Hippocampus Dysfunction in Quinpirole Sensitization Model of Obsessive-Compulsive Disorder

Narušená Funkce Hipokampu u Modelu Obsedantně-Kompulsivní Poruchy Vyvolané Quinpirolem
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Obor: Neurovědy
Předseda oborové rady: Prof. MUDr. Karel Šonka, DrSc.
Školící pracoviště: Oddělení neurofyziologie paměti, Fyziologický ústav AV ČR, v.v.i., Vídeňská 1083, 142 20 Praha 4

Autor: Mgr. Hana Brožka
Školitel: Prof. RNDr. Aleš Stuchlík, Ph.D., DSc.
Oponenti:

Autoreferát byl rozeslán dne: .........................
Obhajoba se koná dne: .........................v .........................hod

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1 ABSTRACT

Obsessive-compulsive disorder (OCD) is a serious psychiatric condition manifested by repeated thoughts followed by stereotypic compulsive behavior. Alterations to cortico-thalamo-striato-cortical circuits are most often implicated in the pathophysiology of OCD. However, many studies have also found a changed volume, shape and activity of the hippocampus in OCD patients. This work focused on the activity of hippocampal CA1 cells during stereotypical checking behavior and on cognitive flexibility in a quinpirole (QNP) sensitization model of OCD.

The activity of CA1 hippocampal cells during stereotypical checking was assessed in an enriched open-field test in QNP sensitized rats. Arc+ (activity-regulated cytoskeletal associated protein, or Arg 3.1) mRNA expression profiles were determined in CA1 coronal hippocampal sections following stereotypical checking. After the establishment of stereotypical checking (10 sessions), rats were exposed to the arena and sacrificed after 5 minutes. QNP sensitized animals visited the same objects with the same frequency as during previous sessions, while control rats did not. Locomotor activity was comparable between QNP treated rats and controls. Following sacrifice, rat brains were flash frozen and sliced to 20 µm thick sections. Sections, mounted on slides, were hybridized with anti-Arc riboprobes, and visualized using tyramide amplification. Both control rats and rats treated with QNP displayed low baseline Arc+ positive cells in CA1. Importantly, there was a significant interaction between QNP and the environment - QNP treated rats displayed a lower number of Arc+ nuclei in CA1 during exploring/checking the open-field compared to control rats; while in the baseline condition there was no significant difference in Arc+ cells in CA1 between QNP treated rats and control rats.

To assess cognitive flexibility, a hippocampus-dependent Carousel arena task with reversal was employed. Animals were to avoid a sector on rotating arena that was not directly perceptible and could only be localized by spatial relationships to distal landmarks. The number of entrances into the sector was used as a measure of learning. Rats treated with QNP displayed a severe, but transient, increase in the number of errors in reversal. Treatment with clomipramine, a drug commonly used to treat OCD, further impaired reversal and impaired acquisition of the Carousel arena task. On the other hand, a combination of clomipramine and risperidone improved the rats’ performance. Furthermore, two-way active avoidance task confirmed hippocampal impairment in QNP treated rats.
2 ABSTRAKT

Obsedantně komulzivní porucha (OCD) je závažné psychiatrické onemocnění, které se projevuje opakovanými nutkavými myšlenkami a následně stereotypním komulzivním chováním. Do patofyziologie OCD jsou zapojeny změny kortiko-thalamo-striato-kortikálních obvodů. Mnoho studií však také zjistilo změněný objem, tvar a aktivitu hipokampu u pacientů s OCD. Tato práce byla zaměřena na aktivitu hipokampálních CA1 buněk během stereotypního kontrolního chování podobného komulzivní kontrole na kognitivní flexibilitu v potkaním modelu sensitizace chinpirolem (QNP).

U sensitizovaných potkanů a kontrol byla hodnocena aktivita hipokampálních buněk v oblasti CA1 během stereotypní kontroly v otevřeném poli s vloženými objekty. Stanovili jsme profily exprese $Arc^+$ (aktivitou regulovaného cytoskeletálního proteinu neboli Arg 3.1) v koronálních hipokampálních řezech v oblasti CA1. Po ustavení stereotypní kontroly (10 sezení) byli potkани vystaveni aréně a přesně po 5 minutách humánně usmrčeni. Sensitizovaná zvířata navštěvovala konkrétní objekty v jednotlivých sezeních s konsistentní preferenci, zatímco kontrolní tuto preferenci měnila od jednoho sezení k druhému. Lokomoční aktivita byla v testovacím sezení mezi oběma skupinami srovnatelná. Po usmrčení byly mozky potkanů bleskově zmrzly a nakrájeny na řezy o tloušťce 20µm. Řezy na sklíčkách byly inkubovány s anti-$Arc$ probami a exprese byla vizualizována pomocí tyramidové amplifikace. Jak kontrolní skupina, tak potkani sensitizovaní QNP, kteří nebyli vystaveni prostředí, vykazovali bazální nízké počty $Arc^+$ buněk v CA1. Důležité je, že došlo k významné interakci mezi QNP a prostředím - potkani sensitizovaní pomocí QNP vykazovaly nižší počet $Arc^+$ jader v CA1 během explorač/kontrole otevřeného pole ve srovnání s kontrolními potkany při této exploraci.

Pro otestování kognitivní flexibilitě byla použita úloha aktivního vyhýbání se místo s přeucením na rotující aréně (Kolotoči) závislá na hipokampu. Zvířata se měla za úkol vyhýbat sektoru na rotující aréně, který nebyl přímo viditelný, a jehož polohu šlo určit pouze prostorovými vztahy vůči vzdáleným orientačním bodům. Počet vstupů do tohoto sektoru byl použit jako měřítko učení a flexibilit. Potkani vystavení QNP vykazoval významné, ale předchůdce zvýšení počtu chyb při přeucení. Léčba klomipraminem, léčivem běžně používaným k terapii OCD, zhoršila početční učení i přeucení na rotující aréně. Na druhou stranu kombinace klomipraminu a risperidonu zachovala výkonnost potkanů na úrovni kontrol. Kromě toho výsledky z neprostorové úlohy aktivního obousměrného vyhýbání se (two-way active avoidance) také podpořily koncept narušení hipokampu u potkanů sensitizovaných ke QNP.
Introduction

Obsessive Compulsive Disorder (OCD) is a serious mental disorder. It is characterized by unwanted intrusive thoughts followed by repetitive physical and/or mental acts, which offer relief to the patients (Van Schalkwyk et al., 2016). OCD is one of the leading causes of disability (Luis Ayuso-Mateos, 2006) and has an immense impact on patients’ wellbeing and productivity (Stein et al., 2000). At present there are several treatments that reduce OCD symptoms, but none cure OCD completely (Hirschtritt et al., 2017). Finding effective treatments is delayed by lack of understanding of OCD etiology; and prevention of OCD is hindered by obliviousness to direct causes. Causes go often unnoticed or cannot be noticed; there are only rare moments when a cause of a mental disease can be confidently pointed at. Here, animal models are indispensable for they allow controllably producing a suspected insult and observing behavioral and neurophysiological changes that follow.

Hippocampus is a well-studied brain region implicated in memory formation, consolidation, retrieval and spatial orientation (Moscovitch et al., 2016). In OCD patients activity of the hippocampus increases after symptom provocation (Adler et al., 2000) and decreases after successful SSRI treatment (Kang et al., 2003). Reduced hippocampal volume and shape deformity was repeatedly observed in patients with OCD (Kang et al., 2003; Hong et al., 2007; Atmaca et al., 2008; Reess et al., 2018). Yet, hippocampal aberrations observed in OCD were never much attended to. A single theory that considers hippocampus in OCD pathology gives it a role of compensating for deficiencies in procedural memory (Ullman and Pullman, 2015). However, since OCD behavior was observed to develop following traumatic brain injury to temporal lobe region (Max et al., 1995), it is possible that hippocampus is involved more directly in OCD as is currently thought.

Hippocampal dysfunction could drive increased reliance on cortico-striato-thalamo-cortical (CSTC) circuits, resulting in observed hyperactivity in these regions in OCD patients (Milad and Rauch, 2012). Voluntary behavior of humans and animals can be either habitual or flexible. CSTC circuits, which involve caudate nucleus, are involved in habitual responses-based learning (Everitt and Robbins, 2013), while hippocampus in tandem with nucleus accumbens (ventral striatum) is implied in flexible goal-directed behaviors (Pennartz et al., 2011). In a seminal study by Packard and McGaugh lesion to the caudate nucleus resulted in goal directed response while lesion of the hippocampus resulted in stereotypical responses in T-maze task (Packard and McGaugh, 1996). In this light, hippocampal deficiency in OCD could lead to overreliance on parts of CSTC circuits.
that involve caudate nucleus – such as orbitofrontal circuit. Primary hippocampal deficiency could manifest as hyperactivity of orbitofrontal cortex, anterior cingulate cortex and caudate - a common finding in OCD patients (Maia et al., 2008).

Quinpirole (QNP) sensitization model is an often-used animal model of OCD that is characterized by a spontaneous stereotypical checking behavior (Szechtman et al., 2001). Irrespective of validity as a model of OCD, QNP sensitization can be very informative of how stereotypical behavior is generated and maintained. Activity of different brain regions in QNP sensitized actively checking animals can be considered as a proxy of symptom provocation state in OCD patients. At present, several studies utilized uptake of radioactive glucose as a measure activity of brain regions in QNP sensitized animals. These studies indicated a trend towards reduced activity of hippocampus in QNP sensitized animals (Carpenter et al., 2003; Servaes et al., 2016, 2017).

Apart from radioactive glucose utilization, expression if immediate early genes (IEGs) can be used as a proxy of plasticity-related activity. *In situ* fluorescent hybridization (FISH) offers high-resolution information regarding plasticity-related neuronal activity (Kubik et al., 2007).

Present work focuses on expression of IEG *Arc* in hippocampus of QNP sensitized actively stereotypically checking rats. This work also assesses a functional hippocampal deficit in QNP sensitized rats using separate hippocampus-dependent and hippocampus-independent behavioral tasks.
4 METHODS

There were six treatment groups used in experiment 1 - a quinpirole-treated group (QNP, n = 15); a saline treated control group (SAL, n = 10); a group receiving quinpirole and clomipramine (QNP+CMI, n = 11); a group receiving quinpirole and risperidone (QNP+RIS, n = 11); a group receiving quinpirole and a combination of clomipramine and risperidone (QNP+CMI+RIS, n = 11); and an additional group receiving only clomipramine (CMI, n = 9). Clomipramine and risperidone were administered 1.5h prior to the experiments; Quinpirole was administered 30-min prior to the experiments. All injections were applied subcutaneously, and appropriate volume of saline was administered to groups not receiving the pharmaceuticals.

4.1 Stereotypical checking in object-enriched open-field

Stereotypical checking and hippocampal activation during stereotypical checking was conducted in large openfield enriched with two three-dimensional object pseudo-randomly placed in arena. Checking behavior was determined from frequency of visits to objects and corners of the arena (zones/locales). To induce stereotypical checking in quinpirole treated rats were exposed to ten 50min long enriched openfield sessions. Quinpirole treated rats received 0.5mg/mL/kg subcutaneous injections 50min prior to placement into the arena. Controls received subcutaneous injection of saline solution at the same time (1 mL/kg). Analysis of visits was automatized using Bioobserve software (Viewer2, Biobserve BmbH).

4.2 Fluorescent in situ hybridization

To determine number of neurons expressing *Arc* following stereotypical checking, animals were exposed to enriched openfield for 5 minutes. Five QNP sensitized rats and three control rats were designated as cage controls (CC groups). These animals were left in the opaque cages throughout the whole experiment. Following openfield exposure, rats were decapitated and their brains were retrieved and flash-frozen. Next, brains were sectioned to 20µm coronal slices, which were
immediately mounted on gelatin-coated slides. Later, sections were processed for fluorescence in situ hybridization as previously described (Vazdarjanova and Guzowski, 2004; Kubik et al., 2012). In short, Arc was visualized by hybridizing Arc mRNA with hapten labeled anti-sense probe followed by tyramide amplification mediated fluorescent dye deposition.

Arc+ nuclei in CA1 area were analyzed using Leica TCS SP8 laser-scanning microscope with apochromatic HCX PL APO 20× immersion objective. Three randomly selected dorsal sections, which included dorsal hippocampus, were selected for analysis. Each stack was composed of 21 horizontal sections. Custom macro for analysis in ImageJ was kindly provided by M.Sc. Stepan Kubik Ph.D. The proportions of Arc+ to Arc- neurons were used to map neurons active during test session. Difference in activities of CA1 neurons between QNP treated and control group was analyzed separately using two-way ANOVA.

4.3 Active Place Avoidance with Reversal on Carousel

Active place avoidance with reversal in a Carousel maze is an established tool to explore spatial learning, cognitive coordination and cognitive flexibility and is hippocampus-dependent. The Carousel maze is an elevated metallic platform that rotates 1 revolution/min. Part of this platform can be assigned as a to-be-avoided sector, where, upon entering, the animal receives a mild electric shock. This electric shock is adjusted individually to elicit an escape response (AC, 50Hz, 0.2 - 0.6 mA), and is administered automatically at 1200-ms intervals up to the time the animal leaves the sector. The to-be-avoided sector is directly imperceptible and stable in the coordinates of the room while the platform rotates constantly at 1 revolution/min. To avoid this sector successfully, the animal has to move in a counter-rotational direction to avoid being dragged into the sector. A camera placed above the arena samples animal movements at a frequency of 25 Hz (the camera detects an infrared light-emitting diode (LED) attached to the back of the animal on a small rubber jacket). In addition to LED, the animal has a subcutaneous needle attached to the nape of the neck that is connected to a current source, to deliver the mild electric shock across the grounded floor. Locomotion and number of errors (entrances into to-be-avoided sector) were extracted for behavioral analysis. The experimental procedure used here can be divided into three principal phases - habituation, acquisition and reversal. In short, the experiment began with 10 sessions of habituation to the arena (every other day), during which rats were sensitized to QNP. Habituation was 30 min long (as are the subsequent acquisition and reversal phases) and there was
no defined to-be-avoided sector. Arena was rotating during habituation. Habituation was relatively long due to the stabilization of sensitization to the QNP after 10 and more injections (Szechtman et al., 1994). Habituation was followed by four or five acquisition sessions. During acquisition 60° wide to-be-avoided sector was arbitrarily defined. Only animals that successfully completed acquisition learning were allowed to proceed to reversal learning. The threshold of acceptance into reversal learning was 10 or fewer errors (on average less than 1 error/3 minutes). Based on results from first experiment number of acquisition sessions in second experiment was increased from four to five since asymptotic performance was desirable during acquisition. In reversal learning the to-be-avoided sector was moved 180°. Reversal learning in first and second carousel experiment consisted of four and three 30min sessions, respectively.

4.4 The Two-Way Active Avoidance Task

The apparatus (Multi Conditioning system, TSE, Germany) was a sound proof, well-lit (10 Lx) and ventilated 90x90x90cm box with plain black nontransparent walls. On the floor was a metallic grid with 0.5cm diameter stainless steel rods, with centers spaced 1.5cm apart. This grid was used to deliver electric shocks to animals. The box was divided into two compartments by a black insert with a 7x7cm cutout door in the middle. In this task, an animal had to make an association between a conditioned stimulus (5s tone; 70db) and an unconditioned stimulus (electric shock; 0.5mA; 500 Hz, AC). Each session began with a 60-second habituation period. Immediately after habituation, the first conditioned stimulus (tone) was sounded. Then there was a 10s delay between the end of the sound and the beginning of the electric shock. The sound and shock were always administered in the compartment where animal was present at that moment. The electric shock was terminated after the animal left the compartment or after 5s. The inter-trial interval (ITI) was variable, with an average of 30s (±60%).

There were 30 trials daily for each animal for 5 consecutive days. We tested naïve animals administered either: quinpirole alone, QNP (n = 6); quinpirole in combination with clomipramine, QNP+CMI (n = 6); or clomipramine alone, CMI (n = 6). The experiment also included control animals administered saline, SAL (n = 7). Prior to experiment, animals received 10 administrations of QNP, CMI or both in their home-cages as sensitization to QNP takes approximately 10 injections (Szechtman et al., 1994). Therefore, the experiment was conducted
during the 11-15th QNP injections. At this time, animals received pharmaceuticals in their home-cage, and were tested 1.5 hour after CMI administration and/or 30 min after QNP administration.

Key parameters measured were the number of escapes after the conditioned stimulus (sound) prior to the unconditioned stimulus (conditioned stimulus escape; CSE) and escapes only after electric stimulation (unconditioned stimulus escape; USE). We also analyzed the ratio of US escapes to CS escapes (USE/(CSE + 1) – one is added to CSE for cases when CSE = 0). We considered this parameter as being an indicator of learning that is independent of freezing behavior or higher locomotion. Also, the number of escape failures – where an animal stayed in the same compartment even during the whole unconditioned stimulus - was recorded. Such ‘freezing’ behavior is related to anxiety, and we wanted to assess how QNP and CMI contribute to the expression of anxious behavior.

4.5 Statistical analyses

All statistical analysis was done using SPSS software (SPSS Inc., version 23, USA) Two-way repeated-measure ANOVA was used to compare all treatment groups in acquisition and reversal phases. Acquisition and reversal were analyzed and interpreted completely separately. Hochberg’s G2 post hoc test was used, which is powerful despite differences between group sizes. Chi square statistics was used to analyze number of animals excluded for reversal training based on less than 10 error criterion. Two-way active avoidance results were analyzed using three-way ANOVA.
#1: To characterize checking behavior in quinpirole treated rats in enriched open-field arena, as described by Szechtmian (1998).

#2: To determine if stereotypical checking in quinpirole treated rats is associated with changes in expression of immediate-early gene *Arc* in the hippocampal CA1 area.

#3: To determine if quinpirole treated rats display inflexible behavior in a hippocampus dependent Carousel maze task.

#4: To determine if clomipramine, risperidone or combination of thereof ameliorates cognitive flexibility deficit in quinpirole treated rats.

#5: To determine how quinpirole and clomipramine treatment affects a performance in a hippocampus independent two-way active avoidance in shuttle boxes.
6 RESULTS

6.1 Rats express stereotypical checking following repeated QNP treatment

In the first experiment we assessed if rats, repeatedly treated with quinpirole (QNP) display stereotypical checking behaviors was described before (Szechtman et al., 2001). We expanded the analysis of stereotypical behavior to include checking patterns of the whole open-filed area, not limiting ourselves to homebase visits as is common in literature (Dvorkin et al., 2010). We found that when animals injected with quinpirole are repeatedly introduced into the environment they develop between-sessions stable checking activity - measured as number of visits to inserted objects and corners of the arena (see figure 1)

Figure 1 | Depicts checking of zones in enriched openfield in 10 sessions. Panel A shows visits to openfield re-coded zones in SAL treated rats, where no pattern of between-session checking
developed. Panel B shows visits to openfield re-coded zones in QNP treated rats. Significant differences between visit frequencies are based on Bonferroni post-hoc test with level of accepted significance set to $p < 0.05$; denoted by asterix (*). Panels C and D show a typical trajectory of SAL treated rats (C) and QNP treated rats (D).

6.2 Intranuclear *Arc* mRNA expression in CA1 area of the hippocampus is lower during stereotypical checking in QNP treated rats compared to control rats

Next, we quantified plasticity related activity in CA1 region of hippocampus using ratio of *Arc+/*Arc- nuclei in samples of CA1 of rats treated with QNP, SAL in openfield and in home-cage conditions. We found that QNP treated rats and SAL treated rats had equally low *Arc* expression when in a home-cage. However, when introduced into familiar enriched openfield (where stereotypical checking in QNP treated animals was present) QNP treated animals had much lower numbers of *Arc*+ neurons than SAL rats (11 ± 4% compared to 33 ± 11%; $p<0.05$). Importantly, QNP treated animals displayed stereotypical behavior from previous session despite session to assess *Arc* expression was much shorter (5-min compared to 50-min). The reduction of session length was important to equilibrate locomotor activity between groups (which did not differ between groups).
6.3 Reversal learning on Carousel arena is impaired in QNP treated rats

Since we show that hippocampus has less $Arc^+$ cells compared to SAL rats in CA1 area of hippocampus, we hypothesized that QNP treated rats may be specifically impaired in hippocampus dependent task such as Carousel arena task. In this experiment we compared
acquisition and reversal learning of Carousel arena task between rats treated with QNP (QNP, n = 10) and control rats treated with saline (SAL, n = 11).

We shown that QNP treated rats displayed a significant increase of number of errors in carousel maze during first day of reversal session (Figure 3). Errors are entrances into to-be-avoided sector on a circular arena that are punished by a mild electric shock when animal enters it. Task is made difficult by the presence of arena rotation while the to-be-avoided sector remains stable in the room coordinates. Rats have to use extra-maze clues to localize to-be-avoided sector. During reversal, this to-be-avoided sector is re-localized to the opposite, previously safe, location on the arena.

Although both SAL treated and QNP treated rats had equal number of errors during four acquisition sessions and equal number of animals reached asymptotic performance; number of entrances during first day of reversal significantly differed between these two groups (SAL: 20 ± 5; QNP: 85 ± 14; p<0.05). Interestingly, QNP treated rats’ performance was not due to increased perseveration as measured by a level of avoidance of previously punished sector.
Figure 3 | Number of errors (entrances into the to-be-avoided sector (#/30 min)) in Carousel arena.

A-B. During acquisition sessions (ACQ1–ACQ4) when all rats are included. (D) Number of errors (#/30-min) in four reversal sessions (REV1–REV4). There was no difference in Carousel arena acquisition learning between QNP and SAL treated rats (A). Also, there was no difference between QNP and SAL treated rats that were tested in reversal (B). C. Displays number of errors during reversal training on Carousel arena. Post-Hoc test revealed a significantly more errors in QNP treated rats compared to Sal treated rats in first day of reversal testing (REV1, p<0.001). D. Percentage of time spent in former shock sector compared to mean time spent in always safe sectors during first 10-min of reversal (REV1). QNP-treated rats shown a significantly higher percentage of time spent in former to-be-avoided sector lower rate of perseveration compared to control rats (SAL).
6.4 Clomipramine and Risperidone combination improves reversal deficit in QNP treated rats

Next, we tested if commonly used OCD treatments can be effective in alleviating observed reversal learning deficit in QNP treated rats. We co-treated QNP treated rats with clomipramine (CMI) - a drug commonly used to treat OCD (Fallon et al., 1998), risperidone - an antipsychotic that is not effective in OCD but is an D2 antagonist (Alevizos et al., 2002) and therefore works in opposite direction to QNP. Lastly, we included QNP rats co-treated with both CMI and RIS – following a rationale that some of the treatment unresponsive OCD patients respond to a combination of antidepressants and antipsychotics (Hollander et al., 2003). Risperidone, in these treatment unresponsive cases was an antipsychotic of choice (Roman et al., 2012). Interestingly, CMI and RIS added alone to QNP treatment impaired acquisition performance. In fact, impairment following CMI augmentation was so severe that it inspired us to do a follow up experiment. Importantly, it was a combination of CMI and RIS that proved superior and restored QNP treated rats’ reversal performance to par with controls (Figure 4).

![Figure 4](image)

**Figure 4** | Number of errors in Carousel arena acquisition (panel A; ACQ1-ACQ5) and reversal (panel B; REV1-REV3). A. Comparisons are made between numbers of errors (entrances to the to-be-avoided sector) between all treatment groups during acquisition. Throughout all sessions the group receiving a combination QNP + CMI made significantly more errors than the control group. Moreover, in the first session the QNP + CMI group made significantly more errors than the group treated with a combination of QNP + CMI + RIS. On the last day of acquisition, the groups treated with QNP + RIS and QNP + CMI made significantly more errors than the SAL group. The average
number of errors is displayed ±SEM. * denotes a significant difference at p < 0.05. B. Comparisons of number of errors (entrances to the to-be-avoided sector) between all treated groups during reversal. In the reversal phase of the experiment there was significant impairment in QNP group compared to the SAL group. The average number of errors is displayed ±SEM. * denotes a significant difference from the control group at p < 0.05.

Lastly, we tested if the CMI augmentation impaired hippocampal function to a degree that was incompatible with Carousel maze learning. We utilized a task - cued active avoidance task in shuttle boxes that has one peculiarity: lesion of hippocampus facilitates learning in this task (Wang et al., 2015). Shuttle boxes are two interconnected compartments with electrified floor. In this task a rat has to pair sound with electric shock. Escape to the unoccupied compartment terminates the shock. Neither of compartments is predictive of shock, and natural pairing of place with the shock is what hinders the performance. For a healthy animal it is a struggle to overcome natural contextual learning. Here we shown that rats treated with QNP display a superior learning compared to control rats when measured by number of conditioned responses (escapes to the sound, see figure 5). This is greatly indicative of postulated functional hippocampal impairment in QNP induced animal model of OCD.
**Figure 5** | Two-way active avoidance in shuttle boxes. A. The number of conditioned escapes (CSE). B. The number of unconditioned escapes (USE). C. The ratio of unconditioned escapes to conditioned escapes (USE/(CSE + 1)). D. The number of escape failures. There was a significant increase in the number of conditioned escapes when quinpirole was included, and significantly reduced freezing when either quinpirole, clomipramine, or both was included. The average number of errors is displayed ±SEM. Significances are not included, because data was analyzed by three-way ANOVA.
Here we confirmed that rats treated with QNP display stereotypical checking as described by the inventor of this OCD animal model – prof. Szechtman (Szechtman et al., 1998). Stereotypical checking, typical of animals repeatedly treated with QNP, is traditionally observed in open-field arena enriched with 2-3 objects. Animals displaying stereotypical checking behavior usually repeatedly visit some of these objects and/or selected corners of the arena (together called locales). In many studies, visits to ‘homebase’ – the most frequently visited locale – is considered as a main measure of stereotypical checking. However, human OCD rituals are usually complex (Swedo et al., 1989). To determine if more complex pattern of stereotypical checking is also present in QNP treated rats we extended the analysis and examined the stability of preference for all locales between all consecutive sessions. We show that the idiosyncratic pattern of visits to locales that developed in each QNP-treated rat was indeed preserved across sessions. We found that QNP treated rats display the same pattern of preference not only for the ‘homebase’ but also for locales in all sessions. Importantly, CA1 hypoactivity emerged only during stereotypical checking and not during rest or during exploration in untreated control rats.

Importantly, QNP treated rats also displayed similar pattern of checking when session in enriched open field was much shorter: during 5-min sessions that preceded sacrifice for establishment of IEG Arc expression.

To measure the activity of hippocampal CA1 region in QNP sensitization model of OCD we used in situ hybridization of Arc mRNA. Although using IEG expression restricts output to a binary characterization of previously active or inactive neurons (pattern, rate and spatial specificity of firing cannot be captured), IEG expression profiles reveal important information about which neurons were plastically recruited during given behavioral experience (Minatohara et al., 2015). In present study we observed a robust decrease of Arc expressing neurons in CA1 of hippocampus - 60% decrease of Arc+ nuclei compared to exploring controls - during stereotypical checking in enriched open-field arena in QNP treated rats. Importantly, rats which were repeatedly treated with QNP, but were resting (cage controls), CA1 activity was comparable to control animals. This finding stresses the importance of environmental dependency of QNP sensitization in the development of checking behavior (Szechtman et al., 1993). As mentioned above, although activity of neurons was measured during shorter period than ‘standard’ sensitization session (5-
min compared to 50-min) pattern of stereotypical checking remained very similar. Moreover, assessing IEG expression following 5-min short sessions had an important advantage in equalizing locomotor activity between groups, as control animals were usually hypoactive later into the session. In other studies that measured regional brain activity using local cerebral glucose utilization possible effect of locomotion on study outcome was not accounted for (Carpenter et al., 2003). Yet, our findings are in line with findings of studies that examined brain activity in QNP treated rats without checking activity. The [18F]-FDG uptake was decreased by 19% in hippocampus in anesthetized QNP treated animals measured by MicroPET/CT imaging (Servaes et al., 2016) and a statistically insignificant decrease of hippocampal cerebral glucose utilization was observed in QNP sensitized rats in locomotor activity boxes (Carpenter et al., 2003).

Next we show that QNP treated rats showed a significant, albeit transient, reversal-learning deficit. This reversal deficit was manifested as an increased number of errors during the first session after reversal of to-be-avoided sector compared to the control group. It should be noted that this deficit was specific only for the beginning of reversal training and was not of perseverative nature. Lack of perseveration is in contrast with repeated findings that chronic administration of quinpirole induces perseverative behavior in alternation tasks (Einat and Szechtman, 1995; Kontis et al., 2008). Similarly, perseverance is indicated by studies documenting enhanced ‘compulsive’ lever pressing after repeated administration of QNP (Joel et al., 2001). Moreover, in lever pressing task a marked reversal learning deficit was associated with a high incidence of perseverative responding following QNP administration (Boulougouris et al., 2009). Despite the oft-reported perseverative behavior, non-perseverative behavior in reversal was also observed following D2-like manipulation. For example, non-perseverative errors in reversal were demonstrated when quinpirole was infused locally into the nucleus accumbens (Haluk and Floresco, 2009). Non-perseverative errors were also observed in spatial reversal learning in humans after systemic administration of another D2-like agonist – bromocriptine (Mehta et al., 2001). Still, the low perseveration in QNP treated rats in our study is intriguing in light of previous studies that have reported high perseverative behavior specifically in chronically QNP treated rats (Kontis et al., 2008; Boulougouris et al., 2009).

In the last two experiments, we investigated the effects of commonly used pharmacological treatments used in OCD on cognitive flexibility performance in QNP treated rats. First, we showed a cognitive flexibility deficit in QNP treated rats. Second, we observed effects of the
tricyclic antidepressant clomipramine (CMI), the antipsychotic risperidone (RIS), and a combination thereof on cognitive coordination and flexibility. We discovered that CMI impaired acquisition learning in QNP treated rats. Moreover, we showed that RIS does not ameliorate the learning impairment of QNP treated rats, but the administration of CMI and RIS together - a combination of pharmaceuticals used in SRI-resistant OCD patients - ameliorates the reversal learning deficit induced by QNP treatment. Last, using a behavioral test that does not require the hippocampus, we discovered that CMI augmentation in QNP treated rats is specific for the hippocampus-dependent Carousel arena task.

Despite the detrimental effect of CMI alone and no positive effect of RIS alone when administered to QNP treated rats, when CMI and RIS are co-administered, the reversal deficit is decreased in QNP treated rats. Offering a potential mechanism of action would be pure conjecture given the complexity of the molecular targets of both CMI and RIS. Yet, our findings still have important implications for OCD treatment. Risperidone is the most effective antipsychotic prescribed to augment the SRI treatment of OCD (Dold et al., 2013), and it is intriguing that risperidone augmentation improved reversal learning in an animal model of OCD. It has been proposed that antipsychotics benefit patients treated with antidepressants because they decrease dopaminergic tone (Chernoloz et al., 2009). This is in line with our proposed mechanism of an acquisition-learning deficit after clomipramine augmentation (which could have aggravated a dopamine neurotransmission reduction). The effectiveness of clomipramine and risperidone in rescuing the reversal deficit in QNP treated rats opens up a possibility that a combination of SRI with an antipsychotic may be the best treatment option for OCD patients with cognitive flexibility deficits.
8 SUMMARY

One goal of this work is to point to new potential directions in the research on Obsessive Compulsive Disorder. Alterations in hippocampal shape and function have often been reported in literature, but were not given much attention. In our research, we may have stumbled onto an important aspect of OCD. Although our findings are solely correlational, we have discovered that an impaired hippocampal state is associated with stereotypical checking and cognitive inflexibility – both hallmarks of OCD. Namely, we found the reduced expression of the immediate-early gene Arc (a marker of plasticity-related neuronal activity) in the main hippocampal output region – the CA1. Also, at the level of behavior, we found impairment in a hippocampus-dependent task and superior performance in a hippocampus-independent task in the QNP sensitization model of OCD. The present study also assessed the effects of common drugs prescribed to OCD patients – clomipramine, risperidone and the combination thereof – on cognitive flexibility in the QNP sensitization animal model of OCD. We found that the combination of clomipramine and risperidone may be the most effective in rescuing QNP induced cognitive deficits, as acquisition and reversal performance were spared in animals receiving this treatment. We propose that QNP sensitization could model a subgroup of OCD patients with cognitive flexibility deficits stemming from hippocampal dysfunction. We further suggest that this subgroup of patients may benefit most from the augmentation of SRI treatment with antipsychotics to improve cognitive flexibility.
9 SHRNUTÍ

Jedním z cílů této práce je poukázat na nové potenciální směry ve výzkumu Obsedantně kompulzivní poruchy. Často byly v literatuře zmíněny změny tvaru a funkce hipokampu, ale nebyl jim věnován dostatek pozornosti. V našem výzkumu se nám možná podařilo narazit na důležitý aspekt OCD. Ačkoli jsou naše nálezy pouze korelační, objevili jsme, že zhoršený hipokampální stav je spojen se stereotypní kontrolou a kognitivní neflexibilitou - tedy dvěma znaky OCD. Konkrétně jsme našli sníženou expresi časného genu Arc (markeru neuronální aktivity související s plasticitou) v hlavní hipokampální výstupní oblasti - CA1. Kromě toho jsme zjistili poškození na úrovni chování v úloze závislé na hipokampe a naopak vynikající výkon v úloze nezávislé na hipokampus v quinpirovém senzitizačním modelu OCD. Tato studie také hodnotila účinky běžných léků předepisovaných pacientům s OCD - klomipraminem, risperidonem a jejich kombinací - na kognitivní flexibilitu u zvířecího modelu senzitizace QNP u OCD. Zjistili jsme, že kombinace klomipraminu a risperidonu může být nejúčinnější při záchrane kognitivních deficitů vyvolaných QNP, protože u modelových zvířat, kterým byla podávána tato léčba, nedošlo k narušení akvizice ani přeučení. Předpokládáme, že senzitizace QNP by mohla modelovat podskupinu pacientů s OCD s deficitem kognitivní flexibility vyplývajícím z hipokampální dysfunkce. Dále předpokládáme, že této podskupině pacientů by nejvíce pomohla léčba SRI doplněná o podávání antipsychotik ke zlepšení kognitivní flexibility.
10 REFERENCES


Publications relevant to the thesis:


Other publication:


