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REVIEW

CHROMOTHERAPY AS A MODULATOR OF ZEBRAFISH BEHAVIOR: A CONCEPTUAL REVIEW

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Abstract: *Chromotherapy, the therapeutic use of colored light, has been shown to influence physiological and behavioral processes, including stress modulation, learning, and memory. Zebrafish (Danio rerio), with their tetrachromatic vision, rapid development, and well-characterized behavioral repertoire, provide an ideal model for studying the effects of color exposure. Innate color preferences in zebrafish, particularly for red and green, significantly affect exploratory behavior, associative learning, and cognitive flexibility. Experimental approaches such as T-maze and Y-maze assays allow the assessment of these preferences and their impact on learning and memory. Overall, zebrafish studies highlight the potential of chromotherapy as a non-invasive tool to modulate behavior and cognitive function, offering insights relevant for broader vertebrate models and translational research.*

Keywords: Chromotherapy; Zebrafish; Color preference; Learning and memory; Behavioral modulation; T-maze; Neurobehavioral research

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1. INTRODUCTION

Nature exerts a profound influence on human physiology, and colors play a crucial role in shaping perception, emotion, and overall behavior [1]. Chromotherapy, or color therapy, utilizes both visible and invisible wavelengths to support health, as specific regions of the body absorb distinct colors and imbalances in these wavelengths can lead to physiological disturbances ([1,2]. Exposure to natural or artificial light can restore this balance, with each color generating electrical impulses and energy that stimulate biochemical and hormonal processes involved in healing [1,3].

Color functions as an essential environmental variable affecting both physiological and behavioral responses in humans and animals, influencing emotional, cognitive, and social processes through subtle visual cues [4]. Variations in facial coloration, such as increased redness or yellowness, have been linked to emotional states including anger, happiness, and surprise, whereas paler tones often correlate with fear, disgust, or sadness [4,5]. Moreover, exposure to red light prior to a painful stimulus increases perceived pain intensity compared to cooler hues like green or blue, highlighting the direct influence of color on sensory perception [6].

The interaction between color and behavior is bidirectional, as colors can modulate cognitive and behavioral processes while behavioral states can influence color perception [7]. Pleasant or calming colors, such as blue, tend to evoke positive memories, while less appealing colors like brown are associated with negative cognitions, and warm colors may enhance alertness whereas cooler shades like blue and green generally reduce stress and anxiety [8]. Colors can also influence other neural processes, including subjective perception of pain, demonstrating their broad impact on brain function [6].

In animals, color perception is critical for detecting food, predators, mates, and suitable shelters, and in zebrafish, color cues play a significant role in learning and memory formation. Zebrafish behavior has been widely used to assess neurotoxic effects of drugs and chemicals, employing tasks such as T-maze, Y-maze, and cross-maze to test responses to visual and color stimuli, and their neural mechanisms for color recognition are evolutionarily conserved [9]. Zebrafish possess four types of cones: UV, short-, medium-, and long-wavelength, allowing perception of a broad range of colors, and larvae begin to respond to light around 3.5 days post-fertilization, distinguishing colors by day five [9,10].

Innate color preferences can influence learning, with effects depending on both the type of task and the animal's evolutionary or developmental background, as animals often detect colors more effectively when they contrast with their environment [11]. Strong innate color biases may limit cognitive flexibility and hinder reversal learning, and studies in zebrafish raised in different environments suggest that these preferences affect associative and reversal learning abilities [12]. Given the strong influence of color on emotional and physiological states, chromotherapy has potential as a behavioral modulator, and zebrafish, with visual and stress systems similar to humans, provide a valuable model for investigating how exposure to specific wavelengths can influence behavior and promote emotional balance [4].

2. THE ZEBRAFISH MODEL FOR STUDYING CHROMOTHERAPY

Nature exerts a profound influence on human physiology, with colors playing a key role in shaping perception, emotion, and behavior. Chromotherapy, or color therapy, uses visible and invisible wavelengths of light to restore physiological balance, as different body regions absorb specific colors, and imbalances may lead to health disturbances. Exposure to light stimulates electrical impulses and biochemical and hormonal processes that support healing [1]. Colors function as crucial environmental cues influencing emotional, cognitive, and social responses; in humans, changes in facial coloration such as increased redness or yellowness reflect emotional states like anger, happiness, or surprise, while paler tones indicate fear or sadness. Additionally, exposure to specific colors modulates sensory perception, for example, red light enhances pain perception compared to cooler hues like green or blue [4].

The bidirectional relationship between color and behavior extends to cognitive processes: pleasant colors like blue evoke positive memories, whereas less appealing colors like brown are linked to negative cognition; warm colors may increase alertness, while cooler shades generally reduce stress and anxiety [7]. Colors also influence neural processes, including subjective pain perception, illustrating their broad impact on the brain. In animals, color perception is essential for detecting food, predators, mates, and shelter, and in zebrafish, it plays a major role in learning and memory formation.

Zebrafish are widely used in behavioral research due to their small size, transparency, fully sequenced genome, and ease of maintenance [13]. They are employed to study associative and reversal learning, visual discrimination, and color preference using assays such as T-maze and Y-maze tasks, and their innate color biases can significantly influence learning outcomes [12]. Research indicates that animals detect colors more effectively when they contrast with their environment, and strong innate color biases may reduce cognitive flexibility, affecting reversal learning. Studies comparing wild-caught and domesticated zebrafish populations show that preference differences for low-opponency colors (blue vs green) and high-opponency colors (red vs green) modulate learning, with green resembling the natural habitat and red resembling food stimuli [12,14].

Light exposure, whether natural sunlight or artificial sources like LEDs, plays a critical role in zebrafish development, regulating growth hormone, feeding, survival, and retinal formation, but excessive or prolonged light can cause stress, photoreceptor damage, immune impairment, altered behavior, and reduced appetite [15]. Different LED wavelengths can also lead to oxidative stress, tissue damage, altered locomotion, apoptosis, and changes in metabolic pathways, effects exacerbated by even slight temperature increases [16].

Although zebrafish lack a hippocampus, they are capable of forming visual associative memories, and pairing preferred colors with rewards can strengthen long-term memory, enhancing their utility as a model for studying cognitive function [17]. Preferred colors influence learning and memory, particularly in tasks involving visual discrimination, as shown by place preference tests where color choice modulates performance [18].

Given the strong effects of color on emotional and physiological states, chromotherapy presents a promising approach to modulate behavior. Zebrafish, with visual and stress systems analogous to humans, provide an ideal model to investigate how specific wavelengths influence behavior, learning, and cognitive performance [4]. Understanding innate color preferences and their interaction with environmental stimuli enhances the use of zebrafish as a translational model for studying neurobehavioral processes and the effects of light-based interventions (**Figure 1**) [9,14].

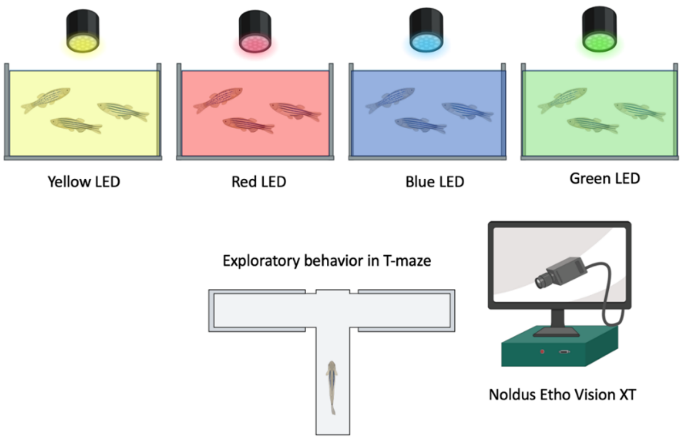

Figure 1. Schematic representation of a theoretical setup for studying zebrafish responses to colored light. The diagram illustrates exposure to four LED lights (yellow, red, blue, and green) and behavioral recording using the EthoVision XT tracking system (Noldus) to analyze locomotor activity.

3. COLOR PREFERENCE IN ZEBRAFISH

The T-maze color preference test in zebrafish involves placing individuals at the base of a T-shaped maze and allowing them to choose between two differently colored arms, with the time spent in each arm reflecting innate or learned color preferences (**Figure 2**) [14]. Studies have shown that zebrafish display strong and consistent preferences for certain colors, particularly red and green, which can significantly influence their performance in associative learning tasks, motivation to approach social rewards, and flexibility during reversal-learning trials. Notably, these preferences vary across wild and domesticated populations, emphasizing the role of environmental and genetic background in shaping innate biases [12].

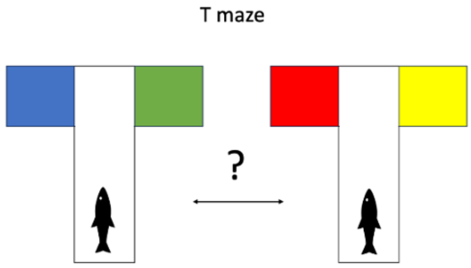

Figure 2. T-Maze Assay for Evaluating Color Preference in Zebrafish

Behavioral testing in maze setups demonstrates that very young zebrafish naturally gravitate toward blue-tinted zones, followed by red and green, whereas yellow consistently attracts them the least [19]. Other studies report that in T-maze assays, zebrafish generally avoid blue while showing similar preferences for red and green, both of which are favored over yellow. These findings highlight that natural color preferences can affect learning and memory outcomes, and should therefore be considered when designing color-based behavioral experiments [14].

Research also indicates that zebrafish exhibit clear innate color preferences that shape exploratory and learning behaviors across different assays. Adults tend to spend more time in blue-colored zones compared to red or yellow, suggesting a natural attraction to cooler hues, although place-preference and T-maze tests often show a general avoidance of blue in favor of red and green, both consistently preferred over yellow. These patterns appear stable across sexes and underline the importance of accounting for innate color biases in behavioral testing, particularly when assessing learning and memory [19].

Sex-specific differences in color preference have also been documented. While zebrafish do not exhibit strong directional biases in maze tasks, females consistently prefer red over blue, yellow, or white, whereas males often show no significant color bias. In contrast, both sexes display a strong preference for black when presented with black and white zones in a T-maze. These inherent preferences are critical to consider in the design and interpretation of behavioral assays, as they can influence learning outcomes and exploratory patterns [20,21].

4. CHROMOTHERAPY AS AN ADJUVANT TREATMENT

Chromotherapy is an ancient healing method that employs the visible spectrum of electromagnetic radiation to modulate physiological and psychological functions [3]. It is based on the principle that different colors can influence the body's energy balance and facilitate recovery, although the precise mechanisms remain unclear. Proposed explanations include photobiological interactions and possibly quantum-level effects, such as the influence of light on the dipole moment of water molecules and the distribution of charges within tissues. Investigating these effects through modern biophysical and electromagnetic research could provide a scientific basis for chromotherapy and support its role as a complementary therapeutic approach [22].

Different light frequencies produce the colors of the visible spectrum, which may affect physiological processes by influencing the internal structure of water, the main component of the human body. Some researchers suggest that water can retain structural "information" through the specific arrangement of its molecules. Chromotherapy is thought to act on neurohormonal pathways, particularly those involving melatonin and serotonin in the brain. Given that the visible spectrum ranges from approximately 400 to 700 nm and that other electromagnetic waves are known to impact human biology, it is plausible that perceived colors can similarly influence health [23].

Chromotherapy has been applied to alleviate various physiological and psychological conditions, including anxiety disorders, which impose substantial individual and societal costs and are a leading cause of disability [24]. Different organs are associated with specific colors that can elicit targeted physiological and psychological effects. For instance, low-energy colors such as blue or violet can help manage migraines induced by stress, hypotension, or reduced blood volume during pregnancy or menstruation. Chromotherapy has also been shown to reduce oxidative stress associated with hyperglycemia and enhance the body's antioxidant defenses. In clinical settings, bright colors in the environment have been used to reduce stress in stroke patients, while specific wavelengths of light influence enzymatic activity, such as glucose oxidase, cholesterol oxidase, and superoxide dismutase. Red light generally has stimulating effects, whereas blue light has calming properties, useful in cases like muscle spasms [1,25].

From a psychological perspective, color exposure plays an important role in mental health. Chromotherapy has been combined with techniques such as Eye Movement Desensitization and Reprocessing (EMDR) to support patients with Post-Traumatic Stress Disorder (PTSD), targeting brain regions like the amygdala and hippocampus to reduce distressing memories and emotional responses [1].

CONCLUSIONS

Chromotherapy, through exposure to specific colors of light, can influence physiological and behavioral processes, including stress responses, learning, and memory. Zebrafish, with their tetrachromatic vision and well-characterized behavior, provide an ideal model to study these effects. Innate color preferences in zebrafish, such as attraction to red and green, significantly impact exploratory behavior and cognitive performance, emphasizing the importance of considering natural biases in experimental design. Overall, chromotherapy offers a promising approach to modulate behavior and cognitive function in zebrafish, providing insights for potential applications in other vertebrates.

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