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Artificial Sweeteners and Hippocampal Neuronal Activation: Molecular Insights from c-Fos Signaling and Oxidative Stress Pathways

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ABSTRACT

***Background*:** Artificial sweeteners are widely used as non-caloric substitutes for dietary sugars and are increasingly incorporated into beverages, processed foods, and pharmaceutical preparations. While these compounds are generally regarded as safe within approved intake levels, growing experimental evidence suggests that certain artificial sweeteners may influence neural activity within brain regions involved in learning and memory, particularly the hippocampus. The hippocampus plays a critical role in cognitive processing and exhibits high metabolic demand, making it especially sensitive to metabolic and oxidative disturbances. Recent experimental studies have explored the effects of artificial sweeteners on hippocampal neurons using molecular markers of neuronal activation, including the immediate early gene **c-Fos**, which is widely used as an indicator of activity-dependent neuronal signaling. Alterations in c-Fos expression provide insight into how external stimuli, including dietary exposures, can modify neural circuit activity. In parallel, several investigations have reported that artificial sweeteners may influence oxidative stress pathways within the brain, potentially affecting mitochondrial function, redox balance, and neuronal survival. These molecular alterations may contribute to changes in synaptic plasticity and hippocampal-dependent cognitive performance observed in experimental models. This review summarizes current knowledge regarding artificial sweeteners and their potential effects on hippocampal neuronal activation, with particular emphasis on c-Fos signaling and oxidative stress mechanisms. By integrating findings from neurophysiology, molecular neuroscience, and experimental nutrition research, this article aims to clarify how dietary sweeteners may influence hippocampal neuronal activity and highlight key pathways that may link metabolic exposures with changes in brain function.

Keywords: Artificial sweeteners; Hippocampus; c-Fos; Immediate early genes; Neuronal activation; Oxidative stress; Neuroplasticity; Aspartame; Non-nutritive sweeteners; Memory circuits; Synaptic signaling; Hippocampal neurons.

INTRODUCTION

Artificial sweeteners, also referred to as non-nutritive or high-intensity sweeteners, are widely used as substitutes for dietary sugars in food, beverages, and pharmaceutical preparations. These compounds provide intense sweetness while contributing little or no caloric value, making them attractive alternatives for individuals attempting to reduce sugar intake or manage body weight. Over recent decades, the global consumption of artificial sweeteners has increased substantially due to the rising prevalence of obesity, diabetes mellitus, and other metabolic disorders. Consequently, these compounds are now present in a broad range of products including diet beverages, processed foods, chewing gum, oral medications, and nutritional supplements [1].

Several artificial sweeteners have received regulatory approval for human consumption, including aspartame, saccharin, sucralose, acesulfame potassium, neotame, and advantame. In addition, naturally derived non-nutritive sweeteners such as steviol glycosides extracted from *Stevia rebaudiana* have gained increasing popularity due to their plant origin and low caloric content. Although these sweeteners differ considerably in their chemical structure and metabolic fate, they share the common feature of activating sweet taste receptors with minimal contribution to energy intake [2].

Despite their widespread use and regulatory approval, the long-term biological effects of artificial sweeteners remain an area of active scientific investigation. While early studies primarily focused on metabolic and toxicological safety, more recent research has explored their potential effects on the central nervous system. This interest arises from evidence that sweet taste receptors and related signaling pathways are not confined to the oral cavity but are also expressed in various tissues, including the gastrointestinal tract and the brain. These findings raise the possibility that artificial sweeteners may influence neural signaling pathways involved in appetite regulation, metabolic control, and cognitive function [3].

Among brain structures potentially affected by dietary factors, the **hippocampus** has attracted particular attention. Located within the medial temporal lobe, the hippocampus plays a central role in memory consolidation, spatial navigation, and contextual learning. Hippocampal neurons exhibit high synaptic activity and metabolic demand, making them especially sensitive to metabolic disturbances, oxidative stress, and neurochemical alterations. Experimental studies have suggested that dietary exposures—including excessive sugar intake and certain artificial sweeteners—may influence hippocampal structure and function through mechanisms involving neurotransmitter modulation, inflammatory signaling, and oxidative stress [4].

A key molecular tool for studying neuronal activation in the hippocampus is the **immediate early gene c-Fos**. c-Fos is rapidly expressed in neurons in response to synaptic stimulation and is widely used as a marker of activity-dependent neuronal signaling. Because c-Fos expression reflects recent neuronal activation, it has become an important indicator for mapping functional neural circuits and identifying brain regions involved in behavioral or metabolic responses. Changes in c-Fos expression patterns within the hippocampus can therefore provide valuable insight into how external stimuli, including dietary components, modulate neural activity within memory-related circuits [5].

In addition to neuronal activation pathways, oxidative stress has emerged as another potential mechanism linking artificial sweeteners with neural effects. Several experimental studies have reported that chronic exposure to certain artificial sweeteners may alter antioxidant defense systems and increase the generation of reactive oxygen species in neural tissues. Because hippocampal neurons are particularly vulnerable to oxidative damage, disturbances in redox homeostasis may influence synaptic plasticity and neuronal survival, potentially affecting cognitive performance and memory processes [6].

Given the increasing global consumption of artificial sweeteners and the growing interest in their neurological effects, understanding their potential impact on hippocampal neuronal activity has become an important research priority. The present review aims to examine current evidence regarding artificial sweeteners and their influence on hippocampal neuronal activation, with particular emphasis on molecular signaling pathways involving **c-Fos expression and oxidative stress mechanisms**. By integrating findings from molecular neuroscience, neurophysiology, and nutritional research, this article seeks to provide a comprehensive overview of how dietary sweeteners may interact with hippocampal circuits that support learning and memory.

Artificial Sweeteners: Classification, Metabolism, and Brain Exposure

Artificial sweeteners, commonly referred to as non-nutritive sweeteners or high-intensity sweeteners, are compounds that provide sweetness far exceeding that of sucrose while contributing minimal or no caloric energy. Because of their strong sweetening capacity, only very small quantities are required to achieve the desired taste in foods and beverages. These compounds have therefore become widely used in dietary products intended for individuals seeking to reduce caloric intake or control blood glucose levels. Despite sharing the common feature of intense sweetness, artificial sweeteners differ substantially in their chemical structures, metabolic pathways, and physiological effects [7].

Artificial sweeteners can generally be classified into two broad groups based on their origin: **synthetic sweeteners** and **naturally derived non-nutritive sweeteners**. Synthetic sweeteners include compounds such as **aspartame, saccharin, sucralose, acesulfame potassium, neotame, and advantame**, which were developed through chemical modification to produce high sweetening potency. Naturally derived alternatives include **steviol glycosides**, extracted from the leaves of *Stevia rebaudiana*, which provide sweetness through plant-derived compounds with minimal caloric value. Each of these sweeteners interacts with sweet taste receptors but undergoes different metabolic processes after ingestion [8].

Among synthetic sweeteners, **aspartame** is one of the most extensively studied. Chemically, it is a methyl ester composed of two amino acids, aspartic acid and phenylalanine. After ingestion, aspartame is rapidly hydrolyzed in the gastrointestinal tract into its constituent components, which are absorbed and metabolized through normal physiological pathways. The metabolites include phenylalanine, aspartic acid, and methanol, each of which may influence metabolic or neural processes under certain conditions. Because of its widespread use in beverages and processed foods, aspartame has been a focus of numerous experimental studies investigating potential neurological effects [9].

Saccharin, one of the earliest artificial sweeteners introduced into the food supply, possesses a chemical structure unrelated to carbohydrates. Unlike aspartame, saccharin is not metabolized extensively in the body and is largely excreted unchanged through the kidneys. Similarly, **sucralose**, a chlorinated derivative of sucrose, is poorly absorbed from the gastrointestinal tract and is primarily eliminated without significant metabolic transformation. These differences in metabolism may influence how various sweeteners interact with physiological systems, including neural pathways [10].

Beyond systemic metabolism, an important question concerns the ability of artificial sweeteners or their metabolites to affect the **central nervous system**. Although most sweeteners are absorbed and metabolized outside the brain, certain metabolites may influence neural signaling either indirectly through systemic metabolic pathways or directly through interactions with neuronal receptors. For example, phenylalanine derived from aspartame metabolism can cross the **blood–brain barrier** via large neutral amino acid transporters. Once in the brain, elevated levels of phenylalanine may influence neurotransmitter synthesis, particularly the production of catecholamines and serotonin, which are involved in mood regulation and cognitive processes [11].

Another mechanism through which artificial sweeteners may influence neural activity involves **sweet taste receptors expressed outside the oral cavity**. Sweet taste receptors belonging to the T1R family have been identified in several tissues, including the gastrointestinal tract, pancreas, and certain brain regions. Activation of these receptors by artificial sweeteners may trigger signaling cascades that influence hormone secretion, metabolic regulation, and possibly neuronal activity. These findings suggest that artificial sweeteners may exert broader physiological effects beyond their role in taste perception [12].

Experimental research has also explored the possibility that chronic consumption of artificial sweeteners could affect brain chemistry through indirect metabolic pathways. Changes in gut microbiota composition, alterations in glucose metabolism, and modifications in insulin signaling have all been proposed as potential mechanisms linking dietary sweeteners to neural function. Because the hippocampus is highly sensitive to metabolic disturbances, such systemic changes may ultimately influence hippocampal neuronal activity and synaptic plasticity [13].

In addition to metabolic influences, certain artificial sweeteners have been investigated for their potential to induce oxidative stress in neural tissues. Experimental studies in animal models have reported increased levels of reactive oxygen species and alterations in antioxidant enzyme activity following prolonged exposure to some sweeteners, particularly aspartame. These biochemical changes may disrupt neuronal homeostasis and contribute to functional alterations within hippocampal circuits that are involved in learning and memory [14].

Taken together, artificial sweeteners represent a diverse group of compounds with distinct metabolic properties and biological effects. Although their primary role is to provide sweetness without caloric intake, growing evidence suggests that they may interact with metabolic and neural signaling pathways through multiple mechanisms. Understanding these metabolic and physiological processes is essential for evaluating how artificial sweeteners may influence hippocampal neuronal activity and molecular signaling pathways within the brain.

c-Fos as a Marker of Neuronal Activation in the Hippocampus

Understanding how external stimuli influence neuronal activity requires reliable molecular indicators that reflect rapid changes in neural signaling. Among these indicators, **immediate early genes (IEGs)** have become essential tools in neuroscience research. Immediate early genes are rapidly and transiently expressed in neurons in response to synaptic stimulation, allowing researchers to identify brain regions that have recently been activated by physiological, behavioral, or environmental stimuli. One of the most widely studied immediate early genes is **c-Fos**, which has become a standard molecular marker for detecting neuronal activation in experimental neuroscience studies [15].

The c-Fos protein is encoded by the **FOS gene**, which belongs to the family of proto-oncogenes involved in regulating gene transcription. When neurons are stimulated, intracellular signaling cascades triggered by neurotransmitter receptors lead to rapid activation of transcription factors that induce c-Fos expression. This process occurs within minutes following neuronal activation, and c-Fos protein levels typically peak within one to three hours after stimulation. Because of this rapid and transient expression pattern, c-Fos serves as a valuable indicator of recent neuronal activity within specific brain regions [16].

Once expressed, the c-Fos protein forms a heterodimer with members of the Jun family to create the **activator protein-1 (AP-1) transcription factor complex**. This complex regulates the transcription of multiple downstream genes involved in cellular processes such as synaptic plasticity, neuronal growth, and metabolic adaptation. Through this mechanism, c-Fos not only functions as a marker of neuronal activation but also participates in the molecular events that support long-term neuronal adaptation and plasticity [17].

Within the hippocampus, c-Fos expression has been widely used to map neuronal activity associated with learning and memory. Experimental studies have demonstrated that hippocampal neurons exhibit increased c-Fos expression following tasks that require spatial navigation, contextual learning, or memory retrieval. These patterns of activation are particularly prominent in hippocampal subfields such as **CA1, CA3, and the dentate gyrus**, which are critical components of the hippocampal memory circuit. The ability to visualize c-Fos expression therefore provides important insight into how hippocampal networks respond to cognitive and environmental stimuli [18].

In addition to behavioral activation, c-Fos expression in the hippocampus can also be influenced by metabolic and physiological factors. Conditions that alter neurotransmitter signaling, synaptic activity, or cellular stress may lead to changes in c-Fos expression patterns. For example, exposure to stress, changes in dietary composition, and pharmacological agents have all been shown to modify c-Fos activation in hippocampal neurons. These findings suggest that c-Fos expression reflects not only cognitive processing but also the broader physiological state of neural circuits [19].

Importantly, the hippocampus is particularly sensitive to metabolic disturbances and oxidative stress, factors that can influence neuronal activity and gene expression. Experimental studies have demonstrated that oxidative imbalance and mitochondrial dysfunction can alter signaling pathways involved in synaptic transmission and neuronal activation. Such disturbances may ultimately affect the expression of immediate early genes, including c-Fos, thereby modifying activity patterns within hippocampal circuits that support memory and learning [20].

Because of these properties, c-Fos has become a valuable experimental tool for investigating how external factors—including dietary components—affect hippocampal neuronal activity. By measuring c-Fos expression in hippocampal neurons, researchers can assess how various stimuli influence neural activation patterns within memory-related circuits. This approach has been applied in studies examining the neurological effects of dietary sugars, metabolic disorders, and artificial sweeteners, providing insight into how nutritional exposures may alter hippocampal neuronal signaling [21].

However, it is important to recognize that c-Fos expression represents an indirect indicator of neuronal activation rather than a direct measure of neuronal firing. While increased c-Fos expression generally reflects increased synaptic activity, the relationship between gene expression and electrophysiological activity can be influenced by multiple factors including stimulus intensity,

duration, and cellular signaling pathways. Therefore, c-Fos data are often interpreted alongside electrophysiological recordings or behavioral assessments to provide a more comprehensive understanding of neural circuit function [22].

In summary, c-Fos is a critical molecular marker that allows researchers to visualize and quantify neuronal activation within the hippocampus. Through its role as both a transcription factor and an indicator of neuronal activity, c-Fos provides valuable insight into how hippocampal circuits respond to environmental, metabolic, and behavioral stimuli. Investigating changes in c-Fos expression therefore offers an important approach for understanding how dietary exposures, including artificial sweeteners, may influence neuronal signaling and functional organization within hippocampal memory networks.

Mechanisms of Artificial Sweetener–Induced Neuronal Activation

Artificial sweeteners may influence neuronal activity through several interconnected biological mechanisms that affect neurotransmission, metabolic signaling, and cellular stress responses. Although these compounds are primarily used for their sweetening properties, experimental evidence suggests that certain artificial sweeteners can modify neural signaling pathways in the central nervous system. Because the hippocampus is highly sensitive to metabolic and neurochemical changes, it represents a key region where such effects may be observed [23].

One of the proposed mechanisms involves **modulation of neurotransmitter systems**. Some artificial sweeteners, particularly aspartame, are metabolized into components that can influence neurotransmitter synthesis. Aspartame hydrolysis produces phenylalanine and aspartic acid, both of which are amino acids capable of affecting neural signaling. Phenylalanine can cross the blood–brain barrier through large neutral amino acid transporters and may influence the synthesis of catecholamines such as dopamine and norepinephrine. Elevated levels of phenylalanine in the brain may also interfere with the transport of other amino acids involved in neurotransmitter production, potentially altering neural activity within hippocampal circuits [24].

Aspartic acid, another metabolite of aspartame, functions as an excitatory amino acid that can activate glutamatergic receptors. Glutamate-mediated signaling plays a central role in synaptic plasticity within the hippocampus, particularly in processes such as long-term potentiation. Excessive stimulation of excitatory receptors, however, may lead to increased neuronal excitability and cellular stress, potentially influencing activity-dependent gene expression including immediate early genes such as c-Fos [25].

Another pathway through which artificial sweeteners may influence neuronal activation involves **metabolic and hormonal signaling**. Sweet taste receptors are expressed not only in the oral cavity but also in other tissues including the gastrointestinal tract and pancreas. Activation of these receptors by artificial sweeteners can stimulate signaling pathways that influence hormone secretion, including insulin and incretin hormones. These systemic metabolic signals may indirectly affect brain function by altering glucose availability, energy metabolism, and neuroendocrine regulation, factors that are known to influence hippocampal neuronal activity [26].

In addition to metabolic effects, artificial sweeteners have been investigated for their potential to induce **oxidative stress** within neural tissues. Oxidative stress occurs when the production of reactive oxygen species exceeds the capacity of antioxidant defense systems, leading to cellular damage and disruption of normal physiological processes. Several experimental studies have demonstrated increased levels of lipid peroxidation and altered antioxidant enzyme activity in the brains of animals exposed to certain artificial sweeteners, particularly after prolonged administration. Because hippocampal neurons are especially vulnerable to oxidative damage, these biochemical changes may influence neuronal signaling and gene expression [27].

Oxidative stress can activate multiple intracellular signaling pathways that regulate gene transcription. Among these pathways are the mitogen-activated protein kinase (MAPK) cascades and other stress-responsive signaling systems that influence the expression of immediate early genes. Activation of these signaling pathways may contribute to increased c-Fos expression in neurons exposed to metabolic or oxidative stress, thereby linking biochemical disturbances with observable changes in neuronal activation patterns [28].

Another possible mechanism involves alterations in **mitochondrial function**. Mitochondria play a critical role in neuronal energy production and regulation of oxidative balance. Disruption of mitochondrial activity can impair ATP generation and increase the production of reactive oxygen species, ultimately affecting neuronal survival and synaptic transmission. Some experimental investigations have suggested that exposure to artificial sweeteners may influence mitochondrial enzyme activity and redox balance in brain tissues, which may in turn affect neuronal excitability and gene expression in hippocampal neurons [29].

Artificial sweeteners may also influence neuronal activity indirectly through **changes in gut–brain communication**. The gut microbiota plays an important role in metabolic regulation and can influence neural signaling through the production of metabolites, immune mediators, and neuroactive compounds. Alterations in microbial composition induced by artificial sweeteners have been reported in experimental studies and may affect systemic metabolism and inflammatory signaling. Because the hippocampus is sensitive to inflammatory mediators and metabolic disturbances, changes in gut microbiota may represent another pathway linking dietary sweeteners to neural activity [30].

Taken together, these mechanisms suggest that artificial sweeteners may influence hippocampal neuronal activity through a combination of neurochemical, metabolic, and oxidative pathways. Modulation of neurotransmitter systems, alterations in metabolic signaling, induction of oxidative stress, mitochondrial dysfunction, and changes in gut–brain interactions may all contribute to changes in neuronal activation patterns. These processes may ultimately influence the expression of activity-dependent genes such as c-Fos within hippocampal circuits involved in learning and memory.

Understanding these mechanisms is essential for interpreting experimental findings that link artificial sweetener exposure with changes in hippocampal neuronal activation. Further investigation is required to clarify how these molecular pathways interact and whether they translate into functional alterations in cognitive performance and memory processing.

Oxidative Stress and Neuroinflammatory Pathways in the Hippocampus

Oxidative stress represents one of the most important cellular mechanisms capable of disrupting neuronal function within the hippocampus. Neurons require substantial amounts of energy to maintain membrane potentials, support synaptic transmission, and sustain plasticity mechanisms associated with learning and memory. Because of this high metabolic activity, hippocampal neurons produce significant quantities of reactive oxygen species as byproducts of mitochondrial respiration. Under physiological conditions, endogenous antioxidant systems—including enzymes such as superoxide dismutase, catalase, and glutathione peroxidase—maintain redox balance and protect neural tissue from oxidative damage. However, when the production of reactive oxygen species exceeds the capacity of antioxidant defenses, oxidative stress can occur, leading to structural and functional alterations in neurons [31].

The hippocampus is particularly vulnerable to oxidative stress compared with many other brain regions. This vulnerability is related to several factors, including its high oxygen consumption, dense concentration of polyunsaturated fatty acids within neuronal membranes, and relatively limited antioxidant capacity in certain neuronal populations. Excessive reactive oxygen species can damage cellular components such as lipids, proteins, and nucleic acids, ultimately impairing neuronal signaling and synaptic function. These processes may contribute to disruptions in hippocampal plasticity and cognitive performance [32].

One of the key cellular targets of oxidative stress is the **mitochondrial system**, which plays a central role in neuronal energy production. Mitochondria generate adenosine triphosphate through oxidative phosphorylation, a process that inevitably produces reactive oxygen species as metabolic byproducts. When oxidative stress increases, mitochondrial function may become impaired, leading to reduced energy production and further accumulation of reactive oxygen species. This cycle of mitochondrial dysfunction and oxidative stress can compromise neuronal viability and disrupt synaptic transmission within hippocampal circuits [33].

Oxidative stress is also closely linked with **neuroinflammatory responses** within the brain. Reactive oxygen species can activate microglial cells and astrocytes, the primary immune-related cells of the central nervous system. Once activated, these cells release inflammatory mediators such as cytokines, chemokines, and additional reactive oxygen species. Although neuroinflammatory responses may initially serve protective functions, chronic activation can lead to sustained inflammatory signaling that contributes to neuronal injury and synaptic dysfunction [34].

Within the hippocampus, neuroinflammatory processes can influence several aspects of neuronal function, including synaptic plasticity, neurotransmitter release, and neuronal survival. Inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-1β have been shown to alter synaptic transmission and impair long-term potentiation, a key mechanism underlying memory formation. Persistent neuroinflammation may therefore interfere with hippocampal circuits involved in learning and memory processes [35].

Oxidative stress and neuroinflammation are also capable of activating intracellular signaling pathways that regulate gene expression in neurons. Among these pathways are stress-responsive transcription factors and kinase cascades that influence the

expression of immediate early genes such as **c-Fos**. Increased oxidative stress can stimulate signaling pathways including mitogen-activated protein kinases and nuclear transcription factors that promote c-Fos expression in neurons experiencing metabolic or inflammatory stress. Consequently, altered c-Fos expression may reflect not only synaptic activation but also cellular responses to oxidative and inflammatory stimuli [36].

Several experimental studies have investigated the relationship between artificial sweetener exposure and oxidative stress in the brain. Chronic administration of certain sweeteners has been associated with increased lipid peroxidation, reduced antioxidant enzyme activity, and structural alterations in neural tissues in animal models. Because hippocampal neurons are particularly sensitive to oxidative damage, these biochemical changes may influence neuronal signaling and gene expression patterns within hippocampal memory circuits [37].

Importantly, oxidative stress may interact with other mechanisms that influence hippocampal neuronal activation. For example, mitochondrial dysfunction may alter calcium homeostasis and neurotransmitter release, further modifying neuronal excitability. Similarly, inflammatory mediators released by activated glial cells can affect synaptic signaling and plasticity within hippocampal networks. These interconnected pathways highlight the complex relationship between metabolic disturbances, oxidative stress, and neuronal activation within the hippocampus [38].

In summary, oxidative stress and neuroinflammatory processes represent key mechanisms that can disrupt hippocampal neuronal function. Through effects on mitochondrial activity, synaptic signaling, and gene expression pathways—including those regulating c-Fos—these processes may contribute to altered neuronal activation patterns within hippocampal circuits. Understanding the interaction between oxidative stress and neuronal signaling is therefore essential for interpreting how dietary exposures, including artificial sweeteners, may influence hippocampal physiology and cognitive function.

Experimental Evidence of Artificial Sweetener Effects on Hippocampal c-Fos Expression

Experimental studies investigating the neurological effects of artificial sweeteners have increasingly focused on neuronal activation markers to better understand how these dietary compounds influence brain function. Among these markers, **c-Fos expression** has become a widely used tool for mapping neuronal activation in response to physiological, behavioral, and metabolic stimuli. Because c-Fos expression reflects recent neuronal activity, measuring its distribution within the hippocampus provides valuable insight into how dietary exposures may alter hippocampal circuit function and memory-related processing [39].

Animal studies have played a central role in exploring these relationships. Rodent models are frequently used because they allow controlled administration of artificial sweeteners and detailed examination of neural tissues using immunohistochemical techniques. In these experiments, animals are typically exposed to artificial sweeteners through drinking water or diet for defined periods, after which brain tissue is analyzed to detect c-Fos expression within specific hippocampal regions. Changes in c-Fos expression patterns may indicate alterations in neuronal activation induced by the dietary intervention [40].

Several studies have reported that exposure to certain artificial sweeteners can modify neuronal activity in brain regions associated with cognition and reward processing. In particular, aspartame exposure has been associated with behavioral and neurochemical changes in experimental animals, including alterations in locomotor activity, anxiety-like behavior, and learning performance. Histological analyses from these experiments have demonstrated modifications in neuronal signaling within the hippocampus and related limbic structures, suggesting that artificial sweeteners may influence neural circuits involved in memory and emotional regulation [41].

In addition to behavioral studies, molecular investigations have examined the effects of artificial sweeteners on immediate early gene expression in the brain. Alterations in c-Fos expression have been observed in hippocampal subfields following exposure to metabolic stressors or neurochemical modulators, indicating that these genes respond to changes in neuronal signaling pathways. Experimental models evaluating dietary exposures have therefore used c-Fos immunostaining to determine whether artificial sweeteners alter activation patterns within hippocampal neurons [42].

Some studies have suggested that artificial sweetener exposure may increase markers of neuronal stress and oxidative damage in hippocampal tissue. These biochemical alterations may influence neuronal excitability and synaptic signaling, potentially leading to changes in activity-dependent gene expression such as c-Fos. Because the hippocampus plays a critical role in spatial learning and contextual memory, disturbances in neuronal activation patterns may contribute to cognitive changes observed in

certain experimental models [43].

Behavioral testing has also been incorporated into experimental designs examining artificial sweetener exposure. Tasks such as the **Morris water maze**, radial arm maze, and object recognition tests are commonly used to evaluate hippocampal-dependent learning and memory in rodents. Alterations in performance during these tasks have been reported in some studies involving dietary sweetener exposure, suggesting possible functional consequences of changes in hippocampal neuronal activity. When combined with c-Fos mapping techniques, these behavioral assessments provide a comprehensive approach for linking molecular changes with cognitive outcomes [44].

Another important aspect of experimental research involves evaluating the distribution of c-Fos expression across different hippocampal subfields. The dentate gyrus, CA3, and CA1 regions each play distinct roles in memory processing and synaptic integration. Changes in c-Fos expression within these subregions may therefore reflect alterations in specific components of hippocampal circuitry. For example, increased c-Fos expression in the dentate gyrus may indicate enhanced neuronal activation associated with pattern separation, whereas changes in CA1 activity may reflect modifications in memory retrieval or information transfer to cortical areas [45].

Despite these findings, it is important to recognize that results from experimental studies are not always consistent. Differences in study design, dosage, duration of exposure, and animal species can influence outcomes and complicate interpretation. Some investigations have reported minimal or no significant effects of artificial sweeteners on neuronal activity or cognitive performance. These discrepancies highlight the need for further well-controlled studies to clarify the relationship between artificial sweetener consumption and hippocampal neuronal activation [46].

In summary, experimental evidence suggests that artificial sweeteners may influence hippocampal neuronal activity under certain conditions. Studies utilizing behavioral testing and molecular markers such as c-Fos provide valuable insights into how dietary exposures can modify neural circuits involved in memory and cognition. However, the complexity of these interactions and variability among experimental findings emphasize the importance of continued research to determine the precise mechanisms through which artificial sweeteners may affect hippocampal neuronal function.

Implications for Hippocampal Plasticity and Cognitive Function

The hippocampus plays a fundamental role in learning and memory through its capacity for synaptic plasticity, a process that allows neuronal circuits to modify their strength and connectivity in response to experience. Mechanisms such as **long-term potentiation (LTP)** and **long-term depression (LTD)** represent key physiological processes underlying hippocampal plasticity. These processes involve activity-dependent changes in synaptic efficacy, dendritic spine remodeling, and alterations in gene expression that ultimately contribute to the formation and stabilization of memory traces. Because immediate early genes such as **c-Fos** participate in transcriptional regulation following neuronal activation, they are closely linked to the molecular events that support synaptic plasticity in hippocampal networks [47].

Activation of c-Fos within hippocampal neurons reflects not only transient neuronal activity but also the initiation of intracellular signaling pathways that influence long-term neuronal adaptation. Through the formation of the AP-1 transcription factor complex, c-Fos regulates the expression of multiple downstream genes involved in synaptic remodeling, neuronal growth, and metabolic regulation. Consequently, changes in c-Fos expression patterns may signal alterations in the cellular processes that maintain hippocampal plasticity and support memory consolidation [48].

Experimental studies examining metabolic and dietary influences on hippocampal function have demonstrated that disturbances in neuronal signaling pathways may impair plasticity mechanisms. Oxidative stress, mitochondrial dysfunction, and inflammatory signaling can disrupt synaptic transmission and interfere with the induction of long-term potentiation. Because these processes influence neuronal excitability and intracellular signaling pathways, they may ultimately affect the expression of activity-dependent genes such as c-Fos and modify neuronal activation patterns within hippocampal circuits [49].

Alterations in hippocampal plasticity may have significant consequences for cognitive function. The dentate gyrus, CA3, and CA1 subfields operate as integrated components of a memory-processing network that enables pattern separation, pattern completion, and memory retrieval. Disturbances in neuronal activity within any of these regions can affect the efficiency of information processing within the hippocampus. Changes in activity-dependent gene expression, including c-Fos activation, may therefore reflect underlying disruptions in the neural circuitry responsible for learning and memory [50].

Animal studies examining dietary exposures have provided additional insights into the relationship between metabolic factors and hippocampal plasticity. In some experimental models, prolonged exposure to certain dietary components has been associated with impaired spatial learning, reduced synaptic plasticity, and alterations in hippocampal neuronal signaling. These findings suggest that metabolic disturbances may influence the molecular pathways that regulate neuronal activation and memory-related processes in the hippocampus [51].

Another important factor influencing hippocampal plasticity is **adult neurogenesis**, which occurs primarily in the subgranular zone of the dentate gyrus. Newly generated neurons integrate into existing hippocampal circuits and contribute to learning, memory formation, and cognitive flexibility. Environmental conditions such as physical activity, enriched environments, and metabolic status can significantly influence the rate of hippocampal neurogenesis. Disturbances in metabolic homeostasis or oxidative balance may therefore affect both neuronal activation and the generation of new neurons within hippocampal networks [52].

Importantly, the relationship between dietary exposures and hippocampal plasticity is complex and influenced by multiple interacting factors. Nutritional composition, metabolic health, oxidative stress, and systemic inflammatory responses may all contribute to alterations in neuronal signaling pathways. Changes in c-Fos expression patterns within hippocampal neurons may therefore represent one component of a broader network of molecular responses that reflect how environmental and metabolic conditions influence brain function [53].

From a translational perspective, understanding the molecular mechanisms that link dietary exposures to hippocampal neuronal activation may have important implications for neurological health. Because the hippocampus is critically involved in cognitive performance and is particularly vulnerable to metabolic disturbances, identifying factors that influence hippocampal plasticity may help clarify how lifestyle and dietary habits contribute to cognitive outcomes across the lifespan. Continued investigation into the molecular pathways regulating neuronal activation—including immediate early gene signaling and oxidative stress mechanisms—may therefore provide valuable insight into the complex relationship between nutrition and brain function [54].

In summary, hippocampal plasticity depends on coordinated interactions between neuronal signaling pathways, gene expression mechanisms, and metabolic conditions. Activity-dependent genes such as c-Fos serve as key indicators of neuronal activation and may participate in the molecular processes that support memory formation. Alterations in these signaling pathways, whether induced by metabolic disturbances or environmental exposures, may influence hippocampal circuit function and cognitive performance. Further research is needed to better understand how dietary factors interact with neuronal signaling mechanisms to shape hippocampal plasticity and brain health.

Conclusion

The hippocampus represents a central structure in the neural circuitry underlying learning, memory formation, and spatial cognition. Its highly organized architecture and dynamic synaptic networks enable the integration and processing of complex sensory and contextual information. Because hippocampal neurons exhibit high metabolic activity and rely on tightly regulated signaling pathways, they are particularly sensitive to metabolic disturbances, oxidative stress, and environmental influences. Understanding the factors that modify hippocampal neuronal activation is therefore essential for interpreting how external exposures may affect cognitive function.

Immediate early genes, particularly **c-Fos**, provide valuable insight into neuronal activation within hippocampal circuits. As a rapidly expressed transcription factor, c-Fos serves as a molecular indicator of neuronal activity and participates in the regulation of downstream genes involved in synaptic plasticity, neuronal adaptation, and memory-related processes. Changes in c-Fos expression within hippocampal neurons therefore reflect alterations in cellular signaling pathways that may influence synaptic strength and network connectivity.

Evidence from experimental studies suggests that artificial sweeteners may interact with neural signaling pathways through multiple mechanisms. These mechanisms include modulation of neurotransmitter systems, alterations in metabolic and hormonal signaling, induction of oxidative stress, and activation of intracellular pathways that regulate gene expression. Because hippocampal neurons are highly susceptible to oxidative imbalance and metabolic disruption, such changes may influence neuronal activation patterns and activity-dependent gene expression within hippocampal memory circuits.

Oxidative stress and neuroinflammatory responses appear to represent particularly important mechanisms linking metabolic

exposures with neuronal signaling changes in the hippocampus. Disruption of mitochondrial function and increased production of reactive oxygen species can impair synaptic transmission, alter intracellular signaling pathways, and influence transcriptional responses such as c-Fos activation. These molecular alterations may contribute to changes in hippocampal plasticity and may potentially affect cognitive performance under certain experimental conditions.

Experimental research using animal models has provided useful insights into these processes by combining behavioral testing with molecular markers of neuronal activation. Observations of altered c-Fos expression patterns in hippocampal regions following exposure to dietary sweeteners suggest that metabolic and nutritional factors can influence neuronal signaling within memory-related circuits. However, the complexity of these interactions and variability among experimental findings highlight the need for further investigation to clarify the precise mechanisms involved.

Overall, current evidence indicates that artificial sweeteners may influence hippocampal neuronal activity through a combination of metabolic, oxidative, and molecular signaling pathways. The use of c-Fos as a marker of neuronal activation has provided an important tool for identifying these changes and for mapping how dietary exposures interact with hippocampal circuits. Continued research integrating molecular neuroscience, nutritional science, and behavioral studies will be necessary to better understand how dietary factors affect hippocampal function and cognitive health.

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