Author's Final Version: Article Published in *Cortex,* 2017.

The mu-rhythm can mirror: Insights from experimental design, and looking past the controversy

Lindsay C. Bowman

University of California, Davis

Marian J. Bakermans-Kranenburg

Leiden University

Kathryn H. Yoo

University of California, Davis

Erin N. Cannon

University of Maryland, College Park

Ross E. Vanderwert

Cardiff University

Pier Ferrari

University of Parma

Marinus H. van IJzendoorn

Leiden University

Nathan A. Fox

University of Maryland, College Park

1. Overview

Hobson & Bishop (2016) present a compelling investigation of whether mu suppression is a useful index of a human mirror neuron system, and parallel the focus of our own recent meta-analysis "Assessing Human Mirror Activity with EEG Mu Rhythm: A Meta-Analysis" (Fox et al., 2015). In their pre-registered study, Hobson and Bishop collected the largest sample yet studied in this area of research and include thoughtful controls and analyses, constituting an admirable and important contribution to the field. Based on this important investigation, Hobson and Bishop conclude that "mu suppression can be used to index the human mirror neuron system, but the effect is weak and unreliable and easily confounded with alpha". In their abstract, they state that their conclusions are in contrast to those of our own meta-analysis; however, we disagree with this statement and show here that their findings converge with our meta-analytic results.

Indeed, contrary to the authors' impressions, we believe our findings and arguments complement those of Hobson & Bishop (2016). Both papers report mu suppression during action-execution and -observation conditions, and conclude these results are not inconsistent with 'neural mirroring'. Further, both papers recommend methodological improvements to more reliably isolate mu suppression that is functionally linked to action. Hobson and Bishop echo our call for caution in interpreting claims about 'mirror system activity' from experiments that (a) do not establish a basic mirroring property by including both action-execution and -observation conditions, (b) do not report EEG activity from multiple electrode sites, and (c) do not evaluate potential domain-general visual and attentional confounds. In spite of these shared concerns, we remain optimistic regarding the utility of mu suppression as an index of mirroring. It is this optimistic view that appears to contrast with Hobson and Bishop. In this response, we defend our original position that, under careful and controlled experimental conditions, mu rhythm is supressed during both action-execution and -observation, making it a useful index for neural mirroring.

2. Isolating action-specific processes in the mu rhythm: The limits of scalp topography and simple comparisons to controls

To exhibit mirroring properties, the mu signal associated with action observation must be demonstrably similar to the signal associated with the actual execution of action. The point of contention between our meta-analysis and the report by Hobson and Bishop (2016) appears to be over the extent to which mu suppression during action observation is a reliable and valid index of neural activity specific to motor/action processes, beyond any domain-general visual attentional processes that may be associated with observing actions more generally.

To accept a reliable separation between action-specific processes and domain-general attentional processes, Hobson and Bishop (2016) set the premise that (a) mu suppression must demonstrate strongest activation at central electrode sites (which overlay the sensorimotor cortex) versus occipital sites (which should predominantly capture domain general processes reflected in the occipital alpha rhythm), and (b) that central suppression must be stronger for action conditions compared to a non-action control condition. While on the surface this premise seems reasonable, a heavy reliance on scalp topography to reveal functional specificity seems inappropriate given EEG's extremely poor spatial resolution. Measurement from a given electrode reflects activity from several populations of underlying neurons whose signals are blurred through volume conduction to the scalp surface (Holsheimer & Feenstra, 1977). Though Hobson and Bishop's use of current source density (CSD) reduces the issue of volume conduction, it by no means eliminates it, and as Tenke and Kayser (2005, pg. 2840) note, "despite the empirical and theoretical elegance of CSD methodology, [it] is restricted to the spatial domain in which the EEG is recorded: the scalp." Thus, though mu suppression may not be specific to or even greatest at central electrode sites, such topographical patterns do not preclude the possibility that activity measured at a scalp electrode could, in part, originate from sensorimotor cortex.

To be clear, an examination of EEG across multiple scalp locations is critical for mu rhythm investigations, as we argue in our original meta-analysis (Fox et al., 2015) and as Hobson & Bishop (2016) also suggest. A comparison of EEG activity across central and occipital electrode sites can shed light on the strength of mu rhythm activity associated

with action-specific processes relative to a confounding occipital alpha rhythm associated with domain-general, attentional processes. Mu suppression in Hobson and Bishop's action-execution condition is clearly greater in central versus occipital electrode sites—a finding that replicates our meta-analysis. Our argument here is that, given the low spatial resolution of EEG, a lack of topographic specificity is not, in and of itself, sufficient to rule out the possibility that EEG scalp signals during action observation reflect, in part, action-specific processes. For example, in some experimental situations, domain-general attentional processes captured in occipital alpha may dwarf mu suppression that is specific to motor/action processes; yet the motor/action processes captured in mu suppression may nonetheless be real and measurable. Indeed, in such situations, for an action-observation condition, we would expect to see some suppression in both central and occipital sites (reflecting both action-specific and domain-general attentional processes). However, the occipital suppression for an action-observation condition should likely still be less than the occipital suppression for a control condition designed to minimally reflect motor/action processes and maximally reflect domain-general visual attention (e.g., Hobson and Bishop's kaleidoscope condition). Hobson and Bishop's findings demonstrate this pattern: across all analyses, suppression in occipital electrodes was smaller in both of the action-observation conditions when compared to the occipital suppression in the non-action kaleidoscope control.

If the action-specific portion of mu suppression in central electrodes is small during action observation and potentially dwarfed by a larger domain-general attentional signal, the key question is how best to isolate those action-specific processes of the mu rhythm. This question was a central focus of Hobson & Bishop's (2016) study as well. Some researchers have used repetition suppression designs to reveal specificity of mu rhythm function (e.g., Coll et al., 2015). As an alternative approach, Hobson and Bishop compared EEG activity during action-observation and -execution conditions to activity in a control condition. We argue that, beyond simple comparison to a control condition designed to capture domain-general attentional processes, data from such a condition could be subtracted from data in the condition of interest in order to help isolate the portion of the neural signal most specific to motor/action processes. Subtraction

procedures across two or more conditions are routinely employed in fMRI analyses (Faro & Mohamed, 2010). Although there are certainly limitations to this approach, it may be useful in disentangling the action-specific aspects of the mu rhythm from the domain-general attentional processes that are also captured in the EEG.

Though Hobson and Bishop (2016) did not subtract the control condition activity from the action-observation condition activity, aspects of their third analysis using static baseline images provide support for the subtraction approach in isolating mu rhythm's action-specific processes. Recall that the calculation of mu suppression is already a subtraction procedure wherein the EEG signal in a 'baseline' period is subtracted from a condition of interest. Hobson and Bishop's first two analyses used baselines that did not closely match the visual scene or attentional demand of the action-observation condition of interest. However, their third analysis used static images of the scene that, when in motion, constituted the action-observation condition of interest. These static image baselines therefore captured the most similar visual and attentional qualities of the event of interest, and thus they were likely able to best isolate the portion of the neural signal most specific to motor/action processes, revealing mu rhythm's mirroring properties. Indeed, with this third, more fine-grained analysis, Hobson and Bishop (2016; pg. 302) agree that mu suppression "did give a pattern of results that was consistent with mirror neuron activity".

Note that subtraction conditions need not be specific to dynamic stimuli. For example, mu suppression could be calculated when viewing static images of a hand performing an action (e.g., a hand grasping a silver handle), as well as when viewing a control condition of static images matched for domain-general visual/attentional content but critically devoid of implied human action (e.g., a silver circle next to a skin colored triangle). Mu-suppression in the control condition could then be subtracted from that in the hand condition to reveal the neural processes specific to the implied object-directed action, beyond the domain-general attentional processes present in both conditions. Designing a control condition that is equal in attentional demands to the experimental condition but devoid of action execution or observation can be difficult, as Hobson & Bishop (2016)

note. Researchers may turn to fMRI studies in which this subtraction procedure has been commonly employed for inspiration and approaches to match attentional demands across conditions.

The notion that a phenomenon is best revealed under a more specific set of parameters and with specific methodological approaches does not necessarily reduce its significance. Researchers must determine what those specific parameters and optimal approaches are. It is clear that to reveal action-specific processes reflected in the mu rhythm and evaluate its mirroring properties, domain-general attentional processes must be controlled. Hobson and Bishop's (2016) findings support this point: in their first two analyses, the action-specific processes associated with observing action were arguably less well isolated, and in these first two analyses central mu suppression in the action-observation conditions was weak and potentially overshadowed by the domain-general attentional signal reflected in occipital alpha. Thus their findings raise a note of caution for interpreting mu rhythm studies that lack necessary domain-general controls.

However, when optimal parameters and approaches are employed, mu rhythm may be a reliable index of neural mirroring. Hobson and Bishop's (2016) findings provide support for this notion as well, and offer an important extension to the results of our meta-analysis. Mu suppression during both action execution and observation was detected in our meta analysis (Fox et al., 2015) across studies with disparate methods and in some cases without optimal controls, demonstrating a degree of robustness to mu rhythm's "mirroring" property. However, we also found that while mu suppression during action execution showed topographic specificity, mu suppression during action observation did not. Hobson and Bishop's large-scale empirical investigation replicated each of these patterns of results, and critically further demonstrated that when attentional and other domain-general processes were better controlled (as in the case of their third analysis with static, within-trial, baseline images), mu suppression during action observation did show topographic specificity, similar to mu suppression during action execution.

Thus, Hobson and Bishop's (2016) approach underscores the importance of rigorous methodological controls to elicit mu rhythm activity reflective of the action-specific processes of interest. Taken together, our meta-analytic findings and the empirical findings from Hobson and Bishop strengthen and complement each other, evincing a detectable mirroring property in the mu rhythm, and laying a foundation for future research to use mu rhythm as a reliable and valid index of neural activity associated with action-specific processes.

Lastly, to address Hobson and Bishop's concern that even under their most optimal condition parameters 16-21% of individual participants did not exhibit significant mu suppression at central electrode sites, we note that it is not uncommon for a particular neural signature to fall short of full statistically significant expression in some individuals in a given study. Drawing from the fMRI literature, when region of interest (ROI) analyses are conducted, not all participants meet criteria for ROI selection. For example, a meta-analysis of fMRI data found that the medial prefrontal cortex (MPFC) and temporoparietal junction (TPJ) are the two regions most consistently involved in mental-state reasoning across studies (Schurz et al., 2014); however, considering one individual study (e.g., Saxe et al., 2009), 15-30% of participants did not exhibit significantly different activation in mental-state-reasoning versus a non-mental control conditions in either the MPFC or TPJ. The lack of significant activation in the mental versus control condition in these regions for some individuals does not discount the regions' involvement in mental-state reasoning more generally, as evinced by the meta-analytic data. This type of individual variation in strength of neural response is not specific to the one example we present; it occurs across fMRI, EEG, and ERP studies alike (e.g., Taylor-Colls & Pasco Fearon, 2015).

Extending this argument to mu rhythm research, even though not every individual may meet criteria for significant mu suppression during action observation and execution, mu suppression may still be considered a reliable and valid index of neural mirroring as a whole. Moreover, such individual differences in presence and strength of mu suppression could be a valuable source of meaningful variation across participants. Recent

developmental literature has capitalized on the relation between individual differences in mu suppression and additional behavioral/cognitive characteristics related to observing and executing actions. For example, individual differences in 9- and 12-month-olds' central mu suppression during action observation was positively related to individual differences in the infants' ability to competently reach for and grasp objects (Cannon et al., 2016). This correlation was specific to mu suppression at central sites, and did not exist for mu suppression at frontal, parietal, or occipital sites (satisfying Hobson & Bishop's, 2016 criteria for topographic specificity). For 3- to 5-year-old children, stronger mu suppression during action execution in central-parietal (but not frontal or occipital) electrode sites was positively associated with advances in behavioral measures of children's motor skill and action-representation (Bowman et al., 2016). Moreover, central mu suppression was not related to domain-general behavioral measures of executive functioning or language skills.

The developmental literature is newly emerging, and the same cautions for interpreting existing mu rhythm research in adults should also be applied to developmental research when methodological controls are absent. Indeed, the issue of confounding domain-general attention could perhaps be particularly pertinent in developmental studies in which young participants may be especially attentive to actions that they are just learning. Nonetheless, when appropriate procedures and corrections are applied, developmental data illustrate the potential importance of individual differences in mu-suppression during action observation and execution, and underscore the utility of using behavioral measures of motor/action performance, as well as behavioral domain general control measures, to help isolate action-specific neural processes reflected in the mu rhythm.

3. On Existing and Future Studies: Cautions and Approaches to Move Ahead

Our meta-analysis (Fox et al., 2015) and the Hobson and Bishop (2016) empirical investigation have each called for implementation of more rigorous methodological approaches to isolate action-specific processes of the mu rhythm, as well as for caution in interpreting existing mu rhythm studies should such controls be absent. We reiterate each

of these points in the present paper. Caution is particularly warranted in studies that attempt to investigate neural mirroring in other complex cognitive processes or conditions (e.g., language, theory of mind, autism) if it is not first demonstrated that the mu rhythm signal is sufficiently similar across both action-execution and -observation conditions to constitute 'mirroring', and further that the signal being modulated in these more complex processes is indeed specific to action-processes.

We have touched on several possible approaches to help isolate action-specific processes of the mu rhythm in section 2 above and refer readers to Hobson and Bishop (2016) and Fox et al. (2015) for a more in depth discussion of these approaches. Briefly, in future investigations of mu rhythm and neural mirroring, researchers should: 1) examine and report mu rhythm activity from multiple electrodes across the scalp, 2) be mindful of baseline effects on calculation of mu suppression and ensure baselines are at very least the same for all conditions, and 3) include controls for confounding domain-general attentional processes to isolate the action-specific processes mirrored in mu suppression. These action-specific processes could be isolated with repetition suppression designs, subtraction of non-action control conditions from action conditions of interest, associations with behavioral measures of action, and statistical control of behavioral measures of domain-general skills. Lastly, for studies in which interpretation is contingent on mu rhythm having accurately indexed a neural mirroring network, both action-observation and -execution conditions should be included in the same experiment and examined across all participants to demonstrate specific mirroring properties of the mu signal that are then further modulated in other constructs of interest.

4. On Monkeys and Humans: Looking Past the Controversy

A final discussion that is separate but related to the current topic centers on the extent to which patterns in the mu rhythm are comparable to those observed from single cell recordings in the rhesus macaque (di Pellegrino, et al., 1992). The discovery of these classic 'mirror neurons' fueled rising interest in the potential 'mirroring properties' found with the mu rhythm. There are many researchers who use mu rhythm to investigate a

'human mirror neuron system', or who assume mu rhythm activity reflects such a system and wish to examine its role in other constructs. We have noted issues associated with these approaches in section 3 above. More generally, the function and nature of a mirror neuron system in humans and monkeys is controversial (e.g., Heyes, 2010, Hickok, 2014; Glenberg, 2015). How we conceptualize 'mirroring' in the human brain may be necessarily more complex, and involve multiple neural systems that are separate but functionally related. As we (Bowman et al., 2016) and others (e.g., Pineda, 2005) have argued, the fluctuations in mu rhythm measured at the scalp surface may reflect activity from multiple functionally related neural systems networked together. While activation of this type of integrated network could still exhibit mirroring (e.g., if activated during observation and execution of action), this more complex conceptualization of mu rhythm may be disparate from the classic mirror neuron system identified with single cell recordings in monkeys. On the other hand, such an integrated neural network in the human brain could be consistent with new views on an extended mirror neuron network in monkeys, in which recent neuroanatomical and neurophysiological research implicates a broad system of cortical regions with reciprocal connections that extend beyond classic mirror neurons in inferior parietal lobule and premotor cortex (Bonini, 2016).

Regardless of where one might fall on the position of a mirror neuron system in humans or monkeys, we urge researchers not to let such controversy overshadow investigations of mu rhythm as an index of sensorimotor function, and of the potential importance and utility of mu rhythm's 'mirroring properties'. We encourage the field to continue the use of mu-rhythm, but to do so in the context of acknowledging and understanding the boundaries of both studying and interpreting mu rhythm. We believe that mu rhythm may indeed be a useful tool to shed light on how humans develop complex actions, and how humans make sense of the complex actions and interactions of others. We argue that with careful and controlled designs–like those demonstrated in Hobson & Bishop's (2016) third analysis–mu rhythm can illuminate mechanisms that support these important functions, and that mu rhythm thus has important implications for research in basic neuroscience, cognition, and development.

Author's Final Version: Article Published in *Cortex,* 2017.

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