

Supplementary Information

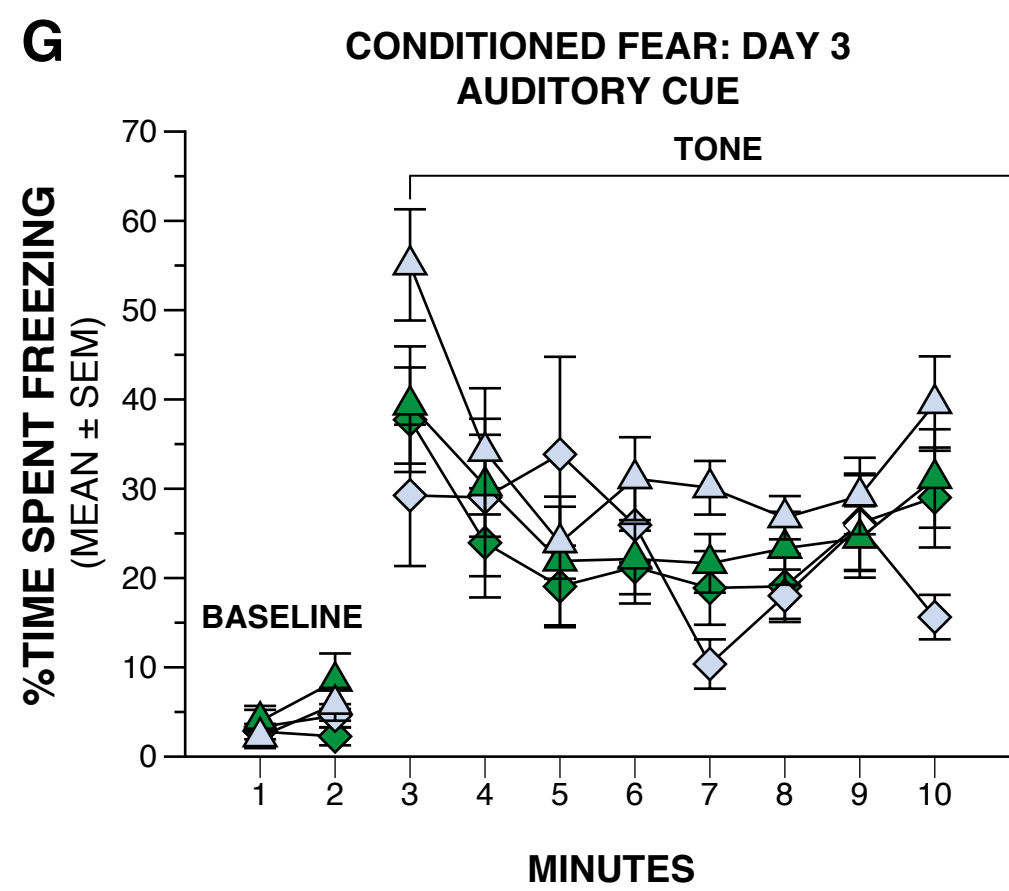
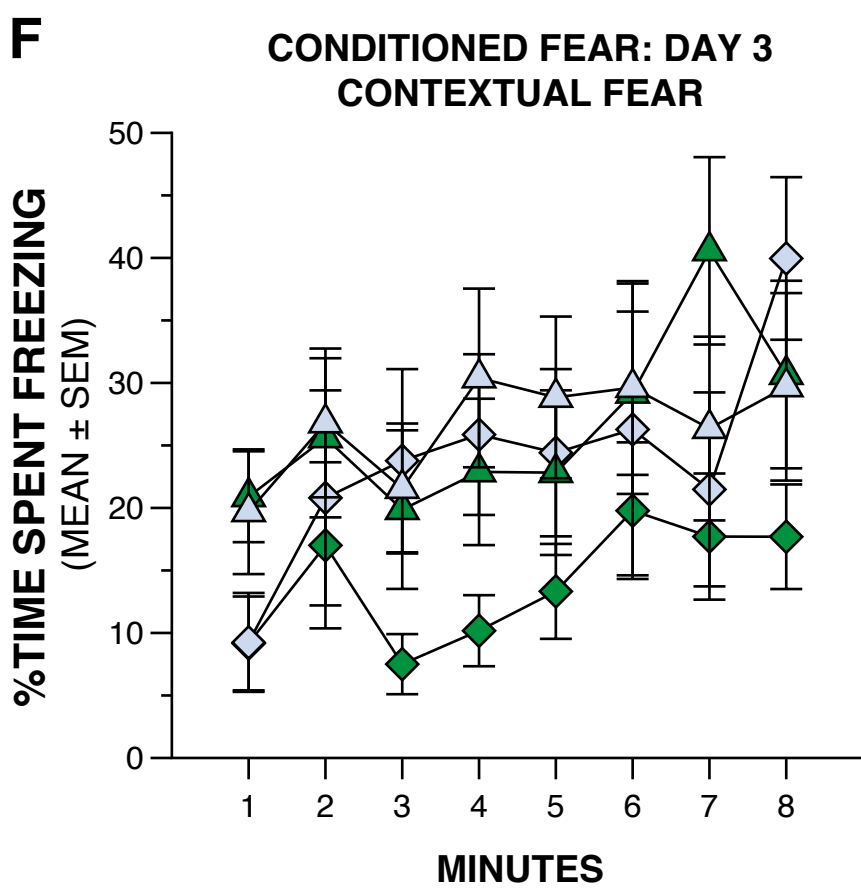
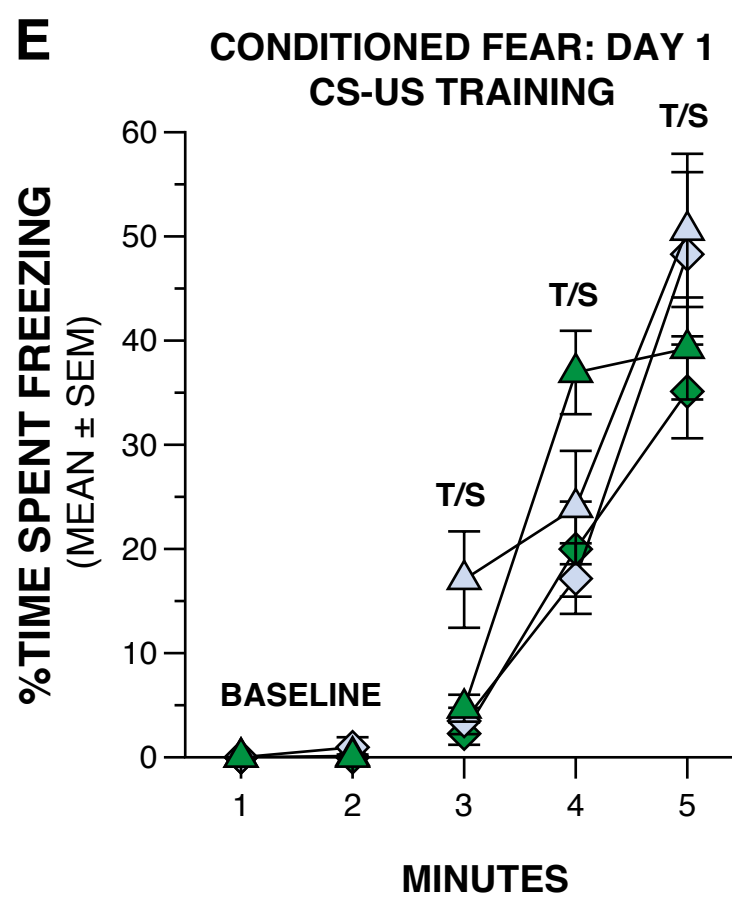
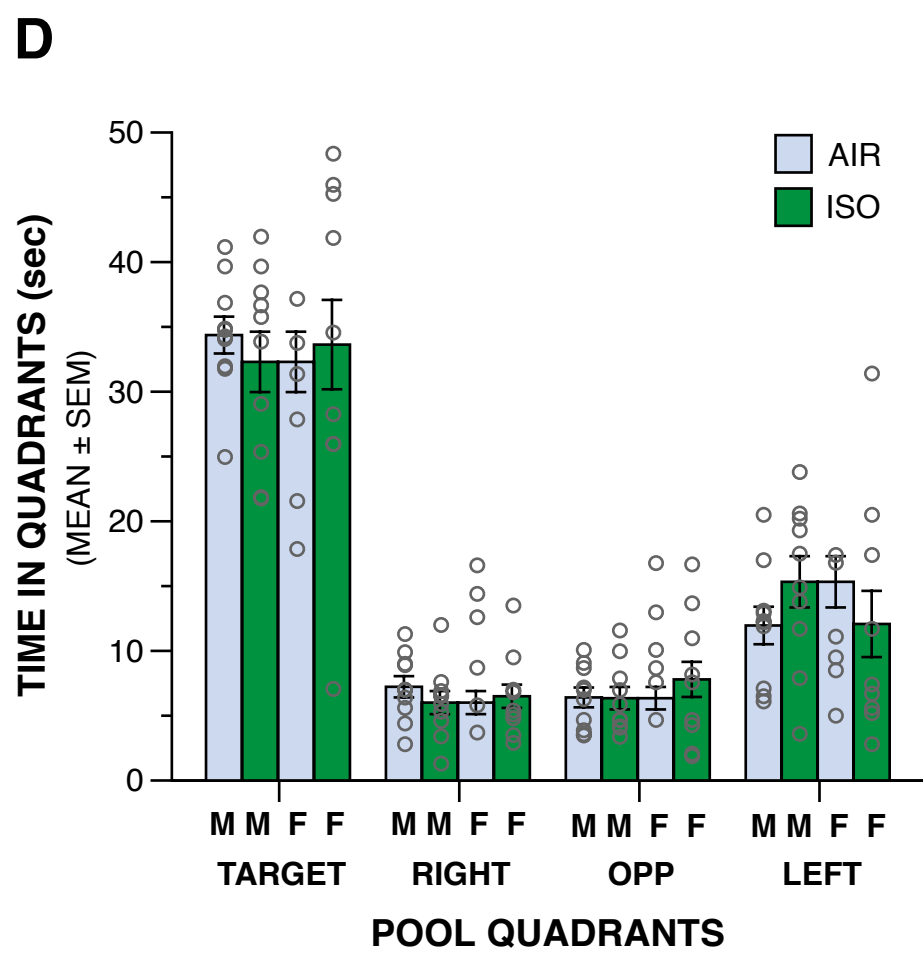
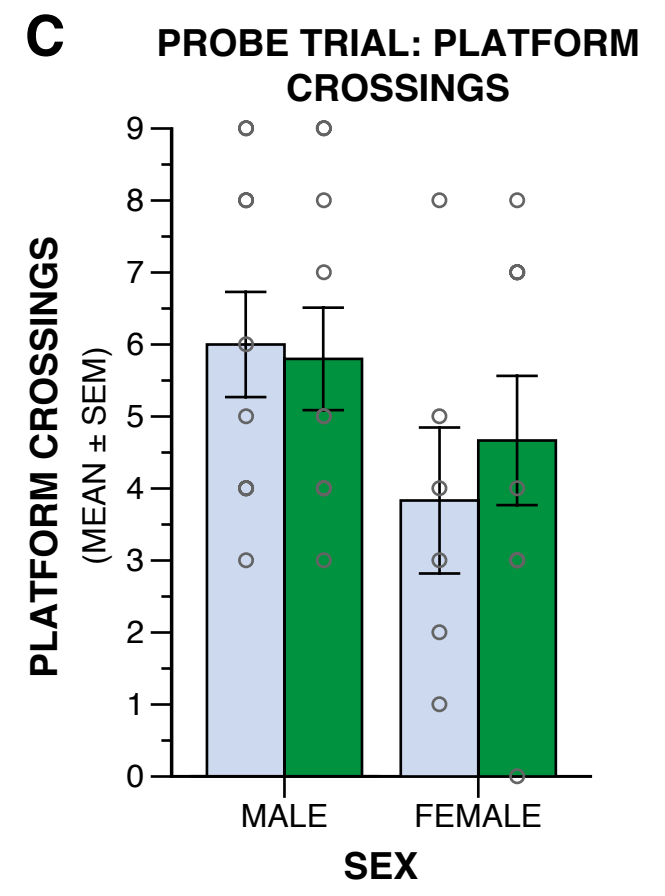
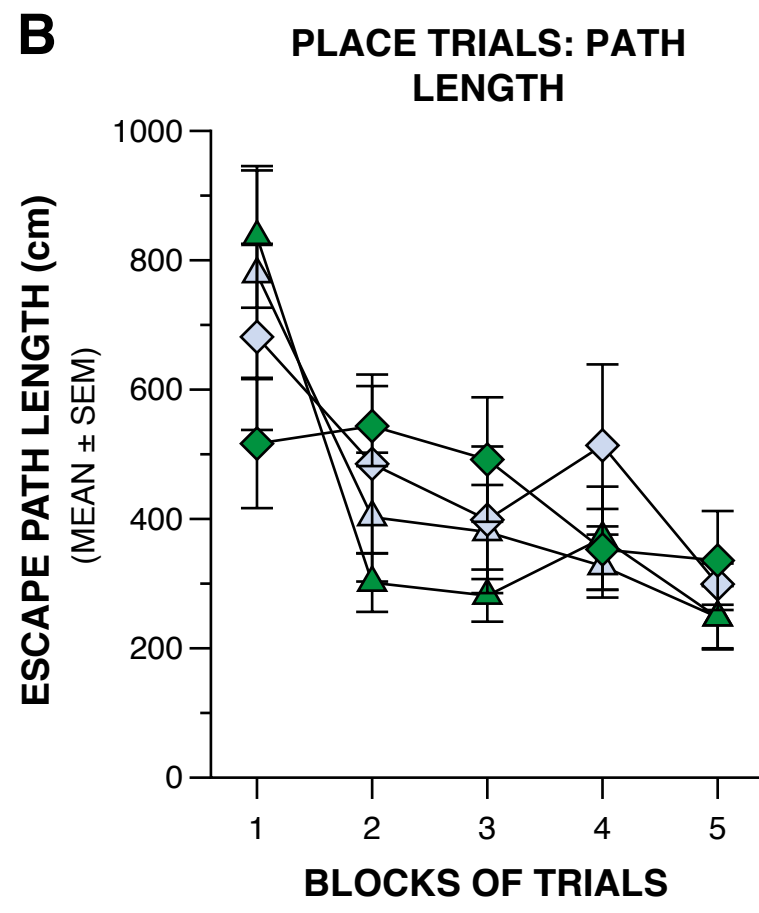
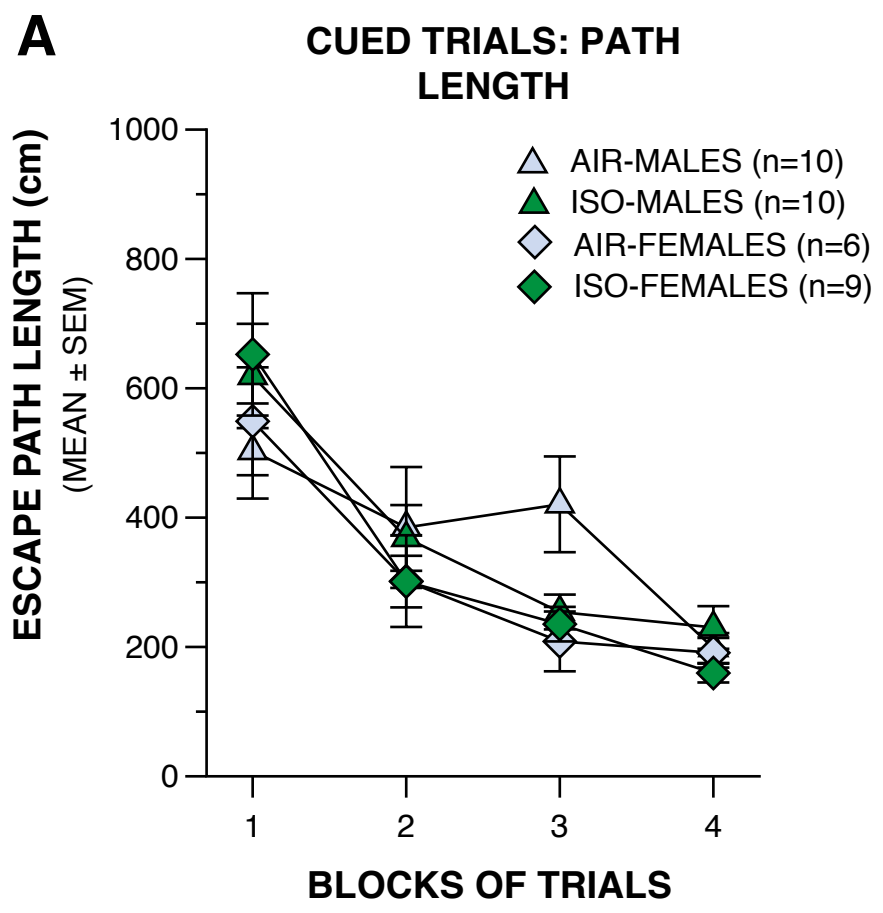
Repeated neonatal isoflurane exposures in the mouse induce apoptotic degenerative changes in the brain and relatively mild long-term behavioral deficits.

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Supplementary Table S1. Statistical results of behavioral testing of P3+5+7 ISO- and AIR-exposed mice.

VARIABLE	COMPARISON	STATISTICAL TEST	OUTPUT	P VALUE	NONPARAMETRIC
Body weights	Age x Drug interaction (P3, P4, P6, P8, P14, P21)	two-way rmANOVA	$F(1,32,40.96)=0.716$	$p=.612$	n/a
	Drug (P30)	two-way ANOVA	$F(1,31)=0.007$	$p=.935$	normal
	Drug (P45)	two-way ANOVA	$F(1,31)=0.230$	$p=.635$	normal
	Drug (P60)	two-way ANOVA	$F(1,31)=0.669$	$p=.420$	normal
Cued, path length (across 4 blocks of trials)	Drug	two-way rmANOVA	$F(1,31)=0.093$	$p=.763$	normal
	Trial	two-way rmANOVA	$F(2.59,80.38)=25.454$	$p<.000005$	normal
	Males only, Drug	rmANOVA	$F(1,18)=0.066$	$p=.800$	n/a
	Females only, Drug	rmANOVA	$F(1,13)=0.391$	$p=.543$	normal
Cued, latency to platform (across 4 blocks of trials)	Drug	two-way rmANOVA	$F(1,31)=0.099$	$p=.755$	normal
	Trial	two-way rmANOVA	$F(2.64,81.67)=29.064$	$p<.000005$	normal
	Males only, Drug	rmANOVA	$F(1,18)=0.388$	$p=.541$	normal
	Females only, Drug	rmANOVA	$F(1,13)=0.612$	$p=.448$	normal
Cued, swim speeds (across 4 blocks of trials)	Drug	two-way rmANOVA	$F(1,31)=0.006$	$p=.939$	normal
	Trial	two-way rmANOVA	$F(1,93)=5.894$	$p=.001$	normal
	Males only, Drug	rmANOVA	$F(1,18)=0.324$	$p=.576$	normal
	Females only, Drug	rmANOVA	$F(1,13)=0.202$	$p=.661$	n/a
Place, path length (across 5 blocks of trials)	Drug	two-way rmANOVA	$F(1,31)=0.320$	$p=.576$	normal
	Trial	two-way rmANOVA	$F(3.37,104.37)=12.152$	$p<.000005$	normal
	Males only, Drug	rmANOVA	$F(1,18)=0.124$	$p=.729$	normal
	Females only, Drug	rmANOVA	$F(1,13)=0.210$	$p=.654$	normal
Place, latency to platform (across 5 blocks of trials)	Drug	two-way rmANOVA	$F(1,31)=0.108$	$p=.744$	normal
	Trial	two-way rmANOVA	$F(3.68,114.19)=8.493$	$p=.000009$	normal
	Males only, Drug	rmANOVA	$F(1,18)=0.167$	$p=.687$	normal
	Females only, Drug	rmANOVA	$F(1,13)=0.002$	$p=.965$	normal
Place, swim speeds (across 5 blocks of trials)	Drug	two-way rmANOVA	$F(1,31)=0.066$	$p=.798$	normal
	Trial	two-way rmANOVA	$F(4,124)=20.016$	$p<.000005$	normal
	Males only, Drug	rmANOVA	$F(1,18)=0.263$	$p=.614$	normal
	Females only, Drug	rmANOVA	$F(1,13)=0.893$	$p=.362$	normal
Probe, target quadrant spatial bias	Drug x Quadrant interaction	two-way rmANOVA	$F(1.64,50.70)=0.849$	$p=.413$	normal
	Males only, Drug x Quadrant interaction	rmANOVA	$F(1.77,31.98)=1.054$	$p=.353$	normal
	Females only, Drug x Quadrant interaction	rmANOVA	$F(1.57,20.36)=0.771$	$p=.446$	normal
Percent time freezing (across test minutes)	Day 1 baseline, Drug	two-way rmANOVA	$F(1,31)=2.652$	$p=.114$	n/a
	Day 1 shock+tone pairing, Drug	two-way rmANOVA	$F(1,31)=1.141$	$p=.294$	n/a
	Day 2 contextual fear, Drug	two-way rmANOVA	$F(1,31)=1.047$	$p=.314$	normal
	Day 3 baseline, Drug	two-way rmANOVA	$F(1,31)=0.064$	$p=.802$	n/a
	Day 3 auditory cued fear, Drug	two-way rmANOVA	$F(1,31)=0.732$	$p=.399$	normal

Equivalent nonparametric test for two-way rmANOVA not available (n/a) for non-normal distributions.



Supplementary Figure S1. Disruptions in learning and memory performance were not observed in mice exposed to neonatal ISO multiple times. Male and female mice were exposed to 1.5% ISO for 3h or AIR only on P3+5+7 and behaviorally characterized across multiple domains beginning during the juvenile stage and continuing into adulthood. **A-B)** ISO and AIR mice of both sexes traveled comparable path lengths to the platform during **(A)** cued and **(B)** place trials of the MWM. **C-D)** During the MWM probe trial, ISO and AIR mice of both sexes demonstrated **(C)** comparable platform location crossings and **(D)** spatial bias for the target quadrant containing the escape platform. **E-G)** ISO and AIR mice spent a comparable percent time freezing during the three days of fear conditioning: **(E)** pairing of shock + tone, **(F)** contextual fear, and **(G)** auditory cued fear. Means \pm SEM are shown. All data points represented by open gray circles.

Supplementary Table S2. Statistical results of behavioral re-testing in later adulthood of P3+5+7 ISO- and AIR-exposed mice.

VARIABLE	COMPARISON	STATISTICAL TEST	OUTPUT	P VALUE	NONPARAMETRIC
Total ambulations (across 10-min blocks)	Drug x Sex x Block	two-way rmANOVA	$F(5,155)=2.626$	$p=.026$	normal
	Males only, Drug	rmANOVA	$F(1,18)=0.374$	$p=.549$	n/a
	Females only, Drug	rmANOVA	$F(1,13)=3.294$	$p=.093$	normal
Time in open arm (across 3 days)	Drug	two-way rmANOVA	$F(1,31)=0.009$	$p=.925$	normal
	Males only, Drug	rmANOVA	$F(1,18)=1.771$	$p=.200$	normal
	Females only, Drug	rmANOVA	$F(1,13)=0.767$	$p=.397$	normal
Percent time in open arm (Aross 3 days)	Drug	two-way rmANOVA	$F(1,31)=0.043$	$p=.837$	n/a
	Males only, Drug	rmANOVA	$F(1,18)=2.066$	$p=.168$	normal
	Females only, Drug	rmANOVA	$F(1,13)=0.687$	$p=.422$	normal
Sociability, investigation zone time (social vs. empty)	Drug x Zone	two-way rmANOVA	$F(1,31)=0.084$	$p=.774$	normal
	AIR, Zone	planned comparison	$F(1,31)=56.521$	$p<.000005$	normal
	ISO, Zone	planned comparison	$F(1,31)=78.950$	$p<.000005$	normal
	AIR males only, Zone	planned comparison	$F(1,31)=57.151$	$p<.000005$	normal
	AIR females only, Zone	planned comparison	$F(1,31)=13.350$	$p=.001$	normal
	ISO males only, Zone	planned comparison	$F(1,31)=45.654$	$p<.000005$	normal
	ISO females only, Zone	planned comparison	$F(1,31)=34.078$	$p<.000005$	normal
Social novelty, investigation zone time (familiar vs. novel)	Drug x Zone	two-way rmANOVA	$F(1,31)=0.846$	$p=.365$	normal
	AIR, Zone	planned comparison	$F(1,31)=28.285$	$p=.00001$	normal
	ISO, Zone	planned comparison	$F(1,31)=12.243$	$p=.001$	normal
	AIR males only, Zone	planned comparison	$F(1,31)=9.678$	$p=.004$	normal
	AIR females only, Zone	planned comparison	$F(1,31)=4.065$	$p=.053$	normal
	ISO males only, Zone	planned comparison	$F(1,31)=18.844$	$p=.0001$	normal
	ISO females only, Zone	planned comparison	$F(1,31)=10.304$	$p=.003$	normal

Equivalent nonparametric test for two-way rmANOVA not available (n/a) for non-normal distributions.