

Glutamatergic ionotropic blockade within accumbens disrupts working memory and might alter the endocytic machinery in rat accumbens and prefrontal cortex

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Summary. Effects of blocking N-methyl-D-aspartic acid (NMDA) and non-NMDA glutamatergic receptors on performance in the hole board test was studied in male rats bilaterally cannulated into the nucleus accumbens (Acc). Rats, divided into 5 groups, received either 1 μ l injections of saline, (\pm) 2-amino-7-phosphonoheptanoic acid (AP-7) (0.5 or 1 μ g) or 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-(f)quinoxaline-7-sulphonamide disodium (NBQX, 0.5 or 1 μ g) 10 min before testing. An increase by AP-7 was observed in ambulatory movements (0.5 μ g; $p < 0.05$), non-ambulatory movements and number of movements (1 μ g; $p < 0.05$); sniffing and total exploration (1 μ g; $p < 0.01$). When holes were considered in order from the first to the fifth by the number of explorations, the most visited holes (first and second) of the AP-7 group were significantly higher than the corresponding holes of saline group ($p < 0.05$ for 0.5 μ g and $p < 0.001$ for 1 μ g). When the second hole was compared with the first of his group, a difference was only observed in the AP-7 1 μ g group ($p < 0.001$). Increasing differences between the other holes and the first were observed by drug treatment. At molecular level, it was observed that AP-7 induced an increase of the coat protein AP-2 expression in Acc, but not AP-180 neither the synaptic protein synaptophysin. The increase of AP-2 was also observed in the medial prefrontal cortex by the action of AP-7 but not NBQX. We conclude that NMDA glutamatergic blockade might induce an activation of the endocytic machinery into the Acc, leading to stereotypies and perseverations, lacking cortical intentional direction.

Keywords: NMDA-glutamatergic transmission; nucleus accumbens; anxiety; hole board; schizophrenia; working memory; perception

Introduction

An important number of studies has been done in recent years paying attention to the nucleus accumbens septi (Acc) of the basal forebrain, that is a major component of the ventral striatum of the rat, and its possible role in the pathophysiology of schizophrenia has been remarked. It receives a large number of projections, some of them of dopaminergic nature coming mainly from the ventral tegmental area, and some cortical glutamatergic afferences from the olfactory and limbic cortex including amygdala, hippocampus and prefrontal cortex (see Grace 2000; Gargiulo et al. 2005). The corresponding receptors are found in the Acc (Albin et al. 1991). In turn, the Acc efferents reach several basal ganglia nuclei, as well as hypothalamic and limbic areas (Pennartz et al. 1994). It strongly suggests that the Acc plays a role as an important interface between the corticolimbic and motor systems.

Acc appears to be involved in several behavioral processes, such as motor activity (Carlsson and Carlsson 1990; Gargiulo 1996), motivation and reward (see Tzschentke and Schmidt 2000), anxiety (Plaznik et al. 1994; Jessa et al. 1996; Martínez et al. 2002a, b) and cognitive functions (Martínez et al. 2002a; Gargiulo et al. 2005; reviewed by Gargiulo 2001, 2003; Gargiulo and Landa 2004), as learning and performance of sensory-motor behaviour in the mammalian Acc (Tzschentke and Schmidt 2000; Roullet et al. 2001). Glutamatergic transmission has been

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pointed as related to learning (Baron and Moersbaeher 1996), and both dopaminergic and glutamatergic transmission appear to participate in these Acc functions (Smith-Roe and Kelley 2000; Di Ciano et al. 2001; Baldwin et al. 2002), and they appear to have effects on different aspects of working memory (Aultman and Moghaddam 2001). The role of the glutamatergic transmission in cognitive functions has also been reported in the Acc of birds (Gargiulo et al. 1998, 2005; Keller and Delius 2001; Acerbo et al. 2002).

Excitatory synaptic transmission within the brain is largely mediated by ionotropic glutamate receptors. It is thought that maintaining and modifying of number of glutamate receptors is regulated by internalization from the synaptic membrane. In the case of AMPA receptors the rapid constitutive internalization is regulated by synaptic activity (Ehlers 2000; Lin et al. 2000), meanwhile NMDA receptors are thought to be stable during synaptic activity. However, certain stimuli can induce rapid internalization of NMDA receptors (Snyder et al. 2001; Nong et al. 2003). Glutamate receptor internalization is thought to occur via clathrin coated vesicles (Martin et al. 1991; Beattie et al. 2000; Ehlers 2000; Lin et al. 2000; Petralia et al. 2003), although the specific mechanisms of glutamate receptor internalization are still poorly understood. A dopamine-mediated internalization of D2-receptors cannot be ruled out, as it has been described in the rat striatum after amphetamine administration (Sun et al. 2003).

Endocytosis is a fundamental mechanism by which neurons control intracellular signalling, nutrient uptake and synaptic transmission. This process is carried out by clathrin coated vesicles (CCV). In most cell types, CCV mediate selective transport from the *trans* Golgi network (TGN), and from the plasma membrane to endosomes (Crowther et al. 1976; Schmid 1997; Kirchhausen 1999). In brain, endocytosis mediated by CCV is required for recycling of membrane proteins after the release of neurotransmitters during synapses (Richards et al. 2000; Slevnev and De Camilli 2000). At the nerve terminals, endocytosis must keep up with exocytosis, otherwise an active presynaptic site would deplete itself of transmitter, and its membrane would swell out of congruence with postsynapse. It has been proposed that membrane of CCV participates in reforming of synaptic vesicles, by direct retrieval or via large cisternal intermediates which pinch off directly from the plasma membrane. The assembly of the coat in clathrin coated pits is regulated by specific adaptors (APs) through interactions with both membrane proteins and clathrin (Kirchhausen 1999). Among the four adaptor complexes described so far, AP-2 appears as the most relevant

component of CCV in endocytosis. This complex is a heterotetramer composed by two large subunits (α and β 2) and two smaller ones (μ 2 and σ 2). In nervous tissue, two types of α subunit have been described so far, α 1 and α 2, although the significance of these two isoforms has not been explained.

Besides the adaptor complexes, other proteins participate in the endocytic machinery mediated by clathrin coated vesicles; e.g. the phosphoprotein AP180, Eps 15, Epsin, and HIP-1 (Huntingtin interacting protein) (Tebar et al. 1996; Slevnev and De Camilli 2000; Cousin and Robinson 2001; Kalthoff et al. 2002). From a body of evidence, it is possible that disturbances in the endocytic process at the nerve terminals are implicated in dysfunctions of the central nervous system and in neurodegenerative diseases.

We have previously reported that NMDA glutamatergic blockade of Acc is an usable model of positive (Gargiulo et al. 1998, 2005; Acerbo et al. 2002; Martínez et al. 2002a) and negative symptoms of schizophrenia (Martínez et al. 2002a, b; reviewed by Gargiulo 2001, 2003; Gargiulo and Landa 2004). Since the NMDA receptor antagonists play an important role on cognitive processes, we decided to comparatively study the action of ionotropic NMDA and non-NMDA (Kainate and AMPA) glutamatergic transmission at molecular level and in a working memory test, taking into account the role of this function in schizophrenia. The aim of the present report is to compare the action of selective blockers of N-methyl-D-aspartic (NMDA) and non-NMDA glutamatergic receptors injected stereotaxically into the Acc on performance in the hole board test, and on expression of coat proteins in areas of rat brain, in order to elucidate if the endocytic machinery is affected and if effects related to exploration strategies and locomotor activity are observed under these experimental conditions.

Materials and methods

Reagents

The monoclonal antibodies against α -adaptin (mAB 100/2), γ -adaptin (mAB 100/3), AP-180, synaptophysin, or the biotinylated anti-mouse IgG, and peroxidase-conjugated avidin were purchased from Sigma Chemicals (St. Louis, MO). Chemiluminescent reagents were from Pearce (Rockford, IL).

Subjects

Male rats from a Holtzman-derived colony, aged 90 days and weighing 240–270 g were used ($n = 141$). They were maintained under controlled conditions of temperature (22–24°C), humidity (20–25%) and light cycle of 12L:12D (7:00–19:00 h). Standard rat chow and water were freely available.

Surgery

Animals were anesthetized with ether and stereotaxically implanted with bilateral stainless-steel cannuli into the Acc. Coordinates for cannulae implantation were: antero-caudal: +3.4; lateral: ± 2.0 ; vertical: -4.5 , according to the Atlas of Pellegrino et al. (1979). The cannuli consisted of an outer guiding cannula stainless-steel tubing (23-gauge, 15 mm in length) and an inner removable stylet (30-gauge, 15 mm in length) to prevent obstruction. After surgery, rats were housed individually and maintained undisturbed for a week-long recovery.

Apparatus

The Hole-Board apparatus is an Opto Varimex (Columbus Instruments) with a $42 \times 42 \times 23$ cm arena, with a plexiglass floor with 5 holes, each one of 3 cm in diameter. The room was illuminated by a 60 W bulb 1.5 m above the apparatus. Testing was performed by direct observation, and the vertical, ambulatory, non-ambulatory and the number of movements were counted automatically by the OVM apparatus.

Behavioral procedures

The animals were injected, while manually restrained, 10 min before testing. A 30 gauge, 17 mm long stainless steel injection cannula (dimensioned to reach precisely the Acc) attached to a 10 μ l microsyringe (Hamilton) was introduced into the guide cannula. Volumes of 1 μ l solution were gradually injected over 2 min periods into both the left and right Acc. The injection cannuli were kept in place for an additional minute in order to allow a diffusion. The rats received bilateral injections of saline or drugs (see below) 10 min before each session.

The rats were placed individually in the center of the OVM apparatus, and allowed 5 min for free exploration. All the sessions took place between 17.00–19.00 h. Each rat was used only one time. The standard measures were: rearing, ambulatory movements, non-ambulatory movements, number of movements, sniffing into the holes, head dipping, total holes exploration (sniffing and head dipping together). The last parameter was studied ordering the holes explored (from the first to the fifth by the number of exploratory behaviors displayed into them by each rat).

Drugs

Groups of animals were injected with either (\pm) 2-amino-7-phosphoheptanoic acid (AP-7, Research Biomedical International) solution (0.5 and 1 μ g/1 μ l), or 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-(f) quinoxaline-7-sulphonamide disodium (NBQX disodium salt, Tocris) (0.5 and 1 μ g/1 μ l) both dissolved in saline. Control rats were injected with 1 μ l saline.

Immunoblotting

All the procedures were carried out as described by Manzur et al. (2001). Briefly, after injections, control or treated rats chosen randomly from the behavioral assays were sacrificed, the brain removed and the medial prefrontal cortex and Acc were dissected on a cold plate and placed in individual tubes with 10 mM Tris-acetate, pH 7.2 containing 0.25 M sucrose, 5 mM EDTA, 1 mM leupeptin, and 0.1 mM PMSF (homogenization buffer). The tissues were homogenized separately with a Teflon pestle homogenizer, and postnuclear supernatants were obtained by centrifugation at $1500 \times g$ for 10 min. Between 30 and 50 μ g of proteins from each homogenate were solubilized by boiling in sample buffer and analyzed by SDS-PAGE on 8% acrylamide minigels according to Laemmli (1977). The proteins were then electrotransferred onto 0.2 μ m pore diameter nitrocellulose membrane (Sartorius) and processed for immunodetection

with either monoclonal antibody against α -adaplin, γ -adaplin, AP180 or synaptophysin (each diluted 1:200 in phosphate buffered saline plus 0.05% Tween 20) according to Manzur et al. (2001). Specific protein bands were detected by the chemiluminescent method, using X-OMAT-LS film (Kodak), and quantified by densitometric scanning of the films, and Scion Image program.

Other procedures

Proteins were measured according to Lowry et al. (1951).

Histology

When the testing was completed, the rats were injected with 1 μ l saturated methylene blue solution into the Acc. Fifteen min later they were sacrificed with an excess of ether. The brains were removed from the skull and fixed in 20% formalin solution. The brains were mounted and frozen in a cryotome and cut into 40 μ m sections. The block face was examined with a $10 \times$ magnifying lens and the sections containing the injection sites were saved. Microscopic inspection of these sections served to ascertain the location of the cannula tips. The locations were transferred to standard sections taken from a brain atlas (34). We only report data for those rats which had correct Acc cannula placements, according to the Atlas of Pellegrino et al. (1979).

Data analysis

The Kolmogorov Smirnov test was used to decide if the distribution of the data was normal. One way Anova followed by Student Newman-Kewls Multiple comparison test was applied. In all cases, a $p < 0.05$ (two tailed) was considered significant. The results are reported as means \pm standard errors ($n = 18-26$).

Results

Histology

Figure 1 shows a schematic representation of the cannula placements for all the experiments. Histological analysis shows that the injection site is located in the center of Acc core for the majority of rats. Only rats showing correct Acc placements were included in statistical analysis (Fig. 1).

Behavioural experiments

As observed in the Fig. 2, the ambulatory movements were increased by the lower dose of AP-7 (0.5 μ g; $p < 0.05$), meanwhile, the non-ambulatory movements and number of movements were only increased by the higher dose of AP-7 (1 μ g; $p < 0.05$). Sniffing and total exploration were also increased by the dose of 1 μ g ($p < 0.01$, Fig. 3). When holes were considered in order (from the first to the fifth by the number of exploratory behaviors displayed into them by each rat), exploration into the most visited holes (first and second hole) was significantly higher in the AP-7 group than the corresponding holes of the control group ($p < 0.05$ for

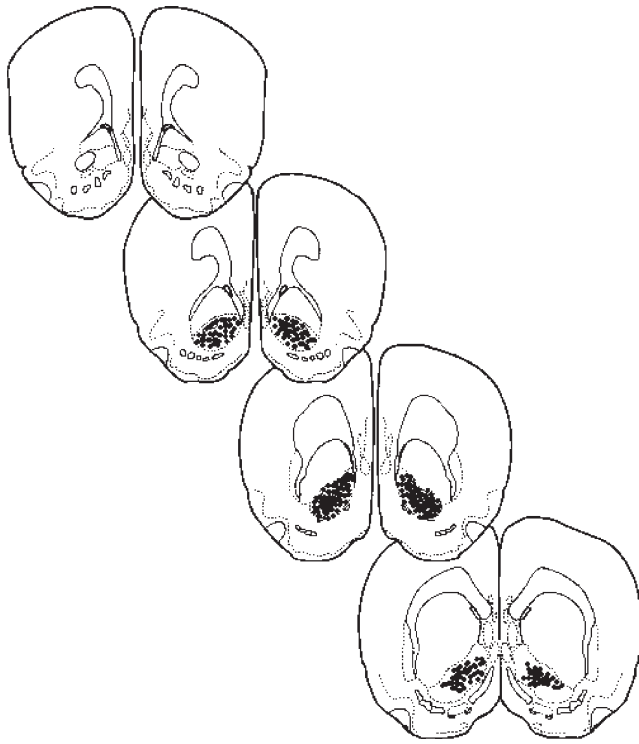


Fig. 1. Frontal brain sections showing the location of the injection site

0.5 μg , and $p < 0.001$ for 1 μg , Fig. 4). When the second hole was compared with the first of the corresponding group, a significant difference was only observed in the AP-7 group with a dose of 1 μg ($p < 0.001$). Increasing differences were observed between the other holes and the first in the experimental rats. In the saline group, fourth and fifth holes

differed significantly of the first ($p < 0.05$). In the lower dose AP-7 treated group, the third hole showed differences with the first ($p < 0.05$), and it was bigger than the third in the fourth and fifth ($p < 0.001$). In the group treated with the higher AP-7 dose, a clear difference in exploratory behaviors was seen between the first and all the other holes ($p < 0.001$). In the NBQX treated groups, the lower dose induced a very significant difference ($p < 0.001$) between all the holes when compared with the first, except the second one. In the case of the higher NBQX dose treatment, no difference was observed between the second and the first hole, but this comparison with the first hole reached significant values when it was compared with the third ($p < 0.05$), fourth and fifth ($p < 0.001$).

Expression of coat proteins is altered in Acc and prefrontal cortex

AP-2 adaptor complexes are involved in internalization of macromolecules via clathrin coated vesicles. Here, we observed that expression of the assembly coat protein AP-2 is altered by the action of the NMDA glutamatergic blockade agent AP-7 injected within the rat Acc. As shown in the Fig. 5, expression of both α -subunits ($\alpha 1$ and $\alpha 2$) is significantly increased by the treatments but not the phosphoprotein AP-180, neither the γ -subunits corresponding to the AP-1 adaptor complex nor synaptophysin (data not shown). Additionally, a similar increase of AP-2 was observed in the prefrontal cortex by the action of AP-7 (Fig. 5) but not by NBQX (data not shown).

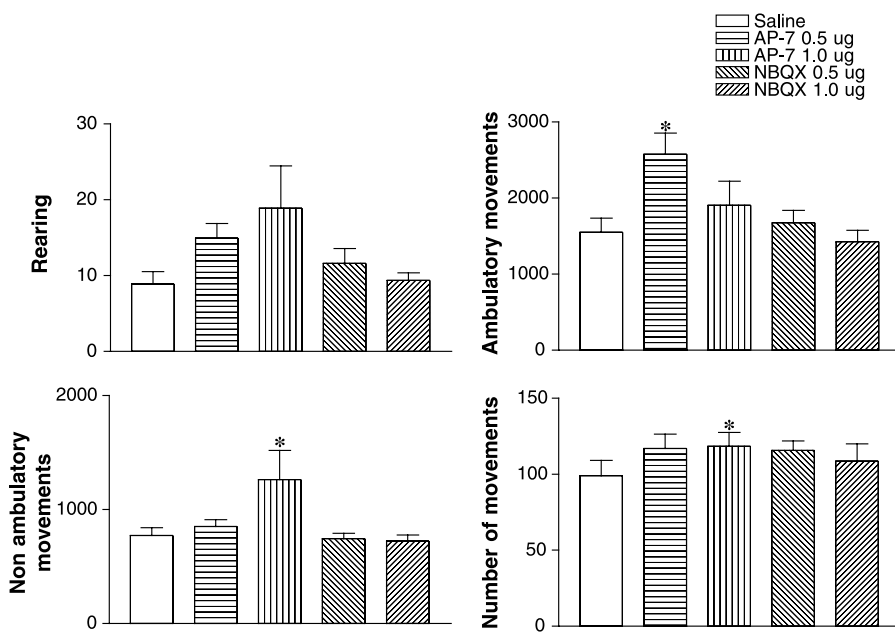


Fig. 2. Top left panel: rearing behavior of rats injected into the Acc with saline (1 μl), 7-aminophosphonoheptanoic acid solution (AP-7, 0.5 and 1.0 $\mu\text{g}/1 \mu\text{l}$) and 2,3 dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-(f)quinoxaline-7-sulphonamide disodium (NBQX) solution (0.5 or 1 $\mu\text{g}/1 \mu\text{l}$) 10 min before testing. Top right panel: ambulatory movements; bottom panel left, non-ambulatory movements; bottom panel right: number of movements of the same groups ($n = 18-26$ rats; means \pm sem; * $p < 0.05$)

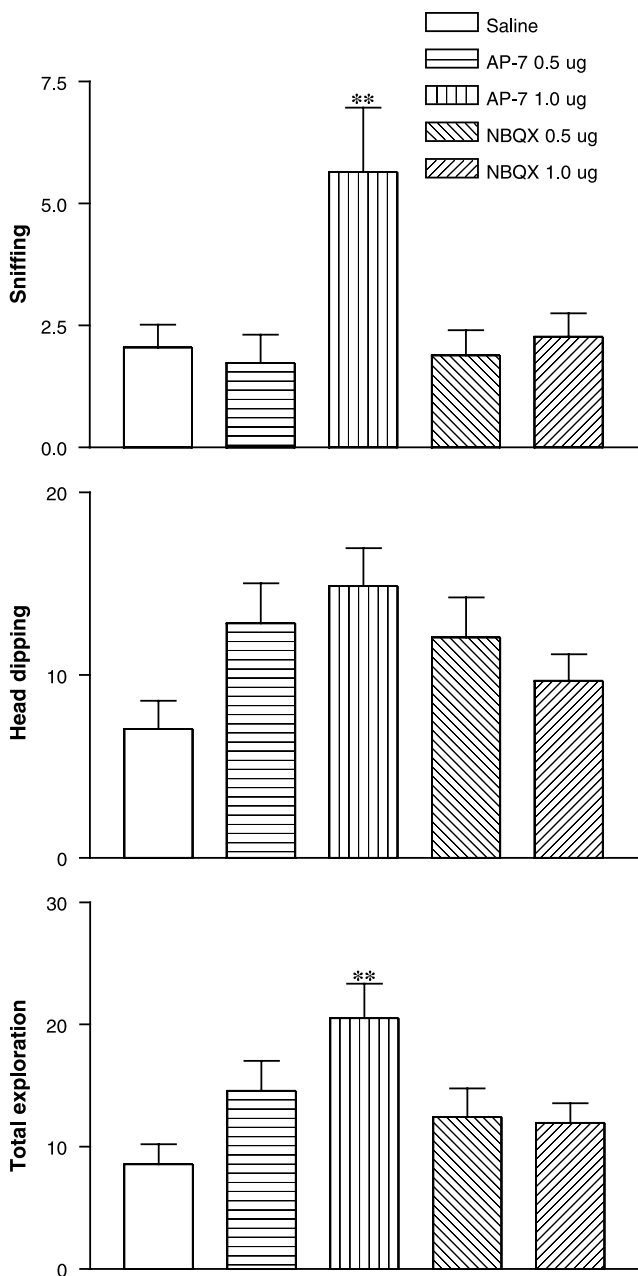


Fig. 3. Top: sniffing behavior of rats injected into the Acc with saline (1 μ l), 7-aminophosphonoheptanoic acid solution (AP-7, 0.5 and 1.0 μ g/1 μ l) and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-(f)quinoxaline-7-sulphonamide disodium (NBQX) solution (0.5 or 1 μ g/1 μ l) 10 min before testing. Medium: head dipping, and bottom, total exploration of movements of the same groups ($n = 18-26$ rats; means \pm sem; * $p < 0.05$)

Discussion

In the present experiments, we explored the effect of the NMDA and non-NMDA glutamatergic blockade of Acc in a working memory test. In line with a previous study, in the plus maze test (Martínez et al. 2002b) ambulatory movements were increased by the lower dose of AP-7 (Fig. 2).

At a higher, we observed an increase in non-ambulatory movements and the total number of movements (Fig. 2). It appears to point that the increasing ambulatory activity displayed with the lower dose of AP-7, ended in the disorganization of behaviour, with the prevalence of stereotyped non ambulatory behaviours, increasing significantly the number of movements. Head dipping was not modified by the treatment, however sniffing was increased significantly, resulting in an increase of total exploration (Fig. 3). However, sniffing, a more stereotyped and incomplete searching behaviour into the holes, is clearly increased by the drug treatment with the higher dose (Fig. 3).

The fact that only the first and the second holes differed significantly of the corresponding saline holes, and not the others, could be considered an important argument in the same way (Fig. 4). Rats of the AP-7 groups display stereotyped exploration mainly in two holes, and in the case of the higher dose, even a clear difference can be observed between the first and the second hole, indicating the prevalence of one hole over all the others. Taking into account that the exploratory strategy used by this group of rats is mainly sniffing, it can be considered that stereotypies result from the pharmacological procedure we used, with the prevalence of mechanic movements over a searching strategy. The impossibility for correct the rat exploration strategy could be related to previous findings; with the same pharmacological procedure an increase in correcting trials was observed in pigeon cognitive tasks (Gargiulo et al. 1998, 2005; Acerbo et al. 2002). At this point we have observed that the mentioned increase could be considered as a stereotyped choice persistence (Robbins 1991). This behaviour could be also related to difficulties in the reversal learning, classically linked to damage in the Acc (Taghzouti et al. 1985; Gal et al. 1997).

The hole board allows the simultaneous assessment of working memory (WM) and reference memory (RM) in rats (Oades and Isaacson 1978; van der Staay 1999). The hole board is classically used with some holes baited with one food pellet each. In these conditions it has been reported that normal rats develop a fixed food search pattern or food search strategy (Oades and Isaacson 1978; Oades 1981a, b, 1982; van der Staay et al. 1990a, b; van der Staay 1999). This schedule enables the differentiation of working memory and reference memory. In our conditions, with only one exposure to the test, we are measuring only working memory. The aim of the present study was to evaluate the effects of glutamatergic blockade without interference of appetitive component. By this reason we did not reinforce the holes with pellets, aiming to study disruptions of searching behaviors with no rewards, and using only one exposition

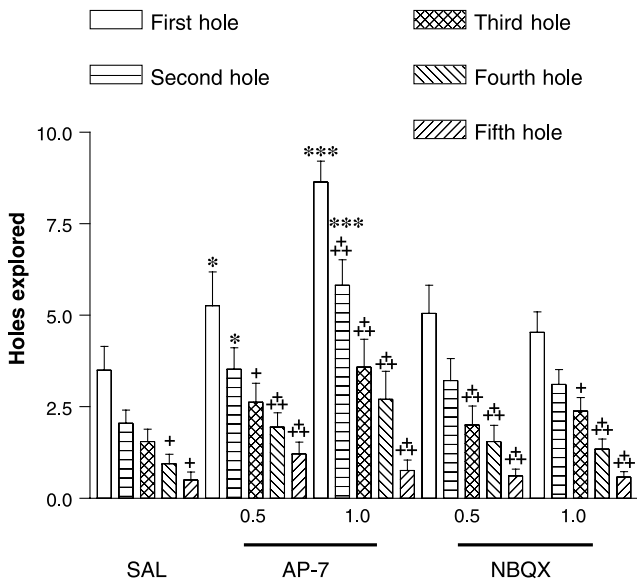


Fig. 4. Comparison of holes exploration in frequency order in rats injected in the Acc with saline (1 µl), 7-aminophosphonoheptanoic acid solution (AP-7, 0.5 and 1.0 µg/1 µl) and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-(f)quinoxaline-7-sulphonamide disodium (NBQX) solution (0.5 or 1 µg/1 µl) 10 min before testing ($n = 18-26$ rats; means \pm sem; * $p < 0.05$; *** $p < 0.001$ compared to the corresponding number hole in saline control group; + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.01$ compared to the first hole of his group or dose)

in conditions of novelty. This situation, with a concomitant Acc glutamatergic blockade, has been reported to produce an increase in dopamine release in this nucleus (Carlsson et al. 1998).

It has been proposed that a glutamate deficiency leads to hiperactivity of monoaminergic systems (Carlsson et al. 2000). This phenomenon may be potentiated by other stim-

ulatory conditions. The mechanisms appears to be that cortical glutamate regulates monoaminergic neurons in two ways, one directly by means of fibers projecting to their somatodendritic areas, or accelerators, and indirectly, projecting to the GABAergic interneurons, or brakes. The balance between the both ways appears to be regulated by feedback loops, and the involvement of the thalamus has been postulated (Carlsson et al. 1998). When the dopamine release is induced by means of amphetamine, the brake relative to the accelerator appears to be strengthened to counteract that release, and a feedback loop, that probably involves the thalamus, appears to be involved. The simultaneous administration of MK-801 enhanced the release of dopamine and the full effect of amphetamine, because of the unconstrained condition of the feedback system (Miller and Abercrombie 1996). Clinically, this phenomenon has been observed in drug free schizophrenic patients using SPECT (Laruelle et al. 1996).

Working memory is a function that has been frequently reported as disrupted in schizophrenic patients (Egan et al. 2001; Keri et al. 2001), and it is a function closely related to frontal cortex (see Goldman-Rakic 1995), and Acc receives glutamatergic projections of this zone (Pennartz et al. 1994; Grace 2000). It has been proposed that the Acc integrates different functions, such as affective facilitation (amygdala), contextual constraints (hippocampus) and goal directed motor plans (prefrontal cortex) (Grace 2000). In Acc limbic structures have overlapping input with the dopamine system, and the result of this integration is a goal-directed movement limited by contextual information and emotional significance of stimuli (Grace 2000), and we are here blocking glutamatergic cortical inputs.

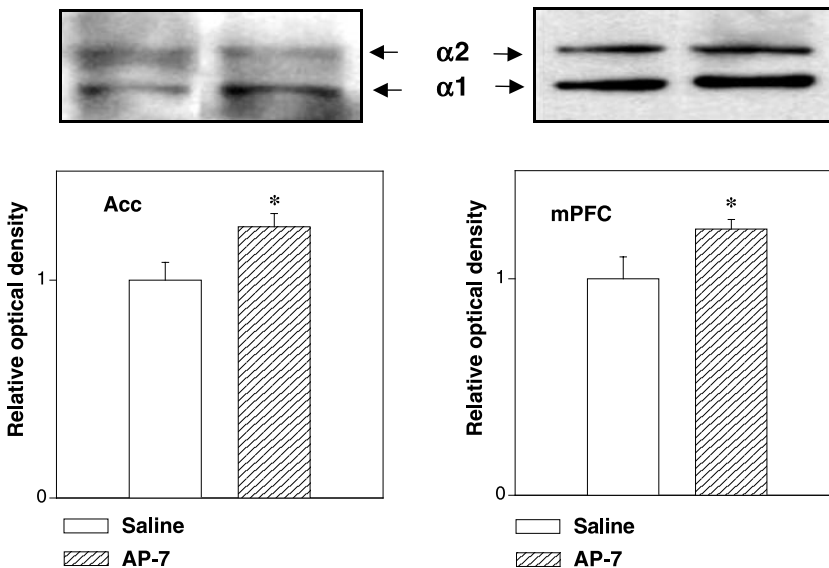


Fig. 5. Expression of AP-2 complexes in nucleus accumbens (Acc) and in medial prefrontal cortex (mPFC) of rat brain after injection with AP-7 or saline within the Acc. Immunoblot analysis of AP-2 from homogenates of the tissues. Bars graphics represent the relative optical density ($\alpha 1 + \alpha 2$ subunits) \pm SD from six experiments ($n = 6$) calculated in arbitrary units from the films (* $p < 0.05$). Top panels: representative pictures of the immunoblots

Stereotyped behaviour has been analysed in terms of cognitive functions (Robbins 1991). The absence of a determined intention could be considered as a failure of goal directed parameters (Grace 2000), or the absence of intentional movements, with the prevalence of spontaneous not directed movements, disrupted by pharmacological blockade. They have been previously linked to dopaminergic stimulation (Le Moal 1995; Lyon and Robbins 1975), however, our results demonstrate a relationship with glutamatergic blockade, and it could be supposed that stereotyped behaviours induced by dopamine could be mediated by inhibitory interactions with glutamatergic terminals, since they have been widely described (Schmidt and Kretschmer 1997; Schmidt 1998; Moore et al. 1999; Nicola et al. 2000). The interference of attention with this pharmacological procedure has been previously postulated by us as a model of schizophrenia, in a perception recognition task (Gargiulo et al. 1998, 2005; Acerbo et al. 2002), starting from our previous clinical findings (Del Vecchio and Gargiulo 1992).

Recovery of membrane proteins following a synaptic process is crucial for maintenance of membrane homeostasis at the nerve terminus and also for the neuronal plasticity. We observed that AP-7 and NBQX induced an increase of AP-2 expression in Acc and in the prefrontal cortex in rats treated with this drug. This could be related with an increase of endocytic activity via clathrin coated vesicles in these areas of the brain. Besides the blockade of glutamate receptors in Acc it is also possible that these drugs may induce internalization and inactivation of these receptors in neurons. On the other hand, the dopamine D3 receptor, which is highly enriched in Acc, has been suggested to play an important role in reinforcement and reward (Levant 1997; Richtand et al. 2001). Activation of D3 receptors regulate GABA_A receptor function through a clathrin coated vesicle endocytosis, suppressing the efficacy of inhibitory synaptic transmission in Acc (Chen et al. 2006). A dopamine-mediated internalization of D2-receptors has been described in the rat striatum after amphetamine administration (Sun et al. 2003). Grace (2000) has proposed that pathological disruptions in one of the glutamatergic afferent system that innervate the Acc (hippocampus, amygdala, or PFC) causes a decrease in tonic DA levels, and a consequence there would be a potent disinhibition of phasic DA release. This phasic DA release could be related to an increased internalization of DA receptors via clathrin coated vesicles. Interestingly, other coat proteins, as AP-180 were not changed by the treatment with the drugs, suggesting that the effect could be directed to certain populations of clathrin coated vesicles. Future evaluations may elucidate

if AP-7 and/or NBQX induce direct internalization of D2/D3 receptors in Acc.

The increase in clathrin activity within the medial prefrontal cortex could be explained by an overloading of cortical activity due to a decrease in the thalamic filter activity, as we hypothesized previously (Gargiulo and Landa 2004), as related to mechanism of schizophrenia (Gargiulo 2001, 2003; Gargiulo and Landa 2004), and leading to judgement disorders, such as schizophrenia, mainly related to perceptual organization deficits (Peters et al. 2002). These cognitive deficits could be considered as central facts of schizophrenia and its neural disorders (Holden 2003a), mainly the glutamatergic neurotransmission dysfunctions (Holden 2003b). A subcortical disbalance between dopaminergic and glutamatergic pathways from brainstem and cortex, respectively, has been proposed as biological base of schizophrenia (Carlsson et al. 2000). Direct and indirect pathways are controlled by glutamatergic corticostriatal fibers, acting as brakes (indirect pathway) or accelerators (direct pathway, see Carlsson et al. 2000). The indirect pathway is a striatal GABAergic system that protects the cortex of a sensory overload and hyperarousal, exerting its inhibitory effect through the inhibition of thalamocortical glutamatergic neurons. Hyperfunction of dopaminergic pathways, or a functional decrease of corticostriatal glutamate pathways leads to schizophrenic psychotic states (Carlsson et al. 1998, 2000). The direct pathway appears to exert an excitatory influence. In our present experimental conditions, the blockade of Acc could be inducing a decrease in the indirect pathway, with a functional decrease of thalamic filter, leading to cortical hyperactivity.

Interestingly, positron emission tomography experiments done in normal subjects with ketamine at subanesthetic doses, studying the brain regions that are involved in NMDA-receptor mediated psychoses, show also frontal cortex hyperfunction (Breier et al. 1997; Vollenweider et al. 1997). The activation was observed within the medial prefrontal cortex and anterior cingulate cortex, and correlate positively with symptom formation (Vollenweider et al. 1997). We used here a highly selective NMDA antagonist injected into the brain area postulated as the neural substrate of phencyclidine psychoses.

We conclude from present results and previous studies (Gargiulo et al. 1998, 2005; Acerbo et al. 2002; Martínez 2002a, b) that a glutamatergic blockade of the Acc appears to lead to cognitive disturbances, such as disruption of working memory, and that here they appear to be related to interference with searching strategies, and by this way with the recognition of novel places, necessary for the acquisition of a new environmental map. The relevance of the

Acc for the goal directed behaviours was here observed. Additionally, this blockade causes disruption of perceptual discrimination, affective flattening and loose of goal directed behaviors, remembering some core symptoms, as the syndromatic essence of schizophrenic psychoses. The relevance of glutamatergic blockade of Acc for the emulation of schizophrenic symptoms is here, once more, strongly suggested. Additionally, an increase in general stereotyped behaviours is showing the relevance of glutamatergic cortical inputs to Acc to direct and manage these movements. Furthermore, activation of synaptic activity within Acc and, even more, in medial PFC, gives additional similarities to this model when compared to human schizophrenia (Breier et al. 1997; Vollenweider et al. 1997).

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References

- Acerbo MJ, Gargiulo PA, Krug I, Delius JD (2002) Behavioural consequences of nucleus accumbens dopaminergic stimulation and glutamatergic blocking in pigeons. *Behav Brain Res* 136(1): 171–177
- Albin RL, Makowiec RL, Hollingsworth ZR, Dure LS IV, Penney JB, Young AB (1991) Excitatory amino acid binding sites in the basal ganglia of the rat: a quantitative autoradiographic study. *Neuroscience* 46: 35–48
- Aultman JM, Moghaddam B (2001) Distinct contributions of glutamate and dopamine receptors to temporal aspects of rodent working memory using a clinical relevant task. *Psychopharmacology* 153: 353–364
- Baldwin AE, Sadeghian K, Holahan MR, Kelley AE (2002) Appetitive instrumental learning is impaired by inhibition of cAMP-dependent protein kinase within the nucleus accumbens. *Neurobiol Learn Mem* 77(1): 44–62
- Baron SP, Moersbaecheer JMJ (1996) Disruption of learning by excitatory amino acid receptor antagonists. *Behav Pharmacol* 7: 573–584
- Beattie EC, Carroll RC, Morishita W, Yasuda H, von Zastrow M, Malenka RC (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat Neurosci* 3(12): 1291–1300
- Breier A, Malhotra AK, Pinals DA, Weisenfeld NI, Pickar D (1997) Association of ketamine-induced psychosis with focal activation of the prefrontal cortex in healthy volunteers. *Am J Psychiatry* 154(6): 805–811
- Carlsson A, Hansson LO, Waters N, Carlsson ML (1998) A glutamatergic deficiency model of schizophren. *Br J Psychiatry* 173 (Suppl 37): 2–6
- Carlsson A, Waters N, Waters S, Carlsson ML (2000) Networks interactions in schizophrenia-therapeutic implications. *Brain Res Rev* 31: 342–349
- Carlsson M, Carlsson A (1990) Schizophrenia: a subcortical neurotransmitter imbalance syndrome? *Schizophrenia Bull* 16: 425–432
- Chen G, Kittler JT, Moss SJ, Yan Z (2006) Dopamine D3 receptors regulate GABA_A receptor function through a phospho-dependent endocytosis mechanism in nucleus accumbens. *J Neurosci* 26(9): 2513–2521
- Cousin MA, Robinson PJ (2001) The dephosphins: dephosphorylation by calcineurin triggers synaptic vesicle endocytosis. *Trends Neurosci* 24: 659–665
- Crowther RA, Finch JT, Pearse BM (1976) On the structure of coated vesicles. *J Mol Biol* 103: 785–798
- Del Vecchio S, Gargiulo PA (1992) Visual and motor function in schizophrenic patients. *Acta Psiquiat Psicol Am Lat* 38: 317–322
- Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. *J Neurosci* 21(23): 9471–9477
- Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA* 98(12): 6917–6922
- Ehlers MD (2000) Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* 28(2): 511–525
- Gal G, Joel D, Gusak O, Feldon J, Weiner I (1997) The effects of electrolytic lesion to the shell subterritory of the nucleus accumbens on delayed non-matching-to-sample and four-arm baited eight-arm radial-maze tasks. *Behav Neurosci* 111: 92–103
- Gargiulo PA (1996) Thyrotropin releasing hormone injected into the nucleus accumbens septi selectively increases face grooming in rats. *Braz J Med Biol Res* 29: 805–810
- Gargiulo PA (2001) Experimental approaches to delusional perception. *Alcmeon Rev Arg Neuropsiqu* 37: 18–30
- Gargiulo PA (2003) Experimental approaches to perceptual dysfunction in schizophrenia. *Rev Neurol* 37(6): 545–551
- Gargiulo PA, Siemann M, Delius J (1998) Visual discrimination in pigeons impaired by glutamatergic blockade of nucleus accumbens. *Physiol Behav* 63: 705–709
- Gargiulo PA, Landa de Gargiulo AI (2004) Perception and psychoses: the role of glutamatergic transmission within the nucleus accumbens septi. *Behav Brain Sci* 27(6): 792–793
- Gargiulo PA, Acerbo MJ, Krug I, Delius JD (2005) Cognitive effects of dopaminergic and glutamatergic blockade in nucleus accumbens in pigeons. *Pharmacol Biochem Behav* 81(4): 732–739
- Goldman-Rakic PS (1995) Architecture of the prefrontal cortex. In: Grafman J, Holyoak KJ, Boller F (eds) *Structure and functions of the human prefrontal cortex*. *Ann NY Acad Sci* 769: 71–83
- Grace AA (2000) Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Res Rev* 31(2–3): 330–341
- Holden C (2003a) Deconstructing schizophrenia. *Science* 299: 333–335
- Holden C (2003b) Excited by glutamate. *Science* 300: 1866–1868
- Jessa M, Nazar M, Plaznik A (1996) Effect of intra-accumbens blockade of NMDA receptors in two models of anxiety, in rats. *Neurosci Res Commun* 19: 19–25

- Kalthoff C, Alves J, Urbanke C, Knorr R, Ungewickell EJ (2002) Unusual structural organization of the endocytic proteins AP-180 and epsin 1. *J Biol Chem* 277: 8209–8216
- Keller S, Delius JD (2001) Discriminative learning occasioned by the administration of a dopamine antagonist. *Psychopharmacology* 157: 320–323
- Keri S, Szendi I, Kelemen O, Benedek G, Janka Z (2001) Remitted schizophrenia-spectrum patients with spared working memory show information processing abnormalities. *Eur Arch Psych Clin Neurosci* 251(2): 60–65
- Kirchhausen T (1999) Adaptors for clathrin-mediated traffic. *Annu Rev Cell Dev Biol* 15: 705–732
- Laemmli UK (1977) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685
- Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdoz J, McCance E, Rosenblatt W, Fingado C, Zoghbi SS, Baldwin RM, Seiby LJP, Krystal JH, Charney DS, Innis RB (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc Natl Acad Sci USA* 93(17): 9235–9240
- Le Moal M (1995) Mesocorticolimbic dopaminergic neurons. Functional and regulatory roles. In: Bloom FE, Kupfer DJ (eds) *Psychopharmacology: the fourth generation of progress*. Raven Press, New York, pp 283–294
- Levant B (1997) The D3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol Rev* 49: 231–252
- Lin JW, Ju W, Foster K, Lee SH, Ahmadian G, Wyszyski M, Wang YT, Sheng M (2000) Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. *Nat Neurosci* 3(2): 1282–1290
- Lowry O, Rosebrough A, Farr R, Randall R (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275
- Lyon M, Robbins TW (1975) The action of central nervous system stimulant drugs: a general theory concerning amphetamine effects. In: Essman W, Valzelli L (eds) *Current developments in psychopharmacology*, vol 2. Spectrum Publications, New York, pp 79–163
- Manfredi JJ, Bazari WL (1987) Purification and characterization of two distinct complexes of assembly polypeptides from calf brain coated vesicles that differ in their polypeptide composition and kinase activities. *J Biol Chem* 262: 12182–12188
- Manzur A, Sosa MA, Seltzer AM (2001) Transient increase in Rab 3 and Synaptobrevin immunoreactivity after mild hypoxia in neonatal rats. *Cell Mol Neurobiol* 21(1): 39–52
- Martin M, Gonzalez-Calero G, Cubero A (1991) Characterization of L-[3H]glutamate binding sites in bovine brain coated vesicles. *Eur J Pharmacol* 207(3): 215–224
- Martínez G, Ropero C, Funes A, Flores E, Landa AI, Gargiulo PA (2002a) AP-7 into the nucleus accumbens disrupts acquisition but does not affect consolidation in a passive avoidance task. *Physiol Behav* 76(2): 205–212
- Martínez G, Ropero C, Funes A, Flores E, Blotta C, Landa AI, Gargiulo PA (2002b) Effects of NMDA and non-NMDA blockade in the nucleus accumbens on the plus maze test. *Physiol Behav* 76(2): 219–224
- Miller DW, Abercrombie ED (1996) Effects of MK-801 on spontaneous and amphetamine-stimulated dopamine release in striatum measured with in vivo microdialysis in awake rats. *Brain Res Bull* 40(1): 57–62
- Moore H, West AR, Grace AA (1999) The regulation of forebrain dopamine transmission: relevance to the pathophysiology and psychopathology of schizophrenia. *Biol Psychiatry* 46: 40–55
- Nicola SM, Surmeier DJ, Malenka RC (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu Rev Neurosci* 23: 185–215
- Nong Y, Huang YQ, Ju W, Kalia LV, Ahmadian G, Wang YT, Salter MW (2003) Glycine binding primes NMDA receptor internalization. *Nature* 422(6929): 302–307
- Oades RD (1981a) Impairments of search behaviour in rats after haloperidol treatment, hippocampal or neocortical damage suggest a mesocorticolimbic role in cognition. *Biol Psychol* 12: 77–85
- Oades RD (1981b) Dopaminergic agonistic and antagonistic drugs in the ventral tegmentum of rats inhibit and facilitate changes of food search behaviour. *Neurosci Lett* 27: 75–80
- Oades RD (1982) Search strategies on a hole-board are impaired in rats with ventral tegmental damage: animal model for tests of thought disorder. *Biol Psychiatry* 17: 243–258
- Oades RD, Isaacson RL (1978) The development of food search behavior by rats: the effects of hippocampal damage and haloperidol. *Behav Biol* 24: 327–337
- Pellegrino LJ, Pellegrino AS, Cushman AJ (1979) *A stereotaxic atlas of the rat brain*. Plenum Press, New York
- Pennartz CMA, Groenewegen HJ, Lopes da Silva F (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. *Prog Neurobiol* 42: 719–761
- Peters ER, Nunn JA, Pickering AD, Hemsley DR (2002) Perceptual organization deficits in psychotic patients. *Psychiatry Res* 110(2): 125–135
- Petralia RS, Wang YX, Wenthold RJ (2003) Internalization at glutamatergic synapses during development. *Eur J Neurosci* 18(12): 3207–3217
- Plaznik A, Palejko W, Nazar M, Jessa M (1994) Effects of antagonists at the NMDA receptor complex in two models of anxiety. *Eur Neuropsychopharmacol* 4: 503–512
- Richards DA, Watimosin C, Betz WJ (2000) Two endocytic recycling routes selectively fill two vesicle pools in frog motor nerve terminals. *Neuron* 27: 551–559
- Richtand NM, Woods SC, Berger SP, Strakowski SM (2001) D3 dopamine receptor, behavioral sensitization, and psychosis. *Neurosci Biobehav Rev* 25: 427–443
- Robbins TW (1991) Cognitive deficits in schizophrenia and Parkinson's disease: neural basis and the role of dopamine. In: Willner P, Scheel Kruger J (eds) *The mesolimbic dopamine system: from motivation to action*. Wiley, Chichester, pp 497–528
- Roulet P, Sargolini F, Oliverio A, Mele A (2001) NMDA and AMPA antagonist infusions into the ventral striatum impair different steps of spatial information processing in a nonassociative task in mice. *J Neurosci* 21(6): 2143–2149
- Schmid SL (1997) Clathrin-coated vesicle formation and protein sorting: an integrated process. *Annu Rev Biochem* 66: 511–548
- Schmidt WJ (1998) Dopamine-glutamate interactions in the basal ganglia. *Amino Acids* 14: 5–10
- Schmidt WJ, Kretschmer BD (1997) Behavioural pharmacology of glutamate receptors in the basal ganglia. *Neurosci Biobehav Rev* 21(4): 381–392
- Schroder S, Ungewickell E (1991) Subunit interaction and function of clathrin coated vesicle adaptors from the Golgi and the plasma membrane. *J Biol Chem* 266: 7910–7918
- Slevnev VI, De Camilli P (2000) Accessory factors in clathrin-dependent synaptic vesicle endocytosis. *Nat Rev Neurosci* 1: 161–172
- Smith-Roe SL, Kelley AE (2000) Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. *J Neurosci* 20(20): 7737–7742
- Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF (2001) Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat Neurosci* 4(11): 1079–1085
- Sun W, Ginovart N, Ko F, Seeman P, Kapur S (2003) In vivo evidence for dopamine-mediated internalization of D2-receptors after amphetamine: differential findings with [3H] raclopride versus [3H] spiperone. *Mol Pharmacol* 63(2): 456–462
- Taghzouti K, Louilot A, Herman JP, Le Moal M, Simon H (1985) Alternation behavior, spatial discrimination, and reversal disturbances in the nucleus accumbens of the rat. *Behav Neural Biol* 44: 354–363

- Tebar F, Sorkina T, Sorkin A, Ericsson M, Kirchhausen T (1996) Eps15 is a component of clathrin-coated pits and vesicles and is located at the rim of coated pits. *J Biol Chem* 271: 28727–28730
- Tzschentke TM, Schmidt WJ (2000) Functional relationship among medial prefrontal cortex, nucleus accumbens, and ventral tegmental area in locomotion and reward. *Crit Rev Neurobiol* 14(2): 131–142
- van der Staay FJ (1999) Spatial working and reference memory of Brown Norway and WAG rats in the holeboard task. *Neurobiol Learn Mem* 71: 113–125
- van der Staay FJ, van Nies J, Raaijmakers WGM (1990a) The effects of aging in rats on working and reference memory performance in a spatial holeboard discrimination task. *Behav Neural Biol* 53: 356–370
- van der Staay FJ, Krechting B, Blokland A, Raaijmakers WGM (1990b) The cone field: a spatial discrimination task for the automatic and simultaneous assessment of working and reference memory in rats. *J Neurosci Methods* 31: 13–22
- Vollenweider FX, Leenders KL, Scharfetter C, Antonini A, Maguire P, Missimer J, Angst J (1997) Metabolic hyperfrontality and psychopathology in the ketamine model of psychosis using positron emission tomography (PET) and [¹⁸F]fluorodeoxyglucose (FDG). *Eur Neuro-psychopharmacol* 7(1): 9–24