PNAS
www.pnas.org

Supplementary Information for

Methylphenidate as a causal test of translational and basic neural coding hypotheses

Amy M. Ni*, Brittany S. Bowes, Douglas A. Ruff, Marlene R. Cohen

*Corresponding author
Email: amn75@pitt.edu

This PDF file includes:

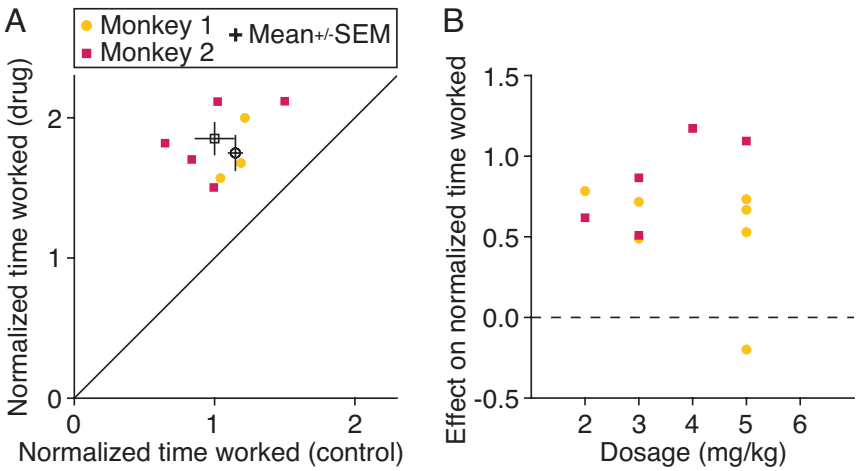

Fig. S1. The effect of methylphenidate on working time does not depend on water consumption or methylphenidate dosage. (*A*) The plot depicts the amount of time the monkey engaged in the change-detection task, normalized to the mean time worked on placebo control days. Each point is the normalized working time for a matched drug day (*y*-axis) and control day (*x*-axis) for each monkey (marker symbols). The open symbols are the mean for each monkey, and error bars represent standard error of the mean (SEM). We subsampled our data so that the mean liquid consumption was indistinguishable before drug and control days for each monkey. In this subset of data, the significant methylphenidate-related increase in working time persists (paired *t*-tests; Monkey 1: n = 3 pairs of days, $t(2) = -6.5$, $p = 0.023$; for Monkey 2 mean liquid consumption was already indistinguishable before drug and control days and thus the data match the data in the main text: n = 5 pairs of days, $t(4) = -6.6$, $p = 2.7 \times 10^{-3}$). (*B*) The effect of methylphenidate on the time the monkey engaged in the change-detection task (*y*-axis; normalized time engaged on the drug day – normalized time engaged on the matched control day) is not consistently related to methylphenidate dosage (*x*-axis; Kendall's rank correlation coefficient; Monkey 1: n = 7 pairs of days, $\tau = -0.17$, $p = 0.49$; Monkey 2: n = 5 pairs of days, $\tau = 0.60$, $p = 0.031$; though see [1]).

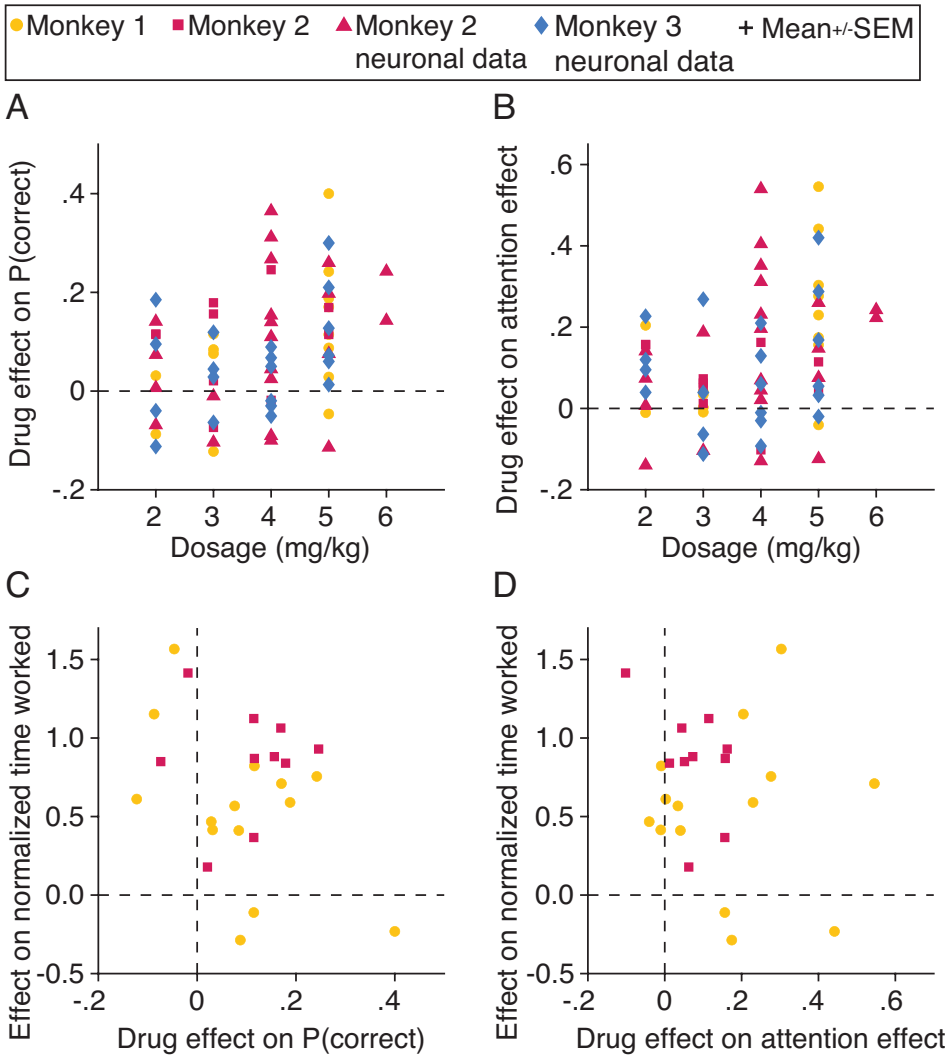

Fig. S2. The effect of methylphenidate on performance does not depend on dosage or on the effect of methylphenidate on working time. (*A*) The effect of methylphenidate on performance at the attended location (*y*-axis; attended hit rate on the drug day – attended hit rate on the paired control day) is not significantly related to methylphenidate dosage (*x*-axis) for each data set (marker symbols; Kendall's rank correlation coefficient; Monkey 1: $n = 14$ [7 pairs of days x 2 stimulus locations per pair], $\tau = 0.45$, $p = 0.054$; Monkey 2: $n = 10$, $\tau = 0.15$, $p = 0.64$; Monkey 2 neuronal dataset: $n = 22$, $\tau = 0.24$, $p = 0.16$; Monkey 3 neuronal dataset: $n = 20$, $\tau = 0.27$, $p = 0.13$). (*B*) The effect of methylphenidate on selective attention (*y*-axis; the difference in hit rate between the attended and unattended locations on the drug day – the difference in hit rate between the attended and unattended locations on the paired control day) is not significantly related to methylphenidate dosage (*x*-axis; Kendall's rank correlation coefficient; Monkey 1: $\tau = 0.45$, $p = 0.054$; Monkey 2: $\tau = -0.25$, $p = 0.40$; Monkey 2 neuronal dataset: $\tau = 0.25$, $p = 0.14$; Monkey 3 neuronal dataset: $\tau = 0.072$, $p = 0.71$). (*C*) There is no detectable relationship between the effect of methylphenidate on performance at the attended location (*x*-axis; attended hit rate at one stimulus location on the drug day – attended hit rate at the same stimulus location on the paired control day) and the effect of methylphenidate on the time the monkey engaged in the

change-detection task (*y*-axis; normalized time engaged at one stimulus location on the drug day – normalized time engaged at the same stimulus location on the matched control day) for each monkey (correlation coefficient; Monkey 1: $R = -0.50$, $p = 0.069$; Monkey 2: $R = 0.035$, $p = 0.92$). Time worked is normalized to the mean time worked on the placebo controls of the pairs. (*D*) There is no detectable relationship between the effect of methylphenidate on selective attention (*x*-axis; the difference in hit rate between attending and not attending one stimulus location on the drug day – the difference in hit rate between attending and not attending the same stimulus location on the paired control day) and the effect of methylphenidate on the time the monkey engaged in the change-detection task (*y*-axis; normalized time engaged at one stimulus location on the drug day – normalized time engaged at the same stimulus location on the matched control day) for each monkey (correlation coefficient; Monkey 1: $R = 0.027$, $p = 0.93$; Monkey 2: $R = -0.45$, $p = 0.19$). It should be noted that it was not our goal to test for dose-dependent effects, and that prior studies have found that the same stimulant can have different effects on different cognitive processes depending on the dosage administered (1-5).

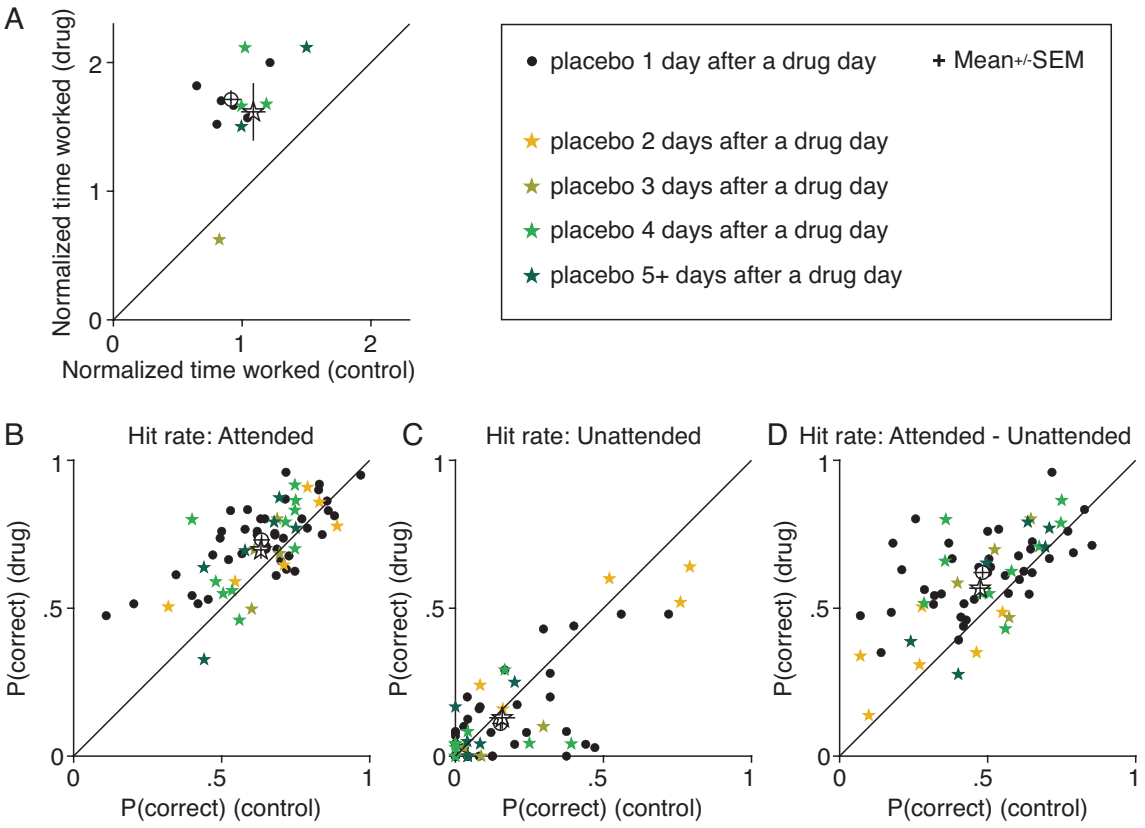

Fig. S3. The number of days after a drug day that a placebo day took place did not affect either motivation or performance. (*A*) The normalized time worked (as illustrated in Fig. 2) is plotted for pairs of days with placebo days that took place one day after a drug day (black circle markers; mean illustrated with an open circle) and for pairs of days with placebo days that took place two or more days after a drug day (filled star markers, color coded yellow to green for 2 to 5+ days after a drug day, as per legend; mean across all pairs with the placebo day 2+ days after a drug day illustrated with an open star). Error bars represent standard error of the mean (SEM). The data from both data sets illustrated in Fig. 2 are combined here. The normalized time worked did not differ between placebo days (illustrated along the *x*-axis) that took place one day after a drug day versus two or more days after a drug day (*t*-test; $t(10) = -1.4$, $p = 0.19$). The time worked was significantly longer on drug than control days both when the placebo day took place one day after a drug day (paired *t*-test; $n = 6$ pairs of days, $t(5) = -9.2$, $p = 2.6 \times 10^{-4}$) and when the placebo day took place two or more days after a drug day (paired *t*-test; $n = 6$ pairs of days, $t(5) = -3.1$, $p = 0.027$). (*B-D*) Conventions as in (*A*). (*B*) The hit rate at the attended location (as illustrated in Fig. 3*A*) did not differ between placebo days that took place one versus 2+ days post-drug (*t*-test; $t(64) = 0.051$, $p = 0.96$). Methylphenidate significantly improved performance both when the placebo day took place one day post-drug (paired *t*-test; $n = 40$ pairs of days, $t(39) = -4.9$, $p = 1.9 \times 10^{-5}$) and when the placebo day took place 2+ days post-drug (paired *t*-test; $n = 26$ pairs of days, $t(25) = -2.8$, $p = 9.5 \times 10^{-3}$). (*C*) The hit rate at the unattended location (as illustrated in Fig. 3*B*) did not differ between placebo days that took place one versus 2+ days post-drug (*t*-test; $t(64) = -0.12$, $p = 0.91$). Methylphenidate did not significantly change performance either when the placebo day took place one (paired *t*-test; $n = 40$ pairs of days, $t(39) = 1.8$, $p = 0.072$) or 2+ days post-drug (paired *t*-test; $n = 26$ pairs of days, $t(25) = 1.2$, $p = 0.24$). (*D*) The attention-related difference in hit rate (as illustrated in Fig. 3*C*) did not differ between placebo days that took place one versus 2+ days post-drug (*t*-test; $t(64) = 0.17$, $p = 0.87$). Methylphenidate significantly increased the selective effect of attention both when the placebo day took place one day post-

drug (paired *t*-test; $n = 40$ pairs of days, $t(39) = -5.2$, $p = 6.1 \times 10^{-6}$) and when it took place 2+ days post-drug (paired *t*-test; $n = 26$ pairs of days, $t(25) = -3.4$, $p = 2.4 \times 10^{-3}$).

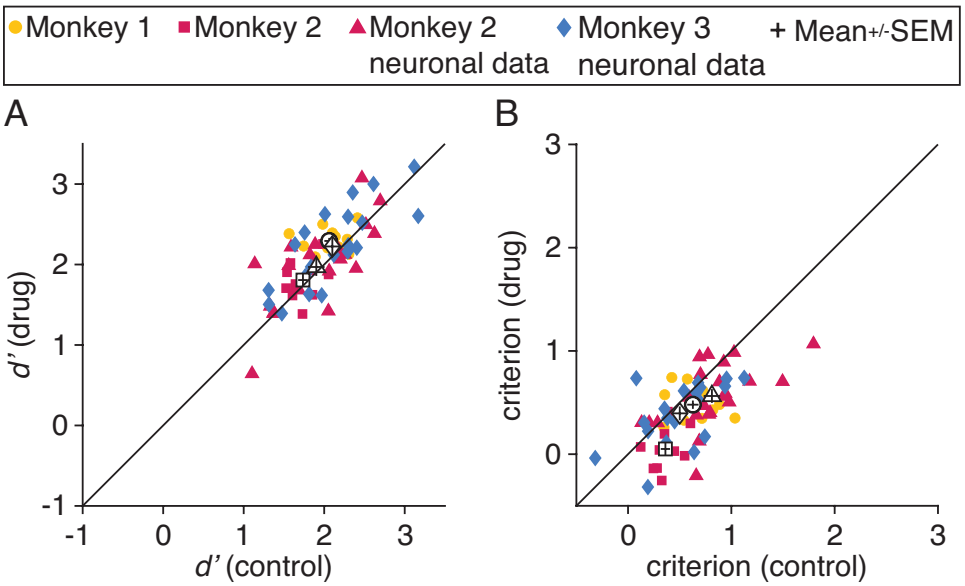

Fig. S4. Methylphenidate increases hit rate at the attended location by both increasing visual sensitivity and decreasing criterion. (*A*) Methylphenidate improved sensitivity (*d'*) at the attended location on drug days (*y*-axis) compared to paired control days (*x*-axis) across the entire data set (paired *t*-test: $t(65) = -3.0$, $p = 3.4 \times 10^{-3}$), though not significantly for all individual data sets (paired *t*-tests; Monkey 1: $n = 14$ [7 pairs of days x 2 stimulus locations per pair], $t(13) = -3.4$, $p = 4.7 \times 10^{-3}$; Monkey 2: $n = 10$, $t(9) = -0.87$, $p = 0.41$; Monkey 2 neuronal dataset: $n = 22$, $t(21) = -0.87$, $p = 0.40$; Monkey 3 neuronal dataset: $n = 20$, $t(19) = -1.6$, $p = 0.12$). The open symbols and error bars depict the mean and standard error of the mean for each data set (marker symbols). (*B*) Methylphenidate decreased criterion at the attended location on drug days compared to paired control days across the entire data set (paired *t*-test: $t(65) = 5.3$, $p = 1.3 \times 10^{-6}$) though not significantly for all individual data sets (paired *t*-tests; Monkey 1: $t(13) = 2.1$, $p = 0.059$; Monkey 2: $t(9) = 4.8$, $p = 9.2 \times 10^{-4}$; Monkey 2 neuronal dataset: $t(21) = 3.6$, $p = 1.8 \times 10^{-3}$; Monkey 3 neuronal dataset: $t(19) = 1.6$, $p = 0.13$). Conventions as in (*A*). It is not surprising that methylphenidate affects both sensitivity and criterion because these measures have been demonstrated to be strongly yoked (6, 7). Attentional measures that improve performance generally affect both sensitivity and criterion (8).

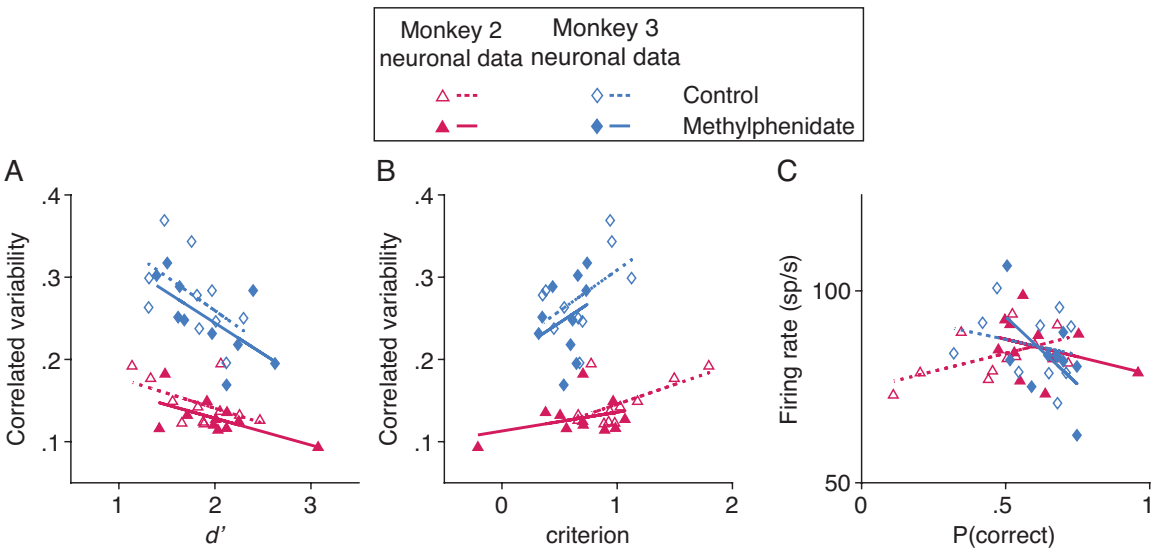

Fig. S5. Methylphenidate both improves visual sensitivity and decreases criterion when it changes correlated variability in V4, but there is no relationship between performance and mean firing rate in V4. (*A*) There was a single relationship between visual sensitivity at the attended location *(d'*; *x*-axis) and attended mean correlated variability (*y*-axis) for Monkey 2 (correlation coefficient; R = -0.59, p = 3.8 x 10^{-3}; correlation was indistinguishable between control and drug conditions, depicted with open and filled symbols, respectively; control: n = 11 days, R = -0.51, p = 0.11; drug: n = 11 days, R = -0.63, p = 0.038; Fisher z PF test of the difference between dependent but non-overlapping correlation coefficients: zpf = 0.40, p = 0.69) and Monkey 3 (correlation coefficient; R = -0.61, p = 4.4 x 10^{-3}; control: n = 10 days, R = -0.54, p = 0.11; drug: n = 10 days, R = -0.65, p = 0.043; Fisher z PF test: zpf = 0.40, p = 0.69). Best fit lines depicted for control (dashed lines) and methylphenidate data (solid lines). (*B*) There was a single relationship between criterion at the attended location *(x*-axis) and attended mean correlated variability (*y*-axis) for Monkey 2 (correlation coefficient; R = 0.57, p = 5.4 x 10^{-3}; control: R = 0.60, p = 0.051; drug: R = 0.36, p = 0.28; Fisher z PF test: zpf = 0.72, p = 0.47) and Monkey 3 (correlation coefficient; R = 0.46, p = 0.041; control: R = 0.51, p = 0.13; drug: R = 0.20, p = 0.42; Fisher z PF test: zpf = 0.89, p = 0.37). Conventions as in (*A*). In analyzing the relationships between correlated variability, sensitivity, and criterion, we did not find that criterion could explain the relationship between correlated variability and sensitivity (partial correlation coefficient controlling for criterion; Monkey 2: R = -0.31, p = 0.17; Monkey 3: R = -0.60, p = 6.9 x 10^{-3}), nor did we find that sensitivity could explain the relationship between correlated variability and criterion (partial correlation coefficient controlling for sensitivity; Monkey 2: R = 0.26, p = 0.26; Monkey 3: R = 0.44, p = 0.058). Sensitivity and criterion are tightly linked in the context of this behavioral task (9); however, experimental paradigms designed to separate the effects of sensitivity and criterion may be able to distinguish potentially distinct roles for these two factors in the effects of methylphenidate. (*C*) Unlike with correlated variability, there was no detectable relationship between performance at the attended location (hit rate; *x*-axis) and attended mean firing rate (*y*-axis) for Monkey 2 (correlation coefficient; R = 0.18, p = 0.42; control: R = 0.54, p = 0.084; drug: R = -0.34, p = 0.30) or for Monkey 3 (correlation coefficient; R = -0.39, p = 0.093; control: R = -0.24, p = 0.51; drug: R = -0.56, p = 0.093). Conventions as in (*A*).

SI References

1. A. Z. Rajala, J. B. Henriques, L. C. Populin, Dissociative effects of methylphenidate in nonhuman primates: trade-offs between cognitive and behavioral performance. *J. Cogn. Neurosci.* **24**, 1371-1381 (2012).
2. R. H. Pietrzak, C. M. Mollica, P. Maruff, P. J. Snyder, Cognitive effects of immediate-release methylphenidate in children with attention-deficit/hyperactivity disorder. *Neurosci. Biobehav. Rev.* **30**, 1225-1245 (2006).
3. A. Z. Rajala, L. C. Populin, R. L. Jenison, Methylphenidate affects task-switching and neural signaling in non-human primates. *Psychopharmacology (Berl)* **237**, 1533-1543 (2020).
4. J. Swanson, R. D. Baler, N. D. Volkow, Understanding the effects of stimulant medications on cognition in individuals with attention-deficit hyperactivity disorder: a decade of progress. *Neuropsychopharmacology* **36**, 207-226 (2011).
5. J. R. Wickens, B. I. Hyland, G. Tripp, Animal models to guide clinical drug development in ADHD: lost in translation? *Br. J. Pharmacol.* **164**, 1107-1128 (2011).
6. T. Z. Luo, J. H. R. Maunsell, Attentional Changes in Either Criterion or Sensitivity Are Associated with Robust Modulations in Lateral Prefrontal Cortex. *Neuron* **97**, 1382-1393 e1387 (2018).
7. D. Sridharan, N. A. Steinmetz, T. Moore, E. I. Knudsen, Does the Superior Colliculus Control Perceptual Sensitivity or Choice Bias during Attention? Evidence from a Multialternative Decision Framework. *J. Neurosci.* **37**, 480-511 (2017).
8. T. Z. Luo, J. H. R. Maunsell, Neuronal Modulations in Visual Cortex Are Associated with Only One of Multiple Components of Attention. *Neuron* **86**, 1182-1188 (2015).
9. A. M. Ni, D. A. Ruff, J. J. Alberts, J. Symmonds, M. R. Cohen, Learning and attention reveal a general relationship between population activity and behavior. *Science* **359**, 463-465 (2018).