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Ovarian Steroids Influence Cerebral Glucose Transporter Expression in a Region- and Isoform-Specific Pattern

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Abstract

Cerebral glucose uptake is mediated by several members of the family of facilitated glucose transporters (protein nomenclature GLUT; gene nomenclature solute carrier family 2 *Slc2a*). Glucose uptake differs between the sexes and also varies with menstrual status in women and across the rodent oestrous cycle. The present study demonstrates the extent to which hormonal variation across the four stages of the rat oestrous cycle affects the mRNA abundance of four members of the GLUT family, including the most well characterised cerebral transporters *Slc2a1* and *Slc2a3*, as well as the insulin-sensitive transporters *Slc2a4* and *Slc2a8* in the hypothalamus, hippocampus and prefrontal cortex. *Slc2a1* varied significantly across the cycle in the hippocampus and prefrontal cortex, and *Slc2a3* and *Slc2a4* also showed significant fluctuation in the hippocampus. Transporter expression significantly increased during pro-oestrus in both the hippocampus and prefrontal cortex. Furthermore, ovarian hormones are critical for normal expression of GLUT mRNA, as demonstrated by reduced expression of *Slc2a1*, *Slc2a3* and *Slc2a8* in the hippocampus after ovariectomy. Collectively, the data reported in the present study demonstrate that glucose transporters are highly sensitive to hormonal variation and that this sensitivity is regionally distinct; thereby fluctuations likely have specific phenotypic implications.

Keywords

oestrous cycle; oestrogen; progesterone; glucose transporter; hippocampus

Glucose is the primary fuel of the brain and alterations in glucose availability directly affect neuronal function. Facilitated glucose transport is mediated by a family of transporters (GLUT) responsible for glucose transport across the endothelial cells of the blood–brain barrier, as well as uptake of glucose into astrocytes and neurones (1,2). The GLUT family is not completely characterised, although the information currently available indicates that at least seven members of the family are expressed in the brain (1). GLUT1 is essential for glucose uptake across the blood–brain barrier (3) and into astrocytes (4), whereas GLUT3 is the primary neuronal glucose transporter (1,5). GLUT4 and GLUT8, which are both insulin-

sensitive glucose transporters (6), have only more recently been recognised for their cerebral expression (1).

The crucial role of GLUT is illustrated by the profound neurological deficits manifested in De Vivo disease, a rare genetic condition in which GLUT1 is not expressed (7). Deficits in the expression and translocation of the GLUT family have also been linked to neuropathological conditions, including Alzheimer's pathology, post-ischaemic/hypoxic brain function and traumatic brain injury (8–10). Less devastating but functionally significant, alterations in cerebral glucose metabolism have also been implicated in several neuropsychiatric disorders, including depression (11), schizophrenia (12) and autism spectrum disorder (13), whereas changes in gene expression of multiple glucose transporters have been linked to chronic stress in animal models (14).

Although extreme changes in GLUTs and glucose uptake have pathological implications for brain function and behaviour, some levels of variation in glucose metabolism and transport are normal. For example, baseline regional cerebral glucose metabolism differs between males and females (15). Neuroimaging studies have demonstrated that both age and sex affect cerebral glucose metabolism (16), and studies in animal models have revealed that age and sex similarly affect cerebral GLUT expression (14). Glucose uptake appears to be highly plastic with fluctuations evident over the rat oestrous cycle (a cycle that repeats every 4–5 days). Ovarian steroids may mediate alterations in glucose uptake because the highest glucose uptake occurs during pro-oestrus (17) and cerebral glucose metabolism decreases in postmenopausal non-users of oestrogen replacement therapy (18).

Despite the evidence of hormonally-induced fluctuations in glucose uptake, the effects of ovarian steroids on expression of glucose transporters have not been fully addressed. In the present study, we determined the effects of hormonal fluctuation across the oestrous cycle on expression of several *Slc2* family genes (solute carrier family 2, the gene family for facilitated glucose transporters). We hypothesised that the increases in oestradiol and progesterone, which peak in subsequent order in pro-oestrus (19), would increase GLUT gene expression, and we focused our assessment on the four members of the family likely to respond to ovarian steroids (Table 1) (1,20–23). Given the variable influence of the oestrous cycle on brain region function, we hypothesised that GLUT gene expression would be differentially affected by the oestrous cycle in the hippocampus, prefrontal cortex and hypothalamus. These regions were selected based on the role of the hypothalamus in both hormonal (24) and metabolic (25) regulation; the known responsiveness of both hippocampal (26) and prefrontal cortex (27) to sex steroids; and the known regulatory circuitry among these regions (28,29). Given that our initial results strongly suggested a role of ovarian steroids in the control of the expression of GLUTs in the hippocampus, we ovariectomised a subset of rats to conclusively determine the impact of ovarian steroids upon hippocampal gene expression of GLUTs.

Collectively, the data reported in the present study demonstrate that glucose transporters are highly sensitive to hormonal variation, and also that this sensitivity is regionally distinct and thereby fluctuations likely have specific phenotypic implications. Establishment of a framework of understanding regarding the relationship between regional brain metabolism

and ovarian hormone influence is critical for an assessment of the potential role of GLUTs in ovarian hormone-dependent changes in behaviour, the consequences of disease states, and the interplay of ovarian senescence, behaviour and ageing.

Materials and methods

Animal husbandry

For the first experiment evaluating GLUT expression across the oestrous cycle, timed pregnant Wistar rats ($n = 16$) were obtained on gestational day 12 from Charles River (Wilmington, MA, USA). Rats were housed under a 14 : 10 h reverse light/dark cycle (lights on 00.00 h; lights off 14.00 h) in a facility controlled for humidity (60%) and temperature (20–23 °C). Pups were culled on postnatal day (PND) 3 to litters of eight per dam (balanced for sex) and weaned on PND 23, and then maintained on standard chow (Lab Diet 5001; PMI International, Richmond, IN, USA) until adulthood (PND 60). Only female offspring were used in the present study. No more than two pups from any one litter were assigned to an experimental group to control for litter effects; a total of 49 females was used. For the second experiment evaluating GLUT expression after ovariectomy, female Wistar rats (PND 56–60) were obtained from Charles River and housed under the same conditions as the rats in the first experiment ($n = 8$ per group). All experiments were approved by the Institutional Animal Care and Use Committee of Emory University and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Cycle staging

Beginning in adulthood between PND 60 and PND 70, vaginal lavage was performed between 10.00 h and 13.00 h each day. Cycle was assessed as dioestrus 1 (D1), dioestrus 2 (D2), pro-oestrus (P) or oestrus (E) based on the methods described by Goldman *et al.* (30) and Westwood (31). Briefly, D1 was typified by leukocytes and sparse round epithelial cells; D2 by the presence of numerous leukocytes with rare larger round epithelial cells; P by the presence of round, nucleated, granular epithelial cells; and E by the presence of needle-like keratinised epithelial cells among more rounded cells. Rats were selected over several days, selecting two or three rats daily per cycle stage. To insure proper staging, only rats with regular 4-day cycles in which cytology, uterine weight, and plasma oestradiol and progesterone concentrations aligned to indicate cycle stage were used in the final analyses (for a final $n = 32$). All rats were rapidly decapitated between 10.00 h and 12.00 h, corresponding to between 2 and 4 h before the end of their light cycle. Rats were collected from the various groups in a counterbalanced manner to guard against circadian effects. Trunk blood was collected for plasma oestradiol and progesterone measurements, uteri were collected to correlate uterine weight to cytological staging, and brains were removed and flash frozen on dry ice before storing at -80 °C. The hippocampus, prefrontal cortex and hypothalamus were dissected from these animals and stored at -80 °C until homogenised for gene analysis.

Plasma hormone analysis

The plasma sex steroids progesterone (sensitivity: 8.57 pg/ml; Enzo Life Sciences, Farmingdale, NY, USA) and oestradiol (sensitivity: 19 pg/ml; Cayman Chemical, Ann

Arbor, MI, USA) were analysed using an enzyme-linked immunoassay. Samples were run in duplicate for all endocrine assays.

Ovariectomy

After acclimatisation to the animal facility, ovariectomy ($n = 8$) or a sham operation ($n = 8$) was performed on the adult female Wistar rats. Rats were dosed 100 μ l per 100 g rat with a ketamine/xylazine/acepromazine cocktail (25 : 5 : 1; original concentrations: ketamine 50 mg/ml; xylazine 20 mg/ml; acepromazine: 10 mg/ml) to induce anaesthesia and then surgery was performed after the rat reached an anaesthetic plane. Rats were maintained under anaesthesia with inhaled isoflurane. Each flank was shaved to the hip and cleaned with betadine and alcohol, and an abdominal incision was made to locate the ovaries. For the ovariectomies, ovaries were gently pulled out, ligated with a 3-0 silk suture and cut. For all animals, the abdominal cavity was closed with a 5-0 absorbable suture and surgical staples were used to oppose the skin. Betadine was applied to the staples, and the rats were placed on a heating pad and monitored. Both sham-operated and ovariectomised rats received fruit loops and oral meloxicam for 1 day, and remained under daily observation until staple removal 5 days post-operation. Sham surgeries were conducted identically to ovariectomy with the exception of the ligation and removal of the ovaries. Rats were rapidly decapitated 7 days after ovariectomy or sham surgery, and plasma and brain were collected as described above.

Quantitative real-time-polymerase chain reaction (qRT-PCR)

Hippocampal RNA was extracted via an RNeasy Mini kit (Qiagen, Valencia, CA, USA). RNA integrity was then assessed with a NanoDrop 2000 spectrophotometer (ThermoScientific, Wilmington, DE, USA). Using the High Capacity RNA to cDNA kit (Applied Biosystems, Foster City, CA), RNA was reverse transcribed and subsequently cDNA was quantified with the PicoGreen Assay (Invitrogen, Carlsbad, CA, USA) and then standardised to 10 pg/ μ l. This allows standardisation at two separate times: once before reverse transcription, and again before qRT-PCR. Rat TaqMan Gene Expression Assays were purchased from Applied Biosystems (Carlsbad, CA, USA) with probes labelled with 6-FAM and MGB (nonfluorescent quencher) at the 5' and 3' ends, respectively: *Slc2a1* (Rn01417099_m1), *Slc2a3* (Rn00567331_m1), *Slc2a4* (Rn00562597_m1) and *Slc2a8* (Rn00585203_m1). The two-step qRT-PCR cycling conditions used on the 7900HT Sequence Detection System (Applied Biosystems) were: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The housekeeping genes, *Ubc* (Rn01789812_g1), *Hprt1* (Rn01527840_m1) and *Tfrc* (Rn01474701_m1) were run as potential endogenous controls. However, when analysed either separately or using the geometric means of the cycle threshold values, which were calculated as described previously (32), the housekeeping gene expression varied by cycle stage, which could confound analysis of the genes of interest. Relative gene expression of individual samples run in triplicate (with coefficient of variation cut-off set to 4%) was thus determined by the comparative C_T quantification method only relative to either dioestrus 1 or sham (2^{-C_T}). Additional precautions of double standardisation were made to ensure consistent loading in the qRT-PCR: once of the RNA before reverse transcription, and again of the cDNA before qRT-PCR. All TaqMan gene expression assays are guaranteed to have 90–100%

amplification efficiency as determined by the genome-aided probe and primer design pipeline and reported in the 'Amplification Efficiency of TaqMan Gene Expression Assays' Application Note 127AP05-03 (Applied Biosystems).

Statistical analysis

Statistical comparisons of differences in gene expression and hormone concentrations were analysed using GRAPHPAD PRISM, version 6.0 (GraphPad Software Inc., San Diego, CA, USA). Hormone concentrations and comparative C_T quantification was analysed by one-way ANOVA followed by the Holm–Sidak post-hoc testing in Experiment 1 (using the factor of cycle stage), and by an unpaired two-sided Student's t-test in Experiment 2 (sham versus ovariectomised). Outliers were assessed by the Grubb's outlier test and removed. $P < 0.05$ was considered statistically significant. Data are presented as the mean $\pm$ SEM.

Results

Oestrous cycle stage was established from vaginal lavage and confirmed with uterine weights and hormone concentrations

Uterine weight was normalised to each rat by dividing uterine weight (mg) by the rat weight (g). Normalised uterine weight varied significantly across the oestrous cycle ($F_{3,28} = 27.35$, $P < 0.0001$), with the greatest uterine weight in pro-oestrus (versus D1: $t_{28} = 8.308$, $P < 0.0001$; versus D2: $t_{28} = 6.796$, $P < 0.0001$; versus E: $t_{28} = 5.176$, $P < 0.0001$; Fig. 1A), as expected based on a review of the literature (33,34). The ratio of progesterone (P4; ng/ml) to oestradiol (E_2 ; pg/ml) was calculated for all samples for which the sex steroid levels fell within the range of the ELISA assay and with the appropriate coefficient of variation for both hormones. This ratio reflects the hormonal state at the end of light cycle for all animals because decapitation and collection occurred for all animals between 2 and 4 h before the beginning of the dark cycle. The ratio varied significantly across the oestrous cycle ($F_{3,12} = 12.19$, $P = 0.0006$; Fig. 1B), with the highest P4 : E_2 ratio in pro-oestrus (versus D1: $t_{12} = 4.564$, $P = 0.0026$; versus D2: $t_{12} = 5.357$, $P = 0.0010$; versus E: $t_{12} = 5.176$, $P = 0.0012$), as expected because samples were collected late in the light cycle or just before the dark cycle during pro-oestrus, and thus would be after the oestradiol surge and during the progesterone surge (19,33,35).

The hippocampus and hypothalamus demonstrate a greater relative abundance of GLUT mRNA than the prefrontal cortex

Relative gene expression in D1 of each GLUT across the three regions studied (hippocampus, prefrontal cortex and hypothalamus) revealed significant regional variation for all GLUT genes analysed (*Slc2a1*: $F_{2,15} = 10.13$, $P = 0.0016$; *Slc2a3*: $F_{2,15} = 5.867$, $P = 0.0131$; *Slc2a4*: $F_{2,15} = 110.6$, $P < 0.0001$; *Slc2a8*: $F_{2,15} = 380.4$, $P < 0.0001$; Fig. 2). Post-hoc testing showed that GLUT gene expression was lowest in the prefrontal cortex for all glucose transporters analysed [*Slc2a1*: prefrontal cortex (PFC) versus hippocampus (HP): $t_{15} = 3.179$, $P = 0.0124$; PFC versus hypothalamus (HYT): $t_{15} = 4.350$, $P = 0.0017$; *Slc2a3*: PFC versus HP: $t_{15} = 3.149$, $P = 0.0197$; *Slc2a4*: PFC versus HP: $t_{14} = 2.620$, $P = 0.0202$; versus HYT: $t_{14} = 14.12$, $P < 0.0001$; *Slc2a8*: PFC versus HYT: $t_{15} = 24.19$, $P < 0.0001$]. Analysis of each region separately across all four glucose transporters in D1 demonstrated

that further significant isoform-specific variation existed throughout the brain (hippocampus: $F_{3,20} = 6.154$, $P = 0.0039$; prefrontal cortex: $F_{3,20} = 30.06$, $P < 0.0001$; hypothalamus: $F_{3,19} = 96.52$, $P < 0.0001$; Fig. 3). *Slc2a1* expression was greatest and *Slc2a4* expression was lowest in all three regions (HP: *Slc2a1* versus *Slc2a4*: $t_{20} = 3.786$, $P = 0.0069$; *Slc2a1* versus *Slc2a8*: $t_{20} = 3.620$, $P = 0.0085$; PFC: *Slc2a1* versus *Slc2a3*: $t_{20} = 3.643$, $P = 0.0048$; *Slc2a1* versus *Slc2a4*: $t_{20} = 9.045$, $P < 0.0001$; *Slc2a1* versus *Slc2a8*: $t_{20} = 6.439$, $P < 0.001$; *Slc2a4* versus *Slc2a3*: $t_{20} = 5.402$, $P < 0.0001$; *Slc2a4* versus *Slc2a8*: $t_{20} = 2.602$, $P = 0.0222$; HYT: *Slc2a1* versus *Slc2a3*: $t_{19} = 12.72$, $P < 0.0001$; *Slc2a1* versus *Slc2a4*: $t_{19} = 12.76$, $P < 0.0001$). Although the hippocampus and prefrontal cortex showed the same general pattern, with gene expression decreasing from *Slc2a1* to *Slc2a3* to *Slc2a8* to *Slc2a4*, the hypothalamus had a different pattern, with gene expression highest in *Slc2a1* and *Slc2a8* and lower in *Slc2a3* and *Slc2a4*.

The hippocampus and prefrontal cortex demonstrate greater cycle-dependent variation in gene expression for GLUTs than the hypothalamus

When analysed by one-way ANOVA within each region and normalising to D1, relative gene expression of *Slc2a1* varied by cycle stage in both the hippocampus ($F_{3,26} = 4.765$, $P = 0.0089$; Fig. 4) and the prefrontal cortex ($F_{3,28} = 4.050$, $P = 0.0165$) but not in the hypothalamus ($F_{3,26} = 0.6155$, $P = 0.6114$). Relative gene expression of *Slc2a3* ($F_{3,25} = 4.110$, $P = 0.0168$) and *Slc2a4* ($F_{3,25} = 3.965$, $P = 0.0193$) also varied significantly across the oestrous cycle within the hippocampus (Fig. 5), although expression of these transporters did not fluctuate in the prefrontal cortex (*Slc2a3*: $F_{3,26} = 2.107$, $P = 0.1219$; *Slc2a4*: $F_{3,26} = 2.378$, $P = 0.0910$) or the hypothalamus (*Slc2a3*: $F_{3,26} = 1.269$, $P = 0.3056$; *Slc2a4*: $F_{3,26} = 0.8541$, $P = 0.4788$). *Slc2a8* expression did not vary by cycle stage in any region analysed (HP: $F_{3,26} = 2.575$, $P = 0.0765$; PFC: $F_{3,26} = 2.374$, $P = 0.0915$; HYT: $F_{3,26} = 0.03024$, $P = 0.9928$).

Expression of GLUT1, 3 and 8 in the hippocampus is directly influenced by ovarian hormones

To determine whether ovarian hormones were necessary for baseline GLUT expression in the hippocampus, the brain region that demonstrated the greatest variation in GLUT expression over the oestrous cycle, female rats were ovariectomised before assessment of GLUT gene expression in the hippocampus. Removal of ovarian hormones was sufficient to cause a reduction in hippocampal gene expression for *Slc2a1* ($t_{13} = 5.581$, $P < 0.0001$), *Slc2a3* ($t_{12} = 4.231$, $P = 0.0012$) and *Slc2a8* ($t_{13} = 7.711$, $P = 0.0001$) but not *Slc2a4* ($P = 0.9692$; Fig. 6).

Discussion

Collectively, the data reported in the present study demonstrate that ovarian hormones directly influence cerebral GLUT gene expression in a region- and isoform-specific manner. During dioestrus 1, differences exist in expression of GLUT genes both by brain region and by isoform. Moreover, GLUT mRNA abundance varies across the stages of the oestrous cycle in an isoform and brain region-dependent manner, with *Slc2a1* varying in the hippocampus, prefrontal cortex and hypothalamus, and *Slc2a3* and *Slc2a4* fluctuating in the

hippocampus only. Maximal mRNA abundance of the GLUTs assessed in the prefrontal cortex and hippocampus occurs primarily in pro-oestrus. Conversely, removal of ovarian hormones via ovariectomy reduced the expression of *Slc2a1*, *Slc2a3* and *Slc2a8* in the hippocampus. These studies indicate that ovarian steroids play a critical role in modulating GLUT expression in limbic brain regions.

In dioestrus, expression of GLUT gene isoforms varied between brain regions in female rats. *Slc2a1*, which encodes for GLUT1 and is expressed in microvessels (3) and glial cells (4), was enriched in hippocampal and hypothalamic tissue (Fig. 2), and was more highly expressed than any other glucose transporter in all regions (Fig. 3). This regional distribution agrees with several previous reports of widespread GLUT1 expression in the rat (4,36). Given the high vascularisation of both the hippocampus and the hypothalamus (37,38), it is not surprising that these regions would be particularly enriched in a blood–brain barrier glucose transporter. Although previous studies suggest greater GLUT activity in the prefrontal cortex than is implied by the present findings of low GLUT gene expression, existing literature on GLUT expression in the cortex has either examined the cortex broadly (39) or specified subregions (40) different from those observed in the present study. One potential explanation for this lower GLUT expression in the prefrontal cortex than the hypothalamus or hippocampus is that the region is relatively less vascularised. Accordingly, exercise is able to induce a stimulatory effect on hippocampal but not prefrontal angiogenesis (41), consistent with potential regional vascular differences.

The high hypothalamic expression during dioestrus 1 of both *Slc2a4* and *Slc2a8*, in addition to *Slc2a1*, is notable. The hypothalamus is a region of both critical hormonal and metabolic control (25), and oestradiol in particular is known to act in the ventromedial hypothalamus to decrease food intake (42). The present findings agree with previous reports of GLUT4 expression in the hypothalamus (43,44). GLUT8, with high hypothalamic expression in the present study, has also been reported in the hypothalamus (6), although this glucose transporter is less well characterised than the other three that were studied in this series of experiments, as a result of its more recent discovery (1). The relatively high expression of GLUT1 (45) may be related to the role of the hypothalamus in glucose-sensing and glucose production and may correspond with the role of the hypothalamus in metabolic regulation.

In addition to determining cerebral regional and isoform-specific GLUT expression during a single stage of the oestrous cycle, the present study established the existence of important fluctuations in transporter expression across the oestrous cycle. *Slc2a1* expression varies over the oestrous cycle in both the hippocampus and the prefrontal cortex, without similar changes in the hypothalamus. The absence of an effect on hypothalamic GLUT expression is in contrast to previous findings. Specifically, Nehlig *et al.* (17) reported changes in hypothalamic glucose uptake, with the greatest increases in pro-oestrus. However, they report this increase in only five of the fifteen hypothalamic subregions analysed (17). Thus, the present study assessing global hypothalamic gene expression may not have assessed the smaller fluctuations apparent in a more subregion specific analysis.

Unlike its lack of effect in the hypothalamus, the stage of the oestrous cycle influenced both the hippocampus and prefrontal cortex, such that *Slc2a1* expression peaked in pro-oestrus

and was significantly lower in oestrus. These data resemble previously reported changes in cerebral glucose uptake across the oestrous cycle (17). Nehlig *et al.* (17) reported fluctuations in glucose uptake for the entorhinal cortex and CA3 region of the hippocampus, with peaks in pro-oestrus as well as metoestrus (synonymous with dioestrus 1) (30), as well as decreases in dioestrus 2 and oestrus, similar to the pattern seen in these studies for *Slc2a1* and *Slc2a3* in the hippocampus. The pattern dissimilarity between the hypothalamus and hippocampus is intriguing given the rich blood supply and hormonal access of both regions. However, these regions have different relative densities of progesterone (46) and oestrogen receptors (47), which have also been shown to vary in expression in response to ovarian hormones (46,48). This variable receptor density could affect the hormonal effect on fluctuations in GLUT expression. Differential expression of receptor and receptor isoforms and/or different regional sensitivity to hormones (46,47) may play a role in the lack of variation observed across the oestrous cycle.

In addition to changes in *Slc2a1* expression, the hippocampus also showed altered *Slc2a3* and *Slc2a4* expression over the oestrous cycle. *Slc2a3* expression followed a similar pattern to *Slc2a1*, as did *Slc2a8* (although *Slc2a8* expression changes were not significant). These findings are consistent with previous reports of increases in GLUT expression in response to ovarian hormone administration. Oestradiol replacement has previously been shown to affect glucose uptake into the rodent brain (49) and also to increase GLUT1 expression in blood–brain barrier endothelial cells (20), as well as GLUT3 and GLUT4 expression in primate cortex (50). Additionally, progesterone may play a key role influencing GLUT1 expression in the hippocampus. Progesterone administration has previously been shown to increase GLUT1 expression in rat endothelial cells and GLUT3 expression in rat hippocampus (21), and increases in glucose uptake have been shown in this region during metoestrus and prooestrus (17). As seen in Fig. 1, and consistent with previous studies (33,51), there is slight surge in the progesterone to oestradiol ratio in D1 in addition to the large surge in the P4 : E2 ratio during pro-oestrus because all of the samples were collected toward the end of the light cycle. It is additionally remarkable the elevations in hippocampal GLUT1 occurred in both dioestrus and pro-oestrus, offering supporting evidence for the regulatory role of progesterone in GLUT1 expression.

By contrast, hippocampal *Slc2a4* showed a distinct pattern from *Slc2a1* and *Slc2a3* across the oestrous cycle, with significant increases in oestrus relative to dioestrus 1. GLUT4 is an insulin sensitive transporter, and oestradiol is known to play a significant role in modulating peripheral insulin sensitivity (22), as well as promoting GLUT4 expression in muscle and adipocytes (52). Progesterone, on the other hand, can promote insulin resistance in various tissues (53). Our pro-oestrus samples were taken during the surge of progesterone, and failure to observe to an increase in *Slc2a4* during pro-oestrus may be a result of the relative ratio and divergent effects of the ovarian hormones in the rat.

The fact that the hippocampus, relative to the prefrontal cortex and hypothalamus, experienced the greatest changes in glucose transporter expression correlates with known changes in hippocampal structure, as well as behavioural changes that occur across the oestrous cycle and in response to ovarian hormone administration. Both exogenous administration of oestradiol and progesterone and fluctuation of these ratios across the

oestrous cycle affect hippocampal spine density, with increases in oestradiol being associated with preservation of spine density and progesterone being associated with a decrease in spine density (54). Pro-oestrus is also associated with altered behaviour, including reduced anxiety-like behaviour and improved performance in hippocampal-dependent tasks (55,56). Changes in hippocampal GLUT3, GLUT4 and GLUT8 expression or function have also been associated with either dexamethasone administration (57) or stress exposure (58,59). Increased cerebral glucose uptake, mediated either by natural fluctuations in ovarian hormone (17) or exogenous administration of ovarian hormones (49), may be mediated by changes in hormonally-dependent GLUT expression (Figs 4, 5 and 6) (20,21,50). These changes in glucose availability may underlie the behavioural and synaptic changes mediated at the level of the hippocampus.

Given the particularly complex degree of changes in GLUT gene expression in the hippocampus over the oestrous cycle, we directly examined the influence of sex steroids on GLUT gene expression in the hippocampus by removing ovarian hormones. As expected based on the oestrous cycle data, ovariectomy reduced the expression of *Slc2a1*, *Slc2a3* and *Slc2a8*. The lack of change in *Slc2a4* may be a result of the fact that it is already expressed at low levels, and therefore may approach a bottoming-out effect, and so the technique used is unable to achieve further reductions. Alternatively, *Slc2a4* may be differentially responsive to ovarian hormones and particularly influenced by oestradiol (60) and, as the data indicated, influenced by its differential pattern of expression across the oestrous cycle. These changes in GLUT expression across the oestrous cycle and after removal of ovarian hormones are particularly relevant given the potential impact of surgical menopause-exacerbated and menopause-related cognitive decline (61,62). This interest has sparked research into the potential mechanisms whereby ovarian hormones modulate neural function (63). The data reported in the present study indicate the important modulatory role that ovarian hormones play in cerebral GLUT expression and, together with both clinical findings and those reported in the literature, indicate the need for future studies aiming to better understand the precise nature of this relationship and its therapeutic implications.

In summary, the present study demonstrated regional and isoform- specificity of cerebral GLUT gene expression in the female rat during a single stage of the oestrous cycle. When expression across the oestrous cycle was assessed, the hippocampus was particularly susceptible to the effects of fluctuating ovarian steroids, which is consistent with cyclic changes in hippocampal-dependent behaviour and structure. In addition, removal of ovarian hormones via ovariectomy reduced the expression of glucose transporters in the hippocampus. These studies demonstrate that ovarian steroids are essential modulators of glucose transporter expression in limbic brain regions, and indicate the need for further research aiming to explore the impact of such modulation on neural function.

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edited the manuscript. GNN designed experiments and revised the manuscript. All authors approved the final manuscript submitted for publication.

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Fig. 1.

Uterine weight and progesterone : oestradiol (P : E) ratio fluctuate across oestrous cycle stages. (A) Uterine weight was normalised to each rat by taking the uterine weight (mg) and dividing by the rat weight (g). Normalised uterine weight varied significantly across the oestrous cycle, with the greatest uterine weight in pro-oestrus. (B) The ratio of plasma progesterone (P4; ng/ml) to plasma oestradiol (E₂; pg/ml) varied significantly across the oestrous cycle, with the highest P4 : E₂ ratio in pro-oestrus. Data are presented as the mean $\pm$ SEM. Letters indicate significant post-hoc differences, with different letters indicating significant differences (P < 0.05).

Fig. 2.

The hippocampus and hypothalamus demonstrate a greater relative abundance of GLUT mRNA than the prefrontal cortex. Fold change in gene expression in dioestrus 1 (D1) of each glucose transporter was calculated by normalising to hippocampal expression and analysed by one-way ANOVA. Analysis revealed significant regional variation for all genes analysed: (A) *Slc2a1*, (B) *Slc2a3*, (C) *Slc2a4* and (D) *Slc2a8*. A Holm–Sidak post-hoc testing showed that glucose transporter gene expression was lowest in the prefrontal cortex for all glucose transporters analysed. Data are presented as the mean $\pm$ SEM. Letters indicate significant post-hoc differences, with different letters indicating significant differences ($P < 0.05$).

Fig. 3.

GLUT1 mRNA abundance is greatest and GLUT4 mRNA abundance is lowest in all regions analysed. Fold change in regional gene expression of glucose transporters in dioestrus 1 (D1) was calculated by normalising to *Slc2a4* and analysed by one-way ANOVA. Analysis demonstrated that significant isoform-specific variation existed throughout the brain. Shown are: (A) hippocampus (HP), (B) prefrontal cortex (PFC) and (C) hypothalamus (HYT). *Slc2a1* expression was greatest and *Slc2a4* expression was lowest in all three regions. Data are presented as the mean $\pm$ SEM. Letters indicate significant Holm-Sidak post-hoc differences, with different letters indicating significant differences ($P < 0.05$).

Fig. 4.

The hippocampus and prefrontal cortex demonstrate greater cycle-dependent variation in gene expression for GLUT1 than the hypothalamus. Fold change in regional expression of *Slc2a1* was calculated by normalising to dioestrus 1 (D1) and analysed by one-way ANOVA. Analysis revealed significant variation by cycle stage in both (A) the hippocampus and (B) the prefrontal cortex but not in (C) the hypothalamus. Data are presented as the mean $\pm$ SEM. Letters indicate significant Holm–Sidak post-hoc differences, with different letters indicating significant differences ($P < 0.05$).

Fig. 5.

Hippocampal mRNA abundance of GLUT3 and GLUT4 varies by cycle stage and peaks in pro-oestrus and oestrus, respectively. Fold change in hippocampal glucose transporter gene expression was calculated compared to dioestrus 1 (D1) and analysed by one-way ANOVA. Relative gene expression of (A) *Slc2a3* and (B) *Slc2a4* varied significantly across the oestrous cycle, although (C) *Slc2a8* expression did not vary by cycle stage. Data are presented as the mean $\pm$ SEM. Letters indicate significant Holm–Sidak post-hoc differences, with different letters indicating significant differences ($P < 0.05$).

Fig. 6.

Expression of GLUT1, 3 and 8 in the hippocampus is directly influenced by ovarian hormones. Fold change in hippocampal glucose transporter gene expression of ovariectomised female rats (OVX) was calculated compared to sham 1 week after surgeries and analysed by an unpaired two-tailed Student's t-test. Relative gene expression was decreased after ovariectomy for (A) *Slc2a1*, (B) *Slc2a3* and (D) *Slc2a8* but not (C) *Slc2a4*. Data are presented as the mean $\pm$ SEM. Asterisks indicate significant differences ($P < 0.05$).

Table 1

Cerebral Glucose Transporter Isoforms Analysed.

Glucose transporter	Gene name	Cerebral cell type	Functional notes
GLUT1-45 kDa	<i>Slc2a1</i>	Astrocytes, neurones (?)	Astrocyte glucose transport
GLUT1-55 kDa		Endothelial cells	Blood–brain barrier glucose transport
GLUT3	<i>Slc2a3</i>	Neurones (somatodendritic)	Primary neuronal glucose transporter
GLUT4	<i>Slc2a4</i>	Neurones (somatodendritic)	Insulin-stimulated glucose transport via plasma membrane
GLUT8	<i>Slc2a8</i>	Neurones (somatodendritic)	Insulin-stimulated translocation to endoplasmic reticulum

Protein and gene names are identified, as well as cell type localisation in the brain and known function.