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Review

Usage of substances and their dosage regimes in pharmacological animal models of schizophrenia

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Abstract

Background: Contrary to what is generally thought schizophrenia is a very common mental health issue. For this, several animal models are used to assess the illness in order to develop a definitive. The most widely spread paradigm is the use of pharmacological models. **Aim:** The aim of this review is to display which are the most used insults for the assessment of social behaviour related negative symptoms in animal models as well as to ascertain which is the most adequate regime. **Design:** Literature review. **Methods:** PubMed database was used for this article by the search of the indexed “schizophrenia”, “animal models”, “social behaviour” and “negative symptoms” descriptors. With the exception of a single article due to it value this review is based on articles from 10 years onwards. Besides, only clinical trials and reviews written in English or Spanish and that had laboratory rodents as target population were accepted. **Results:** The studies assessed agree that pharmacological models (specially those regarding the NMDA receptor antagonists) are a valuable means for the experimental investigation of negative symptoms in schizophrenia with the necessity to emphasise that only some negative symptoms (anhedonia and social interaction, mainly) can be experimentally assessed. **Conclusions:** There is not enough evidence regarding the fours aspects of this review. PCP, Ketamine or MK-801 in sub-acute dosage regimes are currently the most indicated insults to mimic schizophrenic symptoms in rodents, although further research in needed, albeit other substances are valuable as well.

Key Words: schizophrenia, animal models, negative symptoms, social behaviour.

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Introduction

Albeit it is said that schizophrenia is a very uncommon disorder, scientific evidence currently declares that statement as wrong. Over the years, it has been agreed that schizophrenia affects about 0.5-1% of the population worldwide with little incidence shown in genre or ethnicity. (Hida, Mouri, & Noda, 2013). According to CIBERSAM, the Centre of Biomedical Investigation in Mental Health Network 400,000 people suffers schizophrenia in Spain ("Esquizofrenia @ cibersam.es," 2015).

The cause of this disorder remains unsettled and it is unlikely to find a clear, definitive answer to this particular question in the forthcoming few years. The possible given explanations of the origin of the disorder are innumerable; some authors defend the origin as purely genetic while most of the scientific community has arrived to a consensus to declare the origin as multifactorial, provided the available data (Marsden, King, & Fone, 2011). What multifactorialism declares is that, albeit schizophrenia has a clear genetic background, an environmental trigger is necessary to set it out. The possible involved genes are ridiculously abundant with many different of them showing an identical or very similar phenotype while their action mechanism is absolutely non-correlative. At the same time, the necessary secondary (environmental) factors are varied (e.g., perinatal immune activation, adolescent stress, drug use or abuse in certain periods of life (Hida et al., 2013). Therefore, the whole explanation of its origin still remains unclear, thus tremendously broadening the investigation field of the issue to a virtually unmanageable extent (Neill, Harte, Haddad, Lydall, & Dwyer, 2014).

Schizophrenia has a wide range of symptoms, which could be classified into three groups i.e., positive symptoms, negative symptoms and cognitive deficits. We classify into positive symptoms the ones that distort the reality (e.g., delusions, hallucinations, paranoia); into negative symptoms those interfering in movement, speech or interest (e.g., affective flattening, anhedonia, social withdrawal); and into cognitive deficits those consisting in impairments in attention, learning or memory (Porsolt, Moser, & Castagné, 2010).

While there has been an effective treatment for positive symptoms for over half a century i.e., typical and atypical antipsychotics, any pharmacological treatment for negative symptoms and cognitive deficits remains technically undeveloped (Wilson & Koenig, 2014). Most of the antipsychotic drugs used for the treatment of positive symptoms rely on the manipulation of the dopaminergic system (Barzilay et al., 2011), which causes multiple side effects and even the worsening of the negative symptoms and cognitive deficits (Porsolt et al., 2010). The lack of efficacious management for negative symptoms prevents the patient to maintain a normal life because of its multiple social-impeding traits.

As stated before, there are many changes in social behaviour caused directly or indirectly by negative symptoms. Anhedonia refers to a decrease in the capacity to feel pleasure; affective flattening refers to apathy in various situations involving stress; and social interactions refers to the amount of time a group of animals spend having contact or any kind of relationship between each other. It is not difficult to deduct that the impairment of any of these social traits may suppose an enormous handicap in the ability to maintain any kind of competent social behaviour among the intervening sufferers (Wilson & Koenig, 2014).

A large proportion of schizophrenia research is made by the use of animal models and, therefore, the treatments used until current times to diminish the positive symptoms have been discovered thanks to the investigations developed with animal models. It is crucial to know whether the models used for that purpose have a real translational validity, that is, the results shown in animals could happen as well in humans with similar mechanisms. Scientific community all over the world mainly use one or several of the following three models: pharmacological, genetic or environmental model.

Pharmacological models rely on the injection or administration of drugs in the researched animal to provoke schizophrenia-like symptoms; genetic models rely on the manipulation of the genes of the model animal to cause schizophrenia-like symptoms; and environmental models rely upon the manipulation of the social structure of the animal to provoke

schizophrenia-like behaviours in the researched animals. Furthermore, it is of utter importance to comprehend that there is no such thing as a schizophrenic rodent, so the perceived symptoms are only approximations, most of the time very close ones, to those observed in humans (Micale, Kucerova, & Sulcova, 2013; Sarnyai, Jashar, & Olivier, 2014).

The insults utilised in the research of negative symptoms are both pharmacological and environmental. As said before, there are studies regarding positive and negative symptoms. Notwithstanding, negative symptoms don't have a current solution and are worsened by the treatment used for positive symptoms. Although there is no present treatment for negative symptoms, the study of the cluster of manifestations caused by these is of utter, crucial importance for the search of a future treatment. Currently, several insults are used in the research with animals to incite negative symptoms that cause changes in social behaviour typical of schizophrenia. However, of the whole cluster of manifestations seen in schizophrenia and its assessment and study in a laboratory with animal models is, at least, complicated. Of these, anhedonia and social withdrawal are the only ones that, currently, can be measured in an objective, scientific manner (Ellenbroek & Cools, 2000).

It is an undeniable truth that there is not a hundred percent reliability on the research accomplished in animal as their anatomy and physiology are not a hundred percent human alike. Nevertheless, it is generally accepted the research in animal as valid only if those fulfil some requirements. Hereupon the requirements will be presented:

-Construct validity: isolation of the mediating processes and their neural substrates across humans and species used in experimental models for schizophrenia.

-Cross species homology: always having in mind the differences presents between humans and rodents or primates.

- Parametric properties allowing for quantification of deficits and detection of drug defects.

-Reliability, reproducibility across laboratories, potential standardisation and automation of the core paradigm, relatively straightforward introduction

and validation of the procedure.

-Respect for ethics, animal welfare and economic sustainability of the project.

-Translatability: similarities found in common neural substrates engaged in animal and humans as established both by conventional behavioural readouts and by studies of neural substrates with techniques usable in humans (Millan & Bales, 2013; Neill et al., 2010).

This last one is of utter importance to affirm that a study assessed in rodents is valid for humans as it is based on animal models based on the pathways present in human brains and physiology; without this no possible extrapolation would be valid at all.

Since nursing professionals have contact with multiple people in their daily practice, it is very likely to find patients with schizophrenia turning to nursing services. The lack of knowledge and understanding about the subject and his illness might be a big problem, especially if nurses want to provide a good holistic care of the patient. For this reason, and due to there is a substantial knowledge about the treatment to ameliorate the positive symptoms, the main objective of this review is to widen the insight or clarify what is known about the insults for negative symptoms which are the ones that really affect the social behaviour. For this, the work will be based in the search of experiment and studies made with animal models of schizophrenia mainly using rats, mice and/or other kind of rodents. It is expected as the hypothesis for this research that the most typical insult used is pharmacological and environmental ones, especially those that affect hypothalamus or hippocampus.

Objectives

The main objective of this review is to know which are the most used insults for the assessment of social behaviour related negative symptoms in animal models.

Specifically, as one of the secondary objectives, it is targeted to ascertain which dosage regime is the most effective in mimicking the

symptoms seen in laboratory rodents as well as to compile and resolve the different kinds of insults and their effects in anhedonia and social interaction.

Methodology

Article design

It is an evaluative bibliographic review in which light is shed over which are the most used insults for the assessment of social behaviour related negative symptoms in animal models and which dosage regime is the most effective in mimicking the symptoms seen in laboratory rodents, as well as to compile and resolve the different kinds of insults and their effects in anhedonia and social interaction; based on published scientific evidences.

Inclusion criteria

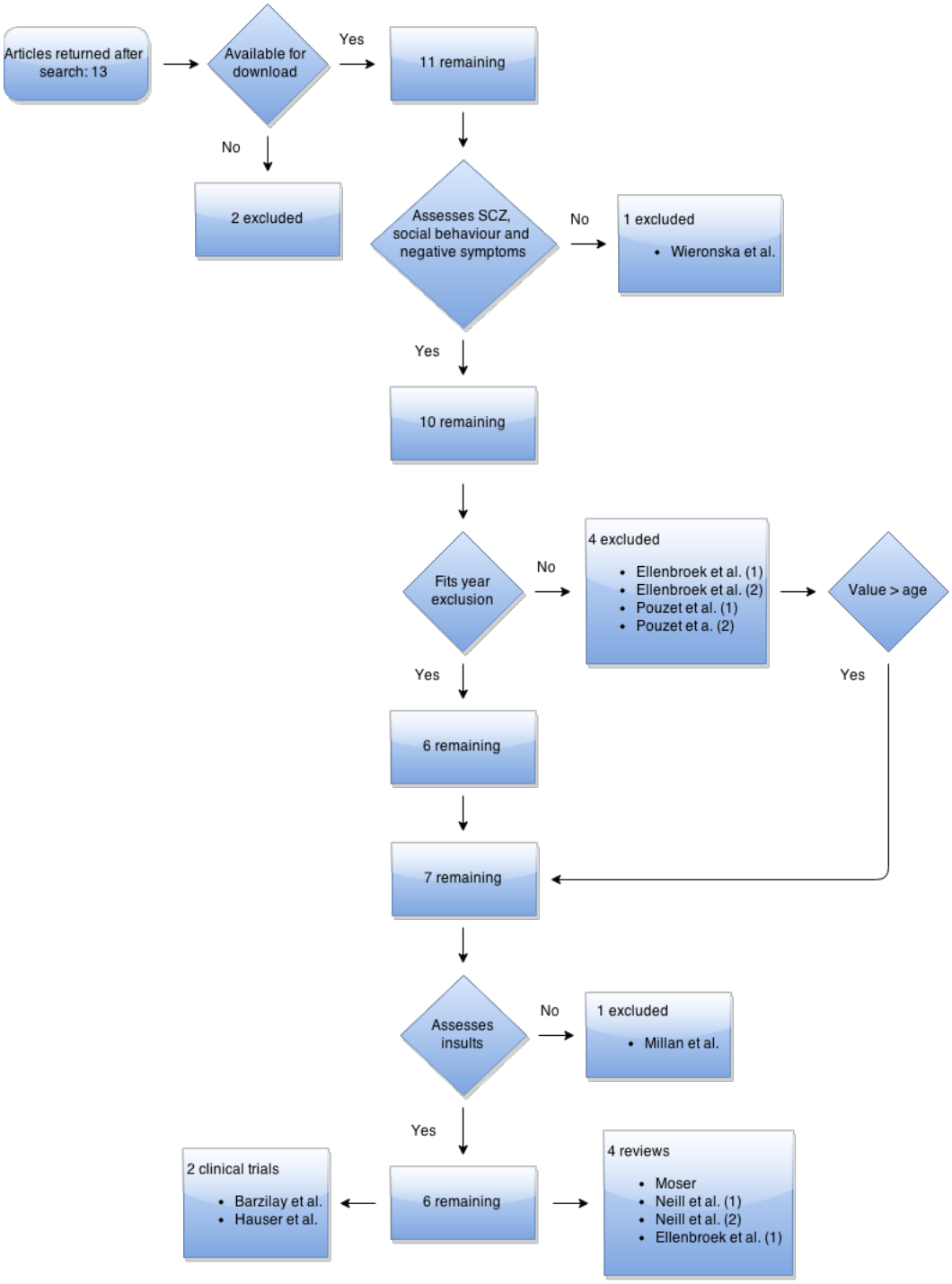
- Accepted publications: original articles (clinical trials)
- Studied population: laboratory rodents (several strains of laboratory mice and rats)
- Variables: different objectively measurable manifestations of negative symptoms
- Social behaviour related publications

Search strategy

In order to perform the search of the information required for this bibliographic review the search engine PubMed was used. Four keyword sets were used “schizophrenia”, “animal models”, “social behaviour” and “negative symptoms” all of them linked with the logic operator “AND” upon their indexation with MeSH controlled vocabulary thesaurus (Figure 1).

This pathway was used: (("schizophrenia"[All Fields] AND "negative symptoms"[All Fields]) AND "animal models"[All Fields]) AND "social behavior"[All Fields] AND ("loattrfull text"[sb] AND English[lang])

The search was limited to those articles available in full text in the UPV/EHU network and to those written in English or Spanish.. Due to the fact that all the articles returned after major searching process were reviews or research reports no exclusion filters were applied.



[1] **Figure 1.** Flowchart describing the selection process.

Authors	Year	Type	Schizophrenia	Animal	Symptoms	Social Behaviour	Selected
Millan et al.	2013	Guide/ review	Yes	Unspecified rodents and primates	Negative symptoms regarding social cognition	Social recognition/ preference	No
Moser (See Table 1)	2013	Review	Yes	Mice and rats of several strains	Negative symptoms	Social interaction	Yes
Neill et al. (A) (See Table 2) (See Table 3)	2010	Review	Yes	Mice and rats of several strains	Negative and cognitive dysfunction	Social interaction	Yes
Neill et al. (B) (See Table 4) (See Table 5)	2014	Review	Yes	Unspecified	Negative symptoms	Social withdrawal and anhedonia	Yes
Ellenbroek et al. (1)	2000	Review	Yes	—	Negative symptoms	Social withdrawal, anhedonia, avolition and apathy	Yes
Wierońska et al.	2013	Clinical trial	Yes	Male albino Swiss mice and male Wistar rat	Positive, negative and cognitive symptoms	Social interaction	No
Barzilay et al.	2011	Clinical trial	Yes	Male C57BL/6 mice	Negative symptoms	Social preference	Yes
Hauser et al.	2009	Clinical trial	Yes	Transgenic th(tk-)/th(tk-) mice, Xenopus lævis oocytes and Sprague-Dawley rats	Positive, negative and cognitive symptoms	Novel object recognition, pre-pulse inhibition.	Yes
Pouzet et al. (1)	2001	Clinical trial	Yes	Wistar rats	Positive and negative symptoms	Social interaction	No
Pouzet et al (2)	2001	Clinical trial	Yes	Wistar rats	Positive and negative symptoms	Social interaction	No
Ellenbroek et al. (2)	1996	Clinical trial	Yes	Macaca fascicularis and Wistar rats	Negative symptoms	Social isolation	No

[1] **Figure 2.** This table shows the articles available to download after the search. It briefly explains the content of each publication. The selected (green) or discarded (red) articles are shown in the last column with a colour code. The possible flaws of each article are marked in purple. Although the first Ellenbroek et al. article has two marked flaws it still has been accepted due to its magnificent value, as it was one of the first reviews explaining the bases of the research of negative symptoms. Moreover, the flawless Wierońska et al. article was discarded due to its lack of value for this review. The (A) and (B) shown in the articles referring to Neill et al. have the only purpose to serve as an accessible visual distinction between them.

A total of eleven documents were available for download. The ones authored by Ellenbroek et al. in 1996 and Pouzet et al. were excluded due to their old publishing date (1996-2001). Concurrently, the two articles released by Pouzet et al. were not useful as they were mere clinical trials of experimental drugs potentially used in schizophrenia if the fact that they were proved useless did not happen. Although the old age of the review released in the year 2000 it has initially been included due to its great value in shedding light to this extensively unaddressed issue. Furthermore, an article authored by Wierońska et al. was excluded because it shows no effects on negative symptoms. Additionally, the article published by Millan et al. was also excluded as it is a guide on how to perform a study in schizophrenia; being of utter interest for the initial search but of none interest for the main body of the review as it gave no evidence of tests accomplished in animals. Once the screening was performed a total of 6 articles were selected and 5 articles rejected. (Figure 2)

Results

Moser accomplished a deep and broad review about negative symptoms based in several strains of rodents in 2014 in which a total of nine paradigms were summarised (methylazoxymethanol (MAM), maternal/foetal malnutrition, maternal infection/immune challenge, ventral hippocampus lesions, prefrontal cortex lesions with nerve growth factor (NGF), post-weaning social isolation rearing, obstetric complications, postnatal polyriboinosinic-polyribocytidilic acid (polyI:C), and glutathione deficiency (GTD)); with only a few being useful for the research of negative symptoms. The publications summarised in this revision are itemised in the following manner (Figure 3):

MAM models were only evaluated on social interaction showing a significant reduction in social interaction after spending 10 minutes in low light in an unfamiliar arena between pairs of rats treated similarly. This model was only supported by a couple of articles. Moreover, each article uses a different strain of rats with similar results.

Maternal/foetal malnutrition models showed no changes in social interaction duration after the subject spending 10 min in interaction test with younger untreated mouse in an unfamiliar arena; ought to be said that this particular paradigm only included a single study, which used two strains (C57BL/6J and 129/SvJ) of unspecified sex.

Authors	Year	Type	Schizophrenia	Animal	Symptoms	Social Behaviour	Selected
Moser	2013	Review *(See Table 1)*	Yes	Mice and rats of several strains	Negative symptoms	Social interaction	Yes
Neill et al. (1)	2010	Review *(See Table 2)* *(See Table 3)*	Yes	Mice and rats of several strains	Negative and cognitive dysfunction	Social interaction	Yes
Neill et al. (2)	2014	Review *(See Table 4)* *(See Table 5)*	Yes	Unspecified	Negative symptoms	Social withdrawal and anhedonia	Yes
Ellenbroek et al. (1)	2000	Review	Yes	—	Negative symptoms	Social withdrawal, anhedonia, avolition and apathy	Yes
Barzilay et al.	2011	Clinical trial	Yes	Male C57BL/6 mice	Negative symptoms	Social preference	Yes
Hauser et al.	2009	Clinical trial	Yes	Transgenic th(tk-)/th(tk-) mice, Xenopus lævis oocytes and Sprague-Dawley rats	Positive, negative and cognitive symptoms	Novel object recognition, pre-pulse inhibition.	Yes

Figure 3. This table shows the articles available to download after the search. It briefly explains the content of each publication. The selected (green) or discarded (red) articles are shown in the last column with a colour code. The possible flaws of each article are marked in purple. Although the first Ellenbroek et al. article has two marked flaws it still has been accepted due to its magnificent value, as it was one of the first reviews explaining the bases of the research of negative symptoms. For more information about the reviews see the figures above marked.

Maternal infection/immune challenge models used three possible insults in the studies: influenza virus in gestational day (GD) 9.5; polyI:C in GD9, 12.5 and 17; and bacterial endotoxin lipopolysaccharide (LPS) in GD9.5. All studies

except one showed some kind of deficit or change in social interaction after spending a ranging time from 5 to 10 minutes in the testing arena. In this particular case 5 studies were reviewed and their concoction included at least 4 (3 mice and 1 rat) different strains (in some cases both sexes were reviewed). In a particular study neither sex nor strains was specified and that was the study that showed no difference compared to saline.

Ventral hippocampus lesions were assessed with a total of seven studies, which used ibotenic acid, lidocaine, polyI:C or tetrodotoxin to cause the lesion in postnatal day (PD) 7. The tests consisted in a period of time ranging between 5 and 10 minutes in the testing arena. The results varied from no changes at all to increases in aggressive interactions of ~290%; has to be said that most of the (6 out of 7) showed a variation in social interaction of at least ~20%, which might be pretty significant. This particular group assessed uniquely rats of Sprague-Dawley and Wistar strains of both sexes (6 studies assessed males and a single study assessed both sexes).

Prefrontal cortex lesions with NGF paradigm was evaluated with a single study utilising only male Sprague-Dawley strains rats, which shown ~18% reduction in active interaction and ~14% reduction in mean proximity duration after spending 10 minutes in low-light unfamiliar arena between pairs of rats similarly treated.

Post-weaning isolation rearing model was examined with a total of four studies using male and female rats of the Sprague-Dawley strain, Lister hooded strain male rats, and male ICR strain mice; which were isolated in a time varying from weaning to PD48 (most of them after PD21). They were tested from 5 to 10 minutes in social interaction testing arenas and thereafter showing changes in contact duration and aggressive behaviour.

No obstetric complication studies have been shown in the table provided by the author. Moreover, neither of the articles reviewed and referenced assessed negative symptoms in schizophrenia.

Postnatal polyI:C was once again evaluated with a single study using male ICR strain mice that showed no deficits in social interaction after the animals were tested fourfold for 5 minutes in a resident-intruder paradigm.

No GTD studies are shown in the table of this review although there is a title dedicated to it. In this case it is not released the strains and sexes of the animal assessed.

This article is useful for this review as it extensively reviews the status of the issue at the moment. Moreover, it shows that pharmacological and environmental insult models are useful to successfully mimic the chemical cerebral disruptions seen in schizophrenia in mammals. It is important to have in mind that the author himself states that very few studies exist to firmly conclude, especially when considering social interaction (Moser, 2014).

There is a review published by Neill et al. in 2010 that focused in the relation between N-methyl-D-aspartate (NMDA) receptor and negative symptoms. In that review, the use of NMDA receptor antagonists assessed shows what causes social deficits is similar to those seen in schizophrenia using PCP, MK-801 or ketamine either acutely or subchronicly in doses ranging from 2 to 10mg/kg daily or bi-daily from 3 to 15 days.

In 5 of the 9 studies rats (male and female (hooded-Lister uniquely) from Sprague-Dawley, Wistar, Long-Evans and hooded-Lister strains, without mixed-strain studies) were administered PCP from 45min to 6 weeks before the test and all of them were tested for 10min in a social interaction arena. PCP was shown to decrease social interaction in all the tests.

In 2 of the 9 studies MK-801 was used in male Sprague-Dawley and Wistar rats (one for each strain, no study assessed both strains). They shown social interaction deficits after being acutely administered 0.2mg/kg MK-801 (Sprague-Dawley) or subchronicly 0.13mg/kg for 14 days (Wistar) and tested 30 (Sprague-Dawley) to 45min (Wistar) later for 10 (Wistar) to 30min (Sprague-Dawley) in a social interaction arena.

Repeatedly, in another 2 of the 9 studies ketamine was used in male Sprague-Dawley and Wistar rats (one for each strain, no study assessed both strains). An acute low dose (7mg/kg) of ketamine has been reported to reduce social interaction in adult male Wistar rats after being tested for 10min in a social interaction arena (30min between the administration and the test). A subchronic administration with ketamine of 30mg/kg for 5 days in Wistar rats shown to reduce nonaggressive behaviour after the subjects being tested for 7min in a social interaction arena (10 days between the last injection and the test).

This publication shows and argues how validity is composed. Concurrently, as some of the other papers do, concludes that the best drug regime is subchronic PCP although this only being a “relatively valid model”. This translatability issue is utterly important for this paper because it shows that rodent models are directly translatable to humans without necessarily requiring specific tests in humans, if proper conditions are fulfilled. In any case, the authors agree that there exist limitations of the NMDA and that further investigation is needed (Neill et al., 2010).

As well as the review published by Neill et al. in 2010 another paper with regard to the subject in 2014. In this extensive review two brief tables appear with numerous data concerning NMDA receptor antagonists and negative symptoms caused by them in testing animal (it is stated that normally rodents are used but it does not specifically say which ones were used). In both tables can be seen the dosage/treatment applied to the animal and the test/task there were put in but in none of them it is found the data supporting the published outcome.

On the one hand, of the two tables appearing in the review, the first one is in connection with cognitive symptoms, which has no relevance for this work. On the other hand, the second has anhedonia (a negative symptom) as an axis. It summarises the outcomes of NMDA receptor antagonist use in relation with anhedonia in four possible treatment lengths: acute, post-acute, chronic and post-chronic. It shows that acutely no used NMDA receptor (PCP, MK-801 or ketamine) causes ant decrement in hedonic behaviours related to consumption. Post-acutely, it displays the reduction of hedonic behaviours related to sucrose consumption 24h after administration of high (15mg/kg and above) doses of PCP. It must be said that these high doses causing anhedonic effects are much higher than the ones needed to cause cognitive effects. Chronically, repeated exposure to low (2mg/kg) dose of PCP reduces reward thresholds. Finally, post-chronically, 5mg/kg of PCP given bi-daily for a week, followed by an unspecified washout period, has no effect of hedonism shown by the test animal. Notwithstanding, a 7 bi-daily 7.5mg/kg dose of PCP without the washout period produced an ephemeral decrease in sucrose consumption.

This study can be considered as a continuation of the previous one as it complements the information given by the aforementioned article in this review. When assessing social behaviour with substances, as it is mainly done, it is utterly important to know which drug dosage regimes and periods (subchronic)

are the most proper; without this being correctly fulfilled none translatability can be possible (due to the excessive or insufficient effects of the employed insults); thus no effect predicted in humans might be valid. At the same time, they state that NMDA model is only validated for asociality (thus for social interaction) and not for anhedonia (Neill et al., 2014).

Barzilay et al. published in 2011 a research report in which some adult intracerebral stem cells were transplanted to male C57BL/6 mice while other received a sham or fake transplantation. After the procedure mice were administered PCP in 0.9% NaCl at 10mg/kg subcutaneously (s.c.) for 14 days. After the last PCP injection mice were tested in the open-field test and showed no difference in total locomotor behaviour. 21 days after the last injection of PCP mice were tested for social preference. Control saline-injected mice showed "significant preference" towards the social stimulus while PCP tested mice that underwent the sham transplantation did not show "significant preference" for the social stimulus. At the same time, mice that were administered with PCP and that underwent the real MSC transplantation did actually show a "significant preference" for social stimulus. Daily clozapine (6mg/kg intraperitoneally (i.p.)) administration did not alter the impaired social behaviour.

The current paper, in this case a clinical trial and not a review, concurrently states, as the previous, that subchronic usage of PCP is the most adequate to mimic the faults acknowledged in humans. Apart from this, it shows that this experimental only treatment might be of great use for investigation until safer methods are developed for humans, should that be possible in the future (Barzilay et al., 2011).

Hauser et al. disclosed in 2009 a report demonstrating that the drug TC-5919 nicotinic receptor-selective agonist was an efficacious means of treating the three symptom groups of schizophrenia in rodents (Sprague-Dawley rats and homozygous transgenic th(tk-)/th(tk-) mice). Rats were tested for pre-pulse inhibition (PPI) and novel object recognition, for this review only the first will be considered as it is the only one of the two assessing models negative symptoms. For PPI, rats were tested in Plexiglas arenas in a lighted, ventilated and sound-attenuated cabinet after being administered TC-5919 (0.1, 0,3 1mg/kg s.c. or saline) and 10min later injected with apomorphine (10mg/kg s.c.). A high-frequency loudspeaker inside the chamber produced a continuous background

noise of 65 dB and a 118-dB startle pulse. Rats' responses were measured with a piezoelectric device.

Mice were tested for social pair, open-field, elevated plus-maze and for PPI as well. PPI test in mice was conducted in a cylindrical Plexiglas arena resting in a ventilated box. A high-frequency loudspeaker inside the chamber produced a continuous background noise of 68 dB and a 120 dB startle pulse. All PPI test sessions consisted of startle trials (pulse-alone), pre-pulse trials (pre-pulse + pulse), and no-stimulus trials. The pulse-alone trial consisted of a 40-ms 120 dB pulse of broadband noise. Injections of saline, TC-5919 (0.1, 0.3mg/kg) or clozapine (3mg/kg) were administered i.p. in a volume of 175 μ l/35g of bodyweight.

Moreover, it was shown that administration of clozapine alone at 3.0 mg/kg had no effect on PPI in control mice. The PPI in th(tk-)/th(tk-) mice was slightly increased by clozapine but this effect did not reach statistical significance. When combined with TC-5619 at 0.1 mg/kg, a dose that did not improve PPI independently in either genotype, there was an improvement in PPI in th(tk-)/th(tk-) mice and no effect in control mice.

Concurrently, for social pair all 16 (8:8) male mice were injected with saline or drug and 45min later they were given two (one with a male and one with a female) 3min social behaviour tests. 30min after the social behaviour test, the animal were placed in the open-field for 10min. 30min after the open-field test; the mice were given a 3min trial on the elevated plus-maze. For the open-field task the mice were transported alone into a clean Plexiglas arena for 10min, after which they were returned to their home cage. For the elevated plus-maze test subjects were placed in the centre of the elevated maze and their activity was videotaped.

Furthermore, the dose of TC-5619 increased the investigation time in the transgenic mice but not in control mice. At the same time clozapine or TC-5619 had no effect on investigation time in any group, but treatment with clozapine+TC-5619 increased the investigation time of a female stimulus in control and transgenic mice. Treatment with clozapine+TC-5919 increased the investigation time of a male stimulus in transgenic mice but not in control. Contrary to the significant effect of TC-5619 on social interaction it had no effect on motor activity in the open-field test. In the elevated plus-maze the transgenic

mice spent more time in the open arms, and consequently less time in the closed arms, than controls did.

Although this article does not review or assess pharmacological social behaviour tests or models it empirically shows the usability of them. This means that it can be used to see what is the real application of these models in real life investigation and not in theoretical conditions as other articles of reviews do. In this particular review acute dosages were applied and not sub-chronic (Hauser et al., 2009).

Ellenbroek et al. published a review in 2000 about animal models for negative symptoms of schizophrenia. It seems to be one of the first reviews in the issue and it has some data that has been proved wrong in recent times. Albeit what has been said before this article plays an important role as it explained the foundations of the animal models for negative symptoms in schizophrenia. It states that negative symptoms and schizophrenia in general can not be assessed with the models used for depression, although signs and symptoms are similar, as the problem does not follow the same physiological pathway. Successively, it affirms that the only negative symptoms that are profitable to study are social withdrawal and anhedonia as they are the only symptoms that can be assessed in an objective way in animals. Other symptoms as alogia, affective flattening, avolition and apathy are virtually impossible to measure in rats, thus making work impossible to fulfil.

Although its old publishing data, it states that amphetamine and PCP (it maintains that amphetamines are not convenient for investigation of anhedonia despite it has been proven wrong by the other, more recent papers) might be useful substances for the modelling of the pathology, concurrently, it also affirms that neonatal lesions, isolation rearing, maternal deprivation and MAM treatment might be used; must be said that those models are still currently employed. Surprisingly, it defends that subchronic PCP treatment as model for social withdrawal in rats as valid (this model is still in use, 15 years later) and current, state-of-the-art models including those involving neonatal and perinatal issues.

The main contradiction to what is currently evidenced is that clozapine may be useful to treat negative symptoms, something that nowadays has been overthrown as it has been proved by the contemporary studies used in this review (Ellenbroek et al., 2000).

Discussion

As previously stated the main objective of this review is to display the possible current evidence surrounding the subject of how schizophrenia is investigated by the usage of diverse kinds of insults. As investigation with humans is practically non-existent and ethically reprehensible, the main current is to research with other mammal animals, primarily with rodents, due to their cheap maintenance cost and their resemblance with humans as they are biologically close to our species (Neill et al., 2014). As a result of this affinity between humans and rodents, animals can be used to build models able to demonstrate how schizophrenia works in humans; this is called validity. For this to be done, mainly, NMDA receptor antagonists (PCP, ketamine, MK-801) are used in a sub-chronic dosage regime (~7 days - ~14 days), as they have been ascertained as the only valid pharmacological insults; in some other cases, other kinds of insults are used in an acute dosage regime that cause brain damage and, thus, schizophrenia-like symptoms e.g. acutely administered ibotenic acid (Moser, 2014; Neill et al., 2010, 2014).

All the studies found firmly agree in utilising rodents as a means for investigation of social behaviours in schizophrenia as a valid model, thus it is presumed that the translatability between rodents and humans is an evidence as all the articles state that they are either using or reviewing at least moderately validated models for their work (Barzilay et al., 2011; Ellenbroek et al., 2000; Hauser et al., 2009; Moser, 2014; Neill et al., 2010b, 2014). Concurrently, a group of four studies agree in regarding sub-chronic dosage of PCP as the most useful substance to create the desired social impairment effects necessary for the research of negative symptoms in schizophrenia (Barzilay et al., 2011; Ellenbroek et al., 2000; Neill et al., 2010, 2014).

It is necessary to emphasise that not all the negative symptoms described in humans can be assessed in rodents due to their difficulty in measuring them. Those symptoms difficult to assess are mainly alogia and affective flattening. Simultaneously, it does not mean that negative symptoms can not be assessed, it only means that there are others (anhedonia and social interaction, mainly) that are much more straightforward and, therefore, easier to investigate (Ellenbroek et al., 2000; Neill et al., 2014).

It is remarkable to say that Neill et al. in their 2010 review affirmed that the use of NMDA antagonists as a model might only provide relatively valid outcomes for schizophrenia. Later in 2014, Neill et al. partially corrected themselves stating that NMDA antagonist model was only well validated and of considerable use to mimic asociality but not anhedonia. It is fiercely important to have this in consideration, as anhedonia is one of the main negative symptoms in humans and, concurrently, one of the most studied negative symptoms. Nevertheless, there is an agreement in using NMDA antagonist model if proper considerations are contemplated (Neill et al., 2010, 2014).

When focusing on which dosage regime is the most proper to mimic many contradictions appear between the different articles. While four of them agree that sub-chronic treatments with insults are the best dosage to mimic social behaviour in rats (Barzilay et al., 2011; Ellenbroek et al., 2000; Neill et al., 2010, 2014) and another two apply acute treatments (Hauser et al., 2009; Moser, 2014). Flaws are found in every article with authors themselves agreeing that either the model is only valid for certain situations or that further research must be done to be able to fully conclude. Must be said that although Moser mostly assesses acute insults it has a reason, most of them only need a single insult dose or trial as they cause permanent changes in the nervous system; a clear example could be causing a ventral hippocampal lesion. In other cases doses are not used as the insult is not a physical or pharmacological one i.e. isolation rearing. In the case of (Hauser et al., 2009) there is no apparent reason or justification for the application of acute insults.

After a thorough reading of multiple papers regarding various aspects and viewpoints of schizophrenia a major drawback attracts attention. Considering that schizophrenia is an incomprehensively multifactorial disorder most papers and models only focus on a single causal factor. Although it is an understandable feature of the papers because in that way the investigation only has scant variables; it does not mimic real nature of the issue with multiple genes, environmental factors and physiologic pathways playing various roles at the same time. Thereafter, I reckon that it would be really profitable, even though arduous, to research considering the broad and ample nature of the problem.

Additionally, it would be of vital value to give answer to how many aspects of negative symptoms are necessary to address an investigation as meaningful; to my knowledge this issue has not been still addressed. As stated before NMDA

antagonist model is only truly valid in some aspects and, according to scientific data, it should not be considered as strong evidence if it is not supported by many other data regarding many other aspects involving schizophrenia, such as genetics.

Conclusions

Currently, there is not enough evidence regarding the fours aspects of this review; schizophrenia, negative symptoms, social behaviour and animal models (specially rodents).

PCP, Ketamine or MK-801 in sub-acute dosage regimes care currently the most indicated insults to mimic schizophrenic symptoms in rodents, although further research in needed. Nevertheless, many other insults seem to be efficacious when trying to mimic schizophrenia like symptoms (MAM, ibotenic acid, etc.) but little evidence has been found to firmly conclude (a single, although extensive, review).

Albeit research is being performed in schizophrenia the current investigation performed by the majority of the scientific community is not being directly focused on this spot. Correlation between the studies and humans seems to be evident if certain aspects are carefully taken into account. Notwithstanding, I acknowledge that profounder investigation respecting this issue would be of great significance for nursing.

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Attachments

Table 1

Study	Insult	Dose	Species, strain and sex	Method used	Measures	Main findings
Flagstad et al. (2004)	MAM	22mg/kg i.p.	Rat, Wistar, male	10' social interaction in low-light in an unfamiliar arena between pairs of rats treated similarly during dark period	Time spent in active and passive social interaction, duration and frequency of proximity (<20cm)	~18% (~32s) reduction in active interaction ~14% (~0.6s) reduction in mean proximity duration
Le Pen et al. (2006)	MAM	25mg/kg i.p.	Rat, Sprague-Dawley, male	10' social interaction in low-light in an unfamiliar arena between pairs of rats treated similarly	Time spent in active and passive social interaction, duration and frequency of proximity (<20cm)	PD:35: ~21% reduction in social interaction (~75s) PD60: ~16% reduction in social interaction (45s)
Harms et al. (2008)	Vitamin D deficiency	N/A	Mouse, C57BL/6J, and 129/SvJ	10' interaction test with younger (4-7 week old) untreated mouse in unfamiliar arena (light level not indicated)	Sniffing and grooming of younger mouse	No change un social interaction duration
Shi et al. (2003)	Influenza virus	$6 \cdot 10^3$ pfu intranasally	Mouse, BALB/c, males and females	5' interaction in familiar arena under normal room lightning	Number of times in contact and latency to first contact	Reduced contact time (3.1s vs. 8.4s) and longer latency to contact (111.4s vs. 24.6s)
Smith et al. (2007)	Polyl:C	20mg/kg i.p.	Mouse, C57BL/6J, sex not explicitly indicated	5' evaluation in 3 compartment test	Social preference: time spent in chamber containing same-sex conspecific minus time spent in empty chamber	Reduced preference for the camber containing the other mouse (~8s vs. 30s)
Nagai et al.	Polyl:C	5mg/kg s.c.	Mouse, ICR,	4 trial of 5' in home cage of test mouse (resident-intruder	Time spent in aggression, social interaction and escape	~25% reduced social interaction time vs. controls. No change in escape behaviour

(2012)			males	paradigm)	behaviour	or aggression.
Bitanihirwe et al. (2010)	Polyl:C	5mg/kg i.v.	Mouse, C57BL/6J, males and females	5' test of preference for social contact with unfamiliar congenic mouse vs. dummy mouse in a Y-maze	Time spent exploring live mouse and dummy object	No deficit in time spent exploring unfamiliar mouse in Polyl:C mice. However, Polyl:C mice spent the same time exploring live mouse and dummy object whereas control mice showed a preference for the live mouse
Abazyan et al. (2010)	Polyl:C	5mg/kg i.p.	Mouse, strain and sex not defined but contained the tetracycline transactivator transgene	10' evaluation in 3 compartment test	Time spent sniffing chamber containing live mouse and empty chamber	No differences compared to saline exposed mice
Kirsten et al. (2010, 2012)	LPS	100µg/kg i.p.	Rat, Wistar, males	PD30 and 31: 10' interaction with non-LPS pup each day. PD60: 5' interaction with non-LPS rat in novel environment. Rats were isolated for 9 or 6 days before testing	Play: incidence of pinning, sniffing the partner, crawling over/under the other animal, partner mounting, following the partner Social interaction: time spent in active social interaction	Reduced juvenile play in isolated but not group-housed LPS-exposed male rats (but very low values in group housed rats) Reduction in frequency but not time spent in active social interaction at PD60. No effects in female offspring in same tests (2012 paper)
Sams-Dodd et al. (1997)	Neonatal VHL	3µg ibotenic acid in .3µl bilaterally	Rat, Sprague-Dawley, males	10' social interaction un low light in an unfamiliar arena between pairs if rats treated similarly	Time spent in active and passive social interaction, duration and frequency of proximity (<20cm)	Active social interaction was reduced at PD35 and PD64 (~22 and 20% respectively; about 45s in both cases). No significant difference at PD96
Becker et al. (1999)	Neonatal VHL	4.5µh/ ibotenic acid in .3µl bilaterally	Rat, Sprague-Dawley, males	7' interaction test in low light, familiar arena with similarly treated rat	Time spent in aggressive and non-aggressive social interaction	Social interaction was modestly reduced by lesion (~27%) but this was composed of a reduction in non-aggressive interactions (~50%) and an increased aggressive interactions (~290% vs.

						control)
Blas-Valdivia et al. (2009)	Neonatal VHL	4µg lidocaine in .3µl bilaterally	Rat, Wistar, males	10' interaction test brightly lit arena. No details on partnering	Number of times in contact and duration of contact	Marked reduction in time spent in contact (~55% and ~48% at PD33 and PD56 respectively) compared to controls. No change in number of contacts
Rueter et al. (2004)	Neonatal VHL	2.5µg ibotenic acid in .3µl bilaterally	Rat, Sprague-Dawley, males	5' interaction in low-light unfamiliar arena between pairs of rats treated similarly	Time spent in social interaction: duration and frequency of proximity (<20cm)	Reduced social interaction time (~42-45% vs. controls). No effect of 22 days treatment with either clozapine (2.5mg/kg/day i.p.) or Risperidone (.1mg/kg/day i.p.)
Vazquez-Roque et al. (2012)	Neonatal VHL	3µg ibotenic acid in .3µl bilaterally	Rat, Sprague-Dawley, males	10' interaction in low-light unfamiliar arena between pairs of rats treated similarly	Number of and time spent in active social interaction	~55% reduction in social interaction; non-significant reduction in number of contacts. Reversal of social interaction deficit following 30 days treatment cerbrolisin (1.076g/kg i.p.)
Silva-Gómez et al. (2003)	Neonatal VHL	3µg ibotenic acid in .3µl bilaterally	Rat, Sprague-Dawley, males and females	10' interaction in low-light unfamiliar arena between pairs of rats treated similarly	Number of and time spent in active social interaction	~55% reduction in social interaction time in males, no change in females; small but significant reduction in number of contacts in females (22 vs. 29) but slight increase in males
Lipska et al (2002)	Reversible neonatal VHL	3ng tetrodotoxin in .3µl bilaterally	Rat, Sprague-Dawley, males	10' interaction in low-light unfamiliar arena between pairs of rats treated similarly	Incidence of aggressive or active social behaviours at 1' intervals	No significant differences (data not shown)
Lazar et al. (2008)	Neonatal prefrontal cortex NGF	93.75µg NGF in 0.75µl bilaterally	Rat, Sprague-Dawley, males	23.5h recording starting at the beginning of light-dark cycle in low-light unfamiliar arena between pairs of rats treated similarly	Mean distance apart, approach/avoidance behaviour, proximity (<20cm)	Greater mean distance apart and less approach and avoidance behaviour

Hermes et al. (2011)	Isolation rearing	N/A	Rat, Sprague-Dawley, females	5' interaction in unfamiliar arena during light-phase	Number of contacts, average length of contacts, total contact time	Reduced contact duration (~4.1 vs. 7s) and reduced total time in social contact (~80 vs. 130s)
Meng et al. (2010)	Isolation rearing	N/A	Rat, Sprague-Dawley, males	10' interaction in low-light familiar arena with a group-reared rat	Time spent in aggression and social interaction. Number of contacts	Marked increase in aggressive behaviours and social interaction after isolation. Differences disappeared after resocialisation (testing on PD78)
Koike et al. (2009)	Isolation rearing	N/A	Mouse, ICR, males	4 trials of 5' test in home caged of test mouse (resident-intruder paradigm)	Time spent in aggression, social interaction and escape behaviour	Marked increase in aggressive behaviours and social interaction after isolation. Clozapine (2.5mg/kg i.p.) almost markedly reduced the aggressive behaviour
Zamberletti et al. (2012)	Isolation rearing	N/A	Rat, Lister-hooded, males	10' interaction in low-light familiar arena with a congener (unclear if isolated or group-reared)	Time spent in active social behaviours and incidence of aggressive behaviours	Increased incidence of aggressive behaviours (0 in group-housed vs. ~7.5 in isolation reared rats). No difference in active social interaction. Aggressive interaction was reduced by repeat but not acute treatment with the CB1 antagonist AM251 (0.5mg/kg i.p.)

Abbreviations: PD, post-natal day; VHL, ventral hippocampal lesion; MAM, methylazoxymethanol; NGF, nerve growth factor; N/A, not-applicable. Changes from control values have been recalculated from data in figures or tables as percent changes for easier comparison between studies.

Table 2

Drug	Dose	Time of testing	Sex/strain	Duration of test	Pairing of animals	References
PCP	Subchronic 2.5mg/kg for 3 days	Tested 45min later	Male Wistar	10min	Saline/saline, drug/drug	**Sams-Dodd, 1995**
PCP	Subchronic 2.5mg/kg for 3 days	Tested 45min later	Male Sprague-Dawley	10min	Saline/saline, drug/drug	**Bruins-Slot et al. (2005)**
PCP	Subchronic 3mg/kg for 14 days	Tested 24h later	Male Sprague-Dawley	10min	Saline/saline, drug/drug	**Lee et al. (2005)**
PCP	Subchronic 10mg/kg for 15 days	Tested 20h later (on days 1, 8 and 15)	Male Long-Evans	10min	Saline/saline, saline/drug	**Audet et al. (2009)**
PCP	Subchronic 2mg/kg, bi-daily for 7 days	Tested 1-6 weeks later	Female hooded-Lister	10min	Saline/saline, saline/drug	**Snigdha and Neill (2008)**
Ketamine	Acute 7mg/kg	Tested 30min later	Male Wistar	10min	Saline/saline, drug/drug	**Silvestre et al. (1997)**
Ketamine	Subchronic 30mg/kg for 5 days	Tested 10 days later	Male Sprague-Dawley	7min	Saline/saline, saline/drug	**Becker et al. (2003)**
MK-801	Acute .2mg/kg	Tested 30min later	Male Sprague-Dawley	30min	Saline/saline, drug/drug	**Rung et al. (2005)**
MK-801	Subchronic .13mg/kg for 14 days	Tested 45min later	Male Wistar	10min	Saline/saline, drug/drug	**Matsuoka et al. (2005)**

A comparative table showing some differences in sex, strain, dosing regime and method of testing used by different groups (using adult rats) in te social interaction test.

Table 3

Drug/dose	Time of testing	Sex/strain	Parvalbumin	Brain region	Behavioural deficits	References
PCP						
Acute (2.58mg/kg)	24h	Male hooded Long-Evans	Deficit in mRNA	Reticular thalamic nucleus	Deficit in attentional set-shifting	**Egerton et al. (2005)**
Chronic intermittent exposure (2.58mg/kg)	72h	Male hooded Long-Evans	Deficit in mRNA	Reticular thalamic nucleus, prefrontal cortex	Metabolic hypofunction in prefrontal cortex	**Cochran et al. (2003)**
Subchronic (2mg/kg bi-daily for 7 days)	6 weeks	Female hooded-Lister	Deficit in PV IR neurons	Hippocampus	Deficits in reversal learning	**Abdul-Monim et al. (2007)**
Subchronic (2mg/kg bi-daily for 7 days)	6 weeks	Male hooded-Lister	Deficit in PV IR neurons	Hippocampus	Disturbances in social interaction	**Jenkins et al. (2008)**
Subchronic (2mg/kg bi-daily for 7 days)	6 weeks	Male hooded-Lister	Deficit in PV IR neurons	Prefrontal cortex	Deficits in novel object recognition	**McKibben et al. (2010)**
Neonatal (10mg/kg on PD7)	PD56	Male Sprague-Dawley	Deficit in PV IR neurons	Cortical deficits	No change in calretinin IR neurons	**Wang et al. (2008)**
MK-801						
Chronic (.02mg/kg for 21 days)	24h	Male Long-Evans	Deficit in PV IR neurons	Hippocampus	No change in calretinin IR neurons	**Braun et al. (2007)**
Chronic (.02mg/kg	24h	Male Long-Evans	Deficit in relative	Hippocampus	Cognitive deficits	**Rujescu et al. (2006)**

for 14 days)			number PV IR neurons		(hole board)	
PD exposure (.2mg/kg on E15-E18)	PD35 and 63	Male and female Sprague-Dawley	Deficit in PV IR neurons	Prefrontal cortex	Enhances PCP induced hyperlocomotion	**Abekawa et al. (2007)**
Ketamine						
Subchronic (30mg/kg for 5 days)	2 weeks	Male Sprague-Dawley	Deficit in PV IR neurons	Hippocampus	—	**Keilhoff et al. (2004)**

Summary of studies evaluating the effect of NMDA receptor antagonists on parvalbumin expression in the rat brain. The table highlights some of the differences in dosing regimens, time of testing following last treatment, sex, strain, and regions tested by a number of research laboratories. It also highlights any behavioural changes reported in the same studies. PD= postnatal day; PV= parvalbumin; IR= immunoreactive; PCP= phencyclidine.

Table 4

Compound	NMDA receptor antagonist	Treatment	Cognitive task	Outcome	Reference
Atypical antipsychotic					
Arsenapine	PCP	2mg/kg i.p. bi-daily for 7 days followed by 7 days washout	Reversal learning and novel object recognition	Improved PCP-induced deficits	**Tarazi and Neill (2013)**
5-TH_6 antagonist					
LU AE58054	PCP	2mg/kg i.p. bi-daily for 7 days followed by 7 days washout	Novel object recognition	Improved PCP-induced deficits	**Arnt et al. (2010)**

MGlu 2/3 agonist					
LY404039	Ketamine	10 and 30mg/kg i.p. for five consecutive days	Odour span task	Failed to reverse ketamine-induced deficits	**Rushforth et al. (2011)**
LY354740	PCP	1 and 5mg/kg i.p. acutely	Discrete-trial delayed alternation task	Improved PCP-induced deficits	**Moghaddam and Adams (1998)**
LY379268	PCP	2mg/kg i.p. bi-daily for 7 days followed by 7 days washout	Novel object recognition	Failed to reverse PCP-induced deficits	**Horiguchi et al. (2011)**

Use of NMDA receptor antagonist models to test the efficacy of different receptor mechanisms for treating cognitive deficits in rodents, compounds or targets that have been progressed into de clinic.

Table 5

Treatment	Summary	Examples
Acute	Acute treatment with PCP, ketamine, or MK-801 tends to produce not decrement in hedonic behaviours related to consumption, and can actually reduce rewards thresholds in intracranial self-stimulation (ICSS) protocols. Acute treatment with ketamine or MK-801 can alleviate deficits in sucrose consumption and in immobility on the force-swimming test. This is particularly clear in the case when deficits are produced by depresogenic treatments such as chronic stress.	**Spielewoy and Markou (2003)** **Lydall et al. (2010)** **Trullas and Skolnick (1990)** **Tizabi et al. (2012) Ye**
Post-acute	Hedonic behaviours related to consumption of sucrose are reduced 24h after high (15mg/kg and above) doses of PCP. However, such doses are an order magnitude higher that those that produce cognitive deficits. Such doses also produce neuronal damage unrelated to that seen in schizophrenia.	**Turgeon and Hoge (2003)** **Baird et al. (2008)** **Turgeon et al. (2010)**

	Alleviation of deficits in sucrose consumption and in mobility on the forced-swimming test persists for several days following a single dose of ketamine. This is especially clear when alleviating deficits produce by depresogenic treatment such as chronic stress.	**Yilmaz et al. (2002)** **Li et al. (2011)** **Ma et al. (2013)**
Chronic	Repeated exposure to low (2mg/kg) dose PCP reduces reward thresholds in the intracranial self-stimulation (ICSS) protocols. Deficits in sucrose consumption and immobility on the forced-swimming test produced by depresogenic treatments can be alleviated by repeated low-dose MK-801 or ketamine.	**Amitai et al. (2009)** **Papp and Moryl (1994)** **Tizabi et al. (2012)**
Post-chronic	5mg/kg of PCP given bi-daily for 7 days, followed by a washout period, has no effect on sucrose consumption, anticipation of future rewards, or the degree of effort expended obtain them. Testing directly after withdrawal following 7 bi-daily 7.5mg/kg PCP injections produced a transient decrease in sucrose consumption. Withdrawal from repeated treatment with PCP persistently decreases reward threshold in the intracranial self-stimulation (ICSS) protocols and increases duration of immobility on forced-swimming test. Pre-natal PCP treatment and withdrawal from repeated ketamine treatment also increase the duration of immobility on forced-swimming test.	**Lydall et al. (2010)** **Jenkins et al. (2010)** **Lydall (2011)** **Baird et al. (2008)** **Corbett et al. (1999)** **Noda et al. (1995)** **Spielewoy and Markou (2003)** **Li et al. (2001)** **Murai et al. (2007)** **Tejedor-Real et al. (2007)**

		Turgeon et al. (2007) **Chindo et al. (2012)**

Summary of anhedonia related behaviours in rodents following various NMDA receptor antagonist treatment and regimes