The massed-spaced learning effect in non-neural human cells

I. Core Energetic and Informational Dynamics

I.1. Primary Energy Transformation & Efficiency

The primary energy transformations involve the chemical energy within the forskolin and TPA molecules, which are used to initiate signaling cascades within the cells. Forskolin activates adenylyl cyclase, leading to increased cAMP levels and consequently PKA activation, while TPA directly activates PKC. These protein kinases phosphorylate downstream targets, including ERK and CREB, ultimately leading to increased transcription of the luciferase reporter gene. The cellular energy for transcription is derived from ATP. The paper itself does not explicitly analyze the energetic implications or efficiency of these steps. However, the system is not energy efficient for material implementation as the energy source is from an external medium via chemical energy of applied stimuli that is not produced by the material itself. There is no inherent feedback loop that reduces the quantity of energy to achieve the same result over time or in function of its own history or a self-contained system as the external source needs continuous feeding.

I.2. Material-Based Memory: Encoding, Storage, and Dynamics

The material basis of memory, in the context of this paper, resides in the sustained changes in the phosphorylation state of CREB and ERK, as well as the levels of total CREB protein, and the resulting elevated expression of the luciferase reporter. These are not inherent within a material itself, but relies on a cascade of biochemical changes within cells, which are metabolic responses of living systems and depend on a cellular homeostasis that is external to material design principle. These protein modifications, while exhibiting some persistence, are still temporal changes in a biological system and do not form a long term *material-based* storage. The system also employs a PEST-tagged unstable luciferase protein, which degrades rapidly, and that is used as a molecular tool that is not necessary for a physical material implementation, but rather to perform time dependent measurement of transcription. This reporter system is designed as a transient proxy for CRE-dependent transcription and does not constitute a material memory element in the sense of an autonomous stable feature embedded in the material structure. The paper notes that differences are accentuated at 24 hours due to changes in CRE promoter activity which suggests a form of long term memory of transcription induction, but that is not a “physical” or material storage of information, but a response of dynamic self regulation (via its homeostatic mechanism) of a biological system. The mechanism is transient phosphorylation, which is not a typical non-volatile state, and the PEST tag ensures that the protein product itself cannot act as a long-term memory medium. The observed “memory” is thus a dynamic pattern within a cascade of biological events, not an intrinsic property of the utilized material itself. The memory is implemented by self regulation of biological homeostatic loops and not an intrinsic “material” response.

I.3. Local, Non-linear Computation and Information Processing

The paper does not provide solid evidence for local material-based computation. Although PKA, PKC, ERK, and CREB are known to contribute to signal processing, the described experiments do not demonstrate these components functioning as local computational units in the sense of processing *material* based signals or implementing *material* based logic operations that goes above of linear responses. The cells process information but that is not a property of a material, but rather their own biological metabolic functions. The interactions among those are likely complex and non linear, but this is not the implementation of computation within a designed material structure, but the cell biochemical pathway which process molecules.The paper shows how the timing of the pulses affects signal integration, but this processing is taking place in a biochemical cascade and requires energy from cellular metabolism. This is not a computation in the sense of embodied physical computation using material properties. There is no evidence in this paper that cells or their components function as logic gates; the study does not demonstrate any complex logic or information processing within the material beyond rate limiting step of signal amplification and protein production and also does not integrate the components based on physical mechanisms to process any external information.

I.4. Dynamic and Multi-Modal Environmental Coupling

The material used in the study does not exhibit active dynamic coupling with the environment. The cells are stimulated with chemical agonists (forskolin and TPA) but there is no evidence of them behaving as dynamic sensors, or generating adaptive responses based on physical interactions with other components. They respond to chemical stimuli but do not “interpret” them beyond this basic chemical input, and there is no evidence of multimodal interaction to extract spatial, temporal information, as it does not present a spatially extended system (such as in living organisms that use mechanical, chemical and optical signal in different ways). The system is not self-sensing, but rather responds to specific inputs of externally applied molecules. There is no modulation of the response in function of a prior experience with a given type of stimuli (other than the spacing effect itself), but instead operates in all contexts in same modality (chemical stimuli alone). Furthermore, the study does not show any use or extraction of any other forms of information from their surroundings (changes in light, temperature, forces etc).

II. Self-Organization, Feedback, and Control

II.1. Hierarchical Modularity and Functional Differentiation

There isn't any clear evidence of hierarchical modular organization within the material system. The cells of the study exhibit common signaling pathways, but there are no specialized modules within the *material itself*, performing distinct interdependent roles as its all driven by shared biochemical pathways. The cellular signaling cascade is not a demonstration of a material based organizational hierarchy, but rather a demonstration of cellular homeostasis and that pathway alone are not related with the concept of material based intelligence. The paper itself did not show that there would be any degree of flexibility to alter these pathways by external material based parameters, where the material structure can be related or have an influence on pathway responses directly using feedback. There was not any spatial distribution or differentiation on cells nor any local feedback mechanism among different cell groups which could lead to functional specialization.

II.2. Local Feedback and Self-Regulation

The paper does describe feedback within the signaling pathways through which the chemical stimulation act. However, there is no demonstration of *material based local feedback loops or self -regulatory mechanisms*. The feedback is occurring at biochemical levels (through activation and deactivation of kinases, and increase in protein production), which occur solely within the cells. This mechanism exists in any living cell with or without any externally imposed stimuli, and represents a cellular feature of adaptation to a chemical stimuli, rather than a material-based self-regulation. The observed regulation is at the level of the protein phosphorylation cascade and is mediated by dynamic changes in CREB activity and protein production, but those mechanisms are not directly implemented by specific material properties or a material feedback loop. The phosphorylation of P-CREB is also shown to be altered via feedback using 666-15 inhibitor, with a decrease of overall protein levels after 24 hours, which suggest a dynamic change, but that change happens by means of biochemical pathways that does not reflect a material behavior. These are feedback loops within a self regulating biochemical system which uses proteins and phosphorylation as means to perform specific adaptation to chemical stimuli, which is well known for all living cells, thus these mechanisms do not reflect a material based self regulation as it uses metabolic reactions as main route to perform the feedback. These mechanisms are not inherent to material properties or their self organizing structures, and do not directly link information derived from the environment to its internal structural dynamics and material behaviour. The described system is not *material based*, but rather biologically based.

III. Adaptability, Learning, and Emergence

III.1. Active Adaptability, Responsiveness to Change, and Learning

The cells show a degree of responsiveness to the temporal pattern of stimulation, as evidenced by the spacing effect. However, this cannot be considered *active adaptation* or *learning* in the context of material intelligence and it only provides a very specific type of responsiveness to a pre-set pulse shape, rather than showing evidence of a material that can use previous history of its interactions to perform better for next steps. The system improves performance by a specific pattern, but that can not be classified as "learning", rather it selects to respond better to that pattern compared to an alternative. There is no evidence that the system dynamically adapts its internal parameters, or operation strategies, in response to a *range of different changes or a diverse set of stimuli*. The temporal-pattern recognition is a form of dynamic response, but not a form of learning in the sense that material is actively changing its own response mechanism, or parameters after receiving an external stimuli, or even that a prior response to a particular input will influence its next response to that same input (other than a better response with repeated stimulation by a specific pattern only). The system does not exhibit any form of self improvement in its dynamics or implementation, but rather operates via a fixed series of biological components or chemical responses. The increase in phosphorylation by specific pattern is more similar to a resonance effect than the concept of learning.

III.2. Temporal Dependence and Memory-Driven Functionality

The system exhibits a limited form of temporal dependence by responding different after repeated pulses and ITI spacing which influences activity, however these are not active responses of material dynamics, but rather biological cell processes. The luciferase expression changes in time depending on previous pulsatile treatment. However, the system uses an unstable reporter protein, which is designed to be transient, therefore it is not the physical element that would retain the information as a material property. There is a response to time, but there is not a material-based mechanism that relies on time (such as phase transitions, mechanical deformations or long range diffusion) to dynamically alter its properties. It acts by a different cellular mechanism as described through time, not by creating a dynamic time-dependent change in a material internal structure (other than transient changes of protein concentration). The temporal dynamic shown in this paper relates with a limited example of time dependency (a selection of ITI) and a decay time, but is not used as a parameter to implement a form of computation and not used (or shown as being able to be) as a parameter to determine a future action.

III.3. Novelty, Unpredictability, and Functional Significance of Emergent Behaviors

The core behaviors found in this publication are well described from a cellular and molecular perspective of biology, and are predictable based on existing knowledge of cellular signaling. There is no evidence of truly novel, unpredictable, or emergent behaviors that are not already described from cellular system and biological memory implementations of living systems. The main finding (spacing effect) reflects a known phenomenon in biology that has a very well established and reproducible experimental output based on an observed chemical pathway. The system displays a response to temporal pattern, but the response itself is not a demonstration of functionality related to information processing in the context of intelligent material behaviours, it is rather described within the context of cellular responses to different chemical stimuli with a specific type of time/pulse shape pattern interaction. The result of this experiment has no demonstrable function for problem-solving, adaptation (other than an imposed response), or any other hallmark of material intelligence besides the specific and limited response in this paper, which is also present in living cellular system. There are no emerging behaviours that are relevant to problem solving or autonomous operations without human interventions. The "emergent" behaviors are limited to well known responses to different patterns of chemical inputs by living cells, and thus can not be considered related to truly emergence in any materials design based concepts.

Measure of Distance from a Truly Intelligent System

Quantitative Assessment

Criterion	Score	Justification
Local Interaction	2	Limited to interaction via cell signaling, no material based local interactions
Local Self-Memory	2	Transient memory based on transient biochemical changes, no inherent physical material storage.
Local Low-Power Computation	1	No evidence of local, material-based computation.
Distributed		No evidence of dynamic distributed sensing or

Sensing/Actuation	1	actuation beyond chemical inputs.
Feedback Loops	3	No inherent material based feedback loops, it only relies on biological based feedback loops.
Adaptability	2	Responsiveness to spacing, but no true adaptation or learning; specific and limited pattern recognition.
Autonomy	2	Minimal self-regulation, relies on external/specific chemical inputs.
Emergence	1	No evidence of true emergent behaviors unrelated to biology.
Hierarchical Organization	1	No material based organization; responses within cells.
Non-Linear Dynamics	2	Response based on nonlinear biochemical pathways, not a physical material system.
Internal Regulation	3	Biological self regulation mechanisms, no material based time-dependent structures.
Multi-Stimuli Integration	1	Single modality, based on chemical stimuli.

Overall Score: 1.83

Specific Deficiencies

The publication's main deficiency is the lack of a *material* basis for the observed intelligent-like behaviors. The core mechanisms and all reported behaviour is purely based on cellular biochemical pathways. While the cells do exhibit a “memory effect” which is a specific type of response to timed input signal, this is fundamentally a function of cellular dynamics (via phosphorylation and protein synthesis/degradation), not inherent material design. The system lacks embodied feedback, local self-memory, local material based computation, distributed sensing that would be required for a truly intelligent material. The most important missing aspect is the active and dynamic connection between material properties, external stimuli, and an internal memory structure that would then lead to system's “own” actions, rather than a reaction, based on a pre programmed cellular-chemical pathway. The system is not self-aware to its own actions, nor it is self-adaptive to perform better in future use, and it does not have a concept of "self" in material sense as has no physically implemented memory or response element using structural parameters. The fundamental conceptual gaps include the absence of any mechanism that would couple information storage to internal structure of material, or to implement feedback through physical means and that all material changes (such as mechanical, electrical, optical and others) should be part of computation and memory. The paper describes a biological response to chemical signaling using biological memory via protein based interactions, not any material based computation or memory based on a physical property that can influence next output. The main missing abstract principles are related to non-equilibrium energy dissipation to allow for long term memory within dynamic system, local self-regulating non-linear feedbacks by means of distributed physical implementation and integration of multiple functionalities using material structural dynamics, which were all absent in the presented work. The lack of material self organization and physical mechanism for non-volatile memory (other than transient phospho-protein states) are also major limitations.

Conclusion

This publication demonstrates an interesting observation that cellular/biological systems exhibit a form of memory similar to the spacing effect and may inform research on learning and memory mechanisms in biology by applying physical design concepts. It represents a limited example of dynamic response in cells. However, it falls *significantly short* of demonstrating any genuine progress towards material intelligence. The study repurposes cellular dynamic functions, as an analog of memory mechanisms but it does not create any *material* embodiment of those functionalities and does not demonstrate any *material* intelligence, nor provides any design to allow material's to work independently from their own cellular mechanisms as it relies on chemical signaling in a biochemical cascade with protein turnover. There is no evidence of self-organization, material-based hierarchical modularity or internal feedback loops. The main finding, while interesting from a cellular perspective is a limited reproduction of biological memory using cellular protein interactions and responses, rather than implementing such mechanism by using structural or material parameters in order to generate an autonomous, self sustained, self adaptive and functionally more complex systems. Moreover, the system does not show any adaptation, or that information derived from environment (other than the chemical pulse pattern) can be used to generate a better response by active dynamic changes of internal material parameters. The lack of material based design or implementations renders the system described in this work as fundamentally limited, providing no clear path towards realizing truly intelligent systems in materials. As such it does not provide any pathway for creation of intelligent material system other than proving that cells respond to temporal stimuli. The conceptual framework is limited to *cellular* memory mechanisms, rather than exploring the fundamental challenges of *material* embodiment for intelligence.

V. Template Refinement

Based on this analysis, several refinements of the template are necessary:

1. **Specificity of "Material Basis":** The template must more rigorously differentiate between biological signaling and true material-based mechanisms. The current phrasing “material basis” was not strict enough and allowed a cell biological responses to be categorized as response by a material. The question needs to further specify that for memory, computation, and self-regulation, the mechanisms must be *inherent material properties* (structural, chemical, mechanical, electrical, optical) rather than functions of other types of components (such as biochemical pathways via phospho-proteins).

2. **"Computation" Definition:** The definition of "local computation" needs to be refined to explicitly exclude biochemical pathway responses. The questions should specify “*material-based* analog computation", clearly distinguishing such operation from external digital control (as was specified earlier but still interpreted in a broad way) and from protein based biological information processing at a biochemical level.

3. **"Adaptation" and "Learning" Critera:** The template needs to be more precise in defining *true active adaptation* and *learning*. Simple responsivity to pre-set inputs (such as a particular set pulses as seen here) needs to be explicitly excluded. The template should request a specific demonstration of a change in material parameters that leads to improved performance or novel behavior, while also showing how those changes are physically retained, which requires material based feedback for self improvement of future operations. The learning should explicitly involve how the material responds to a particular input in function of its prior interactions with a given set of stimuli, using an internal physical memory rather than pre-designed biological responses.

4. **"Multi-Modal" Specificity:** The multi-modal interaction needs to go beyond merely “detecting” multiple inputs of the same type to actively responding to *multiple and different types* of stimuli and extract different type of information encoded by those inputs, to be then used to perform different set of outputs in different modalities. It should require a clear demonstration of how diverse environmental input results in different type of responses which are combined using locally implemented mechanism.

5. **Hierarchical Modularity:** The assessment of hierarchical organization needs a more precise definition, which requires a functional differentiation beyond purely localized responses, which indicates a distributed behaviour of independent but locally connected and cooperating subsystems within a single material structure. It should explicitly request evidence of how the modular organization contributes to enhanced overall capabilities and flexibility, instead of simple replication of similar functional groups. That component must be implemented as part of the material design, rather than relying on cellular interactions which may offer spatial organization and functional differentiation in some very minimal degree of specialization, but it is far from a truly material implementation..

6. **Feedback Loops:** The "feedback loop" question should specify the type of feedback,

requiring that it must be *dynamic, non-linear and hierarchical with material-based implemented components*, excluding any purely biochemical level regulation or cell responses. The question must specify that the material must use a particular form of a physical property to create feedback loops with locally implemented sensors that respond simultaneously with a specific physical effect such as: mechanics, light, temperature, chemical concentration, electrical potential etc. The key concept here is to highlight the necessity of having a non digital control performed by a material without external electronics or any external controller for feedback loops.

7. **Emphasis on Time-Dependence of Material itself:** In particular the template needs to assess how *time itself* is implemented as a parameter for the system to operate: does the system respond only to transient signals, and does the system change if it operates at different time scales? Or can the system make use of time based parameters to define its own memory or action? This should move beyond simple delayed responses, but to change itself due to a property that varies according to time itself using physical system components and their dynamics (such as diffusion, phase transitions, mechanical deformation or stress relaxation).

8. **Distinction between Cellular Response Mechanism and Material System Property:** The template must explicitly state that responses which are only related to cell internal mechanism and do not show a direct influence of physical material changes are not relevant for true material based intelligence. There should be a clear separation between a feature of response from a living cell versus a response from a truly material structure that is working with local physical feedback rather than reliance on biochemical homeostatic pathways and signal transduction, as the main component of system operation.

The most pressing open questions to address include: how to implement non-volatile material based memory that dynamically changes with use, how to create local self regulating physical systems, how to enhance self-organization and internal feedback loops within materials using intrinsic physics as opposed to relying on biochemical or external control systems, how to build analog computation within a material rather than using digital electronics, and how to achieve true autonomy in material devices without a central processing unit. The template needs to address and include those questions for a better evaluation, which can only be achieved by a combination of all points that has been mentioned. The template can evolve, by applying similar feedback as materials that are proposed for intelligent behaviors by making use of its own past experience on previous publications.