Dissection of Hippocampal Dentate Gyrus from Adult Mouse

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Abstract

The hippocampus is one of the most widely studied areas in the brain because of its important functional role in memory processing and learning, its remarkable neuronal cell plasticity, and its involvement in epilepsy, neurodegenerative diseases, and psychiatric disorders. The hippocampus is composed of distinct regions; the dentate gyrus, which comprises mainly granule neurons, and Ammon's horn, which comprises mainly pyramidal neurons, and the two regions are connected by both anatomic and functional circuits. Many different mRNAs and proteins are selectively expressed in the dentate gyrus, and the dentate gyrus is a site of adult neurogenesis; that is, new neurons are continually generated in the adult dentate gyrus. To investigate mRNA and protein expression specific to the dentate gyrus, laser capture microdissection is often used. This method has some limitations, however, such as the need for special apparatuses and complicated handling procedures. In this video-recorded protocol, we demonstrate a dissection technique for removing the dentate gyrus from adult mouse under a stereomicroscope. Dentate gyrus samples prepared using this technique are suitable for any assay, including transcriptomic, proteomic, and cell biology analyses. We confirmed that the dissected tissue is dentate gyrus by conducting real-time PCR of dentate gyrus-specific genes, tryptophan 2,3-dioxygenase (TDO2) and desmoplakin (Dsp), and Ammon's horn enriched genes, Meis-related gene 1b (Mrg1b) and TYRO3 protein tyrosine kinase 3 (Tyro3). The mRNA expressions of TDO2 and Dsp in the dentate gyrus samples were detected at obviously higher levels, whereas Mrg1b and Tyro3 were lower levels, than those in the Ammon's horn samples. To demonstrate the advantage of this method, we performed DNA microarray analysis using samples of whole hippocampus and dentate gyrus. The mRNA expression of TDO2 and Dsp, which are expressed selectively in the dentate gyrus, in the whole hippocampus of alpha-CaMKII+/- mice, exhibited 0.037 and 0.10-fold changes compared to that of wild-type mice, respectively. In the isolated dentate gyrus, however, these expressions exhibited 0.011 and 0.021-fold changes compared to that of wild-type mice, demonstrating that gene expression changes in dentate gyrus can be detected with greater sensitivity. Taken together, this convenient and accurate dissection technique can be reliably used for studies focused on the dentate gyrus.

Protocol

Dissection of hippocampal dentate gyrus

1. In a deeply anesthetized mouse, carefully dissect the brain out from the skull and place it into ice-cold phosphate-buffered saline (PBS).
2. In a Petri dish containing ice-cold PBS, cut the brain along the longitudinal fissure of the cerebrum using a surgical knife, and cut off the regions posterior to lambda (midbrain, hindbrain, and cerebellum).
3. Place the cerebral hemisphere medial side up and, using forceps, carefully remove the diencephalon (thalamus and hypothalamus) under a dissection microscope. This will expose the medial side of the hippocampus, allowing for visualization of the dentate gyrus. The dentate gyrus is distinguishable from Ammon's horn by the gaps between them. Injury to the hippocampus or surrounding area will make it more difficult to isolate the dentate gyrus.
4. Insert a sharp needle-tip (e.g., 27-gauge needle) into each side of the dentate gyrus (boundaries of the dentate gyrus and Ammon's horn; Figure 1), and slide the needles superficially along the septo-temporal axis of hippocampus to isolate the dentate gyrus.
5. Pick up the isolated dentate gyrus using a needle or forceps and place it in a sample tube. The thus-obtained dentate gyrus tissue sample can be used immediately for any assay or stored in a deep-freezer for later use.
6. Isolate the dentate gyrus from the other cerebral hemisphere using the same method.

Quantitative real-time PCR

The dentate gyrus was isolated using the above-mentioned method and the remaining hippocampus was dissected out as the Ammon's horn sample from wild-type mice. Real-time PCR of beta-actin, TDO2, Dsp, Mrg1b and Tyro3 were performed with the dentate gyrus and the Ammon's horn samples as described previously1. Primers 5'-CTGGCGAGATCACGATGACG and 5'-AAGCTACGCTGTTGTCTAACC were used for Mrg1b, and GCCTCCAAAATGCGCCGTCA and 5'-CCAGCCTGGGTACTGAGATCA for Tyro3.

Microarray analysis

Microarray experiments were performed with male wild-type mice and mice heterozygous for the alpha isoform of calcium/calmodulin-dependent protein kinase II (alpha-CaMKII+/+ mice) as described previously1. Briefly, RNA isolated from the whole hippocampus or dentate gyrus of wild-type and mutant mice was hybridized with a Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA), and each GeneChip was scanned by an Affymetrix GeneChip Scanner 3000 (GCS3000). GeneChip analysis was performed with Microarray Analysis Suite version 5.0.
Discussion

The dentate gyrus occupies approximately 25% to 30% of the volume of the hippocampal formation\(^2,3\). It has a unique cell composition and plays crucial roles in various brain functions. Therefore, techniques to isolate the dentate gyrus are useful for analyzing the events that occur specifically in this region.

Here, we demonstrated a procedure to efficiently dissect the dentate gyrus from adult mouse hippocampus and confirmed the precision of the technique. First, histologic study revealed that the dentate gyrus was separated without contamination by other regions (Figure 1), indicating that a pure dentate gyrus sample can be prepared.

Second, we confirmed that the dissected tissue is dentate gyrus by conducting real-time PCR of dentate gyrus-specific genes, TDO2 and Dsp, and Ammon's horn enriched genes, Mrg1b and Tyro3\(^4\) (Figure 2). The mRNA expressions of TDO2 (\(p=0.000023; n's=4\) and 4, respectively) and Dsp (\(p=0.0000030; n's=4\) and 4, respectively) in the dentate gyrus samples were detected at obviously higher levels, whereas Mrg1b (\(p=0.0000080; n's=4\) and 4, respectively) and Tyro3 (\(p=0.000017; n's=4\) and 4, respectively) were lower levels, than those in the Ammon's horn samples. Beta-actin expression levels did not differ in these samples (\(p=0.11; n's=4\) and 4, respectively). Thus, we could check whether or not the dentate gyrus was accurately dissected out by conducting such simple real-time PCR experiments.

Third, to assess the usefulness of this dissection method, we compared the mRNA expression level of whole hippocampus with that of dentate gyrus. Whole hippocampus and dentate gyrus obtained from wild-type (\(n's=9\) and 4, respectively) and alpha-CaMKII\(^{+/-}\) mice (\(n's=18\) and 4, respectively) were processed for microarray analysis, and for all genes scored, the fold-change was calculated by dividing the mutant value by the wild-type value. The results indicated that the changes in mRNA expression, especially of dentate gyrus-specific molecules such as Dsp and TDO2, were detected with up to a 5-fold increase in sensitivity in dentate gyrus samples compared to whole hippocampal samples (Table 1). We previously demonstrated that alpha-CaMKII\(^{+/-}\) mice exhibit behaviors related to human psychiatric disorders such as working memory deficits and an exaggerated infradian rhythm\(^1,5\). Furthermore, morphologic and electrophysiologic features of the dentate gyrus neurons in mutant mice are strikingly similar to those of immature dentate gyrus neurons in normal rodents, indicating that the neurons in these mutant mice fail to develop to maturity\(^1\). The immature dentate gyrus and down-regulated expression of Dsp and TDO2 mRNA in alpha-CaMKII\(^{+/-}\) mice are consistent with the finding that Dsp and TDO2 can be used as markers of mature granule cells in the dentate gyrus (Ohira et al., unpublished data).

Taken together, this convenient and accurate dissection technique can be reliably used for studies focused on the dentate gyrus. Dentate gyrus tissue obtained using this method is applicable to other types of analyses as well, including proteomic and cell biology analyses.
Figure 2. Verification of the isolated dentate gyrus by real-time PCR. The dentate gyrus and the Ammon’s horn obtained from four wild-type mice were processed for real-time PCR of beta-actin, TDO2, Dsp, Mrg1b and Tyro3. Results are presented as means ± SEM. For statistical analysis, Student’s t-test was employed, and p values are followed: beta-actin, p=0.11; TDO2, p=0.000023 (**1); Dsp, p=0.000030 (**2); Mrg1b, p =0.000080 (**3); and Tyro3, p=0.00017 (**4).

Table 1. Microarray analysis of whole hippocampus and dentate gyrus. Genes differentially expressed in dentate gyrus and whole hippocampus of alpha-CaMKII+/- mice were determined by calculating the fold-change from that detected in wild-type mice. Data were analyzed for statistical significance using the Student’s t test between wild-type and alpha-CaMKII+/- mice. Among the genes whose expression exhibited p <0.05 in the dentate gyrus of alpha-CaMKII+/- mice compared to that of wild-type mice, the top 50 genes are listed. Note that the numbers of samples for dentate gyrus are much less than those for whole hippocampus. AffyID, Affymetrix probe identifier; CKII, alpha-CaMKII+/- mice; WT, wild-type mice.

| Gene Title | Genebank | AffyID          | Fold change | p value       | Fold change | p value       |
|------------|----------|-----------------|-------------|---------------|-------------|---------------|
| desmoplakin | AV297961 | 1435494_s_at    | 0.011018913 | 7.02694E-06   | 0.037021003 | 1.86126E-13   |
| desmoplakin | AV297961 | 1435493_at      | 0.014369734 | 7.86747E-06   | 0.04232106  | 1.00579E-12   |
| tryptophan 2,3-dioxygenase | AI098840 | 1419093_at      | 0.020986484 | 5.23546E-09   | 0.10103776  | 4.14823E-13   |
| nephronectin | AA223007 | 1452106_at      | 0.075479901 | 1.05191E-08   | 0.234001154 | 1.66301E-15   |
| nephronectin | AA223007 | 1452107_s_at    | 0.079457767 | 1.40433E-07   | 0.177974715 | 3.9758E-12    |
| thyrotropin releasing hormone receptor | MS9811 | 1449571_at      | 0.103105815 | 0.003093796   | 0.801412732 | 0.283994361   |
| ryanodine receptor 1, skeletal muscle | X83932 | 1427306_at      | 0.104825517 | 3.38513E-07   | 0.650685017 | 0.000308462   |
| nescent helix loop helix 1 | NM_010916 | 1419533_at      | 9.431896    | 6.7979E-06    | 4.078815314 | 5.27E-11      |
| copine family member IX | BB274531 | 1454653_at      | 9.150157    | 7.99492E-06   | 1.797304153 | 0.000269375   |
| doublecortin-like e kinase 3 | BB326709 | 1436532_at      | 0.109336662 | 1.95278E-07   | 0.56697229 | 2.62633E-08   |
| calpain 3 | AF127766 | 1426043_a_at    | 0.111269769 | 8.07053E-06   | 0.370956608 | 2.04421E-14   |
| Adult male corpus striatum cDNA, RIKEN full-length enriched library, clone:CO30023 B07 product:unclassifiable, full | BB357628 | 1460043_at      | 0.118712341 | 6.16926E-07   | 0.682339204 | 2.33001E-06   |
| Gene Name                                      | Accession | Symbol   | Description                                                                 | Gene ID | Description                                                                 | Expression Value | p-Value | Fold Change | p-Value |
|-----------------------------------------------|-----------|----------|------------------------------------------------------------------------------|---------|-----------------------------------------------------------------------------|------------------|---------|-------------|---------|
| insert sequence                               | AV264768  | 1437385_at | collagen and calcium binding EGF domains 1                                   |         |                                                                             | 0.124043978      | 3.65669E-05 | 0.488394112 | 4.05538E-06 |
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| days neonate cerebellum | Mus musculus cDNA clone A730018G18 3', mRNA sequence. |
|------------------------|---------------------------------------------------------|
| FERM domain containing 3 | BB099015 1437075_at 5.860216 0.000345581 2.780297178 1.83072E-06 |
| neuronal pentraxin 2 /// hypothetical protein LOC100044234 | NM_016789 1420720_at 5.7568517 1.34227E-06 2.652516957 0.000206279 |
| Transcribed sequences | BG076361 1460101_at 5.657735 2.5015E-06 1.296248831 0.22870031 |
| spondin 1, (f-spondin) extracellular matrix protein | BC020531 1424415_s_at 0.17783576 1.01658E-06 0.836181248 0.001380141 |
| calbindin-28K | BB246032 1448738_at 0.180317904 1.35961E-05 0.647334052 4.12268E-09 |
| MARCKS-like 1 | AV110584 1437226_x_at 0.186235935 1.47067E-06 0.499291387 2.34984E-08 |
| matriuin 2 | BB338441 1455978_a_at 0.187783528 6.19122E-05 0.915528892 0.35282097 |
| matriuin 2 | BC005429 1419442_at 0.188195795 0.000105295 0.915528892 0.35282097 |
| spondin 1, (f-spondin) extracellular matrix protein | BQ175871 1442613_at 0.189956563 9.41195E-06 0.861033222 0.1394266 |
| arrestin 3, retinal | NM_133205 1450329_a_at 5.2130346 3.944218329 1.07437E-07 |
| RIKEN cDNA A330050F15 gene | AV325555 1457558_at 0.19186781 0.000119342 0.660282035 2.47342E-05 |
| contactin 3 | BB559510 1438628_x_at 0.194404608 4.08641E-07 0.918742591 0.022545297 |
| calbindin-28K | BB246032 1417504_at 0.196381321 2.24182E-05 0.619305124 3.6222E-09 |
| gastrin releasing peptide | BC024515 1424525_at 4.9436426 3.0588E-05 2.752845903 5.72954E-07 |
| sortilin-related VPS10 domain containing receptor 3 | AK018111 1425111_at 4.885766 1.03645E-05 1.29051599 0.029733649 |
| dopamine receptor D1A | BE957273 1455629_at 4.869493 3.77525E-05 1.815881979 0.000516498 |
| proprotein convertase subtilisin/kexin type 5 | BB241731 1437339_s_at 0.210528027 7.8303E-05 0.574126078 9.15496E-05 |
| interleukin 1 receptor, type I | NM_008362 1448950_at 0.210572243 9.64524E-06 0.241135352 2.79816E-08 |
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