this experimental approach, but instead of focusing on total left SMC variance we will focus on the portion left SMC variance related to force output.

Intrinsic Activity Accounts for Behaviorally Relevant Left SMC BOLD Variance

It is clear from the material presented above that intrinsic activity can account for much of the variability in measured left SMC BOLD responses. However, the critical question is whether intrinsic activity can account for the portion of left SMC variance that is related to behavior. To answer this question, we first identified a significant brain-behavior relationship in the left SMC and then applied the above analytic approach to determine how much of this relationship was attributable to intrinsic activity.

The first step was therefore to find some measure of left SMC BOLD variance that was related to natural trial-to-trial variability in force output. For each button press run of each subject, we sorted trials into three bins based on the force of the button press (hard, medium, and soft). To maximize the difference in force, we compared trials in the hard bin (mean = 12.04 N) to trials in the soft bin (mean = 6.91 N). The average left SMC time courses for spontaneous hard and soft button presses are shown in Figure 3A. These time courses were significantly different showing both a main effect of force ($F_{1,16} = 6.14$, $p < 0.03$) and a force × time effect ($F_{7,112} = 3.05$, $p < 0.006$) (Figure 3A). To determine which portion of the left SMC time course was driving this difference, we performed paired t tests on individual time points. Significant differences were observed for time points two and three ($p < 0.05$, Bonferroni corrected for 8 time points). For simplicity, we developed a single quantitative measure of this BOLD-behavior relationship by averaging time points 2 and 3 and subtracting hard from soft. This measure is equivalent to the area between the curves and is shown highlighted in yellow on the time courses themselves and in bar graph form in the inset (Figure 3A). Using this quantitative measure, a robust difference between hard and soft button presses can be appreciated in the left SMC ($p < 0.0025$).

To determine how much of this brain-behavior relationship was attributable to intrinsic activity, we partitioned activity in the left SMC (Figure 3A) into variance attributable to coherent spontaneous activity (i.e., right SMC activity; Figure 3B), and variance specific to the left SMC (i.e., left SMC post-right SMC regression; Figure 3C). BOLD time courses for spontaneous hard and soft button presses as well as the difference between time points 2 and 3 are illustrated. The same BOLD-behavior relationship seen in the left SMC was significantly present in the ongoing spontaneous activity measured in the right SMC (Figure 3B, inset; $p < 0.05$). After spontaneous activity was regressed out of the left SMC (Figure 3C), this BOLD-behavior relationship was no longer significant (Figure 3C, inset; $p = .40$). There was also no longer a significant time course difference as measured by an ANOVA (main effect force $p = 0.254$, force × time $p = 0.138$). Directly comparing the left SMC BOLD-behavior effect before versus after regression (Figure 3D), the majority (74%) of the BOLD-behavior relationship observed in the left SMC can be attributed to ongoing spontaneous activity as measured in the right SMC ($p < 0.05$).

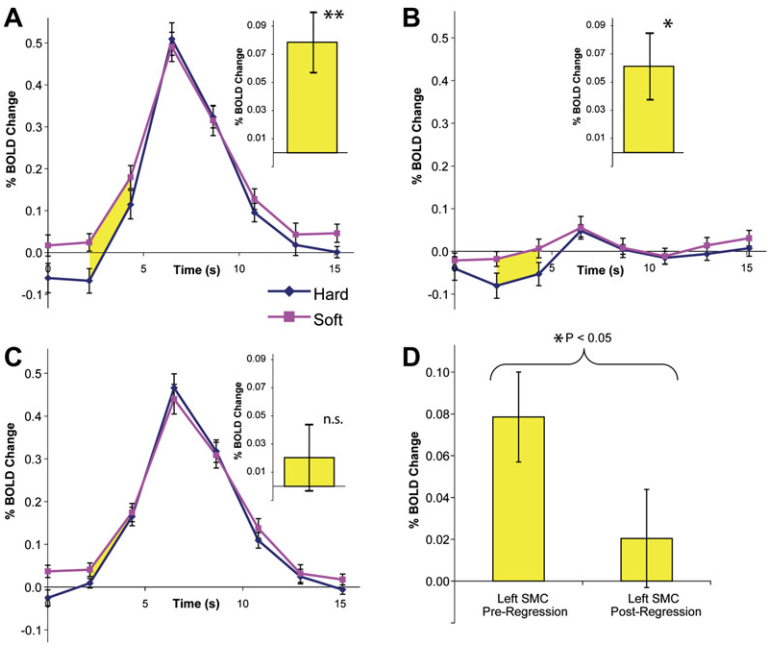

Figure 3. Coherent Spontaneous Activity Accounts for Behaviorally Relevant Left SMC BOLD Variance

(A) Average left SMC BOLD time courses for hard (blue) and soft (magenta) button presses as well as the significant difference between them (yellow area). This area represents the BOLD-behavior effect and is also shown in bar graph form (inset).

(B) The same time courses and area for the right SMC also reveal a significant BOLD-behavior effect.

(C) The same time courses and area for the left SMC post-regression of spontaneous activity do not show a significant BOLD-behavior effect.

(D) The BOLD-behavior relationship in the left SMC is significantly reduced after regression of spontaneous activity. These results show that the majority (74%) of the relationship between spontaneous force variability and BOLD activity in the left SMC can be attributed to ongoing spontaneous activity. *$p < 0.05$, **$p < 0.005$.

Error bars reflect standard error across subjects.

Assessing Spatial Specificity

In addition to the region-based analysis described above, we also applied our BOLD metric on a voxel-wise basis. This identified all brain voxels showing a significant difference between spontaneous hard and soft button presses at BOLD time points 2 and 3 (Figure 4A). As expected from the region-based analysis, we saw a significant BOLD-behavior effect in both the left and right SMC. However, this effect was not present in all brain voxels, but was largely specific to the somatomotor system. We compared the spatial distribution of this BOLD-behavior effect to the distribution of voxels correlated with the left SMC during resting fixation (Figure 4B). The qualitative similarity between these two distributions was evident on examination of the overlap image (Figure 4C).

To quantify the similarity between the BOLD-behavior map (Figure 4A) and the map of correlated intrinsic activity (Figure 4B), we computed the spatial correlation

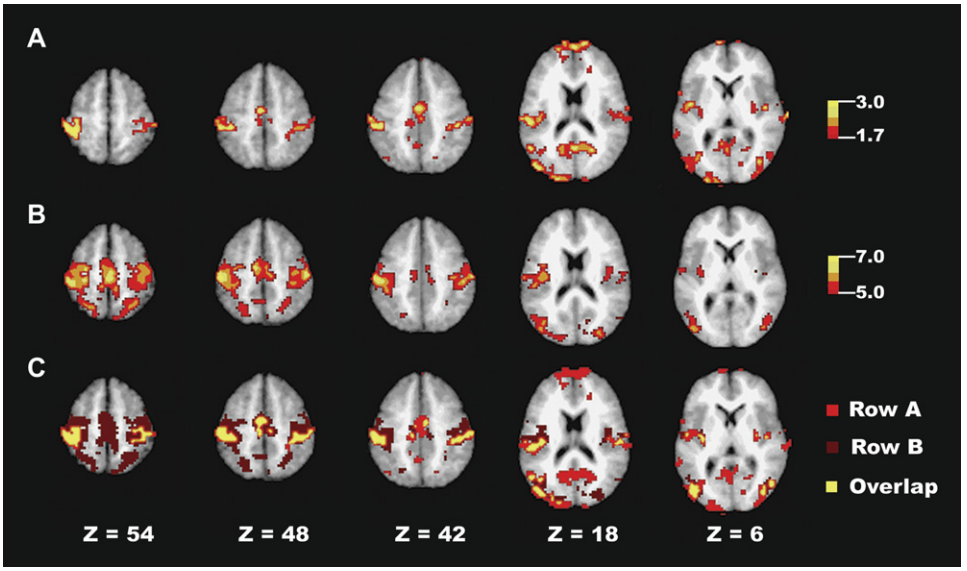

Figure 4. The Distribution of Voxels Showing a BOLD-Behavior Relationship at Time Points 2 and 3 Is Similar to the Distribution of Voxels Correlated with the Left SMC during Resting Fixation

(A) Voxels showing a significant difference between spontaneous hard and soft button presses at time points 2 and 3.

(B) Voxels significantly correlated with the left somatomotor cortex during resting fixation.

(C) The overlap (yellow) between the BOLD-behavior map shown in (A) (red) and the intrinsic correlation map shown in (B) (brown).

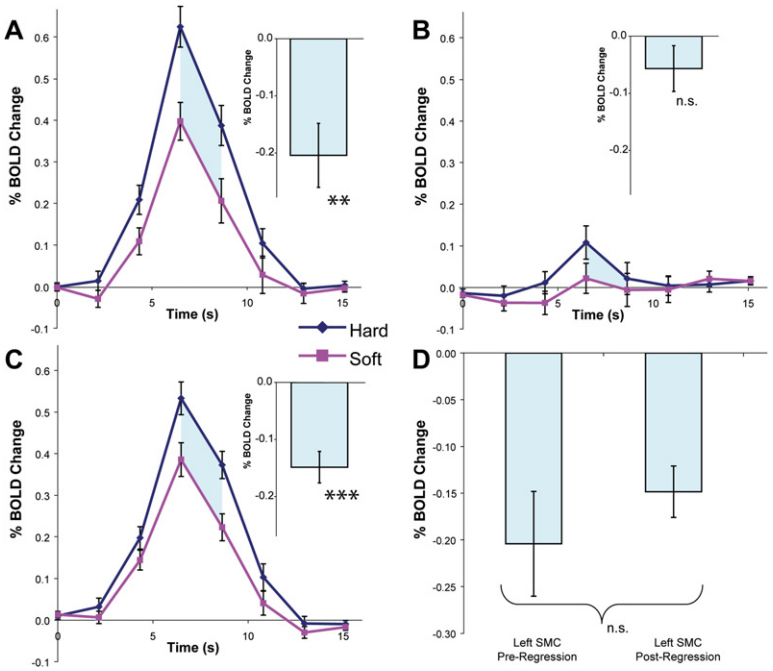

Figure 5. Evaluation of the Left SMC BOLD-Behavior Effect for Instructed Hard and Soft Button-Press Responses

(A) Average left SMC BOLD time courses for hard (blue) and soft (magenta) button presses as well as the significant difference between them (light blue area). This area represents the BOLD-behavior effect and is also shown in bar graph form (inset).

(B) The same time courses and area for the right SMC do not show a significant BOLD-behavior effect.

(C) The same time courses and area for the left SMC post-regression of spontaneous activity show an increase in the significance of the BOLD-behavior effect.

(D) There is no significant difference in the left SMC BOLD-behavior relationship before versus after regression of spontaneous activity. These results contrast sharply with those observed with spontaneous force variability (Figure 3), showing an inversion in the relative magnitude of the hard and soft time courses, a difference in the timing of the significant BOLD-behavior effect, and a difference of the effect of regressing out spontaneous activity on the significance of the BOLD-behavior relationship. **$p < 0.005$, ***$p < 0.0005$.

Error bars reflect standard error across subjects.

coefficient on a single-subject basis (Fox et al., 2006a). There was a significant spatial correlation between the two maps computed across all brain voxels ($p < 0.005$) and computed across only gray matter voxels ($p < 0.02$). To ensure that the significance of this spatial correlation was not driven by the left SMC alone, we also computed the spatial correlation between the BOLD-behavior map and the button press activation map (see Figure 1A). There was not a significant spatial correlation between these two maps computed across all brain voxels ($p = 0.140$) or gray matter ($p = 0.261$).

Ruling Out Evoked Activity

One concern in the present study is that sensory feedback from spontaneous hard versus soft button presses may result in bilateral stimulus-evoked BOLD responses that differ in a manner that accounts for the current BOLD-behavior relationship. To exclude this possibility, 11 of our 17 subjects completed four additional BOLD runs in which they were instructed to press with a "slightly harder" or "slightly softer" force than they had used during the previous runs. Subjects pressed with an average force of 3.19 N during instructed soft runs and 21.15 N during instructed hard runs. The same analysis strategy used to assess spontaneous intertrial variability in force (see Figure 3) was applied to the instructed hard and soft responses (Figure 5).

As expected, there was a significant difference between instructed hard and soft responses in the left SMC (Figure 5A) showing both a main effect of force ($F_{1,10} = 11.08$, $p < 0.01$) and a force × time effect ($F_{7,70} = 7.95$, $p < 0.0001$); however, the direction of this effect was opposite from that seen with spontaneous force variability. As before, we performed paired t tests on all time points and found that two time points (time point 4 and 5) were significantly different ($p < 0.05$, Bonferroni corrected for 8 time points). Similar to our earlier analysis, we developed a single measure of the BOLD-behavior effect by averaging the significant time points (4 and 5) and subtracting hard from soft. Using this measure, there was a robust difference between instructed hard and soft time courses in the left SMC ($p < 0.005$), but again in the opposite direction from that seen with spontaneous force variability. We then applied this metric to the right SMC and the left SMC post-regression of right SMC activity. There was not a significant difference at time points 4 and 5 in the right SMC (Figure 5B; $p = 0.187$). Further, a significant difference remained in the left SMC after regression of spontaneous activity (Figure 5C; $p < 0.0005$). In fact, the significance of the BOLD-behavior effect in the left SMC actually increased after regression of spontaneous activity both at time points 4 and 5 and using an ANOVA (main effect of force, $F_{1,10} = 20.53$, $p < 0.005$; force × time, $F_{7,70} = 9.34$, $p < 0.0001$). Comparing the magnitude of the left SMC BOLD-behavior relationship pre versus post regression of right SMC activity (Figure 5D), there was not a significant difference ($p = 0.182$).

When the instructed hard versus soft results (Figure 5) were compared with the results from the spontaneous force variability experiment (Figure 3) several differences were evident. First, there was a reversal of the hard versus soft contrast. With instructed force variability the hard time

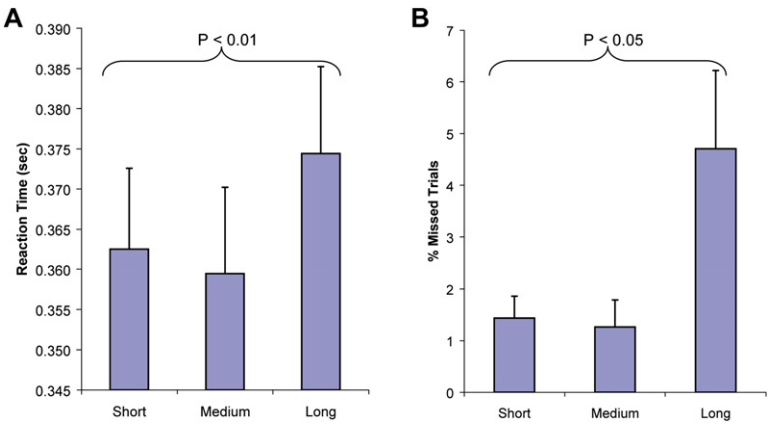

Figure 6. Attention Varies Significantly with ISI

When trials are binned based on the time from the last trial (short, medium, and long ISI) there is a significant influence on reaction time (A) and the percentage of missed trials (B).

Error bars reflect standard error across subjects.

course was higher than the soft (Figure 5), but when force variance occurred spontaneously, the soft time course was higher than the hard (Figure 3). Second, there was a difference in the timing of the two effects. With instructed force variability the significant time course difference was at time points 4 and 5 (6.5 to 8.6 s post button press; Figure 5), but with spontaneous force variability the difference was at time points 2 and 3 (2.2 to 4.3 s post button press; Figure 3). Finally, there was a pronounced difference in the effect of regression on the left SMC BOLD-behavior relationship. When force variability was instructed the BOLD-behavior relationship became more significant (Figure 5), but when force variability was generated spontaneously the BOLD-behavior relationship was largely eliminated (Figure 3). These differences were not due to the different numbers of subjects in the two analyses as they held true even when the analysis of spontaneous force variability was limited to the 11 subjects that also completed the instructed force experiment (see Figure S2 in the Supplemental Data available with this article online). Similarly, these results are unlikely to be due to the difference in button press force between the spontaneous and instructed conditions as the two effects do not converge as the force discrepancy is reduced (Figure S3). These results indicate that instructed and spontaneous force variability give rise to very different BOLD-behavioral relationships. Hence, the BOLD-behavior effect observed with spontaneous force variability is unlikely to be due to hard versus soft button presses per se.

Ruling Out Attention and Anticipation

Another concern that needs to be addressed is the effect of attention or anticipation. Such effects are known to produce changes in BOLD activity and can do so even before the stimulus or event itself. If the underlying mechanism leading to spontaneous force variability was fluctuating attention or anticipation, could this explain the BOLD-behavior effect at time points 2 and 3? We addressed this concern in three ways.

First, we looked for evidence that subjects were differentially attending to or anticipating hard versus soft button presses. If this were the case, one would expect to see a difference in reaction time, i.e., faster responses on strongly attended to or anticipated trials. However, there was no difference in reaction time between hard and soft bins (366 versus 365 ms, $p = 0.88$). Similarly, there was no correlation between force and reaction time on a continuous trial by trial basis ($r = -0.018$, $p = 0.623$).

Second, we looked for evidence that attention or anticipation was responsible for the BOLD modulation at time points 2 and 3 in the left and right SMC. There was no significant correlation between the BOLD metric driving our observed effect (average of time points 2 and 3) and reaction time in the right SMC ($r = 0.041$, $p = 0.46$) or the left SMC ($r = -0.01$, $p = 0.844$). Interestingly, there was a correlation between time points 2 and 3 and reaction time in the left SMC *postregression* ($r = -0.089$, $p < 0.02$). This suggests that while attention does not significantly influence coherent BOLD modulation in the left and right somatomotor cortices, it may affect the BOLD response specifically in the left (contralateral) SMC.

Finally, we looked at the effect of interstimulus interval (ISI) as a specific manipulation of attention. We sorted trials into three bins based on the preceding ISI: short (17.3–19.4 s), medium (21.6 s), and long (23.8–30.2 s). There was a significant effect of ISI on reaction time ($F_{2,32} = 5.506$, $p < 0.01$; Figure 6A) and on the percentage of trials missed ($F_{2,32} = 3.85$, $p < 0.05$; Figure 6B), suggesting that ISI significantly covaried with attention. However, there was no significant influence of ISI on force ($F_{2,32} = 0.067$, $p = .936$) or on our BOLD metric in either the right SMC ($F_{2,32} = 0.582$, $p = .5646$) or the left SMC ($F_{2,32} = 1.34$, $p = 0.276$). Interestingly, there was a significant influence of ISI on points 2 and 3 in the left SMC postregression ($F_{2,32} = 11.5$, $p < 0.0005$), again suggesting that attention influences the BOLD response specifically in the left SMC.

DISCUSSION

There are several noteworthy findings in the current experiment. First, we have replicated our earlier observation that ongoing intrinsic activity accounts for variability in measured BOLD responses (Fox et al., 2006b). Comparing the current results to our previous findings, we see

that ongoing intrinsic activity accounts for more variability in measured brain responses (60% versus 40%) and facilitates a greater improvement in signal to noise (110% versus 60%) than previously appreciated (Fox et al., 2006b). The more robust effects in the present study are likely due to the larger number of subjects, greater number of button press responses, targeted experimental design, and more direct analysis techniques.

Second, we identified a significant BOLD-behavior relationship in the left SMC, showing that the BOLD signal at time points 2 and 3 was related to natural (spontaneous) intertrial variability in button press force. Although previous studies have shown a relationship between left SMC activity and force, these studies were based on instructed force variability with different predetermined force levels set by the experimenter (Cramer et al., 2002; Dettmers et al., 1995). The current results are the first to show a relationship between spontaneous (involuntary) intertrial variability in force and brain activity in contralateral SMC.

Finally, and most importantly, we have shown that the majority (74%) of the BOLD-behavior relationship identified the left SMC can be attributed to ongoing coherent fluctuations as measured in the right SMC. Because the right SMC is only minimally modulated by the button-press task and is coherent with the left SMC during resting fixation, we have referred to activity in the right SMC as reflecting "ongoing intrinsic activity." However, since subjects are not resting but performing a task, we must first consider whether other sources of neuronal and behavioral variability besides ongoing intrinsic activity could possibly account for the present results.

Ruling Out Stimulus-Evoked Activity

It is well established that sensory stimuli can evoke BOLD responses in the human brain, the character of which can vary with the properties of the stimuli. For example, a higher-contrast visual stimulus evokes a larger BOLD response in the visual cortex (Boynton et al., 1999) and a stronger somatosensory stimulus evokes a larger BOLD response in the somatomotor cortex (Kampe et al., 2000). Sensory feedback from hard versus soft button press could conceivably differ such that hard presses resulted in a more negative BOLD deflection at time points 2 and 3 than soft presses. If this evoked activity were present both in the left and right SMC, then hard versus soft button presses alone could account for the current findings.

This concern was directly addressed by comparing spontaneous to instructed hard versus soft button presses. If the BOLD-behavior effect was due to hard versus soft presses per se, then it should be present regardless of whether force variability was spontaneous (involuntary) or instructed (voluntary). However, when subjects were explicitly instructed to press hard versus soft and the data were analyzed in the same way as the spontaneous force variability data, a very different result was observed. First, there was a reversal in the relative magnitude of the hard and soft time courses. Differences in normalization may complicate interpretation of this reversal in the left SMC (see Experimental Procedures), but the relative magnitude can be accurately assessed in the right SMC. This time course reversal suggests that the BOLD-behavior relationship due to evoked responses works in the opposite direction and, if anything, serves to obscure the BOLD-behavior relationship seen with natural force variability.

A second factor that argues against evoked activity as the explanation for the current findings is the timing of the BOLD-behavior effect. The same analysis procedure was applied to all time points in both the instructed and spontaneous conditions but identified a significant difference at time points 2 and 3 in the spontaneous case and time points 4 and 5 in the instructed case. Previous research demonstrates that evoked hemodynamic responses due to discrete neuronal events take around 2 to 2.5 s to develop and peak around 6 s (Boynton et al., 1996). This is consistent with the observation that the peak difference between instructed hard and soft responses occurred 6.5 to 8.6 s post stimulus. In contrast, the BOLD-behavior relationship seen with natural force variability occurred earlier, 2.16–4.32 s after the event (time points 2 and 3). There are two ways to interpret the early timing of the BOLD-behavior effect in the spontaneous condition. First, the effect may be driven by fluctuations in neuronal activity occurring prior to the stimulus, resulting in a peak hemodynamic effect only 2–4 s after the stimulus. Second, the BOLD-behavior effect may be driven by ongoing neuronal activity present at the time of the stimulus, but the effect at later time points (i.e., 4 and 5) is obscured by the influence of evoked activity working in the opposite direction. Regardless of the interpretation, it is clear that the timing of BOLD-behavior effect seen with natural force variability makes evoked activity an unlikely explanation.

Finally, there was a pronounced difference in the effect of regressing out spontaneous activity on the left SMC BOLD-behavior relationship with instructed versus spontaneous force variability. With spontaneous force variability, regression of spontaneous (right SMC) activity all but eliminated the left SMC BOLD-behavior relationship. In contrast, with instructed force variability regressing out spontaneous activity increased the significance of the left SMC BOLD-behavior effect. This improvement in significance suggests that regression of spontaneous activity removed noise that was independent of the BOLD-behavior effect in the instructed condition. This finding is important as it shows that an ipsilateral response alone is not sufficient to eliminate the BOLD-behavior effect by regression as seen with spontaneous force variability.

In summary, there are three pronounced differences between spontaneous and instructed force variability in the current experiment: (1) the reversal of the time course magnitudes, (2) the difference in the timing of the significant BOLD-behavior effect, and (3) the difference in the effect of regressing out spontaneous activity. As such, we can be relatively confident that spontaneous and

instructed force variability represent distinct phenomena in the current experiment. One concern is that these conditions are different because there is a greater hard-soft force difference in the instructed case than in the spontaneous case. However, for this to be the explanation, the properties of the BOLD responses in the somatomotor cortex would have to change quite dramatically over a range of about 10 N. We are aware of no evidence suggesting such an effect in the literature, and analysis of a subset of instructed force responses selected to be closer to the spontaneous hard-soft force difference argues against this as a confounding factor (Figure S3). Another concern is the different number of subjects in the instructed and spontaneous experiments, but the important differences remain when restricted to the same subset of subjects (Figure S2).

If the BOLD-behavior effect seen with spontaneous force variability is due to evoked activity, it must represent a new type of evoked activity that differs in several prominent ways from the evoked BOLD responses commonly reported in the literature and those observed in the present experiment. As such, we believe that the BOLD-behavior effect seen with spontaneous force variability is unlikely to be due to differences in sensory feedback generated by hard versus soft button presses.

Ruling Out Attention and Anticipation

Attention is not a unitary function. Limitations of resources and the need for selection arise at different levels of processing and in different cognitive domains including perception, action, language, and memory (Alport, 1990; Pashler, 1998). One form of attention that has been shown to modulate both neuronal activity and behavior is sensory orienting (Posner and Petersen, 1990). A classic example is the Posner paradigm involving cuing of attention to a region in visual space (Posner, 1980). Subjects respond significantly faster to targets appearing in an attended or anticipated location compared to targets appearing elsewhere (Posner, 1980). There is also an enhancement of neuronal activity in the sensory cortex corresponding to the attended location relative to nonattended regions, the so called attentional "spotlight"(Brefczynski and DeYoe, 1999; Drevets et al., 1995; Kastner et al., 1999; Posner and Petersen, 1990; Tootell et al., 1998). This attentional modulation of neuronal activity can be observed prior to and independent of the stimulus (Kastner et al., 1999; Sapir et al., 2005). Based on these results, the majority of previous experiments showing a correspondence between regional BOLD activity and behavior have cited trial-to-trial changes in attention, not ongoing intrinsic activity, as the most likely source of the variability (Pessoa et al., 2002; Ress et al., 2000; Sapir et al., 2005; Wagner et al., 1998). With such a strong precedent, we felt obliged to show that attention and anticipation cannot account for the current results.

We performed several analyses to exclude attention as the mechanism underlying the observed relationship between spontaneous force variability and BOLD activity at time points 2 and 3 in the left and right somatomotor cortices. The most direct approach was to look at the influence of interstimulus interval. There was a significant effect of ISI on both reaction time and the percentage of missed trials suggesting that attention covaried with ISI. However, there was not a significant relationship between ISI and either force or BOLD activity at time points 2 and 3, suggesting that attention was not responsible for the observed BOLD-behavior effect.

The finding of a significant effect of ISI on reaction time also establishes the validity of our reaction time measurement and, together with previous studies showing a correspondence between attention and reaction time, suggests that reaction time can be used as a marker of attention. We therefore performed additional analyses using reaction time to further exclude attention as a confounding variable. First, we showed that there was no relationship between reaction time and our behavioral measurement of interest (force), either binned into hard and soft bins or on a continuous trial-by-trial basis. Second, we showed that there was no relationship between reaction time and our BOLD measurement of interest (average of time points 2 and 3) in either the left or right SMC. The lack of a relationship between reaction time and either our behavioral or BOLD measurement of interest makes it highly unlikely that attention accounts for the BOLD-behavior relationship in the spontaneous force variability data.

Finally, the spatial distribution of the current effect argues against attention as an underlying mechanism (see Figure 4). The "spotlight" of attention tends to have differential effects on sub-regions within a system (Brefczynski and DeYoe, 1999; Drevets et al., 1995; Kastner et al., 1999; Posner and Petersen, 1990; Tootell et al., 1998), while intrinsic activity tends to be coherent within a system (Biswal et al., 1995; Fox et al., 2005; Greicius et al., 2003; Hampson et al., 2002; Lowe et al., 1998). In the current experiment, attention would be expected to differentially modulate activity in the hand region of the left SMC relative to the rest of the somatomotor system (Drevets et al., 1995). The fact that the current BOLD-behavior effect is present both in the left and right SMC and shows a similar spatial distribution to intrinsic activity measured during resting fixation strongly suggests that ongoing intrinsic activity, not attention, is the underlying mechanism.

Although attention could not account for the BOLD-force relationship in either the left or the right SMC, the influence of attention on brain activity was not absent in the current experiment. Specifically, effects consistent with attention were observed in the BOLD data from the left SMC post right SMC regression (see Figure 2C). We have referred to this as "left SMC-specific activity," and it reflects any modulation that is not coherent or is asymmetric between the left and right SMC. This asymmetric BOLD activity was significantly correlated with both reaction time and ISI, consistent with an influence of attention. Thus, the influence of both intrinsic activity and attention were observed in the present experiment, but these effects were separable based on behavioral measures

(force versus reaction time), interaction with interstimulus interval, and spatial distribution (coherent versus asymmetric).

Ruling Out Other Potential Confounds

While sensory evoked activity and attention/anticipation are the most concerning potential confounds, other mechanisms should be considered. For example, global arousal might cause fluctuations in neuronal activity and behavior. However, our BOLD-behavior effect should then be present in all regions or at least regions implicated in arousal (Critchley et al., 2000), not localized to the somatomotor system. Similarly, after-effects such as the BOLD undershoot could persist from the previous trial, influencing early BOLD time points and confounding our results (Buxton et al., 1998). However, this possibility is excluded by the lack of a relationship between our BOLD measurement and ISI.

To summarize, the factor responsible for the currently observed BOLD-behavior effect in the left SMC is apparent 2 s after an event, is significantly reduced after regression of right SMC activity, is coherent within and largely specific to the somatomotor system, and is independent of both ISI and reaction time. While task-related factors cannot be completely ruled out in any study of awake behavior, no known task-related factor including evoked activity and attention can satisfy these constraints. However, these properties align well with intrinsic fluctuations in BOLD activity repeatedly observed during resting conditions (Biswal et al., 1995; Fox et al., 2005; Greicius et al., 2003; Hampson et al., 2002; Lowe et al., 1998) making intrinsic activity the most parsimonious explanation for the present results.

Implications for Understanding Variability in Human Behavior

It is well known that humans exhibit trial-to-trial variability in their perception and performance even when the task and stimuli remain constant (Gilden, 2001; Gilden et al., 1995). In the current experiment, we investigated variability of a relatively simple behavior, pressing a button with the right index finger in response to a visual cue. We found that intertrial variability in the force of the button press was related to variability in the BOLD signal in the left SMC. This identification of a BOLD-behavior relationship based on intertrial variability is similar to previous studies showing BOLD-behavior relationships with other behaviors and in other brain regions (Boly et al., 2007; Grill-Spector et al., 2004; Pessoa et al., 2002; Pessoa and Padmala, 2005; Ress et al., 2000; Ress and Heeger, 2003; Sapir et al., 2005; Wagner et al., 1998). However, these previous studies did not isolate the source of the variability underlying these BOLD-behavior relationships, often attributing their findings to intertrial fluctuations in attention (Pessoa et al., 2002; Ress et al., 2000; Sapir et al., 2005; Wagner et al., 1998).

The novel contribution of the current study is in directly investigating the source of the variability underlying an identified BOLD-behavior relationship. While task-related factors such as attention undoubtedly do influence brain activity (Drevets et al., 1995; Kastner et al., 1999), the primary source of the variability underlying the BOLD-behavior relationship in the current study was ongoing intrinsic activity. Since intrinsic BOLD activity can be observed during rest (Biswal et al., 1995; Fox et al., 2005, 2006a; Greicius et al., 2003; Hampson et al., 2002; Kenet et al., 2003; Lowe et al., 1998), sleep (Fukunaga et al., 2006), and even under anesthesia (Kiviniemi et al., 2003; Vincent et al., 2007), behavioral variability may be due to factors present independent of task context or even the conscious state. The distinction between task-related factors and intrinsic activity is important because task-related factors such as attention are thought to be under some degree of volitional control. Hence, behavioral variability is often assumed to involve intentionality or at least accountability on the part of the subject. However, if behavioral variability is in part due to intrinsic fluctuations in neuronal activity this assumption may be brought into question.

The idea that ongoing intrinsic activity is responsible for a component of human behavioral variance receives some support from human psychophysics. Variability in human behavior often displays a specific 1/f frequency distribution with greater power at lower frequencies (Gilden, 2001; Gilden et al., 1995; Wagenmakers et al., 2004). Among the types of behavior with this frequency distribution is force output (Gilden, 2001). This observation is interesting given that spontaneous BOLD fluctuations also show a 1/f power spectrum (Figure S4). While the 1/f nature of BOLD fluctuations has been noted previously (Zarahn et al., 1997), we show that the slope is significantly between -0.5 and -1.5 (i.e., 1/f) and that this is significantly different from the frequency distribution of BOLD fluctuations observed in a water phantom.

Although the current study focused on force variability and the somatomotor system, our results could reflect a general property of brain-behavior relationships. Coherent spontaneous activity is not limited to somatomotor cortex, but has been observed in multiple brain systems (Biswal et al., 1995; Fox et al., 2005, 2006a; Greicius et al., 2003; Hampson et al., 2002; Kenet et al., 2003; Lowe et al., 1998). Thus, ongoing intrinsic dynamics could relate to variability in many aspects of perception and behavior (Boly et al., 2007; Gilden, 2001; Gilden et al., 1995; Grill-Spector et al., 2004; Pessoa et al., 2002; Pessoa and Padmala, 2005; Ress et al., 2000; Ress and Heeger, 2003; Sapir et al., 2005; Wagner et al., 1998). The degree to which intrinsic activity plays a role in other types of behavioral variability or is responsible for variability in neuronal activity observed in previous article merits future study.

Spontaneous BOLD Fluctuations and Neuronal Activity

Spontaneous fluctuations in the BOLD signal are increasingly used to study the intrinsic functional architecture of the human and monkey brain (Biswal et al., 1995; Fox

et al., 2005, 2006a; Greicius et al., 2003; Hampson et al., 2002; Lowe et al., 1998; Vincent et al., 2007). However, since the BOLD signal is an indirect measure of neuronal activity, there has been debate as to how these BOLD fluctuations should be interpreted. Nonneuronal factors such as cardiac pulsations, respiratory fluctuations, or subject movement contribute significantly to spontaneous BOLD variance (Birn et al., 2006; Lund et al., 2006; Triantafyllou et al., 2005; Wise et al., 2004), an observation which has lead to the concern that intrinsic BOLD fluctuations are nothing more than nonneuronal noise. The current finding that spontaneous BOLD fluctuations correlate with force output argues strongly that these fluctuations have a neuronal origin.

An important question is how the spontaneous BOLD fluctuations observed in the present study relate to electrophysiological fluctuations. Synchronous fluctuations in electrical activity have been observed in the somatomotor system both in animals and in humans, for examples see Baker et al. (1999), Murthy and Fetz (1996), and Salenius and Hari (2003). These fluctuations have been reported over a range of frequencies, nominally 10–40 Hz, and show a variable degree of task dependence. Coherence has been observed across broad regions of cortex, between cortical hemispheres, and with muscle EMG activity (Baker et al., 1999; Murthy and Fetz, 1996; Salenius and Hari, 2003). Although the precise relationship of these faster electrical oscillations to slow (<0.1 Hz) BOLD fluctuations is unknown, it has been suggested that BOLD fluctuations relate to changes in the power of higher-frequency electrical activity (Bruns et al., 2000; Leopold et al., 2003). Support for this hypothesis comes from correlations between BOLD activity and fluctuations in the band limited power of EEG (Laufs et al., 2003, 2006). Also worth considering may be the coherent low-frequency (<1 Hz) fluctuations in cortical excitability known as "up and down states" seen in various animal preparations (Hahn et al., 2006; Petersen et al., 2003). Future work on the link between BOLD, behavior, and electrophysiology is needed. Specifically, an electrophysiological equivalent of the present results could be investigated by correlating fluctuations in band limited power with force variability.

Conclusions

In the current article, we have shown that intrinsic BOLD fluctuations correlate with variability in human behavior. This finding is important for three reasons. First, it provides strong evidence that spontaneous BOLD fluctuations are more than physiological artifact. Second, it lends new insight into behavioral variability, suggesting that inconsistency in perception or performance should not be automatically attributed to fluctuations in task-related cognitive processes such as attention but could also be due to ongoing fluctuations in intrinsic neuronal activity. Finally, it provides support for the intrinsic perspective on brain function, showing that the brain not only exhibits intrinsic organized fluctuations in neuronal activity, but that these fluctuations impact brain function and behavior in interesting and important ways.

A key objective for future work is to further understand the role of intrinsic activity in the brain and why it may result in variability in behavior. One possibility is that variability in perception and behavior is advantageous (Wiesenfeld and Moss, 1995), either for improved detection of weak signals or to provide a basis for learning and adaptation. Another possibility is that behavioral variability is an undesirable but necessary consequence of some important functionality mediated by intrinsic activity. For example, given its spatial organization spontaneous activity may be important for coordination of neuronal processing (Buzsaki and Draguhn, 2004; Salinas and Sejnowski, 2001; Shatz, 1996). However, this coordination may come at a cost, injecting variability into our perception and behavior. Regardless of what functional role intrinsic activity may serve, our results suggest that it is an essential factor in understanding of the link between brain activity and overt behavior.

EXPERIMENTAL PROCEDURES

Subjects, Task, and Data Acquisition

BOLD sensitized fMRI data (3T, 4 × 4 × 4 mm voxels, TE 25 ms, TR 2.16 s) were acquired in 17 normal right-handed young adults using a 3T Siemens Allegra MR scanner. Subjects were recruited from the Washington University area, consisted of eight males (9 FM), and had an average age of 23.1 years (range 18–27 years).All subjects completed 12 fMRI runs, each 194 frames (7 min) in duration. The first 8 runs for all subjects consisted of two alternating run types, fixation runs and cued button-press runs. The first run type was a resting-state fixation run in which a white crosshair was presented in the center of a black screen. Subjects were instructed to look at a crosshair, remain still, and to not fall asleep. The second run type was a button-press run in which the identical crosshair was presented, but now it occasionally changed from white to dark-gray for a period of 250 ms. Subjects were instructed to press a button with their right index finger as quickly as possible when they saw the crosshair dim. They were told that their reaction times would be recorded. Each of these button press runs contained 20 crosshair dims time-locked to the scanner TR, with an intertrial interval of 8–14 frames (17.3–30.2 s). Subjects practiced this button-press task once in the scanner, prior to the onset of the functional scans. The final four runs were of a third run type, but were not the same for all subjects. The first 11 of the 17 subjects completed four runs in which they were alternately instructed to either to press the button slightly harder or slightly softer than they had during previous runs. The final six subjects completed four runs of a different type that were not used in the present analyses.

Structural data (for definitive atlas transformation) included a high-resolution (1 × 1 × 1.25 mm) sagittal, T1-weighted MP-RAGE (TR = 2.1 s, TE = 3.93 ms, flip angle = 7°) and a T2 weighted fast spin echo scan.

Button-Press Apparatus

The button-press force transducer was similar to that reported by other groups (Ehrsson et al., 2000). It was fabricated in house and designed to operate in the MR environment. Button depression caused graded interruption of an optical beam that was converted to voltage via a photocell. Voltage was recorded using BIOPAC MP150 data acquisition hardware and software at a sampling rate of 200 Hz. The precise relation between applied force (Newtons) and output voltage was calibrated (Figure S5). We fit an equation to this calibration

curve that was a combination of a fourth-order polynomial and an exponential:

$$Y = .0849X^4 - .757X^3 + 2.83X^2 - .774X + \frac{1}{1 - e^{6(X-4.85)}} - 1.23$$

where X is voltage and Y is force in Newtons. The button-press apparatus was mounted to an arch which attached to the bed of the MR scanner and passed over the subject's waist. The apparatus could be moved horizontally, vertically, and angled to optimize comfort for each subject but was then locked into place for the duration of the experiment. Subjects rested their right index finger on the button itself and placed their right thumb along the bottom of the apparatus. They pressed the button using a pinching motion, applying the force between their right index finger and thumb. The displacement of the button was small (<1 mm) and could not be sensed by the subjects. Subjects were told that the button was sensitive and that they need not press it very hard for their response to be recorded. Subjects were familiarized with the apparatus and coached to generate force within a standard range before recording.

Processing of Behavioral Data

For each button press, we determined the peak force and the reaction time. The peak force was simply the peak voltage in a 3 s window following each crosshair dimming converted to Newtons using the calibration curve. The reaction time for each button press was taken as the time between the onset of the crosshair dimming and the first point at which the voltage equaled the voltage baseline + 5% of the peak voltage for that button press. The voltage baseline was the average voltage for the first 200 ms following the onset of the crosshair dimming. Button presses in which the reaction time was greater than three standard deviations above the within-run mean or the force was less than 10% of the within-run mean were counted as miss trials and not included in subsequent analyses.

Processing of Imaging Data

fMRI preprocessing steps included, first, compensation of systematic, slice-dependent time shifts; second, elimination of systematic odd-even slice intensity differences due to interleaved acquisition; and, third, rigid body correction for interframe head motion within and across runs. Step three provided a record of head position within and across all fMRI runs. Each fMRI run was intensity scaled (one multiplicative constant over all voxels and frames) to a yield a whole brain mode value of 1000 (not counting the first four frames; Ojemann et al., 1997). Atlas registration was achieved by computing affine transforms connecting the fMRI run first frame (averaged over all runs after cross-run realignment) with the T2 and average T1-weighted structural images (Ojemann et al., 1997). Our atlas representative template includes MP-RAGE data from 12 normal individuals and was made to conform to the 1988 Talairach atlas (Talairach and Tournoux, 1988). To prepare the BOLD data for the present main analyses, each fMRI run was transformed to atlas space and resampled to 3 mm cubic voxels. This step combined movement correction within and across runs and atlas transformation in one resampling.

At each voxel, linear trends over fMRI runs were removed and the data were spatially smoothed with a 6 mm FWHM Gaussian kernel. Average BOLD intensity for each voxel was normalized to a single value across all runs for each subject. Six movement parameters as well as their temporal derivatives were regressed out of the data on a voxel-wise basis (Fox et al., 2005).

Construction of Subject-Specific Regions of Interest

The first step in our analysis was to identify a LMC region of interest (ROI) in each individual significantly activated by right-hand button presses. We collapsed across all runs and button presses and used conventional linear methods (Friston et al., 1995; Worsley et al., 1996) along with an assumed gamma-type hemodynamic response function (Boynton et al., 1996) with a standard 2.5 s hemodynamic delay to generate t score (converted to equally probable Z score) maps for each individual. These maps were thresholded at Z = 6.0 over contiguous clusters of at least 100 voxels to achieve a multiple comparisons corrected significance level of $p < 0.0001$ (McAvoy et al., 2001). Subject-specific LMC ROIs were created by masking each individual's corrected activation map with a left somatomotor cortex mask. This mask was defined manually using the population averaged activation map as a template.

Next, a RMC ROI was identified for each individual using conventional functional connectivity methodology (Biswal et al., 1995; Fox et al., 2005) applied to the resting-state fixation data. Thus, a map representing the correlation between the time course extracted from the LMC and all other voxels in the brain was created for each individual. The correlation maps were Fisher z transformed, then divided by an approximation of the standard error of z ($1/\sqrt{(n-3)}$ where n is the degrees of freedom in the measurement) to compute a statistical Z score map (Fox et al., 2005). Each individual's Z score map was corrected for multiple comparisons ($p < 0.0001$) at a threshold of 9 and cluster size of 100 (McAvoy et al., 2001), then masked with a RMC mask to create a RMC ROI. The RMC mask was defined manually using the population averaged LMC resting-state correlation map as a template. This identified a ROI in the RMC significantly correlated with the LMC during the resting-state fixation runs.

Regression of Coherent Spontaneous Activity

For each individual, the LMC on RMC regression coefficient, β, was computed using the regional time series derived from the resting fixation runs. This coefficient minimizes the temporal variance of $[f_L(t) - \beta \cdot f_R(t)]$, where $f_L(t)$ and $f_R(t)$ are the regional time series modulations about the mean (computed over the whole run) measured in the somatomotor ROI on the left and right, respectively. For each subject, the β value derived from the fixation runs was applied to the button-press runs to generate the "LMC post-RMC regression" time series $[f_L(t) - \beta \cdot f_R(t)]$.

For each subject, eight frame BOLD response time courses were extracted from $f_L(t)$, $f_R(t)$, and $[f_L(t) - \beta \cdot f_R(t)]$. Time courses were converted to % change from a within-run average baseline. This baseline was computed as the average of the first and last data point of all extracted time courses for a given run from a particular region. To quantify the effect of regressing out the RMC from the LMC responses, we computed signal, noise, and the signal-to-noise ratio for the LMC, RMC, and LMC post-RMC regression as in our previous report (Fox et al., 2006b). Signal power was computed as the mean squared deviation from the baseline of the average response across all button presses. Noise power was computed as the mean squared deviation of the residual. Signal power, noise power, and the signal to noise ratio (S/N) were then compared for the LMC before versus after RMC regression. The significance of changes in these measures was assessed across the population using a Wilcoxon paired nonparametric test.

Assessing the BOLD-Behavior Relationship

For each run of each subject, trials were sorted into three equal-sized bins based on the force of the button press (hard, medium, and soft). The present analyses were based on comparing hard to soft trials. The average BOLD time course for hard trials and soft trials was extracted for each subject from the left SMC, right SMC, and left SMC post-right SMC regression.

To determine if a significant BOLD-behavior relationship was present in the left SMC, hard and soft BOLD time courses were compared using repeated-measures ANOVA. To determine which portion of the left SMC time course contributed to the observed difference, paired t tests on individual time points were computed and the significance assessed after correcting for eight comparisons using Bonferroni's procedure. A single, quantitative measure of the BOLD-behavior relationship was developed by averaging the significant time points (2 and 3) and comparing hard to soft button presses. The significance of this difference was assessed using a paired two-tailed t test.

The significance of this BOLD-behavior metric was also computed for the right SMC and the left SMC postregression. A repeated-measures ANOVA was computed on the left SMC postregression to determine if any significant difference remained between hard and soft time courses. To quantify the impact of right SMC regression on the BOLD-behavior relationship in the left SMC, our quantitative measure of the left SMC BOLD-behavior relationship was applied both pre- and post-regression and the results compared using a Wilcoxon signed-rank test.

Comparing Voxelwise Distributions

To determine which voxels in the brain showed a BOLD-behavior relationship similar to that identified in the left SMC, the average of time points 2 and 3 for hard and soft button presses was subtracted for each subject and each voxel. A population-level random-effects map was computed using a paired two-tailed t test. Resulting t values were converted to Z scores which were corrected for multiple comparisons ($p < 0.05$) at a threshold of $Z = 1.75$ and a cluster size of 150.

To determine which voxels were significantly correlated with the left SMC during resting fixation, a population-level random-effects map was also computed using the single-subject left SMC resting-state z-transformed correlation maps. Resulting t values were converted to Z scores and corrected for multiple comparisons ($p < 0.0001$) at a threshold of $Z = 5.5$ and a cluster size of 100. The false discovery rate for all thresholds and cluster sizes were established through Monte-Carlo simulation (McAvoy et al., 2001).

The spatial distribution of the BOLD-behavior effect and resting state correlation map were qualitatively compared by creating an overlap image of the two population-level random effects maps. The spatial distribution of the two maps was quantitatively compared by computing the spatial correlation between the two maps on a single-subject basis (Fox et al., 2006a). This spatial correlation was assessed both across all voxels within a whole brain mask and all voxels within a gray matter mask. Masks were created using intensity thresholding of a population averaged MP-RAGE anatomical atlas. Resulting correlation coefficients were Fisher z transformed and significance was assessed across the population using a two-tailed t test.

Specifically Instructed Hard versus Soft Button Presses

Eleven of seventeen subjects completed four additional runs in which they were specifically instructed to press the button either slightly harder or softer than they had during previous runs. BOLD data were preprocessed and analyzed the same way as previously described for the other button-press runs. Average eight-frame BOLD time courses following button-press responses were computed for hard runs and soft runs for each individual for the left SMC, right SMC, and left SMC postregression. These time courses from different runs were normalized such that the mean of the first and last time points would be zero both for the hard and soft responses. Note that unlike the analysis of hard versus soft trials within a run, this inter-run normalization prohibits identification of a constant offset between hard and soft responses. Since a constant offset is present in the intertrial hard versus soft responses in the left SMC, it is difficult to compare the result of the inter-run analysis. However, no such offset is present in the intertrial hard versus soft responses in the right SMC, allowing the relative magnitude of the hard and soft time courses in the intertrial and inter-run time courses to be accurately assessed. To ensure that differences between the intertrial and inter-run analyses were not due to the subset of 11 subjects, the analysis of spontaneous (intertrial) force variability was repeated using only the 11 subjects that completed both experiments.

Analysis of Interstimulus Interval

The ISI was varied between 17.3 and 30.2 s at intervals of 2.16 s (1 frame). Since there were relatively few trials at the shortest and longest ISI, we sorted trials into three bins: short ISI (17.3-19.4 s), medium ISI (21.6 s), and long ISI (23.8–30.2 s). The interaction of ISI with reaction time, force, and BOLD data (average of time points 2 and 3) was assessed using repeated-measures ANOVA.

Resting state data from this study are available at www.brainscape.org.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/56/1/171/DC1/.

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