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Research report

The role of nitric oxide in the object recognition memory

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HIGHLIGHTS

- Nitric oxide (NO) is an intra- and inter-cellular messenger.
- Experimental evidence suggests its involvement in recognition memory.
- The object recognition task is sensitive to NO.

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ABSTRACT

The novel object recognition task (NORT) assesses recognition memory in animals. It is a non-rewarded paradigm that it is based on spontaneous exploratory behavior in rodents. This procedure is widely used for testing the effects of compounds on recognition memory. Recognition memory is a type of memory severely compromised in schizophrenic and Alzheimer's disease patients. Nitric oxide (NO) is sought to be an intra- and inter-cellular messenger in the central nervous system and its implication in learning and memory is well documented. Here I intended to critically review the role of NO-related compounds on different aspects of recognition memory. Current analysis shows that both NO donors and NO synthase (NOS) inhibitors are involved in object recognition memory and suggests that NO might be a promising target for cognition impairments. However, the potential neurotoxicity of NO would add a note of caution in this context.

1. Introduction

1.1. Recognition memory

Recognition memory stems from a series of neural processes by which a subject becomes aware that a stimulus has been previously experienced, with recognition as the behavioral outcome of these processes. This type of memory requires that the perceived characteristics of the events are discriminated, identified, and compared with the memory of the characteristics of previously experienced events [73]. Importantly, recognition memory is a type of memory that is impaired in schizophrenia [15,22] and Alzheimer's disease patients [72].

Twenty-six years ago Ennaceur and Delacour [24] introduced a new memory paradigm the novel object recognition task (NORT). NORT is a non-spatial recognition memory task, does not involve at all the learning of a rule since it is based on the spontaneous predisposition of rodents to explore novel objects. In this test, thus, the ability of rodents to recognize a set of novel stimuli in an otherwise familiar environment is considered as a measure of its recognition memory [24].

The standard form of this test involves exposing a rodent to two identical copies of an object (sample trial) for 2–10 min. After a certain delay (intertrial interval), the rodent is then exposed to a novel object and an identical copy of the familiar object (choice trial). Objects can be made of different material (glass, plastic, metal) can have different shape (f.i., cubes, pyramids, cylinders) should have a comparable size and could not be displaced by the rodents. Efforts should be made to equate the pairings of objects in order to avoid any unintentionally induced preference or bias. Attention should be paid to the object odors. Thus, the objects should carefully cleaned before being used for another animal [26].

Exploration of objects was defined as the followings: directing the nose toward the object at a distance of 2 cm or less and/or touching the object with the nose. Turning around or sitting on the object was not considered exploratory behavior. Successful recognition is displayed by the rodent spending a greater amount of time exploring the novel object during the choice trial [24]. Animal's behavior is directly measured by an observer in the testing room or video-recorded and subsequently analyzed.

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Ennaceur and colleagues developed a novel version of this procedure, named the object location task (OLT), aiming to evaluate spatial recognition memory in rodents [25]. Spatial memory is the ability of an organism to acquire a cognitive representation of location in space and the ability to effectively navigate the environment [3]. This task assesses the ability of rodents to discriminate the novelty of the object locations but not the objects itself because the behavioral testing arena is already familiar to the animals [19,25]. During the sample trial of this new paradigm, similarly to NORT, rodents are exposed to two identical objects. After a certain delay, animals are re-exposed to the same two objects, one of which has been displaced to a new location within the apparatus. The definition of exploration is provided above in the context of describing the NORT protocol. Successful recognition is displayed by the animal spending a greater amount of time exploring the object in the new location during the choice trial.

Reportedly, in this context, the duration of the retention interval is of high importance for both the above described recognition memory tasks. The performance of the animals deteriorates as the delay between the sample and the choice trial increases [4]. Moreover, one major challenge in memory research is the question whether a "deficit" is due to an "unspecific" effect on sensory, motor, and/or motivational systems, or actually reflects an effect on the neurobiological substrate of the memory system under question. "Unspecific" effects of the experimental manipulations, such as the application of drugs, brain lesions, genetic manipulations, etc. on these recognition memory paradigms can be potentially detected by a detailed analysis of rodent's behavior in terms of the frequency of contacts with the objects, the time spent in exploring the objects, the distance travelled, the number of rearings, abnormal posture, defecation, etc. [18]. In addition, different studies indicate that the prefrontal cortex, the hippocampus, the parahippocampal regions of the temporal lobe (namely the perirhinal, entorhinal and postrhinal cortices) are brain structures implicated in recognition memory [26,40,79].

Both of these recognition memory tasks do not involve explicit reward or punishment but rely on the natural curiosity of rodents and preference for novelty [69], which do not appear to be influenced by reinforcement/response contingencies [18]. These paradigms are quite similar to procedures used in humans and should have a significant level of construct and predictive validity and probably reflect episodic memory [26]. Moreover, with suitable manipulations, these recognition memory paradigms can evaluate different stages of memory formation such as encoding, storage and retrieval of information. Therefore, either the NORT or the OLT are used for testing putative memory enhancing compounds.

1.2. Nitric oxide (NO)

Nitric oxide (NO), a small, short-lived, and highly diffusible gas, is an important intra- and inter-cellular messenger in the brain [31]. NO originally was identified as endothelium-derived relaxing factor (EDRF) mediating relaxation of blood vessels [28]. NO plays essential roles in the regulation of a wide range of physiological processes, including cellular immunity [34], vascular tone [51], and neurotransmission [30].

1.2.1. Synthesis of NO

NO is originated by the conversion of L-arginine to L-citrulline, with the release of NO. The enzymatic oxidation of L-arginine to L-citrulline occurs in the presence of oxygen (O_2) and nicotinamide adenine dinucleotide phosphate (NADPH) with flavin adenine dinucleotide (FAD) flavin mononucleotide (FMN), henme, thiol and tetrahydrobiopterin as cofactors [38,52].

The enzyme responsible for the generation of NO is NO synthase (NOS). Three NOS isoforms encoded on different distinct genes have been described: neuronal NOS (nNOS, NOS type I) being the isoform found in neuronal tissues, inducible NOS (iNOS, NOS type II) being the isoform which can be synthesized following induction by pro-inflammatory cytokines or endotoxin and endothelial (eNOS, NOS type III) being the isoform expressed in endothelial cells [12]. nNOS and eNOS are constitutively expressed and dependent on the presence of calcium (Ca^{2+}) ions and calmodulin to function, whereas the activity of iNOS is Ca^{2+} independent [for review, see [13]].

NO is formed following activation of glutamate receptors, mainly the *N*-methyl-D-aspartate (NMDA) subtype. After this activation, Ca^{2+} is transiently increased in the cytosol and forms a complex with calmodulin that binds to and activates nNOS [38]. Glial cells (astrocytes and microglia) synthesize NO after the transcriptional expression of a Ca^{2+} independent iNOS isoform [48].

1.2.2. Main physiological targets of NO

NO has been described as an unconventional neurotransmitter because it is not stored in synaptic vesicles and not released upon membrane depolarization but released immediately after its synthesis. NO does not mediates its action by binding to membrane associated receptors but diffuses to adjacent neurons and acts directly to intracellular components [31].The most prominent natural target of NO is soluble guanylyl cyclase (sGC). NO acts as a messenger, activating sGC [2] and participating in the transduction signaling pathway involving cyclic guanosine monophosphate (cGMP). cGMP, in turn, activates cGMP-dependent protein kinase (PKG), which may affect additional second messenger systems. cGMP can also directly activate other protein kinases, such as the cyclic adenosine monophosphate (cAMP)-dependent kinase PKA [49]. Metabolism of cGMP by phosphodiesterase (PDE) suppresses or terminates NO/sCG signaling [37]. Thus, NO is similar to conventional transmitters that act via second messengers to activate protein kinases which may in turn affect transcription factors and protein synthesis.

In this context, current literature indicates that the cGMP should probably no longer be considered the only target of the action of NO. Alternative cGMP-independent mechanisms have recently been proposed. One reaction which is gaining prominence is the *S*-nitrosylation of various proteins such as the NMDA receptor; the caspases 1–4 and 6–8; the cyclic nucleotide-gated (CNG) channels; the large conductance Ca^{2+}-activated potassium (BK_{Ca}) channels and the ryanodine receptor Ca^{2+} release (RyR) channels. Depending upon the protein species, *S*-nitrosylation can either inhibit or up-regulate their activity. The aforementioned opened by *S*-nitrosylation channels and the enzyme mono(ADP-ribosyl) transferase are amongst the cGMP-independent mechanisms by which NO may exerts its action [23].

NO is involved in synaptic activity, neural plasticity and memory functions. It promotes also survival and differentiation of neurons and exerts long-lasting effects through regulation of transcription factors and modulation of gene expression. NO potentially acts among the above described mechanisms, depending on the concentration, with low concentrations being neuroprotective and mediate physiological signaling (e.g., neurotransmission or vasodilatation), whereas higher concentrations mediate immune/inflammatory actions and are neurotoxic [13,14].

Because of its mobility, unconstrained by cell membranes, NO can act across a broad volume and its actions are limited by inactivation (e.g., scavenging or degradation). It has long been postulated that NO can also could act as a retrograde messenger at the synapse, mediating transmission from target neurons back onto the synapse and regulating synaptic plasticity, but the same properties also enable NO to signal to any local compartment and to cells that lack synaptic activity or NOS expression [74].