Amphetamine modulates brain signal variability and working memory in younger and older adults

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Better-performing younger adults typically express greater brain signal variability relative to older, poorer performers. Mechanisms for age and performance-graded differences in brain dynamics have, however, not yet been uncovered. Given the age-related decline of the dopamine (DA) system in normal cognitive aging, DA neuromodulation is one plausible mechanism. Hence, agents that boost systemic DA [such as *d*-amphetamine (AMPH)] may help to restore deficient signal variability levels. Furthermore, despite the standard practice of counterbalancing drug session order (AMPH first vs. placebo first), it remains understudied how AMPH may interact with practice effects, possibly influencing whether DA up-regulation is functional. We examined the effects of AMPH on functional-MRI–based blood oxygen level-dependent (BOLD) signal variability (SD_{BOLD}) in younger and older adults during a working memory task (letter *n*-back). Older adults expressed lower brain signal variability at placebo, but met or exceeded young adult SD_{BOLD} levels in the presence of AMPH. Drug session order greatly moderated change–change relations between AMPH-driven SD_{BOLD} and reaction time means (RT_{mean}) and SDs (RT_{SD}). Older adults who received AMPH in the first session tended to improve in RT_{mean} and RT_{SD} when SD_{BOLD} was boosted on AMPH, whereas younger and older adults who received AMPH in the second session showed either a performance improvement when SD_{BOLD} decreased (for RT_{mean}) or no effect at all (for RT_{SD}). The present findings support the hypothesis that age differences in brain signal variability reflect aging-induced changes in dopaminergic neuromodulation. The observed interactions among AMPH, age, and session order highlight the state- and practice-dependent neurochemical basis of human brain dynamics.

brain signal variability | dopamine | aging | working memory | fMRI

Human brain signals are characteristically variable and dynamic, at a variety of timescales and levels of analysis (1, 2). For decades, age-related cognitive deficits have been conceptualized as due to various forms of "noisy," inefficient neural processing (3, 4). However, the preponderance of available neuroimaging work on brain signal variability and aging indicates that healthy, higher performing, younger adults typically express more signal variability across trials and time in a variety of cortical regions relative to older, poorer performers (2, 5–7). Theoretical and computational explanations of this finding include notions such as flexibility/adaptability, dynamic range, Bayesian optimality, and multistability (2), but empirically supported mechanisms for age and performance-graded differences in human brain signal dynamics are not yet available. Dopamine (DA) neuromodulation may provide one such mechanism.

DA neuromodulation is a leading mechanistic candidate for age-related cognitive losses (8–10). Concurrent with substantia nigra and ventral tegmental DA neuron loss, D_1 and D_2 receptor densities reduce notably from early to late adulthood across various subcortical and cortical regions (9–11). However, it is not yet known whether DA affects brain signal variability in relation to age and performance. DA is generally considered a neuromodulator supporting both system stability (e.g., signal "fidelity," "precision," and "signal-to-noise ratio") and flexibility/adaptability (8–10, 12–14). Single-unit and multiunit computational models demonstrate that simulated aging-related DA losses can yield more inefficient stimulus detection, lower average neuronal firing pattern, and de-differentiation of neural responses in the face of varying stimuli (8). Importantly, adding noise to "older" DA-degraded neurons can improve their relatively poor stimulus detection properties (15). This neurocomputational result highlights the potential benefits of explicitly boosting dynamics in DA-degraded, aging neural systems.

DA may influence in vivo brain dynamics in a variety of ways. DA neurons exhibit dominant low-frequency tonic firing patterns along with intermittent phasic bursts (16), resulting in moment-to-moment variation in neural signaling. At millisecond and second levels, DA release also operates via shorter and longer-term facilitation and depression (e.g., so-called "kick-and-relax" dynamics, 17, 18) that affect subsequent DA-dependent spike dynamics. Mouse data highlight that DA-deficient animals

Significance

Younger, better performing adults typically show greater brain signal variability than older, poorer performers, but the mechanisms underlying this observation remain elusive. We attempt to restore deficient functional-MRI–based blood oxygen level-dependent (BOLD) signal variability (SD_{BOLD}) levels in older adults by boosting dopamine via *d*-amphetamine (AMPH). Notably, older adults met or exceeded young adult SD_{BOLD} levels under AMPH. AMPH-driven changes in SD_{BOLD} also predicted AMPH-driven changes in reaction time speed and variability on a working memory task, but depended greatly on age and drug administration order. These findings (*i*) suggest that dopamine may account for adult age differences in brain signal variability and (*ii*) highlight the importance of considering practice effects and state dependencies when evaluating the neurochemical basis of age- and cognition-related brain dynamics.

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display a complete lack of phasic bursting activity (variable on and off periods of spike trains), exhibiting only single spikes with long interspike intervals (19); notably although, bursting activity in these DA deficient animals can be restored toward normal levels via DA agonism (19). In addition, in line with the contention that brain signal variability can index a healthy neural system (2), animal models indicate that trial-to-trial variability in DA release appears to increase, rather than decrease, with increasing task proficiency (20). From this work, it is plausible that DA may also affect in vivo brain signal variability and its cognitive correlates in humans. Given that normal aging is associated with DA decline and poorer cognitive performance, and that poorer cognitive performance often characterizes generalized aging-related reductions in brain signal variability, lower age-related brain signal variability could reflect DA system degradation. Accordingly, we predicted that pharmacological agents that boost systemic DA, such as amphetamine (AMPH), would restore deficient signal variability levels in older adults. Extant studies have examined the effect of DA agonists on average blood oxygen level-dependent (BOLD) signal responses and cognition (13, 21, 22); however, the effects of DA agonists on BOLD signal variability and cognition, and the moderation of these effects by adult age, have not been investigated thus far.

In the present study, we examine the multivariate effects of AMPH on BOLD signal variability and cognitive performance in younger and older adults during an *n*-back working memory (WM) task. Given impoverished signal variability (5–7) and DA levels (9, 10) in normal aging, we predicted that older adults' BOLD variability, on average, would increase more on AMPH relative to placebo than that of younger adults. We used mixed modeling to examine whether AMPH-related changes in SD_{BOLD} predict AMPH-related changes in performance within individuals, and whether a higher average level of SD_{BOLD} coincides with higher average performance across individuals. In particular, we hypothesized that older adults (typified by lower DA, less BOLD variability, and lower cognitive performance) would show increased BOLD variability and improved cognitive performance under AMPH. Furthermore, recent evidence suggests that drug session order may influence the robustness of drug-related changes in performance (e.g., ref. 23); we thus explored whether administration order (i.e., AMPH first vs. placebo first) would moderate the effects of AMPH on SD_{BOLD}, cognitive performance, or both, thus pointing to state- and practice-dependent aspects of human brain dynamics, and adult age differences therein.

Results

Multivariate Model Linking SD_{BOLD} to AMPH, Age Group, and Task. We first ran a multivariate partial least-squares (PLS) model (see *Methods* and *Supporting Information* for details) that explicitly linked SD_{BOLD} to AMPH, age group, and task condition. We found a single robust latent variable (LV) (cross-block covariance, 60.86%; permuted $P < 0.00001$) representing this relationship. Fig. 1 contains a plot of PLS brain scores for each task condition, AMPH condition, and age group [error bars in Fig. 1 represent 95% bootstrapped confidence intervals; a lack of overlap in interval indicates a reliable difference (24)]. The associated brain pattern (Fig. 1) highlights a unidirectional effect; the higher the brain score, the higher the level of brain signal variability in yellow/red regions. Under placebo, younger adults (YA) expressed more SD_{BOLD} in all task conditions than did older adults (OA; see also *SI Results* and Fig. S1 for confirmation of a placebo-only model supporting this effect). This is in accordance with our previous studies (5–7). However, OA SD_{BOLD} levels dramatically increased under AMPH, whereas YA expressed a more modest increase only at 2-back. For YA and OA on AMPH, SD_{BOLD} appeared to increase with increasing task difficulty until the 2-back condition, and either leveled off or tapered at 3-back; there were no differences between task conditions for OA at placebo. This lack of difference in SD_{BOLD} levels between task conditions in older adults is also in line with previous work (7). Interestingly, under AMPH, older adults became more differentiated across conditions (at least when comparing fixation and 1-back to the 2-back condition).

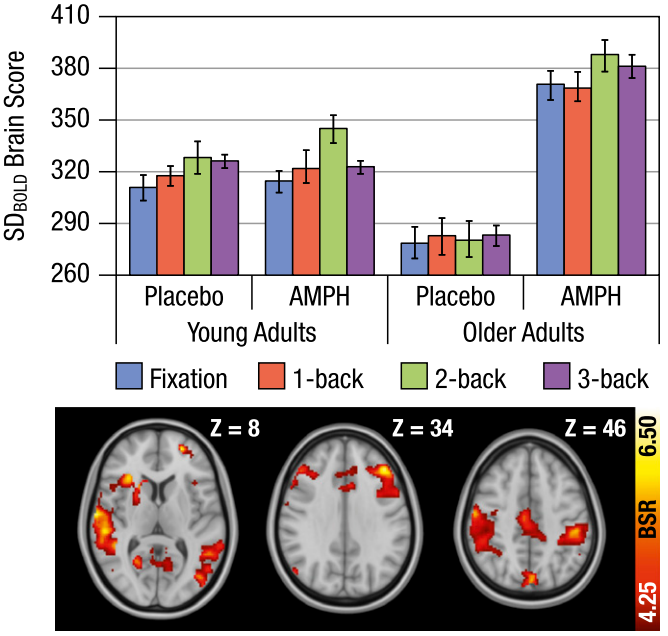

Fig. 1. PLS model of relation between SD_{BOLD}, Age Group, AMPH, and Task Condition. Higher brain scores reflect higher BOLD signal variability. Error bars represent bootstrapped 95% confidence intervals (1,000× with replacement). Brain images are plotted in neurological orientation (left is *Left*). AMPH, amphetamine; BSR, bootstrap ratio.

Spatially, our model revealed several regions typical of WM studies (25, 26), including bilateral middle frontal gyri [dorsolateral prefrontal cortex (DLPFC)], left supplementary motor area (SMA), and bilateral posterior parietal cortices (postcentral gyri). Striatal dopaminergic areas (13, 14, 27) that modulated in SD_{BOLD} were also evident in a left putamen cluster that extended into left caudate nucleus (see Fig. 1 for relevant slices). Other prominent regions included right parahippocampal gyrus (extending into bilateral thalamus and surrounding regions), middle and superior temporal gyri, and inferior occipital gyri. A complete list of bootstrapped cluster peaks (Table S1, model 1), and a complete axial view of brain slices (Fig. S2) can be found in *Supporting Information*.

Although it appears from Fig. 1 that OA SD_{BOLD} levels on AMPH exceed that of YA at both placebo and AMPH, individual slopes reveal a more detailed story. For example, at 2-back, no older adult exceeded the level of the most variable younger adult (Fig. S3). The highest brain scores are similar in both groups, with most OA showing positive slopes on AMPH, and YA showing a mix of positive and negative slopes (and a slight upward mean shift). The OA trend of the slopes is such that those with higher SD_{BOLD} at placebo tended to stabilize or slightly reduce their SD_{BOLD} levels on AMPH, whereas those with lower SD_{BOLD} at placebo tended to increase SD_{BOLD} levels on AMPH. The fact that more older adults increased their SD_{BOLD} levels on AMPH expressed itself as a larger upward AMPH-related group shift in Fig. 1. Such slope effects are consistent with the generic notion of a dopamine-related inverted-U curve, in that those with lower baseline levels of SD_{BOLD} appear to increase the most in SD_{BOLD} on AMPH. Unsurprisingly, statistical examination of this effect indicated that placebo SD_{BOLD} indeed negatively and strongly correlated with AMPH-related change in SD_{BOLD} for each age group across experimental conditions (Fig. S4).

Mixed Models of SD_{BOLD}. The current PLS model represents multivariate, latent-level relations among BOLD, AMPH, task, and age

group. However, such models do not explicitly quantify the unique importance of each effect in the solution. Subsequent mixed models (*SI Methods*) can help parse these various effects, while flexibly accounting for degrees of freedom and model covariances at within- (i.e., AMPH, Task) and between- (i.e., Age Group) subject levels (28). Results revealed robust AMPH [$F_{(1,429.97)} = 52.80$, $P = 1.75 \times 10^{-12}$] and AMPH × Age Group [$F_{(1,429.97)} = 42.15$, $P = 2.33 \times 10^{-10}$] effects (Table S2, model 1). Importantly, Task offered no unique predictive variance. This pattern establishes AMPH as the primary within-subject modulator of SD_{BOLD} in this study. Notably, we found no evidence that these effects were driven by physiological artifacts (for details, see *SI Methods* and *SI Results*, and Table S2, model 2).

Mixed Models Linking AMPH-Related Modulations in SD_{BOLD} to WM Performance. Another goal of the present study was to predict WM performance [reaction time means (RT_{mean}) and SDs (RT_{SD}); *SI Methods*] from SD_{BOLD} (using the PLS brain scores shown in Fig. 1) in the context of AMPH. Because within- and between-person levels were present for both SD_{BOLD} (the key independent variable) and WM (the dependent variable), we used mixed models that separately estimated within- and between-person relations between SD_{BOLD} and WM performance (*SI Methods*). Importantly, for all related model runs, we excluded Task Condition given its lack of unique effects on SD_{BOLD} in the initial mixed model (Table S2, model 1). Furthermore, in light of evidence suggesting that drug session order may influence the robustness of drug-related changes in performance (e.g., ref. 23), we also investigated the effect of Session Order (placebo/AMPH vs. AMPH/placebo).

Of primary importance for the current study, we first fit within-subject–only models to capture whether AMPH-related changes in SD_{BOLD} brain scores (SD_{BOLD}_within) predicted AMPH-related changes in WM (RT_{mean}_within and RT_{SD}_within; models were run separately for each behavioral metric). For the RT_{mean}_within model, results revealed a key three-way interaction between Age Group, Session Order, and SD_{BOLD}_within [$F_{(1,95.57)} = 4.72$, $P = 0.03$; see Table S2, model 3, for all other main effects and lower-level interactions]. This effect (Fig. 2, *Upper*) demonstrates that the relation between AMPH-related changes in SD_{BOLD} and AMPH-related changes in RT_{mean} differs greatly by Age Group and Session Order. Among those receiving AMPH in the first session, RT_{mean} performance improved in the OA group when SD_{BOLD} was higher on AMPH (simple slope: $r = 0.37$); YA showed no effect (simple slope: $r = -0.05$). Conversely, for those who received AMPH in the second session, both YA (simple slope: $r = -0.44$) and OA (simple slope: $r = -0.60$) groups showed improved RT_{mean} when SD_{BOLD} was lower on AMPH relative to placebo. Accordingly, post hoc analyses revealed a significant slope difference between first session YA and OA groups [Age Group × SD_{BOLD}_within interaction, $F_{(1,56.27)} = 4.10$, $P = 0.05$], but not between second session YA and OA groups [$F_{(1,37.96)} = 0.57$, $P = 0.46$]. For the RT_{SD}_within model, we also found an Age Group × Session Order × SD_{BOLD}_within interaction [$F_{(1,81.97)} = 7.31$, $P = 0.01$; see Table S2, model 4, for all model effects]. Here (Fig. 2, *Lower*), only the first-session OA group showed any notable slope (simple slope: $r = 0.48$), and in exactly the same manner as for RT_{mean}_within; when SD_{BOLD} increased on AMPH, first-session OA exhibited more consistent RT performance. Predictably, follow-up analyses again revealed a significant slope difference between first session YA and OA groups [Age Group × SD_{BOLD}_within interaction, $F_{(1,42.58)} = 10.01$, $P = 0.003$], but not between second-session YA and OA groups [$F_{(1,37.31)} = 0.23$, $P = 0.64$]. Finally, we found no reliable between-subject ("level–level") model relations between SD_{BOLD} and RT_{mean} or RT_{SD} (Table S2, models 5 and 6). Behavior-only model results (Table S2, models 7 and 8; and Fig. S5) and behavioral descriptive statistics (Table S3) can also be found in *SI Methods* and *SI Results*.

A Lack of AMPH-Driven $Mean_{BOLD}$ Effects. As in past work (5, 6, 29), we reran the same PLS model as above, but this time using $mean_{BOLD}$ as the voxel measure to compare the relative utility of SD_{BOLD} and $mean_{BOLD}$. Although we found a clear and predictable differentiation between fixation and *n*-back conditions (single LV; cross-block covariance, 58.23%; permuted $P < 0.00001$),

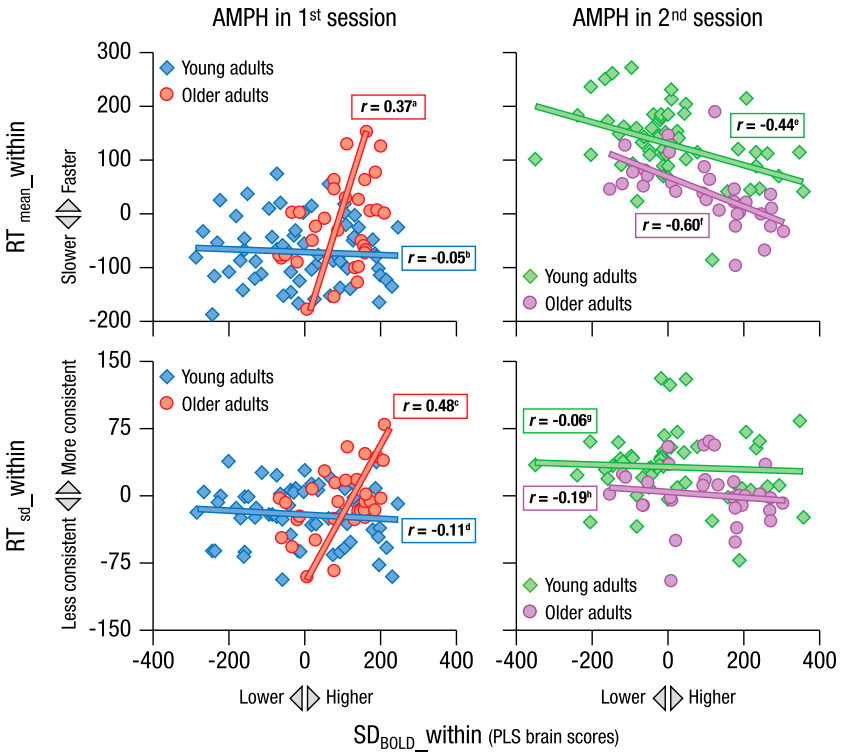

Fig. 2. Relation between AMPH-related changes in SD_{BOLD} PLS brain scores (SD_{BOLD}_within) and (*i*) AMPH-related changes in reaction time means (RT_{mean}_within; *Upper*) and (*ii*) SDs (RT_{SD}_within; *Lower*), moderated by age group and drug session order. Each subject in each group is represented by three points in the scatters, one for each *n*-back condition. Positive SD_{BOLD} values indicate higher BOLD signal variability on AMPH relative to placebo. AMPH, amphetamine. Simple slope *P* values: (a) $P = 0.039$; (b) $P = 0.672$; (c) $P = 0.005$; (d) $P = 0.412$; (e) $P = 0.001$; (f) $P = 0.0002$; (g) $P = 0.682$; (h) $P = 0.311$.

AMPH did not modulate $mean_{BOLD}$, either within or across age groups, precluding the need to further test models linking AMPH-modulated $mean_{BOLD}$ to AMPH-modulated cognition. Full model results can be found in Fig. S6 (PLS bar plots and spatial pattern), Table S2 (model 9, for mixed-model results), and Table S1 (model 2, for bootstrapped cluster peaks).

Discussion

We used multivariate and mixed models to gauge relations between BOLD signal variability, AMPH, age, and WM performance. We obtained two key findings: First, our results support the hypothesis that adult age differences in brain signal variability reflect aging-induced changes in dopaminergic neuromodulation. Second, the substantial interactions between AMPH effects and session order highlight the state- and practice-dependent neurochemical basis of human brain dynamics. These findings are discussed in turn.

The Influence of AMPH on Age-Related SD_{BOLD}. As predicted, AMPH boosted low BOLD signal variability levels in older adults across conditions. Given increasing evidence of higher overall SD_{BOLD} levels in young, high performers in previous work (2, 5–7, 30), we assumed the average YA to begin (at placebo) nearer to optimal signal variability levels, and that the average OA would rise toward YA levels. Accordingly, individual slopes revealed that the majority of OA increased in SD_{BOLD} on AMPH (especially those with the lowest levels of SD_{BOLD} at placebo), whereas YA showed a mix of positive and negative slopes (resulting in a net zero group change). This pattern of results (and our subsequent statistical examination of this effect; Fig. S4) is consistent with the general notion of an inverted-U–shaped DA curve (10, 13, 14), now linking DA and SD_{BOLD}. These AMPH-related effects were found within several brain regions typically associated with WM in mean BOLD activation studies (25, 26) and/or DA (13, 14, 27), including bilateral middle frontal gyri (DLPFC), left SMA, bilateral posterior parietal cortices, and striatum (left putamen/left caudate nucleus). However, no clear AMPH-related modulation of $mean_{BOLD}$ existed for these or any other brain areas in our data, again demonstrating the remarkable sensitivity of BOLD signal variability measures in neuroimaging research (5, 6, 29). AMPH-related regional modulations of SD_{BOLD} extended well beyond typical DA projections, presumably reflecting upstream or downstream effects of DA-related modulation on brain dynamics (e.g., cerebellum, bilateral motor, and inferior occipital cortices), suggesting that DA-related modulation of SD_{BOLD} is widespread and encompasses a broad range of stimulus processing and response execution operations. Notably, AMPH effectively increased SD_{BOLD} in older adults in various regions noted in previous work to show reduced signal variability with age (5–7), including bilateral DLPFC, posterior parietal, and primary visual cortices. However, past work (5, 6, 31) has also sometimes found that select subcortical regions exhibit the inverse effect, such that BOLD signal variability is higher in older adults. Importantly, these previous studies have been primarily between-subject in nature. In the current study, AMPH increased SD_{BOLD} in older adults in all robust regions (cortical and subcortical) in the same direction, within-person. The only other aging-related within-subject study of BOLD signal variability we are aware of is our own (7), which showed that younger adults only increased in SD_{BOLD} level from fixation to task (in a large set of cortical and subcortical regions) to a greater extent than older adults. Thus, although regional points of overlap (or lack thereof) are highly useful to consider, we caution against directly anticipating direct overlap across between- and within-subject results regarding age-related BOLD signal variability. In particular, in this study, within-subject effects statistically dominated over between-subject effects, suggesting that direct manipulation of SD_{BOLD} provides a highly sensitive approach for gauging age-related signal variability (Fig. 1).

One possible mechanism for AMPH-induced increases in SD_{BOLD} in older adults (and in those with lower baseline SD_{BOLD} levels) relates to phasic, oscillatory bursting activity in neurons (trains of spikes followed by off periods that may be rhythmic or arrhythmic), which provides a natural moment-to-moment source of variability. Paladini et al. (19) found that neurons in mice with healthy DA systems exhibited classic bursting activity, whereas DA-deficient mice displayed a complete lack of bursting, showing only single spikes with long interspike intervals. Critically, the authors successfully increased bursting activity in these DA-deficient animals via DA agonism. This finding directly informs the current results. Our presumably DA-degraded older adults (9, 10) may therefore exhibit lower SD_{BOLD} levels due to a lack of DA-driven bursting activity; however, via AMPH, we may have increased bursting in older adults, yielding higher SD_{BOLD} levels. It is thus possible that sparser spiking activity in DA-degraded systems is insufficient to drive BOLD fluctuations overall. Beyond bursting, further delineation of precisely how AMPH may drive DA-based brain signal variability requires specific targeting of D_1 and D_2 systems. One prevailing view (14, 32) assumes that the D_1 system is most important for systemic stabilization and WM maintenance, whereas D_2 signaling enables systemic flexibility and WM updating (13, 33, 34). The task used in this study was indeed a WM updating task, and given that brain signal variability may be a reflection of systemic flexibility (2), the D_2 system may be more relevant in this context than D_1. Should neuronal bursting (19) provide a basis for DA-driven changes in SD_{BOLD}, this same mechanism could influence systemic flexibility via D_2 receptors. However, as AMPH affects both D_1 and D_2 functions concurrently (35), simultaneous PET-fMRI may be required to link both systems to SD_{BOLD}, ideally across multiple sessions in which D_1 (e.g., [^{11}C]SCH 23390)- and D_2 (e.g., raclopride, fallypride)-specific ligands are administered separately.

AMPH-Related Relations Between SD_{BOLD} and WM Performance as a Function of Age and Drug Administration Order. We also applied a series of mixed models in our study to link SD_{BOLD} to within- and between-person WM performance in relation to AMPH. Of particular importance were significant "change–change" relations between AMPH-related SD_{BOLD} and AMPH-related RT (mean and SD) during *n*-back. Critically, we found that age group and order of AMPH administration drove the association between AMPH-related modulations in SD_{BOLD} and RT. This means that whether AMPH-related increases in SD_{BOLD} in cortical (e.g., DLPFC) and subcortical (e.g., striatum) regions noted above were functional for performance depended on age and session order. Older adults who received AMPH in the first session tended to improve in both speed and RT consistency when SD_{BOLD} was greatly boosted on AMPH. Conversely, those who received AMPH in the second session showed either greater performance improvements in the presence of modest reductions in SD_{BOLD} (for RT_{mean}) or no effect at all (for RT_{SD}). How is it possible that AMPH administration order (a typical nuisance regressor) plays such a pivotal role? Order counterbalancing in typical pharmacological imaging studies effectively intermixes practice effects with drug order, and potentially modulates the brain–behavior effects researchers seek to identify. Although practice effects are well acknowledged in the broader DA and cognitive literatures (36–38), understanding of these effects is limited. DA is typically associated with various forms of learning (9, 10, 13, 14, 39), but data on how DA modulation interacts with practice remain sparse. Of the few available studies, Owesson-White et al. (20) demonstrated that DA release increases linearly with reward-based lever press practice in rats. Accordingly, one mechanism to support our findings may be that, by allowing practice, we shifted participants along an inverted-U–shaped DA-performance curve (13) by

increasing baseline DA release. The subsequent administration of a DA agonist (AMPH) when participants are in a more practiced state (as in the placebo-AMPH group) may then have productive or counterproductive effects, to the extent one is already near the apex of the curve. Conversely, those who receive AMPH with limited task practice arguably reflect a purer example of the influence of AMPH alone. Accordingly, for the first-session older group (Fig. 2), those exhibiting the greatest AMPH-related increases in SD_{BOLD} indeed showed the greatest improvements in RT_{mean} and RT_{SD}. However, in this AMPH-placebo group, practice effects unavoidably worked against AMPH-related modulations; as practice-related improvements are expressed at retest (placebo), any AMPH-related effects could then be attenuated due to practice-related increases in DA release (20). Although practice effects are unlikely to confound AMPH effects completely (34), a four-cell study design (placebo/placebo, placebo/drug, drug/placebo, drug/drug) may be needed to separate within-subject practice from drug effects. Although some pharmacological studies have abandoned within-subject designs altogether (in favor of between-subject–only approaches) to guard against potentially problematic practice effects (40, 41), within-subject designs remain a prerequisite for observing drug-related changes in brain activity or performance. The present results show that taking a close look at practice effects in pharmacological imaging research may foster rather than hinder our understanding of DA neuromodulation.

State-dependent learning may be yet another angle from which to understand the importance of drug administration order when interpreting DA-driven relations between brain dynamics, age, and cognitive performance. State-dependent learning is a phenomenon in which learning (or practice) is optimally expressed when one's current state (e.g., on drug) matches the state in which the learning occurred (42). In principle, within-subject placebo vs. drug designs could inherently create a "state mismatch" by only exposing participants to each drug state (placebo and drug) once, a problem compounded by the researcher's decision to deliberately counterbalance the order of that mismatch. Unsurprisingly then, drug effects on brain and behavior could potentially skew or even invert, depending on whether learning occurred on or off drug. In the current study, this could be particularly important for the AMPH-placebo group, who learned the *n*-back task under drug, but were retested under placebo. In combination with practice effects working against drug effects (as noted above), the AMPH-placebo group also undergoes a state mismatch. Consequently, the concurrent AMPH-related gains in SD_{BOLD}, RT_{mean}, and RT_{SD} shown in older adults (and lack thereof in younger adults) may be underestimated. Remarkably, in a striking and rare example of how drug administration order (and concomitant state-dependent learning) can greatly impact drug-related changes in human task performance, a recent study of Parkinson's patients (23) examined retention of daily motor learning in relation to levodopa (L-dopa) for (*i*) an "On–Off" group, who received daily L-dopa the first week but none the following week; and (*ii*) an "Off–Off" group, who were off L-dopa for both weeks. During the second week, the On–Off group demonstrated a remarkable loss of practice-related gains in motor performance that they achieved in the first week, whereas the Off–Off group maintained all performance gains over sessions. Thus, the On–Off group underwent an explicit mismatch that affected subsequent performance in the presence of ongoing practice. Although the present study takes a first step by highlighting the importance and potential driving forces (i.e., practice, state-dependent learning) behind the effects of drug administration order, expanding the four-group study design proposed above (placebo/placebo, placebo/drug, drug/placebo, drug/drug) to also include multiple testing sessions (e.g., for the placebo/drug group, three placebo sessions followed by three drug sessions) would help address the effects of task practice and state-dependent learning, and in turn would elucidate key new parameters for future model development of DA-based relations between brain dynamics, age, and cognition.

Conclusion. The current data provide evidence for AMPH-related changes of SD_{BOLD} levels in relation to aging and working memory. Older adults were capable of expressing or even surpassing younger adult levels of SD_{BOLD} in the presence of a DA agonist (AMPH), providing a first link between manipulation of DA levels, brain dynamics, and age. We also found robust relations between AMPH-driven RT and SD_{BOLD} levels, but only when drug administration order was considered. Accordingly, we encourage researchers pursuing related pharmacological imaging studies to consider session order as an inherently interesting moderator when linking DA, age, cognition, and brain function. To the extent that pharmacological DA studies suffer from the file drawer problem (43), drug administration order may be one culprit in zeroing out inverse behavioral/brain effects resulting from session order. In the present study, this inversion was reflected in RT-SD_{BOLD} relations, serving as a springboard for future work seeking to elucidate the state- and practice-dependent neurochemical basis of brain signal dynamics and its developmental and cognitive correlates (2).

Methods

Participants and Procedure. Our sample ($n = 62$) consisted of 40 younger [YA; age range, 20–30 y; mean (M) ± SD, 25.00 ± 2.95 y; 22 females; years of education, 16.4 ± 3.1] and 22 older [OA; age range, 60–70 y; M ± SD, 63.91 ± 2.77 y; 12 females; years of education, 15.4 ± 3.8] adults. All participants were right-handed, with normal or corrected-to-normal vision, and reported to be psychiatrically and neurologically healthy. All OA were nondemented, community dwelling, and scored ≥28 points on the Mini-Mental Status Examination (44). The Ethics Committee of the State of Berlin approved the study (EudraCT number 2006-002671-40), and written informed consent was obtained from each participant. All participants received financial reimbursement. In a double-blind, counterbalanced (for order of receiving placebo/AMPH) design, participants underwent testing/fMRI scanning on two occasions (1 wk apart), one in which a physician administered a placebo (sugar pill), and another in which they administered low-dose AMPH (0.25 mg/kg body weight). Otherwise, the procedure was identical for the two sessions. Two hours before scanning (to approximate the expected peak AMPH concentration), participants received placebo or AMPH. About 90 min after pill ingestion (and about 30 min before scanning), blood was drawn to later verify serum levels using gas chromatography. Blood pressure and pulse were taken immediately before and 30 min after pill ingestion, and immediately before and after the scanning session.

MRI Task, Acquisition, and Data Preprocessing.

Fixation and cognitive task blocks. While undergoing fMRI, participants performed a letter *n*-back task (blocked-design) adjusted to reduce switch costs in older participants (30). Participants were asked to compare a currently presented single letter with the letter seen one, two, or three letters earlier (i.e., 1-, 2-, and 3-back conditions, respectively). We acquired three runs per participant. Each run consisted of two successive blocks for each condition, with each task block alternating between 20-s-long fixation blocks. We randomized the order of conditions across runs. Fifteen letters were presented during each condition block, resulting in 14 1-back trials, 13 2-back trials, and 12 3-back trials in each block (total number of trials was 84, 78, and 72, respectively). Each block began with 5,000-ms presentation of the condition cue (specifying 1-, 2-, or 3-back). Each letter stimulus was presented for 1,500 ms, separated by a 500-ms fixation cross (a total of 14 fixation crosses were presented in each block). Participants responded "yes" (i.e., the current letter is the same as the *n*-back letter) or "no" (the current letter is not the same as the *n*-back letter) in a two-button forced-choice manner. Before scanning, participants were verbally instructed about the task, practiced three runs, and received feedback on their performance.

MRI scanning and preprocessing. Whole-brain functional MRI data were collected using a 1.5-T Siemens Vision MRI system with a standard echo planar imaging (EPI) sequence [repetition time (TR)/echo time (TE), 2,500/40 ms; flip angle, 90°; voxel size, 4 × 4 × 4.6 mm; interslice gap, 0.15; 26 slices acquired in ascending order approximately axial to the bicommissural plane]. Three dummy volumes preceded each of the three experimental runs to achieve a steady state of tissue magnetization. Each run lasted about 5 min. Two structural scans (proton-density–weighted sequence: TR/TE, 4,350/15 ms; flip angle, 180°; 252 × 256 matrix; 1 × 1 × 4-mm voxel size; and a sagittally oriented high resolution T1-weighted sequence: TR/TE, 20/5 ms; flip angle, 30°; matrix, 256 × 256; voxel size, 1 mm^3) were acquired in the same orientation as the functional EPIs to aid coregistration of the functional images. fMRI data were preprocessed with FSL 5 (45, 46). Preprocessing included the following: motion correction with spatial smoothing

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(8-mm full-width at half-maximum Gaussian kernel) and high-pass filtering (σ = 108 s). Registration of functional images to high-resolution participant-specific T1 images, and from T1 to 2-mm standard space (MNI 152_T1) was carried out using FLIRT. We then masked the functional data with the GM tissue prior provided in FSL (thresholded at probability > 0.37). Extended preprocessing steps were also performed to further reduce data artifacts (5–7, 29), which included ICA denoising (29) and PHYCAA+ (47); see *SI Methods* for details.

Data Analyses.

Behavior. All trials more than ±3 SDs from within-person means on each task condition were dropped before computing reaction time means (RT_{mean}) and SDs (RT_{SD}) for each subject and *n*-back level.

Calculation of SD_{BOLD}. We first performed a block-normalization procedure to account for residual low-frequency artifacts. We normalized all blocks for each condition such that the overall 4D mean (*x**y**z**time) across brain and block was 100. For each voxel, we then subtracted the block mean and concatenated across all blocks. Finally, we calculated voxel SDs across this concatenated time series (5–7, 29).

Partial least-squares analysis. To examine multivariate relations between SD_{BOLD}, AMPH, age group, and task condition during *n*-back, we used a Task PLS analysis (24). Task PLS begins by calculating a between-subject covariance matrix between experimental conditions/groups and each voxel's SD_{BOLD}, which is then decomposed using singular value decomposition. This yields a left singular vector of experimental condition/group weights (*U*), a right singular vector of brain voxel weights (*V*), and a diagonal matrix of singular values (*S*). This analysis produces orthogonal LVs that optimally represent relations between experimental conditions/groups and voxelwise SD_{BOLD} values. To obtain a summary measure of each participant's expression of a particular LV's spatial pattern, we calculated within-person "brain scores" by multiplying the vector of brain weights (*V*) from each LV by within-subject vectors of voxel SD_{BOLD} values (separately for each condition/group within person). Significance of detected relations between multivariate spatial patterns and conditions/groups was assessed using 1,000 permutation tests of the singular value corresponding to each LV. A subsequent bootstrapping procedure revealed the robustness of voxel saliences across 1,000 bootstrapped resamples of the data (48). By dividing each voxel's mean salience by its bootstrapped SE, we obtained "bootstrap ratios" (BSRs) as normalized estimates of robustness. We thresholded BSRs at a conservative value of ±4.25, which exceeds a 99.99% confidence interval. See *SI Methods* for further details.

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