Supplementary Text

Scoring of the behavioral task

We instructed participants to execute the sequence as fast and as accurately as possible, so we tried to score performance in a way that would consider both aspects. Dan and colleagues (77) proposed a measure multiplying the exponential of speed times the exponential of accuracy:

$$ PI = \exp\left( -\left( \frac{BlockDuration}{12} \right) \right) \times \exp\left( -\left( \frac{Errors}{12} \right) \right) \times 100 $$

In their equation, the “12” was the maximum number of correct sequences possible in their task per block, and Errors = 12 - CorrectSequences. We tried using this equation. In our case, it was the time that was fixed (i.e. BlockDuration) and the total number of sequences per block varied. We noticed that the equation weighted errors less severely if made at higher speeds, while weighting participants making mistakes at lower speeds more harshly. As data came from people from multiple age groups, some of them moving more slowly (e.g., older adults) we chose not to apply this asymmetric weighting. Additionally, we noticed that the time constant used greatly changed the shape of the learning curve (e.g., using either 90 seconds or 1.5 minutes).

We thought about a hypothetical case in which two persons generated five correct sequences, with the difference that the first person generated a total of 10 sequences (50% accuracy) and the second a total of 5 sequences (100% accuracy). Based on what we instructed in terms of speed and accuracy, we wanted to give higher credit to the person having higher accuracy. Directly multiplying speed and accuracy, namely 10*0.5 for the first and 5*1 for the second person, would yield scores of 5 for both. Therefore, we chose to multiply the number of correct sequences times the accuracy, which would yield 5*0.5 = 2.5 for the first and 5*1 = 5 for the second, placing the desired prime on both parameters. The resulting equation is:

$$ score = CorrectSequences \times PercentCorrect $$

Correction for individual skill level at the beginning of training

We used a single baseline block as a benchmark (containing an independent sequence, the same for all subjects) for evaluating the initial motor capabilities of each subject. We do not use this block to normalize the performance during training under stimulation, for several reasons. First, it is a different sequence from the sequence people train on, and even if it is similar in structure and equivalent in complexity, previous research shows there is no transfer between different sequences (6), which we also see when comparing the training blocks to the catch blocks in this study (please see Table S1). Second, the training kicks in from the very first execution of a sequence (78). Third, the performance averaged over the whole block might
benefit already from the stimulation, so penalizing it with a different sequence executed under different circumstances (i.e., without stimulation) does not seem adequate.

We used the baseline block as reference for initial skill levels. In the first experiment, we found significant differences in the scores of this block between age groups. Nevertheless, we found no significant differences in accuracy (please see Table S1). In the second experiment (placebo groups), we found no significant differences in neither the scores nor the accuracy in the baseline block, further supporting the notion of participants starting at the same skill level (please see Table S2).

As we confirmed all participants were healthy, we assumed all age groups could improve within the same range of values, allowing us to compare them within the same frame of reference. For this reason, we corrected individual performance by subtracting the score of the first training block. As participants’ improvement is in the same order of magnitude, we consider absolute improvement to be an adequate measure.

Catch block scores

In the main manuscript, we mention that we see no generalization of learning to sequences different from the training sequence. The scores below show average scores of all blocks, including the catch blocks (please see Figure S1).

![Figure S1. Primary outcome of all blocks (i.e. including catch blocks). a) Average score for all blocks of the first experiment (i.e. training without stimulation). The vertical lines represent the standard error of the mean. b) Average scores for all blocks in the second experiment (i.e. training with either verum (V) or placebo (P) stimulation), with vertical bars describing the standard error of the mean. The error bars depict the standard error of the mean.](image-url)
It is clear to see that the improvement seen in the training sequence does not transfer to other sequences. Scores were not significantly different between catch blocks (*Table S1* and *Table S2*).

**Speed and accuracy**

In the main article, we only show the normalized speed and accuracy for each group. The reason is that we were interested in discussing the dynamics of both parameters, and intended to show both processes in the same plot. Here, we show the average speed and accuracy for each group. We describe the speed as the total number of sequences generated within a block (*Figure S2*).

*Figure S2. Group average accuracy and speed. Accuracy (top) and speed (bottom) in the first (a) and second (b) experiments. We defined speeds to be the total number of correct sequences generated within a block, while accuracy corresponds to the ratio of correct sequences to total sequences in each block. The error bars depict the standard error of the mean. V: Verum; P: Placebo.*
Uncorrected scores

In the main manuscript, we present centered average scores for all groups of both experiments, which was necessary to compare the scores statistically. Figure S3 shows the uncentered scores. Interestingly, the unstimulated groups and the groups receiving placebo stimulation look almost exactly the same, and the absence of an effect of atDCS in young and middle-aged adults is more evident.

**Figure S3. Uncorrected scores for both experiments.** The scores obtained by the three groups of the first experiment (a) match those obtained by the groups receiving placebo stimulation in the second experiment (b). Please notice that without the correction, the verum and placebo groups of both young and middle-aged adults are even more similar, highlighting the absence of an effect reported in the main manuscript. In contrast, older adults receiving verum stimulation outscore the unstimulated and the placebo groups of older adults. V: Verum; P: Placebo.
Motor chunk estimation from behavioral data

A common approach at extracting chunking patterns in the execution of discrete sequence production tasks is to average the inter-key intervals (IKIs) and detect statistically significant increases, assumed to separate adjacent chunks (7, 9, 40). Acuna and colleagues (79) implemented a probabilistic approach meant to estimate the likelihood of an individual performing a certain chunking pattern based on the IKIs and the errors generated during the execution of the task, as well as the correlation of these two components. The model they propose uses expectation maximization to estimate the most likely chunking pattern generated on each trial (i.e. sequence execution). For each trial (t), the model uses prior knowledge on the chunking pattern executed in the preceding trial (t-1). This approach stands on an assumption that makes its use difficult in our dataset. The model assumes that improvement is steady and consistent during training, with similar chunking patterns between adjacent trials. This could be the case in young adults, with average scores monotonically increasing between days. In contrast, middle-aged and older adults often show diminished performance at the beginning of a training session with respect to the previous day. On the other hand, the use of a catch block presented in the middle of training sessions often disrupted performance in the subsequent training block (as reported by several participants and as observed in the “dips” in the learning curves of each day, Figure S1), which probably had an impact on the executed chunking pattern. Additionally, errors in older adults, more frequent than in young, would likely obstruct finding the chunking pattern implemented by a participant, as errors cause participants to slow down (80). Furthermore, the probabilistic approach enforces the notion of chunks eventually being fully concatenated by the end of training, while chunk formation is likely constrained by the computational cost of retrieving a certain amount of sequence elements (41).

For these reasons, we decided to use the approach proposed by Song & Cohen (70). In their method, chunking strategies are detected by applying a k-means clustering algorithm to the IKIs, forcing two clusters to label IKIs as either “fast” or “slow”.

Each sequence had nine IKIs, with the first one reflecting the interval between the last key press of the previous sequence and the first key press of the current sequence. After removing incorrect sequences from each block, we normalized the IKIs of each sequence to the total duration the sequence (i.e. divided each IKI by its sequence duration), to account for the gradual increase in speed during training. After normalization, we applied the K-means clustering algorithm to sequences of each block (Sklearn, https://scikit-learn.org/) enforcing the notion of two clusters being present (i.e., “fast” and “slow”), labeling the IKIs of each sequence
based on their proximity to them (Figure S4a). The outcome of this step was a chunking pattern for each individual sequence (Figure S4b).

Figure S4. Extraction of chunking patterns from inter-key intervals (IKIs). a) We applied k-means clustering on IKIs from each block, defining two cluster centroids (i.e. “fast” and “slow”). b) After estimating the centroids, we labelled each IKI of the block as either “1” (i.e. fast) or “0” (i.e. slow), interpreting adjacent “1”’s as intervals belonging to the same motor chunk.
Labelling each sequence in this way results in many different patterns. Figure S5a shows an example of such variability, in which each histogram bin corresponds to a different sequence pattern. As sequences are binary (i.e. consist of ones and zeros), we converted them to decimal to represent them as a single number.

Figure S5. Chunking patterns generated by a single subject. a) Binary chunking patterns (e.g. [011011011]) are represented in decimal form (e.g. to \((0)2^8+(1)2^7+(1)2^6+(0)2^5+(1)2^4+(0)2^3+(1)2^2+(1)2^1+(0)2^0=219\)). b) Output from each criterion for pattern selection. The criteria are called, from top to bottom: “Maximum allocation”, “Reclustering”, “Reclustering top”, “More-often-than-not” and “Highest frequency”. Please refer to the Methods section in the main article for further details.
Because each participant generates multiple sequences, we defined the criteria described in the main text to determine a single pattern for each participant, on each day of training. *Figure S5b* shows the output from the five criteria for a single participant on the first day of training. As some patterns are slightly different from one criterion to the next, we perform a majority vote using the five criteria. In this example, the resulting pattern would be [0 1 1 0 0 1 0 1 1].

**SICI measurements**

In the main text, we mention that we used TMS to measure intra-cortical inhibition at rest in all participants of the second experiment. We applied the SICI paradigm before and after the first training day, to quantify the interneuronal GABAergic inhibition within the primary motor cortex, directly involved in the learning and execution of the motor sequence. We repeated these measurements on the fifth and sixtieth days. We show the group results in *Figure S6*.

*Figure S6. SICI measurements at rest in participants of the second experiment*. Inhibitory efficiency, quantified as the ratio of the amplitude resulting from the short-interval intracortical inhibition (SICI) paradigm divided by the amplitude of the test stimulus (TS). Each marker corresponds to the average ratio per group, with its corresponding standard error. **a)** SICI at rest in all groups receiving placebo stimulation. Panels b to d show SICI measurements at rest in young (b), middle-aged (c) and older (d) adults receiving verum (V) and placebo (P) stimulation. Recordings were performed either before (i.e., "pre") or after (i.e., "pos") performing the finger-tapping task.
Self-reports on attention and fatigue

We used a visual analogue scale (VAS) to inquire about the state of attention and fatigue in all participants before and after each training block. This scale is presented as an ungraded line, with attention ranging from “completely attentive” (0) to “completely inattentive” (10), and fatigue ranging from “awake” (0) to “tired” (10).

In Figure S7 we show the scores reported by the participants over the course of training, averaged per age and stimulation group, with the error bars representing the standard error of the mean. Figure S8 shows the difference in attention and fatigue between the last block of a training day and the first block of the following training day, plotted against the score difference between the same blocks (i.e., offline learning).

Figure S7. Self-reports on the state of attention and fatigue over the course of training. Average attention (a) and fatigue (b) scores provided by the participants of all groups of the first and the second experiments, with young adults at the top and older adults at the bottom panels. The error bars correspond to the standard error of the mean. V: Verum; P: Placebo; NS: No stimulation.
Figure S8. Offline learning as a function of attention and fatigue. The difference in attention and fatigue was calculated between the last block of a training session and the first block of the following training session. Please note that the figures depicting the relationship between offline learning and attention (a) and offline learning and fatigue (b) share the same y-axis. Please note as well that the top row shows the young adults and the bottom row shows the older adults for both attention and fatigue.
Statistical analysis of the main results

This subsection enlists all the statistical tests we ran for both experiments. In each table and for each aspect tested, we specify whether we fitted a linear model (LM) or a linear mixed-effect model (LMER) accounting for individual variation within each group; whenever between-subject variability warranted it, we used a linear mixed-effect model with random intercepts for individuals. The parameter \((1 + 1|\text{ID})\) in the random column is used to specify a random effect of intercept between individuals, allowing the intercept of the fitted model to vary, as defined in the lmer package in R (72). We did not include a random effect of slope per day because inter-individual variability in each group did not justify it. We defined the factor “Day” as categorical, to fit individual lines per training day.

Below, we show tables with the results of all the statistical tests we ran. Table S1 contains a key explaining the different aspects assessed in the execution of our motor task, while Table S2 contains a key with the parameters reported for each statistical test. Table S3 shows the results of the tests ran on the data from the first experiment, in which our groups of young, middle-aged and older adults trained without stimulation. Table S4 contains the tests we ran including all groups of the second experiment and serves as a preamble, alongside the results reported on Table S3, justifying the testing of the effects of the intervention (i.e., atDCS) on each age group separately. The results shown in Table S3 (and confirmed in Table S5) show clear age-related differences in the overall performance, as well as on the speed and on the accuracy, which alongside significant interactions found between the age groups and the intervention over the course of training (Table S4), suggest the mechanisms involved to be different, justifying a separate comparison for each age group. Our tests on the effect of stimulation for each age group are summarized on Table S6, while the subsequent tests done in the groups of older adults taking their generated chunking patterns into account are summarized in Table S7. Table S8 and Table S9 outline the results of the statistical tests we ran on the reported scores for attention and fatigue in the first and in the second experiment, respectively.
Table S1. Key describing the aspects tested for in the assessment of the behavioral and electrophysiological data of both experiments.

| Aspect             | Definition                                                                                                                                 |
|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| Baseline           | First exercise block presented to all subjects, lasting 90 seconds and containing a different sequence to that presented during the training or the catch blocks. It served as a measure of initial skill level. |
| Training           | Set of blocks containing the main training sequence. On each training day, the training sequence was present in six out of seven blocks.          |
| Online learning    | Measure of performance change taking place over the course of a training session, quantified as the difference between the last and the first training block of each session. |
| Online slope       | Slope of the straight lines fitted to the scores of each participant on each training day, reflecting the rate of change in performance on each day.     |
| Offline learning   | Measure of performance change occurring overnight (i.e., between training sessions), quantified as the difference in score between the last block of a training session and the first training block of the following session. |
| Speed              | Speed of execution of the sequences, quantified as the total number of sequences generated per block.                                      |
| Accuracy           | Measure of precision in the execution of the sequence, quantified as the ratio of correct to total number of sequences.                        |
| FU scores          | Scores obtained during the follow-up (FU) sessions.                                                                                       |
| Catch block scores | Scores obtained in the “catch” blocks presented halfway through each training session, containing a sequence different to the training sequence and different on each session. |
| D1 / D2-D5         | Specification of whether an aspect concerns the first training day (D1) or the rest of the training week (D2-D5).                              |
| SICIrest           | Short-interval intracortical inhibition (SICI) measured when individuals where at rest, quantified as the ratio of the magnitude of the conditioning to the test pulse commonly used in the SICI TMS paradigm (please refer to the Methods). The specification “change Tr. D1” refers to the change in SICIrest after the first training session, while “change Tr. Week” refers to the change after the training week. |
| Attention          | Subjective measure of attention reported by each participant before and after each training block, captured using a visual analog scale in the form of an ungraded 10 cm straight line ranging from “completely attentive” (0) to “completely inattentive” (10). |
| Fatigue            | Subjective measure of fatigue reported by each participant before and after each training block, captured using a visual analog scale in the form of an ungraded 10 cm straight line ranging from “awake” (0) to “tired” (10). |
| Label              | The “label” parameter in Table S7 corresponds to a classification we did within older adults receiving verum stimulation, grouping them according to whether they generated “young-like” or “old-like” chunking patterns on day 1. |
Table S2. Key describing the parameters reported for each statistical test.

| **ANOVA parameters** | **Description**                                                                                                                                 |
|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| DF num.              | Numerator degrees of freedom.                                                                                                                   |
| DF den.              | Denominator degrees of freedom. Only applicable when fitting mixed-effects models (i.e., LMER).                                               |
| F                    | F-value in an ANOVA, calculated as the ratio of the variance explained by a model to the unexplained variance.                               |
| p                    | Classical p-value, representing the likelihood of observing the current or more extreme data given that the null hypothesis is true (i.e., the true means of the compared groups are not different). |

| **Post-hoc test parameters** | **Description**                                                                                                                                 |
|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| Estimate                    | Estimated difference between the models fitted to each group.                                                                                 |
| DF                          | Degrees of freedom.                                                                                                                          |
| t                           | T ratio, calculated as the estimate divided by the standard error of the estimate.                                                            |
| p                           | Classical p-value, as described above.                                                                                                         |
| d                           | Effect size, calculated as the difference of the means divided by the standard deviation.                                                     |
| Cl                          | Confidence level, calculated as the estimate ± the value of the inverse cumulative density function of the Student t distribution given the degrees of freedom. |
| Aspect               | Model  | Dependent | Independent | Random | ANOVA params | dof sum | dof Dev | p      | Level | Contrast Estimate | df | t     | p     | d     |
|---------------------|--------|-----------|-------------|--------|--------------|---------|---------|--------|-------|------------------|----|-------|-------|------|
| Baseline            | LM     | Score     | AGE         | NA     | Age          | 2       | 0.79567 | 0.00000 | 0.00452 | 9.12830         | 49 | 1.46524 | 0.00000 | 0.00452 |
|                     |        |           |             |        | 4.90881      | 49      | 1.77121 | 0.00000 | 0.00452 | 9.12830         | 49 | 1.46524 | 0.00000 | 0.00452 |
| Baseline survival   | LM     | Score     | AGE         | NA     | Age          | 2       | 0.44409 | 0.00000 | 0.00452 | 9.12830         | 49 | 1.46524 | 0.00000 | 0.00452 |
|                     |        |           |             |        | 4.90881      | 49      | 1.77121 | 0.00000 | 0.00452 | 9.12830         | 49 | 1.46524 | 0.00000 | 0.00452 |
| training LM        | Score   | AGE DAY   | (1 x 1)     |        |              |         |         |        |       |                  |    |        |       |       |
|                     |        |           |             |        | 0.02431      | 0.00452 | 0.00452 | 9.12830 | 49    | 1.46524 | 0.00000 | 0.00452 |
|                     |        |           |             |        | 4.90881      | 49      | 1.77121 | 0.00000 | 0.00452 | 9.12830         | 49 | 1.46524 | 0.00000 | 0.00452 |
| online slope LM     | Score   | AGE NIGHT | (1 x 1)     |        |              |         |         |        |       |                  |    |        |       |       |
|                     |        |           |             |        | 0.02431      | 0.00452 | 0.00452 | 9.12830 | 49    | 1.46524 | 0.00000 | 0.00452 |
|                     |        |           |             |        | 4.90881      | 49      | 1.77121 | 0.00000 | 0.00452 | 9.12830         | 49 | 1.46524 | 0.00000 | 0.00452 |
| offline learning LM | Score   | AGE NIGHT | (1 x 1)     |        |              |         |         |        |       |                  |    |        |       |       |
|                     |        |           |             |        | 0.02431      | 0.00452 | 0.00452 | 9.12830 | 49    | 1.46524 | 0.00000 | 0.00452 |
|                     |        |           |             |        | 4.90881      | 49      | 1.77121 | 0.00000 | 0.00452 | 9.12830         | 49 | 1.46524 | 0.00000 | 0.00452 |
| speed LM            | Score   | Age Number | (1 x 1)     |        |              |         |         |        |       |                  |    |        |       |       |
|                     |        |           |             |        | 0.02431      | 0.00452 | 0.00452 | 9.12830 | 49    | 1.46524 | 0.00000 | 0.00452 |
|                     |        |           |             |        | 4.90881      | 49      | 1.77121 | 0.00000 | 0.00452 | 9.12830         | 49 | 1.46524 | 0.00000 | 0.00452 |

Table 32. Statistical tests run on behavioral data of the first experiment.
| Age | Day | Growth | Day | Fert. | Day | Growth | Day | Fert. | Day | Growth | Day | Fert. |
|-----|-----|--------|-----|------|-----|--------|-----|------|-----|--------|-----|------|
| AGE:DAY:BLOCK 1 | 183 | 2.518816 | 0.010347 | 183 | 2.748152 | 0.021578 | 183 | 1.115982 | 0.347593 | 183 | 1.311654 | 0.036162 |
| Young | Middle | Older | Young | Middle | Older | Young | Middle | Older | Young | Middle | Older |
| 0.00016 | 0.00197 | 0.00375 | 0.00508 | 0.00638 | 0.00776 | 0.00909 | 0.01042 | 0.01176 | 0.01309 | 0.01442 |
| 0.00162 | 0.00194 | 0.00242 | 0.00276 | 0.00310 | 0.00344 | 0.00378 | 0.00412 | 0.00446 | 0.00480 | 0.00514 |

Accuracy

| LMER | Per. Correct | AGE:DAY:BLOCK |
|------|--------------|----------------|
| 0.95016 | 0.221164 | 0.482348 |

PostHoc

| Term | df | F | p |
|------|----|---|---|
| Day 1 | 3 | 2.0459 | 1.0250 |
| Day 2 | 3 | 4.1222 | 0.0435 |
| Day 3 | 3 | 4.5536 | 0.0305 |
| Day 4 | 3 | 6.4804 | 0.0007 |
| Day 5 | 2 | 6.0532 | 0.0026 |
| Day 1 | 3 | 3.2667 | 0.1013 |
| Day 2 | 3 | 4.8889 | 0.0320 |
| Day 3 | 3 | 5.5444 | 0.0236 |
| Day 4 | 3 | 4.6435 | 0.0310 |
| Day 5 | 2 | 6.3133 | 0.0119 |
| Day 1 | 3 | 3.8889 | 0.1204 |
| Day 2 | 3 | 4.1222 | 0.0449 |
| Day 3 | 3 | 2.4444 | 0.1176 |
| Day 4 | 3 | 1.7111 | 0.1892 |

ANOVA

| Source | Score | AGE:DAY:BLOCK |
|-------|-------|----------------|
| And 2 | 48.500654 | 27.374174 |
| Day 2 | 410 | 10.3645 | 6.088-05 |
| Age 2 | 410 | 1.734182 | 0.14512 |

PostHoc

| Score | Score | AGE:DAY:BLOCK |
|-------|-------|----------------|
| And 2 | 48.500654 | 16.765111 |
| Day 5 | 245 | 1.230156 | 0.29921 |
| Age 5 | 245 | 0.408784 | 0.02185 |

Catch block scores

| Score | Score | AGE:DAY:BLOCK |
|-------|-------|----------------|
| And 2 | 48.500654 | 10.3645 |
| Day 5 | 245 | 1.230156 | 0.29921 |
| Age 5 | 245 | 0.408784 | 0.02185 |
Table S4. Statistical tests run on behavioral data of the second experiment.

| Aspect   | Model | Dependent | Independent | Random | ANOVA param. | DF num. | DF den. | F       | p       |
|----------|-------|-----------|-------------|--------|--------------|---------|---------|---------|---------|
| Baseline | LM    | Score     | AGE,STIM    | NA     | AGE          | 2       | 6.108802| 0.00402 |         |
|          |       |           |             |        | STIM         | 1       | 0.001996| 0.964523|         |
|          |       |           |             |        | AGE:STIM     | 2       | 1.104202| 0.33871 |         |
| Training | LMER  | Score     | AGE,DAY,STIM| (1 + 1|ID) | AGE          | 2       | 54.99973| 12.39233| 3.61E-05|
|          |       |           |             |        | DAY          | 4       | 392.5667| 2.70E-241|         |
|          |       |           |             |        | STIM         | 1       | 1.622449| 0.208107|         |
|          |       |           |             |        | AGE:DAY      | 8       | 22.91452| 1.39E-33 |         |
|          |       |           |             |        | AGE:STIM     | 2       | 0.680037| 0.510806|         |
|          |       |           |             |        | DAY:STIM     | 4       | 2.72677 | 0.027954|         |
|          |       |           |             |        | AGE:DAY:STIM | 8       | 5.243177| 1.67E-06|         |
| Speed    | LMER  | Seq. Number| AGE,DAY,STIM| (1 + 1|ID) | AGE          | 2       | 54.99969| 26.35831| 9.38E-09|
|          |       |           |             |        | DAY          | 4       | 1152.315| 0         |         |
|          |       |           |             |        | STIM         | 1       | 4.571259| 0.036971|         |
|          |       |           |             |        | AGE:DAY      | 8       | 92.13703| 9.04E-128|         |
|          |       |           |             |        | AGE:STIM     | 2       | 0.510373| 0.603087|         |
|          |       |           |             |        | DAY:STIM     | 4       | 11.20811| 5.58E-09 |         |
|          |       |           |             |        | AGE:DAY:STIM | 8       | 2.193037| 0.025422|         |
| Accuracy | LMER  | Per. Correct| AGE,DAY,STIM| (1 + 1|ID) | AGE          | 2       | 55       | 5.36158 | 0.00746 |
|          |       |           |             |        | DAY          | 4       | 7.115792| 1.11E-05|         |
|          |       |           |             |        | STIM         | 1       | 0.029438| 0.864402|         |
|          |       |           |             |        | AGE:DAY      | 8       | 6.245986| 5.45E-08|         |
|          |       |           |             |        | AGE:STIM     | 2       | 7.31263 | 0.001527|         |
|          |       |           |             |        | DAY:STIM     | 4       | 2.190772| 0.067767|         |
|          |       |           |             |        | AGE:DAY:STIM | 8       | 6.361158| 3.66E-08|         |
| | Model | Subject | Independent | Random | GLM (j) | Method | p (j) | p | Level | Cont. Level | '1' | 'p' | 'd' | 'O' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Baseline learning** | LM | Per-Correct | AGE | NA | AGE | 2 | 2 | 3.057761 | 0.064869 | 0.018080 | 0.037315 | 2.066858 |
| | LM | Per-Correct | AGE | NA | AGE | 2 | 24.99996 | 6.075138 | 9.42E‐05 | 0.004783 | 0.008973 | 0.018080 | 0.037315 | 2.066858 |
| | LM | Per-Correct | AGE | NA | AGE | 2 | 2.95E‐05 | 0.004783 | 0.008973 | 0.018080 | 0.037315 | 2.066858 |
| **Training** | LMER | Score | AGE, DAY | 1 (1|ID) | 1 | 2 | 12.1296 | 800 | 0.004374 | 0.014863 | 0.029727 | 0.059454 | 0.118908 | 0.237816 |
| | LMER | Score | AGE, DAY | 1 (1|ID) | 1 | 10.1300 | 800 | 0.001388 | 0.003507 | 0.007014 | 0.014028 | 0.028056 | 0.056112 | 0.112224 | 0.224448 |
| **Online testing** | LM | Score | AGE | NA | AGE | 2 | 3.17298 | 143.2972 | 0.116021 | 0.023204 | 0.046408 | 0.092787 | 0.185574 | 0.371148 |
| | LM | Score | AGE | NA | AGE | 2 | 25.9060 | 143.2972 | 0.116021 | 0.023204 | 0.046408 | 0.092787 | 0.185574 | 0.371148 |
| **Online learning D2-ES** | LMER | Score | AGE, DAY | 1 (1|ID) | 1 | 24.2816 | 32.63027 | 1.46E‐05 | 0.001059 | 0.002119 | 0.004239 | 0.008478 | 0.016956 | 0.033912 |
| | LMER | Score | AGE, DAY | 1 (1|ID) | 1 | 12.1296 | 800 | 0.004374 | 0.014863 | 0.029727 | 0.059454 | 0.118908 | 0.237816 |
| **Online slope D2-ES** | LM | Score | AGE | NA | AGE | 2 | 13.9985 | 32.63027 | 0.000701 | 0.001402 | 0.002805 | 0.005611 | 0.011222 | 0.022448 |
| **Online learning D2-ES** | LMER | Score | AGE, DAY | 1 (1|ID) | 1 | 12.1296 | 800 | 0.004374 | 0.014863 | 0.029727 | 0.059454 | 0.118908 | 0.237816 |

**Table 5.1** Statistical tests on co behavioral and electrophysiological data from the fluid stage of the second experiment.
| Factor          | Model  | Score   | AGE | DAY | AGE x DAY | df    | F    | p    | d    |
|-----------------|--------|---------|-----|-----|-----------|-------|------|------|------|
| Day 1 vs. Day 5 | AGE,   | 1.715866| 0.018783 | 2   | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |
| Day 5 vs. Day 10| AGE,   | 1.715866| 0.018783 | 2   | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |
| Day 1 vs. Day 60| AGE,   | 1.715866| 0.018783 | 2   | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |
| Day 5 vs. Day 60| AGE,   | 1.715866| 0.018783 | 2   | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |
| Day 1 vs. Day 10| AGE,   | 1.715866| 0.018783 | 2   | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |
| Day 5 vs. Day 10| AGE,   | 1.715866| 0.018783 | 2   | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |

### Post-ANOVA Tests

| Test Type | Score | AGE, DAY | df    | F    | p    | d    |
|-----------|-------|----------|-------|------|------|------|
| SICIrest at Baseline | LMER Score | 1.715866 | 0.018783 | 2 | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |
| SICIrest change To D1 | LMER Score | 1.715866 | 0.018783 | 2 | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |
| SICIrest change To Week | LMER Score | 1.715866 | 0.018783 | 2 | 125 | 7.515877 | 0.006331 | 1.394733 | 0.23994 |

### Summary

- **ANOVA Results**: ANOVA was performed to analyze the effects of age, day, and their interaction on the measured variables.
- **Post-Hoc Tests**: Post-hoc tests were conducted to further analyze the differences between specific groups.
- **Significance Levels**: Significance levels for each test are indicated by p-values, with values less than 0.05 typically considered significant.
| Subject       | Age Group | Model | Experiment | Independent | Random | Sessions | DP score | DP score | p       | Level | Contrast Estimate | DF | t       | p       |
|---------------|-----------|-------|------------|-------------|--------|----------|----------|----------|---------|--------|------------------|----|---------|---------|
| Baseline      | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Black 5 accuracy | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Young Online  | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Middle        | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Older         | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Online learning | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Offline learning | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Accuracy      | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Baseline      | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |
| Francis       | Score     | STIM  | NA         | 1           | 1.49297 | 0.22875  | 0.00670 | 0.00670 | 0.81862 |       | 1.25057 1.07630  | 6  | 1.67262 | 0.12939 |

Table 5a. Statistical test on the data from the second experiment, comparing verum and placebo groups for each age group.
**Table S7**: Statistical tests run on data from the second experiment, comparing young-like and old-like older adults in the verum group. Labels are either "Young-like" or "Old-like".

| Aspect          | Model Dependent | Independent | Random | ANOVA | Predict tests
|-----------------|-----------------|-------------|--------|-------|-----------------|
| Baseline speed  | LM Seq. Number  | LMER       | NA     | LMER  | (1920.09, 1.956035, 1.629098) |
| Block 1 speed   | LM Seq. Number  | LMER       | NA     | LMER  | 1.081111 2.956098 1.629098 |
| Block 2 speed   | LM Seq. Number  | LMER       | NA     | LMER  | 0.040252 8.956000 1.629098 |
| Block 3 variance | LM Per. Correct | LMER       | NA     | LMER  | 0.000671 2.956000 1.629098 |

**Training**

| | Score | LMER,DAY (1 + 0) | LMER | | |
| | | | | | |
| Day 1 | 17 | 2.711466 | 0.800000 | Day 1 | 2.711466 5.906421 0.800000 |
| Day 4 | 300 | 84.86827 | 6.000000 | Day 4 | 84.86827 3.875342 6.000000 |
| Label/Day | 4 | 300 | 0.701098 1.705000 | Day 4 | 0.701098 0.305363 1.705000 |
| Day 5 | 737.3941 | 1.705000 | Day 5 | 737.3941 0.305363 1.705000 |

**Online slope D1**

| | Slope | LMER,BLOCK | LMER | | |
| | | | | | |
| Label | 1 | 11.000000 | 11.000000 | Slope | 11.000000 0.000000 11.000000 |
| Block | 1 | 0.000000 | 0.000000 | Block | 0.000000 0.000000 0.000000 |
| Block | 1 | 0.000000 | 0.000000 | Block | 0.000000 0.000000 0.000000 |

**Online slope D2-D5**

| | Slope | LMER,BLOCK | LMER | | |
| | | | | | |
| Label | 1 | 22.5770 | 1.000000 | Slope D1 | 22.5770 0.000000 1.000000 |
| Block | 1 | 0.000000 | 0.000000 | Block | 0.000000 0.000000 0.000000 |
| Block | 1 | 0.000000 | 0.000000 | Block | 0.000000 0.000000 0.000000 |

**Speed**

| | Seq. Number | LMER,DAY (1 + 0) | LMER | | |
| | | | | | |
| Day 1 | 17 | 22.5770 | 1.000000 | Day 1 | 22.5770 0.000000 1.000000 |
| Day 4 | 300 | 16.75607 | 0.000000 | Day 4 | 16.75607 0.000000 0.000000 |
| Day/Label | 4 | 300 | 0.263048 | 1.000000 | Day 4 | 0.263048 0.000000 1.000000 |
| Day 5 | 7.757222 | 1.000000 | Day 5 | 7.757222 0.000000 1.000000 |

**Accuracy**

| | Per. Correct | LMER,DAY (1 + 0) | LMER | | |
| | | | | | |
| Block | 1 | 13.32515 | 0.000000 | Block | 13.32515 0.000000 0.000000 |

**Note:** All tests are performed using LMER with a significance level of 0.05.
| Aspect                        | Age group | Model  | Dependent | Independent | Remainder | Parameters | DF run | DF det | F       | p        | Level | Contrast | DF | Contrast | t     | p       | d     | Q   |
|------------------------------|-----------|--------|-----------|-------------|-----------|------------|--------|--------|---------|----------|-------|----------|----|----------|------|---------|------|------|
| | All | LMER | Offline | AGE, DAY, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |
| | Young | LMER | Offline | AGE, DAY, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |
| | Middle | LMER | Offline | AGE, DAY, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |
| | Older | LMER | Offline | AGE, DAY, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |
| Cor. Attention and offline learning | All | LMER | Fatigue score | AGE, DAY, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |
| | Young | LMER | Fatigue score | DAY, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |
| | Middle | LMER | Fatigue score | DAY, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |
| | Older | LMER | Fatigue score | DAY, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |
| Cor. Fatigue and offline learning | All | LMER | Fatigue score | Fatigue, AGE, BLOCK | (3 + 1) |       |          |        |        |         |          |       |          |    |          |      |         |     |     |

### Statistical Tests

- **Fatigue**
  - Fatigue score: Age, Day, Block
  - ANOVA: F(3, 270) = 6.13, p = 0.002
  - Post-hoc: Tukey's HSD (p = 0.05)

- **Attention**
  - Offline score: Age, Day, Block
  - ANOVA: F(3, 270) = 6.13, p = 0.002
  - Post-hoc: Tukey's HSD (p = 0.05)
| Factor                  | Group | Model  | Independent Variable | Random Effects | Factors  | DF      | F       | p       | Level | Contrast | df | p       | Level | Contrast | df | p       | Level | Contrast | df | p       | Level | Contrast |
|-------------------------|-------|--------|----------------------|----------------|----------|--------|--------|--------|--------|---------|-----|--------|--------|---------|-----|--------|--------|---------|-----|--------|--------|---------|
| Attention               | All   | UNRES | Age, Stim, Day, Block |               |          | 1      | 175.36  | 0.0033  | 0.0033  | 0.17543| 0.0033  | 0.0033 | 0.17543| 0.0033 | 0.0033  | 0.17543| 0.0033 | 0.0033  | 0.17543| 0.0033 |
| Middle LMER Offline     |       |        |                      |                |          |        |        |        |        |         |     |        |        |         |     |        |        |         |     |        |        |         |
| Young LMER              |       |        |                      |                |          |        |        |        |        |         |     |        |        |         |     |        |        |         |     |        |        |         |
| Older LMER Offline      |       |        |                      |                |          |        |        |        |        |         |     |        |        |         |     |        |        |         |     |        |        |         |
| Corr. attention and offline learning | All | UNRES | Age, Stim, Day, Night |               |          | 1      | 251.86  | 0.0005  | 0.0005  | 0.25186| 0.0005  | 0.0005 | 0.25186| 0.0005 | 0.0005  | 0.25186| 0.0005 | 0.0005  | 0.25186| 0.0005 |
| Fatigue                 |       |        |                      |                |          |        |        |        |        |         |     |        |        |         |     |        |        |         |     |        |        |         |
| Young LMER              |       |        |                      |                |          |        |        |        |        |         |     |        |        |         |     |        |        |         |     |        |        |         |
| Older LMER              |       |        |                      |                |          |        |        |        |        |         |     |        |        |         |     |        |        |         |     |        |        |         |
| Corr. fatigue and offline learning | All  | UNRES | Fatigue, Age, Stim, Day, Night |   |          |        |        |        |        |         |     |        |        |         |     |        |        |         |     |        |        |         |
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