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Vulnerable and Resilient Phenotypes in a Mouse Model of Anorexia Nervosa

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ABSTRACT

BACKGROUND: Increased physical activity is a common feature of anorexia nervosa (AN). Although high activity levels are associated with greater risk of developing AN, particularly when combined with dieting, most individuals who diet and exercise maintain a healthy body weight. It is unclear why some individuals develop AN while most do not. A rodent model of resilience and vulnerability to AN would be valuable to research. Dopamine, which is believed to play a crucial role in AN, regulates both reward and activity and may modulate vulnerability.

METHODS: Adolescent and young adult female C57BL/6N mice were tested in the activity-based anorexia (ABA) model, with an extended period of food restriction in adult mice. ABA was also tested in dopamine transporter knockdown mice and wild-type littermates. Mice that adapted to conditions and maintained a stable body weight were characterized as resilient.

RESULTS: In adults, vulnerable and resilient phenotypes emerged in both the ABA and food-restricted mice without wheels. Vulnerable mice exhibited a pronounced increase in running throughout the light cycle, which dramatically peaked prior to requiring removal from the experiment. Resilient mice exhibited an adaptive decrease in total running, appropriate food anticipatory activity, and increased consumption, thereby achieving stable body weight. Hyperdopaminergia accelerated progression of the vulnerable phenotype.

CONCLUSIONS: Our demonstration of distinct resilient and vulnerable phenotypes in mouse ABA significantly advances the utility of the model for identifying genes and neural substrates mediating AN risk and resilience. Modulation of dopamine may play a central role in the underlying circuit.

Keywords: Activity-based anorexia, Anorexia nervosa, Dopamine, Exercise, Food restriction, Hyperdopaminergic, Resilience, Starvation, Vulnerability

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Anorexia nervosa (AN) is characterized by severe restriction of food intake and fear of gaining weight, leading to life-threatening weight loss. The disorder tends to be chronic, resistant to treatment, and associated with high mortality (1–4). Neural mechanisms underlying the disorder remain poorly understood, and there are no approved pharmacological treatments (5).

Excessive physical activity has been associated with AN since its earliest description (6), with 31% to 81% of AN patients exhibiting high activity levels, depending on how it is defined (7,8). Although characterized as compulsive (9–12) or compensatory (13) voluntary exercise, increased nonexercise activity, such as fidgeting, has also been observed (14). Exercise is associated with poorer outcomes, including greater risk of relapse, longer hospitalizations, and increased chronicity (15–19), indicating a role for exercise in maintenance of the disorder. Additionally, higher levels of premorbid activity have been associated with greater risk of developing AN (20–22), even among athletes (23–25), supporting a role for physical activity in the development of AN. However, the majority of individuals who combine diet and exercise do not develop AN, and the underlying factors mediating AN vulnerability or resilience are not understood.

Alterations in dopamine and associated changes in reward have been implicated in AN (26–31). Importantly, dopamine also modulates physical activity (32–34), as exemplified by increased psychomotor activity resulting from increased dopamine transmission with psychostimulants. The relationship between altered dopamine and the increased activity observed in AN has not been empirically characterized, though it is potentially important to understanding the disorder.

Activity-based anorexia (ABA), a widely used rodent model of AN, assesses the interaction between food restriction and physical activity. The model combines limited access to food with unlimited access to a running wheel, leading to hyperactivity, self-starvation, rapid weight loss, and death unless removed from the experiment (35–37). We conducted a detailed analysis of running behavior of adolescent and adult

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female C57BL/6 mice in the ABA model and assessed the impact of genetically increasing dopamine. We discovered distinct vulnerable and resilient phenotypes, with the latter showing adaptation to ABA and weight stabilization. In contrast, vulnerable mice exhibited severely dysregulated running activity, inadequate consumption, and catastrophic weight loss. Vulnerability to ABA was increased in hyperdopaminergic mice, indicating that dopamine may play a central role in the development of AN.

METHODS AND MATERIALS

Animals

Female C57BL/6N mice (Taconic Biosciences, Germantown, NY) were purchased at postnatal day (PND) 21 and PND 56 for the adolescent and early adulthood behavioral studies, respectively. Male and female DAT-cre (dopamine transporter–Cre recombinase) heterozygous mice (DAT knockdown [KD]; $DAT^{cre/+}$; Cat# JAX: 020080; C57BL/6J genetic background) (38) and wild-type (WT) littermates ($DAT^{+/+}$) were bred in-house (New York State Psychiatric Institute, New York, NY; Hunter College, New York, NY). Only female KD and WT mice were used in behavioral studies. Prior to experiments, mice were group housed on a 12-hour light/dark cycle with ad libitum chow (Prolab Isopro 3000 5P75; W.F. Fisher & Son, Somerville, NY). Experiments were approved by the Institutional Animal Care and Use Committees of the New York State Psychiatric Institute, Hunter College, and Queens College.

ABA Procedure

Mice were distributed into 4 groups: ABA (2 hours/day food access; unlimited wheel access), wheel control (WH) (unlimited food and wheel access), food-restricted control (FR) (2 hours/day food access; unlimited access to a locked wheel), and home cage control (HC) (unlimited access to food and a locked wheel). Mice were weighed immediately preceding dark cycle onset and removed when they lost 25% of their baseline weight (Supplemental Methods). Mice that did not require removal after 10 days were characterized as resilient.

Fast-Scan Cyclic Voltammetry

Evoked dopamine release was measured in $DAT^{cre/+}$ and WT littermates of both sexes using slice fast-scan cyclic voltammetry. For adolescent and young adult female mice (C57BL/6N), fast-scan cyclic voltammetry was done in intact, anesthetized animals (Supplemental Methods).

Statistical Analyses

Data were analyzed with a *t* test, analysis of variance with Bonferroni post hoc correction, or Mantel-Cox log-rank test for survival analyses. For analyses across days of restriction, in which mice dropped out on different days, the linear mixed-effects (LME) model was used with the LME4 package (39) in R, version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria), computing *F* values using lmerTest (40). We modeled mouse as a random effect, including intercept and slope across experimental days.

See the Supplement for immunohistochemistry, messenger RNA, and protein quantification methods.

RESULTS

Adolescent C57 Female Mice Are Vulnerable to ABA

We first tested adolescent female mice (PND 43 on ABA day 1). There were no baseline differences in body weight ($F_{3,60} = 0.23$, $p = .87$). During the restriction phase, the FR (locked wheel) and ABA (food restricted; freely moving wheel) groups lost substantial weight compared with non-FR groups (LME model: day × group [$F_{3,66} = 13.89$, $p < .001$]) (Figure 1A). Although ABA and FR groups did not significantly differ in weight loss (LME

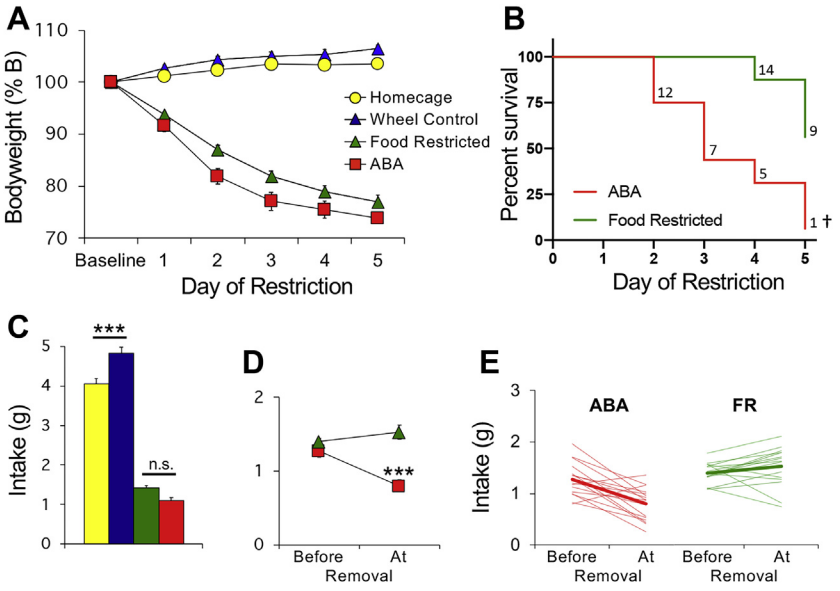

Figure 1. Adolescent female mice are vulnerable to activity-based anorexia (ABA). **(A)** Body weight across days of food restriction. **(B)** Survival curves for ABA and food-restricted control (FR) mice. Numbers indicate number of surviving mice that day. **(C)** Average food intake across 5 days of restriction. **(D)** Average and **(E)** individual (light traces) food intake of ABA and FR mice prior to removal vs. the day of removal (bold lines in E show group average). $n = 16$ per group. ***$p < .001$ vs. FR at removal; †survival curves, $p < .001$. Error bars indicate SEM.

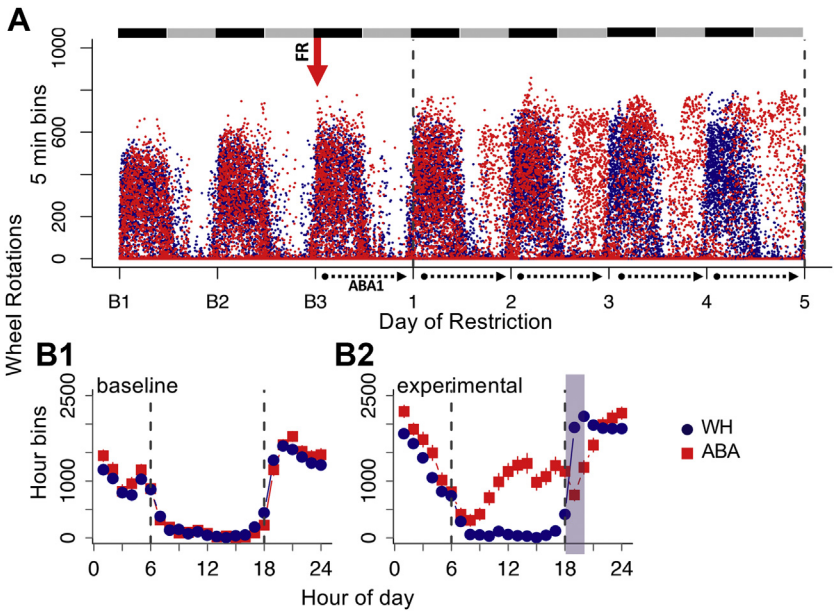

Figure 2. Activity-based anorexia (ABA) increases light cycle running in adolescent mice. **(A)** Dot plot of wheel running across days of experiment for all mice in 5-minute bins. Black and gray bars indicate dark and light cycles, respectively. The red arrow indicates start of food restriction (FR). Dotted lines demarcate data collection days under ABA. **(B)** Group averages in hour bins across a 24-hour period for **(B1)** baseline days and **(B2)** ABA experimental days. The dotted lines mark the light cycle between 6 (lights on) and 18 (lights off) hours, and shading indicates when food was available. Error bars indicate SEM. WH, wheel control.

model [$F_{1,23}$ = 0.75, p = .39]), the survival analysis revealed greater risk for the ABA group (ABA/FR groups: hazard ratio, 3.96; 95% confidence interval [CI], 1.65–9.50; $p < .001$) (Figure 1B). The LME analysis includes data points prior to removal and does not take into account time of removal reflected in the hazard ratio.

Food consumption differed between groups ($F_{3,60}$ = 278.8, $p < .001$) (Figure 1C). Non-FR WH mice increased their consumption compared with HC mice, presumably to compensate for running (Bonferroni $p < .001$) (Figure 1C). In contrast, there was no difference in intake between the ABA and FR groups (Bonferroni p = .29). However, ABA mice exhibited a drop in consumption on the day of removal, which was not observed in the FR mice (time point × group [$F_{1,30}$ = 21.18, $p < .001$]) (Figure 1D, E). That some FR mice required removal indicates that caloric restriction alone can induce life-threatening weight loss.

Abrupt Increase in Light Cycle Running Precedes Removal From the Model in Adolescent Mice

There were no baseline differences between ABA and WH mice in the amount (LME model: group [$F_{1,26}$ = 0.121, p = .73]) (Figure 2A) or circadian distribution (LME model: group × hour [$F_{1,26}$ = 0.28, p = .60]) (Figure 2B1) of wheel running. During food restriction, the ABA mice exhibited a sharp increase in light but not dark cycle running (bar graphs: group × cycle [$F_{1,26}$ = 19.50, $p < .001$]; light cycle: Bonferroni $p < .001$; dark cycle: Bonferroni p = .99) (Figure 3A, B1, and also Figure 2). Though typically considered food-anticipatory activity, this increase started near the onset of the light cycle (LME model: group × hour [$F_{1,26}$ = 8.16, $p < .001$]) (Figure 2B2). This alteration in the distribution of circadian activity increased across days of food restriction (Figure 2A). Plotting the running of individual mice revealed that each mouse exhibited an abrupt increase in light cycle running (Figure 3B1). A comparison of the maximum increase in day-to-day running revealed that the peak change was higher in the ABA group than the WH group during the light cycle (group × cycle [$F_{1,26}$ = 130.4, $p < .001$]) (Figure 3C). This abrupt increase generally preceded removal from the model by 1 day in ABA mice (Figure 3D), suggesting a predictive relationship between peak change in light cycle running and removal. Light cycle running was positively correlated with weight loss in ABA mice (Figure 3E1). Dark cycle running also correlated with weight loss in ABA mice (Figure 3E2), despite similar levels of dark cycle running between groups (Figure 3A, B2). We observed no correlation between running and change in body weight in WH mice (Figure 3F1, F2).

Adult Mice Exhibit Vulnerable and Resilient Phenotypes

We tested ABA in young adult mice (PND 68 on ABA day 1) using the same protocol but extended food restriction to 10 days. Body weight was not different between groups at baseline ($F_{3,60}$ = 2.03, p = .12). In the first 5 days, both ABA and FR mice lost substantial weight, with no group difference (Figure 4A) (LME model: group × day [$F_{1,30}$ = 1.2, p = .23]). In days 6 to 10, weight stabilized for the remaining mice, who survived through the end of the experiment and were characterized as resilient (Figure 4A, C1, C2). Adult mice were less vulnerable to ABA than younger mice (adult vs. adolescent: $p < .001$) (Figure 4B), with ~50% of adults surviving (Figure 4D). Some FR mice (~30%) required removal (Figure 4B, D), indicating that a subset of both adult ABA and FR mice are vulnerable to catastrophic weight loss with prolonged caloric restriction, though ABA increases risk and accelerates progression (ABA/FR groups: hazard ratio, 2.22; 95% CI, 0.82–5.97; p = .10) (Figure 4B). These results demonstrate that both ABA and FR mice exhibit vulnerable (ABA-V, FR-V) and resilient (ABA-R, FR-R) phenotypes. Interestingly, ABA-R mice

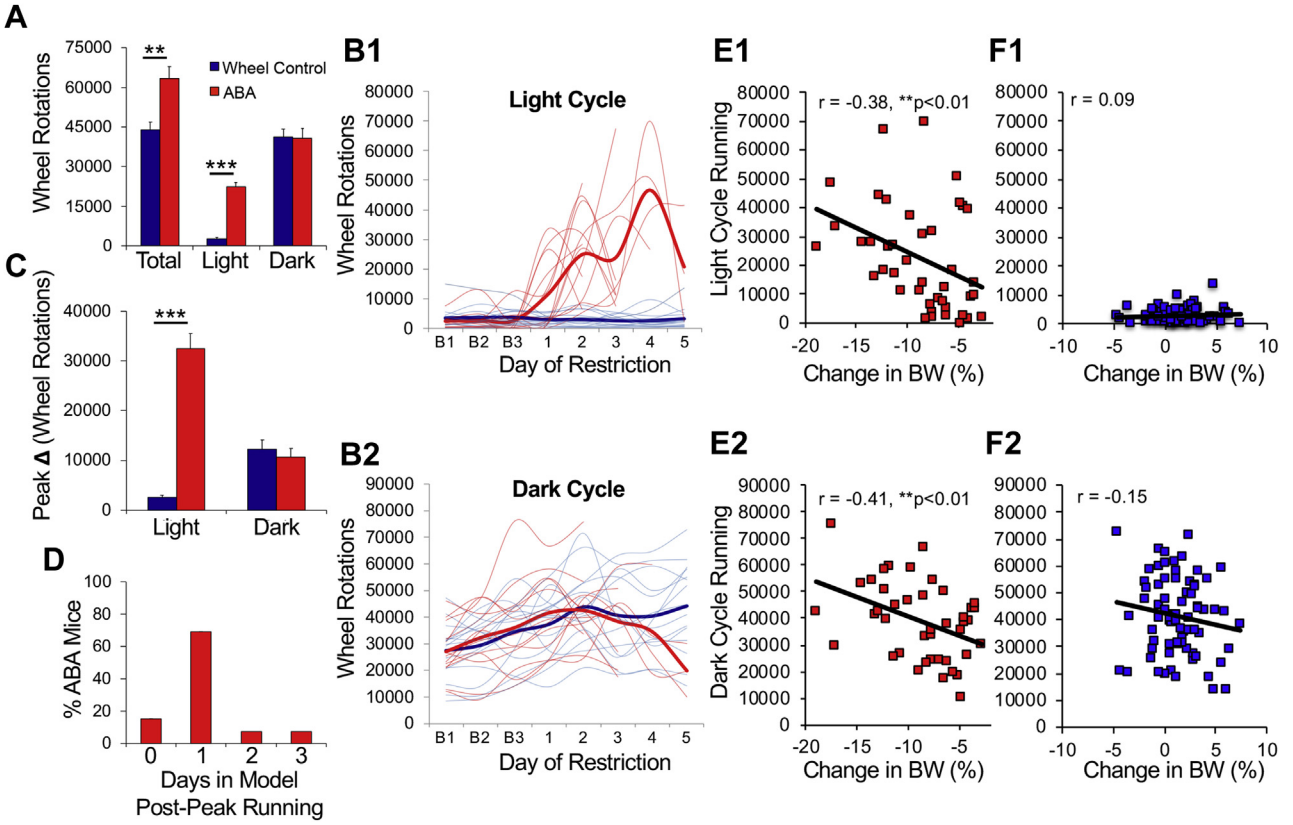

Figure 3. Abrupt increase in light cycle running precedes removal from model. **(A)** Average wheel activity across days of food restriction. **(B)** Wheel running of individual mice (light traces) and group mean (bold traces) across days of food restriction during the **(B1)** light cycle and **(B2)** dark cycle. **(C)** Maximum increase in wheel running across 2 consecutive days (averaged by group) during the light cycle and dark cycle. **(D)** Histogram of number of days mice remained in model after maximum increase in light cycle running. **(E, F)** Correlation between (top panel) light cycle or (bottom panel) dark cycle running and change in body weight (BW) the next day for **(E1, E2)** activity-based anorexia (ABA) mice and **(F1, F2)** wheel control mice. Each symbol represents 1 animal on 1 experimental day. n = 13 (ABA mice), n = 15 (wheel control mice). $^{**}p < .01$; $^{***}p < .001$. Error bars indicate SEM.

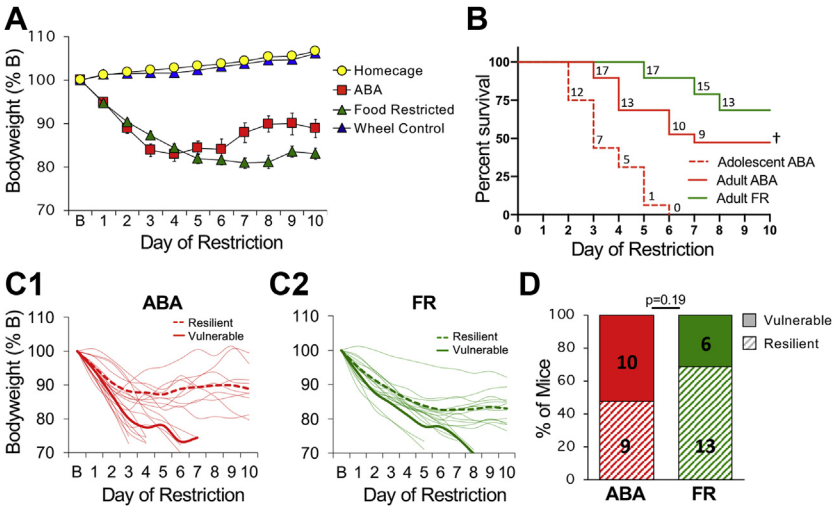

Figure 4. Young adult female mice exhibit vulnerable and resilient phenotypes. **(A)** Body weight across days of food restriction. **(B)** Survival curves for each group. The dotted red line is a replot of adolescent survival from Figure 1 for reference. Numbers indicate the number of surviving mice that day. **(C)** Body weight of individual mice (light traces) across days of food restriction in **(C1)** activity-based anorexia (ABA) and **(C2)** food-restricted control (FR) groups. Group averages for resilient (dashed line) and vulnerable (solid line) mice are shown in bold. **(D)** Percent of vulnerable (solid) and resilient (hatched) mice within ABA and FR groups (number of animals is indicated inside bars). n = 19 (ABA mice), n = 19 (FR mice), n = 12 (home cage control mice), n = 14 (wheel control mice). †Survival curves, difference between adolescent and adult ABA, $p < .0001$. Error bars indicate SEM.

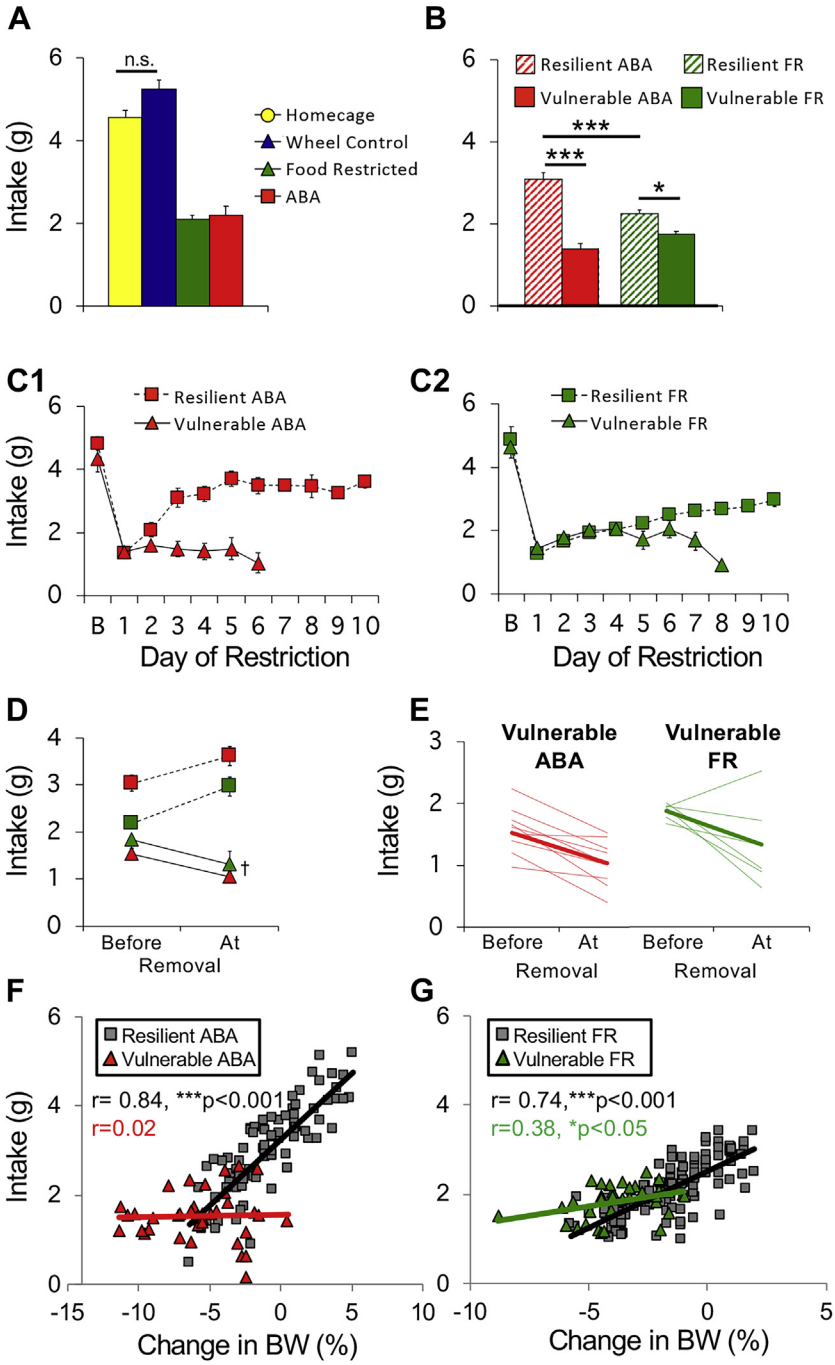

Figure 5. Resilient mice adapt food intake. **(A)** Average food intake across 10 days of restriction for all mice in each group. **(B)** Average food intake of vulnerable (solid) and resilient (hatched) mice in activity-based anorexia (ABA) and food-restricted control (FR) groups. **(C)** Daily food intake of **(C1)** ABA and **(C2)** FR mice across days of restriction. **(D)** Average and **(E)** individual (light traces) food intake of ABA and FR mice prior to removal vs. the day of removal [bold lines in panel **(E)** show group average]. Correlation between consumption during restricted access to food and changes in body weight (BW) the next day for **(F)** ABA and **(G)** FR mice. Each symbol represents 1 animal on 1 experimental day. n = 9 (resilient ABA mice), n = 10 (vulnerable ABA mice), n = 13 (resilient FR mice), n = 6 (vulnerable FR mice), n = 12 (home cage control mice), n = 14 (wheel control mice). $^{*}p < .05$; $^{***}p < .001$; $^{\dagger}p < .01$ vs. vulnerable groups before removal. Error bars indicate SEM.

gained more weight than FR-R mice (LME model: last 3 days, ABA vs FR [$F_{1,17}$ = 7.52, p = .01]) (Figure 4A), suggesting that access to a running wheel promoted an adaptive response in resilient mice.

Resilient Mice Adapt Food Intake

Food restriction similarly reduced consumption in the ABA and FR groups (ABA group vs. FR group: Bonferroni p = .99) (Figure 5A). Resilient mice consumed more food than vulnerable mice in both ABA and FR groups (LME model: ABA phenotype × day [$F_{1,125}$ = 21.28, $p < .001$]; LME model: FR phenotype × day [$F_{1,56}$ = 6.41, $p < .05$]) (Figure 5B–C2). Consistent with increased weight noted above, ABA-R mice exhibited a larger increase in consumption than FR-R mice (LME model: group × phenotype × day [$F_{1,235}$ = 3.94, $p < .05$]) (Figure 5C1, C2). In contrast, vulnerable mice showed a decline

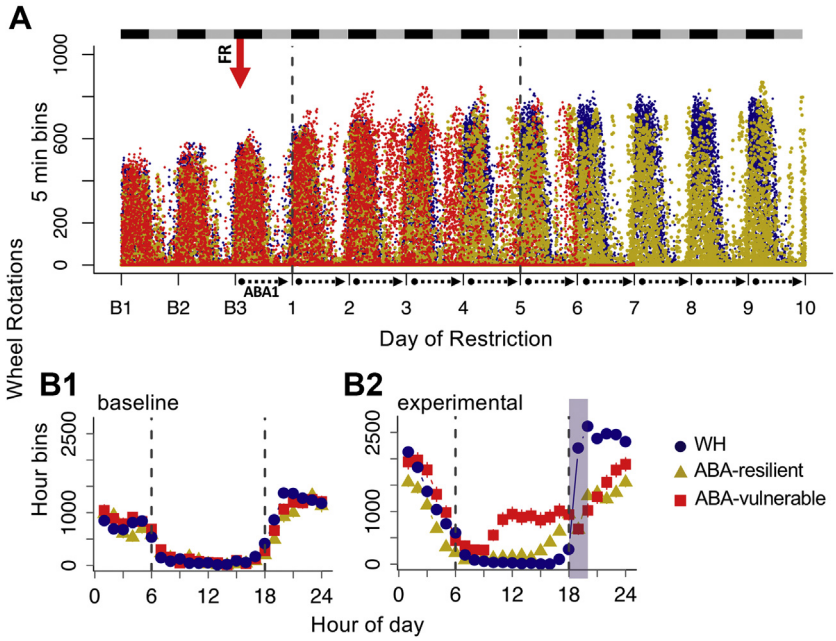

Figure 6. Vulnerable but not resilient activity-based anorexia (ABA) mice exhibit altered distribution of circadian running activity. **(A)** Dot plot of wheel running across days of experiment for all mice in 5-minute bins. Black and gray bars indicate dark and light cycles, respectively. The red arrow indicates start of food restriction (FR). Dotted arrows demarcate data collection days under ABA. **(B)** Group averages in hour bins across a 24-hour period for **(B1)** baseline days and **(B2)** ABA experimental days. The dotted lines mark the light cycle between 6 (lights on) and 18 (lights off) hours, and shading indicates when food was available. Error bars indicate SEM. WH, wheel control.

in consumption that occurred earlier in ABA-V than FR-V mice (days in model [t_{13} = 2.86, p = .01]) (Figure 5C1, C2). This cannot be due to insufficient time to eat because the resilient mice increased food intake in the same amount of time. Similar to adolescent mice, consumption decreased at time of removal for vulnerable mice of both groups (time point × phenotype [$F_{1,33}$ = 54.12, p < .001]) (Figure 5D, E). In resilient mice, consumption correlated with changes in body weight, a correlation reduced in FR-V mice and absent in ABA-V mice (Figure 5F, G).

ABA-V Mice Exhibit Maladaptive Running Behavior

There were no baseline differences between ABA-V, ABA-R, and non-FR WH mice in amount (LME model: group [$F_{2,30}$ = 0.398, p = .67]) (Figure 6A) or circadian distribution of running (LME model: group × hour [$F_{2,30}$ = 2.13, p = .13]) (Figure 6B1). During food restriction, ABA-R mice exhibited an adaptive reduction in total running, while ABA-V mice continued to run as much as WH mice (group [$F_{2,30}$ = 5.21, p = .01]; ABA-R group vs. WH group: Bonferroni p < .05) (Figure 7A). As above, food restriction increased light cycle running, which was modest in ABA-R but dramatic in ABA-V mice (bar graphs [$F_{2,30}$ = 38.05, p < .001]; ABA-V mice vs. ABA-R mice: Bonferroni p < .001; ABA-R mice vs. WH mice: Bonferroni p = .05) (Figure 7B, D1). In contrast, both ABA groups decreased dark cycle running (bar graphs [$F_{2,30}$ = 7.76, p < .01]) (Figure 7C, D2). These changes reflect a shift in running from dark to light cycle, an effect more pronounced in ABA-V mice and partially mitigated in ABA-R mice (LME model, light cycle: ABA phenotype × day [$F_{1,22}$ = 10.03, p < .01]) (Figure 7E1, E2). As observed in adolescent mice, ABA-V mice exhibited an abrupt increase in light cycle running (bar graphs [$F_{2,30}$ = 22.00, p < .001]; ABA-V mice vs. ABA-R mice: Bonferroni p < .001) (Figure 7D1, F) that preceded removal by 1 to 3 days (Figure 7G), an effect greatly reduced in ABA-R mice (ABA-R mice vs. WH mice: Bonferroni p = .19) (Figure 7D1, F). Light cycle running positively correlated with weight loss in the ABA-V group but not in the ABA-R group (Figure 7H1). In contrast, dark cycle running positively correlated with weight loss in both groups (Figure 7H2). Like adolescent ABA mice, the ABA-V mice exhibited an altered distribution of circadian activity (Figure 6A), with running that began 2 to 3 hours into the light cycle (Figure 6B2). In contrast, the ABA-R mice exhibited a modest increase in activity prior to onset of the dark cycle, consistent with adaptive food-anticipatory activity (ABA phenotype × hour [$F_{1,17}$ = 8.92, p < .01]) (Figure 6B2). These data highlight an association between dysregulated increases in light cycle running and vulnerability in the ABA model.

Hyperdopaminergic Mice Show Increased Vulnerability to ABA

To test dopamine's contribution to ABA vulnerability, we used heterozygote DAT-cre mice in which 1 allele of the DAT was replaced with cre ($DAT^{cre/+}$). DAT messenger RNA (genotype [$F_{1,22}$ = 4.7, p < .05]) (Figure 8A) and protein (t_{27} = 2.06, p = .049) (Figure 8B, C) were reduced without affecting expression of other key dopamine-related genes (TH [tyrosine hydroxylase]: p = .81; VMAT2 [vesicular monoamine transporter 2]: p = .50; D2 [dopamine D_2 receptor]: p = .30) (Figure 8A), rendering the mice DAT KDs. Evoked dopamine release measured by fast-scan cyclic voltammetry revealed reduced clearance and increased peak amplitude in KD mice across striatal regions (genotype effects for single stimulation: tau [$F_{1,51}$ = 24.1, p < .001], peak [$F_{1,51}$ = 23.1, p < .001]; genotype effects for burst stimulation: tau [$F_{1.51}$ = 27.6, p < .001], peak [$F_{1,51}$ = 23.7, p < .001]; no genotype × region interactions) (Figure 8D),

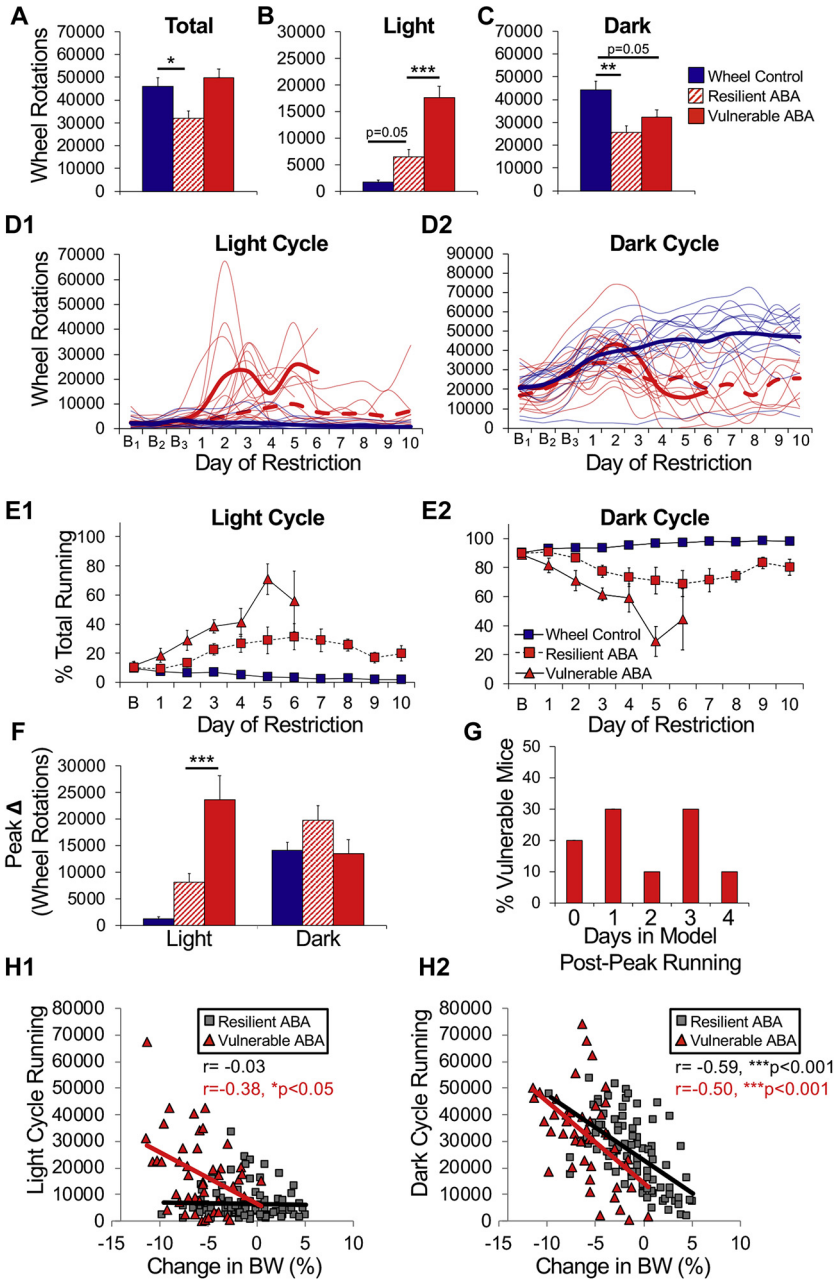

Figure 7. Vulnerable activity-based anorexia (ABA) mice exhibit maladaptive running behavior. **(A)** Total, **(B)** light cycle, and **(C)** dark cycle wheel running averaged across days of food restriction. **(D)** Wheel running of individual mice (light traces) and group mean (bold traces) across days of food restriction during the **(D1)** light cycle and **(D2)** dark cycle (vulnerable ABA mice, solid red; resilient ABA mice, dashed red; wheel control [WH] mice, solid blue). **(E)** Percentage of total running during the **(E1)** light cycle and **(E2)** dark cycle. **(F)** Maximum increase in wheel running across 2 consecutive days (averaged by group) during the light and dark cycles. **(G)** Histogram of the number of days mice remained in the model after maximum increase in light cycle running. Correlation between **(H1)** light cycle or **(H2)** dark cycle running and change in body weight (BW) for vulnerable and resilient ABA mice. Each symbol represents 1 animal on 1 experimental day. $n = 9$ (resilient ABA mice), $n = 10$ (vulnerable ABA mice), $n = 14$ (wheel control mice). $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. Error bars indicate SEM.

indicating mild hyperdopaminergia. We compared young adult (PNDs 62–78, ABA day 1) female KD and WT littermates in ABA, FR, and WH conditions separately.

At baseline, genotypes were similar in weight ($F_{1,72} = 1.07$, $p = .31$), food intake ($F_{1,61} = 0.87$, $p = .35$), and light cycle running ($F_{1,45} = 0.05$, $p = .82$). On the first day of baseline, dark cycle running was 36% higher in $DAT^{cre/+}$ mice than WT mice ($t_{45} = 2.95$, $p < .01$). This difference declined to nonsignificance by the third day of baseline ($t_{45} = 1.24$, $p = .22$), indicating an increased response to novelty rather than sustained elevated running. This interpretation is supported by the lack of a difference between genotypes during 10 additional days of running in WH controls (Figure S1C–E). Combining data across test conditions revealed no main effect of genotype on weight (LME model [$F_{1,48} = 1.51$, $p = .23$]). Significant genotype interactions are described below.

Under ABA conditions, KD mice exhibited accelerated weight loss (LME model: genotype [$F_{1,28} = 6.64$, $p < .05$]) (Figure 9A, B) and poorer survival (KD/WT mice: hazard ratio, 2.23; 95% CI, 0.91–5.47; $p < .05$) (Figure 9C). All KD mice exhibited the vulnerable phenotype, while some WT mice were resilient (Figure 9C). There were no differences between

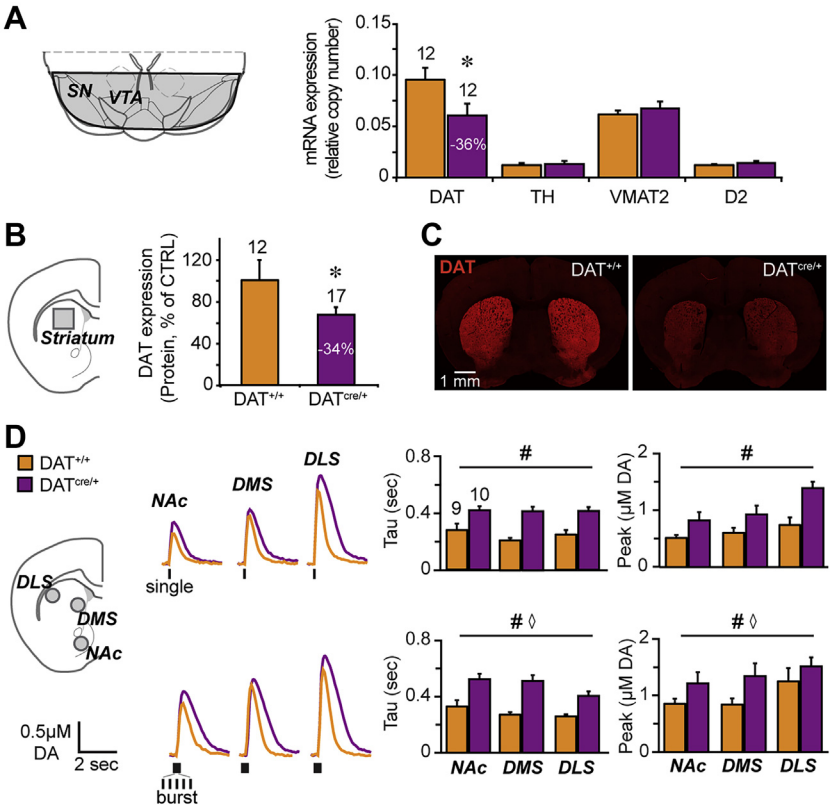

Figure 8. Characterizing hyperdopaminergic $DAT^{cre/+}$ knockdown mice. **(A)** (Left panel) Schematic of a coronal brain slice showing the ventral midbrain dissection (shaded area) used for messenger RNA (mRNA) determinations. (Right panel) Relative messenger RNA expression of dopamine transporter (DAT), tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), and dopamine D_2 receptor (D2) in $DAT^{cre/+}$ and control (CTRL) $DAT^{+/+}$ littermates. n = 5 (female $DAT^{+/+}$ mice), n = 7 (male $DAT^{+/+}$ mice), n = 5 (female $DAT^{cre/+}$ mice), n = 7 (male $DAT^{cre/+}$ mice). **(B)** (Left panel) Schematic of a coronal brain slice showing the striatal dissection (gray square) used for protein determination. (Right panel) Relative DAT protein expression in $DAT^{cre/+}$ compared with $DAT^{+/+}$ control mice. n = 6 (female $DAT^{+/+}$ mice), n = 6 (male $DAT^{+/+}$ mice), n = 7 (female $DAT^{cre/+}$ mice), n = 10 (male $DAT^{cre/+}$ mice). **(C)** Photomicrographs of coronal slices showing that DAT immunofluorescence in the striatum is diminished in the $DAT^{cre/+}$ mice. **(D)** (Left panel) Schematic of a coronal slice showing the fast-scan cyclic voltammetry recordings sites: nucleus accumbens (NAc) core, dorsomedial striatum (DMS), and dorsolateral striatum (DLS) (gray circles). (Middle panel) Representative recordings of evoked DA release following (top) single or (bottom) burst (20 Hz) stimulation (traces) and (right panel) respective average DA clearance (tau) and peak DA release. Number of animals is indicated above the bars. n = 3 (female $DAT^{+/+}$ mice), n = 6 (male $DAT^{+/+}$ mice), n = 4 (female $DAT^{cre/+}$ mice), n = 6 (male $DAT^{cre/+}$ mice). $^{*}p < .05$; $^{\#}$genotype effect $p < .001$; $^{\diamond}$region effect, $p < .05$. Error bars indicate SEM. SN, substantia nigra; VTA, ventral tegmental area.

genotypes in consumption across the experiment (LME model [$F_{1,20}$ = 1.16, p = .21]) (Figure 9D), but resilient WT mice exhibited a compensatory increase in consumption (LME model: WT across days 4–10 [$F_{1,22}$ = 6.18, $p < .05$]) (Figure 9D). Vulnerable mice of both genotypes decreased consumption on the day of removal (time point [$F_{1,15}$ = 49.89, $p < .001$], time point × genotype [$F_{1,15}$ = 3.43, p = .08]) (Figure 9E). Food intake was not correlated with change in body weight in KD mice, like in the vulnerable mice described above (Figure 9F).

During ABA, there were no differences between genotypes when light and dark cycle running were averaged across all days of food restriction (genotype [$F_{1,25}$ = 0.56, p = .46]) (Figure 9G). Vulnerable mice of both genotypes exhibited abrupt increases in light cycle running only (Figure 9H1–2), but these occurred earlier in KD mice. Most KD mice (82%) exhibited peak light cycle running by the second day of food restriction, while peak running was more distributed across days in WT mice (log-rank $p < .05$) (Figure 9J). That is, KD mice responded to food restriction with greater increases in light cycle running between days 1 and 2 (t_{25} = 2.51, $p < .05$) and more total light cycle running on day 2 (t_{25} = 2.02, p = .05). When it did occur, peak change in light cycle running was equally high in both groups (genotype [$F_{1,25}$ = 0.50, p = .49]) (Figure 9I) but preceded removal by 1 to 2 days in most KD mice (73%) and 1 to 4 days in WT mice (data not shown). Like ABA-V mice above, there was a positive correlation between light cycle running and weight loss that was greater in KD than WT mice (Figure 9K1), while the correlation between dark cycle running and weight loss was similar in both genotypes (Figure 9K2). These data demonstrate that increased dopamine promotes the vulnerable phenotype by accelerating increases in running that occur in response to caloric restriction.

In contrast to ABA, under FR conditions, there were no differences between genotypes in weight loss (LME model: genotype [$F_{1,24}$ = 0.19, p = .66], genotype × day [$F_{1,23}$ = 1.20, p = .28]) (Figure 10A) or survival (KD/WT mice: hazard ratio, 1.33; 95% CI, 0.41–4.34; p = .61) (Figure 10B). A subset of both KD and WT FR control mice exhibited resilient phenotypes (Figure 10C), with resilient mice of each genotype exhibiting the same pattern of weight stabilization (LME model: phenotype × day [$F_{1,23}$ = 66.43, $p < .001$], genotype × phenotype × day [$F_{1,23}$ = 0.05, p = .82]) (Figure 10D1, D2) found in the FR-V group (Figure 4C2). Resilient mice consumed more food than vulnerable mice (LME model: phenotype × day [$F_{1,23}$ = 10.42, $p < .01$]), regardless of genotype (LME model: genotype × phenotype × day [$F_{1,23}$ = 1.19, p = .28]) (Figure 10E). In both genotypes, consumption correlated with changes in body weight, a correlation reduced in vulnerable KD mice (Figure 10G1, G2).

Under WH conditions, there were no differences between genotypes in body weight (Figure S1A), food intake (Figure S1B), or wheel running (Figure S1C–E) during 10 additional days of unlimited food access. This indicates that

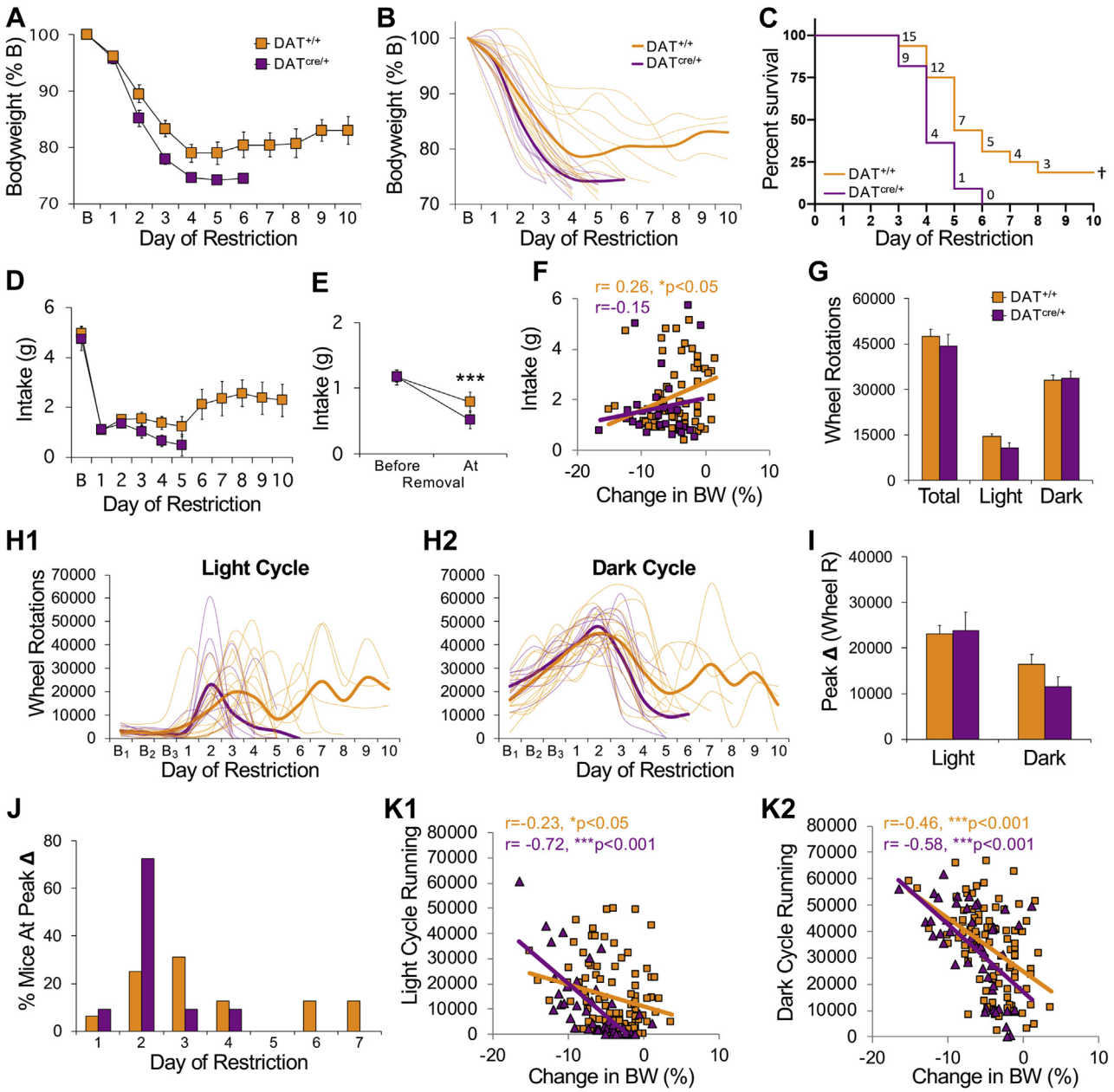

Figure 9. Hyperdopaminergic mice show increased vulnerability to activity-based anorexia. All mice were tested in the activity-based anorexia condition. **(A)** Body weight (BW) across days of food restriction. **(B)** BW of individual mice (light traces) across days of food restriction (bold lines indicate group average). **(C)** Survival curves for each group. Numbers indicate number of surviving mice that day. **(D)** Daily food intake across days of restriction. **(E)** Average food intake prior to removal vs. the day of removal. **(F)** Correlation between consumption during restricted access to food and changes in body weight the next day. Each symbol represents 1 animal on 1 experimental day. **(G)** Average wheel activity across days of food restriction. **(H)** Wheel running of individual mice (light traces) and group mean (bold traces) across days of food restriction for **(H1)** light cycle and **(H2)** dark cycle running. **(I)** Maximum increase in wheel running across 2 consecutive days (averaged by group) during the light and dark cycles. **(J)** Histogram of the percentage of mice that exhibited peak increase in light cycle running on each day of food restriction. **(K)** Correlation between **(K1)** light cycle or **(K2)** dark cycle running and change in BW the next day. Each symbol represents 1 animal on 1 experimental day. Food intake data: n = 12 ($DAT^{+/+}$ mice), n = 8 ($DAT^{cre/+}$ mice); all other data: n = 16 ($DAT^{+/+}$ mice), n = 11 ($DAT^{cre/+}$ mice). †Survival curves, $p < .05$; ***$p < .001$ vs. before removal. Error bars indicate SEM. DAT, dopamine transporter.

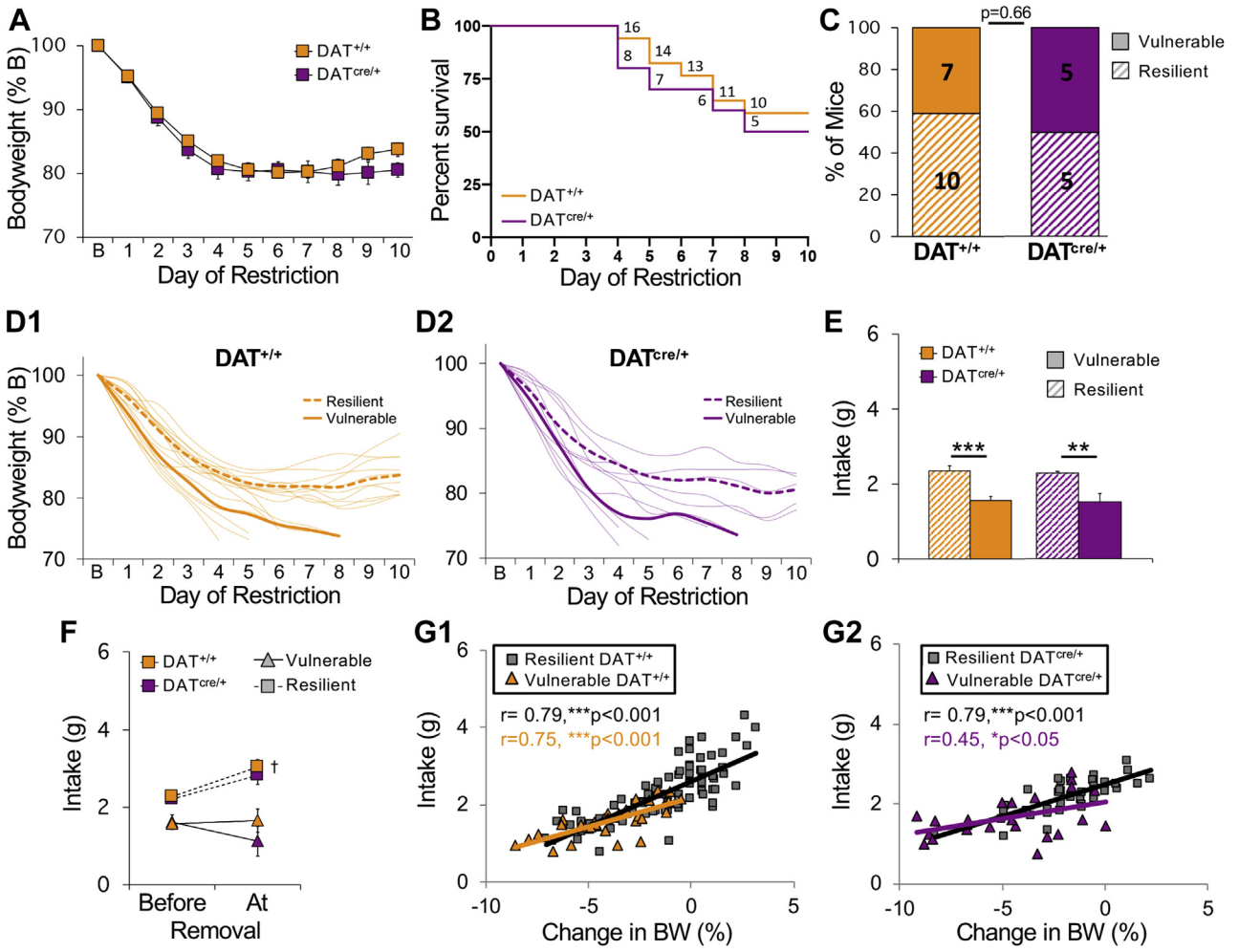

Figure 10. Hyperdopaminergic mice do not show increased vulnerability to food restriction in the absence of a running wheel. All mice were tested in the food-restricted condition. **(A)** Body weight (BW) across days of food restriction. **(B)** Survival curves for each group. Numbers indicate number of surviving mice that day. **(C)** Percent of vulnerable (solid) and resilient (hatched) mice of each genotype (number of animals is indicated inside bars). **(D)** BW of individual **(D1)** control and **(D2)** hyperdopaminergic mice (light traces) across days of food restriction. Group averages for resilient (dashed line) and vulnerable (solid line) mice are shown in bold. **(E)** Average food intake of vulnerable (solid) and resilient (hatched) mice of each genotype. **(F)** Average food intake prior to removal vs. the day of removal. **(G)** Correlation between consumption during restricted access to food and changes in body weight the next day for **(G1)** control and **(G2)** hyperdopaminergic mice. Each symbol represents 1 animal on 1 experimental day. n = 10 (resilient DAT$^{+/+}$ mice), n = 7 (vulnerable DAT$^{+/+}$ mice), n = 5 (resilient DAT$^{cre/+}$ mice), n = 5 (vulnerable DAT$^{cre/+}$ mice). $^{**}p < .01$; $^{***}p < .001$; $^{\dagger}p < .05$ vs. before removal. Error bars indicate SEM. DAT, dopamine transporter.

the observed genotype differences do not result from a generalized, nonspecific elevation in activity, but rather are specific to ABA.

Basal Dopamine Function Is Similar in Adolescent and Young Adult Mice

To assess whether differences in basal dopamine function could account for differences in vulnerability between adolescent (PND 43) and young adult mice (PND 71), we compared striatal dopamine in female C57BL/6N mice at these ages. In adolescent mice, there was increased synaptic DAT in the nucleus accumbens (t_{16} = 3.42, $p < .01$) but not in the dorsolateral striatum (Figure S2). There were no differences between age groups in synaptic D_1 or D_2 receptors or cytosolic TH in either region (Figure S2). Fast-scan cyclic voltammetry revealed no difference between adolescents and adults in evoked dopamine release or clearance in the nucleus accumbens (Figure S3). These data suggest that age-related shifts in vulnerability do not emerge from developmental changes in basal dopamine function. However, this does not rule out differential dopamine response to environmental factors [e.g., (41)].

DISCUSSION

It is unclear why combining diet and exercise leads to AN in some individuals, while most maintain a healthy body

weight. One approach for studying this is to examine individual differences in ABA (42,43). Here, we demonstrate distinct vulnerable and resilient phenotypes, providing a robust animal model for investigating the physiological and neural adaptations underlying resilience and vulnerability to AN.

ABA resilience is associated with a progressive increase in food intake and decrease in overall wheel activity, leading to weight stabilization. Similar decreases have been reported in older C57BL/6 female mice (44,45), possibly reflecting an age-related increase in resilience. Moreover, we found that ABA-R mice actually eat more than FR-R mice and consequently gain more weight, despite the energy expenditure from wheel running. Though counterintuitive, this parallels evidence from clinical studies demonstrating that appropriate exercise may be beneficial in clinical treatment (46–49).

In contrast, vulnerable mice fail to increase their consumption, reducing intake as caloric restriction continues. This failure cannot be attributed to insufficient time to eat, because resilient mice increase consumption in the same 2-hour period of food access. While low food intake correlated with daily weight loss in FR-V mice, this was not found in ABA-V mice. Instead, daily weight loss correlated with amount of wheel activity. Vulnerability did not arise from preexisting differences in activity, as vulnerable and resilient mice ran similarly at baseline. During food restriction, running dramatically increased throughout the light cycle, with vulnerable animals primarily running instead of sleeping. These findings are consistent with prior studies demonstrating that caloric restriction can induce activity that is partially diurnal (50–52) and may reflect exaggerated food-anticipatory activity or a shift in circadian activity to promote foraging (51). Disruption of circadian rhythms has been observed in AN (53). This, as well as hyperactivity, could arise from activation of starvation-induced foraging mechanisms that promote physical activity (26,54–56). Individual differences in this response might arise from genetic variation in a human foraging gene (57).

Our studies with hyperdopaminergic mice indicate that increased dopamine promotes vulnerability to ABA by accelerating increases in activity that occur in response to caloric restriction. Vulnerability was not increased in KD mice tested under FR conditions, suggesting a critical interaction between dopamine, physical activity, and vulnerability to caloric restriction. As the KD is global, such effects may arise from increased striatal dopamine or dopamine actions elsewhere. This could include the hypothalamus or prefrontal cortex, though dopamine reuptake in the prefrontal cortex is mediated primarily by the norepinephrine transporter rather than the DAT (58–60).

ABA studies have primarily used rats, with fewer studies testing mice (44,45,61–63). In contrast to the stable body weight typically found in FR rats [(35,64,65), but see (66,67)], we found that FR control mice can exhibit life-threatening weight loss (Supplemental Discussion). This suggests that caloric restriction is the primary driver of vulnerability, with wheel access accelerating and augmenting expression of the vulnerable phenotype. Increased home cage activity may have promoted weight loss in the FR mice, which would further suggest a central role for activity in the emergence of the vulnerable phenotype.

Consistent with rat studies (68) and AN in humans (69), we find reduced vulnerability to ABA with age. Smaller animals generally have higher metabolic rates, which in combination with less body mass may increase vulnerability in younger mice. The rapidity of decline in adolescent mice may preclude emergence of a resilient phenotype, while greater initial body weight in young adult mice may slow the decline sufficiently for a resilient phenotype to emerge. Extending daily food restriction allowed detection of the vulnerable phenotype in FR mice. Single housing likely exacerbates vulnerability in smaller animals, as the energetic cost of maintaining body temperature is increased without group huddling, compounding increased susceptibility associated with low body mass and high metabolic rate.

Although ABA vulnerability is increased by dopamine, the greater vulnerability we observe in adolescent mice is not due to differences in basal levels of striatal dopamine (Figure S3). Vulnerability during adolescence may instead result from age-related differences in how caloric restriction and exercise induce dopamine adaptations (see below). Alternatively, maturation of dopamine-prefrontal cortical innervation, which continues through young adulthood, may play a role by affecting inhibitory control. The smaller size of the adolescent mouse may be an additional factor.

Dopamine and AN Vulnerability

Dopamine has been implicated in AN, but its contribution remains poorly understood. Recovered AN patients exhibit reduced dopamine metabolites, suggesting decreased turnover (70), but show increased dopamine D_2 receptor binding, reflecting increased D_2 receptor expression and/or decreased dopamine transmission (71). Patients often remain symptomatic after recovery (72), making it difficult to determine whether differences in dopamine observed in clinical studies represent preexisting risk factors or potentially reversible abnormalities induced by starvation (26). Our finding that hyperdopaminergic mice are more vulnerable to ABA suggests that enhanced dopamine increases AN risk. However, this does not necessarily mean that vulnerability is mediated by basal or trait differences in dopamine function. In AN, several factors arising from caloric restriction can upregulate dopamine, including increased glucocorticoids (73–77), enhanced insulin sensitivity (78–81), increased ghrelin (82–84), and decreased leptin (85–90). Indeed, studies have linked decreased leptin with hyperactivity [(87,90–93) but see (94)], with some implicating dopamine (87,90,93). Such adaptations to caloric restriction presumably arise to promote physical activity required to forage for food to obtain needed calories (26), making them potential therapeutic targets for reducing hyperactivity in AN. Dopamine also contributes to thermoregulation (95–97), which is altered in AN (6,98) and ABA (94,99). Hypothermia arising from weight loss may also induce changes in dopamine. Vulnerability to AN might vary due to individual differences in any of these responses to caloric restriction, differences that could result from variation in dopamine genes or genes that modulate dopamine (e.g., leptin, insulin, ghrelin, glucocorticoid, orexin). Of course, factors independent of dopamine may also modulate vulnerability [e.g., (100)].

Dopamine function may change over the course of AN, as observed in addiction, in which dopamine contributes differently in different stages of the disorder (101–104). Such dynamic changes may account for why a recent study found that pharmacogenetic activation of dopamine protects rats from ABA (64). Artificial activation of dopamine cells could have blocked normally occurring changes in dopamine and progressive adaptations to caloric restriction that underlie ABA, dynamic changes facilitated in our hyperdopaminergic mice. The pharmacogenetic activation of some nondopaminergic cells may have also contributed to the discrepancy between our findings. The nature of progressive changes in dopamine across AN/ABA is unknown but merits further study.

A robust model of vulnerability and resilience, as reported here, could aid in the discovery of genes that mediate AN risk and/or contribute to the progression of the disorder. Such work may lead to the identification of biomarkers for early diagnosis and the discovery of novel therapeutic targets.

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