# Glial activation: a driving force for pathological pain

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**Pain is classically viewed as being mediated solely by neurons, as are other sensory phenomena. The discovery that spinal cord glia (microglia and astrocytes) amplify pain requires a change in this view. These glia express characteristics in common with immune cells in that they respond to viruses and bacteria, releasing proinflammatory cytokines, which create pathological pain. These spinal cord glia also become activated by certain sensory signals arriving from the periphery. Similar to spinal infection, these signals cause release of proinflammatory cytokines, thus creating pathological pain. Taken together, these findings suggest a new, dramatically different approach to pain control, as all clinical therapies are focused exclusively on altering neuronal, rather than glial, function.**

From sensation to perception, pain and pain modulation are classically viewed as being mediated solely by neurons. Glia in the CNS have been presumed to have no role because they lack axons and so have not been thought of in terms of cell-to-cell signaling.

For pain, this view is dramatically changing. New research implicates spinal cord glia (microglia and astrocytes) as key players in the creation and maintenance of pathological pain. There is mounting evidence that glia might be key for creating exaggerated pain states of diverse etiologies. This suggests that they might be broadly involved in creating pathological pain and perhaps other sensory phenomena.

The discovery that spinal cord glia have profound effects on pain processing advances our understanding of pain. It also extends current views of glia–neuron interactions[1,2] and provides an excellent model system for exploring glial activation, glia-to-neuron signaling, neuron-to-glia signaling, and the impact of glial activation on behavior. There is no reason to believe that glial modulation of neural function and behavior is restricted to pain.

Furthermore, this new role of glia as pain modulators might have major implications for drug development aimed at controlling clinical pain. Targeting spinal cord glia is a new view of pain control and opens new approaches for pharmacological treatments of pathological pain.

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## Where might glia 'fit' in pain modulation?

Pain transmission from the periphery to higher brain areas is a dynamic process. Pain messages can be suppressed (analgesia), relayed unaltered, or amplified (hyperalgesia). Pain modulation occurs in the spinal cord dorsal horns, where peripheral nerves relay sensory information to pain transmission neurons (PTNs) (Fig. 1a,b). PTNs, in turn, relay pain messages to the brain. It is at the first synapse of the pain circuit, where the periphery meets the CNS, that both analgesia and hyperalgesia are created[3].

Currently, hyperalgesia is thought to be created by a cascade of neuronal events within the spinal cord dorsal horns[4] (Fig. 1a,c). Pain messages are relayed from peripheral nerves to PTNs via release of substance P and excitatory amino acids (EAAs). Under normal conditions (everyday painful events), these excite PTNs by binding NK1 receptors (for substance P) and AMPA receptors (for EAAs). EAAs also bind NMDA receptors on PTNs, but this is normally of no functional consequence because NMDA-linked ion channels are constitutively 'plugged' by $Mg^{2+}$ ions. However, following strong and/or maintained release of substance P and EAAs (e.g. during burns, trauma, etc.), PTNs become sufficiently depolarized so that $Mg^{2+}$ ions exit the channels, allowing extracellular $Ca^{2+}$ to enter (Fig. 1a,c). This $Ca^{2+}$ initiates a variety of events, including production of nitric oxide (NO). This gas diffuses from the neurons, evoking increased release of 'pain' transmitters and further excitation of PTNs (Ref. 4).

Although this neuronal model provided an excellent framework, there are several situations where glia might contribute to pain. First, in infectious diseases, such as AIDS, upwards of 90% of patients suffer from chronic pain yet no bodily source of the pain can be found in nearly half these cases[5,6]. Second, injury often creates pain perceived to arise not only from the injury site but also from healthy surrounding tissues. Such pain cannot be explained by inflammatory mediators or other substances in the skin diffusing to the surrounding areas[7]. Third, pathological pain arising from peripheral nerves (neuropathic pain) is not always perceived as arising only from skin normally innervated by the traumatized nerve (called 'territorial' pain because the pain arises from the skin 'territory' innervated by the affected nerve). Rather, pain is also perceived as arising from skin innervated by other healthy nerves. This so-called 'extra-territorial' pain cannot be explained by communication between peripheral sensory nerves. Last is 'mirror' pain. Here, inflammation or trauma of one body region (e.g. the left foot) does indeed result in pain from that site. However, it might also result in pain perceived as arising from the healthy mirror-image body part (i.e. the right foot)[8,9].

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We will develop the view that spinal cord dorsal horn glia provide an explanation for these pathological pains. As such, this is a dramatic departure from the view that pain is created and modulated purely by neurons.

### Glia as modulators of pain

Glia have long been viewed as static constituents of the CNS, serving primarily support functions[10]. However, CNS synapses are encapsulated by glia, and glia express receptors for many neurotransmitters and neuromodulators[11–13], synthesize and release numerous transmitters[14–16], and produce transporters that uptake or release transmitters from the extracellular and synaptic spaces, respectively[16,17]. Thus, glia possess some of the same characteristics and express many of the same receptors as neurons, so they are well positioned to regulate neuronal functioning[10].

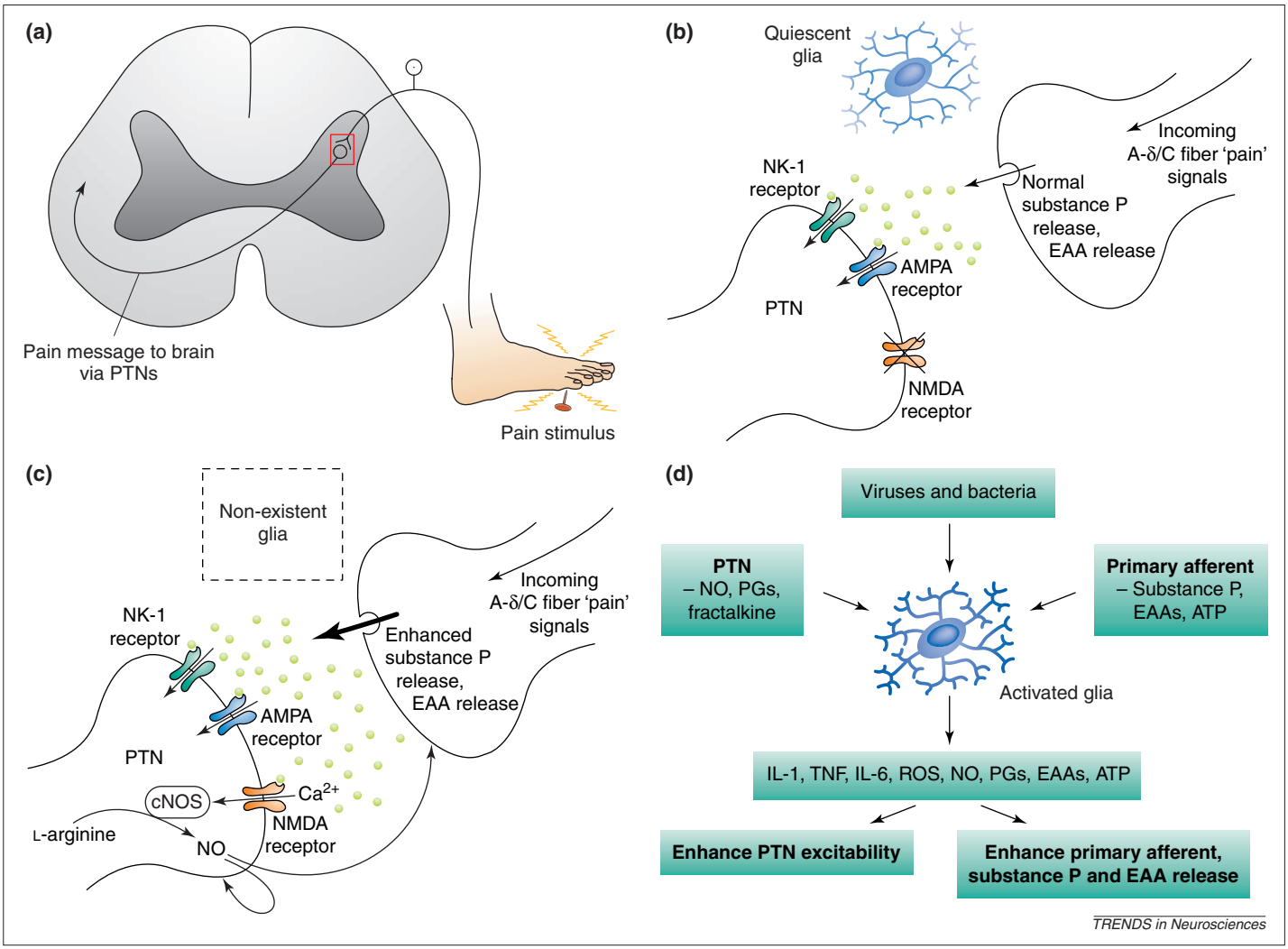


**Fig. 1.** Schematic of pain and pain modulation. (a) Classical 'pain' signaling. When a painful stimulus is encountered (such as stepping on a tack, as shown), peripheral 'pain'-responsive nerves (A-δ and C fibers) are excited. These axons transmit action potentials to their presynaptic terminals in the spinal cord dorsal horn. Neurotransmitters released here bind to and activate postsynaptic receptors on pain transmission neurons (PTNs). In turn, the axons of PTNs ascend contralaterally to the brain, carrying the 'pain' message to higher centers. The box encompassing the sensory presynaptic terminal and the postsynaptic region of the PTN indicates the area shown in detail in (b)–(d). (b) Normal pain. Under normal, every day situations where pain is experienced, glia are present but quiescent. 'Pain' signals arriving from the periphery along A-δ and C fibers cause release of substance P and excitatory amino acids (EAAs) in amounts appropriate to the intensity and duration of the initiating pain stimulus. Activation of NK-1 receptors by substance P and activation of AMPA receptors by EAAs cause transient depolarization of the PTNs, thereby generating action potentials that are relayed to higher brain areas. NMDA-linked channels are inoperative as they are chronically 'plugged' by $Mg^{2+}$. (c) Pathological pain: classic view. In response to intense and/or prolonged barrages of incoming 'pain' signals, the PTNs become sensitized to over-respond to subsequent incoming 'pain' signals. The intense and/or prolonged barrage depolarizes the PTNs sufficiently such that the $Mg^{2+}$ exits the NMDA-linked channel. The resultant influx of $Ca^{2+}$ activates constitutively expressed nitric oxide synthase (cNOS), causing conversion of L-arginine to nitric oxide (NO). Because it is a gas, NO rapidly diffuses out of the PTNs. This NO acts presynaptically to cause exaggerated release of substance P and EAAs. Postsynaptically, NO causes the PTNs to become hyperexcitable. Glia have not been considered as having a role in creating pathological pain by this neuronally-driven model. (d) Pathological pain: new view. Here, glial activation is conceptualized as a driving force for creating and maintaining pathological pain states. The role of glia is superimposed on the NMDA–NO driven neuronal changes detailed in (c), so only the aspects added by including glia in the model are described here. Glia are activated [shown as hypertrophied, relative to (b), as this reflects the remarkable anatomical changes these cells undergo upon activation] by three sources: (1) bacteria and viruses which bind specific activation receptors expressed by microglia and astrocytes, (2) substance P, EAAs, and ATP released by either A-δ or C fiber presynaptic terminals (shown) or by brain-to-spinal cord pain enhancement pathways (not shown), and (3) NO, prostaglandins (PGs), and fractalkines released from PTNs. Following activation, microglia and astrocytes cause PTN hyperexcitability and exaggerated release of substance P and EAAs from presynaptic terminals. These changes are created by the glial release of NO, EAAs, reactive oxygen species (ROS), PGs, proinflammatory cytokines [interleukin-1 (IL-1), IL-6, tumor necrosis factor (TNF)], and nerve growth factor.

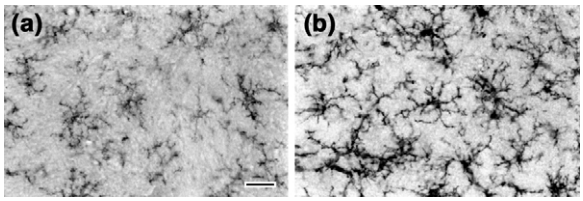


**Fig. 2.** Example of microglial activation in response to a stimulus that creates exaggerated pain states. Peri-spinal administration of HIV-1 envelope glycoprotein gp120 creates exaggerated responses to both thermal and touch/pressure stimuli[27]. Disruption of glial activation abolishes the pain changes[40]. Furthermore, these pain changes are correlated with anatomical evidence of activation of both astrocytes and microglia[40]. An example of such microglial activation is illustrated here. (a) illustrates the dorsal horn of a rat injected peri-spinally with vehicle. (b) is identical except that the rat received peri-spinal HIV-1 gp120 at a dose that creates exaggerated pain states. These photomicrographs are from the tissues collected for analysis reported in Milligan *et al.*[27] Activation of microglia induces these cells to upregulate their expression of complement type 3 receptors. Thus, enzyme-labeled antibodies directed against complement receptor type 3 (OX-42 monoclonal antibodies) can be used to detect microglial activation by light microscopy. By this method, activated microglia appear darker and more densely stained in accordance with their increased expression of OX-42 antibody-bound receptors. Scale bar, 25 μm in (a) and (b).

Glia came to the attention of pain researchers in the early 1990s. At this time, an animal model of neuropathic pain was reported to activate spinal astrocytes[18]. Indeed, drugs that blocked neuropathic pain also blocked astrocyte activation[19]. Thus astrocyte activation appeared, at minimum, to be strongly correlated with neuropathic pain.

Dorsal horn astrocytes and microglia are now known to be activated (i.e. upregulated expression of activation markers) in response to a wide array of conditions that produce hyperalgesia. These include subcutaneous inflammation[20,21], subcutaneous yeast cell walls[21], intraperitoneal bacteria[3], peripheral nerve trauma[22], bone cancer[23], lumbar spinal root constriction[24], spinal nerve transection[25], spinal cord trauma[26], activation of brain-to-spinal cord pain facilitatory circuits[3], and immune activation within the spinal cord[27] (Fig. 2). Glial activation markers are also upregulated by chronic, but not acute, morphine treatment[28]. Glia are physiologically activated (i.e. mobilization of intracellular $Ca^{2+}$, activation of second messengers, glutamate release, etc.) by (1) substance P (Ref. 29), EAAs (Ref. 30), and ATP (Ref. 31), which are released from pain responsive sensory nerve terminals in the dorsal horn, and by (2) NO (Ref. 32), prostaglandins (PGs)[33], and fractalkine[34,35], which are released by PTNs (Fig. 1a,d).

### Glia release classic pain-enhancing substances

Simply because glial activation correlates with pain does not imply that it necessarily mediates pain. However, in this case it does. Glial activation is necessary and sufficient to produce enhanced pain. Glial activation is necessary because disruption of spinal glial function reduces exaggerated pain created by subcutaneous immune challenge[36], subcutaneous inflammation[37], peripheral nerve inflammation[3], spinal nerve transection[38], spinal nerve inflammation[39], and spinal cord immune challenge[40]. Indeed, disrupting glial activation attenuates morphine tolerance, suggesting that substances released by glia counteract the effects of chronic opiates[28]. Notably, disruption of glial activation has no effect on normal responses to acute pain stimuli, supporting that glia are specifically involved in pathological pain processes[36,37,40]. The strategy for testing whether glial activation is sufficient to enhance pain relies on the fact that astrocytes and microglia express certain characteristics in common with immune cells. That is, glia become activated following binding of bacteria or viruses. Indeed, immune activation of glia by peri-spinal administration of either bacterial cell walls[36] or viral envelope proteins[27,40] creates hyperalgesia. Furthermore, glia (but not neurons) bind and become activated by fractalkine, a neuron-to-glia signal released from the surface of strongly activated neurons[34,35]. Fractalkine has just been demonstrated to create exaggerated pain states following spinal administration[41].

Microglia and astrocytes are attractive candidates as mediators of hyperalgesia (Fig. 1a,d). First, activated glia release substances that excite spinal pain-responsive neurons, such as reactive oxygen species (ROS) and NO, PGs and other arachidonic acid products, EAAs, and growth factors[3]. Second, substances released from activated glia cause exaggerated release of 'pain' transmitters from sensory neurons that synapse in the dorsal horn[14,42]. Third, microglia and astrocytes form positive feedback loops, creating perseverative release of pain mediators[3]. Fourth, substances derived from glia exert autocrine and paracrine actions and, as such, are well positioned to globally effect activity in the spinal cord[43,44]. Finally, glia are activated by 'pain' neurotransmitters released in the dorsal horn[12,45–47]. The relative importance of astrocytes versus microglia cannot yet be distinguished for exaggerated pain states. Although these glia are distinct populations of different embryonic origins and response repertoires, each population becomes activated by pain-enhancing stimuli and each excite the other[48,49]. So, these glia will be considered here as a combined functional population whose actions create hyperalgesia.

From the discussion above, activated glia increase exposure of spinal PTNs to EAAs, PGs, NO and substance P (Fig. 1a,d). What is notable about this list is that all are both (1) universally accepted to create exaggerated pain states and (2), in their role in pain modulation, have been classically assumed to derive solely from neurons. The fact that glia dramatically regulate these key pain modulatory factors suggests that the literature on spinal substance P, EAAs, PGs, and NO involvement in exaggerated pain states actually reflects a yet-unrecognized contribution of glia to the effects observed.

### Glia release novel pain-enhancing substances

The impact of glia extends beyond this list of neuroexcitatory substances. Activated glia, similar to other immune cells, release the proinflammatory cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF). Both glia and neurons express receptors for these[50]. Although proinflammatory cytokines have been intracellularly detected in some neurons[22,51], no evidence exists that neurons release these. Notably, IL-1 in spinal neurons is detected in the neuronal nucleus rather than the cytoplasm[21,22]. This suggests that this IL-1 is not made by the neurons (because cytokines are synthesized in the cytoplasm), but rather reflects internalization and nuclear translocation of receptor-bound cytokines. Indeed, nuclear translocation of receptor-bound IL-1 (but not of unbound IL-1) has been suggested to serve as a transcription factor for regulating gene activation[52].

Regarding the effect of proinflammatory cytokines on pain, neither TNF, IL-1 nor IL-6 have been implicated in normal, non-pathological pain[27,53]. By contrast, TNF (Refs 27,54–56), IL-1 (Refs 3,22,27,56–58) and IL-6 (Refs 22,53,59,60) have all been implicated in creating exaggerated pain states by their actions in spinal cord. Because proinflammatory cytokines classically induce the production of each other and act synergistically[61], it is not surprising that all three are involved. These cytokines mediate pain induced by spinal viral challenge (HIV-1 gp120) (Ref. 27), peripheral nerve inflammation[62], peripheral nerve trauma[22,53–56,60], subcutaneous inflammation[3], and spinal dynorphin[57]. These proinflammatory cytokines can also directly induce pain following spinal administration[58,63]. Thus, spinal proinflammatory cytokines might be necessary and are sufficient for exaggerated pain states of diverse etiologies.

### Why is this important?

The emerging story is that spinal cord glia are responsive to signals from the periphery, brain, and spinal immune challenges. Hyperalgesia is created, in part, as a result of substances released by glia that excite PTNs. Given that glia have never before been conceptualized as playing any role in pain modulation, the implications for the understanding of pain and for pain control are great.

The first implication is that substances released by activated spinal cord glia might be an as-yet-unrecognized source of pain in infectious diseases. Many bacteria and viruses 'home' to the CNS. That is, they move from the periphery into brain and spinal cord, taking up residence there. HIV-1 is an example of such a pathogen. HIV-1 enters the spinal cord early in, and continues throughout, disease progression. In turn, glia are activated, releasing proinflammatory cytokines[64]. This has especially insidious implications because drugs used to treat AIDS do not cross the blood–brain barrier[65]. As noted above, a striking observation in the AIDS clinical literature is that most patients suffer from chronic pain, a high percentage of which is of unknown bodily origin[5,6]. This suggests that spinal viral invasion, causing glial activation and proinflammatory cytokine release, might potentially explain such pain. Indeed it predicts that activation of spinal cord glia by viruses or bacteria is sufficient to create exaggerated pain states in the absence of any trauma to the body. Furthermore, it suggests that even when there is an identifiable bodily source of pain (e.g AIDS neuropathies, opportunistic cancers), virally driven spinal glial activation might also exaggerate those sensory signals.

A second implication is that glia are a driving force for creating and maintaining pain following peripheral injury and inflammation. Regardless of whether the peripheral signals arise from inflammation or physical trauma, glia participate in the exaggerated pain states that ensue. This is especially striking for inflammation, constriction or damage of peripheral nerves given that neuropathic pain is a major source of human suffering. Furthermore, such neuropathic pains are, by and large, not relieved by current therapies that target neurons. This suggests that targeting glia might provide a novel approach for treatment of such unremitting pain conditions.

A third implication is that glial activation might create expansions of the body region from which pain is perceived. That is, glial activation might underlie pain perceived from healthy tissues beyond the injury site. It might also underlie extra-territorial pain in which pain arises from regions innervated by distant healthy peripheral nerves. Previous work has concluded that both phenomena arise from excitability changes in pain transmission neurons[7]. They are postulated to arise from neuronally derived modulators, such as NO. These substances are thought to diffusely affect neurons in the dorsal horn region that receive sensory information about the bodily injury. Given the well-defined 'body map' organization of the dorsal horn, diffusion of neuroexcitatory substances would create the perception of pain progressively arising from more distant body sites. It is notable that recent work has shown that extraterritorial pain can be blocked by disruption of spinal IL-1 or glial function[62].

A fourth, and probably related, implication is that glial activation might create 'mirror' pain. As discussed above, mirror image pain is a puzzling pain phenomenon in which damage on one side of the body also results in pain from the corresponding region in the opposite (healthy) side of the body. Little is understood regarding how mirror pain occurs. Both intraspinal and brain-to-spinal cord neuronal circuits have been proposed[9,66]. However, a glial basis for mirror pain has never been considered. Recent work demonstrated that disrupting spinal cord glial and IL-1 function blocks the development and maintenance of mirror pain[62,67].

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## Conclusions

In summary, this body of evidence suggests that the activation of glia in the spinal cord and resultant proinflammatory cytokine release drives exaggerated pain states. It is quite striking that spinal glial activation and proinflammatory cytokine release are key mediators of hyperalgesias ranging from acute peripheral inflammation to chronic nerve trauma to central infection. Given such pervasive involvement, assessing whether glial activation and proinflammatory cytokine release are important contributors to various human clinical pain syndromes appears warranted (for discussion of potential therapeutics, see Refs 62,68). This avenue of investigation is exciting in that it provides a non-neuronal, novel target for pain. Recognition that glial activation is a powerful driving force for exaggerated pain opens up new ways to approach effective clinical pain control.

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# Synaptic reverberation underlying mnemonic persistent activity

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**Stimulus-specific persistent neural activity is the neural process underlying active (working) memory. Since its discovery 30 years ago, mnemonic activity has been hypothesized to be sustained by synaptic reverberation in a recurrent circuit. Recently, experimental and modeling work has begun to test the reverberation hypothesis at the cellular level. Moreover, theory has been developed to describe memory storage of an analog stimulus (such as spatial location or eye position), in terms of continuous 'bump attractors' and 'line attractors'. This review summarizes new studies, and discusses insights and predictions from biophysically based models. The stability of a working memory network is recognized as a serious problem; stability can be achieved if reverberation is largely mediated by NMDA receptors at recurrent synapses.**

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A fundamental ability of the brain is to actively hold an item of information 'on-line' in short-term memory. The stored information can be a sensory stimulus that guides a prospective action, such as a delayed perceptual decision or a delayed behavioral response. It can also be an item retrieved from long-term memory, for example when the memory of a face is activated and used in the visual search of a friend in a crowd. The obligatory physical process underlying active (working) memory is persistent neural activity that is sustained internally in the brain, rather than driven by inputs from the external world. Persistent activity provides the cellular basis of 'a central neural mechanism', as postulated by Hebb, 'to account for the delay, between stimulation and response, that seems so characteristic of thought'[1]. In order for a neural persistent activity to subserve working memory, it must be stimulus-selective, and therefore information-specific. Moreover, it must be able to be turned on and switched off rapidly (≈100 ms) by transient inputs.

For 30 years, persistent activity in the cortex has been documented by numerous unit recordings from behaving monkeys during working memory tasks (Box 1). How does stimulus-selective persistent activity arise in a neural network? Can we explain persistent activity in terms of the biophysics of neurons and synapses, and circuit connectivity? Recent experiments and computational modeling have been devoted to these mechanistic questions; these studies have led to new insights into the cellular basis of mnemonic neural activity in behaving animals.

## Reverberatory excitation: how localized can it be?

The central idea is that recurrent excitatory loops within a neural network can sustain a persistent activity in the absence of external inputs[1,2]. To test this hypothesis, a key issue is to identify the crucial and minimum anatomical substrate for reverberation. In the neocortex, several scenarios are conceivable.

### *Thalamocortical loop*

Persistent activity can arise from a large neural network that involves subcortical systems, through

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