Critical Period Plasticity is Associated with Resilience to Short Unpredictable Stress

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Abstract

Exposure to stress can increase the risk of depression in susceptible individuals, but not in resilient ones. Resilience to stress decreases with age, parallel to drastic changes in the quality of brain plasticity from juvenile to old age, suggesting that the type of plasticity found in the maturing brain promotes resilience. To indirectly test this, we administered short unpredictable stress to adult male and female mice, as well as to adolescent mice and mice that lack SynCAM 1 and display extended adolescent-like, critical period plasticity in the brain. We found that short unpredictable stress robustly increased core body temperature in all groups of mice, indicative of stress-induced hyperthermia (SIH) and confirming the efficacy of the stress paradigm. However, depressive-like behavior as measured though tail suspension test was increased in adult mice only, supporting that the type of plasticity found in the maturing brain promotes resilience to stress. All three groups of mice showed a mild increase in locomotor activity after stress, suggesting that the quality of plasticity does not correlate with resilience to anxiety-like phenotypes. Our study hence provides further evidence for the protective role of developmental plasticity during stress and points to new mechanisms that promote resilience to stress-induced depression.

Introduction

Stressful life events are strongly linked to the onset of depression (McEwen, 2012; McEwen et al., 2015; Czéh et al., 2016), which is one of the leading causes of disability in the USA (WHO). Yet, stress does not increase the risk of depression in individuals that are resilient: capable of successfully adapting to stress exposure (Faye et al., 2018; McEwen and Morrison, 2013; Russo et al., 2012). Stress resilience is thought to decline with age, parallel to changes in the quality of brain plasticity,

which transitions from robust critical period plasticity during in the developing brain to a restricted, top-down modulated process in the adult brain (Ribic, 2020). While the link between plasticity and resilience is still unclear, a previous study demonstrated that chronic restraint stress results in a reversible reduction in the length of dendritic branches in the prefrontal cortex (PFC) of young, but not aged rats, in whom the reduction persists (Bloss et al., 2010). Heightened brain plasticity during postnatal development, as well as interventions that change the quality of plasticity in adulthood, can hence promote resilience to stress (McEwen, 2016, 2012).

In support of this notion, antidepressants markedly increase plasticity in the adult brain and change its quality to a more juvenile/adolescent-like (Maya Vetencourt et al., 2008; Phoumthipphavong et al., 2016). Environmental enrichment, a classical behavioral paradigm that also "rejuvenates" the adult cortex (Greifzu et al., 2016, 2014), mitigates the effects of stress across the lifespan (Macartney et al., 2022; Lehmann and Herkenham, 2011; Hegde et al., 2020). However, both antidepressants and environmental enrichment have systemic effects on the brain, and it is still unclear if they promote resilience and mitigate the effects of stress through altered quality of brain plasticity.

In mice, a species of choice for genetic manipulations of plasticity, stress during late postnatal development has adverse effects on adult behavior and physiology (Peleg□Raibstein and Feldon, 2011; Brydges et al., 2014). However, stress administered during the juvenile period does not negatively impact behavior during adolescence, a period in which plasticity is still heightened, supporting the protective role of developmental plasticity during periods of stress (Peleg□Raibstein and Feldon, 2011). Interestingly, genetic mouse models in which developmental windows of plasticity do not close show altered behavioral responses to adverse experience (Ribic, 2020), from impairments in fear learning (Park et al., 2016; Banerjee et al., 2017) and juvenile-like responses during fear conditioning paradigms (Bhagat et al., 2016; Gogolla et al., 2009), to facilitated fear erasure (Yang et al., 2016) and fear-conditioned response (Miwa et al., 2006). While these studies indicate a complex association between plasticity and adverse experiences, they also point to a protective role of critical period plasticity after fear-inducing events and suggest that reintroducing juvenile/adolescent-like plasticity in adult brain could promote stress resilience.

To begin to address this, we tested the depressive-like and anxiety-like behaviors after a week-long bout of unpredictable stress in mice during early adolescence (postnatal days 28-35), an age at which cortical areas still display critical period plasticity (Ribic, 2020). We further tested if adult mice with extended critical period plasticity through deletion of SynCAM 1 (Synaptic Cell Adhesion Molecule 1)(Ribic et al., 2019) respond to stress in a manner similar to adolescent mice. Our results demonstrate that 1 week of unpredictable stress induces a robust physiological response regardless of age or the level of plasticity in the brain. However, adolescent wild type (WT) and adult SynCAM 1 knock-out (KO) mice are behaviorally resilient to unpredictable stress, unlike adult WT mice that show increased learned helplessness-like behavior after stress. Our study hence provides new evidence in support of the protective role of juvenile/adolescent-like plasticity in the aging brain and suggests that interventions that reintroduce such plasticity in aged brain can promote stress resilience (McEwen, 2016).

Methods

Mice and data analysis. All mice were maintained on C57BL/6 background (The Jackson Laboratory, Bar Harbor, ME) on a reverse 12:12 light:dark cycle, with food and water *ad libitum.* Young animals from both sexes were used during the 4th and 5th week after birth. Adult animals from

both sexes were used from 2-4 months of age. SynCAM 1 KO mice (Fujita et al., 2006) and their WT littermates were maintained on a C57BL/6 background and used at 2-4 months of age. Animals were randomly assigned to experimental groups and littermates were group housed for the duration of experiments, except during social isolation. Animals were treated in accordance with the University of Virginia Institutional Animal Care and Use Committee guidelines. The experimenters were blind to the genotype or group of animals used during data analysis. Both within-subject and between-subject designs were used to allow for the collection of adrenal glands, with physiological and behavioral measures compared using 2-way ANOVA (GraphPad, San Diego, CA). All data are reported as mean ± SEM, where N represents number of animals and infants used, unless indicated otherwise. Target power for all sample sizes was 0.8. In all cases, alpha was set to 0.05.

Unpredictable stress. Mice underwent unpredictable stress over a course of one week, which consisted of the following stressors: physical restraint in a 50 ml falcon tube for adult mice or 20 ml plastic scintillation vial for young mice (3 times a week for 1 hr), placement in a cage with no bedding (2 times a week for 2 hrs) or wet bedding (250 ml of distilled water added to a standard cage with bedding 2 times a week for 2 hrs), tilting the cage at a 20º angle (2 times a week for 2 hrs), shaking the cage lightly on an orbital shaker (150 rpm, 2 times a week for 2 hrs) and exposure to 37 centigrade temperature (40% humidity, 1 time for 10 mins). For males, social defeat (2 times a week for the time needed to defeat) was used as a social stressor, and social isolation (2 times a week for 12 hrs) was used for females. Social defeat was terminated as soon as intruder (test) mice display signs of social avoidance of the resident (dominant) mouse to avoid any physical wounding. Stressors were pseudorandomized (random order that was kept constant between all cohorts of mice). Before the stress week, all mice underwent a control week during which body weight and core body temperatures were measured at the same times as during stress week.

Physiological measurements. Animals were weighed daily at the beginning of the dark cycle. Core body temperature was measured using a rectal probe (Kent Scientific, Torrington, CT) inserted 2 cm deep and read as soon as the temperature stabilized. Temperature measurements were taken before the onset of stress and 15-20 minutes after the onset of stress. For control animals, temperature measurements were taken 2 successive times, 15-20 minutes apart, at the same times of day as during stress. Mice were returned to their home cage in between measurements. All temperature differences were averaged per animal before the statistical comparison.

Adrenal gland isolation. For adrenal gland isolation, mice were anesthetized with a mixture of ketamine and xylazine and transcardially perfused with warm 0.1 M phosphate buffer, followed by warm 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA). Kidneys and adrenals were isolated and postfixed overnight in 4% paraformaldehyde and washed overnight in 0.1 M phosphate buffer. Glands were isolated using a stereomicroscope (Olympus, Tokyo, Japan) and weighed using a precision scale (Mettler Toledo, Columbus, OH). Weight from both glands was averaged and divided by the animal's weight before the statistical comparison.

Tail suspension. Mice were suspended by their tail on a bar 55 cm above a platform for 6 minutes and recorded using a high frame rate camera (GoPro, San Mateo, CA), as previously described (Can et al., 2012). Videos were manually scored by experimenters blind to the experimental group being analyzed. Mice were considered immobile if no movement was detected for 3 seconds.

Open field. Mice were habituated to the room 30 minutes before the behavioral monitoring started. Individual mice were released in a 50x50x50 (all in cm) plexiglass arena with an overhead high frame rate camera (GoPro), and their activity was recorded for 10 minutes. Data was analyzed using MATLAB (Natick, MA)(Zhang et al., 2020).

Results

Short bout of unpredictable stress induces robust hyperthermia

As age correlates with neuronal resilience to stress-induced loss of dendritic complexity (Bloss et al., 2010), we hypothesized that the type of plasticity present in the young brain promotes resilience to stress. To test this, we administered a short bout of unpredictable stress to young mice during the 5th postnatal week, when the levels of cortical developmental plasticity are high (Gordon and Stryker, 1996). We did the same to adult (> 2 months old) mice that do not express SynCAM 1 (Synaptic Cell Adhesion Molecule 1), as they display critical period-like cortical plasticity (Ribic et al., 2019). Our stress paradigm was based on the well-established chronic unpredictable mild stress paradigm (Willner et al., 1987), where male and female adult and adolescent wild type, as well as adult SynCAM 1 knock out (KO) mice underwent a pseudo-randomized array of sex-specific and sex-nonspecific stressors over 1 week (Figure 1A). The sex-specific stressors included social isolation for female mice, i.e. group-housed female mice were separated to individual cages for five hours at a time, and social defeat for male mice, i.e. male mice are placed in the resident cage of an aggressor male mouse until they displayed signs of defeat (Golden et al., 2011). The sex nonspecific stressors included restraint, wet bedding, no bedding, cage tilt, cage shaking (at 150 rpm) and heat exposure (37ºC). The length of stress exposure was kept at 1 week to limit it to the peak of critical period plasticity in the cortex (typically the 5th postnatal week)(Gordon and Stryker, 1996; Ribic, 2020)

To test if our stress paradigm indeed induced a physiological stress response, we compared 3 different physiological measures of stress response using a between-subject design (adrenal gland weight, body weight and core body temperature) across different groups of mice: control and stressed adults, young mice and SynCAM 1 KO mice (Figure 1A-C). For adrenal glands, we normalized their weight to the total body weight of each mouse to account for size differences between young and adult mice, as well as between males and females. We found that the interaction between stress and age/plasticity level was not significant, with neither of the factors having an impact on adrenal gland weight (Figure 1B and Table 1; 2-way ANOVA, p=0.098, $F_{(2, 61)}$=2.42; p_{stress}=0.67, $F_{(1, 61)}$=0.2; $p_{age/plasticity}$=0.078, $F_{(2, 61)}$=2.66), in agreement with a previous study that demonstrated no changes in adrenal weights after 1 week of unpredictable stress (Luo et al., 2025). However, the young mice displayed a trend towards higher values in the stressed group, which became significant after separating the animals by sex (Figure 1C and Table 1; 2-way ANOVA, stress x sex p=0.51, $F_{(1,15)}$=0.47; p_{stress}=0.003, $F_{(1,15)}$=12.6; p_{sex}<0.0001, $F_{(1,15)}$=58.75; Sidak multiple comparison test males p=0.19, females p=0.004). As previously reported (Bielohuby et al., 2007), adrenal glands were larger in females than in males across all groups (Table 1).

It is well established that different types of stress paradigms in rodents result in reduced body weight (Patterson and Abizaid, 2013). As young mice at baseline are significantly smaller than adult mice of both genotypes (young males=18.15±0.8 g, N=3; young females=13.65±0.37 g, N=5; adult males=22.25±0.42 g, N=11; adult females=17.10±0.44 g, N=12; SynCAM 1 KO males=21.34±0.71 g, N=7; SynCAM1 KO females=17.5±0.85 g, N=6), we compared the difference between body weights at the beginning and at the end of the week in control and stressed mice instead of comparing the body weights themselves (Figure 1D). We found that one week of stress did not result in reduced weight gain in any of the groups (Figure 1D; ΔBW adult control=0.33±0.13 g, N=33; adult stress=0.21±0.097 g, N=25; young control=1.5±0.25 g, N=8; young stress=1.33±0.26 g, N=9; SynCAM 1 KO control=0.91±0.13 g, N=16; SynCAM 1 KO stress 0.43±0.33 g, N=14; 2-way ANOVA stress x age/plasticity p=0.56, $F_{(2,99)=}$0.58; p_{stress}=0.12, $F_{(1,99)=}$2.45; $p_{age/plasticity}$<0.0001,

$F_{(2,99)=}15.58$). The effect of age/plasticity was also significant, post-hoc tests (Holm Sidak) revealed that it was mostly due to weight changes in young WT mice, as expected (control adult vs young, p=0.0004; adult vs SynCAM 1 KO, p=0.037; young vs SynCAM 1 KO, p=0.2; stress adult vs young, p=0.0007; adult vs SynCAM 1 KO, p=0.76; young vs SynCAM 1 KO, p=0.02).

Stress induces a robust elevation in core body temperature within 15 minutes, a phenomenon known as stress-induced hyperthermia (SIH)(Vinkers et al., 2008). As our stress paradigm resulted in very small changes in standard measures of bodily stress response, namely adrenal gland weight (Figure 1B) and body weight (Figure 1C), we tested if SIH is present 15-20 minutes after the introduction of each stressor in our paradigm (Figure 1D). Rectal readings of core body temperatures can induce stress as well (Zethof et al., 1994), so we compared the differences in temperature (ΔT) before and after the onset of stress to ΔT between two rectal temperature measurements 15-20 minutes apart taken from control mice not exposed to short unpredictable stress (controls; Figure 1D). As expected, we found a robust elevation of core body temperature in stressed mice of both ages and genotypes that was significantly higher than the ΔT of control mice (ΔT adult control=0.04±0.08 ºC, N=24; adult stress=0.54±0.06 ºC, N=19; young control=0.22±0.13 ºC, N=13; young stress=0.66±0.06 ºC, N=12; SynCAM 1 KO control=-0.07±0.09 ºC, N=6; SynCAM 1 KO stress=0.41±0.04 ºC, N=32; 2-way ANOVA stress x age/plasticity p=0.95, $F_{(2,100)=}0.05$; $p_{stress}<0.0001$, $F_{(2,100)=}42.75$; $p_{age/plasticity}=0.02$, $F_{(2,100)=}4.05$). While the effect of age/plasticity was also significant, post-hoc tests (Holm Sidak) revealed no significant differences between adult, young and SynCAM 1 KO mice within control or stress groups. Altogether, our results demonstrate that a short bout of unpredictable stress induces a robust elevation of core body temperature and an increase in adrenal gland weight in young female mice, indicating the validity of our paradigm.

Stress does not result in depressive-like behaviors in mice with open cortical critical periods

Once we established that our stress paradigm induces a robust physiological response (Figure 1), we tested how mice in whom developmental critical periods of plasticity are still open, namely young wild type (WT) and adult SynCAM 1 KO mice, behaviorally respond to stress compared to adult WT mice. We used two well-established behavioral tests to measure depressive-like and anxiety-like behaviors: tail suspension test (TST) and open field test (OFT; respectively)(Figure 2)(Cryan et al., 2005; Can et al., 2012; Seibenhener and Wooten, 2015).

TST is a measure of learned helplessness-like behavior in rodents that quantifies the fraction of time spent immobilized after exposure to a stressful stimulus, as well as the latency to immobilization. As expected, stressed adult WT mice show a significant increase in the fraction of time spent immobilized (Figure 2A; 2-way repeated measures (RM) ANOVA, stress x age/plasticity $p<0.0001$, $F_{(2,45)}=25.24$; $p_{stress}=0.04$, $F_{(1,45)}=4.11$; $p_{age/plasticity}<0.0001$, $F_{(2,45)}=18.3$; adult WT control=31.79±2.79, stress=53.73±3.5, N=17, p<0.0001; young control=21.74±3.37, stress=16.75±3.22, N=13, p=0.42; SynCAM 1 KO control=26.66±3.29 s, stress=21±3.44, N=18, p=0.18; all values in %, Sidak's post-hoc) and a significant reduction in time lapsed to first immobilization (Figure 2B; 2-way repeated measures (RM) ANOVA, stress x age/plasticity p=0.054, $F_{(2,45)}=3.12$; $p_{stress}=0.002$, $F_{(1,45)}=10.41$; $p_{age/plasticity}=0.0003$, $F_{(2,45)}=9.75$; adult WT control=82.71±10.44 s, stress=37.18±3.63 s, N=17, p=0.0007; young control=108.54±12.04 s, stress=103.46±14.42 s, N=13, p=0.98; SynCAM 1 KO control=75.72±5.55 s, stress=60.06±9.36 s, N=18, p=0.42; Sidak's post-hoc). The effect of age/plasticity level was present in both measures, mostly reflecting the differences in adult WT mice compared to other groups (fraction of time immobilized adult vs young and adult vs SynCAM 1 KO, p<0.0001; young vs SynCAM 1 KO,

p=0.65; latency to first immobilization adult vs young, P<0.0001; adult vs SynCAM 1 KO, p=0.21; young vs SynCAM 1 KO, p=0.007).

To test if our stress paradigm increases anxiety-like behaviors, we compared the preference of mice to stay close to the walls of the open field arena (thigmotaxis, Figure 2C) as well as their locomotor activity (Figure 2D). Interestingly, we found that short unpredictable stress did not impact thigmotaxis (Figure 2C; 2-way repeated measures (RM) ANOVA, stress x age/plasticity p=0.65, $F_{(2,62)}$=0.44; p_{stress}=0.82, $F_{(1,62)}$=0.05; $p_{age/plasticity}$=0.07, $F_{(2,62)}$=2.79; adult WT control=0.83±0.01, stress=0.82±0.01, N=29; young control=0.76±0.01, stress=0.78±0.02, N=9; SynCAM 1 KO control=0.81±0.02, stress=0.81±0.02, N=21). However, as previously reported (Sequeira Cordero et al., 2019), stressed mice from all groups travelled more in the open field arena, with SynCAM 1 KO mice traversing the longest distance (Figure 2D; 2-way repeated measures (RM) ANOVA, stress x age/plasticity p=0.72, $F_{(2,62)}$=0.32; p_{stress}=0.02, $F_{(1,62)}$=5.42; $p_{age/plasticity}$=0.0014, $F_{(2,62)}$=7.35; adult WT control=24.26±0.98 m, stress=26.39±1.53 m, N=29, p=0.51; young control=29.78±1.95 m, stress=32.66±1.88 m, N=9, p=0.72; SynCAM 1 KO control=30.05±2.18 m, stress=34.14±2.21 m, N=21, p=0.07). SynCAM 1 KO mice crossed more distance when compared to adult mice in both control and stress groups (adult vs SynCAM 1 KO control p=0.03, stress=0.002), but not compared to young mice (young vs SynCAM 1 KO control p=0.99, stress p=0.96), confirming previous reports of increased locomotor activity in these mice (Giza et al., 2013). Locomotor activity in all mouse groups did not correlate with the fraction of time spent immobilized in TST, suggesting that increased overall locomotor activity in young and SynCAM 1 KO mice is not the cause of their resilience to stress in TST assay (Pearson r adult=-0.07, p=0.76; young=0.04, p=0.89; SynCAM 1 KO=-0.02, p=0.91). Altogether, our results demonstrated that short unpredictable stress robustly increases depressive-like behaviors in adult mice, but not in young mice or adult mice with critical period-like cortical plasticity.

Discussion

Since Hubel and Wiesel reported a temporally restricted window of plasticity in the kitten cortex during which neurons robustly respond to changes in sensory input (Hubel and Wiesel, 1970), many studies have attempted to restore that type of plasticity to the adult brain aiming to mitigate the damage caused by a myriad of stressors (Ribic, 2020; Hensch and Quinlan, 2018; Levelt and Hübener, 2012). In support of this longstanding notion, antidepressants can restore juvenile-like plasticity to the adult cortex (Maya Vetencourt et al., 2008), but it is still unclear if they promote recovery from stress through changing the quality of plasticity in the adult brain. Our study attempted to shed more light on this issue by testing if short unpredictable stress results in depressive-like behavior in adolescent mice in whom the cortical window of plasticity is still open, as well as in adult mice in whom the plasticity windows are extended into adulthood through deletion of SynCAM 1 (Ribic et al., 2019). We found that this is indeed the case, supporting that interventions that change the quality of plasticity in the adult brain can facilitate stress resilience (Nestler and Russo, 2024; McEwen, 2016; Fuchs et al., 2004).

The duration and type of stressors, along with age and sex of animals, can have a significant impact on physiological measures of stress, as evident in our results as well (Luo et al., 2025; Monteiro et al., 2015). Our stress paradigm did not result in abrogated weight gain in any of the mouse groups, likely because of the short duration of our protocol, as well as the stressors themselves. Adrenal gland weight was, however, increased in young female mice and all 3 groups of mice displayed robust stress-induced hyperthermia, indicating that our protocol was stressful to them. In agreement, adult mice displayed an increase in depressive-like behaviors as measured with tail

suspension test (TST). Interestingly, adolescent mice and adult SynCAM 1 KO mice did not show any changes in immobilization after stress, in support of the notion that juvenile/adolescent-like plasticity promotes resilience to stress. While it is possible that increased locomotor activity of young and adult SynCAM 1 KO mice results in reduced immobilization during TST, we find it unlikely as longer stress duration in adolescent mice results in increased immobilization in female mice (Hu et al., 2025). Further, we found no correlation between immobility and locomotor activity in the open field arena, indicating that young mice and adult SynCAM 1 mice indeed are resilient to short unpredictable stress. Based on our and other studies (Hu et al., 2025), Future studies can now address the impact of longer stress duration or more severe stressors on the behavior of adolescent and adult SynCAM 1 KO mice, and whether juvenile/adult-like plasticity provides a lasting protection from stress.

Unlike immobilization, locomotor activity was mildly impacted by stress, indicated by increased distance travelled in the open field arena in all three groups of mice. While within-group post-hoc comparisons were not statistically significant, our results are in line with previous research that demonstrated stress-associated hyperlocomotion (Sequeira□Cordero et al., 2019). Distance travelled positively correlates with corticosterone levels in mice head-fixed above a treadmill, which itself is stressful (Juczewski et al., 2020), suggesting that increased locomotion is a stress response. Locomotor activity is traditionally classified as anxiety-like behavior, indicating that juvenile/adolescent-like plasticity may not promote resilience to anxiety after stress. However, future studies need to address this issue using paradigms that can assess anxiety-like phenotype independent of locomotion, like novelty suppressed feeding (NSF) (Koskinen and Hovatta, 2023).

While the type of plasticity in our study correlates with resilience to short unpredictable stress, it is possible that the resilience of SynCAM 1 KO mice is due to another mechanism. SynCAM 1 gene expression is not restricted to the brain (Fujita et al., 2006) and, while SynCAM 1 protein is localized to the membrane, it can trigger multiple signaling cascades that promote neuronal resilience to stress (Cheadle and Biederer, 2012; Murakami et al., 2014). However, we find this unlikely, as SynCAM 1 KO mice display overall accelerated learning in spatial tasks and juvenile-like responses to noxious stimuli (Park et al., 2016; Robbins et al., 2010). SynCAM 1 is a potent plasticity brake in the adult brain and even a transient knock-down of its expression can restore juvenile/adolescent like plasticity in the adult primary visual cortex (Ribic et al., 2019). Our study is hence in line with the notion that critical period-like plasticity is protective of stress and provides a rationale for further inquiries into the link between resilience and different types of plasticity.

Our study is limited in its scope, but our findings unambiguously demonstrate the efficacy of short unpredictable stress in inducing depressive-like behaviors in adult mice. The short duration and ease of administering our protocol will undoubtedly facilitate further studies of short unpredictable stress and its long-term effects on brain and behavior. Importantly, our study provides novel evidence that supports the notion that heightened brain plasticity promotes resilience to stress, and provides a rationale for future, more in-depth mechanistic inquiries into the relationship between plasticity and stress.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

AR and RW conceived and designed the study. RW, CVDO, ENL, SCF, GLO and AR collected and analyzed the data. AR wrote the manuscript with input from all authors.

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Data Availability Statement

The datasets generated and analyzed in this study will be provided upon reasonable request.

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Figures and Figure Legends

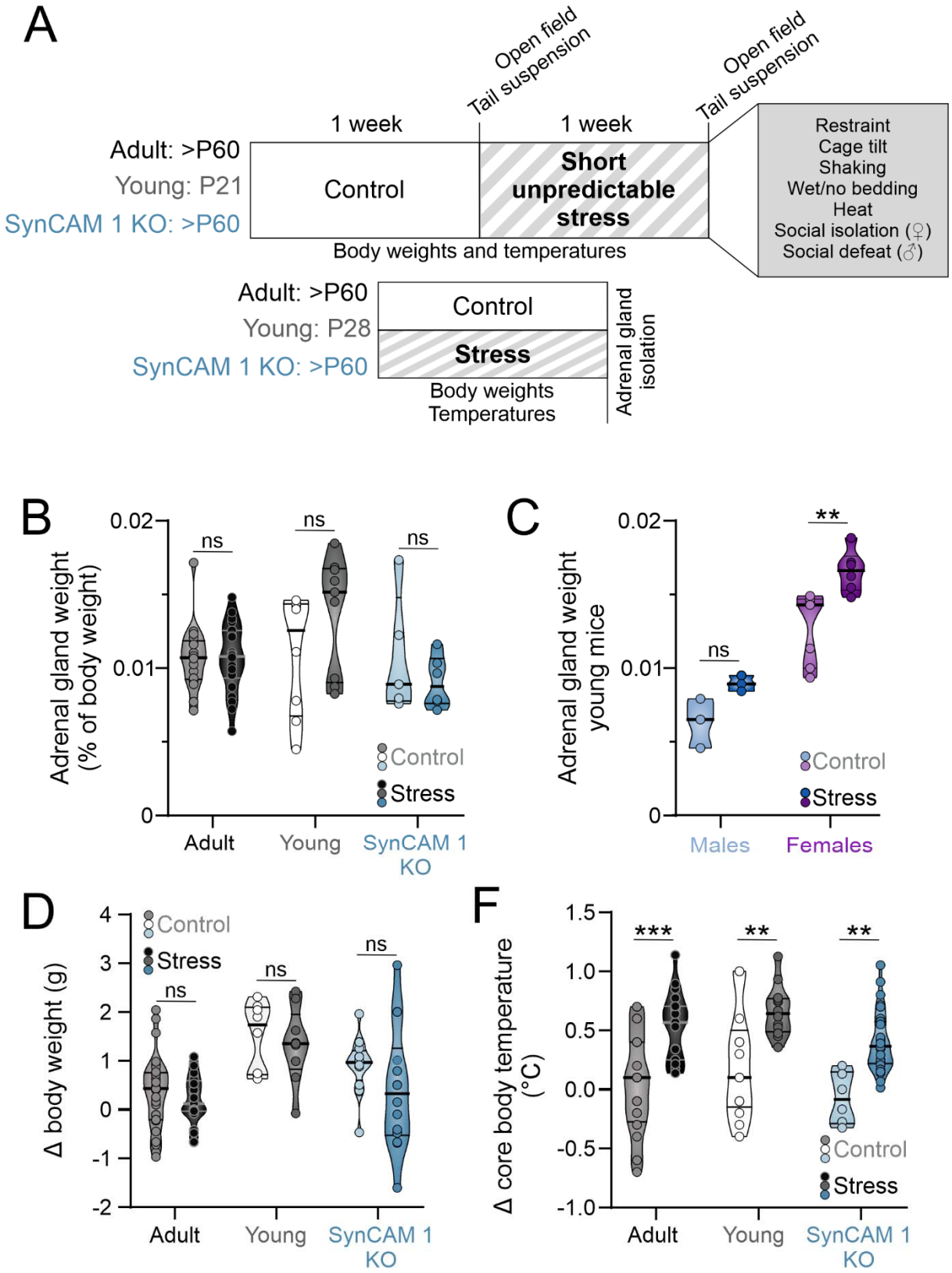


Figure 1. Short unpredictable stress induces robust hyperthermia. A) Experimental schematics. Mice of both sexes (adult, postnatal day/P 60 and older; young, P21-P35; adult SynCAM

1 KO) underwent one week of short unpredictable stress with indicated stressors. Both within-subject (top) and between-subject design (bottom) was used to accommodate for isolation of adrenal glands. **B)** Adrenal gland weight was not significantly increased after stress in any of the groups (2-way ANOVA, $p=0.098$, $F_{(2, 61)}=2.42$; $p_{stress}=0.67$, $F_{(1, 61)}=0.2$; $p_{age/plasticity}=0.078$, $F_{(2, 61)}=2.66$). **C)** After separating the mice by sex, young female mice showed an increase in adrenal gland weight after stress (2-way ANOVA, stress x sex $p=0.51$, $F_{(1,15)}=0.47$; $p_{stress}=0.003$, $F_{(1,15)}=12.6$; $p_{sex}<0.0001$, $F_{(1,15)}=58.75$; Sidak multiple comparison test males $p=0.19$, females $p=0.004$). **D)** Short unpredictable stress did not abrogate weight gain in any of the groups (2-way ANOVA stress x age/plasticity $p=0.56$, $F_{(2,99)=}0.58$; $p_{stress}=0.12$, $F_{(1,99)=}2.45$; $p_{age/plasticity}<0.0001$, $F_{(2,99)=}15.58$). **E)** Short unpredictable stress resulted in robust elevation of core body temperature in all groups of mice (2-way ANOVA stress x age/plasticity $p=0.95$, $F_{(2,100)=}0.05$; $p_{stress}<0.0001$, $F_{(2,100)=}42.75$; $p_{age/plasticity}=0.02$, $F_{(2,100)=}4.05$). Medians and quartiles of the data are indicated by lines and individual points represent mice.

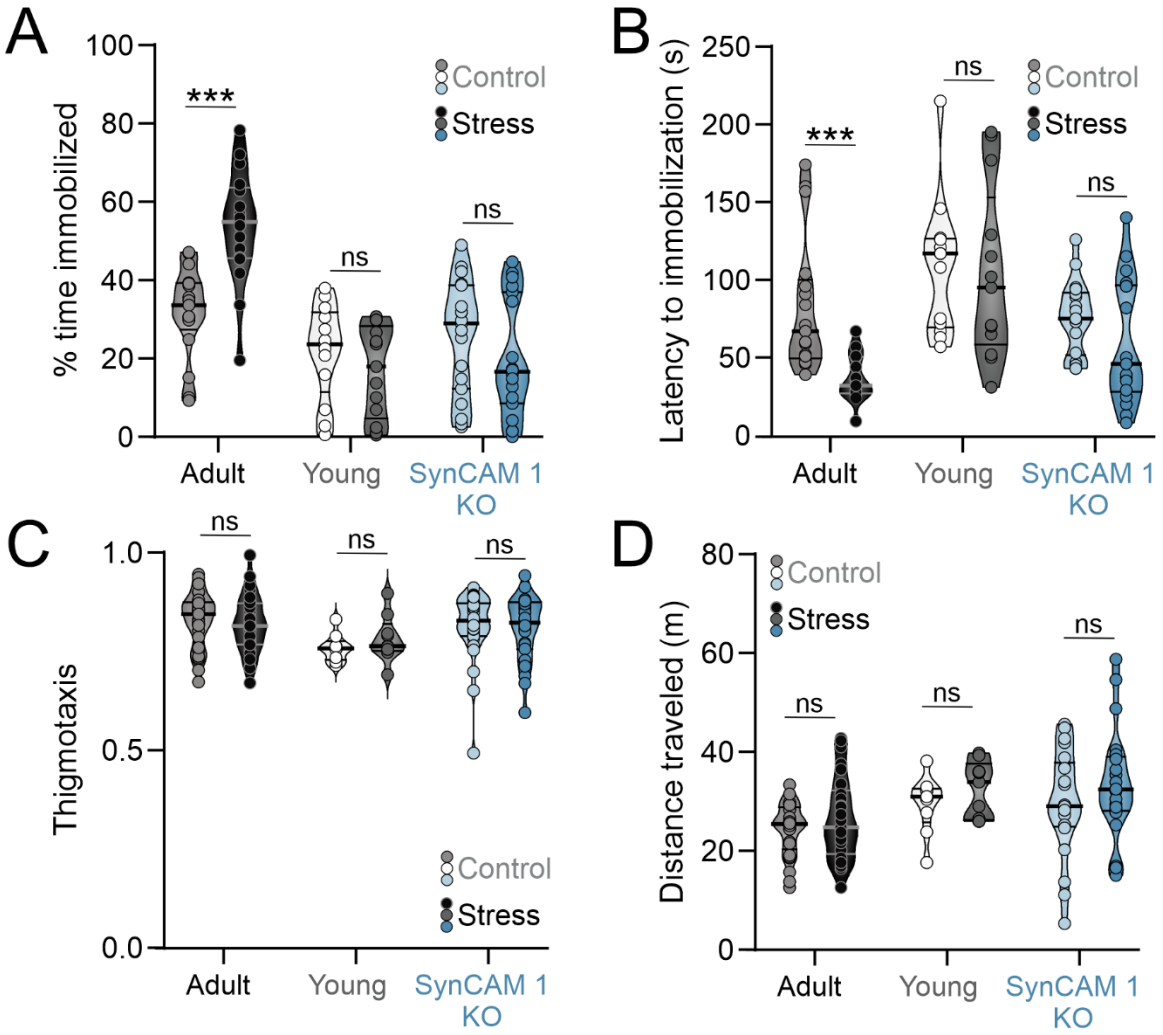
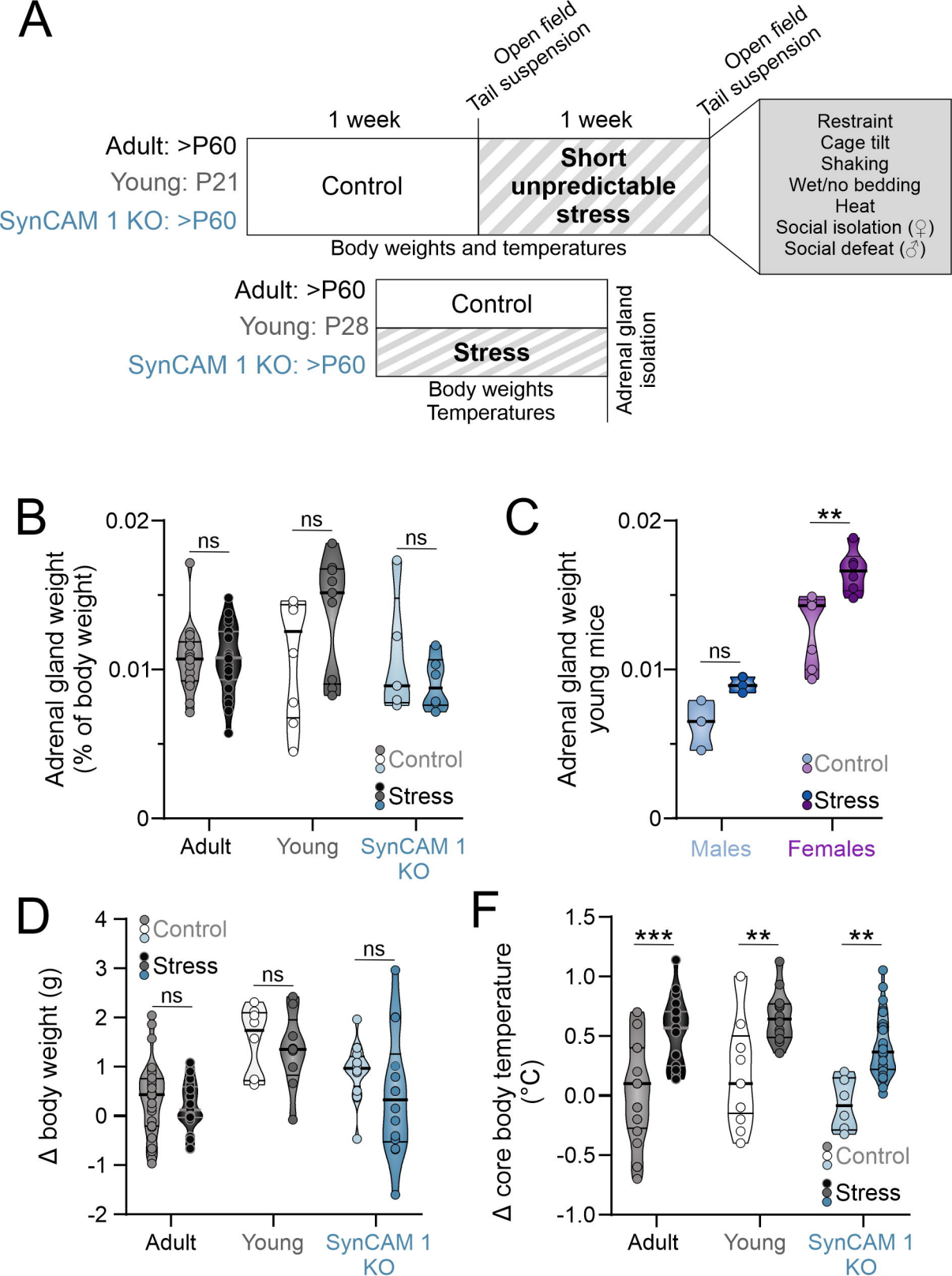
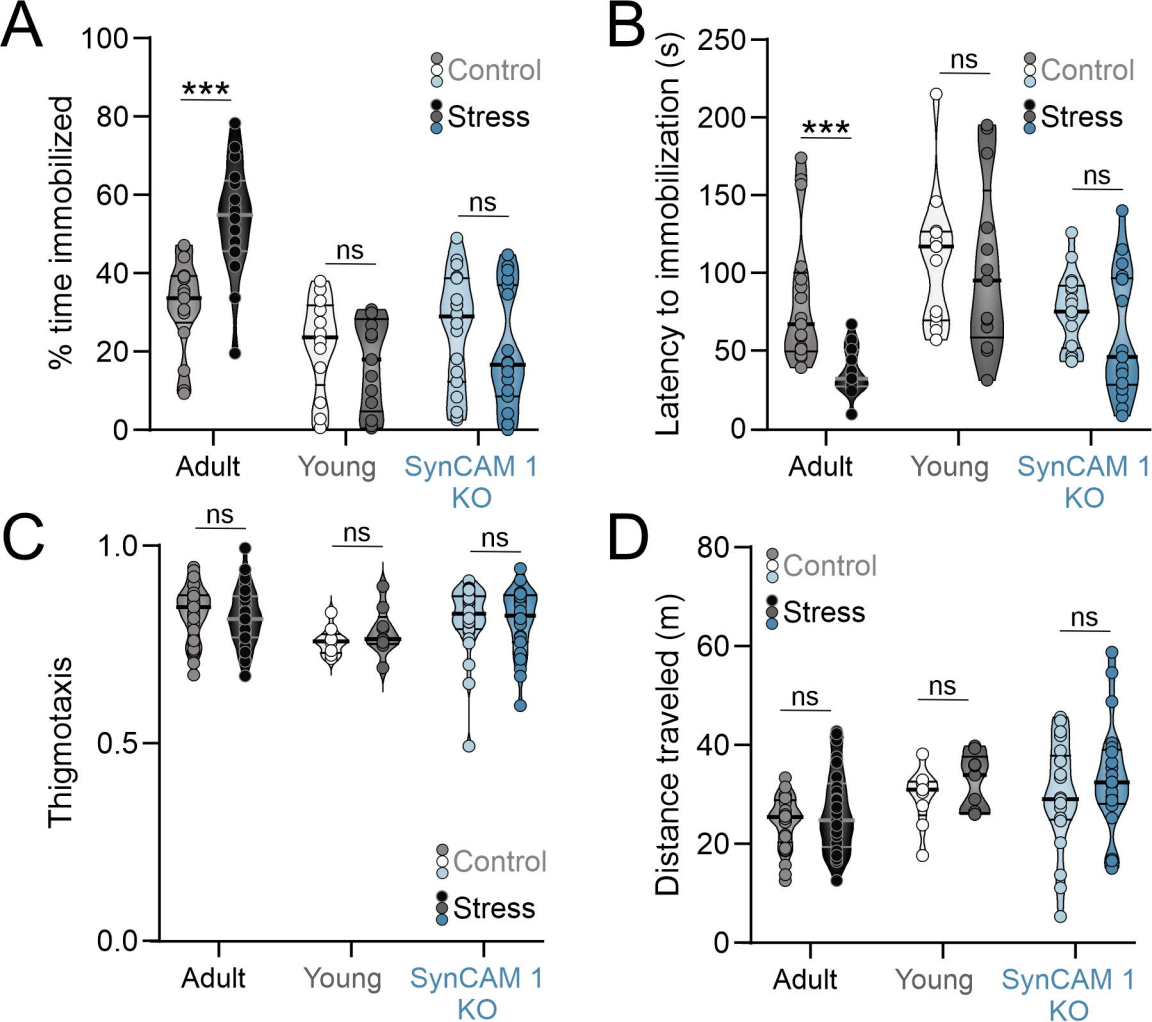


Figure 2. Young mice and adult SynCAM 1 KO mice are resilient to stress-induced increase learned helplessness-like behavior. A) Only stressed adult WT mice show increased immobility after stress in tail suspension test (2-way repeated measures (RM) ANOVA, stress x age/plasticity $p<0.0001$, $F_{(2,45)}=25.24$; $p_{stress}=0.04$, $F_{(1,45)}=4.11$; $p_{age/plasticity}<0.0001$, $F_{(2,45)}=18.3$; adult

WT, $p<0.0001$; young WT, $p=0.42$; SynCAM 1 KO, $p=0.18$; Sidak's post-hoc) and a significant reduction in time lapsed to first immobilization **(B)**(2-way repeated measures (RM) ANOVA, stress x age/plasticity $p=0.054$, $F_{(2,45)}=3.12$; $p_{stress}=0.002$, $F_{(1,45)}=10.41$; $p_{age/plasticity}=0.0003$, $F_{(2,45)}=9.75$; adult WT, $p=0.0007$; young WT, $p=0.98$; SynCAM 1 KO, $p=0.42$; Sidak's post-hoc). **C)** Short unpredictable stress did not impact thigmotaxis in open field test (2-way repeated measures (RM) ANOVA, stress x age/plasticity $p=0.65$, $F_{(2,62)}=0.44$; $p_{stress}=0.82$, $F_{(1,62)}=0.05$; $p_{age/plasticity}=0.07$, $F_{(2,62)}=2.79$). **D)** While stressed mice from all groups travelled more in the open field arena, within-group post-hoc comparisons were not statistically significant (2-way repeated measures (RM) ANOVA, stress x age/plasticity $p=0.72$, $F_{(2,62)}=0.32$; $p_{stress}=0.02$, $F_{(1,62)}=5.42$; $p_{age/plasticity}=0.0014$, $F_{(2,62)}=7.35$; adult WT, $p=0.51$; young WT, $p=0.72$; SynCAM 1 KO, $p=0.07$).


A
Open field
Tail suspension
Open field
Tail suspension
1 week
1 week
Adult: >P60
Young: P21
SynCAM 1 KO: >P60
Control
Short unpredictable stress
Body weights and temperatures
Restraint
Cage tilt
Shaking
Wet/no bedding
Heat
Social isolation (♀)
Social defeat (♂)
Adult: >P60
Young: P28
SynCAM 1 KO: >P60
Control
Stress
Body weights
Temperatures
Adrenal gland isolation
B
Adrenal gland weight (% of body weight)
0.02
0.01
0
ns
ns
ns
Control
Stress
Adult
Young
SynCAM 1 KO
C
Adrenal gland weight young mice
0.02
0.01
0
ns
**
Control
Stress
Males
Females
D
Δ body weight (g)
4
3
2
1
0
-1
-2
Control
Stress
ns
ns
ns
Adult
Young
SynCAM 1 KO
F
Δ core body temperature (°C)
1.5
1.0
0.5
0.0
-0.5
-1.0
***
**
**
Control
Stress
Adult
Young
SynCAM 1 KO


A
% time immobilized
100
80
60
40
20
0
***
ns
ns
Control
Stress
Adult
Young
SynCAM 1 KO
B
Latency to immobilization (s)
250
200
150
100
50
0
***
ns
ns
Control
Stress
Adult
Young
SynCAM 1 KO
C
Thigmotaxis
1.0
0.5
0.0
ns
ns
ns
Control
Stress
Adult
Young
SynCAM 1 KO
D
Distance traveled (m)
80
60
40
20
0
ns
ns
ns
Control
Stress
Adult
Young
SynCAM 1 KO