

Glutamate receptors: variation in structure–function coupling

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Fast excitatory synaptic transmission in the CNS relies almost entirely on the neurotransmitter glutamate and its family of ion channel receptors. An appreciation of the coupling between agonist binding and channel opening has advanced rapidly during the past five years, largely as a result of new structural information about the agonist-binding site. Recent studies suggest that despite many structural similarities different family members use different mechanisms to translate agonist binding into channel opening.

Working hypotheses for glutamate receptor function

Understanding the basic mechanisms by which ligand-gated ion channels translate the free energy obtained from agonist binding to opening the ion channel pore is a central aim in pharmacology. Our current appreciation of the coupling between glutamate receptor structure and function took a quantum leap forward in 1998 when a crystallographic dataset describing the glutamate-binding domain of the GluR2 subunit of AMPA receptors was published [1]. The burst of activity that followed led to the development of new theories relating agonist binding to channel opening. A recent study by Inanobe *et al.* [2] presents structural data describing agonist-binding domains of an NMDA receptor subunit. Understanding the differences between different glutamate receptor subunits offers new insights into the coupling between agonist binding and channel activation.

Glutamate receptors are homo- or heteromeric assemblies of four homologous subunits that each consist of two large extracellular domains attached to a transmembrane domain with an intracellular cytoplasmic tail (Figure 1a). The second extracellular domain contains the agonist-binding site and, when expressed in isolation, this site retains the pharmacological profile of the full-length receptor. All agonist-binding domains derived from NMDA, AMPA and kainate receptor subunits fold into a similar bi-lobed structure that resembles a clamshell, with the ligand-binding site being located within the cleft between the D1 and D2 lobes (Figure 1 and 2).

The agonist-binding domain of the GluR2 subunit of the AMPA receptor is the best characterized glutamate receptor protein, with structures reported for a wide

range of agonists, antagonists and modulators. Comparisons of this domain in the presence and absence of ligands have revealed that agonist binding induces a displacement of D2 that closes the clamshell around the agonist in a manner reminiscent of the induced-fit theory for ligand binding [3]. These structural studies together with mutagenesis and electrophysiological studies have fueled the development of conceptual models of the molecular events that convert glutamate binding to GluR2 channel opening [4]. Several studies have shown that the D1 and D2 domains close ‘tighter’ on agonists that more effectively activate AMPA receptors, suggesting that the degree of domain closure for AMPA receptors is related to agonist efficacy [5–7]. By contrast, antagonists cause D1 and D2 to adopt an open conformation that is similar to the empty *apo* structure (Figure 1c). The clamshell hypothesis is appealing for both its simplicity and its ability to describe a wide range of data. Jin *et al.* [5] have shown that a series of partial agonists, varying by a single atom, induce a differential degree of domain closure that determines the coupling efficiency of the receptor subunits, which contributes to the incremental increase in single channel conductance. This finding, together with important earlier studies [8,9], suggests that tetrameric AMPA receptors can ratchet open their pore as a function of the probability of activation for each contributing subunit, and that this probability increases with the degree of agonist-induced domain closure. This hypothesis describes a non-concerted mechanism by which individual AMPA receptor subunits control channel conductance in a manner related to the nature of the induced fit (i.e. degree of domain closure) of each ligand with its binding site. By contrast, NMDA receptors show a concerted pore opening only after all subunits are bound by agonist and all pre-gating steps have been accomplished [10,11] (Figure 2b).

New structural data reported for the kainate GluR5 and GluR6 subunits and the NMDA receptor subunits NR1 and NR2A show that agonist-binding domains fold in a similar manner [2,12–16]. Consequently, it is reasonable to expect that the structural concepts for subunit control of gating developed for AMPA receptors transfer to the other glutamate receptors. Indeed, the first structures available for the glycine-binding domain of the NR1 subunit complexed with ligands exhibit domain closure that parallels agonist- or antagonist-bound GluR2 structures

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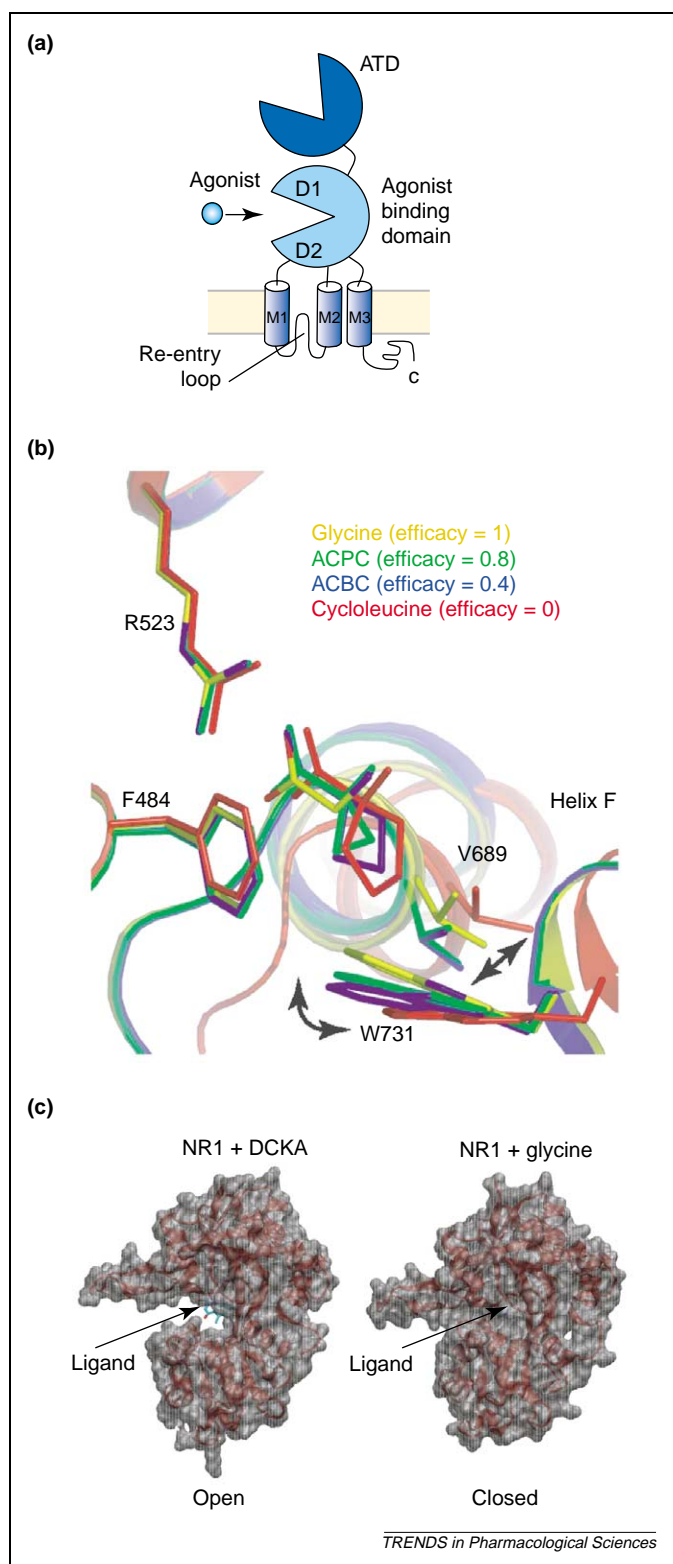


Figure 1. Glutamate receptor structure. **(a)** The domain organization of glutamate receptor subunits. Each glutamate receptor subunit folds into a modular structure of two large extracellular domains [the amino terminal domain (ATD) followed by the agonist-binding domain] connected to a transmembrane domain composed of three membrane-spanning segments (M1–3) and a membrane re-entry loop, and ends in an intracellular C-terminus. **(b)** Superposition of key ligand-binding residues in structures of the NR1 agonist-binding domain in complex with glycine (yellow) and a series of cyclic homologs containing carbocyclic rings of three (ACPC, green), four (ACBC, blue) and five (cycloleucine, red) atoms. The ligand-binding pocket accommodates the incrementally larger rings by adjusting the orientations of V689, W731 and helix F. The movement relative to the position in the glycine complex of these structural elements correlates closely with relative agonist

[2,12]. However, in contrast to GluR2, no substantial difference in the degree of domain closure exists between the full agonist glycine, the partial agonist D-cycloserine or a series of structurally related partial agonists. Thus, the agonist-binding domain of the NR1 subunit shows no relationship between the degree of agonist-induced domain closure and agonist efficacy. This creates an interesting opportunity to compare the activation mechanisms for two structurally related receptors that have long been known to show physiologically relevant differences in function.

The structure–function relationship of glutamate receptors

What can the new structural data for the NR1 subunit tell us about glutamate receptor function? First, comparison of structures for partial agonists and full agonists complexed with the agonist-binding domain might reveal information about regions of the protein that couple agonist binding to gating. Second, evaluation of contact residues in the ligand-binding pocket can suggest whether different agonists can mediate the same long-range intra-protein changes, and whether different receptors show similar agonist-induced long-range intra-protein motions. Several important ideas follow from this approach. For example, the degree of agonist-induced domain closure is closely correlated to the ability of an agonist to open the ion channel within AMPA receptors. Intra-protein strain created by the same closure has been suggested to drive AMPA receptor pore opening. This idea fits well with earlier proposals that individual subunits incrementally contribute to opening of the ion channel pore [8,9]. However, the lack of correlation between agonist efficacy and the degree of domain closure for the NR1 subunit suggests that other structural elements must control agonist efficacy, at least at the glycine-binding site within NMDA receptors.

Additional crystallographic datasets portraying the NR1 agonist-binding domain complexed with a series of constrained partial agonists have recently provided new insight into the structure–function relationship [2]. The complexes suggest a mechanism by which agonists induce an incremental or graded local rearrangement of a few specific contact residues within the NR1-binding pocket without changing the overall orientation of the two clamshell lobes (Figure 1c). The degree of local rearrangement appears to be related to agonist efficacy, suggesting that locally reorganized residues convey conformational changes at the binding site to secondary structure elements, perhaps at the highly flexible hinge region holding D1 and D2 together. Because dynamic behavior of the hinge region is likely to be a key determinant for domain closure, one might speculate that binding of the NR1 glycine site partial agonist shifts the equilibrium between ‘open’ and ‘closed’ conformations of the agonist-binding domain with only the ‘closed’ conformation able to

efficacy. Reprinted, with permission, from [2]. **(c)** The agonist-binding domain can be expressed by recombinant methods as soluble proteins that adopt a bi-lobed, clamshell-like fold. Crystal structures of this domain from NR1 in complex with the competitive antagonist DCKA and glycine are shown. Abbreviations: ACBC, 1-aminocyclobutane-1-carboxylic acid; ACPC, 1-aminocyclopropane-1-carboxylic acid; DCKA, 5,7-dichlorokynurenic acid.

activate the channel. Thus, despite the structural similarities between the agonist-binding domains of AMPA and NR1 subunits, different pictures of how agonists can influence protein structure and channel activation have emerged for AMPA and NMDA receptors [10,11].

Recent progress with respect to the structural basis for other important functional properties of glutamate receptors has been made. Key insights from crystallographic analysis pinpoint the residues that control dimerization of two agonist-binding domains as important determinants for both desensitization and deactivation [4]. Analysis of co-crystallized agonist-binding domains from the NR1 and NR2A subunits has recently demonstrated that the heterodimer interface has a key role in the modulation of NMDA receptor function [16]. More specifically, a single tyrosine residue appears to be important for controlling the rate of deactivation, much like exogenous modulators of deactivation and desensitization for AMPA receptors [17]. These data suggest that NMDA receptors have a built-in modulator that prolongs the time-course of the synaptic signal. The dimer interface between agonist-binding domains is clearly an important region for potential modulation of glutamate receptor function.

Intra-protein glutamate receptor dynamics

Although X-ray crystallographic visualization has transformed our thinking about glutamate receptors, it can only provide a snapshot of the static conformation favored

by a given molecule under the constraints that exist in the crystal lattice at low temperature. How can one derive functional models from static structures? Three points are worth considering. First, comparison of NR1 and GluR2 agonist-binding domains highlights the importance of obtaining additional structural information, even for closely related proteins that on initial examination might appear similar. Furukawa *et al.* [16] illustrate the value of expanded crystallographic datasets that include increasing amounts of the protein of interest. Second, functional studies need to be considered in the context of structural information. Third, additional data from complementary structural methods are needed. Crystallographic data alone do not permit identification of the intra-peptide motions that are responsible for relaying the conformational transitions within the agonist-binding domain and reorganizing the transmembrane helices that form the ion channel.

Other methodologies that probe protein dynamics are poised to become complementary tools to structural methods. Several experimental approaches, including solution X-ray scattering, nuclear magnetic resonance (NMR), ultraviolet and infrared spectroscopies in addition to fluorescence resonance energy transfer (FRET), can be used to study protein structure [18–22]. Such methodologies provide time-resolved monitoring of the dynamic exchanges among conformations of soluble proteins. Recent NMR analysis techniques of the GluR2 agonist-binding domain reveal little motion for the α -carboxyl of

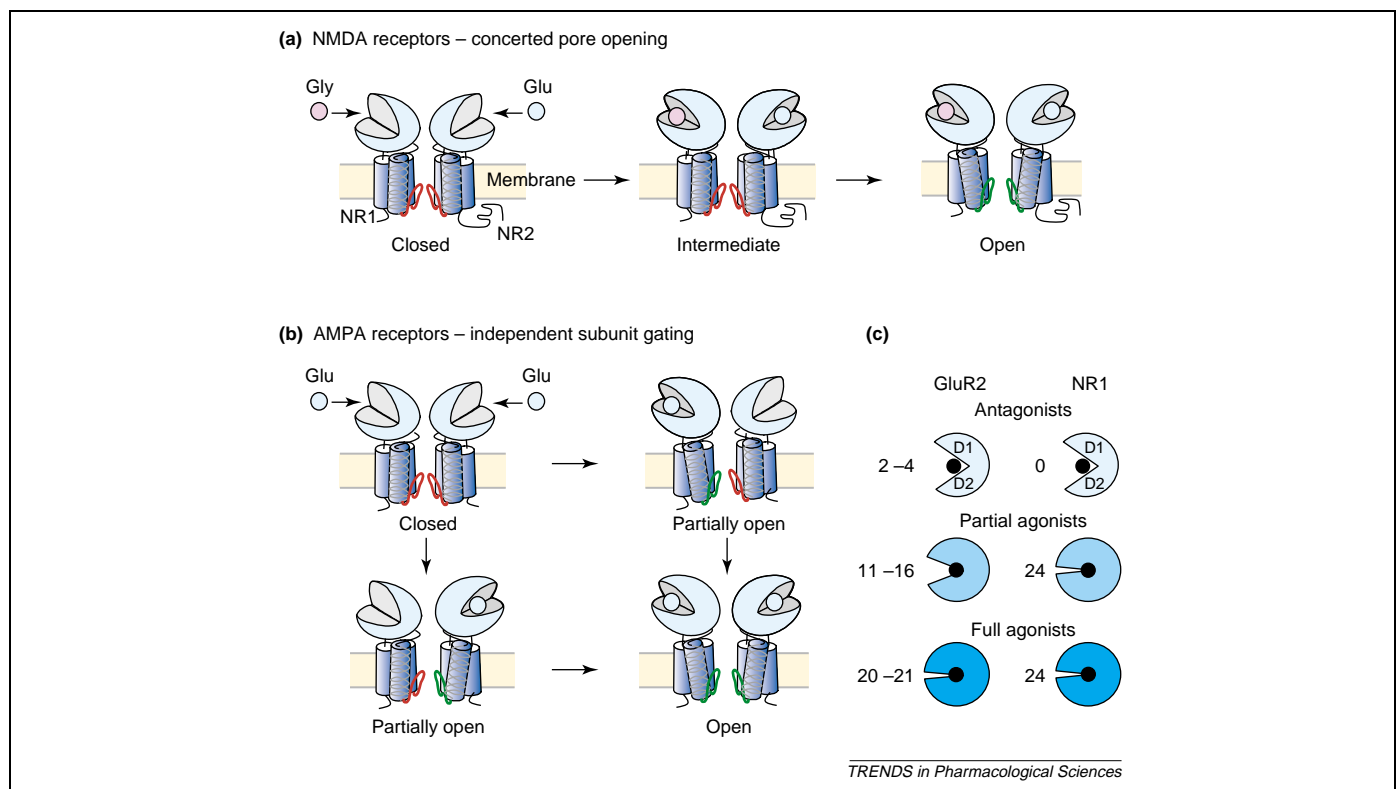


Figure 2. Glutamate receptor function. AMPA and NMDA receptors activate ion channels using different mechanisms. **(a)** NMDA receptors require binding of both glycine and glutamate before the ion channel is activated by a concerted rearrangement involving all four subunits. **(b)** In contrast to NMDA receptors, each subunit within the AMPA receptor can autonomously activate the ion channel. Notice that both cartoons for simplicity depict AMPA and NMDA receptors as dimers instead of tetramers with the ATD omitted. **(c)** Agonist binding to the agonist-binding domain promotes closure of D1 and D2, whereas binding of antagonists stabilizes the open apo conformation. The degree of domain closure correlates with agonist efficacy for AMPA receptors. By contrast, all types of NMDA receptor glycine-site agonists induce a similar degree of domain closure.

glutamate but increased exchange for the γ -carboxyl, indicating that the agonist is dynamic even in a bound state [22]. ^{15}N NMR spectroscopy has also suggested changes in the conformation and dynamics of GluR2 in specific regions that are prime candidates for linking the agonist-binding site to gating elements within the ion channel [23]. In addition, molecular dynamics (MD) simulations of a homology model of the NR2A agonist-binding domain suggests that a differential displacement of helix F occurs for the partial agonist relative to glutamate (Figure 3). The structural reorganization appears to be due to the loss of key interactions between helix F and other sections of the protein (Figure 3b) [24]. This analysis has led to the hypothesis that helix F might be involved in gating, an idea that is supported by both

spectroscopic and crystallographic data [2,16,22]. Following MD simulation, analysis of the trajectory revealed significantly more motion in the pocket around the conformationally restrained partial agonist than for glutamate (Figure 3c). Comparison of the dynamic models implicates the involvement of water in a hydrogen-bonded network for the partial agonist but not for glutamate (Figure 3b). Lastly, purely computational efforts such as homology modeling of closely related family members can provide structural hypotheses before crystallographic data are available, and these models are amenable to MD simulations [25]. All of the non-crystallographic results, although relying on some structural information, provide additional perspectives on glutamate receptor protein structure and, importantly, motion. Together, the crystallographic studies, functional probes and dynamic approaches represent a comprehensive and multi-disciplinary strategy to answer mechanistic questions regarding glutamate receptor function at a level of detail unprecedented for any ligand-gated ion channel at present.

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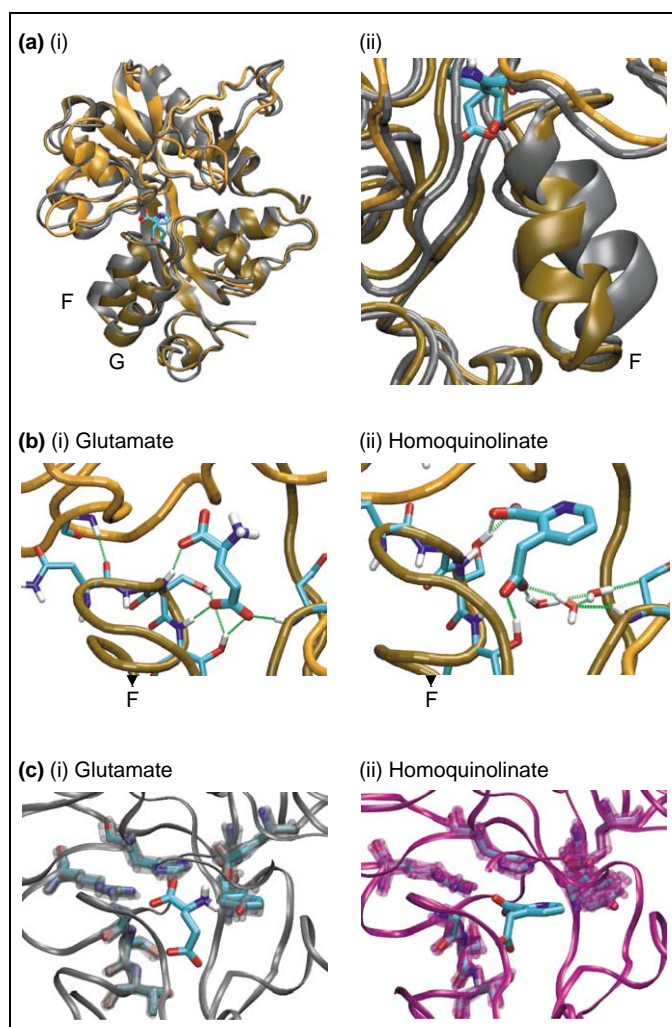


Figure 3. Molecular dynamics simulation of docked agonists in the NMDA receptors. (a) (i) Homology models of the NR2A agonist-binding domain with glutamate or homoquinolinate docked are superimposed. (ii) Molecular dynamics simulation showing a displacement in helix F for a structure with bound partial agonist compared with a structure with bound glutamate. The partial agonist backbone is gray whereas the glutamate-bound structure is orange (D1) and brown (D2). (b) Expanded view of the agonist-binding pocket. Note the differential hydrogen bonding (green broken lines) for glutamate (i) and homoquinolinate (ii). Water forms a hydrogen-bonded network for homoquinolinate. Reprinted, with permission, from [24]. (c) Motion of key binding pocket residues during a molecular dynamics simulation of the NR2A agonist-binding domain is shown by superimposing random frames from a movie of the simulation. The molecular dynamics simulation with docked glutamate (gray) (i) shows less fluctuation for binding site side-chains than for the partial agonist (purple) (ii).

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Stressed-out endogenous cannabinoids relieve pain

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A variety of physical and psychological stressors induce analgesia by activating descending systems that project from the brain to the spinal cord. This stress-induced analgesia (SIA) is mediated by distinct opioid- and non-opioid-dependent mechanisms. New evidence suggests that non-opioid SIA is mediated by two independent endocannabinoids within the midbrain. Furthermore, novel agents that disrupt breakdown of these endocannabinoids enhance non-opioid SIA and pave the way for novel therapies.

Pain modulatory circuits – a target of endogenous cannabinoids and opioids

Noxious information is conveyed via primary afferent fibres to the dorsal horn of the spinal cord and then to the brain via several ascending pain pathways. It is well established that there are descending analgesic systems, one of which projects via the midbrain periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM) to the spinal cord and the medullary dorsal horn [1]. The descending PAG–RVM pathway is thought to form part of an endogenous opioid analgesic system. Exogenous opioid receptor agonists, such as morphine, and endogenous opioids activate this descending analgesic pathway by inhibiting GABA release from interneurons that synapse with descending projection neurons within the PAG and RVM. This process is known as the GABA disinhibition hypothesis.

The main psychoactive constituent of *Cannabis sativa*, Δ^9 -tetrahydrocannabinol (THC), mimicks the activity of endogenous cannabinoids, such as anandamide and

2-arachidonoylglycerol (2-AG), at centrally and peripherally located cannabinoid CB₁ and CB₂ G-protein-coupled receptors (GPCRs) [2]. Numerous animal studies support the idea that THC and synthetic cannabinoid receptor agonists have analgesic activity in acute-pain models [2]. Cannabinoid-induced analgesia is mediated by activation of CB₁ receptors within the dorsal horn, ascending pain pathways and descending analgesic pathways. Thus, similar to opioids, cannabinoids activate the descending PAG–RVM pathway via GABA-mediated disinhibition [3].

Endocannabinoids mediate some forms of stress-induced analgesia

It has long been known that various ‘natural’ and ‘artificial’ stressors induce analgesia via opioid- and non-opioid-dependent descending pathways [4]. Stressors such as brief foot-shock, cold-swim stress and the elevated maze produce opioid-independent analgesia, whereas prolonged foot-shock, conditioned foot-shock and warm-swim stress produce opioid-dependent analgesia. However, the identity of the neuromodulatory transmitter(s) involved in non-opioid SIA has remained a mystery. A recent study in mice that were devoid of the CB₁ receptor suggested that endocannabinoids have a role in SIA [5].

Hohmann *et al.* [6] have now established a role for the endocannabinoid system in non-opioid SIA within the PAG. In their experiments, a non-opioid SIA paradigm was employed [4], in which an electric foot-shock was used as the ‘stressor’ and ‘analgesia’ was measured as the tail-flick latency to a thermal nociceptive stimulus. This form of SIA was reduced by both systemic and intra-PAG administration of the CB₁ receptor antagonist SR141716 (see [Chemical names](#)) but not by the administration of the CB₂ receptor antagonist SR144528 or the nonselective

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