Chapter 6

HSV-1 infection of the brain affects the behavioral and dopaminergic response to ketamine

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Abstract

Dopaminergic and glutamatergic neurotransmission, as well as their interaction, were found to be disturbed in schizophrenia. Herpes viruses have been proposed to play a role in the etiology of schizophrenia and may be responsible for the disturbed neurotransmission. The aim of the present study was therefore to study the effects of herpes virus infection on dopaminergic and glutamatergic neurotransmission, using positron emission tomography (PET).

Rats were intranasally inoculated with the herpes simplex virus type 1 (HSV-1) or PBS (control). Open field and prepulse inhibition (PPI) studies were performed at day -1 pre-inoculation and on day 2 and 4 post-inoculation. Ketamine or saline was administered from day 0 to 4 post-inoculation. PET was used to study neuroinflammation with $[^{11}\text{C}]$-PK11195 and dopamine release with $[^{11}\text{C}]$-raclopride, at day 5 post-inoculation.

HSV-1 infection of the brain resulted in significantly increased exploration and rearing, but did not affect PPI. Administration of ketamine significantly decreased exploration and rearing in HSV-1 infected rats only. Ketamine did not affect PPI. Neuroinflammation was found in the bulbus olfactorius, frontal cortex, striatum, thalamus and brainstem of HSV-1 infected rats. Administration of ketamine did not affect neuroinflammation in control and HSV-1 infected rats. $[^{11}\text{C}]$-Raclopride binding was increased by ketamine in HSV-1 infected rats, but not affected by HSV-1 infection or ketamine alone.

HSV-1 infection and the corresponding neuroinflammation may play a role in the disturbances in dopaminergic and glutamatergic neurotransmission that were seen in schizophrenia. Additional research is necessary to further unravel the role of HSV-1 in the interaction between dopaminergic and glutamatergic neurotransmission.
Introduction

Schizophrenia is a chronic, disabling brain disease that affects approximately 1% of the human population world-wide [1] and typically has its onset during adolescence and early adulthood. The symptoms of schizophrenia are classified into positive symptoms (i.e. psychosis), such as hallucinations and delusions, negative symptoms, such as social withdrawal and flattened emotion, cognitive symptoms and mood symptoms. Although the exact etiology of schizophrenia is unknown, evidence suggests that several neurotransmitter systems are involved in eliciting the clinical symptoms, of which dopamine and glutamate have received the most attention.

Dopamine was the first neurotransmitter that was thought to be involved in schizophrenia, since positive symptoms could be caused by dopamine enhancing drugs, such as amphetamine and cocaine, [2] and reduced by antipsychotic drugs that block dopamine D₂ receptors [3]. It is nowadays proposed that the positive symptoms are due to hyperactivity of the mesolimbic dopamine system (from the ventral tegmental area to the nucleus accumbens), involving D₂ receptors, and that the negative, cognitive and mood symptoms are due to a hypoactivity of the mesocortical dopamine system (from the ventral tegmental area to the prefrontal cortex), involving D₁ receptors [4–7].

Besides dopamine, glutamate has also been suggested to play a role in schizophrenia. This was based on the finding that antagonists of the glutamate N-methyl-D-aspartate (NMDA) receptor, such as phencyclidine and ketamine, caused symptoms in healthy volunteers that mimic the positive, negative and cognitive symptoms of schizophrenia [8,9]. In schizophrenic patients, NMDA receptor antagonists increased the symptoms that these patients were already experiencing. These finding suggests that hypofunction of NMDA receptors plays a role in schizophrenia. However, it has also been proposed that hyperactivity of glutamatergic neurons in several brain areas, including the prefrontal cortex, plays a role in schizophrenia. This was, amongst others, based on the finding that NMDA antagonists increased glutamate levels in the striatum and prefrontal cortex [10].

Both dopaminergic and glutamatergic disturbances have been hypothesized to play an important role in schizophrenia, but these disturbances might be related. Glutamatergic projections from the prefrontal cortex to the ventral tegmental area have been suggested to exert an excitatory influence on the mesocortical dopamine system and an inhibitory influence on the mesolimbic dopamine system [11]. NMDA
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receptor hypofunction, i.e. reduced glutamatergic neurotransmission, would then result in a decreased mesocortical and an increased mesolimbic dopaminergic neurotransmission, resulting in the negative and positive symptoms of schizophrenia, respectively [12].

Although dopaminergic and glutamatergic neurotransmission, and their interaction, may play a role in schizophrenia, it remains to be elucidated what triggers the dysfunction of these neurotransmitters systems. Genetic and environmental factors may be responsible for the development of schizophrenia and therefore dopaminergic and glutamatergic dysfunction. Related to the involvement of environmental factors, it has been hypothesized that herpes viruses play a role in schizophrenia [13]. Herpes viruses are a family of large DNA viruses, of which eight types are known to cause disease in humans [14]. Schizophrenic patients were found to have an increase in the serum antibodies against herpes viruses, when compared to healthy controls, although negative findings have also been reported [13]. Serum antibodies to both the herpes simplex virus type-1 (HSV-1) and the cytomegalovirus (CMV) were associated with cognitive deficits in schizophrenic patients [15,16], and brain morphological changes with exposure to HSV-1 [17,18]. HSV-1 infection of the brain, resulting in herpes encephalitis, results in changes in consciousness, confusion and psychosis, which resembles the positive symptoms seen in schizophrenia [19,20].

Since herpes viruses were hypothesized to play a role in schizophrenia, it would be of interest to determine whether herpes virus infection of the brain could be responsible for dopaminergic and glutamatergic dysfunction. In mice, it has been shown that intracerebral injection of HSV-1 resulted in an increase in the concentration of whole brain homovanillic acid (HVA), the main metabolite of dopamine [21,22]. In addition, it was found that acute infection of rabbits with HSV-1 by corneal inoculation, resulted in dopaminergic activation, whereas chronic infection decreased dopaminergic neurotransmission [23,24]. To our knowledge, there are no studies that report if HSV-1 infection affects glutamatergic neurotransmission.

Of interest is that HSV-1 infection causes a neuroinflammatory response, characterized by the activation of microglia cells, which are the resident macrophages of the brain, and astrocytes. Both activated microglia cells and astrocytes have shown to be involved in the kynurenine pathway of tryptophan. When the immune response is mediated by activated microglia cells, the degradation of tryptophan results in the formation of the NMDA agonist quinolinic acid, while mediation of the immune response by astrocytes results in the formation of the NMDA antagonist kynurenic
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Acid [25]. Neuroinflammation, whether or not caused by HSV-1 infection, may thus affect glutamatergic transmission and consequently dopaminergic transmission.

Dopaminergic and glutamatergic neurotransmission may directly or indirectly be affected by HSV-1 infection of the brain, resulting in the symptoms of schizophrenia. The aim of the present study was therefore to study the effect of HSV-1 infection on dopaminergic and glutamatergic neurotransmission, in a rat model of HSV-1 encephalitis. Dopaminergic neurotransmission was studied using positron emission tomography (PET) with $[11^C]$-raclopride, which binds to the dopamine D$_2$/D$_3$ receptor. To study glutamatergic neurotransmission, the HSV-1 infected rats were injected with the NMDA antagonist ketamine. With $[11^C]$-raclopride, it has been shown that ketamine enhanced the amphetamine induced dopamine release in the striatum [26]. We hypothesized that HSV-1 infection would affect dopaminergic neurotransmission and that this would be enhanced by ketamine administration.

In addition to $[11^C]$-raclopride PET, $[11^C]$-PK11195 PET was used to study the activation of microglia cells in response to HSV-1 infection and ketamine administration. $[11^C]$-PK11195 is a ligand of the peripheral benzodiazepine receptor, which expression is increased in activated microglia cells.

In addition to the PET study, the behavior of the rats was studied in an open field and prepulse inhibition test. In the open field test, the rats are exposed to a novel environment, which is commonly used to test exploration based anxiety (i.e. locomotion and emotion). PPI is used to measure sensorimotor gating in the central nervous system, which refers to the regulation of transmission of sensory information to a motor system to prevent flooding of sensory information. The principle of PPI is that the magnitude of the reaction to a startling stimulus, such as sound, is decreased when it is immediately preceded by a weaker non-startling prestimulus. In schizophrenic patients, PPI is diminished or absent [27] and ketamine was also found to cause a disruption in PPI in rats [28]. Like for $[11^C]$-raclopride, it was hypothesized that ketamine enhanced the HSV-1 induced behavioral changes.

Material and methods

Animals

Male outbred Wistar-Unilever (SPF) rats (279±29 gram) were obtained from Harlan (Lelystad, The Netherlands). The rats were individually housed in Macrolon cages
(38x26x24 cm) on a layer of wood shavings in a room with constant temperature (21±2°C) and fixed, 12-hour light-dark regime (light phase from 7:00–19:00 hours). Food (standard laboratory chow, RMH-B, Hope Farms, The Netherlands) and water were available ad libitum. After arrival, the rats were allowed to acclimatize for at least seven days. During acclimatization and the entire study, all rats were handled daily. All experiments were approved by the Animal Ethics Committee of the University of Groningen, The Netherlands.

**Study design**

The rats were randomly divided into four groups: control rats (control), which were administered with saline (n=8) or ketamine (n=6), and rats inoculated with HSV-1 (HSE), which were administered with saline (n=8) or ketamine (n=6). After acclimatization, the study started with the open field and prepulse inhibition (PPI) studies on day -1 (figure 1). On day 0 the rats were inoculated with HSV-1 (HSE) or PBS (control) and the open field and PPI tests were repeated on day 2 and 4 post-inoculation. On day 5 post-inoculation, [11C]-PK11195 and [11C]-raclopride PET scans were made. The first administration with saline or ketamine was on day 0 (at 1 hour post-inoculation), where after saline or ketamine was administered daily until day 4. All studies were performed between 8:00-12:00 hours, in the light phase of the rats.

**HSV-1 inoculation**

The HSV-1 strain was obtained from a clinical isolate, cultured in Vero-cells and assayed for plaque forming units (PFU) per milliliter. On day 0, the rats were slightly anaesthetized with 5% isoflurane (Pharmachemie BV, The Netherlands) and inoculated with HSV-1 by the application of 100 μl of phosphate-buffered saline with 1x10^7 PFU of virus on the nostrils (50 μl per nostril) with a micro pipette. Control rats were treated similarly by the application of 100 μl PBS without virus. Clinical symptoms in all rats were scored daily post-inoculation by the same observer.

**Drugs and administration**

Ketamine (Ketanest-S®, 5 mg/ml) was obtained from Pfizer B.V. (Capelle a/d IJssel, The Netherlands). The rats were once daily administered with ketamine (16 mg/kg) or saline, from day 0 to 4 post-inoculation. On day 2 and 4, when the open field and PPI tests were carried out, ketamine or saline was administrated before the open field test
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to obtain a direct effect on behavior in addition to the daily (subchronic) administration of ketamine.

**Figure 1** Study design. Rats were inoculated with HSV-1 or PBS at day 0. Open field (OF) and prepulse inhibition (PPI) studies were carried out at day -1 pre-inoculation and at day 2 and day 4 post-inoculation. A [$^{11}$C]-PK11195 small animal PET scan was performed on day 5 post-inoculation. Administration of saline or ketamine started at day 0 and finished at day 4 post-inoculation.

**Open field test**

Open field tests on day -1 pre-inoculation and at day 2 and day 4 post-inoculation, were carried out in the light phase of the cycle. The open field tests on day 2 and day 4 post-inoculation were started at 15 minutes after administration of saline or ketamine. The open field consisted of a circular black arena with a diameter of 1 m. The rats were placed into the open field near the wall and behavior was recorded for 8 minutes using a video tracking system (Ethovision 1.96, Noldus Information Technology, Wageningen, The Netherlands). The total distance moved and the frequencies of grooming, exploration and rearing were analyzed.

**Prepulse inhibition test**

Prepulse inhibition (PPI) tests on day -1 pre-inoculation and at day 2 and day 4 post-inoculation, were carried out in the light phase of the cycle. The PPI tests at day 2 and day 4 post-inoculation started at 40 minutes after administration of saline and ketamine, which was 17 minutes after the open field study was finished. The PPI test was performed using a TSE Startle Response Measuring System. The rats were placed in a small cage (270x100x125 mm) to restrict movement and exploratory behavior. This cage was placed on a transducer platform in the sound-attenuating startle box.
The acoustic stimuli, generated by high-quality and high-linearity speakers, were presented to the rat from both sides of the cages.

After the rats were placed in the startle box, they were allowed to acclimatize for a period of 5 min with a background noise of 70 dB. The PPI test consisted of four different trials: startle pulse alone (SP, 120 dB sound for 40 ms), prepulse alone (PP, 85 dB sound for 20 ms), startle pulse preceded by the prepulse (PP+SP, 85 dB sound for 20 ms followed after 100 ms by 120 dB sound for 40 ms) and nothing (70 dB background noise). Each of the four different trials was presented to the rats 8 times. In addition, the test started with an extra of 3 consecutive SP trials. These extra trials were presented first in order to achieve a relatively stable level of response to the SP trails, since habituation of the reaction is thought to only occur within the first presentations of SP.

The primary outcome measure of the PPI test was the startle response magnitude that was used to calculate the PPI, using the following equation: 100-[100*(mean PP+SP/mean SP)].

**PET study**

[\[^{11}\text{C}\]-PK11195 \[29\] and \[^{11}\text{C}\]-raclopride \[30\] were synthesized as described previously. \[^{11}\text{C}\]-PK11195 and \[^{11}\text{C}\]-raclopride PET scans were performed at day 5 post-inoculation, between 8:00-12:00 hours and 13:00-17:00 hours, respectively. There was always more than two hours (3.5±1.2 hours) between the start of the PET scans to allow for decay of \[^{11}\text{C}\]-PK11195. For each PET scan, the rats were anaesthetized by 5% isoflurane (Pharmachemie BV, The Netherlands) that was mixed with medical air at a flow of 2 ml/min, after which anesthesia was maintained with 2% isoflurane. Following induction of anesthesia, the rats were positioned in the small animal PET camera (Focus 220, Siemens Medical Solutions USA, Inc.) in transaxial position with their heads in the field of view. A transmission scan of 515 seconds with a Co-57 point source was obtained for the correction of attenuation by tissue. After the transmission scan was completed, the PET tracer \[^{11}\text{C}\]-PK11195 (42±12 MBq) or \[^{11}\text{C}\]-raclopride (31±16 MBq) was injected via the penile vein. Simultaneously with the injection of the PET tracer a dynamic emission scan of 60 min was started.

The list-mode data of the emission scans was separated into 21 frames (8x30, 3x60, 2x120, 2x180, 3x300 and 3x600 seconds). Emission sinograms were iteratively reconstructed (OSEM2d, 4 iterations) after being normalized, corrected for attenuation and decay of radioactivity.
PET image analysis was performed using the Clinical Applications Packaging Program (CAPP5). Regions of interest were drawn around the bulbus olfactorius, frontal cortex, striatum, thalamus, parietal/temporal/occipital cortex, midbrain, brainstem and cerebellum in a template PET scan that was co-registered with the PET scan of interest by image fusion.

For $[^{11}\text{C}]$-PK11195, the uptake in these regions of interest in the last 10 min of the PET scan was determined in Bq/cm$^3$ and converted into the standardized uptake value (SUV), which was defined as: [tissue activity concentration (MBq/g)]/[injected dose (MBq)/body weight (g)]. It was assumed that 1 cm$^3$ of brain tissue equals 1 gram.

For $[^{11}\text{C}]$-raclopride the time-activity curves were used to calculate the binding potential (BP) with a reference tissue model, using software developed in Matlab 7.1 (Mathworks, Natick, Massachusets). In the reference tissue model, the BP can be calculated without arterial plasma input, by using the time-activity curve of a reference tissue that is devoid of the target receptor, as the input. Because D$_2$-receptors are not expressed in the cerebellum, this region was chosen as a reference region to calculate the BP in the bulbus olfactorius, frontal cortex, striatum, thalamus, parietal/temporal/occipital cortex, midbrain and brainstem.

**Statistics**

All data are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS for Windows, version 14.0.2. Statistical analysis on the clinical scores was performed with a one-way ANOVA with a Tukey post hoc test to assess group difference on a given day. The pre-inoculation open field data (day -1) was analyzed using a one-way ANOVA with a Tukey post hoc test. Statistical analysis of open field data on day 2 and 4 post-inoculation was performed with a univariate general linear model, with day -1 as a covariate. Day -1 was used as a covariate to correct for individual differences already present before inoculation. The PPI data, the $[^{11}\text{C}]$-PK11195 uptake and the $[^{11}\text{C}]$-raclopride BP were analyzed using a one-way ANOVA with a Tukey post hoc test. Significance for all tests was reached when the p value was <0.05.
Results

Clinical symptoms

Clinical symptoms (figure 2) were scored daily up to five days post inoculation and categorized into the following clinical scores: (0), no symptoms; (1), ruffled fur and irritated mouth, nose and eyes; (2), behavioral signs, like stress and lethargy, and hunched posture; (3), posterior paralysis and impairment of motor function and (4), severe paralysis, labored breathing or death. For rats inoculated with HSV-1 and treated with saline, the first clinical symptoms appeared on day 2 post-inoculation, while the first clinical symptoms in HSV-1 infected rats treated with ketamine were seen on day 4 post-inoculation. In both the rats that received a daily administration of saline and ketamine, the severity of the clinical symptoms increased gradually over time. No statistically significant differences were found between rats that were administered with saline or ketamine. None of the control rats showed any clinical symptoms.

Figure 2 Clinical scores of rats inoculated with HSV-1 on day 1 to day 5 post-inoculation, that received a daily administration of saline (n=8) or ketamine (n=6) from day 1 up until day 4 post-inoculation. The clinical scores represent the following symptoms: (0), no symptoms; (1), ruffled fur and irritated mouth, nose and eyes; (2), behavioral signs, like stress and lethargy, and hunched posture; (3), posterior paralysis and impairment of motor function and (4), severe paralysis, labored breathing or death.

Open field test

The results of the open field tests, which were performed on day -1 pre-inoculation and day 2 and day 4 post-inoculation, are displayed in figure 3. No statistically significant differences between groups on day -1 pre-inoculation were found between
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any of the examined behavioral parameters. To correct for the default behavior on day -1 pre-inoculation, statistical analysis on day 2 and day 4 post-inoculation was performed using day -1 as a covariate.

![Figure 3](image-url)

**Figure 3** Open field study. The total distance (A), the frequency of grooming (B), the frequency of exploration (C) and the frequency of rearing (D) during 8 minute exposure to the open field, on day -1 pre-inoculation and day 2 and 4 post-inoculation of rats inoculated with PBS (control) or HSV-1 (HSE). Rats were administered daily with saline or ketamine from the day of inoculation (day 0) up until day 4 post-inoculation. Data are presented as mean ± standard deviation. *p<0.05 in HSV-1 infected rats when compared to the corresponding control rats and #p<0.05 in rats that were administered with ketamine when compared to the corresponding rats that were administered with saline.

No statistically significant differences in the total distance moved were found between any of the examined groups. No effect of HSV-1 infection on the frequency of grooming was found on day 2 and 4 post-inoculation. Ketamine did not affect the frequency of grooming on day 2 post-inoculation, but on day 4 post-inoculation, a statistically significant lower frequency of grooming was found in HSV-1 infected rats.
that were administered with ketamine, when compared to HSV-1 infected rats administered with saline (0.2±0.4 vs. 2.8±2.1, p=0.037). The frequency of grooming in control rats was not significantly affected by ketamine.

When compared to control rats, a significantly higher frequency of exploration was found for HSV-1 infected rats, when administered with saline, on both day 2 (16.6±8.7 vs. 8.8±10.8, p=0.030) and day 4 (21.9±13.2 vs. 10.1±11.0, p=0.008) post-inoculation. Ketamine did not significantly affect the frequency of exploration on day 2 and day 4 post-inoculation.

The frequency of rearing was statistically significantly higher in HSV-1 infected rats that were administered with saline, when compared to control rats administered with saline (17.6±11.5 vs. 7.9±8.1, p=0.006), on day 4 post-inoculation. In HSV-1 infected rats, administration of ketamine statistically significantly decreased the frequency of rearing, when compared to administration of saline on both day 2 (0.5±1.2 vs. 13.9±7.5, p<0.001) and day 4 (1.5±2.0 vs. 17.6±11.5, p<0.001) post-inoculation. Ketamine did not significantly affect the frequency of rearing in control rats.

**Prepulse inhibition test**

For the prepulse inhibition test, the difference between day -1 pre-inoculation and day 4 post-inoculation were calculated as follows: (day -1/day 4)*100% (figure 4). Regarding prepulse inhibition, no statistically significant differences were found between any of the examined groups. When looking at the startle pulse when preceded by the prepulse, the difference between day -1 pre-inoculation and day 4 was statistically significantly higher for HSV-1 infected rats when administered with ketamine, when compared to saline (110±40% vs. 51±18%, p=0.025). No statistically significant differences were found for the startle pulse alone.

**PET study**

The images of the $^{[11]}$C-PK11195 PET study are displayed in figure 5. In HSV-1 infected rats, increased uptake was mainly seen in the caudal brain areas, when compared to control rats, after both saline and ketamine administration. For quantification of the $^{[11]}$C-PK11195 uptake, the standardized uptake values (SUV) were calculated (table 1). In HSV-1 infected rats, a statistically significant increase in uptake of $^{[11]}$C-PK11195 was found in the bulbus olfactorius (46%, p=0.001), frontal cortex (36%, p=0.002), striatum (24%, p=0.011), thalamus (34%, p=0.001), parietal/occipital/temporal cortex (21%, p=0.039) and brainstem (46%, p=0.017),
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when compared to control rats, after administration of saline. In rats that were administered with ketamine, a statistically significant increased uptake was found only in the bulbus olfactorius (34%, p=0.012) and the frontal cortex (32%, p=0.013) of HSV-1 infected rats, when compared to control rats. Administration of ketamine alone did not affect $[^{11}]$C-PK11195 uptake, since no statistically significant differences were found between control and HSE rats (p>0.9) that were administered with saline or ketamine.

Figure 4 Prepulse inhibition study. The difference between prepulse inhibition (A), prepulse + startle pulse (B) and startle pulse alone (C) on day -1 pre-inoculation and day 4 post-inoculation of rats inoculated with PBS (Con) or HSV-1 (HSE). Rats were administered with saline (Sal) or ketamine (Ket) from the day of inoculation (day 0) up until day 4 post-inoculation. The difference was calculated as follows: (day 4/day -1)*100%. Data are presented as mean ± standard deviation. *p<0.05 when compared to the corresponding rats that were administered with saline.
Figure 5 Full-color in appendix. $[^{11}C]$-PK11195 small animal PET images of control rats (control) and rats inoculated with HSV-1 (HSE), that received a daily administration of saline or ketamine, on day 5 after inoculation. The images display a coronal plane of the rat head at the level of the brainstem, in which the brain is delineated by a dashed line. The images represent brain uptake between 15 and 60 minutes after injection.

Table 1 $[^{11}C]$-PK11195 uptake in control rats (control) and rats inoculated with HSV-1 (HSE), administered with either saline or ketamine, on day 5 post-inoculation. The values represent the standardized uptake value (SUV, mean ± standard deviation) from the last 10 minutes of the 60 minute small animal PET scan. *p<0.05 and **p<0.005 when compared to the corresponding control

<table>
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<th>Region</th>
<th>Saline</th>
<th>Ketamine</th>
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<td></td>
<td>Control (n=7)</td>
<td>HSE (n=7)</td>
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<td>Bulbus olfactorius</td>
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<td>1.19±0.18**</td>
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<td>Frontal cortex</td>
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<td>0.67±0.07**</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Brainstem</td>
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<tr>
<td>Cerebellum</td>
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</table>

P/T/O, Parietal/Temporal/Occipital
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Table 2 $[^{11}\text{C}]-\text{Raclopride binding potential (mean ± standard deviation) in control rats (control) and rats inoculated with HSV-1 (HSE), administered with either saline or ketamine, on day 5 post-inoculation.}$

* $p<0.05$ when compared to saline-control

<table>
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<th>HSE (n=7)</th>
<th>Ketamine Control (n=6)</th>
<th>HSE (n=6)</th>
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<td>Cerebellum</td>
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*P/T/O, Parietal/Temporal/Occipital

**Figure 6** Full-color in appendix. Small animal binding potential (BP) images of the $[^{11}\text{C}]-\text{Raclopride}$ binding potential of control rats (control) and rats inoculated with HSV-1 (HSE), which received a daily administration of saline or ketamine, on day 5 after inoculation. The images display a coronal plane of the rat head at the level of the brainstem, in which the brain is delineated by a dashed line.
The $[^{11}\text{C}]-\text{raclopride}$ BP images are displayed in figure 6. The highest BP of $[^{11}\text{C}]-\text{raclopride}$ can be seen in the striatum, consistent with the highest density of $D_2$ receptors in this area. The $[^{11}\text{C}]-\text{raclopride}$ BP values of all examined brain areas are displayed in table 2. Both infection with HSV-1 and administration of ketamine did not statistically significantly affect the $[^{11}\text{C}]-\text{raclopride}$ BP. The combination of HSV-1 infection and ketamine administration did, however, cause a statistically significant increase in the $[^{11}\text{C}]-\text{raclopride}$ BP in the frontal cortex (65%, $p=0.046$), when compared to control rats that were administered with saline.

**Discussion**

Disturbances in dopaminergic and glutamatergic neurotransmission, as well as their interaction, have been proposed to play an important role in schizophrenia. Environmental factors, such as herpes viruses, may be the cause of these disturbances. In the present study, it was therefore determined if HSV-1 infection of the brain affects dopaminergic and glutamatergic neurotransmission, as well as the behavioral response. It was found that HSV-1 infection significantly increased the frequency of exploration and rearing, but did not affect PPI and $[^{11}\text{C}]-\text{raclopride}$ binding. Administration of ketamine significantly decreased the frequency of exploration and rearing, and increased $[^{11}\text{C}]-\text{raclopride}$ binding, in HSV-1 infected rats only. These finding suggests that HSV-1 infection results in changes in dopaminergic and/or glutamatergic transmission that are inhibited by administration of ketamine.

The intranasal inoculation with HSV-1 resulted in the development of clinical symptoms within two days post-inoculation, with an increase in severity over time. HSV-1 typically infects brain areas along the vomeronasal pathway, such as the prefrontal cortex, piriform cortex, entorhinal cortex and the amygdala, as well as the principle sensory trigeminal nucleus which is located in the brainstem [31,32]. Activation of microglia cells, as shown by $[^{11}\text{C}]-\text{PK11195}$, was found in the bulbus olfactorius, frontal cortex, striatum, thalamus and brainstem, which was consistent with the presence of HVS-1 in these areas. Administration of ketamine (16 mg/kg) did not cause activation of microglia cells in control rats, suggesting that this dose of ketamine used did not cause neuronal damage. Higher dosages of ketamine (80-140 mg/kg) were found to increase the number of activated microglia cells [33]. In HSV-1 infected rats, ketamine did not affect the activation of microglia cells either. This does, however, not exclude that ketamine affects the expression of pro- and/or anti-
inflammatory cytokines. It has been shown that ketamine inhibited the expression of pro-inflammatory cytokines (tumor necrosis factor-alpha and nitric oxide) by microglia cells that were activated by bacterial lipopolysaccharide [34,35].

HSV-1 infection of the brain resulted in an increased frequency of exploration and rearing, but did not change the total distance that was travelled in the open field. This suggests that HSV-1 infection of the brain did not affect locomotion per se, but increased hyperactive and stereotype behavior, as shown by a continuous repetition of exploration and rearing behavior. This was consistent with the increase in motor activity of HSV-1 infected rats in their home cage that was found by Ben Hur et al. [36,37], although in that study rearing was not reported separately from general motor activity. While HSV-1 infection did cause hyperactivity and stereotype behavior in the open field, PPI inhibition was not affected by HSV-1 infection. Since the present study investigated for the first time the effect of acute HSV-1 infection on PPI, the negative finding could not be compared to other data. It has, however, been shown that postnatal HSV-1 infection reduced PPI in the adult rat, when infection occurred at postnatal day 2 [38]. This could be explained by the interference of HSV-1 with brain development, which was not the case in the present study.

The hyperactivity and stereotype behavior in HSV-1 infected rats may represent behavior relevant to the positive symptoms of schizophrenia, rather than the negative, cognitive and mood symptoms, and could be mediated by increased mesolimbic dopaminergic neurotransmission. However, no changes in the dopamine D2 receptor binding of [11C]-raclopride were found. This is in accordance with earlier studies, in which HSV-1 infection of the mice brain was found to cause an increase in the whole brain concentration of the main metabolite of dopamine, homovanillic acid (HVA), but did not affect dopamine concentration [22]. In addition, no change was found in the amount or affinity of dopamine D1 and D2 receptors in the striatum of HSV-1 infected rabbits. These findings suggest that there is an increase in the dopamine metabolism, without affecting concentrations in dopamine. This could explain the hyperactivity and stereotype behavior found in the presents study, without changes in D2 receptor binding. While dopamine was thought to mediate the hyperactivity and stereotypy in HSV-1 infected rats, PPI inhibition was not affected. PPI involves a complex brain circuitry, involving the frontal cortex, limbic system, basal ganglia and pons, and is thought to be mainly mediated by dopaminergic neurotransmission at the dopamine D2 receptor. The absence of PPI inhibition is therefore in agreement with the lack of changes in D2 receptor binding.
The HSV-1 induced hyperactivity and stereotypy were significantly reduced after ketamine administration. A similar trend was seen in control rats, but this was not statistically significant. The reduction of activity and stereotypy in HSV-1 infected rats was accompanied by an increase in the binding of $[^{11}\text{C}]-\text{raclopride}$, in the frontal cortex. Glutamate could play a role in the observed HSV-1 induced behavioral changes and $[^{11}\text{C}]-\text{raclopride}$ binding.

In this study, we found that ketamine reduced the HSV-1 induced hyperactivity and stereotypy, which is in contrast with the generally reported hyperactivity after administration of ketamine. In addition, ketamine did not induce hyperactivity in control rats. The discrepancy could be related to the treatment protocol. Because of the possible effects of ketamine on HSV-1 infection, a subchronic treatment protocol was chosen, in which rats were treated with ketamine during the entire disease process. In the majority of the studies that show hyperactivity, behavioral tests were performed immediately after a single injection of ketamine in control rats, while open field behavior in the present study was studied at 15 minutes after injection. It has been shown that injection of control rats with 16 mg/kg of ketamine, resulted in a statistically significant increase in open field locomotion that persisted until 25 minutes after injection, after which it normalized to pre-treatment levels of locomotion [39]. This was not found in the present study, despite the same dose of ketamine was used. Chronic administration may thus have different effects on activity. Indeed, both subchronic (5 days) [40] and chronic (90 days) [41] ketamine administration did not affect activity in control rats. In addition, it has been reported in control rats that chronic administration with the NMDA antagonist PCP caused a decrease in dopamine release in the prefrontal cortex, while dopamine is increased after a single injection of PCP [42]. This could explain the lack of ketamine induced hyperactivity in control rats and the decrease in activity and stereotypy that was found in the present study, as well as the increased binding of $[^{11}\text{C}]-\text{raclopride}$ in the prefrontal cortex of HSV-1 infected rats, due to a decrease in dopamine release. This increased binding of $[^{11}\text{C}]-\text{raclopride}$ could be an indirect effect of ketamine administration, since the $[^{11}\text{C}]-\text{raclopride}$ PET scan was performed 1 day after the last ketamine administration.

However, it still remains to be elucidated why these effects were found in HSV-1 infected rats, but not in control rats. Perhaps the enhancement of glutamatergic neurotransmission by activated microglia cells plays a role. Activated microglia cells were found to induce glutamate production, via pro-inflammatory cytokines. In
addition, pro-inflammatory cytokines caused an increase in the expression of indoleamine dioxygenase (IDO) in activated microglia cells, which results in an increased degradation of tryptophan into the NMDA agonist quinolinic acid. Ketamine administration blocks the NMDA receptor and may therefore interfere with the quinolinic acid induced glutamatergic hyperfunction, resulting in reduced activity and stereotypy, as well as reduced dopamine in the prefrontal cortex. In addition, it was found that ketamine inhibits the expression of pro-inflammatory cytokines by microglia, resulting in reduced expression of IDO. Although disturbances in glutamatergic neurotransmission, and consequently, dopaminergic neurotransmission by HSV-1 induced activation of microglia cells were found, the exact mechanism needs to be further elucidated. The results do, however, suggest that HSV-1 induced activation of microglia cells induce changes in dopaminergic and glutamatergic neurotransmission.

**Conclusion**

Ketamine reduced the HSV-1 induced hyperactivity and stereotypy and affected dopaminergic neurotransmission in HSV-1 infected rats. HSV-1 infection and the corresponding activation of microglia cells may thus play a role in the disturbances in dopaminergic and glutamatergic neurotransmission that were seen in schizophrenia. However, the relation between dopaminergic and glutamatergic neurotransmission is complex, as well as the role in HSV-1 infection on this relation. Thus although the presents study indicated, for the first time, that HSV-1 may play a role in the disturbances in dopaminergic and glutamatergic neurotransmission, additional research is necessary. Especially direct measurements of dopamine and glutamate release may be of particular interest.

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