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Chronic Stress Modulates Regional Cerebral Glucose Transporter Expression in an Age-Specific and Sexually-Dimorphic Manner

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Abstract

Facilitative glucose transporters (GLUT) mediate glucose uptake across the blood-brain-barrier into neurons and glia. Deficits in specific cerebral GLUT isoforms are linked to developmental and neurological dysfunction, but less is known about the range of variation in cerebral GLUT expression in normal conditions and the effects of environmental influences on cerebral GLUT expression. Knowing that puberty is a time of increased cerebral plasticity, metabolic demand, and shifts in hormonal balance for males and females, we first assessed gene expression of five GLUT subtypes in four brain regions in male and female adolescent and adult Wistar rats. The data indicated that sex differences in GLUT expression were most profound in the hypothalamus, and the transition from adolescence to adulthood had the most profound effect on GLUT expression in the hippocampus. Next, given the substantial energetic demands during adolescence and prior demonstrations of the adverse effects of adolescent stress, we determined the extent to which chronic stress altered GLUT expression in males and females in both adolescence and adulthood. Chronic stress significantly altered cerebral GLUT expression in males and females throughout both developmental stages but in a sexually dimorphic and brain region-specific manner. Collectively, our data demonstrate that cerebral GLUTs are expressed differentially based on brain region, sex, age, and stress exposure. These results suggest that developmental and environmental factors influence GLUT expression in multiple brain regions. Given the importance of appropriate metabolic balance within the brain, further assessment of the functional implications of life stage and environmentally-induced changes in GLUTs are warranted.

Keywords

Stress; age; sex; brain; glucose transporter

1.0 INTRODUCTION

Adequate glucose transport is essential to brain function and survival. The adult brain accounts for 20% of total resting oxygen consumption in humans [1–3] and 4–6% in rats [1],

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which is almost entirely utilized for the oxidation of carbohydrates [4]. While the cerebral metabolic demand is substantial in the adult, the metabolic rate in children is much higher, calculating to as much as 50% of whole-body glucose utilization by the developing human brain [4]. Glucose transporters (protein symbol GLUT, gene symbol *Slc2a*) facilitate glucose transport across the blood-brain-barrier and the uptake of glucose into neurons and glia [5–7]. The crucial role of GLUTs is illustrated by the profound neurological deficits manifested in De Vivo disease, a rare genetic condition in which GLUT subtype 1 is not expressed [8]. Milder deficits in the expression and translocation of GLUTs have been linked to neuropathology including Alzheimer's pathology, post-ischemic/hypoxic brain function, and following traumatic brain injury [9–13]. Less is known about the range of variation in cerebral GLUT expression in normal conditions and the effects of environmental influences on cerebral GLUT expression.

The GLUT family is not fully characterized, however, current literature indicates at least nine GLUT isoforms are expressed throughout the brain [6, 14], and five of the nine have a plausible role in development [15, 16] and a potential role in the effects of chronic stress [16–19]. Here we focus on transporter isoforms GLUT 1, 3, 4, 5, and 8. The specific functions of these GLUTs and their involvement in neurologic and stress-related disorders are outlined in Table 1. Few studies have examined multiple cerebral GLUTs in the same animal, and little attention has been given to the influences of sex, age, or stress exposure on the expression of these essential transporters.

Rapid developmental periods, such as puberty, are characterized by both increased cerebral plasticity and augmented demand for metabolic energy. Therefore, changes in GLUT during the adolescent developmental period could lead to longstanding changes in cerebral metabolism and neuron function. Although there is some evidence that stress can modify cerebral GLUT in the adult rat [16, 19, 20], it is unknown whether stress during adolescence can alter cerebral GLUT expression, or whether stress affects GLUT expression similarly in males and females. Thus, the present study examined the interactions among sex, age, and chronic mixed modality stress on cerebral GLUT mRNA abundance. Because of the dearth of information available regarding cerebral GLUTs during adolescent development and minimal information regarding sex differences, we initially assessed expression of the five GLUT subtypes outlined in four brain regions in male and female adolescent and adult rats. We hypothesized that developmental sex differences in GLUT mRNA abundance would exist in brain regions involved in regulating hormonal secretion and affective behavior, particularly the hypothalamus. In addition, given the substantial energetic demands during adolescence and previous demonstrations of the adverse effects of adolescent stress [21, 22], we determined the extent to which chronic stress altered GLUT mRNA abundance in males and females in both adolescence and adulthood. Collectively, our data demonstrate that cerebral GLUTs are expressed differentially based on brain region, sex, age, and exposure to stress. Furthermore, the timing of the stress exposure interacts with sex to determine the changes in GLUT mRNA abundance. This study is one of the first reports of multiple cerebral GLUTs with attention to age, sex, environment, and brain region. Regional changes in GLUT expression may impact brain function and thereby behavior.

2.0 MATERIALS AND METHODS

2.1 Animals

Timed pregnant Wistar rats (Charles River, Wilmington, MA) arrived on gestational day 12. This timing of shipping stress is not associated with changes in developmental outcomes [23], whereas shipping stress during puberty can have enduring effects on behavior [24, 25]. Rats were housed on a 14:10 reverse light:dark cycle in a facility controlled for humidity (60%) and temperature (20 °C–23 °C). Rodent diet 5001 chow (Purina Mills, Richmond, IN)

and water were maintained *ad libitum* throughout the study. Three days after birth litters were culled and weaned on postnatal day (PND) 23. On PND 36, rats were assigned groups and housed in same-sex pairs. Littermates were assigned to adolescent or adult control or stress groups with no more than two pups per litter assigned to each group. All groups contained between 10 and 12 rats. All animal experiments were approved by Emory University's Institutional Animal Care and Use Committee and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Resources, 1996). All efforts were made to minimize animal suffering and to reduce the number of rats used.

2.2 Experimental Design

For the cohort of rats used to assess regional differences in cerebral GLUT expression as a function of age and sex, rats were maintained in standard colony conditions and adolescent animals were euthanized via rapid decapitation at PND 51 and adult animals were euthanized via rapid decapitation at PND 100. To account for potential variation in circadian or diurnal rhythms in GLUT expression [26, 27], all animals were sacrificed between 10am and 12pm, two to four hours before the beginning of their dark cycle. Brains were removed, flash frozen on dry ice, and stored at −80°C until dissection. The hypothalamus, hippocampus, amygdala, and prefrontal cortex were dissected and stored at −80°C until used for quantitative RT-PCR analysis of glucose transporters *Slc2a1*, *Slc2a3*, *Slc2a4*, *Slc2a5*, and *Slc2a8*.

For the assessment of chronic stress effects on cerebral GLUTs, control animals were treated as described above. Rats in the chronic stress cohort were exposed to the chronic mixed modality stress paradigm in either adolescence (PND 37–48) or adulthood (PND 86–97). While adolescence is difficult to define precisely in rats as in humans, it is usually accepted that infancy and early childhood end at weaning and that adulthood begins at PND60. Sex organ maturation occurs roughly from PND35 to PND49, and initiation of sexual behavior as well as additional neurodevelopmental and cognitive changes occur from PND49 to PND60 [28, 29]. The adolescent stress paradigm thus spans the hormonal surges that occur during the course of development, while the adult paradigm is well past these developmental changes.

To ensure that all effects measured were due to the chronic nature of the stress, and not an acute stress, a three-day waiting period was maintained before euthanizing the animals. After rapid decapitation, brains from the chronic stress cohort were treated as described for the control cohort.

2.3 Mixed Modality Chronic Stress

The mixed modality chronic stress paradigm has previously been described and elicits sex-specific behavioral changes in adolescent rats [21, 22, 30]. Briefly, adolescent and adult stress was defined as individual housing beginning at PND37 (adolescents) or PND 86 (adults) and continuing throughout the study combined with randomly alternating daily exposure to either 6 social defeat episodes and 6 restraint sessions (PND 37–48 adolescent cohort and PND 86–97 adult cohort; 6 days each of social defeat and restraint). Social defeat occurred by pairing the rats with a same sex aggressive Long Evans rat (female aggressors were ovariectomized). Pairing lasted for 30 minutes on each social defeat day. Rats interacted for 3 pins or 5 minutes and were then separated by a screen for the remainder of the 30 minutes. The control groups remained pair-housed with a same sex littermate throughout the study. The study was not designed to assess the specific effects of individual housing, restraint, or social defeat, but was designed to use this combination of established

stressors to induce chronic stress during adolescence or adulthood. Body mass was monitored throughout the study for all groups.

2.4 Vaginal Lavage

Beginning at PND94, vaginal lavage was performed on adult females to determine the estrous cycle stage at time of death. Cycle was characterized as diestrus 1 (D1), diestrus 2 (D2), proestrus (P) or estrus (E) based on methods previously described [31, 32]. There were no significant differences in distribution of animals in a given cycle stage among the different experimental cohorts, therefore, reported group effects are not the result of a confounding effect of estrous cycle stage. However, this study design does not rule out the possibility of effects of estrous cycle stage on mRNA abundance of GLUTs.

2.5 Quantitative RT-PCR

RNA was extracted from frozen rat tissue consisting of hypothalamus, hippocampus, amygdala, and prefrontal cortex using the mirVana PARIS KIT (Ambion Life Technologies, Carlsbad, CA). RNA quantity and integrity was assessed using a NanoDrop 2000 spectrophotometer (ThermoScientific, Wilmington, DE). RNA was then standardized so all samples started reverse transcription with 1ug RNA. Then, RNA was reverse transcribed using High Capacity RNA to cDNA Kit (Applied Biosystems, Foster City, CA). Several rat endogenous control plates (*Tfrc*, *Hprt1*, *Ubc*, and *Ppia*; Applied Biosystems, Foster City, CA) were run in order to determine the optimal endogenous control, but none of the endogenous controls nor their geometric means tested remained invariant among all eight experimental groups. Thus, to ensure standardization in total cDNA across groups, cDNA was quantified via the PicoGreen Assay (Invitrogen, Carlsbad, CA) and then standardized so that all samples started quantitative RT-PCR with 1ug cDNA. TaqMan gene expression master mix and rat TaqMan Gene Expression Assays were purchased from Applied Biosystems (Carlsbad, CA) with probes labeled for *Slc2a1* (GLUT1; assay ID Rn01417099_m1), *Slc2a3* (GLUT3; assay ID Rn00567331_m1), *Slc2a4* (GLUT4, assay ID Rn01752377_m1), *Slc2a5* (GLUT5; assay ID Rn00582000_m1), and *Slc2a8* (GLUT8; assay ID Rn00585203_m1). The universal two-step RT-PCR cycling conditions used on the 7900HT Sequence Detection System (Applied Biosystems) were: 50^{C} (2 min), 95^{C} (10 min), 40 cycles of 95^{C} (15 s) and 60^{C} (1 min). Relative gene expression of individual samples were run in triplicate and calculated by the comparative Ct quantification method relative to the same-sex no stress saline control group or the male adolescent no stress saline group ($2^{-\Delta CT}$) with a Grubb's correction for statistical outliers. The coefficient of variance cut off was set to 4%.

2.6 Statistical Analysis

To determine the effects of sex and developmental stage on GLUT gene expression, statistical comparisons of differences in RT-PCR for the hypothalamus, hippocampus, amygdala, and prefrontal cortex were analyzed using Two-Way ANOVA followed by Tukey's post-hoc testing, with all groups normalized to adolescent male values. Percent change in body mass before and after stress was also analyzed by two-way ANOVA with the factors of sex and stress, with Tukey's post-hoc testing when appropriate. The effect of chronic stress on weight gain was separately assessed in adolescence and adulthood by two-way ANOVAs due to the large disparity in adolescent versus adult weight at baseline. To determine the effects of stress and developmental stage on GLUT gene expression, all groups were analyzed within their given sex (male or female) and normalized to the adolescent control group. Two-way ANOVA was performed using the factors of stress and developmental stage followed by Tukey's post-hoc testing. All statistical analysis was performed using GraphPad Prism v.6 (La Jolla, CA). All data were determined to be

statistically significant when $p < 0.05$ and are presented as a mean ± standard error of the mean (SEM).

3.0 Results

3.1 Hypothalamic GLUT1 and GLUT4 mRNA Abundance Increases with Age and is Sexually Dimorphic

Hypothalamic expression of *Slc2a1*, as measured using RT-PCR, increased with age for male, but not female rats (interaction of sex and age: $F_{1,34} = 4.286$; $p = 0.046$; main effect age: $F_{1,34} = 4.395$; $p = 0.043$; Figure 1a). A sex difference in hypothalamic *Slc2a1* expression also existed (main effect sex: $F_{1,34} = 4.392$; $p = 0.043$) and was specific to lower expression of hypothalamic *Slc2a1* expression in male than female adolescent rats ($p = 0.028$). A similar sex and age dependent pattern of gene expression was observed for *Slc2a4* in the hypothalamus such that *Slc2a4* expression increased with age for male but not female rats (interaction of sex and age: $F_{1,34} = 6.332$; $p = 0.0167$; main effect of sex: $F_{1,34} = 4.787$; $p = 0.035$; Figure 1b). mRNA transcripts that encode for GLUT 3 (sex: $F_{1,32} = 2.968$; $p = 0.0946$; age: $F_{1,32} = .067$; $p = 0.796$), GLUT 5 (sex: $F_{1,34} = 0.955$; $p = 0.335$; age: $F_{1,34} = 0.163$; $p = 0.688$), and GLUT 8 (sex: $F_{1,33} = 2.767$; $p = 0.105$; age: $F_{1,33} = 1.121$; $p = 0.297$) in the hypothalamus did not differ based on either sex or age.

3.2 Hippocampal mRNA Abundance of GLUT 3, 4, and 8 Decreases with Age in Males and Females

Hippocampal expression of *Slc2a3*, *Slc2a4*, and *Slc2a8* was reduced in male and female adults (main effects of age: *Slc2a3*: $F_{1,35} = 4.211$, $p = 0.047$, Figure 2a; *Slc2a4*: $F_{1,35} = 4.328$, $p = 0.044$, Figure 2b; and *Slc2a8*: $F_{1,34} = 5.415$, $p = 0.026$, Figure 2c). No effect of sex or age on expression of Slc2a1 (sex: $F_{1,35} = 0.030$; $p = 0.861$; age: $F_{1,35} = 0.899$; $p = 0.349$) or *Slc2a5* (sex: $F_{1,36} = 0.0008$; $p = 0.977$; age: $F_{1,36} = 2.070$; $p = 0.158$) was observed in the hippocampus.

3.3 Neither Sex nor Age Impact mRNA Abundance of GLUTs in the Amygdala or Prefrontal Cortex

Unlike the hypothalamus and hippocampus, expression of genes encoding for GLUT 1, 3, 4, 5, and 8 did not differ between adolescent and adult rats ($p > 0.05$) or between male and female rats ($p > 0.05$) within the amygdala or the prefrontal cortex.

3.4 Chronic Stress Reduced Weight Gain

Exposure to chronic stress during adolescence reduced the percent weight gained (main effect of stress: $F_{1,39} = 204.8$; $p<0.001$) and preferentially reduced male weight gain (main effect of sex $F_{1,39} = 21.34$; $p < 0.001$; interaction of sex and stress $F_{1,39} = 6.608$; $p = 0.014$; mean percent change ± standard deviation: adolescent male control: 49.29% ± 4.22%; adolescent male stress: 40.38% ± 4.61%; adolescent female control: 28.37% ± 4.11%; adolescent female stress: 25.834 ± 3.23%). Among the adult rats, exposure to chronic stress reduced percent weight gain in both males and females (main effect of stress: $F_{1,38} = 15.57$; $p < 0.001$) without a significant sex effect ($F_{1,38} = 0.008$; $p=0.927$; mean percent change ± standard deviation: adult male control: 4.10% ± 3.31%; adult male stress: 1.22% ± 2.24%; adult female control: 4.79% ± 2.16%; adult female stress: 0.37% ± 3.74%). Prior to stress, body masses for each cohort were (in grams; mean ± standard deviation): male adolescent control, 178.7 ± 14.2, male adolescent pre-stress, 177.8 ± 11.2; female adolescent control, 143.7 ± 8.3; female adolescent pre-stress, 143.2 ± 8.8; male adult control, 456.3 ± 52.6; male adult pre-stress, 459.7 ± 38.1; female adult control, 257.7 ± 18.4; and female adult pre-stress, 258.8 ± 10.7. Post-stress, body masses for each cohort were (in grams; mean ±

standard deviation): male adolescent control, 266.5 ± 18.9; male adolescent post-stress, 249.5 ± 15.1, female adolescent control, 184.6 ± 14. 3, female adolescent post-stress, 180.1 ± 10.9; male adult control, 475.4 ± 60.1; male adult post-stress, 465 ± 41.2; female adult control, 270.1 ± 20.6; and female adult post-stress, 259.7 ± 12.0.

3.5 Chronic Stress and Age Interact to Alter Hypothalamic GLUT mRNA Abundance

Hypothalamic expression of *Scl2a1* (Figure 3a and Figure 3b) and *Scl2a8* (Figure 3g and Figure 3h) was not altered after chronic stress in either males or females at either age assessed ($p > 0.05$). In contrast, hypothalamic expression of *Slc2a3* decreased after chronic stress for female, but not male rats (main effect of stress: $F_{1,37} = 5.67$; $p = 0.022$; Figure 3d). While *Slc2a4* expression remained unchanged among females (stress: $F_{1,39} = 1.910$; $p = 0.174$; age: $F_{1,39} = 0.101$; $p = 0.751$), chronic stress and age interacted to affect *Slc2a4* gene expression in males such that stress increased expression in adolescent males and decreased expression in adult males (interaction of stress and age: $F_{1,33} = 6.980$; $p = 0.012$; Figure 3e). However, consistent with the data presented in Figure 1b, post-hoc testing revealed significant increases in *Slc2a4* expression in male adult controls relative to male adolescent controls ($p = 0.048$) indicating that this effect may be due to differential baseline expression across age.

3.6 Chronic Stress and age Interact to Increase Hippocampal GLUT mRNA Abundance

Male hippocampal *Slc2a1* expression increased after exposure to chronic stress, irrespective of age (main effect of stress: $F_{1,33} = 4.9$; $p = 0.033$; Figure 4a). Expression of *Slc2a1* was the only gene assessed that was altered by chronic stress in the male hippocampus. Female expression of hippocampal *Slc2a3* decreased in adulthood (main effect of age: $F_{1,39} = 4.871$; $p = 0.033$; Figure 4d), but exposure to chronic stress altered this pattern to increase *Slc2a3* expression in adulthood, nullifying the age-related reduction in gene expression. Chronic stress also increased expression of *Slc2a5* in the female hippocampus irrespective of age (main effect of stress: $F_{1,40} = 4.06$; $p=0.036$; Figure 4f). Female expression of hippocampal *Slc2a8* showed an overall decrease with age ($F_{1,34}=5.415$; $p=0.036$; Figure 4h) and as with *Slc2a3*, chronic stress altered this pattern to increase *Slc2a8* expression in adulthood, nullifying the age-related reduction in gene expression. Hippocampal expression of *Slc2a4* was not altered by stress in either males ($F_{1,32}=0.150$; $p=0.700$) or females ($F_{1,40}=0.003$; $p=0.955$) at any age.

3.7 Chronic Stress and age Interact to Increase GLUT mRNA Abundance in the Male Amygdala

In the male amygdala, the increase in *Slc2a3* expression with age (main effect of age: $F_{1,37} = 5.07$; $p = 0.030$; Figure 5c) was further exacerbated after chronic adult stress, as determined in post-hoc analysis ($p= 0.045$). Chronic stress also interacted with age to alter amygdalar expression of *Slc2a5* (interaction of age and stress: $F_{1,33}=6.601$; $p=0.014$; Figure 5e) such that expression increased with chronic stress exposure in adolescent males, but decreased with chronic stress exposure in adult males. Amygdalar expression of *Slc2a1* ($F_{1,34}=1.053$; $p=0.312$), *Slc2a4* ($F_{1,37}=1.572$; $p=0.217$), and *Slc2a8* ($F_{1,32}=0.059$; $p=0.808$) was unaltered by chronic stress in males, regardless of age. Likewise, no GLUT gene isoforms were altered by stress in the female rat amygdala, regardless of age: *Slc2a1* ($F_{1,38}=1.297$; $p=0.261$), *Slc2a3* ($F_{1,39}=1.635$; $p=0.208$), *Slc2a4* ($F_{1,37}=1.572$; $p=0.217$), *Slc2a5* ($F_{1,37}=0.013$; $p=0.909$), *Slc2a8* ($F_{1,36}=0.348$; $p=0.558$).

3.8 Chronic Stress Does Not Alter GLUT mRNA Abundance in the Prefrontal Cortex

In the prefrontal cortex, gene expression of *Slc2a1*, *Slc2a3*, *Slc2a4*, *Slc2a5*, and *Slc2a8* were also analyzed using the same parameters as for the other brain regions. No significant effects

of stress, age, or sex were found for any GLUTs analyzed in the prefrontal cortex ($p > 0.05$ in all cases).

4.0 DISCUSSION

Sex and age modify regional and isoform-specific GLUT expression in the brain. Sex differences in GLUT mRNA abundance were most profound in the hypothalamus, while the transition from adolescence to adulthood appeared to be most influential on GLUT transcript expression in the hippocampus. Exposure to chronic stress impacted GLUT expression differentially depending on age and sex, and effects of chronic stress on GLUT expression were evident in the hypothalamus, hippocampus, and amygdala – but not in the prefrontal cortex. In summary, GLUT mRNA abundance is plastic in the adolescent and adult brain and plasticity in GLUT isoform expression differs by brain region. Furthermore, sex is an important variable in assessing the impact of stress on GLUT transcript expression. Given the critical role of GLUT in supplying energy to the brain, understanding the effects of sex, development, and stress on GLUT expression is an essential first step in developing a comprehensive knowledge regarding the potential role of pathological changes in GLUTs in neuronal and behavioral dysfunctions.

In the hypothalamus, *Slc2a1* and *Slc2a4* expression increased with age in males. Female adolescent rats had higher expression of *Slc2a1* and *Slc2a4* than adolescent males and no effect of age was observed in the females. The increase in male hypothalamic *Slc2a1* expression is consistent with developmental patterns of GLUT1 identified in the hypothalamus and other regions of the rodent brain [33, 34]. The fact that the female hypothalamus did not undergo similar increases in *Slc2a1* may be due to sex differences in developmental timing, given the potential for females to undergo puberty earlier in life [35]. Moreover, significant sexual dimorphism exists in the hypothalamus [36, 37], and others have reported similar divergence in the onset at which males and females reach adult levels of other hypothalamic neuropeptides [38].

Expression of GLUTs in the hippocampus was more sensitive to the effects of the adolescent to adult transition than to sex differences. Hippocampal expression of *Slc2a3, Slc2a4, and Slc2a8* decreased in adulthood in both sexes. This decrease in mRNA abundance is consistent with the peak and subsequent decrease in GLUT4 and GLUT8 protein seen in mouse whole brain homogenate between PND21 and PND60 [39]. To understand this developmental shift, it is crucial to understand the cellular and regional distribution of these transporters. GLUT4 [40] and GLUT8 [41] have previously been shown to be enriched in the hippocampus. GLUT3, GLUT4, and GLUT8 are primarily expressed in neurons [7], with GLUT4 primarily dendritic and GLUT8 localized in both the soma and neuronal processes [42]. This localization and redundancy is suggestive of a role in neurotransmission. During puberty, the hippocampus undergoes significant dendritic pruning and reorganization [43, 44] that plays a critical role in regulating cognitive and affective behavior in adulthood [35, 45]. Reductions in hippocampal neuronal GLUTs may reflect this pruning and represent an overall shift toward an adult dependence on neuronal-glial interactions for nutrient transport.

The shift to increased hypothalamic GLUT1 gene expression with age, but the decrease in hippocampal expression of GLUT3, GLUT4, and GLUT8 is additionally intriguing in light of the developmental shift from the use of ketones to glucose as a metabolic substrate [46]. Throughout the suckling phase, a high level of β-hydroxybutyrate as well as monocarboxylate transporters, found in endothelial cells and astrocytes, are maintained to allow for metabolism and transport of ketone bodies due to the high fat content of milk [47]. Given GLUT1's expression on similar cell types to monocarboxylate transporters, it is not

surprising that the data reflect an age-dependent increase in GLUT1 expression coincident with the known developmental transition to preferentially transport glucose over ketone bodies and alternate substrates such as pyruvate and lactate. The other glucose transporters shown here to decrease in expression with age have different cell type expression [7], have lower affinity for glucose [6], and have further not been shown to play the same critical role in glucose sensing as hypothalamic GLUT1 [48].

We next assessed the effects of chronic mixed modality stress on regional GLUT expression in male and female adult and adolescent rats to determine whether chronic stress would alter cerebral GLUT expression. The data presented here show that chronic stress exposure modulates GLUT mRNA abundance in a sexually-dimorphic manner in the hypothalamus, hippocampus, and amygdala, but not in the prefrontal cortex. Chronic stress significantly altered cerebral GLUT transcription in males and females throughout both developmental stages but in a sexually dimorphic and region-specific manner. Broadly speaking, in the hypothalamus, chronic stress altered expression of specific transporters in a sexually dimorphic manner; in the hippocampus, chronic stress increased expression of specific transporters in both males and females; and in the amygdala, stress affected only male transporter expression.

Consistent with previous data indicating a male bias to the metabolic effects of stress [30, 49], male glucose transporter expression increased in most cases after chronic stress: hypothalamic *Slc2a4* increased after adolescent stress; hippocampal *Slc2a1* increased after stress at both ages; and amygdalar *Slc2a3* and *Slc2a5* after adult and adolescent stress, respectively. Collectively, these data suggest an overall increase in cerebral glucose requirement after chronic stress, similar to the increase in glucose demand and GLUT1 expression in states of physiological stress including hypoxia [50], immune cell activation [51], or vascular injury [52]. Regional differences in isoform-specific regulation in response to stress may be due to relative baseline expression of the transporters in each region as well as each transporter's specific role. For example, regional cerebral blood flow is tightly linked to energy metabolism [53, 54] and highly vascularized regions may be more susceptible to changes in expression of the blood-brain-barrier and astrocyte transporter GLUT1. The hypothalamus is a region of both critical hormonal and metabolic control [55] and may play an important role in glucose and insulin-sensing [56]; thus, GLUT4 may be particularly susceptible to stress influence in this region. Peripherally, GLUT4 has been shown to be upregulated in response to prenatal stress in male, but not female, placenta [57]. In addition, other GLUT isoforms may be affected by stress paradigms under specific metabolic constraints based on each isoform's functional role. For example, others have shown that male hippocampal *Slc2a8* levels are relatively unchanged when exposed to short-term restraint stress [20], consistent with the male hippocampal *Slc2a8* expression data shown here. However, streptozotocin-induced diabetes dramatically increases hippocampal *Slc2a8* expression [20], yet this affect is significantly attenuated when exposed to the short-term restraint stress [20]. Thus, stress effects on GLUT isoforms can be environmentally dependent, as demonstrated in our results by the sexual dimorphism in stress-induced GLUT gene expression.

Though chronic stress induced primarily upregulatory effects on GLUT mRNA abundance in males, it did not have such a consistent effect in females. Chronic stress reduced *Slc2a3* expression in the hypothalamus in both adult and adolescent females. Stress is known to alter the HPG axis, to the extent of causing irregular estrous cycles in female rats [58], and this reduction in the primary neuronal transporter GLUT3 in the hypothalamus in females may be part of this interaction between the HPA and HPG axes. To control for cycle stage, females were examined using vaginal cytology for several days prior to tissue collection to permit for a balance of cycle stage at endpoint collection in both the stressed and control

groups. Although the current study does not address the question of whether or not GLUT expression varies across the estrous cycle, the design used does rule out the possibility that the reported effects in females are due to unequal representation of estrous cycle stages among the groups.

Collectively, the data presented establish that sex, age, and stress all impact gene expression of GLUTs in an isoform- and brain region- specific manner. The data demonstrate global increases in hypothalamic GLUT mRNA abundance in adulthood in males, while female hypothalamic GLUT expression is less plastic from adolescence to adulthood. Consistent with the literature on hippocampal reorganization, we demonstrate decreases in hippocampal transporter expression from the adolescent to adult transition, regardless of sex. Chronic stress effects are complex and affect expression profiles in region- and isoform-specific ways. Stress robustly increased male hippocampal *Slc2a1* expression and altered male amygdala expression, while females showed more regional variability between the hypothalamus and hippocampus in response to stress with both increased and decreased expression depending on the isoform. If these differences in mRNA abundance translate into differences in GLUT protein and function, then these variations may represent alternate energetic strategies which could underlie sex-specific differences in developmental and neurological outcomes and susceptibilities to stress-induced alterations in physiology and behavior.

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Highlights

1. Sex differences in GLUT expression are greatest in the hypothalamus.
2. The transition to adulthood decreases expression of specific hippocampal GLUTs.
3. Stress alters cerebral GLUTs across development in a sex- and region-specific way.
4. Cerebral GLUT expression differs based on region, sex, age, and stress exposure.

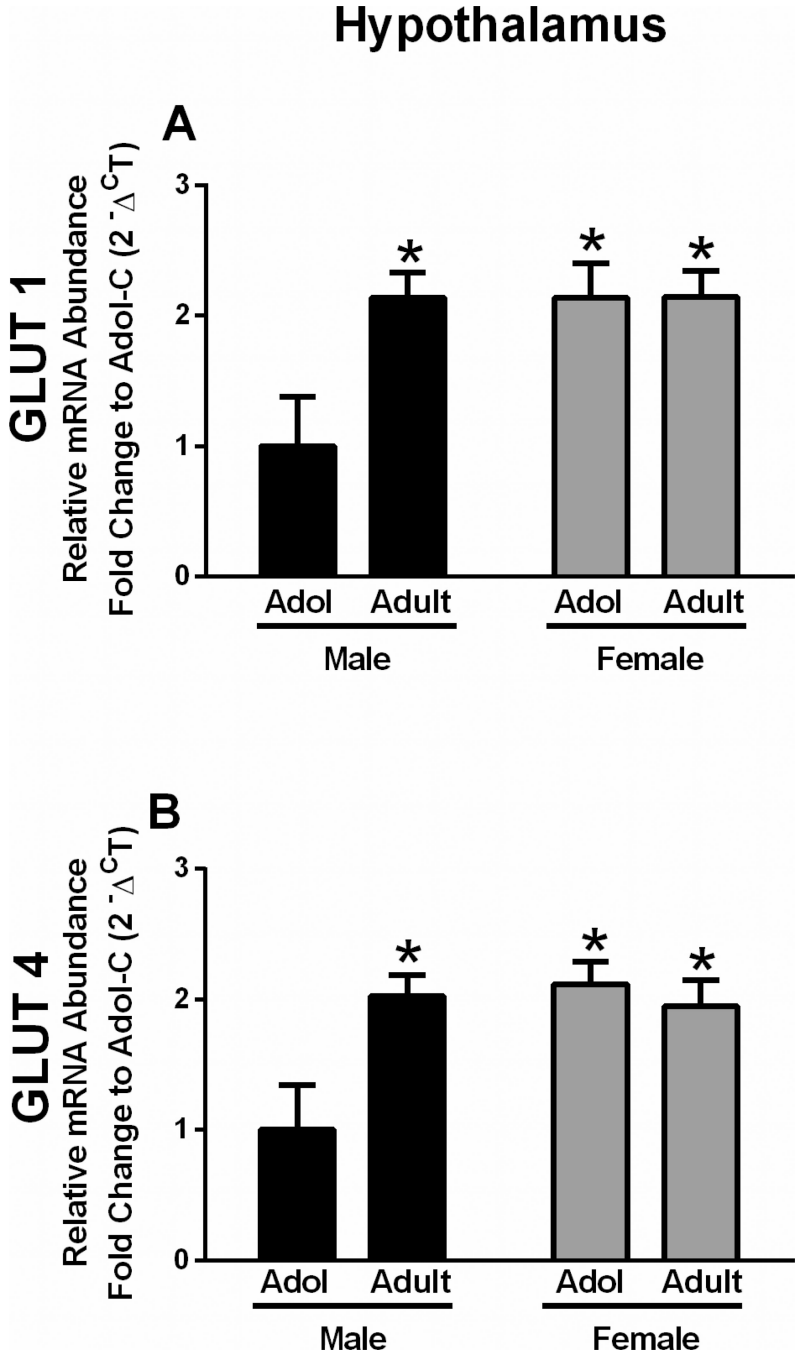

Figure 1. Adolescent male rats express less GLUT1 and GLUT4 in the hypothalamus than either adult males or females

(A) qRT-PCR analysis demonstrated that GLUT1 (*Slc2a1*) expression increased with age (interaction of age and sex: $F_{1,34} = 4.286$; $p = 0.0461$; main effect of age: $F_{1,34} = 4.395$; $p = 0.0436$). In addition, sex dictated expression of *Slc2a1* (main effect of sex $F_{1,34} = 4.392$; $p = 0.0436$), such that male adolescents had lower expression relative to female adolescents, as determined by a Tukey's post-hoc analysis ($p= 0.0282$). (B) GLUT4 *(Slc2a)4* gene expression also increased with age in males (interaction of age and sex: $F_{1,34} = 6.332$; $p = 0.0167$). Similar to *Slc2a1*, a sex difference existed in *Slc2a4* expression (main effect of sex: $F_{1,34} = 4.787$; $p = 0.0356$). Data shown represent mean fold change $2^{-\Delta CT} \pm$ SEM. Figure

symbols: Tukey's post hoc analysis is represented by an asterisk (*) in graphs A and B, which indicates an increase in gene expression relative to the male adolescent control group ($p<0.05$).

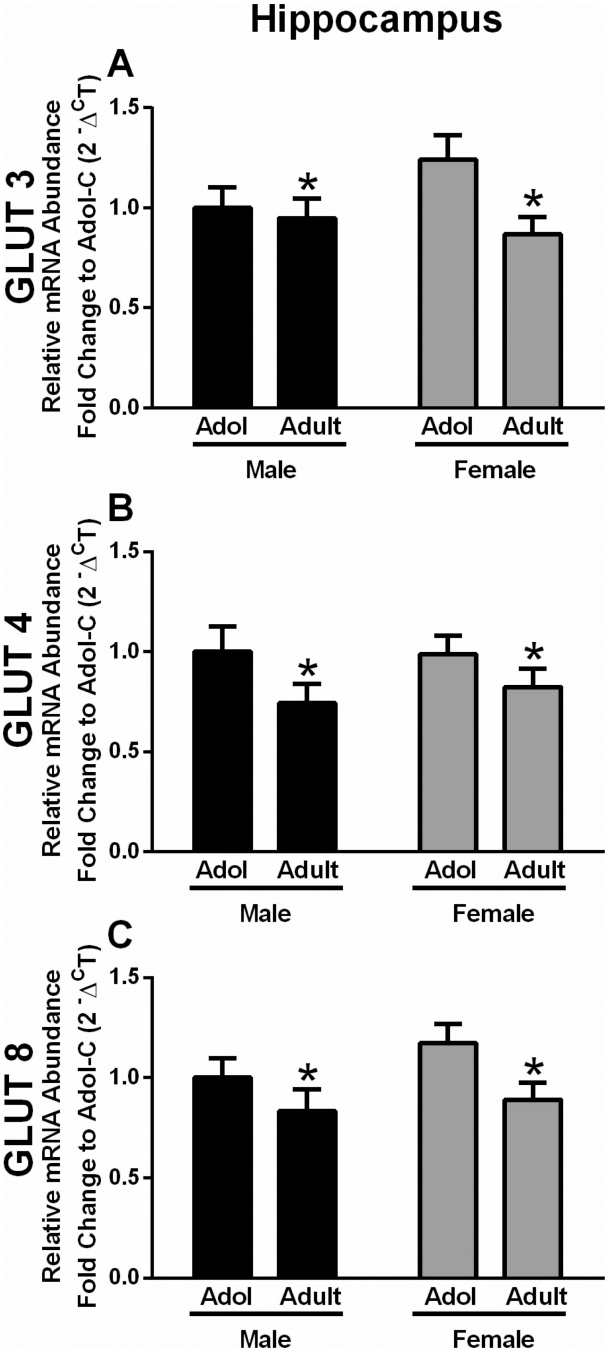

Figure 2. Age determines gene expression of GLUT3, GLUT4, and GLUT8 in the hippocampus, regardless of sex

qRT-PCR analysis demonstrated a decrease in gene expression in the hippocampus of male and female adults compared to adolescents for (A) GLUT3 (*Slc2a3*) (main effect of age $F_{1,35} = 4.211$; $p = 0.0477$), (B) GLUT4 (*Slc2a4*) (main effect of age $F_{1,35} = 4.328$; $p = 0.0449$), and (C) GLUT8 (*Slc2a8*) (main effect of age $F_{1,34} = 5.415$; $p = 0.0261$). Data shown represent mean fold change $2^{-\Delta CT} \pm$ SEM. Figure symbols: In graphs A, B, and C a significant main effect of age is indicated by asterisk (*) relative to male and female adolescent control groups ($p<0.05$).

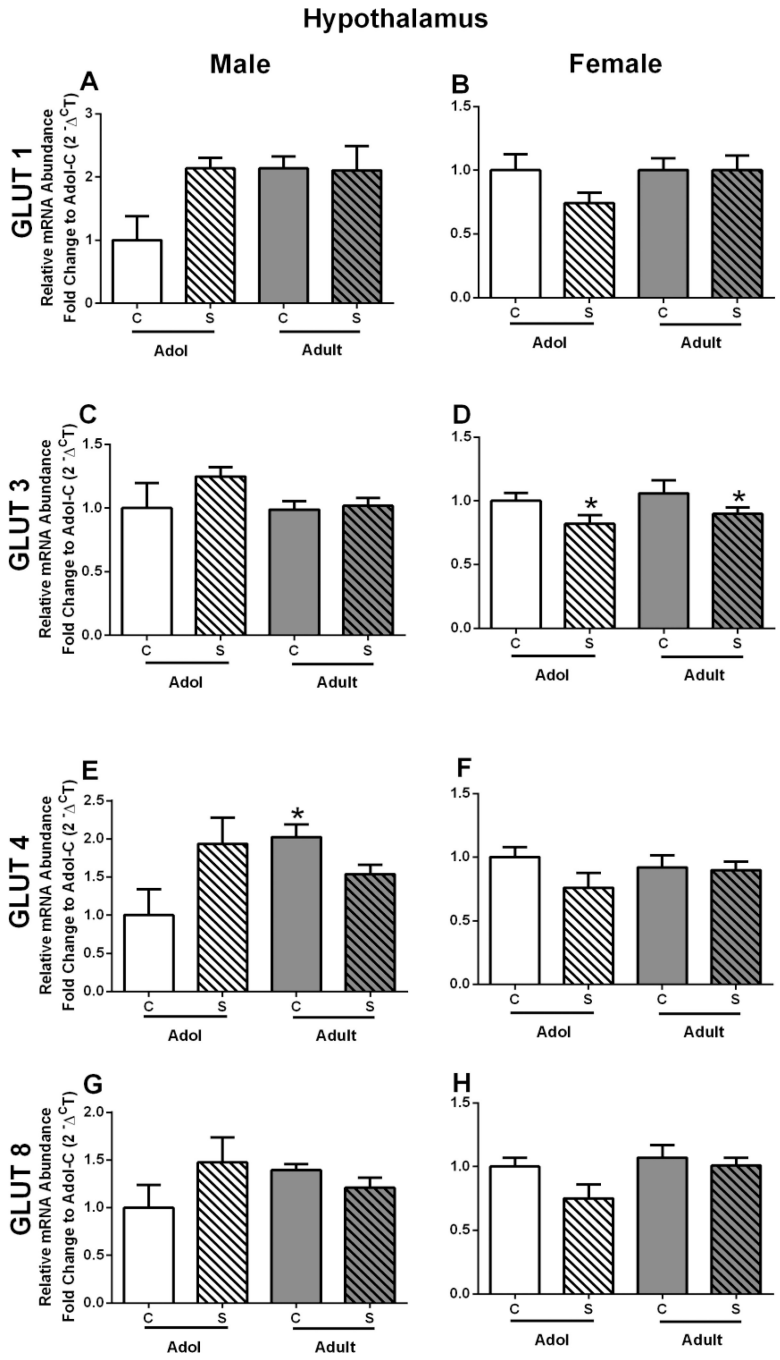

Figure 3. Stress differentially alters GLUT expression in the hypothalamus of male and female rats

qRT-PCR analysis of GLUT1 (*Slc2a1*) revealed no difference for age or stress in (A) males or (B) females ($p>0.05$). (C) While males did not demonstrate an effect of stress or age on expression of GLUT3 (*Slc2a3*) ($p>0.05$), (D) *Slc2a3* gene expression decreased in adolescent and adult females when exposed to stress (main effect of stress $F_{1,37} = 5.668$; $p = 0.0225$) regardless of age ($p>0.05$). (E) GLUT4 (*Slc2a4*) expression in males increased with stress in adolescents and decreased with stress in adults (interaction of age and stress $F_{1,33} = 6.980$; $p = 0.0125$). In addition, expression of *Slc2a4* in adult males in the control group was elevated relative to adolescent males in the control group ($p= 0.0480$). (F) For females there

was no effect of age or stress on *Slc2a4* ($p>0.05$). *Slc2a8* expression was unaffected by either age or stress in males (G) or females (H) ($p>0.05$). Data shown represent mean fold change $2^{-\Delta CT} \pm$ SEM. Figure symbols: In graph D a significant main effect of stress is indicated by asterisk (*) relative to the adolescent and adult control groups ($p<0.05$). In graph E Tukey's post hoc analysis is represented by asterisk (*), which indicates and increase in gene expression relative to the adolescent control group ($p<0.05$).

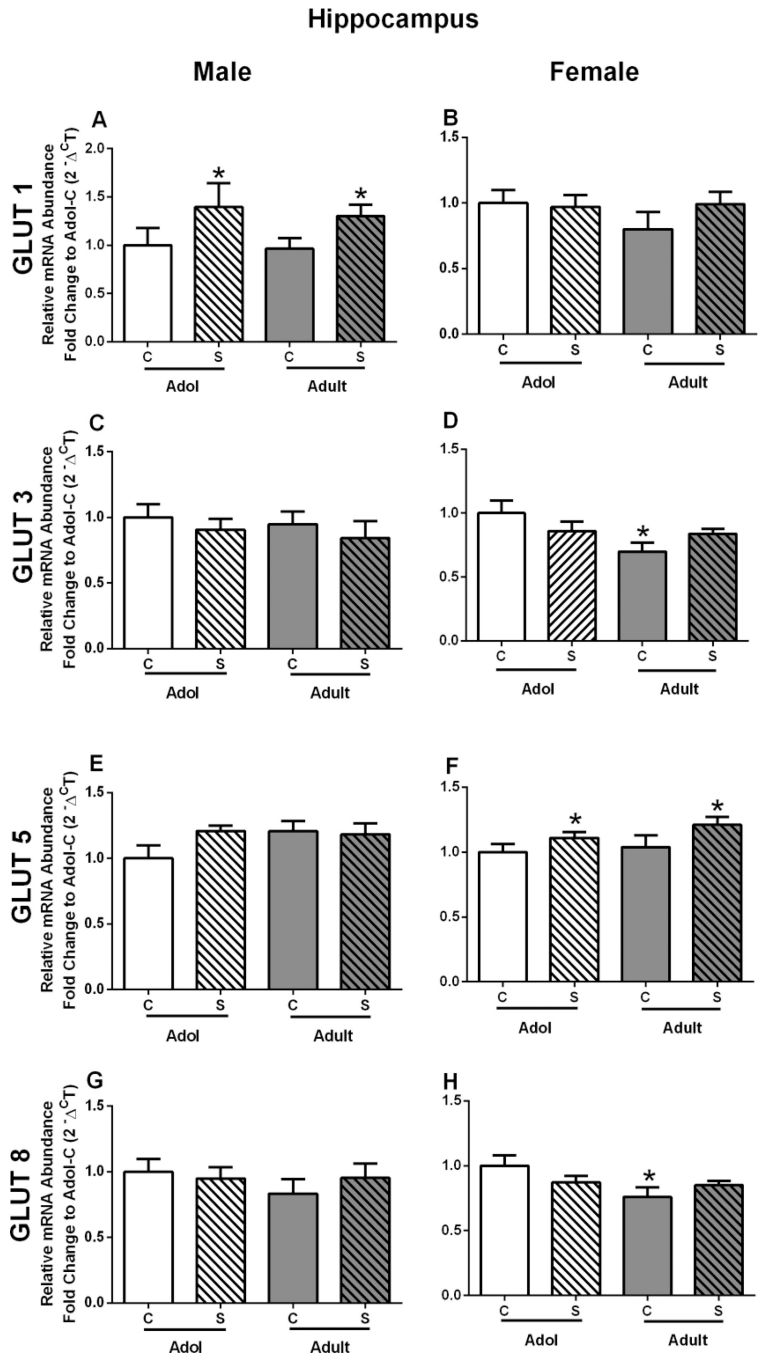

Figure 4. Chronic stress, age, and sex interact to dictate GLUT expression in the hippocampus

(A) qRT-PCR analysis of GLUT1 (*Slc2a1*) demonstrated that chronic stress increased expression of *Slc2a1* in the hippocampus of male rats, regardless of age (main effect of stress $F_{1,33} = 4.906$; $p = 0.0338$). (B) Expression of *Slc2a1* was unaltered by stress or age in females ($p>0.05$). (C) Expression of GLUT3 (*Slc2a3*) in the male hippocampus was unaltered by age or stress ($p>0.05$). (D) In contrast, female rats exhibited a decrease in expression of *Slc2a3* with increasing age (main effect of age $F_{1,39} = 4.871$; $p = 0.0333$) and this effect was specific to the control groups ($p = 0.0353$). (E) In male rats, GLUT5 (*Slc2a5*) expression did not differ due to age or stress ($p>0.05$). (F) In females, *Slc2a5* expression

increased following chronic stress, regardless of age (main effect of stress $F_{1,40} = 4.706$; $p = 0.0361$). (G) Expression of GLUT8 (*Slc2a8*) was not dependent on either age or stress for male rats ($p>0.05$). (H) Similar to *Slc2a3*, *Slc2a8* expression decreased in female adults as compared to adolescent females (main effect of age $F_{1,39} = 4.701$; $p = 0.0361$), which was specific to lower *Slc2a8* expression in female adult controls relative to adolescent controls (Tukey's: $p=0.0483$). Data shown represent mean fold change $2^{-\Delta CT} \pm$ SEM. Figure symbols: In graphs A and F a significant main effect of stress is indicated by asterisk (*) relative to adolescent and adult control groups ($p<0.05$). Tukey's post hoc analysis is represented by asterisk (*) in graphs D and F, which indicates a decrease in gene expression relative to the adolescent control group ($p<0.05$).

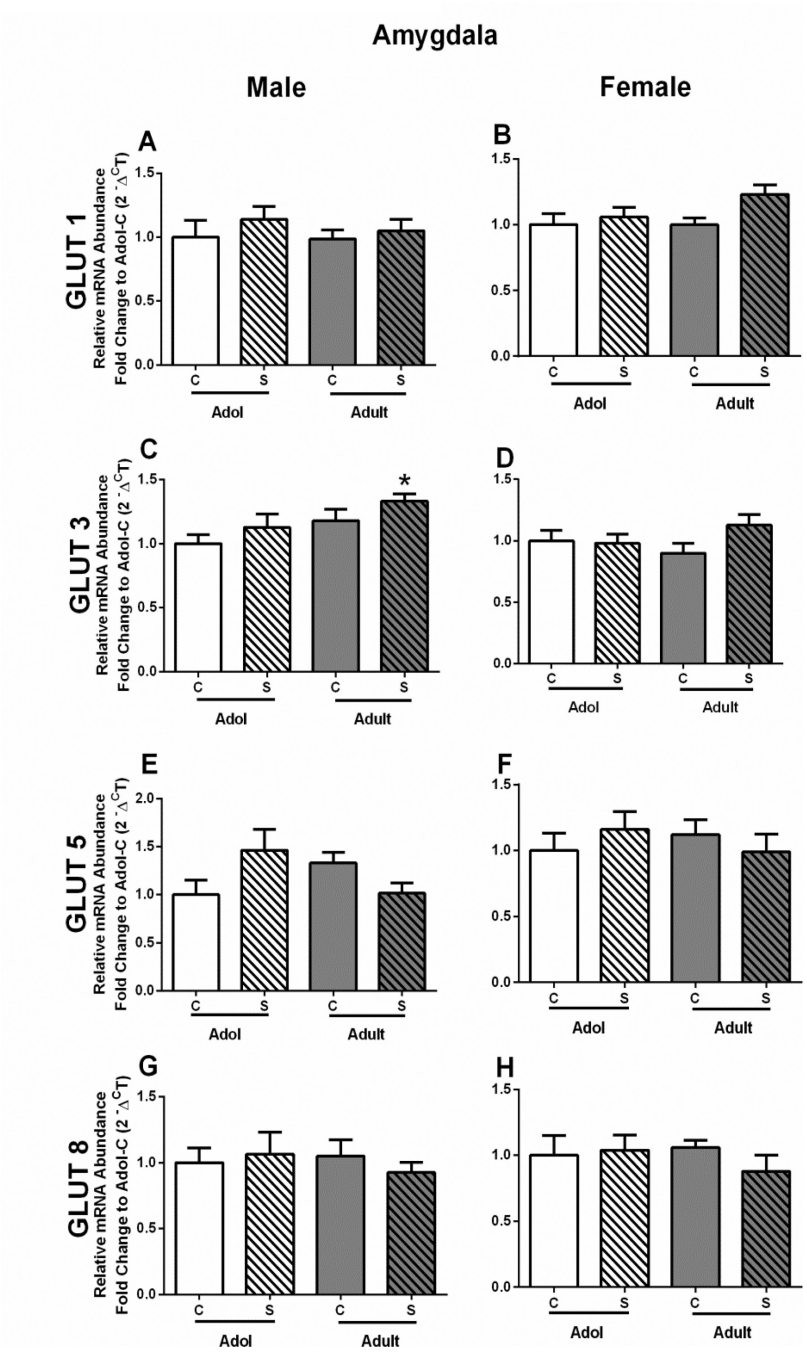

Figure 5. Amygdalar expression of GLUTs is largely impervious to sex, age, and stress

Gene expression of GLUT1 (*Slc2a1*) in (A) male and (B) female rats did not differ due to either age or stress ($p>0.05$). (C) An age difference in GLUT3 (*Slc2a3*) expression existed (main effect of age $F_{1,37} = 5.066$; $p = 0.0304$) specific to increased expression in male adults exposed to stress relative to male adolescent controls, as determined by a Tukey's post-hoc analysis ($p= 0.0465$). (D) *Slc2a3* expression was unchanged by either age or stress in female rats ($p>0.05$). (E) Expression of GLUT5 (*Slc2a5*) was impacted by the combination of age and stress (interaction of age and stress $F_{1,33} = 6.601$; $p = 0.014$), but post hoc analysis did not attribute this effect to any specific group differences ($p > 0.05$). (F) Expression of *Slc2a5*

was not impacted by either age or stress in female rats ($p>0.05$). GLUT8 (*Slc2a8*) expression in the amygdala was impervious to both age and sex for (G) male and (H) female rats ($p>0.05$). Data shown represent mean fold change $2^{-\Delta CT} \pm$ SEM. Figure symbols: Tukey's post hoc analysis is represented by asterisk (*) in graph C, which indicates an increase in gene expression relative to the adolescent control group ($p<0.05$).

Table 1

Summary of cerebral glucose transporters potentially involved in neurological disease or stress responses.

Protein	Gene	Cerebral Location	Involvement in Neurologic Disease and the Stress Response	References
GLUT1	*Slc2a1*	Astrocytes; neurons Endothelial cells	Astrocyte transporter (45 kDa); Blood brain barrier transporter (55 kDa) Deficiency in De Vivo's Disease Decreased expression in Alzheimer's Disease, traumatic brain injury, ischemia	[8–10, 12, 59–62]
GLUT3	*Slc2a3*	Neurons; neurophil	Decreased expression in Alzheimer's Disease, traumatic brain injury, ischemia Reduced expression in rat forebrain after chronic stress exposure	[9, 10, 12, 13, 16, 59]
GLUT4	*Slc2a4*	Neurons; somatodendritic	Insulin sensitive glucose transporter Impaired insulin-induced translocation after corticosterone treatment in rat hippocampus	[5, 7, 63] [17, 64]
GLUT5	*Slc2a5*	Microglia	Microglial localization; involvement in inflammatory response	[7, 65]
GLUT8	*Slc2a8*	Neurons; somatodendritic	Depletion results in increased neurogenesis, hyperactivity, and reduction in risk assessment behaviors without memory alteration in mice	[66, 67]

Table 2

Summary of qRT-PCR analysis addressing factors of age and sex

Cerebral Location	Sex	Age	*Slc2a1*	*Slc2a3*	*Slc2a4*	*Slc2a5*	*Slc2a8*
Hippocampus	**Male**	**Adolescent**	1.00 ± 0.18	1.00 ± 0.10	1.00 ± 0.12	1.00 ± 0.09	1.00 ± 0.09
		Adult	0.96 ± 0.11	0.95 ± 0.09	0.74 ± 0.09	1.20 ± 0.07	0.83 ± 0.10
	Female	**Adolescent**	1.12 ± 0.11	1.24 ± 0.12	0.98 ± 0.09	1.07 ± 0.06	1.17 ± 0.09
		Adult	0.89 ± 0.15	0.87 ± 0.08	0.82 ± 0.09	1.12 ± 0.09	0.89 ± 0.08
Hypothalamus	**Male**	**Adolescent**	1.00 ± 0.37	1.00 ± 0.19	1.00 ± 0.34	1.00 ± 0.15	1.00 ± 0.23
		Adult	2.13 ± 0.26*	0.98 ± 0.06	2.02 ± 0.16*	1.10 ± 0.11	1.39 ± 0.06
	Female	**Adolescent**	2.13 ± 0.19*	1.17 ± 0.07	2.11 ± 0.17*	0.88 ± 0.22	1.30 ± 0.09
		Adult	2.14 ± 0.19*	1.25 ± 0.12	1.95 ± 0.20*	0.91 ± 0.10	1.40 ± 0.12
Amygdala	**Male**	**Adolescent**	1.00 ± 0.13	1.00 ± 0.07	1.00 ± 0.14	1.00 ± 0.15	1.00 ± 0.11
		Adult	0.98 ± 0.07	1.17 ± 0.09	1.26 ± 0.22	1.33 ± 0.11	1.05 ± 0.12
	Female	**Adolescent**	1.00 ±0.08	1.11 ± 0.09	1.22 ± 0.18	1.14 ± 0.15	1.02 ± 0.15
		Adult	1.00 ± 0.05	1.00 ± .09	1.33 ± 0.12	1.28 ±0.12	1.09 ± 0.05
Prefrontal Cortex	**Male**	**Adolescent**	1.00 ± 0.11	1.00 ± 0.04	1.00 ± 0.11	1.00 ± 0.06	1.00 ± 0.10
		Adult	1.03 ± 0.07	0.99 ± 0.07	1.09 ± 0.11	0.88 ± 0.06	1.01 ± 0.07
	Female	**Adolescent**	1.09 ± 0.05	1.08 ± 0.06	1.01 ± 0.08	0.97 ± 0.08	1.14 ± 0.09
		Adult	0.9 ± 0.11	0.91 ± 0.09	0.74 ± 0.11	0.90 ± 0.05	1.18 ± 0.10

Data shown represent mean fold change ($2^{-\Delta CT}$) ± SEM. Significant main effects are shown only in corresponding graphs.

Tukey's post hoc analysis is represented in the table by an asterisk (*), which indicates a decrease in gene expression relative to the male adolescent cohort.

Table 3

Summary of qRT-PCR analysis addressing factors of stress and age in males

Cerebral Location	Age	Group	*Slc2a1*	*Slc2a3*	*Slc2a4*	*Slc2a5*	*Slc2a8*
Hippocampus	**Adol**	**Control**	1.00 ± 0.18	1.00 ± 0.10	1.00 ± 0.12	1.00 ± 0.09	1.00 ± 0.09
		Stress	1.39 ± 0.24	0.90 ± 0.08	0.86 ± 0.071	1.20 ± 0.04	0.94 ± 0.08
	Adult	**Control**	0.96 ±0.11	0.94 ± 0.09	0.74 ± 0.09	1.20 ± 0.07	0.83 ± .010
		Stress	1.30 ± 0.11	0.84 ± 0.13	0.79 ±0.09	1.18 ± 0.08	0.95 ± 0.10
Hypothalamus	**Adol**	**Control**	1.00 ± 0.37	1.00 ± 0.19	1.00 ± 0.34	1.00 ± 0.15	1.00 ± 0.23
		Stress	2.13 ± 0.16	1.24 ± 0.07	1.93 ± 0.34	0.98 ± 0.12	1.47 ± 0.26
	Adult	**Control**	2.13 ± 0.19	0.98 ± 0.06	2.02 ± 0.16*	1.10 ± 0.11	1.39 ± 0.06
		Stress	2.10 ± 0.38	1.01 ± 0.06	1.53 ± 0.12	0.92 ± 0.10	1.21 ± 0.10
Amygdala	**Adol**	**Control**	1.00 ± 0.13	1.00 ± 0.07	1.00 ± 0.14	1.00 ± 0.15	1.00 ± 0.11
		Stress	1.13 ± 0.10	1.12 ± 0.10	1.06 ± 0.16	1.46 ± 0.21	1.06 ± 0.16
	Adult	**Control**	0.98 ± 0.07	1.17 ± 0.09	1.26 ± 0.22	1.33 ± 0.11	1.05 ± 0.12
		Stress	1.05 ± 0.08	1.33 ± 0.05*	0.77± 0.12	1.01 ± 0.10	0.92 ± 0.07
Prefrontal Cortex	**Adol**	**Control**	1.00 ± 0.11	1.00 ± 0.04	1.00 ± .011	1.00 ± 0.06	1.00 ± 0.10
		Stress	1.03 ± 0.08	0.95 ± 0.04	0.87 ± 0.10	0.93 ± 0.04	1.00 ± 0.06
	Adult	**Control**	1.03 ± 0.07	0.99 ± 0.07	1.09 ± 0.11	0.88 ± 0.06	1.01 ± 0.07
		Stress	1.12 ± 0.05	1.07 ± 0.04	1.07 ± 0.10	0.98 ± 0.05	1.11 ± 0.03

Data shown represent mean fold change ($2^{-\Delta CT}$) ± SEM. Significant main effects are shown only in corresponding graphs.

Tukey's post hoc analysis is represented in the table by an asterisk (*), which indicates a decrease in gene expression relative to the male adolescent control group.

Table 4

Summary of qRT-PCR analysis addressing factors of stress and age in females

Cerebral Location	Age	Group	*Slc2a1*	*Slc2a3*	*Slc2a4*	*Slc2a5*	*Slc2a8*
Hippocampus	**Adol**	**Control**	1.00 ± 0.09	1.00 ± 0.10	1.00 ± 0.093	1.00 ± 0.06	1.00 ± 0.08
		Stress	0.97 ± 0.08	0.86 ± 0.07	0.94 ± 0.11	1.11 ± 0.04	0.87 ± 0.05
	Adult	**Control**	0.80 ± 0.13	0.70 ± 0.06*	0.84 ± 0.09	1.04 ± 0.08	0.76 ± 0.07*
		Stress	0.99 ± 0.09	0.84 ± 0.03	0.89± 0.03	1.21 ± 0.06	0.85 ± 0.03
Hypothalamus	**Adol**	**Control**	1.00 ± 0.12	1.00 ± 0.06	1.00 ± 0.08	1.00 ± 0.25	1.00 ± 0.09
		Stress	0.74 ± 0.08	0.82 ± 0.06	0.76 ± 0.11	0.86 ± 0.11	0.75 ± 0.11
	Adult	**Control**	1.00± 0.09	1.06 ± 0.10	0.92 ± 0.09	1.02 ± 0.11	1.07 ± 0.09
		Stress	1.00 ± 0.11	0.90 ± 0.05	0.90 ± 0.06	1.21 ± 0.15	1.01 ± 0.05
Amygdala	**Adol**	**Control**	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.15	1.00 ± 0.13	1.00 ± 0.51
		Stress	1.06 ± 0.07	0.98 ± 0.07	0.90 ± 0.14	1.16 ± 0.13	1.04 ± 0.11
	Adult	**Control**	1.00 ± 0.05	0.09 ± 0.08	1.09 ± 0.10	1.12 ± 0.11	1.06± 0.05
		Stress	1.23 ± 0.07	1.13 ± 0.08	0.97 ± 0.12	0.99 ± 0.13	0.88 ± 0.12
Prefrontal Cortex	**Adol**	**Control**	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.08	1.00 ± 0.08	1.00 ± 0.08
		Stress	0.91 ± 0.12	0.91 ± 0.07	0.87 ± 0.11	0.85 ± 0.11	0.83± 0.15
	Adult	**Control**	0.82 ± 0.10	0.84 ± 0.08	0.73 ± 0.11	0.92 ± 0.06	1.03 ± 0.08
		Stress	0.98 ± 0.06	0.98 ± 0.03	0.89 ± 0.07	0.96 ± 0.05	0.98 ± 0.05

Data shown represent mean fold change ($2^{-\Delta CT}$) ± SEM. Significant main effects are shown only in corresponding graphs.

Tukey's post hoc analysis is represented in the table by an asterisk (*), which indicates a decrease in gene expression relative to the female adolescent control group.