Supplementary material:

Isometric handgrip exercise speeds working memory responses in younger and older adults

Shelby L. Bachman, Sumedha Attanti, and Mara Mather

Davis School of Gerontology, University of Southern California

Supplementary Methods

Section 1. Exclusion of handgrip participants based on EMG

To confirm that participants in the handgrip group squeezed as instructed and rested as instructed during handgrip runs, we computed the mean of each participant's integrated EMG signal (computed during data collection in Acqknowledge) during each squeeze and rest event, for the left and right arms separately. At an individual level, we tested whether the mean EMG signal from the relevant arm during all squeeze phases was numerically greater than mean EMG averaged across both arms during all rest phases. This resulted in 2 older participants, for whom mean EMG during all squeeze events was not greater than mean EMG during all rest events, being excluded from all analyses.

Section 2. Details of target trial placement in n-back task blocks

Each n-back block consisted of 18 trials, with the number of target trials per block differing by working memory load: 0-back blocks contained 6 target trials each, 1-back blocks contained 6 target trials, 2-back blocks contained 5 target trials, and 3-back blocks contained 4 target trials. Constraints placed on target trial placement within blocks are described below.

0-back blocks

Of 6 target trials per block, 3 occurred in the first half of the block, and 3 occurred in the second half of the block. Target trials could occur back-to-back, but only a maximum of 2 times.

1-back blocks

Of 6 target trials per block, 3 occurred in the first half of the block, and 3 occurred in the second half of the block. There were no back-to-back target trials.

2-back blocks

Of 5 target trials per block, 2-3 target trials occurred in the first half of the block, and the remaining 2-3 occurred in the second half of the block. Target trials could occur back-to-back, but only once per block.

3-back blocks

Of 4 target trials per block, 2 occurred in the first half of the block, and 2 occurred in the second half of the block. There were no back-to-back target trials.

Section 3. Details of excluded ECG segments

Table S1

Summary of ECG data segments included based on visual inspection

Age group	Task	N (%) segments excluded
Younger	Baseline	2 (0.003%)
Younger	Handgrip	2 (0.003%)
Younger	N-back	5 (0.006%)
Older	Baseline	0 (0%)
Older	Handgrip	3 (0.004%)
Older	N-back	4 (0.005%)

Note. Signals with artifact and/or noise such that QRS complexes were not visually distinguishable were excluded from analysis.

Section 4. Parameters for QRS detection on ECG segments

QRS detection on ECG data segments was performed with the Physionet Cardiovascular Signal Toolbox (Version 1.0.2; Vest et al., 2018). All parameters were set to the default values except that we changed the window length for QRS detection to 10 seconds to avoid missing values that sometimes arose for the SQIjw index with longer windows. In addition, because we performed ECG quality control outside of Physionet, we changed the rejection threshold to 1 and the low-quality threshold to 0, so that QRS detection would be performed for all segments.

Section 5. Details of pupil diameter data preprocessing and exclusions

While preprocessing eyetracking data segments, we recorded the fraction of missing samples within each event of interest. Events with greater than 70% missing samples were excluded from analysis. A total of 2 participants had greater-than-threshold missingness in baseline eyetracking data segments, thus 4 (0.02%) segments (1 segment per eye, per participant) were excluded from analysis. Handgrip and n-back fixation pupil data segments for these participants were also excluded from analysis. For all remaining handgrip segments, excluded events are described in Table S2. Excluded events in remaining n-back pupil segments are described in Table S3.

Table S2

Summary of events excluded from analysis, for eyetracking data segments from handgrip runs

Event	N (%) excluded
Rest1	166 (0.27%)
Rest2	172 (0.28%)
Rest3	200 (0.32%)
Rest4	210 (0.34%)
Squeeze1	166 (0.27%)
Squeeze2	172 (0.28%)
Squeeze3	200 (0.32%)
Squeeze4	218 (0.35%)

Table S3

Summary of events excluded from analysis, for eyetracking data segments from n-back runs

Event	N (%) excluded
Pre-block fixation: block 1 relative to handgrip offset	300 (0.49%)
Pre-block fixation: block 2 relative to handgrip offset	170 (0.28%)
Pre-block fixation: block 3 relative to handgrip offset	244 (0.39%)
Pre-block fixation: block 4 relative to handgrip offset	106 (0.32%)
Trials: 0-back	3844 (0.34%)
Trials: 1-back	3810 (0.34%)
Trials: 2-back	3620 (0.32%)
Trials: 3-back	1866 (0.31%)

Supplementary Results

Section 1. Results of test-retest reliability analyses of arousal measures across runs of the handgrip protocol

Intra-class correlation analysis was performed to examine test-retest reliability of mean pupil diameter, mean heart rate, and mean sympathetic tone across runs of the handgrip protocol. results of these analyses are presented in Table S4.

Table S4

Intraclass correlation coefficients reflecting test-retest reliability of mean pupil diameter, heart rate, and sympathetic tone values across runs of the handgrip task

Measure	Event	ICC	95% CI	$F(df_1, df_2)$	p
Mean pupil diameter	Rest	0.797	0.648 - 0.879	$F(73, 38.4) = 5.97$	**<.001**
	Squeeze	0.864	0.744 - 0.922	$F(77, 28.3) = 9.47$	**<.001**
Mean heart rate	Rest	0.868	0.812 - 0.909	$F(92, 155.2) = 7.94$	**<.001**
	Squeeze	0.908	0.864 - 0.938	$F(92, 107.7) = 11.83$	**<.001**
Mean sympathetic tone	Rest	0.891	0.847 - 0.924	$F(94, 188.3) = 9.09$	**<.001**
	Squeeze	0.834	0.767 - 0.884	$F(94, 190) = 6.04$	**<.001**

Section 2. Results of pairwise comparisons of arousal measures during the handgrip protocol

We performed pairwise comparisons of each arousal measure (pupil diameter, heart rate, and sympathetic tone) for the squeeze compared to the rest phases, for each group and age group separately. Results of these comparisons for pupil diameter are shown in Table S5, for heart rate in Table S6, and for sympathetic tone in Table S7.

Table S5

Results of pairwise comparisons of pupil diameter during squeeze versus rest phases of the handgrip protocol, by group and age group

Age group	Group	Contrast	Estimate	SE	df	*t*	*p*
Younger	Handgrip	Squeeze - Rest	0.881	0.086	88	10.214	**<.001**
Younger	Control	Squeeze - Rest	0.105	0.090	88	1.168	.983
Older	Handgrip	Squeeze - Rest	0.244	0.098	88	2.477	.061
Older	Control	Squeeze - Rest	0.305	0.094	88	3.252	**.006**

Table S6

Results of pairwise comparisons of heart rate during squeeze versus rest phases of the handgrip protocol, by group and age group

Age group	Group	Contrast	Estimate	SE	df	*t*	*p*
Younger	Handgrip	Squeeze - Rest	4.711	0.551	92	8.544	**<.001**
Younger	Control	Squeeze - Rest	0.290	0.540	92	0.537	1.000
Older	Handgrip	Squeeze - Rest	2.193	0.563	92	3.893	**<.001**
Older	Control	Squeeze - Rest	-0.096	0.551	92	-0.174	1.000

Table S7

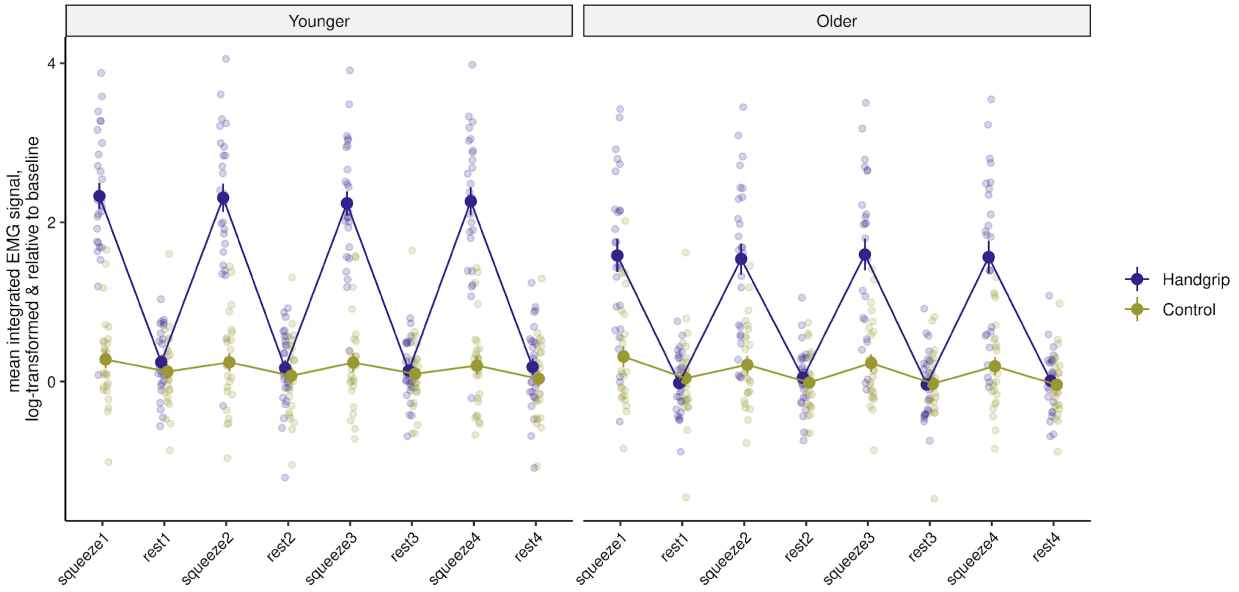
Results of pairwise comparisons of sympathetic tone during squeeze versus rest phases of the handgrip protocol, by group and age group

Age group	Group	Contrast	Estimate	SE	df	*t*	*p*
Younger	Handgrip	Squeeze - Rest	0.002	0.001	92	3.922	**<.001**
Younger	Control	Squeeze - Rest	0.000	0.001	92	0.381	1.000
Older	Handgrip	Squeeze - Rest	0.002	0.001	92	4.129	**<.001**
Older	Control	Squeeze - Rest	-0.001	0.001	92	-1.716	.358

Section 3. Mean integrated EMG during the handgrip (control) protocol

Figure S1

Mean integrated EMG signals across the course of the handgrip protocol



Note. Values were baseline corrected using mean values from a 4-minute baseline period at the beginning of the experiment. Figure reflects data averaged over all 3 runs of the handgrip protocol.

Section 4. Results of repeated measures correlation analyses examining intraindividual associations between handgrip effort and arousal increases during handgrip

Repeated measures correlation analyses indicated that at an intra-individual level, there was a trend toward handgrip effort being positively correlated with increases in pupil diameter, $r_m(305) = 0.108$, 95% CI = 0.009 - 0.209, $p = .058$ (Figure S2A). Handgrip effort was positively correlated with increases in heart rate during handgrip, $r_m(460) = 0.137$, 95% CI = 0.051 - 0.224, $p = .003$ (Figure S2B). However, the correlation between handgrip effort and increases in sympathetic tone was not significant, $r_m(472) = 0.064$, 95% CI = -0.017 - 0.150, $p = .164$ (Figure S2C).

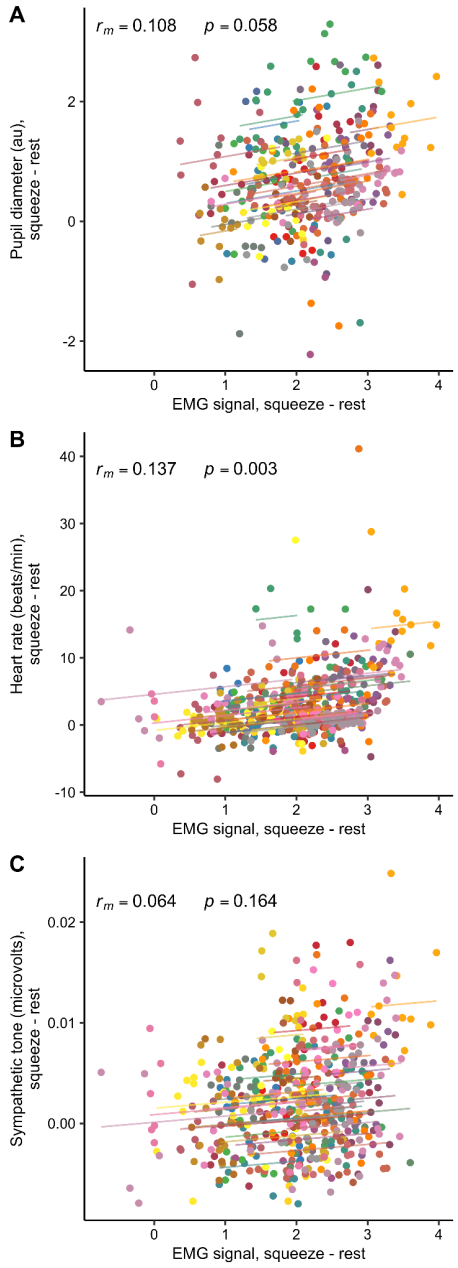


Figure S2. Scatterplots depicting repeated measures correlation between handgrip effort and increases in pupil diameter (A), heart rate (B), and sympathetic tone (C) during the handgrip protocol, for participants in the handgrip group only. Observations from the same participant are presented in the same color, with lines depicting the repeated measures correlation fit for each participant.

Section 5. Results of pairwise comparisons of contralateral versus ipsilateral pupil diameter during the handgrip protocol

We performed pairwise comparisons of mean contralateral versus ipsilateral pupil diameter (laterality relative to the squeezing hand), during squeeze phases of the handgrip protocol, for each group and age group separately. Results of these comparisons for pupil diameter are shown in Table S8.

Table S8

Results of pairwise comparisons of mean diameter of pupil ipsilateral vs. contralateral to squeezing hand during handgrip protocol, by group and age group

Age group	Group	Contrast	Estimate	SE	df	*t*	*p*
Younger	Handgrip	Ipsilateral - Contralateral	-0.076	0.027	90	-2.78	**.027**
Younger	Control	Ipsilateral - Contralateral	0.010	0.029	90	0.335	1.000
Older	Handgrip	Ipsilateral - Contralateral	-0.235	0.030	90	-7.88	**<.001**
Older	Control	Ipsilateral - Contralateral	-0.010	0.030	90	-0.349	1.000

Section 6. Results of test-retest reliability analyses of n-back accuracy and reaction times

Intra-class correlation analysis was performed to examine test-retest reliability of mean accuracy and reaction time across runs of the n-back task. Results of these analyses are presented in Table S9.

Table S9

Intraclass correlation coefficients reflecting test-retest reliability of mean accuracy and reaction time across runs of the n-back task

Measure	Working memory load	ICC	95% CI	*F*(df1, df2)	p
Mean accuracy	0-back	0.675	0.553 - 0.768	*F*(107, 216) = 3.08	<.001
	1-back	0.529	0.352 - 0.664	*F*(107, 215.9) = 2.12	<.001
	2-back	0.620	0.476 - 0.729	*F*(107, 214.9) = 2.62	<.001
	3-back	0.601	0.382 - 0.752	*F*(55, 109.7) = 2.55	<.001
Mean reaction time	0-back	0.826	0.760 - 0.876	*F*(107, 215.7) = 5.73	<.001
	1-back	0.832	0.767 - 0.882	*F*(103, 188.6) = 6.14	<.001
	2-back	0.651	0.515 - 0.754	*F*(100, 202) = 2.87	<.001
	3-back	0.792	0.665 - 0.876	*F*(47, 93.6) = 4.91	<.001

Section 7. Results of pairwise comparisons of n-back accuracy and reaction times

We performed pairwise comparisons of each n-back performance measure (mean accuracy, mean reaction time) for the handgrip versus control group, for each working memory load and age group separately. Results of comparisons of n-back accuracy are shown in Table S10 and those of n-back reaction times in Table S11.

Table S10

Results of pairwise comparisons of mean accuracy on the n-back task for the handgrip versus control group, by working memory load and age group

Age group	Contrast	Load	Bootstrapped estimate	95% CI	Bootstrapped *p*	Bootstrapped *p*, adjusted
Younger	Handgrip - Control	0-back	0.026	0.008 - 0.053	**.002**	**.008**
Younger	Handgrip - Control	1-back	0.022	-0.029 - 0.098	.470	1.000
Younger	Handgrip - Control	2-back	-0.018	-0.09 - 0.068	.675	1.000
Younger	Handgrip - Control	3-back	-0.014	-0.117 - 0.092	.807	1.000
Older	Handgrip - Control	0-back	-0.030	-0.101 - 0.014	.276	.827
Older	Handgrip - Control	1-back	0.029	-0.042 - 0.106	.456	1.000
Older	Handgrip - Control	2-back	0.033	-0.056 - 0.126	.464	1.000

Note. Because mean accuracy data deviated from normality, pairwise comparisons were performed using a bootstrapping procedure. 95% confidence intervals (CIs) and *p*-values were calculated from bootstrap samples.

Table S11

Results of pairwise comparisons of mean reaction times on the n-back task for the handgrip versus control group, by working memory load and age group

Age group	Contrast	Load	Bootstrapped estimate	95% CI	Bootstrapped *p*	Bootstrapped *p*, adjusted
Younger	Handgrip - Control	0-back	-0.079	-0.156 - -0.001	**.048**	.192
Younger	Handgrip - Control	1-back	-0.070	-0.142 - 0.005	.064	.256
Younger	Handgrip - Control	2-back	-0.106	-0.202 - -0.006	**.040**	.160
Younger	Handgrip - Control	3-back	-0.094	-0.218 - 0.027	.144	.575
Older	Handgrip - Control	0-back	-0.056	-0.121 - 0.002	.066	.264
Older	Handgrip - Control	1-back	-0.077	-0.16 - 0.014	.092	.368
Older	Handgrip - Control	2-back	-0.114	-0.191 - -0.031	**.008**	**.032**

Note. Because mean reaction time data deviated from normality, pairwise comparisons were performed using a bootstrapping procedure. 95% confidence intervals (CIs) and *p*-values were calculated from bootstrap samples.

Section 8. Results of pairwise comparisons of salivary alpha amylase and cortisol levels

We performed pairwise comparisons of each salivary assay (alpha amylase, cortisol) across relevant timepoints, for each group and age group separately. Results of comparisons of salivary alpha amylase are shown in Table S12 and those for comparisons of salivary cortisol in Table S13.

Table S12

Results of pairwise comparisons of salivary alpha amylase levels before and after the first handgrip (control) run, for each group and age group

Age group	Group	Contrast	Bootstrapped estimate	95% CI	Boot-strapped *p*	Boot-strapped *p*, adjusted
Younger	Handgrip	Post-handgrip #1 - Pre-handgrip #1	-11.866	-24.243 - 0.765	.058	.232
Younger	Control	Post-handgrip #1 - Pre-handgrip #1	-10.066	-21.075 - -0.451	**.040**	.160
Older	Handgrip	Post-handgrip #1 - Pre-handgrip #1	-19.403	-46.918 - 6.077	.132	.527
Older	Control	Post-handgrip #1 - Pre-handgrip #1	-11.883	-41.951 - 11.745	.364	1.000

Note. Because salivary alpha amylase values deviated from normality, pairwise comparisons were performed using a bootstrapping procedure. 95% confidence intervals and *p*-values were calculated from bootstrap samples. CI = confidence interval.

Table S13

Results of pairwise comparisons of salivary cortisol levels before the baseline period and after all n-back and handgrip (control) runs, for each group and age group

Age group	Group	Contrast	Bootstrapped estimate	95% CI	Boot-strapped *p*	Boot-strapped *p*, adjusted
Younger	Handgrip	Post-tasks - Pre-baseline	-0.031	-0.056 - -0.006	**.020**	.080
Younger	Control	Post-tasks - Pre-baseline	-0.026	-0.047 - -0.002	**.028**	.112
Older	Handgrip	Post-tasks - Pre-baseline	0.000	-0.01 - 0.01	.975	1.000
Older	Control	Post-tasks - Pre-baseline	0.005	-0.007 - 0.016	.442	1.000

Note. Because salivary cortisol values deviated from normality, pairwise comparisons were performed using a bootstrapping procedure. 95% confidence intervals and *p*-values were calculated from bootstrap samples. CI = confidence interval.

Section 9. Results of test-retest reliability analyses of tonic arousal measures during n-back task

Intra-class correlation analysis was performed to examine test-retest reliability of mean pupil diameter during fixation periods, and mean heart rate and sympathetic tone during n-back task blocks, across runs of the n-back task. Results of these analyses are presented in Table S14.

Table S14

Intraclass correlation coefficients reflecting test-retest reliability of measures of tonic arousal across runs of the n-back task

Measure	ICC	95% CI	$F(df_1, df_2)$	*p*
Mean pupil diameter during n-back fixation periods	0.805	0.716 - 0.869	$F(78, 155.2) = 5.19$	**<.001**
Mean heart rate during n-back task blocks	0.886	0.839 - 0.921	$F(92, 185.9) = 8.75$	**<.001**
Mean sympathetic tone during n-back task blocks	0.829	0.759 - 0.881	$F(92, 184.8) = 5.89$	**<.001**

Section 10. Results of pairwise comparisons of tonic arousal measures during the n-back task

We performed pairwise comparisons of arousal measures during the n-back task (fixation pupil diameter, heart rate, and sympathetic tone) for the handgrip versus control group, for each n-back block relative to handgrip offset and age group separately. Results of comparisons of fixation pupil diameter are shown in Table S15, those of heart rate are shown in Table S16, and those of sympathetic tone are shown in Table S17.

Table S15

Results of pairwise comparisons of pupil diameter during n-back fixation periods for the handgrip versus control group, for each n-back block relative to handgrip offset and age group

Age group	Contrast	Block, relative to handgrip offset	Estimate	SE	df	*t*	*p*
Younger	Handgrip - Control	1	-0.442	0.293	33	-1.512	.140
Younger	Handgrip - Control	2	0.315	0.304	33	1.036	.308
Younger	Handgrip - Control	3	0.286	0.300	33	1.954	.347
Younger	Handgrip - Control	4	0.185	0.284	33	0.650	.520
Older	Handgrip - Control	1	0.859	0.362	26	2.371	**.025**
Older	Handgrip - Control	2	0.284	0.368	26	0.773	.446
Older	Handgrip - Control	3	0.587	0.338	26	1.735	.095

Table S16

Results of pairwise comparisons of heart rate during n-back task blocks for the handgrip versus control group, for each n-back block relative to handgrip offset and age group

Age group	Contrast	Block, relative to handgrip offset	Estimate	SE	df	*t*	*p*
Younger	Handgrip - Control	1	0.648	1.459	46	0.444	.659
Younger	Handgrip - Control	2	1.176	1.338	46	0.879	.384
Younger	Handgrip - Control	3	0.679	1.215	46	0.559	.579
Younger	Handgrip - Control	4	0.026	1.480	46	0.018	.986
Older	Handgrip - Control	1	-1.986	1.839	44	-1.080	.286
Older	Handgrip - Control	2	-0.516	1.668	44	-0.309	.758
Older	Handgrip - Control	3	-0.898	1.301	44	-0.691	.493

Table S17

Results of pairwise comparisons of sympathetic tone during n-back task blocks for the handgrip versus control group, for each n-back block relative to handgrip offset and age group

Age group	Contrast	Block, relative to handgrip offset	Estimate	SE	df	*t*	*p*
Younger	Handgrip - Control	1	0.001	0.001	47	1.077	.287
Younger	Handgrip - Control	2	0.001	0.001	47	1.003	.321
Younger	Handgrip - Control	3	0.001	0.001	47	0.940	.352
Younger	Handgrip - Control	4	0.001	0.001	47	0.895	.375
Older	Handgrip - Control	1	-0.001	0.001	44	-0.755	.454
Older	Handgrip - Control	2	0.000	0.001	44	-0.366	.716
Older	Handgrip - Control	3	0.001	0.001	44	0.391	.698

Section 11. Results of pairwise comparisons of phasic pupillary responses during the n-back task

We performed pairwise comparisons of maximum pupil diameter and the onset time of maximum pupil diameter on n-back trials for the handgrip versus control group, for each working memory load and age group separately. Results of comparisons of maximum pupil diameter are shown in Table S18, and results of comparisons of onset time of maximum pupil diameter are shown in Table S19.

Table S18

Results of pairwise comparisons of maximum pupil diameter during n-back trials for the handgrip versus control group, for each working memory load and age group

Age group	Contrast	Load	Estimate	SE	df	*t*	*p*
Younger	Handgrip - Control	0-back	-0.269	0.269	39	-1.001	.323
Younger	Handgrip - Control	1-back	0.257	0.223	39	1.155	.255
Younger	Handgrip - Control	2-back	-0.062	0.262	39	-0.237	.814
Younger	Handgrip - Control	3-back	-0.443	0.300	39	-1.476	.148
Older	Handgrip - Control	0-back	-0.335	0.197	31	-1.701	.099
Older	Handgrip - Control	1-back	0.372	0.256	31	1.455	.156
Older	Handgrip - Control	2-back	-0.288	0.322	31	-0.897	.377

Table S19

Results of pairwise comparisons of onset time of maximum pupil diameter during n-back trials for the handgrip versus control group, for each working memory load and age group

Age group	Contrast	Load	Estimate	SE	df	*t*	*p*
Younger	Handgrip - Control	0-back	-0.010	0.042	39	-0.237	.814
Younger	Handgrip - Control	1-back	-0.033	0.047	39	-0.697	.490
Younger	Handgrip - Control	2-back	-0.089	0.053	39	-1.665	.104
Younger	Handgrip - Control	3-back	-0.057	0.038	39	-1.494	.143
Older	Handgrip - Control	0-back	0.002	0.055	31	0.037	.971
Older	Handgrip - Control	1-back	-0.038	0.054	31	-0.710	.483
Older	Handgrip - Control	2-back	-0.045	0.059	31	-0.754	.457

Section 12. Results of test-retest reliability analyses of phasic pupillary measures during n-back task

Intra-class correlation analysis was performed to examine test-retest reliability of maximum pupil diameter and onset time of maximum pupil diameter during n-back trials, across runs of the n-back task. Results of these analyses are presented in Table S20.

Table S20

Intraclass correlation coefficients reflecting test-retest reliability of phasic pupillary values across runs of the n-back task

Measure	Working memory load	ICC	95% CI	$F(df_1, df_2)$	*p*
Maximum pupil diameter during trials	0-back	0.335	-0.121 - 0.633	$F(34, 66.1) = 1.55$	.064
	1-back	0.201	-0.284 - 0.520	$F(50, 100.1) = 1.25$	.175
	2-back	0.290	-0.170 - 0.591	$F(41, 84) = 1.42$	.090
	3-back	0.253	-0.525 - 0.671	$F(20, 41.6) = 1.34$	.208
Onset time of maximum pupil diameter during trials	0-back	0.566	0.249 - 0.765	$F(34, 69.4) = 2.34$	**.001**
	1-back	0.685	0.497 - 0.811	$F(49, 99.7) = 3.16$	**<.001**
	2-back	0.655	0.421 - 0.804	$F(41, 82.2) = 2.86$	**<.001**
	3-back	0.138	-0.827 - 0.628	$F(20, 40.5) = 1.16$	.338

Supplementary References

Vest, A. N., Da Poian, G., Li, Q., Liu, C., Nemati, S., Shah, A. J., & Clifford, G. D. (2018). An open source benchmarked toolbox for cardiovascular waveform and interval analysis. *Physiological Measurement*, *39*(10), 105004. https://doi.org/10.1088/1361-6579/aae021