

Research report

# Inactivation of the rat dorsal striatum impairs performance in spatial tasks and alters hippocampal theta in the freely moving rat

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## Abstract

We analysed the interaction between the dorsal striatum (motor coordination and planning) and the hippocampus (sensory information processing and integration) during performance of goal-directed tasks. The performance of rats that had been injected with different doses of the D<sub>2</sub>-antagonist Sulpiride into the dorsal striatum was tested in an egocentric 4-arm maze task that tests striatal functions. Furthermore, hippocampal EEGs were recorded before, during and after inactivation of the dorsal striatum via injections of Sulpiride of rats that were performing a continuous alternation task. Injection of 5 µl of 100 mM Sulpiride increased the number of errors committed in the egocentric 4-arm maze ( $p < 0.01$ ), indicating that the dorsal striatum is involved in motor control and motor memory recall in such a task. In the recording study, the same dose of Sulpiride injected into the dorsal striatum had powerful effects on the hippocampal EEG. The main activity in the theta range (5–10 Hz) was shifted from higher frequencies in the 8–10 Hz range to lower frequencies in the 5–7 Hz range ( $p < 0.005$ ). The impairment in the behavioural egocentric task after Sulpiride injection, and the effects of Sulpiride on hippocampal theta shows that there is a functional interaction between the dorsal striatum and the hippocampus. While the dorsal striatum coordinates the execution of complex motor programs, the hippocampus integrates spatial and other sensory information required for the planning and execution of goal-directed movements.

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## 1. Introduction

The hippocampus of the rat is known to process sensory information, in particular spatial information required for guiding goal-directed behaviour in complex spatial tasks. Lesions or pharmacological inhibition of hippocampal functions impair the ability of rats to integrate multimodal information, to accurately navigate in spatial tasks, or to bridge temporal delays within complex tasks [45,63,11,41]. Results from single cell recording studies in the hippocampus have demonstrated that neurons encode spatial, temporal, and goal-related information [46,30,22,19], (see [24] for review). In addition, motor information also appears to be encoded in

the hippocampal neuronal activity. Several studies have found that neuronal firing activity correlated with the direction of movement [44,49,8], with the movement speed of the animal [8,44], with the preparedness for movement [12,15], or with the execution of complex motor tasks [55,14]. Neurons have been found that fire in relation to the movement sequence that had been learned by the animals to navigate towards a goal. For example, Frank et al. [14] have described hippocampal neurons that fire when the animal is turning at a particular location in a W-shaped maze. These cells fire only when the particular turning movement is executed at the particular location in the maze (so-called trajectory cells). It appears that such neurons integrate motor movements with spatial information. Similar cells have been described by others in similar types of goal-directed arm maze tasks [16,61]. Another indication that the hippocampus receives motor information is

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the fact that theta rhythm in the hippocampal EEG is activated by motor activity [57,8]. It has been debated for many decades what the functional role of this type of theta activity might be. Bland has postulated that sensorimotor integration of motor information in the basal ganglia and sensory information from the hippocampus are functionally integrated by synchronisation of theta rhythm [13,3].

Motor coordination is primarily controlled by other parts of the brain, in particular the basal ganglia. The dorsal striatum is the part of the basal ganglia that is involved in storing and retrieving learned motor programs [43,48]. While the hippocampus is part of a system that underlies sensory ('declarative') learning, or stimulus-response associations, the basal ganglia are part of a motor learning ('habit learning') system [29,43,10,48,59].

There is convincing evidence for functional connections between the hippocampus and the striatum and that activity in the striatum reaches the hippocampus. Studies of single cell recordings in the dorsal striatum described neurons that encode spatial information. These neurons had similar 'place cell'-type firing properties that hippocampal neurons have during the performance of spatial tasks [38,50,52]. Also, synchronisation of theta activity recorded in the dorsal striatum and simultaneously in the hippocampus was observed in animals that were performing tasks in a T-maze. When the animal did not perform a goal-directed task, no such synchronisation was seen [2,9]. Furthermore, stimulation of the hippocampus induced oscillations in the dorsal striatum, and electrical stimulation of the dorsal striatum induced hippocampal theta rhythm [18], or increased hippocampal neuronal firing activity to the point of inducing epileptic seizures [37,58]. This indicates that there is a functional integration of information processed in the basal ganglia with information processed in the hippocampus in order to execute goal-directed movements.

We therefore propose that the activity of the hippocampus during goal-directed movements reflects the integration and synchronisation of spatial sensory information with motor information. We propose that the motor information reaches the hippocampus from the dorsal striatum, and further, that this motor information that includes current and future motor activities, is required to efficiently plan routes and to navigate successfully and quickly through space. In order to plan the next movements in relation to the current spatial context, it is necessary to integrate current spatial information with current and planned motor activities, and to plan ahead the future body movements required to reach the goal. It is further suggested that for this integration process, theta is a necessary mechanism required to synchronise the information processing of the dorsal striatum and the hippocampus. In order to test this hypothesis, we analysed the behaviour and the EEG in the hippocampus of navigating rats before and after blocking the dorsal striatum. The dorsal striatum was inactivated by injections of the D<sub>2</sub>-antagonist Sulpiride [56,1], and an effective dose of Sulpiride was first determined by establishing a dose-response relationship of Sulpiride on the performance of

a basal ganglia-dependent egocentric 4-arm maze task. Using this effective dose, we analysed the behaviour and the EEG in the hippocampus of rats that performed a reinforced continuous alternating T-maze task before and during the inhibition of the dorsal striatum. We have shown previously that in rats that perform this task, hippocampal neurons develop firing properties that correlate with motor activity [27].

We hypothesise that firstly, this inactivation should affect performance in a spatial maze task, and secondly, if there is a functional connection between the dorsal striatum and the hippocampus, changes in the EEG of navigating animals should become visible.

## 2. Methods

- Experiment 1: Performance in a 4-arm maze task after Sulpiride injections.
- Experiment 2: Theta recordings of the hippocampus during an alternating T-maze task after Sulpiride injections.

### 2.1. Subjects

Adult male Long-Evans rats (Charles River, Germany), 1–4 months of age, were used for this experiment (eight animals for experiment 1, and four for experiment 2). The rats were housed individually with a reversed 12:12 h light:dark cycle. The training and testing was conducted in the dark period of the light cycle. A diet of 15 g rodent food pellets per day was imposed on the rats.

All experiments have been licensed by the German authorities according to German and EU law.

### 2.2. Apparatus

*Experiment 1:* The experiment was conducted in a 4-arm maze (cross maze; Fig. 1). The runways were 70 cm long and 17 cm wide and possessed 33 cm high walls. In three of the arms, a black cup (4 cm × 4 cm) for food reward was located in the external ends of the arms; only the start arm (=south arm) did not possess a foot cup. The maze was elevated 55 cm from the ground on wooden boxes. It was located in a sound-attenuated room and divided by a white curtain from the rest of the room. The compartment was dimly lit by four 20 W lights located in the corners of the compartment. Several landmarks were attached on the south sidewall of the compartment to enable the animals to navigate in the room.

*Experiment 2:* The training and recordings were conducted in a figure-8-shaped maze (Fig. 2) (see also [27] for further details). The maze consisted of elevated running alleys without walls. The dimension and lengths of the different running alleys and the configuration is shown in Fig. 2. Two black food cups were located at the northern ends of the two outer runways. The maze was elevated 55 cm from the ground and was located in a compartment of a sound-attenuated room that was also used for experiment 1.

### 2.3. Surgery

*Experiment 1:* The technique has been described in detail elsewhere [28]. The rats had been handled 2 weeks before surgery. Animals were anaesthetised with 75 mg/kg ketamine (Ketamin;

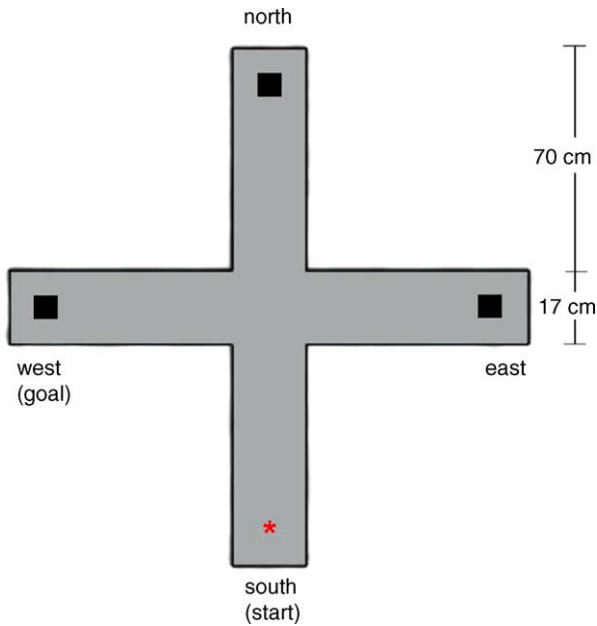


Fig. 1. Schematic drawing of the 4-arm maze. The three black rectangles represent the food cups. The rats started in the south arm (asterisk) and the west arm was baited, any visit of different arms was counted as an error.

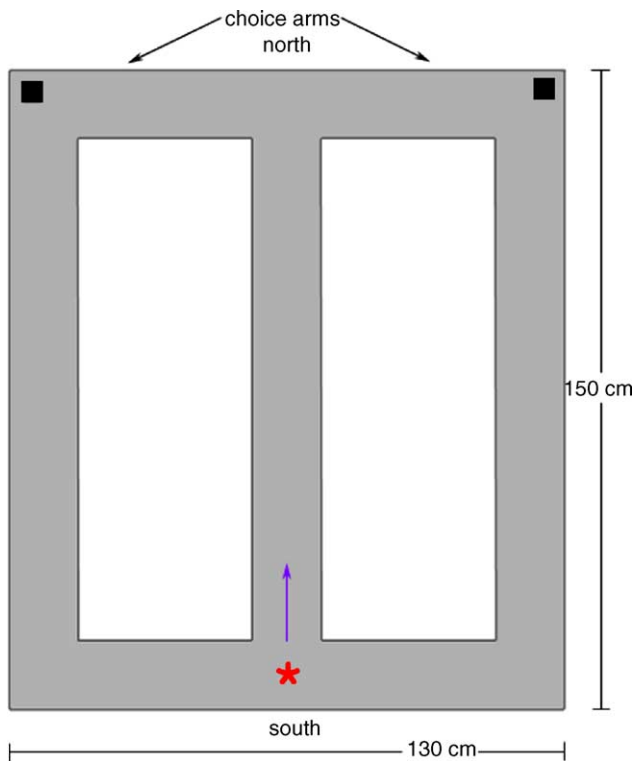


Fig. 2. Schematic drawing of the figure-8-shaped maze. Rats performed a continuous alternation task in which they traversed the central stem of the apparatus and then alternated between left and right turns at the upper junction. Rewards for correct alternations were provided at the black food cups on the end of each choice arm (north side). The rat returned to the starting position via the connecting arms, and then traversed the central stem again on the next trial. The asterisk indicates the starting position. For details see [27].

WDT, Garbsen, Germany) plus 5 mg/kg xylazine (Rompun; Bayer, Leverkusen, Germany) administered subcutaneously. After deep anaesthetisation, the rats were transferred to a stereotaxic frame. The skull was exposed and the bregma suture intersection was identified. Two 0.9 mm wide holes were drilled bilaterally, 1.7 mm anterior and  $\pm 2.5$  mm lateral to bregma. A 3.5 mm long guide cannulae was inserted via this hole into the dorsal striatum. Two stainless steel screws were inserted in the skull, and guide cannulae were fixed to the screws with dental cement. To prevent clogging of the guide cannulae, a wire was fitted in the cannulae and kept in place by a dust cap.

*Surgery for Experiment 2:* The technique has been described in detail before [26,27]. Units that contained four recording wires ('Tetrodes' [47]) were made of four twisted 25  $\mu$ m heavy formvar-coated platinum–iridium (90/10%) wires (Cal-Wire Company, Vernon, CA, USA). Tetrodes were soldered to a headstage that allowed controlled lowering of tetrodes after implantation. The impedance of electrodes was 400–800 k $\Omega$ . The rats were operated after 4 weeks of training and after performing the task well. Animals were anaesthetised with 75 mg/kg ketamine/5 mg/kg xylazine. A local injection of lidocaine was given into the scalp, and the skin was cut and pulled back. Animals were mounted in a stereotaxic frame. A 1.5 mm diameter hole was drilled into the skull (4 mm post bregma, 3 mm lateral to midline) to allow the positioning of a microdrive with four tetrodes above the dorsal hippocampus. Two additional 0.9 mm wide holes were drilled bilaterally, 1.7 mm anterior and  $\pm 2.5$  mm lateral to midline, into the skull. Through every hole a 3.5 mm long injection guide cannulae was inserted into the dorsal striatum. Four 1 mm stainless steel screws were inserted into the skull. One of the screws served as a ground electrode. The microdrive and the injection guide cannulae were fixed to the screws by dental acrylic cement. The sides of the scalp skin were treated with antibiotics. To prevent clogging of the guide cannulae, a wire was fitted in the cannulae and kept in place by a dust cap.

#### 2.4. Behavioural training

*Experiment 1:* Three days after recovery from the surgery the rats were habituated to the 4-arm maze and to rewards of chocolate flavored cereals (Coco Krispie, Kellogg's) by letting them freely explore the maze in which Choco Krispies were scattered randomly. There were two daily training sessions, in the morning (between 9 and 12 O'clock) and in the afternoon (between 2 and 5 O'clock). A trial consisted of 20 runs. At the start of each run, the rat was placed at the end of the start arm (=south arm) and had free access to all arms, but only the goal arm (=west arm) was baited. Every visit of a non-goal arm was counted as an error. A run was finished when the rat consumed the Coco Krispie.

*Experiment 2:* Before the implantation of the recording electrodes, the rats were trained in the figure-8-shaped maze to perform a reinforcement-alternation task. In this task the rats had to learn to choose the opposite choice arm to that chosen in the previous run. At the beginning of each training session the rat was put into the far end of the start arm. At the first run, rats had to choose one of the arms to get a food reward. The animals then had to return to the starting arm by running down the outside alley that takes them to the starting arm. Animals then had to run up the starting arm again and choose the arm not chosen previously in order to get a reward. The animals had to return to the starting arm again using the outside alleyway and make a new choice, which again had to be the arm not chosen in the previous run. Hence, the rats had to perform a figure-8-

shaped path. The duration of one training session was about 10 min per rat. At one training day two training sessions were conducted. A week of training consisted of five trainings days. Following erroneous choices, the rats had to continue the run without reward and were rewarded in the next run if they chose the choice arm opposite the wrongly chosen arm. After 4 weeks of training, the animals had learned the task well (<90% errors).

## 2.5. Drugs/injection procedure

*Experiment 1:* To investigate which concentrations of Sulpiride (Tocris Cookson, UK) are effective in blocking dorsal striatal functions, a dose-response relationship of Sulpiride was established by testing the concentrations of 5  $\mu$ l injections per hemisphere of a 1, 10, 50 or 100 mM Sulpiride solution. Animals were trained to criterion (<90% errors). Three saline injections were given as a control. About 45 min after injection, rats were tested in the 4-arm maze with one trial. After the saline injections, the different concentrations of Sulpiride were tested on their effect on movements and behaviour. Injections were separated by 24 h. The Sulpiride injections were 5  $\mu$ l per hemisphere of a 1, 10, 50 or 100 mM solution. To avoid possible accumulation effects, the doses used were not in ascending order of concentration but chosen at random. About 45 min after injection, rats were tested in the 4-arm maze. The location of injection in the brain was 4.5 mm ventral, 1.7 mm anterior and  $\pm$ 2.5 mm lateral to bregma. After the injection cannulae had been inserted into the guiding cannulae, the solutions were slowly applied using a 5  $\mu$ l Haemilton syringe connected to the injection cannulae by a silicon tube. The duration of injection was about 10–20 s. When the injection process was finished, the dummy cannulae were re-inserted into the guide cannulae.

*Experiment 2:* After the tetrodes had been lowered into the hippocampus and a good theta signal was recorded, injection trials were given. At four different days, 5  $\mu$ l of the 100 mM Sulpiride solution was injected bilaterally into the dorsal striatum via the injection guide cannulae. As a control, 5  $\mu$ l of saline were injected into the hemispheres of the dorsal striatum on two different days. EEGs of rats were recorded in two runs of 3 min each, then the injections were made, and after a diffusion time of 45 min, two additional runs of 3 min were given.

## 2.6. Recording procedure (Experiment 2)

After recovery, the rats were trained (see above) once again for 1 week. Over the next 3 weeks the animals were recorded 5 days per week with the data acquisition system Dacq (Axona, London, UK) while the rats performed the alternating T-maze-task. All EEG recordings were done while the animals were in motion to ensure equal recording conditions. Technical details of the recording system have been published elsewhere [26,27]. The recording sessions took place in the morning (between 9 and 12 O'clock) and consisted of two runs that took 3 min each. During this period of 3 weeks the tetrodes were also regularly lowered in the brain's dorsoventral plane by turning the headstage screws, in steps of about 300  $\mu$ m. This was done to adjust the tetrode tip location within the hippocampus. Before a recording, the rat's headstage was connected to a headstage preamplifier (Axona, London, UK). The amplifier was connected via cables to a main AC-coupled amplifier (10,000–25,000 amplification) and to the recording system (Axona). The cables were suspended by rubber bands to prevent the animals

trailing the wires during runs. The locations of the animals were tracked using a camera-computer system (Axona, London). The headstage that the animals wore during the runs contained an LED light that was tracked by the system. X–Y coordinates were stored for analysis off-line. Speed of animals was analysed by measuring the distance traveled of six runs per group and was divided by time.

Signals from 15 of the 16 electrodes of a headstage were sampled with 48 kHz and bandpass-filtered between 600 Hz and 6 kHz to collect single unit data (not presented in this study). The signals recorded on the remaining electrode were lowpass filtered >200 Hz and sampled with 960 Hz for the local field potential-channel and stored for off-line analysis. The EEG channels of the tetrodes were recorded differentially to a reference stainless steel anchoring screw on the skull. Data was analysed using TINT software (Axona, UK), and EEG spectral analysis and statistics were computed with MatLab (MathWorks Inc., USA).

## 2.7. Statistics

*Experiment 1:* Given are the mean errors  $\pm$  standard errors of the mean (S.E.M.) ( $n=6$ ).

Task performances in training trials were compared with saline or injections of different Sulpiride concentrations. Data was analysed by a Kruskal–Wallis Analysis of Variance (ANOVA) with an Scheffe post hoc test. In order to compare the individual trials after Sulpiride injection with that after saline injection the Wilcoxon rank sum test was used.

*Experiment 2:* For the analysis after Sulpiride injections only those trials were used in which the rats performed the task and moved in the maze. To visualise the distribution of power at the different frequencies of the theta range (5–10 Hz), the EEG power spectral density analysis was computed for each trial. The frequency of the peak power value in the theta range in the power spectral density was specified. An ANOVA with Scheffe post hoc test was conducted to compare the mean frequencies of the hippocampal theta after saline injection, after Sulpiride injection and without injection. For the trials without injection, trials were used which were performed before the Sulpiride injection.

## 2.8. Histology

The animals were anaesthetised with injections of 1–2 ml of a 10% urethane-solution. Brains were removed and cut into blocks and fixed for 2 h in a phosphate buffered 4% formaldehyde solution. Brains were then cut in 10–20  $\mu$ m sections on a vibratome. Sections were put on microscope slides with a paintbrush and stained using the cresyl violet staining technique [28].

## 3. Results

### 3.1. Experiment 1: Performance in a 4-arm maze task after Sulpiride injections

#### 3.1.1. Acquisition phase of the task

All rats learned the task and improved continuously during the acquisition phase of the task (Fig. 3; Kruskal–Wallis Analysis of Variance ANOVA;  $p \leq 0.01$ , d.f. = 20). A Scheffe

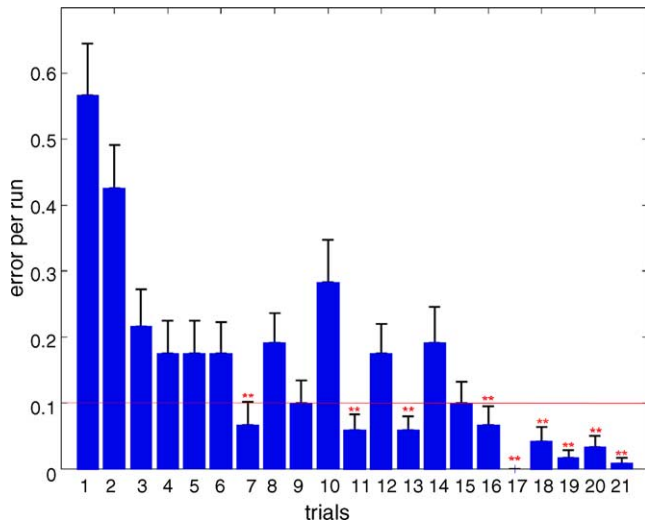


Fig. 3. Task acquisition in the 4-arm maze,  $n=6$ . The horizontal line represents the criterion (mean error of 0.1 per trial) used to evaluate whether the rats had learned the task. Significant trials compared to the trials 1 and 2 are labeled with two asterisks (Scheffe post hoc test after a Kruskal–Wallis Analysis of Variance;  $p \leq 0.01$ ). After the 16th trial the rats performed at criterion and had learned the task.

post hoc test indicated that the trials 7, 11, 13 as well as all trials above trial 15 were better in performance than the first two trials ( $p \leq 0.01$ ). Performance in all trials after trial 15 was better than criterion (error per run  $\leq 0.1$ ), reflecting that the rats had learned the task at this stage (Fig. 3);  $n=6$ .

### 3.2. Sulpiride dose response evaluation after dorsal striatal injections

Sulpiride affected performance in this task. Analysis of the different Sulpiride concentrations (0, 1, 10, 50 and 100 mM) was conducted with concentrations as the independent variable and performance in the task after the appropriated Sulpiride injections as the dependent variable ( $p \leq 0.01$ , d.f. = 3; Fig. 4). A Scheffe post hoc test revealed that the performance of the rats after 5  $\mu$ l/100 mM Sulpiride injections was significantly worse than the performance after the 1, 10 and 50 mM Sulpiride injections. The 100 mM Sulpiride group committed more errors when searching for the reward than the control group or the low Sulpiride concentration groups ( $p \leq 0.01$ ) (Fig. 4). This result is consistent with the observations made after the Sulpiride injections. No conspicuous behavioural changes were visible 45 min after the injections of the 1, 10 and 50 mM Sulpiride injections, while all of the 5  $\mu$ l/100 mM Sulpiride injected rats showed obvious behavioural changes such as continuous head tremor, and jerky and slowed down movements, all typical symptoms of dopamine inhibition in the basal ganglia [33]. Rats showing these symptoms were tested in the task after the symptoms attenuated (approximately 90–100 min after injections). In some trials, Sulpiride injected rats ceased task performance

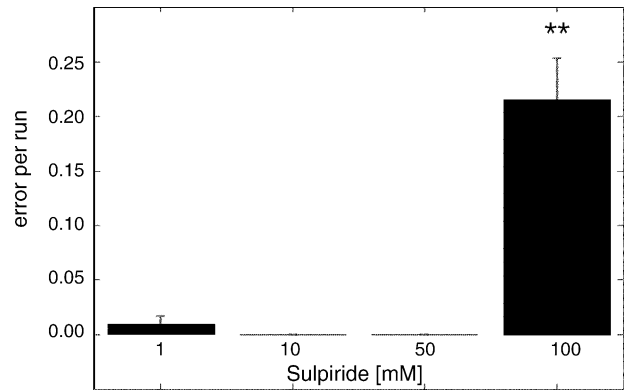


Fig. 4. Dose-response effect for Sulpiride. Shown are mean errors  $\pm$  S.E.M. committed in the 4-arm maze task. Injection of 5  $\mu$ l of a 100 mM solution per hemisphere impaired performance significantly. A Kruskal–Wallis ANOVA revealed a highly significant difference between groups ( $p \leq 0.01$ , d.f. = 3). The Scheffe post hoc test showed that the 100 mM Sulpiride injected animals performed significantly worse than the 1, 10 and 50 mM Sulpiride injected animals ( $p \leq 0.01$ ).

after some runs and developed a rigid body posture (rigor) and the trial had to be aborted.

A comparison of the task performance between rats injected with 100 mM Sulpiride (trials 25, 26, 27, 37) and rats injected with saline (trials 17, 19, 21) with a Wilcoxon rank sum test showed a highly significant worse performance ( $p \leq 0.01$ ,  $z = 5.614$ , rank sum = 132720) after Sulpiride injection (see Fig. 5).

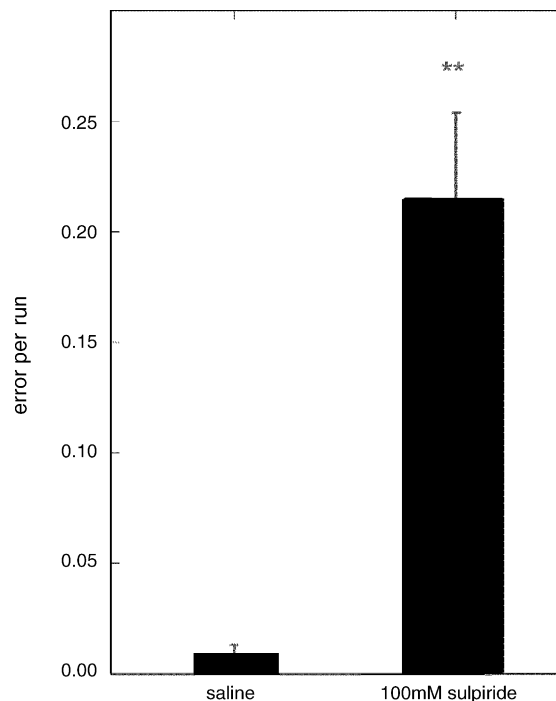


Fig. 5. Comparison between the saline trials (trials 17, 19, 21) and the Sulpiride trial results (trials 25, 26, 27, 37). A Wilcoxon rank sum test showed that the Sulpiride group performed worse compared to the saline group ( $p \leq 0.01$ ,  $z = 5.614$ , rank sum = 132720).

### 3.3. Experiment 2: Theta recording in the hippocampus during a reinforced alternating T-maze task after Sulpiride injections

#### 3.3.1. Histology

The brain slices (not shown) confirmed that the injection sides were in the dorsal striatum and that the recording area was the hippocampal area CA1.

**3.3.1.1. Hippocampal theta recordings after Sulpiride injections.** Spectral density estimations of the EEG after 5  $\mu$ l/100 mM Sulpiride injections into the dorsal striatum had great effects on theta (4–10 Hz), and a wider dispersion of high-amplitudes peaks in the theta range was induced after drug injection compared with saline injections. In the control group, the peaks with high-amplitudes in the theta range were located within a much narrow band of theta (see Fig. 6).

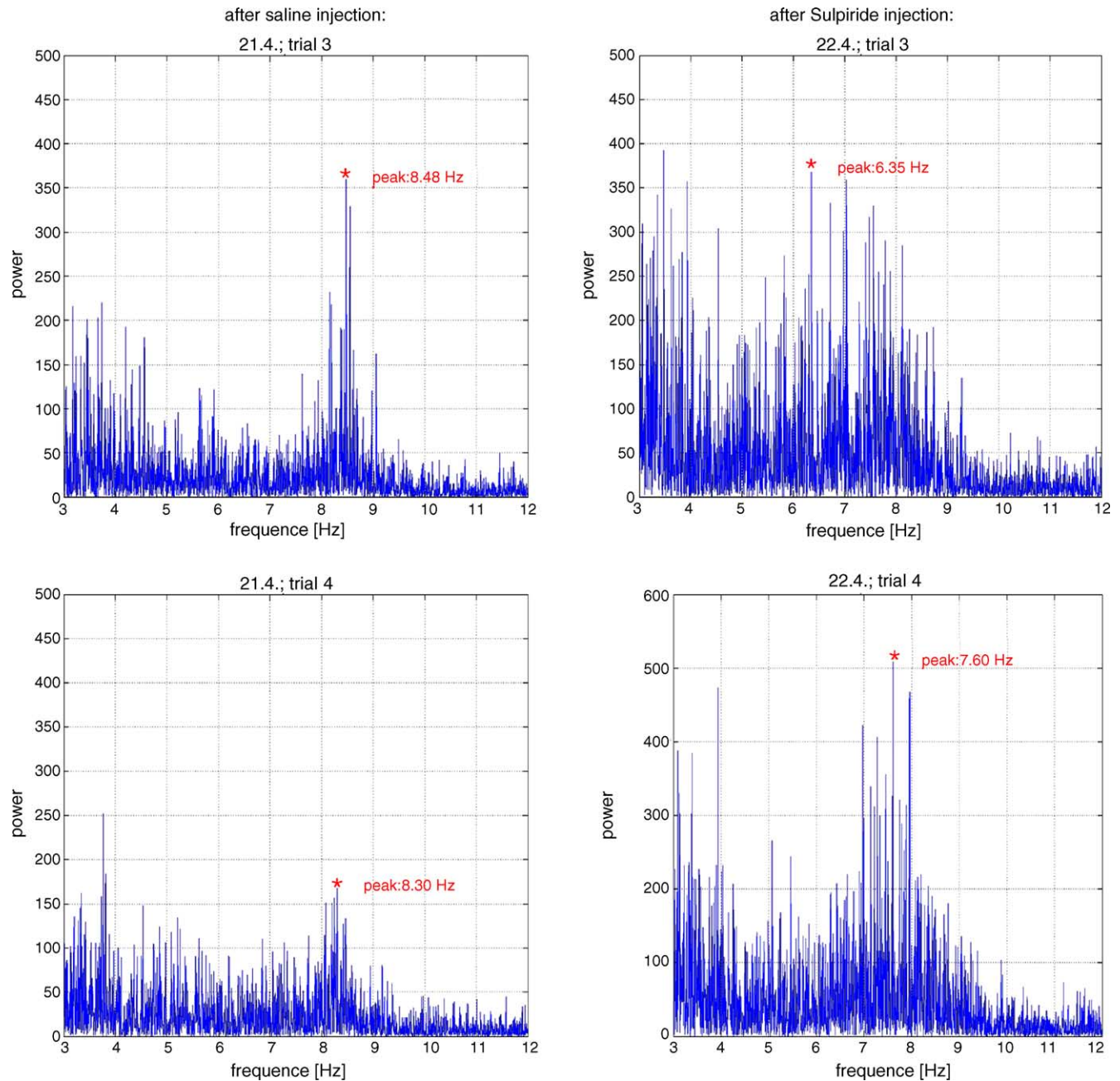


Fig. 6. Sample plots of the EEG power spectral analysis in the theta range after saline and Sulpiride injection. Left: power spectral density after saline injections from trials 3 and 4 are shown; right: power spectral density after Sulpiride injections from trials 3 and 4. Shown is the frequency range from 3 to 12 Hz. The distribution of high-amplitude peaks in the theta range (4–10 Hz) after the Sulpiride injections is much more dispersed than after saline injections, where the high-amplitude peaks are located tightly together. The peak power value of the theta range between 4 and 10 Hz are labeled with a star. Frequencies of the hippocampal theta after saline injections of 8.48 and 8.30 Hz are shifted to lower frequencies of 6.35 and 7.60 Hz after Sulpiride injections (see also Fig. 7b). All power units are in  $V^2$ .

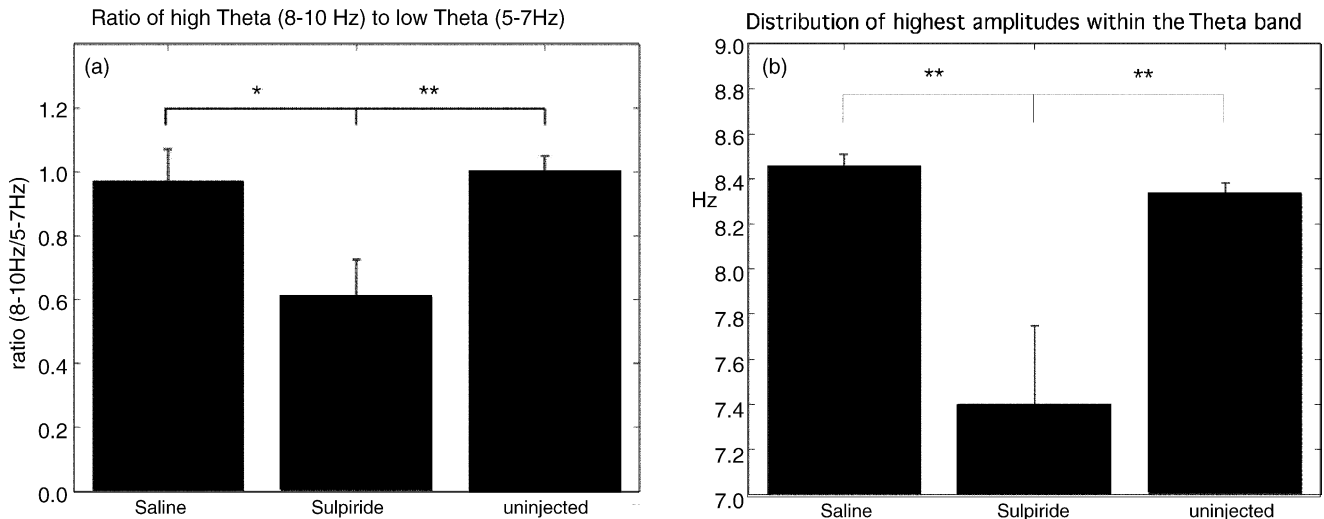


Fig. 7. (a) Comparing the ratio between the low frequency band of theta (5–7 Hz) to the high frequency theta band (8–10 Hz). Injection of Sulpiride shifted the ratio from the higher frequency theta band to the lower band. An ANOVA detected an overall difference between groups ( $p < 0.005$ ,  $F = 7.36$ , d.f. = 2). A post hoc Scheffe test showed a difference between injected control and Sulpiride ( $p < 0.05$ ) and between Sulpiride and uninjected control ( $p < 0.01$ ). There was no difference between saline-injected and uninjected controls. (b) Comparison of the mean frequencies of hippocampal theta after saline injection, after Sulpiride injection, and without injection. An ANOVA with Scheffe post hoc test comparing the means of the frequencies of the hippocampal theta after Sulpiride injection (7.40 Hz), after saline injection (8.46), and without injection (8.33 Hz) showed a highly significant difference between groups ( $p < 0.01$ ,  $F = 9.683$ , d.f. = 2). The post hoc test reveals significant differences between Sulpiride and saline runs ( $p < 0.01$ ) and between Sulpiride runs and runs without injections ( $p < 0.01$ ), but not between saline-injected runs and runs without injection.

The frequencies of the hippocampal theta showed a shift to lower frequencies in the Sulpiride injected trials in comparison with the saline-injected trials conducted 1 day before the Sulpiride injected trials (see Fig. 6). Comparing the ratio between the low frequency bands of theta (5–7 Hz) to the high frequency theta band (8–10 Hz) showed that injection of Sulpiride shifted the ratio from the higher frequency theta band to the lower band. An ANOVA detected an overall difference between groups ( $p < 0.005$ ,  $F = 7.36$ , d.f. = 2). A post hoc Scheffe test showed a difference between injected control and Sulpiride ( $p < 0.05$ ,  $n = 4$ ) and between Sulpiride and uninjected control ( $p < 0.01$ , see Fig. 7a). There was no difference between saline-injected and uninjected controls. An ANOVA with Scheffe post hoc test that compared the means of the frequencies of the peak power values after Sulpiride injection (7.40 Hz,  $n = 4$ ), after saline injection (8.46 Hz,  $n = 4$ ), and without injection (8.33 Hz,  $n = 6$ ) showed a significant difference ( $p < 0.01$ ,  $F = 9.68$ , d.f. = 2; see Fig. 7b). The post hoc test revealed differences between theta activity after Sulpiride and after saline injection ( $p < 0.01$ , mean difference = 1.060, S.E.M. = 0.269) and between theta recordings after Sulpiride injection and theta without injections ( $p < 0.01$ , mean difference = 0.935, S.E.M. = 0.246), but not between saline-injected runs and runs without injection ( $p = 0.88$ , mean difference = 0.125, S.E.M. = 0.246). The dispersion of the high-amplitude peaks in the theta range and the shift of the hippocampal theta, both seen after Sulpiride injections into the dorsal striatum, reflect a redistribution of power in the theta range, whereby high frequency peaks are reduced, while lower frequencies appear that previously had not been seen in the control runs. The analysis of travel speed showed that

there was no difference between groups (ANOVA  $p > 0.05$ ). Sulpiride group  $25 \text{ cm} \pm 7 \text{ cm/s}$ , saline group  $24 \pm 7.4 \text{ cm/s}$ , uninjected group  $28 \text{ cm} \pm 6 \text{ cm/s}$ ;  $n = 6$  per group.

#### 4. Discussion

Injections of the D<sub>2</sub>-antagonist Sulpiride (0.5  $\mu\text{l}/100 \text{ mM}$  per hemisphere) into the rat dorsal striatum impaired performance in the egocentric 4-arm maze task. This result underscores the importance of the dorsal striatum in such a task. In the second experiment, injections of this dose of Sulpiride into the dorsal striatum led to a redistribution of power within the theta range of the hippocampal EEG field potential, recorded while the rats were performing a continuous alternating T-maze task. The dopamine D<sub>2</sub> receptor density is high in the dorsal striatum, but only modest in surrounding areas such as the anterior cingulate cortex and the frontal cortices [42]. Therefore, the behavioural effects found after Sulpiride injection are most likely caused by a block of dorsal striatal actions.

The results shown here support the hypothesis that the hippocampus integrates motor information from the dorsal striatum with spatial sensory information from the temporal cortex in order to plan goal-directed movements. Thus, the inactivation of the dorsal striatum leads to an impairment of this integration, and thus impaired performance in the alternation task. Blocking the dorsal striatum also induced a redistribution of the power in the theta range, with less activity in the higher theta frequencies, and more activity in the lower range. A similar finding has been observed in EEG record-

ings in humans that had to solve working memory tasks of increasing difficulty. It was shown that the higher frequency range within the theta spectrum was increased in correlation with increasing working memory load [32,31]. This suggests that the increase in workload and input affects the higher frequency band of theta frequencies more than the lower band.

How does striatal activity affect hippocampal theta? The power at the different frequencies in the EEG reflects the activities of neuronal circuits that are specifically tuned to those frequencies [36,17,62]. It appears that the input from the intact dorsal striatum activates hippocampal neuronal populations oscillating at higher frequencies, and the reduction of this input shifts the main activity towards the lower frequencies. Previous studies have shown that dopamine antagonists reduce oscillation frequencies in the striatal EEG [51] and this reduction appears to affect hippocampal EEG directly by reducing the overall excitatory input affecting the high frequency oscillators. This concept would also explain why theta in the immobile rat has a lower frequency than in the moving rat, because in the immobile state there are mainly sensory excitatory inputs while in the moving rat, there are both sensory and motor excitatory inputs available. Additionally, previous work has shown that a reduction of stimulatory input reduces the frequencies of EEG oscillations [36,17,62,25]. It would further explain why there appear to be two types of theta. It is known that sensory input can induce theta (type 1) and that motor activity activates theta, known as type 2 theta [3,4,6]. These two types of theta might reflect the two types of inputs that each independently activate theta.

Our results further support the theory of Bland [3] who postulates a functional sensorimotor integration of different brain areas by synchronisation of theta rhythm in these areas. He explicitly states that hippocampal EEG activity is a result of sensory and motor related inputs via the ascending brainstem pathways, and that a diminished excitatory input to the hippocampus due to an inactivation of a hippocampal input structure would result in a frequency shift of hippocampal theta from higher to lower frequencies [3,4]. This suggests that the motor activity related activation of hippocampal theta during goal-directed movements is partly due to excitatory input from the dorsal striatum (type 2 theta).

An important question that arises from our proposed model is by which, anatomical pathway motor information might be relayed to the hippocampus. While the hippocampus as part of the temporal lobe receives information from nearly all sensory modalities via the superficial layers of the entorhinal cortex (perforant path), there is no direct afferent projection from the brain areas that control motor activity. However, there are several anatomical studies that suggest possible pathways by which the hippocampus might receive information from the dorsal striatum. One possible pathway could be via efferent connections of the globus pallidus with the perirhinal and temporal cortices in the rat [53]. Since the globus pallidus is the main output structure of the basal ganglia, and the hippocampus receives strong inputs from the temporal cortices (especially the entorhinal cortex

[40,60]), information from the dorsal striatum could reach the hippocampus via this route. Another pathway could be the reciprocal connection between the dorsal striatum and the pedunculopontine tegmental nucleus (PPT) [18], a part of the ascending brainstem synchronising pathways, to which the hippocampus is connected via the medial septum [35,4,18]. Finally, connections exist from the hippocampus to the striatum via the projection of the ventral hippocampus to the ventral striatum [5,38], and these might also play a role in the transfer of information.

In fMRI studies of subjects learning novel routes have shown that the greatest activity was in the basal ganglia and the hippocampus [20]. This supports the idea that there is a flow of information between the basal ganglia and the hippocampus that is used for developing behavioural routines and stereotypes in navigation.

Taken together, the experiments presented here support the theory that striatal and hippocampal brain areas cooperate in the processing of motor and sensory information, and that this information is integrated to develop navigational strategies and efficient goal-directed motor programs, which can be adapted in a quick and flexible manner while the animal performs a task.

What functional role might theta rhythm and the synchronisation of theta between different brain areas play?

- Theta and gamma rhythms modulate the firing probability of neurons, and thereby establish the precise timing of neuronal spikes in relation to theta [47,6].
- Theta and gamma act as ‘internal clocks’ that superimpose a phase code on spiking neurons. This way, theta and gamma can synchronise the activity of neuronal networks in different brain areas that cooperate in information processing. This way, neuronal networks can be functionally coupled [54,7].
- Neuronal networks that do not cooperate in information processing are kept separate in time by at least one phase of gamma rhythm, as shown in the temporal separation of place cell action potentials in the hippocampus [47]. This way, multiple representations of information can be processed and stored in the same brain area without interference between the networks [39].
- The synchronisation of neuronal activity and excitatory synaptic activity facilitates the induction of synaptic plasticity (“neurons that fire together, wire together”) [21,23].
- Theta rhythm modulates local inhibition and greatly facilitates the induction of synaptic plasticity in the hippocampus during periods of low inhibition [25]. In this way, theta might play an important role in the support of working memory [34,39].

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