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ORIGINAL INVESTIGATION

Opposing effects of glutamatergic and GABAergic pharmacological manipulations on a visual perception task with relevance to schizophrenia

John C. Talpos[1] · John Riordan[1] · Joseph Olley[1] · Jason Waddell[2] · Thomas Steckler[1]

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Abstract
Rationale Numerous psychiatric disorders and neurodegenerative diseases have been associated with differences in visual perception, and it has been proposed that the treatment of these differences may represent a novel means to treat disorders like schizophrenia. Unfortunately, few methods exist to study visual perception in pre-clinical species.
Objective The purpose of the present study was to adapt a task of visual integration by proximity with relevance to schizophrenia to a rodent touchscreen environment to determine the effects of glutamatergic and GABAergic compounds. In this way, we could evaluate the effects of common models of cognitive impairment, as well as the effects of net excitation versus inhibition, on a task of visual integration.
Method Rats were trained to perform a visual discrimination where the stimuli were composed of rows of dots differing only in there horizontal and vertical proximity. Once stable performance had been achieved, animals were tested under the influence of glutamatergic or GABAergic drugs (ketamine, MK-801, PCP, memantine, chlordiazepoxide, or diazepam) while attempting to perform a visual discrimination with altered stimuli.
Results Ketamine appeared to impair perceptual grouping in this paradigm, while the GABA agonist chlordiazepoxide enhanced grouping even in the presence of non-selective effects.
Conclusions In general, these findings support the theory that NMDA antagonists may disrupt visual grouping by proximity and highlight a potential beneficial effect of enhanced GABA activity in perception. However, additional research will be required to confirm the stimulus selectivity of this effect, and the clinical significance of this approach.

Keywords Visual integration · Autism · NMDA receptor · Visual cortex · Rat · Touchscreen · Schizophrenia

Introduction

Many disorders of the central nervous system are associated with changes in visual perception. For instance, Alzheimer's has been associated with changes in light contrast sensitivity and 3D coherent motion (Kirby et al. 2010; Lemos et al. 2012; Risacher et al. 2013), autism spectrum disorder (ASD) with changes in perceptual grouping and coherent motion (Farran and Brosnan 2011; Robertson et al. 2014; Scherf et al. 2008), and schizophrenia with visual hallucinations and differences in contour integration (Green et al. 2009). Surprisingly, these differences and the potential contribution of these differences to symptom severity have remained largely unexplored. As vision is the primary sensory modality in most humans, understanding these changes in perceptual ability may provide a novel window into studying the etiology of schizophrenia, and "normalizing" differences in visual perception may also be a novel means of improving daily functioning in certain patient groups.

Schizophrenia is one of the few CNS disorders where a change in visual perception has been given serious consideration as a core symptom (Barch et al. 2009; Green et al. 2009). It is well established that some schizophrenic patients suffer from visual hallucinations. However, schizophrenic patients also show more subtle changes in visual perception that may be more pervasive and just as important in regards to quality of life. This was highlighted by the recent Cognitive

✉ John C. Talpos
jtalpos@its.jnj.com

[1] Department of Neuroscience, Janssen Research and Development, 30 Turnhoutseweg, 2340 Beerse, Belgium

[2] Open Analytics, 20 Jupiterstraat, 2600 Antwerp, Belgium

Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS) initiative that identified seven key cognitive domains that were disrupted in schizophrenia, including visual perception (Barch et al. 2009). Of specific interest was the process of visual integration, the process by which local attributes of scene are used to create a cohesive whole that can then be used to drive behavior (Green et al. 2009). To put it simply, this is the perceptual equivalent of seeing a group of trees as a forest, rather than just a series of trees. Despite the fact that examining differences in visual perception are seen as being key to better understanding and treat schizophrenia, few techniques are available to study visual perception in a pre-clinical setting (Siegel et al. 2013).

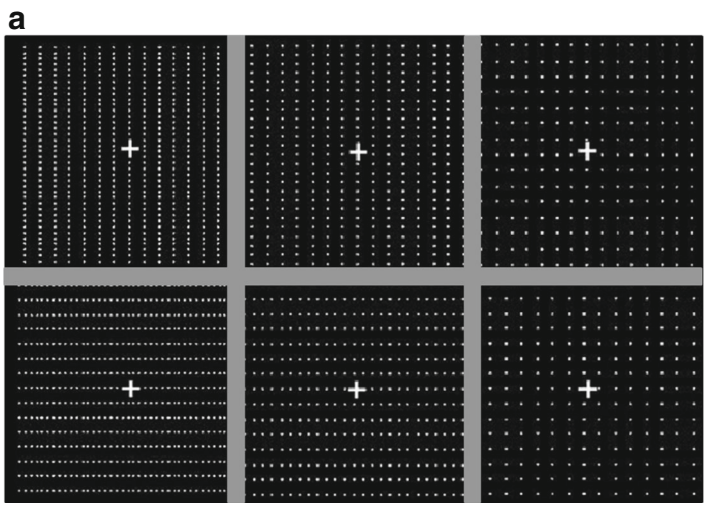
Human tasks of visual perception are typically based off of two choice visual discriminations or go/no-go tasks, both of which can easily be adapted for rodents in a touchscreen environment. Touchscreen-equipped operant boxes are popular to model aspects of human cognition in the rodent, especially because they allow the possibility of using the identical task and stimuli in rodents as in humans (Talpos and Steckler 2013). If the rodent visual system has some homology with the human visual system, then rodent tasks of visual perception may prove to be a highly accurate translational model of human perception. One task of particular interest for schizophrenia is grouping by spatial proximity (Kurylo et al. 2007).

Perceptual grouping by spatial proximity takes advantage of the natural phenomena by which dots in rows can be perceived as "lines" dependent upon their relative horizontal and vertical arrangement (see Fig. 1a). As the horizontal to vertical ratio approaches one, the ability to perceive these lines disappears. Work by Kurylo et al. (2007) has shown that medicated schizophrenic patients require more "signal" than typical patients to observe these lines, a finding in line with similar perceptual tasks like contour integration or coherent motion (Green et al. 2009). While contour integration, coherent motion, and perceptual grouping by proximity are all subtly different, they share in common an integration element that is thought to be dependent upon areas early in the visual cortex (V1-V2). Moreover, Kurylo and Gazes (2008) has reported that rodents can learn to discriminate based upon stimuli of this type and that the discrimination is dependent upon an intact visual cortex as well.

Here, we adapt the perceptual grouping paradigm used by Kurylo et al. to a touchscreen environment (Kurylo et al. 2007; Kurylo and Gazes 2008; Ward et al. 2013). To do this, we initially trained rats to discriminate horizontal versus vertical dot lines. Once animals were performing this discrimination, they were presented with stimuli of varying difficulty (12 levels; Fig. 1b) where difficulty increased as the horizontal-to-vertical ratio became closer to zero. Having established that animals could learn the task and that performance was dependent upon the horizontal to vertical ratio (Fig. 2), we then performed several pharmacological challenges. With these pharmacological challenges, we wanted to test two hypotheses: (1) NMDA-R antagonism will cause a shift in threshold for grouping by proximity (requiring more signal for grouping to occur), and (2) in accordance with the theory of excitation/inhibition balance, that decreasing neuronal excitation via stimulation of the GABAergic system will result in an improvement in perceptual grouping.

It has been previously reported that ketamine may selectively disrupt grouping by proximity in the rodent, albeit only at high doses (20–30 mg/kg) and only when using a best-dose procedure (Kurylo and Gazes 2008). These results are partially supported by a report claiming impaired performance with stimuli made of degraded glass patterns (a pattern of random dots that is then super imposed upon a rotated copy of itself and known to activate area V1 of the primate brain (Glass and

Fig. 1 Example stimuli pairs (**a**) used in this study, and a table (**b**) of all ratios used with their non-log equivalents



Examples of the full (0.44), a medium (0.20), and the lowest (0.01) signal conditions used.

b

Log Horizontal / Vertical Ratio	Horizontal / Vertical Ratio
0.01	1.03
0.04	1.1
0.08	1.2
0.12	1.32
0.16	1.44
0.2	1.58
0.24	1.74
0.28	1.91
0.32	2.09
0.36	2.29
0.4	2.51
0.44	2.76

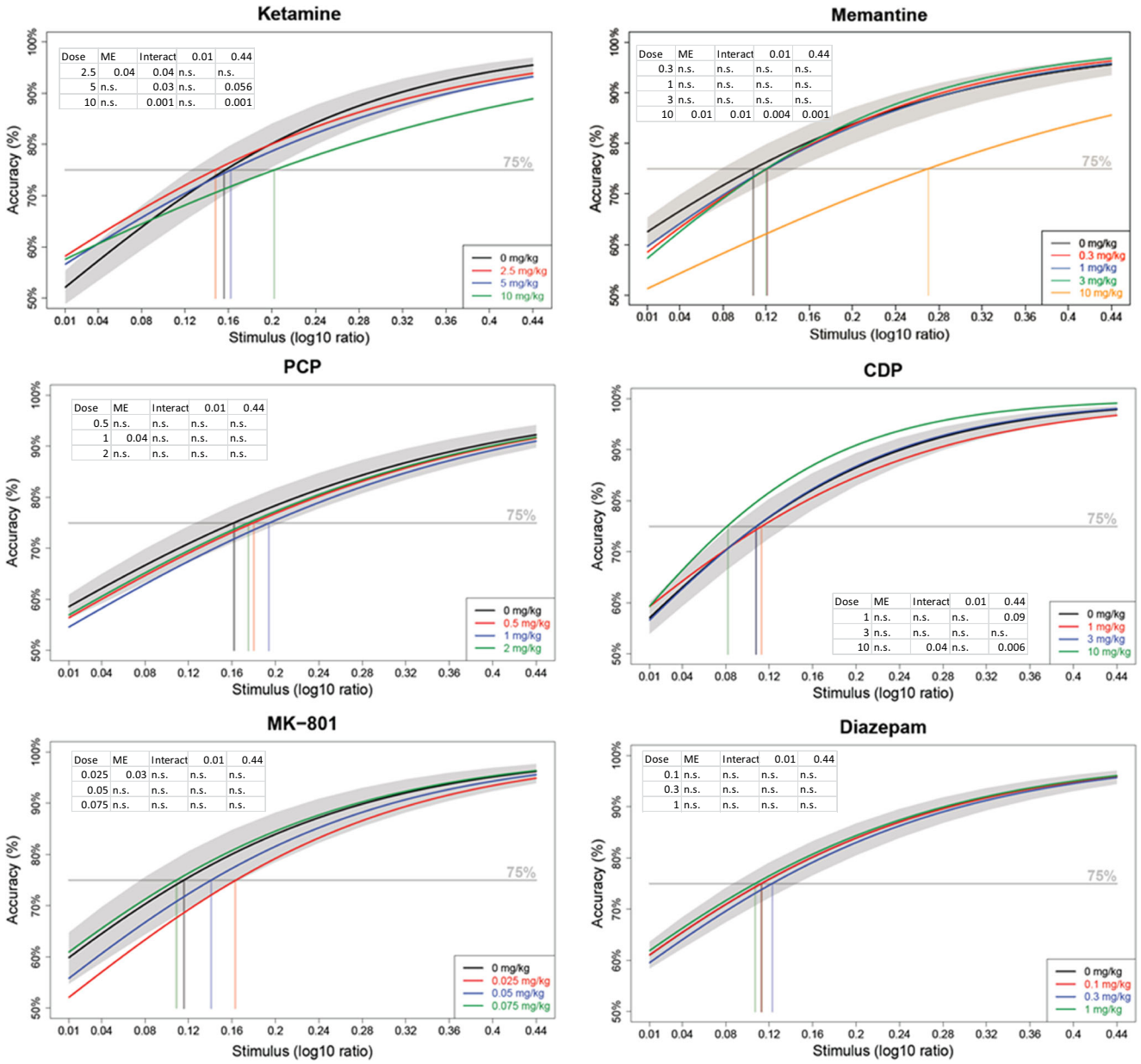


Fig. 2 The effects of compounds on accuracy. Estimated mean values are presented and the *gray area* represents 1 standard error of the vehicle condition. *Tables* highlight the presence of main effects (ME) and interactions (Interact.) between compound on stimulus ratio, as well as effects of compounds on performance at the 0.01 and 0.44 ratios. *Vertical lines* represent the stimulus ratio needed to induce a 75 % accuracy under different drug conditions

Perez 1973) after administration of ketamine or PCP, whereas performance with non-manipulated stimuli was not affected (Ward et al. 2013). Accordingly, we have attempted to replicate and extend this work by testing the effects of ketamine, PCP, MK-801, and memantine to more completely investigate the effects of NMDA-R antagonists on perceptual grouping. Multiple NMDA antagonists were selected as they are known to have different effects on behavior. Moreover, these compounds were of specific interest as ketamine, PCP, and MK-801 are popular models of disease and cognitive impairment while memantine and ketamine are both used to treat disorders of the central nervous system, Alzheimer's, and depression, respectively. Moreover, we also tested the GABAergic positive allosteric modulators (PAMs) diazepam and chlordiazepoxide (CDP) and hypothesized that these compounds may enhance grouping under more demanding conditions. This hypothesis is based off the assumption that visual perception is controlled in part by competitive inhibition (Wyatte et al. 2012), and functioning of the visual cortex appears to be in part regulated by inhibitory GABAergic neurons (Zhang et al. 2014). Accordingly, we speculate that an increase in inhibition will enhance perceptual grouping by proximity. We have

previously shown that acute administration of NMDA antagonists may have some effect on accuracy of a visual discrimination. To distinguish effects on the requisite performance of a visual discrimination from perceptual grouping abilities, we used mixed effects logistic regression models to determine when the shape of the curve is altered (interaction) as opposed to when the curve is merely shifted (main effect) to determine if treatment is interacting with the stimuli. An interaction between treatment and stimuli can then be used as evidence for an effect of treatment on perceptual grouping.

Materials and methods

Subjects

All testing and handling performed in this study complied with relevant EU ethics committee directives (86/609/CEE), along with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioural Research (National Research Council 2003), and the study protocol was approved by the Janssen Research & Development animal experimental ethics committee (Beerse, Belgium).

The same group of 24 Lister Hooded rats (from Harlan, Netherlands, weight approximately 200 g on arrival) was used for the entirety of the study. All rats were group housed in individually ventilated cages of four rats each, measuring 48 cm in width, by 37.5 cm in length, and 21 cm in height—a total volume of 1400 cm^2, with temperature kept at 22 °C, and humidity at 55 %. Subjects were kept on a 12-h light/dark cycle, with lights on at 07:00, and testing was confined to a 2-h window between 09:00 and 11:00 wherever possible. Animals were kept on a restricted diet intended to maintain body weight at 85 % of free-feeding weight. Free access to water was given except during testing. Wood chew blocks and plastic tunnels were also included in the housing to provide environmental enrichment.

Apparatus

All experiments were performed in modified Med Associates operant chambers (Med Associates Inc., Fairfax, VT), measuring 33.5 cm high, 32.5 cm wide, and 40 cm long. Each chamber contained a 3-W speaker, a small house light at the back, and a food pellet receptacle beneath equipped with a reward light and an infra-red nose-poke detector. The food pellet receptacle was connected to a reward pellet dispenser. The floor consisted of stainless steel bars, spaced roughly 1 cm apart. The wall opposite the pellet receptacle was replaced with a 26 cm by 36 cm LCD computer monitor and fitted with an infrared touch detection system. Touchscreens were controlled with Klimbic software (K Limbic, Sawbridgeworth, UK, version 1.21.3.3). The screen was separated into four distinct horizontal regions by a steal mask placed over the screen (only the center two locations used). In front of this was a counterweighted steel flap of 5 cm by 36 cm, designed to slow the response of the rat. Each chamber was housed inside a larger sound attenuated box, fitted with a fan to ensure ventilation and to provide background white noise.

Compounds

Compounds were administered via subcutaneous injection in a physiological saline vehicle, at a volume of 1 ml/kg, with the exceptions of CDP, diazepam, and memantine HCl. CDP was in a physiological saline vehicle, but in a volume of 10 ml/kg due to difficulty dissolving the higher doses at a lower volume, and diazepam had to be given in a 20 % cyclodextrin vehicle. Memantine HCl was administered via an intraperitoneal injection to align with our previous work with the compound in a standard visual discrimination (Talpos et al. 2012). All compounds but ketamine were administered 30 min before testing—ketamine was given 5 min before testing because of the fact that its effects rapidly diminish post-injection. Each compound was tested with a within-subject design, meaning that all subjects received all doses of each compound, and drugs were administered on Tuesdays and Fridays. Doses of MK-801 used were 0.025, 0.05, and 0.075 mg/kg (Tocris Cookson); PCP doses were 0.5, 1, and 2 mg/kg (synthesized internally); ketamine 2.5, 5, 10, and 20 mg/kg (synthesized internally); memantine HCl 0.3, 1.0, 3.0, and 10 mg/kg (Sequoia Research Products); CDP 1, 3, and 10 mg/kg; and diazepam 0.1, 0.3, and 1 mg/kg (Sequoia Research Products). Doses were based on previously published findings (NMDA antagonists (Gilmour et al. 2009; Talpos et al. 2012; Ward et al. 2013) or unpublished internal results (CDP and diazepam)).

Stimuli

The perceptual grouping task is a simple visual discrimination in which subjects are required to select one of two stimuli. The stimuli are simple dot lattices in which dots are either presented closer together in the horizontal plane or the vertical plane. The stimuli can be expressed as a ratio of horizontal distance between dots against vertical distance, or spaced apart. As the space between horizontal dots and between vertical dots becomes smaller, the difference between horizontal and vertical presentations becomes greater and the stimuli become easier to discriminate. Conversely, as the distance between horizontal and vertical spacing between dots becomes more comparable, the stimuli are harder to discriminate (Figs. 1 and 2). This ratio is expressed as Log10 (Ratio), in order to enable results to be expressed graphically in regular steps. Twelve different horizontal and vertical stimuli, ranging from Log10

(Ratio)=0.01 (hardest) to Log10 (Ratio)=0.44 (easiest), were used in this study (Fig. 1b).

Pre-training

Rats were acclimatized to the operant chambers by placing them inside while the fans were running (with a peanut butter and reward pellet mix being placed onto the screen). The peanut butter and pellet mixture was used to encourage exploration of the screen, a necessity for eventual task acquisition. Animals were then trained to associate the sounding of a tone and the illumination of the reward magazine with the delivery of a food pellet. To do this, a pellet was delivered into the pellet receptacle in conjunction with the sounding of a short tone and the activation of the pellet receptacle light. Once the pellet was collected, the light would turn off, and after 10 s, another pellet would be delivered in conjunction with the activation of the receptacle light and the sounding of the tone. The session ended after the delivery of 60 pellets or once 45 min had passed, whichever came first. Animals were judged to be ready for the next training once they had completed 60 trials for two consecutive sessions.

Once rats had made the association between a tone and the delivery of a food reward, they were trained to touch the screen to earn a reward. In the initial touch training phases, the entire touchscreen displayed a white image, and touching any part of it resulted in a tone sounding and a food pellet being dispensed. Once the pellet was collected, a 5-s inter-trial interval occurred, and then the next trial began. After 60 trials or 45 min (whichever occurred first), the session ended. Once again, rats needed to complete all 60 trials on two consecutive sessions in order to move to the next stage.

The final pre-training phase was the same as the previous, except that only one location on the screen was lit, and this location changed randomly every trial. Animals were again required to complete 60 trials within 45 min on two consecutive days to reach criteria for training on the visual discrimination.

Perceptual grouping task training

Rats were initially trained on stimuli with the greatest ratio (Log10 (Ratio)=0.44). Half of the rats were trained to select the horizontal “line” stimulus, and half the vertical line stimulus. The two stimuli were displayed in the two central windows of the four-window steel mask, with location varied across trials within a session. On selection of the correct stimulus, rats were rewarded with a food pellet. If the incorrect stimulus was selected, all lights were extinguished for 10 s and the trial was subsequently repeated until completed correctly. To reach criterion during this phase of training, rats had to complete 72 trials within 45 min, with rats required to get an average of 80 % of trials correct over 3 days. This was achieved in 16 sessions.

The final stage of training was to ensure that rats could successfully complete discriminations with the modified stimuli. During a session, all 12 levels of difficulty were presented eight times in a pseudo-random fashion (96 total trials). The sessions lasted for a maximum of 45 min. This phase lasted five sessions, simply to ensure that rats were able to complete the task as expected, and to ensure that % correct was dependent on stimulus ratio.

Statistics

Data was generated via K-limbic (Conclusive Solutions, Sawbridge, UK) and then formatted with Microsoft Excel 2013. Trials completed and response latencies were analyzed with Statistica (Version 12; StatsSoft), whereas accuracy was investigated using R. Animals had to reach at least 40/96 trials completed to be included for analysis (except for the analysis of the trials completed, which was performed on all subjects).

In order to test for effects of treatment on latencies, all data were transformed into log10 (latency), and repeated measures ANOVAs were performed, with post hoc analysis being Dunnett’s *t* tests against vehicle. A log10 transformation was performed to account for the skewed nature of latency data. The number of trials completed were all analyzed with *t* tests. This was done because in some instances, there was not suitable variance (all animals completed all trials) to perform ANOVAs.

For analysis of percent correct, we have made use of a mixed effects logistic regression model. The logistic model is more powerful and less biased and accounts for the variance structure of percentage-based data more accurately than an ANOVA based on summarized values (for additional information on the merits of this approach, see (Zhao et al. 2001). Moreover, this approach allows us to take advantage of the clear hierarchy within the stimuli, something that is not possible with an ANOVA. In interpreting the nature of an observed effect, it is useful to know what is occurring under the minimum and maximum stimuli conditions. Here we are able to model the maximum (0.44) and minimum values (0.01) while taking into consideration the whole data set in conjunction with the logistic regression model. The approach used here is actually very similar to what might be used in a bio-assay (four parameter logistic regression) except that we have used a goodness of fit model to determine interactions and significance is eventually determined via a chi-square.

To discover the effects of treatment on perceptual grouping, in each trial, the stimulus was treated as a numeric variable (0.01–0.44, corresponding to difficulty), and each dose as a factor variable. As the result was a binary variable, giving a value of 1 on correct completion of a trial, or 0 otherwise, a modified logistic regression model was applied. In lieu of available software for fitting mixed effects models on four-

parameter logistic data, we test the following mixed effects logistic regression models:

Model 1 Results ~ stimulus + (1| subject)
Model 2 Results ~ stimulus + dose + (1| subject)
Model 3 Results ~ stimulus * dose + (1| subject)

First, we use a likelihood ratio test to test for a significant difference between models 1 and 2. This indicated whether a significant overall dose effect in the data existed. Second, we tested models 2 and 3 against each other. If this test was significant, it indicated a statistically significant interaction between dose and stimulus. Effectively, the presence of a significant interaction means that the fitted curves for each dose group can be non-parallel, each taking a different shape. Without a significant interaction, the fitted curves will merely be shifted horizontally. As the experiment was in a repeated measures form, each model was a mixed effects model, with "subject" (animal) serving as the random factor. Using this model, the main effects and interactions were calculated, as were effects under the 0.01 and 0.44 stimulus condition (values determined at 0.01 and 0.44 as determined by the logistic regression). The independent analysis at the 0.01 and 0.44 stimulus conditions was included to explicitly test if the compounds evaluated caused a specific change in behavior under either the easiest or hardest test conditions.

Results

MK-801

No primary treatment effect of MK-801 was seen on accuracy (P=0.06), nor was there a statistically significant interaction between treatment and difficulty (P=0.175). However, a significant dose effect was observed at 0.025 mg/kg (P=0.03). Moreover, no significant differences were seen in the estimated performances at stimulus ratios of 0.01 or 0.44 (see Fig. 2).

MK-801, at the highest dose tested (0.075 mg/kg), caused a significant decrease in trials completed (P=0.002). No main effect of MK-801 was observed on response latency (F(3, 57)=0.98, P=0.41), although a main effect of level (F(11, 209)=3.23, P<0.001) was observed, as was a modest interaction between MK-801 and level (F(33, 627)=1.58, P=0.021). A main effect of MK-801 was observed on collection latency (F(3, 54)=18.92, P<0.001), with 0.025 and 0.05 mg/kg causing a decrease in latency. No effect of level (F(11, 198)=0.94, P=0.505) or level by MK-801 interaction (F(33, 594)=0.78, P=0.81) was detected (see Fig. 3).

PCP

No overall treatment effect of PCP was seen on accuracy (P=0.217), and there was no interaction between treatment and level (P=0.713). However, the model table revealed a just significant dose effect at 1 mg/kg (P=0.04). Moreover, no significant differences were seen in the estimated performances at stimulus ratios of 0.01 or 0.44 (see Fig. 2).

PCP had no effect on trials completed. However, significant effects of PCP (F(3, 54)=24.37, P<0.001) and level (11, 198)=2.41, P=0.007) were observed on response latency. No significant interaction between PCP and level was observed (F(33, 594)=1.08. A Dunnett's t test indicated a significant increase in latency at the highest dose of PCP (2.0 mg/kg; P<0.001). PCP (F(3, 51)=10.09, P<0.001) and level (F(11, 187)=2.30, P=0.011) both had a significant effect on collection latency. However, PCP and level did not significantly interact (F(33, 561)=1.35, P=0.096). A Dunnetts t test indicated that the 0.5 and 1.0 mg/kg treatment conditions were associated with significant decreases (P<0.001) in collection latency (see Fig. 3).

Ketamine

Ketamine had a large overall treatment effect on performance (P<0.001), along with a significant interaction between treatment and difficulty (P<0.001) (see Fig. 2).

Ketamine, at 10 and 20 mg/kg, caused significant decreases in trials completed (P<0.001). Because of the extreme reduction in trials completed at 20 mg/kg (vehicle=93.3, 20 mg/kg=6.7), the 20 mg/kg condition was not included within future analysis of the effects of ketamine. Ketamine caused a significant increase in response latency (F(11, 132)=26.48, P<0.001). A Dunnett's t test indicated significant increases from vehicle at 5 and 10 mg/kg. No effect of level (F(11, 132)=1.19, P=0.30) or level by MK-801 interaction (F(13, 396)=0.75, P=0.839) was detected. Only the lowest dose of ketamine showed a statistically significant main effect on accuracy (P=0.04); however, all doses showed significant interactions with difficulty (2.5–5.0 mg/kg, P<0.01; 10 mg/kg, P<0.001; 20 mg/kg, P<0.01). While no significant differences were detected at the stimulus ratio of 0.01, significant differences in performance were detected at the 0.44 ratio condition (10–20 mg/kg, P<0.001; see Fig. 3).

Memantine

Memantine had a main effect of treatment on accuracy (P<0.001), and there was a significant interaction between treatment and difficulty (P<0.001). However, significant differences from vehicle and an interaction with stimulus difficulty were only seen at the 10 mg/kg condition (P<0.001; main effect and interaction). Significant differences from vehicle were also seen at the 0.01 (P<0.001) and 0.44 (P<0.001) stimulus level after treatment with 10 mg/kg memantine (see Fig. 2).

Treatment	Latency (mean log10 msec)				Trials		75%
(mg/kg)	Response	SE	Collection	SE	Completed	SE	Ratio
Ketamine	***		***				
Veh	3.47	0.03	3.15	0.03	93.35	2.29	0.157
2.5	3.47	0.03	3.13	0.03	95.60	0.40	0.148
5	3.71***	0.04	3.20**	0.03	85.85	3.79	0.162
10	3.73***	0.03	3.20**	0.02	49.85***	6.25	0.203
20					6.75***	1.39	0.365
PCP	***		***				
Veh	3.41	0.02	3.17	0.01	94.05	1.38	0.162
0.5	3.37	0.02	3.10***	0.02	95.48	0.52	0.18
1	3.39	0.02	3.10***	0.01	96.00	0.00	0.194
2	3.63***	0.05	3.15	0.02	84.80#	5.34	0.175
MK-801			***				
Veh	3.50	0.04	3.18	0.01	94.59	0.87	0.116
0.025	3.52	0.03	3.10***	0.01	96.00	0.00	0.163
0.05	3.59	0.04	3.11***	0.02	95.14	0.48	0.141
0.075	3.54	0.04	3.15 #	0.02	79.36**	4.58	0.109

Treatment	Latency (mean log10 msec)				Trials		75%
(mg/kg)	Response	SE	Collection	SE	Completed	SE	Ratio
Memantine	***		***				
Veh	3.40	0.02	3.14	0.02	95.40	0.41	0.108
0.3	3.40	0.02	3.12	0.02	95.59	0.41	0.121
1	3.38	0.02	3.10	0.02	94.86*	0.89	0.121
3	3.46	0.03	3.10	0.02	93.31*	1.89	0.12
10	4.03***	0.04	3.26***	0.03	48.65***	4.76	0.27
CDP	***		***				
Veh	3.45	0.03	3.17	0.02	93.88	1.20	0.108
1	3.41	0.02	3.15	0.02	96.00	0.00	0.113
3	3.42	0.02	3.14	0.02	96.00	0.00	0.108
10	3.62***	0.04	3.30*	0.02	63.83	6.06	0.082
Diazepam	***		***				
Veh	3.40	0.02	3.14	0.01	96.00	0.00	0.113
0.1	3.39	0.02	3.15	0.01	95.10	0.51	0.113
0.3	3.37*	0.02	3.14	0.01	96.00	0.00	0.123
1	3.49***	0.03	3.23***	0.02	93.38***	2.25	0.107

Fig. 3 The effects of compounds on secondary measures. The presence of an *asterisk* indicates a significant effect where $^{\#}P<0.1$, $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$. *Gray areas* indicate where a comparison could not be performed because of a lack of variance (trials completed), or because of a too few trials completed. The estimated 75 % accuracy ratio is included for illustrative purposes

The highest dose of memantine (10 mg/kg) caused a significant reduction in trials completed ($P<0.001$). A main effect of memantine was also seen on response latency ($F(4, 64)=162$), $P<0.001$), but no interaction was seen between mematine and stimulus level ($F(44, 704)=1.06$, $P=0.36$). A Dunnett's *t* test indicated a significant increase in response latency at 10 mg/kg ($P<0.001$). A main effect of memantine was also seen on collection latency ($F(4, 64)=61.75$, $P<0.001$) and memantine also interacted with stimulus level ($F(44, 440)=1.62$, $P=0.009$). A Dunnett's *t* test indicated a small, but significant decrease in collection latency at 1.0 and 3.0 mg/kg ($P<0.05$), whereas 10 mg/kg caused a highly significant increase in response latency ($P<0.001$). Fewer subjects were included in the analysis of collection latency as animals did not always earn a pellet reward, and in some instances, this resulted in recorded collection latency (see Fig. 3).

CDP

Treatment with CDP resulted in a significant main effect of treatment on accuracy ($P=0.006$) and in a significant treatment by difficulty (stimulus level) interaction ($P<0.001$). Individually, the highest dose (10 mg/kg) significantly interacted with stimulus level ($P<0.05$) and had a significant effect at the 0.44 stimulus condition ($P<0.001$; see Fig. 2).

At the highest dose tested, CDP (10 mg/kg) caused a significant decrease in trials completed ($P<0.001$). Furthermore, a significant effect of CDP ($F(3, 48)=31.46$, $P<0.001$) and a significant CDP by level interaction were observed ($F(11, 176)=5.94$, $P<0.001$) on response latency. A Dunnett's *t* test indicated that 10 mg/kg caused a significant increase in response latency ($P<0.001$). No effect of level was observed on response latency ($F(33, 528)=5.94$, $P<0.001$). A main effect of CDP was seen on collection latency ($F(3, 45)=52.54$, $P<0.001$). A Dunnett's *t* test indicated a significant increase in collection latency at 10 mg/kg ($P<0.001$). No significant effects of level ($F(11, 165)=1.52$, $P=0.12$) or CDP by level interactions ($F(33, 495)=1.07$, $P=0.36$) were observed (see Fig. 3).

Diazepam

Diazepam had no treatment effect on accuracy ($P=0.433$) and did not interact with stimulus level ($P=0.721$). Furthermore, no effect was seen under any individual treatment condition or under the 0.01 and 0.44 stimulus conditions (see Fig. 2).

No effect of diazepam was seen on trials completed. However, diazepam did cause a significant increase in response latency ($F(3, 60)=9.62$, $P<0.001$). A Dunnett's *t* test collapsed across groups indicated a significant increase in latency at the highest dose tested (1 mg/kg). No statistically significant effect of level ($F(11, 220)=1.77$, $P=0.06$) or level by diazepam interaction ($F(33, 660)=1.06$, $P=0.37$) was detected on response latency. Similarly, diazepam caused a significant increase in collection latency ($F(3, 60)=15.00$, $P<0.001$) at 1 mg/kg. However, no interaction with level ($F(33, 660)=1.04$, $P=0.40$) or main effect of level ($F(11, 220)=1.33$, $P=0.21$) was detected (see Fig. 3).

Discussion

In agreement with previous work, we have demonstrated that rodents can solve a visual discrimination using spatial grouping stimuli. Moreover, performance was grouping dependent, where altering the vertical to horizontal ratio would influence performance. While performance was generally stable under these mixed ratio conditions, a small increase in performance was seen over time. Ketamine, PCP, MK-801, and memantine all decreased accuracy in a task designed to evaluate visual perceptual ability. Interestingly, only ketamine and memantine appeared to have a standard dose-dependent effect on

performance; MK-801 and PCP both appeared to follow U-shaped curves, with the significant effects being observed at lower doses but not at higher doses. While memantine did eventually decrease perceptual grouping, this effect was only seen at the highest dose tested, a dose that increased all response latencies and nearly halved trials completed. As predicted, the GABAergic PAM CDP increased perceptual grouping accuracy; however, no effect was observed with diazepam. These results suggest that manipulations of the glutamatergic and GABAergic system may influence perceptual grouping by proximity, although this may not occur in the expected difficulty-dependent fashion.

It has been previously shown that schizophrenic patients are worse on multiple measures of visual integration, including perceptual grouping by proximity (Green et al. 2009; Uhlhaas et al. 2006). Moreover, two previous studies, one using stimuli similar to those used here and another using glass patterns, demonstrate that NMDA-R antagonists (ketamine or PCP) disrupt grouping by proximity under demanding (low signal) conditions. Our findings are generally in line with these previous studies; however, we only partially replicated the previously observed effects. While ketamine, PCP, MK-801, and memantine did disrupt performance, no significant interaction was observed after PCP or MK-801 treatment. The lack of an interaction, in conjunction with our previous findings that NMDA antagonists may induce small impairments in performance of a visual discrimination (Talpos et al. 2012), leads us to believe that MK-801 and PCP are more likely to be influencing the performance of the visual discrimination, rather than perceptual grouping. However, we cannot rule out that the underlying change in behavior could still be grouping-related as data by Ward et al. (2013) would suggest.

Significant interactions were observed with ketamine and mematine, suggesting that these compounds may influence perceptual grouping. Of greatest interest are the results observed with ketamine, the first compound tested in this study (with slightly lower baseline values). Ketamine appeared to disrupt performance under the maximum ratio conditions but had a tendency toward actually enhancing performance under the more difficult conditions meaning that ketamine may have been altering visual perception at 10 mg/kg. While this enhancement at the 0.01 signal condition was not significant, the significant interaction highlights the fact that treatment with ketamine alters the response to varying levels of grouping. Studies by Ward et al. (2013) and Kurylo and Gazes (2008) also suggest that ketamine can interact with perceptual grouping abilities. Our results agree very well with these findings. One possible point of contention is that in this study, ketamine did disrupt performance under the maximum signal condition, suggesting the presence of a main effect of ketamine which could influence the interpretation of the data. However, our statistical model does not indicate a main effect suggesting that the effects observed are best explained by an effect on perceptual grouping and, specifically, that ketamine will alter the influence of varying levels of proximity on accuracy in a visual discrimination. Moreover, we have previously demonstrated that ketamine has no effect on accuracy during performance of a visual discrimination using other stimuli (Talpos et al. 2009). We have also demonstrated that ketamine does not disrupt performance in a visuo-spatial paired associates learning task (Talpos et al. 2014) that likely also requires a visual discrimination. These data further support the notion that ketamine is influencing perceptual grouping. While an interaction was observed with memantine, impairments at the 0.01 and 0.44 condition suggest that while treatment may have caused a statistical interaction, it is not entirely clear if this translates to a biological interaction.

There are some important differences between the study design used here and those used by Ward et al. (2013) or Kurylo and Gazes (2008). First, Ward et al. used glass patterns for their discrimination, a type of stimuli known to activate area V1 of the visual cortex. While a discrimination using glass patterns may be solved via grouping by proximity, there are alternative ways by which such a discrimination could be completed. For example, by focusing on only part of the glass pattern image, it could be that the task was completed by a discrimination based on shape alone. Moreover, assuming animals were completing the glass pattern discrimination task by grouping by proximity, the degradation process by Ward et al. would be increasing signal noise by disrupting the pattern of dots, while also disrupting signal strength. In this regard, our stimuli are more in line with that used by Kurylo and Gazes (2008).

Kurylo and Gazes (2008) used dot patterns that only differed in their horizontal-to-vertical ratio. Accordingly, it seems that rodents could only solve this task via a grouping by proximity, or by somehow measuring the horizontal and vertical separation of dots within the stimuli. We used stimuli very similar to those used in Kurylo and Gazes, except that our stimuli ratio ranged from 1.03 to 2.76, as opposed to 1.2 to 5.9. This reduced stimulus range could contribute to the lack of interaction between PCP and stimulus ratio, although clearly asymptotic performance had been reached. Another important difference between the two studies is the doses used and the number of treatments given. In the study reported here, each animal received each treatment once. However, in the study of Kurylo and Gazes (2008), high doses of ketamine were given on numerous occasions and this resulted in no effect of drug: When the maximum sub-anesthetic dose, as determined by an a priori criterion, was given, an effect of ketamine on accuracy was observed after multiple testing sessions were pooled to ensure completion of an adequate number of trials. We did not attempt to replicate this finding because repeated high-dose administration of ketamine has been shown to have long-lasting impacts on an animal's behavior and is used as an animal model of psychosis (Neill et al. 2010).

Accordingly, this would cloud interpretation of the observed findings. Future work with the procedure used here should include a wider range of stimuli (up to a 5.9 ratio) to determine if this would alter the effects seen. Moreover, this work, as well as future work, will be aided by the inclusion of a vertical/horizontal line control condition that will facilitate determining if an observed effect can be attributed to changes in perceptual grouping, or is better attributed to alteration in performance of a visual discrimination. Despite the differences in procedures and dosing regimens used, these three studies are in basic agreement that ketamine does cause alterations in perceptual grouping. What is unclear is if this effect is suitably robust to serve as pharmacological model of impaired perceptual abilities.

Confirming that NMDA antagonists are generally associated with a net-neuronal excitation, we anticipated that the GABAergic PAMs, CDP, and diazepam would potentially cause an enhancement in perceptual grouping under the more demanding conditions. While diazepam seemingly had no effect on performance in this test, a main effect of CDP, and a treatment by stimulus interaction (10 mg/kg) was observed. These data suggest that GABAergic stimulation may indeed result in enhanced grouping by proximity. However, it is unclear why this effect should also be observed under levels of high accuracy, and why the lowest dose tested (1 mg/kg) might even disrupt performance. An alternative, non-perceptual explanation could be that higher doses of CDP caused a delayed response owing to sedative effects of the drug, thus forcing the animal to take longer in responding and resulting in a more accurate choice. However, the lack of effect with diazepam, which also caused sedation, would suggest otherwise. Moreover, we can offer no straightforward explanation as to why an effect was seen with CDP but not diazepam, but this may be related to intrinsic activity at the GABA A receptor, differences in potency, receptor subtype selectivity, off-site activity, bioavailability, or even vehicle. However, some support for this dissociation can be found within the human literature: For example, Wagemans and colleagues found that lorazepam, but not diazepam, disrupted performance on tasks of countour integration, suggesting that benzodiazepines differ in their properties to effect perceptual abilities in humans (Beckers et al. 2001; Wagemans et al. 1998). However, as in the rodent, very little work has been performed in humans investigating the influence of pharmacological manipulations on perceptual grouping. Additional research will clearly be required to explain the potential biphasic nature of these effects, and biological meaningfulness of the observed effects, and to confirm the stimulus-dependent nature of the observed effects. Importantly, however, the observed effects at 10 mg/kg (increased performance under the high signal condition, and lowered performance under the low signal condition) would be very difficult to attribute to a main effect on performance of a visual discrimination. This is important because despite the findings of Kurylo and Gazes (2008) and Ward et al. (2013), it could be argued that the effects observed here with ketamine are grouping-related. Accordingly, this unusual pattern of performance driven by CDP, which cannot be explained by a simple main effect on accuracy, supports the notion that this procedure is dependent on perceptual grouping. Still, this work would benefit greatly from either pre-clinical imaging studies, or direct injection work, to improve the construct validity of this approach as a translational measure of grouping by proximity.

In summary, visual discriminations based upon spatial proximity represent an exciting avenue for future research. It seems likely that perceptual abilities in the rodent can be studied using the same stimuli and experimental designed as used in humans. Hence, these rodent data may contribute to understanding the fundamentals of human visual perception. The visual cortex is often used as a model of synaptic plasticity. While many in vitro and ex vivo methodologies exist to study changes in plasticity, none allows for the analysis of changes in visual cortex plasticity on a cognitive readout. Accordingly, this task and other tasks of rodent visual perception may be extremely powerful tools in understanding the relevance of local biochemical changes on global cognitive functioning. Perceptual grouping has been shown to be disrupted in schizophrenia indicating that research in the area can be applied to disease. This work could also be of relevance for ASD, where a large number of risk genes have been discovered, many of which are associated with alterations in excitation and inhibition (Luckhardt et al. 2014; Yizhar et al. 2011). Moreover like schizophrenia, ASD is associated with abnormalities in visual perception (Farran and Brosnan 2011; Frith 1996; Scherf et al. 2008), and the two disorders carry many overlapping genetic risk factors (Ayalew et al. 2012; Fromer et al. 2014). However, care should be taken to avoid over-interpretation of these data as small effects with NMDA-R antagonists have previously been reported on performance of a visual discrimination (Fellini et al. 2014; Talpos et al. 2012). As such, it will be necessary to confirm that the effects observed here are dependent upon the visual cortex, and to further quantify what changes are stimulus dependent as opposed to simply disrupting the performance of an operant discrimination. Furthermore, it will be necessary to determine if perceptual grouping occurs in an all-or-none fashion (either the process is engaged, or is not engaged) or is more akin to working memory and is load-dependent (more difficult conditions cause more engagement). Regardless, future work should focus on developing perceptual grouping by proximity as a translational measure, by further investigating the effects of pharmacological manipulations in the rodent as well as in man to determine the construct and predictive validity of this approach.

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