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Molecular orbital differentiation of agonist and antagonist activity in the Glycine$_B$-iGluR-NMDA receptor

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ABSTRACT

We present various molecular electronic descriptors of agonists and antagonists for Glycine$_B$-iGluR-NMDA receptor with a view to identify computational measures that help differentiate between these two classes of biologically active compounds. We use as reference the glycine site in the NR1 subunit of the NMDA receptor (Glycine$_B$-iGluR-NMDA). Glycine$_B$-iGluR-NMDA receptor is important in learning and memory, and it is involved in various neurodegenerative diseases such as Alzheimer, Parkinson, and Huntington as well as in neuropathies such as schizophrenia and depression. We carried out quantum calculations at two levels, (1) B3LYP Density Functional (6-311G**), and (2) PM3 Hamiltonian for 168 molecules, of which 22 are agonists and 146 are antagonists. Regardless of the quantum mechanical level used we found a consistent signature of agonists versus antagonist action, the energy of the lowest unoccupied molecular orbital (LUMO). Effective differentiation of agonists and antagonists by a single molecular descriptor is seldom seen. We present a plausible electronic structure argument to rationalize these results.

1. Introduction

The members of the Glutamate family of receptors predominantly mediate excitatory neurotransmission in the mammal brain. These receptors comprise two families: (i) metabotropic receptors (mGluRs), not associated with ion channels, and (ii) ionotropic receptors (iGluRs), associated with cation channels. The latter have two sub-families, non-NMDA type, which are activated by AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) and Kainate ((2*S*,3*S*,4*S*)-3-(carboxymethyl)-4-prop-1-en-2-ylpyrrolidine-2-carboxylate), and the receptors activated by NMDA (*N*-methyl-D-aspartate).

iGluR-NMDA is of great pharmacologic interest because it is implicated in various neurodegenerative disorders, among others: Alzheimer's [1], Huntington [2], and Parkinson's diseases [3]. It is also implicated in neuropsychiatry illnesses such as schizophrenia [4] and depression [5], as well as in disorders provoked by alcohol [6] and psychotropic drugs [7]. iGluR-NMDA also takes part in pain sensation [8]. It is also one of the most important receptors for synaptic plasticity, essential for the formation of memories [9]. iGluR-NMDA is activated by glutamate, its natural agonist, but it requires the presence of glycine as co-agonist [10,11] and of modulators such as polyamines, Zn^{2+}, and the redox potential of the surrounding medium. When iGluR-NMDA is activated, the ion channels open and become highly permeable to calcium [12], but regulated by voltage and blocked by magnesium (Mg^{2+}) ions [13,14]. Additionally, this channel is a sodium (Na^+) symporter and potassium (K^+) antiporter.

iGluR-NMDA is a complex containing from 3 to 5 subunits [15], and in most configurations include at least one copy of NR1 subunit in addition to NR2 (A–D) [16] or NR3 (A–B) [17]. Subunit NR1 appears to be the key for the formation of the channel. NR1 contains the glycine or glycine$_B$-binding site, insensible to strychnine. NR2 contains the glutamate-binding site.

iGluR-NMDA contains several modulating sites that allow an ample number of allosteric interactions [18–21], polyamine sites, a Zn^{2+} site, a phosphorylation site, and a site modulated by different states of pH and redox potential. Various substances such

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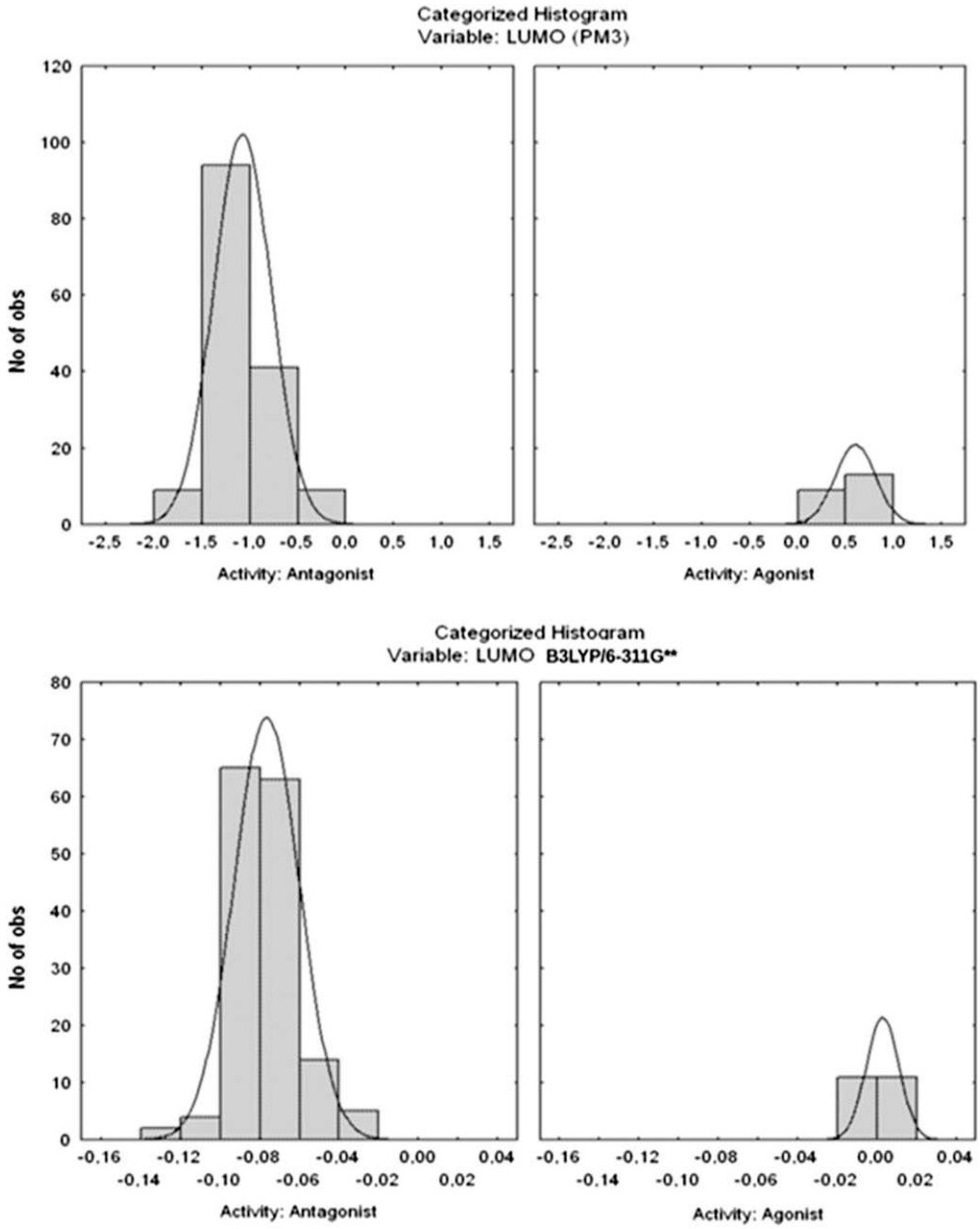

Fig. 1. Categorized histograms for LUMO energies calculated with PM3 and B3LYP/6-311G**. A clear separation between the group of agonist and antagonist molecules is observed, independently of the level of theory employed.

as Phencyclidine (PCP) and MK-801 [22,23] can block the ion channel.

This study is centered primarily on how molecular characteristics of chemical compounds that interact with the glycine$_B$ site can be used to discriminate between agonist and antagonist activity. Our primary objective is to identify possible factors that regulate both types of activity that could shed additional light into agonist and antagonist activity.

We selected from the literature 168 compounds with different activities (22 agonist and 146 antagonists) [24–46]. We conducted PM3 (Parametric Model 3) and Density Functional calculations of molecular properties.

2. Methods

We used as base the studies of Szymoniak and Gómez-Jeria [47,48] who found that an important QSAR molecular descriptor for *antagonists* of the glycine$_B$ site and of dopamine receptors is the energy of the lowest unoccupied molecular orbital (LUMO). We proceeded to determine if this held also for agonists and if LUMO energies could be used to discriminate between both classes of compounds. The LUMO parameter and others, such as HOMO and the total electronic energy were evaluated. We chose for this work the NR1 subunit of the ionotropic glutamate receptor activated by

Table 1
Kolmogorov–Smirnov and Shapiro–Wilk *W* normality test result to variables: Total Energy, HOMO and LUMO for PM3 and B3LYP/6-311** calculations.

Variable	Ks-d	*p*	Shapiro–Wilk *W*	*p*
Total Energy (PM3)	0.078	>0.2	0.977	0.00538
HOMO (PM3)	0.103	<0.1	0.953	0.00001
LUMO (PM3)	0.238	<0.01	0.801	0.00000
Total Energy (B3LYP/6-311**)	0.110	<0.05	0.954	0.00002
HOMO (B3LYP/6-311**)	0.077	<0.20	0.990	0.20976
LUMO (B3LYP/6-311**)	0.200	<0.01	0.847	0.00000

Table 2
Mann–Whitney *U* test, Wald–Wolfowitz runs test and Kolmogorov–Smirnov test to two-independent samples, results calculated with PM3 and B3LYP/6-311G**.

Variable	Wald–Wolfowitz runs test		Kolmogorov–Smirnov test	Mann–Whitney *U* test	
	Z	*p*-Level	*p*-Level	*U*	*p*-Level
HOMO (PM3)	−11.63	2.3E−30	$p < 0.001$	37	1.3E−13
LUMO (PM3)	−13.02	0.0	$p < 0.001$	0	3.6E−14
HOMO (B3LYP/6-311G**)	−0.86	4.9E−01	$p > 0.10$	1443	2.8E−01
LUMO (B3LYP/6-311G**)	−13.02	0.0	$p < 0.001$	0	3.6E−14

N-methyl-D-aspartate, iGluR-NMDA ($Glycine_B$). For this purpose we collected compounds that bind specifically to this site [24–46], agonist and antagonists of iGluR-NMDA-$Glycine_B$. We chose this due to the great diversity and complexity of the available compounds' structures, in such manner as to include a wide sample of molecular species. The only inconvenience is the few number of agonists (22) compared to antagonists (146). Furthermore, only 3 are total agonists and 19 are partial agonists. The search for agonists and antagonists appears to be exacerbated by the fact that no model exists that can predict a priori a compound's activity. This failure creates hurdles for commercial interests in creating drugs that can inhibit the receptor to protect against the noxious effects of over expression or over excitation.

All molecular structures were originally modeled with the commercial software WebLab Viewer Pro version 4.0 from Accelrys [49]. The structures were initially optimized with the MM2 force field [50] using Molecular Modeling Pro version 5.2.4. Then we optimized the geometry for each structure quantum mechanically by DFT using B3LYP/6-311G**. Subsequently we calculated the HOMO and LUMO orbital energies and total electronic energy. All calculations were done for the neutral molecules.

The data for each of the calculated parameters is evaluated for normal distribution statistics. This was done with two types of statistical tests conducted with the statistical package STATISTICA 6.0 [51] Shapiro–Wilk [52] and Kolmogorov–Smirnov [53]. The general idea is to investigate if the data sets calculated for agonists and antagonists are a type of bimodal distribution, which may allow the differences of the means to be used to distinguish between agonists and antagonists. The vast majority of variables do not follow a normal distribution.

Consequently, two tests were applied to each of the distributions as a means to evaluate them as independent samples. These tests were: Wald–Wolfowitz runs, Kolmogorov–Smirnov two-independent samples [54] and Mann–Whitney [53]. The tests were conducted with the non-parametric statistical package program in STATISTICA 6.0. Before the tests were applied, bioactivity was assigned as a categorical variable, either agonists or antagonists, taken the partial agonists as belonging to the agonist set.

Structures were optimized at each level of theory PM3 semi-empirical Hamiltonian [55] and B3LYP Density Functional Theory 6-311G** basis set using Jaguar 6.5 [56] we optimized the structures and calculated the total energy, the HOMO and LUMO and other molecular descriptors than shown in Supplementary material.

3. Results and discussion

Normality and variable correlations for LUMO energies are shown in Fig. 1. This variable clearly has a non-Gaussian distribution. Shapiro–Wilk and Kolmogorov–Smirnov tests were used to evaluate whether or not the samples are normally distributed. The null hypothesis H_0, that the samples are normally distributed, is shown in Table 1, the hypothesis H_0 is rejected. The data is not normally distributed (Shapiro–Wilk $p < 0.02$, Kolmogorov–Smirnov $p < 0.2$). Thus, all the variables are not normally distributed. Instead in what follows we conduct a non-parametric statistical test. As shown in Fig. 1 the LUMO energy distribution, with PM3 and B3LYP/6-311G**, are clearly bimodal distributions. According to the alternate hypothesis H_1; the data is not normally distributed. For the HOMO energy, calculated with B3LYP/6-311G** (see Table 1), the null hypothesis H_0 is accepted due to its low statistical significance, for this variable the data distribution corresponds to a normal distribution. All other variables, i.e., LUMO energy, Total energy calculated with PM3 and B3LYP/6-311G** and HOMO energy calculated with PM3, do not have a normal distribution. As shown in Table 1 the LUMO energy is very significant in these tests, in such a way that it appears that its distribution, which is confirmed by the graph, is bimodal.

The non-parametric statistics, groups-independence tests, were performed and the variables proved not to be normal. The null hypothesis is rejected, H_0, when the probability for significant differences is $p > 0.05$ and the two subsets are identical and therefore belong to a single population. When the probability for significant differences is $p < 0.05$ the alternate hypothesis is

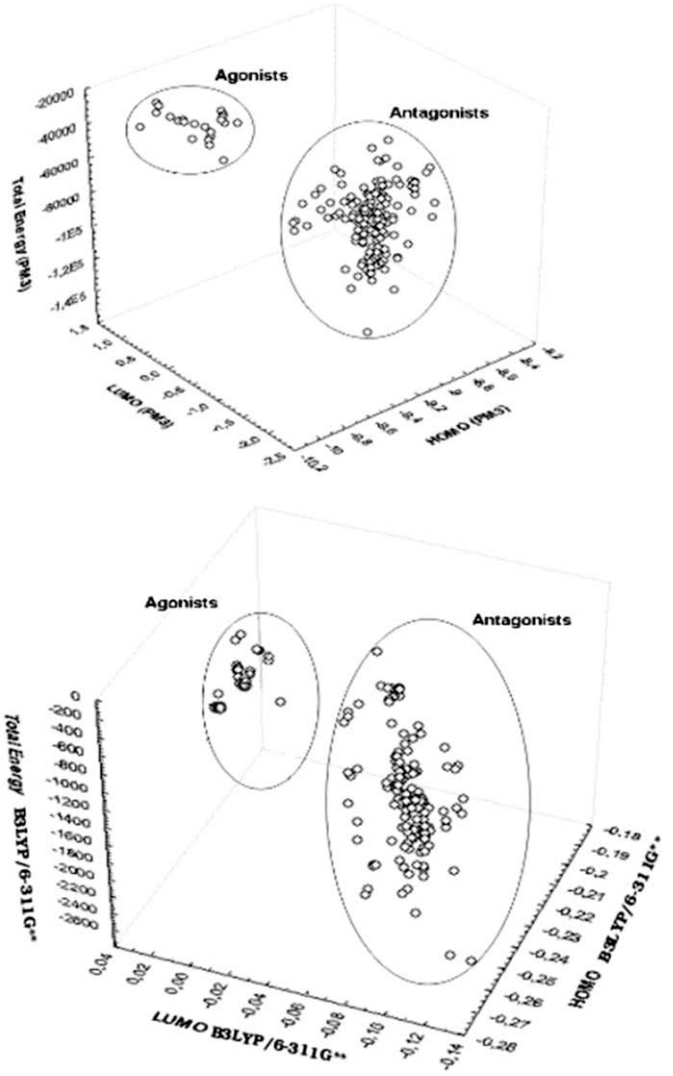

Fig. 2. 3D scatterplot Total E, LUMO and HOMO calculated at the PM3 and B3LYP/6-311G** levels.

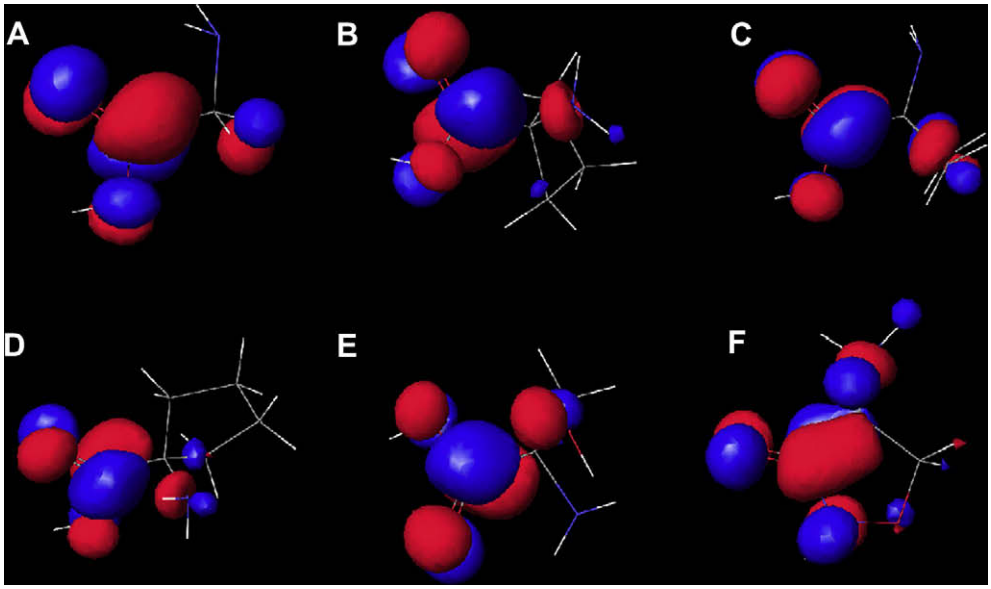

Fig. 3. LUMO orbitals for agonist molecules: (A) Glycine, (B) ACBC, (C) ACPC, (D) Cycloleucine, (E) Alanine and (F) D-cycloserine. See additional material.

accepted; the two subsets come from two different populations. Table 2 shows again that the variable that best discriminates between the two groups, agonist and antagonists, remains the LUMO energy. This can be seen in the categorized histograms Fig. 1, where for the LUMO variable, using various levels of theory (PM3 and B3LYP/6-311G**), yields two very distinct groups. In Fig. 2 we present three-dimensional plots in which it is also possible to observe two groups (agonists and antagonists), well differentiated for the calculated variables with PM3 and for the calculated variables with B3LYP/6-311G**.

These results might be used to support an electronic hypothesis regarding the site of $Glycine_B$ on NMDA receptors, and the role that such electronic effects play on the agonistic and antagonistic activities of the various compounds on the receptor. One might

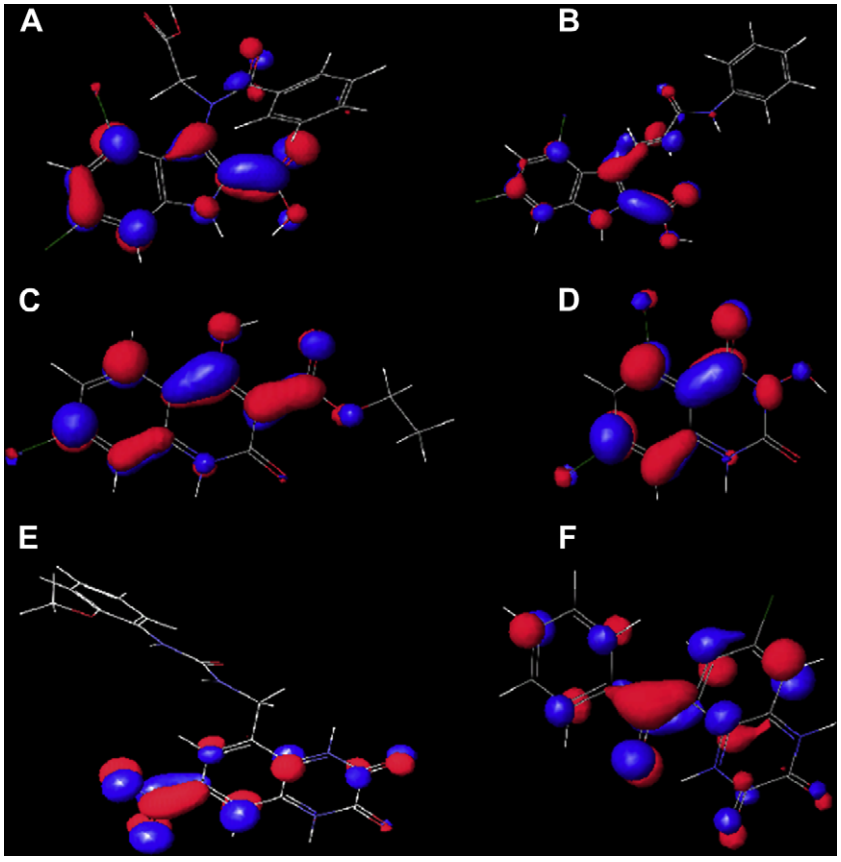

Fig. 4. LUMO orbitals for antagonist molecules: (A) 3aceg, (B) 1thq, (C) AAA, (D) b2hqd, (E) bgaqdad8b and (F) e7eqx. See additional material.

venture to say that it is a necessary, but not sufficient, condition to possess LUMO energy significantly higher than the average antagonist LUMO energy for agonist activity to be displayed by a molecule. This assertion does not depend on the specific level of theory used to calculate the LUMO energies. However, one must also consider such a necessity to be correlated to other factors, such as the structural dissimilarity between the two sets of compounds as discussed in the conclusion section.

According to other authors [57–61] the results presented here show that the activation of the NR1 subunit (agonist activity) is possibly mediated by the LUMO orbital. In this work using multivariate statistical models, the variable LUMO energy was an important factor in the correlation functions obtained for agonistic activity in this model. Here we prove that the LUMO energy is a relevant factor for the discrimination between agonists and antagonists activity. Unlike previous studies, the results of our study of the Glycine$_B$ site indicate that the LUMO is an important factor for either agonist or antagonist affinity for its pharmacological target. For activation between the Glycine$_B$ site, it appears necessary that the ligand contains a high lying orbital to receive electrons from the receptor, and that most likely the mechanism involved is affected by a high-energy LUMO, at least higher in energy than the highest LUMO of all known antagonists.

Finally, we look for geometric patterns in the LUMO orbital contours for each set, to determine the orbital position of these molecules within agonists and antagonists. As shown in Figs. 3 and 4, for agonist and antagonists there is a very distinct LUMO energy pattern. Antagonists' LUMO orbitals are generally present in the ring system for each molecule, thus, generally the LUMO are the delocalized π orbitals of these rings. The LUMO energy varies depending on the substituent present around these rings, as indicated in the crystal structure and in other previous studies [43,48]

Antagonists have π–π stacking interaction with the receptor [48]. It is quite possible that π–π stacking interactions are relevant for antagonists, possibly through the LUMO orbital in the antagonist interacting with the low energy HOMO orbital in the receptor. Thus, this interaction might be a key feature for antagonists binding with the receptor. So if molecules are designed with these conditions and with the appropriate substituent effects to fine-tune the interaction energy with the receptor HOMO orbital energy, it is possible to obtain new substances with higher values of activity.

In addition agonists also have a clear pattern. LUMO orbitals are located in the carbonyl and carboxyl groups of the agonist molecules. Accordingly, interaction might be due to electron exchange, with proton transfer probably playing a dominant role in the activation. Is clear that besides the LUMO energy other parameters, such as the proper sterics, shape effects, solvation effects, are necessary for the orbital interaction to take place.

As with the antagonists, it is vital to discover the full set of necessary agonist features, for designing new drugs that interact with the pharmacological target more effectively. In general, it appears that for glycine$_B$ sites, the lowest unoccupied molecular orbital is a regulating factor for ligand activity type. This result should be investigated in other receptor types and their ligands, and it is the subject of future research.

4. Conclusions

The present work points to the importance that unoccupied molecular orbitals may have on the type of activity, agonistic versus antagonistic, exhibited by a compound in the glycine$_B$ site. Lower energy LUMOs are a feature of antagonists, while agonists clearly have distinctly higher level LUMOs. The result is independent of the level of theory used in calculating energies.

LUMO energies for antagonists are lower than for agonists by about 0.08 Hartrees (50 kcal/mol) on average using the B3LYP method (see Fig. 2), indicating that a given range of orbital energy matching is required for electron density to be shared with and to activate the glycine$_B$ site of NMDA. The LUMOs of agonists are also more tightly localized than those of antagonists (Figs. 3 and 4), perhaps indicative of a more specific type of electronic interaction is at play. Both features find correspondence in the standard expression for a first order quantum mechanical perturbation. Thus, if we view the unperturbed glycine$_B$ site of NMDA electronic wavefunction as the n-th eigenstate of the unperturbed Hamiltonian

$$H_0\Psi_n^0 = E_n^0\Psi_n^0 \tag{1}$$

we can write the presence of the agonist as a perturbation V on the Hamiltonian H_0 of the active site, and the new energy levels and eigenstates of the perturbed Hamiltonian are again given by the Schrödinger equation:

$$(H_0 + V)\Psi_n = E_n\Psi_n \tag{2}$$

The first order correction to the localized glycine$_B$ site electronic wavefunction

$$\Psi_n = \Psi_n^0 + \Psi_n^1 \tag{3}$$

is given by

$$\Psi_n^1 = \sum_{k\neq n} \frac{\left\langle \Psi_k^0 | V | \Psi_n^0 \right\rangle}{E_n^0 - E_k^0}\Psi_k^0 \tag{4}$$

and its new electronic energy is given by

$$E_n = E_n^0 + E_n^1 = E_n^0 + \left\langle \Psi_n^0 | V | \Psi_n^0 \right\rangle \tag{5}$$

Eq. (4) shows that the largest contribution to the perturbation of the original active site wavefunction comes from the lowest lying excited electronic state whose overlap with the agonist perturbation V (numerator) is the largest. Since glycine and the majority of agonists are electrophilic compounds we can argue that such localized glycine$_B$ active site excited state is electron rich and its local energy E_k^0 should closely match the LUMO energy of the agonist. Finally, Eq. (5) suggests that for the perturbation to be significant the original wavefunction must yield a good overlap matrix element with the agonist perturbation Hamiltonian V.

In conclusion, amongst molecules that show biological activity in the glycine$_B$ receptor, the LUMO energy is sufficient to discriminate between agonistic versus antagonistic activity. However, the LUMO energy is not a sufficient condition to guarantee biological activity, as other factors, such as solvation and sterics often play an important role. This point was tested with 7 inactive compounds resembling one antagonist family, including their LUMO energies, which show no biological activity whatsoever (see Supplementary material).

The data included here provides some basis for the idea that in order for a molecule to bind to the site of Glycine$_B$ and activate it, its LUMO energy must be higher than the LUMO energy for the average antagonist. One possible interpretation is that for receptor activation to take place electron density exchange between the receptor and the ligand, with a specific LUMO energy, might be necessary. One might speculate that the activity of the agonist might be modulated by the higher LUMO in such a way that interaction with the receptor's HOMO leads to excess energy that can be spent in searching for the correct conformer leading to strong binding geometries to the active site. An alternate

explanation is that the two classes of compounds included here, agonist versus antagonist compounds, have significantly different chemical structures, as separate chemical compound classes, that no structural overlap exists between them to allow for similar LUMOs to be found amongst the two populations. To tests this alternate explanation we searched the literature exhaustively for antagonists that chemically resemble the existing agonists set. The search for this counter-example was, however, unsuccessful. Perhaps in a different class of receptors such test can be carried out. Nevertheless, the present results might shed some light into the mechanism of receptor activation by agonists.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.01.013.

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