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First Faculty of Medicine

Summary of the Dissertation


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Cognitive flexibility in selected animal models of psychiatric disorders

Kognitivní flexibilita ve vybraných animálních modelech psychiatrických onemocnění

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

Abstrakt

Kognitivní flexibilita představuje schopnost adaptivně měnit své chování či způsob přemýšlení v závislosti na vnějších podmínkách. Kognitivní rigidita byla popsána u řady psychiatrických a neurovývojových onemocnění, včetně její role v rozvoji a udržování některých symptomů. V této práci jsme se proto zaměřili na studium kognitivní flexibility a dalších behaviorálních charakteristik v několika animálních modelech relevantních ke schizofrenii, obsedantně-kompulzivní poruše a poruše autistického spektra. V myším "two-hit" modelu schizofrenie jsme našli rozdíl mezi skupinami v set-shiftingu a snížený počet parvalbuminových interneuronů v hipokampu u stresovaných myších samic. Překvapivě jsme nezjistili zhoršení v žádném jiném behaviorálním testu. Ve dvou farmakologických potkaních modelech relevantních k OCD jsme ukázali, že agonista dopaminových D2/D3 receptorů quinpirole a agonista serotoninových A1/7 receptorů 8-OH-DPAT způsobily natolik závažné poruchy prostorového učení a paměti v úloze aktivního vyhýbání se místu, že nebylo možné testovat reversal. Léky snižující glutamátergní neurotransmisi, memantin a riluzol, překvapivě ještě prohloubily tento deficit, přestože žádný takový efekt nebyl pozorován v případě, kdy byly aplikovány samostatně. Dále jsme ukázali, že *knockout* CRMP2 (collapsin response mediator proteinu 2) způsobuje behaviorální i neurobiologické změny relevantní k poruchám autistického spektra. Myši s delecí CRMP2 měly defekty v navádění axonů a prořezávání a remodelaci dendritických trnů. Objevila se u nich snížená sociální interakce v postnatálním období i v dospělosti, zvýšená perseverace ve Y-maze a snížená úzkost ve vyvýšeném křížovém bludišti. Překvapivě měli normální výkon v úloze aktivního vyhýbání se místu, což naznačuje zachovanou prostorovou paměť a kognitivní flexibilitu.

Klíčová slova: Kognitivní flexibilita, animální modely, schizofrenie, obsedantně kompulzivní porucha, porucha autistického spektra

Abstract

Cognitive flexibility is the ability to adjust thinking and behavior based on changing conditions. Cognitive rigidity has been described in various psychiatric and neurodevelopmental disorders and has been suggested to contribute to symptom maintenance. Therefore, we aimed to study cognitive flexibility and other behavioral characteristics in several rodent models relevant to schizophrenia, obsessive-compulsive disorder, and autism spectrum disorder. In a two-hit mice model relevant to schizophrenia, we found the between-group difference in set-shifting and decreased number of parvalbumin interneurons in the hippocampus of stressed female mice. Interestingly, we found no impairment in any other behavioral task. In two pharmacological rat models relevant to OCD, we showed that sensitization to D2/D3 receptor agonist quinpirole and serotonin 1A/7 agonist 8-OH-DPAT produced severe spatial learning and memory impairment in the Active Allothetic Place Avoidance task. The impairment was so severe that the reversal couldn't be tested. Surprisingly, drugs decreasing glutamatergic neurotransmission, memantine and riluzole, further impaired the performance in both models, although no such effect was observed when they were applied alone. Lastly, we showed that the knockout of a collapsin response mediator protein 2 (CRMP2) produced behavioral and neurobiological impairments relevant to autism spectrum disorder. CRMP2 knockout mice had defects in axonal guidance, pruning, and dendritic spine remodeling, decreased social interaction in the postnatal period and adulthood, increased perseveration in the Y-maze, and decreased anxiety in the Elevated Plus Maze. Surprisingly, they had normal spatial memory and reversal learning in the Active Allothetic Place Avoidance task.

Keywords: Cognitive flexibility, animal models, schizophrenia, obsessive-compulsive disorder, autism spectrum disorder

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Introduction

Cognitive flexibility plays an important role in our everyday lives. It helps us when we need to switch from one activity to another, modify our behavior according to situational changes or understand the point of view of others. It is a complex, higher-order cognitive skill mainly related to executive functions (Dajani & Uddin, 2015). Executive functions include a wide array of psychological functions, such as attention, planning, task initiation, ongoing process monitoring, task completion, and inhibition (Friedenberg & Silverman, 2006). In this sense, cognitive flexibility refers to the ability to switch between multiple cues, goals, tasks, or activities based on their changing importance.

However, cognitive flexibility is a broader concept that also incorporates flexibility in reasoning. For example, explanatory flexibility is related to attributional styles and plays a role in explaining events in our life, especially whether we consider specific situational factors (Zhu et al., 2021). Higher explanatory flexibility has been associated with better adjustment to adverse life events (Fresco et al., 2007). Belief flexibility is a willingness to change one's beliefs when encountering new evidence and acknowledging the possibility of being mistaken (Zhu et al., 2021). Decreased belief flexibility has been linked to more delusional thinking, persecutory ideation, and schizotypy, even in the clinically healthy population (Bronstein et al., 2017; Bronstein et al., 2019).

Impaired cognitive flexibility has been described in several psychiatric or neurodevelopmental disorders, and inflexibility in beliefs, thinking style, and specific cognitive biases have been suggested by some researchers to be an important factor in the development and maintenance of delusions in psychotic disorders and symptoms of depression, post-traumatic stress disorder, or obsessive-compulsive disorder (Davis et al., 2016; Jelinek et al., 2016; Miegel et al., 2021; Moritz & Woodward, 2007). Importantly, cognitive rigidity has been suggested as one of the hallmark features of obsessive-compulsive disorder (OCD) and autism spectrum disorder (ASD) (Gruner & Pittenger, 2017; Scarpa et al., 2021). Decreased cognitive flexibility has also been described in healthy relatives of patients with OCD, showing that cognitive rigidity might be part of the OCD endophenotype (Chamberlain et al., 2007).

This thesis is focused on executive cognitive flexibility and spatial learning and memory in animal models relevant to schizophrenia, OCD, and ASD – three disorders where cognitive rigidity has often been implicated. Furthermore, other related behavioral and neurobiological findings are described. The thesis is based on four original articles published in journals with an impact factor.

Aims of the thesis

1. To assess (spatial) cognitive flexibility in several rodent models relevant to schizophrenia, obsessive-compulsive disorder, and autism spectrum disorder, as well as other related behavioral characteristics, especially perseveration, spatial learning, and memory.

2. To assess cognitive deficits and the role of parvalbumin interneurons in a two-hit mice model relevant to schizophrenia.

3. To assess the role of antiglutamatergic agents on spatial learning and cognitive flexibility in two pharmacological rat models relevant to OCD.

4. To assess a behavioral phenotype in mice CRNP2 knockout model.

Methods

Two-hit model of schizophrenia based on the prenatal poly(I:C) application and mild chronic stress in periadolescence

Induction of a model

Female C57BL/6N (Charles River) mice (N = 16) were timed-mated and, on gestational day (GD) 9, subjected to a single subcutaneous application of poly(I:C) (potassium salt, Tocris) at a dose of 5 mg/kg (injection volume of 1 ml/kg) or vehicle control (sterile 0.9% NaCl, 1 ml/kg). Offspring were weaned and sexed on postnatal day (PD) 21. They were kept in the IVC system until adulthood (approximately 60 days of age) and transferred to standard open cages (26 × 20.5 × 13.5 cm) one week before the start of behavioral testing. Both male (N = 15) and female (N = 21) offspring were used for behavioral experiments and quantification of PVIs in the hippocampus. Half of the pups (N = 19) underwent mild chronic stress every other day between PD 30 and 40. Five different stressors were used: three mild shocks (0.3 mA, PD 30), restraint stress for 45 min (PD 32), water deprivation for 16 h (PD 34), two one-minute long forced swimming sessions (PD36), and repeated changes of home cages during the dark phase of the light/dark cycle (PD38). Mice in the control group (N = 17), which did not undergo the stress protocol, were taken out of the cage and briefly handled (15 s) by the same experimenter.

Behavioral methods and immunohistochemistry

Locomotion and sensitivity to amphetamine in the open field

Mice were tested in a square white Plexiglas apparatus (50 × 50 cm) in two daily sessions. On the first day, mice were injected with saline solution and placed into the maze for 30 min to assess locomotion. 24 h later, they were injected with amphetamine (D-amphetamine sulfate, Sigma-Aldrich; 2.5 mg/kg, subcutaneously) and immediately placed into an open-

field arena (90 min) to monitor the locomotor response to amphetamine. Both sessions were recorded and analyzed with an Ethovision system (Noldus, Wageningen, The Netherlands).

Anxiety in Elevated Plus Maze

Elevated Plus Maze (EPM) was used to assess anxiety according to a previously described protocol (Walf & Frye, 2007). The behavior of mice was recorded for 5 min by a camera mounted above the maze. Videos were analyzed manually in BORIS software. The number of entries and total time spent in open arms, closed arms, and the central area were calculated.

Spatial set-shifting task

Set-shifting was tested in a non-transparent, white cross maze with enclosed arms and one arm blocked by a removable wall, creating a T-maze with the possibility to block different arms across trials. Mice underwent a spatial version of set-shifting based on switching between egocentric and allocentric navigation, as described in Torres-Berrío et al. (2019). Prior to the experiment, animals were food restricted and kept at 95% of their original body weight until the last experimental session. Condensed milk in water (50:50) was used as a reward, and mice were familiarized with this before the experiment. The whole procedure consisted of one habituation session and 10 experimental sessions: five sessions of learning in the egocentric condition and five sessions of the allocentric condition (with a switch on day 6). During the habituation session, small plastic containers with condensed milk were placed in each arm, and animals were allowed to freely explore the whole maze for 15 min. The first experimental session started the next day. Colorful geometrical shapes that served as visual cues were attached above each arm, and arms were marked according to cardinal points (N = north, S = south, E = east, W = west) (see figure 1). Each session consisted of 20 trials. In the egocentric condition, mice were placed into the S-arm and the N-arm in a pseudorandom order and trained to make a left turn to find a reward hidden at the end of the E or the W arms. In the allocentric condition, mice again started from the S and the N arms, but the reward was always hidden in the E-arm. To avoid the possibility of mice navigating by the aroma of the milk, we put a small drop of condensed milk on the wall of both correct and incorrect arms. The results were calculated as the % of incorrect trials from each session for both parts. For the second part, never-reinforced (old information) and perseverative (new information) errors were also calculated because one of the allocentric conditions required the animal to turn left as in the egocentric part (never-reinforced errors), and the other one demanded turning to the right (perseverative errors).

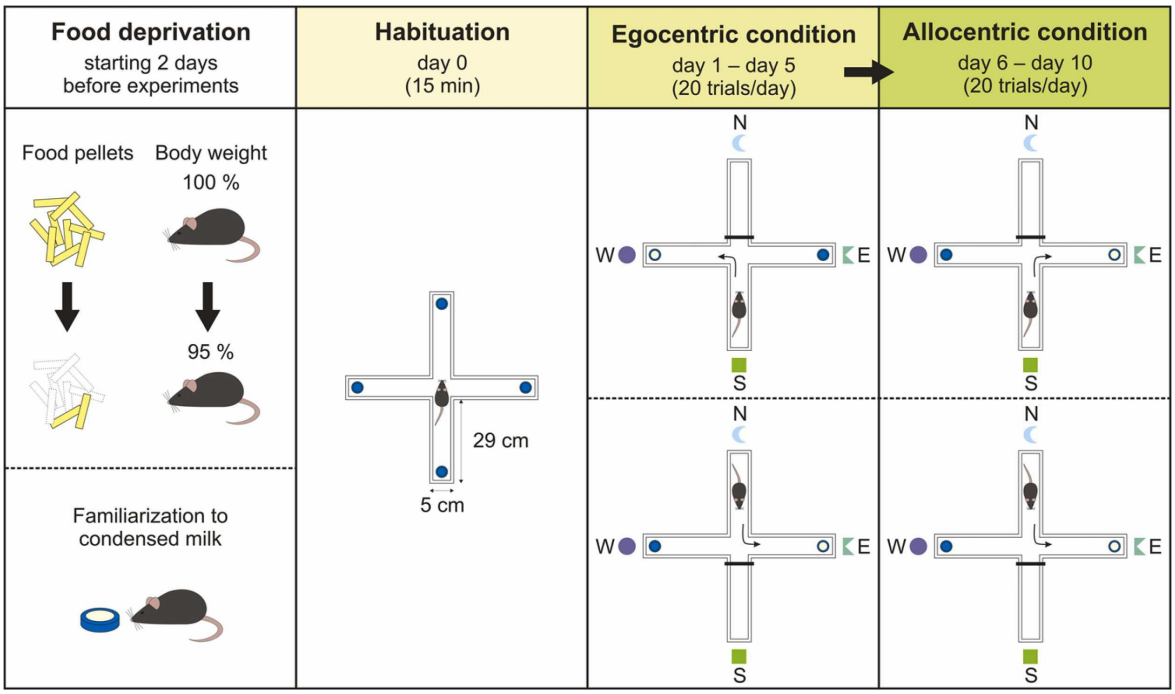

Figure 1. Schematic illustration of set-shifting procedure and apparatus.

Prepulse inhibition

Prepulse inhibition of the startle reflex (PPI) is considered a measure of sensorimotor gating and early information processing, and its impairment has been repeatedly described in schizophrenia (San-Martin et al., 2020). In our study, we adapted the protocol described in Meyer et al. (2005). Animals were tested in an acoustic startle response chamber (Coulbourn Habitest, Pennsylvania, USA). The percent difference between prepulse-pulse and pulse-alone trials was counted for each animal and each intensity according to the formula: (startle response to pulse alone - startle response to prepulse-pulse)/startle response to pulse alone) x 100.

Verification of cytokines (IL-6)

IL-6 levels were measured in six non-pregnant female mice sacrificed 3 h after the application of poly(I:C) (5 mg/kg) or saline (controls). Mice were anesthetized with isoflurane, and blood was collected by cardiac puncture into an anticoagulant-containing tube. Samples were centrifuged at 2500 g, 4 ◦C for 10 min, and separated plasma was used for further analysis. A mouse IL-6 ELISA kit (Sigma-Aldrich, RAB0308) was performed according to the manufacturer's instructions.

Immunohistochemistry

Immunohistochemistry and brain preparations were done as described earlier in Brozka et al. (2017). A rabbit PV antibody (1:1000, Abcam, ab11427) was used as the primary antibody, and an Alexa Fluor Plus 555 antibody (1:1000, Invitrogen, A32732) as a secondary antibody. PVIs in the CA1, CA2, CA3, and dentate gyrus of the hippocampus were counted, and the size of the areas was measured on a BX53 microscope (Olympus, Japan). Analyzed sections ranged from -1.36 to -2.36 relative to *bregma*.

Pharmacological models of OCD based on quinpirole and 8-OH-DPAT sensitization

Induction of models

The Long-Evans rats were injected subcutaneously with D2/D3 receptor agonist quinpirole (0.25mg/kg), serotonin 1A/7 agonist 8-OH-DPAT (0.25 mg/kg) or saline (1 ml/kg) every other day (10 injections in total). Immediately after the injection, the rats were put into the rotating Carousel maze without the activated shock sector, so they could explore freely and habituate to the rotating arena for 50 min. In the acquisition phase, they were injected with either memantine, riluzole (1mg/kg or 5mg/kg in the quinpirole study and 1mg/kg in the 8-OH-DPAT study) or saline 30 minutes before the session and with quinpirole, 8-OH-DPAT or saline right before the experimental session. Fifty-six rats were tested in the quinpirole study and ninety-eight rats in the 8-OH-DPAT study.

Behavioral methods

Spatial memory and cognitive flexibility in the Active Allothetic Place Avoidance task (AAPA)

In the AAPA, the rodents must learn to coordinate arena and room frames and use only extra-maze cues (e.g., posters on the wall, door, etc.) to navigate the maze and avoid a 60° unmarked to-be-avoided sector. The carousel maze rotates clockwise at one rotation per min and consists of an elevated metallic disc surrounded by a transparent plastic wall. The visually imperceptible 60° to-be-avoided sector is defined inside the arena, where rats receive mild foot shocks at intervals of 1200 ms until they leave the sector. The intensity of foot shocks is adjusted individually (0.2–0.6 mA) and kept to the minimum possible level to elicit an escape response and motivate the animal to learn the sector position. The foot shocks are administered through a cable attached to a harness on the back of the rat and connected to the conductive subcutaneous implant. An overhead camera was used to monitor the rat, which had an infrared light-emitting diode (LED) attached to its back with a rubber harness. A software program detects the rat's movements by monitoring the x, y coordinates of the LED. The training schedule consisted of 10 habituation and 10 acquisition sessions (every other day).

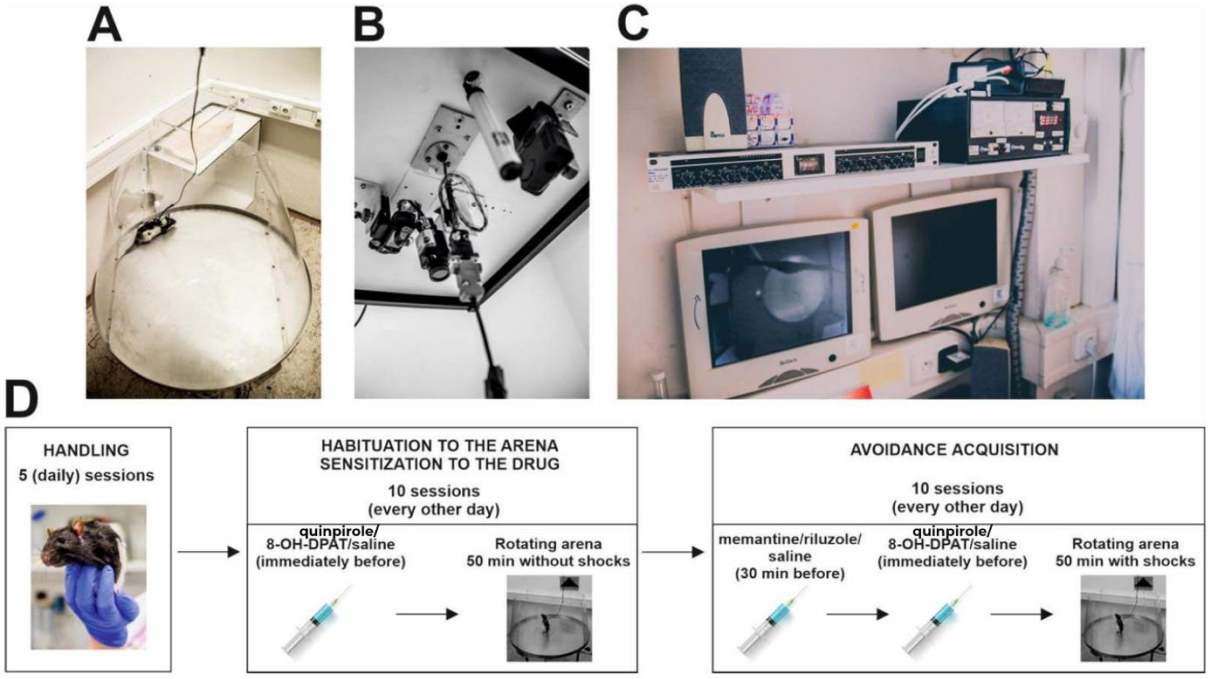

Figure 2. Experimental setup and design. **A.** Rotating Carousel maze. **B.** Camera recording the movement of rats and cable with an infrared light-emitting diode attached to the ceiling above the Carousel maze. **C.** Experiments were monitored on computers from an adjacent room. **D.** Design of the experiment.

CRMP2 knockout model

Crmp2-/- mice were developed in the Laboratory of Molecular Neurobiology, as described in Žiak et al., 2020.

Behavioral methods

Ultrasonic vocalization

Ultrasonic vocalizations emitted by socially isolated pups (WT n = 14, *crmp2-/-* n = 13) were recorded on postnatal days (PD) 6, 8, and 12. Each pup was taken from its home cage, put into a Styrofoam box, and recorded for 5 min with a microphone (Dodotronic Ultramic 250K, Italy) placed at the top of the box. Audacity software (freely available) was used for recordings with a sampling frequency set to 250 kHz. The vocalizations were analyzed automatically using Avisoft-SASLab Pro; however, the automatic analysis was checked and manually corrected if necessary. The main parameters measured were the number of vocalizations and their total length.

Anxiety in the Elevated Plus Maze (as described in a previous section)

Social approach and social novelty in the Three-chamber task

Sociability and social novelty preference were tested in the tree-chamber apparatus (54 x 20 x 33 cm) made from clear plexiglass. The chambers were divided by transparent walls with squared openings (5 x 5 cm) and sliding doors. 24 male mice were tested (WT n = 11, *crmp2-/-* n = 13). Each mouse was first placed in the middle compartment for 10 min. After the

habituation, an unknown male mouse (stranger 1) was enclosed in a little wire cage and placed in either the left or right compartment. A black plug (4.5 cm in diameter) was used as an object and placed in the opposite compartment inside an identical wire cage. The position of the stranger mouse and object was counterbalanced between trials, and stranger mice were previously habituated to the cage. Sliding doors were then opened, and the test mouse was allowed to freely explore the apparatus for 10 min. After this part of the experiment ended, the object was removed, and another unknown mouse (stranger 2) was put inside the same chamber. The test mouse was then allowed to explore all chambers for another 5 min. The behavior was recorded by a camera placed above the apparatus. Time spent in each chamber and time spent sniffing the wire cages were analyzed manually in BORIS software.

Working memory and perseveration in the Y-maze

Spontaneous alternation was tested in the Y-maze with each arm 35 cm long, 6 cm wide, and 18 cm high. The mice were left free to explore the empty apparatus for 8 min. Between trials, the apparatus was cleaned with ethanol and then wiped clean and dry to erase any scent marks. The number of arm visits was counted, indicating the exploratory activity. Spontaneous alternation was measured as the ratio of actual triads (three different arms entered in three subsequent visits) to potential triads (theoretical maximum performance). In the Y-maze task, 17 male mice (9 WT, 8 KO) were tested.

Spatial memory and cognitive flexibility in the Active Allothetic Place Avoidance task for mice

A Carousel maze for mice consists of a circular arena (56 cm in diameter) with an electrified grid floor, surrounded by a transparent plexiglass wall, rotating at approximately one rotation per minute. A computer-based tracking system (Tracker, Biosignal Group, USA) recorded the positions of the mouse and the arena at a sampling rate of 25 Hz. A 60° unmarked to-be-avoided sector was defined in the coordinate frame of the room, and each entry into the sector was punished by mild electric foot shocks (scrambled; 100 Hz alternating current; 40–80 V) delivered by the tracking system into the grid floor. Each shock lasted 0.5 s and was repeated after 0.9 s if the mouse failed to escape the sector in time. The shock intensity was individualized for each mouse (0.2–0.4 mA) to ensure an escape reaction while avoiding excessive pain. The training schedule consisted of five acquisition sessions and four reversal sessions, where the sector position was changed by 180°. Two 10-min sessions were scheduled for each experimental day, separated by approximately 3h of rest in the home cage. 21 male mice (11 KO, 10 WT) were tested in this task.

Results

The two-hit model based on prenatal immune activation with poly(I:C) and chronic stress-induced selective deficit in spatial set-shifting and decreased number of parvalbumin-positive interneurons in the hippocampus.

We found a deficit in the spatial version of set-shifting in the group exposed to poly(I:C) in the prenatal period. However, the groups did not differ in other behavioral tasks (prepulse inhibition, amphetamine sensitivity, locomotion in the open field, and anxiety in the EPM). The analysis further showed a reduced number of PVIs in the dorsal hippocampus of females who underwent stress compared to females in the control group. We also found a significant effect of stress in females on the number of parvalbumin-positive interneurons in the CA1 and CA3 regions of the hippocampus but no effect of poly(I:C).

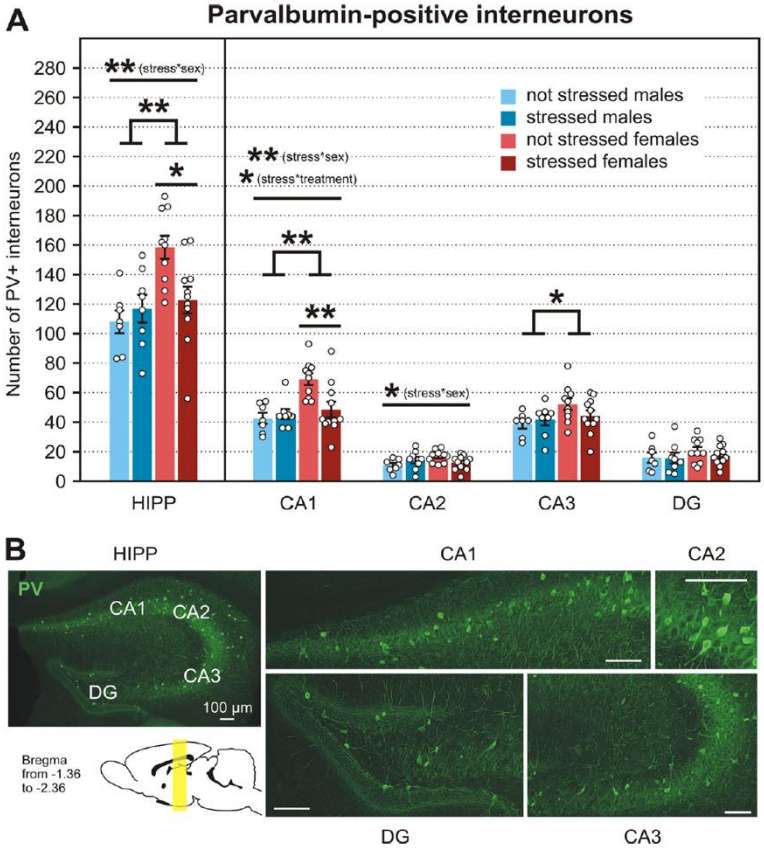

Figure 3. Parvalbumin-positive interneurons (PVIs) in the dorsal hippocampus of adult mice. **A.** Non-stressed female mice in the control group had the highest number of PVIs. Particularly, female mice had a significantly higher number of PVIs in the CA1 and CA3 regions compared to male mice. In the CA1 region, non-stressed female mice had significantly more PVIs compared to stressed female mice regardless of the treatment group.
B. Immunohistochemically stained PVIs in the hippocampus. Scale bars (for all images) = 100 µm. CA = *Cornu Ammonis*; DG = dentate gyrus; HIPP = hippocampus; PV = parvalbumin. All data are presented as mean ± SEM, *p < 0.05, **p < 0.01.

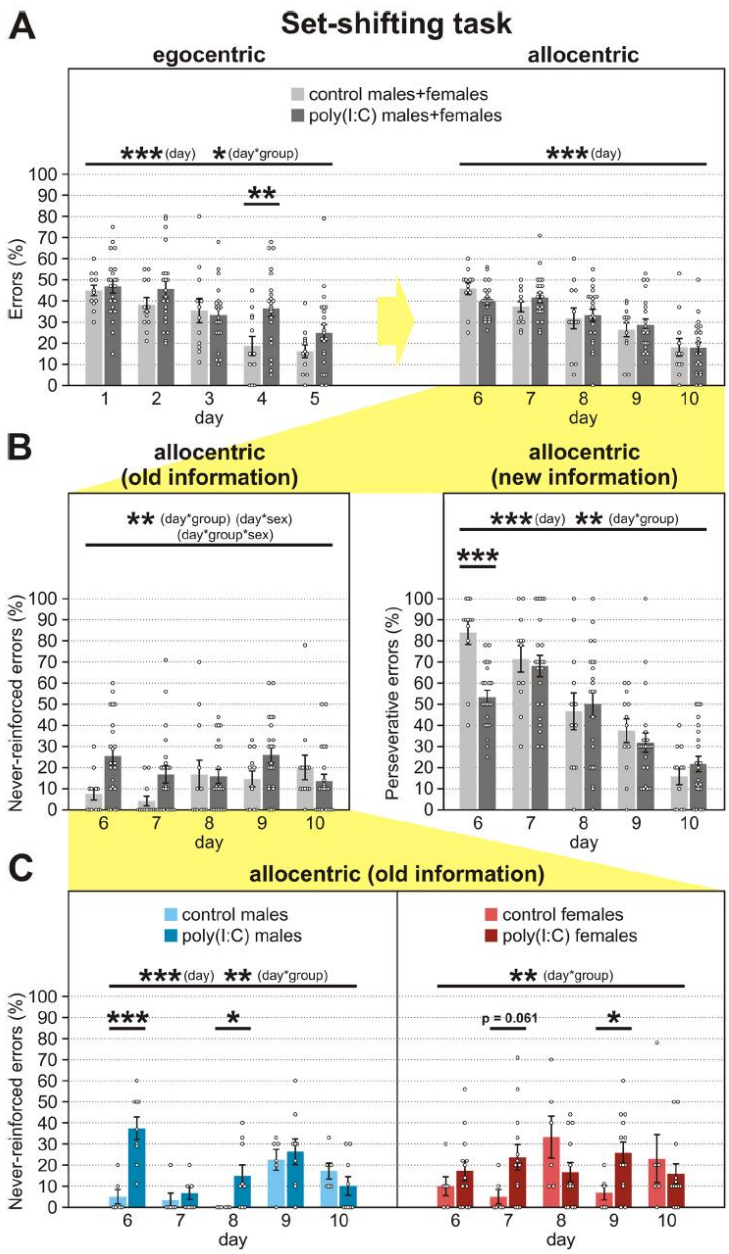

Figure 4. Results from attentional set-shifting. **A.** Mice in the poly(I:C) group had significantly more errors on the 4th day of the egocentric condition. **B.** Mice in the control group had significantly more perseverative errors on the 1st day of the allocentric condition (6th day of the experiment). **C.** Male mice in the poly(I:C) group had significantly more never-reinforced errors than the control group on the 1st and 3rd day of the allocentric condition (6th and 8th day of the experiment). Female mice in the poly(I:C) group had significantly more never-reinforced errors on the 4th day of the allocentric condition (9th day of the experiment). All data are presented as mean ± SEM, *p < 0.05; **p < 0.01; ***p < 0.001.

Acute and chronic administration of D2/D3 agonist quinpirole and 5-HT1A/5-HT7 agonist 8-OH-DPAT produced hyperlocomotion and impaired spatial learning in the Active Allothetic Place Avoidance task in rats.

We found that repeated treatment with quinpirole (0.25 mg/kg) and acute treatment with 8-OH-DPAT (0.25 mg/kg) produced significant hyperlocomotion in Long-Evans rats (see figure 4). Furthermore, the treatment with both drugs impaired spatial learning in the AAPA in a rotating arena, where rats had to avoid an invisible „shock“ sector with the help of spatial cues. Surprisingly, the administration of quinpirole and 8-OH-DPAT impaired spatial learning so much that reversal couldn’t be tested (see figures 5 and 7). In addition, rats that received 8-OH-DPAT did not accelerate the escape reaction throughout the training as did the control groups. Visual inspection of their reaction revealed they had intact

responsiveness to electrical shocks, but their escape route was less spatially organized, so they were less able to leave the "shock" sector quickly. It also suggests poor spatial knowledge of the environment. Notably, the correlation between the number of entrances into the „shock" sector and locomotion in the quinpirole study was significant only for a quinpirole group pretreated with riluzole (1mg/kg), suggesting that a high number of errors in all other groups was not just a byproduct of hyperlocomotion. Similarly, in the 8-OH-DPAT study, the animals acutely treated with 8-OH-DPAT had more entrances to the „shock" sector per unit of distance, suggesting that the spatial impairment was largely independent of hyperlocomotion.

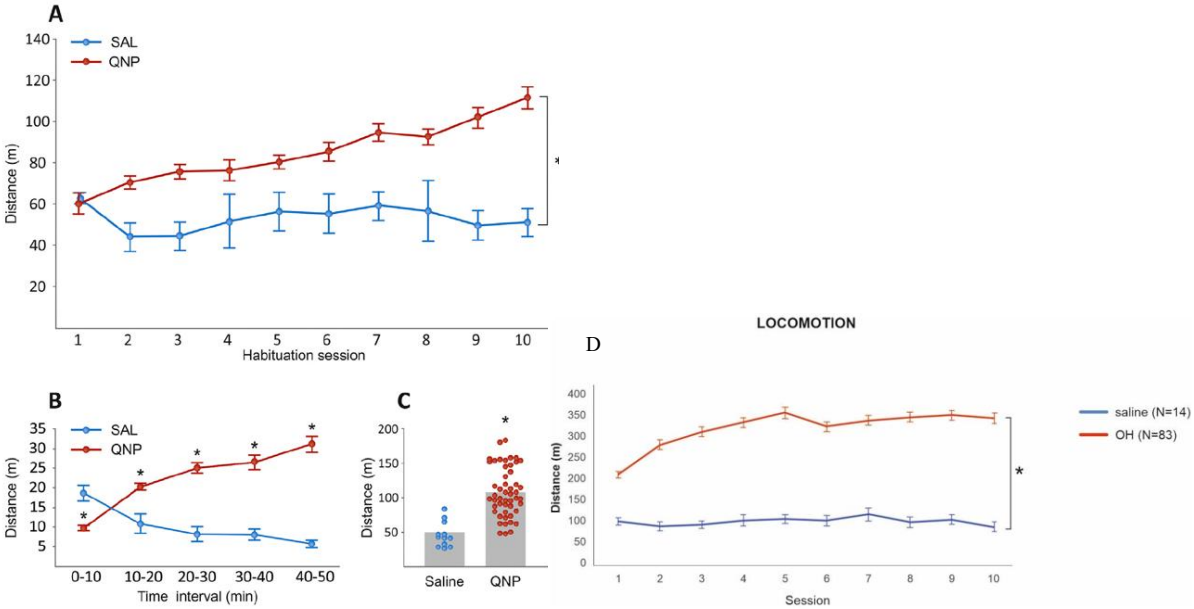

Figure 4. A. Locomotion of the quinpirole and saline groups during habituation (meters/50 min). On the last day of habituation, there was a two-fold increase of locomotion in the QNP group (QNP = 112 ± 33m, SAL = 53 ± 19 m). **B.** Mean changes in the locomotion of QNP and saline groups during the 10th session. The control group locomoted significantly more in the first 10-min interval, and their locomotion continued to decrease during the whole session, while the locomotion of the QNP group continued to increase. Differences between both groups were significant in each time interval ($p \leq 0.001$). **C.** Significant difference between the QNP and saline group locomotion on the 10th day of habituation ($p < 0.001$). **D.** Locomotion in the 8-OH-DPAT group was significantly higher from the first day of sensitization/habituation throughout all 10 sessions. * denotes a significant difference at $p = 0.01$. Data are presented as mean values ± SEM.

Antiglutamatergic agents, memantine and riluzole, further exacerbated the cognitive deficits produced by quinpirole and 8-OH-DPAT administration.

In our study, neither memantine nor riluzole alleviated the deficit in spatial learning induced by quinpirole and 8-OH-PAT. Memantine at the dose of 5mg/kg significantly aggravated the deficit caused by quinpirole, and both memantine and riluzole (1mg/kg) further exacerbated the learning deficit caused by 8-OH-DPAT, as well as hyperlocomotion (see figures 5, 6 and 7). Importantly, memantine and riluzole had no effect on spatial learning or locomotion in saline control groups. Therefore, the detrimental effect was caused by the interaction of memantine and riluzole with the quinpirole or 8-OH-DPAT.

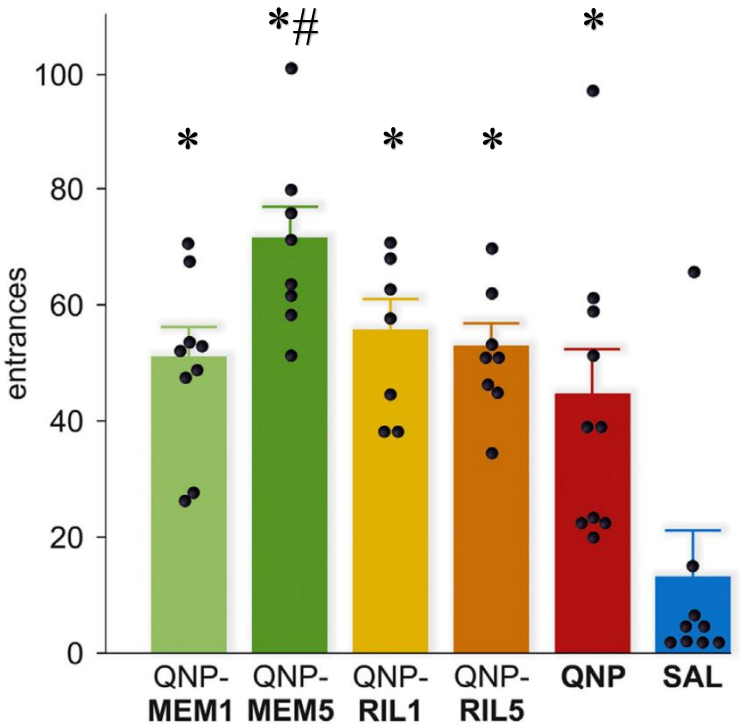

Figure 5. Results from the acquisition. Number of entrances into the to-be-avoided sector of all treatment groups. All groups made significantly more errors than the control group ($p < 0.01$). The QNP-memantine 5 mg/kg group made the highest number of errors, even significantly more than the QNP-saline group ($p < 0.05$). * denotes a significant difference from the saline control group at $p < 0.01$. # denotes a significant difference from the QNP-saline group at $p < 0.05$. Data are presented as an average number of errors ± SEM.

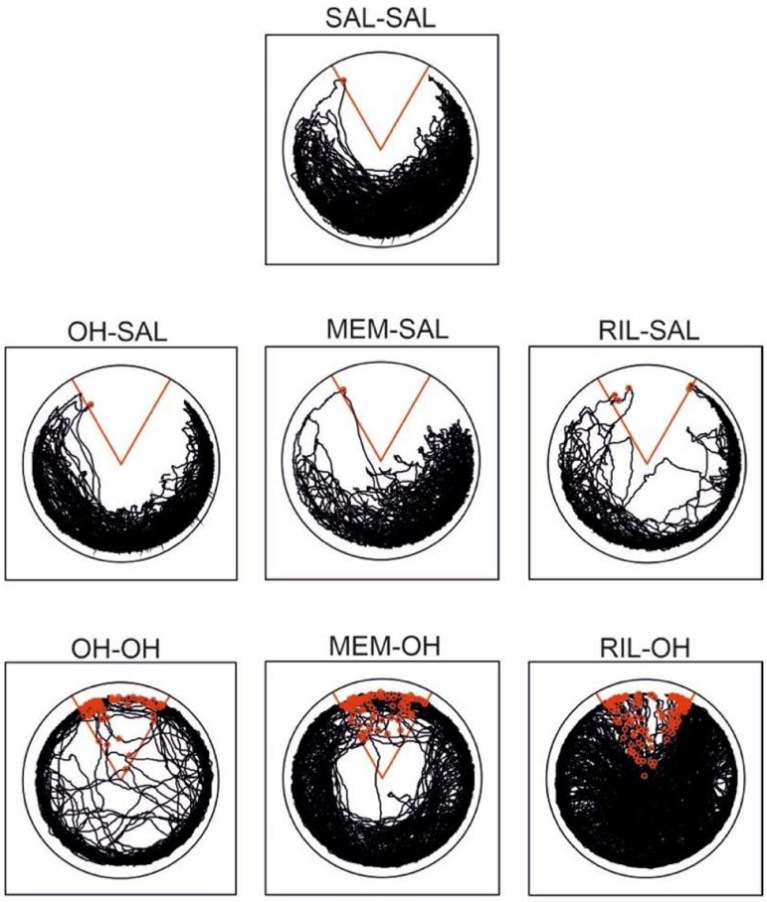

Figure 6. Typical trajectories of treatment groups on the 10th day of acquisition in the 8-OH-DPAT study. The "to-be-avoided" sector and shocks received are marked in red. The SAL-SAL (control group) and OH-SAL groups avoided the sector well. Similarly, the MEM-SAL and RIL-SAL groups had a comparably good performance. All groups that received 8-OH-DPAT during acquisition were more active and did not avoid the shock sector efficiently. The RIL-OH group had the highest locomotion and number of entrances to the to-be-avoided sector

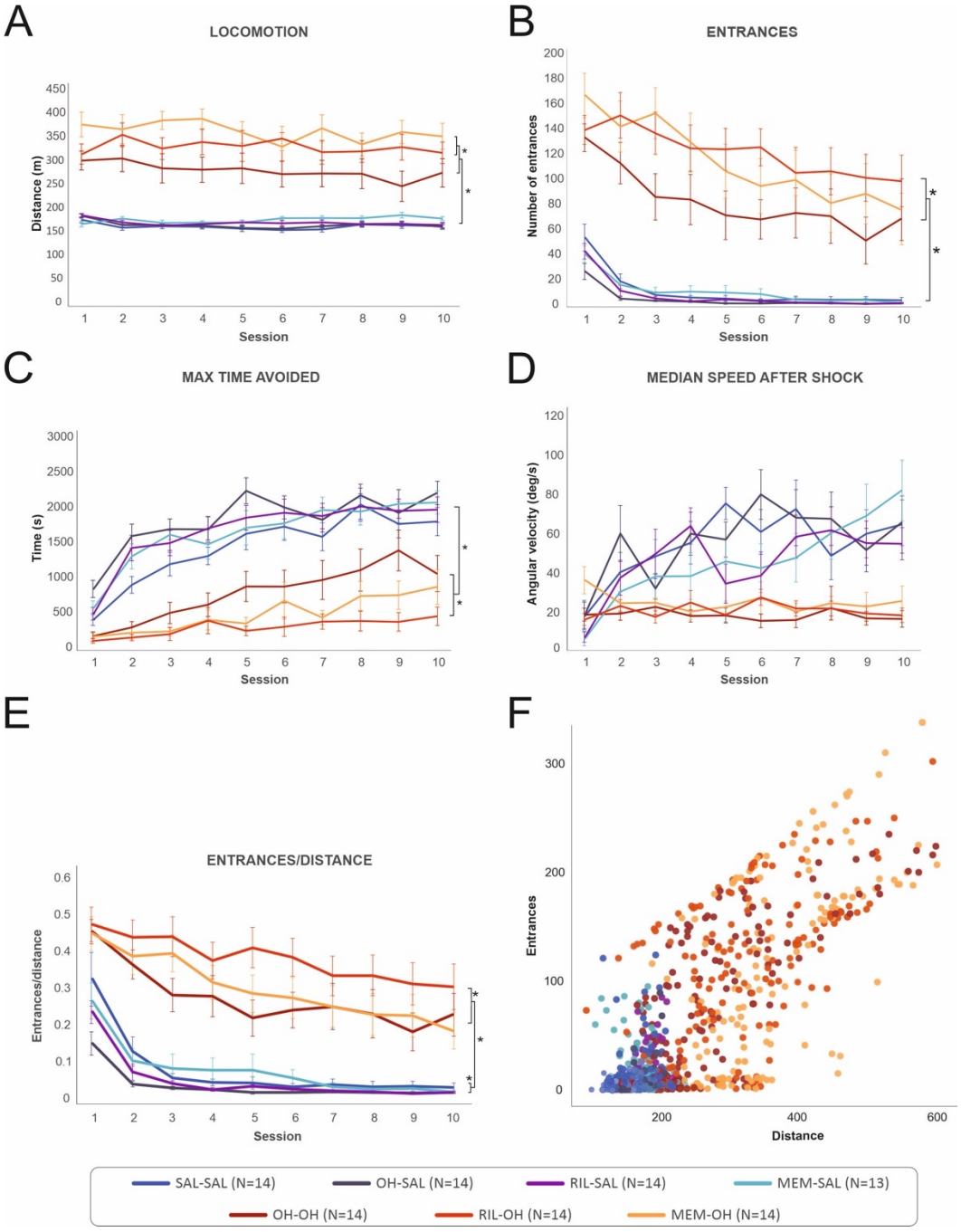

Figure 7. The behavior of all treatment groups during the 10 acquisition sessions in the five measured parameters. **A.** Locomotion was stable during all 10 sessions for each group, although it was significantly higher in the "OH" compared to the "saline" groups. The MEM-OH and RIL-OH groups had significantly higher locomotion than the OH-OH groups. **B.** The number of entrances to the shock sector decreased across sessions, but it was significantly higher in the "OH" groups compared to the "saline" groups during all 10 sessions. The RIL-OH and MEM-OH groups had the highest number of entrances to the shock sector. **C.** Maximum time avoided increased from the first to the last session and was significantly higher in the "saline" groups than in the "OH" groups. **D.** Median speed after shock did not change in the "OH" groups and only slightly increased in the "saline" groups, but with noticeable variation across sessions. **E.** The entrances/distance parameter showed that the "OH" groups had a higher number of entrances compared to the "saline" groups, and the RIL-OH group had the highest number of entrances even when controlled for locomotion. The OH-SAL and RIL-SAL groups had the lowest number of entrances per distance. **F.** Correlation of locomotion and number of entrances. A higher number of entrances correlated with hyperlocomotion in some animals from the "OH" groups. * denotes a significant difference at p = 0.05. Data are presented as mean values ± SEM with the exception of Figure 3F, which presents each trial for each animal.

CRNP2 knockout in mice produces behavioral deficits and neuronal alterations similar to those in ASD patients.

We showed that CRMP2 has a role in both Sema3A and Sema3F signaling, and its knockout produces both behavioral and neurobiological impairments relevant to autism spectrum disorder. CRMP2 knockout mice had defects in axonal guidance, pruning, and dendritic spine remodeling. Furthermore, we found decreased social interaction (fewer USV calls) in pups, which is in line with impaired social interaction in ASD early in life. Importantly, reduced sociability endured into adulthood, as adult CRMP2 knockouts showed no preference for a social partner in the three-chamber test. Moreover, CRMP2 knockouts had increased perseveration in the Y-maze and decreased anxiety in the EPM (see figure 9). Interestingly, we found no impairment in cognitive flexibility. Also, spatial learning and long-term memory were preserved, suggesting no severe cognitive deficit (see figure 8).

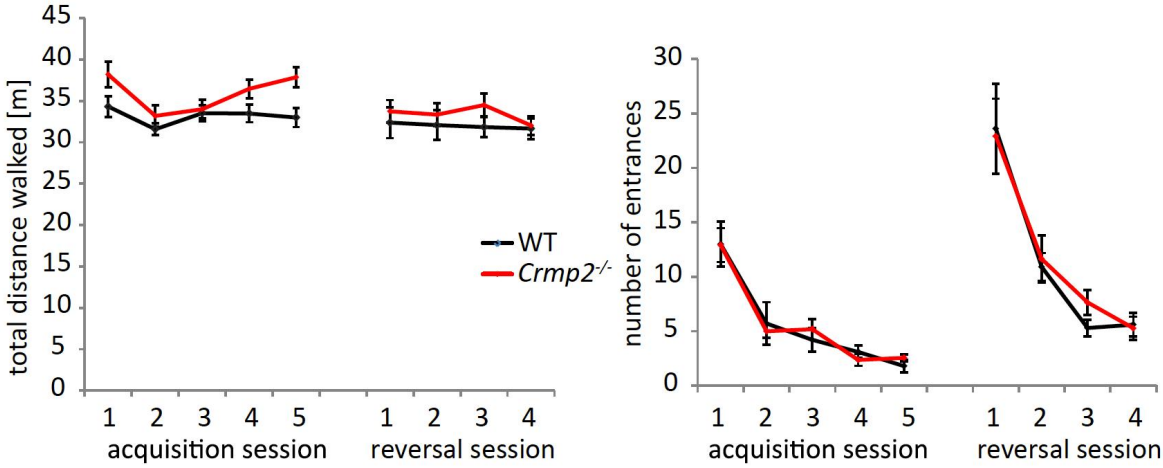

Figure 8. Results from the AAPA task. WT and knockouts learned the task successfully, as demonstrated by a decreasing number of entrances into the sector during both the acquisition and reversal phases. No significant difference was measured between WT and *crmp2-/-* mice in either acquisition or reversal setup, suggesting preserved spatial learning, memory, and cognitive flexibility. Mean ± SEM.

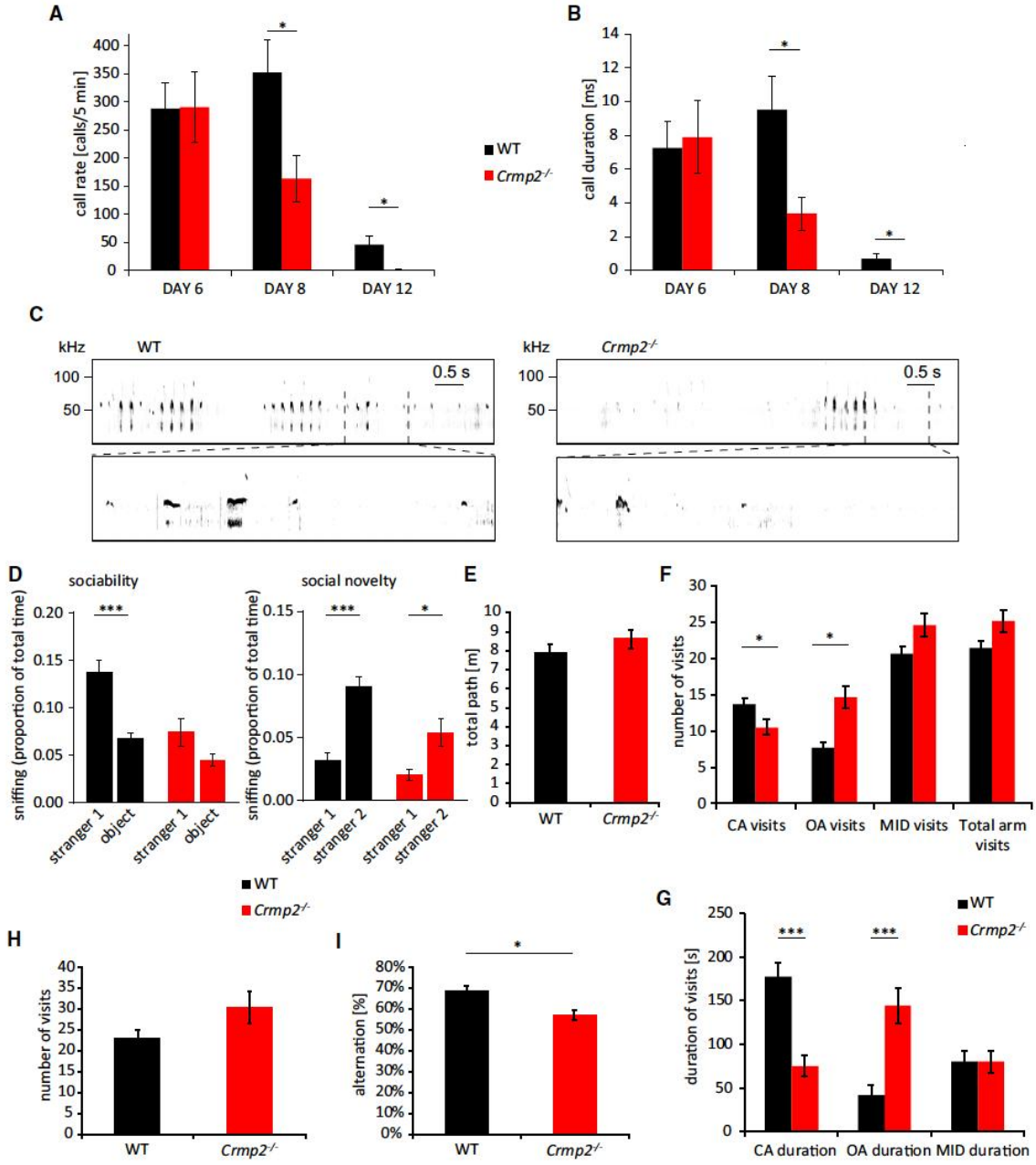

Figure 9. A, B. Ultrasonic vocalization was measured at PD6, PD8, and PD12 (WT = 14 pups, *crmp2-/-* = 13 pups). In *crmp2-/-* mice, there was a significant decrease in the rate and duration of calls at P8 and P12. Mean ± SEM, *p < 0.05. **C** Representative sonograms of the PD8 mice. **D** Three-chamber test (WT n = 11, *crmp2-/-* = 13). In the sociability phase, WT mice spent significantly more time with a social partner (p = 0.0001), while the difference was not significant in *crmp2-/-* mice (p = 0.07). In the social novelty phase, when an object was substituted with a second social partner (stranger 2), both WTs and knockouts preferred novel mice to known mice. Mean ± SEM, *p < 0.05, ***p < 0.001. **E–G** Elevated plus maze test (n = 10 mice/genotype). **E.** Total distance walked is similar in WT and *crmp2-/-(*p = 0.3). **F, G**. Frequency and duration of the open arms (OA) visits are increased in *crmp2-/-* mice suggesting decreased anxiety. CA denotes closed arms, MID denotes the transition zone between arms, and total arm visits represent a sum of visits in all four arms, mean ± SEM, *p < 0.05, ***p < 0.00. **H, I.** Y-maze (WT n = 9 mice, *crmp2-/-* n = 8 mice). Decreased alternations between arms of the Y-maze indicate impaired working memory or increased perseveration. Mean ± SEM, *p < 0.05.

Discussion

In the mice two-hit model of schizophrenia, we found impairment in the poly(I:C) group in a spatial set-shifting task with no effect of stress on set-shifting performance. Surprisingly, no difference between groups was found in prepulse inhibition, anxiety, locomotion, or sensitivity to amphetamine. Our finding of selective impairment in cognitive flexibility contrasts with previous works that described broader behavioral impairment. Mainly, a decreased prepulse inhibition was often found (e.g., Carreño et al., 2020; Giovanoli et al., 2013, 2016; Li et al., 2009; Meyer, Murray, et al., 2008; Vuillermot et al., 2010) together with higher sensitivity to amphetamine (e.g., Borçoi et al., 2015; Meyer, Murray, et al., 2008; Meyer, Nyffeler, et al., 2008) and social behavior impairment (e.g., Labouesse et al., 2015; Richetto et al., 2017; Vuillermot et al., 2017). However, some studies also failed to find a difference between groups. For example, Deane et al. (2021) found no PPI impairment in the poly(I:C) induced MIA rat model, and in a study by Goh et al. (2020), PPI was not influenced by either poly(I:C) exposure or social isolation. Several factors might mediate the lack of significant differences between groups in these behavioral tasks. Firstly, some recent studies showed that contrary to two-hit (or multiple-hit) hypotheses, multiple adversities may increase resilience (Goh et al., 2020; Gomes et al., 2015). Apart from that, housing conditions may be an important factor (Logge et al., 2014; Mueller et al., 2018). Furthermore, we found a decreased number of parvalbumin-positive interneurons in the CA1 and CA3 areas of the hippocampus of a group that underwent mild chronic stress but with no effect of poly(I:C). Interestingly, further analysis by sex showed that the effect was mediated solely by female mice. Our results are in line with findings of decreased number of parvalbumin and somatostatin-positive interneurons, as well as lower parvalbumin and somatostatin mRNA expression in the hippocampus of patients with schizophrenia (Konradi et al., 2011) and with findings of an effect of chronic stress on PVIs in all areas of the dorsal hippocampus (Czéh et al., 2015).

In the quinpirole and 8-OH-DPAT pharmacological rat models of OCD, we showed impaired spatial learning and memory in the Active Allothetic Place Avoidance task. Surprisingly, the impairment was so severe that the reversal couldn't be tested. Contrary to our expectations, memantine, and riluzole exacerbated this deficit, although no such effect was observed when they were applied alone. Interestingly, a recent study that used fMRI to look at the effect of memantine in rat quinpirole model showed that memantine increased activity in the frontal cortex in saline control animals, while it had no such effect in quinpirole-treated rats. This finding points toward the interaction between dopaminergic and glutamatergic receptors and suggests that glutamatergic treatment might be unsuitable for patients with altered dopaminergic neurotransmission (Straathof et al., 2022). In conclusion, 8-OH-DPAT and quinpirole sensitization probably influence glutamatergic neurotransmission through different pathways and induce a decrease of glutamatergic neurotransmission in the hippocampus, which subsequently impairs learning and memory. Memantine and riluzole interact with quinpirole and 8-OH-DPAT to produce further deficits that are not seen when both drugs are administered alone.

In the study on the role of collapsin response mediator protein 2 (CRMP2), we showed that mice with CRMP2 deletion had neurodevelopmental and behavioral abnormalities relevant to autism spectrum disorder. CRMP2 knockouts had altered axon guidance, pruning, and dendritic spine remodeling. Importantly, changes in both Sema3A and Sema3F signaling pathways have been implicated in ASD pathogenesis (Calderon de Anda et al., 2012; Degano et al., 2009; Li et al., 2019; reviewed in Carulli et al., 2021). Furthermore, CRMP2 knockouts had reduced social interaction, decreased spontaneous alternation in the Y-maze, and anxiety in the EPM. The impairments in social communication in juvenile mice resemble impaired social interaction in ASD, which typically develops in the first three years of life and is considered one of the key diagnostic criteria of ASD (WHO, 2019). Surprisingly, we found no impairment in cognitive flexibility. However, other cognitive functions, such as long-term memory and spatial learning, were not impaired either. In contrast with the apparent tendency to rigidity displayed by people with ASD in everyday life, studies on executive cognitive flexibility showed contrasting results. Some studies reported deficits in set-shifting tasks (Brady et al., 2013; Miller et al., 2015; Yerys et al., 2009), while others showed ASD patients had normal performance when controlled for IQ (de Vries & Geurts, 2012; Di Sarro et al., 2022; Dirks et al., 2020; Yerys et al., 2015). Some authors suggested that people with ASD might be more impaired in cognitive flexibility tasks under specific conditions, especially in tasks with less predictable changes or when explicit instructions are not provided (Van Eylen et al. 2011). It is, therefore, possible that even in rodent models with high face and construct validity regarding ASD, deficits in cognitive flexibility may not be detected.

Conclusions

In a two-hit mice model of schizophrenia, we showed that set-shifting seems to be a highly sensitive task with regard to prefrontal alterations and can uncover even subtle neurobiological disruptions. Our results also point toward the impact of chronic stress on hippocampal PVIs.

In pharmacological rat models relevant to OCD, we showed that sensitization to quinpirole and 8-OH-DPAT produced severe impairment in spatial learning and memory, probably induced by changes in glutamatergic neurotransmission. However, drugs decreasing glutamatergic neurotransmission, memantine and riluzole, further impaired the performance in a spatial memory task in both models. Because no such effect was seen when administered alone, memantine and riluzole probably interacted with quinpirole and 8-OHDPAT. The results suggest an interaction between the glutamatergic, dopaminergic, and serotoninergic systems, which should be considered when choosing pharmacological treatment.

Lastly, we showed that CRMP2 has a role in both Sema3A and Sema3F signaling, and its knockout produced both behavioral and neurobiological impairments relevant to autism spectrum disorder. In conclusion, the disruption of CRMP2 seems to be an important factor in ASD pathophysiology, and CRMP2 knockout mice are a promising model for exploring neurobiological mechanisms.

Summary in Czech

Kognitivní flexibilita v myším „two hit“ modelu schizofrenie a role parvalbumin-pozitivních interneuronů

V této studii jsme se zaměřili na prozkoumání behaviorálních deficitů a změn v parvalbumin-pozitivních interneuronech (PVIs) v myším „two-hit“ modelu schizofrenie. Nejprve jsme aplikovali poly(I:C) březím samicím, jejichž mláďata dále podstoupila stresování v období adolescence. Zjistili jsme deficit v prostorové verzi set-shiftingu ve skupině, která byla vystavena poly(I:C) v prenatálním období, zatímco v žádné další úloze (prepulzní inhibice, senzitivita na amphetamin, lokomoce v open fieldu, úzkost v EPM) se skupiny nelišily. Analýza dále ukázala snížený počet PVIs v dorzálním hipokampu u samic, které prošly stresováním ve srovnání se samicemi, které stresováním neprošly. Výsledky naší studie ukázaly, že set-shifting může být považován za vysoce senzitivní úlohu ve vztahu k prefrontálním deficitům, která je schopná odhalit i malé změny, jež se v jiných úlohách neprojeví. Poukázaly také na efekt opakovaného, nepředvídatelného stresu na pokles PVIs v hipokampu, zejména u samic.

Efekt látek snižujících glutamátergní neurotransmisi na kognitivní deficit v potkaních modelech obsedantně-kompulzivní poruchy

V této práci jsme sledovali efekt memantinu a riluzolu, léků snižujících glutamátergní neurotransmisi, ve dvou potkaních modelech OCD založených na quinpirolové a 8-OH-DPAT senzitizaci. Opakovaná aplikace quinpirolu a 8-OH-DPAT narušila prostorové učení a paměť v úloze aktivního vyhýbání se místu, a to do takové míry, že nebylo možné testovat reversal. Memantin i riluzol překvapivě ještě prohloubily tento deficit, přestože žádný takový efekt nebyl pozorován v případě, kdy byly aplikovány samostatně. Senzitizace quinpirolem a 8-OH-DPAT pravděpodobně různými cestami ovlivňuje glutamátergní neurotransmisi a vyvolává pokles glutamátu v hipokampu, což následně zhoršuje učení a paměť. Memantin a riluzol interagují s quinpirolem a 8-OH-DPAT, čímž dochází k dalšímu prohloubení kognitivního deficitu, který se neprojevuje v případě samostatného podání obou léčiv.

Role CRMP2 v neurovývoji a jeho význam v rozvoji poruch autistického spektra

V této studii jsme se zaměřili na roli collapsin response mediator proteinu 2 (CRMP2) v neurovývoji a na jeho potencionální roli v rozvoji neurovývojových onemocnění, zejména poruchy autistického spektra. V laboratoři Molekulární Biologie ve Fyziologickém ústavu AV ČR byly vyvinuty myši s delecí CRMP2, u kterých se ukázaly neurovývojové i behaviorální abnormality. CRMP2 knockouti měli sníženou sociální interakci a snížená sociabilita přetrvala až do dospělosti, kdy nevykazovali preferenci pro sociálního partnera v three-chamber testu. Kromě toho měli zvýšenou perseveraci ve Y-maze a nižší úzkost v EPM. Překvapivě jsme nezjistili žádné zhoršení v kognitivní flexibilitě. Nebyly však porušeny ani další kognitivní funkce, jako dlouhodobá paměť a prostorové učení. Dále se

ukázalo, že CRMP2 má důležitou roli v Sema3A a Sema3F signalizaci a s tím souvisejícím navádění axonů, prořezávání a remodelaci dendritických trnů, které byly u CRMP2 knockoutů pozměněné. Lze tedy říci, že narušení CRMP2 se zdá být důležitým faktorem v patofyziologii poruch autistického spektra.

Summary in English

Cognitive flexibility in the mice two-hit model relevant to schizophrenia and the role of parvalbumin-positive interneurons

In this study, we aimed to investigate behavioral deficits and changes in parvalbumin-positive interneurons (PVIs) in a two-hit model of schizophrenia. We first administered poly(I:C) to pregnant mice whose offspring underwent stress in periadolescence. We found a deficit in the spatial version of set-shifting in the group exposed to poly(I:C) in the prenatal period. However, the groups did not differ in other behavioral tasks (prepulse inhibition, amphetamine sensitivity, locomotion in the open field, and anxiety in the EPM). The analysis further showed a reduced number of PVIs in the dorsal hippocampus of females who underwent stress compared to females in the control group. Our study showed that set-shifting could be considered a highly sensitive task in relation to prefrontal deficits, capable of detecting even small changes that are not apparent in other tasks. Our results also pointed to the effect of chronic, unpredictable stress on the reduction of PVIs in the hippocampus, especially in female mice.

Effect of antiglutamatergic agents, memantine and riluzole, on the cognitive deficit in rat model relevant to obsessive-compulsive disorder

In the present study, we looked at the effect of memantine and riluzole, drugs decreasing glutamatergic neurotransmission, in two rat models relevant to obsessive-compulsive disorder. We used repeated administration of quinpirole and 8-OH-DPAT and showed that it impaired spatial learning and memory in the Active Allothetic Place Avoidance task. The impairment was so severe that the reversal couldn't be tested. Surprisingly, memantine and riluzole exacerbated this deficit, although no such effect was observed when they were applied alone. Sensitization with quinpirole and 8-OH-DPAT likely affect glutamatergic neurotransmission through different pathways and decrease hippocampal glutamate transmission, which subsequently impairs learning and memory. Memantine and riluzole interact with quinpirole and 8-OH-DPAT to cause additional deficits that do not manifest when the two drugs are administered alone.

The role of CRMP2 in neurodevelopment and its relevance for autism spectrum disorder.

In this study, we focused on the role of collapsin response mediator protein 2 (CRMP2) in neurodevelopment and its potential function in neurodevelopmental disorders, particularly autism spectrum disorder. Mice with CRMP2 deletion were developed in the Laboratory of

Molecular Biology at the Institute of Physiology CAS and indeed showed neurodevelopmental and behavioral abnormalities. CRMP2 knockouts had reduced social interaction, and the reduced sociability persisted into adulthood when they showed no preference for a social partner in the three-chamber test. In addition, they had decreased spontaneous alternation in the Y-maze and anxiety in the EPM. Surprisingly, we found no impairment in cognitive flexibility. However, other cognitive functions, such as long-term memory and spatial learning, were not impaired either. Furthermore, CRMP2 appeared to have an important role in Sema3A and Sema3F signaling and associated axon guidance, pruning, and dendritic spine remodeling, which were altered in CRMP2 knockouts. It can be concluded that CRMP2 disruption appears to be an important factor in the pathophysiology of autism spectrum disorder.

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