# Integrated Undergraduate Research Experience for the Study of Brain Injury

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We developed a series of hands-on laboratory exercises on "Brain Injury" designed around several pedagogical goals that included the development of: 1) knowledge of the scientific method, 2) student problem solving skills by testing cause and effect relationships, 3) student analytical and critical thinking skills by evaluating and interpreting data, identifying alternative explanations for data, and identifying confounding variables, and 4) student writing skills by reporting their findings in manuscript form. Students, facilitated by the instructor, developed a testable hypothesis on short-term effects of brain injury by analyzing lesion size and astrocytic activity. Four sequential laboratory exercises were used to present and practice ablation techniques, histological processing, microscopic visualization and image-capture, and computer aided image analysis. This exercise culminated in a laboratory report that mimicked a research article. The effectiveness of the laboratory sequence was assessed by measuring the acquisition of 1) content on anatomical, physiological, and cellular responses of the brain to traumatic brain injury, and 2) laboratory skills and methods of data-collection and analysis using surgical procedures, histology, microscopy, and image analysis. Post-course test scores, significantly greater than pre-course test scores and greater than scores from a similar but unstructured laboratory class, indicated that this hands-on approach to teaching an undergraduate research laboratory was successful. Potential variations in the integrated laboratory exercise, including multidisciplinary collaborations, are also noted.

*Key words: neuroanatomy; histology; microscopy; image analysis; brain injury; experimental design*

Active learning methods such as hands-on laboratories and independent research have been shown to be excellent ways of developing a variety of cognitive, problem solving and critical thinking skills (Czaja et al., 1998; Levin, 1993; Maloy, 1993) whether the students intend to become scientists or only consumers of scientific knowledge. Besides the effects on cognitive development, direct involvement in the research process can enhance a student's enthusiasm for a discipline, reinforce confidence in their abilities, and encourage students to delve deeper into a subject. This type of involvement can be particularly effective in engaging students who are exploring a discipline such as neuroscience as a career. We have found that many of our students interested in neuroscience and psychology are fascinated by the effects of traumatic injury on the brain. To capitalize on this interest, we developed an integrated "hands-on" neuroscience exercise for entry-level undergraduates that encourage students to integrate and apply lecture material in a problem-solving "hands-on" laboratory setting. This exercise engaged students by getting them to utilize lecture material to design an experiment examining the effects of surgically-induced cortical injury in rats and then acquiring laboratory skills as they executed their experiment.

We focused on several distinct yet partially overlapping pedagogical goals as we created the exercise. Some of these goals were primarily cognitive and included the development of: 1) knowledge of the scientific method, 2) student problem solving skills by testing cause and effect relationships, 3) student analytical and critical thinking skills by evaluating and interpreting data, identifying alternative explanations for data, and identifying confounding variables, and 4) student writing skills by reporting their findings in manuscript form. Another set of skills were more content or technique oriented and included: 1) mastery of content on anatomical, physiological, and cellular responses of the brain to traumatic brain injury, and 2) acquisition of laboratory skills and methods of data-collection and analysis using surgical procedures, histology, microscopy, and image analysis. The resulting exercise was developed for students at a sophomore level or higher, taking their first neuroscience course. Students, facilitated by the instructor, designed an experimental hypothesis to test two short-term effects of brain injury by analyzing lesion size and astrocytic activity. This exercise spanned a five-week period of preliminary lectures and laboratory assignments.

## MATERIALS AND METHODS

*Participants*

The class had six students, four sophomores and two juniors, who were taking their first neuroscience course, Biological Basis of Behavior, with the co-requisite laboratory course, Introduction to Neuroscience Methods.

*Animal Subjects*

Adult male albino rats between 300-375 g were used for the experiment. They were maintained in individual cages with food and water available *ad libitum*. Colony lighting was maintained on a 12:12 h light-dark cycle. All procedures were approved by the Regis University IACUC.

*Apparatus*

For the ablation procedure, an inexpensive device designed after Kaplan et al. (1983) was used as a head holder. A hand-held dental drill (Foredom, Model 73) was used to open the skull during surgery and a 1/20 hp vacuum pump (General Electric) was used to provide suction for the ablation of cortical tissue. Surgery was conducted under sodium pentobarbital (IP, 50 mg/kg body weight, Abbott Laboratories) anesthesia, supplemented as needed with isoflurane inhalant (AErrane, Fort Dodge Animal Health). For the histology laboratory, tissues were cut with a Leica (Model 1850) cryostat and mounted on 3 x 1 in slides (Rite-on, Gold Seal) frosted on one side for labeling. The microscopy laboratory featured a Nikon Labophot with 2x, 4x, 10x, and 20x objectives. Microscopic images were captured using a Pixera digital camera mounted to the trinocular tube of the Labophot with a universal C-mount. Camera functions were controlled by Pixera Viewfinder Image Capture software. Once the images were captured, students used SimplePCI Image Analysis software (C-Imaging Systems) to analyze and quantify the objects in which they were interested.

## INSTRUCTIONAL PLAN

*Preparatory Lecture & Lab Exercises*

Prior to the start of the integrated laboratory, the students received seven hours of classroom lectures on neurophysiology and general neuroanatomy using Kolb and Wishaw (1995) as the required textbook. Following this, there were four hours of lectures and class discussion on the effects of traumatic impact injury to the head (e.g., head striking a windshield during a car accident; Kolb and Wishaw, 1995, Finnie and Blumbergs, 2002). These lectures included topics such as a description of physiological, glial, and neuronal responses at the injury site, shearing, diaschisis, and other secondary effects of injury, and a description of the longer term responses such as phagocytosis, capillary proliferation, and scarring. This was followed by an examination of factors that affect recovery of behavioral and cognitive functions. In addition, the students had completed two laboratory exercises, one in which they received hands-on experience with a perfusion technique and a second involving psychopharmacology, before the laboratory series described below. These lecture and laboratory exercises were coordinated to present the essential background material on the effects of brain injury in lecture first before presenting the students with a general condition to test in lab.

To generate “ownership” in the project, students were told that they could test any variable that they wanted and was reasonable within the capacity of the laboratory. From that point forward, the instructor functioned as a group facilitator to guide the students through experimental design issues and as an “expert consultant” when it was necessary to instruct the students in the methods needed to complete the next phase of the experiment. Details of the experiment were developed incrementally from session to session. Each student team was expected to have completed their portion of the experiment by the next lab session. At the beginning of each lab session, each team reported their progress, any problems encountered, and how the problems were solved. After this the instructor explained the basic method required for the next step of the experiment (e.g., how to use a cryostat) and then the students had to determine how they wanted to use that method in their experiment. Interactions between student teams were encouraged throughout the project.

**Laboratory 1: Development of experimental design and ablation method**

The instructor (ERD) opened the session with, “In lecture, you have learned about immediate and long term effects of traumatic injury to brain tissue. Today, you will identify the effects of brain injury that you want to study, and then develop an experiment to test the validity of elements of those effects.” At this point the instructor guided the students as they:

1. Identified all questions and variables that the class could study within a four-week period (four three-hour lab sessions and independent time) and the capacities of the laboratory facilities.
2. Narrowed these to two questions and identified the experimental variables the students wanted to test.
3. Generated hypotheses based on their existing knowledge.
4. Designed an experiment to test these hypotheses. This included identifying potentially confounding variables such as how each team would produce a lesion at the same location and of the same size as other teams.
5. Assigned each test condition to two-person teams.

The students designed an experiment with three independent variables: 1) recovery time: 3, 40, and 120 hours, 2) lesion condition: unilateral lesion with the opposite hemisphere treated as a no lesion control condition, and 3) sample site (astrocyte only): wall and floor of the lesion and mirrored locations of intact hemisphere. The students also chose to measure two variables: astrocyte populations in the sample areas and the size of lesion vacuole. To accomplish this, they decided to create lesions using specific criteria. The lesions were to be 3 x 3 x 3 mm created by suction. The medial edge of the lesion was to be 2 mm left of the midsagittal suture. The anteromedial corner of the lesion was to be located posterior to bregma at a point 60% of the distance between bregma and lambda. At the end of the assigned recovery period, each team would sacrifice its rat and prepare for the next step of the exercise, histological staining. Students then viewed a video, made by the authors, that demonstrated how to perform a cortical ablation, provide postoperative care, and clean the surgical instruments. The students then completed their assignment.

The surgical procedure for this exercise involved a craniotomy and a suction ablation of cortical tissue. Rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and placed in a head holding device. The skin, followed by the connective tissue, was opened exposing the skull and bony landmarks. Using the bony landmarks, the students removed the bone over the area of interest. The dura was opened and a suction ablation was performed. Following the ablation, a small piece of Gelfoam was inserted into the vacuole. The surgical incision was closed by layer, connective tissue followed by skin using 3/0 silk. The students were responsible for post-surgical care. This was initiated by giving the rat a prophylactic injection of the antibiotic gentamycin (50mg/ml, Vedco, Inc, 0.1 ml SC). The rat was placed in a clean cage under a heat lamp and monitored until it exhibited clear mobility. Following recovery to this point, the rat was monitored for behavioral and surgical complications every eight hours until sacrificed. The rats were sacrificed by an overdose of sodium pentobarbital, perfused intracardially with saline followed by 10% formalin. The brain tissue was removed and stored in formalin. All student surgeries were done under the supervision of the authors. Regis University IACUC had previously approved the procedure.

**Laboratory 2: Histological Methods**

Before class, students were given a written description of histological methods, their strengths and limitations, and the procedures for Nissl and Fast-blue staining. At the beginning of class, the instructor guided the class through a discussion of their progress, then encouraged the class to analyze what their next steps in the experiment should be and why. The students were then given hands-on demonstrations of the procedures for using a cryostat and staining their tissue. Afterwards, the class decided on the specific procedures (e.g., to cut their sections at 30 μm) to use in their experiment. Between lab sessions, each team had to section and stain their tissue with Cresyl Violet and Fast-Blue for examination.

**Laboratory 3: Microscopy Methods**

The class began with a discussion of their progress and determined what their next steps should be and why. A description of application of light microscopy and the basic function and anatomy of a microscope (e.g., light path) and of light microscopy was then given (by CLB) on topics such as the light path of a microscope. Students received instruction on Kohler illumination adjustments, image acquisition using the Pixera camera mounted on the Nikon microscope, and image quantification with SimplePCI software. Students then decided what procedures would be used in their experiment. This included how they would estimate the size and lesion locations using the 2x images and the criteria for identifying and counting astrocytes using 20x images of the middle section of each lesion. Between lab sessions, each team had to obtain the data for their own tissue.

**Lab Session 4: Data Analysis and Interpretation.**

Once again the class began the session by discussing their progress and problems with data acquisition. Then they shared cell counts, measurements of their lesions, and computerized images from each of their samples. The class discussed (with minimal guidance by the instructor) the possible interpretations of their data, whether their data agreed with lecture material and library resource material, what the limitations of their data might be, and what the implications of their data were for understanding brain injury. The instructor then described the function, the needed information, and the format of each component of the scientific manuscript, as well as APA citation and reference formatting. Each student's report was due at the next lab session

## STUDENT RESULTS

Students obtained counts of astrocyte populations that supported their predictions, especially at the 120-hour recovery period (Table 1). Students measured the maximum lesion width and height, and obtained area measurements using the SimplePCI software (Table 2).

**Table 1.** Astrocyte count at each recovery time.

| | | Recovery Period (hours) | | |
|---|---|---|---|---|
| | | 3 | 40 | 120 |
| Wall | Intact | 2 | 4 | 5 |
| | Lesion | 3 | 9 | 34 |
| Floor | Intact | 2 | 6 | 12 |
| | Lesion | 2 | 11 | 87 |

**Table 2.** Measurements of lesion size

| | Recovery Period (hours) | | |
|---|---|---|---|
| | 3 | 40 | 120 |
| Width (μm) | 3497 | 2704 | 3728 |
| Height (μm) | 1426 | 1284 | 2278 |
| Area ($\mu m^2$) | 3309985 | 441146 | 4768248 |

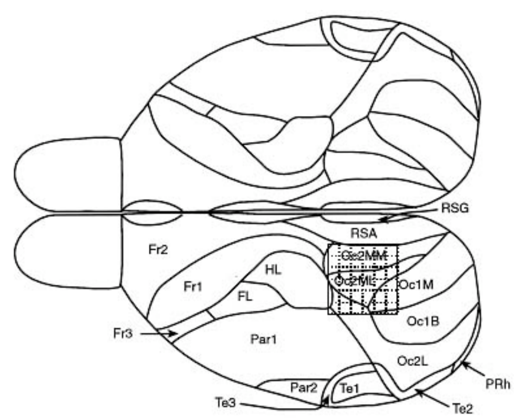


**Figure 1.** Schematic map of rat brain (dorsal view). The proposed lesion area is indicated by the hatched box.

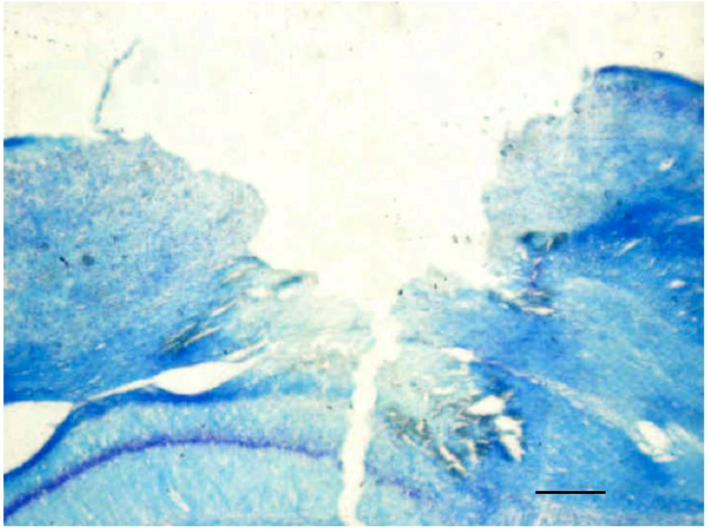

**Figure 2**. Example of coronal section through the site of an ablation lesion stained with cresyl violet and Luxol Fast Blue (2x magnification). The lesion site is in area Oc2ML (see Figure 1). Scale bar = 500 µm

The proposed extent of the 3 x 3 x 3 mm lesion, represented by a hatched box, is indicated on a schematic drawing of the dorsal surface of the rat brain, inclusive of cytoarchitectural areas (Figure 1). The lesion for the rat given a 40 hour recovery time, had an irregular shape and thus appeared to have a larger area than anticipated by the other measures. Since there was only one rat at each recovery point and since several of the students had not yet taken a course in statistics, the students decided not to attempt any statistical analysis. Samples of captured images obtained by the students with the 2x and 20x objectives are shown in Figures 2 and 3. The students were instructed on the differences between neurons and glial cells. The neurons would be identified by large triangular or tear-drop shaped cells with pale purple staining cytoplasm and the possibility of a clear central area representing the nucleus. The glial cells would be elliptical in shape, smaller, and darker purple staining indicating a high degree of protein synthesis as a response to the repair process going on at the edge of the lesion.

## RESULTS

This five-week experience was positioned at the beginning of the semester and thus formed a foundational aspect to the entire course. Pre-lab and post-lab examinations and a final laboratory report in manuscript form were used to assess the outcomes of this experience. At the end of each laboratory session, the discussion that ensued addressed the specific pedagogical objectives of this exercise. For example, What have we learned about the scientific method to this point? What have we learned about cause and effect relationships to this point? What have we learned about designing an experiment with respect to variables and alternate interpretations of data to this point? How is the information best presented in a written format?

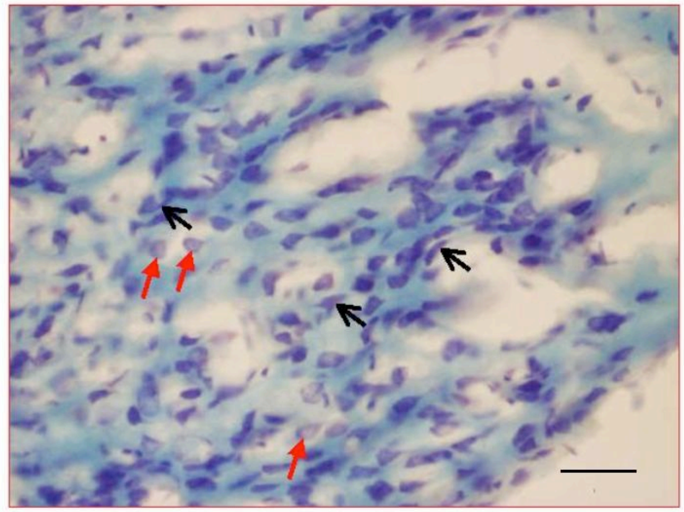

**Figure 3**. Example of region along the edge of lesion that students counted to assess astrocyte population stained with cresyl violet and Luxol Fast Blue. The edge at the bottom right is on the lateral edge of the lesion vacuole (20x magnification). (Red arrows = neurons, Black arrows = astrocytes). Scale bar = 500 µm

Each of these questions is addressed in the context of the lab assignment for that particular day. The ultimate outcome of this exercise is seen in the increased scores on the post-lab exam compared to the pre-lab exam (see next section). The increase in problem solving skills and critical thinking skills are seen in the answers to the more open-ended questions on the post-lab exam. These questions were left blank on the pre-lab exams and have been answered in a complete and thoughtful manner following this experience. The manuscript style report required at the end of this exercise is the first lab report required in this course, as well as a first lab report for most of these students, and thus forms a foundational learning experience. While it was graded, it in essence forms a baseline learning experience.

**Pre-Lab and Post-Lab test Assessment**

As part of the assessment process, each student took a test the first day of the laboratory and a similar test as part of the final exam on the last day of the class. Each test had several parallel questions to assess gains from the course. The questions most relevant to this project were:

A. "You hypothesize that (drug name) might have detrimental effects on recovery of behavioral and neurological function after traumatic brain injury. 1) Design and outline the experimental procedures you would employ to test this hypothesis. 2) Critique your design. 3) Are there limitations on the interpretation of your findings related to your experimental methods?"

B. "Assume that your experiment was a success. What would be the next experimental question that you would address with your project and how would you design the experiment (you may wish to use the same technique or use a new technique to answer this question)?"

The mean pre-lab test score was 3.8% and the mean post-lab test score was 87.7% ($F(1,5) = 225.31$, $p < 0.001$). Anecdotally, a group of students that was presented with similar topics but did not have the benefit of this organized series of labs scored a mean of 78.4% on somewhat similar questions as part of a final exam.

## DISCUSSION

This exercise was intended to promote student involvement as a means of developing cognitive, problem solving and critical thinking skills. In addition to the acquisition of cognitive skills, the students learned and implemented a number of fundamental laboratory skills. Choosing traumatic brain injury as the topic provided a backdrop for integrating lectures and laboratory exercises from which students could develop testable hypotheses. Students with minimal neuroscience background were guided through a series of exercises to help them understand and apply the scientific method while testing a student-generated hypothesis and to gain theoretical and hands-on experience with animal surgery, histology, microscopy, and manuscript writing. The images of the lesions and the data collected as part of their analyses are evidence that the students had acquired the skills set as goals for the project. The experimental design developed during the project, the increase in scores on the posttest questions relative to the pretest scores, and relative to scores of another class responding to similar, although not identical, questions are evidence of growth in cognitive skills.

From a pedagogical perspective, this exercise was designed to introduce structure and function of the central nervous system to sophomores and juniors as their first neuroscience course. This exercise was based on three specific educational components that included a 1) presentation of lecture material prior to either demonstration or video instruction of specific related laboratory techniques, 2) guided discussion format, and 3) laboratory write-up in the style of a research manuscript. The lecture material presented as introduction to the laboratory experience served as an anchoring point for students with primarily a general psychology and biology background. These lectures covered basic neuroanatomy and neurophysiology, followed by an introduction to the mechanism of injury, as well as short-term and long-term effects of trauma to the central nervous system. This information provided a reference point which the class could move forward together in the development of an experimental question and a focal point for facilitating discussions by the instructor. This format challenged the students to take an active role in all discussion and set the tone by which they were engaged in the development of an experimental question. For example, a guided discussion format was used to help the students design an experiment to test the theories on the effects of traumatic lesions of the brain. As a part of the discussion, the instructor introduced fundamental concepts of experimental design and basic experimental tools used to investigate nervous system organization, and then guided the discussion on lecture material while enabling students to determine their own question about the effect of central nervous system injury that they could test. For the introduction of laboratory techniques, a series of videotapes on perfusion and ablation techniques made by the authors, and prescribed exercises on histological, microscopic, and image analysis techniques preceded the actual practice of each technique. These pedagogical methods allowed for questions to be answered at each step of the design prior to the student actually using an experimental animal. This minimized the use of animals in this exercise and assured that student hands-on involvement was a positive experience. As the students became involved in the design of the experiment and acquired a set of experimental tools, they carried out their experiment on tissue they prepared themselves. The design of this exercise was to culminate in a laboratory write-up that mimicked the preparation of a manuscript.

The discussion that preceded the finalization of the laboratory report focused on analysis and interpretation of the data. As students discussed the implications of their data with respect to the original question, they were introduced to the specific parts of a research manuscript. This discussion was facilitated by questions such as: What do you see in the captured image? What does it mean? Do your observations fit previously reported information [presented in lecture and text]? How do your observations relate or extend the foundational information used to generate the original design? In this case, the students chose to look at astrocyte proliferation following an experimental ablation. The analysis also included documentation of the lesion size compared to the planned size. The facilitation that followed included how to interpret their numbers, how to relate their data to published works, and how the presence of astrocytes related to migration, repair, and function. The analysis of astrocyte number was expressed as a population within a standard area and then compared by number of days from lesion. The results of size of the lesion were more problematic but were used by the instructor to illustrate the relationship between experimental design and data interpretation. For example, the number of rats ($n = 1$) for each time point and lack of experience of students were possible explanations for the variability noted. Students were also guided to consider other secondary brain injury sequelae such as vascular proliferation or ischemic changes that might be responsible for the variation in lesion size and that these variables could be the basis of future experiments. This discussion style yielded a positive outcome to the students' experiences, giving them a feeling of accomplishment from their first practice at designing, performing, and analyzing an experimental process.

The proposed outcome of this exercise was to increase the learning of students through hands-on exercises that support development of cognitive, problem solving, and critical thinking skills through facilitated discussions. As a mechanism to gauge the success of this exercise, pre- and post-lab exams were given to the students to assess improvement of cognitive skills. Some questions were more factually oriented such as: If you were designing an experiment, how and/or why would you choose histology as part of your data analysis? Other questions were open-ended and asked for more use of cognitive skills through the use of integration: 1) How would you demonstrate the effects of an experimental lesion of the central nervous? 2) How would you analyze and interpret data obtained from a captured image involving counterstains for Nissl substance and myelin? 3) Assuming your current experiment supports your hypothesis, what would be the next question that you would ask? 4) Outline the main features of an experiment to test your question.

This cascade of thinking, demonstrated by the different question styles on the exams, was the process around which the integrated laboratory exercise was developed, and formed the basis and sequence for the facilitated discussions during the labs. This development of cognitive, problem-solving, and critical thinking skills allows the students to consider where this experience might lead and gives them the opportunity to express themselves and the level of their knowledge at each time point. During lab discussions, the students identified a number of extensions of this exercise. The students questioned whether the anatomical changes documented in their experiment have a behavioral correlate? They also questioned whether the anatomical changes were correlated with biochemical changes in neural activity, whether it was possible to correlate biochemical changes with behavioral changes, and whether behavioral changes could be ameliorated with pharmacological means?

While these questions indicate developing cognitive skills and a broadening, multidisciplinary perspective, they also suggest exciting potential variations in this exercise. These questions could be incorporated by adding components, e.g. behavioral tests sensitive to functions affected by lesions of specific cortical areas. Another approach might be to add collaborations between this course and a Psychology course studying relevant behavioral functions. The addition of similar collaborative interactions with Biochemistry courses and/or Biology courses could look at specific biochemical markers; neurotransmitters, membrane receptors, and/or intracellular signaling compounds. In a truly integrated curriculum, each of these parameters of CNS injury would be integrated across courses with all groups meeting to discuss and correlate their findings. It would also be possible to develop an individual course with students from each discipline contributing to the design of the question and participating in the entire exercise. Any expansion or integration would necessarily require an extension of the exercise as it is designed. Relevant facilitated discussions would need to be included and laboratory sessions would need to be added to provide students the opportunity to acquire the necessary skills and experience. However, these added labs would provide students with a rich, highly integrated course across multiple scientific disciplines. The experience would expose the student to the scientific process, while teaching them a topic that is truly interdisciplinary and of interest to the entire neuroscience community.

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